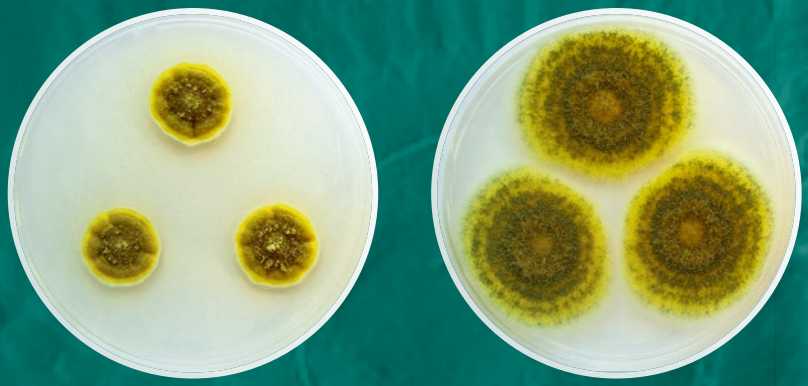
John I. Pitt
Ailsa D. Hocking

Fungi and Food Spoilage

Fourth Edition



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Fungi and Food Spoilage

John I. Pitt • Ailsa D. Hocking

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Fourth Edition

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This book is dedicated to John Pitt, who passed away during the final stages of its production; it stands as a tangible and enduring legacy of his passion for his science.

Ailsa D. Hocking

Preface to the Fourth Edition

It is now 40 years since we set out to write the first edition of this book, which was published in 1985. During the late 1980s and early 1990s many papers were published on the physiology and ecology of foodborne fungi, which permitted considerable expansion in the second edition. In addition, the second edition drew on our own experiences in studying the mycobiota of Southeast Asian commodities and permitted a large increase in included species. That list had changed little since. The third edition we regarded as "evolutionary, rather than revolutionary" as knowledge of species specificity in substrate and in mycotoxin production improved greatly in the preceding years. Early steps in molecular taxonomy were incorporated. At that time, taxonomy appeared quite stable. However, that stability lasted only a few years. The decision by the Melbourne Botanical Congress to implement an immediate end to dual nomenclature had a major impact on accepted names in several important food spoilage genera – an impact not yet fully resolved. Improvements in molecular methods saw changes to the circumscription of some genera and a large increase in molecular information resulted in the addition of many species to all of the major genera. Names of a number of species included here were changed – apart from the actual name changes, the overall list of species that are of importance in food spoilage has been relatively little affected. However, the very important disciplines of physiology and ecology of foodborne fungi have been neglected since 2012, with a great emphasis on molecular taxonomy instead. The information provided by the great increase in molecular information sheds no light at all on physiology or ecology. Major changes in the fourth edition come from decisions made at the Melbourne Congress and the expansion in molecular information, as noted above. However, we have incorporated other changes as well. We have included a new chapter on "Mycotoxins", designed to provide basic information related to food spoilage fungi in particular. We have incorporated colour figures where that improves clarity of descriptions and ease of use. We have also upgraded other information on species in this edition where new information has been published. We thank Dr Tom May of the Royal Botanic Gardens, Melbourne, Victoria, for invaluable advice on thorny nomenclatorial and taxonomic questions, and Dr David Miller of Carleton University, Ottawa, Canada, for reviewing the mycotoxin chapter. We also thank Mr Nick Charley and Mr Mark Wilson for curation of the FRR fungal culture collection at CSIRO, North Ryde, NSW. We also thank Dr Paul Kirk of CABI, UK,

for establishing and maintaining the authoritative *Index Fungorum*, which we have used extensively as a nomenclatural guide.

With the closure of the North Ryde Laboratory, curation, maintenance and distribution of cultures from the FRR collection has been transferred to the DAR Plant Pathology and Mycology Herbarium held at the NSW Dept of Primary Industries, Orange, NSW 2800, Australia. The FRR collection will be maintained under original FRR numbers and will be the only source of FRR cultures in the future. For enquiries please contact the Curator, Dr J. Bailey, email: jordan.bailey@dpi.nsw.gov.au, or Assistant Curator, Ms K. Cowan, email karren.cowan@dpi.nsw.gov.au.

Preface to the Third Edition

In contrast to the second edition, the Third Edition of "Fungi and Food Spoilage" is evolutionary rather than revolutionary. The second edition was intended to cover almost all of the species likely to be encountered in mainstream food supplies, and only a few additional species have been included in this new edition. The third edition represents primarily an upgrading – of taxonomy, physiology, mycotoxin production and ecology. Changes in taxonomy reflect the impact that molecular methods have had on our understanding of classification but, it must be said, have not radically altered the overall picture. The improvements in the understanding of the physiology of food spoilage fungi have been relatively small, reflecting perhaps the lack of emphasis on physiology in modern microbiological science. Much remains to be understood about the specificity of particular fungi for particular substrates, of the influence of water activity on the growth of many of the species treated, and even on such simple parameters as cardinal temperatures for growth and the influence of pH and preservatives. Since 1997, a great deal has been learnt about the specificity of mycotoxin production and in which commodities and products specific mycotoxins are likely to occur. Changes in our understanding of the ecology of the included species is also in most cases evolutionary. A great number of papers have been published on the ecology of foodborne fungi in the past few years, but with few exceptions the basic ecology of the included species remains.

Recent changes in our understanding of foodborne fungi include the realisation that *Aspergillus carbonarius* is a major source of ochratoxin A in the world food supply, that *Aspergillus westerdijkiae* and not *A. ochraceus* is the other common *Aspergillus* species making this toxin and that these species are responsible for ochratoxin A in foods outside the cool temperate regions, where *Penicillium verrucosum* is the important species. In recent years a number of new species have been found to be capable of producing aflatoxin, but the fact remains that most aflatoxin in the global food supply is produced by *Aspergillus flavus* and *A. parasiticus*. The taxonomy of *Fusarium* species is still undergoing major revision. However the renaming of *Fusarium moniliforme* as *F. verticillioides* is the only change of importance here. Recent publications have improved our understanding of species – mycotoxin relationships within *Fusarium*.

Among the colleagues who helped us to prepare this edition, we wish to particularly thank Dr A.-L. Markovina, now of the University of Sydney, who assisted in literature searches and some cultural and photographic work, and Mr N.J. Charley who has continued his excellent work of curating the FRR culture collection, on which so much of the descriptive work in this book is based.

Preface to the Second Edition

In planning for the second edition of *Fungi and Food Spoilage*, we decided that the book would benefit from a larger format, which would permit improved illustrations, and from some expansion of the text, in both numbers of species treated and overall scope. These aims have been realised. The Crown Quarto size has allowed us to include substantially larger, clearer illustrations. Many new photographs and photomicrographs have been added, the latter taken using a Zeiss Axioscop microscope fitted with Nomarski differential interference contrast optics. We have taken the opportunity to include more than 40 additional species descriptions, to add a new section on mycotoxin production for each species, and to update and upgrade all of the text.

Since the first edition, changes in climate for stabilising fungal nomenclature have resulted in development of a list of "Names in Current Use" for some important genera, including *Aspergillus* and *Penicillium.* Names of species used in the second edition are taken from that list, which was given special status by the International Botanical Congress, Tokyo, 1994. Names used in this edition have priority over any other names for a particular species. Publication of a list of "Authors of Fungal Names" (P. M. Kirk and A.E. Ansell, Index of Fungi, Supplement: 1–95, 1992) has also stabilised names of authorities for all fungal species. Abbreviations of authors' names used in this edition conform to those recommended by Kirk and Ansell. Some progress in standardisation of methods and media has also been made, primarily through the efforts of the International Commission on Food Mycology.

The first edition included some 400 references. When we began revisionary work, we felt that the number of references in the area of food mycology had probably doubled or increased by perhaps 150% during the intervening years. In fact, this second edition includes over 1900 references, almost a 5 fold increase over the 1985 edition! This provides a clear indication that interest in, and study of, food mycology has greatly increased in recent years. Modern referencing systems have enabled us to expand information from tropical sources, especially in Asia and Africa, but we are conscious of the fact that treatment of fungi found in foods on a worldwide basis remains rather incomplete.

We gratefully acknowledge support and assistance from colleagues who have contributed to this new edition. Ms J.C. Eyles formatted and printed the camera ready copy, Ms C. Heenan collated, arranged and formatted the illustrations, and Mr N.J. Charley looked after the culture collection, culture growth and colony photography. Without this level support, the book would not have been completed.

Preface to the First Edition

This book is designed as a laboratory guide for the food microbiologist, to assist in the isolation and identification of common foodborne fungi. We emphasise the fungi which cause food spoilage, but also devote space to the fungi commonly encountered in foods at harvest and in the food factory. As far as possible, we have kept the text simple, although the need for clarity in descriptions has necessitated the use of some specialised mycological terms.

The identification keys have been designed for use by microbiologist with little or no prior knowledge of mycology. For identification to genus level, they are based primarily on the cultural and physiological characteristics of fungi grown under a standard set of conditions. The microscopic features of the various fungi become more important when identifying isolates at the species level. Nearly all of the species treated have been illustrated with colony photographs, together with photomicrographs or line drawings. The photomicrographs were taken using a Zeiss WL microscope fitted with Nomaski interference contrast optics. We are indebted to Mr W. Rushton and Ms L. Burton, who printed the many hundreds of photographs used to make up the figures in this book.

We also wish to express our appreciation to Dr D.L. Hawksworth, Dr A.H.S. Onions and Dr B.C. Sutton of the Commonwealth Mycological Institute, Kew, Surrey, UK, Professor P.E. Nelson and the staff of the Fusarium Research Center, University of Pennsylvania, USA and Dr L.W. Burgess of the University of Sydney, who generously provided facilities, cultures and advice on some of the genera studied.

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Introduction

1

From the time when primitive man began to cultivate crops and store food, spoilage fungi have demanded their share. Fuzzes, powders and slimes of white, black, green, orange, red or brown have silently invaded – acidifying, fermenting, discolouring and disintegrating, rendering nutritious commodities unpalatable or unsafe.

Until comparatively recently, fungi have generally been regarded as causing only unaesthetic spoilage of food, despite the fact that *Claviceps purpurea* was linked to human disease more than 250 years ago, and the acute toxicity of macrofungi has long been known. Japanese scientists recognised the toxic nature of yellow rice 120 years ago, but 70 years elapsed before its fungal cause was confirmed. Alimentary toxic aleukia killed many thousands of people in the USSR in 1944–47: although fungal toxicity was suspected by 1950, the causal agent, T-2 toxin, was not clearly recognised for another 25 years (Pitt and Miller 2017).

Forgacs and Carll (1952), in a prophetic article, warned of the danger from common spoilage fungi, but it was not until 1960, when the famous Turkey "X" disease killed 100,000 turkey poults in Great Britain, and various other disease outbreaks followed in rapid succession, that the Western world became aware that common spoilage moulds could produce dangerous toxins. Since 1960 a stream of toxigenic fungi and potentially toxic compounds has been discovered (Pitt and Miller 2017). On these grounds alone, the statement 'It's only a mould' has become no longer acceptable to food microbiologist, health inspector or consumer. The demand for accurate identification and characterisation of food spoilage fungi has become urgent.

In the rush of research into mycotoxins, however, it must not be forgotten that food spoilage as such remains an enormous problem throughout the world. Figures are difficult to obtain. However, even given a dry climate and advanced technology, losses of food to fungal spoilage in Australia must be in excess of $10,000,000 per annum: losses in humid tropical climates and countries with less highly developed technology remain staggering. An estimate 10–20% of all food production lost to fungal growth is not unrealistic. Research into fungal food spoilage and its prevention is clearly an urgent necessity: lacking in spectacular appeal, it is, however, often neglected. A further point, of the highest significance, needs emphasis here. Research on the fungi which cause food spoilage, and the mycotoxins they produce, can only be carried out effectively if based on accurate identification of the microorganisms responsible. Taxonomy and nomenclature (systematics) make up the vital root system of all the trees of biological science.

The prevention of fungal food spoilage as an art is old, but as a discipline, young. Drying, the oldest method of food preservation, has been practiced for millennia, and is still the most common, effective and cheap technique for preserving

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food. Only recently have we been able to identify with certainty the fungal species which cause spoilage of dried foods. Prediction of their responses to a given environment, specified by physico-chemical parameters such as water activity, temperature, pH and oxygen tension, even now is sometimes uncertain.

Within historic times, newer methods of food preservation have been introduced – salting, curing, canning, refrigeration, freezing, the use of preservatives, irradiation and most recently, high hydrostatic pressure. Freezing excepted, each new technique has selected for one or more fungal species resistant to the process applied. As examples we can take *Polypaecilum pisce* on salt fish, *Xeromyces bisporus* on fruit cake, *Cladosporium herbarum* on refrigerated meat, *Zygosaccharomyces bailii* in preserved juices, *Z. rouxii* in jams and fruit concentrates, *Aspergillus flavus* on peanuts, *A. chevalieri* on hazel nuts, *Penicillium commune* on cheeses, *Byssochlamys fulva* in acid canned foods ..... the list of quite specific food – fungus associations is extensive. The study of such associations is one of the more important branches of food mycology.

This book sets out to document current knowledge on the interaction of foods and fungi, in the context of spoilage and toxicity, not food production or biotechnology. Four aspects are examined. First, ecology: what factors in foods select for particular kinds of fungi? A chapter is devoted to the physical and chemical parameters which influence the growth of fungi in foods. Second, methodology: how do we isolate fungi from foods? Which are the best media to use? A chapter is devoted to that topic. Third, the commodity: which fungi are usually associated with a particular food or method of preservation? Here ecological factors interact to produce a more or less specific habitat. Major classes of foods and their associated spoilage fungi are described. Next, the fungus: what fungus is that? How do we go about identifying food spoilage and mycotoxigenic fungi? In a series of chapters, the main food spoilage moulds and yeasts are described and keyed, together with others commonly associated with food but not noted for spoilage. Where possible, further information is given on known habitats and sources, physiology, heat resistance etc., together with a selective bibliography. Finally, is the identified fungus likely to be toxic. As well as a general chapter on mycotoxins – toxicity, formation and methods of control, accurate information on mycotoxin production by many specific fungi is also included.

As far as possible, the precise terminology for fungal structures used by the pure mycologist and indeed most necessary for him has been avoided in these chapters. Some concepts and terms are of course essential: these have been introduced as needed and are listed in a glossary.

The taxonomic sections of this book are designed to facilitate identification of food spoilage and common food contaminant fungi. A standardised plating regimen is used, originally developed for the identification of *Penicillium* species (Pitt 1979) and extended here to other genera relevant to the food industry. Under this regimen, cultures are incubated for one week at 5, 25 and 37 °C on a single standard medium and at 25 °C on two others. In conjunction with the appropriate keys, this system will enable identification of most foodborne fungi to species level in just seven days. For a few kinds of fungi, notably *Fusarium* species, yeasts and xerophiles, subsequent growth under other more specialised conditions will be necessary.

Finally, this book is dedicated to the general food microbiologist. May it help to restore equilibrium and assist in continued employment, when the quality assurance manager demands: 'What is it?'..... 'How did it get in?'..... 'What does it do?'..... 'How do we get rid of it?'..... and, worst of all..... 'Is it toxic?'

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Ecology of Fungal Food Spoilage

2

Food is not commonly regarded as an ecosystem, perhaps on the basis that it is not a "natural" system. Nevertheless an ecosystem it is and an important one, because food plants and the fungi that colonise their reproductive parts (seeds and fruit), have been co-evolving for millennia. The seed and nut caches of rodents have provided a niche for the development of storage fungi. Fallen fruit, as they go through the cycle of decay and desiccation, have provided substrates for a wide range of pathogenic and spoilage fungi also. Humans have aided and abetted the development of food spoilage fungi through the setting up of vast and varied food stores. It can be argued, indeed, that rapidly evolving organisms, such as haploid asexual fungi, are moving into niches created by man's exploitation of certain types of plants as food.

Food by its very nature is expected to be nutritious: therefore food is a rich habitat for microorganisms – in contrast with the great natural systems, soil, water and plants. Given the right physico-chemical conditions, only the most fastidious microorganisms are incapable of growth in foods, so that factors other than nutrients usually select for particular types of microbial populations.

Perhaps the most important of these factors relates to the biological state of the food. Living foods, particularly fresh fruits, vegetables, and also grains and nuts before harvest, possess powerful defence mechanisms against microbial invasion. The study of the spoilage of such fresh foods is more properly a branch of plant pathology than food microbiology. The overriding factor determining spoilage of a fresh, living food is the ability of specific microorganisms to overcome defence mechanisms. Generally speaking, then, spoilage of fresh foods is limited to particular species. Such specific relationships between fresh food and fungus are discussed in Chap. 11 and under particular species.

Other kinds of foods are either moribund, dormant or nonliving, and the factors which govern spoilage are physical and chemical. There are eight principal factors:

1. Water activity;
2. Hydrogen ion concentration – pH;
3. Temperature – of both processing and storage;
4. Gas tension, specifically of oxygen and carbon dioxide;
5. Consistency – solid or liquid;
6. Nutrient status;
7. Specific solute effects; and
8. Preservatives.

Each will be discussed in turn below.

2.1 Water Activity

Water availability in foods is most readily measured as water activity. Water activity (a_w), is a physico-chemical concept, introduced to

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microbiologists by Scott (1957), who showed that a_w effectively quantifies the relationship between moisture in foods and the ability of microorganisms to grow on them.

Water activity is defined as a ratio:

$$a_w = p / p_o$$

where p is the partial pressure of water vapour in the test material and p_o is the saturation vapour pressure of pure water under the same conditions.

Water activity is numerically equal to equilibrium relative humidity (ERH) expressed as a decimal. If a sample of food is held at constant temperature in a sealed enclosure until the water in the sample equilibrates with the water vapour in the enclosed air space (Fig. 2.1a), then

$$a_w(\text{food}) = \text{ERH}(\text{air}) / 100.$$

Conversely, if the ERH of the air is controlled in a suitable way, as by a saturated salt solution, at equilibrium the a_w of the food will be numerically equal to the generated ERH (Fig. 2.1b). In this way a_w can be experimentally controlled, and the relationship of a_w to moisture (the sorption isotherm) can be studied. For further information on water activity, its measurement and significance in foods see Duckworth (1975), Pitt (1975), Troller and Christian (1978) and Rockland and Beuchat (1987). For sorption isotherms of a wide range of foods see Iglesias and Chirife (1982). For an understanding of the mathematical relationships underlying the shape of sorption isotherms see Timmermann et al. (2001).

In many practical situations, a_w is the dominant environmental factor governing food stability or spoilage. A knowledge of fungal water relations will then enable prediction both of the shelf life of foods and of potential spoilage fungi. Although the water relations of many fungi will be considered individually in later chapters, it is pertinent here to provide an overview.

Like that of all other organisms, growth of fungi is profoundly affected by the availability of water. On the a_w scale, life as we know it exists over the range 0.9999+ to 0.60 (Table 2.1) or possibly as low as 0.585 (Stevenson et al. 2017). Growth of animals is virtually confined to 1.0–0.99 a_w; the permanent wilt point of mesophytic plants is near 0.98 a_w; and most microorganisms cannot grow below 0.95 a_w. A few halophilic algae, bacteria and archaea can grow in saturated sodium chloride (0.75 a_w) and even lower in mixed salt solutions (Stevenson et al. 2015), but are confined to salty environments. Ascomycetous fungi and conidial fungi of ascomycetous origin comprise most of the organisms capable of growth below 0.9 a_w. Fungi capable of growth at low a_w, in the presence of extraordinarily high solute concentrations both inside and out, must be ranked as among the most highly evolved organisms on earth. Even among the fungi, this evolutionary

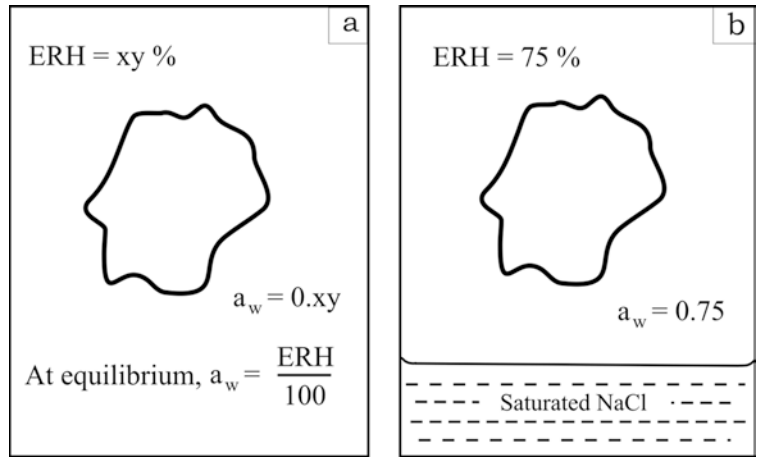

Fig. 2.1 The concept of water activity (a_w) (**a**) the relationship between a_w and equilibrium relative humidity (ERH); (**b**) one method of controlling a_w by means of a saturated salt solution, which generates a specific ERH at a specific constant temperature

Table 2.1 Water activity and microbial water relations in perspective[a]

a_w	Perspective	Foods	Moulds	Yeasts
1.00	Blood Plant wilt point Seawater	Vegetables Meat, milk Fruit		
0.95	Most bacteria	Bread	Basidiomycetes Most soil fungi	Basidiomycetes
0.90		Ham	Mucorales *Fusarium*	Most ascomycetes
0.85	*Staphylococcus aureus*	Dry salami	*Rhizopus* *Cladosporium*	*Zygosaccharomyces rouxii* (salt)
0.80			*Aspergillus flavus* Xerophilic Penicillia	*Zygosaccharomyces bailii*
0.75	Salt lake Halophiles	Jams Salt fish Fruit cake	Xerophilic Aspergilli *Wallemia* *Eurotium*	*Debaryomyces hansenii*
0.70		Confectionery Dried fruit Dry grains	*Xerochrysium* *Eurotium halophilicum*	
0.65			*Xeromyces bisporus*	*Zygosaccharomyces rouxii* (sugar)
0.60	DNA disordered			

[a]Modified from data of J.I. Pitt as reported by Brown (1974). Water activities shown for microorganisms approximate minima for growth reported in the literature

path must have been of the utmost complexity: the ability to grow at low a_w is confined to only a handful of genera (Pitt 1975).

The degree of tolerance to low a_w is most simply expressed in terms of the minimum a_w at which germination and growth can occur. Fungi able to grow at low a_w are termed xerophiles: one widely used definition is that a xerophile is a fungus able to grow below 0.85 a_w under at least one set of environmental conditions (Pitt 1975). Xerophilic fungi will be discussed in detail in Chap. 9.

Information about the water relations of many fungi remains incomplete, but where it is known it has been included in later chapters.

2.2 Hydrogen Ion Concentration

At high water activities, fungi compete with bacteria as food spoilers. Here pH plays the decisive role. Bacteria flourish near neutral pH and fungi cannot compete unless some other factor, such as low water activity or a preservative, renders the environment hostile to the bacteria. As pH is reduced below about 5, growth of bacteria becomes progressively less likely. Lactic acid bacteria are exceptional, as they remain competitive with fungi in some foods down to about pH 3.5. However, most fungi are little affected by pH over a broad range, commonly 3–8 (Wheeler et al. 1991). Some conidial fungi are capable of growth down to pH 2, and yeasts down to pH 1.5. However, as pH moves away from the optimum, usually about pH 5, the effect of other growth limiting factors may become apparent when superimposed on pH. Figure 2.2 is an impression of the combined influence of pH and a_w on microbial growth. The diagram is schematic: wide variation exists among organisms.

For heat processed foods, pH 4.5 is critical: the heat processing necessary to destroy the spores of *Clostridium botulinum* also destroys all fungal spores. In acid packs, below pH 4.5, less severe processes may permit survival of heat resistant fungal spores (Sect. 2.3).

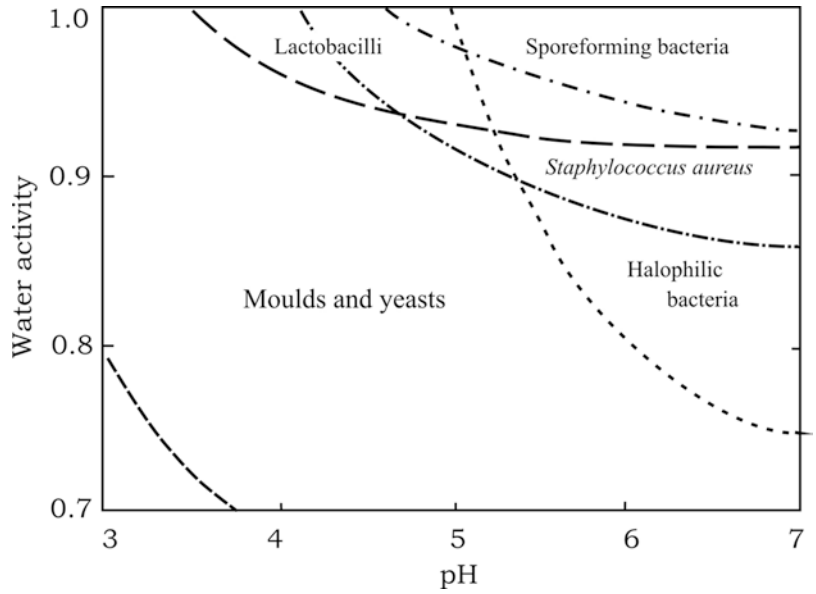

Fig. 2.2 A schematic diagram showing the combined influence of water activity and pH on microbial growth

2.3 Temperature

The influence of temperature in food preservation and spoilage has two separate facets: temperatures reached during processing and those existing during storage.

As noted above, heat resistant fungal spores may survive pasteurising processes given to acid foods. Apart from a few important species, little information exists on the heat resistance of fungi. Much of the information that does exist must be interpreted with care, as heating menstrua and conditions can vary markedly, and these may profoundly affect heat resistance. High levels of sugars are generally protective (Beuchat and Toledo 1977). Low pH and preservatives increase the effect of heat (Beuchat 1981a, b; Rajashekhara et al. 2000) and also hinder resuscitation of damaged cells (Beuchat and Jones 1978).

Ascospores of filamentous fungi are more heat resistant than conidia (Pitt and Christian 1970; Table 2.2). Although not strictly comparable, data of Put et al. (1976) indicate that the heat resistance of yeast ascospores and vegetative cells is of the same order as that of fungal conidia.

Among the ascomycetous fungi, *Byssochlamys* species are notorious for spoiling heat processed fruit products (Olliver and Rendle 1934; Richardson 1965). The heat resistance of *B. fulva* ascospores varies markedly with isolate and heating conditions (Beuchat and Rice 1979): a D value between 1 and 12 min at 90 °C (Bayne and Michener 1979) and a z value of 6–8 °C (King et al. 1969; Kotzekidou 1997) are practical working figures. The heat resistance of *B. nivea* ascospores is marginally lower (Beuchat and Rice 1979; Kotzekidou 1997). Species of *Talaromyces*, *Hamigera* and *Thermoascus* seem to be emerging more commonly as heat resistant spoilage species in fruit juices and purees. D values of 1.6–2.6 min at 82 °C have been reported for *Talaromyces trachyspermus*, and 2.7–4.1 min at 88 °C for *T. bacillisporus* in blueberry and grape juice (Tranquillini et al. 2017). *Hamigera avellanea* was more heat resistant than *Talaromyces* species, with reported D values up to 0.43–1.25 min at 93 °C, and *Thermoascus crustaceus* the most heat resistant with D_{95} values between 1.11 and 2.53 min in blueberry or grape juice (Scaramuzza and Berni 2014).

Ascospores of *Neosartorya fischeri* have a similar heat resistance to those of *B. fulva*, but have been reported less frequently as a cause of food spoilage, although they occur commonly in raw materials such as berries. Heat resistant fungi are discussed further in Chap. 4.

Table 2.2 Comparative heat resistance of ascospores and conidia[a]

Fungus	Spore type	Initial viable count/ml	Survivors (%)		
			50 °C	60 °C	70 °C
Eurotium amstelodami	Ascospores Conidia	5.0×10^2 7.3×10^2	93 107	85 0.3	3 0
Eurotium chevalieri	Ascospores Conidia	1.0×10^3 8.9×10^2	103 128	62 0.1	21 0
Xeromyces bisporus	Ascospores	1.0×10^3	93	30	0.3
Aspergillus candidus	Conidia	3.8×10^2	102	0	0
Wallemia sebi	Conidia	7.1×10^2	42	0	0

[a]Heated at temperatures shown for 10 min. Data from Pitt and Christian (1970)

Food products may be stored under refrigeration, where temperature is expected to play a preservative role, or at ambient temperatures, in which case prevention of spoilage relies on other parameters. Food frozen to −10 °C or below appears to be microbiologically stable, despite some reports of fungal growth at even lower temperatures. The lowest reliably reported temperatures for fungal growth are in the range −7 to 0 °C, for species of *Fusarium*, *Cladosporium*, *Penicillium* and *Thamnidium* (Pitt and Hocking 1997). Nonsterile food stored at ca 5 °C in domestic refrigerators, where conditions of high humidity prevail, will eventually be spoiled by fungi of these genera. At high a_w and neutral pH, psychrophilic bacteria may also be important (mostly *Pseudomonas* species).

Thermophilic fungi, i.e. those which grow only at high temperatures, are rarely of significance in food spoilage. If overheating of commodities occurs, however, in situations such as damp grain, thermophiles can be a very serious problem.

Thermotolerant fungi, i.e. species able to grow at both moderate and high temperatures, are of much greater significance. *Aspergillus flavus* and *A. niger*, able to grow between ca 8 and 45 °C, are among the most destructive fungi known.

2.4 Gas Tension

Food spoilage moulds, like almost all other filamentous fungi, have an absolute requirement for oxygen. However, some species appear to be efficient oxygen scavengers, so that the total amount of oxygen available, rather than the oxygen tension, determines growth. The concentration of oxygen dissolved in the substrate has a much greater influence on fungal growth than atmospheric oxygen tension (Miller and Golding 1949). For example, *Penicillium expansum* has been reported to grow quite normally in only 2.1% oxygen over its entire temperature range (Golding 1945), and many other common food spoilage fungi are inhibited only slightly when grown in nitrogen atmospheres containing approximately 1.0% oxygen (Hocking 1990). *Paecilomyces variotii* (=*Byssochlamys spectabilis*) produced normal colonies when grown in our laboratory at 25 °C under 650 mm of vacuum.

Most food spoilage moulds appear to be sensitive to high levels of carbon dioxide, although there are notable exceptions. When maintained in an atmosphere of 80% carbon dioxide and 4.2% oxygen, *Penicillium roqueforti* still grew at 30% of the rate in air (Golding 1945), provided that the temperature was above 20 °C. At 25 °C, in 40% CO_2 and 1% O_2, *P. roqueforti* grew at almost 90% of the rate in air (Taniwaki et al. 2001a). *Xeromyces bisporus* was reported to grow in high levels of carbon dioxide (Dallyn and Everton 1969), but Taniwaki et al. (2009) could not repeat those observations.

Byssochlamys species are particularly tolerant of reduced oxygen and/or elevated carbon dioxide levels. Growth of *B. nivea* was little affected by replacement of nitrogen in air by carbon dioxide, and growth in carbon dioxide – air mixtures was proportional only to oxygen concentration, at least up to 90% carbon dioxide (Yates et al. 1967). Both *B. nivea* and *B. fulva* were capable of

growth in atmospheres containing 20%, 40% or 60% carbon dioxide with less than 0.5% oxygen, though inhibition increased with increasing carbon dioxide concentration (Taniwaki et al. 2001, 2009). Indeed *Byssochlamys* species can be correctly described as facultative anaerobes, growing under similar conditions to the obligate anaerobe *Clostridium sporogenes* (Taniwaki et al. 2009). *B. fulva* is capable of growth in 0.27% oxygen, but not in its total absence (King et al. 1969). It is also capable of fermentation in fruit products, but presumably only if some oxygen is present.

At least some species of *Mucor*, *Rhizopus* and *Fusarium* are able to grow and ferment in bottled liquid products and sometimes cause fermentative spoilage. Growth under these conditions may be yeast-like. Species of *Mucor*, *Rhizopus* and *Amylomyces* used as starter cultures in Asian fermented foods can grow under anaerobic conditions, demonstrated by growth in an anaerobe jar with a hydrogen and carbon dioxide generator (Hesseltine et al. 1985). Other authors have reported growth under anaerobic conditions of *Mucor* species, *Absidia spinosa*, *Geotrichum candidum*, *Fusarium oxysporum* and *F. solani* (= *Neocosmospora solani*) (Stotzky and Goos 1965; Curtis 1969; Taniwaki 1995; Taniwaki et al. 2009). The yeast-like fungus *Moniliella acetoabutans* can cause fermentative spoilage under totally anaerobic conditions (Stolk and Dakin 1966).

As a generalisation, however, it is still correct to state that food spoilage problems due to filamentous fungi usually occur under aerobic conditions, or at least where oxygen tension is appreciable, due to leakage or diffusion through packaging.

In contrast, *Saccharomyces* species, *Zygosaccharomyces* species and other fermentative yeasts are capable of growth in the complete absence of oxygen. Indeed, *S. cerevisiae* and *Z. bailii* can continue fermentation under several atmospheres pressure of carbon dioxide. This property of *S. cerevisiae* has been harnessed by mankind for his own purposes, in the manufacture of bread and many kinds of fermented beverages. *Z. bailii*, on the other hand, is notorious for its ability to continue fermenting at reduced water activities in the presence of high levels of preservatives. Fermentation of juices and fruit concentrates may continue until carbon dioxide pressure causes container distortion or explosion. The closely related species *Z. rouxii* is a xerophile, and causes spoilage of low moisture liquid or packaged products such as fruit concentrates, jams and dried fruit. The difference in oxygen requirements between filamentous fungi and fermentative yeasts is one of the main factors determining the kind of spoilage a particular commodity will undergo.

2.5 Consistency

Consistency, like gas tension, exerts considerable influence over the kind of spoilage to which a food is susceptible. Generally speaking, yeasts cause more obvious spoilage in liquid products, because single celled microorganisms are able to disperse more readily in liquids. Moreover, a liquid substrate is more likely to give rise to anaerobic conditions and fermentation is more readily seen in liquids. In contrast, filamentous fungi are assisted by a firm substrate and ready access to oxygen, but may grow on the surface of liquids.

The foregoing is not intended to suggest that yeasts cannot spoil solid products nor moulds liquids: merely that all other factors being equal, fermentative yeasts have a competitive advantage in liquids and cause more obvious spoilage under these conditions. In solid foods, filamentous fungi have the advantage of producing hyphae, capable of penetrating and utilising substrates, while yeasts (and bacteria) are confined to growing on surfaces only.

2.6 Nutrient Status

As noted in the preamble to this chapter, the nutrient status of most foods is adequate for the growth of any spoilage microorganism. Generally speaking, however, it appears that fungal metabolism is best suited to substrates high in carbohydrates, whereas bacteria are more likely to spoil proteinaceous foods. Lactobacilli are an exception.

Most common mould species appear to be able to assimilate any food-derived carbon source with the exception of hydrocarbons and highly condensed polymers such as cellulose and lignin. Most moulds are equally indifferent to nitrogen source, using nitrate, ammonium ions or organic nitrogen with equal ease. Some species achieve only limited growth if amino acids or proteins must provide both carbon and nitrogen. A few isolates classified in *Penicillium* subgen. *Biverticillium* (= *Talaromyces* in current taxonomies) are unable to utilise nitrate (Pitt 1979).

Some xerophilic fungi are known to be more demanding. Ormerod (1967) showed that growth of *Wallemia sebi* was strongly stimulated by proline. Xerophilic *Chrysosporium* species (= *Bettsia* and *Xerochrysium*) and *Xeromyces bisporus* also appear to require complex nutrients, but the factors involved have not been defined (Pitt 1975).

Yeasts are often fastidious. Many are unable to assimilate nitrate or complex carbohydrates; a few, *Zygosaccharomyces bailii* being an example, cannot grow with sucrose as a sole source of carbon. Some require vitamins. These factors limit to some extent the kinds of foods susceptible to spoilage by yeasts. This point is worth emphasising. Certain foods (or nonfoods) lack nutrients essential for the growth of *Z. bailii*. Addition of nutrient, for whatever reason, can turn a safe product into a costly failure. Two cases from our own experience illustrate this point. In the first, a highly acceptable (and nutritious) carbonated beverage containing 25% fruit juice was eventually forced from the Australian market because it was impractical to prepare it free of occasional *Z. bailii* cells. Effective levels of preservative could not be added legally and pasteurisation damaged its flavour. Substitution of the fruit juice with artificial flavour and colour removed the nitrogen source for the yeast. A spoilage free product resulted, at the cost of any nutritional value and a great reduction in consumer acceptance.

The other case concerned a popular water-ice confection, designed for home freezing. This confection contained sucrose as a sweetener and a preservative effective against yeasts utilising sucrose. One production season the manufacturer decided, for consumer appeal, to add glucose to the formulation. The glucose provided a carbon source for *Z. bailii*, and as a result several months production, valued at hundreds of thousands of dollars, was lost due to fermentative spoilage.

2.7 Specific Solute Effects

As stated earlier, microbial growth under conditions of reduced water availability is most satisfactorily described in terms of a_w. However the particular solutes present in foods can exert additional effects on the growth of fungi. Scott (1957) reported that *Aspergillus amstelodami* (= *A. montevidensis*) grew 50% faster at its optimal a_w (0.96) when a_w was controlled by glucose rather than magnesium chloride, sodium chloride or glycerol. Pitt and Hocking (1977) showed a similar effect for *A. chevalieri*, and reported that the extreme xerophiles *Chrysosporium fastidium* (= *Bettsia fastidia*) and *Xeromyces bisporus* grew poorly if at all in media containing sodium chloride as the major solute. In contrast Pitt and Hocking (1977) and Hocking and Pitt (1979) showed that germination and growth of several species of *Aspergillus* and *Penicillium* were little affected when medium a_w was controlled with glucose–fructose, glycerol or sodium chloride.

Zygosaccharomyces rouxii, the second most xerophilic organism known, has been reported to grow down to 0.62 a_w in fructose (von Schelhorn 1950). Its minimum a_w for growth in sodium chloride is reportedly much higher, 0.85 a_w (Onishi 1963).

Some fungi are halophilic, being well adapted to salty environments such as salted fish. *Phialosimplex halophilus* and *Polypaecilum pisce* grow more rapidly in media with NaCl as controlling solute, rather than glucose or glycerol (Andrews and Pitt 1987; Wheeler et al. 1988). Such fungi have been called halophilic xerophiles to distinguish them from obligately halophilic bacteria.

2.8 Preservatives

Obviously, preservatives for use in foods must be safe for human consumption. Under this constraint, food technologists in most countries are limited to the use of weak acid preservatives: benzoic, sorbic, nitrous, sulphurous, acetic and propionic acids – or, less commonly, their esters. In the concentrations permitted by most food laws, these acids are useful only at pH levels up to their pK_a plus one pH unit. Classical theory indicates that their effect is due to entry to cells as the undissociated acid, followed by dissociation and disruption of cell metabolism, however, that has been disputed recently (Stratford and Anslow 1998; Stratford et al. 2013). For earlier studies of the mechanism of action of weak acid preservatives see Warth (1977, 1991), Brul and Coote (1999) and Stratford and Lambert (1999).

The use of chemical preservatives in foods is limited by law in most countries to relatively low levels, and to specific foods. A few fungal species possess mechanisms of resistance to weak acid preservatives, the most notable being *Zygosaccharomyces bailii*. This yeast is capable of growth and fermentation in fruit based cordials of pH 2.9–3.0, of 45 °C Brix and containing 800 mg/L of benzoic acid (Pitt and Hocking 1997). The yeast-like fungus *Moniliella acetoabutans* can grow in the presence of 4% acetic acid, and survive in 10% (Pitt and Hocking 1997).

Of the filamentous fungi, *Penicillium roqueforti* appears to be especially resistant to weak acid preservatives, causing spoilage of preserved European bread (Suhr and Nielsen 2004). Weak acid resistance has been suggested as a useful aid to isolation and identification (Engel and Teuber 1978).

2.9 Conclusions: Food Preservation

It is evident from the above discussion that the growth of fungi in a particular food is governed largely by a series of physical and chemical parameters, and definition of these can assist greatly in assessing the food's stability. The situation in practice is made more complex by the fact that such factors frequently do not act independently, but synergistically. If two or more of the factors outlined above act simultaneously, the food may be safer than expected. This has been described by Leistner and Rödel (1976) as the "hurdle concept". This concept has been evaluated carefully for some commodities such as fermented sausages and is now widely exploited in the production of shelf stable bakery goods and acid sauces.

For most fungi, knowledge remains meagre about the influence of the eight parameters discussed here on germination and growth. However, sufficient information is now available that some rationale for spoilage of specific commodities by certain fungi can be attempted, especially where one or two parameters are of overriding importance. This topic is considered in later chapters devoted to particular commodities.

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Naming and Classifying Fungi

3

As with other living organisms, the name applied to any fungus is a binomial, a capitalised genus name followed by a lower case species name, both written in italics. The classification of organisms into genera and species was a concept introduced by Linneaus in 1753 and it is the keystone of biological science. The concept of a species is as fundamental to the biologist as Arabic decimal numeration is to the mathematician. Here the analogy ends: the concept of "base 10" is rigorous; the concept of a species, fundamental as it is, is subjective, and dependent on the knowledge and concepts of the biologist who described it.

3.1 Taxonomy and Nomenclature: Systematics

Once biologists began to describe species, and to assemble them into genera, questions about their relationships began to arise: is species x described by Jones in 1883 the same as species y described by Smith in 1942? Does species z, clearly distinct from x and y in some characters, belong to the same genus? The study of these relationships is termed **taxonomy**. Modern taxonomy is based on sound scientific principles, but still involves subjective judgment.

When the decision is made that species x and species y are the same, however, the taxonomist must follow clearly established procedures in deciding which name must be used ("has priority"). The application of these procedures is termed **nomenclature** and for our purposes is governed by the the International Code of Nomenclature for algae, fungi and plants (ICN; McNeill et al. 2012), known as "the Melbourne Code". Note that, as would be expected, "International Code of Nomenclature" is written with capitals, but "algae, fungi and plants" is not, as the ICN authors recognised that these names are terms of convenience rather than strict taxonomic entities.

The ICN is a relatively complex document of 62 Articles dealing with all aspects of correctly naming plants, algae and fungi. It is amended every 6 years by special sessions at each International Botanical Congress and is republished thereafter. The ICN impinges only indirectly on the work of the practicing mycologist or microbiologist. It is nevertheless of vital importance to the orderly naming of all plant life. To ignore the ICN is to invite chaos.

Where confusion arises over the correct name for a botanical species – a constant source of irritation to the nontaxonomist – it stems usually from one of three causes: indecision by, or disagreement among, taxonomists on what constitutes a particular species; incorrect application of the provisions of the ICN; or ignorance of earlier literature.

To return to our example: when species x and species y are seen to be the same, x has priority

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because it was published earlier; y becomes a **synonym** of x. Important synonyms are often listed after a name to aid the user of a taxonomy, and this procedure has been followed here.

Through ignorance, the same species name may be used more than once, for example, *Penicillium thomii* Maire 1915 and *P. thomii* K.M. Zalessky 1927. The name *P. thomii* has been given to two quite different fungi. Clearly *P. thomii* Maire has **priority**; the later name is not **valid**. To avoid ambiguity, correct practice in scientific publication is to cite the author of a species at first mention, and before any formal description.

The ICN provides rules to govern change of genus name also. In our example, if species z is transferred to the genus to which species x and y belong, it retains its species name but takes the new genus name. The original author of the name z is placed in brackets after the species name, followed by the name of the author who transferred it to the correct genus. For example, *Citromyces glaber* Wehmer 1893 became *Penicillium glabrum* (Wehmer) Westling 1893 on transfer to *Penicillium* by Westling in 1911. Note the use of Latinised names: *glaber* (masculine) became *glabrum* (neuter) to agree with the gender of the genus to which it was transferred.

Further points on the use of the ICN arise from this example. *Penicillium glabrum* retains its date of original publication, and therefore takes priority over *P. frequentans* Westling 1911 if the two species are combined. When Raper and Thom (1949) combined the two species, a taxonomically correct decision at that time, they retained the name *P. frequentans*, which was nomenclaturally incorrect, causing confusion when Subramanian (1971) and Pitt (1979) took up the correct name. It is worth pointing out that the confusion in this and similar situations arose from Raper and Thom's action in ignoring the provisions of the ICN, not from that of later taxonomists who correctly interpreted it. When, as the result of molecular studies, Houbraken et al. (2014) decided that *P. frequentans* was a distinct species from *P. glabrum*, it was reinstated, as it was still a valid name.

3.2 Hierarchical Naming

A given biological entity, or **taxon** in modern terminology, can be given a hierarchy of names: a cluster of related species is grouped in a genus, of related genera in families, of families in orders, orders in classes, and classes in phyla (singular phylum), in our case, all within the kingdom Fungi. Similarly a species can be divided into smaller entities: subspecies, varieties and *formae speciales* (a term usually reserved for plant pathogens).

Modern molecular methods have shown that fungi are more closely related to animals than to plants, but the transfer of fungal nomenclature from the plant to the animal code of nomenclature would have caused chaos, so has not been contemplated. Molecular methods have also shown that some organisms traditionally studied by mycologists – some so-called "lower fungi" – are not really fungi at all, but are included under fungal nomenclature in the ICN and no doubt will continue to be studied by mycologists.

Traditionally, kingdom Fungi has been divided into several phyla, based on spore type and some environmental considerations. Again molecular methods have revolutionised this hierarchical structure. From the point of view of the food mycologist, one important change introduced in the previous edition of this work (Pitt and Hocking 2009) was the demise of the old phylum Deuteromycota, absorbed almost entirely into the phylum Ascomycota. The connection between the Ascomycota, the fungi that produce sexual spores in enclosed bodies, and the Deuteromycota, where spores are always asexual, has been known for a long time. However, molecular taxonomy has provided a sound basis for the assurance needed to discontinue the use of Deuteromycota, by positively linking sexual and asexual stages in many species in a number of genera.

The hierarchical subdivisions in Kingdom Fungi of interest in the present context are shown below, using as examples three genera and species important in food spoilage. The ranks above Family are not controlled under the ICN: the rankings adopted by Kirk et al. (2008) have been followed here.

Kingdom	**Fungi**	**Fungi**	**Fungi**
Phylum	Zygomycota	Ascomycota	Basidiomycota
Subphylum	Mucoromycotina	Ascomycotina	
Class	Zygomycetes	Eurotiomycetes	Wallemiomycetes
Order	Mucorales	Eurotiales	Wallemiales
Family	*Mucoraceae*	*Trichocomaceae*	*Wallemiaceae*
Genus	*Rhizopus*	*Aspergillus*	*Wallemia*
Species	*stolonifer*	*glaucus*	*sebi*

Note that names of families, genera, species and varieties are italicised, while higher taxonomic ranks are not.

Three phyla of the kingdom Fungi include genera of significance in food spoilage. As indicated in the examples above, these are Zygomycota, Ascomycota and (much less commonly) Basidiomycota. Fungi from each of these phyla have quite distinct properties, shared with other genera and species from the same phylum. Unlike other texts, this book will not rely on initial recognition of a correct phylum before identification of genus and species can be undertaken. Nevertheless, identification of the phylum can provide valuable information about a fungus, so the principal properties of these three phyla are described below.

3.3 Zygomycota

Fungi of interest within the phylum Zygomycota belong in the class Zygomycetes. Fungi in this class possess three distinctive properties:

1. **Rapid growth.** Most isolates grow very rapidly, often filling a Petri dish of malt extract agar with loose mycelium in 2–4 days.
2. **Nonseptate mycelium.** Actively growing mycelia are without septa (cross walls) and are essentially unobstructed. This allows rapid movement of cell contents, termed "protoplasmic streaming", which can be seen readily by transmitted light under the binocular microscope. In wet mounts the absence of septa is usually obvious (Fig. 3.1a).
3. **Reproduction by sporangiospores.** The reproductive structure characteristic of Zygomycetes is the **sporangiospore**, an asexually produced spore which in genera of interest here is usually produced inside a sac, the **sporangium**, on the end of a long specialised hypha. Sporangiospores are produced very rapidly.

From the point of view of food spoilage, the outstanding properties of Zygomycetes are: very rapid growth, especially in fresh foods of high water activity; inability to grow at low water activities (no Zygomycetes are xerophiles); and lack of resistance to heat and chemical treatments. From the point of view of food safety, Zygomycetes have rarely been reported to produce mycotoxins.

3.4 Ascomycota

The phylum Ascomycota is distinguished from the phylum Zygomycota by a number of fundamental characters, the most conspicuous being the production of septate mycelium (Fig. 3.1b). Consequent on this, growth of fungi in this phylum is usually slower than that of those in Zygomycota, although there are some exceptions.

Fungi in the phylum Ascomycota, commonly called "ascomycetes", characteristically produce their reproductive structures, **ascospores**, within a sac called the **ascus** (plural, **asci**, Fig. 3.2a, b). In most fungi, nuclei normally exist in the haploid state. At one point in the ascomycete life cycle, diploid nuclei are produced by nuclear fusion, which may or may not be preceded by fusion of two mycelia. These nuclei undergo meiosis within the ascus, followed by a single

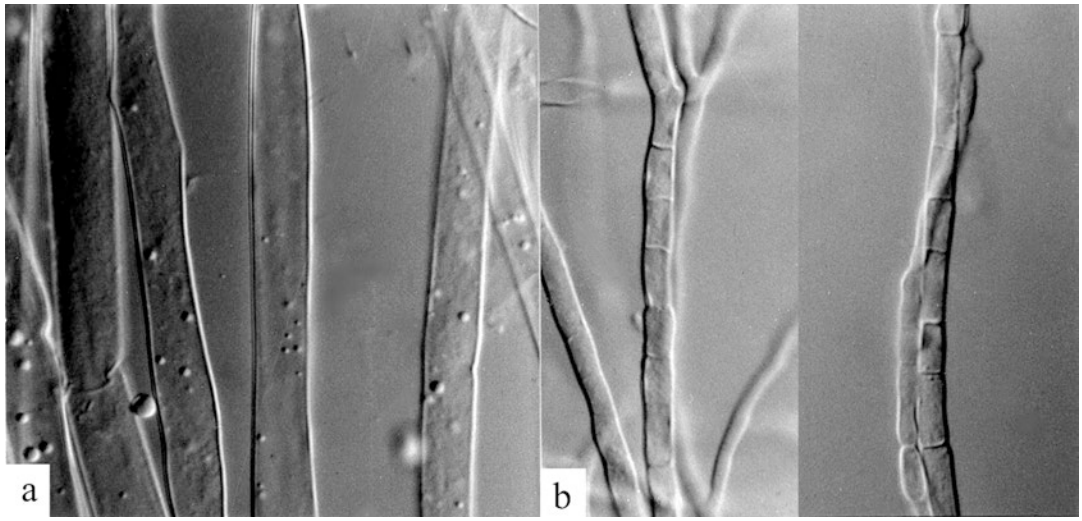

Fig. 3.1 (**a**) Nonseptate mycelium of *Syncephalastrum racemosum*; (**b**) septate mycelium of *Fusarium equiseti*

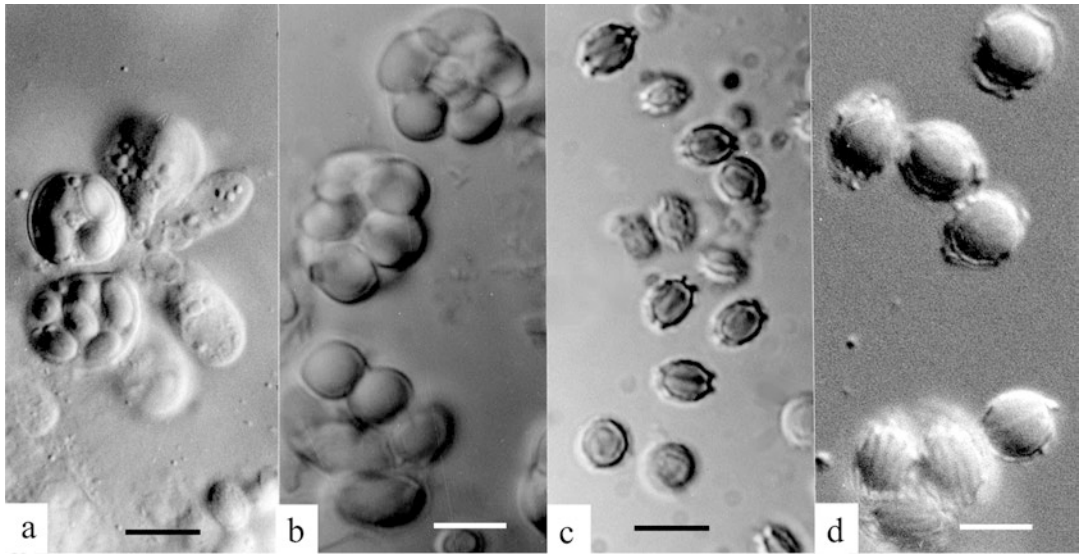

Fig. 3.2 Asci and ascocarps (**a**) asci of *Talaromyces* species; (**b**) asci of *Byssochlamys fulva*; (**c**) ascospores of *Penicillium alutaceum*; (**d**) ascospores of *Neosartorya quadricincta*. Bars = 5 μm

mitotic division, and then differentiation into eight haploid ascospores. In most genera relevant to this work, asci can be recognised in stained wet mounts by their shape, which is spherical to ellipsoidal and smoothly rounded; size, which is generally 8–15 μm in diameter; and the presence when maturity approaches of eight ascospores tightly packed within their walls. At maturity asci often rupture to release the ascospores, which are thick walled, highly refractile, and often strikingly ornamented (Fig. 3.2c, d).

Two other characteristics of asci are significant: generally they mature slowly, after incubation for 10 days or more at 25 °C, and they are usually borne within a larger, macroscopic body, the general term for which is **ascocarp**. Genera of interest here usually produce asci and ascospores within a spherical, smooth walled body, the **cleistothecium** (Fig. 3.3a) or a body with hyphal walls, the **gymnothecium** (Fig. 3.3b).

Ascospores are highly condensed, refractile spores, which are often resistant to heat, pressure

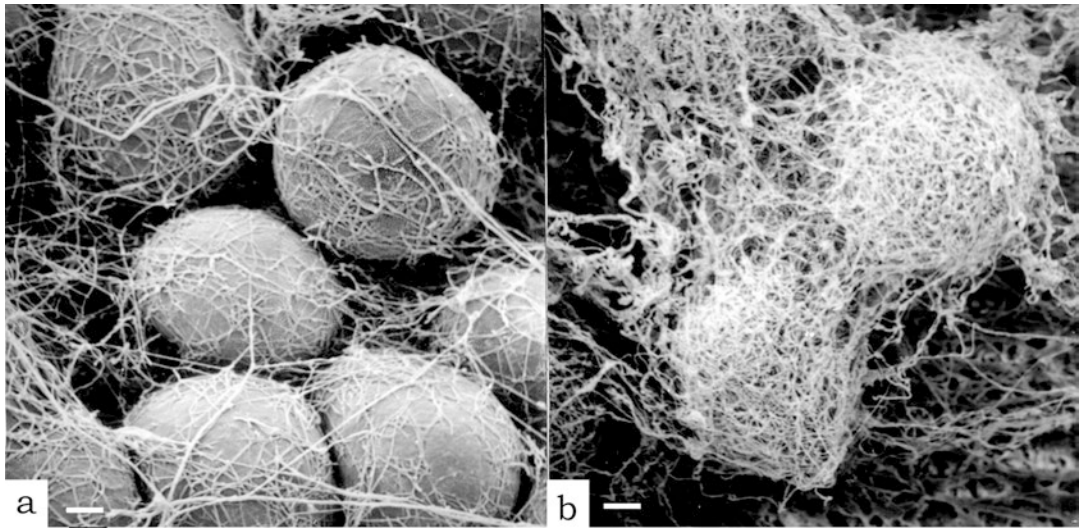

Fig. 3.3 (**a**) cleistothecia of *Penicillium*; (**b**) gymnothecia of *Talaromyces*. SEM. Bars = 50 µm

and chemicals. Almost all xerophilic fungi are ascomycetes.

Besides their sexual spores, ascospores, ascomycetes commonly produce haploid asexual spores. Formed after mitotic nuclear division, these spores are borne singly or in chains, in most genera of interest here from more or less specialised hyphal structures. The general term for this type of spore is **conidium** (plural, **conidia**), but other more specialised terms exist for specific kinds of conidia. During evolution, some ascomycetes with well developed asexual stages have lost the ability to produce ascospores, and appear to rely entirely on conidia for survival and dispersal.

Conidia, and the specialised hyphae from which they are borne, are astonishingly diverse in appearance. Especially with fungi that no longer produce the ascosporic state, the size, shape and ornamentation of conidia, and the complexity of the structures producing them, can be of great value in taxonomy. However, conidial fungi are classified as ascomycetes – or, rarely, basidiomycetes. Lacking ascospores, conidial fungi are not usually heat resistant, but conidia may be quite resistant to chemicals. Some conidial fungi are xerophilic.

3.5 Basidiomycota

The phylum Basidiomycota includes mushrooms, puffballs and the plant pathogenic fungi called rusts and smuts. Until recently basidiomycetes were considered to be of no interest to the food mycologist. However, molecular studies have indicated that the small brown fungus *Wallemia sebi*, long a curiosity because of its lack of resemblance to any other species, is a basidiomycete. It has no obvious phylogenetic affinity with any other genus, and has now been classified in its own order, Wallemiales (Zalar et al. 2005). Only one other species, the very rarely isolated foodborne fungus *Trichosporonoides nigrescens* A.D. Hocking & Pitt (Hocking and Pitt 1981) has a known affinity with the phylum Basidiomycota.

3.6 One Name – One Fungus

Although it was established about 150 years ago that fungal species may carry the genetic information necessary to produce both ascospores and conidia, these two kinds of spores are produced by different mechanisms and have different

functions. The two types of spores may be produced under different environmental or cultural conditions, so it is not surprising that mycologists have sometimes given different names, generic and/or species, to a single fungus. Under earlier systems of classification, using ascomycetous and deuteromycetous names, both names were accepted, under a system known as "dual nomenclature". The ascomycete state, commonly referred to as the **teleomorph**, was the name applied to the fungus when the ascomycete state was present. If the conidial state occurred in the absence of the ascomycetous state, it was known as the **anamorph**. If it was given a separate name, this applied only to the conidial state, and was used only when the ascomycete state was absent, or to refer specifically to the conidial state if the ascomycete was present. From the food mycologist's viewpoint, dual nomenclature was of great value, as use of a teleomorph generic name implied that the fungus in question had the properties associated with the ascospore – such as heat and/or chemical resistance. Strains identified by the asexual name could be readily destroyed by heat or chemical means.

Dual nomenclature worked very effectively until the advent of molecular methods showed unequivocally that two names were being applied to a single genome in many common fungi. In consequence, the Melbourne Botanical Congress in 2011 moved to abolish dual nomenclature, and declared that from 2013 each fungus could only bear one name. The Congress left open the crucial question "What criteria should be used to choose the appropriate name for genera long treated by dual nomenclature?" In line with the prevailing view that sexual states are of greater evolutionary significance than asexual states, the ICN states that teleomorph names are to be favoured over anamorph names in making this choice. However, leading mycologists quickly decided that this was not necessarily the best approach. For example, it was found quite recently that certain species of the common anamorph genus *Alternaria* produced ascocarps and ascospores. The teleomorph genus was named *Lewia*, but has been observed only in the laboratory and its occurrence in nature is likely to be quite rare. Why should *Lewia* take precedence over *Alternaria*? Also, some mycologists have taken the view that the more commonly cited name in the literature should be chosen, but what measure of the literature will provide the best answer? Others have suggested always using the earlier name – but again that is not necessarily in line with common usage.

It was hoped that all of the necessary decisions would have been made by the time of the Botanical Congress in Shenzhen, China, in 2017, but that did not occur. Where such agreements have been reached, the recommended names has been applied in this book, and the previously used dual name, be it teleomorph or anamorphh, has been placed in synonymy. In the example above, *Alternaria* is the recommended name, and *Lewia* is listed as a synonym. In the case of the asexual genus *Penicillium*, it was recommended by Houbraken and Samson (2011), Samson et al. (2011) and Visagie et al. (2014) that *Penicillium* be given priority over the sexual genus *Eupenicillium.* At the same time, species in *Penicillium* subgenus *Biverticillium* were separated from *Penicillium* and placed in the sexual genus *Talaromyces*. In one case, the older asexual name has been preferred, but in the other the much newer sexual generic name. This has been accepted by taxonomists, so these changes have been made in Chap. 7.

In many cases, specialist groups have made sound scientific decisions on the one name to be used for a genus, but in other cases vested interests have interfered with science. It is unlikely that concensus on the one name for all genera of relevance to food mycologists will be established by the time this volume goes to press. Occasions where such an eventuality may occur will be discussed under the relevant genera. In particular, the nomenclature of *Aspergillus* and some teleomorph genera associated with it has not been clarified. This problem will be discussed further under Chap. 8, *Aspergillus*.

3.7 Practical Classification of Fungi

Fungi are classified into a vast array of orders, families, genera and species. Among natural organisms, the numbers of taxa of fungi are

rivalled only by those of flowering plants and insects. Estimates of fungal species range as high as 1.5 million; only 5% of this number have so far been described (Hawksworth 1991).

Many fungi are highly specialised. Some will grow only in particular environments such as soil or water; many are obligate parasites and require a specific host, such as a particular plant species, and will not grow in artificial culture; many grow in association with plant roots or as commensals within the plant itself. From the point of view of the food mycologist, these kinds of fungi are irrelevant. However, fungi which spoil foods are also highly specialised, their speciality being the ability to obtain nutrients from, and hence grow on, dead, dormant or moribund plant material more or less regardless of source. The principal factors influencing food spoilage by fungi are physico-chemical, and have already been outlined in Chap. 2. The point being made here is that food spoilage fungi are classified in just a few orders and a relative handful of genera. For this reason there is much to be said for food mycologists avoiding the use of a traditional, hierarchical classification as outlined above, and employing a less formal approach to the identification of the fungi of interest to them.

In the present work, this pragmatic approach has been followed as far as possible:

* The use of specialised terms has been kept to a minimum, while being cognisant of the need for clarity of expression:
* Hierarchical classification has been avoided as far as possible, consistent with retaining a logical approach to the presentation of fungi which are related;
* Identification procedures used have been designed to be simple and comprehensible, avoiding the use of specialised equipment or procedures unavailable in the routine laboratory. To this end, identification of most species included in this work is based entirely on inoculation of a single series of Petri dishes, incubation under standardised conditions, and examination by traditional light microscopy;
* A standard plating regimen has been used for the initial examination of all isolates (except yeasts), so that identification procedures can be carried out without foreknowledge of genus or even subkingdom;
* Cultural characters, which can be broadly defined as the application of microbiological techniques to mycology, have been used throughout.

Once fungi began to be studied in pure culture, in the early 20th Century, it became possible to use cultural characteristics in taxonomy. Charles Thom and Kenneth B. Raper introduced semidefined media in studying the taxonomy of *Aspergillus* and *Penicillium*, genera of paramount importance in food spoilage. Pitt (1973, 1979), refined their concepts, by standardising time and temperature of incubation, and using the measurement of colony diameters as a taxonomic criterion in *Penicillium* taxonomy. This approach has been found to have a much broader applicability, and has been extended here to the examination of every isolate (excluding yeasts) by a single system: inoculation onto a standard set of Petri dishes, and examination of them culturally and microscopically after 7 days incubation. Most of the genera and species included in this book can be identified immediately, at that point. Only in exceptional cases has it been found necessary to reinoculate isolates onto a further set of media in order to complete identification. The exceptional fungi are firstly the xerophiles, many of which grow poorly if at all on the standard media, and secondly genera such as *Fusarium* and *Trichoderma*, in which some species cannot readily be differentiated on the standard regimen. Details of the techniques used are given in Chap. 4.

Food microbiologists, the primary audience for this book, are familiar with cultural techniques and the use of a wide range of media and varied incubation conditions, so the authors make no apology for the taxonomic approach used here. In the field of mycology, different genera have been studied by many different people of varied backgrounds, and for different reasons. Consequently, keys and descriptions have been based on a wide variety of media, often traditional formulations incorporating all sorts of

natural products. This heterogeneity makes comparisons difficult and adds unnecessary complexity to the task of the nonspecialist confronted with a range of fungal genera.

No doubt, rapid improvements taking place in the scope and precision of molecular methods will mean the classical taxonomic methods will be superceded in the coming years. However, classical methodology will always be important for work in culture – studies on physiology, ecology and mycotoxicology, for example.

3.8 Phylogeny Versus Taxonomy

Phylogeny has been defined as "the development or evolution of a group of organisms". It is usually depicted in a "phylogenetic tree" which may be based on any characteristic, but is now used specifically for a diagram based on a comparison of the genetic code from similar lengths of DNA amplified from specimens in the group. A great deal of experimentation and discussion has occurred over the past 30 years as to which lengths of DNA are most suitable for such comparisons. For the fungi, the most valuable (in terms of distinguishing among species) has been the internal transcribed spacer region (ITS) of ribosomal DNA. This particular region of genomic DNA has been shown to give good definition to both genera and species, and has now become the standard on which the system of "barcoding" fungi is being developed (Schoch et al. 2012; Xu 2016). As the ITS region does not discriminate among some species in *Penicillium* and sometimes other genera, a number of other genes have also been used to produce trees. The most commonly used in the genera of interest have been β-tubulin and calmodulin. Several algorithms are in common use for such comparisons, of which "Nearest Neighbour Joining" and "Maximum Likelihood" have been the most frequent.

Taxonomy can be defined as "the science dealing with the identification, naming and classification of organisms". In a paper delivered but only partly published, Pitt (1990) stated "Taxonomy can be good or bad, effective or ineffective, ... but phylogeny can only be right or "wrong". Too often mycologists confuse the two disciplines, apparently believing that "good" taxonomy results from "right" phylogeny. This thinking involves two major errors. First, we often remain uncetain whether a particular phylogenic tree is "right", as the trees produced from different lengths of DNA or using different algorithms often show variation in species proximity. Second, phylogenies to date have been derived from only a few genes, making up just a very small part of the genome. The ITS region approximates 500 base pairs, while genomes of the fungi of interest are usually over 30 million base pairs. Rapidly mutating genes, including those involved in morphology or secondary metabolite production, are unsuitable for use in phylogenetic comparisons. It is generally agreed that taxonomy should follow phylogeny, a philosophy with which we are in agreement. However, blindly following phylogenies which ignore morphology, at least in genera such as *Penicillium* and *Aspergillus* where morphological differences clearly have phylogenetic significance, does no appear to be sensible.

Which are the "right" genes for phylogeny? Some are too stable, leading to lack of discrimination of genera or species. Some, suitable for particular genera, are of little value for others. Some contain introns, or are hypervariable. Papers on barcoding referred to earlier (Schoch et al. 2012; Xu 2016) provide excellent overviews of that issue.

A further issue with phylogenetic analysis is that DNA is a much more sensitive measure of variation in a genome than classical taxonomic criteria. This has led, and will continue to lead, to a rapid increase in the number of published fungal species. It might be hoped that most of the increases in numbers would have resulted from exploration of the vast areas of the globe with scant fungal records or study of the fungal species associated in one way or another with plants or animals, especially insects. However, too often increases in species numbers have resulted from splitting existing species into smaller units with

limited differences in important characters, including morphology, physiology and ecology. In a recent communique with worldwide medical mycologists, the Nomenclature Committee of the International Society for Human and Animal Mycology has councelled caution in accepting new phylogenies, and in some cases recommended the continued use of existing classical taxonomies.

At the same time, it is acknowledged that molecular methods and phylogenetic analyses have been of great importance to fungal taxonomy in recent years and the trend to reliance on molecular taxonomy will continue into the future. Is there still a place for traditonal taxonomy? Absolutely. Traditional taxonomy provides an entry point into physiology, biochemistry and ecology, fundamentally important areas about which genetic studies provide little or no information. What we might describe as visual taxonomy, as set out in this book, provides an invaluable insight into the important features of any fungus.

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Methods for Enumeration, Isolation and Identification

4

This chapter describes techniques and media suitable for the enumeration, isolation and identification of fungi from foods. Some techniques are similar to those used in food bacteriology, others have been developed to meet the particular needs of food mycology. Most of the media have been specifically formulated for foodborne fungi. The approach taken here is designed to provide a systematic basis for the study of food mycology.

In 1984 a group of about 30 of the world's foremost scientists in food mycology met in Boston, Massachusetts, USA, to hear and discuss a wide range of presentations that explored many aspects of methodology in food mycology. Agreement was reached on broad issues, and areas requiring further work pinpointed. The proceedings were published as "Methods for the Mycological Examination of Food" (King et al. 1986). At a second workshop, held in Baarn, the Netherlands, in 1990, results of a number of collaborative studies on media and methods were presented, and some standardised protocols developed. The proceedings, published as "Modern Methods in Food Mycology" (Samson et al. 1992) provided a comprehensive overview of current thinking in this field.

The working group which organised those two workshops was then formalised as the International Commission on Food Mycology (ICFM), a Commission under the auspices of the Mycology Division of the International Union of Microbiological Societies (IUMS). ICFM is dedicated to international standardisation of methods in food mycology. Subsequent ICFM workshops have been held in Copenhagen, Denmark (1994), Uppsala, Sweden (1998), Samsø, Denmark (2003), Key West, Florida (2007) and in Freising, Germany in 2010, 2013, 2016 and 2019. Papers from the 1994 and 1998 workshops were published in the *International Journal of Food Microbiology* and the proceedings of the 2003 (Samsø) workshop were published as "Advances in Food Mycology" (Hocking et al. 2006).

The methodology described below is based on recommendations from ICFM, and represents current thinking within the food mycology community. However, no formal endorsement from ICFM is implied.

4.1 Sampling

It must be emphasised at the outset that results from mycological assays of foods are only as good as the samples used. However, sampling is beyond the scope of this text. Excellent treatises on sampling plans for food bacteriological purposes have been produced by the International Commission on Microbiological Specifications for Foods (ICMSF 1986, 2002), and are generally applicable to food mycology. Part 2 of this publication, Specific Proposals for Sampling and Sampling Plans, is now available as a free download from the ICMSF website (http://www.icmsf.org/publications/books.html).

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4.2 Enumeration Techniques

Quantification of the growth of filamentous fungi is more difficult than for bacteria or yeasts. Vegetative growth consists of hyphae, which are not readily detached from the substrate, and which survive blending poorly. When sporulation occurs, very high numbers of spores may be produced, causing sharp rises in viable counts, often without any great increase in biomass.

The estimation of fungal growth or biomass is not easy, because no primary standard exists (i.e., such as cell numbers used for yeasts and bacteria). Although techniques for quantifying biomass have improved in recent years, most food laboratories continue to rely on viable counting (dilution plating) for detecting and quantifying fungal growth in foods.

As well as dilution plating, a second standard method, known as direct plating, has been developed for estimating fungal numbers and growth in foods. Both methods are described in detail below. Techniques for biomass estimation will be discussed later.

4.2.1 Direct Plating

Contributions at the international workshops mentioned above emphasised the use of direct plating as the preferred method for detecting, enumerating and isolating fungi from particulate foods such as grains and nuts. In direct plating, food particles are placed directly on solidified agar media. In most situations, particles should be surface disinfected before plating, as this removes the inevitable surface contamination arising from dust and other sources, and permits recovery of the fungi actually growing in the particles. This process provides an effective measure of inherent mycological quality, and permits assessment of the potential presence of mycotoxins as well.

Surface disinfection should be omitted only where surface contaminants become part of the downstream mycoflora, for example in grain intended for flour manufacture. Even here, surface disinfection before direct plating provides the most realistic appraisal of actual grain quality.

Results from direct plating analyses are expressed as percentage infection of particles. The technique provides no direct indication of the *extent* of fungal invasion in individual particles. However, it is reasonable to assume that a high percentage infection is correlated with extensive invasion in the particles, and a higher risk of mycotoxin occurrence.

The standard protocol recommended by the ICFM (Hocking et al. 2006, p. 344) is given below, with amplification where necessary.

Surface disinfection Surface disinfection is carried out by immersing particles in a chlorine solution. Household chlorine bleach, nominally 4–5% active chlorine, is effective. Dilute the chlorine 1 to 10 with water before use, to provide an approximately 0.4% solution. Immerse particles for 2 min, stirring occasionally, then drain the chlorine. Chlorine solutions are rapidly denatured by organic matter, so it is important to use a surplus of chlorine solution (10 times the volume of the particles) and to use the solution only once.

This process is conveniently carried out in 250 to 500 mL beakers. Place 50 or more particles in the beaker, and add chlorine. To dislodge air bubbles, immediately stir with a pair of forceps, leaving them in the solution, and preferably cover the beaker with a watch glass. The watch glass simplifies decanting of the chlorine, and the forceps, disinfected by the chlorine, may be used to plate the particles.

Studies in our laboratory have shown that the treatment outlined here may be inadequate under some conditions. In commodities such as peanuts or maize where high levels of *Aspergillus flavus* or *Penicillium* species may be present, surface disinfection may be difficult. Here 2 min immersion in 70% ethanol followed by 2 min in 0.4% chlorine is recommended.

Rinsing After the chlorine is poured off, particles may be rinsed once with sterile water. Use a one minute treatment, with stirring, then pour the water off. Again a watch glass should cover the beaker during this period, and the sterile forceps should be used for stirring. It is not clear

whether rinsing fulfils any essential function. Early direct plating regimens used agents such as mercuric chloride for disinfection and rinsing was essential to remove such toxic materials before they penetrated the particles too deeply. However, chlorine is effectively denatured by the particles and is believed to penetrate very little. In our opinion, the rinsing step can be omitted without loss of efficacy of the treatment, with savings in time and materials, and reduced risk of recontamination from the air.

Plating After disinfection and the optional rinse, particles should be plated onto solidified agar, at the rate of 6 to 20 particles per plate, depending on particle size. Use the disinfected forceps. Plating should be carried out immediately: keep the watch glass on the beaker if this is not possible.

Incubation Incubate plates upright, under normal circumstances for 5 days at 25 °C. See more detailed notes below.

Examination After incubation, examine plates visually, count the numbers of infected particles, and express results as a percentage. Differential counting of various genera is often possible. Correct choice of media, a stereomicroscope and experience will all assist in this process.

4.2.2 Dilution Plating

Dilution plating is the appropriate method for mycological analysis of liquid or powdered foods. It is also suitable for grains intended for flour manufacture, and other situations where total fungal contamination is relevant.

Sample preparation The two most common methods of sample preparation for dilution plating are stomaching and blending: stomaching is recommended by ICFM (King et al. 1986). The Colworth Stomacher (Sharpe and Jackson 1972), or equivalent equipment (e.g. BagMixer®, Interscience, Saint Nom La Bretèche, France), is a very effective device for dispersing and separating fungi from finely divided materials such as flour and spices, and soft foods such as cheeses and meats, and its use is strongly recommended. Treatment time in the Stomacher should be 2 min. Harder or particulate foods such as grains, nuts or dried foods such as dried vegetables, should be soaked before stomaching. Soaking times from 30 to 60 min are generally sufficient, but for extremely hard particles such as dried legumes, soaking for up to 3 hr may be required. Comminution in a Waring Blendor or similar machine is a suitable alternative for these types of samples, and may give a more satisfactory homogenate. Blending times should not exceed 60 sec, as longer treatments may fragment mycelium into lengths too short to be viable or overheat the homogenate.

The sample size used should be as large as possible. If a Stomacher or BagMixer 400 is used, a sample size of 10 to 40 g is suitable.

Diluents The recommended diluent is aqueous 0.1% peptone (Hocking et al. 2006a, p. 344), suitable for both filamentous fungi and yeasts. Saline solutions, phosphate buffer or distilled water are no longer recommended by ICFM as they may be deleterious to yeasts (Mian et al. 1997). The addition of a wetting agent such as polysorbitan 80 (Tween 80) may be desirable for some products, but the natural wetting ability of the peptone is usually adequate.

Special diluents may be necessary in some circumstances. If yeasts are to be enumerated from dried products or juice concentrates, the diluent should also contain 20–30% sucrose, glucose or glycerol, as the cells may be injured or be susceptible to osmotic shock.

Dilution Serial dilutions of fungi are carried out by the same procedures as those used in bacteriology, and the recommended dilution rate is 1:10 (= 1 + 9). Fungal spores sediment more quickly than bacteria, so it is important to draw aliquots for dilution or plating as soon as possible, preferably within one minute (Beuchat 1992).

Plating Spread (surface) plating is recommended. When pour plates are used, fungi develop more slowly from beneath the agar surface and may be obscured by faster growing colonies from surface spores. Hence spread plating allows more uniform colony development, improves the accuracy of enumeration of the

colonies and makes subsequent isolation of pure cultures easier.

The optimum inoculum for surface plating is 0.1 mL. Best results will be obtained if plates are dried slightly before use. After adding the inoculum, spread it evenly over the agar surface with a sterile bent glass rod ("hockey stick"). Sterilise the rod by flaming it with ethanol before use. A plate spinner is a useful accessory. It is usually possible to enumerate plates with up to 150 colonies, but if a high proportion of rapidly growing fungi are present, the maximum number which can be distinguished with any accuracy will be lower. Because of this restriction on maximum numbers, it may be necessary on occasion to accept counts from plates with as few as 10 to 15 colonies. Clearly, such limitations on numbers per plate and the overgrowth of slow colonies will result in higher counting errors than are usually achieved with bacteria or yeasts.

Enumerating yeasts is less difficult. In the absence of filamentous fungi, from 30 to 300 colonies per plate can be counted and errors will be comparable with those to be expected in bacterial enumeration.

Incubation The standard incubation conditions are 25 °C for 5 days. However other conditions may be more suitable in some circumstances (see notes below).

Reporting results As in bacteriology, results from dilution plating are expressed as viable counts per gram of sample. Note that such results are not directly comparable with those obtained from direct plating, and may not offer a direct indication of the extent of fungal growth.

4.2.3 Incubation Conditions

As noted above, the standard incubation conditions specified by ICFM are 25 °C for 5 days (Hocking et al. 2006a). Undoubtedly, 25 °C is the most suitable temperature for routine work in temperate to subtropical environments. Few if any common fungi are sensitive to this temperature, even those that grow readily under refrigeration. Higher temperatures are unacceptable in the temperate zone: 30 °C is close to the upper limit for growth of some important *Penicillium* species. In tropical regions, incubation at 30 °C is recommended, as a more realistic temperature for enumerating fungi from commodities stored at ambient temperatures. In cool temperate regions such as Europe, 22 °C may be a more suitable incubation temperature.

When used for fungi, Petri dishes should be stored upright. The principal reason is that some common fungi can shed large numbers of spores during handling, which in an inverted dish will be transferred to the lid. Reinversion of the Petri dish for inspection or removal of the lid may liberate spores into the air or onto benches and cause serious contamination problems.

4.3 Sampling Surfaces

Methods are outlined below for directly sampling the mycoflora of surfaces of commodities such as fruits, meats, cheeses, salamis and dried fish, and also packaging materials, machinery and walls. The techniques are based of those described by Langvad (1980) for studying the fungal flora of leaves.

If samples are particulate, or can be cut up, sterile forceps can be used to press pieces of a suitable size (up to about 10 mm square) onto a suitable medium in a Petri dish. The sample is then removed, leaving an impression, and any spores or mycelium transferred will form colonies within a few days. This technique is known as press plating.

For packaging materials such as cardboard, an alternative method is to cut a piece which will fit in a standard Petri dish. The dish is prepared by adding a sterile filter paper moistened with 10% glycerol and then placing a bent glass rod on it as a separator. After adding the sample, a thin layer of an appropriate agar medium is poured over its surface. To reduce evaporation, the dish should be sealed with Parafilm or a similar material or placed in a polyethylene bag before incubation at 25 °C for a few days. If contamination levels are not too heavy, the number and types of moulds present can be effectively estimated by this method. Colonies may be subcultured for identification.

For sampling walls or other surfaces, or for nondestructive sampling, impressions may be taken using adhesive tape. Carefully handled, tape coming from the roll will be virtually sterile. Press a short length of tape firmly onto the surface to be sampled, adhesive side down, then transfer it, still with the same side down, onto a suitable growth medium. After 1–2 days incubation at 25 °C, the tape may be removed to allow development and sporulation of colonies.

Surface sampling techniques for assessment of sanitation in food production and processing areas are discussed by Evancho et al. (2001). Surfaces may be sampled by using sterile swabs or by agar contact methods. The swab method involves rubbing a moistened sterile cotton swab over the test surface and placing the swab in a dilution bottle to be subsequently diluted and plated on appropriate media. Agar contact plates, also known as RODAC (Replicate Organism Direct Agar Contact) plates are restricted to use on smooth or semi-smooth flat surfaces. An alternative to agar contact plates is the agar slice technique, where agar is filled into a syringe-like apparatus or into an artificial sausage casing. The solidified agar can be pushed out onto the surface to be sampled, then the portion making contact sliced off with a sterile scalpel or wire, and placed in a Petri dish for incubation.

4.4 Air Sampling

Air sampling in the food processing environment is discussed by Evancho et al. (2001). The simplest method of air sampling is by sedimentation or settle plates. A Petri dish containing an appropriate agar medium is exposed to the atmosphere for a fixed period of time (usually 15–60 min), then closed and incubated at 25 °C (Samson et al. 2004a). This method can be useful in food production areas, as it gives a direct indication of the number and types of fungi likely to come into contact with exposed product. However, the settle plate technique lacks precision, and volumetric air sampling is a much more reliable indicator of air quality.

A number of air sampling devices are commercially available. Of these, the Anderson Sampler (either the Two Stage or Six Stage model; Anderson Instruments Inc, Atlanta, GA, USA) is probably the best, giving accurate and consistent results (Buttner and Stetzenbach 1993). However, the Anderson Sampler is expensive and requires mains power or a large battery for operation. In factory situations hand held instruments that are small and readily portable are more convenient. There are many such air samplers on the market, including the dry cell battery operated Biotest Hycon RCS and RCS High Flow centrifugal air samplers (Biotest, Solihull, UK), the MAS-100 air sampler (Merck, Darmstadt, Germany) or the Oxoid Air Sampler (ThermoFisher Scientific) and the SAS range of samplers (Cherwell Laboratories, UK) which can impact directly onto 90 mm agar plates or 55 mm RODAC plates. The Biotest RCS Plus sampler was reported to give comparable results to larger and more sophisticated machines, but its sampling efficiency gradually decreased for particle sizes below 4 μm (Benbough et al. 1993). Haig et al. (2016) have published a comprehensive review on bioaerosol sampling, sampling equipment and methodology.

4.5 Isolation Techniques

The term “isolation” is used here in its strict sense: the preparation of a pure culture, free from any contamination and ready for identification.

4.5.1 Yeasts

Streaking techniques commonly used for bacterial purification are equally suitable for the isolation of yeasts (Fig. 4.1). A method widely used by yeast specialists is to disperse a portion of a colony in 2 to 3 mL of sterile water, then streak a single loop of this suspension over the whole surface of a plate, moving the loop slowly down

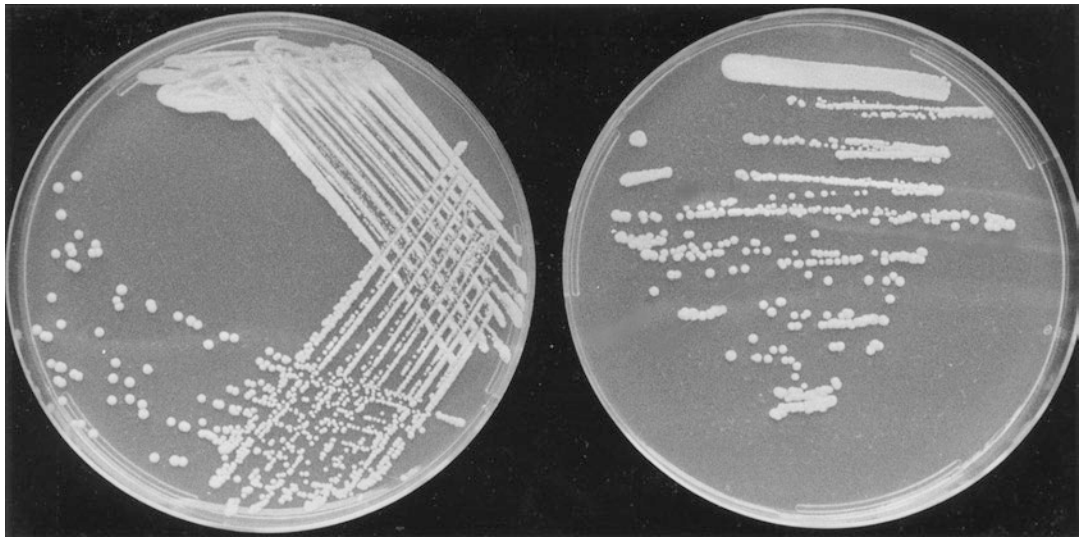

Fig. 4.1 Petri dishes of yeasts growing on malt extract agar, showing streaking methods suitable for producing isolated colonies

from top to bottom while simultaneously moving it rapidly across the plate from side to side. After suitable incubation, well separated single colonies should appear in the lower half of the plate (Fig. 4.1).

If all of these single colonies appear to be of similar size and appearance (taking into account the effect of crowding), the culture may be judged to be pure. Microscopic checks of some single colonies are also desirable. Disperse a needle point of cells from a colony in a drop of water, add a cover slip and examine by bright field illumination at about 400×. Cell outlines will be clearly visible. Note that, unlike those of bacteria, yeast cell sizes often vary considerably in a pure preparation. Purity is indicated not so much by uniformity of cell size within a preparation as by similarity in microscopic cell appearance from colony to colony. When a culture is considered to be pure, streak it onto an appropriate slant.

4.5.2 Filamentous Fungi

Streaking techniques are ineffective for filamentous fungi and are not recommended. Isolation depends on picking a small sample of hyphae or spores – judged to be pure by eye, by hand lens or preferably under the stereomicroscope – and placing this sample on a fresh plate as a point inoculum. Purity is subsequently judged by uniformity in appearance of the colony which forms after incubation. The appearance of a mixed culture depends on the growth rates of the fungi present. If rates are diverse, a mixed culture is often indicated by a clump of dense hyphae at the inoculum point, surrounded by looser wefts of spreading hyphae. With fungi of approximately equal growth rates, mixtures are often indicated by colonies with sectoring growth: sectors will show differences in mycelial, spore or reverse colours or in radial growth rates.

The simplest starting place for isolating fungi is an enumeration plate with well separated colonies. Use a needle of platinum or nichrome, preferably cut to a chisel point with a pair of pliers, or a steel sewing needle. Sterilise it by heating, then plunge the tip into cold agar and leave until cool – with nichrome or steel this will require several seconds. With the tip of the cold, wet needle pick off a few spores or a tuft of mycelium – just enough to be visible – and inoculate a single point on a plate or slant.

The same procedure can be applied to mixed cultures arising from direct plating or surface sampling techniques. It is advisable to keep notes

on the appearance of the colony area sampled, as this will give an indication of whether the culture which grows up is the same as that intended to be isolated.

It is generally easy to isolate rapidly growing fungi from those which grow more slowly. The outermost hyphal tips are usually free of contamination. The reverse process is often much more difficult. The isolation of slowly growing fungi in the presence of rapidly growing "weeds" often requires skill, patience and ingenuity. It is desirable to watch the point inocula daily over several days at least, because a particular stage in the life cycle may give some advantage. The slow colony may germinate more rapidly, or have a sector accessible to a needle, or spore more freely. The use of higher or lower incubation temperatures, or media of low a_w or low nutrient status, or the addition of dichloran or rose bengal (see below) may all be of value in this process.

Freeing fungi from bacteria, long considered to be a very difficult procedure, has been greatly simplified in recent years with the advent of media containing antibiotics. With use of the media recommended in the next section, bacterial contamination of cultures should be a rare event.

When a pure colony is obtained, it should be inoculated onto a slant of an appropriate medium and incubated until ready for identification. Again, filamentous fungi should always be inoculated at a single point, preferably near the centre of the slant. This permits the best colony development and sporulation in most fungi.

4.5.3 Slants

For short to medium term storage, fungi are usually stored on slants, most commonly in McCartney or Universal bottles which have the advantages of being free standing and having caps that can be sealed to retard drying during storage. During incubation and until colonies are fully mature, however, caps must be kept loose on slants in bottles. Moulds require free access to oxygen for typical growth and sporulation. Oxygen starvation during growth will at best lead to retarded sporulation or, at worst, death of the culture.

Long term preservation of fungi is dealt with later in this chapter.

4.6 Choosing a Suitable Medium

Food laboratories often rely on a single all purpose medium to produce a "yeast and mould" count in everything from raw material to finished product. But just as the food bacteriologist uses selective media for particular purposes, so too food mycologists have developed a range of media suited to specific applications. It is plainly unrealistic to expect a single medium to answer all questions about fungal contamination in all foods. The fungi that spoil meats or fresh vegetables are not the same as those that grow on dried fish. Although often used for this purpose, very dilute media such as potato dextrose agar are of little or no value for enumerating fungi from dried foods.

The most important division in types of enumeration media lies between those suitable for high water activity foods such as eggs, meat, vegetables and dairy products, and those suited to the enumeration of fungi in dried foods. The most suitable media for dried foods depend on the type of food, the major categories being foods low in soluble solids such as cereals, high sugar foods such as confectionery and dried fruit, and salt foods. A second consideration lies in whether the primary interest is in filamentois fungi, or yeasts, or both; and a third concerns the presence or absence of preservatives. Finally, media are available for specific mycotoxigenic fungi, notably *Aspergillus flavus* and related species and *Penicillium verrucosum* plus *P. viridicatum*.

An overview of media considered most suitable for particular purposes is given in Table 4.1. The table is derived from Pitt and Hocking (1997), together with recent recommendations from ICFM (Samson et al. 1992; Hocking et al. 2006).

Table 4.1 Recommended media for fungal detection, enumeration and isolation[a]

Type of food	Selecting for	Medium	Remarks
Fresh foods: milk and milk products, fruit, cheese, seafoods	Moulds Yeasts General	DRBC TGY, MEA, OGY DRBC	Blend (where necessary) and dilution plate
Freshly harvested grains, nuts	General Dematiaceous Hyphomycetes *Fusarium* Yeasts	DRBC DRBC, CZID CZID TGY, MEA, OGY	Direct plate Direct plate Direct plate Dilution plate
Fruit juices, fresh	Yeasts	TGY, MEA, OGY	Dilution plate
Fruit juices, preserved	Preservative resistant yeasts	TGYA, Malt Acetic Agar	Dilution plate
Fruit juices, to be pasteurised, or pasteurised products	Heat resistant moulds	PDA, MEA	Special protocol
Fruit juice concentrates	Xerophilic yeasts	MY50G	Special diluents
Dried foods in general	General	DG18	Direct plate
Stored cereals, nuts	General Dematiaceous Hyphomycetes *Fusarium*	DG18 DRBC, CZID CZID	Direct plate Direct plate Direct plate
Grain for milling into flour	General	DG18	Stomach or blend and dilution plate
Dried fruit, confectionery, chocolate, etc	Xerophilic moulds and yeasts	MY50G	Direct plate
	Fastidious xerophiles	MY50G	Direct plate
	- in presence of *Eurotium* spp.	MY70GF	Direct plate
Salt foods, e.g. salt fish	General	DG18	Direct plate or press plate
	Halophilic xerophiles	MY5-12, MY10-12	Direct plate or press plate
General	Fungi producing aflatoxins	AFPA	Direct or dilution plate
General	Fungi producing ochratoxins	DRYS	Direct or dilution plate

[a] For medium acronyms, see Sect. 4.6

4.6.1 General Purpose Enumeration Media

To be effective, a general purpose enumeration medium must fulfil several requirements (Pitt 1986). As these are sometimes overlooked, they are listed here:

- To inhibit bacterial growth completely, without affecting growth of foodborne fungi (filamentous or yeasts);
- To be nutritionally adequate and support the growth of fastidious fungi;
- To suppress the growth of rapidly spreading fungi, especially the Mucorales, but not to prevent their growth entirely, so they too can be enumerated;
- To slow radial growth of all fungi, to permit counting of a reasonable number of colonies per plate, without inhibiting spore germination;
- To promote growth of relevant fungi; and
- To suppress growth of soil fungi or others generally irrelevant in food spoilage.

Fulfilling the above requirements necessitates the use of inhibitory compounds, and a fine line sometimes exists between inhibition of undesirable microorganisms and suppression of growth of those being sought. Modern fungal enumeration media rely on the use of antibiotics at neutral pH for the inhibition of bacteria. Such media allow better recovery of moribund and sensitive fungi than the acidified media commonly used in the past. For many years rose bengal has been added to media to slow colony spread, while more recently 2,6-dichloro-4-nitroaniline (dichloran) has been added to inhibit rapidly spreading moulds. Many common spoilage fungi, *Aspergillus* and *Penicillium* species in particular, develop better on media with adequate nutrients. Low nutrient media of very high a_w, such as potato dextrose agar, have lost favour because they are selective against some species in these genera.

The media described below are considered to be the most satisfactory general purpose enumeration media available (Hocking et al. 2006a). Formulations are given in Appendix 1.

Dichloran rose bengal chloramphenicol agar (DRBC) DRBC (King et al. 1979; Pitt and Hocking 1997) is recommended for both moulds and yeasts. It is particularly suited to fresh and high a_w foods (Hocking et al. 1992). This medium contains both rose bengal (25 mg/kg) and dichloran (2 mg/kg), which restrict colony spreading without affecting spore germination unduly. Compact colonies allow crowded plates to be counted more accurately. This combination of inhibitors also effectively restricts the rampant growth of most of the common Mucoraceous fungi such as *Rhizopus* and *Mucor* (Fig. 4.2), although it does not completely control some other troublesome genera such as *Trichoderma*.

In routine use, it is recommended that DRBC plates be incubated away from light at 25 °C for 5 days.

Dichloran 18% glycerol agar (DG18) Hocking and Pitt (1980) developed DG18 to be selective for xerophilic fungi from low moisture foods such as stored grains, nuts, flour and spices. DG18, a_w 0.955, was designed for enumeration of a range of nonfastidious xerophilic fungi and yeasts. However it has been shown since that it supports growth of the common *Aspergillus*, *Penicillium* and *Fusarium* species, as well as most yeasts, and many other common foodborne fungi found in foods.

DG18 can now be described as a general purpose medium with emphasis on enumeration of fungi from dried foods. DG18 is also recognised to be a very useful medium for enumeration of airborne fungi (Wu et al. 2000; Samson et al. 2004a). It is an effective inhibitor of Mucoraceous fungi, and bacteria are totally suppressed. However, growth of *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species is still somewhat too rapid, and colonies may have diffuse margins. The addition of detergent to DG18 has been reported to be an improvement in this respect (Beuchat and de Daza 1992).

Although DG18 is a satisfactory isolation medium for *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species, it is not suitable for their identification. Species from this subgenus are usually identified on Czapek Yeast Extract Agar with 20% Sucrose (CY20S), described later in this chapter.

Other general purpose media Under circumstances where rapidly spreading moulds do not cause problems, two alternative general purpose enumeration media are satisfactory. These are Rose Bengal Chloramphenicol Agar (RBC; Jarvis 1973), from which DRBC was developed, and Oxytetracycline Glucose Yeast Extract Agar (OGY; Mossel et al. 1970). OGY has been found to be very suitable for yeasts in the absence of moulds (Andrews 1992a).

Most of the media discussed above are available in ready to use dehydrated form from commercial media suppliers.

4.6.2 Selective Isolation Media

Although considerable progress has been made in the past 20 years, the formulation of selective media for foodborne fungi still requires a great deal of research. The availability of effective media can greatly simplify the isolation and identification of significant food spoilage and

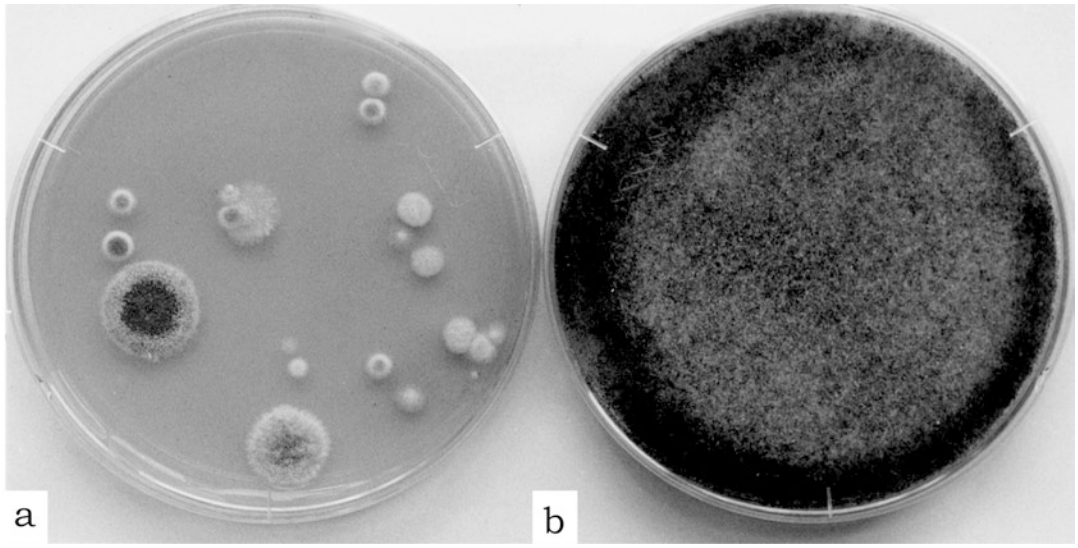

Fig. 4.2 Petri dishes of (**a**) DRBC and (**b**) RBC showing effective control of *Rhizopus* growth by dichloran in DRBC

mycotoxigenic fungi. Most attention has been paid so far to the requirements of xerophilic fungi because of their failure to develop on standard high a_w media. For mycotoxigenic fungi, satisfactory selective media exist only for *Aspergillus flavus* and closely related species, *Penicillium verrucosum* and *P. viridicatum*, and the genus *Fusarium*. These media are considered below.

Media for *Aspergillus flavus* and related species Bothast and Fennell (1974) developed Aspergillus Differential Medium after finding that *Aspergillus flavus* and *A. parasiticus* produced conspicuous, diagnostic orange-yellow colours in the colony reverses in the presence of an appropriate nitrogen source and ferric salts. Few other fungi produced a similar colouration. It was later shown that these species produce aspergillic acid or noraspergillic acid, which react with ferric ammonium citrate to form the colour complex (Assante et al. 1981). However, development of the orange-yellow reverse pigment is not indicative of aflatoxin production.

This medium was reformulated by Pitt et al. (1983) to produce Aspergillus Flavus and Parasiticus agar (AFPA). As well as more effective concentrations of the active ingredients, AFPA contains dichloran and chloramphenicol to inhibit spreading fungi and bacteria, respectively.

When incubated on AFPA at 30 °C for 42–48 hr, colonies of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* are distinguished by bright orange-yellow reverse colours. Only *A. niger* can be a source of error: it grows as rapidly as *A. flavus* and sometimes produces a yellow, but not orange, reverse. After 48 hr *A. niger* colonies begin production of their diagnostic black or dark brown heads, which provide a ready distinction from *A. flavus*. Prolonged incubation of AFPA, beyond four days, is not recommended because *Aspergillus ochraceus* and closely related species may also produce a yellow reverse after this time. AFPA is recommended for the detection and enumeration of potentially aflatoxigenic fungi in nuts, maize, spices and other commodities. Its advantages include rapidity, as 48 hr incubation is usually sufficient, specificity, and simplicity, as little skill is required in interpreting results. In consequence, it can be a simple, routine guide to possible aflatoxin contamination (Pitt 1984). This medium is also very effective for enumerating *Aspergillus flavus* in soils, where levels down to 5 spores per gram can be detected. Where soils carry a heavy bacterial load, doubling the quantity of chloramphenicol or adding other antibiotics may be of value.

Detection of aflatoxin production using Coconut Cream Agar Dyer and McCammon

(1994) developed Coconut Cream agar (CCA) to detect aflatoxin production in *Aspergillus flavus* and *A. parasiticus.* CCA can be made using any brand of commercial canned coconut cream (available from Asian food stores in many places). Dilute 50:50 with water, add agar (1.5%) and autoclave. Inoculate solidified plates with up to four colonies (picked with a wet needle) and incubate at 30 °C for 5–7 days. Examine, reverse upmost, under long wave length UV light. Colonies producing aflatoxins will fluoresce bluish white or white, especially in the centres. Ignore fluorescence from the Petri dish itself. Use an uninoculated coconut cream agar plate as a control (Dyer and McCammon 1994). Plates inoculated with known nontoxigenic and toxigenic strains are also useful controls. *A. parasiticus* isolates almost always produce aflatoxins.

Media for fungi producing ochratoxin A Although ochratoxin A was first described from *Aspergillus ochraceus*, recent molecular studies indicate that *A. westerdijkiae* is the major ochratoxin A producing species in *Aspergillus* Section *Circumdati* (Frisvad et al. 2004). *Aspergillus carbonarius* is also an important source of ochratoxin A, particularly in grape products (Abarca et al. 2004; Leong et al. 2006 and references therein). However, there are no selective or indicative media for these fungi. When DG18 is used as the isolation medium, selection of colonies with light brown (ochre) or black sporulation is a good starting point for detecting *A. westerdjikiae* or *A. carbonarius* respectively. Coconut cream agar (Dyer and McCammon 1994) can also be used to screen for ochratoxin production in *A. carbonarius* and species in *Aspergillus* Section *Circumdati* (Heenan et al. 1998). Plates are best incubated at 25 °C rather than 30 °C for potentially ochratoxigenic species of *Aspergillus*.

Penicillium verrucosum is the most important ochratoxin A producer in the genus *Penicillium* (Pitt 1987), although the closely related *P. nordicum* can also produce this mycotoxin (Frisvad and Samson 2004). Frisvad (1983) developed Dichloran Rose Bengal Yeast extract Sucrose Agar (DRYS) for selective enumeration of *Penicillium verrucosum*. *P. nordicum, P. viridicatum* and *P. aurantiogriseum* are also selected by DRYS. *P. verrucosum* and *P. nordicum* produce ochratoxin A, *P. verrucosum* also produces citrinin, while the latter two species produce xanthomegnin and viomellein. According to Frisvad (1983), *P. verrucosum* colonies on DRYS have a violet brown reverse, and the latter two species produce yellow colonies with a yellow reverse. The incubation regimen recommended by Frisvad (1983) is 7–8 days at 20 °C. Subsequently, Frisvad et al. (1992) developed Dichloran Yeast extract Sucrose 18% Glycerol agar (DYSG) on which *P. verrucosum* produces a red brown reverse. Because of its lower a_w, DYSG inhibits rapidly growing fungi such as *Rhizopus* and *Mucor* more effectively than DRYES. In a study of 76 samples of wheat, rye and barley, Lund and Frisvad (2003) found that DYSG was more effective than DRYES for screening grain samples for the presence of *P. verrucosum* and potential ochratoxin A contamination.

Media for *Fusarium* species Dichloran Chloramphenicol Peptone agar (DCPA; Andrews and Pitt 1986) can be used to isolate *Fusarium* species from grains and other substrates. The medium was developed from Nash-Snyder medium (Nash and Snyder 1962) a medium for enumeration of *Fusarium* species from soils. DCPA uses a low level of dichloran as a substitute for the high level of pentachloronitrobenzene (PCNB), and chloramphenicol rather than the antibiotic mixture used by Nash and Snyder (1962).

When *Fusarium* species are dominant, DCPA is effective for their isolation from grains, animal feeds and soil. However, DCPA has been found to be less effective in mixed populations. DCPA is a very useful medium for the identification of *Fusarium* species because it often induces the abundant formation of macroconidia (Hocking and Andrews 1987).

A much more stringent medium, effective for isolation of most *Fusarium* species occurring in foods, is Czapek Iprodione Dichloran Agar (CZID; Abildgren et al. 1987). As well as dichloran, this medium contains the fungicide iprodione. CZID is highly selective for *Fusarium* species, and is probably the best medium for *Fusarium* enumeration and isolation. CZID is

suitable for isolation of *Fusarium* species by direct plating of surface disinfected grains and other commodities, or dilution plating of homogeneous samples such as flour. Some questions remain concerning whether CZID may be too selective and not support growth of all foodborne *Fusarium* species. However, the common species all grow well.

Castellá et al. (1997) developed a *Fusarium* selective medium (MGA 2.5) using 2.5 µg/L malachite green as the selective agent. They reported that MGA 2.5 was more selective than Nash-Snyder medium as it did not allow development of colonies of other fungal genera. Bragalat et al. (2004) compared the efficacy of MGA 2.5 medium with DCPA, CZID and several other *Fusarium* media, using pure cultures of twelve *Fusarium* species commonly found in foods as well as naturally contaminated samples. They reported that there was no statistically significant difference in colony counts of the *Fusarium* spp. tested, but that colonies on MGA 2.5 were smaller than the other media. MGA 2.5 did not allow growth of other fungi such as Zygomycetes and yeasts from naturally contaminated samples, thus providing better selectivity than the other media.

Media for dematiaceous Hyphomycetes Although designed primarily for *Fusarium* isolation, CZID has been found in our laboratory to be very useful for isolating dematiaceous Hyphomycetes, provided that iprodione is added at half the usual concentration, as full strength iprodione tends to restrict colony diameters too severely. *Alternaria, Bipolaris, Curvularia and Stemphylium* species will grow and sporulate well when incubated for 5 days at 25 °C with a 12 hr photoperiod. *Drechslera* species will grow but will not sporulate on this medium.

DRBC is also of value for isolating these fungi, but some species do not sporulate readily on it.

DCPA, originally developed for isolation of *Fusarium,* may also be used as an isolation medium for the dematiaceous Hyphomycete genera mentioned above. *Alternaria, Curvularia* and related genera usually grow rapidly and sporulate well on DCPA. However, fungi should not be maintained or stored on DCPA for more than two weeks, as ammonia is produced by aging cultures.

Media for xerophilic fungi Xerophilic fungi are of great importance in food spoilage, and hence media and techniques for their enumeration and isolation have received much attention. Xerophiles range from those which grow readily on normal media and which are only marginally xerophilic, such as many *Aspergillus* and *Penicillium* species, to those, such as *Xeromyces bisporus*, which will not grow at all on high a_w media. It is not surprising that no single medium is suitable for quantitative estimation of all xerophilic fungi found causing food spoilage. As noted earlier, DG18 was developed as a general medium for xerophiles, and remains the medium of choice for this purpose. DG18 should be used in any general examination of the mycoflora of dried foods.

Media for extreme xerophiles Fungi discussed here include *Xeromyces bisporus*, *Xerochrysium* and *Bettsia* species, *Eremascus* species, *Aspergillus halophilicus* and the halophilic xerophiles *Polypaecilum pisce* and *Phialosimplex halophilus* (=*Basipetospora halophila*). These extreme xerophiles grow slowly if at all on DG18, and are quickly overgrown by rapidly spreading xerophiles such as common *Eurotium* species. Such species require special media and techniques.

Since the early 1950s, studies on extremely xerophilic fungi have been carried out in our laboratory (Scott 1957). A very wide variety of media have been used for their cultivation. The most effective of these, suitable for all except the halophilic xerophiles, is Malt Extract Yeast Extract 50% Glucose Agar (MY50G, a_w 0.89; Pitt and Hocking 1997).

Isolation techniques for extreme xerophiles As well as special media, extreme xerophiles require special techniques for isolation. If a low a_w commodity such as ground spice, dried fruit, fruit cake, confectionery or dried fish show signs of white mould growth, it is likely that the fungus responsible is an extreme xerophile. Fungi of this kind are usually sensitive to diluents of high a_w, and often cannot be isolated by dilution plating. For direct plating, a convenient tech-

nique is to place small pieces of sample, without surface disinfection, onto a rich, low a_w medium, such as MY50G. Alternatively, examine the food under the stereomicroscope, which will often provide useful information in any case, and use an inoculating needle to pick off pieces of mycelium or spores from the surface of the spoiled food. Place these pieces (three to six per plate) directly onto MY50G. Colonies should develop after 1–3 weeks incubation at 25 °C. Quite frequently extreme xerophiles will be present in pure culture and can be readily isolated. Examination with the stereomicroscope will usually indicate whether this is the case. Selection of growth which appears to cover the range of types seen will assist isolation of the principal fungi present.

Direct sampling by the press plating technique described earlier in this chapter can be useful for isolating xerophiles.

Sometimes extreme xerophiles are accompanied by xerophilic *Aspergillus* (=*Eurotium*) species, also capable of growth at very low a_w. The latter are identifiable under the stereomicroscope by their *Aspergillus* heads. Under these circumstances, isolation of extreme xerophiles is more difficult. A medium of sufficiently low a_w to slow the growth of xerophilic Aspergilli (*Eurotium*) species is necessary. The most satisfactory medium is Malt Extract Yeast Extract 70% Glucose Fructose Agar (MY70GF; a_w 0.76).

MY70GF is of similar composition to MY50G, except that it contains equal parts of glucose and fructose to prevent crystallisation of the medium at the concentrations used (70% w/w). It is made in a similar manner to MY50G.

Growth of even the extreme xerophiles on MY70GF is extremely slow, and plates should be incubated for at least 4 weeks at 25 °C. Once growth is apparent, pick off small portions of colonies and transfer them to MY50G, to allow more rapid growth and sporulation.

Media for halophilic xerophiles Some xerophilic fungi from salted foods such as salt fish grow more rapidly on media containing NaCl and hence are correctly termed halophilic xerophiles. Malt extract Yeast extract 5% salt 12% glucose agar (MY5-12, a_w 0.93), and Malt extract Yeast extract 10% salt 12% glucose agar (MY10-12, a_w 0.88) are suitable for these fungi. Techniques for enumeration and isolation are similar to those described above for other extreme xerophiles.

4.6.3 Techniques for Yeasts

The simplest enumeration and growth medium for most food spoilage yeasts is Malt Extract Agar (MEA). Although originally introduced as a growth medium for filamentous fungi, its rich nutritional status makes it very suitable for yeasts, and its relatively low pH (usually near 5.0) reduces the possibility of bacterial contamination.

More recently, Tryptone Glucose Yeast extract agar (TGY) has been recommended for enumeration of yeasts (Hocking et al. 1992, 2006). Due to a higher glucose concentration (10%) and higher pH (5.5–6.0), this medium is more effective in recovery of stressed yeast cells, and colony development is usually faster than on MEA. However, its higher pH means that an antibiotic should be incorporated for enumeration of yeasts from food samples which may contain bacteria. Recommended antibiotics are chloramphenicol or oxytetracycline at the concentrations used in DRBC and OGY.

Both MEA and TGY are suitable for enumeration of yeasts in products such as fruit juices, fruit purees and yoghurt, where moulds are usually present only in low numbers.

If large numbers of moulds are present, often the case with solid products such as cheese, a general purpose enumeration medium such as DRBC or DG18 should be used.

For circumstances where yeast enumeration remains difficult on media such as DRBC, a valuable technique is that of de Jong and Put (1980), which involves a three day anaerobic incubation. They recommended using pour plates or streak plates of Mycophil Agar (BBL Microbiological Systems, Cockeysville, Maryland, U.S.A.), but malt extract agar should be equally suitable. Antibiotics may be added if necessary. Plates are incubated at 25 °C for 3 days in an anaerobic jar, then taken out and incubated aerobically for

another 2 days at 25 °C. As many spoilage yeasts are able to grow anaerobically, colonies will commence development without the possibility of overgrowth by moulds. This technique is unlikely to be suited to the enumeration of yeasts on surfaces of fresh fruit or vegetables, as yeasts which colonise these surfaces are often strict aerobes.

Enrichment for yeasts in liquid products In liquid food products, low numbers of yeasts may be difficult to detect, but may have a serious potential to cause spoilage. Enrichment techniques are the only satisfactory way of monitoring products in these circumstances.

For products or raw materials free from suspended solids and of low viscosity, standard membrane filtration techniques are a satisfactory method for detecting low numbers of yeasts. The filter can be placed directly onto a suitable medium such as MEA or TGY and staining can be carried out subsequently. Centrifugation may also be used, but has the disadvantage that only relatively small volumes of product can be screened.

If products or raw materials are viscous, of low a_w, or contain pulps and cannot be filtered efficiently, other enrichment techniques are needed. In many cases, the best enrichment medium is the product itself, diluted 1:1 with sterile water. A 1:1 dilution increases the a_w of juice concentrates or honey to a level which will allow growth of potential spoilage yeasts without causing a lethal osmotic shock to the cells. If the product contains preservative, dilution will reduce the concentration and permit cells to grow.

To detect low numbers of spoilage yeasts in cordials, fruit juice concentrates and similar materials, simply decant half the product from the container and replace it with sterile water. Leave the cap loose, incubate at room temperature or 30 °C and watch for evidence of fermentation. Shaking the container daily will help to detect gases resulting from fermentation.

Classical enrichment techniques used in bacteriology can also be used for yeasts. TGY broth has been used very successfully in our laboratory for enrichment of low numbers of yeasts in liquid products. Add 10 mL of product to 90 mL TGY broth and incubate at 25 °C for 3–4 days, or 30 °C for 2–3 days. Look for signs of fermentation, and streak out onto TGY agar.

Detection of preservative resistant yeasts A few species of yeasts are able to grow in products containing preservatives such as sorbic, benzoic or acetic acids or sulphur dioxide. The most important of these is *Zygosaccharomyces bailii*. The simplest and most effective way to screen for preservative resistant yeasts is to spread or streak product onto plates of Malt Acetic Agar (MAA), which is MEA with 0.5% acetic acid added (Pitt and Richardson 1973), or TGY with 0.5% acetic acid added (TGYA, Hocking 1996).

MAA and TGYA are made by adding glacial (16N) acetic acid to melted and tempered basal medium to give a final concentration of 0.5%. Mix and pour immediately. These media cannot be held molten for long periods or remelted because of their low pH (approximately 3.2 and 3.8 for MAA and TGYA respectively). The acetic acid does not need sterilisation before use.

MAA and TGYA are suitable media for monitoring raw materials, process lines and products containing preservatives for resistant yeasts. They are also effective for testing previously isolated yeasts for preservative resistance.

Erickson (1993) developed a selective medium for *Zygosaccharomyces bailii.* Zygosaccharomyces bailii Medium (ZBM) is based on Sabauroud Dextrose Agar amended with fructose, NaCl, tryptone, yeast extract and trypan blue dye, then acetic acid (0.5%) and potassium sorbate (0.01%) are added to make the medium selective. It is designed as a plating medium for detection of *Z. bailii* in acidified ingredients in conjunction with hydrophobic grid membrane filtration (Erickson 1993).

When compared with MAA and TGYA in an interlaboratory study, ZBM was found to be highly selective for *Zygosaccharomyces bailii*, to the exclusion of other important preservative resistant yeasts such as *Schizosaccharomyces pombe* and *Pichia membranaefaciens*. In addition, recovery of *Z. bailii* cells sublethally injured by lyophilisation was significantly lower on ZBM

than on TGYA or MAA (Hocking 1996). Its high selectivity and complex formulation make ZBM unsuitable for routine laboratory use as a medium for detection of preservative resistant yeasts.

Enrichment of preservative resistant yeasts A technique capable of detecting yeast numbers as low as 1 cfu/mL within 4 days has been developed in our laboratory (Hocking et al. 1996). This method is particularly suitable for the detection of *Zygosaccharomyces bailii* in raw materials or finished product, but can also be used for the detection of *Schizosaccharomyces pombe, Pichia membranaefaciens* and other species of preservative resistant yeasts. The method involves a 2 to 3 day enrichment step followed by a plating step with a further 2 days incubation. Triplicate 20 mL Tryptone Glucose Yeast extract (TGY) broths containing 0.5% acetic acid (TGYA) are each inoculated with 1 g or 1 mL of product, and the broths incubated at 30 °C. After incubation for 48 and 72 hr, 0.1 mL from each broth is spread-plated onto TGY agar containing 0.5% acetic acid, and the plates incubated at 30 °C.

The detection time of the method is shortened by incubating broths and plates at 30 °C rather than the traditional temperature of 25 °C, as the optimum growth temperature for *Zygosaccharomyces bailii* is 30–32 °C (Jermini and Schmidt-Lorenz 1987). The sensitivity of the method is greatly increased by using triplicate broths instead of single or duplicate broths, and by spread plating 0.1 mL from each broth instead of streaking a loopful onto TGYA agar.

This method has been used to detect low numbers of cells of *Zygosaccharomyces bailii* in experimentally inoculated cordial syrup, mayonnaise, salad dressing and barbecue sauce, and other preservative resistant yeasts such as *Schizosaccharomyces pombe, Pichia membranifaciens* and some preservative resistant strains of *Saccharomyces cerevisiae*. Yeasts of intermediate preservative resistance (e.g. *Debaryomyces hansenii, Pichia kudriavzevii* and *D. delbrueckii*) can also be detected by this method. Better recoveries were obtained using TGY than a Malt Extract agar and broth system, possibly due to the fact that the pH of TGY broth +0.5% acetic acid is 3.8, compared with pH 3.2 for MEA + 0.5% acetic acid, and there is a lower concentration of glucose (2%) in the ME system compared with 10% in TGY. Yeasts which are unable to grow in the presence of acetic acid or other weak acid preservatives (sorbic or benzoic acids and their salts) are not detected by this method.

4.6.4 Techniques for Heat Resistant Fungi

Heat resistant spoilage fungi, such as *Byssochlamys*, *Talaromyces*, *Neosartorya* and ascosporic *Penicillium* species can be selectively isolated from fruit juices, pulps and concentrates by laboratory pasteurisation using various methods (Beuchat and Rice 1979; Hocking and Pitt 1984; Beuchat and Pitt 2001; Houbraken and Samson 2006). Three methods are described here: a plating method based on that of Murdock and Hatcher (1978), a direct incubation method and a filtration method for liquid samples such as liquid sugar.

Plating method If the sample to be tested is more concentrated than 35°Brix, it should first be diluted 1:1 with 0.1% peptone or similar diluent. For very acid juices such as passionfruit, normally about pH 2.0, the pH should be adjusted to 3.5–4.0. Two 50 mL samples are taken for examination. Erlenmeyer flasks (250 mL) or polyethylene Stomacher bags may be used as heat penetration into these containers will be rapid. If using Stomacher bags, the tops should be heat sealed. If a heat sealer is not available, the tops may be folded over and secured with a clip, and should not be fully immersed. The two samples are heated in a closed water bath at 80 °C for 30 min, then rapidly cooled. Each 50 mL sample is then mixed with an equal volume of double strength MEA distributed over four 150 mm Petri dishes. The Petri dishes are loosely sealed in a plastic bag to prevent drying and incubated at 30 °C for up to 30 days. Plates are examined weekly for growth. Most moulds will produce visible colonies within ten days, but incubation for up to 30 days will allow for the possible presence of badly heat damaged spores, which may

germinate very slowly. This long incubation time also allows most moulds to mature and sporulate, aiding their identification.

The main problem associated with this technique is the possibility of aerial contamination of the plates with common mould spores, which will give false positive results. The growth of green *Penicillium* colonies, or colonies of common *Aspergillus* species such as *A. flavus* and *A. niger*, is a clear indication of contamination as these fungi are not heat resistant. To minimise this problem, plates should be poured in clean, still air or a Class 2 biohazard cabinet if possible. If a product contains large numbers of heat resistant bacterial spores (e.g. *Bacillus* species), antibiotics can be added to the agar. The addition of chloramphenicol (100 mg/L of medium) will prevent the growth of these bacteria.

Direct incubation method A more direct method used for screening fruit pulps and other semisolid materials avoids the problems of aerial contamination. Place approximately 30 mL of pulp in each of three or more flat bottles such as 100 mL medicine flats. Heat the bottles in the upright position for 30 min at 80 °C and cool, as described previously. The bottles of pulp can then be incubated directly, without opening and without the addition of agar. They should be incubated flat, allowing as large a surface area as possible, for up to 30 days at 30 °C. Any mould colonies which develop will need to be subcultured onto a suitable medium for identification. If containers such as Roux bottles are available, larger samples can be examined by this technique, but heating times must be increased. Bottle contents should reach at least 75 °C for 20 min when checked by a thermometer suspended near the centre of the pulp.

For further details of the above methods see Hocking and Pitt (1984) or Beuchat and Pitt (2001).

Filtration method for liquid sugars This method permits the detection of very low numbers of cells in clear liquids such as liquid sugar. Sample size should be at least 100 g, taken after vigorously shaking the container from which the sample is drawn. Add 100 mL diluent (0.1% aqueous peptone) to 2 × 50 g samples and mix well to dissolve. Filter both samples sequentially through the same sterile 0.45 µm membrane filter. After both samples have passed through the filter, rinse the interior of the funnel with 3 × 20–30 mL volumes of sterile diluent. Remove the filter from the filter holder using sterile forceps, and place it in a sterile bottle or Stomacher bag. Add 10 mL diluent to the bottle or bag containing the filter, and place in a water bath at 75 °C for 30 min. Ensure the sample is submerged in the water bath (weigh down if necessary). Cool rapidly to room temperature, shake well, then divide the 10 mL of diluent between 3 Petri dishes. Add a generous portion of MEA with antibiotics to each plate, mix the agar and sample well, then let the plates solidify. Incubate at 30 °C for up to 30 days, examining weekly. Count colonies and report count per 100 g. This method was developed by BCN Research Laboratories, Rockford, Tennessee, USA.

4.6.5 Other Plating Techniques

Three other techniques which were developed for counting bacteria have been applied to fungal enumeration.

Spiral plate count Zipkes et al. (1981) evaluated the application of the spiral plate procedure to the enumeration of fungi. They compared this procedure with the traditional pour plate and streak plate methods, and found that spiral plating gave a higher overall recovery and lower replicate plating errors than the other two methods. The medium they used was potato dextrose agar, but the technique should be no less efficient using the media recommended here. Automation of spiral plate counting was studied by Manninen et al. (1991). They compared counts of pure bacterial, yeast and mould cultures using standard plating methods and spiral plate counts determined both manually and with a laser colony detector. They concluded that counts were not significantly different except where large colonies (10–15 mm diameter) of *Rhizopus oligosporus* were enumerated. Other *Rhizopus* and *Mucor* species are also likely to interfere with this method unless a suit-

able plating medium (such as DRBC) is used. Alonso-Cajella et al. (2002) found that the spiral plate technique compared well with standard plate counting on OGYE agar for enumeration of yeasts and moulds in goats milk cheeses, however García-Armesto et al. (2002) found the method unsuitable for enumeration of yeasts in raw ewe's milk.

Hydrophobic grid membrane filters Membrane filters overprinted with a square hydrophobic grid have been developed for rapid enumeration of bacteria. The hydrophobic grid membrane filter (HGMF) system is marketed as ISO-Grid™ by Neogen (Lansing, MI, USA). The HGMF "count" is determined by a most probable number (MPN) calculation. Brodsky et al. (1982) applied the HGMF technique to counting fungi in foods. They compared it with spread plating on potato dextrose agar amended with antibiotics, and found that the HGMF technique produced higher counts in 2 days than the traditional method did after 5 days. The HGMF method could find use for evaluating the quality of raw materials before incorporation into a product. For example, Erickson (1993) has proposed a method using HGMF in conjunction with a selective medium for detection of the preservative resistant yeast *Zygosaccharomyces bailii* in acidified ingredients. The HGMF method was collaboratively trialled by 20 laboratories using six naturally contaminated foods. Although there were some differences between counts obtained by HGMF and the reference method (5 day pour plate), the differences were not significant, and the HGMF method was adopted by the AOAC International (Entis 1996). Spangenberg and Ingham (2000) found that the HGMF method gave equivalent results to DRBC for enumeration of yeasts and moulds in grated low-moisture Mozzarella cheese.

Use of HGMF requires special holders for the square membrane filters, and an automated counting system is necessary to take full advantage of the method. The number of colonies on an HGMF can be counted visually, but it is a relatively slow process.

Petrifilm™ Yeast and Mold Petrifilm YM (3M Company, St. Paul, MN, USA) is a proprietary system for enumerating fungi on a layer of medium enclosed in a plastic film, which eliminates the use of Petri dishes. A collaborative study carried out by the AOAC (Knight et al. 1997) concluded that Petrifilm YM gave comparable results with the BAM method (USFDA 1992) for yeast and mould counts in five different food types inoculated with a cocktail of several species of *Aspergillus* and *Penicillium* and two species of yeast. Consequently, Petrifilm YM was adopted first action by AOAC International. Other workers (Beuchat et al. 1990, 2007; Taniwaki et al. 2001b; Ferrati et al. 2005) have shown that Petrifilm YM performed satisfactorily compared with conventional cultural methods when tested on a range of foods. However, Petrifilm was not particularly effective in inhibiting the growth of spreading moulds such as *Rhizopus* and *Mucor*. In addition, subculturing colonies for identification was more difficult from Petrifilm YM than from traditional Petri dishes. Petrifilm YM has a high a_w and we do not recommend it for analysis of foods of less than 0.95 a_w.

4.7 Estimation of Fungal Biomass by Chemical Methods

A deficiency in all of the enumeration techniques which rely on culturing fungi is that the result is at best poorly correlated with growth or **biomass**. Biomass is usually regarded as the fundamental measure of fungal growth in biotechnology, but it is not easy to quantify under the conditions existing in foods. The most fundamental method for estimating biomass is measurement of hyphal length, but the process is so tedious, even in pure culture, that it has seldom been attempted. Taniwaki et al. (2006) compared estimates of colony diameters, ergosterol and mycelial dry weight with hyphal length. Least useful was colony diameter which showed little correlation with hyphal length. The correlations with mycelial dry weight and ergosterol were better, but not for all species. Mycelial dry weight is most

commonly used as a biomass estimate, but its relationship to mycelial wet weight and to metabolism vary widely in foods, due to the great influence of a_w on both parameters. Fungi growing at reduced a_w can be expected to be more dense than at high a_w due to increased concentrations of internal solutes, though this is exceptionally difficult to measure experimentally. The question of a satisfactory fundamental measure of fungal biomass remains unanswered.

Two chemical techniques have been used to estimate the extent of fungal growth in commodities. These techniques, estimating chitin or ergosterol, rely on a unique component of the fungus that is not found in other microorganisms or foods. Neither has attracted much attention in the past 10 years, but are included here as the only useful methods for estimating fungal biomass.

4.7.1 Chitin

Chitin is a polymer of N-acetyl-D-glucosamine, and is a major constituent of the walls of fungal spores and mycelium. It also occurs in the exoskeleton of insects but is not present in bacteria or in foods. Hence the chitin content of a food or raw material can provide an estimate of fungal contamination.

Chitin is most effectively assayed by the method of Ride and Drysdale (1972). Alkaline hydrolysis of the food sample at 130 °C or at 121 °C in an autoclave (Jarvis 1977) causes partial depolymerisation of chitin to produce chitosan. Treatment with nitrous acid then causes partial solubilisation and deamination of glucosamine residues to produce 2,5-anhydromannose, which is estimated colorimetrically using 3-methyl-2-benzothiazolone hydrazone hydrochloride as the principal reagent. Improved assay sensitivity can be achieved by derivatisation of glucosamine and other products with *o*-phthalaldehyde, separation by high performance liquid chromatography and detection of fluorescent compounds with a spectrofluorimeter (Lin and Cousin 1985). Ekblad and Nasholm (1996) also described an HPLC method which measured fluorescence of a 9-fluorenylmethylchloroformate derivative of glucosamine. The chitin assay remains rather complex and slow, usually requiring about 5 hr.

The chitin assay can be a valuable technique for estimating the extent of fungal invasion in foods such as maize and soybeans (Donald and Mirocha 1977), wheat (Nandi 1978) and barley (Whipps and Lewis 1980), to estimate mycorrhizal fungi and fungal pathogens in plant material and soil (Ekblad and Nasholm 1996; Ekblad et al. 1998; Penman et al. 2000; Singh 2005) and measure wood-rotting fungi (Nilsson and Bjurman 1998). Particular attention has been paid to the possibility of developing the chitin assay as a replacement for the Howard mould count for tomato products (Jarvis 1977; Bishop et al. 1982; Cousin et al. 1984; Potts et al. 2002).

The chitin assay has some shortcomings. The relationship between dry weight and chitin content varies at least twofold for different food spoilage fungi (Cousin 1996). Some foods contain naturally occurring amino sugars such as glucosamine and galactosamine, which should be removed by acetone extraction prior to hydrolysis (Whipps and Lewis 1980). Insect contamination of grain samples can produce grossly misleading results, so materials such as stored grains frequently contain insect fragments and need to be checked before chitin assays are attempted. Because of these difficulties, the use of chitin as a chemical assay for fungi in foods has largely been superseded by the ergosterol assay.

4.7.2 Ergosterol

Ergosterol is the major sterol produced by fungi, but at most is a minor component of plant sterols (Weete 1974). Ergosterol occurs as a component of fungal cell membranes, so is inherently likely to be correlated with hyphal growth and biomass. It is therefore a good candidate as a chemical for measuring fungal growth in foods and raw materials. Methodology for estimating ergosterol in cereals was developed by Seitz et al. (1977, 1979) and refined by Newell et al. (1988). Samples are

blended with methanol, saponified with strong alkali, extracted with petroleum ether, and fractionated by high pressure liquid chromatography. Ergosterol is detected by ultraviolet absorption, optimally at 282 nm, a wavelength at which other sterols exhibit little or no absorbance. Liquid chromatography or gas chromatography with mass spectrometric detection (LC-MS; GC-MS) have also been used to estimate ergosterol (Headley et al. 2002; Dong et al. 2006; Varga et al. 2006). GC-MS in combination with non-discriminating flash pyrolysis (Py-GC-MS) provides the advantages of little sample preparation and small sample size (Parsi and Gorecki 2006).

The ergosterol assay provides a useful method for quantifying fungal growth, so numerous studies have assessed the relationship between fungal growth and ergosterol production, using both liquid and solid substrates.

Ergosterol content of fungal hyphae may be affected by diverse factors including growth conditions, hyphal age and fungal species. Using liquid cultures, Zill et al. (1988) showed a correlation between ergosterol production, mycelial wet weight and mycelial protein in *Fusarium graminearum*. Torres et al. (1992) reported that *Aspergillus ochraceus* grown in liquid culture showed a three fold increase in ergosterol concentration in relation to mycelial dry weight as the culture aged from 2 to 26 days. Other reports (Matcham et al. 1985; Newell et al. 1987; Seitz et al. 1979) have indicated smaller variations, only one to two fold. Marfleet et al. (1991) showed that fungal biomass and ergosterol levels were correlated for three representative fungal species over a range of a_w on solid substrates but not in liquid media. Nout et al. (1987) showed that the ergosterol content of *Rhizopus oligosporus* varied widely, from 2 to 24 µg/mg mycelial mass, and varied with substrate, aeration and growth phase, tending to increase as growth slowed. Taniwaki (1995) demonstrated that in fungi growing in atmospheres low in O_2 and with elevated CO_2 levels, the ergosterol content of hyphae was significantly reduced. In atmospheres containing 60% CO_2, ergosterol content per unit of hyphal length was up to six times less than in air. Growth medium also affected ergosterol concentrations: on average, seven foodborne fungal species grown on PDA produced more than twice as much ergosterol per unit hyphal length as when grown on CYA (Taniwaki 1995; Taniwaki et al. 2006). However, there was a reasonable correlation between ergosterol and mycelium dry weight for seven of the eight species tested. *Aspergillus (Eurotium) chevalieri* was the exception: this species produced little ergosterol and appeared to produce several other sterols (Taniwaki et al. 2006). Marin et al. (2005) examined sixteen species of food spoilage fungi, and concluded that ergosterol content and colony diameters were better correlated to fungal biomass than fungal counts were. Marin et al. (2008) showed that, for fourteen common food spoilage fungi, correlation coefficients between ergosterol and colony diameters were sufficiently significant over a range of a_w values (0.95–0.85), pH (5–7) and potassium sorbate concentrations (0.5–1.5%) for both parameters to be useful in growth modelling.

Quantifying ergosterol production in foods has proved more difficult. Seitz et al. (1977) showed a good correlation between damage in rice grains and their ergosterol content, between ergosterol in wheat and rainfall during the growing season, and between ergosterol content and fungal invasion in several sorghum hybrids. Matcham et al. (1985) reported good correlations between linear extension of *Agaricus bisporus* grown on rice grains and chitin, ergosterol and laccase production. Ergosterol content correlated with colony counts of fungi on wheat grains at 0.95 a_w but not at 0.85 a_w (Tothill et al. 1992). Using a stereomicroscope for visual examination, they concluded that sound grain contained up to 6 µg/g ergosterol, microscopically mouldy grain 7.5–10 µg/g, and visibly mouldy grain more than 10 µg/g ergosterol. From studies on ergosterol levels, colony counts and mould growth in a variety of grain samples, Schnürer and Jonsson (1992) concluded that ergosterol correlated with colony counts better on DG18 ($r = 0.77$) than on MEA ($r = 0.69$). Ergosterol levels of food grade wheat ranged from 2.4–2.8 µg/g dry weight, samples from

field trials (of unspecified quality) from 3.0–5.6 µg/g and feed grains from 8–15 µg/g dry weight.

After an extensive survey of ergosterol levels in Danish crops, Hansen and Pedersen (1991) concluded that the normal levels of ergosterol in barley were 7.6 ± 2.8, in wheat for bread making, 5.0 ± 1.5, rye for bread making 6.8 ± 2.2, peas 2.2 ± 2.7 and rapeseed 2.4 ± 1.3 µg/g dry weight respectively. Ochratoxin A in barley correlated well with ergosterol content and reached significant levels when ergosterol increased to 25 µg/g dry weight. However, aflatoxin B_1 became detectable in cottonseed meal when ergosterol reached only 4 µg/g. "Burned" rapeseed, a measure of quality, became significant when ergosterol reached 1.4 µg/g dry weight. Lamper et al. (2000) found that ergosterol content correlated well (r = 0.87) with deoxynivalenol levels in wheat inoculated with *F. graminearum* or *F. culmorum*. Moraes et al. (2003) found good correlation between mould counts and ergosterol content of Brazilian maize (r = 0.94) but a poor correlation (r = 0.4) between ergosterol and aflatoxin content. Pietri et al. (2004) found significant correlation between ergosterol content of Italian maize and the major mycotoxins, fumonisin B_1(1995 crop) or zearalenone and deoxynivalenol (1996 crop). Hossain et al. (2015) found no correlation between ergosterol and aflatoxin in maize from four different geographic locations, but a significant correlation between ergosterol and zearalenone was reported by these authors. Ergosterol content correlated strongly with fat acidity values and germination ability of stored canola (Pronyk et al. 2006). These authors also noted that *Penicillium* and *Aspergillus* species contributed more to ergosterol than *Aspergillus* subgenus *Aspergillus (Eurotium)* species. Ergosterol levels in sound canola were between 1.46–1.67 µg/g, whereas levels above 2 µg/g indicated significant levels of spoilage (Pronyk et al. 2006).

Karaca and Nas (2006) examined ergosterol content of dried figs, and found good correlation (r = 0.92) between aflatoxin and ergosterol in reject figs which were fluorescent, but no significant correlation with patulin content. Kadakal et al. (2005) found good correlation (r = 0.98) between ergosterol and patulin in apple juice, and that both patulin (r = 0.99) and ergosterol (r = 0.99) were linearly related to the proportion of decayed apples used to make the juice. Ergosterol has been used to assess mould growth in cheese with variable results (Pecchini 1997; Taniwaki et al. 2001a).

Ergosterol content has also been investigated as an indicator of the mycological status of tomato products. Battilani et al. (1996) found a significant correlation between ergosterol, Howard Mould Count and fungal growth, but with a high level of uncertainty. Kadakal et al. (2004) found a linear relationship between degree of decay in tomato pulp and Howard Mould Count (r = 0.97) and ergosterol (r = 0.96), and concluded that ergosterol has the potential to be used in quality assessment of tomatoes. Sio et al. (2000) described an improved method for extraction of ergosterol from tomato products.

Other applications of ergosterol as a measurement of fungal biomass include estimation of mould spores in indoor air and aerosols (Miller and Young 1997; Robine et al. 2005; Lau et al. 2006), estimation of wood decay by fungi (Eikenes et al. 2005) and estimation of fungi in soil and wetlands (e.g. Headley et al. 2002; Zhao et al. 2005).

The ergosterol assay is reported to have a high sensitivity and, in contrast to the chitin assay, requires only one hour for completion (Seitz et al. 1979). Despite its limitations, it appears to be a useful indicator of fungal invasion of foods and to hold promise as a routine technique for quality control purposes.

4.8 Other Methods for Detecting or Measuring Fungal Growth

4.8.1 Impedimetry and Conductimetry

Metabolites produced by growth of microorganisms in liquid media alter the medium's impedance and conductance. The use of changes in these properties as a measure of bacterial growth was suggested by Hadley and Senyk (1975), and was first applied to yeasts by Evans (1982) and to

moulds by Jarvis et al. (1983). Most of the subsequent work on fungi has been carried out with yeasts, but the methodology is often applicable to moulds also.

During the 1980s there were a number of studies aimed at optimising media for inducing detectable and reproducible changes in either conductance or capacitance during fungal growth (see Pitt and Hocking 1997). For example, Owens et al. (1992) reported that media high in ammonium ions and glucose, with added yeast extract and peptone, decreased the influence of product variability and induced higher conductance changes. An impedimetric method for detection of heat resistant fungi in fruit juices was described by Nielsen (1992). The detection limit in artificially contaminated juices was one *Neosartorya* ascospore per millilitre, detectable in 100 hr. Huang et al. (2003) reported an impedance method for detection of bacteria and fungi in bottled water which shortened the detection time from 5 days to 27 h for fungi and from 48 h to 11 h for bacteria.

Although impedimetry and conductimetry promised to be effective rapid methods when used under well defined conditions for a specific purpose with a particular kind of food, the methodology has not been broadly taken up for food mycology applications.

4.8.2 Fungal Volatiles

Methods for detection and characterisation of fungal volatiles are finding increasing applications in food mycology, for the detection of fungal deterioration of grain and other food products, for the identification of particular fungi, and for assessing potential mycotoxin contamination. Over recent decades there has been rapid growth in the development of gas sensor arrays. Gas sensors are chemical sensors that produce an electronic signal which is used as input into a pattern recognition system in order to recognise different volatiles and odours. This integrated system of gas sensor and pattern recognition is often called an 'electronic nose' or e-nose' (Gardner and Bartlett 1994; Schaller et al. 1998). Arrays comprising equipment to collect headspace volatiles, analysis by GC-MS or other sensitive analytical methods, coupled with computer analysis are also commonly used.

Deterioration of stored grain results from a combination of chemical and biological changes, with changes due to fungal growth often predominant. Deterioration is marked by off odour development, loss of germinability, caking, rancidity and sometimes mycotoxin development (Abramson et al. 1980). Fungi produce volatile chemicals during growth and particular chemicals may be associated with grain deterioration (Kaminski et al. 1974, 1975). R.N. Sinha et al. (1988) monitored production of 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone in stored grain and found that the presence of these volatiles usually correlated with seed infection by *Alternaria alternata*, *Aspergillus (Eurotium) repens*, *Emericella (Aspergillus) versicolor* and several *Penicillium* species. Octenol and octanone in particular seemed to be associated with deterioration in grain due to fungal growth. Barkat et al. (2017) studying Australian wheat identified particular compounds that were indicative of the presence of *A. alternata* (cyclooctasiloxane, hexadecamethhyl) and *A. infectoria* (pentadecane). Adamek et al. (1992) identified methylfuran, 2-methylpropanol and 3-methylbutanol as the most important metabolites from *Aspergillus pseudoglaucus*, *A. flavus*, *Penicillium cyclopium* and *Fusarium culmorum* growing on wheat. The volatiles produced by several *Penicillium* species were also studied. Börjesson et al. (1990) collected volatiles produced by several food spoilage fungi grown in pure culture on wheat grains and found that some compounds, especially 3-methyl-1-butanol, were produced early in fungal growth and could be used as an early warning of potential deterioration, before fungal growth became visible. The production of volatile metabolites by *Penicillium* species in grain correlated well with carbon dioxide production and ergosterol formation (Börjesson et al. 1990, 1992). The use of volatile compound production as an indicator of mould deterioration in grains has been extensively assessed and reviewed (Schnürer et al. 1999;

Magan and Evans 2000; Paolesse et al. 2006; Balasubramanian et al. 2007; Orina et al. 2017).

Fungal volatiles can be used to detect potential mycotoxin contamination, to discriminate between fungal species (Sunesson et al. 1995; Keshri et al. 1998), and even between toxigenic and nontoxigenic strains of particular fungi (Sahgal et al. 2007). Karlshøj et al. (2007a) used an electronic nose to differentiate between closely related *Penicillium* species (*P. camemberti, P. nordicum, P. paneum, P. carneum, P. roqueforti* and *P. expansum*) from cheese. Volatile profiles can be used to predict mould spoilage in bakery products (Vinaixa et al. 2004; Marin et al. 2007a) and to detect and differentiate between toxigenic and nontoxigenic *P. verrucosum* strains in bakery products (Needham and Magan 2003). Volatile profiles have also been used to differentiate between toxigenic and nontoxigenic *Fusarium* stains (Keshri and Magan 2000; Demyttenaere et al. 2004), to identify cultures of *Fusarium* species (Infantino et al. 2017), to identify mycotoxins (aflatoxins, ochratoxin A and deoxynivalenol) in durum wheat (Tognon et al. 2005) and to detect and quantify ochratoxin A and deoxynivalenol in barley (Olsson et al. 2002). This technology has also been applied to predict the presence of *P. expansum* and patulin in apple products (Karlshøj et al. 2007), and to detect and discriminate diseases of potato tubers (Kushalappa et al. 2002) and stem-end rot and anthracnose in mangoes (Moalemiyan et al. 2006). Electronic nose technology has also been used for early detection of moulds in libraries and archives (Pinzari et al. 2004).

4.8.3 Immunological Techniques

Cell wall proteins of fungi produce antigens which can be detected by immunological methods. Some antigens are derived from components common to a wide range of fungi, and hence are indicative of general fungal growth, whereas others are genus or even species specific. Although a variety of methods have been developed to take advantage of these fungal antigens, none is in widespread commercial use, having been largely overtaken by molecular detection methods.

Enzyme-linked immunosorbent assay (ELISA) The most commonly applied immunological technique is ELISA. The technique relies on production of immunoglobulin antibodies against particular fungal antigens. These antibodies are then used in the ELISA assay. A considerable body of research during the 1980s to the 2000s (see Pitt and Hocking 2009) showed that it was possible to produce genus-specific antibodies, but there was some cross-reaction between closely related genera such as *Penicillium* and *Aspergillus,* or *Mucor* and *Rhizopus* (Notermans and Heuvelman 1985).

ELISA techniques were studied as a potential replacement for the Howard mould count. Antigens from tomato moulds (*Alternaria alternata*, *Geotrichum candidum* and *Rhizopus stolonifer*) were used to produce an ELISA test sensitive to 1 μg/g of mould in tomato. A correlation was observed between antigen formation and mould added to tomato puree, and background interference was very low (Lin et al. 1986). The method was tested against a broader range of foods, with encouraging results (Lin and Cousin 1987). Robertson and Patel (1989) improved the sensitivity of the method for tomato paste by using a polyclonal antibody against *Botrytis cinerea*, *Mucor piriformis* and *Fusarium solani* in addition to the three species used by Lin et al. (1986). ELISA-based methodology was reported for the detection of *Fusarium* species in corn (Meirelles et al. 2006), cornmeal (Iyer and Cousin 2003) and grain (Rohde and Rabenstein 2005). Correlations with other measures of fungal growth (ergosterol, colony count, mycotoxin levels) were variable. ELISA tests have been applied to detection of yeasts in dairy products (Garcia et al. 2004) and fruit juice (Yoshida et al. 1996). *Aspergillus* species have also been examined as targets for immunological detection because of their importance in mycotoxin contamination. See Pitt and Hocking (2009) or the review by Li et al. (2000) for more details on application of ELISA methods for detection of fungi in foods.

ELISA methodology has been applied most successfully to the detection of mycotoxins, including aflatoxins, ochratoxin A, deoxynivale-

nol and other trichothecenes, fumonisins and some *Alternaria* toxins, and many ELISA-based kits are commercially available for mycotoxin detection and quantification (Turner et al. 2009).

Latex agglutination A different approach to the immunological detection of fungi in foods has been the coating of latex beads with antibodies and detection of agglutination of the beads in the presence of antigens (Kamphuis et al. 1989; Notermans and Kamphuis 1992; Stynen et al. 1992). It was found that 0.8 μm latex beads coated with antibodies from the extracellular polysaccharide produced by *Penicillium digitatum* specifically detected *Aspergillus* and *Penicillium* species (Kamphuis et al. 1989). Detection limits were as low as 5 to 10 ng/mL of the purified antigen. Commercially produced latex agglutination tests were of value for screening the mycological quality of grains and processed foods (Braendlin and Cox 1992; van der Horst et al. 1992), although one kit performed poorly in detection of mould in tomato products (van der Horst et al. 1992).

Kesari et al. (2004) used a latex agglutination text to detect teliospores of Karnal bunt (*Tilletia indica*) in single grains of wheat and were able to detect a few as 750 teliospores, which they reported as suitable for single seed analysis.

No commercial applications of this technology have been found relating to detection of fungi in foodstuffs.

Fluorescent antibody techniques Fluorescent antibody techniques have also been used directly for the detection of mould in foods. Warnock (1971) detected *Penicillium aurantiogriseum* in barley by this method, while Robertson et al. (1988) used antisera from five fungi to visualise moulds and simplify their detection in the Howard mould count technique. No further advances have been reported.

4.8.4 Molecular Methods

The first techniques for detecting DNA sequences using specific probes were devised more than 40 years ago (Southern 1975). With the development of the polymerase chain reaction (PCR), gene cloning and oligonucleotide synthesis, DNA sequences can now be prepared in large quantities for use in probes. Depending on its role in the genome, DNA may be specific at almost any taxonomic level. Molecular methods can be used in both detection and identification of fungi, and real-time PCR methods even allow quantitative detection. This field of research continues to develop very rapidly, and significant advances may be expected.

There have been rapid advances in molecular methods for the detection of mycotoxigenic fungi. Probes have been developed that target genes in the mycotoxin biosynthetic or regulatory pathways for many species of *Aspergillus, Penicillium* and *Fusarium,* enabling these fungi to be detected and/or quantified in grains, grapes, apples, coffee and other food matrices (Edwards et al. 2002; Paterson 2006; Geisen 2007; Niessen 2007a, b; Rossi et al. 2007).

Molecular methods have also been developed for the detection of spoilage fungi, particularly yeasts, e.g., *Brettanomyces/Dekkera* in grapes and wine (Phister and Mills 2003; Agnolucci et al. 2007; Hayashi et al. 2007), *Hanseniaspora* in wine (Phister et al. 2007); *Zygosaccharomyces bailii* in wine and fruit juices (Rawsthorne and Phister 2006) and *Kluyveromyces marxianus* in yoghurt (Mayoral et al. 2006). More general techniques have been described for detection of yeasts in juices (Casey and Dobson 2003; Ros-Chumillas et al. 2007), milk, yoghurt and cheese (Cocolin et al. 2002; Bleve et al. 2003; Garcia et al. 2004; Lopandic et al. 2006; Gente et al. 2007), sour dough fermentation (Meroth et al. 2003), table olives (Arroyo-Lopez et al. 2006) and vacuum package ham (Sanz et al. 2005). PCR-based methods have also been described for detection of moulds in orange juice (Wan et al. 2006), *Alternaria* in cereal grains (Zur et al. 2002) and *Botrytis* in onion seeds (Walcott et al. 2004).

As gene sequencing has become more readily available and cheaper, along with freely accessible databases such as GenBank (http://www.ncbi.nlm.nih.gov/Genbank/), sequence analysis and comparison is becoming a routine method for identification of yeasts and filamentous fungi.

For yeasts, the most common target region is the D1/D2 domain of the 26s rDNA (Kurtzman et al. 2003), but the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) may also be used (van der Vossen et al. 2003; Pulvirenti and Giudici 2003; Solieri et al. 2006; Villa-Carvajal et al. 2006).

Sequencing of the ITS region along with 'housekeeping genes' such as calmodulin, β-tubulin and elongation factor 1-α is now commonly applied for purposes of identification and phylogenetic analysis of important food spoilage and mycotoxigenic fungi in the genera *Aspergillus* (Varga 2006; Geiser et al. 2007; Peterson 2008), *Penicillium* (Peterson 2004, 2006; Samson et al. 2004b; Wang and Zhuang 2007; Serra et al. 2008) and *Fusarium* (O'Donnell et al. 2004; Scott and Chakraborty 2006; Leslie et al. 2007). Details of more recent analyses utilising the ITS region will be found under individual genera.

Despite the apparent power of molecular techniques, they need to be applied with some caution, particularly when comparing DNA sequences with those in the publicly available databases to identify yeast or mould isolates. Correct identification relies on the database sequences having the correct name attached to them by the depositor, which is not always the case. If the identification makes sense, the percent homology is 98% or greater, and the number of base pairs on which the homology is scored is high, then the answer is probably correct.

4.9 Identification Media and Methods

4.9.1 Standard Methodology

The identification keys in this book are based primarily on the standardised procedure described for the identification of *Penicillium* species by Pitt (1979). Cultures are grown for 7 days on three standard media at 25 °C, and on one of these at 5 and 37 °C also. The three media are Czapek Yeast Extract Agar (CYA; Pitt 1973) used at all three temperatures; Malt Extract Agar (MEA; Raper and Thom 1949) and 25% Glycerol Nitrate Agar (G25N; Pitt 1973). Their formulae are given in Appendix 1. Preparation time of CYA and G25N is reduced by the use of Czapek concentrate (Pitt 1973), which is added to the media at the rate of 1% of the aqueous portion.

As media ingredients have become more purified in recent years, difficulties with extent and colour of sporulation on CYA have been encountered, especially with some *Penicillium* species. To overcome this problem, Czapek concentrate has been reformulated (Pitt 2000) by the inclusion of traces of zinc and copper (Smith 1949) (See Appendix 1).

4.9.2 Plating Regimen

As noted above, cultures for identification are grown on three media and at three temperatures. Maximum efficiency in time, incubator space and materials is achieved by inoculating two cultures on a single Petri dish of G25N, and at 5 and 37 °C, as shown in Fig. 4.3. Cultures are rarely mutually inhibitory under these conditions, although overgrowth of one culture by another is occasionally a problem at 37 °C. Standard sized Petri dishes (90–100 mm) are used, except that at 5 °C, smaller plates (50–60 mm in diameter) can be an advantage. The smaller size is easier to examine under the low power microscope. All plates are incubated for a standard time of 7 days.

Plates incubated at 37 °C should be enclosed in polyethylene bags to prevent evaporation and drying of the medium. Unless the humidity is very low, plates at 25 °C will not dry excessively in 7 days. If no 5 °C incubator is available, use a polyethylene food container or insulated box in a household refrigerator. The box should be equipped with a thermometer, and its location moved by trial and error until a place with a 5 °C average temperature is located. Temperatures at 5 and 37 °C should ideally be ±0.5 °C, and be checked frequently; at 25 °C, ±2 °C control is adequate (Okuda 1994).

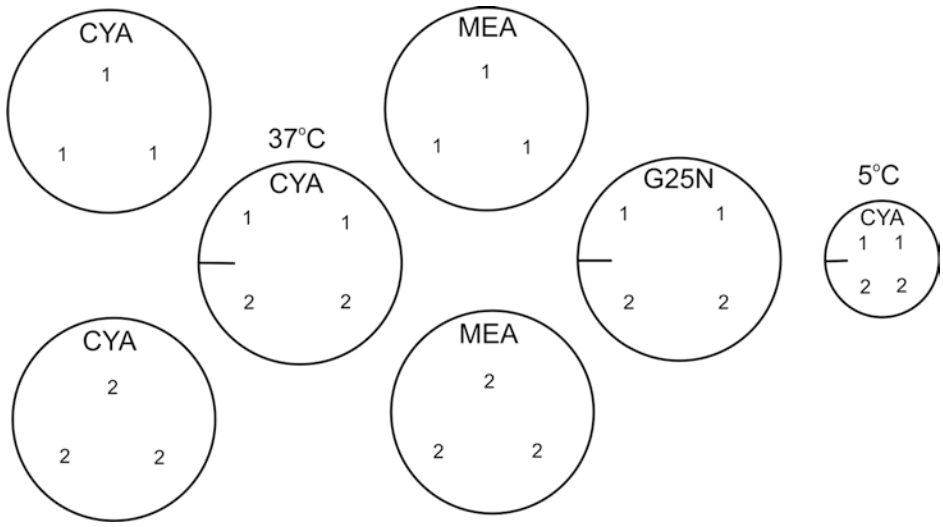

Fig. 4.3 Schematic of regimen used for culturing fungal isolates for identification

4.9.3 Inoculation

As shown in Fig. 4.3, Petri dishes of CYA and MEA for incubation at 25 °C are inoculated with a single culture at three points, equidistant from the centre and the edge of the plate, and from each other. Plates of the other media are inoculated with two points per culture, as illustrated.

With some fungi, especially *Penicillium* and *Aspergillus*, it is important to minimise colonies from stray spores. The most satisfactory technique is to inoculate plates with spores suspended in semisolid agar (Pitt 1979). Dispense 0.2–0.4 mL of melted agar (0.2%) and detergent (0.05%), such as polysorbitan 80 (Tween 80), in small vials and sterilise. To use, add a needle point of spores and mycelium to a vial and mix slightly. Then, before flaming the needle, use it to stab inoculate the 5 °C plate; residual spores on the needle make a good inoculum. Next, take a sterile loop, mix the vial contents thoroughly, and inoculate the standard plates. Used vials can be sterilised by steaming, and reused several times before being washed or discarded.

4.9.4 Additional Media and Methods

The plating regimen outlined above can be used to identify most of the fungi described in subsequent chapters of this book. Some exceptions exist, because certain genera either grow poorly or fail to sporulate on the standard media. As noted earlier in this chapter, fastidious xerophiles are identified on MY50G agar. *Aspergillus* subgen *Aspergillus* (*Eurotium* species), traditionally identified on Czapek Agar with 20% sucrose, are identified here on Czapek Yeast Extract Agar with 20% Sucrose (CY20S; see Appendix 1). *Trichoderma* species are best identified on Potato Dextrose Agar (PDA) after a relatively short incubation time (3–4 days), as the structures tend to autolyse as cultures mature.

Penicillium **subgenus** ***Penicillium*** Many species classified in subgenus *Penicillium* are morphologically similar, and identification using traditional morphological techniques remains difficult. These Penicillia are very common in foods, and many produce mycotoxins, so correct identification is often critical. Species within

subgenus *Penicillium* fall into two groups: those with an affinity for proteinaceous foods and those which grow more vigorously in foods high in carbohydrate. Frisvad (1981, 1985) introduced creatine sucrose agar (CREA) to permit differentiation between these two groups. Creatine (as the sole nitrogen source) permitted growth of the former group while inhibiting the latter. Incorporation of bromocresol purple enabled visualisation of pH changes, either acid or alkaline, depending on the creatine or sucrose metabolism of a particular species. However, discrimination between positive and negative responses was not always clear, and the main tabulation of species reactions to CREA (Table 1 in Frisvad 1985) was difficult to interpret. Frisvad (1993) subsequently produced a number of variations of CREA, including acid and neutral pH formulations, and substitution of sucrose with fructose or lactose. Although the merits of each formulation were discussed, no firm recommendation resulted from this exercise.

Pitt (1993) modified Frisvad's strongly alkaline CREA medium by studying several sucrose and creatine concentrations over a wide pH range. The result was Neutral Creatine Sucrose agar (CSN), a medium producing eight different reactions among the 20 *Penicillium* subgenus *Penicillium* species tested. When included in the normal plating regimen for identification of *Penicillium* cultures from the subgenus *Penicillium*, CSN provides a very useful aid to distinguishing between these difficult and closely related species. See Chap. 7 for details of use of CSN, its reactions and interpretation. Its formulation is given in Appendix 1.

Dematiaceous Hyphomycetes The natural habitats for many dematiaceous Hyphomycetes are plants or plant material, so finding suitable laboratory media and conditions to induce typical sporulation can be difficult. *Alternaria*, *Curvularia* and *Stemphylium* species are best identified from Dichloran Chloramphenicol Malt extract Agar (DCMA; Andrews 1992b) plates incubated for 7 days at 25 °C under lights. Conidial characteristics of *Bipolaris* species vary with type of medium. Species described here are best identified from Tap Water Agar (TWA) containing a natural substrate such as sterilised wheat or millet seeds, or wheat straw. *Drechslera* species will grow well on DCMA but sporulation is poor. The best sporulation is achieved with V-8 Juice (V-8 J) agar or TWA with one of the natural substrates mentioned above, incubated at 25 °C for 7–10 days under lights with a 12 hr photoperiod. Light/dark periodicity is important as *Drechslera* cultures require light to produce conidiophores and darkness to produce conidia, possibly due to light inactivation of flavine necessary for conidial formation (Knan 1971; Platt et al. 1977).

For the identification of *Trichoderma* and *Fusarium* species, Potato Dextrose Agar (PDA) is used. *Fusarium* species also require additional methods and media as outlined below.

4.9.5 *Identification of* Fusarium *Species*

Fusarium isolates exhibit unusually high variability in colony morphology and also may deteriorate rapidly in culture. Thus, they should be identified as soon as possible after initial isolation with a minimum of subculturing to avoid deterioration. It is common practice to prepare cultures of Fusaria from single spores for growth on identification media, as this reduces both of these problems.

Single sporing The technique for preparing single spore cultures is as follows (Nelson et al. 1983; Leslie and Summerell 2006). Pour about 10 mL of 2% water agar into unscratched glass or plastic Petri dishes and allow to dry, either by holding the plates at room temperature for several days, or by placing them inverted in an oven at 37–45 °C for about 30 min. Prepare a suspension of conidia in a 10 mL sterile water blank so that it contains 1–10 spores per low power (10×) microscope field when a drop from a 3 mm loop is examined on a slide. With experience, this concentration can be gauged simply by observing the turbidity of the suspension. Pour the suspension of spores onto a dried water agar plate, drain off the excess liquid, and incubate in an inclined position at 20–25 °C for 18–20 hr.

After incubation, open the Petri dish, shake off any accumulated moisture droplets, and examine under a stereomicroscope using transmitted light. The germinating conidia should be visible under 25× magnification. A dissecting needle with a flattened end and sharpened edges is used to cut out small squares of agar containing single, germinating conidia. These single conidia are then transferred on the agar blocks to the desired growth medium.

If the original culture is contaminated with bacteria, a drop of 25% lactic acid may be added to the water blank. Allow this acidified spore suspension to stand for 10 min before pouring onto a water agar plate. Germination of acid-treated *Fusarium* conidia may be delayed by 24 hr or more.

Media Two media have been used in this book for the identification of *Fusarium* isolates: Potato Dextrose Agar (PDA) for colony characteristics and colour; and Dichloran Chloramphenicol Peptone Agar (DCPA) for the development of diagnostic macro-, micro- and chlamydoconidia.

A third medium, Carnation Leaf Agar (CLA) is recommended by some *Fusarium* specialists for *Fusarium* cultivation and identification (Nelson et al. 1983; Leslie and Summerell 2006). CLA is an excellent medium, on which most *Fusarium* species readily produce their diagnostic macroconidia. Production of macroconidia on DCPA is usually comparable with that on CLA, but microconidia and chlamydoconidia are often more plentiful on CLA due to greater production of aerial hyphae. DCPA is used in the present work rather than CLA, however, because dried, gamma-irradiated carnation leaves are difficult to obtain in many localities.

Inoculation and incubation For identification by the methods used in this book, single spore cultures of *Fusarium* isolates should be prepared on agar blocks as outlined above, inoculated, one per plate, onto two plates each of PDA and DCPA, and incubated at 25 °C for 7 days. Individual plates may be used for each medium, or alternatively divided plates may be used, with one medium on each half of the plate. Illumination during incubation is essential for the production of macroconidia. The light source may be diffuse daylight (not direct sunlight) or light from a bank of fluorescent tubes. A photoperiod of 12 hr per day is normally used. Alternating temperatures of 20 and 25 °C have been recommended (Nelson et al. 1983; Leslie and Summerell 2006) but are not essential.

A simple light bank may be constructed from a standard 40 watt fluorescent fixture with two cool white tubes, suspended 0.5-l m above the laboratory bench or shelf supporting the cultures. The addition of a black light tube (e.g. Phillips TL 40W/80 RS F40BLB) is also desirable and in some cases essential to induce macroconidial or chlamydoconidial production.

4.9.6 Yeasts

Yeast identification systems Identification of foodborne yeasts remains a difficult task, as colony characteristics and microscopic morphology are of limited value. Generally it has been necessary to use biochemical and physiological tests such as fermentation of carbohydrates, assimilation patterns for a range of carbon and nitrogen sources, and growth at various temperatures. Details of these methods and media may be found in Kurtzman and Fell (1998), Kurtzmann et al. (2003) or Barnett et al. (2000). Molecular methods (see below) are being used increasingly in yeast identification, replacing biochemical testing.

Identification using systems based on biochemical and physiological testing is complex and time consuming. However, a number of attempts have been made to assist those who wish to persevere with yeast identification in this manner. Several simplified systems have been published in the literature, and both automated and manual yeast identification systems are now commercially available.

Deák and Beuchat (1987) published a Simplified Identification Key (SIK) which included 215 species of foodborne yeasts. They subsequently modified their system, restricting it to the 76 species most frequently occurring in foods (SIM), and reported that it was much

more successful than the API 20C system (BioMérieux, Marcy-l'Etoile, France) for identification (Deák 1993). The SIM uses only two Petri dishes and three test tubes to examine each strain for ability to assimilate 10 carbon sources, fermentation of glucose, assimilation of nitrate and splitting of urea. These biochemical tests are supplemented by morphological observations. SIM separates the yeasts into six groups by a dichotomous key utilising the results of five key tests. Further tests are used to differentiate the yeasts in these six groups using secondary dichotomous keys.

A dichotomous key to 25 common species of foodborne yeasts was published by Smith and Yarrow (1995), who used 17 biochemical and physiological tests to distinguish the species.

Of the commercial systems available, the most widely used for foodborne yeasts are the Biolog, which is an automated system, and the BioMérieux ID32C Yeast Identification strips which can be read manually or automatically using the ATB system. BioMérieux also markets the fully automated VITEK 2 system, based on a card containing 64 tests. However, the database comprises only 46 clinically important species (Aubertine et al. 2006) so has limited application to foodborne yeasts.

The Biolog (Biolog Inc., Hayward, CA, USA; http://www.biolog.com/microID.html) utilises a Yeast Identification Test Panel (YT MicroPlate™) consisting of a matrix of 8 × 12 wells. The first three rows contain 35 carbon source oxidation tests using tetrazolium violet as an indicator of oxidation. The next five rows contain carbon assimilation tests which are scored turbidimetrically against a negative control panel containing only water. The last row contains two carbon sources, and tests for the co-utilisation of various carbon sources with D-xylose. The hardware (Biolog MicroStation Reader) consists of an automated plate reader coupled with a computer, which interprets the results and compares them with the resident database which currently includes 267 species. Manual interpretation of the Biolog plates is not recommended. This system has been designed with the food industry in mind, and the database contains all the common foodborne yeasts, unlike other systems which are usually aimed at the clinical market.

The ATB ID32C system (BioMérieux, Marcy-l'Etoile, France) is an automated system utilising the BioMérieux ID32C Yeast identification strips. These strips contain 30 assimilation tests, plus a positive (glucose) and a negative control well, all of which are inoculated with a yeast suspension of specified density. The strips are incubated at 30 °C for 48–72 hr. As with the Biolog, the ATB automated system consists of a plate reader attached to a computer. The database associated with the ATB system contains 63 yeast species.

The BioMérieux ID32C strips can be read manually, and the results enable identification of yeasts using published keys, or computer identification programmes such as that of Barnett et al. (1996). This system is may be used with reasonable success, particularly when the test results from the ID32C strip are supplemented with extra tests (glucose fermentation, urease production, nitrate utilisation, growth in 0.5% and 1% acetic acid, and in 50% and 60% glucose, growth at 37 °C, production of pseudohyphae, ascospore formation and morphological observations) to give a more comprehensive base for the identification. Growth in 0.5% and 1% acetic acid indicates preservative resistance, and growth in 50% and 60% glucose gives an indication of ability to grow at reduced a_w. Both these parameters are important in determining a yeast's ability to cause spoilage in particular products.

Identification using DNA sequencing is increasingly becoming the method of choice, as extensive databases such as GenBank are freely available for identification purposes. The 600–650 nucleotide D1/D2 region of the large subunit (26S) ribosomal DNA is the most widely targeted section of the genome, and sometimes the ITS region may also be used (Kurtzman et al. 2003). Sequencing of the D1/D2 region, along with some supplementary physiological and biochemical tests, is the identification method currently used in our laboratory.

Even using the available systems, identification of yeasts still requires some specialist

knowledge and interpretation, and remains time consuming.

Our experience indicates that no more than twelve species of spoilage yeasts are of real concern in foods. It is possible to differentiate these few species by relatively simple techniques, i.e. colony and microscopic morphology, growth on the standard media used for filamentous fungi, growth on other media which test for preservative resistance, ability to use nitrate as a nitrogen source and adaptation to high NaCl concentrations. Details of these techniques are given in Chap. 10.

4.10 Examination of Cultures

As noted above, all cultures for identification should first be grown on the standard regimen described earlier. After 7 days incubation, the following examination should be carried out, and then the general key to fungi in Chap. 5 should be used. That key will be of assistance even in the event of cultures failing to grow under one or more of the standard conditions.

4.10.1 Colony Diameters

Measure the diameters of macroscopic colonies in millimetres from the reverse side (Fig. 4.4). Microscopic growth or germination at 5 °C is assessed by low power microscopy (60–100×), by putting the 5 °C Petri dish on the microscope stage and examining by bright field, transmitted light. Growth at 37 °C is assessed macroscopically only; germination of spores at 37 °C is an unreliable character.

4.10.2 Colony Characters

Colony appearance can be judged by eye or with a hand lens, but examination is more effective if a stereomicroscope is used. Magnifications in the range of 5× to 25× are the most useful. Characters such as type and location of sporing structures and extent of sporulation are best gauged with the stereomicroscope. Reflected light is more effective than transmitted light.

To determine colony colours, examine colonies by daylight or by daylight-type fluorescent light. In some genera, reference to a colour dictionary is helpful. The Methuen "Handbook of Colour" (Kornerup and Wanscher 1978) has been used in this work and is highly recommended.

4.10.3 Preparation of Wet Mounts for Microscopy

Fungi should always be examined microscopically as wet mounts rather than heat fixed and stained like bacteria. To prepare a wet mount, use

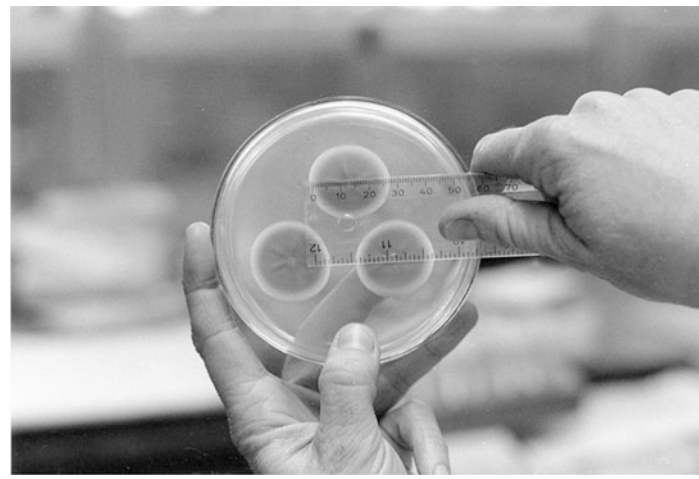

Fig. 4.4 Technique for measuring colony diameters by transmitted light

an inoculating needle to cut out a small portion of the colony which includes sporing structures. Examination with the stereomicroscope can be an invaluable aid here. For freely sporing fungi with little mycelium, cut a piece of colony near the edge, where fruiting structures are young and spore numbers not excessive. Take structures which may enclose spores, i.e. cleistothecia, etc, from near colony centres, where the probability of mature spores is highest. If the only differentiated parts of the colony appear to be buried in the agar, e.g. pycnidia, take a sample of these with a small piece of the agar. Float the cut colony sample from the needle onto a slide with the aid of a drop of 70% ethanol. It may be necessary to tease out the specimen with the needle and the corner of a cover slip (square cover slips are best). Fungal specimens may be highly hydrophobic: the ethanol helps to wet the preparation, minimising the amount of entrapped air. When most of the ethanol has evaporated, add a drop of lactic acid (for phase or interference contrast optics) or lactofuchsin stain (see below) for bright field. Add a cover slip; if necessary remove excess liquid from the preparation by gently blotting with facial tissue or similar absorbent paper. The preparation is now ready for examination.

4.10.4 Staining

A wide variety of stains are in use for mycological work. However most are time consuming to prepare, or to use, or are slow to act, because fungal walls and spores are highly resistant to stains. By far the most effective stain for use in food mycology is lactofuchsin (Carmichael 1955), which suffers from none of these faults. It consists of 0.1% acid fuchsin dissolved in lactic acid of 85% or higher purity. Young actively growing fungal structures are preferentially coloured bright pink, so sporing structures can usually be readily distinguished against a background of older mycelium. Cleistothecial initials, developing asci and maturing ascospores are also seen more readily in preparations stained with lactofuchsin.

Like most other mycological stains, lactofuchsin is corrosive. Take care to clean it off microscope parts or skin! Be especially careful of the objective faces, because lactic acid will slowly corrode the relatively soft glass used in lenses.

4.10.5 Microscopes and Microscopy

Choice of microscope Fungal identifications inevitably involve microscopy. A high quality, binocular compound microscope is essential for serious mycological work. Bright field, phase contrast and interference contrast optics are all suitable. If bright field optics are used, preparations should be stained. Phase contrast optics avoid the need for staining, although as explained above, staining has merit for certain structures in any case. Resolution under phase contrast sometimes suffers from excessive halo effects because fungal structures are highly refractile. Experience suggests that bright field optics are more satisfactory than phase contrast for examining most fungi. Interference contrast is superior to both bright field and phase contrast, and is strongly recommended. The very thin optical section cut by this system provides higher resolution of dense structures and the relatively low contrast is restful to use.

The microscope should be equipped with at least four objectives: 6× or 10× for examining Petri dishes under low power (for germination, etc.); 16× or 20× for searching fields for sporing structures; 40× for examining structural details and measuring stipes, fruiting structures and large conidia; and 100× oil immersion for observation of details of spore attachment, surface texture, ornamentation of hyphae and spores and fine measurements. Oculars should be 10× or 12.5×, and may with advantage be wide field and have a high eye point suitable for use with spectacles. One should be a focusing ocular, equipped with an eyepiece micrometer, which is essential for measuring dimensions of spores and sporogenous structures.

In the examination of fungal mounts, it is stressed that it is most important to use low power

optics before succumbing to the temptation to use oil immersion. The principal reason is that fungal preparations usually remain as small clumps and do not disperse as bacteria do. Only under low power is the search for the optimal area of the slide for the observation of fruiting structures likely to be rewarded. Once a suitable area is located under the 16× or 20× objective, move to the 40×. This should be the lens most used; microscope optics are such that only the finest details of ornamentation can be observed more effectively under oil immersion than at this magnification.

Aligning the microscope Correct alignment of the microscope is essential, so that its resolution is as high as possible and it can be used for long periods without discomfort. An incorrectly aligned microscope will lead to poor observation, discomfort, fatigue, headache and eye-strain. A person of normal visual acuity should be able to use a correctly aligned instrument throughout a whole working day without discomfort. The steps to correctly align a microscope are given below. They should be read in conjunction with the microscope manufacturer's instructions.

1. Mount a slide on the stage, and bring it into approximate focus. If a prepared slide is not available, a slide marked with a marking pen or ink line is a satisfactory substitute.
2. Close the microscope's field diaphragm (the one at the microscope base nearer the light source). The image of the diaphragm opening should now be visible in the microscope field. If it is, first focus it with the condenser focusing knob and then centre it in the field with the condenser centring screws. If the diaphragm opening cannot be seen, first rack the condenser up and down and watch to see if the opening becomes visible; if it does not, rack the condenser to its highest position, and then slowly open the field diaphragm until the opening comes into view. Centre the diaphragm approximately and proceed as above.
3. For bright field optics, the condenser diaphragm should be adjusted each time the objective power is changed. Remove one ocular; close the condenser diaphragm so that the field seen down the open tube is about two-thirds its maximum size. With phase contrast and interference contrast systems, this adjustment is less critical.

The preceding steps align the microscope itself, and should be checked frequently. If optimal illumination is desired, each step should be carried out for each new slide, and each objective change. As a routine habit, the whole process should take only a few seconds.

If the available microscope is not equipped with a built in light source, a field diaphragm and a fully centring condenser, it is unlikely to be satisfactory for the identification of small spored fungi such as *Penicillium* species.

The following steps are designed to align the observer with the microscope, compensating for individual differences in sight. Provided settings on the microscope are remembered, these steps need be carried out only occasionally, to check that visual acuity has not altered. Different settings will be needed for an individual with and without spectacles or contact lenses.

4. Assuming the microscope is binocular, pull the oculars out to their greatest distance apart and then, while watching a focused field, move them gradually together until a single circular field is seen without strain or head movement. Note the distance on the scale between the oculars; this is the individual's interpupillary distance. Repeat this operation two or three times until satisfied that the correct distance has been found. This distance should always remain the same and be similar on any microscope.
5. Under the 40× or 100× objective, locate a tiny, readily recognised point on the slide and focus on it. Take a piece of white card and place it between the focusing ocular and the corresponding eye. Leave the eye open. Now focus the tiny point with the other eye, carefully, with the microscope fine focus. Next, transfer the white card to the other ocular and, using the focusing collar beneath the ocular, refocus the tiny point. Remove the card and note the

setting on the scale. Repeat until satisfied the correct setting has been found.

6. On some microscopes, the eyepiece micrometer can be focused independently. Use the focusing system on the ocular itself to focus the micrometer. Note the setting on the scale on the side of the ocular.

Always check the settings on the microscope before use, and after making measurements with the micrometer. It is very easy to upset the ocular alignment when measuring.

4.11 Preservation of Fungi

Many fungi are stable in culture, and can be subcultured repeatedly without apparent change or deterioration. Others, especially *Fusarium* species and other plant pathogens will degenerate rapidly after only a few transfers.

For stable isolates that are used routinely in the laboratory, storage on agar slopes is satisfactory. Many freely sporing fungi will survive for several months, and sometimes much longer, when stored at 1–4 °C on a medium such as CYA. One of the hazards of storage at these temperatures is contamination by psychrophilic *Penicillium* or *Cladosporium* species. Storage at freezer temperatures (−18 to −20 °C) prevents such contamination, but some fungi do not survive well under these conditions.

Storage at room temperatures, at 10 °C or above, for long periods is not advisable because of the likelihood of invasion by culture mites (see below).

4.11.1 Lyophilisation

For unstable cultures, and indeed for the long term storage of any food spoilage fungi, freeze drying or *lyophilisation* is the best method of preservation. Many commercial systems are now available for carrying out this process.

A satisfactory menstruum for lyophilisation of most fungi is l.5× normal strength reconstituted nonfat milk powder (15% in distilled water). For fungi with hydrophobic conidia, such as *Aspergillus* and *Penicillium*, a small amount of detergent (0.05%) such as polysorbitan 80 (Tween 80) should be added to the milk. Dispense the milk in 10 mL lots in small tubes or 12.5 mL (0.5 oz) McCartney bottles, and sterilise by steaming for 20 min on three successive days (the Tyndallisation process). The milk must be stored at 20 °C or above between steamings, to permit bacterial spores to germinate. Occasionally bacterial spores will survive this process: it is advisable to store the milk at room temperature for some days after Tyndallisation. Any bottles which show clotting or other breakdown should be autoclaved and discarded. Autoclaving at 121 °C, even for 10 min or less is not recommended, as browning will occur and browning compounds are known to be inhibitory to microorganisms.

Most common spoilage fungi survive lyophilisation well. However, in our experience and that of others (Mikata et al. 1983; Smith and Onions 1994), some yeasts, plant pathogens and xerophiles do not. Storage of lyophilised ampoules at refrigeration temperatures (0–4 °C) is recommended, but room temperature is probably satisfactory provided sunlight is avoided and temperatures do not exceed 30 °C. Some laboratories routinely store lyophilised cultures at −18 to −20 °C.

Strains being maintained for a particular trait or utilised for a specific purpose such as system testing or metabolite production should always be lyophilised. Continued subculturing often leads to deterioration or loss of the desired character. The ability of a strain to produce a particular mycotoxin, for example, may decrease with each transfer. Isolates should be lyophilised as soon as possible after primary isolation to prevent degeneration.

4.11.2 Other Storage Techniques

A variety of systems other than lyophilisation have been proposed for long term storage of fungi. Of these, liquid nitrogen storage has found most acceptance with major culture collections. This type of storage appears to be superior to any

other for plant pathogens and fungi which will not sporulate in pure culture. However, liquid nitrogen systems are expensive to establish and maintain and are only suitable for large collections.

Freezer units which run at very low temperatures (−80 °C or below) are available and are well suited to the needs of culture collections. In our laboratory we routinely store cultures at −80 °C, using glycerol (60–80%) as a cryoprotectant. Spore suspensions are prepared by taking conidia or ascospores from a freely sporulating sector of the colony, dispersing them in the glycerol then freezing in screw-capped cryovials. An 80% solution of glycerol remains viscous at −80 °C, which enables cultures to be removed from the freezer for subculture without the need for defrosting.

For smaller laboratories that do not have access to lyophilisation, liquid nitrogen or ultra-low temperature storage, some simple techniques exist that can be used to maintain fungal cultures over relatively long periods (e.g. 1–10 years) without the need for subculturing.

Water storage A simple and inexpensive method of fungal culture preservation is storage of agar blocks in water (Smith and Onions 1994). Small agar blocks (7–10 mm square) are cut from the growing margin of a young fungal colony and placed in sterile water in a bottle such as a Bijou bottle (6.25 mL or 0.25 oz McCartney bottle). The rubber lined cap is screwed down, and the bottles stored in a cool room (1–10 °C). Cultures may be revived by removal of a block and placing it on a suitable growth medium. Using this method, cultures have been reported to remain viable and retain their characteristics for up to 7 years (Boeswinkel 1976; Smith and Onions 1994).

Some yeasts may be maintained by storing as suspensions in water (Kirsop 1984). Growth from a late logarithmic slant culture is suspended in sterile distilled water and transferred to a sterile container so that 90% of the volume is filled with the suspension. Containers are stored at room temperature. Survival of some *Candida, Saccharomyces, Cryptococcus, Rhodotorula* and *Schizosaccharomyces* species for up to 4 years has been reported (Kirsop 1984).

Silica gel storage Many fungal cultures may be maintained for long periods (often more than 10 years) by drying spore suspensions onto silica gel (Smith and Onions 1983; Smith 1984). This method is not suitable for mycelial cultures, but can be used with some success for yeasts (Kirsop 1984). As silica gel liberates heat when moistened, the technique depends on keeping the cultures cool enough to avoid damaging the spores during preparation. Medium grain plain (non-indicating) silica gel of 6–22 mesh is placed in suitable glass bottles (Bijou or McCartney bottles) to a depth of about 1 cm and sterilised, either by dry heat at 180 °C for 2–3 hr, or by autoclaving at 121 °C for 15 min. Autoclaved silica gel must be thoroughly dried in an oven before use. Bottles are precooled by placing in a tray of ice or refrigerating for 24 hr, then transferring to an ice tray for inoculation.

Suspensions of fungal spores or yeasts are prepared in sterile skimmed milk (as for lyophilisation, above) and the suspension added to the cooled silica gel to wet three-quarters of it. The bottles and gel are allowed to dry at room temperature for 10–14 days with caps slightly loosened. Caps are then screwed down and the bottles stored at 4 °C (storage at room temperature is also satisfactory) in air tight containers over indicating silica gel to absorb any moisture.

Cultures are revived by shaking a few crystals of silica gel onto a suitable growth medium (broth culture may be better for yeasts). Survival varies according to species or even strain, but survival of yeasts for up to 5 years (Kirsop 1984) and fungi for more than 10 years (Smith and Onions 1983) has been reported.

4.12 Housekeeping in the Mycological Laboratory

Like any other microbiological laboratory, a mycological laboratory should be kept in a clean condition. Discard unwanted cultures regularly and dispose of them by steaming or autoclaving. Wipe bench tops regularly with 70% ethanol. Floors should be wet-mopped, or polished only

with machines equipped with efficient vacuum cleaners and dust filters. Where possible store food and plant materials away from the laboratory. Open Petri dishes carefully. Use small inocula on wet needles. Transport Petri dishes to the stereomicroscope stage before removing lids. Do not bump cultures during transport.

Contrary to popular belief, a well run mycological laboratory is not a source of contamination to bacteriological laboratories. The air in a mycological laboratory should not carry a significant population of fungal spores. The reverse problem can occur, however, because bacteria multiply more rapidly than do fungi. Bacterial spores are often present in food laboratories, readily infect fungal plates, and can rapidly outgrow and inhibit fungal mycelia, especially at 37 °C.

If for any reason fungal spore concentrations do build up in a laboratory and cause an unacceptable level of contamination, the air should be purified. The simplest technique is to spray the air throughout the laboratory with an aerosol before it is closed in the evening. Any aerosol spray, such as a room deodoriser or air freshener, is effective. Aerosol droplets entrain fungal spores very efficiently and carry them to the floor.

A more drastic and effective treatment in cases of severe contamination is to spray a solution of 2% thymol in ethanol around the room and close it for a weekend. The spray is rather pungent, and while not harmful to humans, it effectively kills fungal spores and mites (see below). Do not leave cultures on benches before fumigation.

4.12.1 Culture Mites

A major hazard in growing and maintaining fungal cultures is the culture mite. Many species of mites live on fungal hyphae as their main or sole diet in nature, and find culture collections an idyllic environment. Mites crawl from culture to culture, contaminating them with fungi and bacteria as they go or, given long enough, may eat them out entirely.

Mites are very small (0.05–0.15 mm long), usually just visible to the observant naked eye. They are arachnoids, related to spiders, and hermaphroditic. Each mite leaves a trail of eggs about half adult size as it goes. Eggs hatch with 24 hr and reach adulthood within two or three days. The damage an unchecked mite plague can do to a fungal culture collection has to be experienced to be believed.

The most common sources of mites are plant material, soil, contaminated fungal cultures and mouldy foodstuffs. Mites can also be carried on large dust particles. Building work near a laboratory almost always induces a mite infestation.

The avoidance of losses due to mites requires constant vigilance. Always watch for tell-tale signs, such as contaminants growing around the edges of a Petri dish, a “moth-eaten” appearance to colonies or “tracks” of bacterial colonies across agar. Examination of suspect material or cultures under the stereomicroscope will readily reveal the presence of mites and mite eggs.

Adult mites are rapidly killed by freezing, and mite eggs will only survive 48–72 hr at −20 °C. Cultures contaminated by mites can often be recovered by freezing for 48 hr, then subculturing from uninfected portions of the culture with the aid of the stereomicroscope. Suspect food and other samples being brought into the laboratory should also be frozen for 24 hr to destroy mites before enumeration or subculturing is carried out.

Infestation by mites can be minimised by good housekeeping, i.e. by avoiding accumulation of dust or old cultures in the laboratory. It is also good practice to handle and store food and plant samples well away from areas where fungi are inoculated and incubated.

To control a mite infestation, remove all contaminated material, including cultures. Freeze Petri dishes and culture tubes which must be recovered; autoclave, steam or add alcohol to all others. Clean benches thoroughly with sodium hypochlorite (household bleach) or 70% ethanol. Incubators can be disinfested with aerosol insecticides.

4.12.2 Problem Fungi

There are three fungal invaders which should be watched for carefully in a food mycology laboratory: *Neosartorya fumigata*, *Rhizopus stolonifer* and *Neurospora (Chrysonilia) sitophila*. The first is a human pathogen; the others can cause a contamination chain which is difficult to break.

Neosartorya fumigata may cause invasive aspergillosis in the lungs or serious allergenic responses in some individuals. It is sound practice to immediately kill and discard cultures of this fungus as soon as it is recognised. On no account should it be used for experimental studies in food spoilage or biodeterioration without precautions to prevent dissemination of spores. The morphology of *N. fumigata* is described in detail in Chap. 8, but it is readily recognisable in the unopened Petri dish:

- colonies are low, dull blue and broadly spreading, with a velvety texture;
- growth is very rapid at 37 °C, covering a Petri dish in two days;
- long columns of blue conidia are readily seen under the stereomicroscope.

Rhizopus stolonifer is a ubiquitous fungus in many kinds of foods. It grows rampantly at 25 °C, filling a Petri dish with sparse, dark mycelium in two days. It produces barely macroscopic aerial fruiting structures which are at first white, then become black. Given seven undisturbed days, it sheds dry, black spores outside the Petri dish, providing an effective inoculum for a continuous chain of future contamination. Once such an infection occurs, it is essential to carefully place the contaminated plate in a suitable container such as a plastic bag before transporting it to steamer or autoclave. Then use 70% ethanol to clean areas on which the plate had been incubated or placed. At daily intervals, carefully examine all plates inoculated subsequently, discarding any which show *Rhizopus* contamination, until infection ceases. Spraying the air with aerosols or thymol in ethanol will assist. Unlike *Neosartorya fumigata*, *R. stolonifer* is not pathogenic.

The third problem fungus, *Neurospora sitophila*, is known as "the red bread mould". It used to be common, but due to changes in manufacturing practice, it is now encountered less frequently, either in the bakery or the laboratory. Like *Rhizopus stolonifer*, it grows with great rapidity at 25 °C. It forms a thin, pink mycelial growth across a Petri dish, clearly following the oxygen gradient which leads to the open air. It will force its way unerringly between dish and lid and, once outside, will produce masses of pink spores, which are quickly shed and build up around the base of the Petri dish. Decontamination relies on the same techniques as for *Rhizopus*: *N. sitophila* is probably the more difficult fungus to eradicate. Again, apart from its nuisance value in the laboratory, it is harmless.

4.12.3 Pathogens and Laboratory Safety

While it must be said that any fungus which is capable of growth at 37 °C is a potential mammalian pathogen, the physiology of the healthy human is highly resistant to nearly all of the fungi encountered in the food laboratory. Nevertheless, fungi which can grow at 37 °C should be treated with caution. In particular, the habit of sniffing cultures is to be avoided wherever possible. It is true that odours produced by fungi have been used quite frequently as taxonomic criteria, especially in older publications, but their subjective and ephemeral nature makes them of little value for this purpose, and the risks involved are serious. Some laboratories regard fungal volatiles as such a serious risk that cultures such as *Penicillium* and *Aspergillus* species are handled in a biohazard cabinet. Many types of fungal spores are allergenic or carry mycotoxins. Inhalation of fungal spores should be avoided as far as possible.

Of the fungi described in this book, only the Aspergilli normally pose any direct threat to health. *N. fumigata* has already been mentioned, and care should also be taken when handling cultures of *Neosartorya* species,

which are closely related to *N. fumigata* and also grow prolifically at 37 °C. Other *Aspergillus* species, particularly *A. flavus*, *A. niger* and *A. terreus*, have been isolated from human specimens from time to time. For more detail see de Hoog et al. (2000). These species appear to be mainly opportunists and pose little threat to healthy people. Careful handling and good housekeeping are all that are required.

However, immunocompromised individuals are in a different category. It is increasingly evident that such people have little resistance to fungal infection. Persons suspected to be immunocompromised, regardless of the cause, should not work in a mycology laboratory, nor indeed be permitted knowingly to enter one, unless all work is carried out in biosafety cabinets.

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5 Primary Keys and Miscellaneous Fungi

Principles underlying fungal classification have been outlined in Chap. 3, including a brief overview of the relevant divisions of the Kingdom Fungi and their principal methods of reproduction. Some further detailed information is necessary to assist in use of the keys which follow in this chapter.

Ascomycetes As discussed in Chap. 3, ascomycetes produce **ascospores** in **asci** (Fig. 3.2). One genus, *Byssochlamys*, produces asci which are unenclosed; all other genera of relevance here produce asci in some kind of fruiting body, or ascocarp. The two kinds of ascocarp commonly seen in food spoilage fungi, the cleistothecium and the gymnothecium, have been described and illustrated in Chap. 3 (Fig. 3.3). Both types of ascocarp are usually pale or brightly coloured and release ascospores by rupturing irregularly. Of genera relevant here, cleistothecia are produced by *Aspergillus*, *Emericella, Monascus, Penicillium* and *Neosartorya* and gymnothecia by *Talaromyces*.

A third class of ascocarp, less commonly encountered in foodborne fungi, is the perithecium. Perithecia have cellular walls like cleistothecia, but are distinguished by the presence of an apical pore (ostiole) through which asci or ascospores are liberated; also asci are long and clavate (club shaped) with ascospores arranged in a line within them. In the one perithecial genus of interest here, *Chaetomium*, the perithecia are black and have stout hyphae attached to the walls.

Anamorphic or conidial fungi Many fungi do not usually produce sexual state spores (ascospores) but reproduce by asexual spores termed **conidia**. Strictly conidial fungi, which used to be classified separately as "*Fungi imperfecti*" or "Deuteromycetes" possess an amazing variety of ways of producing conidia. Terminology for structures bearing conidia and for conidia themselves has become astonishingly complex in recent years; fortunately most of it is not essential for the recognition of the genera discussed in this text. Terms which are important in the keys which follow are described below.

Anamorphic or asexual fungi have traditionally been divided by the method of spore formation. Molecular information has now rendered this classification obsolete, but it remains useful in the present context, where identification is primarily morphological. The traditional approach separates genera which form conidia aerially, grouped in the artificial class Hyphomycetes, from those in which conidia are borne in some sort of enveloping body, the class Coelomycetes.

Hyphomycetes Fungi have developed seemingly endless ways of extruding or cutting off conidia, solitarily or in chains, from fertile cells which themselves may be borne solitarily or aggregated into more or less ordered structures. Hyphomycete taxonomy attempts to thread a way through this maze. In general, type and degree of aggregation of the fertile cells, and type of conidium, provides the basis for generic

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classification, while details of these characters and of spore size, shape and ornamentation are more commonly used to distinguish species.

Features of conidia used in the keys in this work are length, septation, ornamentation and colour, particularly whether walls are light or dark. The method of conidium formation (ontogeny) is seldom emphasised here, because terminology is complex and distinctions may not be obvious. The principal point to note is the disposition of conidia: they may be borne **solitarily**, i.e. just one conidium per point of production; **singly**, i.e. successively from a single point, but unattached to each other; or **in chains**. Solitary conidia are borne on a relatively broad base and usually adhere to the fertile cell. Conidia formed in chains are usually extruded from a small cell of determinate length, often a **phialide**, which in most genera narrows to a distinct neck (collulum). Conidia borne singly may be extruded in this same manner, or be borne by extrusion from a pore in a hypha or fertile cell, or be cut off by hyphal fragmentation.

Phialidic Hyphomycetes Hyphomycetes may produce phialides solitarily (the genus *Acremonium*) or in loosely ordered structures (*Trichoderma*) or highly ordered structures (*Aspergillus*, *Penicillium* and *Talaromyces*). Genera of interest here with less ordered phialidic structures can mostly be differentiated by macroscopic characters, e.g. colony diameters and colours. However, differentiating genera with highly ordered phialidic structures will necessitate careful microscopic examination. Phialides in *Aspergillus*, *Penicillium* and *Talaromyces* are sometimes borne directly on a stalk or **stipe** which arises from a hypha; in other species, however, the phialides are borne from supporting cells, termed **metulae** (sing. **metula**) and in some species the metulae may in turn be supported by other cells, termed branches or **rami** (sing. **ramus**). The whole structure, including the stipe, is called a **conidiophore.**

In *Aspergillus*, stipes are always robust, with thick walls and usually without septa; the stipe terminates in a more or less spherical swelling, the **vesicle**, which bears phialides, or metulae and phialides, over most of its surface. In *Aspergillus*, phialides (and metulae) are always produced simultaneously, and this feature can readily be recognised by examining young developing conidiophores (Fig. 8.1a). Similar structures, though smaller, are produced by some *Penicillium* species: these are clearly distinguished from *Aspergillus* species by stipes which are septate, and by phialides which are produced over a period of time (successively; Fig. 8.2b). Most *Penicillium* species, and all of those in *Talaromyces*, do not produce phialides on vesicles, but in a cluster directly on a stipe, or on metulae and/or rami. The fruiting structure in *Penicillium* and *Talaromyces* is termed a **penicillus**, while that in *Aspergillus* (for want of a better term) is called a **head**, or less commonly, an **aspergillum** (Klich and Pitt 1988).

Coelomycetes As noted earlier, genera grouped here in the obsolete order Coelomycetes produce conidia within an enveloping body, termed a **conidioma** (pl. **conidiomata**). In Petri dish culture, conidiomata are produced on or just under the agar surface, and are macroscopically visible, usually being 100–500 μm in diameter. Two kinds of conidioma are important here: the **pycnidium**, a more or less spherical body with one or more pores (ostioles) through which conidia are released; and the **acervulus**, a flat body from which conidia are released by lifting or rupturing of a lid. The majority of Coelomycetes are pathogens on plants and many have not been studied in pure culture. In consequence, their taxonomy is difficult and genera and species are often poorly delimited. For a complete account of traditional Coelomycete taxonomy see Sutton (1980).

Yeasts Yeasts are fungi which have developed the ability to reproduce by forming single vegetative cells by budding or, in a few species, by fission, in a manner similar to bacteria. Like bacteria, and unlike fungal spores, such cells are metabolically active and may in turn reproduce by budding (or fission). Yeast cells may survive for long periods both in culture and in nature. In consequence many yeasts produce true spores rarely or not at all.

Yeasts are readily distinguished from filamentous fungi on the agar plate by their soft textured

colonies and limited growth. They are usually also readily distinguished from bacteria by their raised and often hemispherical colonies, white or pink colours and lack of "bacterial" odour. If in doubt, make a simple wet mount of a colony in water or lactofuchsin, add a cover slip and examine with the oil immersion lens. Yeast cells are larger than bacteria, measuring at least 3 × 2 µm and are not uniform in size. If the culture is not too old, some cells will usually show developing buds.

Yeasts cannot be classified solely by morphological features or growth on the standard media, and so are considered in a separate chapter (Chap. 10).

5.1 The General Key

The taxonomic terms discussed above will enable use of the general and miscellaneous keys which follow, although some other taxonomic terms may be introduced in discussions of particular genera. **It is emphasised that these keys are designed for use on isolates which have been incubated for 7 days on the standard plating regimen outlined in Chap. 4.** Colony diameters should be measured in millimetres from the reverse side by transmitted light. The general key has been designed to be as simple as possible and suitable for routine use, but it should be read in conjunction with the notes below it.

General key to food spoilage fungi

1	No growth on any standard medium in 7 days	Chapter 9 – Xerophilic fungi
	Growth on one or more standard media	2
2 (1)	Colonies yeasts, either recognisably so on isolation or in culture, i.e. colonies soft, not exceeding 10 mm diam on any standard medium	Chapter 10 – Yeasts
	Growth filamentous, exceeding 10 mm diam on one or more standard media	3
3 (2)	Growth on CYA and/or MEA faster than on G25N	4
	Growth on G25N faster than on CYA and MEA	Chapter 9 – Xerophilic fungi
4 (3)	Hyphae frequently and conspicuously septate	5
	Hyphae lacking septa, or septa rare	Chapter 6 – Zygomycetes
5 (4)	No mature spores present in 7 days	6
	Mature spores present in 7 days	9
6 (5)	Immature fruiting structures of some kind present	7
	No fruiting structures (or spores) detectable by low power microscopy or wet mounts from CYA or MEA	
	Continue incubation; when immature structures or spores are visible, reenter key	
7 (6)	Colonies and fruiting structures white or brightly coloured	8
	Colonies or fruiting structures dark	Chapter 5 – Miscellaneous fungi
8 (7)	Colonies exceeding 50 mm diam on CYA or MEA	Chapter 8 – *Aspergillus* and its teleomorphs
	Colonies not exceeding 50 mm diam on CYA or MEA	Chapter 7 – *Penicillium* and *Talaromyces*
9 (5)	Spores (conidia) less than 10 µm long, borne in chains on clustered fertile cells (phialides), on well-defined stipes	10
	Spores (conidia) of various sizes, borne singly or solitarily, or if borne in chains, then chains not grouped together	16
10 (9)	Mature conidia truncate or flattened at the base; colonies white, buff or brown	*Scopulariopsis*
	Conidia not truncate at the base, symmetrical from end to end; colony colour various	11

(continued)

11 (10)	Phialides or metulae and phialides borne on more or less spherical swellings on the stipe apices	12
	Phialides borne on penicilli, i.e. on unswollen stipes with or without intervening metulae and rami	13
12 (11)	Conidia blue or green, phialides produced successively on vesicles, vesicles less than 10 µm diam, stipes usually septate	Chapter 7 – *Penicillium* and *Talaromyces*
	Conidia variously coloured, phialides and/or metulae produced simultaneously on vesicles, vesicles larger than 10 µm diam, stipes nonseptate	Chapter 8 – *Aspergillus* and its teleomorphs
13 (11)	Mature conidia spherical to ellipsoidal, in shades of blue, green and/or grey	Chapter 7 – *Penicillium* and *Talaromyces*
	Mature conidia ellipsoidal to fusiform or cylindroidal, not blue, green or grey	14
14 (13)	Phialides cylindrical, rough walled, necks truncate, mature conidia cylindroidal	*Geosmithia*
	Phialides gradually tapering, smooth walled, often with long necks angled away from the phialide axis, conidia ellipsoidal to fusiform	15
15 (14)	Colonies white or brown	*Byssochlamys*
	Colonies red or purple	*Purpureocillium*
16 (9)	Colonies on CYA and MEA not exceeding 60 mm diam in 7 days	17
	Colonies on CYA or MEA exceeding 60 mm diam in 7 days	28
17 (16)	Conidia borne within a fruiting body on or beneath the agar surface	*Epicoccum sorghinum*
	Conidia borne from aerial or surface hyphae	18
18 (17)	Mycelium and conidia hyaline or brightly coloured	19
	Mycelium and/or conidia dark coloured	27
19 (18)	Conidia with a single lateral septum (cross wall)	*Trichothecium*
	Conidia nonseptate or with more than one septum	20
20 (19)	Conidia borne from gradually tapering fertile cells (phialides)	21
	Conidia borne directly on hyphae, or by budding or hyphal fragmentation	23
21 (20)	Colonies exceeding 50 mm diam on CYA	22
	Colonies not exceeding 50 mm diam on CYA	*Acremonium*
22 (21)	Colonies on CYA or MEA at 7 days showing yellow, red or purple in obverse or reverse	*Fusarium*
	Colonies white on CYA and MEA at 7 days	*Neocosmospora*
23 (20)	Colonies exceeding 45 mm diam on MEA; conidia borne solely by the break up of hyphae to form arthroconidia	*Galactomyces*
	Colonies not exceeding 40 mm diam on MEA; conidia not exclusively arthroconidia	24
24 (23)	Conidia exceeding 12 µm long; developing cleistothecia, fist-like on arm-like stalks, also present	*Monascus*
	Conidia not exceeding 12 µm long; no evidence of cleistothecial development	25
25 (24)	Conidia yeast-like, borne on spicules (small projections) from hyphae, or by budding; conidia 5 µm or less long	*Endomyces*
	Some conidia yeast-like, borne by budding; hyphal fragments, arthroconidia and/or chlamydoconidia also present; conidia >5 µm long	26
26 (25)	Budding hyphal fragments (10–50 µm long) present	*Hyphopichia*
	Hyphal fragments absent; or if present, not budding	*Moniliella*
27 (18)	Colonies low, mucoid and yeast-like, becoming grey to black in both obverse and reverse	*Aureobasidium*
	Colonies dry and velutinous, obverse green, reverse olive or deep blue black	*Cladosporium*
28 (16)	Spores borne within an enclosed fruiting body on or under the agar surface	29
	Spores borne from aerial or surface hyphae	33

(continued)

29 (28)	Spores consistently less than 15 µm long	30
	The larger or all spores more than 15 µm long	31
30 (29)	Fruiting bodies perithecia containing asci and ascospores, with stout, black hyphae attached to the walls	*Chaetomium* and related genera
	Fruiting bodies pycnidia, containing conidia, without attached hyphae	*Epicoccum sorghinum*
31 (29)	Fruiting bodies pycnidia, roughly spherical	*Lasiodiplodia*
	Fruiting bodies flat (acervuli)	32
32 (31)	Conidia hyaline or brightly coloured, nonseptate, without terminal appendages	*Colletotrichum*
	Conidia dark, with three or four septa and with spike-like, sometimes branched, terminal appendages	*Pestalotiopsis*
33 (28)	Colonies and conidia hyaline or brightly coloured	34
	Colonies and/or conidia dark coloured	39
34 (33)	Colonies with grey or green areas	35
	Colonies white, orange, pink or purple	36
35 (34)	Colonies green	*Trichoderma*
	Colonies grey	*Botrytis*
36 (34)	Colonies low and persistently white	*Galactomyces*
	Colonies floccose, white or becoming brightly coloured	37
37 (36)	Colonies predominantly orange, orange conidia shed profusely around the Petri dish rim	*Neurospora*
	Colonies white, pink or purple, sporulation on MEA weak or absent, better on DCPA under lights	38
38 (37)	Colonies on CYA or MEA at 7 days showing yellow, red or purple in obverse or reverse	*Fusarium*
	Colonies white on CYA and MEA at 7 days	*Neocosmospora*
39 (33)	Conidia consistently less than 15 µm long	40
	Conidia frequently exceeding 15 µm long	42
40 (39)	Conidiophores long, branched, apically swollen, bearing closely packed pale brown conidia	*Botrytis*
	Conidiophores short or ill-defined, dark brown or black conidia borne irregularly	41
41 (40)	Conidia dark brown, often with a lighter coloured band around the periphery	*Arthrinium*
	Conidia uniformly jet black	*Nigrospora*
42 (39)	Conidia approximately spherical	*Epicoccum*
	Conidia elongate	43
43 (42)	Conidia with transverse septa (or thick walls between cells) only	44
	Larger conidia with both transverse and longitudinal septa, or irregularly septate	47
44 (43)	Conidia clavate (club shaped), often with long hyphal appendages at the apices; found almost exclusively on rice	*Trichoconiella*
	Conidia cylindroidal, ellipsoidal or an elongate “D” shape; source more general	45
45 (44)	Conidia cylindroidal, with parallel sides except at the terminal cells	*Pyrenophora*
	Conidia fusoid, narrowing from the central cells to the terminal cells, often bent or “D” shaped	46
46 (45)	Conidia not exceeding 40 µm long	*Curvularia*
	Conidia usually exceeding 40 µm long	*Bipolaris*
47 (43)	Conidia clavate (club shaped)	*Alternaria*
	Conidia spherical to roughly ellipsoidal or short cylindrical	*Stemphylium*

5.1.1 Notes on the General Key

Couplet 1 No growth on any standard medium indicates an extreme xerophile, i.e. a *Xeromyces, Bettsia* or *Xerochrysium* species, or a nonviable culture. Next, inoculate culture on to MY50G for 7 days at 25 °C. If growth occurs, enter the key in Chap. 9, "Xerophilic Fungi"; no growth on MY50G indicates a nonviable culture. Isolates of *Xeromyces, Bettsia* and *Xerochrysium* are usually white, sometimes with pure black areas, or rarely golden brown. If the original culture used as inoculum is coloured either uniformly black, or other than pure white or golden brown, it is probably nonviable.

Couplet 2 Yeasts are usually readily distinguished by slow growth, soft, easily sampled colonies, small spherical to ellipsoidal cells, often of variable size and shape and by reproduction by budding. See Chap. 10, "Yeasts" for identification procedures.

Couplet 3 The ability to grow more rapidly on G25N than on CYA or MEA indicates a xerophile (at least for keying purposes here). Check the key in Chap. 9, "Xerophilic Fungi". Some isolates can be identified from the standard plates, while others will require growth on CY20S or MY50G for identification.

Couplet 4 The absence of septa in young, growing hyphae indicates an isolate belongs to subkingdom Zygomycotina, discussed here in Chap. 6, "Zygomycetes".

Couplets 5, 6 Some isolates from a variety of fungal genera will not produce spores on the standard media in 7 days. Continue to incubate such cultures, preferably in diffuse daylight such as a laboratory window sill, at temperatures near 25 °C. Also inoculate such cultures onto two or three plates of DCMA and incubate these at 25 °C or thereabouts in darkness and in diffuse daylight or, if possible, under fluorescent illumination (see Chap. 4). After 1–2 weeks, check again for spores or fruiting bodies. If such structures are not seen, the isolate is unlikely to be significant in foods. If identification is still desired, molecular analysis of the ITS region is recommended.

Apparently asporogenous cultures should also be checked with a stereomicroscope while scraping up a sector of the colony with a needle. Fruiting bodies submerged in the agar will sometimes become visible with this technique.

Couplet 7 Some isolates which produce white or brightly coloured fruiting bodies also produce very sparse aerial conidial structures which are easily overlooked. Check such cultures carefully with the stereomicroscope; if conidial structures are found, make a wet mount and reenter the key at Couplet 5. A finely drawn glass needle will sometimes be of assistance in making mounts from delicate conidial structures on the colony.

Nearly all dark fruiting structures encountered will mature at 25 °C within 2 weeks. Light does not usually influence this process. When mature spores are formed, refer to the following section.

5.2 Miscellaneous Fungi

In this chapter are considered the genera which do not logically fit into some larger grouping considered elsewhere. Some are important in specific food spoilage problems, others are found in particular habitats such as cereals, while still others represent the aerially dispersed fungal flora found as ubiquitous contaminants or saprophytes. As will be seen, they are a very heterogeneous collection.

Most fungi significant in food spoilage or food contamination and not treated in other chapters are included in this chapter. It is inevitable, though, that occasional isolates from foods will not belong to the genera considered in this section. The key has not been designed to take account of this, as it would be a practical impossibility. When an isolate appears to key out satisfactorily, it must be checked against the description to confirm the identification. Some isolates will of course belong to a recognisable genus, but not the species described; in that case the references indicated will provide further information.

The miscellaneous fungal genera are considered in alphabetical order below.

5.3 Genus *Acremonium* Link

Commonly referred to as *Cephalosporium* Corda in pre 1970 literature, *Acremonium* was redefined by Gams (1971) as a large and varied genus characterised by the production of small, hyaline, single celled conidia borne singly, i.e. successively but not connected to each other, from solitary phialides. Molecular taxonomy has shown that *Acremonium* is polyphyletic and indeed the described species come from at least three orders, associated with a dozen or more teleomorphs (Summerbell et al. 2011). In a study involving more than 150 taxa, they concluded that the genus *Acremonium* could be maintained, in the sense originally described by Link and adopted by Gams (1971), by selecting a new type (an epitype) for Link's original species, *A. alternatum.*

A variety of species have been recorded from foods from time to time, most often as "*A. strictum*", a name not recognised by Summerbell et al. (2011). However, the illustration of *A. alternatum* in Seifert et al. (2011) is sufficiently similar to the following description to be provided as representative of foodborne isolates. Only molecular analysis is likely to provide a definitive species name for a given isolate. In the species described here, the phialides gradually taper to the apices without basal thickening or formation of a distinct neck, and conidia aggregate in balls of slime. Under the stereomicroscope, the slime balls look like large single spores, but their true nature becomes evident in wet mounts. *A. alternatum* occurs in pre 1970 literature under the name *Cephalosporium acremonium* and mostly as *A. strictum* in more recent works. As noted by Domsch et al. (1980), this name has been used for a variety of species, so that reports on physiology and occurrence are unreliable. For a detailed bibliography see Seifert et al. (2011).

Acremonium alternatum Link **Fig. 5.1**

Acremonium strictum (name of uncertain application; no valid authority)
Cephalosporium acremonium (name of uncertain application; no valid authority)

Colonies on CYA 20–30 mm diam, white or orange to pink, dense to floccose or funiculose; reverse pale or with orange to pink tones. Colonies on MEA 13–20 mm diam, similar to those on CYA or of slimy texture. Colonies on G25N less than 5 mm diam, usually 1–2 mm, of white mycelium. Sometimes growth at 5 °C. No growth at 37 °C.

Conidia borne successively but singly from the apices of long, solitary, usually unbranched phialides, aggregating in a slime ball at the phialide tip, cylindrical to ellipsoidal, hyaline, 3–6 × 1–2 µm, smooth walled.

Distinctive features See genus preamble.

Taxonomy As noted above, *Acremonium alternatum* is the correct name for the fungus commonly called *Cephalosporium acremonium* in early food literature (Gams 1971) and later *Acremonium strictum* (Summerbell et al. 2011).

Identifiers MycoBank MB143790; epitype CBS H-20525; ex-type strain CBS 407.66; nuclear LSU barcode HQ231988 (Summerbell et al. 2011).

Physiology Mycelial growth and sporulation of *"A. strictum"* were found to be enhanced by the addition of mannitol to MEA (Teixeira et al. 2005).

Mycotoxins *Acremonium alternatum* and other *Acremonium* species encountered in foods are not known to produce mycotoxins.

Ecology The main foodborne source of *Acremonium* species is maize (Williams et al. 1992; Pitt et al. 1993; Pitt and Hocking 1997). *Acremonium* species have been isolated from black bean seeds in Argentina (Castillo et al. 2004) and raw cork (*Quercus suber*) in Spain (Alvarez-Rodriguez et al. 2002). Other records, including the earlier names *A. strictum* and *Cephalosporium acremonium*, include wheat, barley and rice, bananas showing crown rot, fresh vegetables, peanuts, pecans, hazelnuts and walnuts, soybeans, frozen meat, salami and biltong (Pitt and Hocking 1997).

References Gams (1971), Domsch et al. (1980), Summerbell et al. (2011), and Seifert et al. (2011).

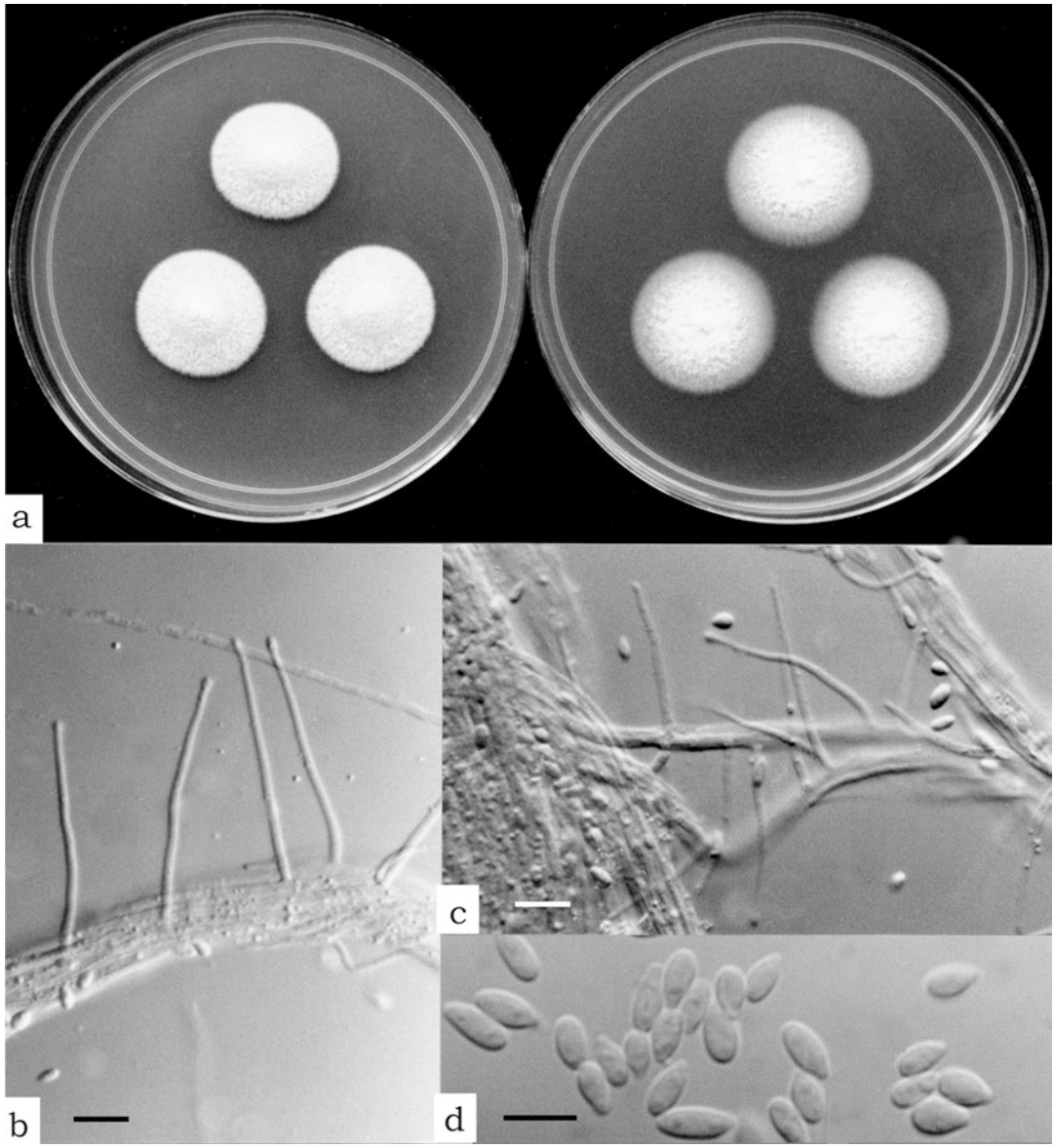

Fig. 5.1 *Acremonium alternatum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) phialides, bar = 10 µm; (**d**) conidia, bar = 5 µm

5.4 Genus *Alternaria* Nees

A*lternaria* produces large brown conidia with conspicuous transverse and, usually less pronounced, longitudinal septa, borne from inconspicuous conidiophores, and with a distinct conical narrowing or "beak" at the apical end. These conidia are often formed in chains. *Stemphylium* Wallroth produces conidia which are similarly septate, but are roughly ellipsoidal, and this genus is less common isolated from foods. *Alternaria* produces new conidia by lateral growth of the conidiophore from near the apex, or through the beak area. New *Stemphylium* conidia are produced from the spore base, through the pore from which a conidium has already been

produced. Simmons (1967) revived a third related genus, *Ulocladium* Preuss, accepted by Pitt and Hocking (2009) but synonymised with *Alternaria* by Woudenberg et al. (2013). *Trichoconiella* forms conidia similar to those of *Alternaria*, but with transverse septa only. That genus is associated with rice.

Many *Alternaria* species are important plant pathogens, causing both plant diseases and postharvest spoilage, with a very wide range of plant hosts (Lawrence et al. 2013). Some species can be pathogenic and allergenic (de Hoog et al. 2000). A great deal of the modern taxonomic work on *Alternaria* and related genera was carried out by Emory Simmons and his associates, culminating in a monograph of 276 species (Simmons 2007). This taxonomy supplanted the earlier concept that species were host specific, i.e. Simmons showed that *Alternaria* from citrus fruits may be *A. citri,* but may also be one of several other species.

Molecular studies summarised by Lawrence et al. (2013) and and extended by Woudenberg et al. (2013) have led to redefinition of the genus, including merging several genera established, or accepted, by Simmons (2007). *Alternaria* is accepted (Rossman et al. 2015) as the oldest and most appropriate name for all species, both anamorph and teleomorph, that lie within the broad concept of the genus put forward by Woudenberg et al. (2013). In addition, many of the species erected on morphological grounds by Simmons (2007) and earlier papers have been shown to be very closely related by molecular methods (Lawrence et al. 2013; Woudenberg et al. 2013). In practice, most foodborne isolates can be assigned to *A. alternata* or *A. infectoria* with little loss of physiological or ecological information – or as far as is known, information about mycotoxin production.

The differences between infection of wheat grains by these two species warrant further attention. *A. alternata* produces a range of mycotoxins, whereas *A. infectoria* does not. However, *A. infectoria* is the main cause of discolouration of wheat grains known as "black point", whereas infection by *A. alternaria* produces little observable change in grain appearance. So the downgrading of wheat on colour, while ensuring that high quality wheat is of good colour, has no effect on possible mycotoxin contamination. Put another way: removal of possible mycotoxin contamination in wheat cannot be achieved by colour grading (Webley et al. 1997; Pitt et al. 1998b).

The distribution of these two species is also of interest. In Australian wheat, *A. alternaria* and *A. infectoria* are of roughly equal occurrence in wheat from northern states, New South Wales and Queensland, loosely defined as warm temperate zones with generally dry and high temperature maturation conditions for wheat crops, whereas *A. infectoria* is dominant in the southern states of Victoria and South Australia, cool temperate zones with generally damper and lower temperature maturation conditions (Pitt et al. 1998b). More specifically, over the three seasons 1993–1996, 139 samples, each of 100 grains, from northern states showed 27% of kernels infected by *A. alternata* and 28% infected by *A. infectoria*, whereas 215 similar sized samples from the southern states showed 28% and 62% infection, respectively.

While 25 samples of Eastern Canadian wheat showed higher infection by *A. alternata* (60%) than *A. infectoria* (15%), 100 samples of Western Canadian wheat showed equal, low level (<20%) infection by either species. Five hundred samples from the 1993 and 1994 seasons of all grades of US wheat showed no marked differences in levels of infection by these two species, however, 69 samples of European wheat from the UK and France showed <2% infection by *A. alternata* but 60% infection by *A. infectoria* (Pitt et al. 1998b).

Analysis of selected samples for tenuazonic acid showed that low levels of this toxin were present in many samples which also showed high levels of infection with *A. alternata*, but not detected in samples from Australia and the UK that had high levels of *A. infectoria* infection and low levels of *A. alternata* infection (Pitt et al. 1998b).

Generic ITS barcode: AF347031 (EGS 34-016) (Seifert et al. 2011).

Key to included species

1	Conidia in unbroken chains, i.e. narrow hyphal elements between spores lacking; species occurring on wheat and many other substrates	*A. alternata*
	Primary conidia producing secondary conidia, i.e. conidia often separated by short hyphal lengths; species usually occurring on wheat	*A. infectoria*

Alternaria alternata (Fr.) Keissl. Fig. 5.2

Alternaria tenuis Nees

Colonies on CYA and MEA 50–70 mm diam, or covering the whole Petri dish, plane, of deeply floccose off-white to grey brown mycelium; reverse brown to nearly black. Colonies on G25N 10–15 mm diam, low and dense, olive brown or grey; reverse brown to almost black. At 5 °C, at least microcolonies, often colonies up to 4 mm diam. Usually no growth at 37 °C, occasionally colonies up to 10 mm diam, similar in appearance to those at 25 °C, or white.

Colonies on DCMA 60–70 mm diam or covering the whole Petri dish, plane, floccose but not sparse, mycelium grey to dark grey; reverse grey to dark grey or black, less commonly bluish black.

Conidia blown out from the apices of undistinguished conidiophores as short, irregularly branched chains of up to 10 units, and then septating both laterally and longitudinally, with up to 6 transverse and 2–3 longitudinal or oblique septa, usually of clavate or pyriform shape overall, tapering towards the apices, forming a short beak, in culture usually 20–40 × 8–12 µm, with walls smooth to conspicuously roughened.

Distinctive features *A. alternata* (and very close relatives not distinguished from it here) is the most common saprophytic *Alternaria* species, occurring on a wide variety of sources. On DCMA, conidia are produced more or less uniformly above the agar surface, in quite lengthy chains. The larger conidia have both longitudinal and transverse septa.

Identifiers MycoBank MB119834; holotype CBS 916.96; representative strains CBS 196.96 = FRR 5009; ITS barcode AF347031, alternative markers *gadph* AY278808, *rpb2* KC584376, *tef1* KC584634 (Woudenberg et al. 2013).

Physiology Optimum growth of *Alternaria alternata* is near 25 °C with minima variously reported as −5 to 6.5 °C and maxima near 36 °C (Hasija 1970; Domsch et al. 1980). The minimum a_w for growth at 25 °C is 0.86 (Hocking et al. 1994). The pH range for growth is 2.7–8.0, with optimal growth occurring at pH 4.0–5.4 (Hasija 1970). *A. alternata* is able to grow in oxygen concentrations as low as 0.25% (v/v) in N_2, with growth rates being proportional to oxygen concentration (Follstad 1966; Wells and Uota 1970). The presence of the volatile compounds 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone in stored wheat grains is usually correlated with infection by *A. alternata*, amongst other fungi (K.K. Sinha et al. 1988; R.N. Sinha et al. 1988).

Mycotoxins *Alternaria alternata* produces several mycotoxins. Alternariol and alternariol monomethyl ether have been reported to be genotoxic (Bottalico and Logrieco 1998; EFSA 2011), and therefore of greater concern for human health than other compounds, of which tenuazonic acid is the most important (Logrieco et al. 2003). Tenuazonic acid is toxic to a wide range of plants and animals, particularly mice, chick embryos and chickens (Logrieco et al. 2003; EFSA 2011). Sorghum grains containing high levels of tenuazonic acid were associated with the human haematological disorder known as onyalay (Bottalico and Logrieco 1998). Less toxic compounds include altenuene and altertoxins (Logrieco et al. 2003; EFSA 2011).

Estimates by EFSA (2011) indicated that reported levels of exposure to alternariol and alternariol monomethyl ether in Europe can be higher than the threshold of toxicological concern and indicated the need for further work. Levels of the nongenotoxic compounds reported in foods have been below the level of concern (EFSA 2011).

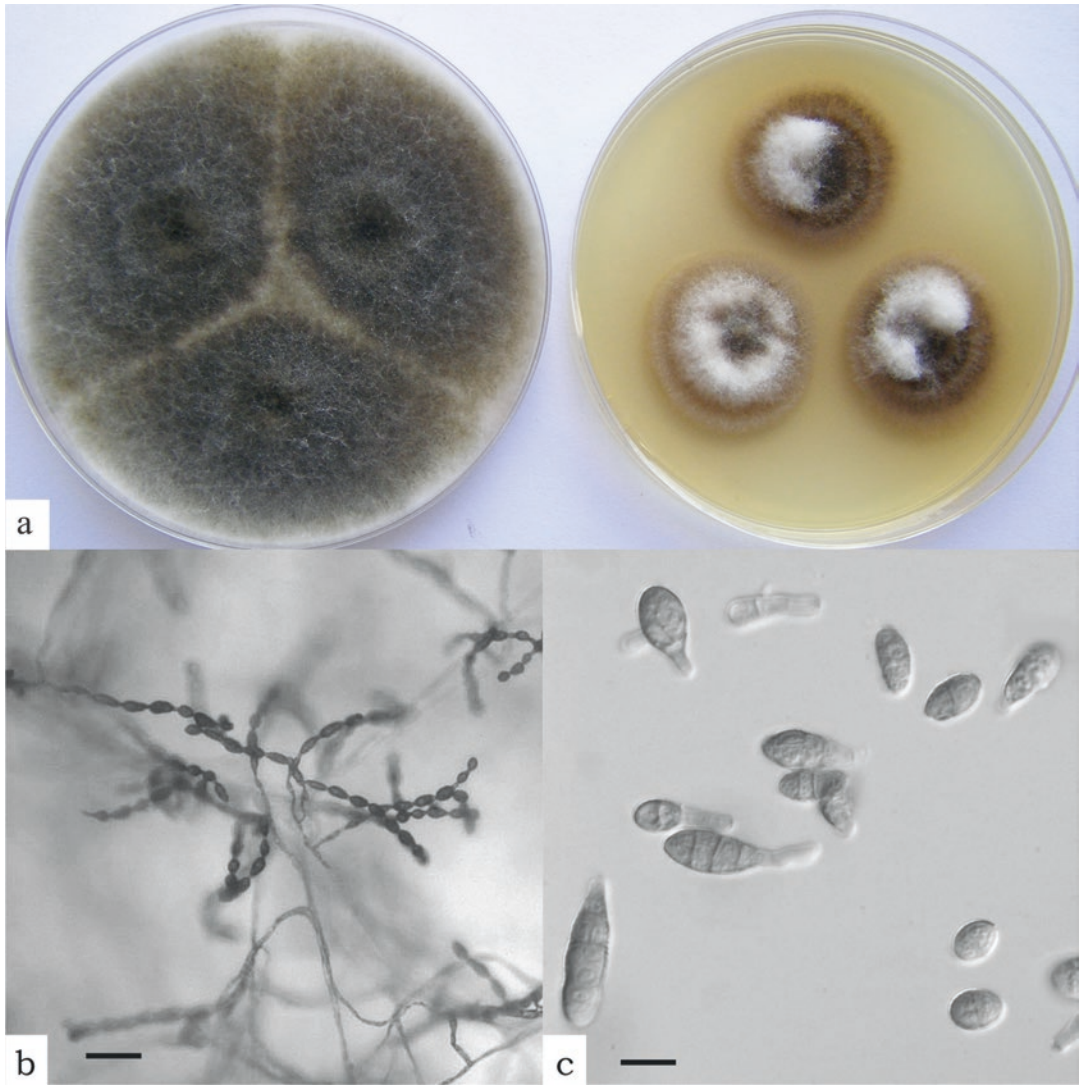

Fig. 5.2 *Alternaria alternata* (**a**) colonies on PDA and DCMA, 7 d, 25 °C; (**b**) conidia *in situ*, bar = 50 µm; (**c**) conidia, bar = 20 µm

The production of one or more of these toxins has been reported from a very wide range of foods, including tomatoes, citrus fruits, apples, cereal grains and cereal products, legumes, nuts and oilseeds and vegetables (EFSA 2011).

Maximum production of alternariol, alternariol monomethyl ether and altenuene occurred at 25 °C and 0.98 a_w (Magan et al. 1984). However, the optimum for tenuazonic acid production was reported as 0.90 a_w at 25 °C (Etcheverry et al. 1994).

It has been known for a long time that *Alternatia alternata* forma specialis *lycopersici* was able to produce toxic compounds, known as AAL toxins, which assisted its role as a tomato pathogen (Siler and Gilchrist 1983). Soon after the discovery of the fumonisins as major toxic metabolites of certain *Fusarium* species, it was found that AAL toxins are related to the fumonisins (Shephard et al. 1993). Several compounds are now known, of varying toxicity (Cole et al. 2003). These toxins are mentioned here only to point out that they are not known to occur in

foods, and indeed were below the limit of detection in 853 samples of German foods, mostly cereals, legumes and nuts (EFSA 2011).

Ecology Under the names *Alternaria alternata* and *A. tenuis*, this species has been reported from a very wide range of foods. However, the following reports undoubtedly include a number of other species. *A. alternata* is a major pathogen of fresh tomatoes (Harwig et al. 1979; Zitter and Wien 1984; Pose et al. 2004), though other *Alternaria* species are probably involved here. Spoilage of egg plants and peppers (Snowdon 1991), apples (Combrink et al. 1985) and bananas stored under modified atmospheres (Wade et al. 1993) have been reported. Damage may occur also to a wide variety of fresh vegetables including beans, cauliflowers, cucumbers, melons, peas and potatoes (Webb and Mundt 1978; Snowdon 1991). *A. alternata* has been recorded frequently from a wide range of cereals, particularly wheat (Pitt et al. 1998b; Clear et al. 2005, Fakhrunnisa et al. 2006; Lugauskas et al. 2006; see also Pitt and Hocking 1997), barley (Andersen et al. 1996; Fakhrunnisa et al. 2006; Medina et al. 2006; see also Pitt and Hocking 1997), but also in rice, rapeseed, sunflower seed (Pozzi et al. 2005) and sorghum (Fakhrunnisa et al. 2006). In Australia and Southeast Asia, *A. alternata* has caused severe damage, spoilage and mycotoxin production in wheat and sorghum (Pitt et al. 1994, Webley et al. 1997 and our unpublished observations). This species has been recorded quite frequently from nuts, including peanuts, hazelnuts and pecans (see Pitt and Hocking 1997). Other sources include soybeans, cold stored and frozen meat, biltong, bottled water and spices (Pitt and Hocking 1997; Cabral and Fernandez Pinto 2002). This species has occasionally been associated with gushing in beer (Niessen et al. 1992).

Closely related species *Alternaria tenuissima* (Kunze) Wiltshire differs from *A. alternata* by the formation of longer conidia (up to 60 µm) in unbranched chains (Simmons 2007). The metabolite profiles of *A. alternata* and *A. tenuissima* are very similar. In one study, it was found that most *A. tenuissima* isolates examined produced alternariol, alternariol monomethyl ether and tenuzoic acid, whereas three of the *A. alternata* isolates produced altenuene, alternariol, alternariol monomethyl ether and altertoxin I (Andersen et al. 2002). Andersen and Frisvad (2004) reported *A. tenuissima* from mouldy tomatoes. In our experience this species is of common occurrence in cereals. Identifiers: MB28005; type not known; representative culture CBS 117.44.

Alternaria infectoria E.G. Simmons **Fig. 5.3**

Lewia infectoria (Fuckel) M.E. Barr & E.G. Simmons (teleomorph)
Pleospora infectoria Fuckel

Colonies on CYA and MEA 60 mm or more diam, on CYA plane, low to moderately deep, mycelium dark grey to almost black, sometimes with a paler grey floccose overlay; reverse bluish black; on MEA moderately deep to deep, dense to floccose, of dark grey mycelium sometimes overlaid with lighter grey hyphae; reverse bluish black. On G25N, colonies 8–15 mm diam, of grey or greenish grey mycelium; reverse pinkish, grey or black. At 5 °C, at least germination, usually colonies of 2–5 mm diam formed. At 37 °C, colonies 5–25 mm diam, low to deep, grey, reverse dark.

Colonies on DCMA 60 mm or more diam, usually with wide margins, low to subsurface, centrally usually sparse and floccose, aerial mycelium white to pale olive brown; reverse pale brown, brown or olive brown, sometimes darker in annular or irregular patches.

Conidia on DCMA observed under the stereomicroscope characteristically formed in short, irregularly branched chains, forming clusters, with primary conidia borne from knobby hyphae, usually bearing secondary conidia borne from the tips, at the end of short (30–100 µm) hyphal lengths; conidia characteristically clavate (club-shaped) 20–50 × 7–14 µm, with up to 7 transverse septa and 1 or more longitudinal septa as well, or sometimes irregularly septate, walls and septa thick and dark brown, and with walls sometimes conspicuously roughened.

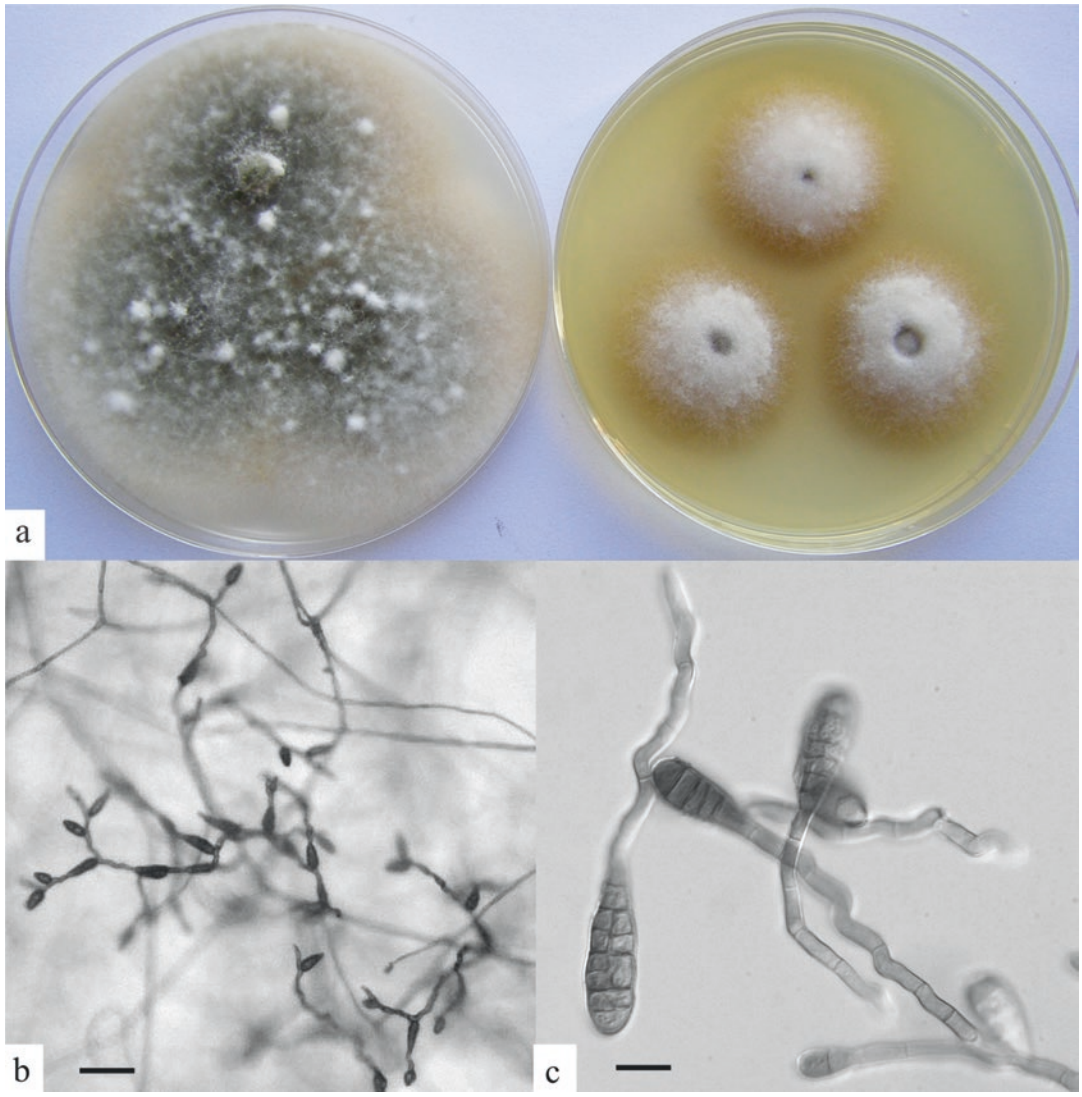

Fig. 5.3 *Alternaria infectoria* (**a**) colonies on PDA and DCMA, 7 d, 25 °C; (**b**) conidia, bar = 50 µm; (**c**) conidia, bar = 20 µm

Distinctive features After growth on DCMA, *Alternaria infectoria* is readily distinguished from *A. alternata* and other *Alternaria* species under the stereomicroscope. *A. infectoria* forms clusters of conidia borne in irregularly branching chains that, unlike those of *A. alternata,* have short hyphal segments between the conidia. Colonies on DCMA have low, wide margins, are less floccose than those of *A. alternata* and (apart from conidia) have paler colours.

Taxonomy This species was commonly known as the *Alternaria* state of *Pleospora infectoria* until Simmons (1986) provided a binomial name, *Alternaria infectoria*. He showed that its teleomorph does not belong in *Pleospora*, as had been widely accepted, and erected a new genus, *Lewia,* for it (Simmons 1986). Simmons (1986) reported that *Alternaria* species related to *A. infectoria* have very rarely been seen to produce a teleomorph; however on several occasions we

have seen *Lewia* teleomorphs in isolates of *A. infectoria* grown on autoclaved wheat grains. The older name *Alternaria* has priority and has been accepted (Rossman et al. 2015).

Identifiers MycoBank MB103987; holotype CBS 210.86; ex-type strains CBS 210.86 = FRR 5010; ITS barcode FB958526; alternative markers *gapdh* AY278793, *rpb2* KC584404, *tef1* KC584662 (Woudenberg et al. 2013).

Physiology No physiological studies on this species have been reported.

Mycotoxins *Alternaria infectoria* is chemically very different from other *Alternaria* species (Andersen et al. 2002). *A. infectoria* does not produce any of the known *Alternaria* metabolites (Andersen and Thrane 1996) and shares only a few metabolites with other *Alternaria* species (Andersen et al. 2002, 2015). We have confirmed the absence of any toxin production by this species (unpublished).

Ecology *A. infectoria* is associated with grains, having been reported from small grains in the USA (Bruce et al. 1984), Australian and Argentinian wheat (Pitt et al. 1998b; Andersen et al. 2015), Danish barley (Andersen et al. 1996) and Norwegian grains (Kosiak et al. 2004). It is associated with black point discolouration in wheat (Webley et al. 1997; Perelló et al. 2008). This species may be confined to small grains.

References Simmons (1986, 2007), Lawrence et al. (2013), and Woudenberg et al. (2013).

5.5 Genus *Arthrinium* Kunze

Apiospora Sacc. (teleomorph)
Papularia Fr.

Arthrinium, in earlier literature often known by its synonym *Papularia*, is not a common genus in foods, but has occasionally caused spoilage. *Arthrinium* produces relatively large, dark walled (but not black) conidia borne solitarily, both terminally and laterally, on short, narrow conidiophores. A comprehensive study of this genus was carried out by Crous and Groenewald (2013) who accepted 15 species and indicated that it of widespread occurrence, mostly as epiphytes on various plant species. Some species produce a sexual state, *Apiospora*, but Crous and Groenewald (2013) considered that as *Arthrinium* was both older and more widely used, it was the logical choice as genus name. Two species are treated here, *A. phaeospermum* and *A. arundinis,* distinguished from each other by conidial size.

Arthrinium phaeospermum (Corda) M.B. Ellis **Fig. 5.4**

Papularia sphaerosperma (Pers.) Höhn.
Gymnosporium phaeospermum Corda

Colonies on CYA and MEA covering the whole Petri dish, mycelium low or floccose, coloured white or grey, sometimes with conspicuous areas of pink, darkening in age; reverse yellow or brown. Colonies on G25N 10–18 mm diam, of white mycelium. Sometimes germination at 5 °C. No growth at 37 °C.

Reproduction by solitary conidia, blown out from the ends of, or from denticles on the sides of, short, narrow, sometimes sinuous conidiophores, themselves borne in clusters from mother cells on natural substrates, but often singly in culture; conidia circular in plan view but elliptical from the side, 8–12 × 5–7 µm, dark brown, smooth walled, often with a narrow, hyaline band around the longest periphery.

Distinctive features The conidium of *Arthrinium* is distinctive: solitary, dark walled, circular in plan but elliptical from the side, and often with a hyaline peripheral band. Cultivation on DCPA and under lights may assist sporulation. In the present context, *Arthrinium* is distinguished from *Nigrospora* by the latter's production of jet black conidia entirely devoid of ornamentation.

Identifiers MycoBank MB326468; type unknown; representative cultures CBS 114314 (from barley leaf), FRR 4452 (from Australian rice).

Physiology Conidia of *Arthrinium* appear to be highly heat resistant. In apple juice, conidia survived a pasteurising process of 88 °C for 1.5 min and, in water, heating at 105 °C for 2.5 min (Anon 1967). No experimental details, i.e., numbers heated or come-up time, were

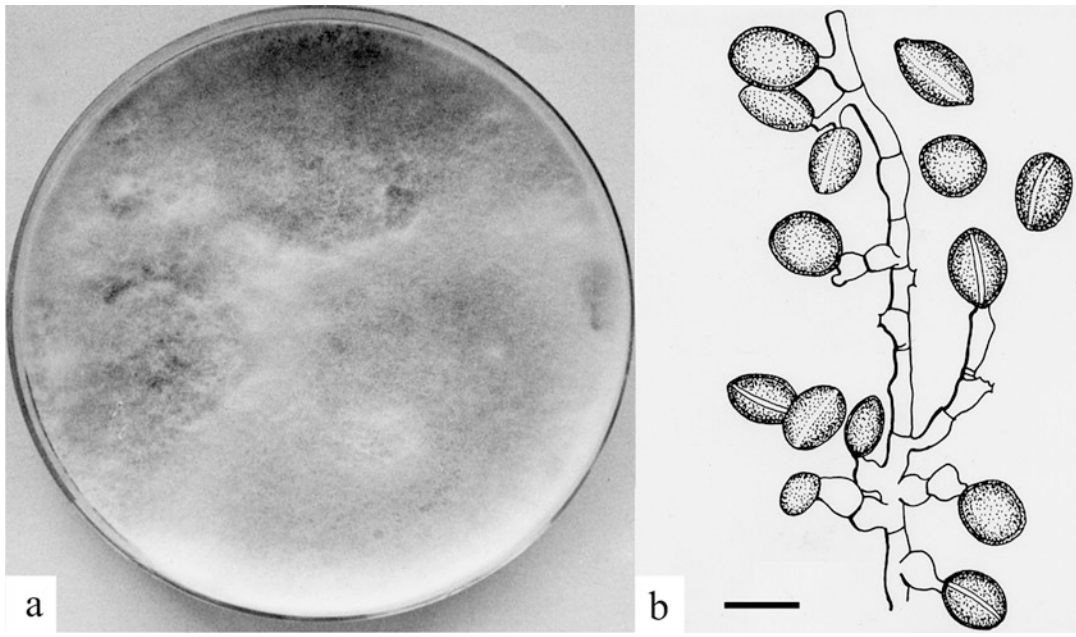

Fig. 5.4 *Arthrinium phaeospermum* (**a**) colony on CYA, 7 d, 25 °C; (**b**) conidia, bar = 10 µm

reported, however, and a decimal reduction time cannot be calculated. Even so, survival of a heat treatment at 88 °C indicates that *A. phaeospermum* is uncommonly heat resistant for a conidial fungus. Optimum germination of *A. phaeospermum* occurs when the conidia are suspended in sterile saline solution at 20 °C for 15 minutes before plating onto 2% malt extract agar (Agut and Calvo 2004). The authors also noted that the viability of *Arthrinium* conidia was low, making this genus relatively rare in the environment.

Mycotoxins *Arthrinium* species are not known to produce mycotoxins, however, reports of secondary metabolites with antimicrobial properties have been found for some *Arthrinium* species (quoted in Agut and Calvo 2004).

Ecology *Arthrinium phaeospermum* (reported as *Papularia sphaerosperma*) has been recorded as a cause of spoilage of pasteurised apple juice (Anon 1967). It has also been reported on barley, wheat flour, rice, pecans and airline meals (see Pitt and Hocking 1997). It was isolated from Morelo cherries (Olszak 1994) and Iranian barley (Asgari et al. 2004). *Arthrinium phaeospermum* has also been recorded as a pathogen on sweet potatoes (Ravichandran and Sullia 1983). We isolated *A. phaeospermum* at low levels from paddy rice, mung beans, soybeans, black beans and cashews in Thailand (Pitt et al. 1993).

Additional species *Arthrinium arundinis* (Corda) Dyko & B. Sutton (= *Apiospora montagnei* Sacc., named for the sexual state, now a synonym), also known as *Papularia arundinis* (Corda) Fr., is similar to *A. phaeospermum* in all characters examined, except for the production of smaller conidia, 6–8 µm long. Data sheets at the International Mycological Institute, Egham, Surrey, U.K., record its isolation from white flour and molasses; it has also been reported from wheat (Pelhate 1968), barley (Flannigan 1969) and cashews (Pitt et al. 1993). Identifiers: MycoBank MB308985; type not known; representative cultures CBS 133509 (= NRRL 13883), FRR 3750.

References Ellis (1971) and Crous and Groenewald (2013).

5.6 Genus *Aureobasidium* Viala & G. Boyer

Growth of *Aureobasidium* isolates is at first yeast-like, but, while remaining very low and mucoid, colonies spread rapidly and turn black in patches. Microscopically, hyphae as well as budding yeast-like cells are present; the latter are actually conidia. The conidia are borne from small denticles (minute projections) directly from the hyphal walls or from short lateral protrusions on the hyphae; characteristically 2–4 denticles on one cell will produce conidia synchronously. There may be more than one species (Seifert et al. 2011) but the most common and widespread is *A. pullulans*, described below.

Aureobasidium pullulans (de Bary) G. Arnaud Fig. 5.5

Dematium pullulans de Bary
Pullularia pullulans (de Bary) Berkhout

Colonies on CYA and MEA 25–35 mm diam, low and mucoid, faintly pink, becoming grey to black in areas at 7–10 days; reverse in similar colours. Colonies on G25N 10–12 mm diam, similar to those on CYA. At 5 °C, microcolonies to colonies up to 3 mm diam. No growth at 37 °C.

Conidia borne on denticles directly from hyphae or sometimes small lateral protrusions; conidia yeast-like, primary ones borne from the denticles, usually measuring 10–16 × 3–6 µm, and secondary ones by budding from the

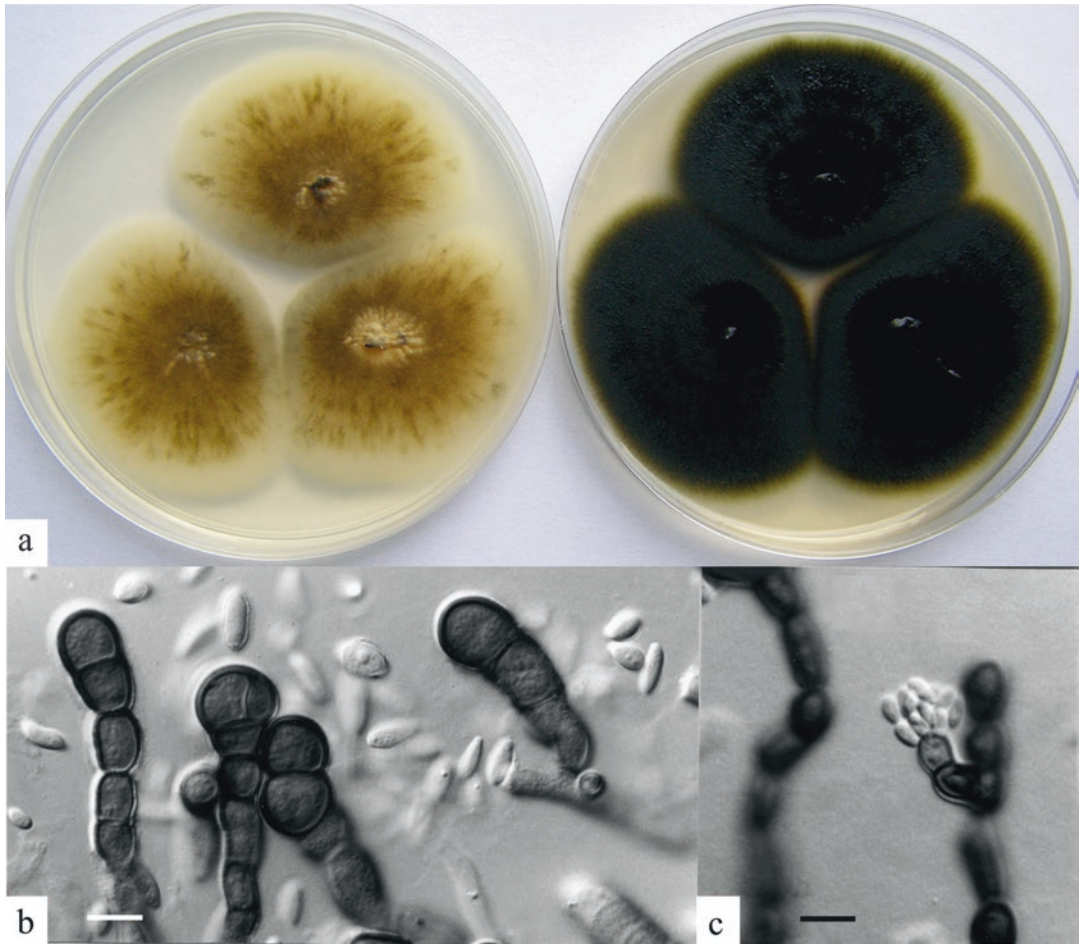

Fig. 5.5 *Aureobasidium pullulans* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) conidia, bar = 10 µm

primaries, 7–10 × 3–5 µm, not adhering to each other, smooth walled.

Distinctive features *Aureobasidium pullulans* is readily recognised by its characteristic low, mucoid, white then pink to black colonies and yeast-like conidia. Several other genera have a similar appearance (Hermanides-Nijhof 1977; Beh 2007; Seifert et al. 2011) but they rarely occur in foods.

Taxonomy Earlier literature discusses this species under the names *Dematium pullulans* and *Pullularia pullulans*.

Identifiers MycoBank MB429267; neotype CBS 584.75; ex-type culture CBS 584.75; ITS barcode AJ244232; alternative markers 26S rDNA DQ321374, 18S rDNA DQ471004, *RPB2* DQ470906.

Mycotoxins Mycotoxins are not known to be produced.

Physiology The temperature range for growth of *Aureobasidium pullulans* has been reported as 2–35 °C, with an optimum of 25 °C (Skou 1969), but some earlier data, together with its abundance in low temperature habitats, suggests that some strains grow down to −5 °C (Michener and Elliott 1964). It is of common occurrence in salterns (Butinar et al. 2005), so is likely to be a xerophile. Heat resistance is very low (Skou 1969). The ability of *A. pullulans* to grow on sugars used in yeast taxonomy has been reported by de Hoog and Yurlova (1994) and Beh (2007).

Ecology A ubiquitous saprophyte from all sorts of moist and decaying environments, *Aureobasidium pullulans* has been reported from a very wide range of foods, but only rarely as a cause of spoilage. Its prevalence in frozen foods is noteworthy, being the predominant mould isolated from blueberry, apple and cherry pies by Kuehn and Gunderson (1963). It was the most frequently isolated mould in Canadian icewine must, but played no role in fermenting must (Subden et al. 2003). The surface of healthy grapes is predominately colonised by *A. pullulans* (Fleet 2003; Prakichaiwattana et al. 2004; Beh 2007). *Aureobasidium pullulans* has been reported in yoghurt in a survey of a Slovakian dairy plant (Pieckova et al. 2002), and has been associated with the spoilage of cold stored meat and cheese (see Pitt and Hocking 1997). *A. pullulans* has been recorded in a variety of fresh foods and commodities including fresh vegetables, cabbage, strawberries, grapes, citrus and pasteurised orange juice (see Pitt and Hocking 1997). Other records include shrimp, green olives, barley, wheat and flour, oats and nuts (see Pitt and Hocking 1997). *A. pullulans* can occasionally be pathogenic and molecular methods have been developed to aid identification (Chan et al. 2011).

References Hermanides-Nijhof (1977), Domsch et al. (1980), and de Hoog and Yurlova (1994).

5.7 Genus *Bipolaris* Shoemaker

In separating unrelated species from *Helminthosporium*, Shoemaker (1959) revised the genus *Drechslera* and erected *Bipolaris*. *Bipolaris* species were shown to be associated with two distinct teleomorphs and as that correlated with morphological differences, Leonard and Suggs (1974) segregated *Exserohilum* from *Bipolaris*. These genera produce long, large conidia with transverse septa only; the septa are thick and quite different from those in e.g. *Trichoconiella*. In *Drechslera*, now known by its teleomorph name *Pyrenophora*, conidia are cylindrical and germinate at any cell, whereas conidia of *Bipolaris* and *Exserohilum* are fusoid, i.e. gradually narrowing, and conidia germinate only from the end cells (bipolar germination). In *Bipolaris*, germ tubes develop roughly along the line of the conidium axis, whereas in *Exserohilum*, the germ tube is often offset from the axis, and develops at an angle (Alcorn 1983). *Exserohilum* species are apparently rare in foods, although *E. rostratum* (Drechsler) K.J. Leonard & Suggs has been reported from sorghum (Usha et al. 1994).

Species from these genera are important plant pathogens, so the literature is voluminous and complex. The taxonomy of *Bipolaris* and another closely related genus, *Curvularia*, was comprehensively overhauled by Manamgoda

et al. (2014), and that publication should be consulted for further details. Manamgoda et al. (2014) accepted 47 species, three of which – *B. maydis, B. oryzae* (Breda de Haan) Shoemaker and *B. sorokiniana* (Sorokin) Shoemaker – occur frequently on cereal crops, so occur as contaminants in foods. *Bipolaris maydis* is treated here.

Generic ITS barcode DQ491489 (Seifert et al. 2011).

Bipolaris maydis (Nishik. & C. Miyake) Shoemaker Fig. 5.6

Cochliobolus heterostrophus (Drechsler) Drechsler (teleomorph)

Colonies on CYA and MEA 45–55 mm diam, plane, deeply floccose, of light to dark grey mycelium; reverse dark brown to dark blue black. On G25N, colonies 2–6 mm diam. At 5 °C, germination. At 37 °C, colonies 5–15 mm diam, usually low, dense and wrinkled, of grey mycelium, reverse deep blue black.

Colonies on DCMA 30–40 mm diam, plane, of sparse to dense, floccose grey mycelium, reverse brown, grey or black.

Conidia borne singly from nodes on knobby or geniculate mid to dark brown hyphae, usually slightly curved, (40–)50–80 × 12–16 µm, with 6–9 inconspicuous septa, and with smooth, dark, thick walls.

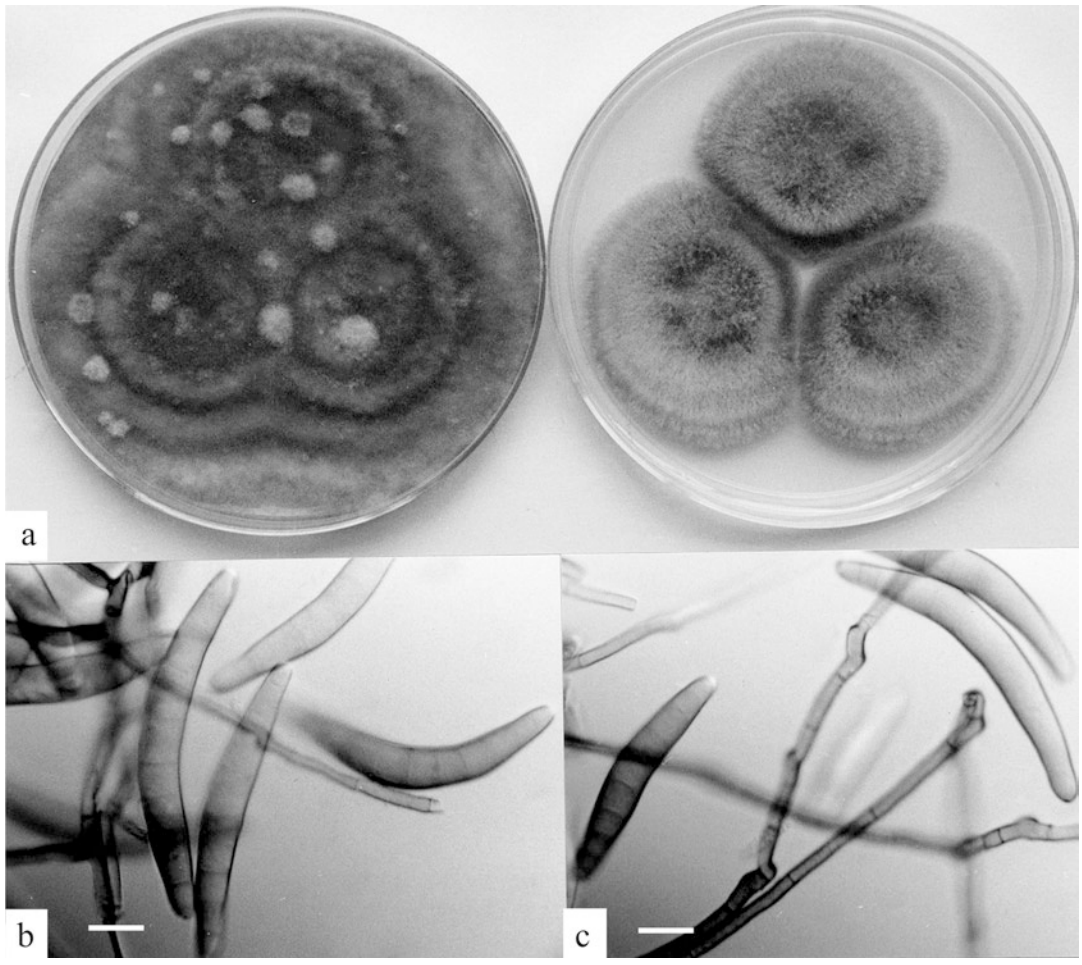

Fig. 5.6 *Bipolaris maydis* (**a**) colonies on MEA and DCMA, 7 d, 25 °C; (**b**, **c**) conidiophores and conidia, bar = 10 µm

Distinctive features Large conidia, often slightly curved, of fusoid shape, and with several thick transverse septa are characteristic of this genus. Conidial curvature and shape distinguish it from *Pyrenophora*, while conidial length and the presence of more than three, thick septa allow distinction from *Curvularia*. The species treated here is common on maize.

Taxonomy Some species of *Bipolaris* produce teleomorphs classified in the genus *Cochliobolus* Drechsler. However, Rossman et al. (2015) recommended that *Bipolaris* be given priority and that recommendation has been followed here.

Identifiers MycoBank MB293684; neotype CBS 137271, ex-type culture CBS 137271 = ATCC 48332, ITS barcode AF071325, alternative markers *gapdh* KM034846, *tef1* KM093794 (Manamgoda et al. 2014; Marin-Felix et al. 2017).

Physiology Species of *Bipolaris* sporulate significantly better when grown on substrates containing cellulose (Pratt 2006). Manamgoda et al. (2014) used sterilised maize leaves placed on water agar or slide cultures of half strength potato dextrose agar.

Mycotoxins No significant mycotoxins are known.

Ecology We isolated *Bipolaris maydis* from 1% of Thai maize kernels, and less frequently from Thai sorghum and mung bean samples (Pitt et al. 1993, 1994). It was also found at similar levels in commodities from Indonesia and the Philippines (Pitt et al. 1998a). Other records of *Bipolaris* in foods are rare. However, references to *Drechslera* or *Pyrenophora* species in foods often have referred to taxa correctly classified in *Bipolaris*, e.g. in rice, *B. oryzae* (Breda de Haan) Shoemaker; in durum wheat, *B. sorokiniana* Shoemaker (Nirenberg et al. 1995); in barley, *B. australiensis* (M.B. Ellis) Tsuda & Uemaya, *B. maydis* and *B. setariae* (Sawada) Shoemaker; in peanuts, *B. spicifera* (Bainier) Subram.; in coriander seed, *B. bicolor* (Mitra) Shoemaker; and in avocados, *B. setariae* Shoemaker (see Pitt and Hocking 1997).

References Alcorn (1983, 1990), Sivanesan (1987), and Manamgoda et al. (2014).

5.8 Genus *Botrytis* P. Micheli: Fr.

Botrytis is a very widespread genus in the temperate zones, where it occurs as a pathogen causing gray mould rot on over 200 plant species, including cultivated fruits, vegetables, and ornamental flowers (Plesken et al. 2015). Invasion may occur before maturity or postharvest, both in transport and in storage. Onions and other *Allium* species and grapes are the most susceptible crops. In the latter, it is notable that the disease is sometimes encouraged. Grapes affected by *Botrytis*, in this circumstance called "the noble rot", are used in the production of certain high quality sweet wines in France, Germany, Australia and other countries.

Botrytis species are characterised by the production of conidia on pegs from spherical swellings. The taxonomy of species occurring in Brazil has recently been studied by molecular analysis (Azevedo et al. 2020), and the results and conclusions are probably of wide applicability. The most commonly encountered species was *Botrytis cinerea*, in agreement with many earlier authors. *B. pseudocinerea* A.-S. Walker et al. was segregated from *B. cinerea* by Plesken et al. (2015). These authors reported that several distinct lineages exist in *B. cinerea*, but further separation would be difficult.

Generic ITS barcode: DQ491491 (Seifert et al. 2011).

Botrytis cinerea Pers. Fig. 5.7

Colonies on CYA and MEA covering the whole Petri dish, floccose, growth sometimes patchy or irregular, mycelium white, becoming grey to dark grey as conidiogenesis proceeds; reverse pale to grey. On G25N, colonies 10–18 mm diam, irregular in outline, floccose centrally or in patches, becoming grey; reverse grey. At 5 °C, colonies up to 5 mm diam produced, low and sparse. No growth at 37 °C.

Conidiophores borne from aerial hyphae, stipes of indeterminate length, each bearing terminally an irregular cluster of short branches, 10–30 µm long, with swollen spherical apices, 8–10 µm diam; conidia borne singly from these

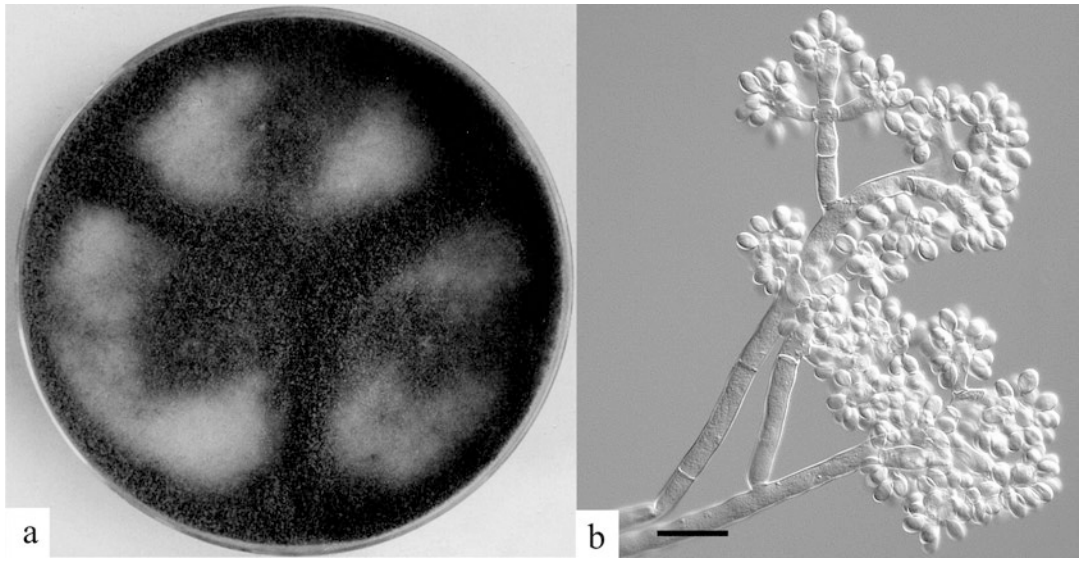

Fig. 5.7 *Botrytis cinerea* (**a**) colony on CYA, 7 d, 25 °C; (**b**) conidiophores and conidia, bar = 25 µm

apices on denticles (small pegs), ellipsoidal, 8–12 µm long, smooth walled, not released at maturity.

Distinctive features Solitary conidia borne on denticles from terminally swollen, short branches are characteristic of *Botrytis*. Colony growth is grey.

Identifiers MycoBank MB217312; type not known. A recent taxonomic study (Azevedo et al. 2020) did not record depositing any isolates in an international repository.

Physiology Growth has been reported at 0.93 a_w (Snow 1949) and at 0.90 a_w (W.R. Jarvis 1977). Lahlali et al. (2007) observed no growth of *Botrytis cinerea* at 0.93 a_w in the presence of NaCl or at 0.89 a_w in the presence of a non-ionic solute. Reported growth temperatures are rather variable, with minima from −2 to 5 °C or even 12 °C, maxima 28–35 °C, and optima of 22–25 °C (Domsch et al. 1980). *B. cinerea* will grow from pH 2 to 8 (W.R. Jarvis 1977) and in O_2 concentrations down to 1% (Follstad 1966). The effect of low temperatures and controlled atmosphere storage on the growth of *B. cinerea* on susceptible fruits and vegetables has been detailed (Reyes 1990). Conidia of *B. cinerea* were inactivated by 1.4 min at 48 °C in either synthetic medium or strawberry puree (Villa-Rojas et al. 2012).

Mycotoxins Mycotoxin production has not been reported – fortunately, in view of this species role in speciality wine production.

Ecology *Botrytis cinerea* is a virulent pathogen, the cause of rots in many kinds of fresh fruits (Snowdon 1990). It is the most important cause of disease in grapes, both before harvest and in storage and the most common cause of spoilage in berries such as strawberries, blueberries, raspberries and blackberries (Snowdon 1990; Tournas and Katsoudas 2005), and also causes large losses in apples and pears, tomatoes, stone fruits and kiwi fruit (see Pitt and Hocking 1997). A gel-like turbidity can be caused in raspberry juice, making filtration difficult (Will et al. 1992). For a review of many aspects of occurrence, pathogenicity and control, see Elad et al. (2004).

Although vegetable spoilage is perhaps less important, the range of crops affected is very large and includes asparagus, beans, cabbages, carrots, celery, melons and potatoes (Snowdon 1991; Lugauskas et al. 2005; Tournas 2005). It has also been isolated from a wide variety of dried or processed foods, but here its role is uncertain and it is probably present only as a contaminant.

Additional species Several other species of *Botrytis* are commonly occurring vegetable pathogens. Of interest here are *B. allii* Munn, *B. byssoidea* J.C. Walker [now known as *Ciborinia allii* (Sawada) L.M. Kohn] and *B. porri* N.F. Buckw. [now *Botryotinia porri* (J.F.H. Beyma) Whetzel], all of which cause spoilage of onions and leeks (Ellis 1971; Snowdon 1990) and *B. pseudocinerea* A.-S. Walker et al. which like *B. cinerea* is a broad spectrum pathogen (Plesken et al. 2015).

Botrytis allii grows optimally at 20–25 °C, with a minimum below 5 °C and a maximum near 35 °C. The optimum a_w for growth is in excess of 0.99. Growth occurs down to 0.96 a_w in media containing NaCl and 0.93 a_w in media containing KCl or sucrose, or on onion leaves (Alderman and Lacy 1984). Identifiers: MycoBank MB237131; type not known.

References Ellis (1971), Coley-Smith et al. (1980), Domsch et al. (1980), Elad et al. (2004), and Plesken et al. (2015).

5.9 Genus *Byssochlamys* Westling

Byssochlamys is an ascomycete genus characterised by the absence of cleistothecia, gymnothecia or other bodies enveloping asci during development. Asci in *Byssochlamys* are borne in open clusters, in association with, but not surrounded by, unstructured wefts of fine, white hyphae. Common *Byssochlamys* species are associated with the spoilage of heat processed, acid foods. Species of *Byssochlamys* produce an asexual state, *Paecilomyces,* and under the Code of Nomenclature for algae, fungi and plants only one of these generic names can be valid. At this time, no decision has been made as to which genus should be accepted. Therefore in this work both names will be used as appropriate, with alternative names in brackets giving the correct name if the other genus becomes accepted.

Techniques for isolating *Byssochlamys* rely on a heat treatment to inactivate other fungal spores which are less heat resistant. These techniques have been described in Chap. 4.

The genus was monographed by Samson et al. (2009) using a combination of morphological and molecular characters. They accepted five species with a known sexual state and included four others that are known only from an asexual *Paecilomyces* state.

Four *Byssochlamys* species are significant in food spoilage: *B. fulva, B. nivea*, *B. spectabilis* and *B. lagunculariae*. *B. nivea* and *B. lagunculariae* are readily distinguished from the others by white colonies. *B. nivea* forms ascospores readily in the laboratory, when incubated for 10 days at 25 or 30 °C (but not 37 °C). *B. fulva* and *B. spectabilis* form brown colonies of quite similar appearance. *B. fulva* usually forms ascospores in the laboratory when grown at 30 °C (but not at 25 or 37 °C). *B. spectabilis* does not usually make ascospores in laboratory culture, as it is heterothallic, i.e. usually two isolates must be mated to form the teleomorph.

How do we know we have a *Byssochlamys* if it does not produce ascospores? Presumptive evidence of a *Byssochlamys* can be made from plates at 25 or 37 °C if the isolate has come from heat processed foods or raw materials. If a brown fungus with a *Paecilomyces* asexual state is isolated after a sample has been heat treated at 75 °C, it is probably *B. spectabilis.* This aspect is discussed further under "Heat processed acid foods" in Chap. 12.

The following key assumes that the sample from which a potential *Byssochlamys* isolate has been recovered has been heat treated at 75 °C.

Key to *Byssochlamys* species included here

1	Colonies on CYA and MEA predominantly buff to brown	2
	Colonies on CYA and MEA persistently white to cream	*B. nivea* *B. lagunculariae*
2	White asci and ascospores produced on MEA at 30 °C after 7–10 days; conidia mostly cylindroidal	*B. fulva*
	Colonies remaining buff to brown, teleomorph not present in pure culture; conidia mostly ellipsoidal	*B. spectabilis*

Byssochlamys fulva Olliver & G. Sm. Fig. 5.8

Paecilomyces fulvus Stolk & Samson

Colonies on CYA and MEA at least 60 mm diam, often covering the whole Petri dish, relatively sparse, low or somewhat floccose; conidial production heavy, uniformly coloured olive brown or paler; reverse in similar colours or pale. Colonies on G25N 5–10 mm diam, texture variable, low and sparse to deep and floccose, coloured white or as on CYA. No growth at 5 °C. At 30 and 37 °C, colonies usually covering the whole Petri dish, low and sparse, coloured as on CYA or brighter; reverse in similar colours.

Teleomorph single asci borne from, but not enveloped by, wefts of contorted white hyphae, best developed at 30 °C on MEA, maturing in 7–12 days, occasionally formed at 25 °C in fresh isolates but maturing slowly if at all; asci spherical to subspheroidal, 9–12 µm diam; ascospores ellipsoidal, 5–7 µm long, smooth walled. Asexual reproductive structures penicilli, best observed at 25 °C, borne from surface hyphae or long, trailing, aerial hyphae; stipes 10–30 µm long; phialides of nonuniform appearance, flask shaped or narrowing gradually to the apices, 12–20 µm long; conidia mostly cylindroidal or doliiform (barrel shaped), usually narrow and 7–10 µm long, but sometimes longer, wider or ellipsoidal from individual phialides, smooth walled.

Distinctive features In culture at 30 °C, *Byssochlamys fulva* is distinguished by rapidly growing brown colonies with areas of fine white hyphae, in which asci are produced in open clusters. At 25 °C, colonies may not produce the white hyphae, and then they closely resemble the asexual state of *B. spectabilis*. However, under the microscope *B. spectabilis* produces ellipsoidal not cylindroidal conidia.

Identifiers MycoBank MB265519; neotype IMI 40021; ex-type cultures CBS 146.48 = IMI 40021 = ATCC 10099 = NRRL 1125 = FRR 1125.

Physiology The major physiological characteristic making *Byssochlamys fulva* significant in food mycology is the heat resistance of its ascospores. First noted by Olliver and Rendle (1934), who described this species, and very carefully documented by Hull (1939), this property has been extensively studied. Early studies were comprehensively reviewed by Beuchat and Rice (1979).

Many variables can affect the heat resistance of *Byssochlamys fulva*, which can vary markedly from isolate to isolate (Bayne and Michener 1979; Hatcher et al. 1979). Factors such as pH, water activity (Dijksterhuis and Samson 2006), and the presence of preservatives also have an effect. Ascospores are more susceptible to heat if the pH is low and/or if preservatives such as sodium benzoate or potassium sorbate are present (El-Geddawy 2005). On the other hand, high levels of sugar have a protective effect (El-Geddawy 2005). For *Byssochlamys fulva*, a D value of between 1 and 12 min at 90 °C (Bayne and Michener 1979) and a z value of 6–7 C° (King et al. 1969) are practical working values. Ascospores of *Byssochlamys fulva* isolated from canned tomato paste were more heat resistant in tomato juice than in phosphate buffer (Kotzekidou 1997). Treatment at 80 °C for 1 hour had no effect on the viability of ascospores, however, in the presence of CO_2, a considerable reduction (80%) in ascospore numbers was observed (Ballestra and Cuq 1998).

B. fulva ascospores are also very resistant to high pressure, with resistance increasing with ascospore age (Chapman et al. 2007). After treatment at 600 MPa for 10 min, a 4–5 log reduction was achieved for 3 week old ascospores, whereas the same treatment applied to 9 week old ascospores resulted in a 1 log reduction, and 15 week old ascospores were activated after pressure treatment (Chapman et al. 2007).

The second physiological characteristic which makes *Byssochlamys fulva* an outstanding spoilage fungus is its ability to grow at very low oxygen tensions. This ability, shared with other *Byssochlamys* species, but not with other common heat resistant fungi, provide them with a selective advantage in products such as canned, bottled or cartoned fruits and fruit juices. Our observations suggest that in the presence of very low levels of oxygen, these species grow anaerobically and produce CO_2. A small amount of oxygen contained in the headspace of a jar or bottle,

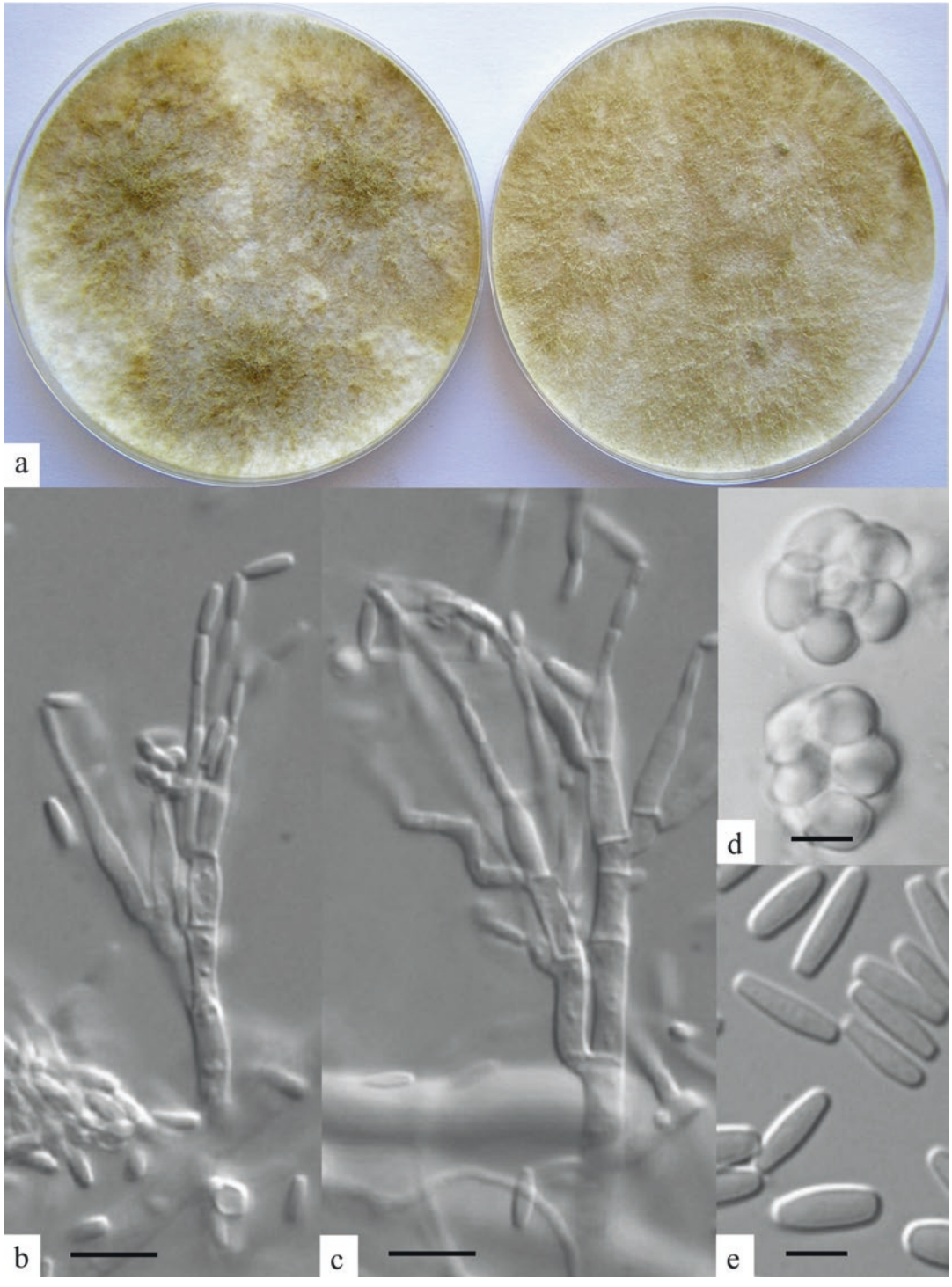

Fig. 5.8 *Byssochlamys fulva* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) asci and ascospores, bar = 5 µm; (**e**) conidia, bar = 5 µm

or the slow leakage of oxygen through a package such as a Tetra-Brik can provide sufficient oxygen for these fungi to grow. The production of gas may cause visible swelling and spoilage of the product.

Byssochlamys fulva can grow extensively in atmospheres containing 20, 40 or 60% CO_2 and less than 0.5% O_2. In 20% CO_2, colonies reached 40–80 mm diameter in 15–30 days, depending on the medium. In 60% CO_2 and less than 0.5% O_2, colonies of 20–40 mm diameter were formed in 30 days; colonies in 80% CO_2 and 20% O_2 were similar (Taniwaki 1995; Taniwaki et al. 2001a).

The small amount of growth formed while residual oxygen is consumed in canned or bottled fruit may be sufficient to allow the production of pectinolytic enzymes. The first sign of spoilage by *Byssochlamys* species is usually a slight softening of the fruit. This progresses until total disintegration takes place, due to the production of powerful pectinases by the fungus (Hull 1939; Beuchat and Rice 1979). Off odours, and a slightly sour taste may develop and, as noted above, gas production may occur. It is rare for canned and bottled fruit to be spoiled by fungi other than *Byssochlamys* species.

Mycotoxins Patulin production by some isolates of *Byssochlamys fulva* has been known for many years (Rice et al. 1977). However, most isolates are not producers (Puel et al. 2007; Houbraken et al. 2006; Luangsa-ard et al. 2011) and there is no published evidence that patulin has been produced by growth of *B. fulva* in commercially processed fruit juices. Very low levels of patulin were produced under atmospheres containing less than 0.5% O_2 and 20–60% CO_2 (Taniwaki 1995). *B. fulva* synthesises byssochlamic acid (Houbraken et al. 2006), a toxin of low potency.

Ecology Spoilage caused by *Byssochlamys fulva* was first recognised in canned strawberries in England in the 1930s. Olliver and Rendle (1934) investigated the incidence of *B. fulva* in fruits, in other canning ingredients and in packing materials. Although strawberries and plums seemed to be particularly affected, *B. fulva* was also found on other fruits such as berries, currants and apples (Olliver and Rendle 1934). Hull (1939) found *B. fulva* on leaves, fruits and straw from strawberry fields, and also on mummified fruit. Olliver and Rendle (1934) and Hull (1939) concluded that soil acts as the primary reservoir for *Byssochlamys* ascospores and that fruit which came in contact with soil directly or from rain splash were susceptible to contamination.

Byssochlamys fulva has been reported from several other areas of the world, notably the United States, where it has occurred in grape products (King et al. 1969; Splittstoesser et al. 1971), and Australia, as the cause of spoilage of canned strawberries (Richardson 1965), fruit juices and fruit based baby foods. The main Australian source of *B. fulva* has been shown to be passionfruit juice (Hocking and Pitt 1984). Effective measures to overcome this problem include washing of fruit before juice extraction, rejection of difficult to clean wrinkled fruit and screening of juices for heat resistant ascospores (Cartwright and Hocking 1984; Kotzekidou 1997). For details of screening methods see Chap. 4.

The high heat resistance of *B. fulva* and *B. nivea* ascospores has led to the study of other approaches for deactivating them. Intermittent use of dimethyldicarbonate was suggested (van der Riet and Pinches 1991) after it was found that a single high dose (1000 mg/l) had no effect on ascospores of *B. fulva* (van der Riet et al. 1989). Approximately 1.2 kGy of ionising radiation produced one decimal reduction in viable ascospores of two resistant strains of *B. fulva*; it was estimated that more than 7 kGy would be needed for an effective pasteurising process. Apple juice containing high numbers of spores and treated with 5 kGy spoiled after 3 months (van der Riet and van der Walt 1985).

References Beuchat and Rice (1979), Hocking and Pitt (1984), and Houbraken and Samson (2006, 2011).

Byssochlamys nivea Westling **Fig. 5.9**

Paecilomyces niveus Stolk & Samson

Colonies on CYA 40–50 mm diam, low and quite sparse, white to slightly grey; reverse pale to mid brown. Colonies on MEA covering the whole Petri dish, low and sparse, white to creamish, with small knots of dense hyphae; reverse pale to brownish. On G25N, usually only microscopic growth. No growth at 5 °C. At 30 °C on CYA, colonies covering the whole Petri dish, similar to on MEA at 25 °C, but often more dense, envelop-

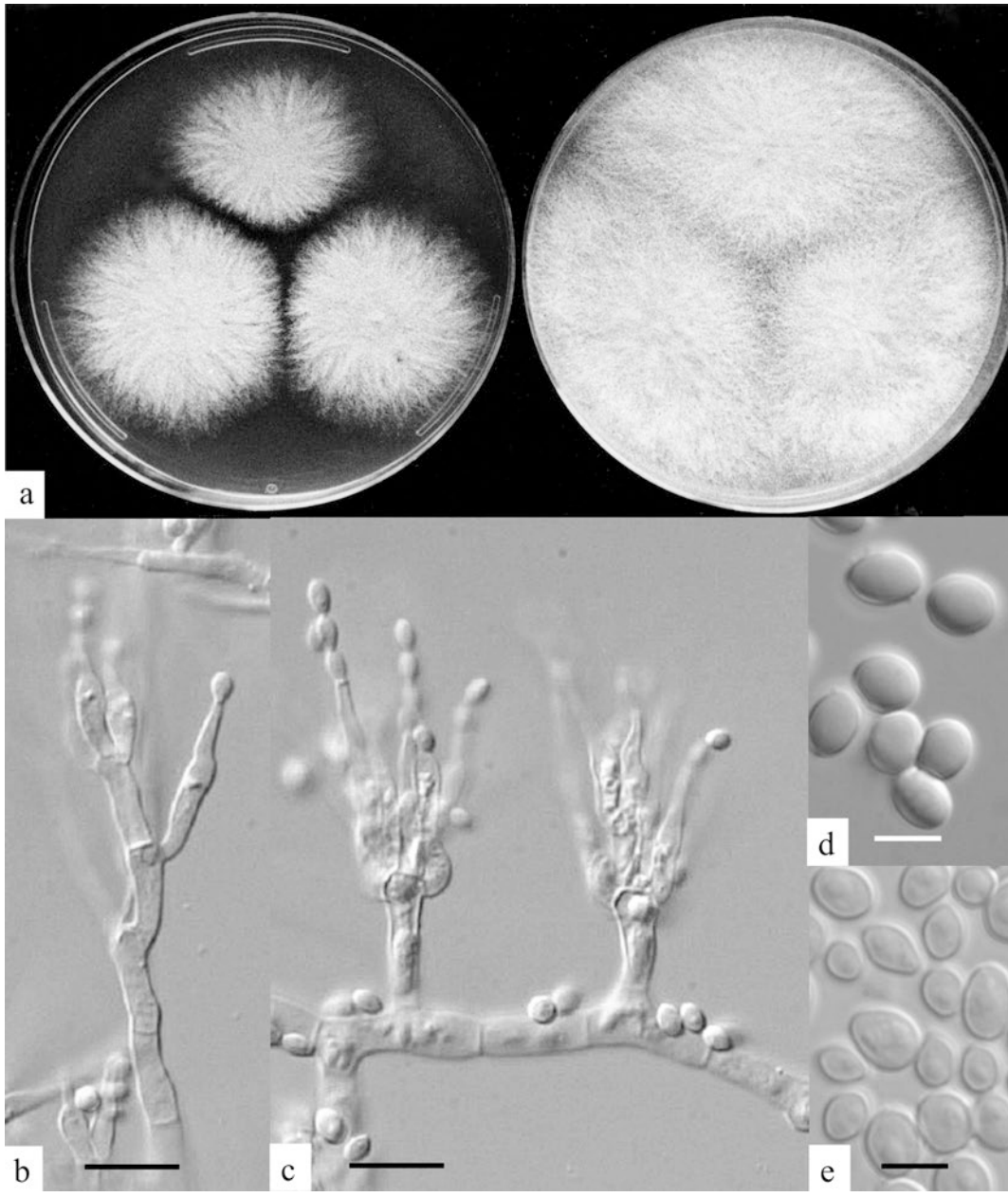

Fig. 5.9 *Byssochlamys nivea* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) ascospores, bar = 5 µm; (**e**) conidia, bar = 5 µm

ing distinct knots of dense hyphae. At 37 °C, colonies 50–70 mm diam, low to floccose, moderately dense, white to cream, reverse pale to brown.

Sexual state similar to that of *B. fulva* except for slightly smaller asci (8–11 µm diam) and ascospores (4–6 µm diam), maturing in 10–14 days at 25 °C and in 7–10 days at 30 °C, but rarely found at 37 °C. Asexual states of two kinds produced, aleurioconidia and penicilli; aleurioconidia borne singly, common at 30 and 37 °C, spherical to pyriform, 7–10 µm diam; penicilli sparsely produced, with short stipes bearing irregular metulae and phialides or phialides alone, and with phialides sometimes borne solitarily from hyphae as well; phialides 12–20 µm long, cylindrical then gradually tapering; conidia ellipsoidal to pyriform, 3–6 µm long, smooth walled.

Distinctive features *Byssochlamys nivea* and *B. lagencularia* are readily distinguished from *B. fulva* by persistently white colonies They differ from other fungi by forming three characteristic types of reproductive structures: aleurioconidia, penicilli and solitary asci. *B. lagencularia* differs from *B. nivea* by the formation of smaller ascospores and conidia (Samson et al. 2009).

Identifiers MycoBank MB171153; lectotype CBS 100.11; ITS sequences DQ464362, FJ389934; alternative markers 18S rDNA FJ225775, FJ358345, 26S rDNA AY176750 (CBS 2016). Note: CBS states that CBS 100.11 is a neotype; however the culture was deposited by Westling in the year of description, so it is a more corrcctly a lectotype.

Physiology The minimum a_w for growth of *Byssochlamys nivea* was 0.92 at 21 °C, 0.89 at 30 °C and 0.87 a_w at 37 °C (Roland and Beuchat 1984). So far as has been established, the physiology of *B. nivea* is similar to that of *B. fulva*. Like *B. fulva*, it causes spoilage of heat processed acid foods under conditions of low oxygen tension. *B. nivea* ascospores are reported to be marginally less heat resistant than those of *B. fulva* (Put and Kruiswijk 1964; Beuchat and Rice 1979). A D value at 88 °C of 0.75–0.8 min, with *z* values ranging from 4.0–6.1 C°, was determined by Casella et al. (1990). See also Engel and Teuber (1991) and Quintavalla and Spotti (1993). *B. nivea* ascospores exhibited pressure resistance similar to that reported for *B. fulva* (Butz et al. 1996; Chapman et al. 2007). The utility of combined heat and pressure processing was demonstrated by Evelyn and Silva (2015).

Remarks about the physiology of *B. fulva* probably apply equally well to *B. nivea*. Growth of *B. nivea* in atmospheres containing 0.5% O_2 and 20, 40 or 60% CO_2 was somewhat slower than observed for *B. fulva*, while growth on 20% O_2 and 80% CO_2 was rather faster (Taniwaki 1995). Taniwaki et al. (2001a) showed that *B. nivea* was capable of continued growth at 40% CO_2 with <0.5% O_2.

Mycotoxins *B. nivea* may produce patulin (Rice et al. 1977) but that species is not known to be a source of patulin in foods. Several studies have investigated the effect of physical parameters on patulin production, including the effect of headspace in glass jars of heat processed grape juice (Rice 1980), controlled atmospheres (Orth 1976; Taniwaki 1995), temperature, a_w and preservatives (Roland and Beuchat 1984; Roland et al. 1984). Minimum a_w values for patulin production were 0.92 at 21 °C and 0.87 at both 30 and 37 °C (Roland and Beuchat 1984). Ayesha and Viswanath (2006) reported the production of patulin in sugar cane juice inoculated with *B. nivea*. Patulin synthesis was greater at 20 °C than 35 °C, and more patulin was produced in juice than in commercial broth. However, no patulin was found in commercial packs.

B. nivea also produces byssochlamic acid and mycophenolic acid (Houbraken et al. 2006) but these compounds are not of significance in foods.

Ecology *Byssochlamys nivea* appears to be a less common problem in foods than *B. fulva*. It has mostly been reported from European sources: from sweet cider in Switzerland, from fruit juices in Denmark, from bottled strawberries in the Netherlands and packaged ravioli in Italy (see Pitt and Hocking 1997). Spoilage of the latter product was prevented by packing in an atmosphere of 60% CO_2, 39.4% N_2 and 0.05% O_2 (Dragoni and Cantoni 1988). Microwave heating (used to pasteurise packaged ravioli) had little effect on *B. nivea* (Dragoni et al. 1990). *B. nivea*

has also been isolated from passionfruit juice and from cream cheese in Australia (A.D. Hocking unpublished).

Additional species *Byssochlamys lagunculariae* (C. Ram) Samson et al. differs from *B. nivea* by smaller ascospores and conidia and slower growth on CYA (Samson et al. 2009). It can be expected to have similar physiological properties to *B. nivea*. It does not produce patulin. It has been isolated from pasteurised strawberries and aloe juice (Samson et al. 2009). Identifiers: MycoBank MB512557; holotype CBS 373.70; ex-type culture CBS 373.70 (CBS 2016).

References Beuchat and Rice (1979), Hocking and Pitt (1984), Houbraken et al. (2006), and Samson et al. (2009).

Byssochlamys spectabilis (Udagawa & Shoji Suzuki) Houbraken & Samson Fig. 5.10

Talaromyces spectabilis Udagawa & Shoji Suzuki

Paecilomyces varioti Bainier (asexual state)

Colonies on CYA of variable size, 30–70 mm diam, plane, of low to floccose appearance, usually coloured uniformly brown or olive brown from conidia; reverse pale. Colonies on MEA 70 mm diam or more, otherwise very similar to those on CYA. Colonies on G25N 8–16 mm diam, similar to on CYA or of white mycelium only. No germination at 5 °C. At 37 °C, colonies growing very rapidly, 60 mm or more diam, similar to those at 25 °C or with sporulation reduced; reverse pale.

Sexual state not seen in pure culture, but after mating two appropriate strains, asci borne singly, 9–12 µm diam, ascospores ellipsoidal, 5–7 µm long, with smooth to finely roughened walls. Asexual state borne from aerial hyphae on short stipes, of irregular pattern, a cluster of phialides alone or with metulae and phialides or occasionally rami; phialides 12–20 µm long, tapering gradually, with collula often bent away from the axis; conidia mostly subspheroidal to ellipsoidal, sometimes cylindroidal or pyriform, usually 3.0–5.0 µm long, smooth walled.

Distinctive features With its rapidly growing colonies, coloured uniformly brown to olive brown at both 25 and 37 °C, and irregular penicilli with long phialides, the asexual state of *Byssochlamys spectabilis, Paecilomyces variotii,* is a readily recognised fungus. Only the asexual state of *B. fulvus, P. fulvus,* is similar: *P. variotii* is distinguishable by its broadly ellipsoidal rather than cylindrical conidia. When grown on MEA at 30 °C for 10 days, *B. fulvus* usually produces its sexual state, whereas isolates identifiable as *P. variotii* do not produce asci. However, if *P. variotii* is isolated from a heat treated food or beverage, it can be confidently assumed to have grown from an ascospore of *Byssochlamys spectabilis* (Houbraken et al. 2008, our unpublished observations).

Taxonomy Houbraken et al. (2006) transferred *Talaromyces spectabilis,* described by Udagawa and Suzuki (1994), to *Byssochlamys* and reported that it is the teleomorph of *P. variotii.* The teleomorph is rarely seen in laboratory culture because *B. spectabilis* is heterothallic: production of ascospores requires co-culturing of (+) and (−) strains (Houbraken et al. 2008).

Identifiers MycoBank MB 512558; holotype CBS 101075; ex-type culture CBS 101075; ITS sequences AY753330, EU037051; alternative markers 18S rDNA AY526473 (CBS 2016).

Physiology *Byssochlamys spectabilis* is a xerophilic fungus, with growth recorded down to 0.80 a_w (Wheeler and Hocking 1988, as *P. variotii*). It grows between about 5 and 45–48 °C, with an optimum at 35–40 °C (Samson 1974, as *P. variotii*). This speces is able to grow under low oxygen tensions (Samson et al. 2009; our unpublished observations).

Mycotoxins Houbraken et al. (2006) reported viriditoxin production by *B. spectabilis*, but this compound is not significant in foods.

Ecology This species is a ubiquitous contaminant of foods and raw materials. It has a strong association with edible oils, raw materials containing oil, bacon, peanuts and peanut cake (see Pitt and Hocking 1997), margarine (Demirci and Arici 2006) and cocoa beans (Wojcik-Stopczynska 2006). Amongst many other reports, it has been recorded from cereals (Loiveke et al. 2004), bread, meat products, biltong, health

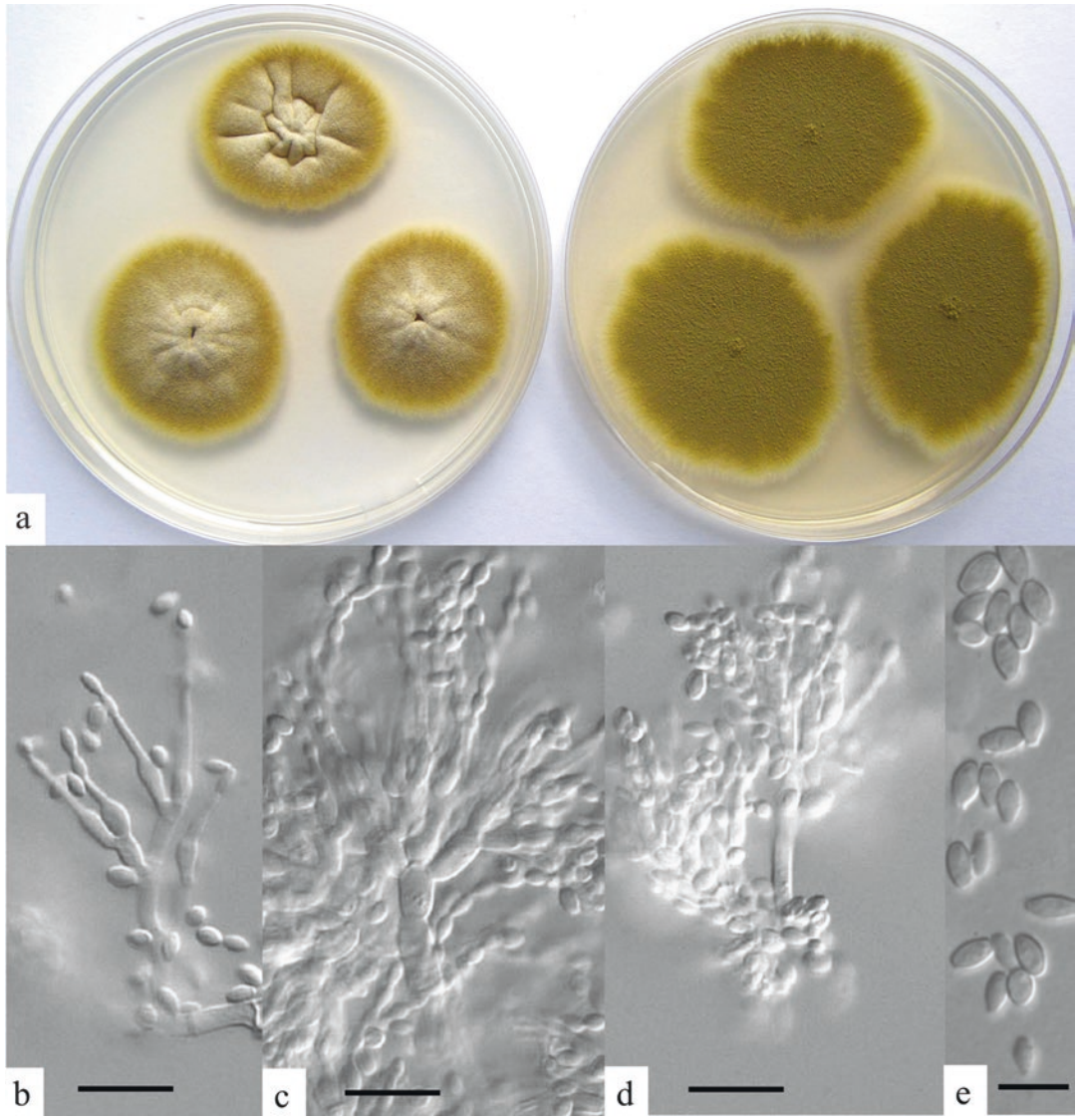

Fig. 5.10 *Byssochlamys spectabilis* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

foods and airline foods (see Pitt and Hocking 1997). In our laboratory we have frequently isolated *B. spectabilis* (as *P. variotii*) from heat processed fruit products and beverages, including sports drinks. It was found in 7% of all black peppercorns we examined from Indonesia (Pitt et al. 1998a), and at low levels in black beans, cassava, cashews and copra from Thailand (Pitt et al. 1994).

Byssochlamys spectabilis is notable for sorbate resistance, and causes spoilage of margarine and processed cheeses due to production of 1,3-pentadiene from the degradation of sorbate (Sensidoni et al. 1994). It is also one cause of spoilage in dried fruits and other products. This is due to the "mouldy leather" odour of chloroanisole produced from chlorophenols (Tindale et al. 1989; Whitfield et al. 1991).

References Samson (1974), Udagawa and Suzuki (1994), and Houbraken et al. (2006, 2008).

5.10 Genus *Chaetomium* Kunze and Related Genera

Chaetomium and related genera are ascomycetes which produce black ascocarps and which are commonly encountered in foods. The ascocarps are perithecia, similar in appearance to cleistothecia, but asci are clavate (club shaped), and have an apical opening (ostiole) through which the ascospores are released. There is a superficial resemblance to the genus *Phoma*, which produces asexual conidia in black pycnidia; ascospores in *Chaetomium* and related genera are often freed from the ascus before being discharged singly from the perithecium, and hence could be mistaken for conidia. *Chaetomium* and related genera are readily distinguished from *Phoma* by the presence of stout black or dark coloured hyphae attached to the perithecial walls, which are visible in culture under the low power microscope.

The species of *Chaetomium* and related genera considered here are notable as producers of cellulases and so commonly occur on dung, wood and paper products. They are relatively common in some kinds of foods and are often isolated from indoor environments. *Chaetomium* has become a large genus and was split into several genera by Wang et al. (2016). Many authors have contributed to the taxonomy. *C. globosum* is the most frequently encountered species of *Chaetomium* and is described here as representative of the genus. In addition descriptions of *Ovatospora brasiliense* and *Dichotomopilus funicola* (= *Chaetomium brasiliense* and *Chaetomium funicola* from previous editions) are included here. Both species are quite common in tropical commodities and have been separated from *Chaetomium* by small differences.

Key to *Chaetomium* and related species included here

1	Colonies on CYA at 37 °C much larger than at 25 °C	*Ovatospora brasiliense*
	Colonies on CYA at 37 °C smaller than at 25 °C, or absent	2
2(1)	Colonies on CYA and MEA at 25 °C less than 50 mm diam, ascospores 5–7 µm long	*Dichotomopilus funicola*
	Colonies on CYA and MEA at 25 °C usually covering the whole Petri dish, ascospores 8–10 µm long	*Chaetomium globosum*

Chaetomium globosum Kunze Fig. 5.11

Colonies on CYA and MEA covering the whole Petri dish, on CYA low and sparse, of scanty white mycelium and conspicuous though usually sparse black perithecia, ca 0.2 mm diam; on MEA growth more dense but still low, coloured grey or greenish black from hyphae enveloping abundant perithecia; reverse on both media usually brown. On G25N, colonies less than 5 mm diam produced. No growth at 5 °C. At 37 °C, colonies usually 20–30 mm diam, of white mycelium.

Reproductive structures perithecia, black, 150–200 µm diam, with numerous stout, dark hyphae appended; ascospores produced after 1–2 weeks, spheroidal, broadly ellipsoidal or apiculate, commonly 8–10 µm long, smooth walled. Conidia not generally produced.

Distinctive features In contrast to *Ovatospora brasiliense*, *C. globosum* grows much more rapidly at 25 °C than 37 °C. Ascospores are larger than those of *Dichotomopilus funicola.*

Identifiers MycoBank MB172545; neotype CBS H-22185; ex-type culture CBS 160.62 (CBS 2016).

Physiology Chapman and Fergus (1975) reported that *Chaetomium globosum* ascospores germinated from a minimum temperature of 4–10 °C to a maximum of 38 °C, and most rapidly at 24–38 °C. They also reported germination over the whole pH range tested, 3.5–7.0. Heat resistance was low: 1% of ascospores survived for 10 min at 55 °C, but not at 57 °C. Ahammed et al. (2005) observed maximum sporulation at 28 °C and pH 5.0 on a medium containing sucrose and thiamine. *C. globosum* can grow down to 0.94 a_w in soil (Kouyeas 1964).

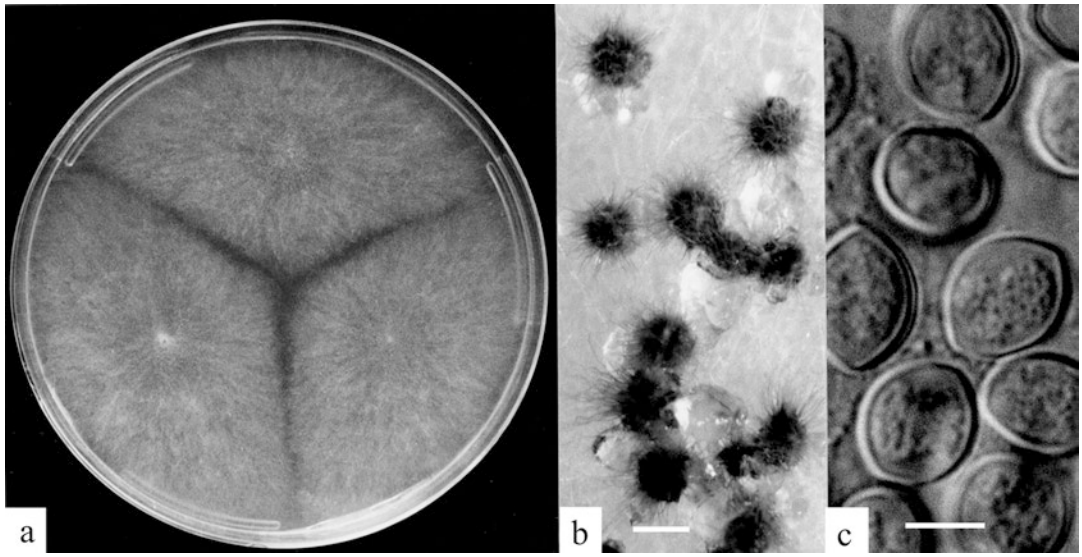

Fig. 5.11 *Chaetomium globosum* (**a**) colony on CYA, 7 d, 25 °C; (**b**) perithecia *in situ*, bar = 100 µm; (**c**) ascospores, bar = 5 µm

Mycotoxins *Chaetomium globosum* produces chaetoglobsins A and C, moderately toxic compounds reported to be produced in building materials (Fogle et al. 2007). Their relevance to foods is unknown.

Ecology *Chaetomium globosum* has been isolated from a variety of commodities, particularly wheat, barley, rice, beans and soybeans. It has also been recorded from nuts (see Pitt and Hocking 1997) and spices (Mandeel 2005). We isolated it frequently from tropical commodities: from 12–17% of maize samples, 14–32% of mung bean samples and 33–35% of soybean samples from Thailand, Indonesia and the Philippines, with overall infection rates of 1–4% of all particles examined (Pitt et al. 1994, 1998a).

Relatively high infection levels were also encountered in Thai cashews, copra and paddy rice. Kemiri (candle) nuts from Indonesia were highly infected: 37% of samples, up to 40% in infected samples and 9% of nuts overall (Pitt et al. 1998a). This species has not been reported to cause food spoilage, although it has been implicated in causing disease in pears in Egypt (Ismail and Abdalla 2005).

Dichotomopilus funicola (Cooke) X. Wei Wang & Samson

Fig. 5.12

Chaetomium funicola Cooke

Colonies on CYA 20–25 mm diam, plane, of low and sparse white mycelium, sometimes pale grey centrally, enveloping abundant developing perithecia, reverse pale or greyish brown. Colonies on MEA 35–40 mm diam, plane, sparse, low to somewhat floccose, mycelium white or grey, enveloping developing perithecia; reverse pale to greyish olive or pale brown. On G25N, usually no growth. At 5 °C, no germination. At 37 °C, usually no growth.

Perithecia borne in a layer on the agar surface, 120–160 mm diam, surrounded by a mix of dichotomously branched hyphae and almost straight, dark walled hyphae; asci maturing in 14 days, breaking down immediately, ascospores ellipsoidal, 5–7 × 3.0–5.0 µm, grey or brown, sometimes with one or two internal oil droplets visible, often collapsing in age, smooth walled.

Distinctive features *Dichotomopilus funicola* grows only slowly at 25 °C and usually not at 37 °C. Ascospores are smaller than those of *Chaetomium globosum*.

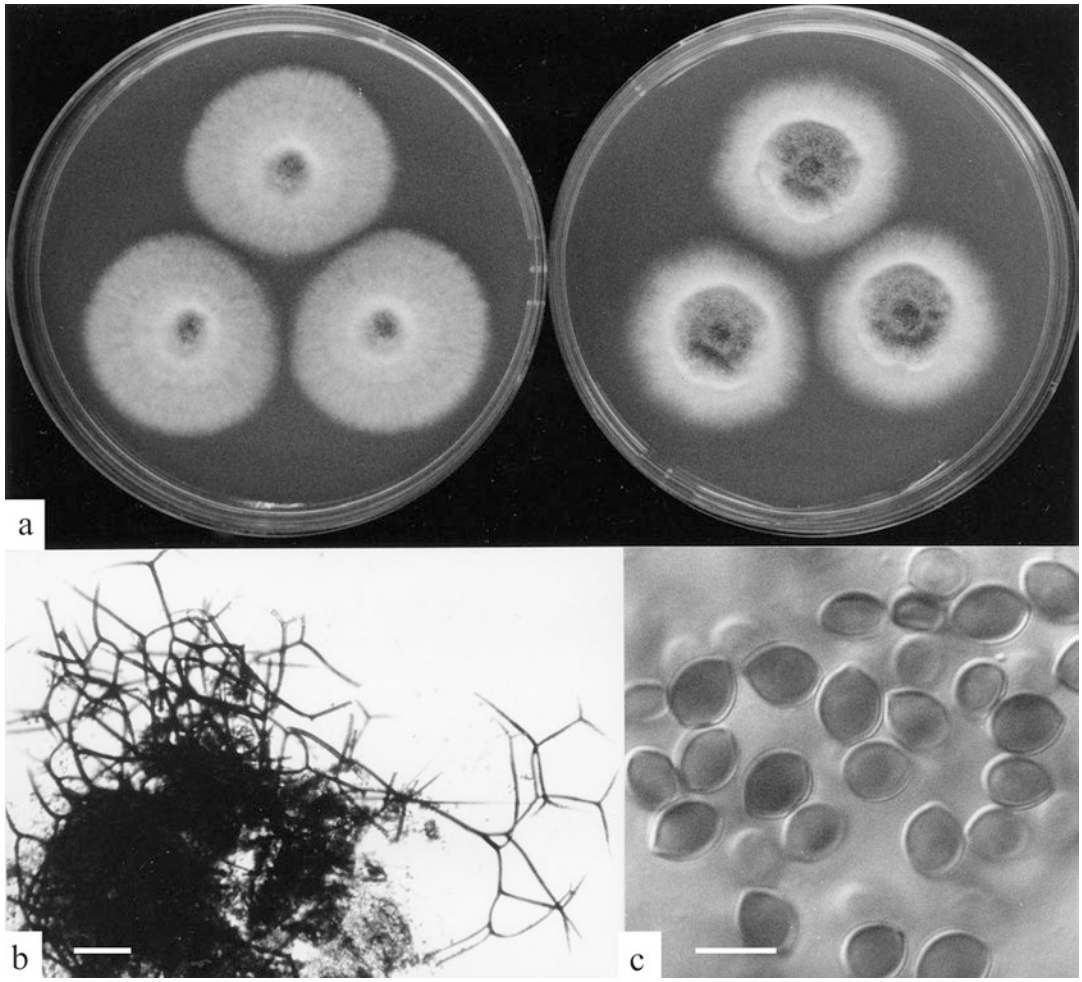

Fig. 5.12 *Dichotomopolus funicola* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) perithecium *in situ*, bar = 50 µm; (**c**) ascospores, bar = 5 µm

Identifiers MycoBank MB818841; epitype HMAS 244231 (Wang et al. 2014); ex-epitype culture CBS 159.52 (CBS 2016).

Physiology No studies are known to us. Domsch et al. (1980) indicated that perithecial production is maximal at about 20 °C, indicating that this species is better adapted to low than high temperatures.

Mycotoxins Mycotoxin production has not been reported.

Ecology Despite its low temperature optimum, we repeatedly isolated *Dichotomopilus funicola* (as *Chaetomium funicola*) from tropical commodities. It was present in 23% of Indonesian soybean samples, ranging up to 50% infection in some samples, and in 3% of beans overall (our unpublished data). Lower levels of infection (1% or more overall) were observed in peanuts, cashews, copra, mung beans and sorghum from Thailand (Pitt et al. 1993, 1994), in mung beans and maize from Indonesia, and paddy rice from the Philippines (Pitt et al. 1998a and our unpublished data).

Ovatospora brasiliensis (Bat. & Pontual) X. Wei Wang & Samson **Fig. 5.13**

Chaetomium brasiliense Bat. & Pontual

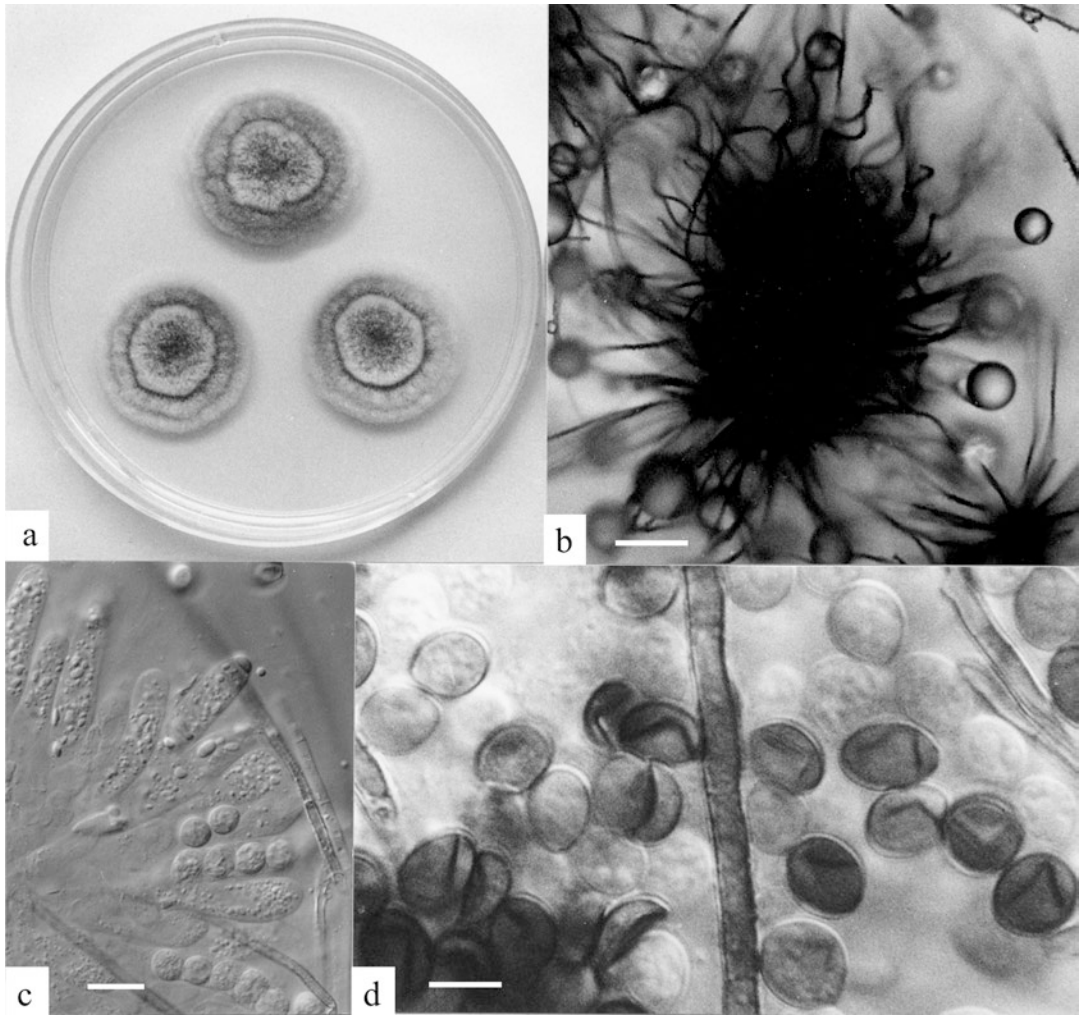

Fig. 5.13 *Ovatospora brasiliensis* (**a**) colony on CYA, 7 d, 25 °C; (**b**) perithecium *in situ*, bar = 25 µm; (**c**) asci, bar = 10 µm; (**d**) ascospores, bar = 5 µm

Colonies on CYA and MEA 25–35 mm diam, on CYA low to moderately deep and dense, of white to grey mycelium; reverse sometimes pale, more commonly dark grey to almost black; on MEA low and sparse, dark grey, with some paler grey aerial hyphae; reverse dark grey. No growth on G25N or germination at 5 °C. Colonies at 37 °C covering the whole Petri dish, 65 mm or more diam, general appearance similar to those on CYA at 25 °C; reverse very dark grey to greyish black.

Reproductive structures perithecia, not observed on CYA or MEA at 25 °C, but on CYA at 37 °C abundant, forming a deep, contiguous layer on the agar surface, most readily observed at colony margins, especially between colonies, surrounded by radiating long, stout hyphae, straight at first but at maturity curling into long corkscrew shapes, 75–100 µm diam, maturing in 7 days at colony centres; asci clavate (club-shaped), 60–70 µm long, ascospores subspheroidal to broadly ellipsoidal, 6–8 µm long, brown, with smooth walls. Conidia not produced.

Distinctive features Unlike the other *Chaetomium* and related species considered here, *O. brasiliensis* grows much more rapidly at 37 °C than 25 °C, and perithecia are formed in much greater numbers at the higher temperature. Perithecia are black, but under the stereomicro-

scope appear surrounded by grey hyphae. Ascospores are smaller than those of *C. globosum*.

Identifers MycoBank MB818851; holotype not identified here, in Batista collection at University of Recife, Recife, Brazil; no ex-type culture known; representative cultures CBS 122.65 = FRR 4062.

Physiology No studies on physiology are known to us. However, the description above indicates that this species has a high temperature optimum for growth.

Mycotoxins Significant mycotoxins are not known to be produced.

Ecology We have isolated *Ovatospora brasiliensis* not infrequently from tropical sources. From Thailand, 1% of mung beans, black beans, paddy rice grains and cashew kernels, and 7–10% of soy bean and sorghum samples, but less than 1% of individual seeds, contained *C. brasiliense* (Pitt et al. 1993, 1994). Similar figures were obtained from Indonesian commodities (Pitt et al. 1998a). In samples from the Philippines, this species was found in maize, peanuts, soybeans, mung beans, black pepper, paddy and milled rice, all near 1% of total particles examined (our unpublished data).

References Von Arx et al. (1986), Doveri (2013), Wang et al. (2014), and Zhang et al. (2017).

5.11 Genus *Cladosporium* Link

Cladosporium is a very commonly isolated genus and is often dominant in studies of airborne microbiota. Species occur both as saprophytes and as plant pathogens. Conidia of *Cladosporium* species are particularly well adapted to aerial dispersal, being small, dry, heavily pigmented and apparently highly resistant to sunlight. In culture, *Cladosporium* is readily recognised. Colonies are 15–40 mm in diameter, low, dense and velvety, and coloured olive. Colony reverses on CYA are often a deep iridescent blue black, uncommon among slowly growing genera.

Reproductive structures are fragile, tree-like conidiophores, which branch irregularly by budding from the youngest cells. The structures can be seen by examination of colonies under the stereomicroscope. They disintegrate partially or totally in wet mounts, leaving masses of conidia, which may show buds or bud scars. In shape, but not colour, the conidia often resemble yeast cells; however walls are thick, coloured olive and often roughened.

Cladosporium species occur as pathogens on fresh fruit and vegetables (Snowdon 1990, 1991) and may cause spoilage of strawberries (Beneke et al. 1954; Dennis et al. 1979) or tomatoes (Harwig et al. 1979). On other foods, *Cladosporium* species usually occur as contaminants rather than as spoilage fungi. However, all common species grow at temperatures near 0 °C, and Cladosporia have been reported to cause spoilage of chilled meats, cheese and other refrigerated commodities from time to time (see Pitt and Hocking 1997).

Cladosporium, its teleomorphs and related genera have been studied extensively over the past 15 years (Crous et al. 2007; Bensch et al. 2010, 2012, 2015). As now circumscribed, *Cladosporium* includes about 170 species. Many are associated with the sexual genus *Davidiella*, but *Cladosporium* is the preferred name (Rossman et al. 2015). The most frequently reported saprophytic species, *Cladosporium herbarum*, *C. cladosporioides* and *C. sphaerospermum* are treated here: each is now considered a major lineage which includes many other species.

Key to *Cladosporium* species included here

1	Single celled conidia small, less than 4 µm wide	2
	Single celled conidia often exceeding 4 µm wide	*C. herbarum*
2(1)	Single celled conidia ellipsoidal or apiculate (lemon-shaped)	*C. cladosporioides*
	A high proportion (greater than 40%) of single celled conidia roughly spherical or pyriform	*C. sphaerospermum*

Cladosporium cladosporioides (Fresen.) G.A. de Vries Fig. 5.14

Colonies on CYA and MEA 25–40 mm diam, low and dense, lightly wrinkled or plane, surface velutinous or lightly floccose; mycelium inconspicuous; conidia abundant, coloured olive; reverse bluish grey. Colonies on G25N 5–12 mm diam, plane, sometimes centrally raised, velutinous, coloured as on CYA; reverse bluish black. At 5 °C, colonies usually 1–2 mm diam, occasionally only germination occurring. No growth at 37 °C.

Conidiophores *in situ* dendritic (treelike), closely packed, with stipes bearing branching structures of acropetally produced cells, all functioning as conidia at maturity and separating in liquid mounts; conidia heavy walled, pale olive brown, larger ones non or singly septate, 10–30 × 2–5 µm, smooth walled, smaller ones nonseptate, ellipsoidal to apiculate, 3–7 × 2–4 µm, with walls smooth to finely roughened.

Distinctive features *Cladosporium cladosporioides* produces smaller conidia than *C. herbarum*; unlike *C. sphaerospermum*, the majority

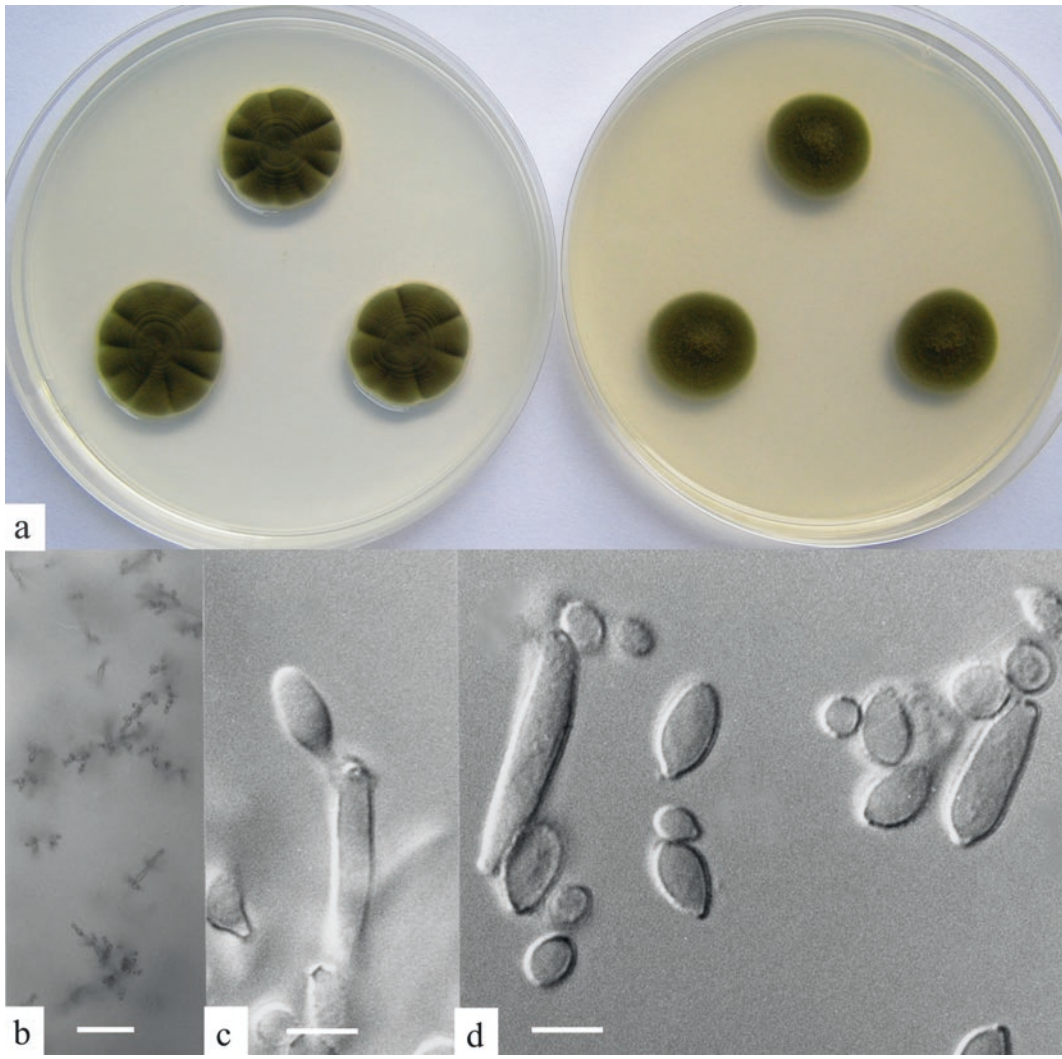

Fig. 5.14 *Cladosporium cladosporoides* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) fruiting structures *in situ*, bar = 50 µm; (**c**) conidiophores, bar = 5 µm; (**d**) conidia, bar = 5 µm

of conidia are ellipsoidal. Growth on CYA and MEA is much faster than that of *C. sphaerospermum*.

Taxonomy Bensch et al. (2010) studied the *Cladosporoum cladosporioides* complex and recognised 22 species within a monophyletic clade; Bensch et al. (2012) increased that number to 39. All species are closely related and can be expected to share major physiological properties.

Identifiers Mycobank MB294915; neotype CBS 112388; ex-type strain CBS 112388; ITS barcode HM148003, alternative markers *act* HM148490, *tef1* HM148244 (Marin-Felix et al. 2017).

Physiology Gill and Lowry (1982) reported a minimum growth temperature of −5 °C for *Cladosporium cladosporioides*; the maximum is near 32 °C (Domsch et al. 1980). It has been reported to grow down to 0.86 a_w (Hocking et al. 1994) or 0.87 a_w at 25 °C (Segers et al. 2015), and is relatively resistant to microwave heating (Dragoni et al. 1990). The minimum inhibitory concentration of sorbic acid is 160 mg/kg at pH 5 (Skirdal and Eklund 1993).

Mycotoxins This species is not known to produce mycotoxins.

Ecology *Cladosporium cladosporioides* has been isolated from a very wide variety of foods, including wheat and flour, barley, rice, sorghum, fresh and frozen meat and fresh vegetables (see Pitt and Hocking 1997). It occurred on 16% of 74 dried fish samples from Indonesia (Wheeler et al. 1986), and was common on Egyptian walnuts and hazelnuts (Abdel-Hafez and Saber 1993). It has also been found in peanuts (El-Magraby and El-Maraghy 1988).

C. cladosporioides is not as common a cause of spoilage in fresh fruit as *C. herbarum*, but can cause rots of raspberries and melons, and sooty discolouration of bananas (Snowdon 1990). Because of its psychrophilic nature, *C. cladosporioides* causes spoilage of refrigerated foods such as cheese (Northolt et al. 1980) and meat (Gill et al. 1981). It was the dominant fungus causing losses in vacuum packed Australian cheese blocks (Hocking and Faedo 1992).

C. cladosporioides is also very common in major Southeast Asian commodities: we isolated this species from 33% of mung bean, 14% of peanut, 13% of soybean and 10% of maize samples from the Philippines and 21% of soybeans and 14% of mung bean samples from Indonesia (Pitt et al. 1998a and our unpublished data).

References Ellis (1971), Domsch et al. (1980), Crous et al. (2007), Bensch et al. (2010), and Marin-Felix et al. (2017).

Cladosporium herbarum (Pers.) Link — Fig. 5.15

Davidiella tassiana (de Not.) Johanson (teleomoph)

Colonies on CYA and MEA 18–32 mm diam, velutinous to lightly floccose, plane or slightly wrinkled, coloured olive; reverse olive grey to dark greenish grey. Colonies on G25N 5–10 mm diam, low and sparse to deep and dense, coloured as on CYA, or paler. At 5 °C, colonies usually 1–2 mm diam; occasionally only germination. No growth at 37 °C.

Conidiophores *in situ* sparsely and irregularly branched dendritic structures, borne on long, dark stipes, separating in fluid mounts; conidia ellipsoidal to cylindroidal, extremities sometimes irregular due to bud scars, usually nonseptate but larger cells with 1–2 septa, commonly 8–15(−20) × 4–6 µm, pale brown, with densely roughened walls.

Distinctive features The conidia of *Cladosporium herbarum* are 4–6 µm wide, wider than the other commonly occurring species, and have distinctly roughened walls.

Taxonomy The teleomorph of *C. herbarum* is *Davidiella tassiana* (De. Not.) Johansen (Bensch et al. 2012). This is not usually seen in pure culture. *Cladosporium herbarum* is a complex of over 20 species according to Bensch et al. (2012).

Identifiers MycoBank MB231458; epitype CBS 121621; ex-type culture CBS 121621 = ATCC MYA-4682; ITS barcode EF679363; alternative markers *act* EF679516, *tef1* EF679440 (Marin-Felix et al. 2017).

Physiology Growth of *Cladosporium herbarum* has been reported down to 0.88 a_w (Snow 1949) and down to −10 °C (Joffe 1962). The maximum growth temperature is 28–32 °C (Domsch et al. 1980). This

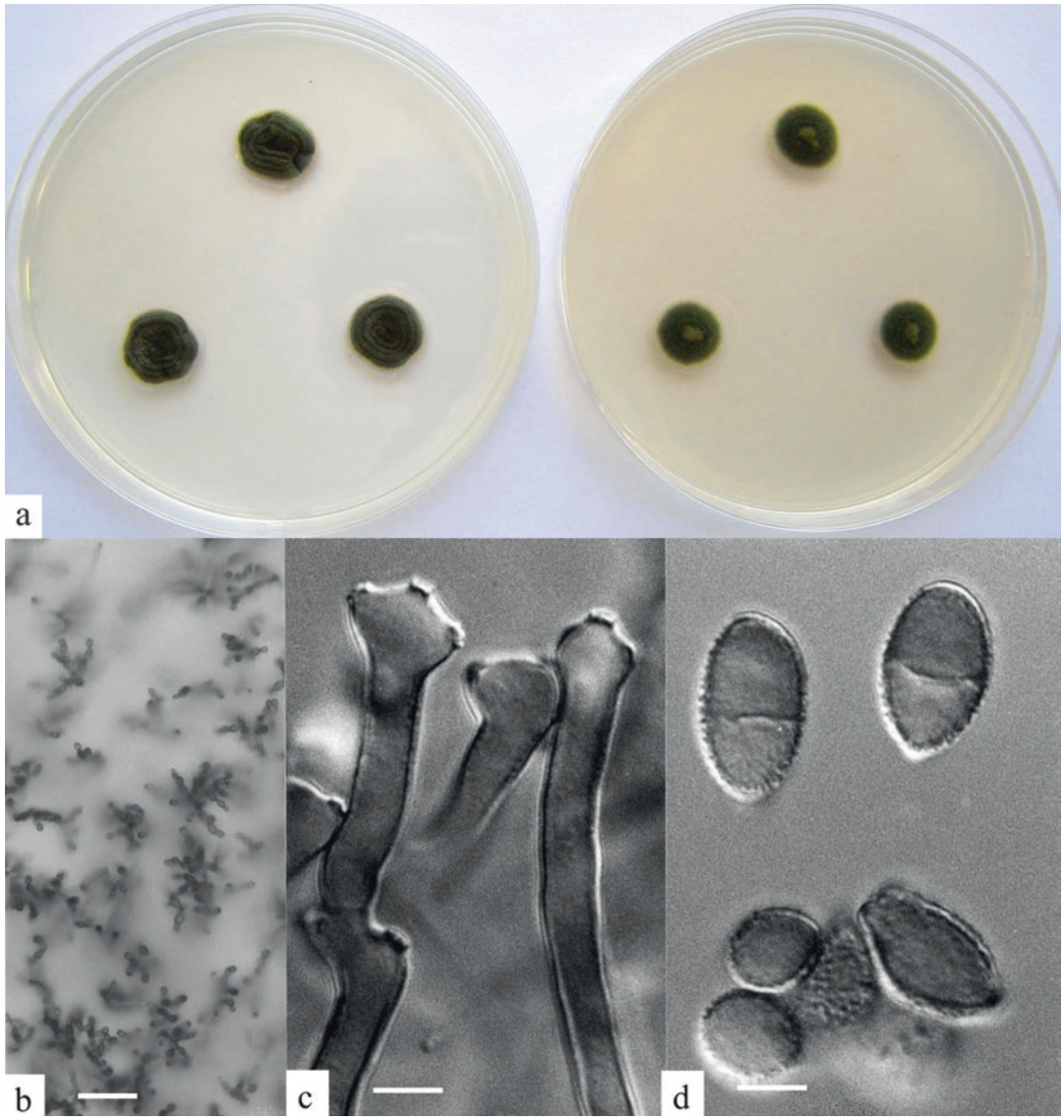

Fig. 5.15 *Cladosporium herbarum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) fruiting structures *in situ*, bar = 50 µm; (**c**) conidiophores, bar = 5 µm; (**d**) conidia, bar = 5 µm

species can grow and sporulate in an atmosphere containing 0.25% oxygen (Follstad 1966).

Mycotoxins No mycotoxins are known to be produced.

Ecology Because *Cladosporium herbarum* has been the best known *Cladosporium* name for a long time, it seems likely that some reports of this species really refer to other Cladosporia, especially *C. cladosporioides,* in our experience the more common species in foods. For example, Waghray et al. (1988) reported *C. herbarum* as common on Indian rice, but according to Pitt et al. (1994, 1998a, unpublished), the common *Cladosporium* species on Southeast Asian rice is *C. cladosporioides*. In contrast to *C. cladosporioides, C. herbarum* causes spoilage of fresh fruits and vegetables: rots in fresh yams (*Dioscorea* sp.; Adeniji 1970a), stored apples (Snowdon 1990; Kalafatoglu and Karapinar 1991), stone fruit, tomatoes, melons, grapes (Snowdon 1990,

1991; Camili and Benato 2005), passionfruit (Ribeiro Jr and Dias 2005) and pawpaw (papaya) (Echerenwa and Umechuruba 2004). Its common occurrence on fresh apples can lead to contamination of apple juice and high acid fruit based products. As a psychrophile, *C. herbarum* causes "black spot" spoilage of meat in cool stores (Gill et al. 1981), of cheese during ripening (Gueguen 1988) and in storage under vacuum (Hocking and Faedo 1992). It has been isolated from fresh and frozen meat and processed meat products (see Pitt and Hocking 1997). *C. herbarum* has also been isolated from eggs, peanuts, hazelnuts, walnuts, cereals, chickpeas, soybeans and frozen fruit pastries (see Pitt and Hocking 1997; Kuehn and Gunderson 1963; Arya 2004). Growth on dough carrying machinery (Gradel and Müller 1985), and on wine corks (Daly et al. 1984) has been reported.

References Schubert et al. (2007) and Bensch et al. (2012).

Cladosporium sphaerospermum Penz. Fig. 5.16

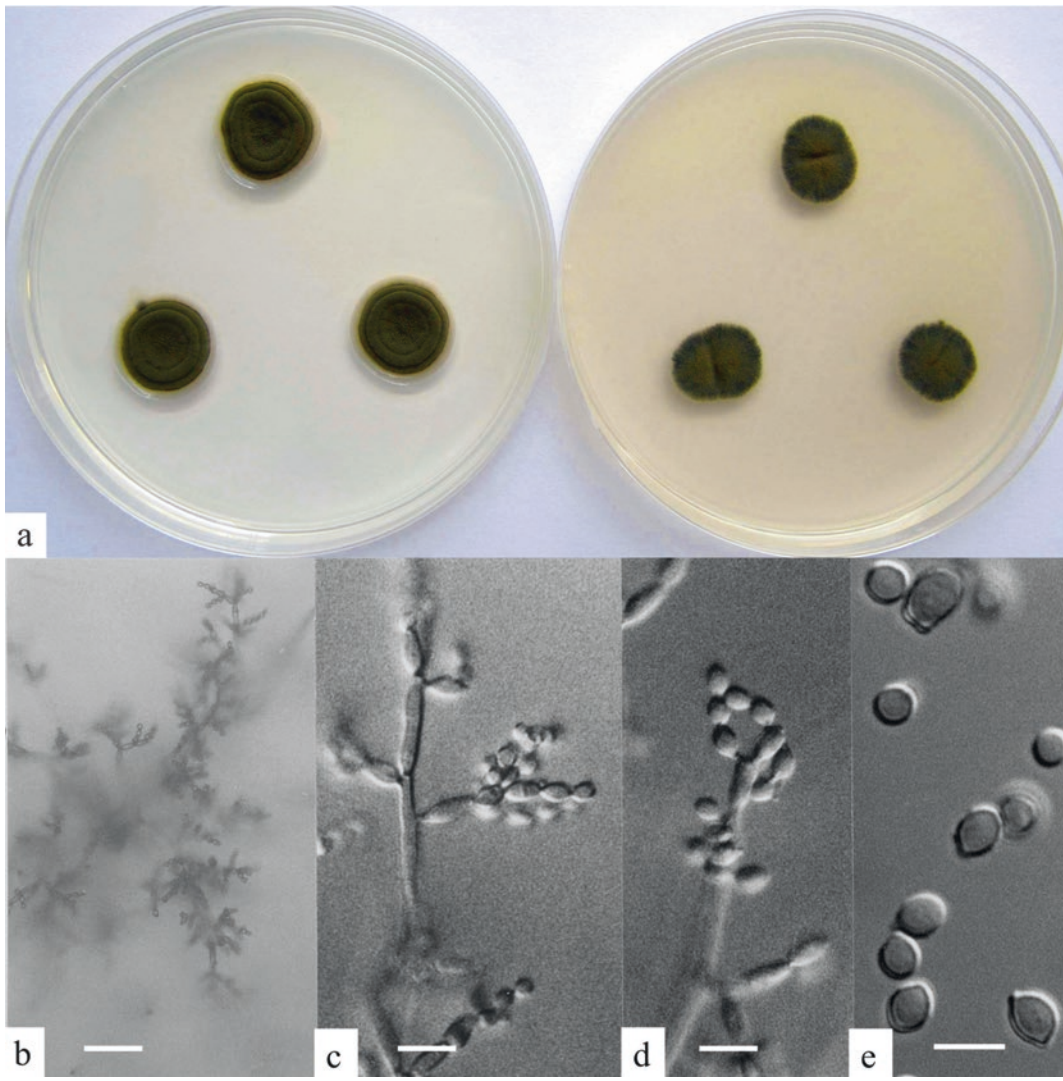

Fig. 5.16 *Cladosporium sphaerospermum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) fruiting structures *in situ*, bar = 50 µm; (**c**, **d**) conidiophores and conidia (slide culture), bar = 10 µm; (**e**) conidia, bar = 5 µm

Colonies on CYA 18–25 mm diam, low and dense, usually plane, surface velutinous; conidia abundant, coloured olive to dark olive; reverse bluish grey. Colonies on MEA 12–20 mm diam, of similar appearance to those on CYA, sometimes lighter in colour. Colonies on G25N 8–12 mm diam, plane or centrally raised, velutinous, coloured as on CYA; reverse almost black. At 5 °C, sometimes germination. No growth at 37 °C.

Conidiophores under the stereomicroscope dendritic (tree-like), closely packed, with stipes bearing branching structures of acropetally produced cells, all functioning as conidia at maturity, separating in liquid mounts; conidia heavy walled, pale olive brown, larger ones 10–30 × 2–4 µm, only occasionally septate, smooth walled, smaller ones nonseptate, subspheroidal, ogival or apiculate, 4–6(−10) × 3–4 µm, with walls smooth to definitely roughened.

Distinctive features Similar to *Cladosporium cladosporioides* in most respects, *C. sphaerospermum* is distinguished by slower growth on CYA and MEA and by production of a high proportion (40% plus) of more or less spherical conidia.

Taxonomy Molecular analyses identified several species as closely related to *C. sphaerospermum* (Zalar et al. 2007; Bensch et al. 2012), but none is of common occurrence.

Identifiers MycoBank MB119529; neotype CBS 193.54**;** ex-type strains CBS 193.54 = ATCC 11289 = IMI 049637; ITS barcode DQ780343; alternative markers *act* EF101380, *tef1* EU570261 (Marin-Felix et al. 2017).

Physiology *Cladosporium sphaerospermum* has an optimum a_w for growth near 0.97 at 25 °C. This species is a xerophile, able to germinate and grow slowly at 0.815 a_w (Hocking et al. 1994) or 0.82 a_w at 25 °C (Segers et al. 2015). The optimum temperature for growth is 25 °C, with a maximum at 30 °C (Bensch et al. 2012).

Mycotoxins No mycotoxins are known to be produced.

Ecology Although less common than *Cladosporium cladosporioides, C. sphaerospermum* has been isolated from a wide range of foods, including barley and peanuts in Egypt, U.S. pecan nuts, European meat products, spoiled cheese in Europe and Australia, stored apple fruit in Turkey (see Pitt and Hocking 1997) and carbonated soft drinks in Nigeria (Odunfa 1987) and Argentina (Ancasi et al. 2006). We have isolated this species at low frequency from peanuts from Thailand (Pitt et al. 1993), rice and soybeans from the Philippines, and maize and mung beans from Indonesia (Pitt et al. 1998a).

References Zalar et al. (2007) and Bensch et al. (2012).

5.12 Genus *Colletotrichum* Corda

Colletotrichum is an asexual genus with many species, widespread as plant pathogens. In this genus, conidia are borne inside an **acervulus**, a flat structure with a more or less closed lid which eventually ruptures. Acervuli are readily seen on the agar surface with the unaided eye or by low power microscope. Conidia of *Colletotrichum* are single celled and hyaline or brightly coloured. They may be cylindrical or pointed, straight or curved.

A number of *Colletotrichum* species cause spoilage of fresh fruits and vegetables. Examples include *C. acutatum* J.H. Simmonds on strawberries, *C. circans* (Berk.) Voglino on leeks and onions, *C. coccodes* (Wallr.) S. Hughes on potatoes, tomatoes and eggplants, *C. higginsianum* Sacc. on cabbage and other cucurbits, *C. lindemuthianum* (Sacc. & Magnus) Briosi & Cavara on beans and *C. musae* (Berk. & M.A. Curtis) Arx on bananas (Snowdon 1990, 1991). A number of *Colletotrichum* species have sexual states described in the genus *Glomerella* but the asexual generic name *Colletotrichum* has been preferred (Weir et al. 2012).

One species, *C. gloeosporioides*, a common cause of fruit spoilage, is treated here as an example of this important genus. Sutton (1980, 1992) considered that *C. gloeosporioides* was an agglomerate of several species with a similar appearance. Weir et al. (2012) studied this species complex in detail and concluded that *C. gleosporioides* included 22 species. Marin-Felix et al. (2017) accepted approximately 200 species

and provided details of types and molecular markers. The species description given below is taken from a limited number of isolates and undoubtedly does not cover the full range of variation.

Colletotrichum gloeosporioides (Penz.) Sacc. Fig. 5.17

Glomerella cingulata (Stonemason) Spauld. & H. Schrenk. (teleomorph)

On CYA and MEA, colonies 60 mm diam or more, often covering the whole Petri dish, with a dense basal layer of hyphae and conidial fruiting bodies (acervuli) overlaid by areas of floccose white, orange or grey mycelium; acervuli up to 500 µm long, pale, grey or orange; reverse with pale grey or orange areas. On G25N, colonies 2–5 mm diam, pale or black. At 5 °C, no growth to germination. No growth at 37 °C.

Reproductive structures flat, lidded acervuli, opening irregularly, containing a single closely packed layer of phialides, of irregular dimensions; conidia borne singly, cylindroidal, with rounded ends, nonseptate, 12–18 × 3–3.5 µm, hyaline and smooth walled.

Distinctive features In the present context, *Colletotrichum gloeosporioides* is distinguished by producing conidia in acervuli and by

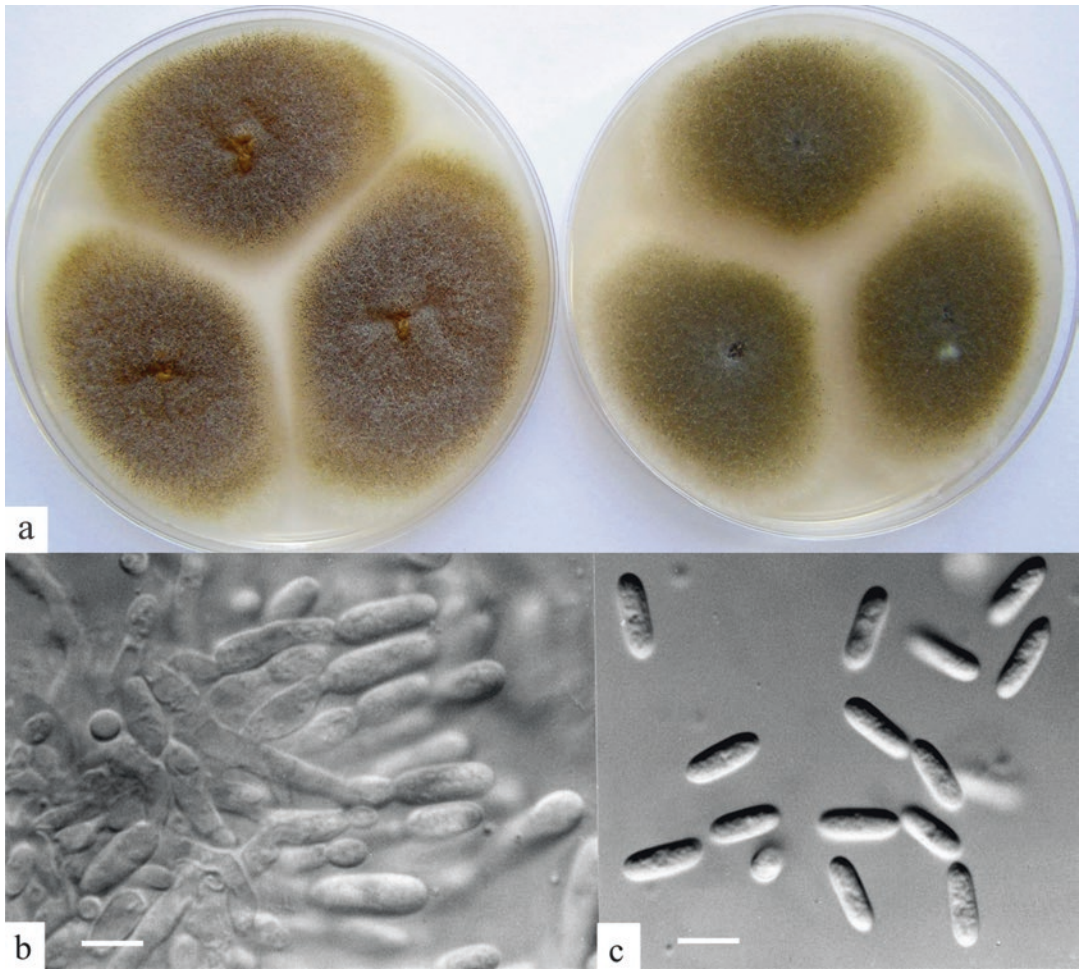

Fig. 5.17 *Colletotrichum gloeosporioides* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) immature conidiophores, bar = 10 µm; (**c**) conidia, bar = 5 µm

pathogenicity on tropical fruits; conidia are aseptate cylinders with rounded ends, 12–18 µm long.

Taxonomy This species as circumscribed here is broadly defined and should more correctly be described as "*Colletotrichum gloeosporioides* species complex" (Sutton 1992; Weir et al. 2012). For a more detailed taxonomy see Cannon et al. (2008) or Weir et al. (2012). *Glomerella cingulata*, the sexual state of *Colletotrichum gloeosporioide* is not found in foods.

Identifiers MycoBank MB158410; epitype IMI 356878; ex-epitype strains CBS 953.97 = CBS 112999 = IMI 356878 (Cannon et al. 2008); ITS barcode JQ005152, alternative markers *gapdh* TQ005239, *act* JQ005500 (Marin-Felix et al. 2017).

Physiology *Colletotrichum gloeosporioides* was insensitive to storage in 10–13% CO_2 in the presence of 3–7% O_2 (Wade et al. 1993).

Mycotoxins Mycotoxins are not known to be produced.

Ecology According to Sutton (1980), the International Mycological Institute Herbarium has records of *Colletotrichum gloeosporioides* species complex from 470 different host genera – a remarkable host range, even taking into account that numerous phylogenetic species may be included.

Colletotrichum gloeosporioides and closely related species cause anthracnoses of tropical fruits, including avocados, bananas, pawpaws (papayas) and mangoes (Kamle et al. 2013), and of temperate fruits, including *Citrus* (Weir et al. 2012) apples, stone fruits and grapes. *Colletotrichum* species cause damage to bananas (*C. musae*), strawberries (*C. acutatum*; Gunnell and Gubler 1992), grapefruit, tomatoes and other fruits (Snowdon 1990; Pitt and Hocking 1997).

Anthracnoses on fruit are dark, relatively dry, shrunken skin blemishes which expand rapidly as the fruit ripens. Except in advanced stages, the blemishes are only skin deep, and the fruit is edible, if unsightly. Advanced lesions may develop pinkish masses of conidia. Control in bananas is generally possible with benzimidazole or similar fungicides; in mangoes, pawpaws and avocados, hot water dips, with or without fungicide, can be beneficial (Smoot and Segall 1963; Hall and Scott 1977). Gamma radiation can be used to control *C. gloeosporioides* in paw-paws (papayas) (Cia et al. 2007).

Colletotrichum species were isolated from 40% of paddy rice samples examined from Thailand (Pitt et al. 1994). *C. dematium* (Pers.) Grove was found at low levels in several Southeast Asian commodities, including soybeans, cowpeas, peanuts and black pepper (Pitt et al. 1998a).

References Von Arx (1957), Sutton (1980, 1992), Weir et al. (2012), and Kamle et al. (2013).

5.13 Genus *Curvularia* Boedijn

In *Curvularia*, conidia are long and ellipsoidal, with three to four transverse septa, borne on short knobby conidiophores. As the name implies, conidia are often curved due to an asymmetrically swollen central cell. Most species are plant pathogens. Some species have sexual states in the genera *Cochliobolus* and *Pseudocochliobolus*: *Curvularia* is the preferred name (Rossman et al. 2015). The taxonomy of *Curvularia* was revised by Manamgoda et al. (2015), while types and molecular markers for more than 80 species were established by Marin-Felix et al. (2017). The species most common in foods is *C. lunata*; *C. pallescens* is described here as a second foodborne species.

Key to *Curvularia* species included here

1	Mycelium on CYA and MEA grey to dark grey; reverse often blue black; conidia with central cells dark walled	*C. lunata*
	Mycelium on CYA and MEA pale to mid grey; reverse brown; all cells in conidia with uniform wall colour	*C. pallescens*

Curvularia lunata (Wakker) Boedijn

Fig. 5.18

Cochliobolus lunatus R.R. Nelson & F.A. Haasis (teleomorph)

On CYA and MEA, colonies at least 60 mm diam, often covering the whole Petri dish, usually deep, moderately dense and floccose, mycelium off-white to grey, often approaching black, in age sometimes developing orange or salmon coloured areas; reverse usually grey to bluish black, sometimes with areas of salmon. On G25N, colonies 5–15 mm diam, low and dense, grey to black with reverse similar. At 5 °C, usually germination. At 37 °C, colonies (5–)20–40 mm diam, of similar appearance to those at 25 °C.

Colonies on DCMA 55–65 mm diam or covering the whole Petri dish, plane, sparse, velutinous, reverse brown or dark grey.

Conidia, best seen in mounts from growth close to the agar surface on MEA or on DCMA, borne from pores along the sides of short knobby conidiophores, elongate, smooth walled, with 3 septa, almost always curved at an asymmetric cell third from the base, 16–25(−30) × 8–14 µm, end cells pale brown, central cells darker.

Distinctive features *Curvularia lunata* is the species of *Curvularia* commonly isolated from foods. It is distinguished from *C. pallescens* by darker colonies, a blue black reverse, and nonuniform pigmentation in conidial walls. Conidial production usually occurs on MEA, but culturing on DCMA and/or under lights may assist recognition if MEA plates are sterile.

Taxonomy When grown in pairs, some isolates of this species mate to produce an ascomycetous state, *Cochliobolus lunatus* R.R. Nelson & F.A. Haasis. This state is not encountered in agar culture of single isolates or in foods.

Identifiers MycoBank MB429823; neotype CBS 730.96; ex-type culture CBS 730.96; ITS barcode JX256479; alternative markers *gapdh* JX276441, *tef1* JX266596 (Marin-Felix et al. 2017).

Physiology *Curvularia lunata* was able to germinate at 0.86 a_w, but only grew down to 0.89 a_w at 25 °C (Hocking et al. 1994).

Mycotoxins No reliable reports of mycotoxin production by this species are known to us.

Ecology As *Curvularia lunata* is primarily an invader of monocotyledon plants (Domsch et al. 1980), the most common food sources are cereals: records include rice, barley, wheat, maize and sorghum (Pitt and Hocking 1997; Mtisi and McLaren 2003; Echemendia 2005; Fakhrunnisa et al. 2006; Hussaini et al. 2009).

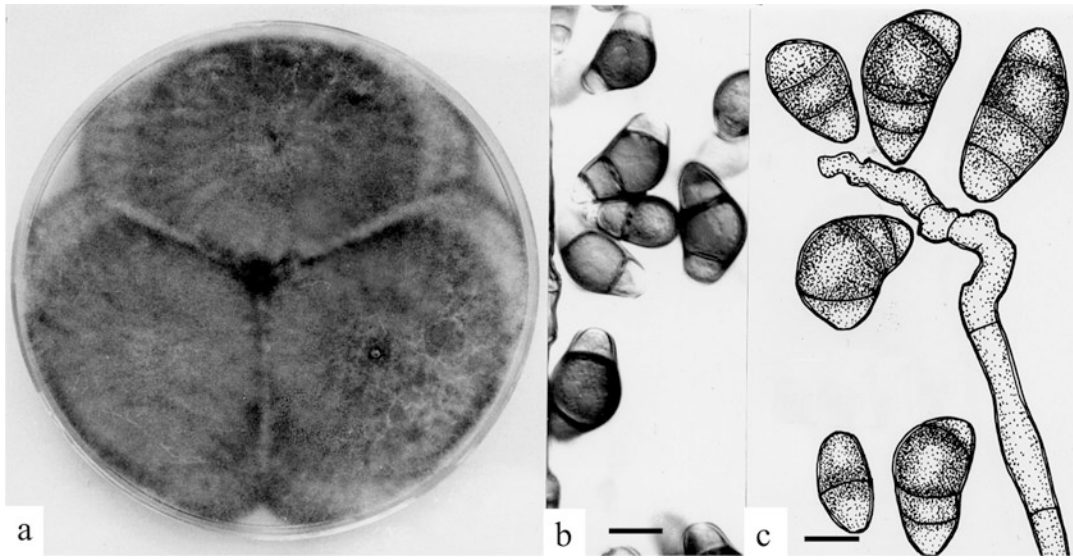

Fig. 5.18 *Curvularia lunata* (**a**) colony on CYA, 7 d, 25 °C; (**b**, **c**) conidiophore and conidia, bar = 10 µm

It has been reported as one cause of spoilage of fresh tomatoes in Nigeria (Muhammad et al. 2004), and has also been found on litchi fruit (Wells et al. 1981), soybeans (Ahammed et al. 2006), hazelnuts and walnuts (Abdel-Hafez and Saber 1993), peanuts and spices (see Pitt and Hocking 1997).

In Thailand, *Curvularia lunata* is a major invader of sorghum: we isolated it from 56% of samples and 8% of all grains examined. It was also prevalent in paddy rice samples, where 28% of samples and 3% of all grains examined were infected (Pitt et al. 1994). It was found in 9–10% of paddy rice samples from Indonesia and the Philippines (Pitt et al. 1998a, unpublished data).

Like many fungi able to grow at 37 °C, *Curvularia lunata* has been reported as an opportunistic pathogen for many years, in a wide variety of infections. For reviews see Manamgoda et al. (2015) and Bengyella et al. (2017).

Additional variety *Curvularia lunata* var. *aeria* (Bat.) M.B. Ellis is a floccose taxon accepted as a variety of *C. lunata.* Colonies of *C. lunata* var. *aeria* on CYA 50–65 mm diam, plane, floccose, of pale to mid grey mycelium, reverse deep blue black. Colonies on MEA covering the whole Petri dish, plane, deeply floccose, especially so at colony to colony and colony to Petri dish junctions, mycelium white to orange grey, reverse yellow brown, dark brown or blue black. Colonies on G25N 4–6 mm diam, of dark grey mycelium. No growth at 5 °C. At 37 °C, colonies 50–65 mm diam, similar to those on CYA at 25 °C, sometimes less floccose, reverse dark blue black. Colonies on DCMA 55–65 mm diam, plane, sparse, floccose, especially at colony junctions, reverse brown or dark grey. Conidia are similar to those of *C. lunata*.

The variety is distinguished from *Curvularia lunata* var. *lunata* by a more floccose growth habit and faster growth at 37 °C. It occurs in similar foods and commodities to *C. lunata*, the highest level we have encountered being 2% of all Thai rice grains and 1% of all Thai sorghum grains examined (Pitt et al. 1994). Identifiers: MycoBank MB353427; ex-type culture CBS 294.61 = IMI 83444.

Curvularia pallescens Boedijn Fig. 5.19

Colonies on CYA 50–65 mm diam, on MEA covering the whole Petri dish, plane, of low to floccose mycelium, pale grey to mid grey, reverse brown to dark brown. On G25N, colonies 3–6 mm diam, brown or grey. At 5 °C, usually

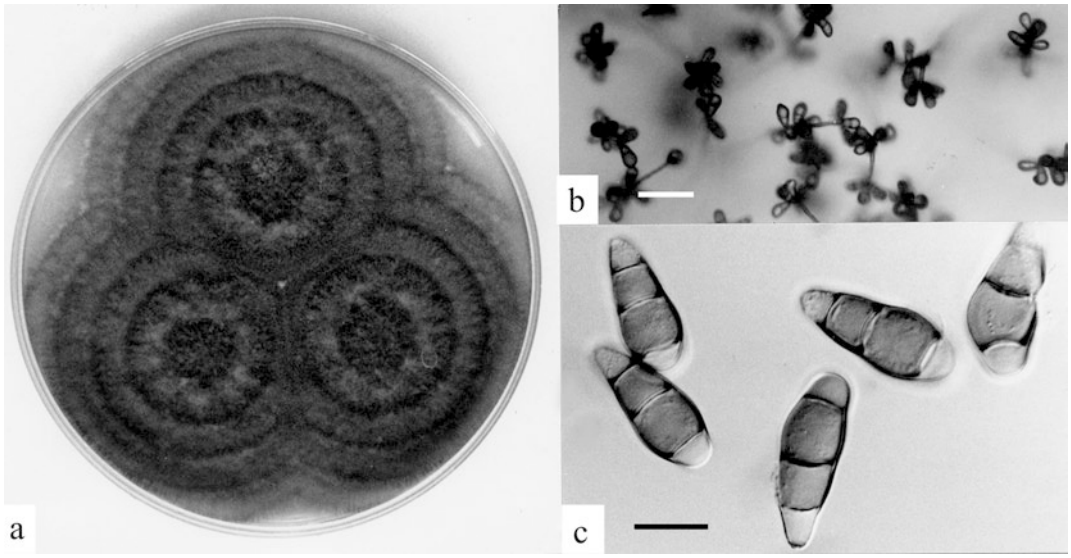

Fig. 5.19 *Curvularia pallescens* (**a**) colony on MEA, 7 d, 25 °C; (**b**) fruiting structures *in situ*, bar = 50 µm; (**c**) conidia, bar = 10 µm

germination. At 37 °C, colonies 40–50 mm diam, of floccose grey mycelium, sometimes brown soluble pigment, reverse dark brown.

On DCMA, colonies 50–65 mm diam, plane, with pale brownish grey to mid brown mycelium, reverse reddish brown to dark brown.

Conidia borne from pores in geniculate hyphae, ellipsoidal, with 3 septa, sometimes inconspicuous, almost straight along one side but with the eccentric swelling of the penultimate cell characteristic of the genus, 18–25 × 9–12 µm, with walls smooth, pale to mid brown, and with all cells in each conidium of a similar colour.

Distinctive features This species lacks the abrupt curvature in the conidia observed in other foodborne species of *Curvularia*; conidia are relatively light in colour and characterised by an even colour in all cells. Colony reverses on CYA and MEA are brown, not blue black.

Identifiers MycoBank MB273299; holotype CBS 156.35; ex-type culture CBS 156.35; ITS barcode KJ922380, alternative markers *gapdh* KM083606, *tef1* KM196570 (Marin-Felix et al. 2017).

Physiology Like *Curvularia lunata, C. pallescens* was able to germinate at 0.86 a_w, but only grew down to 0.89 a_w at 25 °C (Hocking et al. 1994).

Mycotoxins This species is not known to produce mycotoxins.

Ecology The major food source of *Curvularia pallescens* is sorghum (Pitt et al. 1994; Ishrat et al. 2005). However, it has also been recorded on rice (Pitt et al. 1994; Gutierrez et al. 2002) and causing rots in melons (Sharma et al. 2002).

References Ellis (1971), Domsch et al. (1980), Sivanesan (1987), Freire et al. (1998), Manamgoda et al. (2015), and Bengyella et al. (2017).

5.14 Genus *Endomyces* Reess

Endomyces is a genus of yeast-like ascomycetes, indeed often classified with the yeasts, in *Saccharomycopsis* (Kurtzman and Fell 1998; Barnett et al. 2000; Kurtzman et al. 2011). However, as growth is filamentous and spreading on agar plates, it is more readily recognised here. Yamada et al. (1996) considered that differences in ITS DNA were sufficient to separate *Endomyces* from *Saccharomycopsis*. The genome was sequenced by Choo et al. (2016) who indicated that this species is deeply ancestral to the true yeasts. The conidial state consists of yeast-like cells borne on spicules or small projections. The ascomycete state occurs sometimes in culture: the production of unenclosed, evanescent asci is suggestive of a relationship with the true yeasts. The only species common in foods is *E. fibuliger*. See also *Hyphopichia*, which appears to be a closely related genus.

Endomyces fibuliger Lindner Fig. 5.20

Endomycopsis fibuliger (Lindner) Dekker
Saccharomycopsis fibuligera (Lindner) Klöcker

Colonies on CYA 18–30 mm diam and on MEA 15–25 mm diam, of low and sparse to moderately dense white to pale grey mycelium, sometimes centrally umbonate; reverse pale, off-white or very pale yellow. Colonies on G25N 8–15 mm diam, similar to those on CYA. No germination at 5 °C. At 37 °C, colonies usually 5–20 mm diam, similar to at 25 °C; reverse off-white to dull yellow brown.

Conidia borne from spicules (small projections) along the length of undifferentiated hyphae, yeast-like, spherical, ellipsoidal or pyriform, 3–6 µm long, with thin, smooth walls. Asci sometimes produced in culture on CYA or MEA, evanescent, ascospores observed singly or more commonly in tight clusters of 2–4, ellipsoidal, with a longitudinal flange, offset from the spore axis to give a "bowler hat" appearance, 7–8 µm long, including the flange.

Distinctive features This species produces white, powdery, filamentous colonies which bear conidia from spicules on vegetative hyphae, and sometimes also by budding (yeast-like cells).

Taxonomy This species has been described under both *Endomycopsis* and *Saccharomycopsis*; in the latter case the epithet is spelled "*fibuligera*".

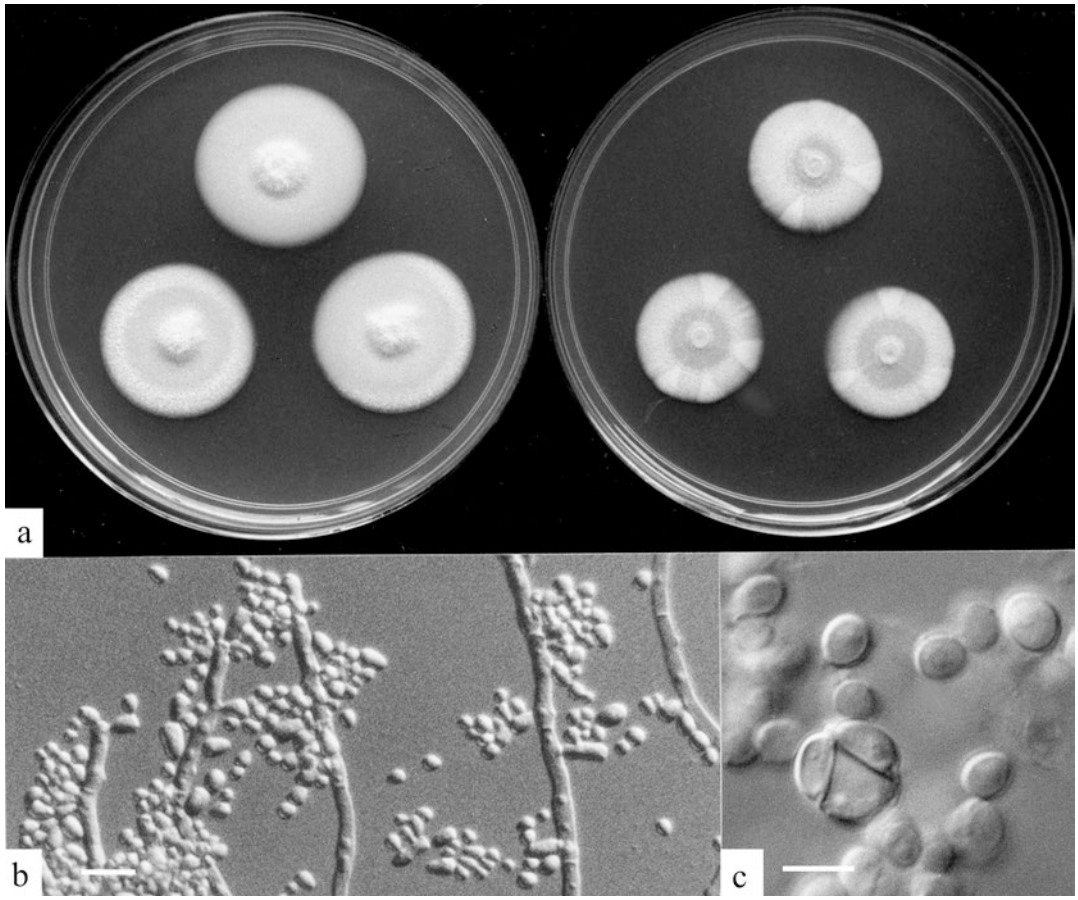

Fig. 5.20 *Endomyces fibuliger* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) conidiophores and conidia (slide culture), bar = 10 µm; (**c**) adherent ascospores, bar = 5 µm

As explained above, we consider *Endomyces fibuliger* to be the valid name. Recent taxonomies list this species under *Saccharomycopsis fibuligera* (Lindner) Klöcker (Kurtzman and Fell 1998; Barnett et al. 2000; Kurtzman et al. 2011) without taxonomic justification.

This species and *Hyphopichia burtonii* have a close resemblance in culture. Both grow at more or less the same rates and have similar habitats. The fragmenting of fertile hyphae in *H. burtonii* is the only feature which provides a characteristic difference in morphology.

Identifiers MycoBank MB214707; type CBS 329.83, ex-type strains CBS 329.83, NRRL Y-2388 (as *S. fibuligera*).

Physiology The physiology of *Endomyces fibuliger* has not been studied in any detail. A minimum growth temperature around 5 °C has been reported (Spicher 1986). *E. fibuliger* on wheat and rye bread was less affected by reduced O_2 levels than other spoilage moulds and its growth could not be prevented by modified atmosphere packaging (Suhr and Nielsen 2005).

Mycotoxins This type of fungus is unlikely to produce toxic compounds.

Ecology This species is not uncommon in cereals and cereal products, especially packaged bread (Spicher 1984, 1985). In Europe, along with *Hyphopichia burtonii*, it is known as "chalky mould" of bread (Spicher 1986). It produces powerful amylases (Gogoi et al. 1987; Manilal et al. 1991). *Endomyces fibuliger* (*Saccharomycopsis fibuligera*) was found in fermented potato pulp used in an Indonesian starter

Ragi tape (Abe et al. 2004) and in 'Marcha', a traditional amylolytic starter used to produce sweet-sour alcoholic drinks in the Himalayan regions of India, Nepal, Bhutan and Tibet (Tsuyoshi et al. 2005).

References Von Arx (1981b), Barnett et al. (1990, 2000), Kurtzman et al. (2011), and Choo et al. (2016), as *Saccharomycopsis fibuligera*.

5.15 Genus *Epicoccum* Link

Epicoccum is a hyphomycete genus, a saprophyte or secondary invader of senescent plant tissue. It is characterised by the production of masses of large, stalked, spherical, irregularly septate conidia, borne on rapidly growing multicoloured colonies. *Epicoccum* is very widely distributed in air, in soil and on decaying vegetation, one particular source being dying grass (Kilpatrick and Chilvers 1981). Its ubiquity in the environment means it is commonly found on foods but it is an uncommon cause of spoilage.

Epicoccum was long considered to include only a single species, *E. nigrum*. Indeed, Kilpatrick and Chilvers (1981) examined the variability of 2000 isolates of *Epicoccum* and concluded that all belonged to a single, genetically variable species. A study of the 5.8S and ITS regions of DNA confirmed this (Wang and Guo 2004). However, more recent, wider ranging studies have indicated that this species should be split and the genus now includes several species (Aveskamp et al. 2010; Fávaro et al. 2011; Jayasiri et al. 2017). *E. nigrum* is by far the most commonly occurring. One other species, long known as *Phoma sorghina*, has been moved to *Epicoccum* (Aveskamp et al. 2010). Although, in our hands, this species shows no similarity to the genus description or type species, it has been included here. It has been keyed separately in the general key, however.

Key to *Epicoccum* species included here

1	Colonies on CYA and MEA multicoloured, conidia large and spherical, borne aerially	*E. nigrum*
	Colonies on CYA and MEA dark, fruiting structures subsurface	*E. sorghinum*

Epicoccum nigrum Link **Fig. 5.21**

Epicoccum purpurascens Ehrenb. ex Schltdl.

Colonies on CYA 60 mm diam or more, often covering the whole Petri dish, low and dense or funiculose to floccose; mycelium orange brown, brown, or sometimes reddish or greenish, in fresh isolates enveloping or surmounted by brown black clusters of conidia, sometimes dominating colony appearance; clear to red brown exudate sometimes produced; reverse usually orange brown to black or with pink, red or green areas. On MEA colonies generally similar to on CYA, sometimes with a different colour combination, or occasionally with surface slimy. On G25N colonies 3–10 mm diam, low to deep, yellow to dark brown; reverse similar, sometimes with yellow soluble pigment. At 5 °C, response variable, no growth to colonies up to 8 mm diam formed. No growth at 37 °C.

Conidia borne solitarily on short conidiophores, usually in dense clusters, spherical with a broad, tapering, truncate base; brown, irregularly septate when mature, commonly 15–25 (−30) µm diam, with rough walls obscuring numerous septa.

According to Schol-Schwarz (1959), conidia on stems of sterile lupins measure 7–65 × 6–54 µm. As has been observed with other genera, conidia on agar media are much less variable in size and often smaller than on natural substrates.

Distinctive features See the genus preamble.

Taxonomy In early literature this species is known as *E. nigrum* Link. A change in the 1905s in the nomenclatural rules for naming fungi described in early days resulted in substitution of *E. nigrum* with *E. purpurascens*, and this name is still found in the literature. A more recent change in nomenclatural rules has seen *E. nigrum* as the correct name once more.

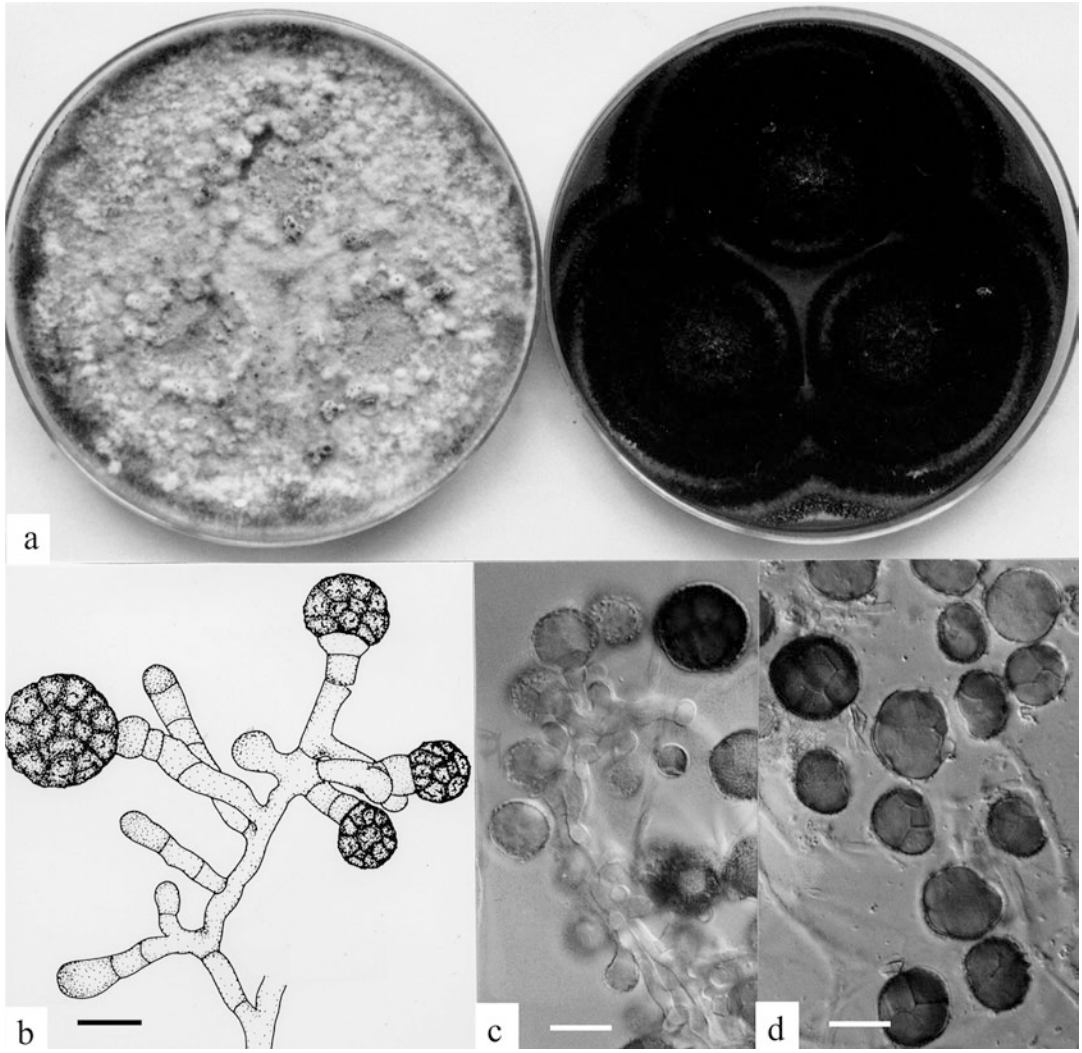

Fig. 5.21 *Epicoccum nigrum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) conidiophores and conidia, bar = 10 µm; (**c**, **d**) conidia, bar = 10 µm

Identifiers MycoBank MB226758; type not known; representative strain CBS 173.73 = ATCC 24428 = IMI 164070 (Aveskamp et al. 2010).

Physiology According to Kilpatrick and Chilvers (1981), maximum growth rates vary widely among isolates of this species. However, optimal temperatures for growth are usually 20–25 °C, with a maximum at 30–35 °C and a minimum below 5 °C. The optimum water potential for growth is −20 bars (0.98 a_w), and minimum −120 bars (0.91 a_w) (Kilpatrick and Chilvers 1981).

Mycotoxins Mycotoxin production has not been recorded.

Ecology As noted in the genus preamble, *Epicoccum* is common in the general environment and hence readily finds its way onto foods such as cereals and nuts. It has been reported to cause spoilage of cantaloupes, in which it produces a red discolouration (Snowdon 1991), and as a pathogen on cucumbers, tomatoes, apples and pears (Bruton et al. 1993). Among other fungi, *E. nigrum* can produce core rot of apples (Combrink et al. 1985). It has been associated

with spoilage of pecans and is sometimes present in barley after harvest and during malting, but is not a cause of gushing (see Pitt and Hocking 1997; Niessen et al. 1991; Hudec 2007). *E. nigrum* has been reported on fresh vegetables, nuts and cereals. Other records include rice, maize and wheat; gourds and muskmelons, pecans, peanuts, frozen and cured meats and biltong (see Pitt and Hocking 1997; Gros et al. 2003).

Epicoccum nigrum was isolated from 29% of soybean samples and 7% of cashew samples in Thailand, with up to 12% infection in infected soybean samples, 15% in cashews and 1% infection overall in both (Pitt et al. 1994).

Epicoccum sorghinum (Sacc.) Aveskamp et al. Fig. 5.22

Phoma sorghina (Sacc.) Boerema et al.

Colonies on CYA and MEA variable, usually 50–55 mm diam, of dense to floccose grey green or olive mycelium, with characteristic white to salmon pink tinges; reverse salmon or reddish. Colonies on G25N 8–10 mm diam, of sparse brown mycelium. No growth at 5 °C or 37 °C.

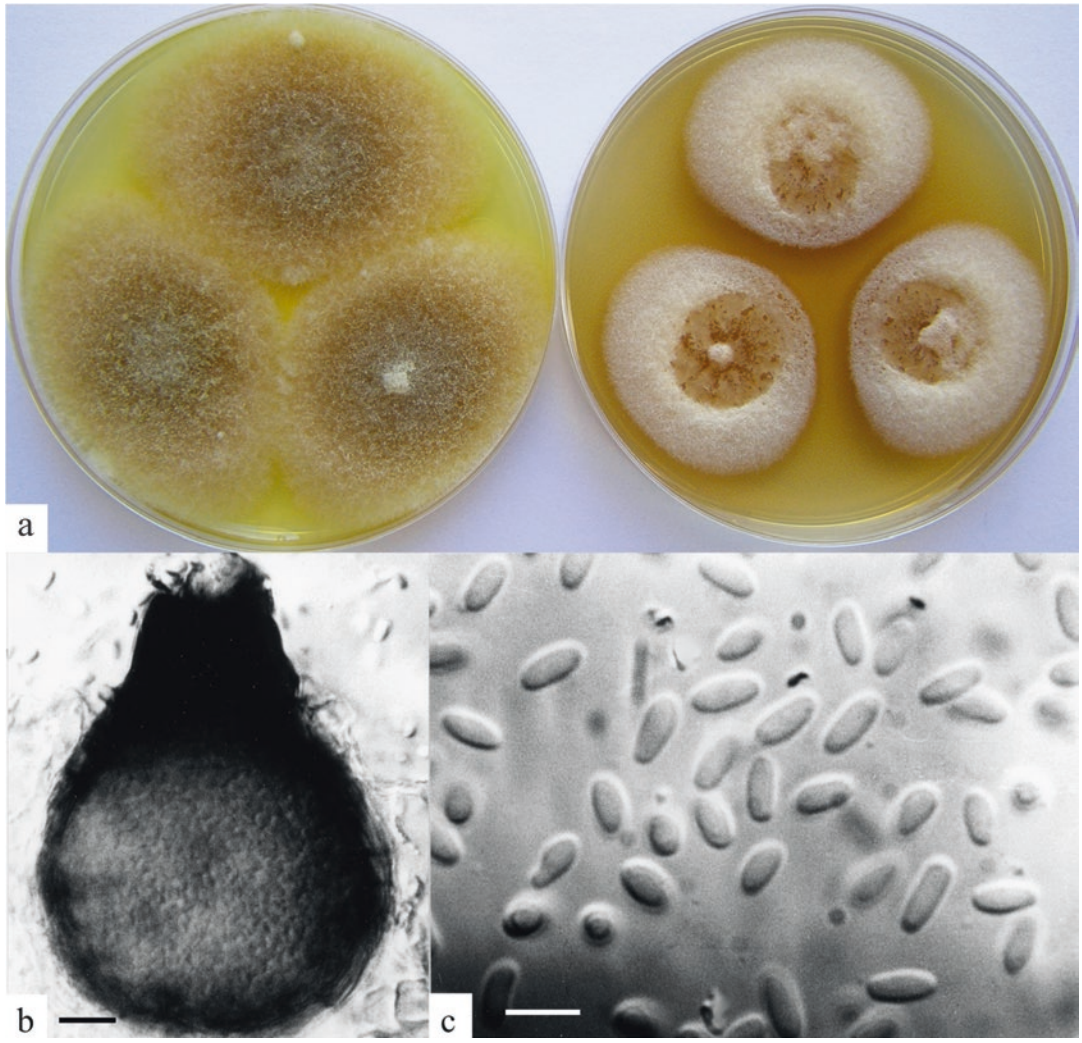

Fig. 5.22 *Epicoccum sorghinum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) pycnidium, bar = 10 µm; (**c**) conidia, bar = 5 µm

Pycnidia produced abundantly on MEA, just beneath the agar surface, 300–400 µm diam, with one or more inconspicuous ostioles exuding conidia in a slimy matrix; conidia cylindroidal, 4–5 × 2–2.5 µm, hyaline, with thin, smooth walls. Chlamydoconidia *fide* Oliveira et al. (2019) brown, variable in shape and size, 5–10 µm long, irregularly septate.

Distinctive features This species resembles a *Phoma* species: *Phoma* species produce black subsurface pycnidia which exude small cylindrical conidia in slime. However, molecular data indicate that it is correctly placed here.

Taxonomy As described above, this species has the characteristics of a *Phoma* species. However it has been shown (Aveskamp et al. 2010) that *Phoma* as traditionally known is polyphyletic and that *Epicoccum* is the appropriate genus for this species. Aveskamp et al. (2010) used the name "*Epicoccum sorghi*" but this has since been treated as an orthographic variant, with *E. sorghinum* accepted as the correct name.

Identifiers MycoBank MB544157; type not known; representative strains CBS 627.68, 179.80 (Jayasiri et al. 2017).

Physiology The optimum temperature for growth of *Epicoccum sorghinum* was reported to be 28 °C (Schémaeza et al. 2013). Sporulation by *E. sorghinum* may be stimulated by growth on DCPA or PDA.

Mycotoxins *Epicoccum sorghinum* (as *Phoma sorghina*) has been reported to produce tenuazonic acid (Shephard et al. 1991; Oliveira et al. 2017) and is the major source of this toxin in sorghum (Oliveira et al. 2019). The toxicity of tenuazonic acid was investigated by EFSA (2011) who concluded that although data was limited, this toxin was probably not a cause for concern for human health.

Ecology The major source of *Epicoccum sorghinum* is sorghum grain (González et al. 1997; Hussaini et al. 2009; Makun et al. 2009; Oliveira et al. 2017). It occurs wherever sorghum is grown. It has been recorded as a pathogen on a variety of other plant crops, but has rarely if ever been recorded from other foods.

References Kilpatrick and Chilvers (1981), Aveskamp et al. (2010), Fávaro et al. (2011), Jayasiri et al. (2017), and Oliveira et al. (2018, 2019).

5.16 Genus *Fusarium* Link

The character which defines the genus *Fusarium* is the production of septate, fusiform to crescent shaped conidia, termed **macroconidia**, with a foot shaped basal cell and a more or less beaked apical cell. Macroconidia may be produced in discrete pustules, called **sporodochia**, or in confluent, slimy masses, known as **pionnotes**. Mounts from these areas, which are usually cream, salmon pink or orange, will reveal masses of these spores. However, they are rarely widely distributed over the colony and frequently are entirely absent. A light bank is invaluable for promoting formation of macroconidia in *Fusarium* cultures.

Many species of *Fusarium* also produce smaller 1–2 celled conidia, **microconidia**, of various shapes. **Chlamydoconidia**, either terminal or intercalary, are characteristic of some species also. *Fusarium* colonies are usually fast growing and consist of felty aerial mycelium which is usually brightly coloured in shades of pink, red, violet or brown.

Fusarium species are renowned for their role as plant pathogens, causing a wide range of diseases such as vascular wilts, root and stem rots, pre- and post-emergence blight and many others. *Fusarium* species are widely distributed in soils, particularly cultivated soils, and are active in the decomposition of cellulosic plant materials. They are a major cause of storage rots of fruits and vegetables and are commonly associated with cereals and pulses, which they usually invade before harvest. De Nijs et al. (1996) have published a compendium of the occurrence of more than 60 *Fusarium* species in raw materials, foods and feeds but, in our experience, a relatively small number of species are important in food spoilage.

Taxonomy The taxonomy of *Fusarium* has been unsettled and sometimes controversial for a long time, due to many factors including invasion of so many plant hosts, few distinctive morphological characters, instability in culture and, perhaps most important, formation of teleomorphs in several genera. The milestone work of Wollenweber and Reinking (1935) distinguished 65 species which was followed by the gross oversimplification of the genus by Snyder and

Hansen, who accepted only nine species in a series of papers in the 1940s. This wholly unworkable system was revised and enlarged by Booth (1971) and Nelson et al. (1983). As the result of a number of international meetings, Leslie and Summerell 2006 published a practical laboratory manual which includes 70 species.

The advent of sophisticated methods of molecular analysis led to realisation that morphological techniques failed to distinguish adequately the very high degree of variability within particular species and so the concept of "species complexes" emerged (O'Donnell et al. 1998b). Establishing "phylogenetic species" within each complex, sometimes only distinguishing them by numbers, led to a much greater discrimination of important properties between related species. Species complexes are strongly supported phylogenetically and bring together taxa that share many phenotypic characters including toxin production (Summerell 2019). In time all species became incorporated into complexes (O'Donnell et al. 2013), now numbering 23 (Summerell 2019). These roughly correspond to clades in other genera. However, it now seems apparent that horizontal transfer of genes in *Fusarium* species is not uncommon (Ma et al. 2010; Laurence et al. 2015), due no doubt to proximity of mycelia in plant tissue. That greatly complicates attempts at speciation within a complex.

Since the last edition of this book the number of accepted *Fusarium* species has greatly increased. During 2000 to 2009, 46 new species were described: from 2010 to 2019, a further 69 (Summerell 2019). However, the species in the text which follows are still recognised and are still the major species within the complexes and in the real world. The laboratory manual of Leslie and Summerell (2006) is still in common use and it has been followed here, with minor changes due to improved taxonomy.

A number of *Fusarium* species produce teleomorphs described in several genera – *Gibberella* Sacc., *Nectria* Fr., *Calonectria* De Not., *Neocosmospora* E.F. Sm. and others. The move to "one fungus – one name" inevitably led to discussion of whether such teleomorph names or the single asexual name *Fusarium* should prevail. Gräfenhan et al. (2011) used a phylogenetic analysis to remove a number of *Fusarium* species from the genus and provided a new, much narrower circumscription of *Fusarium*. Rossman et al. (2013) proposed the conservation of some generic names, including keeping *Fusarium* instead of *Gibberella*, the teleomorph associated with some species. In broadening the circumscription of *Fusarium* again and advocating against the use of teleomorph names, Geiser et al. (2013) (in a paper which included most *Fusarium* scientists), laid the groundwork for stable *Fusarium* taxonomy into the future. In an excellent review paper, the current state of *Fusarium* taxonomy has been aptly summed up by Summerell (2019) as follows: "Since the mid-1980s, definitions of *Fusarium* species have seen the use of three predominant species concepts—morphological, biological, and phylogenetic—to differentiate *Fusarium* species. …most current workers now use a combination of morphological, biological, and phylogenetic markers in a polyphasic approach based on a relative weighting of the available data and markers." For the reader who wishes to explore the intricacies and difficulties of *Fusarium* taxonomy in more detail, the paper by Summerell (2019) is a recommended starting point.

Mycotoxins *Fusarium* is one of the three major fungal genera producing mycotoxins. The most widespread *Fusarium* mycotoxins are the trichothecenes, a family of sesquiterpenes. More than 50 such compounds are known to be produced by species in this genus: trichothecene formation has been shown recently to be confined to species in the *F. sambucinum* and *F. acuminatum-equiseti* complexes (O'Donnell et al. 2018). Some are highly toxic: none appears to be benign. Trichothecenes are often produced as mixtures even under pure culture conditions and are very difficult to separate, so the toxicity of many compounds remains uncertain. The most important is deoxynivalenol (and sometimes nivalenol), produced by *F. graminearum, F. culmorum* and related species. T-2 toxin, produced primarily by *F. sporotrichioides,* caused the deaths of many people and animals during the twentieth century, but is of uncommon occurrence now.

The second important family of toxins produced by *Fusarium* species are the fumonisins, produced by *F. verticillioides, F. proliferatum* and related species. Fumonisins are sphingosine analogues, interfering with membrane function in man and animals (Miller and Trenholm 1994). Zearalenone, not a true mycotoxin, really an oestrogen analogue produced by fungi, is formed by the same species as make deoxynivalenol. Further details will be found in Chap. 13, "Mycotoxins". Other less toxic or less important compounds, such as moniliformin and fusaric acid, are discussed briefly under the appropriate species.

Cultural instability Many *Fusarium* species are notorious for their instability in culture. Isolates of some species will degenerate quickly, often after only one or two transfers. For this reason, it is important to identify Fusaria as soon as possible after primary isolation. Pure cultures for identification are traditionally started from a single germinated spore, as the mass transfer of Fusaria appears to increase the rate of deterioration of strains in culture.

Identification procedures Identification of *Fusarium* isolates is often difficult, but the task can be made easier by observing a few basic rules:

- Identify cultures as soon as possible after primary isolation;
- Always grow cultures for identification from single germinated conidia;
- Use standardised media and incubation conditions;
- Use a light bank (Chap. 4) whenever possible.

Diagnostic features. The main characters used to distinguish species of *Fusarium* are (1) the size and shape of the macroconidia; (2) the presence or absence of microconidia; (3) the manner in which microconidia are produced; (4) the type of phialide on which microconidia are produced; (5) the presence or absence of chlamydoconidia; and (6) the colours and morphology of colonies on PDA.

The morphology of macroconidia is a principal diagnostic feature for *Fusarium* species. Macroconidia generally have at least three septa, with a differentiated apical cell which may be pointed, rounded, hooked or filamentous, and a basal cell which may be foot-shaped, with a distinct heel, or just slightly notched. *Fusarium* macroconidia generally exhibit some degree of curvature, the convex and concave sides being referred to as the dorsal and ventral sides, respectively. Although some macroconidia are usually produced in the aerial mycelium, the shape and size of those in sporodochia are more regular and are used for identification purposes where possible.

Microconidia are usually produced in the aerial mycelium and their shape can be very important in *Fusarium* identification. Most species which produce microconidia form only a single type, the most common shape being ellipsoidal to clavate. However, *F. poae* produces spherical to apiculate microconidia and *F. sporotrichioides* produces a variety of shapes: ellipsoidal, pyriform and spherical. The method of production of microconidia and the types of phialides on which they are borne are also useful diagnostic criteria. *Fusarium verticillioides* produces its microconidia in long, delicate, dry chains, which are best observed by using the 10x objective of the compound microscope. Some species produce microconidia in **false heads** (small, mucoid, adherent balls of conidia), and others produce them singly. In some species, microconidia are produced on phialides with only one pore, and these are termed **monophialides,** but a few species produce phialides with more than one pore (**polyphialides**). Species which produce polyphialides usually produce monophialides as well.

Descriptions Descriptions of the microscopic features of species in this book are based on structures formed in cultures grown on DCPA at 25 °C, with a 12 h. photoperiod, under a light bank consisting of two cool white fluorescent tubes and one black light tube (see Chap. 4). Cultures should be examined at 7 days, then at 10 days and 14 days if sporulation is poor. Aerial mycelium often develops better after 10 days incubation on DCPA and chlamydoconidium production is more reliable in older cultures.

Descriptions of the colony characteristics are taken from cultures grown on PDA at 25 °C for 7 days, also with a 12 h photoperiod. Additional information may be gained by recording the growth rates of colonies on PDA at 25 °C and 30 °C after 3 days (Burgess et al. 1994). Growth rates at 30 °C on PDA are particularly helpful in distinguishing isolates of *Fusarium avenaceum* from those of *F. acuminatum*.

Key to *Fusarium* species included here

1	Microconidia abundant	2
	Microconidia rare or absent	9
2 (1)	Colonies on PDA with mycelium and/or reverse coloured greyish rose or burgundy	3
	Colonies on PDA in shades of cream, pale salmon or violet	5
3 (2)	Microconidia spherical to apiculate, borne singly on monophialides	*F. poae*
	Microconidia ellipsoidal, clavate, fusiform and/or pyriform, borne on polyphialides, or both polyphialides and monophialides	4
4 (3)	Microconidia clavate only, produced profusely, giving colonies on PDA a powdery appearance	*F. chlamydosporum*
	Microconidia various shapes: clavate, pyriform and spindle shaped (check PDA cultures also)	*F. sporotrichioides*
5 (2)	Microconidia produced in long or short chains (some false heads may also be present)	6
	Microconidia produced singly or in false heads	7
6 (5)	Microconidia produced from monophialides only	*F. verticillioides*
	Monophialides and polyphialides both present	*F. proliferatum*
7 (5)	Colonies cream or bluish, sporodochia cream	*Neocosmospora solani*
	Colonies pale salmon or violet, sporodochia salmon	8
8 (7)	Microconidia borne on short, stout monophialides; chlamydoconidia usually produced	*F. oxysporum*
	Microconidia borne on polyphialides and slender monophialides; chlamydoconidia not produced	*F. subglutinans*
9(1)	Colonies cream, pale salmon or brown	10
	Colonies greyish-rose to burgundy	11
10(9)	Macroconidia cigar- or spindle-shaped, produced in the aerial mycelium	*F. incarnatum*
	Macroconidia obviously curved, produced in sporodochia	*F. equiseti*
11(9)	Macroconidia robust, ventral side straight; aerial mycelium tan to brown	12
	Macroconidia delicate and slender, slightly or definitely curved; aerial mycelium white or pinkish	13
12(11)	Macroconidia short and stout, up to 7 µm wide	*F. culmorum* (See *F. graminearum*)
	Macroconidia longer and narrower, maximum width 5.5 µm	*F. graminearum*
13(11)	Macroconidia with elongated basal cells and long, with whip-like apical cells	*F. longipes*
	Macroconidia with basal and apical cells not obviously elongated	14
14(13)	Macroconidia delicate and needle-like, with sides almost parallel	*F. avenaceum*
	Macroconidia with slight to definite curvature	*F. acuminatum*

Fusarium acuminatum Ellis & Everh. Fig. 5.23

Gibberella acuminata Wollenw. (teleomorph)

Colonies on CYA 40–50 mm diam, of dense, felty mycelium, white to greyish rose or greyish magenta; reverse uniformly pale or with areas of greyish rose. Colonies on MEA 45–65 mm diam, yellow brown centrally, greyish rose at the margins; reverse deep brownish yellow to brownish orange, occasionally pale. Colonies on G25N 9–15 mm diam. At 5 °C, colonies 7–12 mm diam. No growth at 37 °C.

On PDA, colonies usually covering the whole Petri dish, of dense to floccose white to pale salmon mycelium, sometimes greyish rose at the margins; reverse dark ruby centrally, greyish ruby at the margins. On DCPA, colonies sparse, of floccose to funiculose white to pale salmon mycelium; reverse pale or with brownish red annular rings.

Macroconidia relatively slender, usually with 5 septa, but 3 and 4 septa not uncommon, with a long, tapering apical cell and foot shaped basal cell, distinctly but not highly curved, with the widest point often one third of the distance from the base, giving a "bottom heavy" appearance; microconidia produced sparsely by some isolates; chlamydoconidia produced, relatively slowly.

Distinctive features Ruby to dark ruby reverse colours on PDA, and relatively slender, slightly curved macroconidia, usually with 5 septa, are the distinctive features of *Fusarium acuminatum*. However, unless chlamydoconidia are present, this species can be confused with

Fig. 5.23 *Fusarium acuminatum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**, **c**) macroconidia, bar = 10 µm

F. avenaceum. *F. armeniacum* (see below) is also very closely related and is distinguished by differences in morphology, molecular markers and mycotoxin profiles (Burgess and Summerell 2000).

Taxonomy Perithecia of the teleomorph of *Fusarium acuminatum* can be produced in the laboratory by inoculating opposite mating types onto sterile wheat straws (Booth 1971). Variability of *F. acuminatum* in culture, which correlated with secondary metabolite production (Logrieco et al. 1992), was shown to be due to the inclusion of *Fusarium armeniacum* in that species (Burgess and Summerell 2000).

Identifiers MycoBank MB219366; type not known.

Physiology Some isolates of *Fusarium acuminatum* have antioxidant enzyme activity (Kayali and Tarhan 2005).

Mycotoxins Most of the mycotoxin production reported from *Fusarium acuminatum* precedes the splitting off of *F. armeniacum*, an important mycotoxin producer (Desjardins 2006; Leslie and Summerell 2006). However more than half of the *F. acuminatum* strains examined produced deoxynivalenol at low levels (Marín et al. 2012). *F. acuminatum* also produces moniliformin (Chelkowski et al. 1990; Logrieco et al. 1992) and enniatins (Logrieco et al. 1992; Kononeko et al. 1993; Desjardins 2006; Leslie and Summerell 2006) as well as some other minor toxins (Desjardins 2006).

Ecology *Fusarium acuminatum* has been isolated from a wide variety of plants throughout the world. Although some isolates may cause severe root rot in particular legume species (Leslie and Summerell 2006), *F. acuminatum* is generally regarded as a saprophyte. It has been reported to cause rot in pumpkins (Elmer 1996), is one cause of rot in stored potatoes (Theron and Holz 1990), of stored kiwifruit (Wang et al. 2015) and is weakly pathogenic in bananas (Jiménez et al. 1993). It is quite common in poor quality wheat from cool temperate zones (Mills and Wallace 1979; Abramson et al. 1987). It has been isolated from developing peanut pods (Barnes 1971), barley (Abdel-Kader et al. 1979) and, in our laboratory, from rain-damaged sorghum and soybeans. The incidence of *F. acuminatum* in tropical commodities was low (Pitt et al. 1993, 1994).

Additional species *Fusarium armeniacum* (G.A. Forbes et al.) L.W. Burgess and Summerell is distinguished from *F. acuminatum* by rapid production of chlamydoconidia, bright orange sporodochia, less curvature in macroconidia and more distinct footcells (Leslie and Summerell (2006). It. produces T-2, HT-2 toxins and deoxynivalenol (Desjardins 2006; Burgess and Summerell 2000). It was found in Korean rice at low levels (1% of *Fusarium* isolates; Hong et al. 2015), levels too low to indicate significant mycotoxin production.

Identifiers: MycoBank MB467535; holotype DAR 67507.

References Nelson et al. (1983), Desjardins et al. (2006), Burgess and Summerell (2000), and Leslie and Summerell (2006).

Fusarium avenaceum (Fr.) Sacc. Fig. 5.24

Gibberella avenacea R.J. Cooke (teleomorph)

Colonies on CYA covering the whole Petri dish, moderately deep to deep, of open, floccose mycelium coloured white, very pale rose or deeper greyish rose; reverse varying from pale to pale yellow, or with areas of greyish rose or sometimes uniformly deep burgundy. Colonies on MEA 45–55 mm diam, low to moderately deep, of open floccose to funiculose mycelium, coloured white, pale rose or greyish rose, sometimes brown centrally; reverse brownish orange, sometimes paler centrally or at the margins. Colonies on G25N 9–15 mm diam. At 5 °C, colonies 10–12 mm diam. No growth at 37 °C.

On PDA, colonies moderately deep to deep, of dense mycelium coloured white, pale salmon, or sometimes dark brownish red, with central masses of reddish orange sporodochia, sometimes surrounded by an outer ring of paler sporodochia; reverse greyish red, with darker annular rings, paler towards the margins. On DCPA, colonies deep, of moderately dense white to pale salmon mycelium with a central mass of orange to salmon sporodochia, often surrounded by concentric rings of sporodochia; reverse pale.

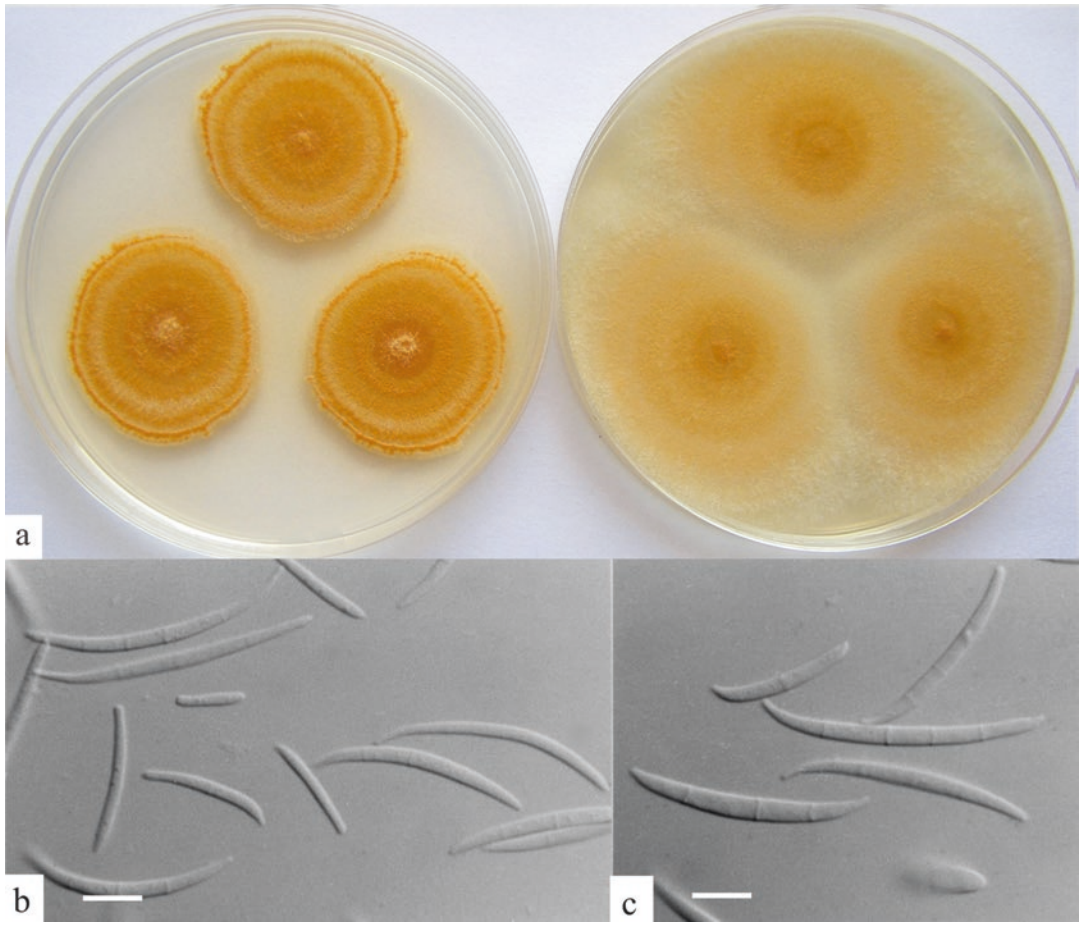

Fig. 5.24 *Fusarium avenaceum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**, **c**) macroconidia, bar = 10 µm

Macroconidia long, slender, with 4–7 septa, thin walled, straight or slightly curved, with a tapering apical cell and a notched or foot-shaped basal cell; microconidia produced sparsely by some isolates; chlamydoconidia absent.

Distinctive features *Fusarium avenaceum* is distinguished by thin walled, needle-like macroconidia and by the absence of chlamydoconidia. Despite the fact that *F. avenaceum* and *F. acuminatum* are not considered by *Fusarium* taxonomists to be closely related, these two species can be difficult to distinguish, as isolates with macroconidia of intermediate form are not uncommon. Colony diameters on PDA at 30 °C after 3 days can be a useful differentiating feature: under these conditions colonies of *F. avenaceum* are usually 8–15 mm diam, whereas those of *F. acuminatum* are 15–28 mm diam (Burgess et al. 1994). Isolates of *F. avenaceum* show an unusually broad range of colours on PDA, and also have a very broad host range. However, extensive genetic analysis has shown no bases for splitting the species, and pathogenicity tests on single strains have confirmed the broad host range (Nalim 2004).

Taxonomy The teleomorph of *Fusarium avenaceum* is *Gibberella avenacea* R. J. Cook. It is not usually seen in culture on the media used here.

Identifiers MycoBank MB161610; type not known.

Physiology The optimum growth temperature for *Fusarium avenaceum* is 35 °C, the minimum near −3 °C and the maximum 31 °C

(Domsch et al. 1980). The minimum a_w for growth is approximately 0.90 at 25 °C (Magan and Lacey 1984c), and the pH optimum ranges between 5.4 and 6.7 (Domsch et al. 1980).

Mycotoxins This species has been reported to produce a variety of trichothecene and other mycotoxins. However Nelson et al. (1983) regarded only reports of moniliformin production as accurate. Later reports have confirmed this (Chelkowski et al. 1990; Abbas et al. 1991; Bosch and Mirocha 1992), though levels found in northern European cereals were low (Uhlig et al. 2007). Reports of production of fusarin C (Farber and Sanders 1986; Thrane 1988; Leonov et al. 1993) and enniatins (Blais et al. 1992; Kononeko et al. 1993; Uhlig et al. 2007) also appear to be reliable (Desjardins 2006; Leslie and Summerell 2006). Production of any trichothecene toxin has not been confirmed, and *F. avenaceum* does not carry the *tri*5 gene which is essential for trichothecene production (Tan and Niessen 2003).

Ecology *Fusarium avenaceum* has a world wide distribution wherever crops are grown, but is relatively uncommon in food commodities, except in northern Europe, where it dominates (Uhlig et al. 2007). It is a major component of Fusarium head blight in cereals in Europe, the US Pacific Northwest region, and Canada (Desjardins 2006). Logrieco et al. (2002) identified *F. avenaceum* as a component of Fusarium ear rot of maize in Europe and it was one of the most prevalent *Fusarium* species in Norwegian oats and spring wheat (Hofgaard et al. 2016). It has been reported from barley (Flannigan 1969; Petters et al. 1988; Stenwig and Liven 1988) where it may inhibit germination of malting grains (Hudec 2007), but is of minor importance in gushing of beer (Niessen et al. 1992). Other reported sources are sorghum (Onyike and Nelson 1992), peanuts (Joffe 1969), pigeon peas (Maximay et al. 1992) and, in our laboratory, triticale. *Fusarium avenaceum* has been reported to cause spoilage of cool stored broccoli (Mercier et al. 1991), dry rot of stored carrots in Italy (Marziano et al. 1992) and dry rot of rutabaga (swede turnip) in Canada (Peters et al. 2007). It has occasionally caused spoilage of apples, pears, asparagus, tomatoes, eggplant and potatoes (Snowdon 1990, 1991) and has been reported as a postharvest pathogen of stonefruit in New Zealand (Hartill and Broadhurst 1989).

References Domsch et al. (1980), as *Gibberella avenacea*, Nelson et al. (1983), and Leslie and Summerell (2006).

Fusarium chlamydosporum Wollenw. & Reinking **Fig. 5.25**

Fusarium fusarioides (Gonz. Frag. & Cif.) Booth

Colonies on CYA covering the whole Petri dish, of low to moderately deep floccose mycelium, coloured white to pale rosy pink, often with surface appearing powdery due to production of microconidia; reverse pale to greyish rose or brownish red. Colonies on MEA 55–70 mm diam, of low, moderately dense mycelium in shades of yellow brown, or greyish rose to greyish ruby, paler at the margins; reverse deep yellow brown to orange brown. Colonies on G25N 15–20 mm diam. At 5 °C, colonies 1–2 mm diam. At 37 °C, colonies 5–15 mm diam.

On PDA, colonies covering the whole Petri dish, of felty mycelium, coloured pale salmon, sometimes browner, or with patches of greyish red, often with a powdery appearance from profuse microconidial production; reverse deep violet brown to dark ruby, paler at the margins. On DCPA, colonies of sparse, floccose, pale salmon mycelium, often powdery with microconidia, showing poorly defined annulations; macroconidia occasionally produced near the colony centres in salmon sporodochia; reverse pale.

Macroconidia often rare, relatively short and stout, usually with 3–5 septa, slightly curved; microconidia produced abundantly from polyphialides in the aerial mycelium, with 0–2 septa, fusiform to slightly clavate. Chlamydoconidia usually abundant in older cultures, produced singly, in pairs or in clumps.

Distinctive features The presence of abundant fusiform microconidia borne on polyphialides is the most outstanding feature of *Fusarium chlamydosporum*. Also colonies on PDA have dark violet brown to dark ruby reverse colours. The newly described *F. atrovinosum* L. Lombard & Crous is very closely related,

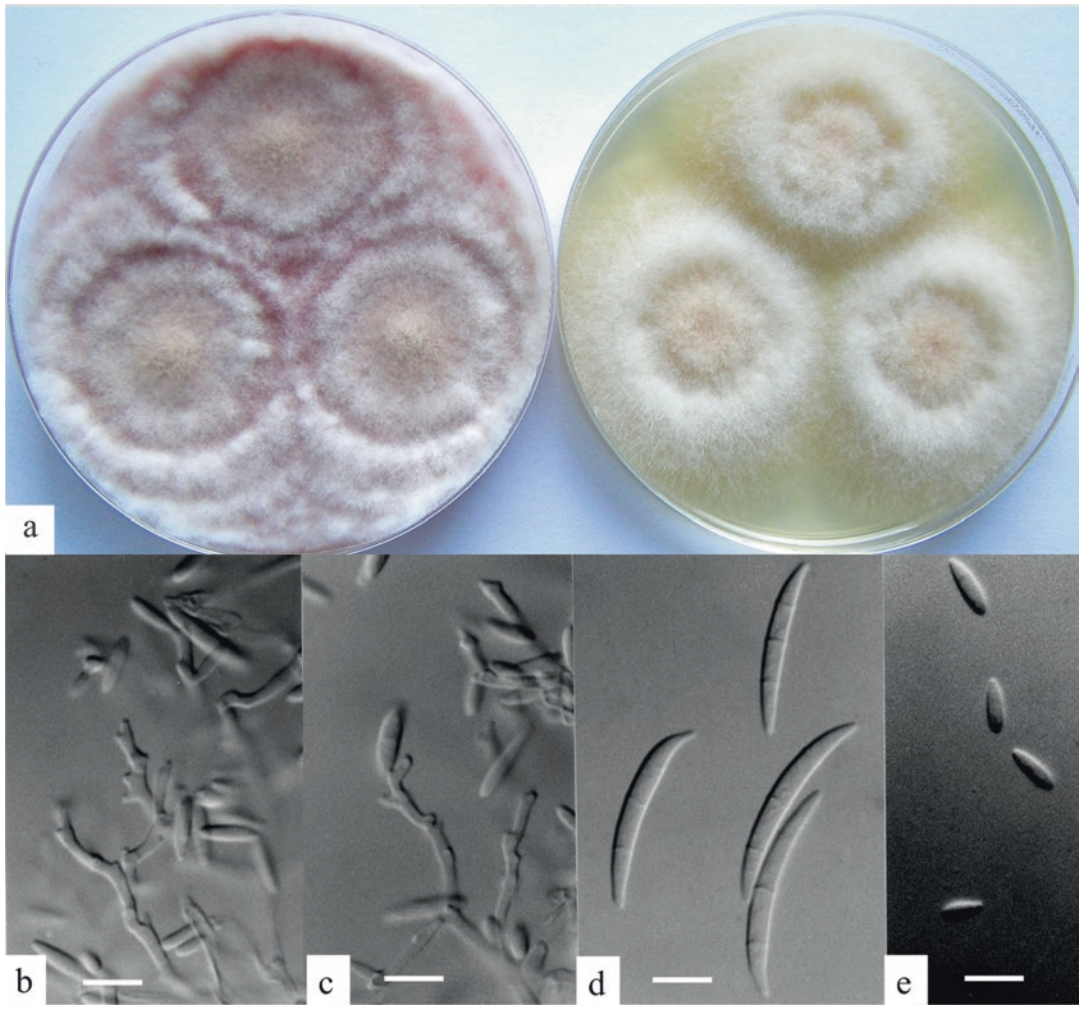

Fig. 5.25 *Fusarium chlamydosporum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**, **c**) polyphialides, bar = 10 µm; (**d**) macroconidia, bar = 10 µm; (**e**) microconidia, bar = 10 µm

differing by production of microconidia with none or one septum only (Lombard et al. 2019).

Taxonomy *F. chlamydosporum* has priority over *F. fusarioides* as the correct name for this species (Domsch et al. 1980; Nelson et al. 1983; Leslie and Summerell 2006). The taxonomy of this and surrounding species has been comprehensively reviewed by Lombard et al. (2019).

Identifiers MycoBank MB260522; neotype CBS 243.25 (Lombard et al. 2019); ex-neotype strains CBS 243.25, NRRL 26912.

Physiology This species has minimum, optimum and maximum temperatures for growth of 5, 27 and 37 °C (Seemüller 1968).

Mycotoxins Production of type A trichothecenes (including T-2 toxin, HT-2 toxin, monoacetoxyscirpenol, neosolaniol and iso-neosolaniol) by *Fusarium chlamydosporum* was reported by Park and Chu (1993), however subsequent studies have found no evidence of trichothecene production in this species (Desjardins 2006). Moniliformin is the major mycotoxin produced by *F. chlamydosporum* (Marasas et al. 1984; Desjardins 2006).

Ecology *Fusarium chlamydosporum* is mainly an inhabitant of soils in warmer climates (Domsch et al. 1980; Leslie and Summerell 2006), and is not regarded as a plant pathogen or

spoilage fungus. However, it is commonly isolated from grains in drier areas, particularly in the Middle East, southern Europe, central Asia and Australia (Leslie and Summerell 2006), and has also been isolated from pearl millet (Wilson et al. 1993; Jurjevic et al. 2007), pecans (Huang and Hanlin 1975), and sorghum (Rabie et al. 1975; Onyike and Nelson 1992). A low incidence of *F. chlamydosporum* was found in peanuts from both Indonesia and the Philippines (Pitt et al. 1998a) and from mung beans and sorghum in Thailand (Pitt et al. 1994). Involvement in dry rot of potatoes has also been reported (Somani 2004; Esfahani 2006).

References Nelson et al. (1983), Leslie and Summerell (2006), and Lombard et al. (2019).

Fusarium culmorum (Wm.G. Smith) Sacc. Fig. 5.26

Colonies on CYA covering the whole Petri dish, of dense felty mycelium, often with a floccose overlay, sometimes reaching the Petri dish rim, pale red to pastel red; reverse pastel red to deep red. Colonies on MEA 60 mm or more diam, floccose, in age often reaching the Petri dish lid, pale red to pastel red, commonly with a greyish orange to yellowish brown overlay; reverse brown to reddish brown. Colonies on G25N usually 5–10 mm diam, mycelium orange white, reverse yellow to orange. At 5 °C, germination. No growth at 37 °C.

On PDA, colonies covering the whole Petri dish, of dense to floccose mycelium, pale red and pale yellow brown; reverse red to deep red. On DCPA, colonies 50–65 mm diam, of sparse mycelium, orange to pinkish white, bearing abundant macroconidia in orange sporodochia; reverse dull orange brown.

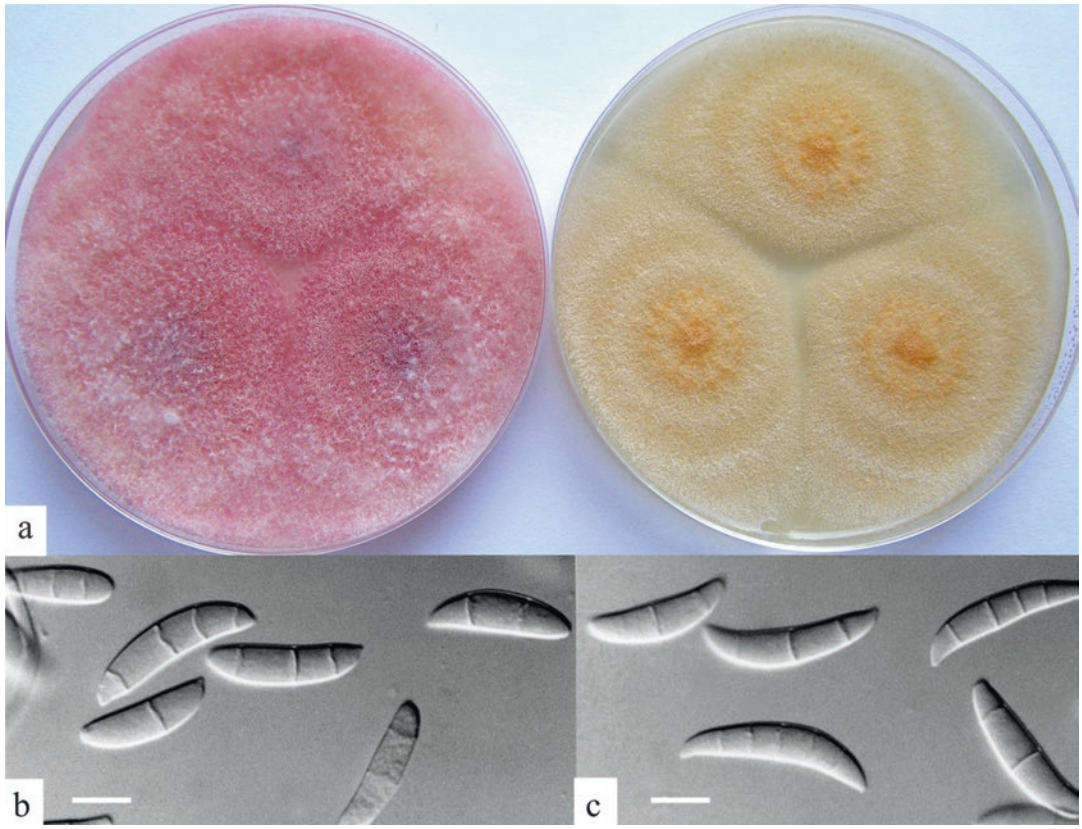

Fig. 5.26 *Fusarium culmorum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**, **c**) macroconidia, bar = 10 µm

Macroconidia relatively short, wide and only slightly curved, with 4–5 septa, 30–45 µm long, with rounded or sometimes papillate apical cells; basal cells with a slight to definite notch, sometimes papillate. Microconidia not produced. Chlamydoconidia sometimes formed, in conidia, or intercalary in the hyphae, singly or in chains, 9–14 µm diam, smooth walled.

Distinctive features Short, stout macroconidia are the prime feature distinguishing *Fusarium culmorum* from most other species. *F. culmorum* may be confused with *Fusarium crookwellense* L.W. Burgess et al., but macroconidia of the latter species have a distinctly foot-shaped basal cell, whereas those of *F. culmorum* are shorter and stouter, and the basal cell is not distinctly foot-shaped.

Taxonomy No teleomorph is known for *F. culmorum*. Mating types have been observed and it is assumed that the species is heterothallic. Overall taxonomy of this species has been reviewed by Scherm et al. (2013).

Identifiers MycoBank MB196997; type not known.

Physiology *Fusarium culmorum* has been reported to be psychrotrophic, growing down to 0 °C, with an optimum at 21 °C and a maximum of 31 °C (Arsvoll 1975); however Magan and Lacey (1984c) reported growth at 35 °C. The minimum a_w for growth is 0.87 at 20–25 °C and pH 6.5: at pH 4.0, growth did not occur below 0.90 a_w (Magan and Lacey 1984a). *F. culmorum* is very tolerant of low O_2 tensions (Magan and Lacey 1984b). Radiation resistance of *F. culmorum* was relatively high: up to 0.8 kGy were needed for a tenfold reduction in spore numbers on grain, and up to 1.39 kGy on media (O'Neill et al. 1991).

Mycotoxins *Fusarium culmorum* produces a variety of mycotoxins. The most important are deoxynivalenol, nivalenol and their derivatives (Abramson et al. 2001; Hestbjerg et al. 2002; Chandler et al. 2003; Jennings et al. 2004) and zearalenone (Bakan et al. 2001; Hestbjerg et al. 2002; Llorens et al. 2004a; Brinkmeyer et al. 2005). Moniliformin production was reported by Scott et al. (1987) but was not detected in 42 isolates of *F. culmorum* from Canada by Abramson et al. (2001). Reports of production of type A trichothecenes (T-2 toxin, HT-2 toxin) have not been substantiated (Leslie and Summerell 2006).

The existence of two chemotypes of *F. culmorum*, those that produce deoxynivalenol and those that produce nivalenol and/or fusarenone-X (Miller et al. 1991) has been confirmed by molecular studies. The occurrence of these two chemotypes in Europe has been summarised by Scherm et al. (2013). In addition, Lauren et al. (1992) examined 45 isolates of *F. culmorum* from New Zealand soil and pasture and found none produced deoxynivalenol or its monoacetyl isomers. Within the trichothecene gene cluster, isolates possessing the *Tri7* and *Tri13* genes produce nivalenol and related compounds, whereas sequences in the *Tri3, Tri5* and *Tri6* genes are associated with deoxynivalenol production (Chandler et al. 2003; Jennings et al. 2004; Quarta et al. 2005; Quarta et al. 2006).

A strain of *F. culmorum* produced deoxynivalenol optimally at 25 °C, but only between 0.995 and 0.97–0.96 a_w (Hope and Magan 2003; Hope et al. 2005). At 15 °C, deoxynivalenol was produced in lower concentrations later in the growth cycle, but over a slightly greater a_w range (0.995 to 0.95–0.94 a_w). The dynamics of nivalenol production for this strain were similar (Hope and Magan 2003). Zearalenone production by *Fusarium culmorum* was reported to be optimal above 25 °C (Bottalico et al. 1982).

Ecology This species has a world wide distribution in soil and as a pathogen of cereals and other hosts, with a higher incidence in temperate climates (Domsch et al. 1980; Nelson et al. 1983; Leslie and Summerell 2006). It is an important component of the cohort of *Fusarium* species that cause head blight of wheat and associated cereal crops in Europe, Canada, China and other areas with cool weather during the growing season (Desjardins 2006). In wheat it causes extensive internal damage to the grain, and reductions in flour yield and baking quality (Meyer et al. 1986). *F. culmorum* was reported as the dominant *Fusarium* species in barley in Europe (see Pitt and Hocking 1997). It also occurs in triticale (Perkowski et al. 1988). *F. culmorum* has been identified as a component of Fusarium ear rot of maize in Europe (Logrieco

et al. 2002). *F. culmorum* was one cause of crown rot in bananas (Wade et al. 1993), and is a minor cause of spoilage of apples and pears (Snowdon 1990).

References Nelson et al. (1983), Desjardins et al. (2006), Leslie and Summerell (2006), and Scherm et al. (2013).

Fusarium equiseti (Corda) Sacc. Fig. 5.27

Gibberella intricans Wollenw. (teleomorph)

Colonies on CYA filling the whole Petri dish, often to the lid, of dense to floccose white mycelium; reverse pale or pale salmon. Colonies on MEA covering the whole Petri dish, of open, floccose white to pale brown mycelium; reverse pale, or sometimes showing areas of pale greyish red. Colonies on G25N 12–20 mm diam. At 5 °C, colonies of 1–4 mm diam produced. At 37 °C, usually no growth, although in isolates from the tropics, colonies up to 35 diam.

On PDA, colonies of dense to floccose mycelium, white to pale salmon, becoming brown with age, with a central mass of orange to brown sporodochia, sometimes surrounded by poorly defined sporodochial rings; reverse pale salmon, often with a brown central area and brown flecks.

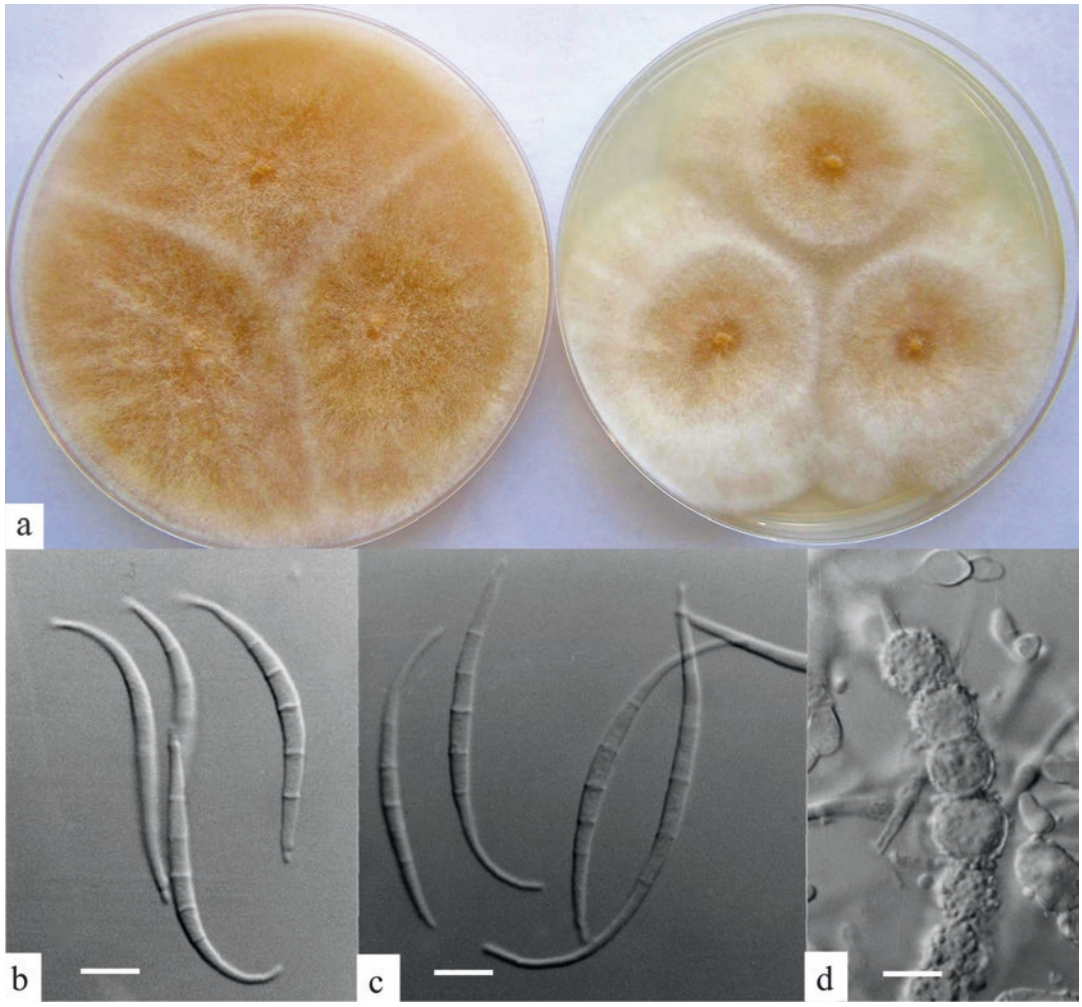

Fig. 5.27 *Fusarium equiseti* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**, **c**) macroconidia, bar = 10 µm; (**d**) chlamydoconidia, bar = 10 µm

On DCPA, colony appearance usually dominated by salmon, orange or brownish sporodochia centrally and in poor to well defined concentric rings; mycelium low and sparse, coloured white or pale salmon; reverse pale.

Macroconidia distinctly curved, often with a "hunchbacked" appearance, with 5–7 septa, ranging from relatively short in some isolates to very long in others, with a wide range of sizes often present in a single isolate, basal cell distinctly foot-shaped, often with an elongated heel, apical cell elongated and curved, in long spores becoming filamentous; chlamydoconidia usually produced abundantly, in chains or clumps; microconidia absent.

Distinctive features *Fusarium equiseti* produces distinctly curved macroconidia which are often elongate, especially in the basal and apical cells. Colonies on PDA are floccose, usually with at least a central mass of orange sporodochia, and with reverse pale salmon, often flecked with brown. Chlamydoconidia are usually abundant; microconidia are not produced.

Taxonomy The teleomorph of *Fusarium equiseti* is *Gibberella intricans* Wollenw. However, there are few records of its occurrence in nature (Booth 1971). *F. equiseti* is very closely related to *F. incarnatum*. Together they form a complex believed to include 33 phylogenetically distinct species, few of which have binomial names (Villani et al. 2019).

Identifiers Mycobank MB 199819; neotype CBS H-5570; cultures ex-neotype CBS 307.94, NRRL 26419 (Xia et al. 2019).

Physiology *Fusarium equiseti* grows strongly at 30 °C (Burgess et al. 1994) but, from observations in our laboratory, most isolates grow poorly or not at all at 37 °C. The minimum a_w for growth has been reported to be 0.92 a_w (Chen 1966). Growth occurred at pH 3.3 but not 2.4, and was still rapid at pH 10.4 (Wheeler et al. 1991). Tripathi et al. (1999) reported growth between pH 2.0 and pH 11.00. Growth in N_2 with <1% O_2 was 90% of that in air (Hocking 1990).

Mycotoxins *Fusarium equiseti* has been reliably reported to produce a number of mycotoxins. Trichothecene production in *F. equiseti* is variable, with both type A trichothecenes (diacetoxyscirpenol and related compounds, T-2 toxin, HT-2 toxin and neosolaniol) and type B trichothecenes (deoxynivalenol, 15-acetyldeoxynivalenol, fusarenone X and nivalenol) reliably reported (see Pitt and Hocking 1997; Langseth et al. 1998; Hestbjerg et al. 2002; Kosiak et al. 2005; Desjardins 2006). Zearalenone, moniliformin, beauvericin, fusarochromanone and related compounds have also been reported (see Pitt and Hocking 1997; Logrieco et al. 1998a; Desjardins 2006), but production of butenolide is questionable (Desjardins 2006).

Ecology A cosmopolitan soil fungus, *Fusarium equiseti* has a distribution extending from Alaska to the tropics (Domsch et al. 1980). It has been isolated from a variety of plants, particularly cereals, where it may cause stem and root rots (Leslie and Summerell 2006). *F. equiseti* has been identified as a minor component of maize ear rot in Europe, and head blight of wheat and associated grains in Europe and North American (Desjardins 2006). It has been reported from various cereal grains including wheat, maize, barley, rye and rice (see Pitt and Hocking 1997; Pitt et al. 1998a) and also from peanuts, walnuts, soybeans, cowpeas, sorghum, oilseeds and herbs (see Pitt and Hocking 1997). It has been isolated from tomatoes (Okoli and Erinle 1989) and capsicums (Adisa 1983; Hashmi and Ghaffar 1991), and has been reported as contributing to soft rot of bell peppers in India (Shukla and Sharma 2000) and crown rot of bananas (Wallbridge 1981; Jiménez et al. 1993). It has also been implicated as a cause of rots of cucurbit fruits in contact with soil (Burgess et al. 1994), dry rots of potato tubers (El-Hassan et al. 2004) and soft rot of pumpkins (Elmer 1996).

Fusarium equiseti has caused spoilage of UHT processed fruit juices, due to its ability to grow in very low O_2 tensions (Hocking 1990).

References Domsch et al. (1980) as *Gibberella intricans*, Nelson et al. (1983), Desjardins et al. (2006), Leslie and Summerell (2006), and Villani et al. (2019).

Fusarium graminearum Schwabe

Fig. 5.28

Gibberella zeae (Schwabe) Petch (teleomorph)

Colonies on CYA filling the whole Petri dish, often to the lid, of dense, floccose mycelium coloured greyish rose, greyish yellow or paler; reverse usually orange red to greyish ruby, though sometimes pale brownish pink. Colonies on MEA filling the whole Petri dish, often reaching the lid at the margins at least, of dense to openly floccose mycelium, in shades of greyish rose and greyish yellow to golden brown; reverse orange brown to yellowish brown, sometimes paler at the margins. Colonies on G25N 20–30 mm diam, occasionally more. At 5 °C, colonies of 5–12 mm diam produced. No growth at 37 °C.

On PDA, colonies filling the whole Petri dish, of dense, floccose mycelium coloured olive brown, yellowish brown, reddish brown or pale salmon, or in combinations of those colours; sometimes with a central mass of red brown to orange sporodochia; reverse ruby to dark ruby centrally, sometimes violet brown. On DCPA, colony appearance dominated by salmon to orange sporodochia in concentric rings, overlaid by sparse, floccose, pale salmon mycelium.

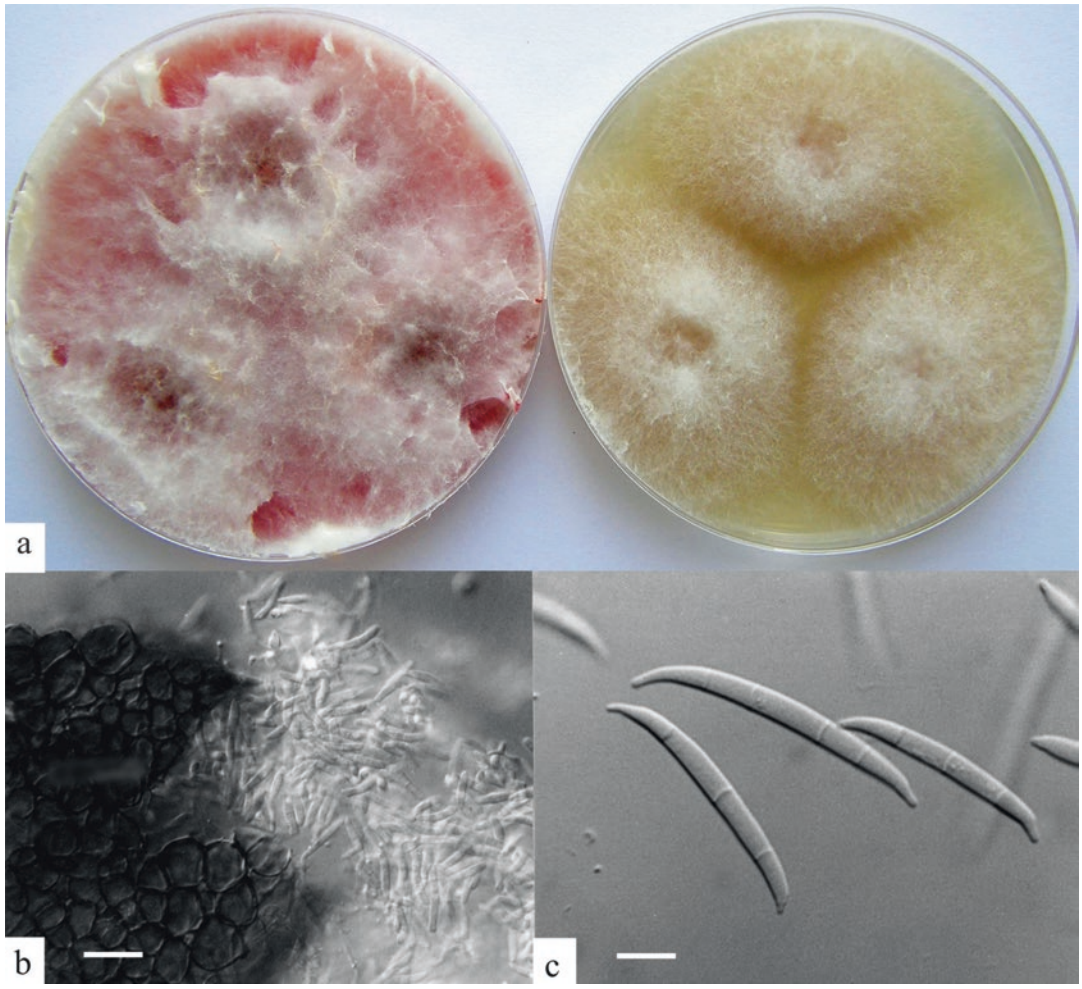

Fig. 5.28 *Fusarium graminearum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) perithecium and ascospores, bar = 25 µm; (**c**) macroconidia, bar = 10 µm

Macroconidia usually with 5 septa, sometimes less, thick walled, straight to moderately curved, with the ventral surface almost straight and a smoothly arched dorsal surface; basal cell distinctly foot-shaped, apical cell tapered; chlamydoconidia formed tardily in some isolates; microconidia absent.

Distinctive features On PDA, colonies of *Fusarium graminearum* are usually highly coloured, with dense to floccose greyish rose to golden brown mycelium and dark ruby reverse. Macroconidia are relatively straight and thick walled, with a foot-shaped basal cell. Microconidia are not produced.

Taxonomy The teleomorph of *Fusarium graminearum* is *Gibberella zeae* (Schwabe) Petch. Francis and Burgess (1977) described two populations of *F. graminearum*, which they termed Group 1 and Group 2. Group 2 isolates, the real *F. graminearum*, are responsible for head scab of wheat and also cause destructive ear rot of maize (Burgess et al. 1981; Desjardins 2006; Leslie and Summerell 2006). Group 1 isolates, which cause root and crown rot of wheat, were given species status and described as *Fusarium pseudograminearum* O'Donnell & T. Aoki (teleomorph *Gibberella coronicola* T. Aoki & O'Donnell) by Aoki and O'Donnell (1999a, b). *F. graminearum* is homothallic and forms perithecia readily in nature and in culture on Carnation Leaf Agar (Leslie and Summerell 2006), whereas *F. pseudograminearum* is heterothallic and does not. O'Donnell et al. (2004) proposed that the '*F. graminearum* clade' be split into nine different species, based on geographical and molecular data, however the morphological distinctions between these species are small. This proposal has not been taken up by all *Fusarium* researchers (Leslie and Summerell 2006). The complete genome of *Fusarium graminearum* has been sequenced and is available (Broad Institute 2003). Laurent et al. (2017) compared the genomes of six French isolates of this species and discovered over 240,000 base pair differences from that reference isolate, of which 96% were single nucleotides polymorphisms. About 80% of all the *F. graminearum* protein-coding genes were found to be polymorphic. The genetic variants discovered and annotated will be valuable resources for further genetic and genomic studies.

Identifiers Mycobank MB200256; type not known.

Physiology The optimal temperature for growth of *Fusarium graminearum* is between 24 and 26 °C on both liquid and solid media at pH 6.7–7.2 (Booth 1971), and 25 °C on irradiated wheat grains (Ramirez et al. 2006a). The minimum a_w for growth is close to 0.90 at 15–25 °C (Cuero et al. 1987; Ramirez et al. 2006a). The minimum pH for growth is temperature dependent, near pH 3.0 at 25 and 37 °C, and pH 2.4 at 30 °C. The maximum is near pH 9.5 at 37 °C, but greater than pH 10.2 at the lower temperatures (Wheeler et al. 1991).

Mycotoxins *Fusarium graminearum* produces numerous mycotoxins: almost 50 toxic compounds have been reported. The most important economically are type B trichothecenes – deoxynivalenol (DON) and its derivatives 3-acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV) and its derivatives and zearalenone (see Marasas et al. 1984; Pitt and Hocking 1997; Desjardins 2006; Leslie and Summerell 2006). See Desjardins et al. (2006) for a comprehensive review of trichothecene mycotoxins. Other toxins reported include aurofusarin, culmorins, fusarin C and steroids (see Pitt and Hocking 1997; Leslie and Summerell 2006). Type A trichothecenes, including T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol, have been reported from some isolates. However, production of these toxins by *F. graminearum* was queried by Nelson et al. (1983) and remains unverified.

Based on production of the different trichothecenes, *F. graminearum* has been divided into two chemotypes, chemotype I for deoxynivalenol producers and chemotype II for nivalenol producers. Strains producing 3-acetyldeoxynivalenol are classified as chemotype IA and those producing 15-acetyldeoxynivalenol as chemotype IB (Ichinoe et al. 1983; Logrieco et al. 1988; Miller et al. 1991). North American strains are predominantly chemotype IB (Abbas et al. 1989b; Mirocha et al. 1989b; Miller et al. 1991;

Abramson et al. 1993; Desjardins 2006). This chemotype also predominates in the Ukraine (Leonov et al. 1990) and New Zealand (Lauren et al. 1992). Chemotype IA predominates in China (Miller et al. 1991; H.Y. Zhang et al. 2007; J.B. Zhang et al. 2007), Argentina (Ramirez et al. 2006b) and Italy (Logrieco et al. 1988) while in Japan (Sugiura et al. 1990) and Poland (Visconti et al. 1990) both chemotype IA and IB are commonly isolated. Chemotype II occurs only rarely in North America (Miller et al. 1991) but is more common elsewhere, including New Zealand (Lauren et al. 1992), Australia (Blaney and Dodman 1988, 2002; Tan et al. 2004), Japan (Sugiura et al. 1990), Korea (Lee et al. 1986; Kim et al. 1993), Italy (Logrieco et al. 1988; Somma et al. 2014) and South Africa (Sydenham et al. 1991).

Mycotoxin production by *F. graminearum* has been shown to be more complicated by Crippin et al. (2019) who showed that *F. graminearum* isolates from Ontario sometimes did not produce deoxynivalenol but the structurally related 7-α hydroxy, 15-deacetylcalonectrin and its hydrolysis product 7-α hydroxy, 3,15-dideacetylcalonectrin, in addition to the usual metabolite 15-acetyldeoxynivalenol. No relationship was seen between production of these particular toxins and genotype assessed using published genetic probes (Crippin et al. 2019, 2020).

Ramirez et al. (2006a) reported that deoxynivalenol production occured most rapidly at 25 °C, but the maximum amount was produced at 30 °C. The a_w range for deoxynivalenol production was 0.95–0.995 a_w. Llorens et al. (2004b) reported that 28 °C was optimal for deoxynivalenol production, but nivalenol and 3-acetyldeoxynivalenol were produced optimally at 20 °C and 15 °C respectively. Zearalenone production was optimal at 20 °C (Llorens et al. 2004a).

Other toxins produced by *F. graminearum* include butenolide, culmorin, sambucinol, calonectrin and related compounds, fusarins and a number of other minor metabolites (Desjardins 2006).

Ecology *Fusarium graminearum* is primarily a pathogen of gramineous plants, particularly wheat, in which it causes crown rot and head scab. Reports have come from Australia, Canada, the United States, Japan, Korea, South Africa and many European countries (see Pitt and Hocking 1997; Desjardins 2006; Leslie and Summerell 2006). *F. graminearum* also causes cob rot and stalk rot of maize in many countries leading to DON and zearalenone formation in maize (see Pitt and Hocking 1997; Leslie and Summerell 2006; Sampietro et al. 2013). Occurrence of *F. graminearum* in barley is less frequent, but it is believed to be one cause of gushing in beer (Niessen et al. 1992). Other sources include sugar beets (Bosch and Mirocha 1992), soybeans (Clear et al. 1989; Jacobsen et al. 1995), sorghum (Onyike and Nelson 1992) and triticale (Perkowski et al. 1988). *F. graminearum* has been reported to cause dry rot in potatoes (Ali et al. 2005) and postharvest rots in pumpkins (Elmer 1996). Crown rot of bananas may also be due to *F. graminearum* (Wallbridge 1981; Jiménez et al. 1993).

Quorn, a mycoprotein produced for human consumption in the United Kingdom, is produced from a strain of *Fusarium* which was originally identified as *F. graminearum*. This strain, which does not produce any trichothecene toxins, has since been assigned to a different species, *F. venenatum* Nirenburg (O'Donnell et al. 1998a). For reviews of this topic see Edwards (1993), Trinci (1994), and Wiebe (2004).

References Domsch et al. (1980) (as *Gibberella zeae*), Nelson et al. (1983), Desjardins et al. (2006), and Leslie and Summerell (2006).

Fusarium incarnatum (Desm.) Sacc. Fig. 5.29

Fusarium pallidoroseum (Cooke) Sacc.
Fusarium semitectum Berk. & Ravenel

Colonies on CYA filling the whole Petri dish, sometimes to the lid at the edges, of dense, floccose, white, pale salmon or pale brown mycelium, sometimes powdery from macroconidia produced in the aerial mycelium; pale salmon sporodochia occasionally produced at the inoculation points; reverse pale salmon, yellowish, greyish yellow or with brown patches. Colonies on MEA filling the whole Petri dish, of floccose

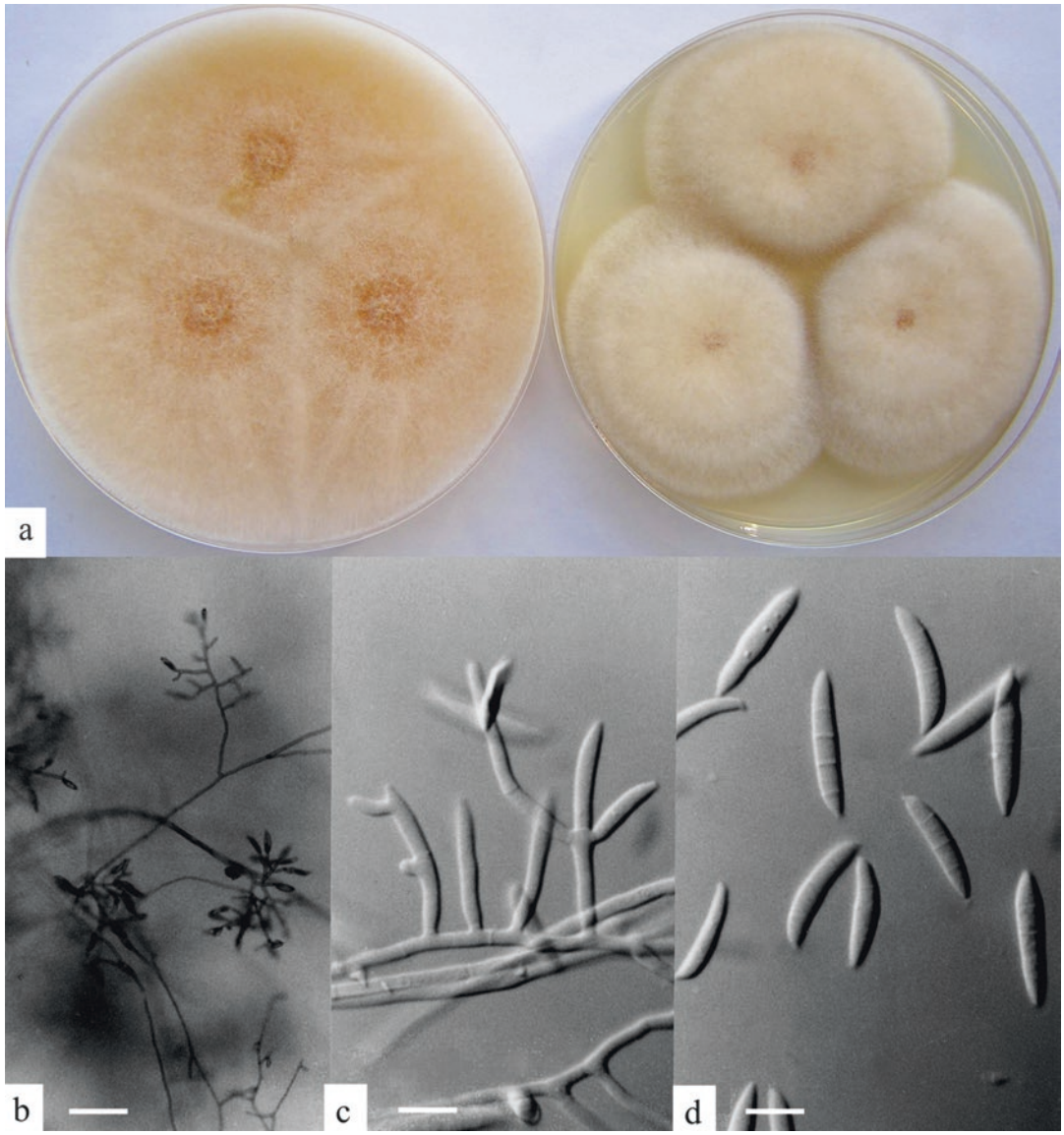

Fig. 5.29 *Fusarium incarnatum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) phialide**s** bearing macroconidia in aerial mycelium *in situ*, bar = 50 µm; (**c**) phialides, bar = 10 µm; (**d**) macroconidia, bar = 10 µm

or funiculose white to pale greyish yellow mycelium; reverse pale, sometimes greyish orange centrally or in patches. Colonies on G25N 15–20 mm diam. At 5 °C, usually only microcolonies produced. Usually no growth at 37 °C, but occasionally colonies of 2 mm diam formed.

On PDA, colonies of dense, floccose mycelium, coloured pale salmon, brown or yellow, sometimes with a powdery appearance from macroconidia in the aerial mycelium; reverse pale salmon, often brownish centrally. On DCPA, colonies in similar colours to those on PDA, but less dense, aerial mycelium often powdery with macroconidia, and occasionally a central area of salmon to orange sporodochia; reverse pale.

Macroconidia of two types produced: the first in the aerial mycelium, often from polyphialides,

cigar or spindle shaped, straight or slightly curved, with 4–5 septa, and with poorly differentiated basal and apical cells; the second in sporodochia, more curved, with a foot-shaped basal cell and curved apical cell. Chlamydoconidia usually produced; microconidia not produced.

Distinctive features The production of spindle or cigar shaped macroconidia from polyphialides in the aerial mycelium is the most distinctive feature of *Fusarium incarnatum*. These macroconidia can be observed *in situ* with the low power microscope, and are often arranged in pairs in "V" shapes, resembling rabbit ears.

Taxonomy This species was commonly known as *Fusarium semitectum* for many years. However, Nirenberg (1990) pointed out that the species epithet *incarnatum* predates *semitectum* by 40 years, and the name *F. incarnatum* has slowly been accepted, though both Desjardins et al. (2006) and Leslie and Summerell (2006) used *F. semitectum.* Using molecular analyses, O'Donnell et al. (2009) showed that this species and the closely related *F. equiseti* included up to 30 phylogenetic species, which in many cases were given numbers not binomial names and the list has been added to since. Xia et al. (2019) have stabilised nomenclature in this complex by naming and typifying many of these species. All are closely related and some cannot be distinguished by morphological means.

Identifiers Mycobank MB231142; epitype CBS H-24060; cultures ex-epitype CBS 132.73 = ATCC 24387 = IMI 128222 = NRRL 25478 (Xia et al. 2019).

Physiology The temperature range for growth of *Fusarium incarnatum* is 3 °C to ca 37 °C, with the optimum near 25 °C (Kakker and Mehrotra 1971; S. Marín et al. 1996; Shukla and Sharma 2000). Cherian (2007) reported maximum rotting of bananas by *F. incarnatum* occurred at 30 °C.

Mycotoxins Mycotoxin production by *Fusarium incarnatum* and other members of the *Fusarium incarnatum-equiseti* complex remains unclear. Indeed Villani et al. (2019) showed that the complete sequences of 13 genomes from the complex revealed a complicated pattern of mycotoxin gene clusters which correlated poorly with phylogenetic species. Zearalenone production has been reported (see Marasas et al. 1984; Pitt and Hocking 1997; Desjardins 2006; Leslie and Summerell 2006). Production of T-2 toxin has also been reported, but is perhaps uncommon (Marasas et al. 1984; Desjardins 2006). Japanese isolates of *F. incarnatum* have been reported to produce diacetoxyscirpenol, nivalenol, fusarenone X and neosolaniol (Suzuki et al. 1980, 1981). Diacetoxyscirpenol production has also been reported by Molto et al. (1997). Diacetylnivalenol or T-2 toxin and neosolaniol were produced by 18 of 21 *Fusarium* isolates in the *F. incarnatum-equiseti* complex taken from soybean roots in Ethiopica and Ghana (Hartman et al. 2019). In a study of this complex in Brazilian rice, Avila et al. (2019) identified isolates which produced zearalenone and deoxynivalenol and less commonly nivalenol, and 4- and 15-acetyldeoxynivalenol. T-2 and HT-2 mycotoxins were not detected.

Ecology *Fusarium incarnatum* is widespread in tropical and subtropical countries, although reports from foods are relatively uncommon. It has been found at low levels in maize in Italy (Logrieco et al. 1995) and the USA (Katta et al. 1995), and we have found high levels of *F. incarnatum* in Thai maize (45% of samples, up to 22% infected grains in infected samples and 4% of all kernels examined; Pitt et al. 1993). Incidence in Indonesian maize was similar, but levels in Philippine maize lower (Pitt et al. 1998a). *F. incarnatum* is one of the dominant fungi in pearl millet (Wilson 2002; Jurjevic et al. 2007) and is common in peanuts (Joffe 1969; Gilman 1969; Oyeniran 1980; Pitt et al. 1993; Dharmaputra and Retnowati 1996). It has also been reported from rice (Desjardins et al. 2000a) and sorghum (Onyike and Nelson 1992; Usha et al. 1994; Shabbir and Rajasab 2004). It causes storage rots of various fruits (Snowdon 1990), especially crown rot of bananas (see Pitt and Hocking 1997; Vesonder et al. 1995; D.H. Marin et al. 1996; Cherian 2007) and soft rot of bell peppers (Shukla and Sharma 2000; Sharma and Shukla 2003). It has also been reported to cause postharvest spoilage of tomatoes (Muniz et al. 2003; Chaturbhuj and Rai 2005), passionfruit (Muniz et al. 2003), mushrooms (Kang et al. 2002) and

disease in walnuts (Belisario et al. 1999). This species has also been isolated from pigeon peas (Maximay et al. 1992), soybeans (Vaamonde et al. 1987; and our unpublished data), black gram (Goyal and Jain 1998), black beans (Castillo et al. 2004), coriander (Hashmi and Thrane 1990; Hashmi and Ghaffar 1991), and sunflower seeds (Shahnaz and Ghaffar 1991), though with few reports in each case. Earlier reviews indicate isolations from citrus fruits, tomatoes, melons, cucumbers and potatoes (Booth 1971; Domsch et al. 1980).

In our experience, *F. incarnatum* is much more common in tropical commodities than the above citations would indicate. This species appears to be endemic in paddy rice from Thailand (in 94% of samples examined, up to 44% of grains infected in infected samples and a very high 20% of all grains examined; Pitt et al. 1994) and Indonesia (60% of samples, up to 60% of grains in infected samples and 7% of all grains; Pitt et al. 1998a). It was the most common fungus in Thai mung beans (55% of samples; up to 76% infection in infected samples, and 15% infection overall). It was also common in Thai black beans (6% of all beans examined), sorghum (4% of all grains) and soybeans (2% of all beans; Pitt et al. 1994). Generally similar figures were found for infection in rice, soybeans, black beans and mung beans in Indonesia and the Philippines (Pitt et al. 1998a). Indeed, *F. incarnatum* was by far the most common fungal invader of beans that we encountered in Southeast Asia.

References Desjardins et al. (2006), Leslie and Summerell (2006), and Xia et al. (2019).

Fusarium longipes Wollenw. & Reinking Fig. 5.30

Colonies on CYA and MEA covering the whole Petri dish, plane, deep and floccose or sometimes funiculose, mycelium pale orange to pale red or dull reddish, reverse on CYA brownish orange to light brown, on MEA deep red to brownish red. On G25N, colonies 15–22 mm diam, of floccose, pale orange mycelium, reverse in similar colours. No germination at 5 °C. At 37 °C, colonies of variable size, up to 65 mm diam, of white to very pale pink mycelium, reverse dull yellow to pinkish brown.

On PDA, colonies covering the whole Petri dish, similar to those on CYA, reverse dull red to dark red. On DCPA, colonies 55–65 mm diam, sparse, mycelium pale pink, showing abundant orange sporodochia containing macroconidia, reverse dull yellow to brown.

Macroconidia on DCPA strongly curved, 50–80 × 4–6 µm, with 4–6 septa; apical cells usually with a long papilla or a long slender whip-like end; basal cells with a deep notch and long "foot". Microconidia not produced. Chlamydoconidia produced by some isolates, in chains or small clusters, spherical to ellipsoidal or irregular in shape, 7–12 µm diam, with thin smooth walls, becoming rough in age.

Distinctive features Pale orange to pale red mycelium on CYA and MEA, fast growth at 37 °C and very long, slender, strongly curved macroconidia are the main distinguishing features of *Fusarium longipes*.

Taxonomy No teleomorph is known for this species.

Identifiers Mycobank MB269717; type not known.

Physiology No studies on the physiology of this species are known to us.

Mycotoxins This appears to be a nontoxigenic species (Wing et al. 1993; Nelson et al. 1994), although Logrieco et al. (1998a) reported beauvericin production by one isolate.

Ecology *Fusarium longipes* principally comes from tropical soils (Burgess et al. 1994; Sangalang et al. 1995). It has been reported from peanuts from West Java (Dharmaputra and Retnowati 1996), and we isolated it from 13% of Indonesian peanut samples, at up to 16% of kernels in infected samples and 1% of all kernels examined (Pitt et al. 1998a). It was also found at low levels in Indonesian maize and cowpeas, Thai maize, mung beans, cassava and sesame seed, and paddy rice from the Philippines (Pitt et al. 1993, 1998a). *F. longipes* has also been reported from cereals in Iran (Zare and Ershad 1997).

References Nelson et al. (1983) and Leslie and Summerell (2006).

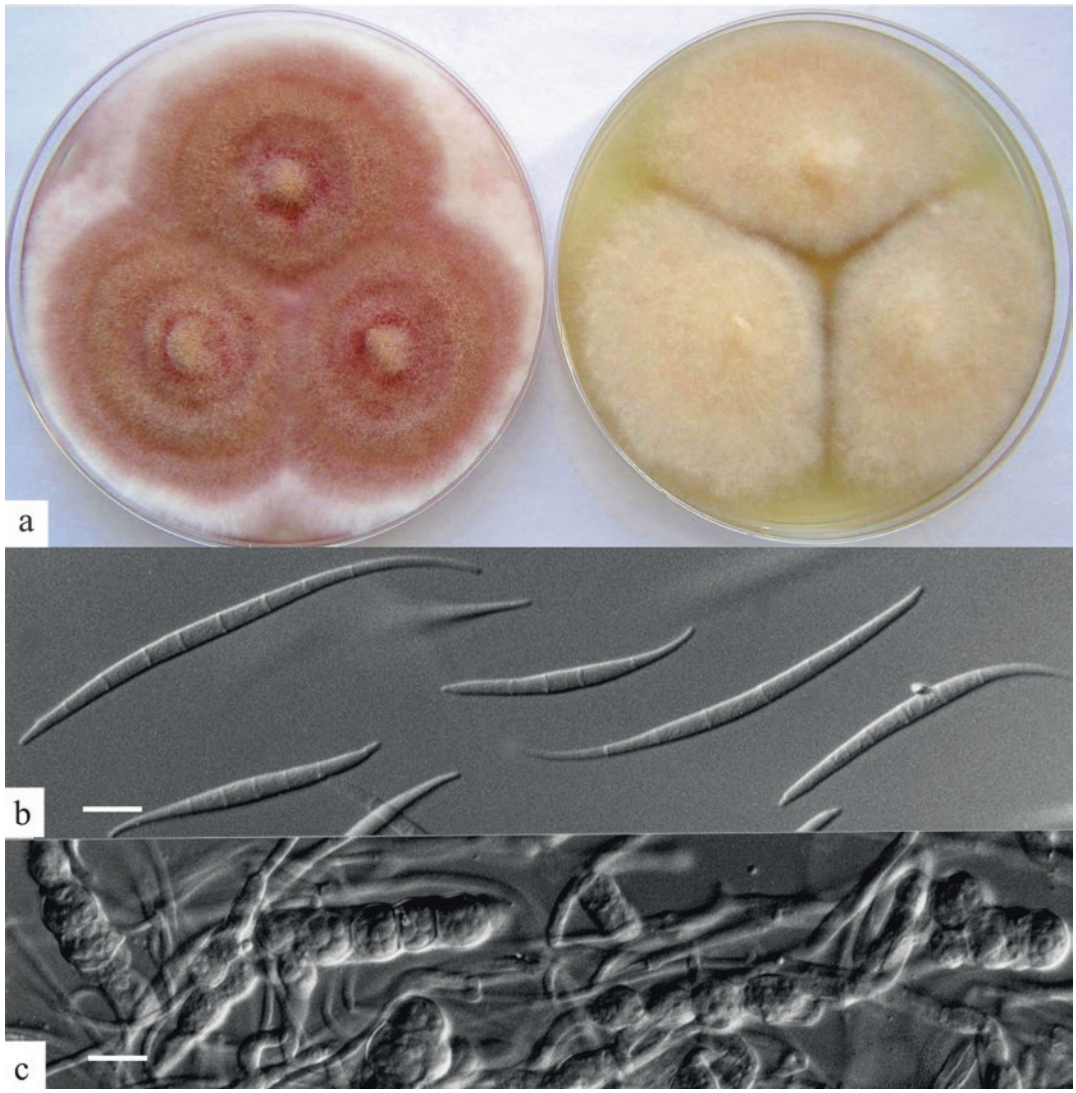

Fig. 5.30 *Fusarium longipes* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) macroconidia, bar = 10 µm; (**c**) chlamydoconidia, bar = 10 µm

Fusarium oxysporum Schltdl. Fig. 5.31

Colonies on CYA 50–70 mm diam, sometimes covering the whole Petri dish, moderately deep, of floccose white to greyish mycelium; reverse pale to pale greenish grey. Colonies on MEA 65–70 mm diam, often covering the whole Petri dish, of floccose white to pale greyish magenta mycelium; reverse greyish magenta to dark purple, often paler at the margins. Colonies on G25N 12–16 mm diam, occasionally larger. At 5 °C, germination to formation of microcolonies. At 37 °C, no growth, or colonies up to 5 mm diam formed.

On PDA, colonies of white, pale salmon or pale mauve mycelium, sometimes dense and floccose, sometimes low, often with salmon sporodochia in a central mass, and sometimes in one or two poorly defined concentric rings also; reverse pale salmon, often mauvish centrally, sometimes dark magenta. On DCPA, colonies low, sometimes with concentric rings of sparse, floccose

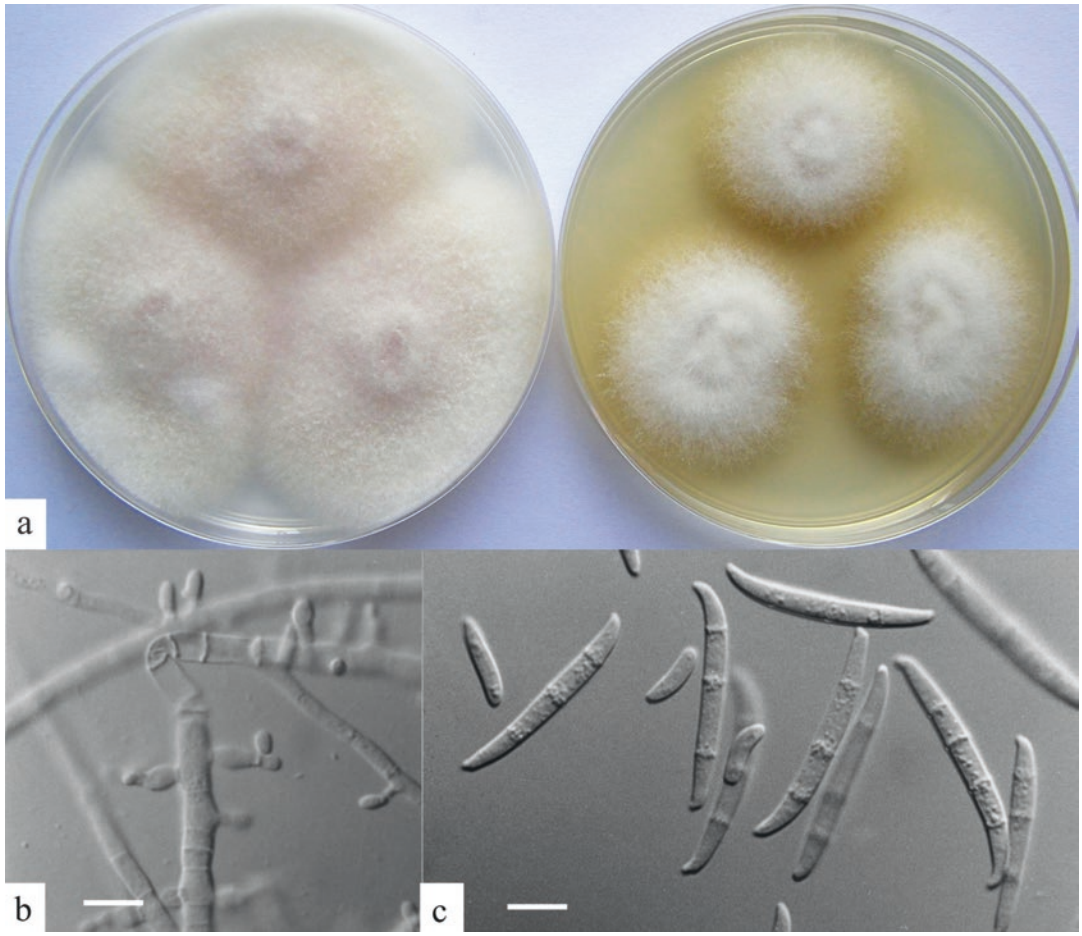

Fig. 5.31 *Fusarium oxysporum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) phialides, bar = 10 µm; (**c**) phialides, bar = 10 µm; (**d**) macroconidia and microconidia, bar = 10 µm

aerial mycelium, powdery with microconidia on the agar surface, or sometimes uniformly low with little aerial mycelium and then with appearance dominated by surface macroconidia.

Macroconidia only slightly curved, usually with 3 septa, occasionally more, thin-walled, with notched or foot-shaped basal cells and short, sometimes hooked, apical cells; microconidia abundant, fusiform to kidney shaped, produced in false heads from short, stout monophialides. Chlamydoconidia produced singly or in pairs.

Distinctive features *Fusarium oxysporum* produces abundant fusiform to kidney shaped microconidia in false heads from short, stout, flask shaped phialides in the aerial mycelium. The colony reverse on PDA is usually mauve, violet or greyish magenta.

Taxonomy In his review of the current state of *Fusarium* taxonomy, Summerell (2019) singled out *Fusarium oxysporum* as being especially difficult taxonomically. It has been known for some time that this species includes multiple clades (Gordon and Martyn 1997; Laurence et al. 2014) and is now classed as a species complex. It includes many plant pathogenic races called *formae speciales* (abbreviated f. sp.), at last count over 140 (Lombard et al. 2019). In a noteworthy attempt to clarify the taxonomy surrounding this species, Lombard et al. (2019) selected an epitype and described 11 new species, some of

importance in foods. During examination of more than 160 strains of *F. oxysporum,* Lombard et al. (2019) found that all strains from veterinary sources clustered together with some strains from food processing equipment in their new species *F. veterinarium*. Three strains from contaminated dairy products and fruit juice clustered together in their new species *F. contaminatum*. The majority of the isolates collected from tomato clustered together in *F. languescens* (Lombard et al. 2019).

No teleomorph is known, however substantial evidence exists that horizontal gene transfer has occurred in this complex, especially important in the transfer of pathogenic traits (Laurence et al. 2015). For further information about this species complex, see Lombard et al. (2019) and Summerell (2019).

Identifiers MycoBank MB218372; epitype CBS H-23620; ex-epitype culture CBS 144134 (Lombard et al. 2019).

Physiology Domsch et al. (1980) reported an optimum growth temperature between 25 and 30 °C for *Fusarium oxysporum*, with a minimum above 5 °C, and a maximum at or below 37 °C. The optimum pH for growth is 7.7 with the wide range of pH 2.2 to 9.0 being tolerated (Domsch et al. 1980). The minimum a_w for growth is 0.89 at 20 °C, after a germination time of 2 months (Schneider 1954). Growth in N_2 with <1% O_2 was 97% of that in air (Hocking 1990). Some growth occurred in 99% CO_2, with only trace amounts of O_2 and N_2 present (Hocking 1990). Atmospheres of 20–40% CO_2 with 5% or 1% O_2 reduced colony growth rate of *F. oxysporum* by 40–50%, but ergosterol content was reduced by 80–90% under these conditions (Taniwaki et al. 2001a). *F. oxysporum* chlamydoconidia do not exhibit exception heat resistance, despite the ability of this species to cause spoilage in beverages (see below). In experiments in our laboratory, survival was recorded from mixed suspensions of microconidia and chlamydoconidia after heating at 63 °C for up to 48 s, but not after exposure to 65 °C for 6 s in 20° Brix sucrose solution at pH 4.2. *F. oxysporum* was more resistant to dry heat, with sporadic survival detected after exposure to 140 °C for 60 s (our unpublished data).

Mycotoxins *Fusarium oxysporum* has been widely reported to produce moniliformin (Marasas et al. 1979; Abbas et al. 1989b, 1991; Mirocha et al. 1989a; Chelkowski et al. 1990) and zearalenone (Jiménez et al. 1997; see also Pitt and Hocking 1997), although production of the latter compound appears to be in doubt as *F. oxysporum* does not possess the *tri5* gene required for trichothecene biosynthesis (Tan and Niessen 2003). *F. oxysporum* isolates reported to produce wortmannin (Abbas and Mirocha 1988; Abbas et al. 1989a, 1992; Gunther et al. 1989) have been reexamined and classified as *F. torulosum* (Thrane 2001). Other mycotoxins reported to be produced by *F. oxysporum* are beauvericin (Logrieco et al. 1998a; Moretti et al. 2002), type B fumonisins (Abbas et al. 1995; Proctor et al. 2004) and type C fumonisins (Seo et al. 1996).

Ecology *Fusarium oxysporum* is the most economically important and commonly encountered species of *Fusarium* and is considered to be one of the 10 most destructive pathogenic fungi (Geiser et al. 2013; Lombard et al. 2019). It is a serious wilt pathogen of many crop plants, including sweet potatoes, cabbage and other crucifers, cucumbers and melons, oil and date palms, tomatoes, peas, soybeans and cowpeas, clovers, cotton and a variety of others (Booth 1971; Nelson et al. 1981) and is geographically widespread (Sangalang et al. 1995; Leslie and Summerell 2006). Long known as a cause of crown rot of bananas (Wallbridge 1981; Jiménez et al. 1993; Boruah et al. 2004), one race of the causal *formae speciales F. oxysporum* f. sp. *cubensis* is now devastating Cavendish bananas across the world (Pegg et al. 2019; Summerell 2019).

In that light, the common occurrence of *Fusarium oxysporum* in foods is not unexpected. It occurs in cereals, including maize in the United States, Australia, Turkey, South Africa and the Philippines; rice in India, Egypt and Indonesia; barley in Egypt and sorghum from Africa (see Pitt and Hocking 1997). It is widespread on nuts: peanuts, pecans, hazelnuts and walnuts (see Pitt and Hocking 1997).

This species is one cause of crown rot of bananas (Wallbridge 1981; Jiménez et al. 1993; Boruah et al. 2004), and also occurs as a minor pathogen on citrus, pome fruits, tomatoes, melons (Snowdon 1990) and pineapples (Biswal et al. 2007). Other sources include coriander seeds (Hashmi and Ghaffar 1991), cacao beans (Ribeiro et al. 1986), navy beans (Tseng et al. 1996), adzuki and mung beans (Tseng and Tu 1997), garlic (Rath and Mohanty 1986), capsicums (Hashmi and Thrane 1990), truffles (Bokhary et al. 1990), asparagus (Weber et al. 2006), cheese (Hocking and Faedo 1992; Lund et al. 1995) and potatoes (Vrany et al. 1989; Kim and Lee 1994; Venter and Steyn 1998). *F. oxysporum* has been implicated as a cause of gill-blackening disease in Kumma prawns (*Penaeus japonicus*) (Souheil et al. 1999).

Due to its ability to grow in low O_2 tensions, *F. oxysporum* has caused spoilage of UHT processed fruit juices (Hocking 1990). This problem became widespread from 2001 onwards, and our laboratory investigated several spoilage incidents occurring in Australia and New Zealand in fruit, dairy- and soy-based aseptically filled beverages. We had anecdotal evidence of similar problems in Europe, Asia and North America. *F. oxysporum* appeared to be colonising the aseptic area of the packaging equipment, and once established, was very difficult to eliminate.

References Booth (1971), Nelson et al. (1981, 1983), Leslie and Summerell (2006), Lombard et al. (2019), and Summerell (2019).

Fusarium poae (Peck) Wollenw. Fig. 5.32

Colonies on CYA filling the whole Petri dish, deep, of moderately dense white to pale rose mycelium; reverse unevenly coloured, pale to rose red or deep red. Colonies on MEA filling the whole Petri dish, deep, of sparse, pale rose mycelium; reverse brownish orange or paler. Colonies on G25N 8–12 mm diam. At 5 °C, germination to colonies up to 2 mm diam formed. No growth at 37 °C.

On PDA, colonies moderately deep, of floccose, pale salmon to pale rose mycelium, darker centrally, reverse varying from pale salmon at the margins to greyish ruby centrally, or entirely dark ruby to dark magenta. On DCPA, colonies moderately deep, of floccose pale salmon mycelium in poorly defined concentric rings; sporodochia rarely present; reverse pale or, in highly pigmented isolates, with annular brownish red rings.

Macroconidia usually sparsely produced, varying in shape, mostly with 3 septa, occasionally more, slightly curved, with a foot shaped basal cell. Microconidia abundant, spherical, often with a distinct papilla, occasionally also lemon shaped, aseptate or with one septum, produced in the aerial mycelium from short flask shaped phialides on compact branched stipes, often appearing like bunches of grapes when examined *in situ* under the low power microscope.

Distinctive features The abundant production of spherical microconidia borne from flask-shaped phialides on compact, branched stipes distinguishes *Fusarium poae* from other species. It also has a distinctive odour, described as 'fruity' or 'sweet' (Leslie and Summerell 2006).

Taxonomy *Fusarium poae* is a distinctive species. Molecular analysis indicated a high genetic variability in 173 isolates collected from 13 countries world wide. However, most of the variability resulted from differences within, rather than between, American and European populations (Dinolfo et al. 2014). A group of cultures with morphology similar to *F. poae*, referred to as "powdery" *F. poae*, are now considered to be the separate species *F. langsethiae* Torp & Nirenburg (Torp and Nirenberg 2004). This species has been isolated from oats, wheat and barley in cooler areas of Europe and Scandinavia. *F. langsethiae,* which does not have the characteristic sweet odour of *F. poae,* is also distinguished by its metabolic profile (Thrane et al. 2004) and by molecular techniques (Schmidt et al. 2004). It appears to be more closely related to *F. sporotrichioides* than *F. poae* (Niessen et al. 2004; Dinolfo et al. 2014). Although no teleomorph is known, Vanheule et al. (2017) identified hallmarks of both sexual recombination and clonal spread of successful genotypes among a large number of European isolates of *F. poae*.

Identifiers Mycobank MB119380; type unknown.

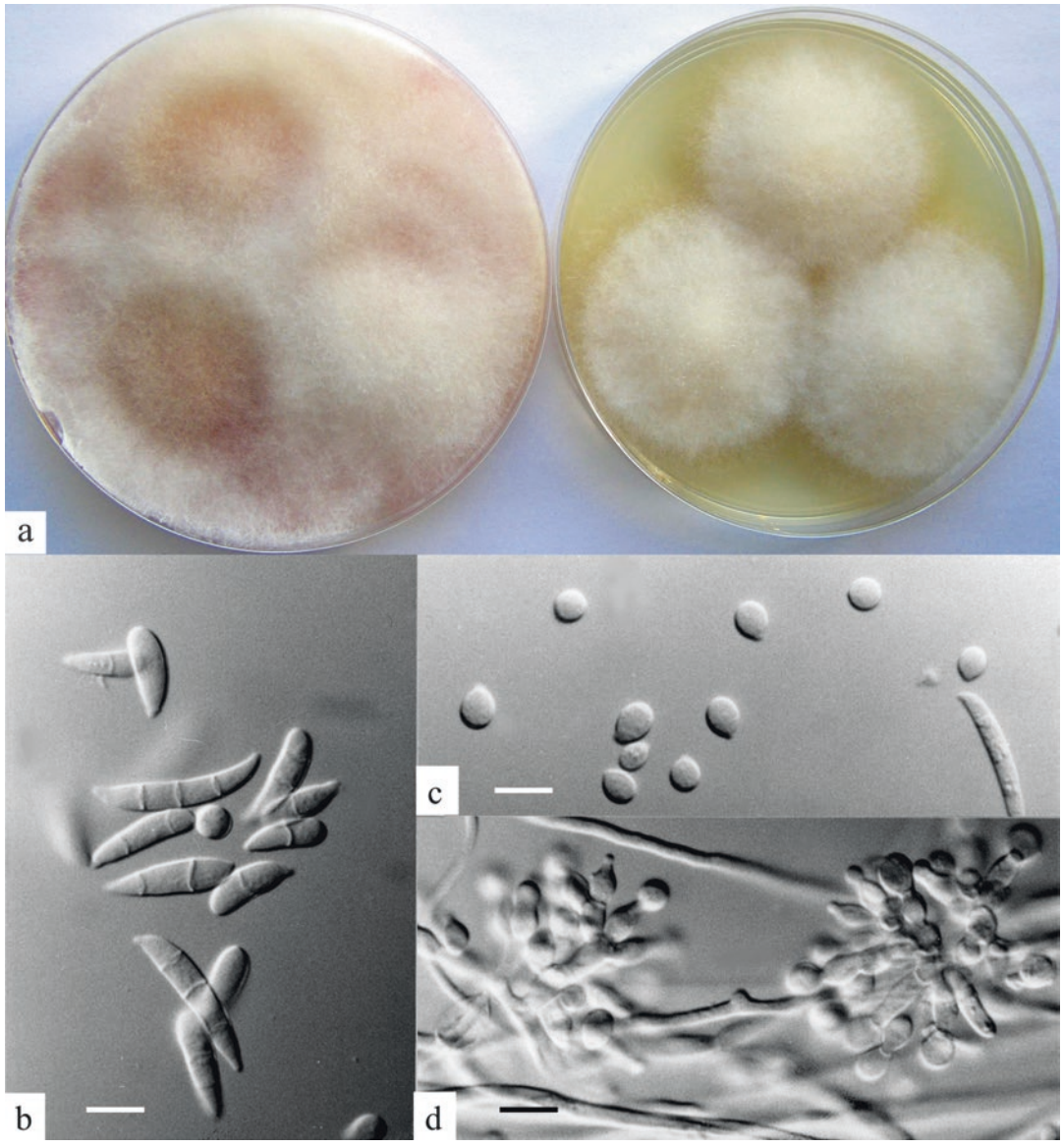

Fig. 5.32 *Fusarium poae* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) macroconidia, bar = 10 µm; (**c**) microconidia, bar = 10 µm; (**d**) phialides, bar = 10 µm

Physiology The optimum temperature for growth of *Fusarium poae* is near 25 °C, with a minimum near 2.5 °C and a maximum of 35 °C (Kvashnina 1976; Torp and Nirenberg 2004). The minimum a_w for growth is near 0.90 between 17 °C and 25 °C (Magan and Lacey 1984c).

Mycotoxins Reports of toxin production by *Fusarium poae* have been very variable. Reports that it was perhaps responsible for Alimentary Toxic Aleukia due to the production of T-2 toxin have largely been discounted, as *F. sporotrichioides*, the major producer of that toxin, was also commonly present during that disease syndrome, and in the haemorrhagic diseases that occurred in cattle, pigs and poultry in the USA in the 1960s (Marasas et al. 1984). However, it has been concluded more recently that certain strains of *F. poae* can indeed produce T-2 toxin (De Nijs et al.

1996; Langseth et al. 1998; Thrane et al. 2004). In looking at the loci responsible for trichothecene production, Vanheule et al. (2017) showed evidence of a transposable element at one locus that was partly responsible for a complex distribution of the various trichothecenes among *F. poae* isolates from Europe.

Most strains of *F. poae* produce nivalenol, diacetoxyscirpenol, monoacetoxyscirpenol, fusarenone-X and scirpentriol (Thrane et al. 2004), with some strains also producing beauvericin and enniatins (Thrane et al. 2004; Chelkowski et al. 2007). Logrieco et al. (1998a) also reported beauvericin production by *F. poae*. T-2 toxin is more often produced by *F. sporotrichioides* and *F. langsethiae* (Thrane et al. 2004; Desjardins 2006).

Ecology *Fusarium poae* mainly occurs in temperate regions, where it is found on woody seedlings or herbaceous and gramineous hosts (Booth 1971; Leslie and Summerell 2006). From foodstuffs, it has been reported most commonly from grains, especially wheat, from France, Canada, Japan, Ethiopia, Hungary, Poland, Germany and Norway (see Pitt and Hocking 1997; Birzele et al. 2002; Bottalico and Perrone 2002; Ioos et al. 2004; Kosiak et al. 2003, 2004; Stenglein et al. 2014). It has been implicated as a cause of panicle blight of oats in Poland (Mielniczuk 2001), head blight of oats in Canada (Tekauz et al. 2004) and as a pathogen of barley in Argentina (Barreto et al. 2004), where it commonly occurs in malting barley (Nogueira et al. 2018). It has been associated with maize in cooler climates, where it may be involved in ear rot (see Pitt and Hocking 1997; Logrieco et al. 2002; Chelkowski et al. 2007). *F. poae* has also been recorded from soybeans in the USA (Abbas and Bosch 1990), heart rot of sugar cane in South Africa, rice in Australia and decayed stored citrus fruit in Georgia (Booth 1971).

References Nelson et al. (1983), Desjardins et al. (2006), and Leslie and Summerell (2006).

Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach & Nirenberg **Fig. 5.33**

Gibberella intermedia (Kuhlman) Samuels et al. (teleomorph)

Colonies on CYA covering the whole Petri dish, plane, floccose, of pale orange mycelium, reverse light orange to pastel red. Colonies on MEA similar to those on CYA but much less dense, mycelium in similar colours, reverse orange, greyish red or violet brown. On G25N, germination or colonies up to 5 mm diam. At 5 °C, no germination. At 37 °C, colonies 5–20 mm diam, of pale orange mycelium, reverse usually orange.

On PDA, colonies covering the whole Petri dish, similar to on MEA, reverse light orange to greyish red. On DCPA, colonies 45–55 mm diam, of pale orange mycelium, plane, floccose, similar in texture to on CYA, orange sporodochia usually evident on the agar surface, reverse orange brown.

Macroconidia on DCPA slightly curved, with 3–4 septa, 40–50 × 3.0–4.0 µm, rounded or papillate at both ends, or sometimes with a notch at the basal end, with thin, smooth walls. Microconidia borne in chains and false heads from monophialides or polyphialides, ellipsoidal or bean shaped, commonly 8–12 × 2.5–4.0 µm and nonseptate, occasionally longer and with one or more septa, with thin smooth walls. Chlamydoconidia not produced.

Distinctive features Similar to *Fusarium verticillioides* in most respects, *F. proliferatum* is distinguished by the formation of microconidia from polyphialides. Microconidial chains are usually shorter in *F. proliferaturm* than *F. verticillioides*. Freshly isolated cultures form abundant macroconidia in sporodochia, but this feature is often lost after repeated transfer (Leslie and Summerell 2006).

Taxonomy Morphologically, *F. proliferatum* is indistinguishable from *F. fujikuroi* Nirenburg.

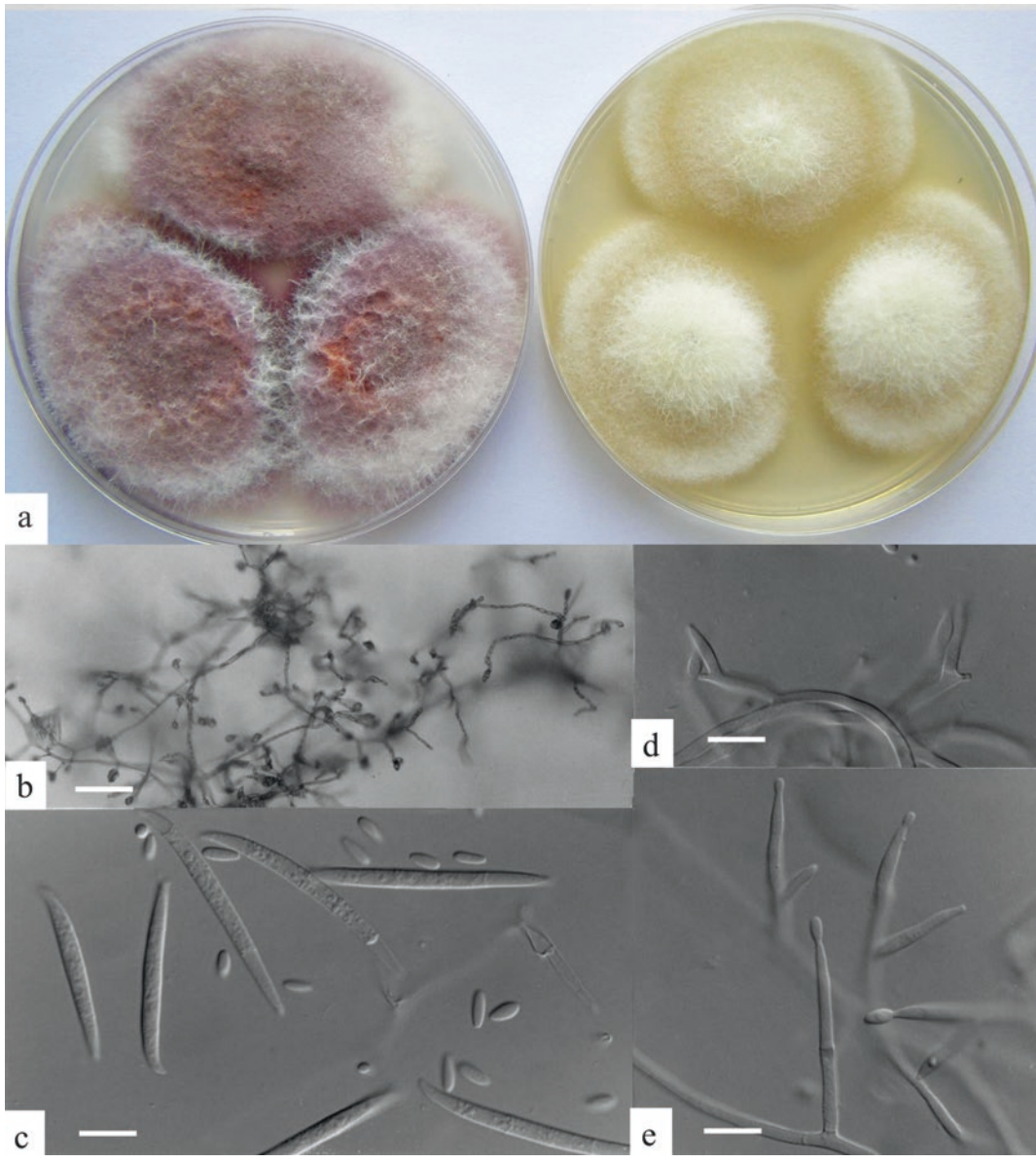

Fig. 5.33 *Fusarium proliferatum* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) phialides bearing microconidia in chains and false heads *in situ*, bar = 50 µm; (**c**) macroconidia and microconidia, bar = 10 µm; (**d**) polyphialides, bar = 10 µm; (**e**) monophialides, bar = 10 µm

These two species can be differentiated using mating tests or by DNA sequencing. *F. fujikuroi*, which is associated mainly with rice, does not produce fumonisins, whereas most isolates of *F. proliferatum* do.

Identifiers Mycobank MB509381; type not known.

Physiology The optimum pH and temperature for growth of *Fusarium proliferatum* are 5.5 and 25 °C respectively, with growth occurring at 5 °C and 37 °C, but not 40 °C on maize meal (S. Marin et al. 1996). The minimum a_w for germination reported by S. Marin et al. (1996) was 0.88, with no germination occurring at 0.85 a_w

after 40 days. However, Samapundo et al. (2005a) reported a minimum a_w of 0.87 for growth of *F. proliferatum* on maize, with optimum growth occurring at 30 °C at 0.97 a_w, the highest a_w tested. *F. proliferatum* was able to grow on irradiated maize kernels at 0.98 and 0.93 a_w in atmospheres containing up to 60% CO_2 with 20% O_2, although the lag time was longer and the growth rate slower than the controls (Samapundo et al. 2007a). Reduction of headspace oxygen from 20% to 2% (without elevated CO_2) had no effect on growth of *F. proliferatum,* but vacuum packaging and the incorporation of oxygen scavenging sachets prevented growth (Samapundo et al. 2007b). Application of 1% ammonium bicarbonate to maize at a_w values between 0.99 and 0.92 prevented growth and fumonisin production by *F. proliferatum* and was proposed as a possible treatment for stored maize (Samapundo et al. 2007c). The antioxidants propylparaben and butylated hydroxyanisole (BHA) can also inhibit growth and fumonisin production by *F. proliferatum* under some conditions (Etcheverry et al. 2002)

Fumonisin B_1 production was inhibited at headspace CO_2 concentrations of 40%, 30% and 10% at 0.98, 0.95 and 0.93 respectively (Samapundo et al. 2007a). When headspace oxygen was controlled, optimum fumonisin production occurred with 20% O_2 at 0.98 a_w, but at 0.95 and 0.93 a_w, more fumonisin was produced with 10% headspace O_2 (Samapundo et al. 2007b). Marín et al. (1995) reported fumonisin production was higher at 25 than at 30 °C, and highest at the highest a_w tested (0.97), with low levels still produced at 0.92 a_w. Samapundo et al. (2005b) found that there was little difference in fumonisin production at 25 and 30 °C, but lower amounts were produced at 15 °C, irrespective of a_w value (between 0.97 and 0.92). Conversely, Melcion et al. (1998) observed maximal fumonisin production at 15 °C on maize at very high a_w.

Mycotoxins *Fusarium proliferatum* is a major producer of fumonisins B_1, B_2 and B_3 (Ross et al. 1990; see also Pitt and Hocking 1997; Desjardins 2006), and indeed strains of *F. proliferatum* are among the highest fumonisin producers (Leslie et al. 2004). Of 38 isolates taken from a wide variety of plant sources and grown on a rice substrate, 16 (42%) produced >1000 µg/kg fumonisin B_1, with no relationship found between host species and the amount of toxin produced (Stępień et al. 2011). The highest producers were taken from maize, asparagus, pineapple, and garlic. Other mycotoxins produced include moniliformin, beauvericin, enniatins and fusarin (Marasas et al. 1986; Desjardins et al. 2000a; see also Desjardins 2006).

Ecology Reporting of *Fusarium proliferatum* in foods and feeds has increased sharply with the discovery of fumonisins and that this species is an important source of these toxins. It has been shown to colonise maize plants throughout the world, and is increasingly important in maize ear rot in Europe (see Bacon and Nelson 1994; Desjardins 2006; Leslie and Summerell 2006). It was reported from sorghum in Nigeria by Onyike and Nelson (1992), pine nuts in Spain (Marín et al. 2007) and onions in Finland (Haapalainen et al. 2016). We have encountered this species in maize, mung beans, paddy rice and occasionally sorghum in Southeast Asia (Pitt et al. 1998a). *F. proliferatum* has also been reported as a cause of black point in wheat (Conner et al. 1996; Desjardins et al. 2007). In contrast with *Fusarium verticillioides, F. proliferatum* has a very wide host range. It is a pathogen of bananas (Jiménez et al. 1993), onions (Toit et al. 2003; Stankovic et al. 2007), garlic (Dugan et al. 2003, 2007; Stankovic et al. 2007) and asparagus (Elmer 2000; Gossmann et al. 2005; Corpas-Hervias et al. 2006; Weber et al. 2006; C. Liu et al. 2007). Fumonisins have been reported in infected garlic (Seefelder et al. 2002) and asparagus (Logrieco et al. 1998b; Seefelder et al. 2002; Weber et al. 2006; C. Liu et al. 2007).

References Nelson et al. (1983), Desjardins et al. (2006), and Leslie and Summerell (2006).

Fusarium sporotrichioides Sherb. Fig. 5.34

Colonies on CYA filling the whole Petri dish, deep, of floccose white to pale pink and brown mycelium; reverse pale salmon centrally, brownish at the margins. Colonies on MEA similar to those on CYA except more highly coloured; reverse violet brown centrally, paler at the margins. Colonies on G25N 12–15 mm diam. At 5 °C, colonies 5–10 mm diam. No growth at 37 °C.

On PDA, colonies of dense, floccose, salmon to pink mycelium, sometimes with a central mass of orange sporodochia; reverse greyish rose to burgundy, occasionally paler. On DCPA, colonies of floccose pale salmon mycelium in concentric rings; orange sporodochia sometimes present on the agar surface; reverse pale.

Macroconidia abundant, moderately curved, with 3–5 septa and with a curved, pointed apical cell and a notched or foot-shaped basal cell. Microconidia abundant, produced from polyphialides in the aerial mycelium, fusiform,

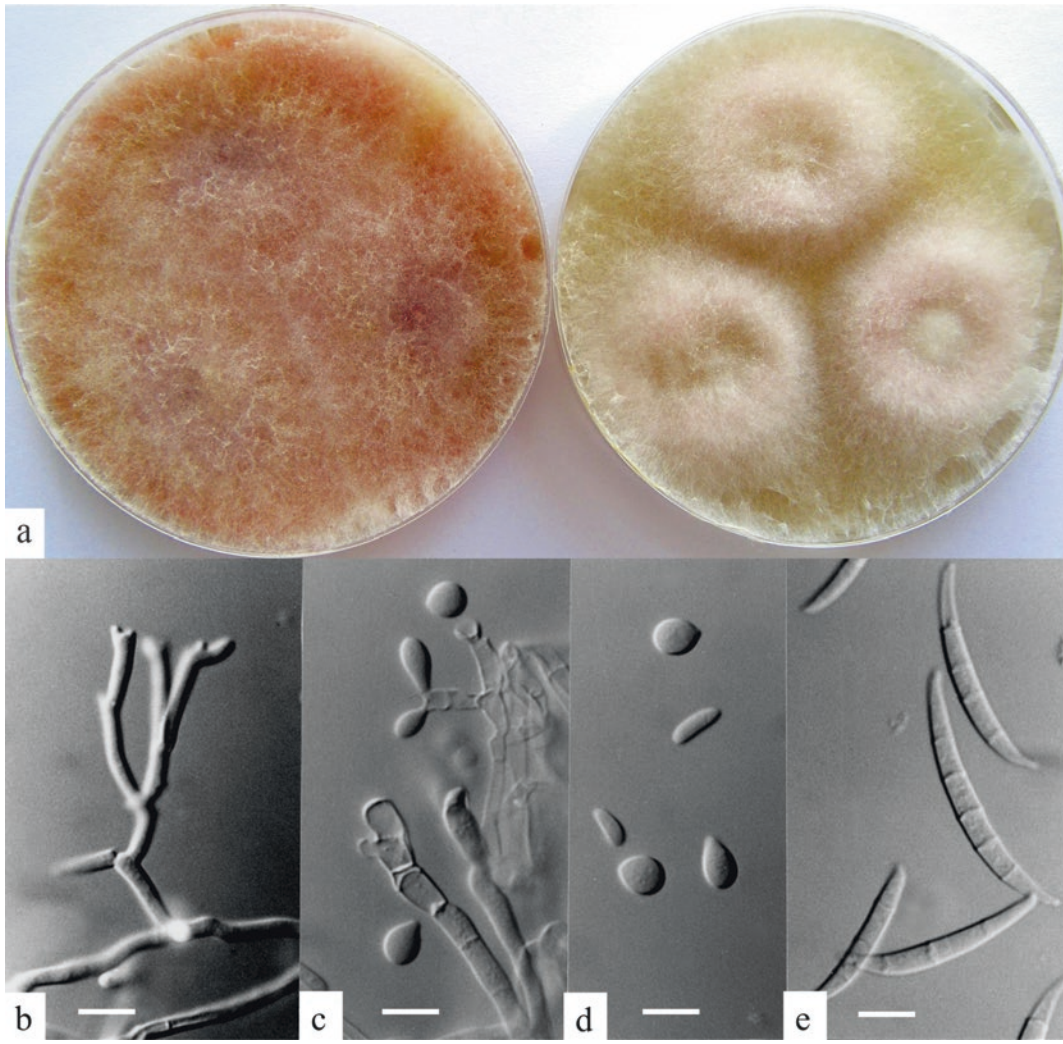

Fig. 5.34 *Fusarium sporotrichioides* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**, **c**) polyphialides, bar = 10 µm; (**d**) microconidia, bar = 10 µm; (**e**) macroconidia, bar = 10 µm

broadly ellipsoidal or pyriform, the latter often produced only on PDA, often with a papilla at the base. Chlamydoconidia formed abundantly, singly or in chains or clumps, as cultures age.

Distinctive features On PDA, *Fusarium sporotrichioides* has a simlar appearance to *F. poae, F. chlamydosporum* and *F. tricinctum.* The production of both fusiform and pyriform microconidia from polyphialides distinguishes *F. sporotrichioides* from these closely related species. Pyriform microconidia are produced more commonly, and sometimes exclusively, on cultures on PDA. Colony reverses on PDA are always greyish rose or burgundy.

Identifiers Mycobank MB145064; type not known.

Physiology Domsch et al. (1980) gave the optimum growth temperature of *Fusarium sporotrichioides* as 22.5–27.5 °C, with a maximum of 35 °C. Joffe (1962) reported the growth of toxigenic isolates of this species down to −2 °C. Schneider (1954) reported 0.88 a_w as the minimum for growth, after 8 weeks incubation at 20 °C.

Mycotoxins *Fusarium sporotrichioides* is regarded as the major cause of alimentary toxic aleukia (ATA) a devastating disease which occurred in the USSR during and after World War II, in times of extreme food shortage resulting in consumption of overwintered cereals (Joffe 1978). Hundreds of thousands of people died from T-2 toxin produced by this species and *F. poae* (Marasas et al. 1984). *F. sporotrichioides* also produces a number of T-2 toxin derivatives, including HT-2, T-2 triol and T-2 tetraol, and biosynthetic intermediates such as neosolaniol, diacetoxyscirpenol and 15-monoacetoxyscirpenol (Thrane et al. 2004; Abramson et al. 2004; see also Pitt and Hocking 1997; Desjardins 2006; Leslie and Summerell 2006). *F. sporotrichioides* has been used to elucidate the biochemistry and genetics of trichothecene biosynthesis (Desjardins et al. 1993), with the first trichothecene biosynthetic gene, trichodiene synthase (*TRI5*) cloned from *F. sporotrichioides* in 1989 (see Desjardins 2006; Leslie and Summerell 2006). Early reports of deoxynivalenol and nivalenol production have not been confirmed and may be due to misidentification of isolates (Desjardins 2006). Other mycotoxins produced by *F. sporotrichioides* include aurofusarin, beauvericin and occasionally enniatins, but not nivalenol, fusarenon-X or fumonisins (Thrane et al. 2004; Desjardins 2006).

Fusarium sporotrichioides has been implicated as the cause of mouldy corn toxicosis (haemorrhagic syndrome) in cattle, pigs and poultry in the USA and elsewhere, fescue foot in cattle feeding on winter pastures in the USA, Australia and New Zealand, akakabi-byo (scabby grain intoxication) and bean hull poisoning in animals in Japan (Marasas et al. 1984; Wu et al. 1997).

Ramakrishna et al. (1996) reported that T-2 toxin formation by *Fusarium sporotrichioides* on barley grain occurred optimally at 20 °C and toxin production was stimulated by the presence of *Aspergillus flavus* or *Penicillium verrucosum.*

Ecology While not so commonly isolated as some other species described here, *Fusarium sporotrichioides* is important because of its toxicity. It occurs more commonly in cool climates and, in foods, is almost entirely confined to grains. Historically, this species has been associated with rye in Europe (Joffe 1978; Matossian 1989). More recently, it has been reported most frequently from wheat, in Japan, Spain, Hungary, Poland, Canada, Ethiopia (see Pitt and Hocking 1997) and Finland and Russia (Yli-Mattila et al. 2004). Other grain sources include maize (Logrieco et al. 1995; Logrieco et al. 2002; Adejumo et al. 2007; and see Pitt and Hocking 1997), barley (Yli-Mattila et al. 2004; Bourdages et al. 2006), sorghum, wild rice (Nyvall et al. 1999) and cereal grains in general (see Pitt and Hocking 1997; Langseth et al. 1998; Kosiak et al. 2003). This species has also been isolated from peanuts (Austwick and Ayerst 1963), sugar beets (Bosch and Mirocha 1992) and in our laboratory from Australian soybeans.

References Domsch et al. (1980), Nelson et al. (1983), Desjardins et al. (2006), and Leslie and Summerell (2006).

Fusarium subglutinans (Wollenw. & Reinking) P.E. Nelson et al. Fig. 5.35

Fusarium moniliforme J. Sheld. var. *subglutinans* Wollenw. & Reinking
Fusarium sacchari (Butler) W. Gams var. *subglutinans* Wollenw. & Reinking
Gibberella subglutinans (Edwards) P.E. Nelson et al. (teleomorph)

Colonies on CYA covering the whole Petri dish, of dense white to very pale salmon mycelium, in degenerate cultures of low and sparse funiculose white mycelium; sometimes with a central orange spore mass; reverse pale, salmon or yellowish. Colonies on MEA less dense than on CYA, of white to pale salmon mycelium, sometimes powdery with microconidia; reverse pale yellow, salmon or violet grey. Colonies on G25N 5–12 mm diam, occasionally larger. At 5 °C, germination to microcolony formation. No growth at 37 °C.

On PDA, colonies low to moderately deep, of floccose to funiculose mycelium coloured white, pale salmon, pale pink or mauve, sometimes powdery with microconidia, reverse violet grey to deep violet centrally, paler at the margins, or uniformly pale. On DCPA, colonies low at the margins, floccose centrally, of pale salmon mycelium, sometimes overlying a thin layer of pale orange macroconidia on the agar surface; reverse pale.

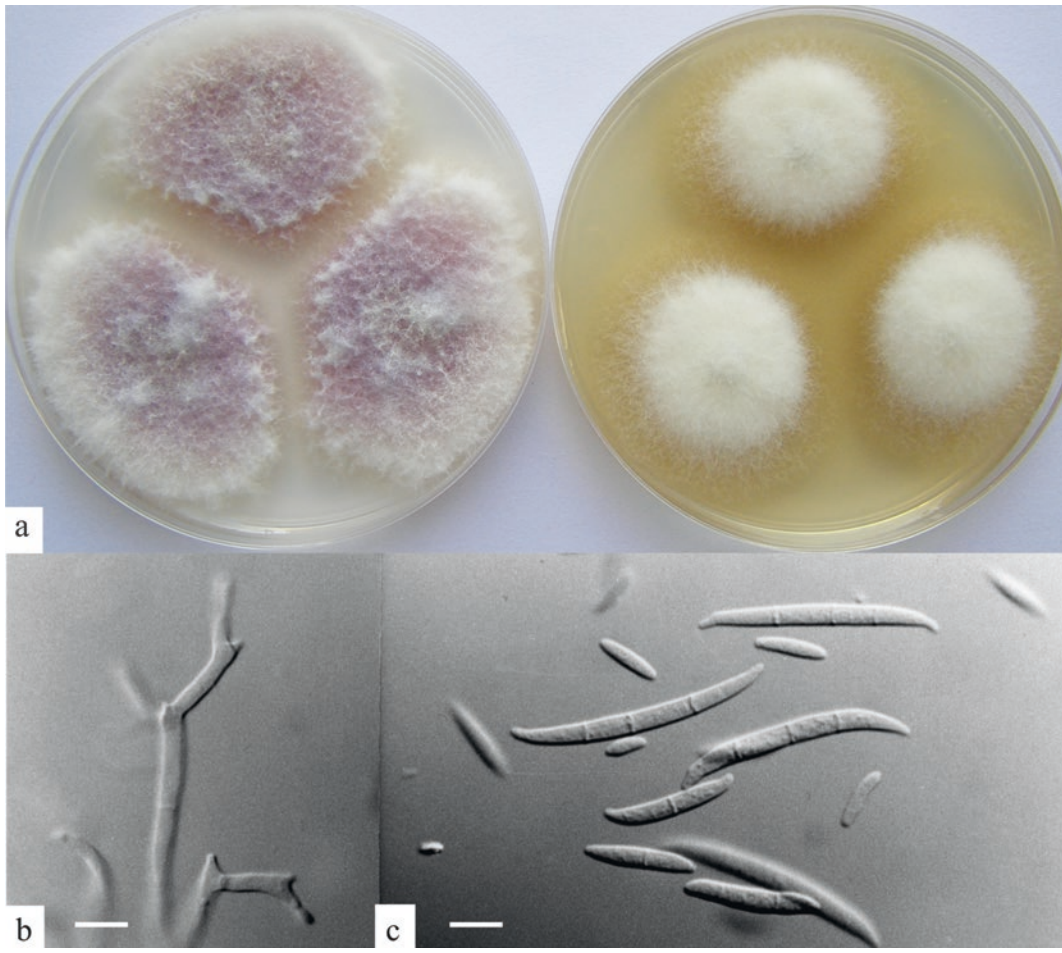

Fig. 5.35 *Fusarium subglutinans* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) polyphialides, bar = 10 µm; (**c**) macroconidia amd microconidia, bar = 10 µm

Macroconidia slightly curved to almost straight, with 3–5 septa and thin, delicate walls, narrow, tapered apical cells and foot shaped basal cells. Microconidia abundant, fusiform or broadly ellipsoidal, aseptate or with a single septum, produced in false heads from polyphialides and also from simple phialides. Chlamydoconidia not produced.

Distinctive features The production of microconidia in false heads from polyphialides and the absence of chlamydoconidia distinguish *Fusarium subglutinans* from otherwise similar species such as *F. verticillioides* and *F. oxysporum*.

Taxonomy *Fusarium subglutinans* was raised to species status by Nelson et al. (1983), previously having been regarded as a variety of *F. moniliforme* or *F. sacchari*. It is a major species in the *Fusarium fujikuroi* complex. The teleomorph of *F. subglutinans* is *Gibberella subglutinans* (Edwards) P.E. Nelson et al. At least two cryptic species are included in the concept of *F. subglutinans* (Steenkamp et al. 2002; Desjardins et al. 2006). One was described as *F. temperatum* by Scauflaire et al. (2011), from Belgian maize. This species has also been recorded from Korean maize (Tagele et al. 2019).

Identifiers Mycobank MB115356; type not known.

Physiology Martelleto et al. (1998) reported growth of *Fusarium subglutinans* between 10 and 30 °C, with 25 °C being optimal. Castellá et al. (1999b) found growth of *F. subglutinans* was optimal between 20–25 °C on maize cultures, but on rice, growth was best at 15 °C. Highest production of fusaproliferin was at 20 °C on maize after 6 weeks (Castellá et al. 1999b).

Mycotoxins One of the less toxic of major *Fusarium* species, *F. subglutinans* has been reported to produce moniliformin as its major toxin (see Pitt and Hocking 1997; Kostecki et al. 1999; Sewram et al. 1999; Logrieco et al. 2002; Desjardins 2006; Desjardins et al. 2006; Leslie and Summerell 2006; Sørensen et al. 2007). Production of fumonisin B_1 has also been reported (Visconti and Doko 1994), but levels are generally low (Reynoso et al. 2004). Other toxins reported are beauvericin and fusaproliferin (Moretti et al. 1995; Logrieco et al. 1998a; Castellá et al. 1999b; Kostecki et al. 1999; Shephard et al. 1999; Torres et al. 2001; Reynoso et al. 2004).

Ecology Although it has been reported from a wide range of substrates, the main source of *Fusarium subglutinans* is maize. It has been isolated from Europe (Italy, Logrieco et al. 1995; Austria, Lew et al. 1991; Poland, Logrieco et al. 1993b), the United States (Abbas et al. 1988, 1989b; Katta et al. 1995), Canada (Neish et al. 1983), the Caribbean (Julian et al. 1995), Peru (Logrieco et al. 1993a), Argentina (Torres et al. 2001; Reynoso et al. 2004, 2006), Mexico (Desjardins et al. 2000b; Morales-Rodriguez et al. 2007), South Africa (Marasas et al. 1978, 1979; Rheeder et al. 1995), Zimbabwe (Mubatanhema et al. 1999), Indonesia (Pitt et al. 1998a), Australia (Burgess et al. 1981; Blaney et al. 1986) and New Zealand (Hussein et al. 1991; Hussein et al. 2002). *F. subglutinans* has also been isolated in Mexico from teosintes, the nearest wild relative of maize (Desjardins et al. 2000b). *F. subglutinans* has been reported from wheat in Europe (Bottalico and Perrone 2002; Ioos et al. 2004; Cosic et al. 2007), South Africa (Boshoff et al. 1998), Tanzania (van Dyk 2004), Egypt (Gherbawy et al. 2006) and Iran (Moosawi-Jorf et al. 2007), but it does not appear to play an active role in head blight.

Fusarium subglutinans is a pathogen of pineapples (Bolkan et al. 1979; Rohrbach and Taniguchi 1984; Matos et al. 2000), bananas (Jiménez et al. 1993, 1997; Wade et al. 1993; Vesonder et al. 1995) and capsicums (bell peppers) (Hashmi and Thrane 1990; Utkhede and Mathur 2004). Other sources have been sorghum (Onyike and Nelson 1992), sugar beets (Bosch and Mirocha 1992), traditional African vegetables (van der Walt et al. 2006), tomatoes and lemons (Muniz et al. 2003), and black pepper (Pitt et al. 1998a).

References Booth (1971), Nelson et al. (1983), Desjardins et al. (2006), and Leslie and Summerell (2006).

Fusarium verticillioides (Sacc.) Nirenberg

Fig. 5.36

Fusarium moniliforme J. Sheld. (in part)
Gibberella moniliformis Wineland (teleomorph)

Colonies on CYA usually covering the whole Petri dish, low at the margins to moderately deep centrally, of floccose to funiculose white mycelium; reverse pale, or in shades of pale salmon or violet. Colonies on MEA usually covering the whole Petri dish, low to moderately deep centrally, of white or pale salmon funiculose mycelium, sometimes powdery with microconidia; reverse of some isolates pale, of others violet or greyish magenta. Colonies on G25N 5–12 mm diam. At 5 °C, either germination or no growth. At 37 °C, colonies of 4–10 mm diam produced.

On PDA, colonies of low, densely funiculose mycelium coloured white to pale salmon, usually powdery with chains of microconidia; reverse varying from isolate to isolate, pale salmon, greyish violet, brownish violet or deep violet; dark blue sclerotia produced by some isolates. On DCPA, colonies low, of thin, funiculose white to pale salmon mycelium in concentric rings, powdery with microconidia, and sometimes alternating with rings of macroconidia produced on the agar surface; reverse pale.

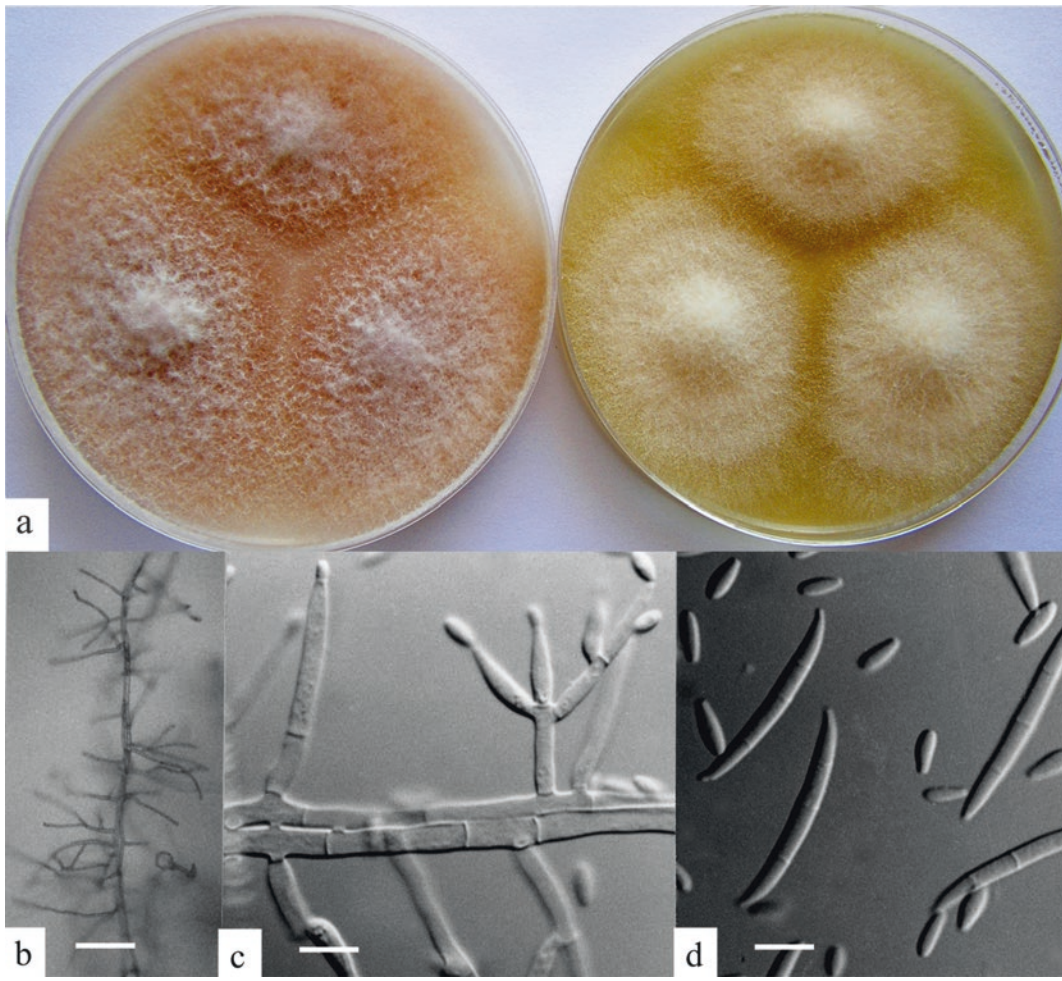

Fig. 5.36 *Fusarium verticillioides* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) phialides bearing chains of microconidia, bar = 50 µm; (**c**) phialides, bar = 10 µm; (**d**) macro- and microconidia, bar = 10 µm

Macroconidia usually long and slender, almost straight, thin walled, with foot shaped basal cells; microconidia fusiform to clavate, produced from long monophialides, forming chains readily seen *in situ* under the low power microscope (6× to 10×); chlamydoconidia not produced.

Distinctive features The production of microconidia in long chains from relatively long phialides distinguishes *Fusarium verticillioides* from most other common *Fusarium* species. *F. proliferatum* is very similar to in appearance to *F. verticillioides* but is distinguished by the production of polyphialides bearing microconidia in short chains.

Taxonomy In 1877, Saccardo described *Oospora verticillioides* from maize in Italy. In 1904, John Sheldon described *Fusarium moniliforme* from maize that was associated with animal toxicosis in the United States (see Desjardins 2006), and for most of the twentieth century, the epithet *F. moniliforme* was used for this fungus. From an extensive study of the group of *Fusarium* species which included *F. moniliforme*, Nirenberg (1976) concluded that this species should be called *F. verticillioides*, but at the time, this proposal was not generally accepted. However, Seifert et al. (2003), representing the International Society for Plant Pathology and the International Committee on the Taxonomy of Fungi (ISPP/ICTF) Subcommittee on *Fusarium* Systematics, proposed that the name *Fusarium verticillioides* be applied to the fungus that produces fumonisin in maize, on the grounds that the name *F. moniliforme* represented an unacceptably broad species concept and that *F. verticillioides* was the older name which had priority. This name is now generally accepted for isolates from maize and a few other sources (Leslie and Summerell 2006). Subsequently, morphologically similar species within the *Fusarium fujikuroi* complex, associated with different plant hosts and different mating types, have been described: *F. thapsinum* from sorghum, *F. sacchari* from sugar cane, *F. mangiferae* from mango and *F. fujikuroi* from rice. These names have been accepted (Leslie and Summerell 2006).

Identifiers Mycobank MB314223; type not known.

Physiology The maximum temperature for growth of *Fusarium verticillioides* has been reported to be 32–37 °C, the minimum as 2.5–5 °C, and the optimum near 25 °C (Nirenberg 1976). The minimum a_w for growth was 0.87 at 25 °C, after a 4 month germination time (Armolik and Dickson 1956). Reduction of headspace oxygen from 20 to 2% had no effect on growth of *F. verticillioides*, but vacuum packaging with oxygen absorbing sachets completely inhibited growth (Samapundo et al. 2007b). Fumonisins B_1 and B_2 were produced down to at least 0.92 a_w (Marín et al. 1995) but were not detected at 0.89–0.91 a_w on irradiated maize grains (Marín et al. 1999). Maximum fumonisin production occurred with 15% oxygen at 0.976 a_w, but with 5% O_2, 0.93 was the optimum a_w (Samapundo et al. 2007b).

Mycotoxins The major mycotoxin produced by *Fusarium verticillioides* is fumonisin B_1, the cause of leucoencephalomalacia in horses, pulmonary oedema in pigs and liver cancer in rats. It is a possible cause of human oesophageal cancer (Gelderblom et al. 1988; Sydenham et al. 1990; Shephard et al. 2007) and neural tube defects (Desjardins 2006). For further information see Chap. 13, "Mycotoxins". Isolates of *F. verticillioides* from maize from many countries have been shown to produce fumonisins B_1 and B_2 (Bezuidenhout et al. 1988; Laurent et al. 1989; Desjardins 2006; see also Pitt and Hocking 1997). This species also commonly produces fusarins, fusaric acid and naphthoquinones, but not beauvarin, moniliformin or fusiproliferin (Desjardins 2006; Leslie and Summerell 2006). Reports of zearalenone, diacetoxyscirpenol and deoxynivalenol production by *F. verticillioides* were discounted by Nelson et al. (1983). *F. verticillioides* isolates from banana rots (Mirete et al. 2004; Moretti et al. 2004), which produce moniliformin but not fumonisins (Patiño et al. 2006) have now been characterised as *F. musae* (van Hove et al. 2011) and may be one cause of human ketatitis (Triest and Hendrickx 2016).

Ecology *Fusarium verticillioides* is widespread in the tropics and humid temperate areas of the world (Leslie and Summerell 2006). This species is an endemic pathogen of maize, causing both stalk rot and cob rot which can be present at

all stages of plant development. It has been isolated from maize kernels in every major location where it has been sought, including the United States, Canada, Mexico, Honduras, Ecuador (Pacin et al. 2002), Peru and Argentina; from Iran (Ghiasian et al. 2004, 2006), India, China, Nepal (Desjardins and Busman 2006) and Taiwan; from Europe (Logrieco et al. 2002), and from Australia, South Africa and other countries on the African subcontinent (Adejumo et al. 2007; Bigirwa et al. 2007) (see also Pitt and Hocking 1997; Desjardins 2006; Leslie and Summerell 2006). Incidence in individual maize kernels, where reported, has almost always been very high, e.g. 100% in Sardinia (Bottalico et al. 1995), 54% in mainland Italy (Logrieco et al. 1995), 97% in Thailand (Pitt et al. 1993), 92% in the Philippines and 73% in Indonesia (Pitt et al. 1998a).

Fusarium moniliforme has been reported from a very wide range of crops, but *F. verticillioides* has a much narrower circumscription. Reports from sorghum (Leslie et al. 2005) are likely to due to *F. thapsinum*, from rice (Desjardins et al. 2000a) to *F. fujikuroi,* from bananas, *F. musae* (van Hove et al. 2011) while millet (Leslie et al. 2005) may have been *F. pseudonygamai* O'Donnell & Nirenberg (Vismer et al. 2019). Other reports which follow may require verification. *F. verticillioides* (or *F. moniliforme*) has been reported from several types of nuts, i.e. hazelnuts, pecans, peanuts and kola nuts (see Pitt and Hocking 1997). This species has also been reported from oilseeds: sunflower, amaranth, and soybeans; also spices, including coriander, fenugreek, cardamom and pepper (see Pitt and Hocking 1997). It has also been found in mung beans (Pitt et al. 1994), biltong (van der Riet 1976) and cheeses (Northolt et al. 1980).

F. verticillioides (or *F. moniliforme*) has been reported to cause storage rot of oranges and other citrus (Snowdon 1990), garlic (Gargi and Roy 1988), yams (Ogundana 1972), pineapples (Lim 1983), asparagus (Snowdon 1991) and dry rot of passion fruit (Lutchmeah 1993). Reports from bananas (Chorin and Rotem 1961; Jiménez et al. 1993; Vesonder et al. 1995) have probably been due to *F. musae* (van Hove et al. 2011).

References Desjardins et al. (2006) and Leslie and Summerell (2006).

5.17 Genus *Galactomyces* (E.E. Butler & L.J. Peterson) Redhead & Malloch

The genus *Geotrichum* was an asexual genus distinguished by its method of reproduction, the formation of arthroconidia and no other spore type. Arthroconidia are cylindrical spores formed by septation of vegetative hyphae into short segments which separate at maturity. After the discovery of a sexual state, von Arx (1977) transferred this genus to *Dipodascus* Lagerh. as *D. geotrichum* (E.E. Butler & L.J. Peterson) Arx, and in the same year this species was transferred to *Galactomyces* Redhead & Malloch by Redhead and Malloch (1977). These genera are closely related (de Hoog and Smith 2004) and both names are currently in use: the taxonomy of de Hoog and Smith (2004, 2011), who retained *Galactomyces*, is preferred here. Only one species, *G. candidus*, is significant in foods.

Galactomyces candidus de Hoog & M.Th. Smith **Fig. 5.37**

Geotrichum candidum Link: Fr.
Oidium lactis Fresen.
Oospora lactis (Fresen.) Sacc.

Colonies on CYA of variable size, 20–45 mm diam, very low and quite sparse, plane, of white mycelium, often leathery and difficult to dissect with a needle; reverse pale. Colonies on MEA 50–65 mm diam, similar to on CYA but of softer, yeast-like texture. On G25N, no growth to germination. At 5 °C, no germination to colonies up to 4 mm diam, of dense white mycelium. At 37 °C, usually no growth, occasionally sparse colonies up to 10 mm diam formed.

Conidiophores undifferentiated hyphae, at maturity fragmenting almost entirely to form arthroconidia; arthroconidia hyaline, cylindrical, sometimes developing rounded ends and thickened walls, commonly 5.0–8.0 × 2.0–5.0 µm, smooth walled.

Ascospores not seen under these standard cultural conditions: ascospores borne singly *fide* de Hoog and Smith (2004) "broadly ellipsoidal, 4.0–5.5 × 6–8 µm, with an echinate inner wall and an irregular exosporium wall, often with a hyaline equatorial furrow".

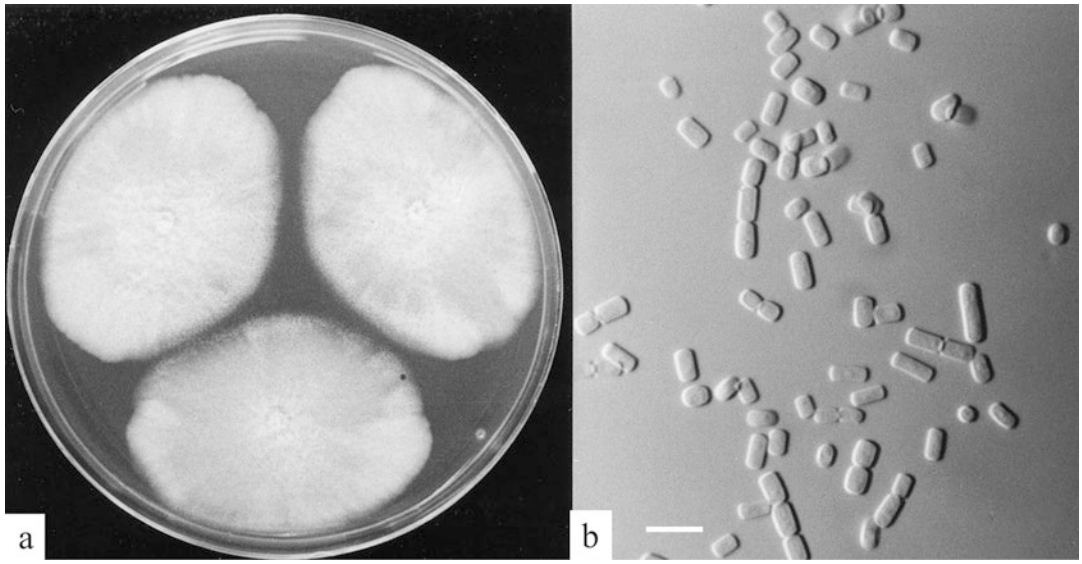

Fig. 5.37 *Galactomyces candidus* (**a**) colonies on MEA, 7 d, 25 °C; (**b**) arthroconidia, bar = 10 µm

Distinctive features See genus description.

Taxonomy Redhead and Malloch (1977) and later authors considered that *Galactomyces geotrichum* (E.E. Butler & L.J. Petersen) Redhead & Malloch was the correct name for the common species previously known as *Geotrichum candidum*, however de Hoog and Smith (2004) indicated that this was incorrect and introduced *Galactomyces candidus* as a new name for that species.

Identifiers Mycobank MB500129; holotype CBS 178.71 (= CBS H-13664); ex-type culture CBS 178.71.

Physiology *Galactomyces candidus* is restricted to habitats of high water availability, its minimum a_w for growth being 0.90 (Heintzeler 1939). Growth occurred at 0.95 but not at 0.90 a_w (Plaza et al. 2003). Optimal growth temperatures are 25–30 °C (Plaza et al. 2003), with maxima of 35–38 °C (Domsch et al. 1980). The conidia have a very low heat resistance, with a D value of 30–40 min at 52 °C (Beuchat 1981). Miller and Golding (1949) reported that *G. candidum* was able to grow at very low oxygen tensions, but not anaerobically.

Mycotoxins This species is not known to produce any toxic compounds.

Ecology *Galactomyces candidus* is a significant pathogen of citrus fruits during postharvest storage, causing sour rots (Butler et al. 1965; Hall and Scott 1977; Snowdon 1990). It occurs in fruit weakened by over maturity and long storage. Lemons and grapefruit are particularly susceptible, but a variety of other fruit can also be affected (Butler 1960). Initial infection is mainly through injuries. The primary control measure is preventative, and lies in choosing sound, young fruit for long term storage (Hall and Scott 1977). As *G. candidus* grows poorly below 10 °C, cold storage can assist in control (Rippon 1980; Plaza et al. 2003).

Galactomyces candidus causes spoilage of tomatoes (Okoli and Erinle 1989; Snowdon 1991), dried capsicums (Atanda et al. 1990) and sapodillas (Kusum and Geeta 1990) in tropical countries. A wide variety of vegetables, including carrots, cucumbers, onions, peas and potatoes are also susceptible (Snowdon 1991).

Galactomyces candidus (as *Geotrichum candidum*) was long a problem in the canning and freezing industries. Known as "machinery mould" (Eisenberg and Cichowicz 1977), it is a frequent contaminant of processing lines, and consequently of products such as frozen foods (Pitt and Hocking 1997). Standard methods have

been established for estimating *G. candidus* in food processing machinery, by physical counting rather than microbiological techniques (Cichowicz and Eisenberg 1974). Improvements in sanitation procedures have reduced this problem in recent years. Kure et al. (2004) concluded that air was the major source of contamination in cheese factories in Norway.

This mould is a very common problem in raw milk in Europe especially when it is used in the manufacture of soft, fresh cheeses such as quarg or Roblochon (see Pitt and Hocking 1997; Lopandic et al. 2006). Contamination of surface ripened cheeses such as Brie and Camembert can also be a problem (Pitt and Hocking 1997). However, more recent surveys suggest that *G. candidus* may play an important role in the ripening of many soft and semi-hard cheeses as well as contributing desirable cheese flavours (Boutrou and Gueguen 2005; Boutrou et al. 2006; Ghosh et al. 2006).

Galactomyces candidus has been isolated from a variety of other foods, including meats, raw and Parma hams, hard cheeses and traditional fermented foods, and Nigerian alcoholic beverages (see Pitt and Hocking 1997). Low levels were found in maize, peanuts, sorghum, soybeans and black rice in Thailand (Pitt et al. 1993, 1994). It is of quite common occurrence in barley during malting (see Pitt and Hocking 1997).

References von Arx (1977) and de Hoog and Smith (2004, 2011).

5.18 Genus *Geosmithia* Pitt

Geosmithia was split from *Penicillium* by Pitt (1979) on the basis of differences in phialide shape and wall texture, and conidial shape and colour: conidia are strictly cylindrical and borne from usually rough walled, cylindrical phialides. Molecular studies (Ogawa et al. 1997 and later papers) have shown that the concept was polyphyletic, so many of the species then known were relocated to *Penicillium* or *Rasamsonia* Houbraken & Frisvad. The type species, *G. lavendula* (Raper & Fennell) Pitt was placed in the Hypocreales, distant from most genera of relevance here. The species treated here, *G. putterillii,* remains in *Geosmithia* with *G. lavendula*.

The main habitat of *Geosmithia* species, including *G. putterillii*, is in association with bark beetles (Kolarík et al. 2004) and many new species have been described from that habitat (Kolarík et al. 2017), including *G. morbida* Kolařík et al., the cause of a destructive canker disease in walnut trees. *G. putterillii* is quite common on cereal products, and may cause spoilage occasionally.

Geosmithia putterillii (Thom) Pitt Fig. 5.38

Penicillium putterillii Thom

Colonies on CYA 25–35 mm diam, velutinous, floccose or somewhat funiculose, mycelium white or buff, conidial production moderate to heavy, off white to buff or very pale yellow; reverse dull yellow or olive brown. Colonies on MEA 20–30 mm diam, usually markedly funiculose, otherwise as on CYA. Colonies on G25N 15–18 mm diam, similar to those on CYA. At 5 °C, no germination to formation of microcolonies. At 37 °C, usually no growth, sometimes colonies to 8 mm diam.

Conidiophores borne from surface or aerial hyphae, stipes 20–100 µm long, with rough walls; penicilli often complex, with 2, 3 or more branch points; phialides rough walled, cylindrical, 8–10 µm long; conidia cylindrical, 4.0–5.0 × 2.0–2.5 µm, smooth walled.

Distinctive features *Geosmithia putterillii* forms quite slowly growing off white to buff colonies, with irregular penicilli and distinctive cylindrical conidia.

Taxonomy Kolarík et al. (2004) published a comprehensive molecular and morphological study of *G. putterillii* and related taxa. For the most recent full taxonomy of the genus see Huang et al. (2019).

Identifiers Mycobank MB314415; holotype IMI 40212; ex-type cultures IMI 40212 = CBS 233.38 = NRRL 2024 = FRR 2124 = ATCC 10487.

Physiology Information on the physiology of *Geosmithia putterillii* is lacking. However, judged by its isolation from a variety of dry substrates, this species is xerophilic.

Mycotoxins Mycotoxin production has not been reported.

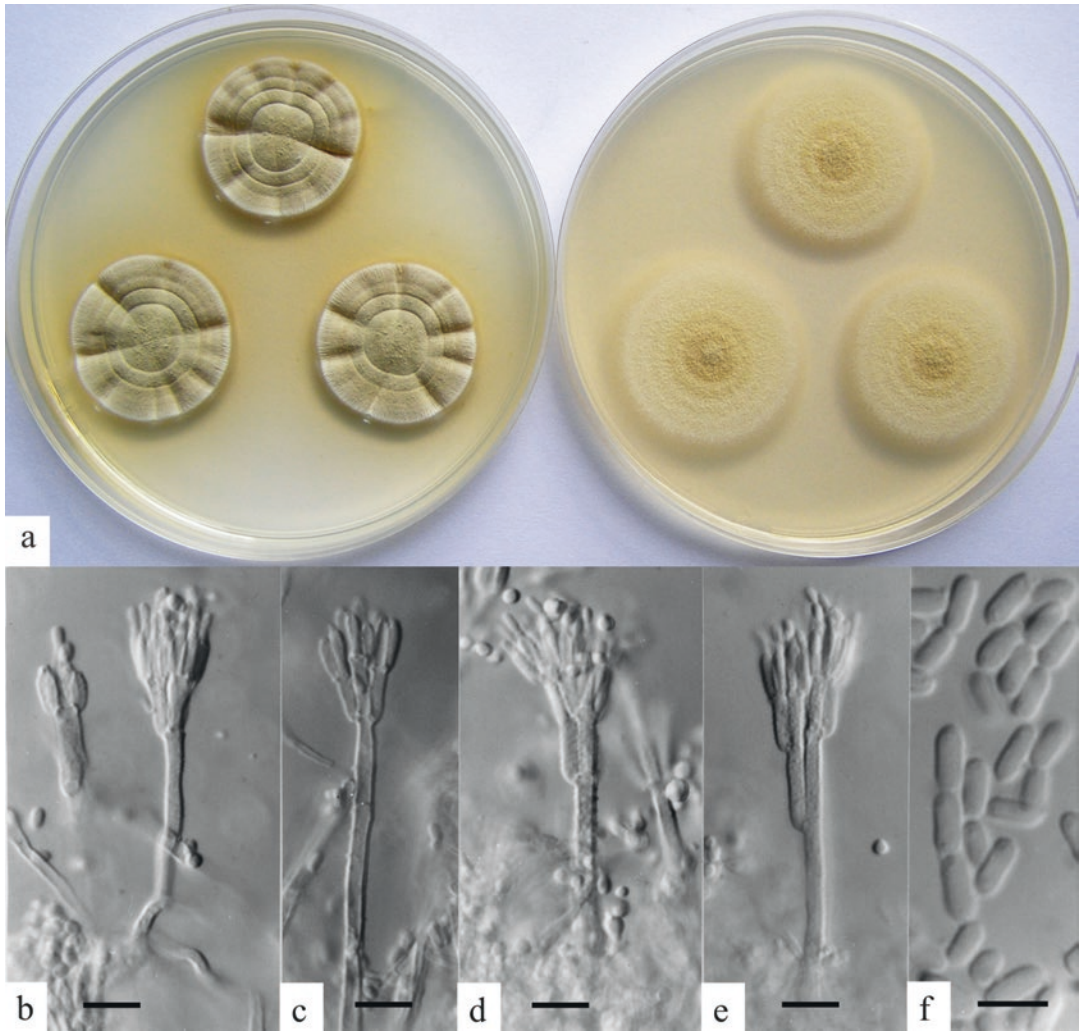

Fig. 5.38 *Geosmithia putterillii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**e**) penicilli, bars = 10 µm; (**f**) conidia, bar = 5 µm

Ecology Most foodborne isolations of this species have come from cereals and cereal products, katsuobushi and dried peas (see Pitt and Hocking 1997), although Kolarík et al. (2004) report that it is normally associated with bark beetles. In our laboratory it has twice been recorded as the cause of spoilage of Lebanese bread, on which it produces a profuse white growth. We have also isolated it from Indonesian soybeans (Pitt et al. 1998a). It has been reported (as *Penicillium pallidum*) as one cause of rots in melons (Snowdon 1991).

Reference Pitt (1979), Kolarík et al. (2004), and Huang et al. (2019).

5.19 Genus *Hyphopichia* Arx & van der Walt

Hyphopichia is a genus of yeast-like fungi, probably closely related to *Endomyces*, from which it differs by the production of hyphal fragments which produce yeast-like conidia by budding from spicules. There is a single species, *H. burtonii*.

Hyphopichia burtonii (Boidin et al.) Arx & van der Walt Fig. 5.39

Candida chodatii (Nechitsche) Berkhout
Candida variabilis (Lindner) Kerkhout
Cladosporium chodatii (Nechitsche) Sacc.
Cladosporium fermentans Goto et al.
Pichia burtonii Boidin et al.
Endomycopsis burtonii (Boidin et al.) Kreger-van Rij
Endomycopsis chodatii Wick.
Trichosporon behrendii Lodder & Kreger-van Rij
Trichosporon variabile (Lindner) Delitsch

Colonies on CYA 22–25 mm diam and on MEA 25–30 mm diam, of low and sparse to moderately dense white to pale grey mycelium, sometimes centrally umbonate; reverse pale. Colonies on G25N 10–12 mm diam, similar to those on CYA. No germination at 5 °C. At 37 °C, colonies 5–10 mm diam, similar to at 25 °C; reverse pale.

Conidia borne from spicules (small projections) along the length of undifferentiated hyphae, yeast-like, ellipsoidal, pyriform or near cylindroidal, 2.5–5.0 × 1.5–2.5 µm, with thin, smooth walls; hyphal fragments also evident, 10–50 × 2.5–3.0 µm, still functioning as fertile hyphae, producing conidia (blastospores) from lateral spicules and terminal buds. Ascospores not observed in pure culture, two mating strains needed; ascospores hat-shaped, 1–4 per ascus.

Distinctive features This species produces white, powdery, filamentous colonies which bear conidia from spicules on vegetative hyphae, and also by budding (yeast-like cells). It is distinguished from *Endomyces fibuliger* by the fragmentation of hyphae into short lengths; these can also act as reproductive structures.

Taxonomy This species and *Endomyces fibuliger* have rarely been placed in the same genus by taxonomists, but in culture the resemblance is unmistakable. Both grow at more or less the same rates and have similar habitats. One (*E. fibuliger*) is homothallic and the other heterothallic; however, the fragmenting of fertile hyphae in *Hyphopichia burtonii* is the only obvious difference in morphology. Molecular studies indicated that this species is can be split into three phylogenetic clusters; one of them was named *H. pseudoburtonii* Groenew. & M.T. Sm. (Groenewald and Smith 2010). It can be expected to be similar physiologically to *H. burtonii.*

The teleomorphs of *Hyphopichia burtonii* and *H. pseudoburtonii* have been seen only in the laboratory, after mixing of two mating strains. However, the teleomorph name is used here, both because it has become accepted in the recent literature and because there are several confusing anamorph names, at both genus and species level. Some taxonomies have included this species in the yeasts, as *Pichia burtonii* Boidin et al. (Kurtzman and Fell 1998; Barnett et al. 2000) or as *H. burtonii* (Kurtzman et al. 2011).

Identifiers MycoBank MB315565; holotype CBS 2352; ex-type cultures ATCC 13169 = NRRL Y-1933.

Physiology *Hyphopichia burtonii* grows more rapidly at 30 °C than 20 °C (Ramakrishna et al. 1993). The minimum is >5 °C and growth occurs at 37 °C. Growth occurs at least down to 0.90 a_w on barley grains (Ramakrishna et al. 1993).

Mycotoxins This type of fungus is unlikely to produce toxic compounds.

Ecology This species is not uncommon in cereals and cereal products, especially packaged bread (Spicher 1984, 1985). In Europe, along with *Endomyces fibuliger*, it is known as "chalky mould" (Spicher 1986). It also forms part of the flora of Parma hams during ripening (Simoncini et al. 2007).

Reference Kurtzman et al. (2011).

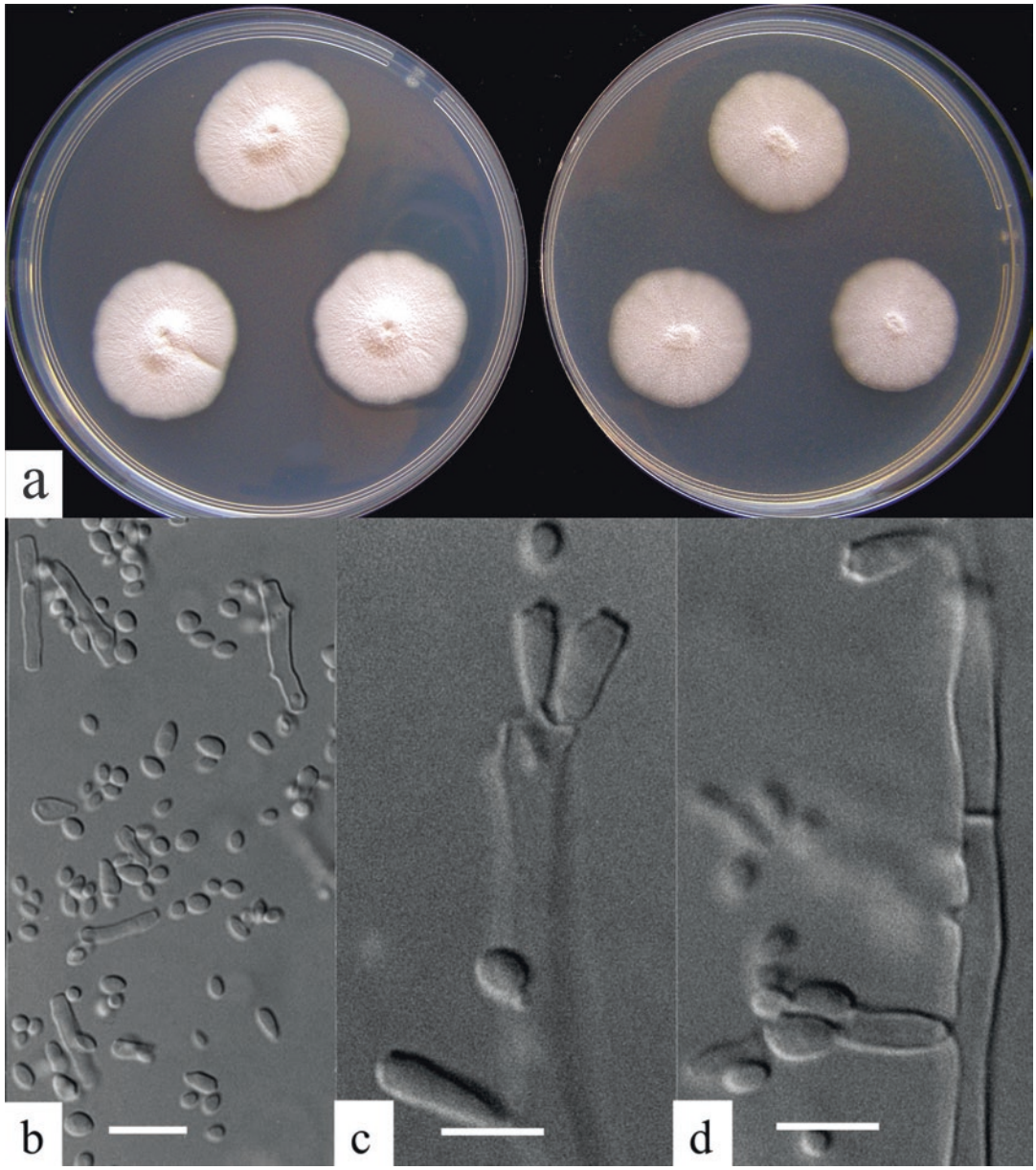

Fig. 5.39 *Hyphopichia burtonii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) vegetative cells, bar = 10 µm; (**c**,**d**) vegetative cells, bar = 5 µm

5.20 Genus *Lasiodiplodia* Ellis & Everh

The genus *Lasiodiplodia,* previously known as *Botryodiplodia* Sacc. (Sutton 1980), includes about 20 species, many of them plant pathogens. Morphologically, the genus is characterised by distinctive large, striate conidia, as described below. A molecular overview is provided by de Silva et al. (2019). The most common species is *L. theobromae*, which has a very wide host range, including many economically important plants and food crops.

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. Fig. 5.40

Botryodiplodia theobromae Pat.
Botryosphaeria rhodina (Berk. & Curt.) Arx

Colonies on CYA and MEA usually filling the whole Petri dish with a loose to moderately dense weft of light to dark grey hyphae, adhering to the Petri dish lid; pycnidia borne beneath the agar surface (visible from the reverse), and sometimes at the base-lid interface or on the lid itself; reverse pale, black near pycnidia, uniformly grey black elsewhere. On G25N, colonies 30–50 mm diam, occasionally more, low and spreading, with sparse white aerial mycelium; reverse pale or grey. No growth at 5 °C. At 37 °C, colonies greater than 50 mm diam, of low, dense mycelium, coloured grey or deep red; red brown soluble pigment and occasionally exudate produced; reverse deep red brown to almost black.

Reproductive bodies on CYA pycnidia, grey black and roughly spherical, 200–400 µm diam, but on other media reportedly up to 5 mm diam (Punithalingam 1976), or forming a stroma (Alasoadura 1970), easily ruptured, of dark brown to black pseudoparenchymatous cells; conidia at 7 days ellipsoidal, 15–20 × 9–12 µm, with smooth, brownish walls and little ornamentation, at maturity (18–)20–30 × 10–15 µm, with a transverse septum and ornamented by longitudinal striations.

Distinctive features *Lasiodiplodia theobromae* is distinguished in culture by fast growth on G25N and at 37 °C: on G25N growth is sparse and usually colourless, but at 37 °C it is dense, with mycelium grey and/or red. Microscopically, this species is distinguished by large ellipsoidal conidia which are borne in black pycnidia, and are ornamented with a transverse septum and longitudinal striations.

Taxonomy A molecular study using a combined analysis of the ITS region and the translation elongation factor 1-α indicated that *L. theobromae* as currently understood includes three species, distinguishable by differences in the size of their conidia (Alves et al. 2008).

This species is sometimes known by its teleomorph name, *Botryosphaeria rhodina*, but this state is not usually seen in culture.

Identifiers Mycobank MB188476.

Physiology Alasoadura (1970) reported cardinal temperatures for *Lasiodiplodia theobromae* as minimum 15 °C, optimum 28 °C and maximum 40 °C. This is difficult to reconcile with statements by Uduebo (1974) that "The vegetative

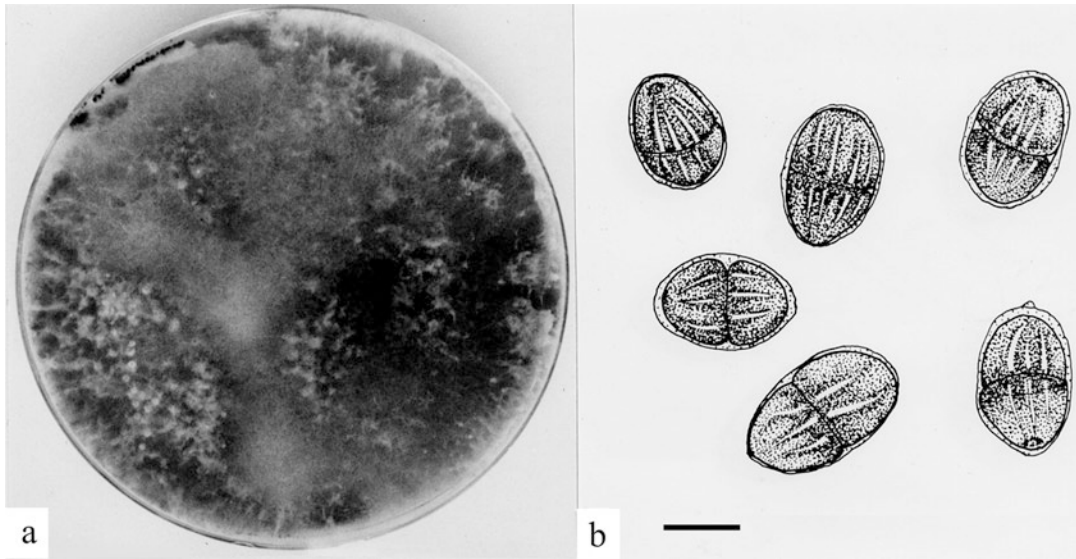

Fig. 5.40 *Lasiodiplodia theobromae* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) conidia, bar = 10 µm

growth at 37°C is scanty", or that this species does not grow at 35 °C on potato dextrose agar (Adeniji 1970b). Isolates of this species which we have studied grew rapidly at 37 °C, and our observations are consistent with the statement that distribution of *L. theobromae* is mainly confined to the area between 40°N and 40°S (Punithalingam 1976). Several references suggest that germination and growth of *L. theobromae* is confined to high water activities: vigorous growth observed on G25N in this study is at variance with this.

Mycotoxins Mycotoxin production has not been reported.

Ecology Punithalingam (1976) aptly described *Lasiodiplodia theobromae* as "an unspecialised virulent rot pathogen causing numerous diseases dieback, root rot, rot or decay of various fruits and storage rot of yams." Spoilage by *L. theobromae* of avocados, bananas, grapes, guavas, mangoes, okra, papayas, passionfruit, traditional Nigerian foods and yams has been reported (see Pitt and Hocking 1997). Control measures for fruits are well documented by Punithalingam (1976).

Lasiodiplodia theobromae has also been reported from pecans, peanuts, wheat and soybeans (see Pitt and Hocking 1997). In our experience, it is of common occurrence in many tropical food commodities. We isolated it from 58% of Thai maize samples, at up to 46% of kernels in infected samples and in 7% of all kernels examined (Pitt et al. 1993). It was also common in Thai peanuts, mung beans and sorghum, with 30–33% of samples showing infection (Pitt et al. 1993, 1994). Indonesian crops were equally affected, particularly peanuts, where 47% of samples were positive, with up to 64% of kernels affected in those samples, and overall 9% of the more than 12,000 peanut kernels examined were infected with this species. More than 25% of maize, talo bean, velvet bean, black soybean and cowpea samples also showed infection (Pitt et al. 1998a).

References Punithalingam (1976), Domsch et al. (1980), under *Botryosphaeria*, and Silva et al. (2019).

5.21 Genus *Monascus* Tiegh

Monascus is a genus of ascomycetes characterised by the production of colourless to pale brown cleistothecia and aleurioconidia. Each cleistothecium is borne from a knot of hyphae on a well defined stalk, in 7 day old cultures resembling a clenched fist on a narrow forearm. Aleurioconidia occur singly or in short chains. Asci break down rapidly so that, when ascospores are mature, the impression under the microscope is of a sac filled with a mass of ellipsoidal, smooth walled, refractile spores.

Species of *Monascus* are best known for their role in the fermentative production of Oriental foods, of which red rice (ang-kak), rice wine and kaoliang brandy are the best known (Hesseltine 1965; Lin 1975; Chen et al. 2015). Of 11 described species, three were accepted by Hawksworth and Pitt et al. (1983): two, *M. purpureus* Went and *M. pilosus* K. Saito ex D. Hawksw. & Pitt, were considered to be associated almost exclusively with fermented foods and the third, *M. ruber* Tiegh., to be of widespread occurrence and sometimes caused food spoilage. The discovery that *Monascus* species are associated with stingless bees led to a complete taxonomic revision of the genus by Barbosa et al. (2017). Nine species were accepted, with *M. ruber,* treated below, remaining the only species commonly associated with foods. For a complete taxonomy of the genus see Barbosa et al. (2017).

Monascus ruber Tiegh. **Fig. 5.41**

Monascus pilosus K. Saito ex D. Hawksw. & Pitt

Colonies on CYA 20–32 mm diam, occasionally only 15 mm, plane, sparse, surface texture floccose to deeply floccose; mycelium white at first, then becoming pale brown as cleistothecia and aleurioconidia develop, in age sometimes becoming dark brown; brown soluble pigment sometimes produced; reverse sometimes uncoloured, usually brown to dark brown near sepia. Colonies on MEA 30–42 mm diam, plane and sparse,

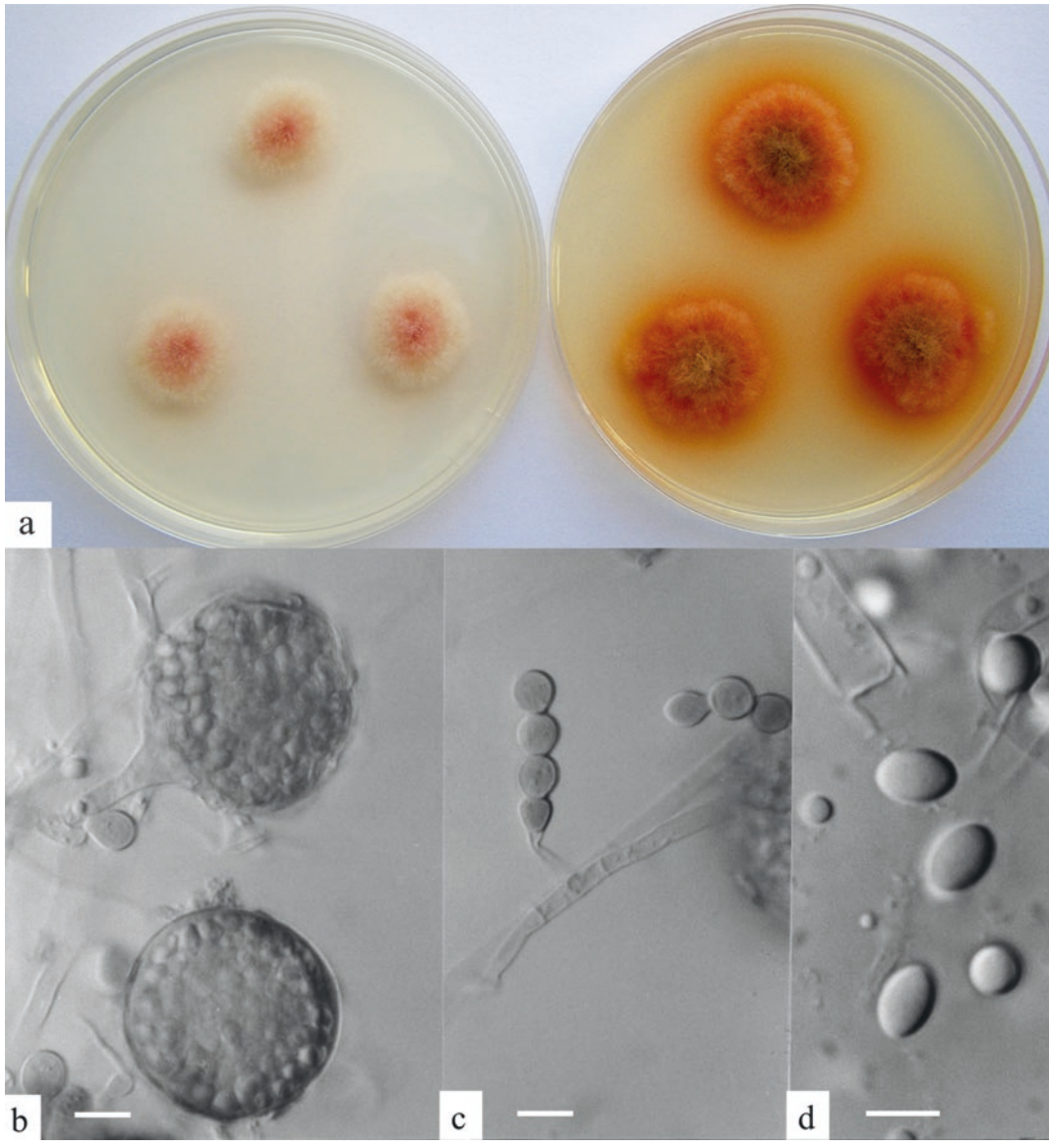

Fig. 5.41 *Monascus ruber* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) cleistothecia, bar = 10 µm; (**c**) aleurioconidia, bar = 10 µm; (**d**) ascospores, bar = 5 µm

sometimes with little aerial growth but usually with deeply floccose mycelium, white at the margins, becoming brown to orange brown as cleistothecia and aleurioconidia develop; orange or brown soluble pigment sometimes produced; reverse pale at the margins, khaki or brownish orange at the centres. Colonies on G25N usually 16–20 mm diam, sometimes only 10 mm, plane, sparse and floccose; mycelium white; reverse uncoloured or brown. No growth at 5 °C. At 37 °C, colonies 12–30 mm diam, low and sparse, coloured as on CYA at 25 °C, or with reverse deeper brown and sometimes with a reddish tinge.

Cleistothecia spherical, 30–60 µm diam, borne as a hyphal knot from a well defined stalk, with cellular walls, becoming brown during maturation; ascospores ellipsoidal, hyaline, 5–7 × 4.0–4.5 µm, smooth walled. Aleurioconidia sometimes borne on pedicels from the sides of

hyphae, but more commonly terminally, sometimes borne singly but more often in chains of up to 10 cells, spherical to pyriform, often rounding at maturity, 10–14 µm diam or 10–18 × 8–14 µm, with thick, smooth, brown walls. Chlamydoconidia and arthroconidia produced by most isolates also.

Distinctive features The cleistothecium produced by *Monascus* is distinctive, being borne as a fist-like hyphal knot on an arm-like stalk. *M. ruber* is distinguished from other *Monascus* species by relatively rapid growth, especially on MEA, and by brown pigmentation in the walls of cleistothecia and aleurioconidia.

Taxonomy Sequencing of the ITS region and part of the β-tubulin gene indicated that *M. ruber* and *M. pilosus* were conspecific (Park et al. 2004) and that was confirmed by Barbosa et al. (2017). Phylogenetic analysis showed that *Monascus* was closely related to the xerophilic genera *Xeromyces* and *Xerochrysium* (Barbosa et al. 2017).

Identifiers MycoBank MB234876; neotype CBS 135.60; ex-type strains IMI 81596 = ATCC 15670 (Barbosa et al. 2017).

Physiology The cardinal temperatures reported for *Monascus ruber* are minimum 15–18 °C, optimum 35 °C and maximum near 45 °C (Manandhar and Apinis 1971; Panagou et al. 2003). *M. ruber* has been isolated from relatively concentrated substrates (such as high moisture prunes, *ca* 0.85 a_w) and is probably weakly xerophilic. However, an isolate failed to grow in a solution of NaCl at 0.92 a_w (Panagou et al. 2003). Growth of *M. ruber* was little affected over the pH range 3.0–5.0 (Panagou et al. 2003) and was able to grow down to pH 2.2 at 30 °C in high a_w (Panagou et al. 2005). The combined effect of temperature (25–35 °C), water activity (0.999–0.92 a_w) and pH (2.0–6.8) was studied on gradient plates by Panagou et al. (2005), and also modelled against several equations (Panagou et al. 2003, 2005, 2007).

Ascospores of *M. ruber* are heat resistant. D_{80} in citrate buffer (pH 4) was 2.1 min. NaCl provided some protection: D_{80} values were 0.88 min in 5.6% brine, but 1.04 min in 10.5% brine. *z* values over the temperature range 70–80 °C varied from 7.4 to 7.9 C° under the conditions used (Panagou et al. 2002). They concluded that a heat process of 5 min at 80 °C would assist the olive industry in producing a stable product.

Pigmentation has received a great deal of attention, having been used to distinguish a number of species utilised in Oriental fermentations. However, Carels and Shepherd (1977) showed that red pigments form during growth near neutral pH values, whereas cultures become orange if the pH of the fermenting food becomes strongly acid. Pigment stability is affected by pH, temperature, light, oxygen and water activity (Dufosse et al. 2005). The production of pigments by *Monascus* species has been reviewed by Miyake et al. (2008) and Chen et al. (2015).

Mycotoxins *Monascus ruber* (and *M. purpureus*) produce citrinin (Blanc et al. 1995a, b) both in submerged and solid state culture. This is of importance as red pigments produced by *Monascus* species are used as food colourants. Recent studies indicate that high pigment production can be achieved without citrinin synthesis by incorporating histidine in the growth media (Hajjaj et al. 2000; Xu et al. 2003). Wang et al. (2005) surveyed type cultures of 23 species of *Monascus* (most now regarded as synonyms) for their ability to synthesise citrinin and found that all, including *M. ruber*, produced citrinin.

Ecology A widespread species, *Monascus ruber* has caused spoilage of high moisture Australian prunes (Hawksworth and Pitt 1983). It was isolated quite frequently from Indonesian dried fish (Wheeler et al. 1986). Other sources include mayonnaise (Muys et al. 1966a), bread (Spicher and Isfort 1988), table olives (Panagou et al. 2002), and minced meat, soft cheese, fruit sauce, spices, honey, cacao beans, soy beans, peas, palm kernels, maize and various animal feeds, and silage (data sheets, International Mycological Institute, Egham, Surrey; Hawksworth and Pitt 1983).

References Hawksworth and Pitt et al. (1983) and Barbosa et al. (2017).

5.22 Genus *Moniliella* Stolk & Dakin

Moniliella is a genus of yeast-like fungi, characterised by the production of budding cells, thin walled arthroconidia and, in one species, by relatively large chlamydoconidia (up to 3× hyphal diameter). In some respects it is similar to *Hyphopichia*, but conidia are larger, and the hyphal fragments bearing conidia on spicules, characteristic of *Hyphopichia,* are absent. The taxonomic review by de Hoog (1979), accepted two species, *M. suaveolens* and *M. acetoabutans*, and these remain the species of importance in foods. *M. suaveolens* is found in butter and margarine, and other substrates rich in oil, while *M. acetoabutans* is of interest because of its resistance to acetic acid and hence potential ability to spoil mayonnaise and other products preserved with acetic acid. New species *M. carnis* and *M. dehoogii* were described from a meat fermentation process by Thanh et al. (2012), and more recently *M. sojae* was described from a Vietnamese soy paste fermentation (Thanh et al. 2018). All three are related to *M. suaveolens*, which is also known as *Saprochaete suaveolens* and *Vanriji humicola*. See Taxonomy under the species name below.

Key to *Moniliella* species included here

1	Spherical chlamydoconidia produced, 8–12 µm diam, with thick, brown walls; growth on malt acetic agar	*M. acetoabutans*
	Thick walled chlamydoconidia not produced; no growth on malt acetic agar	*M. suaveolens*

Moniliella acetoabutans Stolk & Dakin Fig. 5.42

Colonies on CYA and MEA 22–30 mm diam, deeply floccose, especially on CYA, mycelium pure white; reverse yellow brown. Colonies on G25N 5–10 mm diam, deep but often mucoid, uncoloured. No growth at 5 °C. Usually no growth at 37 °C, occasionally colonies up to 5 mm diam.

Conidia of three types produced, budding cells from hyphal extremities, arthroconidia by differentiation of hyphal tips, and chlamydoconidia, in intercalary or terminal positions on hyphae, solitary or in short chains; budding conidia ellipsoidal, arthroconidia cylindroidal, both 5–9 µm long, chlamydoconidia spherical, 8–12 µm diam, with thick brown walls.

Taxonomy A primer set for rapid detection of *Moniliella acetoabutans* (and *M. suaveolens*) in food raw materials by PCR was developed by Nakayama et al. 2016.

Distinctive features *Moniliella acetoabutans* is morphologically distinguished by its three types of conidia: budding conidia like a hyaline *Cladosporium*; arthroconidia like *Galactomyces*, but not so extensive; and relatively large, brown walled chlamydoconidia. In culture, the definitive test for *M. acetoabutans* is its ability to produce quite rapidly growing white colonies on malt acetic agar – or on MEA containing 2% acetic acid.

Identifiers MycoBank MB334423; holotype CBS 169.66; ex-type strains CBS 169.66 = IMI 159918 = ATCC 18455.

Physiology This species appears to be unique in its tolerance of acetic acid and other weak acid preservatives. Adaptation to 1.75% acetic acid by the type strain of *M. acetoabutans* in "PDB" medium (perhaps potato dextrose broth) was reported by Nakayama et al. (2016). In our laboratory, *M. acetoabutens* was able to grow in MEA with 4% added acetic acid, to our knowledge a unique property. This species is capable of fermentative growth, like a true yeast, and also appears to be highly tolerant of acid pH.

Mycotoxins Toxic compounds are not produced.

Ecology Dakin (Stolk and Dakin 1966) isolated *Moniliella acetoabutans* from spoiling sweet fruit sauce, and then a variety of other similar products. It also caused fermentative spoilage of a large production run of mayonnaise in Australia in 1971. The source was wooden vinegar tanks, where the fungus was surviving in 10%

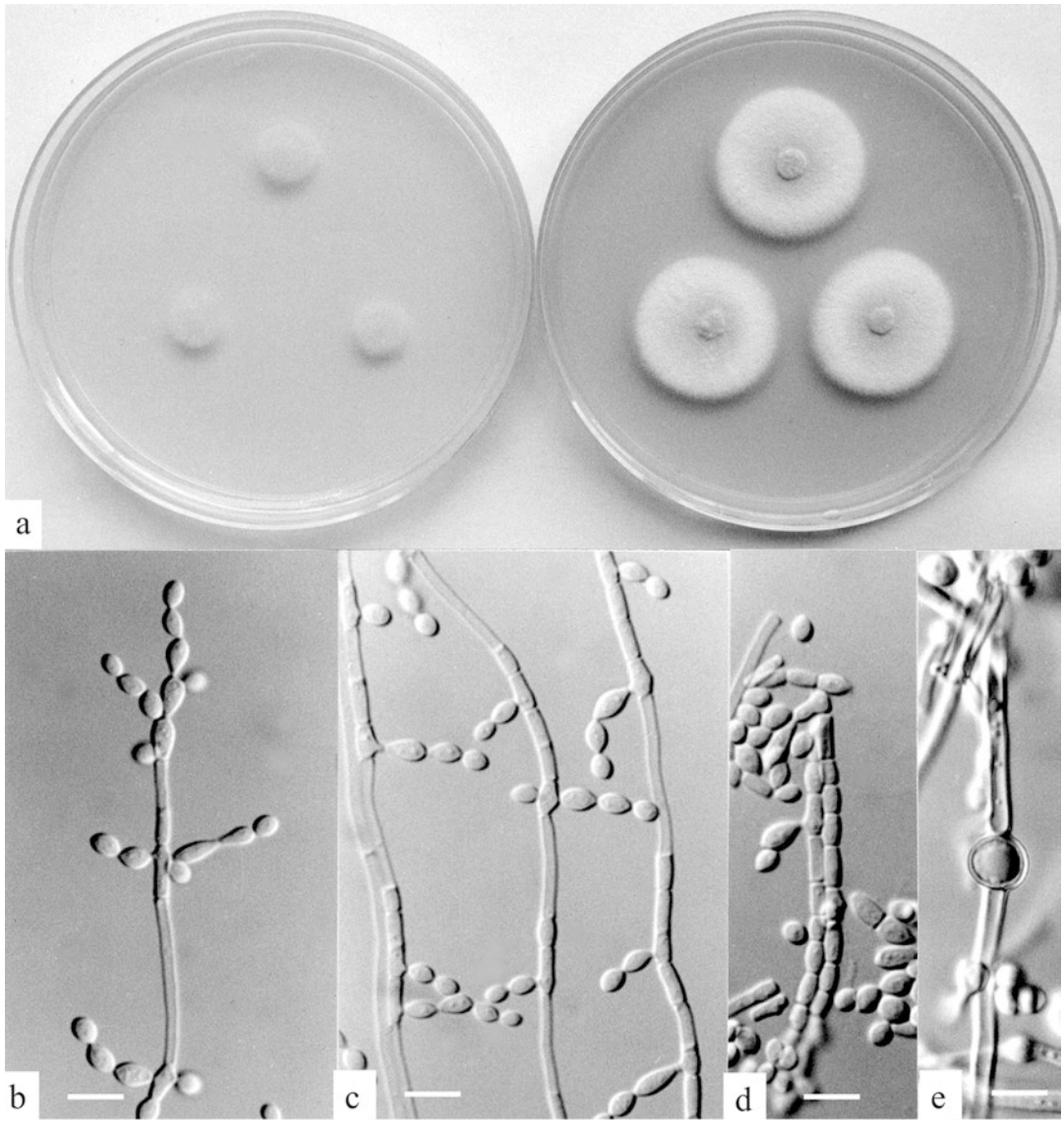

Fig. 5.42 *Moniliella acetoabutans* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) conidiophores producing budding cells (slide culture), bar = 10 µm; (**d**) arthroconidia, bar = 10 µm; (**e**) chlamydoconidia, bar = 10 µm

acetic acid, then growing on the wood as the tank level lowered and the acetic acid became diluted by evaporation. Data sheets from the International Mycological Institute, Egham, Surrey, record isolations from various acetic acid preserves.

Moniliella acetoabutans infections appear to be rare, though perhaps increasing (Nakayama et al. (2016). Always associated with vinegar, they may be overcome either by holding vinegar or acetic acid in stainless steel tanks, or by pasteurising this ingredient before addition to product.

References Stolk and Dakin (1966), Dakin and Stolk (1968), de Hoog (1979), and Nakayama et al. (2016).

Moniliella suaveolens (Lindner) Arx

Fig. 5.43

Cladosporium suaveolens (Lindner) Delitsch
Cladosporium butyri O.S. Jensen
Saprochaete suaveolens (Krzemecki) de Hoog & M.T. Sm.
Vanrija humicola (Dasz.) R.T. Moore

Colonies on CYA and MEA highly variable in character, 15–40 mm diam, either white, floccose, sparse and persistently white, or low, dense, velutinous and olive, lightly to heavily sporing; reverse colourless or olive. Colonies on G25N 3–8 mm diam, of white mycelium. At 5 °C, sometimes germination. At 37 °C, no growth.

Conidiophores undifferentiated, bearing conidia in short, sometimes branched, acropetal chains (the youngest spore at the end), breaking up in liquid mounts; conidia subspheroidal or ellipsoidal to cylindroidal, or somewhat irregular, nonseptate, when ellipsoidal 9–13 × 7–10 µm, when cylindroidal commonly 15–20 × 5–7 µm, with smooth, slightly thickened walls.

Distinctive features Unlike *Moniliella acetoabutans*, this species does not make chlamydoconidia. It is not preservative resistant, and is normally associated with oils and oil-based foods.

Taxonomy *Moniliella suaveolens* as defined by de Hoog (1979) included two varieties which culturally bear little resemblance to each other. One (*M. suaveolens* var. *suaveolens*) produces low, spreading colonies, which remain white; the other (*M. suaveolens* var. *nigra*) forms compact, dense colonies, which rapidly become olive. Thanh et al. (2018) segregated some isolates of *M. suaveolens* into new species, but failed to identify their relationship with the varieties accepted by de Hoog (1979).

In recent years, the taxonomy and nomenclature of this species have become confused. *Index Fungorum* lists this species under the name *Saprochaete suaveolens* (Krzemecki) de Hoog & M.T. Sm., using the taxonomy of de Hoog and Smith (2004), while CBS uses *Vanrija humicola* (Dasz.) R.T. Moore (Moore 1980), following the more recent taxonomy of Liu et al. (2015). In the latter case, no obvious connection exists between the name used and the name in *Moniliella. S. suaveolens* is more common in the literature, often relating to flavour compounds produced by this species, but *Moniliella suaveolens* is also still used. We have decided to retain the latter name here.

Identifiers MycoBank MB317819; holotype CBS 126.42; ex-type culture CBS 126.42.

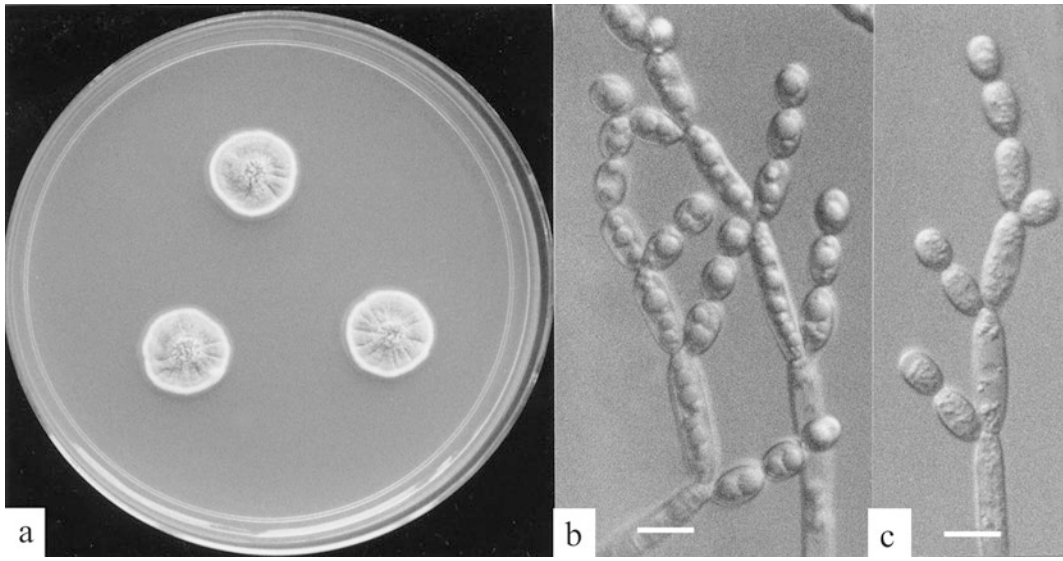

Fig. 5.43 *Moniliella suaveolens* (**a**) colonies on MEA, 7 d, 25 °C; (**b**, **c**) vegetative cells (slide culture), bar = 10 µm

Physiology No physiological studies are known to us.

Mycotoxins Mycotoxins are not produced.

Ecology This species has caused spoilage of margarine in Europe (Muys et al. 1966a, b; Bours and Mossel 1973) and Australia (Hocking and Pitt, unpublished). It has been reported as one of the causes of chalky bread in Europe (Spicher 1985, 1986; Spicher and Isfort 1987). It has occasionally been isolated from cheese (Chabalier et al. 1997; Torkar and Vengust 2008).

References de Hoog (1979), de Hoog and Smith (2004), Liu et al. (2015), and Thanh et al. (2018).

5.23 Genus *Neocosmospora* E.F. Sm

The genus *Neocosmospora* E.F. Sm. 1899 was formally revived for the very well known species *Fusarium solani* by Lombard et al. (2015) on the basis of a systematic molecular review of the family *Nectriaceae*. That review indicated that *Neocosmospora* was separated from *Fusarium* by other genera: although all of these genera were related, the morphological and phylogenetic differences were sufficient to indicate that separation was in the best interests of long term stability in these important and taxonomically difficult genera. Sandoval-Denis and Crous (2018) provided a detailed taxonomy of *Neocosmospora*, describing and accepting 15 species.

Neocosmospora is better known as the "*Fusarium solani* complex" and includes species and clades of great importance as both plant and animal pathogens (Sandoval-Denis and Crous 2018). *Neocosmospora* species produce colonies which are predominantly white or cream coloured on PDA or DCPA, lacking the red or purple pigments characteristic of *Fusarium* species. As is the case in other species complexes considered here under *Fusarium,* physiological and molecular species are known by a mix of formal species and informal numbers. *Neocosmospora solani* is described here as the major species within the genus and as a representative of this group of fungi.

Neocosmospora solani (Mart.) L. Lombard & Crous Fig. 5.44

Fusarium solani (Mart.) Sacc.
Haemanectria haematococca (Berk. & Broome) Samuels & Rossman

Colonies on CYA 60–65 mm diam, low, of moderately dense white mycelium, often covered in very fine droplets of clear exudate; a central, cream spore mass sometimes present; reverse pale, sometimes with bluish or greenish areas. Colonies on MEA 50–60 mm diam, low to moderately deep, of sparse, often slightly funiculose, white to pale violet mycelium; reverse pale, sometimes bluish grey centrally. Colonies on G25N 3–8 mm diam. No growth at 5 °C. At 37 °C, either no growth, or colonies up to 10 mm diam formed.

On PDA, colonies low to moderately deep, of white to cream mycelium in concentric rings, often alternating with rings of cream or bluish grey sporodochia; in some isolates the teleomorph produced, of dark orange perithecia scattered over the central area of the colony; reverse pale or with areas of turquoise grey or pale violet brown. On DCPA, colonies low, of sparse, white mycelium in annular rings, with a central mass of cream sporodochia; reverse pale to pale yellow brown.

Macroconidia abundant, stout, thick walled, with 3–4, or less commonly 5 septa, straight, parallel sided for most of the length, apical cells blunt and rounded, basal cells either rounded, notched or sometimes distinctly foot-shaped. Microconidia usually abundant, ellipsoidal, fusiform or kidney-shaped, produced in false heads on very long, straight phialides. Chlamydoconidia produced singly or in pairs.

Distinctive features *Neocosmospora solani* produces colonies which are predominantly white or cream coloured on PDA and DCPA, lacking the red or purple pigments characteristic of *Fusarium* species. Sporodochia are cream coloured rather than the salmon or orange types produced by *Fusarium* species. The macroconidia are stout and straight or only slightly

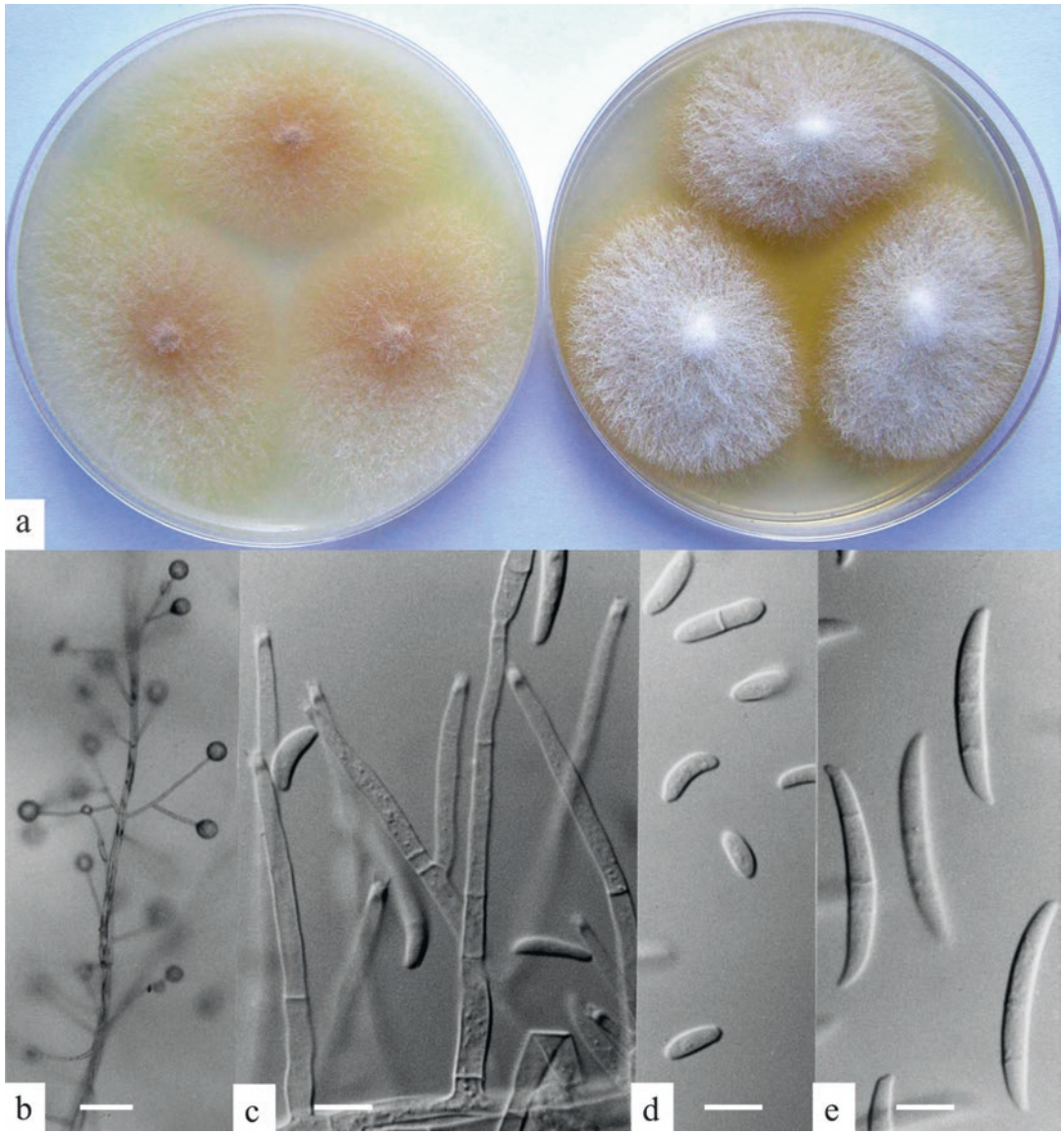

Fig. 5.44 *Neocosmospora solani* (**a**) colonies on PDA and DCPA, 7 d, 25 °C; (**b**) phialides bearing macroconidia in false heads *in situ*, bar = 50 µm; (**c**) phialides, bar = 10 µm; (**d**) microconidia, bar = 10 µm; (**e**) macroconidia, bar = 10 µm

curved. Microconidia are produced in false heads (mucoid balls) on very long, slender phialides. In micromorphology, this species is very similiar to *Fusarium oxysporum*, but *N. solani* has much longer phialides.

Taxonomy Schroers et al. (2016) provided a detailed taxonomy of *Neocosmospora solani* and related species.

Identifiers Mycobank MB810964; lectotype MBT203352; epitype CBS H-22335; ex-epitype cultures NRRL 66304, CBS 140079. Additional markers ITS/28S KT313633, *tef* KT313611, *RPB2* KT313623 (Schroers et al. 2016).

Physiology Domsch et al. (1980) noted that various authors have reported the optimum growth temperature for *Neocosmospora solani* to

be between 27 and 31 °C, with strong growth at 37 °C. However, our observations indicate only weak growth at this temperature, and Chaturvedi et al. (2003) reported optimum growth at 25 °C. Schneider (1954) reported growth of *N. solani* down to 0.90 a_w after a germination time of 8 weeks at 20 °C.

Mycotoxins *Neocosmospora solani* produces neither trichothecene nor fumonisin toxins, likely because this genus separated from *Fusarium* before those groups of toxins arose (O'Donnell et al. 2013). Mouldy sweet potatoes (*Ipomoea batatas*) or extracts from them have been shown to be toxic to a variety of animals, including cattle (Wilson et al. 1970; Doupnik et al. 1971), chickens (Doupnik et al. 1971; Peckham et al. 1972) and mice (Boyd and Wilson 1972). The toxic principles are believed to be furanoterpenoids, ipomeanols and ipomeanine (Nelson et al. 1983, 1994; Mawhinney et al. 2008). These compounds are not mycotoxins, but result from fungal metabolism of phytoalexins produced by the plant (see Leslie and Summerell 2006). *N. solani* has been reported to produce fusaric acid (Bacon et al. 1996) and the immunosuppressive compound cyclosporin (Sugiura et al. 1999; Coleman 2016).

Ecology A cosmopolitan soil fungus, *Neocosmospora solani* has frequently been isolated from subterranean crops such as potatoes, sweet potatoes, yams and peanuts (see Pitt and Hocking 1997; Ismail 2001; Peters et al. 2008). Hot-water dipping (57.5 °C, 20–30 min) can reduce the incidence of storage rots of potatoes caused by *N. solani* (Ranganna et al. 1998). *N. solani* may also invade a wide variety of other vegetable crops (Snowdon 1991), especially cucurbits including muskmelons (Champaco et al. 1993) and squash (Assawah and Al-Zarari 1984), and legumes such as beans, soybeans and peas (see Pitt and Hocking 1997; Tseng and Tu 1997). Other sources are diverse, including small grains, where it may be part of the cohort of *Fusarium* species causing head blight (Bottalico and Perrone 2002), maize, sorghum, bananas, guavas, cassava, sugar beets, capsicums, garlic, and seeds of sunflower, sesame and coriander (see Pitt and Hocking 1997). We have isolated *N. solani* from beer, shortening flakes, chilled water lines in a beverage production plant, the inside of a juice filling machine, and an air conditioning duct.

This species was widespread in Indonesian crops, especially beans, usually at about 1% of all seeds examined, in paddy rice, maize, peanuts and pepper (Pitt et al. 1998a).

Fusariosis is an uncommon disease in humans, but about 50% of cases of fusariosis are due to *Neocosmospora* species (Slavin et al. 2015; Sandoval-Denis and Crous 2018). *N. solani* can attack keratin, and is a principal cause of human keratinitis of fingernails and eyes (Domsch et al. 1980; de Hoog et al. 2000; Leslie and Summerell 2006; He et al. 2011). *Neocosmospora* species are also among the most significant pathogens associated with severe infections in transplant recipients (Slavin et al. 2015; Sandoval-Denis and Crous 2018). *N. solani* can be a serious pathogen of crustaceans, attacking and destroying the chitinous exoskeleton (Fisher et al. 1978; Gonzalez 1995; Le et al. 2005) and has also been reported to cause disease in sea turtles (Cabañes et al. 1997; Castellá et al. 1999a; de Hoog et al. 2000).

References Desjardins et al. (2006), Leslie and Summerell (2006), Lombard et al. (2015), Schroers et al. (2016), and Sandoval-Denis and Crous (2018).

5.24 Genus *Neurospora* Shear & B.O. Dodge

Chrysonilia Arx

The best known species in this genus is *Neurospora sitophila*, an ascomycete which has been of great value in genetic studies. It is not found in foods, as it is heterothallic. However, the common pest species known as "red bread mould" is the asexual state of this species. It was known for a long time as *Monilia sitophila*, where it was an outlier, so was transferred to *Chrysonilia,* a genus erected by von Arx (1981a) to accommodate it. The asexual state of *N. sitophila* is characterised by very rapid growth and the production of conidia in chains, cut off

from the apices of undifferentiated hyphae. This species is readily recognisable in Petri dish culture: sparse, white to pink mycelium spreads rapidly across the dish and up the walls, then within 3 days sheds enormous numbers of orange conidia *outside* the dish. This attribute makes it a particularly troublesome laboratory contaminant. Cultivation in Petri dishes is therefore not recommended: observation of these characters in a culture tube is sufficient for positive identification.

Neurospora sitophila Shear & B.O. Dodge Fig. 5.45

Chrysonilia sitophila (Mont.) Arx
Monilia sitophila (Mont.) Sacc.

Colonies in 28 ml McCartney bottles or test tubes distinguished by pale pink floccose growth, filling the entire bottle, then turning salmon, first at the bottle neck or tube plug and subsequently throughout, as sporulation occurs.

On CYA and MEA, colonies covering the whole Petri dish, mycelium pink, reaching the lid in tufts or patches and all around the rim, producing vast numbers of salmon conidia at or near the rim and sometimes for several millimetres beyond it, and shedding them profusely; reverse salmon or pink. On G25N, colonies up to 30 mm diam produced, low, dense and mucoid. No growth at 5 °C. At 37 °C, colonies covering the whole Petri dish, similar to at 25 °C.

Reproductive structures arthroconidia, cut off in succession from the apices of branching hyphae; conidia at maturity variable in size and shape, spherical to ellipsoidal, pale orange, 6–15 µm diam, with thin smooth walls.

Distinctive features See remarks above.

Taxonomy As noted above, the species seen in the laboratory is the asexual state of the well known species used in many genetic studies. Known as *Chrysonilia sitophila* in previous editions of this book, it has been reduced to synonymy under the ICN.

Identifiers MycoBank MB278268; presumed holotype CBS 178.27.

Physiology According to Panasenko (1967), *Neurospora sitophila* is capable of growth down to 0.88–0.90 a_w.

Mycotoxins No mycotoxins have been recorded for this species.

Ecology For long a common sight in bakeries and on bread, the asexual state of *Neurospora sitophila* is less commonly encountered now, but can still be a great source of trouble as a persistent contaminant in laboratories (see Chap. 4). It has sometimes been reported from other foods: pastries, hazelnuts, beans and meat products (see

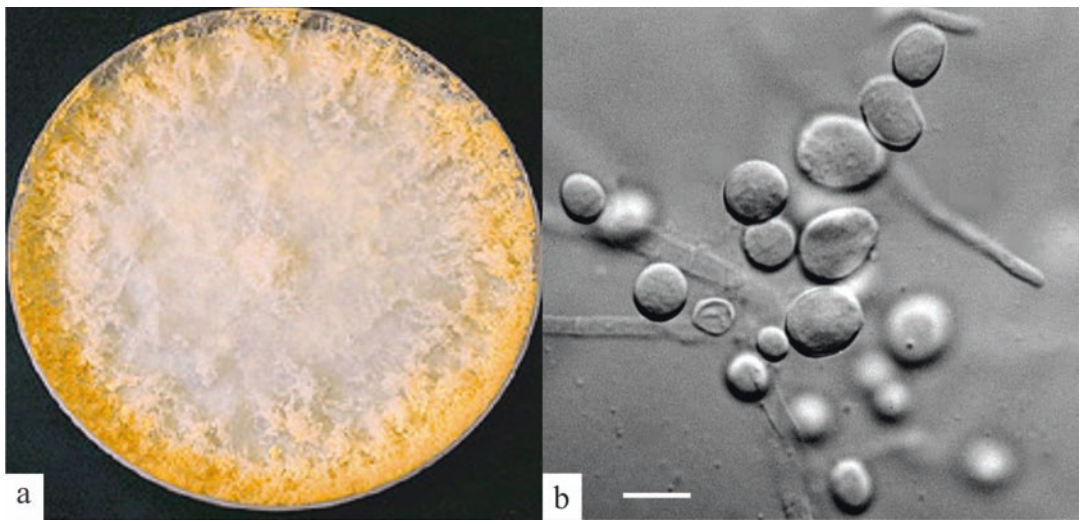

Fig. 5.45 *Neurospora sitophila* (**a**) colony on CYA, 7 d, 25 °C; (**b**) conidia, bar = 10 µm

Pitt and Hocking 1997). We isolated it frequently from Thai cassava, where it infected 36% of all pieces examined, and Philippine peanuts, where it infected up to 90% of nuts in some samples, and was present in 1% of nuts overall. It was also present in 1% of all Indonesian peanuts, sorghum and maize grains examined (Pitt et al. 1998a). Infection rates in maize and peanuts ranged up to 40% in some samples (our unpublished data). We also found it at lower levels in Thai soybeans, black beans and cashews (Pitt et al. 1994). *N. sitophila* is also known as a contaminant of cork slabs (Oliveira et al. 2003; Pereira et al. 2006).

Reference Von Arx (1981a).

5.25 Genus *Nigrospora* Zimm

Characterised by the production of relatively large, solitary, jet black, smooth walled, oblate conidia, *Nigrospora* occurs in nature mainly as a plant and seed pathogen, but is found also in air, soil and water (Wang et al. 2017). Two species are of significance in foods: *N. oryzae* and *N. spherica*.

Nigrospora oryzae (Berk. & Broome) Petch Fig. 5.46

Khuskia oryzae H.J. Huds. (teleomorph)

Colonies on CYA and MEA covering the whole Petri dish, low to moderately deep, dense to floccose, mycelium flesh coloured or pale orange to pure grey; black conidia conspicuous at low magnifications; reverse pale, greyish orange or grey to deep bluish grey. Colonies on G25N usually 10–15 mm diam, white or mucoid. No growth at 5 °C or 37 °C.

Conidiophores borne from aerial hyphae, short, dark walled, bearing conidia in isolation or in clusters from groups of irregular cells; conidia solitary, jet black, oblate, sometimes collapsing, mostly 12–15 µm long, with smooth, featureless walls, remaining attached or (on natural substrates) violently discharged.

Distinctive features See genus description. *Nigrospora* is clearly distinguished from *Arthrinium*, which it superficially resembles, by its jet black conidia which lack surface features or markings.

Taxonomy *Nigrospora oryzae* is the asexual state of an ascomycete, *Khuskia oryzae*, which is found only as a pathogen on certain plants. The older name has been given preference under the ICN (Wang et al. 2017).

Identifiers Mycobank MB253729; holotype IMI 99832 (slide of holotype) (Wang et al. 2017).

Physiology Optimum growth for *Nigrospora oryzae* has been recorded at 0.995 a_w and 25 °C. *N. oryzae* sporulated at 0.98 and 0.995 a_w but not

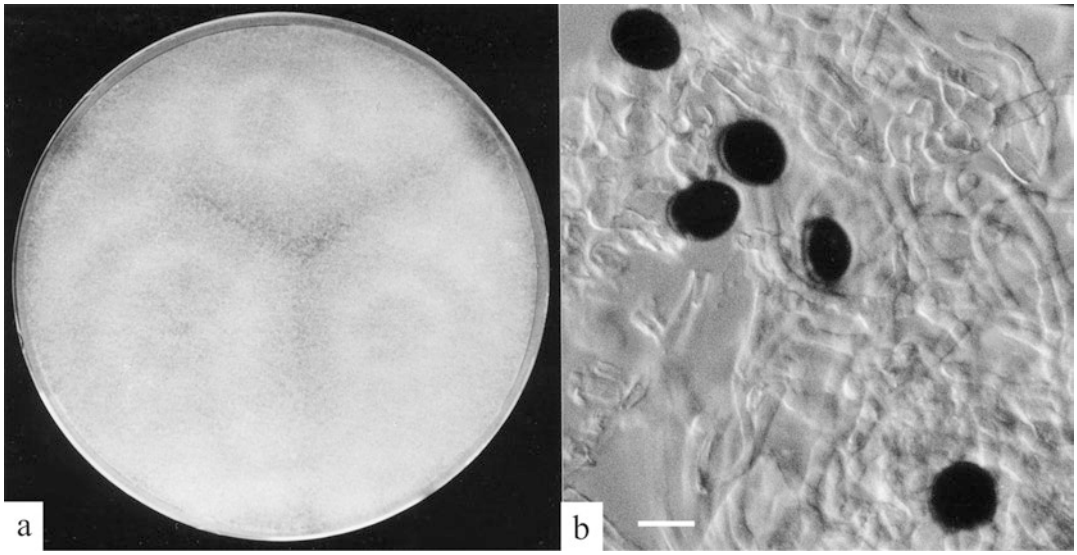

Fig. 5.46 *Nigrospora oryzae* (**a**) colonies on MEA, 7 d, 25 °C; (**b**) conidia, bar = 10 µm

at lower a_w values (0.95, 0.90 or 0.85) at 25 °C (Sempere and Santamarina 2006). Inability to grow at low or high temperatures has been noted above.

Mycotoxins Mycotoxin production has not been reported for *Nigrospora* species.

Ecology As a plant pathogen on cereal crops, the widespread distribution of *Nigrospora oryzae* in cereal grains is to be expected. It has been reported from barley (Fakhrunnisa et al. 2006), wheat, other leguminous and cereal foods, pecans and various health foods (see Pitt and Hocking 1997). It also causes a storage disease of apples in India (Khanna and Chandra 1975).

We isolated *Nigrospora oryzae* frequently in surveys of Southeast Asian food commodities (Pitt et al. 1993, 1994, 1998a and unpublished). The highest level of occurrence was on paddy rice, where it was isolated from an average of 40% of samples, with up to 68% of infected kernels in infected samples and in 10% of more than 7000 grains examined from Thailand, Indonesia and the Philippines. This species was isolated from high percentages of samples (25% or more) of maize, sorghum, soybeans, cashews and copra from Thailand, with overall infection rates of 2–5%. In Indonesia, kemiri nuts showed infection at 5% overall, while maize, sorghum, soybeans, kidney beans, cowpeas and talo beans were all infected at the rate of 1% or more of all seeds examined (Pitt et al. 1998a). Maize, mung beans and black pepper from the Philippines showed similar levels of infection (our unpublished data).

Additional species *Nigrospora sphaerica* (Sacc.) E.W. Mason is similar in culture to *N. oryzae*, but produces larger conidia, mostly 15–18 µm long. This species is associated with two diseases of bananas, crown rot (Wallbridge 1981) and squirter (Hall and Scott 1977; Snowdon 1990), and *Nigrospora* rot of apples (Snowdon 1990). It has also been recorded from wheat, peanuts, pecans, maize and biltong (see Pitt and Hocking 1997).

Although less common in Southeast Asian food commodities than *N. oryzae*, we isolated *N. sphaerica* from kemiri nuts, maize, peanuts, milled rice, cowpeas and black soybeans in Indonesia (Pitt et al. 1998a), and from paddy rice, maize, soybeans and black pepper in the Philippines (our unpublished data). Identifiers: MycoBank MB253729, holotype IMI 103253 (slide of holotype, Wang et al. 2017).

References Ellis (1971), Domsch et al. (1980), and Wang et al. (2017).

5.26 Genus *Pestalotiopsis* Steyeart

Pestalotiopsis and the closely related genus *Truncatella* were split from *Pestalotia* de Not. by Steyaert (1949), who confined *Pestalotia* to a single species, not found in foods. These genera are all characterised by the formation of black acervuli containing relatively large fusiform conidia with transverse septa and spiky appendages from one or both ends. *Truncatella* species have four transverse septa, *Pestalotiopsis*, more common in foods, has five and *Pestalotia*, six. *Pestalotiopsis* and *Truncatella* are not encountered frequently in foods other than cereals or nuts, where they can be spoilage agents. In our experience, direct plating of cereals will frequently detect *Pestalotiopsis*, but dilution plating techniques are ineffective.

Sutton (1980) accepted a single species in *Pestalotiopsis*, *P. guepinii*. However this genus is a wide spectrum plant pathogen, now with hundreds of described species, many of which were based on host specificity. This is no longer considered to be a sound basis for species delimitation (e.g. Jeewon et al. 2004). The state of *Pestalotiopsis* taxonomy remains confused because of the plethora of species based on host and equally because many of them lack types (Maharachchikumbura et al. 2011). That paper recommended that authors should not put species names on *Pestalotiopsis* isolates until species had been epi- or neo-typified. Subsequent papers (e.g. Maharachchikumbura et al. 2014) have improved the situation, but the process is far from complete. The species described here is the type species and is used here as an example of the genus.

Pestalotiopsis guepinii (Desm.) Steyeart Fig. 5.47

Colonies on CYA and MEA growing rapidly, covering the whole Petri dish, plane and floccose; mycelium usually white, sometimes off-white to pale brown; reverse pale or in similar colours to the mycelium. Colonies on G25N 10–16 mm diam, of low, white mycelium. Sometimes germination or growth at 5 °C. Usually no growth at 37 °C.

Conidia produced in flat, black acervuli, borne just beneath the agar surface, opening irregularly at maturity, filled with a dense layer of conidia; conidia fusiform, five-celled (four-septate), 20–28 × 6–9 µm, the central 3 cells brown, 15–20 µm long, the apical and basal cells hyaline, the basal one with a single usually unbranched spike-like appendage and the apical one with two or more simple or branched spiky appendages.

Distinctive features *Pestalotiopsis* shares with *Truncatella* the production in subsurface acervuli of relatively large fusiform conidia with appendages. In *Pestalotiopsis*, conidia have four septa, in *Truncatella*, three.

Identifiers MycoBank MB289201; type not known.

Physiology Optimum sporulation of *Pestalotiopsis guepinii* was achieved on media containing 3% sucrose. In addition, reduced nitrogen concentration also favoured sporulation (Ebenezer et al. 2002).

Mycotoxins Mycotoxin production has not been reported.

Ecology There are few records under the name *Pestalotiopsis* in the food literature. Most relate to fruit spoilage: *Pestalotiopsis* sp. from crown rot of bananas and rots in litchis, canker in guavas, and tarry rots in pomegranates (Snowdon 1990). Most reports of *Pestalotia* species, which are likely to refer to *Pestalotiopsis*, have come from pecans. Other *Pestalotia* records include wheat, rice, almonds and hazelnuts (see Pitt and Hocking 1997).

We isolated *Pestalotiopsis guepinii* at low levels from Thai copra, maize, black beans and red beans (Pitt et al. 1993, 1994).

Additional genus *Truncatella* Steyaert produces colonies similar to those of *Pestalotiopsis*, and produces similar conidiomata. Conidia are fusiform with three septa, and measure 15–20 × 6–8 µm; the two median cells are brown and 11–14 µm long; apical and basal cells are hyaline; and the basal cell is without appendages. Appendages from the apical cell are variable in number and branching.

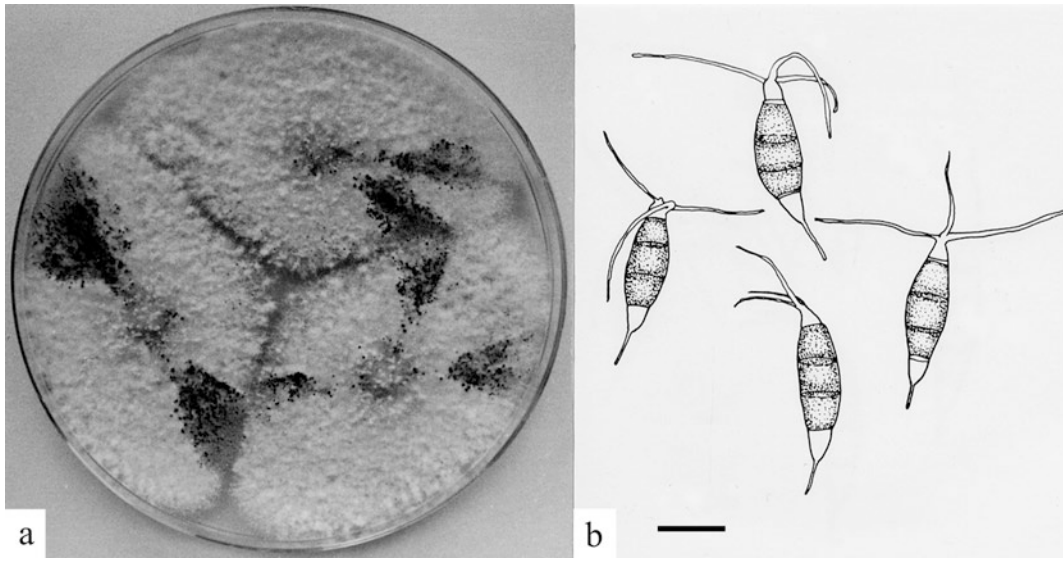

Fig. 5.47 *Pestalotiopsis guepinii* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) conidia, bar = 10 µm

No records of *Truncatella* in foods were found and it is unknown whether some references *Pestalotia* or *Pestalotiopsis* include *Truncatella* species.

Reference Sutton (1980) and Maharachchikumbura et al. (2011, 2014).

5.27 Genus *Purpureocillium* Luangsa-ard et al.

The asexual genus *Paecilomyces* was split from *Penicillium* by Bainier (1907a) on the basis of differences in phialide shape and conidial colour. As monographed by Samson (1974), *Paecilomyces* was an extensive genus, with species common in soil, in food spoilage, as insect pathogens and of medical importance. A variety of evidence, but especially molecular information (e.g. Luangsa-ard et al. 2004), has resulted in splitting of the genus until few species are left, most of which are related to the sexual genus *Byssochlamys*, as described there. One other species, *Paecilomyces lilacinus*, important in both medicine and insect biocontrol, was separated into the new genus *Purpureocillium* by Luangsa-ard et al. (2011), as described below.

Purpureocillium lilacinum (Thom) Luangsa-ard et al. Fig. 5.48

Penicillium lilacinum Thom
Paecilomyces lilacinus (Thom) Samson

Colonies on CYA 25–35 mm diam, plane, dense to floccose; mycelium in marginal areas white, elsewhere pale pink or pinkish-grey, sometimes also areas of bright pale yellow; conidial production sparse, pinkish brown; reverse pale, yellow or brown. Colonies on MEA 25–32 mm diam, low and sparse, mycelium uniformly pinkish-grey; reverse pale or centrally brown. Colonies on G25N 3–6 mm diam, low and dense. No germination at 5 °C. At 37 °C, responses ranging from no growth to colonies up to 5 mm diam.

Conidiophores borne from aerial or surface hyphae, 200–600 µm long, with finely roughened walls, bearing irregular verticils of metulae both terminally and subterminally; phialides 7–10 × 2.5–3.0 µm, tapering to long, narrow collula; conidia ellipsoidal to fusiform, 2.5–3.0 × 2.0–2.2 µm, with smooth to finely roughened walls.

Distinctive features This species forms discrete colonies like a *Penicillium,* but colonies are lilac or pink and produce long, slender phialides and ellipsoidal conidia.

Identifiers MycoBank MB519529; type not known.

Physiology *Purpureocillium lilacinus* grows from 8–38 °C and between pH 2 and 10 in submerged culture (Duncan 1973). It is capable of growth down to at least 0.90 a_w in NaCl (Tresner and Hayes 1971) or carbohydrate media (Panasenko 1967).

Mycotoxins No mycotoxins of significance are produced by this species.

Ecology Although a contaminant rather than a food spoilage fungus, *Purpureocillium lilacinus* has been isolated from a variety of foods: cereals, including wheat, flour and barley, peanuts and pecans, fresh figs, beans, frozen meat, salami (see Pitt and Hocking 1997) and in our laboratory from bottled water and pandanus nuts from Papua New Guinea.

References Samson (1974) and Luangsa-ard et al. (2011).

5.28 Genus *Pyrenophora* Fr.

Drechslera S. Ito

Shoemaker (1959) revised the genus *Drechslera*, provided a clear separation from *Helminthosporium*, and erected *Bipolaris.* Leonard and Suggs (1974) segregated *Exserohilum* from *Bipolaris*. These three asexual genera produce long, large conidia with transverse septa, which are much thicker than those in e.g. *Trichoconiella*. In *Drechslera*, conidia are cylindrical and germinate at any cell, whereas in *Bipolaris* and *Exserohilum*, conidia narrow gradually and germinate only from the terminal cells. These three genera are associated with different teleomorphs: *Bipolaris* with *Cochliobolus, Exserohilum* with *Setosphaeria* K.J. Leonard & Suggs and *Drechslera* with *Pyrenophora.* For the first two, it has been recommended (Rossman et al. 2015) that the asexual names be provided

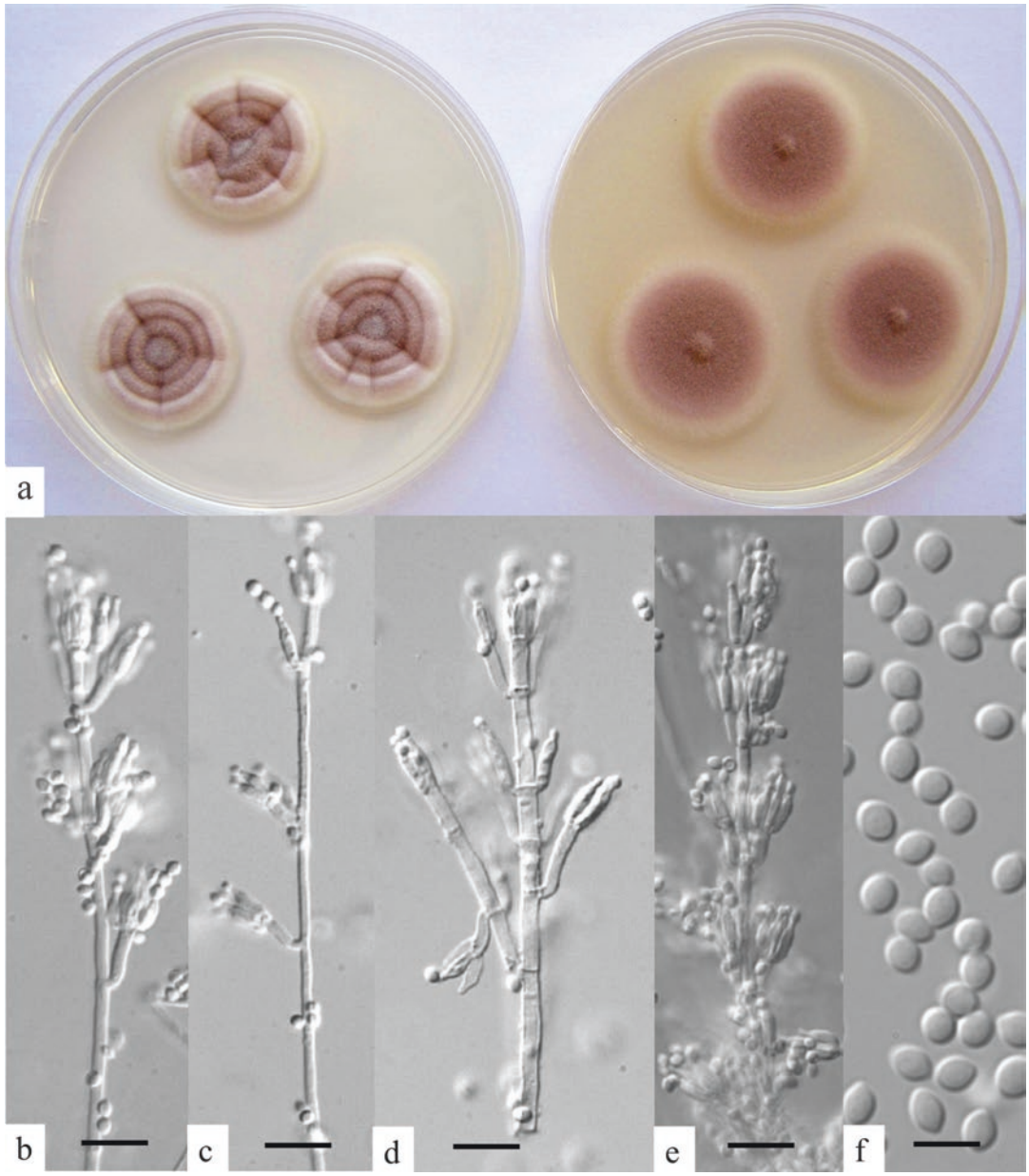

Fig. 5.48 *Purpureocillium lilacinum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**e**) penicilli, bars = 10 µm; (**f**) conidia, bar = 5 µm

priority, but that *Pyrenophora* be accepted rather than *Drechslera* – used in the earlier editions of this book.

Pyrenophora species are serious pathogens on cereal plants, and a number of species have been recorded to occur on grains from time to time. However, they appear to have little or no role in food spoilage. Species are mostly distinguished by differences in spore size; one, *P. tritici-repentis*, is treated here as an example.

Pyrenophora tritici-repentis (Diedicke) Drechsler

Fig. 5.49

Drechslera tritici-repentis (Diedicke) Shoemaker (anamorph)

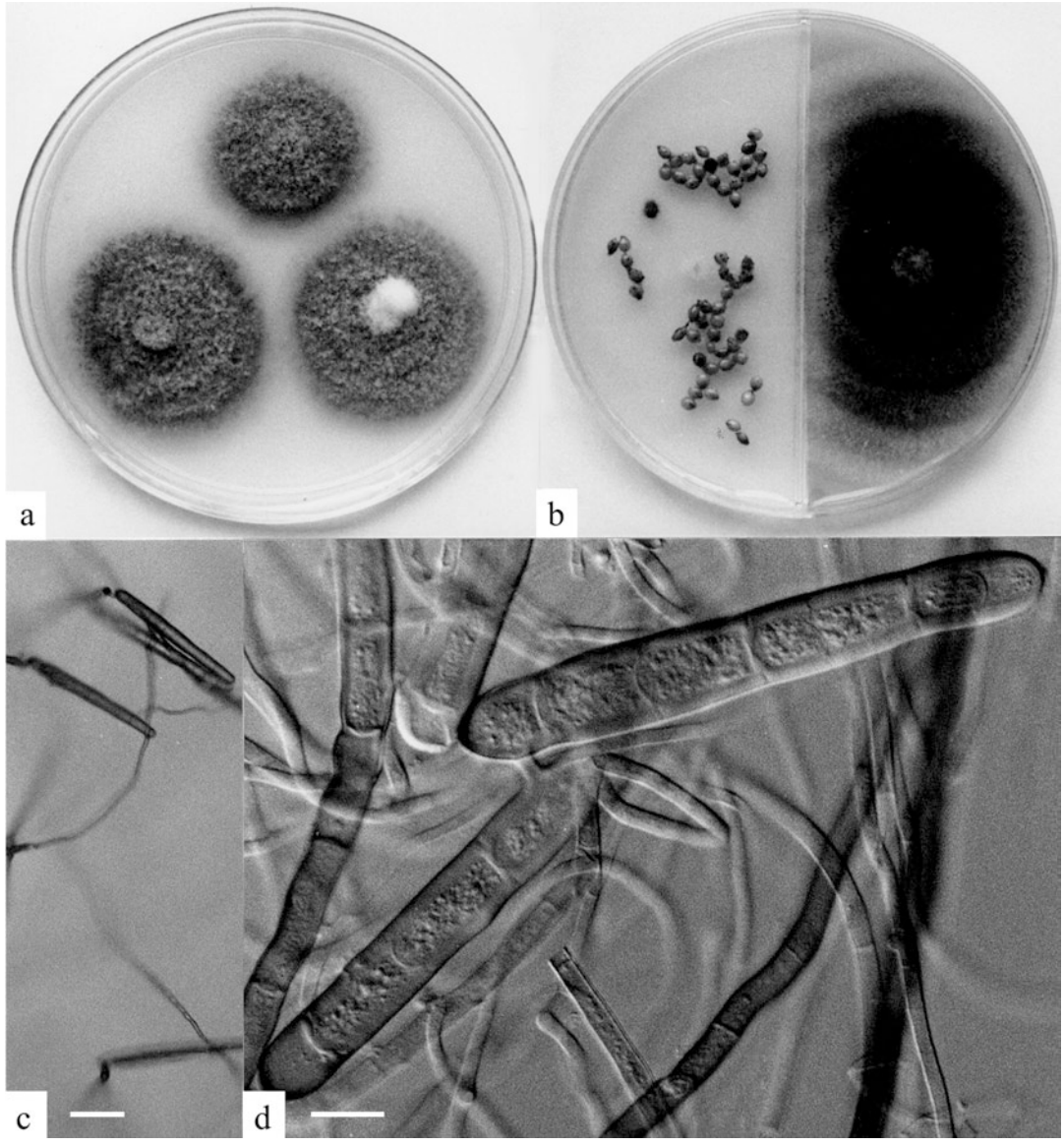

Fig. 5.49 *Pyrenophora tritici-repentis* (**a**) colony on MEA, 7 d, 25 °C; (**b**) colonies on tap water agar plus millet seed and on V-8 juice, 7 d, 25 °C; (**c**) conidia *in situ*, bar = 50 µm; (**d**) conidia, bar = 10 µm

Colonies on CYA 55–60+ mm diam, sometimes covering the Petri dish, of low to deep, floccose, pale to mid grey mycelium, sometimes lightly sporing in upper mycelial layers; exudate and soluble pigment absent; reverse dark grey to blue black. Colonies on MEA covering the Petri dish, similar to on CYA but sporulation usually absent; reverse brownish grey to bluish black. Colonies on G25N 4–8 mm diam, of sparse, pale grey mycelium. At 5 °C, sometimes germination. At 37 °C, no growth.

Colonies on DCMA when grown under lights 45–60 mm diam, similar to those on CYA, sometimes lightly sporing; reverse pale to dark grey.

Conidiophores 150–250(−400) µm long, straight or sinuous, sometimes knobby terminally, bearing solitary conidia; conidia cylindroi-

dal, commonly 70–120 × 14–17 µm, with 5–7 septa, brown, smooth walled.

Distinctive features See genus preamble.

Taxonomy The state seen in pure culture is usually the asexual form, though sometimes immature perithecia may be observed (Sivanesan 1987). Moolhuijzen et al. (2018) have described this species in detail.

Physiology No physiological studies on *Pyrenophora* (or *Drechslera*) species are known to us. In common with most other plant-borne dematiaceous hyphomycetes, growth is unlikely to occur below about 0.90 a_w. Species appear to be mesophilic.

Mycotoxins No mycotoxins are known to be produced.

Ecology *Pyrenophora tritici-repentis* is one cause of "pink tip" in wheat. We isolated it from 56% of 344 surface disinfected North American wheat samples, where it was present at up to 27% in infected samples and 2% of all grains examined (unpublished data). Occurrence in Australian wheat was much lower (5% of samples; unpublished data), and it was not isolated from Southeast Asian commodities (Pitt et al. 1994, 1998a).

Several other *Pyrenophora* or *Drechslera* species have been reported from grains and seeds. However most records examined refer to species now included in *Bipolaris* or, less commonly, *Exserohilum*.

References Alcorn (1983), Sivanesan (1987), and Moolhuijzen et al. (2018).

5.29 Genus *Scopulariopsis* Bainier

Like *Paecilomyces*, *Scopulariopsis* was segregated from *Penicillium* by Bainier (1907b); it is generally agreed that the differences from *Penicillium* are more fundamental. In *Scopulariopsis*, conidia are not extruded from phialides, but are cut off from annelides. The difference from a phialide is clearly evident: as each successive conidium is cut off from the tip of an annelide, a small amount of wall material remains, so that the annelide elongates, and in age shows a succession of faint rings or scars. A consequence is that conidia borne from annelides possess distinctive broad bases. In *Scopulariopsis*, this characteristic is readily seen under the microscope at high magnification.

Colonies of *Scopulariopsis* species range in colour from white to brown, but are never green or blue. Most species are broad ranging saprophytes, of common occurrence in decaying vegetation and soil. In comparison with their ubiquity in these habitats, they must be classed as relatively uncommon in foods. Only one species, *S. brevicaulis*, warrants description here.

Scopulariopsis brevicaulis (Sacc.) Bainier **Fig. 5.50**

Microascus brevicaulis S.P. Abbott (teleomorph)

Colonies on CYA usually 40–50 mm diam, low, dense and velutinous, often irregularly wrinkled, coloured orange grey to brownish orange (M. 5B2-C4); reverse bright yellow to orange brown. Colonies on MEA usually 40–50 mm diam, sometimes much smaller, 15–30 mm, low and sparse at the margins, sometimes centrally floccose, plane, coloured brownish orange (M. 6C4-5); reverse yellow brown. Colonies on G25N 15–20 mm diam, low and dense, white or centrally yellow, reverse white to bright yellow. No growth at 5 °C. At 37 °C, colonies 7–20 mm diam, dense, often centrally raised, white or brown; reverse dull yellow.

Reproductive structures varying from single conidiogenous cells (annelides) to irregular penicilli, sometimes with well defined metulae and rami; conidia pyriform with a distinctly truncate base, clearly visible before release from the annelide, 5–8 µm diam, brown, with rough walls, sometimes adhering in short chains.

Distinctive features Small single celled conidia with truncate bases are characteristic of *Scopulariopsis*. Colonies of *S. brevicaulis* at 25 °C are low, dense and brown. Growth at 37 °C is slow. The brown, rough walled conidia are distinctive.

Taxonomy When grown on PDA for an extended period, some isolates of *Scopulariopsis brevicaulis* were shown to produce a perithecial sexual state, which was named *Microascus*

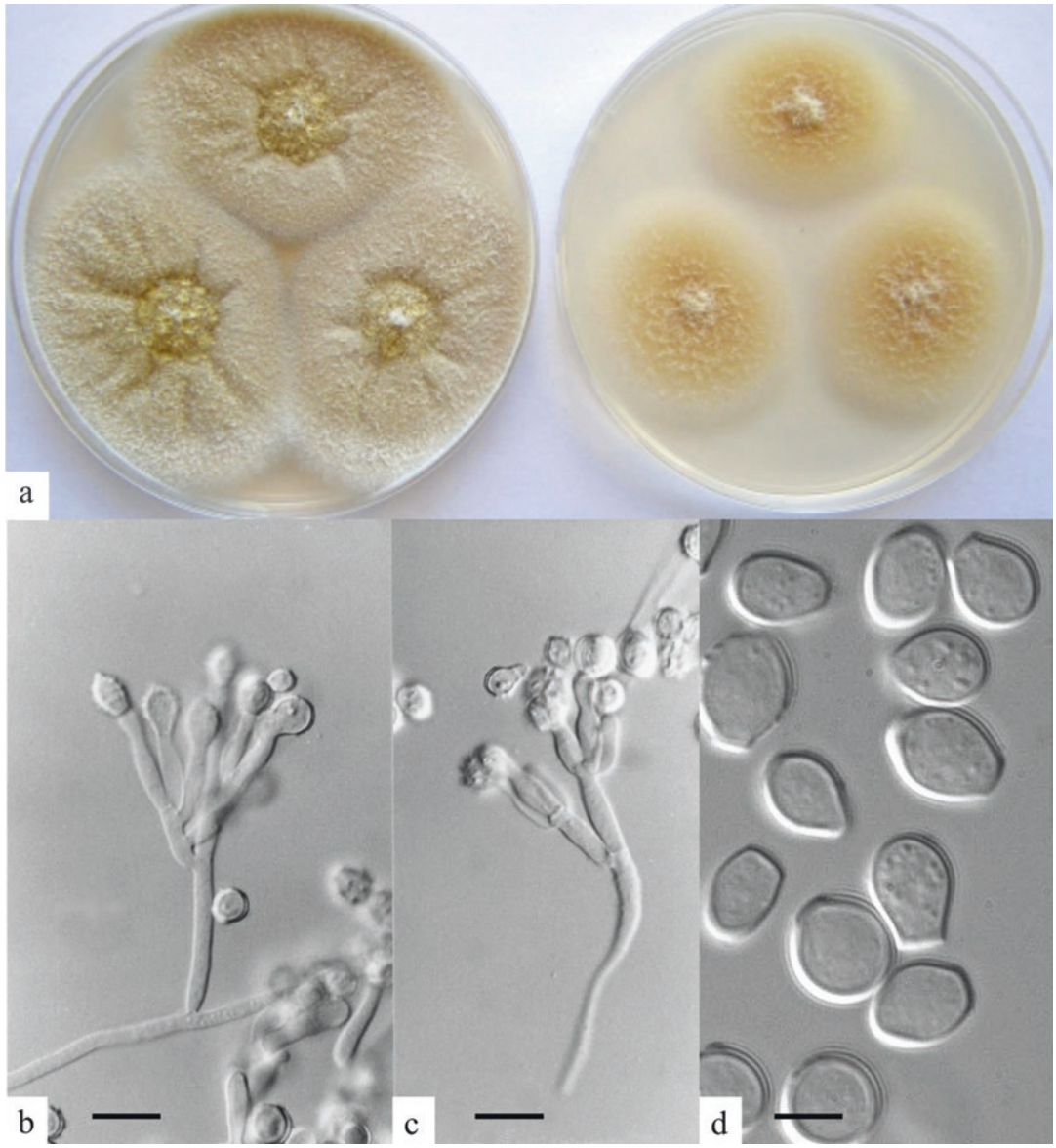

Fig. 5.50 *Scopulariopsis brevicaulis* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

brevicaulis, and showed that *Scopulariopsis* belonged in the family *Microascaceae* (Abbott et al. 1998). However, most isolates do not produce the sexual state in culture and the *Scopulariopsis* name is still in common use. We support that useage.

Identifiers MycoBank MB154310, type not known.

Physiology The minimum a_w for growth of this species is 0.90 (Galloway 1935). Better growth was observed in media supplemented with 0.1 to 1 M NaCl (Yoder et al. 2003). The optimum pH for growth was found to be pH 10 (Bothast et al. 1975).

Mycotoxins Mycotoxins are not produced.

Ecology *Scopulariopsis brevicaulis* was reported to be a common contaminant on freshwater dried shrimp in Bangladesh (Ara et al. 2020). Inagaki (1962) isolated this species from rice grains and rice flour, nonfat dried milk and

butter. This species was reported to be a cause of spoilage in cheese (Northolt et al. 1980), along with other rarer species of *Scopulariopsis*. Other sources have been barley, wheat, salami, matured cheese, bacon and biltong (see Pitt and Hocking 1997) and luncheon meats (Mohamed and Hussein 2004). We have isolated this species from soybeans and black pepper from the Philippines, and peanuts and mung beans from Indonesia (Pitt et al. 1998a).

Reference Morton and Smith (1963) and Abbott et al. (1998).

5.30 Genus *Stemphylium* Wallr.

Like *Alternaria, Stemphylium* produces conidia with both longitudinal and transverse septa, however those of *Stemphylium* are more or less ellipsoidal to short cylindroidal, not pointed. In extensive molecular studies of *Alternaria, Stemphylium* and related genera (*Embellisia* and *Nimbya*), Pryor and Gilbertson (2000) and Pryor and Bigelow (2003) provided strong molecular evidence that the genus *Stemphylium* is distinct from these other genera. The genus was revised and updated using multigene molecular analyses by Woudenberg et al. (2017), who accepted 28 phylogenetic species. See also Marin-Felix et al. (2019).

Species of *Stemphylium* occur commonly as weak parasites or saprophytes on a variety of plants and plant materials. Several species have been reported from foods, but as taxonomy was confused until recently, the species involved are unclear. For simplicity, the type species, *S. botryosum* is described here. In the study by Woudenberg et al. (2017), it was found only on *Mendicato sativa* (lucerne or alfalfa) plants, so other species undoubtedly have been found on other food materials.

Stemphylium botryosum Wallr. Fig. 5.51

Pleospora tarda E.G. Simmons (teleomorph)

Colonies on CYA 50–65 mm diam, of low to somewhat floccose, rather sparse mycelium, olive to olive brown; reverse olive grey to dark grey. Colonies on MEA 50–70 mm diam, low to floccose, in the latter case sometimes with areas of white or pale yellow mycelium, elsewhere pale to dark grey; reverse light to dark grey, sometimes pale yellow or pinkish. Colonies on G25N 6–15 mm diam, pale to dark grey. At 5 °C, usually germination. At 37 °C, no growth.

Colonies on DCMA 35–45 mm diam, low to somewhat floccose, sparse, grey to dark grey in areas; reverse in similar colours.

Conidiophores undifferentiated, of variable length, with slightly swollen (up to 7 µm) tips bearing conidia successively, blown out from a pore as a hyaline cell, then septating both longitudinally and transversely, becoming thick walled and detaching with maturity; the smaller conidia more or less spherical, with 1–2 septa, the larger ones at maturity usually short cylindrical, with rounded ends and straight or irregularly swollen sides, with 2–4 transverse and 1–2 longitudinal septa, sometimes near spherical or irregular in shape, overall ranging in size from 13 µm diam up to 30–32 × 16–20 µm, with brown walls, slightly roughened or with short spines, or occasionally with larger dark warts.

Distinctive features As noted above, *Stemphylium* is distinguished by dark conidia with both transverse and longitudinal septa, with ends rounded, symmetrical from end to end. *S. botryosum* conidia characteristically are slightly constricted in the middle.

Taxonomy The teleomorphs of this and other *Stemphylium* species have been described in the ascomycete genus *Pleospora* (Simmons 1969, 1985). However, *Pleospora* has been shown to be polyphyletic, so the asexual name *Stemphylium* is preferred (Rossman et al. 2015). Metabolite profiles have been used to distinguish several *Stemphylium* species, including *S. botryosum* (Andersen et al. 1995).

Identifiers MycoBank MB218021; holotype on a stem in Wallroth collection; ex-type culture CBS 714.68; ITS barcode KC584238; alternative

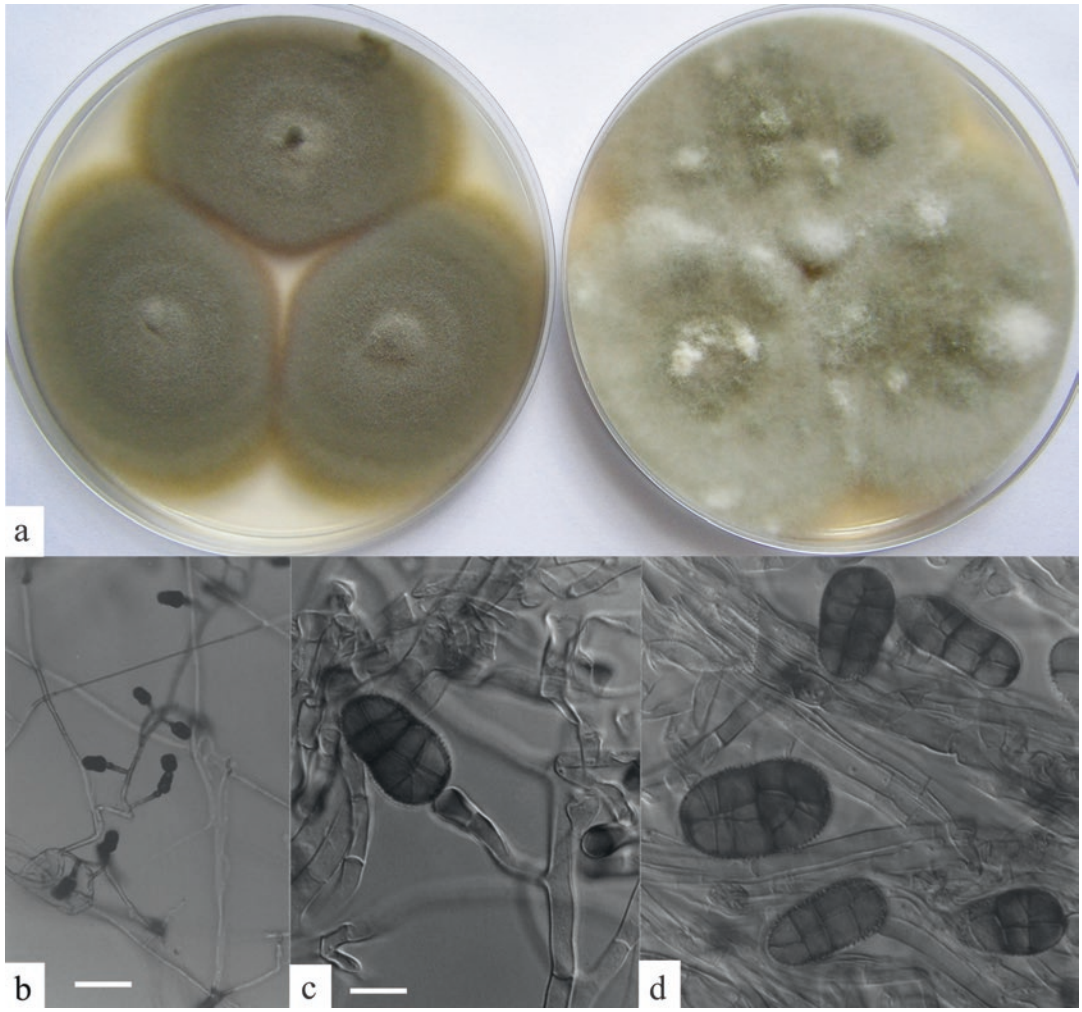

Fig. 5.51 *Stemphylium botryosum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) conidiophores and conidia *in situ*, bar = 50 µm; (**c**, **d**) conidia, bar = 10 µm

markers *gapdh* AF443881, *cmd4* KC850826 (Woudenberg et al. 2017)

Physiology Potato dextrose agar supported abundant mycellial growth, whereas conidiogenesis was highest on Sabouraud maltose agar (Susuri and Doda-Gashi 2003).

Mycotoxins This genus is not known to produce significant mycotoxins.

Ecology This and other *Stemphylium* species have been reported as pathogens on mangoes (Johnson et al. 1990), squash (Assawah and Al-Zarari 1984), sweet potatoes (Ravichandran and Sullia 1983), asparagus and lettuce (Snowdon 1991), tomatoes (Dodds et al. 1991; Muniz et al. 2003) and peppers (Muniz et al. 2003). *Stemphylium* species have also been isolated from malting barley (Sepitkova and Jesenska 1986), lucerne seeds (Susuri and Doda-Gashi 2003) and soybeans (our unpublished data).

References Simmons (1967, 1969, 1985), Woudenberg et al. (2017), and Marin-Felix et al. (2019).

5.31 Genus *Trichoconiella* B.L. Jain

This genus was established to accommodate the fungus more commonly known as *Alternaria padwickii* (Ganguly) M.B. Ellis. Conidia of this species produce only transverse septa, excluding it from *Alternaria* (Jain 1975).

Trichoconiella padwickii (Ganguly) B.L. Jain Fig. 5.52

Alternaria padwickii (Ganguly) M.B. Ellis

Colonies on CYA 30–45 mm diam, plane, dense to floccose, mycelium yellowish grey to light orange, sometimes with pale grey areas; yellow exudate sometimes produced; reverse pale or orange at the margins, centrally very dark brown to bluish black. Colonies on MEA 45–55 mm diam, plane, low to floccose, mycelium pale orange or grey; reverse pale or pinkish at the margins, overall bluish black. No growth on G25N or at 5 °C. At 37 °C, colonies up to 5 mm diam formed, or no growth.

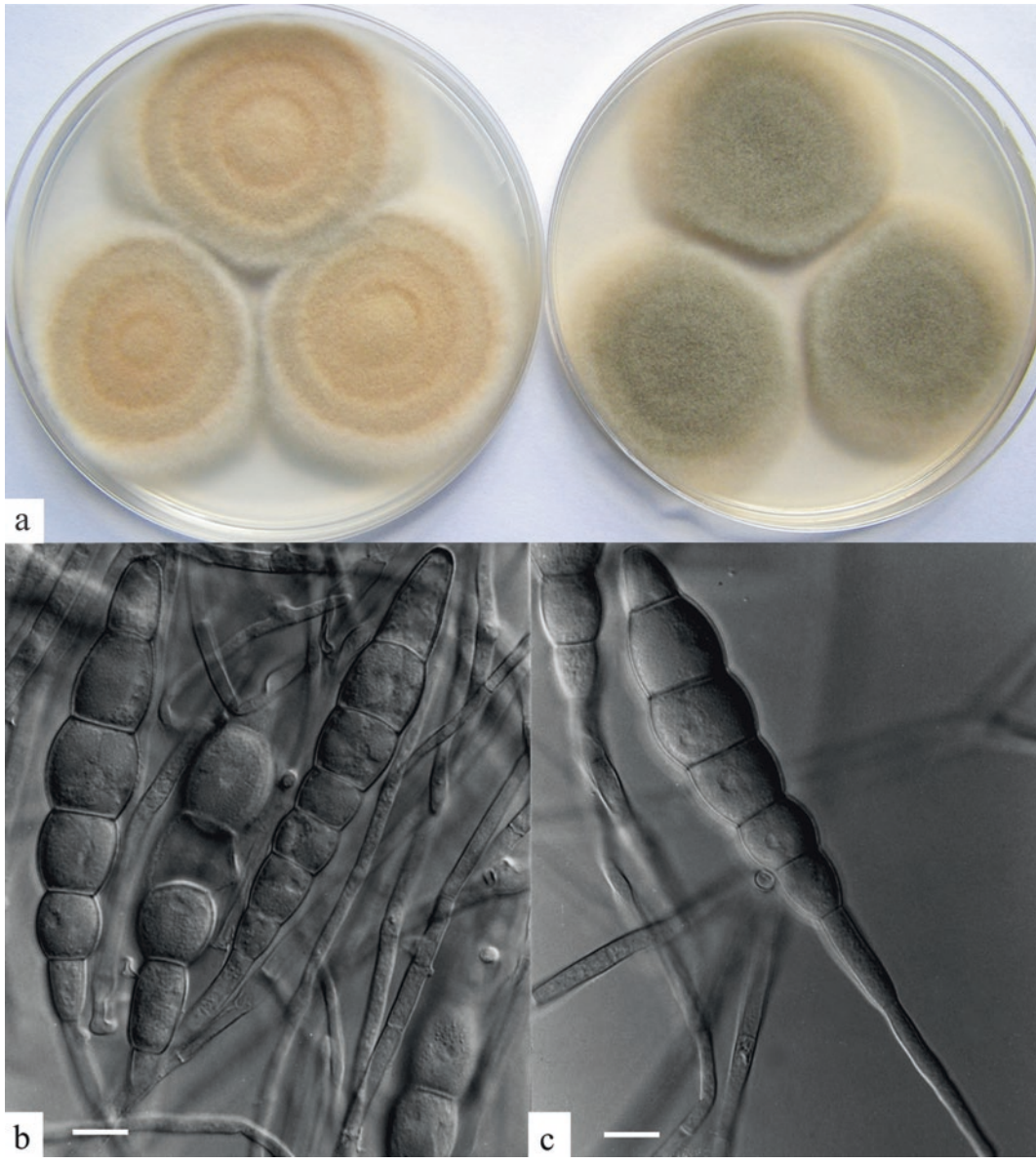

Fig. 5.52 *Trichoconiella padwickii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) conidia, bar = 10 µm

Colonies on DCMA 35–55 mm diam, similar in appearance to on MEA, but mycelium mid to dark grey; reverse grey, dark grey or bluish black.

Conidia best observed on colonies grown on sterile wheat grains, borne singly from long hyphae, with 5–6 transverse septa, 40–55 × 9–11 µm between the tip and last septum, with the central cells larger, giving a narrowly ellipsoidal shape, and with smooth, brown walls; hyphae sometimes remaining attached to the conidium and then giving the impression of long appendages.

Distinctive features This genus superficially resembles *Alternaria*, but produces conidia with transverse septa only. Long, whiplike hyphal appendages are also distinctive. *Trichoconiella padwickii* appears to be almost exclusively associated with rice.

Identifiers MycoBank MB324891; type not known.

Physiology No reports of physiological studies are known to us.

Mycotoxins Mycotoxins are not known to be produced.

Ecology This species (by either generic name) has rarely if ever been reported from the food literature. However, we have found *Trichoconiella padwickii*, reported as *Alternaria padwickii*, to be endemic in rice from Southeast Asia. It was present in 9 of 18 samples of Thai paddy rice, with up to 60% of grains in individual samples showing infection, and was found in 12% of all individual grains examined (Pitt et al. 1994). The incidence in paddy rice from Indonesia (71% of samples, 19% of all particles) and the Philippines (72% of samples, 23% of all particles) was even higher. Individual rice samples from Indonesia had up to 69% of particles infected by *T. padwickii*, while incidence was up to 90% of grains in samples from the Philippines (Pitt et al. 1998a and our unpublished data).

Ellis (1971) reported *Trichoconiella padwickii* (as *Alternaria padwickii*) from rice grains from Egypt, India, Malaysia, Nigeria, Pakistan and Sabah, indicating that this species is very widespread. Occurrence on crops other than rice has rarely been reported.

References Ellis (1971), Jain (1975), and Domsch et al. (1980).

5.32 Genus *Trichoderma* Pers.

In this ubiquitous genus, reproduction is by small single celled conidia produced from phialides which are arranged in irregular verticils, with the subterminal phialides borne more or less at right angles to the stipe. Colonies are low and spread rapidly. Mycelial growth is loose textured and characteristically develops irregularly, often appearing in tufts or isolated patches. Conidia, green in the common species, sometimes develop only after exposure to light.

Trichoderma species have usually been considered to be soil fungi, but the genus is considered now to comprise largely plant symbionts or endophytes and parasites on other fungi (mycoparasites) (Chaverri and Samuels 2013). Some are also of increasing importance as human pathogens. Interest in the genus has increased sharply in recent years, as some species have been proposed as biocontrol agents against plant pathogenic fungi, either directly or by enhancing plant defenses (Sharma et al. 2009). Some species produce chitinases and cellulases of industrial importance.

Speciation in *Trichoderma* has proved to be exceptionally difficult. The most common species name in the literature, *T. viride* Pers., has often been incorrectly used (Rifai 1969) and it is now considered to be a rare species (Jaklitsch et al. 2006). Conidia produced by *T. viride* have rough walls, whereas the majority of *Trichoderma* isolates produce conidia with smooth walls. Reports in the literature on *Trichoderma* species should be treated with caution, as green spored *Trichoderma* isolates have often been called *T. viride* regardless of the texture of their conidial walls.

Because most *Trichoderma* isolates have similar morphology, taxonomy proved very difficult until the advent of molecular methods. The use of sequences from multiple gene loci resulted in a large increase in species numbers, from nine in 1970 to over 80 (Samuels 2006). Many species have been shown to have teleomorphs in *Hypocrea*, though few are found in laboratory culture (Samuels 2006). The taxonomy of *Trichoderma* is still complex: Samuels (2006) remains the most comprehensive overview. The genomics of the 12 most common *Trichoderma*

species were discussed with regard to the evolution of mycoparasitism (Kubicek et al. 2019).

The most commonly isolated *Trichoderma* species has been regarded as *T. harzianum* Rifai (Bisset 1991a; Samuels 2006). This was the species we reportedly encountered most frequently from foods, and the name we used in previous editions. However, in a major revision of *T. harzianum* and related species, Chaverri et al. (2015) concluded that *T. harzianum* is a rare species found only in Europe and North America. They described several new species, separated by molecular differences, especially in the *tef* gene, and slight differences in micromorphology. It is likely that the isolates we have encountered, which came mostly from tropical sources, belong to *T. afroharzianum*, which is described below as representative of this genus.

It is worth noting that Chaverri et al. (2015) examined four cultures available commercially for biocontrol and purported to contain "*T. harzianum*" as the active fungus and concluded that none did, and that the most widely distributed contains *T. afroharzianum*. Care should be exercised in handling *Trichoderma* isolates in the laboratory. Conidia are small, are shed and dispersed readily, and contaminant colonies grow rapidly. Many Trichodermas are mycoparasites, producing powerful chitinases and cellulases which, in time, can overrun and destroy other cultures completely.

Trichoderma afroharzianum Chaverri et al. Fig. 5.53

Colonies on CYA and MEA generally covering the whole Petri dish, often irregular in outline or with isolated tufts evident, of white to yellow mycelium, with bright to dull yellow green conidia developing over the whole surface or in patches or tufts; reverse pale or yellowish. Colonies on G25N less than 5 mm diam, with growth weak. At 5 °C, usually no growth, occasionally germination. No growth at 37 °C.

Conidiophores consisting of highly branched structures, with a stipe bearing branches and the branches rebranching, all approximately at right angles, to form a pyramidal shape, with each branch bearing phialides irregularly; phialides ampulliform, commonly 5–7 × 3.0–3.5 µm, larger when borne apically, bearing conidia singly, not in chains; conidia often adhering in small clusters, spheroidal, subspheroidal, or sometimes broadly ellipsoidal, 2.5–3.2(−4.0) µm diam or in

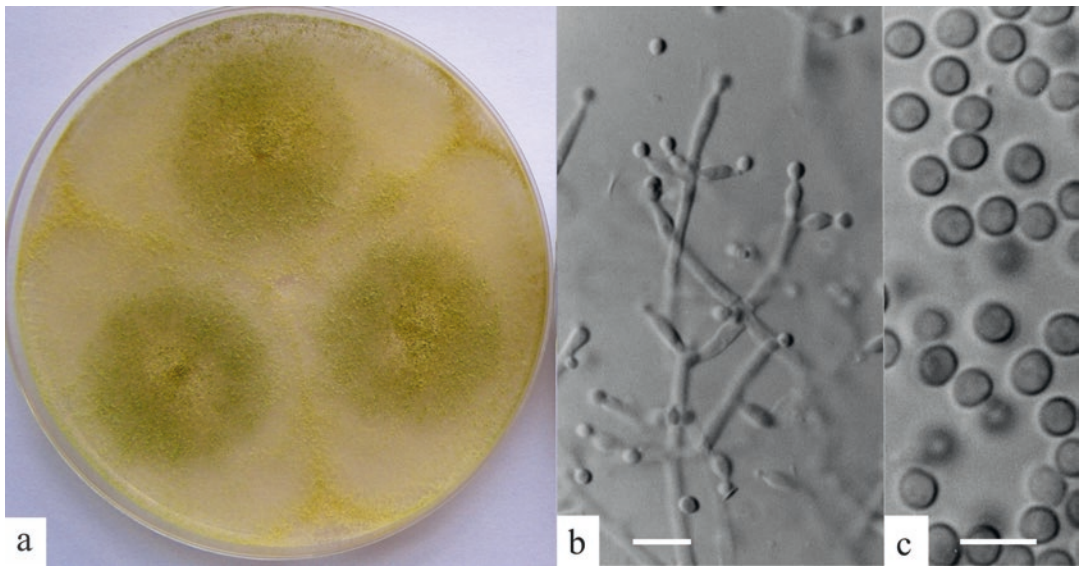

Fig. 5.53 *Trichoderma harzianum* (**a**) colonies on MEA, 7 d, 25 °C; (**b**) conidiophores, bar = 10 µm; (**c**) conidia, bar = 5 µm

length, smooth walled. Solitary aleurioconidia also formed by some isolates, spherical to broadly ellipsoidal, 6–8 µm diam.

Distinctive features *Trichoderma afroharzianum* produces conidiophores which are compactly branched in a pyramidal shape, the base being highly branched, and the apex usually bearing a solitary phialide. Conidia are smooth walled, nearly spherical and less than 3.5 µm long.

Taxonomy *Trichoderma afroharzianum* is one of several species closely related to *T. harzianum*. See genus preamble.

Identifiers MycoBank MB809945; holotype BPI 881096; ex-type culture CBS 124620 (Chaverri et al. 2015).

Physiology Because of the confusion in the literature over the identity of *Trichoderma* isolates, it is likely that much of the information reported in earlier literature for *T. viride* actually is based on studies on *T. harzianum* or a closely related species. Domsch et al. (1980) reported the optimum growth temperature for *T. harzianum* as approximately 30 °C, with a maximum near 36 °C, in agreement with the description above. However, Chaverri et al. (2015) indicated that *T. harzianum* and related species mostly grow at 37 °C or above. Our observations indicate a minimum growth temperature at or slightly above 5 °C. The minimum a_w for growth is 0.91 at 25 °C (Griffin 1963).

Mycotoxins Mycotoxin production by *Trichoderma afroharzianum* and closely related species has not been reported. However, some *Trichoderma* species produce trichothecene or other mycotoxins. However, much of the information is old and speciation unreliable.

Ecology The species complex centred on *Trichoderma harzianum* has been isolated frequently from cultivated and forest soils in all parts of the world (Domsch et al. 1980). It has been reported from rotting tubers of cassava (Ekundayo and Daniel 1973) and as a cause of spoilage in apples (Penrose et al. 1984). It has been reported from salmon and peas (data sheets, International Mycological Institute, Egham, Surrey). We found *T. harzianum,* at levels up to 1% of all grains or nuts examined, in maize from Thailand and Indonesia, and sorghum from Indonesia. It was encountered at low levels in Philippine maize, Thai peanuts and Indonesian cowpeas (Pitt et al. 1993, 1994, 1998a). *T. viride* was not encountered at all.

Trichoderma species are no doubt widely distributed in foods, but which species remains unclear.

References Bissett (1984, 1991a, b), Samuels (2006), and Chaverri et al. (2015).

5.33 Genus *Trichothecium* Link

Trichothecium is a distinctive genus with a single common species, *T. roseum,* characterised by sparse, pinkish colonies and conidia formed in a unique V-formation on long stipes. *T. roseum* is a common saprophyte in damp and decaying habitats and is sometimes a weak pathogen of both plants and animals.

Trichothecium roseum (Pers.) Link Fig. 5.54

Cephalothecium roseum Corda

Colonies on CYA and MEA 50–60 mm diam, low and often sparse, characteristically coloured orange pink near salmon; reverse similarly coloured, or less intense, or brownish. Colonies on G25N barely macroscopic, 1–2 mm diam at most. At 5 °C, no germination to germination. No growth at 37 °C.

Conidiophores long, simple hyphae, bearing conidia at the tip successively, each formed as a blown out cell below the previous one, offset from the hyphal axis and adhering loosely to form characteristic short, V-shaped chains; conidia approximately ellipsoidal to pyriform, with a single transverse septum, 16–20 × 8–12 µm, and with thin, smooth walls.

Distinctive features See genus preamble.

Identifiers MycoBank MB164181; type not known.

Physiology Domsch et al. (1980) reported growth temperatures for *Trichothecium roseum* as minimum 15 °C, optimum 25 °C and maximum 35 °C. Our data on growth at low temperature is at variance with this, indicating a

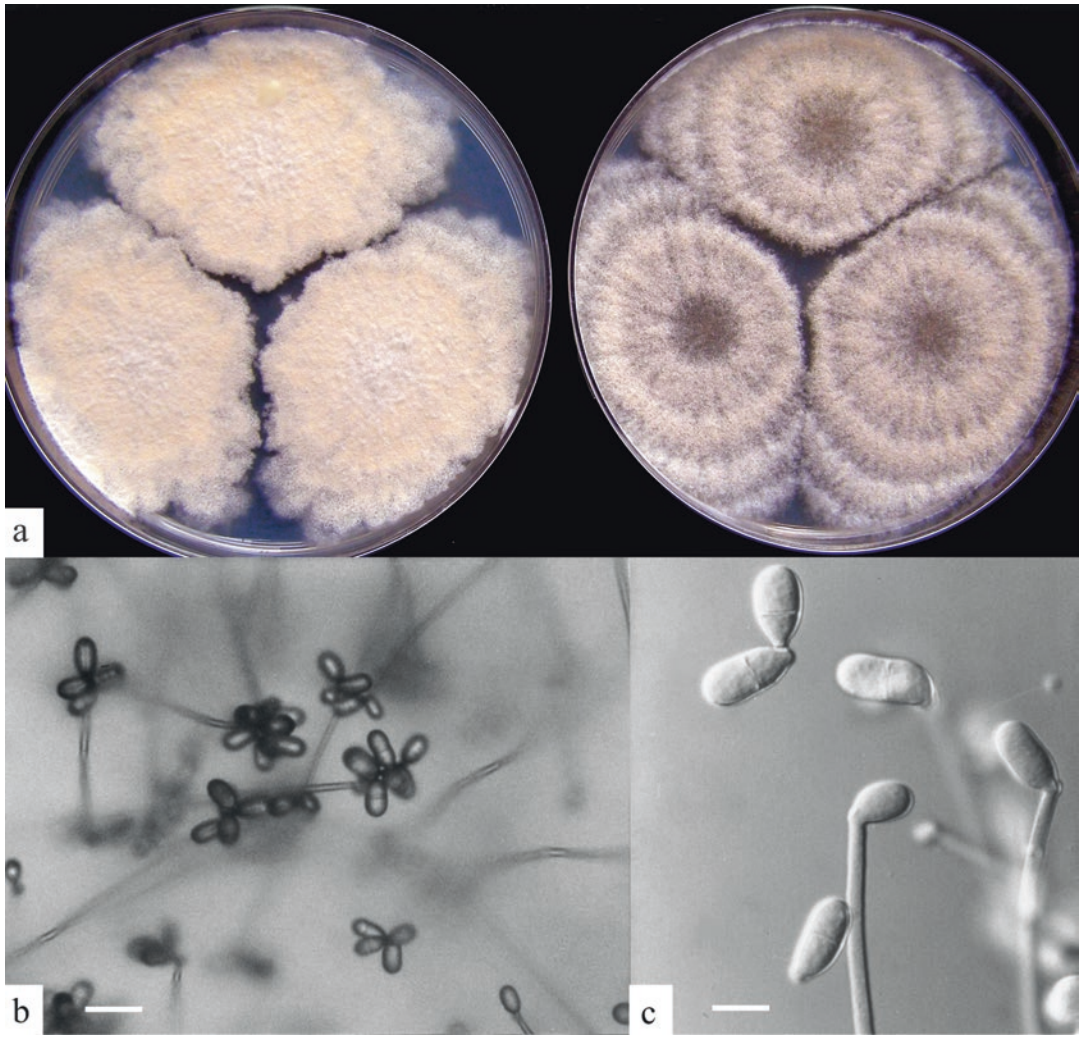

Fig. 5.54 *Trichothecium roseum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) conidiophores and conidia *in situ*, bar = 25 µm; (**c**) conidia, bar = 10 µm

minimum growth temperature of 5 °C or near. Growth occurs down to 0.90 a_w (Snow 1949).

Mycotoxins *Trichothecium roseum* has been reported to produce trichothecene toxins, including trichothecin, trichothecolon and 12,13-epoxy-4-(1-oxobut-2-enyloxy)-trichothec-9-ene (Konishi et al. 2003). Some of these toxins have been found in grapes and wheat in measurable quantities, but as heavy growth by *T. roseum* in foods is rare, they are unlikely to be significant in human health. However, their presence at quite low levels in grape musts has been reported to inhibit wine yeasts (Flesch et al. 1986).

Ecology As a ubiquitous and readily recognised saprophyte, *Trichothecium roseum* has been isolated from a variety of foods. Cereals are a common source, including barley, wheat, maize, sorghum and paddy rice (see Pitt and Hocking 1997). Other important sources include apples (Valletrisco and Niola 1983), grapes (see Pitt and Hocking 1997; Serra et al. 2005; Blancard et al. 2006) and tomatoes (Dal Bello 2008). This species has caused spoilage of a wide variety of other fruits and vegetables from time to time, but is usually only a minor pathogen (Snowdon 1990, 1991; Hamid et al. 2014). It has

also been isolated from meat products, cheese, beans, hazelnuts, pecans and pistachios (see Pitt and Hocking 1997). We isolated *T. roseum* from sorghum, cashews, paddy and milled rice, peanuts, maize and soybeans in Southeast Asia, but always at a low incidence (Pitt et al. 1998a). In our experience, levels of *T. roseum* in foods other than fruits are usually low, and spoilage exceptional.

Reference Domsch et al. (1980).

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Zygomycetes

6

Zygomycetes (the phylum Zygomycota) are among the most ancient lineages in the true fungi (Hoffmann et al. 2013). They are characterised by the production of solitary spores, **zygospores**, as their sexual state. Zygomycetes of significance here belong in the subphylum Mucoromycotina, the order Mucorales and most in the family *Mucoraceae*. These fungi are characterised by hyphae with few if any cross walls (septa): the hyphae are essentially unobstructed tubes. Absence of septa facilitates rapid translocation of nutrients and organelles such as mitochondria and nuclei between sites of growth, nutrient adsorption and spore formation. In consequence, Zygomycetes are also characterised by rapidity of growth. Many species are able to fill a Petri dish with loosely packed mycelium and to produce mature spores within two days of inoculation.

Zygospores are large (usually greater than 30μm diam), dark-walled, distinctive bodies (Fig. 6.1) on which Zygomycetes may rely for long term survival. Formation of zygospores by the majority of species encountered in food spoilage requires mating by two strains, so zygospores are not commonly observed in the pure cultures used for identification. A few species such as *Rhizopus sexualis* do produce zygospores in pure culture, and this is a valuable taxonomic aid. Perhaps because they are physically difficult to separate from other more abundant spore types, little is known about the physiological properties of zygospores, such as resistance to heat and chemicals.

Zygomycetes embrace a wide variety of fungi, with diverse habitats. Almost all require high water activities for growth. In damp situations, their rapid growth habit provides a selective advantage over most, more advanced fungi with septate hyphae. Many are found on dung or as insect pathogens. For a guide to genera of Zygomycetes which can be grown in the laboratory see O'Donnell (1979).

Order Mucorales The order Mucorales is monophyletic (having a single evolutionary origin) with all genera closely related genetically (White et al. 2006). However, early molecular studies indicated poor correlation with traditional morphological classification within the order (Voigt et al. 1999; O'Donnell et al. 2001; Kwasna et al. 2006). When taken together with more recent studies (Hoffmann et al. 2013), phylogenetic relationships within the order have been clarified. The ITS region has been shown to discriminate among most species in the order that have been examined (Walther et al. 2013). These authors have provided ITS barcodes which will be of value for discriminating among some of the species described in this chapter.

Although some species within this order produce zygospores as a sexual state in culture, traditional classification has been based on asexual (anamorphic) reproduction. Asexual reproduction in the order Mucorales is primarily by

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sporangiospores, which are typically borne within sporangia. **Sporangia** (Fig. 6.2a) are closed sacs, borne on stipes (stalks). Stipes are often termed **sporangiophores**, although this name is more appropriately applied to the whole fruiting structure in line with conventional terminology in other asexual fungi. Stipes may be borne singly or in clusters from fertile hyphae, may be branched or unbranched, or may grow out from beneath a terminal sporangium to produce a succession of sporangia (sympodial branching, Fig. 6.2b).

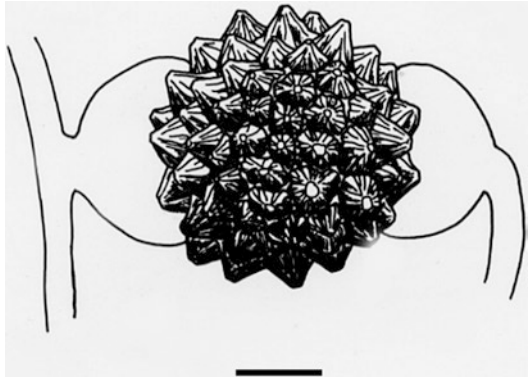

Fig. 6.1 Zygospore of *Rhizopus sexualis*, bar = 50μm

Sporangia appear under the low power microscope as small (less than 1 mm diam) brown to black spheres distributed on long stipes throughout the aerial mycelium. Sporangial walls disintegrate in age, releasing the sporangiospores.

In microscopic mounts from 7 day old cultures, intact sporangia are rare: the structures mostly seen at stipe apices are **columellae** (Fig. 6.2c), formed within the sporangia and remaining intact in age. The manner in which columellae collapse after sporangial disintegration provides useful taxonomic information.

In one genus, *Syncephalastrum*, sporangiospores are borne in cylindrical **merosporangia** in a radial array on the columella surface (Fig. 6.2d). Under low magnifications, these structures have some resemblance to the fruiting structures of *Aspergillus*. In another genus, *Thamnidium*, sporangiospores are borne both in typical sporangia and also in small **sporangioles**, formed in clusters (Fig. 6.2e), often on the same stipe as a sporangium. In *Cunninghamella*, sporangioles are also borne in clusters, on spicules, but never produce sporangiospores. Here the sporangiole itself acts as the reproductive unit.

Some species in the Mucorales also produce **chlamydoconidia** as a second type of anamorph.

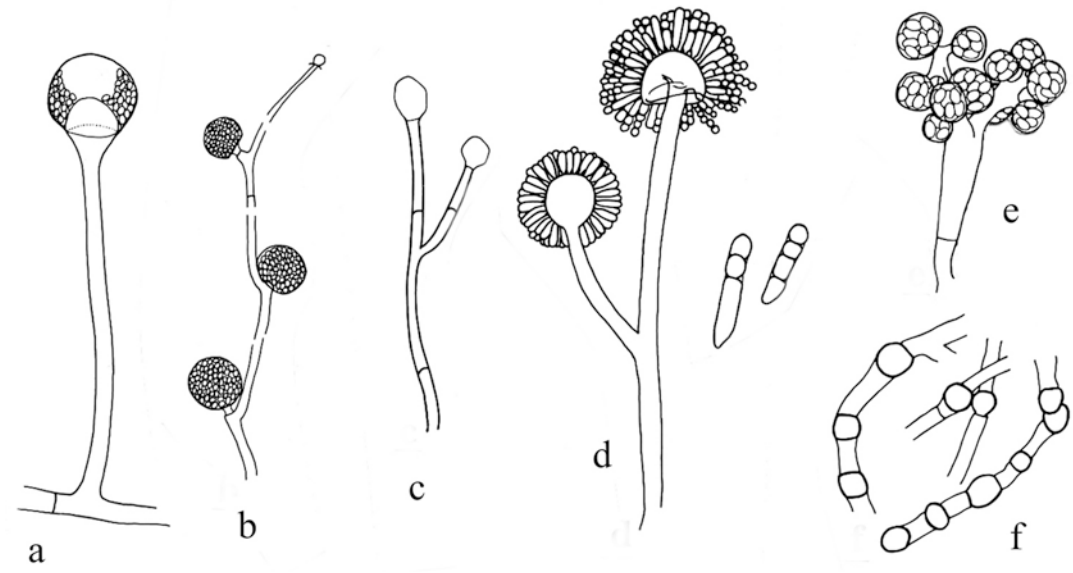

Fig. 6.2 Anamorphic reproductive structures in genera of Mucorales (**a**) sporangium; (**b**) sympodial branching; (**c**) collumellae; (**d**) merosporangium; (**e**) sporangioles; (**f**) chlamydoconidia

These are cylindrical to spherical cells with relatively thick walls formed in hyphae and stipes, sometimes in great numbers (Fig. 6.2f). They probably function as a resting stage, more resistant to light, heat and desiccation than sporangiospores, and are probably analogous to similar structures formed by Ascomycetes.

Identifying genera in Mucorales One theme of this book is to provide a standardised system for identification of food spoilage fungi. Where possible, identification is achieved after seven days incubation, by a single macroscopic and microscopic inspection of a standard set of Petri dishes. The keys to Mucorales hereunder are accordingly based on this system.

To the experienced mycologist it will be obvious that because they grow so rapidly, isolates of Mucorales can be identified much earlier than seven days. However, a second theme of this book is the facilitation of fungal identifications by the bacteriologist who does not instinctively recognise a *Mucor* from a *Monascus*, so the standard seven day schedule has been maintained here. There is an unexpected bonus: the characteristic shapes of columellae collapsing in age simplifies recognition of some genera (Fig. 6.3).

Significant genera Seven genera in the order Mucorales are treated here: *Cunninghamella*, *Lichtheimia*, *Mucor*, *Rhizomucor*, *Rhizopus*, *Syncephalastrum* and *Thamnidium*. These genera are differentiated here primarily by the morphology of their sporangiophores and sporangia.

Rhizoids, mucous and contamination Other useful features for distinguishing some of these genera are the production of rhizoids and the secretion of mucous material. Rhizoids are short root-like structures produced by *Rhizopus* at the base of each sporangiophore (Fig. 6.3c). *Mucor* species do not produce rhizoids, whereas *Lichtheimia* and *Rhizomucor* species produce them irregularly.

As the name implies, *Mucor* produces sporangiospores in a layer of mucous which causes them to adhere to the colony when disturbed or picked with a needle. *Lichtheimia* and *Rhizomucor* are similar, but *Rhizopus* produces dry spores.

If a *Mucor* culture fills the Petri dish the lid can be removed with minimal disturbance to the culture; however, the rhizoids of *Rhizopus* adhere to the lid and part of the culture will detach with it. Beware! Carry out this operation away from your inoculating area. Dry spores, which are aerially dispersed when plates are opened, and very rapid growth, make *Rhizopus* species a serious source of laboratory contamination.

The seven genera of Mucorales considered here are keyed below and then treated in alphabetical order.

Key to genera of the order Mucorales included here

1	All reproductive cells borne on spicules (spikes) around vesicles	2
	Some or all reproductive cells sporangiospores, borne within sporangia (Fig. 6.2a–c)	3
2 (1)	Reproductive cells sporangiospores, borne in cylindrical sacs (merosporangia; Fig. 6.2d)	*Syncephalastrum*
	Reproductive cells single celled spherical sporangioles	*Cunninghamella*
3 (1)	As well as sporangia, clusters of smaller sacs (sporangioles, Fig. 6.2e) present	*Thamnidium*
	Only sporangia, or columellae derived from sporangia, present	4
4 (3)	Columellae retaining approximately spherical shape after sporangiospore discharge (Fig. 6.3a), sporangiospore walls smooth or spiny	5
	Columellae collapsing to form funnel or umbrella shapes, sporangiospore walls smooth or striate	6
5 (4)	Sporangiospores rarely exceeding 5µm in long axis	*Rhizomucor*
	Sporangiospores commonly exceeding 5µm in long axis	*Mucor*
6 (4)	Columellae collapsing inwardly from the apex to form a funnel shape (Fig. 6.3b), sporangiospore walls smooth	*Lichtheimia*
	Columellae collapsing outwardly to form an umbrella shape (Fig. 6.3c), sporangiospore walls striate	*Rhizopus*

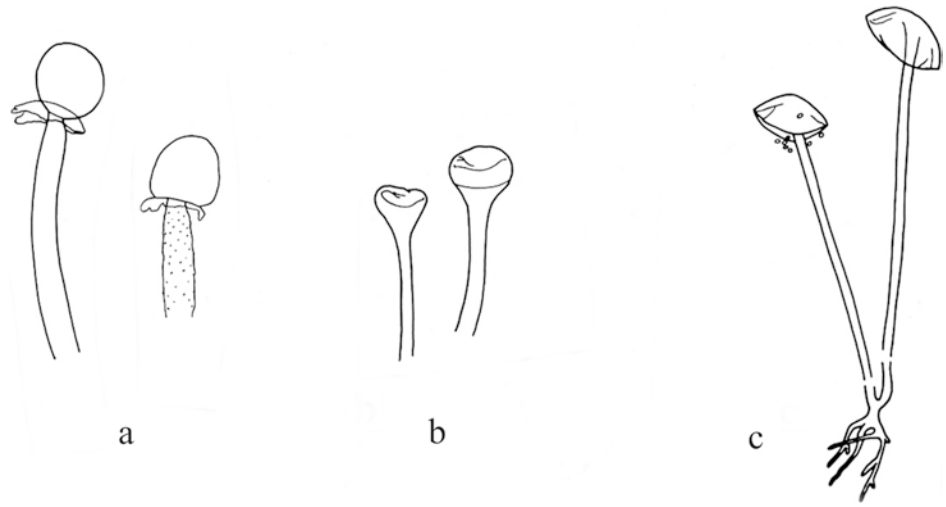

Fig. 6.3 Collapse of columellae in age (**a**) little collapse (*Mucor, Rhizomucor*); (**b**) funnel shapes (*Lichtheimia*); (**c**) umbrella shapes (*Rhizopus*)

6.1 Genus *Cunninghamella* Matr.

Cunninghamella is unusual in that sporangiophores give rise to sporangioles (small sporangia) which do not differentiate into sporangiospores but themselves act as the conidial stage. The sporangioles are borne on spicules (spikes) from vesicles; the vesicles are borne terminally or irregularly on the sporangiophores. This genus is now classified in the family *Cunninghamellaceae* along with *Lichtheimia* (Walther et al. 2013). Zheng and Chen (2001) listed 15 species, and a molecular study of 12 of these showed good agreement with traditional speciation (Liu et al. 2001). DNA barcodes for all *Cuninghamella* species have been provided by Yu et al. (2015).

Zheng and Chen (2001) reported that a great deal of literature confusion exists over the names of species, in particular with the common species *C. bertholletiae* and *C. elegans.* These two species are distinguishable by small morphological differences, and by the fact that *C. bertholletiae* grows to 42 °C or more, while the maximum growth temperature for *C. elegans* is usually 35 °C. Although the name *C. elegans* is most commonly seen in the food literature, our experience indicates that *C. bertholletiae* is the most common species, along with *C. echinulata*. *C. bertholletiae* is described below: *C. echinulata* differs by the production of sporangioles with conspicuously spiny walls (Zheng and Chen 2001).

Cunninghamella bertholletiae Stadel Fig. 6.4

Colonies on CYA and MEA covering or filling the whole Petri dish, sparse, mycelium off white to beige, reverse colourless to pale yellow. On G25N, ranging from no growth to colonies 15 mm diam, of sparse, translucent mycelium. At 5 °C, no germination. At 37 °C, colonies similar to those on CYA at 25 °C, mycelium off white to grey, reverse pale yellow to brown.

Sporangiophores long, bearing solitary vesicles terminally and in irregular verticils subterminally or along the length; sporangioles borne from spicules, breaking off at maturity, spherical to ellipsoidal, 7–11μm diam or 9–13μm long, with smooth to finely roughened walls.

Zygospores not commonly seen in pure culture, *fide* Zheng and Chen (2001) irregularly ellipsoidal to spherical, brownish, 20–45μm diam, with rough walls.

Distinctive features See genus description.

Taxonomy Most *Cunninghamella* species are heterothallic, producing zygospores only when two mating types from the same species are grown together.

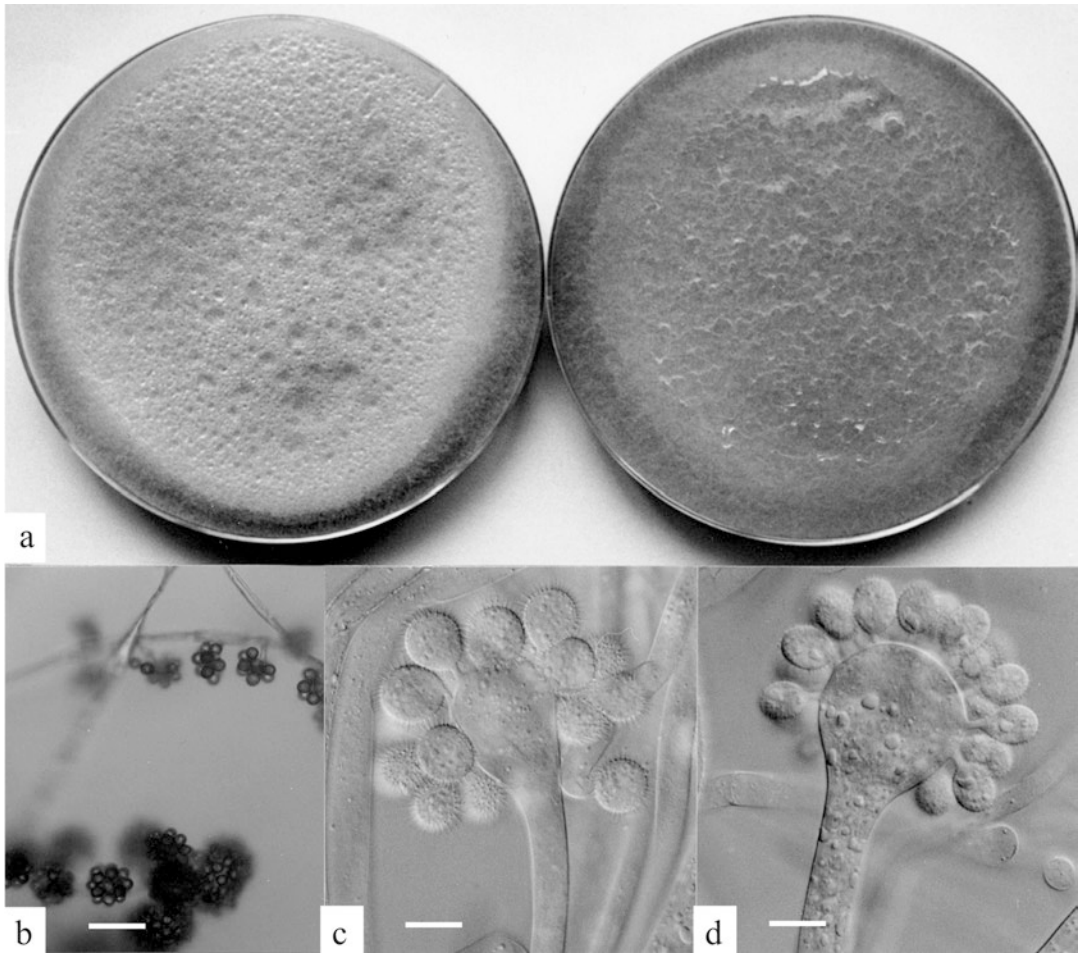

Fig. 6.4 *Cunninghamella bertholletiae* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) developing sporangioles *in situ*, bar = 50μm; (**c**, **d**) vesicles and mature sporangioles, bar = 10μm

Identifiers MycoBank MB230361; neotype NRRL 1380; ex-type strain NRRL 1380; ITS barcode AF254930 (Yu et al. 2015).

Physiology *C. bertholletiae* grows up to 42–45 °C. Growth in soil is confined to above −90 bars (ca 0.93 a_w) (Kouyeas 1964).

Mycotoxins Mycotoxins are not produced.

Ecology In common with other *Cunninghamella* species, *C. bertholletiae* mostly occurs in tropical and subtropical soils (Zheng and Chen 2001). *C. echinulata* was a cause of rotting in kola nuts (Adebajo 1994). We have seen *C. bertholletiae* at low levels in maize from the Philippines and peanuts from Indonesia (Pitt et al. 1998 and unpublished).

References O'Donnell (1979); Zheng and Chen (2001); Yu et al. (2015).

6.2 Genus *Lichtheimia* Vuill.

The genus *Lichtheimia* produces a distinctive type of columella which widens gradually at the junction with the stipe, outside the circumference of the sporangium (Fig. 6.5b). In other Zygomycete genera considered here, the junction of stipe and columella is abrupt, and the columella is wholly within the sporangial wall. In age, columellae of *Lichtheimia* frequently collapse inward from the apex to form funnel shaped structures.

Lichtheimia species form rhizoids, irregular root-like outgrowths at the bases of the stipes, but these are less conspicuous and less regular than in *Rhizopus* species. Only one *Lichtheimia*

species, *L. corymbifera,* is at all common in foods. Until recently, *L. corymbifera* was classified in *Absidia*, but molecular studies cited in the preamble to this chapter indicated that *Absidia* was polyphyletic, so the old name *Lichtheimia* was revived to accommodate *A. corymbifera* and the closely related *A. ramosa* (Hoffmann et al. 2007, 2009). The genus was monographed by Alastruey-Izquierdo et al. (2010), who accepted five species.

Lichtheimia corymbifera (Cohn) Vuill. Fig. 6.5

Absidia corymbifera (Cohn) Sacc. & Trotter

On CYA, colonies covering the Petri dish, low and sparse, white to pale brown or grey; reverse colourless. On MEA, colonies filling the whole Petri dish with deep floccose mycelium, coloured mid grey by sporangia; reverse pale. On G25N, colonies 10–30 mm diam, sparse and floccose, coloured as on MEA. No growth at 5 °C. At 37 °C, colonies covering the whole Petri dish, similar to those at 25 °C.

Sporangiophores borne from aerial hyphae, stipes sometimes irregularly branched; sporangia hyaline, 15–50μm diam, appearing pyriform due to conical columellae; columellae pyriform 10–30μm diam, sometimes with small projections on the apices or with collarettes above the base, in age often collapsing inward from the apex to form funnel shaped structures; sporangiospores hyaline, broadly ellipsoidal to spheroidal, 3–6μm long, smooth walled.

Distinctive features See genus description. The ability to grow at 42 °C or above was used as a character to separate *Lichtheimia* from *Absidia* by Hoffmann et al. (2007). *Lichtheimia ramosa* (Zopf) Vuill. is closely related to *L. corymbifera*, differing by faster growth at 42 °C and by producing predominantly cylindroidal to ellipsoidal conidia (Alastruey-Izquierdo et al. 2010).

Identifiers MycoBank MB416447; neotype CBS 429.75; ex-type strain CBS 429.75; ITS barcode GQ342878 (Alastruey-Izquierdo et al. 2010).

Physiology Evans (1971) recorded growth temperatures for *Lichtheimia corymbifera* as minimum 14 °C, maximum 50 °C and optimum near 40 °C, while a maximum of 53 °C was recorded for one of three isolates by Hoffmann et al. (2007). This species is able to germinate and grow down to 0.88 a_w (Hocking and Miscamble 1995). Growth in nitrogen (<1% O_2) was similar to that in air (Hocking 1990).

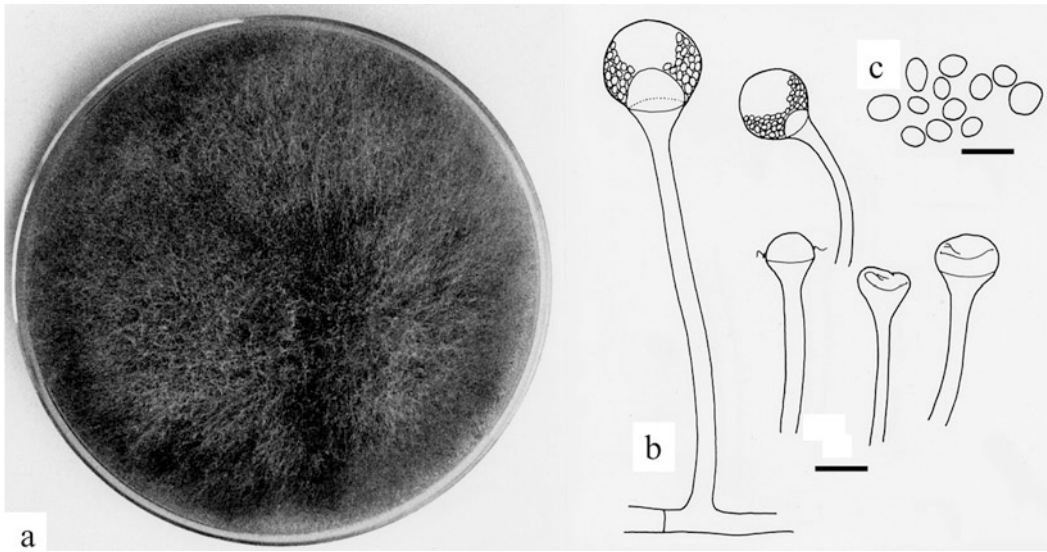

Fig. 6.5 *Lichtheimia corymbifera* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangia and columellae, bar = 25μm; (**c**) sporangiospores, bar = 10μm

Mycotoxins Mycotoxin production has not been reported.

Ecology *Lichtheimia corymbifera* is a weak human and animal pathogen, with a very wide host range and capable of infecting many body organs (de Hoog et al. 2000). Almost all isolations from foods have been under the name *Absidia corymberifa*. Most isolations have been from wheat, barley, malted barley, and cereal products such as flour and bran. This species has also been found in meat products and biltong, cassava, hazelnuts and sunflower seeds (see Pitt and Hocking 1997). It has been recorded as a pathogen of peaches (Singh and Prashar 1988). *L. corymbifera* is probably widespread in the tropics: Oyeniran (1980) recorded isolations from cocoa, palm kernels and maize. It was the dominant species found on cocoa beans in Brazil during drying (Copetti et al. 2011). We have found it, always at low levels, in sorghum and mung beans from Thailand (Pitt et al. 1993, 1994), in peanuts, kemiri nuts, milled rice and coriander from Indonesia (Pitt et al. 1998) and peanuts, paddy and milled rice, soybeans and black pepper from the Philippines (our unpublished data).

References De Hoog et al. (2000); Hoffmann et al. (2007, 2009).

6.3 Genus *Mucor* P. Micheli ex L.

As traditionally described, *Mucor* is a very common and widespread genus in nature, occurring in soils, decaying vegetation, dung and many other moist habitats where rapidly growing fungi have an advantage. Molecular studies (Voigt et al. 1999; O'Donnell et al. 2001; Kwasna et al. 2006; Hoffmann et al. 2013) have concluded that *Mucor* is polyphyletic, however the species included here appear to fall into the major *Mucor* clade, so the traditional description is used here. Unlike those of *Lichtheimia*, sporangia of *Mucor* have columellae borne wholly within the sporangial wall; the columellae collapse irregularly, if at all, in age (Fig. 6.3a). Unlike *Rhizopus* species, most Mucors do not produce rhizoids. In species of interest here, sporangiospores are longer than 5µm and have walls which are smooth or spiny, but not striate. This is in contrast to *Rhizopus* species, which commonly produce sporangiospores with striate walls.

Some *Mucor* species are able to grow and weakly ferment under anaerobic conditions and occasionally cause spoilage of beverages in this manner. Under these circumstances, growth is yeast-like in appearance (Fig. 6.6), although individual cells are much too large to be mistaken for a true yeast. Inoculation of such cells onto aerobic media produces normal growth. Yeast-like growth has also been reported to occur when *Mucor* and some related genera grow in the presence of high sodium chloride concentrations (Tresner and Hayes 1971).

At least 20 species of *Mucor* have been reported from foods. Five appear to be most significant: *M. circinelloides*, *M. hiemalis*, *M. piriformis*, *M. plumbeus* and *M. racemosus*. These species are keyed and described below. The ITS region is very informative for *Mucor* species, and many species can be recognised from ITS data alone (Walther et al. 2013).

Key to *Mucor* species included here

1	Strong growth at 37 °C	*M. circinelloides*
	No growth at 37 °C	2
2 (1)	Columellae frequently with small irregular projections on the apices; sporangiospores with minute spines	*M. plumbeus*
	Columellae without apical projections; sporangiospores smooth walled	3
3 (2)	Columellae 50–100µm diam; growth on G25N weak or absent	*M. piriformis*
	Columellae usually less than 50µm diam; colonies on G25N greater than 10 mm diam	4
4 (3)	Chlamydoconidia abundant, often dominating microscopic appearance	*M. racemosus*
	Chlamydoconidia present in low numbers or absent	*M. hiemalis*

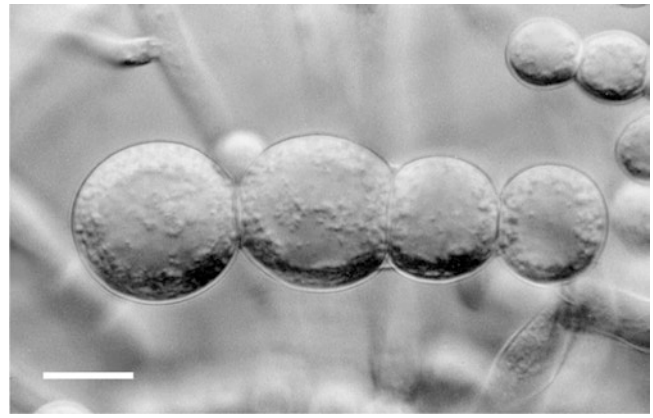

Fig. 6.6 *Mucor plumbeus* showing yeast-like growth, in liquid culture under anaerobic conditions, bar = 25µm

Mucor circinelloides Tiegh. Fig. 6.7

On CYA, colonies 60 mm diam or more, often spreading across the whole Petri dish, but growth relatively low and sparse, appearing pale grey or yellowish; reverse uncoloured. On MEA, colonies filling the whole Petri dish, in colours similar to those on CYA. On G25N, colonies 15–25 mm diam, low and relatively dense, golden yellow in both obverse and reverse. At 5 °C, colonies 4–10 mm diam, low and sparse. At 37 °C, colonies 20–40 mm diam, sparse and floccose, with colours more brown than at 25 °C.

Sporangiophores borne from aerial hyphae, stipes commonly branched, often sympodially, sporangia spherical, 25–50µm diam, sometimes up to 80µm; columellae roughly spherical, up to 50µm diam; sporangiospores hyaline, ellipsoidal, mostly 4.5–7µm long, smooth walled. Chlamydoconidia uncommon, spherical, cylindrical or rather irregular, up to 15µm diam. Zygospores not formed in pure culture.

Distinctive features *Mucor circinelloides* has numerous characters in common with *M. hiemalis*. Unlike *M. hiemalis*, *M. circinelloides* grows well at 37 °C (20 mm or more in 7 days), grows weakly at 5 °C (less than 10 mm v. greater than 20 mm in 7 days), and commonly produces sympodially branched stipes.

Taxonomy On the basis of mating studies, Schipper (1976) synonymised several species with *M. circinelloides*, then divided the species into several "forma". That taxonomy has been confirmed by molecular information (Walther et al. 2013), but identification to species is sufficient here, as the physiology of the various forma is similar so far as is known.

Identifiers MycoBank MB198947; neotype CBS 195.68; ex-type strain CBS 195.68; ITS barcode JN205961 (Walther et al. 2013).

Physiology Tresner and Hayes (1971) reported that *Mucor circinelloides* grew in media containing 15% (w/v) NaCl (= 0.90 a_w) but not 20% (= 0.86 a_w). On a medium with glucose as controlling solute, this species germinated and grew down to 0.90 a_w (Hocking and Miscamble 1995).

Mycotoxins This species is not known to produce mycotoxins.

Ecology *Mucor circinelloides* has been reported frequently as an animal pathogen and occasionally as a human pathogen (de Hoog et al. 2000). It is the dominant fungus in traditional Slovak sheep cheese (Laurencik et al. 2008) and is common in French cheese (Hermet et al. 2012). However, this species can also cause spoilage in cheese and yams (see Pitt and Hocking 1997), and can be pathogenic on mangoes (Johnson et al. 1990) and peaches (Restuccias et al. 2006). It is associated with meji, a Korean fermented soybean base used in soy sauce and soybean paste manufacture (Hong et al. 2012). It has also been isolated from meat, hazelnuts and walnuts, from maize, mung beans, soybeans and barley (Pitt et al. 1993, 1994, 1998; see Pitt and Hocking 1997).

References Schipper (1976); Domsch et al. (1980); Walther et al. (2013).

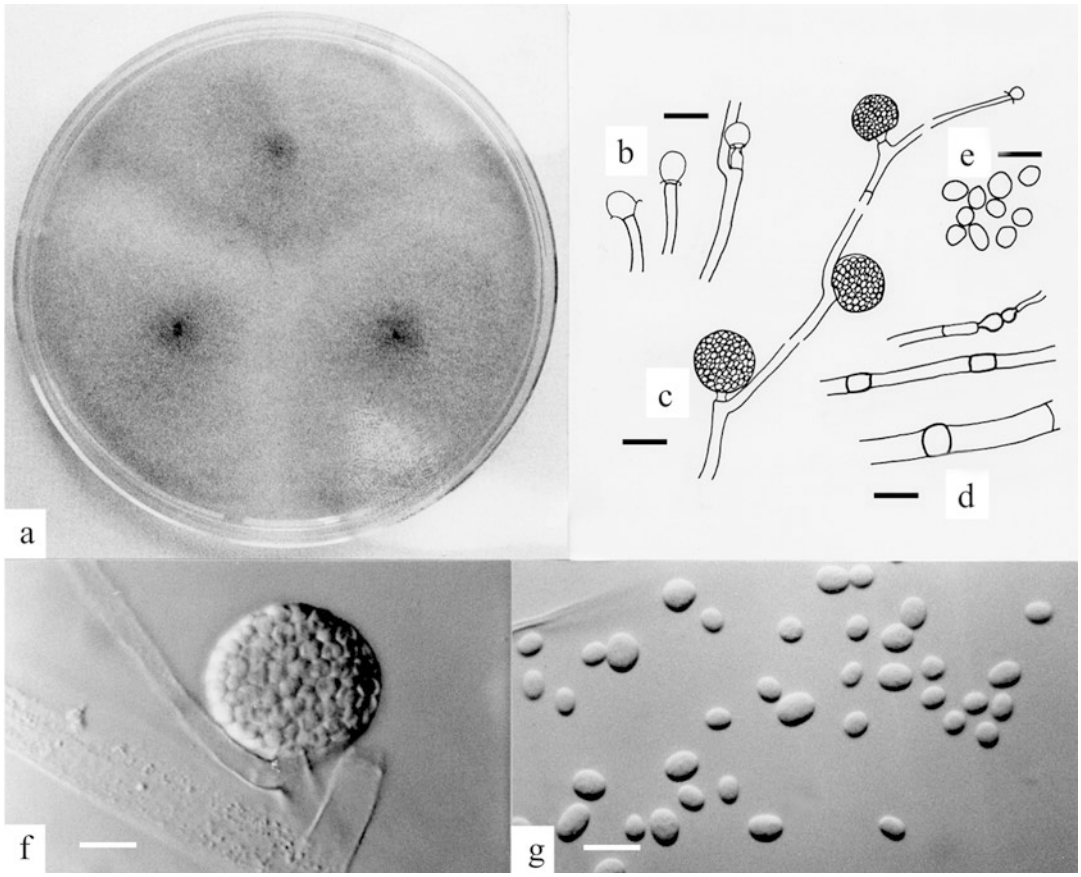

Fig. 6.7 *Mucor circinelloides* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) columellae; (**c**) sporangia; (**d**) chlamydoconidia; (**e**) sporangiospores; (**f**) sporangium; (**g**) sporangiospores, all bars = 10µm

Mucor hiemalis Wehmer Fig. 6.8

On CYA, colonies spreading across, and sometimes filling, the whole Petri dish, growth relatively sparse, greyish; reverse pale. On MEA, colonies filling the whole Petri dish, relatively dense, greyish to distinctly yellow; reverse yellow to golden yellow. On G25N, colonies 10–15 mm diam, moderately dense, bright yellow in both obverse and reverse. At 5 °C, colonies 20–30 mm diam, low and sparse. No growth at 37 °C.

Sporangiophores borne aerially, stipes generally unbranched, less commonly sympodially branched; sporangia up to 60µm diam; columellae ellipsoidal, 15–30µm diam; sporangiospores hyaline, narrowly to broadly ellipsoidal or reniform (kidney shaped), 5–11µm long, smooth walled. Chlamydoconidia uncommon, spherical to cylindrical or irregular, up to 15µm diam. Zygospores not formed in pure culture.

Distinctive features *Mucor hiemalis* is similar to *M. circinelloides*, but grows more rapidly at 5 °C and does not grow at 37 °C. Moreover, it produces larger sporangiospores which are sometimes reniform, and usually has unbranched stipes.

Taxonomy On the basis of mating studies, Schipper (1973) synonymised several species with *M. heimalis*, then divided the species into several "forma". That taxonomy has been confirmed by molecular information (Walther et al. 2013), but identification to species is sufficient here, as the physiology of the various forma is similar so far as is known.

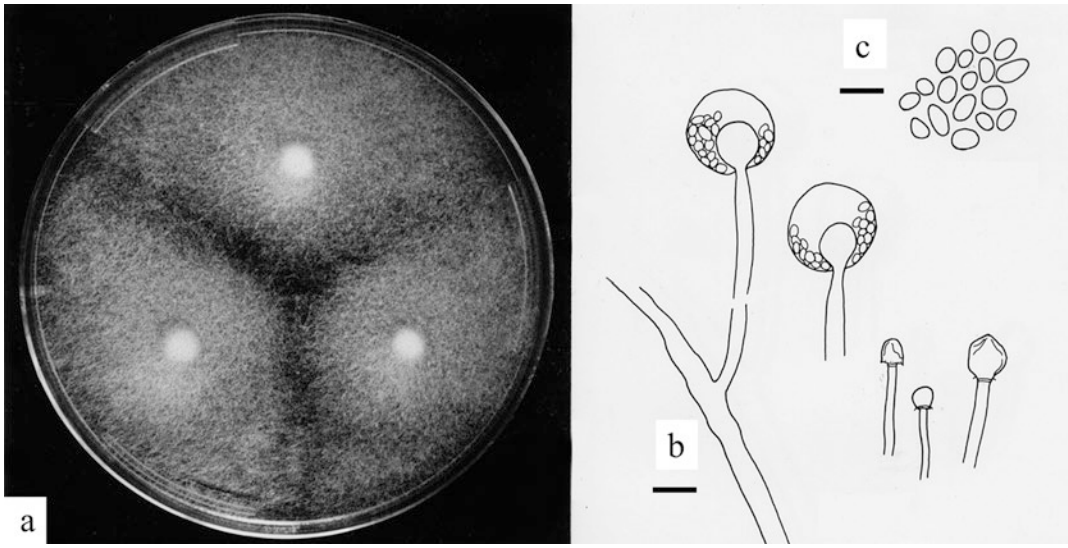

Fig. 6.8 *Mucor hiemalis* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangia and columellae, bar = 25µm; (**c**) sporangiospores, bar = 10µm

Identifiers MycoBank MB249401; neotype CBS 201.65; ex-neotype strain CBS 201.65; ITS barcode JN206125 (Walther et al. 2013).

Physiology Growth of some *Mucor hiemalis* isolates occurs at temperatures below 0 °C (Joffe 1962).

Mycotoxins Mycotoxin production has not been reported.

Ecology *Mucor hiemalis* has been reported as a rare cause of cutaenous mycosis (de Hoog et al. 2000). This species causes rots in guavas (Ito et al. 1979), carrots and cassava (Snowdon 1991). It has been reported from spoilage of cheese (Hayaloglu and Kirbag 2007) and of yoghurt due to inward collapse of containers (Foschino et al. 1993). It has also been reported from fresh vegetables (Lugauskas et al. 2005), from chestnuts (Jermini et al. 2006), hazelnuts and soybeans (see Pitt and Hocking 1997), from wheat based fast foods in Nigeria (Fapohunda and Ogundero 1990), from preprepared airline food in Egypt (Saudi and Mansour 1990), and from chocolate confectionery in Italy (Dragoni et al. 1989). We have isolated it at low levels from Thai and Indonesian maize and paddy rice (Pitt et al. 1993, 1998).

Reference Schipper (1973); Walther et al. (2013).

Mucor piriformis Scop. Fig. 6.9

On CYA, colonies low and sparse, spreading across the Petri dish or discrete; mycelium colourless, overall colour buff from sporangia; reverse pale. On MEA, colonies 35–60 mm diam, coloured grey or brownish; reverse pale. On G25N, colonies less than 7 mm diam, or growth absent. At 5 °C, colonies 30–45 mm diam, low and sparse, or with some aerial hyphae. No growth at 37 °C.

Sporangiophores on CYA borne from surface hyphae, stipes short, broad, often sympodially branched and with encrusted walls; sporangia up to 150µm diam, with spinulose walls; columellae spheroidal to short cylindroidal, 25–80(−100) µm diam or in length, the larger ones collapsing irregularly; sporangiospores hyaline, spherical to broadly ellipsoidal, 6–12(−20) µm diam or in long axis, smooth walled. Chlamydoconidia uncommon; zygospores not formed in pure culture.

Distinctive features *Mucor piriformis* grows rapidly on CYA at 5 °C, but relatively poorly under the standard 25 °C incubation conditions. This is especially noticeable on G25N, where growth is weak or absent. Mycelium on CYA at 25 °C is often contorted and highly branched.

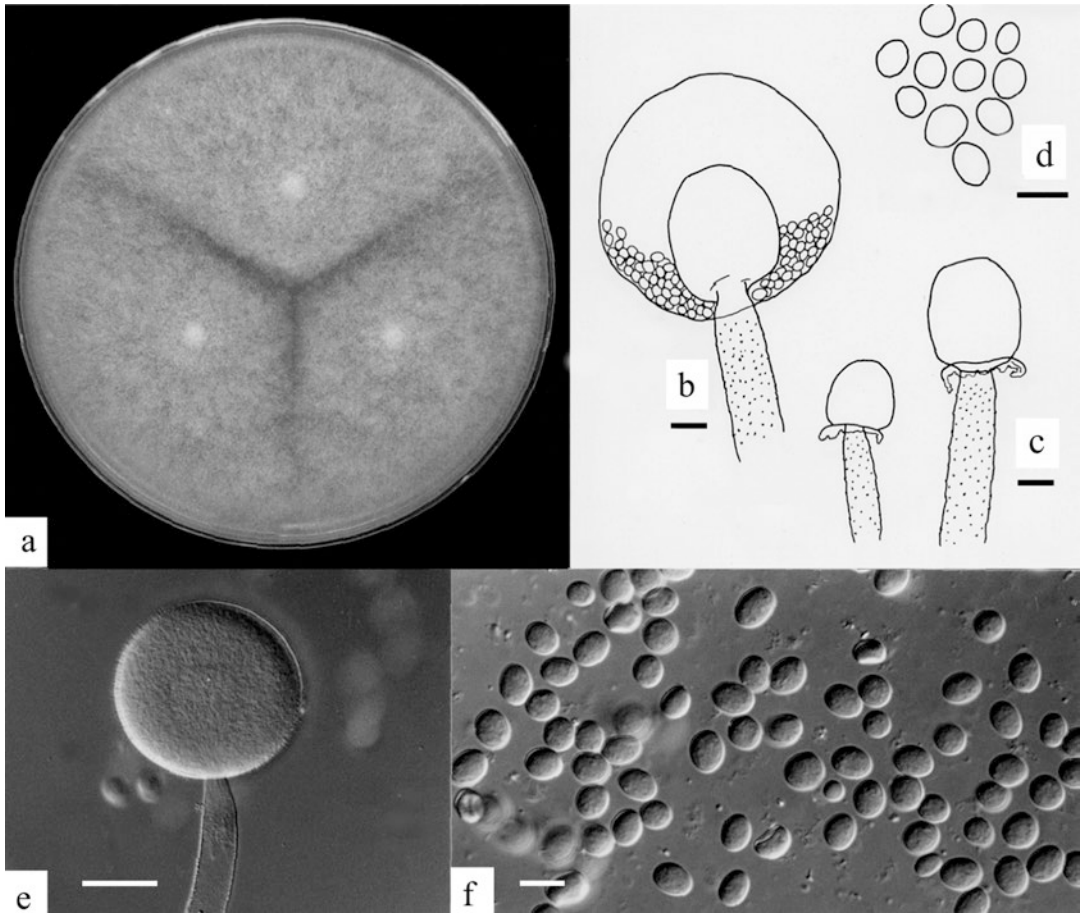

Fig. 6.9 *Mucor piriformis* (**a**) colonies on MEA, 7 d, 25 °C; (**b**) sporangia, bar = 25μm; (**c**) columellae, bar = 25μm; (**d**) sporangiospores, bar = 10μm; (**e**) developing sporangium, bar = 25μm; (**f**) sporangiospores, bar = 10μm

Sporangia, columellae and many sporangiospores are larger than those of other common *Mucor* species.

Identifiers MycoBank MB271881; neotype CBS 169.25; ex-type strain CBS 169.25; ITS barcode JN206028 (Walther et al. 2013).

Physiology *Mucor piriformis* is a psychrophile. Cardinal temperatures are: minimum, near 0 °C, optimum 20–21 °C, maximum 26 °C (Michailides and Spotts 1990). Mycelium and sporangiospores were inactivated by heating to 43–46 °C and 52–55 °C, respectively (Michailides and Ogawa 1989).

Mycotoxins Mycotoxin production has not been reported.

Ecology *Mucor piriformis* is a destructive pathogen of fresh strawberries (Snowdon 1990; Pitt and Hocking 1997), and causes rotting of cold stored pears, apples and tomatoes (see Pitt and Hocking 1997), plums (Borve and Vangdal 2007), mandarins (Saito et al. 2016) and yams (Amusa and Baiyewa 1999; Iwata 2006). A variety of treatments for control of pear spoilage have been proposed: dips in hot water (Michailides and Ogawa 1989), a hot water pressure process (Spotts et al. 2006), salt and surfactant solutions (Spotts and Cervantes 1989), prestorage drench in thiabendazole (Lennox et al. 2004) or treatment of wash water with chlorine dioxide or peracetic acid (Roberts and Reymond 1994; Mari et al. 2003). This species has been reported as dominant in the mycoflora of cassava and yam chips (Gnonlonfin et al. 2008).

References Schipper (1975); Michailides and Spotts (1990); Walther et al. (2013).

***Mucor plumbeus* Bonord.** **Fig. 6.10**
Mucor spinosus Tiegh.

On CYA and MEA, colonies at least 50 mm diam, low to deep, often spreading across the Petri dish; mycelium colourless, overall colour pale to deep grey from sporangia; reverse colourless. Colonies on G25N 20–35 mm diam, low, moderately dense, white to pale yellow brown; reverse pale. At 5 °C, colonies 8–15 mm diam, low and sparse. No growth at 37 °C.

Sporangiophores borne from surface or aerial hyphae, stipes unbranched or branched sympodially, sporangia dark greyish brown, up to 80µm diam, with spiny walls; columellae pyriform to ellipsoidal or short cylindroidal, up to 50 × 30µm, often with irregular projections at the apices; sporangiospores brown, spheroidal, commonly 7–8(−12) µm diam, with walls rough or minutely spiny. Chlamydoconidia uncommon; zygospores not formed in pure culture.

Distinctive features *Mucor plumbeus* is distinguished by its grey colour, columellae with irregular apical projections, and brown sporangiospores with rough or spiny walls.

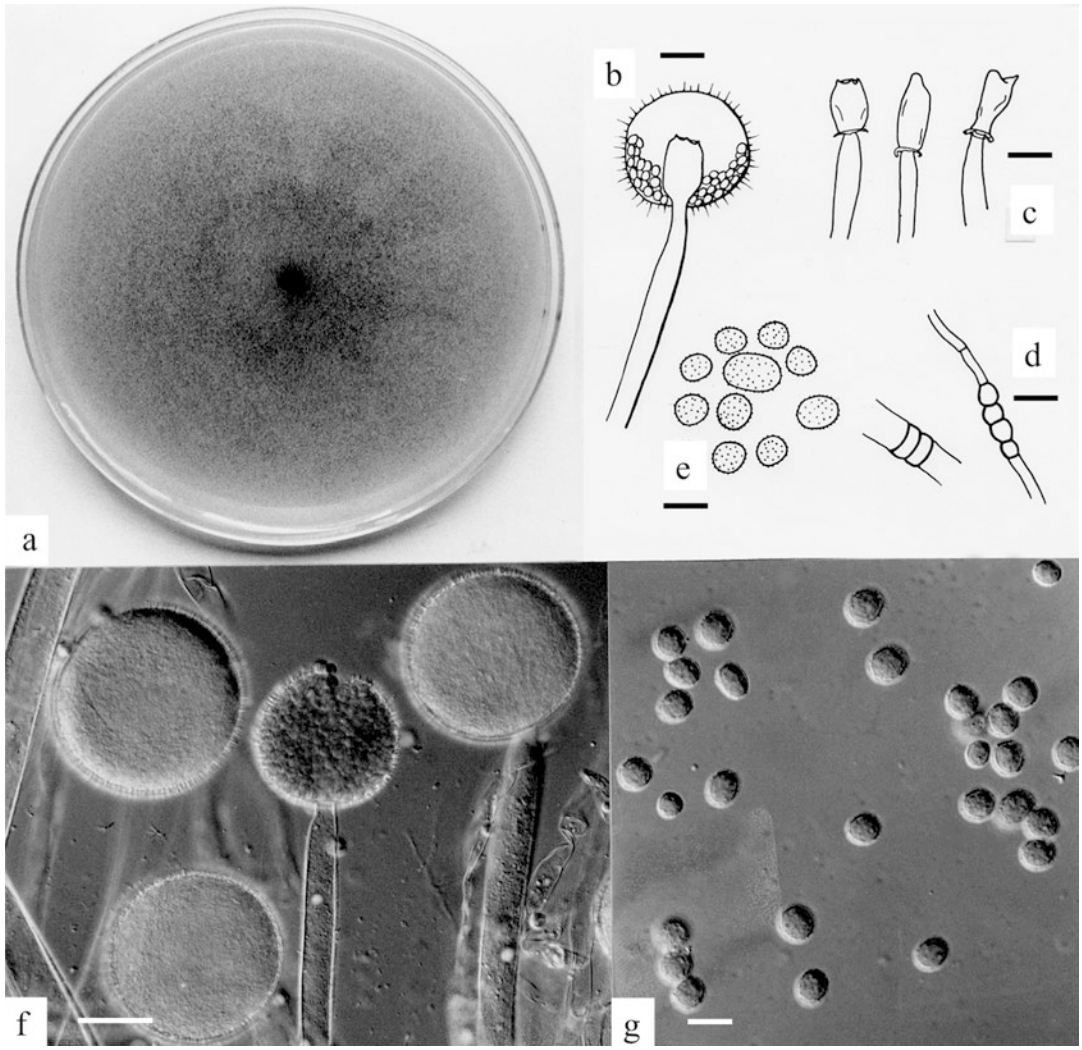

Fig. 6.10 *Mucor plumbeus* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangia, bar = 25µm; (**c**) columellae, bar = 25µm; (**d**) chlamydoconidia, bar = 25µm; (**e**) sporangiospores, bar = 10µm; (**f**) sporangia, bar = 25µm; (**g**) sporangiospores, bar = 10µm

Taxonomy Molecular data indicate that *M. plumbeus* is correctly placed in the genus *Mucor* (Kwasna et al. 2006; Walther et al. 2013).

Identifiers MycoBank MB237923; type unknown; representative culture CBS 129.49, IMI 116944, ATCC 18418; ITS barcode JN205912 (Walther et al. 2013).

Physiology Panasenko (1967) reported growth of *Mucor plumbeus* from 4 or 5 to 35 °C, with an optimum of 20–25 °C. The minimum a_w for growth was reported to be 0.93 by Snow (1949). Growth in N_2 (<1% O_2) was 80% of that in air; some growth occurred in an atmosphere of ≥97% CO_2, with only trace amounts of O_2 (Hocking 1990). As measured by colony diameters, growth on cheddar cheese in an atmosphere of 20% or 40% CO_2 and 5% O_2 was 50% of that in air, and still 30 or 40% of that in air in 20% or 40% CO_2 and 1% O_2 respectively (Taniwaki et al. 2001). *M. plumbeus* was still able to grow, albeit slowly, in 60% CO_2 and <0.5% O_2 (Taniwaki et al. 2009).

Mycotoxins Mycotoxins are not known to be produced.

Ecology *Mucor plumbeus* has been reported to commonly occur on cheese (Hermet et al. 2012; as *M. spinosus*) and to cause spoilage (Northolt et al. 1980; Devoyod 1988); this species has been observed to cause anaerobic spoilage of apple juice in our laboratory. Meat (Gros et al. 2003; Pitt and Hocking 1997), nuts and cereals are other commodities from which this species, and its synonym *M. spinosus*, have been reported (see Pitt and Hocking 1997). It was isolated at low levels from black rice in Thailand, soybeans in the Philippines and coriander in Indonesia (Pitt et al. 1994 1998).

Reference Schipper (1976); Walther et al. (2013).

Mucor racemosus Bull. Fig. 6.11

On CYA and MEA, colonies spreading across the Petri dish, low to moderately deep; mycelium colourless, overall colour light to mid brown from sporangia and chlamydoconidia; reverse light brown. On G25N, colonies 25–40 mm diam, low, moderately dense, similar in colour to those on CYA. At 5 °C, colonies 12–25 mm diam, low and sparse. No growth at 37 °C.

Sporangiophores borne from surface or aerial mycelium, stipes branched sympodially or irregularly, sporangia up to 80µm diam, light brown, with encrusted walls; columellae ellipsoidal to

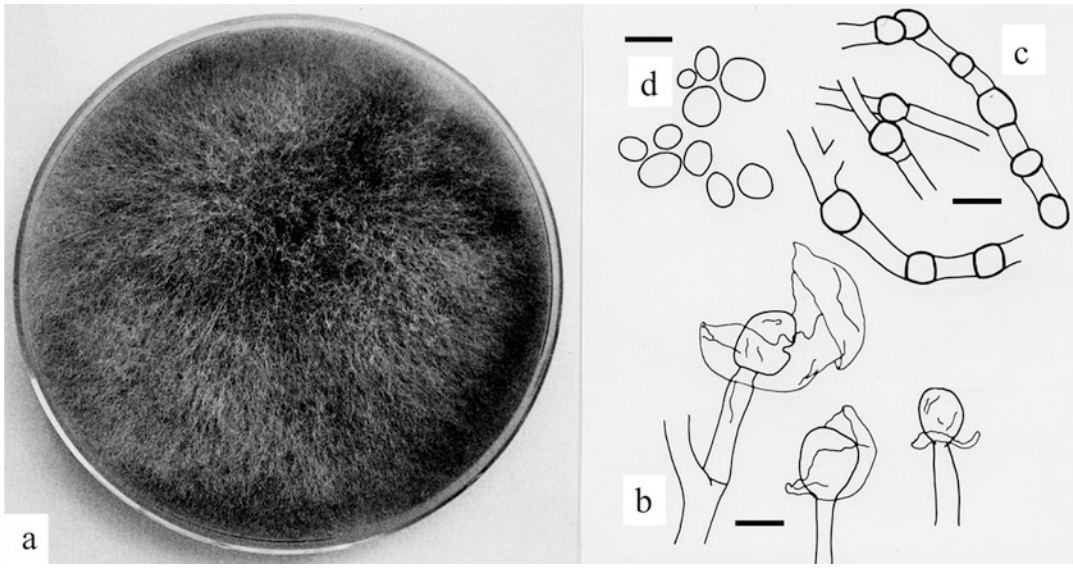

Fig. 6.11 *Mucor racemosus* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) columellae, bar = 25µm; (**c**) chlamydoconidia, bar = 25µm; (**d**) sporangiospores, bar = 10µm

pyriform, up to 40µm long; sporangiospores hyaline to pale brown, broadly ellipsoidal to subspheroidal, commonly 5–8µm diam, smooth walled. Chlamydoconidia and arthroconidia formed abundantly, 5–20µm or more in diam or long axis. Zygospores not formed in pure culture.

Distinctive features *Mucor racemosus* is similar in many respects to *M. plumbeus*. *M. racemosus* differs by faster growth on G25N; brown not grey colony colouration; the much greater abundance of chlamydoconidia; smooth sporangiospores; and the absence of irregular projections on columellae.

Taxonomy Molecular data indicate that *M. racemosus* is correctly placed in the genus *Mucor* (Kwasna et al. 2006; Walther et al. 2013).

Identifiers MycoBank MB247402; neotype CBS 260.68; ex-type strain CBS 260.68; ITS barcode JN205898 (Walther et al. 2013).

Physiology *Mucor racemosus* grows between −3 or −4 °C and 30–35 °C, with an optimum of 20–25 °C (Panasenko 1967). The minimum a_w for growth is 0.92 (Panasenko 1967).

Mycotoxins No mycotoxins are produced.

Ecology *Mucor racemosus* is responsible for a spongy soft rot of cool stored sweet potatoes, potatoes and citrus (Chupp and Sherf 1960). It has been reported, along with *M. hiemalis*, from spoilage of yoghurt due to inward collapse of containers (Foschino et al. 1993), and as a cause of spoilage of cheese (Devoyod 1988; and in our laboratory) and cheesecake (Piskorska-Pliszczynska and Borkowska-Opacka 1984). It is associated with meji, a Korean fermented soybean base used in soy sauce and soybean paste manufacture (Hong et al. 2012). It has also been found contaminating cheese (Kivanç 1992; Lund et al. 1995; Cantoni et al. 2003; Hermet et al. 2012), salami and other processed meats (Cantoni et al. 2007; Castellari et al. 2010; Canel et al. 2013), raisins (Youssef et al. 2000), frozen and processed meats, salted horse meat, fermenting cacao beans, maize, barley, soybeans and paddy rice (see Pitt and Hocking 1997).

Reference Schipper (1976); Walther et al. (2013).

6.4 Genus *Rhizomucor* Lucet & Costantin

The genus *Rhizomucor* was revived by Schipper (1978a, b) for three species previously accepted in *Mucor*, but distinguished by the production of stolons and by their thermophilic nature. Molecular studies (Voigt et al. 1999; Hoffmann et al. 2013) have support this concept. Zheng et al. (2009) accepted seven species and provided a key. *Rhizomucor* species are significant in foods mainly in tropical regions. The most common species in foods is *R. pusillus*; *R. miehei* is mentioned also.

Rhizomucor pusillus (Lindt) Schipper **Fig. 6.12**

Mucor pusillus Lindt

On CYA, colonies sometimes 25–35 mm diam, of colourless to white floccose mycelium surmounted by brown sporangia, or sometimes covering the whole Petri dish and then similar to on MEA. On MEA, colonies covering the whole Petri dish, low and relatively sparse, pale to mid grey; reverse pale, yellowish or greenish grey. On G25N, growth sparse, not exceeding 5 mm diam, or absent. No growth at 5 °C. At 37 °C, colonies similar to those at 25 °C on MEA, but more dense, brown to dark grey.

Sporangiophores borne from surface hyphae, stipes sometimes appearing unbranched, but usually extensively and irregularly branched; poorly formed rhizoids sometimes apparent but not adjacent to the stipe bases, a distinction from *Rhizopus*; sporangia spherical, brown or grey, 40–60(−80) µm diam; columellae spherical, ellipsoidal or pyriform, 20–45µm diam, sometimes collapsing irregularly (similar to *Mucor* species); sporangiospores hyaline, spherical to broadly ellipsoidal, 3–4µm diam, smooth walled. Zygospores produced by occasional isolates, black, broadly ellipsoidal, 60–70µm diam.

Distinctive features *Rhizomucor* species are most readily distinguished by rapid growth at 37 °C and by their sporangiospores, which are small (less than 5µm diam) and smooth walled.

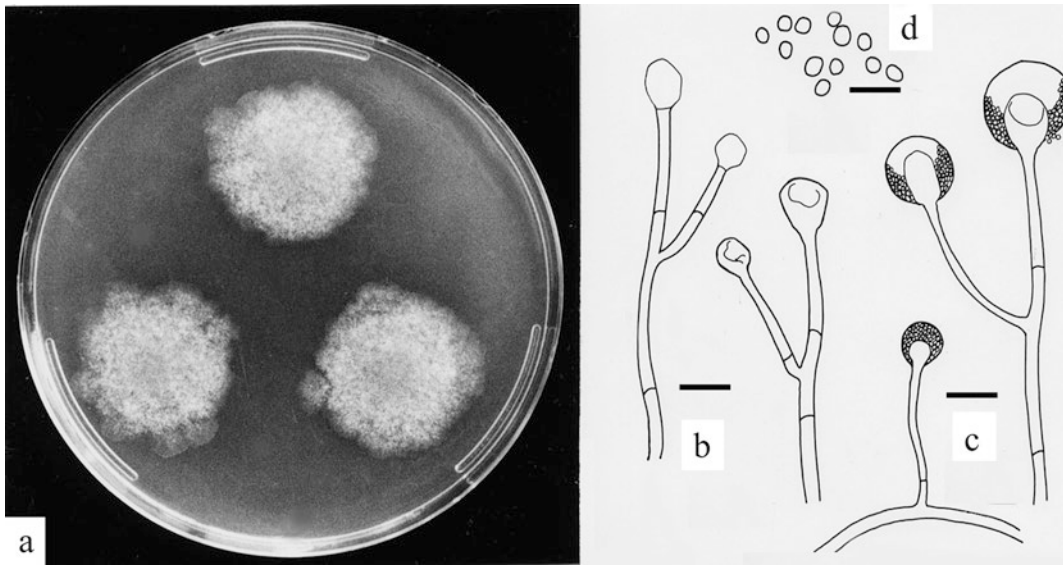

Fig. 6.12 *Rhizomucor pusillus* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) columellae, bar = 25µm; (**c**) sporangia, bar = 25µm; (**d**) sporangiospores, bar = 10µm

Identifiers MycoBank MB322484; epitype CBS 354.68; ex-type strain CBS 354.68; ITS barcode JN206312 (Walther et al. 2013).

Physiology *Rhizomucor pusillus* is a thermophile. Growth has been reported at temperatures as high as 60 °C (Crisan 1973). Optimum growth conditions are 37–42 °C, with a lower limit of 20 °C (Panasenko 1967; Evans 1971).

Mycotoxins Mycotoxin production has not been reported.

Ecology *Rhizomucor pusillus* is a frequent agent of bovine mycotic abortion and a rare cause of human zygomycosis (de Hoog et al. 2000). This species appears to be of widespread though uncommon occurrence in foods. Reported sources include cereals (Lugauskas et al. 2006), kola nuts (Adebajo and Popoola 2003), various spices (Mandeel 2005), olives (Roussos et al. 2006), meat products, pecans, hazelnuts and walnuts, sunflower seeds, and various tropical products (see Pitt and Hocking 1997). It is used in cheese manufacture in Japan (Koizumi 2001). We isolated *Rhizomucor pusillus* from mung beans in Thailand and kemiri nuts in Indonesia (Pitt et al. 1994, 1998).

Additional species *Rhizomucor miehei* (Cooney & R. Emers.) Schipper (synonym *Mucor miehei* Cooney & R. Emers.) has occasionally been isolated from foods (Kuthubutheen 1979; Ogundero 1981). It is similar in most characters to *R. pusillus*, including thermophily. *R. miehei* produces sporangia up to 50(−60) µm diam with spiny walls, with columellae rarely larger than 30µm diam. Identifiers: MycoBank MB322483; holotype CBS 182.67; ex-type cultures CBS 182.67; IMI 126334ii; ATCC 16457; ITS barcode GQ118995, markers 18S and 26S GQ118995.

Reference Schipper (1978a, b); Walther et al. (2013).

6.5 Genus *Rhizopus* Ehrenb.

The genus *Rhizopus* needs little introduction to food microbiologists, because *R. stolonifer* is among the most obvious moulds encountered on Petri dishes inoculated with food materials. Coarse, rampant growth and rapidly maturing spores make this species a source of endless contamination to the unwary laboratory worker. Other *Rhizopus* species are less common in foods, and cause less nuisance.

Rhizopus species, especially *R. oligosporus*, have been used for millennia to modify basic foods in the Orient. The subject of fermented foods is outside the scope of this book: for further information see for example Hesseltine (1965), Gray (1970), Beuchat (1987) or Rombouts and Nout (1995).

Rhizopus is distinguished from other genera in the order Mucorales by the formation of rhizoids (short root-like appendages), which are conspicuous at the base of the sporangiophores; by columellae which often collapse into umbrella shapes in age; and by dry sporangiospores with striate walls. The most recent complete taxonomic treatment is by Zheng et al. (2007) who accepted 10 species. Molecular phylogenetic analyses have indicated that *Rhizopus* is a monophyletic genus, though some disagreement on speciation still exists (Abe et al. 2006, 2010; Liou et al. 2007; Hoffmann et al. 2013). Five species are considered here and keyed below: *R. arrhizus*, *R. microsporus*, *R. oligosporus, R. sexualis* and *R. stolonifer*. Because of its ubiquitous nature, *R. stolonifer* is described first.

Key to *Rhizopus* species included here

1	Abundant zygospores present on CYA and MEA	*R. sexualis*
	Zygospores not produced	2
2 (1)	Rapid growth at 37 °C; spores commonly less than 8µm diam	3
	Growth at 37 °C weak or absent; spores commonly 8–20µm diam	*R. stolonifer*
3 (2)	Columellae up to 100µm diam; spores 5–8µm long	*R. arrhizus*
	Columellae not more than 80µm diam; spores less than 5µm long	4
4 (3)	Columellae up to 75µm diam; spores less than 4µm long, with spinose walls; source food fermentations and fermented foods	*R. oligosporus*
	Columellae less than 40µm diam; spores 3–5µm long, with finely striate walls; source other types of foods	*R. microsporus*

Rhizopus stolonifer (Ehrenb.) Vuill. Fig. 6.13

Rhizopus nigricans Ehrenb.

On CYA, colonies covering the whole Petri dish, sometimes low and sparse and with black sporangia only at the margins, sometimes filling the whole Petri dish and then similar to those on MEA; reverse pale. On MEA, colonies filling the whole Petri dish, often reaching and adhering to the lid, with floccose white mycelium bearing conspicuous sporangia, at first white, then with maturation rapidly becoming black, either distributed uniformly or concentrated at dish peripheries; reverse uncoloured. On G25N, colonies similar to on MEA but less dense. At 5 °C, spores barely germinating. At 37 °C, usually no growth, sometimes colonies up to 15 mm diam, very thin and sparse.

Sporangiophores borne in groups of 3 to 5 from clusters of rhizoids, stipes unbranched, robust and up to 3 mm long, with brown walls; sporangia 100–350µm diam, usually spherical; columellae roughly spherical, up to 200µm diam, in age often collapsing downwards and outwards to produce umbrella shapes; sporangiospores commonly 8–20µm in long axis, pale brownish, with striate walls.

Distinctive features *Rhizopus stolonifer* is distinguished by its habit, with coarse hyphae and rampant growth at 25 °C, and by sporangia which are white when first formed but which change to black with maturity. Sporangiospores are large, with striate walls. In contrast to that at 25 °C, growth at 5 and 37 °C is weak or absent.

Taxonomy Twenty nine isolates identified as *R. stolonifer* or possibly related species were phylogenetically analysed by RAPD analysis (Vagvolgyi et al. 2004) and *ca* 30 different isolates by Liou et al. (2007) using DNA sequences from the D1/D2 of rDNA. These analyses divided *R. stolonifer* and some varieties into two or four clades. In each case, the isolates commonly seen in foods remained in *R. stolonifer.*

Identifiers MycoBank MB119545; type not known; representative strain CBS 382.52, IMI 57762, ATCC 6227b; ITS barcode AB113022 (Abe et al. 2010).

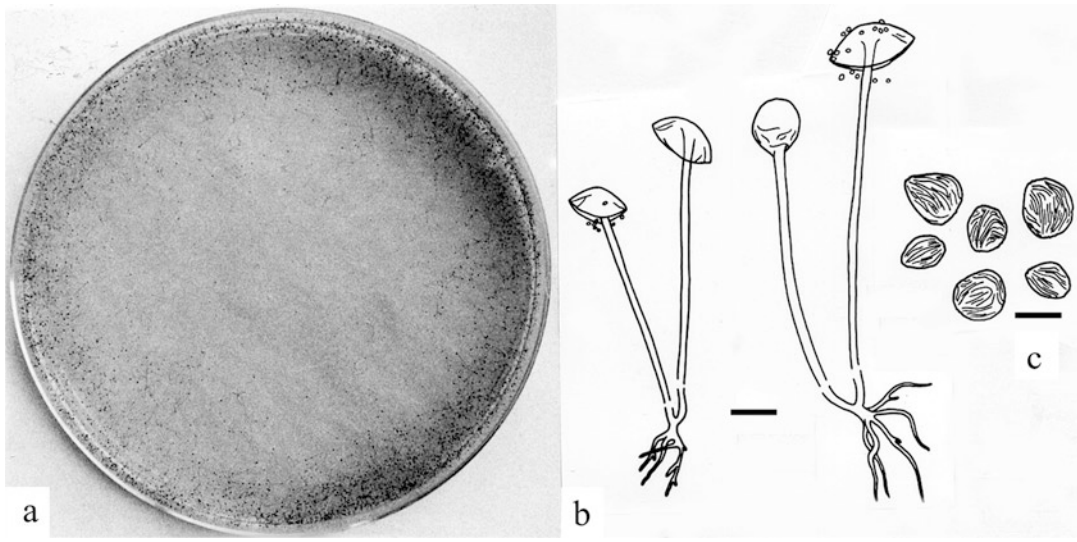

Fig. 6.13 *Rhizopus stolonifer* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangiophores with rhizoids and collapsed columellae, bar = 25μm; (**c**) sporangiospores, bar = 10μm

Physiology *Rhizopus stolonifer* has been reported to grow from 4.5 or 5 °C up to 30 °C (Schipper 1984) or 35–37 °C (Pierson 1966, and our observations), with an optimum near 25 °C. However, Zheng et al. (2007), who tested 36 isolates of *R. stonolonifer*, reported maximum growth temperatures of only 30–32 °C. This species germinated down to 0.84 a_w at 25 °C, but growth was very slow, and was absent at 30 °C (Hocking and Miscamble 1995). It produced the fastest mycelial growth we have ever recorded: at 25 °C and an a_w in excess of 0.99, the radial growth rate reached 2 mm/hr (i.e. nearly 5 cm/day). Like some other species in the Mucorales, *R. stolonifer* can grow under anaerobic conditions (Stotzky and Goos 1965), however an atmosphere of 80% O_2 plus 20% CO_2 halved its growth rate at 25 °C (Hoogerwerf et al. 2002).

Mycotoxins *Rhizopus stolonifer* (a single isolate tested) was moderately toxic to ducklings (Rabie et al. 1985). The possible toxins involved have not been elucidated.

Ecology *Rhizopus stolonifer* is by far the most commonly occurring species of the order Mucorales in foods. It causes destructive rots of a wide range of fruits (Snowdon 1990, 1991). The most important are fresh berries, especially strawberries (Benecke et al. 1954; Dennis et al. 1979; Harris and Dennis 1980), and stone fruits of all kinds (Snowdon 1990; Singh and Shukla 2005). Tomatoes, eggplants (aubergines), capsicums and guavas are also seriously affected in some countries (Kassim 1987; Snowdon 1991; Bautista-Banos et al. 2014; Embaby and Hassan 2015). These diseases occur postharvest and are commonly known as "transit rot". Whole cases of fruit can decay in just a few days. Spoilage is usually initiated through damage, and then spreads by contact. Control usually relies on preharvest or postharvest sprays or dips of fungicides. Thiabendazole (Benlate) or 2,6-dichloro-4-nitroaniline (Dichloran or Botran) provide reasonable control (Snowdon 1990, 1991 and see Pitt and Hocking 1997). Other treatments suggested include hot water dips and irradiation (Nicoue et al. 2004), ultraviolet light (Pan et al. 2004) and chlorine dipping (Sabaa-Srur et al. 1993). Control of postharvest rot has been satisfactorily achieved with applications of yeasts such as *Metschnikowia fructicola* Kurtzman & Droby (Kurtzman & Droby 2001; Karabulut et al. 2004; Spadaro et al. 2013) and *Cryptococcus laurentii* [= *Papiliotrema laurentii* (Kuff.) Xin Zhan Liu et al.] (Zhang et al. 2007). Transgenic tomatoes with reduced polygalacturonase activity appear to have a higher resistance to transit rot (Sanders et al. 1992).

Pectic enzymes of *Rhizopus stolonifer* survive the canning process normally applied to fruit. If even a small proportion of infected fruit are processed, these enzymes can cause softening and spoilage of canned apricots (Harper et al. 1972).

Many types of vegetables are also susceptible to spoilage by *Rhizopus stolonifer* (Snowdon 1991). Beans and peas, carrots, sweet potatoes, yams and cassava (see Pitt and Hocking 1997) are all highly susceptible.

Isolation of this species has been reported from many other food sources, including wheat and barley (Lugauskas et al. 2006) soybeans (Tariq et al. 2005), peanuts (Gachomo et al. 2004), hazelnuts (Senser 1979), pecans and bran (see Pitt and Hocking 1997), spices (Mandeel 2005), cheese (Hayaloglu and Kirbag 2007) and meat products (see Pitt and Hocking 1997). *R. stolonifer* is a cosmopolitan fungus, particularly in tropical and subtropical regions, in almost all kinds of fresh, moist, or partially dried foods.

References Schipper (1984); Zheng et al. (2007).

Rhizopus arrhizus A. Fisch. Fig. 6.14

Rhizopus oryzae Went & Prins. Geerl.

On CYA and MEA, colonies filling the whole Petri dish with fine greyish mycelium and small blackish grey sporangia; reverse pale. On G25N, colonies 30–60 mm diam, or occasionally filling the whole Petri dish, relatively low and sparse. No growth at 5 °C. Colonies at 37 °C covering and sometimes filling the Petri dish, similar to on CYA or more sparse.

Sporangiophores borne in clusters of l–3 from rhizoids, with stipes up to 1500µm long, usually unbranched; sporangia spherical, up to 150µm diam, white at first then becoming greyish black at maturity; columellae usually spherical, up to 100µm diam, pale brown, in age often collapsing downwards to form umbrella shapes; sporangiospores brown, of variable shape, ellipsoidal to broadly fusiform or irregularly angular, commonly 5–8µm long, with striate walls.

Distinctive features *Rhizopus arrhizus* is distinguished from *R. stolonifer* by its smaller

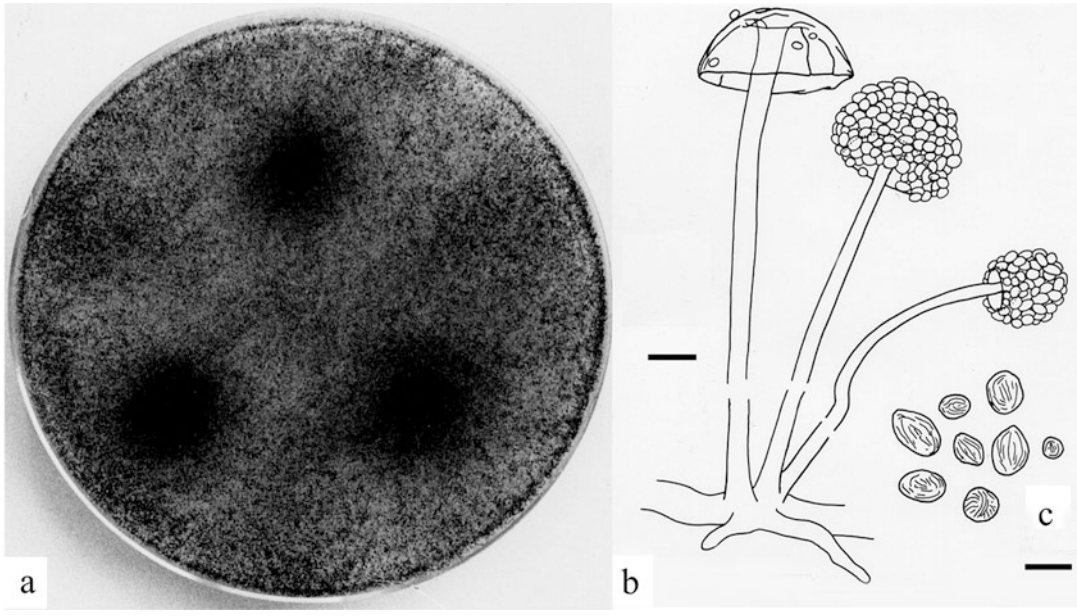

Fig. 6.14 *Rhizopus arrhizus* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangia and columellae, bar = 25µm; (**c**) sporangiospores, bar = 10µm

sporangia and spores, and by much faster growth at 37 °C. It differs from *R. microsporus* and *R. oligosporus* by longer stipes, larger collumellae and larger spores.

Taxonomy Schipper and Stalpers (1984) concluded that *R. oryzae* was the correct name for this species, considering *R. arrhizus* to be a doubtful name and placed this latter species in synonymy with *R. oryzae*. In the latest *Rhizopus* taxonomy, Zheng et al. (2007) have taken up *R. arrhizus* based on neotypification by Ellis (1985), and that nomenclature has been accepted in the detailed study by Dolatabadi et al. (2014).

Identifiers MycoBank MB167790; neotype NRRL 1469 (Ellis 1985); ex-type strain NRRL 1469; ITS barcode AB097334 (CBS 112.07, ex-type of *R. oryzae*, Abe et al. 2010).

Physiology *Rhizopus arrhizus* (often as *R. oryzae*) has been reported to grow from 7 °C to 42 or 45 °C, with the optimum near 37 °C (Panasenko 1967; Gleason 1971; Schipper 1984; Zheng et al. 2007). Its minimum a_w for growth is 0.88 (Hocking and Miscamble 1995). Growth under optimal conditions is exceptionally fast (up to 1.6 mm/hr), although slower than that of *R. stolonifer* (Hocking and Miscamble 1995). *R. arrhizus* produces lipase D, an enzyme which hydrolyses triglycerides of fatty acids and acts as a catalyst for the esterification of fats and oils to improve their physical properties. The toxicity of lipase D produced by *R. arrhizus* was examined in rats for 13 weeks and no ill effects were detected (Flood and Kondo 2003).

Mycotoxins Maize meal on which isolates of *Rhizopus arrhizus* had been grown was toxic to ducklings and rats, causing growth depression (Rabie et al. 1985). The toxin responsible has not been elucidated.

Ecology *Rhizopus arrhizus* is an important agent of human zygomycoses, causing mainly rhinocerebral infections (de Hoog et al. 2000). It has been isolated quite frequently from foods, sometimes as *R. oryzae*. It appears to be associated with many of the rots in fruits and vegetables usually attributed to *R. stolonifer* (Snowdon 1990, 1991). From our observations, it is more common in tropical commodities than is *R. stolonifer*. It was especially common in peanuts, being isolated from 21% of all peanut kernels examined from Indonesia, 15% of those from Thailand, and 14% of those from the Philippines. It was growing in 25% of all copra pieces from Thailand and 9% of kemiri nuts from Indonesia. It was present in lower numbers in maize, various kinds of beans, sorghum and cowpeas (Pitt et al. 1993, 1998). Other reports include maize (Askun 2006), peanuts, pecans, hazelnuts, pistachios, wheat, barley, potatoes, sapodillas and various other tropical foods (see Pitt and Hocking 1997).

References Schipper (1984); de Hoog et al. (2000); Zheng et al. (2007); Dolatabadi et al. (2014).

Rhizopus microsporus Tiegh. Fig. 6.15

On CYA and MEA, colonies covering or filling the whole Petri dish, of fine, pale grey mycelium, becoming dark grey as sporangia mature; reverse pale to dull yellow. On G25N, germination to microcolony formation. No growth at 5 °C. At 37 °C, colonies filling the whole Petri dish, similar to those on CYA or MEA at 25 °C.

Sporangiophores borne singly or in groups of 2–3 from clusters of poorly formed rhizoids, stipes unbranched, relatively short, commonly 200–500µm long, with brown walls; sporangia black, 100–200µm diam, usually spherical; columellae 20–35µm diam, spherical, some remaining so in age, others collapsing downwards, to form umbrella shapes; sporangiospores ellipsoidal, 3–5µm long, with thin, striate walls.

Distinctive features *Rhizopus microsporus* differs from *R. stolonifer* by shorter stipes and smaller columellae, by the absence of white immature sporangia and by strong growth at 37 °C. It is distinguished from *R. arrhizus* by production of smaller columellae and smaller spores. *R. oligosporus* is very similar to *R. microsporus*, but produces larger columellae and smooth to spinose spores.

Taxonomy Sporangia up to 80µm and spores up to 6.5 (−7.5) µm long were described by Schipper and Stalpers (1984). This species was divided into six varieties by Zheng et al. (2007), however a detailed polyphasic study of morphology, mating, physiology and molecular

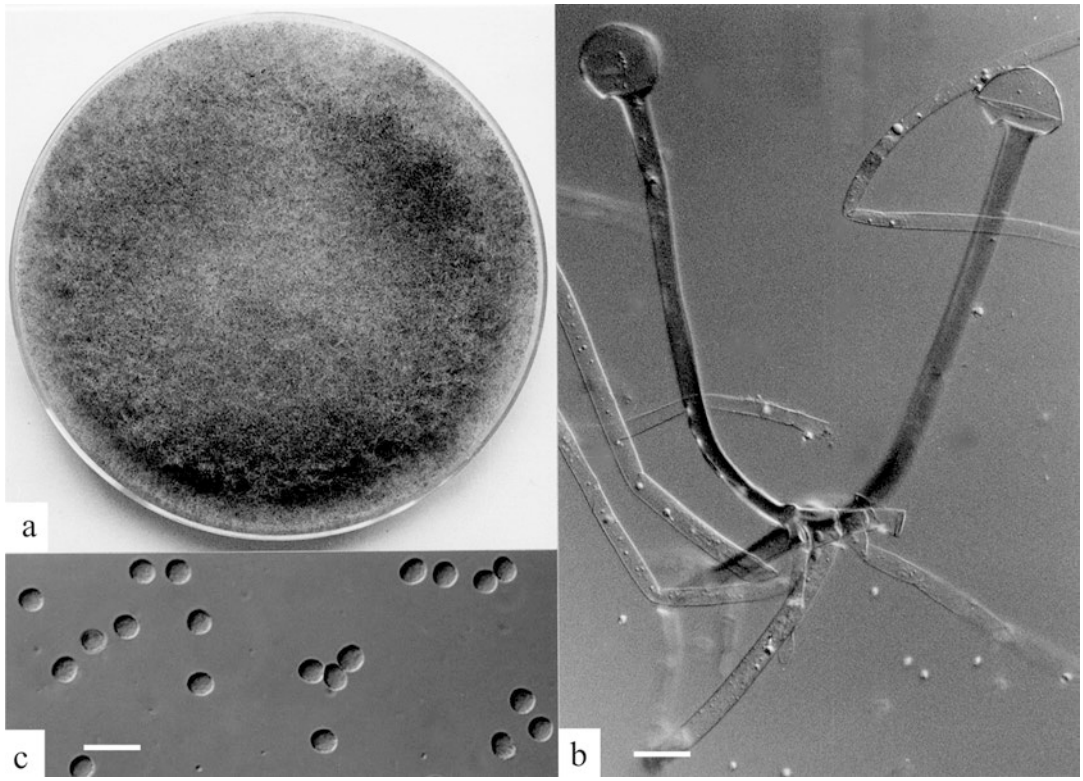

Fig. 6.15 *Rhizopus microsporus* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangiophores with rhizoids, bar = 25µm; (**c**) sporangiospores, bar = 10µm

characters has concluded that all varieties belonged to a single, well circumscribed species (Dolatabardi et al. 2014b).

Identifiers MycoBank MB177331; type not known; representative strains CBS 700.68, IMI 190511, ATCC 52804; ITS barcode AB097386 (CBS 700.68, Abe et al. 2010).

Physiology *Rhizopus microsporus* was reported to grow up to 46–48 °C by Schipper and Stalpers (1984) and Zheng et al. (2007) but to 52 °C by Dolatabardi et al. (2014b), with an optimum temperature near 45 °C. The lower limit for growth is 0.90 a_w (Hocking and Miscamble 1995).

Mycotoxins Wilson et al. (1984) reported *R. microsporus* produced rhizonin A, a nonspecific hepatotoxin. However, the toxin is not synthesised by the fungus, but by a bacterial symbiont belonging to the genus *Burkholderia* (Partida-Marinez et al. 2007). This finding is of interest as species of *Rhizopus* are frequently used in the food industry for the preparation of fermented foods such as tempeh and sufu.

Ecology *Rhizopus microsporus* causes human zygomycoses, particularly cutaneous and gastrointestinal infections (de Hoog et al. 2000). This species appears to be uncommon in foods from temperature zones, however we isolated it quite frequently from tropical commodities. It was found in 18% of maize samples from the Philippines, and from 3% of all kernels examined. It was also present, at lower numbers, in peanuts, paddy rice, soybeans and black pepper from the Philippines, and maize, peanuts, paddy and milled rice, soybeans, mung beans and coriander from Indonesia (Pitt 1998 and our unpublished data). It was found to be common in sorghum in Nigeria (Jidda and Anaso 2014; reported as *R. oligosporus*).

References Schipper and Stalpers (1984); Zheng *et al*, (2007); Dolatabardi et al. (2014b).

Rhizopus oligosporus Saito Fig. 6.16

Colonies on CYA 50–60 mm diam, not usually covering the whole Petri dish, low, plane and sparse, with ill-defined margins, pale brown, sporangia sparsely produced, brown, sometimes enclosed in clear to brown droplets; reverse uncoloured to pale brown. Colonies on MEA covering the whole Petri dish, sometimes reaching, and adhering to, the lid, coloured dark grey to black, sporangia abundant, black; reverse uncoloured. On G25N, sometimes germination. At 5 °C, no germination. At 37 °C, colonies covering the whole Petri dish in a low, very sparse, often cobwebby growth; reverse uncoloured.

Sporangiophores best observed on MEA, stipes 150–400µm long, usually with well developed short rhizoids at or near the base, terminating in dark sporangia, 80–120µm diam, at 7 days usually broken with spores dispersed; columellae persistent, often split, but remaining spheroidal to pyriform, 25–75µm diam or long; sporangiospores spherical to subspheroidal, 3.0–3.5µm diam, with thin, finely spinose walls.

Distinctive features In common with *Rhizopus microsporus, R. oligosporus* differs from *R. stolonifer* by shorter stipes and smaller columellae, by the absence of white immature sporangia and by strong growth at 37 °C. It is

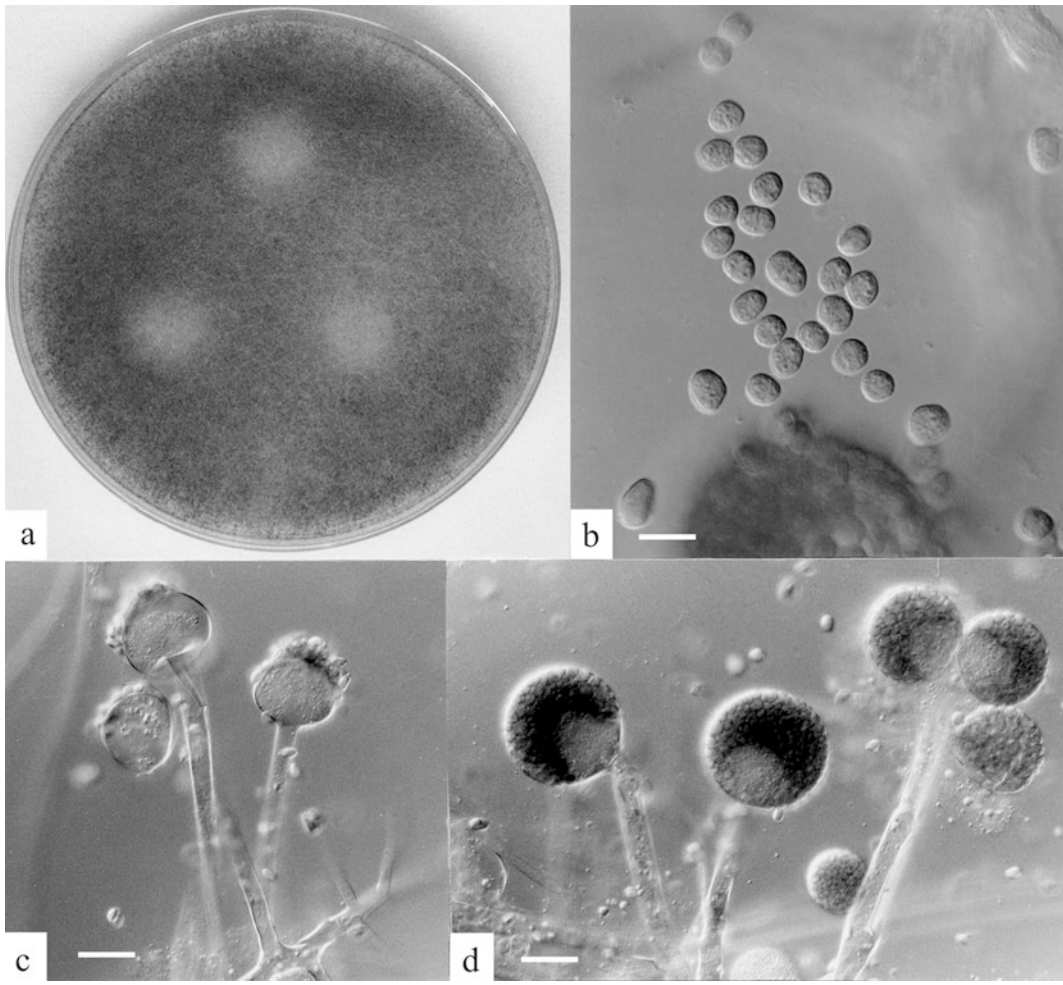

Fig. 6.16 *Rhizopus oligosporus* (**a**) colonies on MEA, 7 d, 25 °C; (**b**) sporangiospores, bar = 10µm; (**c**) developing sporangia, bar = 25µm; (**d**) mature sporangia, bar = 25µm

distinguished from *R. arrhizus* by production of smaller columellae and smaller spores. *R. oligosporus* produces larger columellae than do isolates of *R. microsporus* and forms smooth to spinose spores.

Taxonomy Schipper and Stalpers (1984) reduced *Rhizopus oligosporus* to the status of a variety of *R. microsporus*, and this was maintained by Zheng et al. (2007). However, this taxon deserves species status as a domesticated species used in, and probably confined to, food fermentations. Its derivation from *R. microsporus* seems likely, as the two taxa share many properties. A detailed light and scanning electron microscopy study by Jennessen et al. (2008) provided criteria for separating *R. oligosporus* from related species. Sporangiospores of *R. oligosporus* are often larger and less regularly shaped than those of other *Rhizopus* species. This is likely to be the result of domestication (Jennessen et al. 2008).

Identifiers MycoBank MB155475; neotype CBS 337.62; representative strains CBS 338.72, IMI 174457, ATCC 22959, NRRL 2710; ITS barcode AB097395 (CBS 337.62, Abe et al. 2010).

Physiology Maximum growth temperature of thirteen isolates was 45–49 °C (Zheng et al. 2007).

Mycotoxins No mycotoxins are known to be produced. Weak toxicity to ducklings was reported by Rabie et al. (1985). However, only a single isolate was tested.

Ecology *Rhizopus oligosporus* is used in food fermentations, the most notable product being tempeh, which is produced in Southeast Asian countries, especially in Indonesia. Cooked, dehulled soybeans are soaked for 2–3 days, then inoculated from a previous batch or, more commonly now, with a starter culture, usually *R. oligosporus*. Fermentation takes place under ambient (tropical) conditions, preferably with air temperatures of 25–28 °C for 36–48 hours (Hesseltine 1965, 1991; Ko and Hesseltine 1979; Beuchat 1987; Nout and Rombouts 1990). Feng et al. (2007) reported the production of volatiles by *R. oligosporus* during the fermentation of soybean and barley tempeh. *R. oligosporus* has also been used to increase the functional properties of foods and reduce the levels of allergenic proteins in buckwheat (Handoyo et al. 2006). As noted above, *R. oligosporus* appears to be a domesticated fungus. It has rarely, if ever, been reliably reported from sources other than fermented foods.

References Hesseltine (1965); Schipper and Stalpers (1984); Zheng et al. (2007).

Rhizopus sexualis (G. Sm.) Callen Fig. 6.17

On CYA, colonies covering the whole Petri dish, very low and sparse, of white to greyish mycelium; sporangia in limited numbers, pale; black zygospores conspicuous; reverse uncoloured. On MEA, colonies similar to on CYA, though more dense. On G25N, colonies at least 40 mm diam, sometimes covering the whole Petri dish, often as vigorous as on CYA or MEA, and of similar appearance, but zygospores usually absent. No growth at 5 or 37 °C.

Sporangiophores borne from rhizoids, 1–3 per cluster, stipes up to 1500µm long, usually unbranched; sporangia spherical, 50–150µm diam, white, becoming grey; columellae spherical to ellipsoidal, up to 100µm diam or long, in age collapsing to umbrella shapes; sporangiospores subspheroidal, ellipsoidal or angular, 5–12(−25) µm long, with thick, grey, striate walls. Zygospores commonly occurring, 80–180µm diam, black, with adjacent cells (suspensors) approximately spherical, but at maturity appearing hemispherical.

Distinctive features *Rhizopus sexualis* is distinguished from other foodborne species of *Rhizopus* and *Mucor* by the profuse production of conspicuous black zygospores.

Taxonomy Abe et al. (2010) considered *R. sexualis* to be a synonym of *R. stolonifer*. It seems likely that this species is only a variant of *R. stolonifer* that produces zygospores homothallically, i.e. without mating.

Identifiers MycoBank MB290490; lectotype CBS 336.39; representative strains CBS 123.64, IMI 103481; ITS barcode AB113020 (Abe et al. 2010).

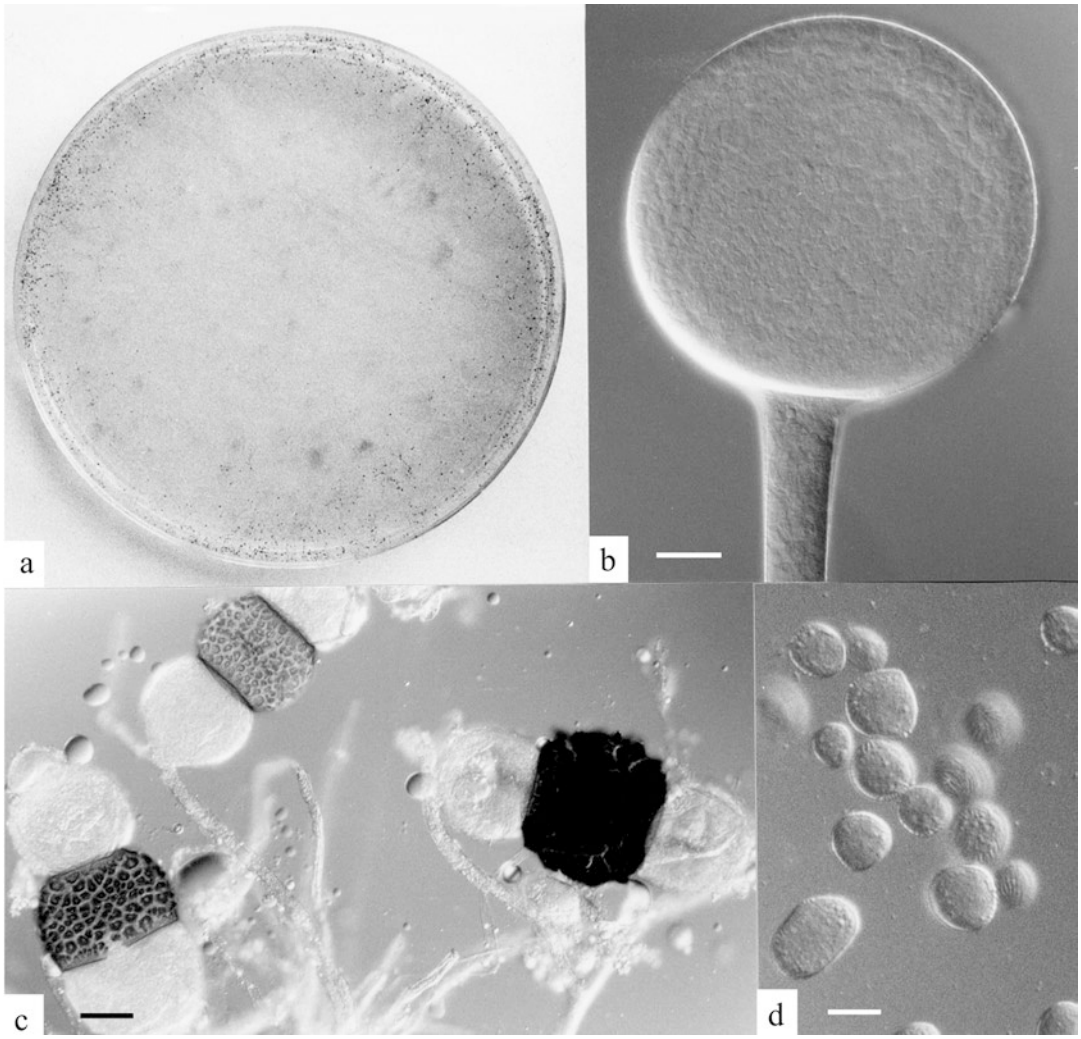

Fig. 6.17 *Rhizopus sexualis* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangium, bar = 25µm; (**c**) zygospores, bar = 50µm; (**d**) sporangiospores, bar = 10µm

Physiology Schipper (1984) reported that this species only grows below 30 °C and Zheng et al. (2007) reported 26–27 °C as the maximum growth temperature. Relatively fast growth on G25N perhaps indicates a relatively low minimum a_w for growth.

Mycotoxins Mycotoxin production has not been reported.

Ecology This species causes a soft rot of strawberries (Harris and Dennis 1980) and occasionally of other fruit.

References Schipper (1984); Zheng et al. (2007); Abe et al. (2010).

6.6 Genus *Syncephalastrum* J. Schröt.

Syncephalastrum produces sporangiospores in cylindrical sacs (merosporangia) attached around spherical vesicles, providing a superficial resemblance to *Aspergillus niger* at low magnifications. In other respects, this genus resembles *Mucor*. There is one species, *S. racemosum*.

Syncephalastrum racemosum Cohn

Fig. 6.18

On CYA and MEA, colonies covering the whole Petri dish, mycelium sparse, pale to dark grey; reverse pale or yellowish brown. On G25N, colonies 20–30 mm diam, dense to floccose, grey; reverse pale. No growth at 5 °C. Colonies at 37 °C filling the whole Petri dish, dark grey; reverse yellow brown.

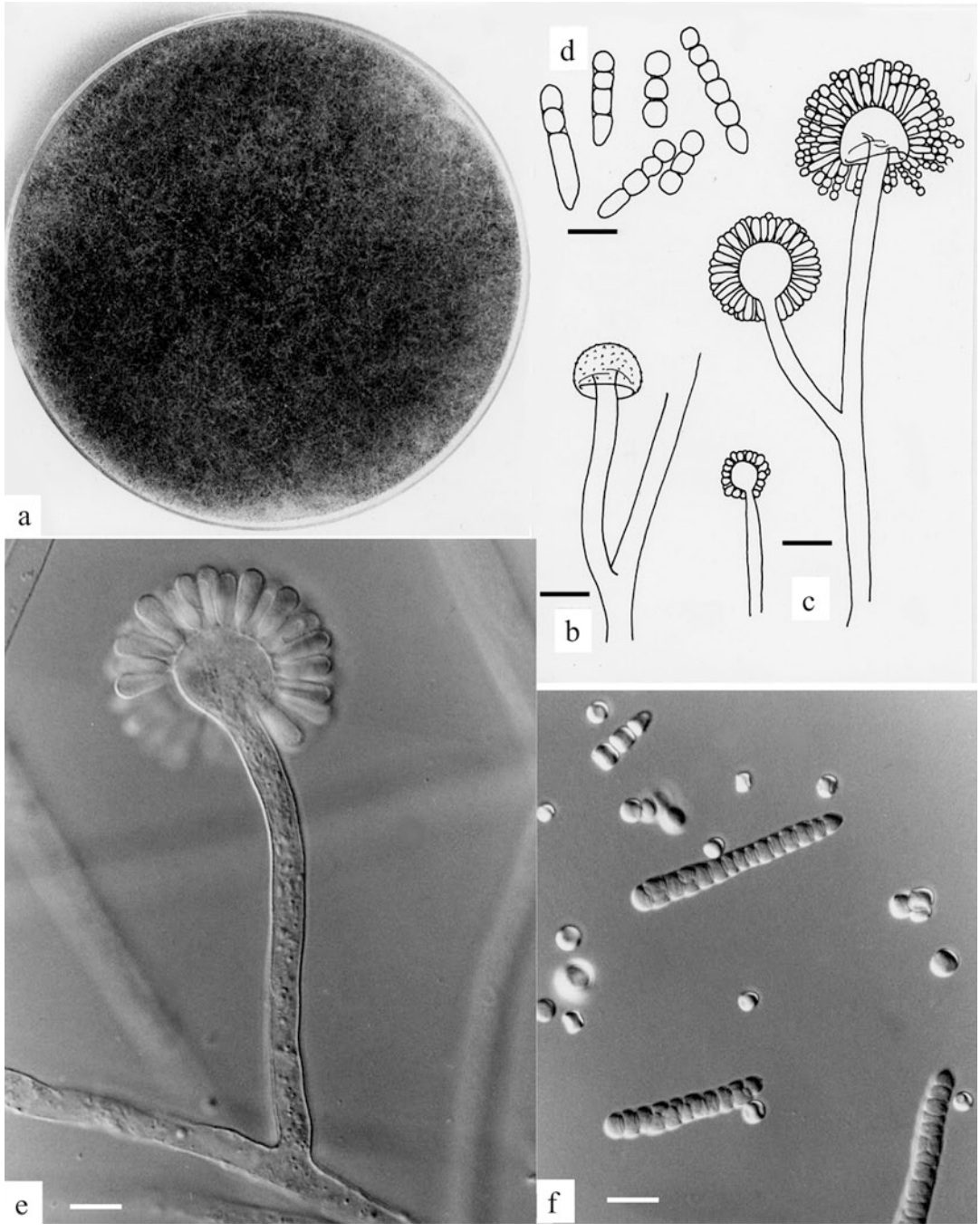

Fig. 6.18 *Syncephalastrum racemosum* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) columellae, bar = 25µm; (**c**) stages in merosporangium formation, bar = 25µm; (**d**) sporangiospores, bar = 10µm; (**e**) developing merosporangium, bar = 10µm; (**f**) sporangiospores, bar = 10µm

Sporangiophores borne from aerial hyphae, stipes long and branched or produced as short side branches from fertile hyphae; sporangial heads 30–80µm diam, with sporangiospores formed in a single line within cylindrical sacs (merosporangia) borne on spicules around the vesicle; vesicles spherical or nearly so, 10–50µm diam, brown, with walls smooth except at merosporangium attachment points, usually collapsing irregularly; sporangiospores adhering in chains of up to 10, becoming brown, irregular in size and shape, spherical to cylindrical, 3.0–5.0(−10) µm diam or long, smooth walled.

Distinctive features See genus description.

Taxonomy This genus was maintained in a separate family, *Syncephalastraceae,* by Hoffmann et al. (2013).

Identifiers MycoBank MB201627; type not known; representative strains CBS 440.59, IMI 77601, ATCC 14831, NRRL A5990.

Physiology Good growth of *Syncephalastrum racemosum* has been reported between 17 and 40 °C (Domsch et al. 1980): no doubt growth limits are rather wider than these figures. As the minimum for growth is 0.84 a_w (Hocking and Miscamble 1995), this is one of the most tolerant species to low a_w in the Mucorales.

Mycotoxins It was reported by Makun et al. (2007a) that two of three isolates of *Syncephalastrum* were mildly toxic to mice (one of three injected with extracts died). No other details are known and toxicity has not been reported elsewhere.

Ecology Reported isolations from foods have not been numerous, but *S. racemosum* is of widespread occurrence nevertheless. Principal reported sources have been from nuts (see Pitt and Hocking 1997), young coconuts (Waje et al. 2005), cereals, spices (Elshafie et al. 2002), fermented foods, processed meats and capsicums (see Pitt and Hocking 1997). We isolated this species from 10% of all coriander seeds examined from Indonesia, and 4% of all kemiri nuts. It was present in 2% of all sorghum grains from Thailand, and 1% of Thai mung beans, Philippine maize, soybeans and black pepper, and Indonesian peanuts. It was present at lower levels in soybeans, black and red beans, cassava and copra from Thailand, and mung beans, maize and milled rice from Indonesia (Pitt et al. 1994, 1998). More recent reports include cashews (Adebajo and and Diyaolu 2003), millet (Makun et al. 2007b) and chickpeas (Dawar et al. 2007).

References Benjamin (1959); O'Donnell (1979); Hoffmann et al. (2013).

6.7 Genus *Thamnidium* Link

As well as large collumellate sporangia, *Thamnidium* produces sporangioles, small sporangia without columellae, borne on highly branched structures. Sporangioles in *Thamnidium* contain sporangiospores, similar to those borne in sporangia. There is a single species, *T. elegans*.

Thamnidium elegans Link Fig. 6.19

On CYA, colonies usually covering the whole Petri dish, sparse but quite deep due to the production of long sporangiophores, coloured grey to pale brown; reverse pale. On MEA, colonies 30–50 mm diam, similar to those on CYA but with longer, larger sporangiophores. On G25N, colonies variable, from germination only to colonies 10 mm diam, low and dense. At 5 °C, colonies 15–35 mm diam, of low mycelium. No growth at 37 °C.

Sporangiophores very long, borne from surface hyphae, stipes bearing sporangia or sporangioles or both; sporangia brown, 150–250µm diam; columellae roughly spherical, 40–80µm diam, or sometimes larger, collapsing or tearing irregularly; sporangioles 12–25µm diam, rough walled; sporangiospores narrowly to broadly ellipsoidal, 6–15µm long, with thin, smooth walls.

Distinctive features See genus description.

Taxonomy *Thamnidium* lies within the family *Mucoraceae*, but its precise phylogenetic relationship remains unclear (Hoffmann et al. 2013).

Identifiers MycoBank MB249697; type not known; representative strains CBS 341.55, IMI 283871, ATCC 11808, NRRL 1613.

Physiology *Thamnidium elegans* is psychrophilic, growing down to at least 1 °C (Brooks and

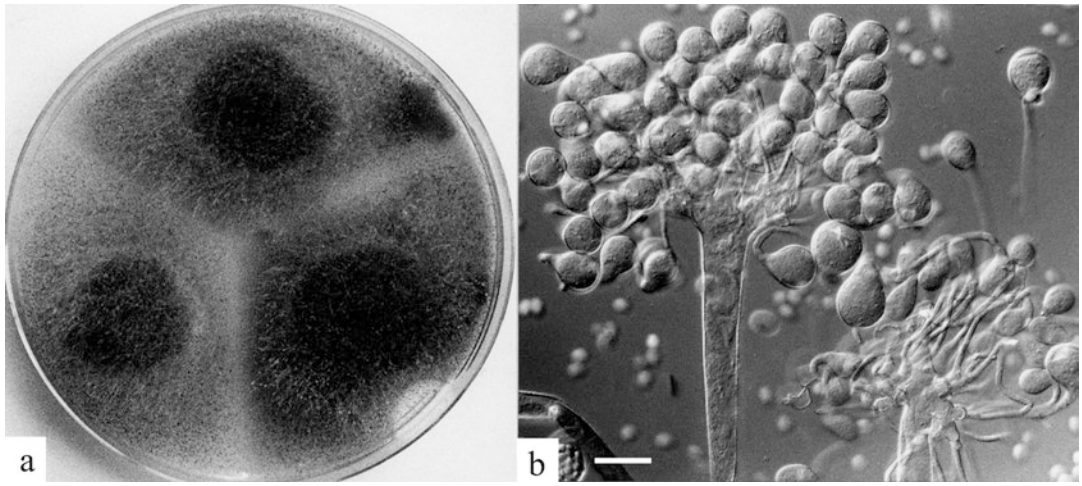

Fig. 6.19 *Thamnidium elegans* (**a**) colonies on CYA, 7 d, 25 °C; (**b**) sporangiophore, developing sporangioles, and sporangiospores, bar = 25μm

Hansford 1923). The maximum temperature for growth is about 27 °C (Gleason 1971).

Mycotoxins Mycotoxin production has not been reported.

Ecology *Thamnidium elegans* has traditionally been associated with cool stored meat, on which it occurs as long "whiskers". Improved temperature control of meat storage, particularly the use of cool stores, has virtually eliminated this problem in developed countries. However, where meat is traditionally hung for curing, festoons of *T. elegans* can still sometimes be seen. Whether this constitutes "spoilage" depends on definition. Spoilage of Taleggio cheese (a traditional, washed rind, soft, red pigmented cow's milk cheese from Italy) has been reported (Dragoni et al. 1997). We isolated *T. elegans* from coriander from Indonesia, where it was found in 1% of seeds examined (Pitt et al. 1998).

References Hesseltine and Anderson (1956); O'Donnell (1979); Hoffmann et al. (2013).

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Penicillium and *Talaromyces*

7

The genus *Penicillium* was described by Link in 1809, and named for the tiny brushlike fruiting structure, termed a **penicillus** (Latin, little brush; Fig. 7.1), that is characteristic of species in this genus. No doubt exists over the use of this name, as one species described by Link was *P. expansum*, the common apple rot fungus, now designated as type of the genus (Hawksworth et al. 1976). It has long been known that *Penicillium* as originally described is associated with two quite different types of ascocarps. One is a very hard (sclerotioid) **cleistothecium** (Fig. 3.3). The second quite different type is composed of fine hyphae woven into a more or less closed structure of indeterminate size, known as a **gymnothecium** (Fig. 3.3). Benjamin (1955) described this sexual state under the name *Talaromyces*, derived from the Greek word for a basket, which aptly describes the body in which asci are produced by this genus. Benjamin (1955) also used the name *Carpenteles* Langeron 1922 for the *Penicillium* species producing cleistothecia, but Stolk and Scott (1967) showed that the obscure *Eupenicillium* F. Ludw. 1892 was the correct name. Scott and Stolk transferred all *Carpenteles* species to *Eupenicillium*, and described 16 new species (Scott and Stolk 1967; Stolk and Scott 1967). To make this taxonomy more accessible, Pitt (1974) developed a synoptic key to the genus.

In the first half of the twentieth century, it was common practice to name both teleomorph and anamorph species producing a *Penicillium* anamorph by their *Penicillium* name. This practice conflicted with provisions of the International Code of Botanical Nomenclature and also ignored the role played by the teleomorph on cultural appearance, longevity, heat and chemical resistance, etc. These important characteristics are readily overlooked if the *Penicillium* name is used. Benjamin (1955) argued forcefully for adoption of the teleomorph names where appropriate, so that nomenclature would be in conformity with the Botanical Code. Despite vigorous opposition from some taxonomists (Raper 1957; Fennell 1973) the use of teleomorph names for *Penicillium* (and *Aspergillus*) species where ascomycete states are present became firmly established (Pitt 1979b; Samson and Pitt 1985; Pitt and Hocking 1985, 1997; Pitt and Samson 1993; Pitt et al. 2000; Samson et al. 1981, and subsequent editions).

Early molecular studies (LoBuglio et al. 1993; Berbee et al. 1995) indicated that although not distantly related, the two sexual states with *Penicillium* anamorphs were not sister genera, being separated by *Aspergillus* (among other genera). To coincide with the changes to the Botanical Code in 2012 (McNeill et al. 2012), that established that each fungus could have only one name, Samson et al. (2011) split *Penicillium* into two genera, using the sexual name *Talaromyces* for the new genus which also accommodated all species classified in *Penicillium* subgen. *Biverticillium*. At the same

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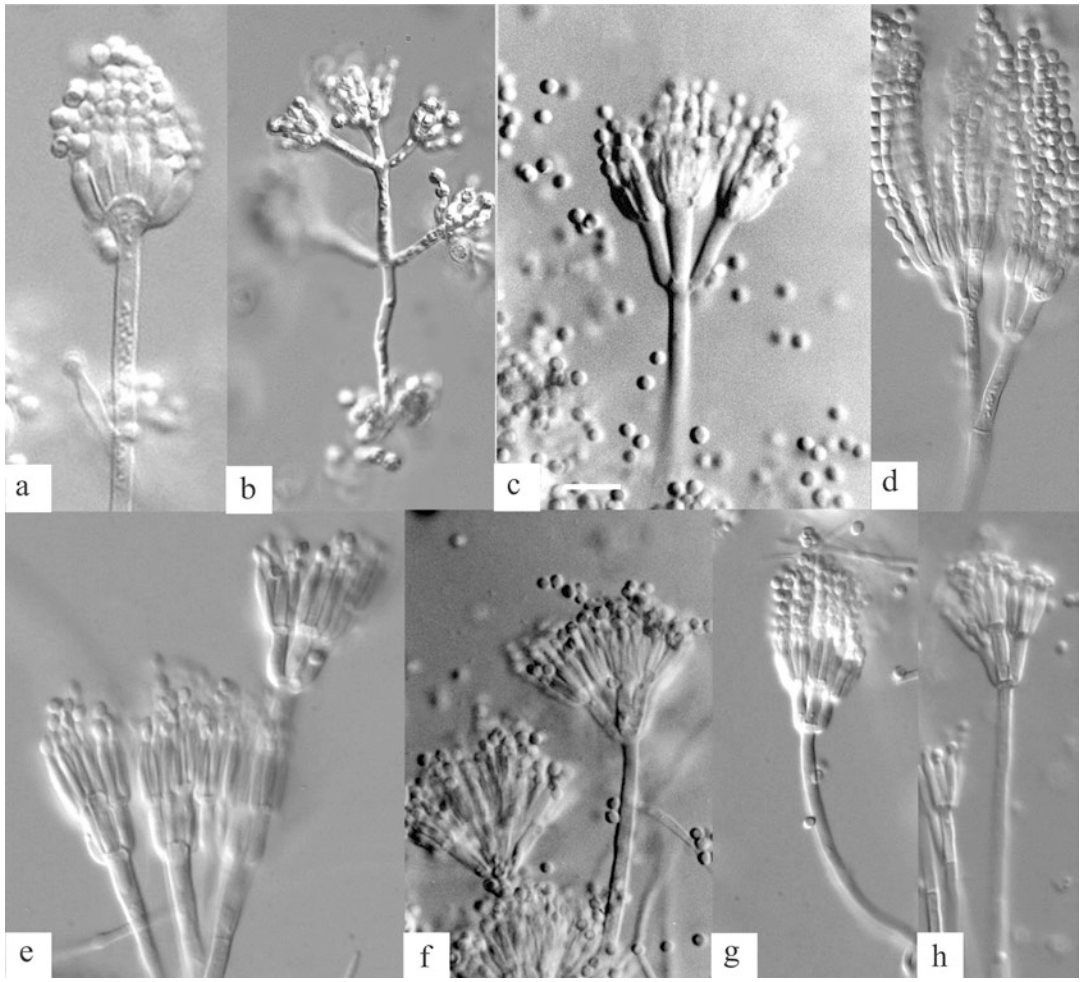

Fig. 7.1 Penicilli characteristic of *Penicillium* and *Talaromyces* species (**a**) monoverticillate, *Penicillium* subgenus *Aspergilloides*; (**b**, **c**) biverticillate, *Penicillium* subgenus *Furcatum*; (**d**) terverticillate, *Penicillium* subgenus *Penicillium*; (**e**–**h**) biverticillate, *Talaromyces*

time, Houbraken and Samson (2011) synonymised *Eupenicillium* with *Penicillium*. The taxonomy of the greatly expanded genus *Talaromyces* was monographed by Yilmaz et al. (2014), while Visagie et al. (2014) provided a checklist of all species accepted in *Penicillium*, of reduced size but now monophyletic. These nomenclatural and taxonomic changes have been followed here.

7.1 Distinguishing *Penicillium* from *Talaromyces*

If an isolate apparently belonging to *Penicillium* or *Talaromyces* produces immature ascocarps, distinguishing the appropriate genus is straightforward. Hard bodies with solid walls (sclerotia or cleistothecia) are characteristic of *Penicillium*, while soft hyphal bodies of indeterminate size

(gymnothecia) are characteristic of *Talaromyces*. These structures can usually be distinguished by disturbing a colony with a needle under the stereomicroscope, when partially hidden ascocarps will be observable more readily, or by making a wet mount. In the absence of a teleomorph or sclerotial state, the morphology of the penicillus provides the basis for distinguishing between these two genera. Count the number of branch points between phialide (or conidial chain) and stipe, down the main axis of the penicillus. If characteristic penicilli having one or three branch points are observed, the isolate will usually belong to *Penicillium* (Fig. 7.1a, d). Note that some *Talaromyces* species that make gymnothecia may produce small penicilli with only one branch point: the presence of the sexual state positively indicates *Talaromyces*.

If biverticillate penicilli are observed (Fig. 7.1b, c, e, f, g, h) the clearest way to distinguish between *Penicillium* and *Talaromyces* is by observing the following characters: the relative lengths of phialides and their supporting cells (metulae); the number of metulae per stipe; and colony diameters on G25N. The shape of the phialides is often diagnostic too. The differences between these two genera are shown in Table 7.1. These genera will be treated separately below.

7.2 Genus *Penicillium* Link

While it is arguable whether *Aspergillus* or *Penicillium* is of greater economic importance as a cause of food spoilage, it is certain that *Penicillium* is the more diverse genus, in terms of numbers of species and range of habitats. Raper and Thom (1949) accepted 137 species in *Penicillium* (including some now moved to *Talaromyces*). Pitt (1979b) examined the original protologues of the exactly 1000 *Penicillium* species that had been described until that time, and accepted 150 of them (again including *Talaromyces*). With the advent of molecular taxonomy, species numbers have increased rapidly. The list of species accepted in the new, narrower concept of *Penicillium* published by Visagie et al. (2014) included 354 species, with 88 species accepted in *Talaromyces* (Yilmaz et al. 2014). More have been described since that time.

Penicillium species are ubiquitous, opportunistic saprophytes. Nutritionally, they are supremely undemanding, being able to grow in almost any environment with a sprinkling of mineral salts and all but the most complex forms of organic carbon, over a wide range of physicochemical environments, i.e. a_w, temperature, pH and redox potential. They occur more commonly in temperate zones than in the tropics, indeed they are rare in the more arid tropical regions, where *Aspergillus* dominates.

A majority of the described species are soil fungi, and their occurrence in foods is more or less accidental and rarely of consequence. However, a number of species are closely associated with human food supplies. In particular, many species in section *Penicillium* appear to have their primary natural habitat on cereal grains. Some species are more specialised: several are destructive pathogens on fruit (e.g. *P. digitatum*, *P. expansum, P. italicum*); a few grow below 0.80 a_w (e.g. *P. brevicompactum*, *P. chrysogenum*, *P. implicatum*), at low oxygen tension (e.g. *P. roqueforti)* or are preservative resistant (*P.*

Table 7.1 Characters distinguishing between biverticillate *Penicillium* and *Talaromyces* species

	Penicillium	*Talaromyes*
Ascocarps	Cleistothecia, sclerotioid	Gymnothecia, hyphal
Ratio of metula length to phialide length	Much greater than one (Fig. 7.1b, c)	Approximately one (1–1.2; Fig. 7.1e, f, g, h)
Metulae per stipe	Not exceeding five	Usually exceeding five
Colony diam on G25N	9–18 mm	Less than 10 mm
Phialide shape	Flask shaped, gradually tapering to neck (ampulliform)	Parallel sided, abruptly tapering to neck (acerose)

roqueforti). Many are psychrotrophic and capable of causing food spoilage at refrigeration temperatures. None is regarded as a serious human or animal pathogen (De Hoog et al. 2000).

A problem with attempting to establish the incidence of particular *Penicillium* species in foods is that so many accounts have not identified Penicillia to species level; as a result many surveys of Penicillia in foods are of little value in the present context. Reports on the occurrence of *Penicillium* species in this book have therefore relied on a relative handful of papers which have provided detailed species lists. Given the taxonomic difficulties of the genus, it is unlikely that these reports are entirely accurate, but in our view they provide the best information available.

Taxonomy *Penicillium* taxonomy has evolved rapidly since the last monograph was published (Pitt 1979b). Publication of a list of "Names in Current Use" (NCU) was developed to ensure that names in *Penicillium*, *Aspergillus* and related genera would enjoy protection from earlier names under the International Code of Botanical Nomenclature (Pitt and Samson 1993; Greuter et al. 1994: p. *x*), so that Pitt and Hocking (2009) indicated that the taxonomy of *Penicillium* had become quite stable. Although the relevance of that NCU list has been questioned by some taxonomists, most common species names remain unaffected, apart from those in *Penicillium* subgenus *Biverticillium*, now all transferred to *Talaromyces*.

The advent of molecular methodology has resulted in a major revision of taxonomy within the genus, as well as greatly increasing species numbers. While accepting *Penicillium* subgenera *Penicillium* and *Biverticillium* (now in *Talaromyces*), Houbraken and Samson (2011) combined the subgenera *Aspergilloides* and *Furcatum* as defined by Pitt (1979b) into one subgenus, *Aspergilloides*. These authors also completely revised the existing classification below the subgeneric level, producing a phylogenetic classification that sometimes conflicts with the primarily morphological classification of Pitt (1979b) used in previous editions of this book. This creates a dilemma. The new classification is based entirely on molecular characteristics and, unfortunately, penicillus compexity and structure below subgenus level are sometimes not well supported by the phylogeny. This is surprising, as morphology is influenced by many genes in any genome so phylogenetic trees usually reflect morphology.

This lack of concordance means that it is unclear whether the phylogenetic classification of Houbraken and Samson (2011) is the optimal one. Three of the four genes used by them, *RPB1, Cct8* and *Tsr1* have rarely been used in other studies of *Penicillium* taxonomy. The more recent, authoritative work on *Penicillium* taxonomy (Visagie et al. 2014) recommends using ITS and ß-tubulin as the best genes for distinguishing *Penicillium* species, with calmodulin and *RPB2* (used by Houbraken and Samson 2011) where necessary. In addition, the new subgen. *Aspergilloides* is very large, with 13 clades, but with few or no cultural or morphological characters to support them, so classical techniques cannot be used to follow the phylogenetic classification. This book is designed for users of classical, cultural characteristics for taxonomy, so we are continuing to follow the classification of Pitt (1979b) in this work.

Penicillium taxonomy is not easy for the inexperienced. The species commonly occurring in foods are mostly similar in colour and general colony appearance. Reproductive structures are small and often ephemeral. However it is the authors' belief that a high percentage of isolates from foods can be identified to species level if they are grown under standardised conditions of medium and temperature, and examined after a relatively short time (7 days), so that fruiting structures and colony colours are at their best. Colony diameters are readily measured and provide very valuable information. The standard conditions used throughout this book were originally developed specifically for *Penicillium* taxonomy. The cultural conditions used and the general principles of colony examination, etc., have been outlined in Chap. 4.

Identification To determine the subgenus to which a *Penicillium* isolate belongs, micro-

scopically examine a mount made from a 7 day old culture grown on CYA at 25 °C, and examine several well defined penicilli. Count the number of branch points along the main axis of these penicilli. In the simplest subgenus, *Aspergilloides*, one branch point will be observed in a majority of structures: conidia are borne from phialides (the primary conidiogenous cell), which in turn are borne directly from the stipe as a single whorl or verticil (Fig. 7.2a, b). Such penicilli are termed monoverticillate.

In its most complex form, the penicillus characteristically has three branch points (terverticillate), and in some species not infrequently four branch points (quaterverticillate), between phialide and stipe (Fig. 7.2f–h). Species with such penicilli are classified in subgenus *Penicillium*. As a useful check, nearly all the commonly encountered species in this subgenus grow to 18 mm in diameter or more on G25N medium in 7 days at 25 °C.

Of intermediate complexity are biverticillate penicilli, i.e. ones having two branch points. As described above (Sect. 7.1) species showing one

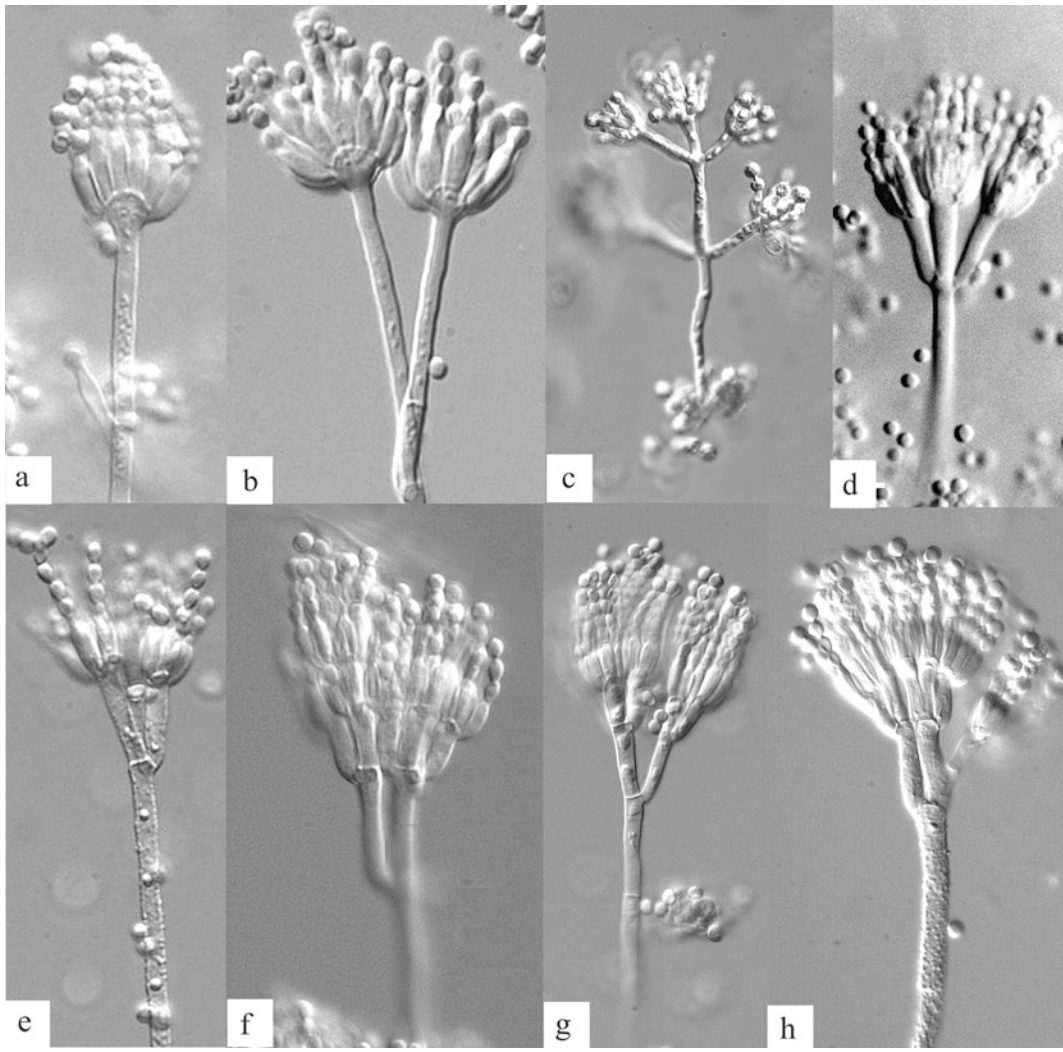

Fig. 7.2 Penicilli characteristic of *Penicillium* species (**a**, **b**) monoverticillate, *Penicillium* subgenus *Aspergilloides*; (**c**–**e**) biverticillate, *Penicillium* subgenus *Furcatum*; (**f**–**h**) terverticillate, *Penicillium* subgenus *Penicillium*

type of biverticillate penicillus (Fig. 7.1f) belong in the genus *Talaromyces*. Species with the other penicillus types (Fig. 7.2c–e) belong in *Penicillium* subgen. *Furcatum*.

Cleistothecia and sclerotia In *Penicillium* (and *Aspergillus*), only a proportion of species produce the sexual state in the laboratory, and probably in nature too. Some species produce cleistothecia that develop asci and ascospores only under prolonged incubation and often not at all. Such immature cleistothecia are termed **sclerotia**, for want of a better name. Species producing cleistothecia or sclerotia occur quite rarely in foods and are of relatively low importance to the food microbiologist. However, mature ascospores from *Penicillium* species with a sexual state are usually heat resistant, while it seems likely that sclerotia can also act as a heat resistant body, as they have occurred from time to time as survivors of heat processing. Williams et al. (1941) recorded that a new species, *Penicillium lapidosum* (stone-like, an apt name), was causing spoilage of canned blueberries. It possessed highly heat resistant sclerotia, and was later shown to produce a sexual state. Cleistothecial *Penicillium* species have also been isolated as heat resistant contaminants of fruit juices on several occasions (Anon 1967; Van der Spuy et al. 1975; Hocking and Pitt 1984, and unpublished). No particular species appears to be responsible, and growth of the fungus in the product has been rare. As a cause of food spoilage, ascospores from *Penicillium* species can be safely ignored unless an unusual set of circumstances leads to excessive contamination of some raw material or product.

On the other hand, if a *Penicillium* isolate in cultivation is found to produce a sclerotial state, it is often easy to identify. Hence a key to such species is provided here. Some of these species are relatively common. As noted above, Houbraken and Samson (2011) synonymised *Eupenicillium* with *Penicillium*, so species known by *Eupenicillium* names in previous editions of this book are known by the appropriate *Penicillium* name.

Key to cleistothecial and sclerotigenic *Penicillium* species included here

1	Colonies on CYA less than 20 mm diam, coloured deep brown	*P. cinnamopurpureum*
	Colonies on CYA more than 20 mm diam, not coloured deep brown	2
2 (1)	Colonies with bright yellow or orange mycelium and/or reverse colours	3
	Colonies with mycelium and reverse uncoloured, or in colours other than bright yellow or orange	5
3 (2)	Penicilli strictly monoverticillate	4
	Penicllli biverticillate or sometimes irregular	*P. ochrosalmoneum*
4 (3)	Growth on CYA at 37 °C similar to at 25 °C	*P. hirayamae*
	No growth at 37 °C	*P. sclerotiorum*
5 (2)	Penicilli monoverticillate	6
	Penicilli biverticillate	7
6 (5)	Growth at 37 °C	*P. javanicum* *P. brefeldianum*
	No growth at 37 °C	*P. thomii*
7 (5)	Usually growth at 37 °C, conidial walls rough	*P. simplicissimum*
	No growth at 37 °C, conidia with smooth walls	*P. raistrickii*

Because of the size and complexity of the genus, no general key to *Penicillium* is given. Taxonomy is simplified by providing a key to subgenera here, and then keys to species in the preamble to each subgenus.

Key to subgenera of *Penicillium*

1	Penicilli monoverticillate or with only a minor proportion bearing metulae	Subgenus *Aspergilloides*
	Penicilli commonly biverticillate or more complex	2
2 (1)	Penicilli predominantly biverticillate or irregularly monoverticillate and biverticillate; colonies on G25N rarely exceeding 18 mm diam	Subgenus *Furcatum*
	Penicilli predominantly terverticillate; colonies on G25N not usually less than 18 mm diam	Subgenus *Penicillium*

7.3 *Penicillium* subgenus *Aspergilloides* Dierckx

In *Penicillium* subgen. *Aspergilloides* conidiophores are strictly or predominantly monoverticillate, i.e. with phialides borne directly on the stipe, with only one branch point between stipe and conidial chain. In a few species, metulae may be present on a proportion of stipes (less than 50%).

The distinction between species considered to produce monoverticillate or biverticillate penicilli is not always obvious and indeed is not absolute. Some species produce penicilli on hyphal branches which can be interpreted either as short monoverticillate stipes or as metulae. The distinction made here is that if such a hypha, as well as producing intercalary branches, terminally produces a cluster of two or more branches at an acute angle, these are interpreted as metulae, the penicillus as biverticillate, and the species is placed in subgen. *Furcatum*. Where the hypha gives rise only to branches at right angles along its length and neither hypha nor branches have terminal clusters of metulae, the hypha is interpreted as a fertile hypha, each branch as a stipe, the penicilli as monoverticillate, and the species is classified in subgen. *Aspergilloides*.

One character used to differentiate species in subgen. *Aspergilloides*, but not elsewhere in the genus, is the presence or absence of terminal swellings (vesicles) on the stipes. A stipe is considered to be vesiculate when the terminal swelling is twice the stipe diameter or more. If in doubt, examine colonies grown on MEA, as vesicles are sometimes more obvious on this medium.

Key to *Penicillium* subgenus *Aspergilloides* species included here

1	Conspicuous orange, yellow, brown or purple mycelium, soluble pigment or reverse colours on CYA	2
	Colonies on CYA lacking bright colours	5
2 (1)	Colonies on CYA coloured brown and/or purple	*P. cinnamopurpureum*
	Colonies on CYA coloured orange or yellow	3
3 (2)	Stipes usually vesiculate	*P. sclerotiorum*
	Stipes not or rarely vesiculate	4
4 (3)	Colonies at 37 °C more than 15 mm diam	*P. hirayamae*
	Colonies at 37 °C less than 15 mm diam or growth absent	*P. citreonigrum*
5 (1)	Colonies on CYA greater than 30 mm diam	6
	Colonies on CYA not greater than 30 mm diam	8
6 (5)	Stipes rough walled	*P. thomii*
	Stipes smooth walled	7
7 (6)	Growth at 37 °C	*P. decumbens*
	No growth at 37 °C	*P. glabrum*
		P. spinulosum
8 (5)	Stipes usually vesiculate, conidia dark green	*P. implicatum*
	Stipes usually nonvesiculate, conidia grey green to grey	*P. restrictum*

Penicillium cinnamopurpureum S. Abe ex Udagawa **Fig. 7.3**

Eupenicillium cinnamopurpureum D.B. Scott & Stolk

Colonies on CYA 15–20 mm diam, of closely textured, sulcate or wrinkled white to brown mycelium, usually enveloping numerous brown to pinkish cleistothecia; conidial production sparse, coloured Greyish Green (25C-D3, 26D2); clear exudate and purple soluble pigment typically produced; reverse pink, purple or cinnamon, rarely pale or dull orange. Colonies on MEA 13–15 mm diam, plane, usually with a central area of cinnamon to brown cleistothecia surrounded by white mycelium and few to numerous penicilli; colours as on CYA except reverse sometimes dull yellow or orange, usually also with some purple areas. Colonies on G25N 8–12 mm diam, of dense white mycelium, conidial production light to moderate, grey green; reverse purple, pink or cinnamon. At 5 °C, usually no germination. At 37 °C, colonies 5–8 mm diam, of white mycelium or, rarely, no growth.

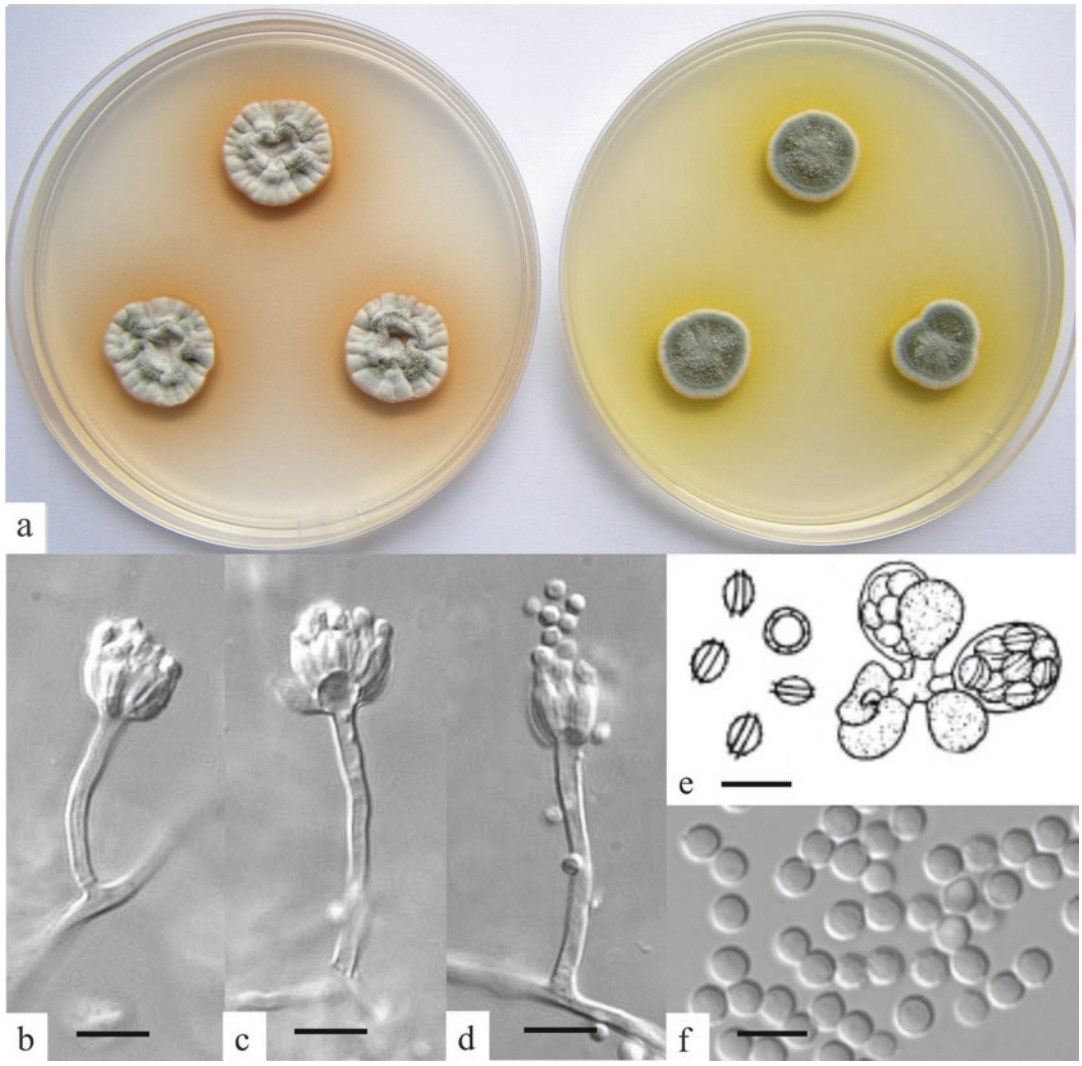

Fig. 7.3 *Penicillium cinnamopurpureum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) ascospores, bar = 5 µm; (**f**) conidia, bar = 5 µm

Cleistothecia 150–250 µm diam, pinkish cinnamon to brown, becoming hard, maturing very slowly; ascospores ellipsoidal, pale yellow, 3.0–3.5 µm long, with spinulose walls and with two low longitudinal flanges. Conidiophores borne from surface mycelium, stipes 20–150 µm long, strictly monoverticillate, smooth walled, terminating in enlarged (4–5 µm) apices; phialides ampulliform to acerose, 8–12 µm long, gradually tapering; conidia subspheroidal to ellipsoidal, 2.0–3.5 µm long, with smooth or finely roughened walls.

Distinctive features *Penicillium cinnamopurpureum* is a distinctive and readily recognised species. It grows slowly on CYA and MEA, stipes are strictly monoverticillate, are apically enlarged, and bear long, gradually tapering phialides. Most isolates produce brown cleistothecia, which mature very slowly.

Taxonomy Changes to the Botanical Code (McNeill et al. 2012) mean that the name *Eupenicillium cinnamopurpureum* used in earlier editions of this work is now correctly *Penicillium cinnamopurpureum* following synomymy of *Eupenicillium* (Houbraken and Samson 2011). Some isolates fail to produce cleistothecia at all, but are still readily recognisable as belonging here. Peterson et al. (2015) expanded the concept of *P. cinnamopurpureum* and recognised a number of new species. None has any apparent connection with food.

Identifiers Mycobank MB302386; holotype NHL No. 6359 (Udagawa 1959); ex-type strains CBS 425.65, NRRL 162, FRR 162, ATTC 18489; ITS barcode EF626950, alternative markers *BenA* EF626948, *RPB2* JN406533, *CaM* EF626949 (Visagie et al. 2014).

Physiology This species is among the most xerophilic of the Penicillia, growing down to 0.78 a_w in glycerol based media (Hocking and Pitt 1979), or 0.82 a_w in salt (25% w/v; Udagawa and Tsuruta 1973). From growth data in Pitt (1979b: 67), this species will grow at temperatures between 4–6 °C and 35–38 °C, with some isolate to isolate variation.

Mycotoxins Mycotoxin production has not been reported.

Ecology A very widely distributed species, *Penicillium cinnamopurpureum* has been isolated from flour (Graves and Hesseltine 1966), rice (Udagawa 1959), dried beans and rice, especially after long term storage (Udagawa and Tsuruta 1973; Tsuruta and Saito 1980), dried peas (Smith 1939) and stored maize kernels (Wicklow et al. 1998). It was isolated from a soybean fermentation starter (meju) in Korea (Jung et al. 2012). We isolated this species quite frequently from Southeast Asia: from soybeans, red beans, black rice and copra in Thailand; from maize, peanuts, soybeans and black pepper in the Philippines; and from maize, peanuts, kemiri nuts, mung beans, soybeans, milled rice and red kidney beans in Indonesia (Pitt et al. 1994, 1998). *P. cinnamopurpureum* was amongst the most commonly isolated Penicillia in Australian wheat and various milling fractions (flour, semolina, bran and wheat germ) during a survey of the microbiology of flour milling (Hocking, unpublished).

Related species A closely related species, *P. gravinicasei*, was described recently from cheese matured in a cave in Apulia, Italy (Anelli et al. 2018). It is very closely related to *P. cinnamopurpureum.* Identifiers: Mycobank MB823510; holotype BPI 910534; ex-type culture NRRL 66733; ITS barcode MG600580; alternative markers *BenA* MG600656, *RPB2* MG600575, *CaM* MG600570 (Anelli et al. 2018).

References Udagawa (1959), Pitt (1979b, 2000), and Visagie et al. (2014).

Penicillium citreonigrum Dierckx Fig. 7.4

Penicillium citreoviride Biourge
Penicillium toxicarium L. Miyake
(invalid name)

Colonies on CYA 20–28 mm diam, radially sulcate and often centrally wrinkled, dense and velutinous; mycelium white to bright yellow; conidia sparse to moderately abundant, Greenish Grey (27C-D2); exudate present only rarely, clear to pink; yellow soluble pigment typically produced; reverse usually brilliant yellow, occasionally yellow brown. Colonies on MEA 22–26 mm diam, plane to lightly sulcate, low, dense and velutinous; mycelium white, becoming yellow or buff centrally; conidial production moderate, in colours similar to those on CYA or slightly

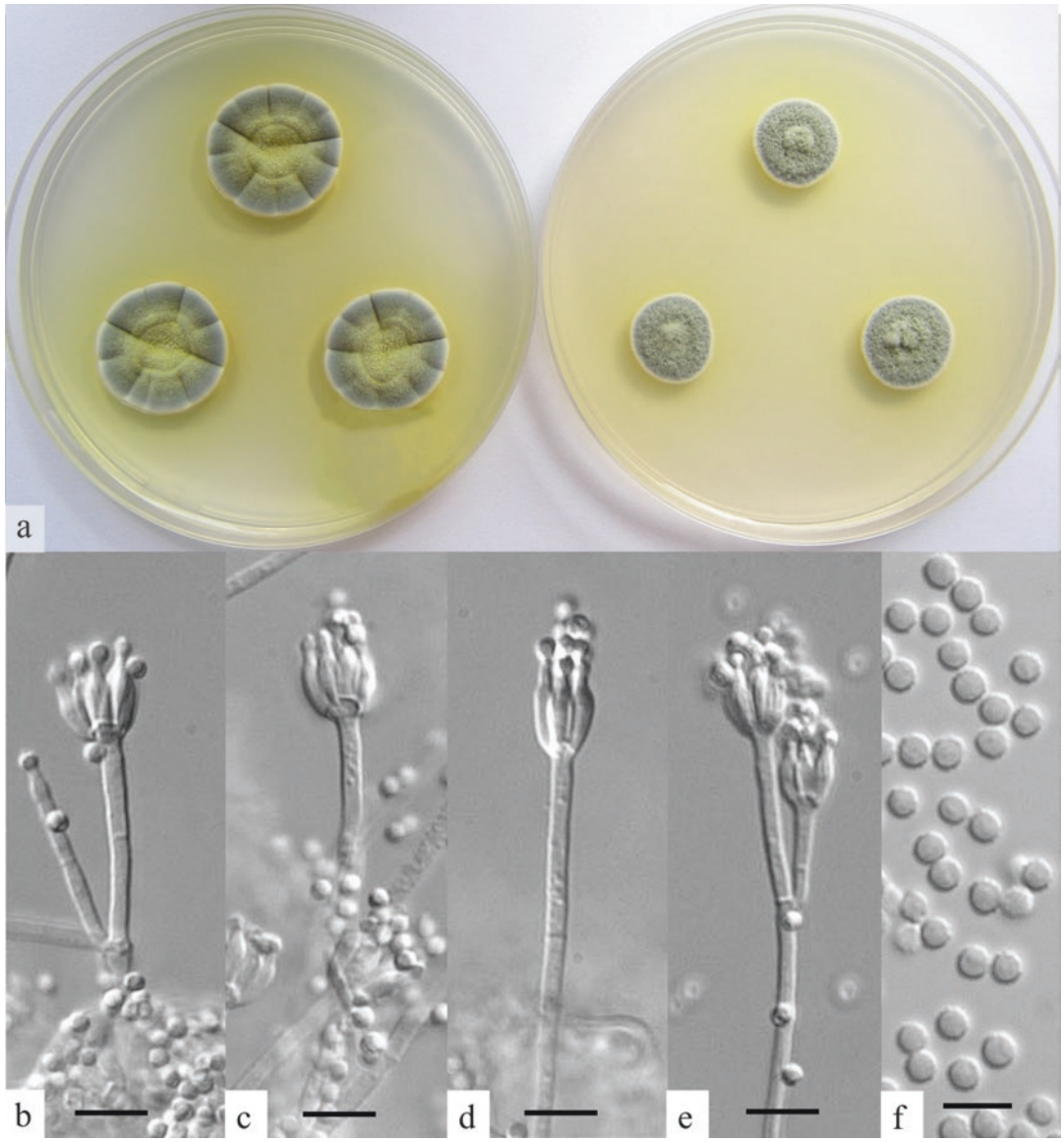

Fig. 7.4 *Penicillium citreonigrum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–e**) penicilli, bars = 10 µm; (**f**) conidia, bar = 5 µm

bluish; exudate produced rarely, clear to yellow; brown soluble pigment sometimes produced; reverse pale, brown or deep reddish brown. Colonies on G25N 11–14 mm diam, coloured similarly to those on CYA; reverse pale to brilliant yellow or brown. At 5 °C, germination to formation of microcolonies. At 37 °C, typically no growth, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from floccose or less commonly funiculose aerial hyphae, stipes slim and delicate, 60–100 µm long, smooth walled, monoverticillate, occasionally with two metulae, nonvesiculate; phialides ampulliform, length varying with isolate, 5–12 µm long; conidia spherical or near, 1.8–2.8 µm diam, with walls smooth to very finely roughened, borne in short disordered chains.

Distinctive features *Penicillium citreonigrum* produces compact yellow colonies which at most grow weakly at 5 and 37 °C; stipes are slender, not apically enlarged; conidia are tiny and smooth walled.

Taxonomy Raper and Thom (1949) used the name *Penicillium citreoviride* for this species. Pitt (1979b) took up the earlier valid name *P. citreonigrum*, and this has been accepted.

Identifiers Mycobank MB165197; neotype IMI 92209i; ex-type strains CBS 258.29, IMI 92209, NRRL 761, FRR 761, ATCC 48736; ITS barcode AF033456; alternative markers *BenA* EF198621, *RPB2* JN121474, *CaM* EF198628 (Visagie et al. 2014).

Physiology The physiology of *Penicillium citreonigrum* has been little studied. Cardinal temperatures for growth have been reported as, minimum, below 5 °C, optimum, 20–24 °C and maximum, 37–38 °C (ICMSF 1996). However, Ji et al. (2007) reported an optimum growth temperature of 27–30 °C for *P. citreonigrum* (as *P. citreoviride*). They recorded growth at 0.80 a_w at 20, 25 and 30 °C, but no growth occurred at 15 °C at a_w values from 0.80 to 0.90. However, the authors allowed only 24 h for equilibration of the rice cakes (1.5 cm thick) from an initial a_w of 0.92, and as the actual a_w did not appear to have been measured, we would consider their data on effect of a_w on growth to be unreliable. The minimum a_w for growth is not known, but this species is probably a xerophile.

Mycotoxins *Penicillium citreonigrum* is the major source of citreoviridin (El-Banna et al. 1987), the cause of the Oriental disease known in Japan as acute cardiac beriberi. Recognised for the past three centuries (Ueno and Ueno 1972), this disease frequently occurred in young healthy adults and death could occur within a few days. The disease in Japan is now only of historical interest. However, it reappeared in northern Brazil in 2006, where more that 1000 cases occurred and resulted in at least 40 deaths (Rosa et al. 2010). The disease occurred among subsistence farmers cropping rice on recently cleared land and was more common in young healthy males. Although other mycotoxins were found, the presence of citreoviridin was confirmed and symptoms of the disease indicated this toxin was the cause (Almeida et al. 2012). For further details, see Chap. 13.

Penicillium citreonigrum produces citreoviridin on rice from 10 to 37 °C, with a maximum near 20 °C (Ueno 1972).

Ecology Although not a commonly isolated species, *Penicillium citreonigrum* is very widely distributed. Its occurrence as a cause of spoilage of rice in Japan from time to time has been well documented. It was found at low levels in Brazilian rice (Rosa et al. 2010) and isolated from one of 10 rice samples from Thailand (Shiratori et al. 2017). *P. citreonigrum* has been reported from other cereals by several authors, including Graves and Hesseltine (1966), Saito et al. (1971b) and Basu and Mehrotra (1976). It was isolated very occasionally in our laboratory during a survey of the microbiology of Australia wheat and flour milling (Hocking, unpublished). It has been isolated much less frequently from other foods: mung beans (Pitt et al. 1994), spices (Takatori et al. 1977) and jam (Udagawa et al. 1977). Magnoli et al. (2005) isolated this species from pig feed, which consisted of 60% maize and also from rabbit feed in which maize was replaced by oats, alfalfa and barley.

References Uraguchi (1971) and Pitt (1979b, 2000).

Penicillium decumbens Thom Fig. 7.5

Colonies on CYA commonly 20–30 mm diam, occasionally 40 mm, low and dense, velutinous to lightly floccose; mycelium white to cream; conidial production light to moderate, Greyish Green to Dull Green (25C-D3); reverse pale, dull yellow brown or olive. Colonies on MEA 25–40 mm diam, usually plane, typically low and relatively sparse, less commonly floccose; moderate numbers of conidia produced, coloured as on CYA. Colonies on G25N 11–16 mm diam, usually rather sparse, in general terms similar to those on CYA. At 5 °C, germination by a proportion of conidia up to formation of microcolonies. At 37 °C, colonies 5–20 mm diam, velutinous to floccose, coloured white to grey green; reverse pale or brownish.

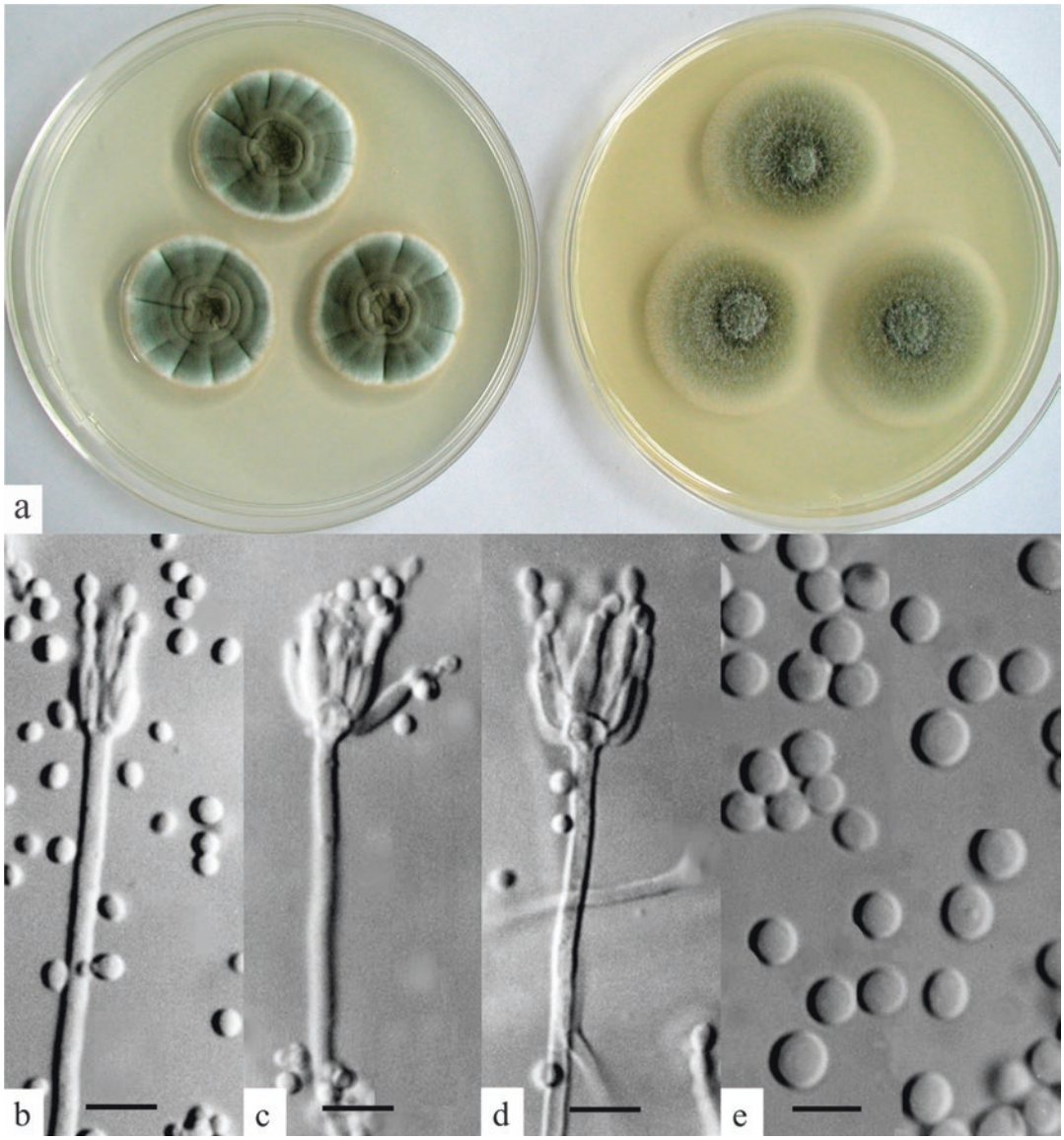

Fig. 7.5 *Penicillium decumbens* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Conidiophores borne from aerial hyphae, stipes short, 20–60(−100) µm long, with thin, smooth walls, monoverticillate, nonvesiculate to somewhat enlarged; phialides ampulliform, long and slender, 8–11(−14) µm long; conidia ellipsoidal, in some isolates also pyriform, smooth walled, 2.5–3.0(−4.0) µm long, borne in short, loose columns.

Distinctive features *Penicillium decumbens* produces short, somewhat apically swollen, monoverticillate conidiophores from aerial hyphae, and smooth walled, distinctly ellipsoidal, dull green conidia. Colonies usually do not show any other colours. This is one of the few *Penicillium* species which typically grows at both 5 and 37 °C.

Identifiers Mycobank MB156582; lectotype IMI 190875; ex-type cultures CBS 230.81, IMI 190875, NRRL 741, FRR 741; ITS barcode AY157490; alternative markers *BenA* KJ834446, *RPB2* JN406601 (Visagie et al. 2014).

Physiology In the only recorded physiological study, *Penicillium decumbens* grew down to 0.87 but not 0.82 a_w at both 20 and 30 °C (Valero et al. 2007a).

Mycotoxins No mycotoxins are known.

Ecology A ubiquitous fungus, *Penicillium decumbens* has been isolated from a wide variety of foods. These include dried peas and beans (see Pitt and Hocking 1997); several kinds of nuts (Pitt et al. 1998); flour milling equipment (Hocking, unpublished), flour, rice, infant cereal preparations, soybeans, meat products and fresh vegetables (see Pitt and Hocking 1997). This fungus was also isolated from Spanish grapes and dried vine fruits, although spoilage was not recorded (Valero et al. 2007a, b). Spoilage of food by this species appears to be unusual.

References Pitt (1979b, 2000).

Penicillium glabrum (Wehmer) Westling Fig. 7.6

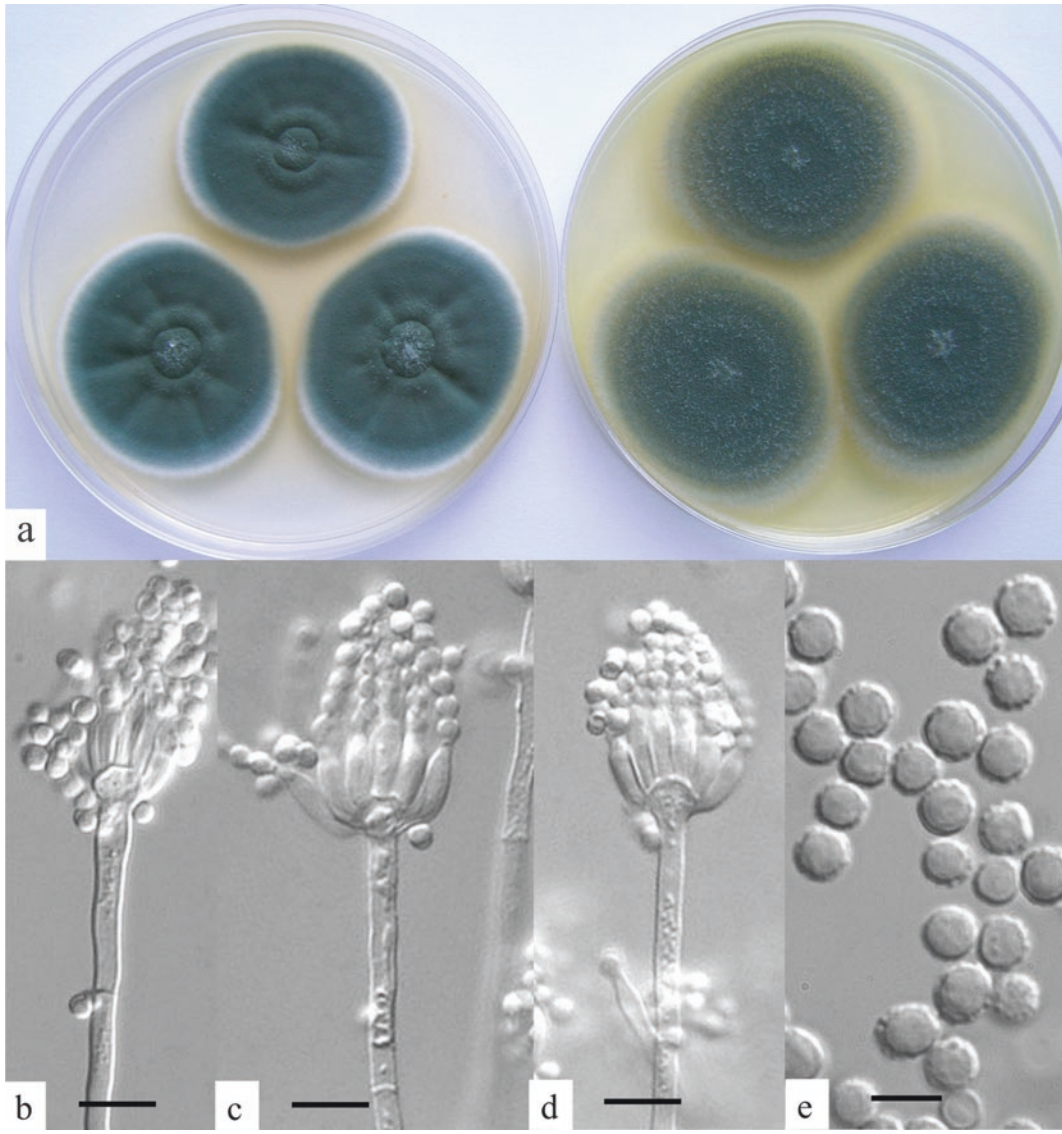

Fig. 7.6 *Penicillium glabrum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Colonies on CYA 40–50 mm diam, low, plane to radially sulcate; surface texture strictly velutinous; white mycelium sometimes visible at the centres; conidial production moderate to heavy, Dull Green to Dark Green (26-27E-F3-5); exudate produced centrally by some isolates, clear, yellow or brown; bright yellow soluble pigment sometimes produced; reverse varying from pale or greenish to bright yellow, deep orange, brown or occasionally reddish. Colonies on MEA 40–55 mm diam, low, strictly velutinous, plane or centrally umbonate; white mycelium visible at the centres only; conidial production moderate, coloured as on CYA; yellow soluble pigment sometimes produced; reverse sometimes pale, but more commonly strongly coloured, greenish, olive, yellow or brown. Colonies on G25N 17–24 mm diam, sulcate or wrinkled, velutinous; mycelium white. At 5 °C, at least germination, usually microcolonies or colonies up to 4 mm diam formed. No growth at 37 °C.

Conidiophores borne from subsurface or surface mycelium or to a limited extent from aerial hyphae, stipes (25–)50–100(−200) µm long, with walls smooth to finely roughened, monoverticillate or occasionally with two metulae, commonly vesiculate up to 6 µm diam; phialides numerous, ampulliform, 8–12 µm long; conidia spherical to subspheroidal, 3.0–3.5 µm diam, with walls smooth or very finely roughened, borne in long well defined columns.

Distinctive features *Penicillium glabrum* grows rapidly at 25 °C, producing dark green colonies with a strictly velutinous texture. Stipes are vesiculate; conidia are smooth walled and borne in long columns. This species is distinguishable from *P. spinulosum* and *P. frequentans* only with difficulty (*See* Additional Species below).

Taxonomy The relationship of *Penicillium glabrum* with *P. spinulosum* was examined using classical techniques by Pitt et al. (1990) who reported that these two species were distinct, but very closely related. Using molecular methods, Barreto et al. (2011) studied *P. glabrum* and related species isolated from cork and kept the separation of *P. glabrum* from *P. spinulosum.* Houbraken et al. (2014) accepted this separation, and indeed named separate clades after these two species while agreeing that morphological distinctions were small and not absolute.

Pitt (1979b) regarded *P. frequentans* as a synonym of *P. glabrum* and that judgement was followed in earlier editions of this book. However, Houbraken et al. (2014) recognised *P. frequentans* as a separate, though closely related, species.

Identifiers Mycobank MB120545; neotype IMI 91944 (Pitt 1979b); ex-type cultures CBS 125543, IMI 91944, ATCC 10448, NRRL 939; ITS barcode GU981567; alternative markers *BenA* GU981619, *RPB2* JF417447, *CaM* GQ367545 (Visagie et al. 2014).

Physiology *Penicillium glabrum* is a psychrotroph, able to grow down to at least 0 °C (Mislivec and Tuite 1970). Maximum temperatures for growth are near 30 °C, not, as reported by Domsch et al. (1980), above 40 °C.

Mycotoxins Mycotoxins are not produced.

Ecology *Penicillium glabrum* has been reported to cause rots in pomegranate fruit postharvest in Greece (Bardas et al. 2009) and Italy (Spadaro et al. 2010). This species has caused spoilage of cheese and margarine (see Pitt and Hocking 1997). It has been isolated frequently from a wide range of foodstuffs, in earlier literature under the name *P. frequentans*: from dried and concentrated products such as maize, peanuts, rice and jam; from fermented and cured meats and from fresh cabbage and yams (see Pitt and Hocking 1997), from wheat, flour and other milling fractions (Hocking, unpublished) and canned carbonated beverage (our data and Ancasi et al. 2006). We have isolated *P. glabrum* from bottled water, sports beverages, fruit juices, fruit purées and a variety of spoiled dairy products. *P. glabrum* has also been reported from chestnuts (Overy et al. 2003) and grapes (Serra et al. 2006).

Additional species *Penicillium spinulosum* Thom closely resembles *P. glabrum*, the main difference being that *P. spinulosum* produces spinose conidia, is more floccose and produces grey green rather than dark green conidia (Pitt et al. 1990; Houbraken et al. 2014). *P. frequentans*, also closely related, resembles *P. glabrum* morphologically. *P. spinulosum* has been reported to be a xerophile, germinating down to 0.80 a_w at

22–25 °C (Pelhate 1968; Hocking and Pitt 1979) and the other species are likely to be similar. Like *P. glabrum*, these species are psychrotrophs, and have not been reported to produce mycotoxins. *P. spinulosum* has been reported most frequently from wheat and flour and meat products (see Pitt and Hocking 1997). We have isolated it from soybeans and peanuts in Southeast Asia (Pitt et al. 1998). From the food microbiologist's viewpoint, differentiation of these three species is usually unnecessary. It is likely that all are common in foods. Identifiers of *P. spinulosum*: Mycobank MB215401; lectotype IMI 24316i (Pitt 1979b); ex-type cultures CBS 374.48, IMI 24316, ATCC 10498, NRRL 1750, FRR 1750; ITS barcode AF033410; alternative markers *BenA* KJ834493, *RPB2* JN406558, *CaM* GQ367524; of *P. frequentans* Mycobank MB152118; holotype CBS 105.11; ex-type culture CBS 105.11; ITS barcode KM189525; alternative markers *BenA* KM088762, *RPB2* KM089534, *CaM* KM089147 (Visagie et al. 2014).

References Pitt (1979b, 2000), Barreto et al. (2011), and Houbraken et al. (2014).

Penicillium hirayamae Udagawa

Eupenicillium hirayamae D.B. Scott & Stolk

Fig. 7.7

Colonies on CYA usually 22–28 mm diam, but up to 45 mm diam if cleistothecia absent, radially sulcate, of dense, brilliant yellow or orange mycelium usually enmeshing cleistothecia and overlaid by funicles (ropes) of fertile hyphae; conidial production moderate, Dull Green (28D3); exudate clear to pale yellow; reverse usually Apricot to Deep Orange (5B6-A8). Colonies on MEA usually 15–22 mm diam, but up to 35 mm if cleistothecia absent, plane, otherwise similar to those on CYA. Colonies on G25N 8–14 mm diam, similar to those on CYA. No germination at 5 °C. At 37 °C, colonies 20–30 mm diam, mycelium centrally brown, otherwise similar to those produced at 25 °C.

Cleistothecia buff to yellow, appearing orange or brown from adherent hyphae, 250–300 µm diam or up to 400 µm long, hard, maturing after 4–6 weeks or more; ascospores small and ellipsoidal, yellow, 2.2–3.0 µm long, with rough walls and two small longitudinal flanges. Conidiophores borne from ropes of aerial hyphae, with stipes 10–50 µm long, smooth walled, strictly monoverticillate; phialides ampulliform, 6–8(−10) µm long; conidia subspheroidal, minute, 1.8–2.8 µm long, smooth walled.

Distinctive features Relatively slowly growing, brilliant yellow and orange colonies make *Penicillium hirayamae* a readily recognised species. Confirmation is provided by the formation of very similar colonies at 25 and 37 °C and by the production of short monoverticillate conidiophores, mostly from aerial hyphae.

Taxonomy Changes to the Botanical Code (McNeill et al. 2012) mean that the name *Eupenicillium hirayamae* used in earlier editions of this work is now correctly *Penicillium hirayamae* following synomymy of *Eupenicillium* (Houbraken and Samson 2011). Most isolates produce cleistothecia and in due course ascospores. A few isolates have been encountered which do not – interestingly colony growth is significantly faster. Molecular relationships of several species with *P. hirayamae* were studied and illustrated by Visagie et al. (2013). They retained *P. hirayamae* as a distinct species. The other species are not relevant here.

Identifiers Mycobank MB302402; holotype NHL 6046; ex-type cultures CBS 229.60, IMI 78255, ATCC 18312, NRRL 143, FRR 143; ITS barcode JN626095; alternative markers *BenA* JN625955, *RPB2* JN121459, *CaM* JN626003 (Visagie et al. 2014).

Physiology We are not aware of any physiological studies on this species.

Mycotoxins Mycotoxin production has not been reported.

Ecology *Penicillium hirayamae* has mostly been isolated from cereals, from Thailand, USA, South Africa and India (Pitt and Hocking 1997).

References Udagawa (1959) and Pitt (1979b, 2000).

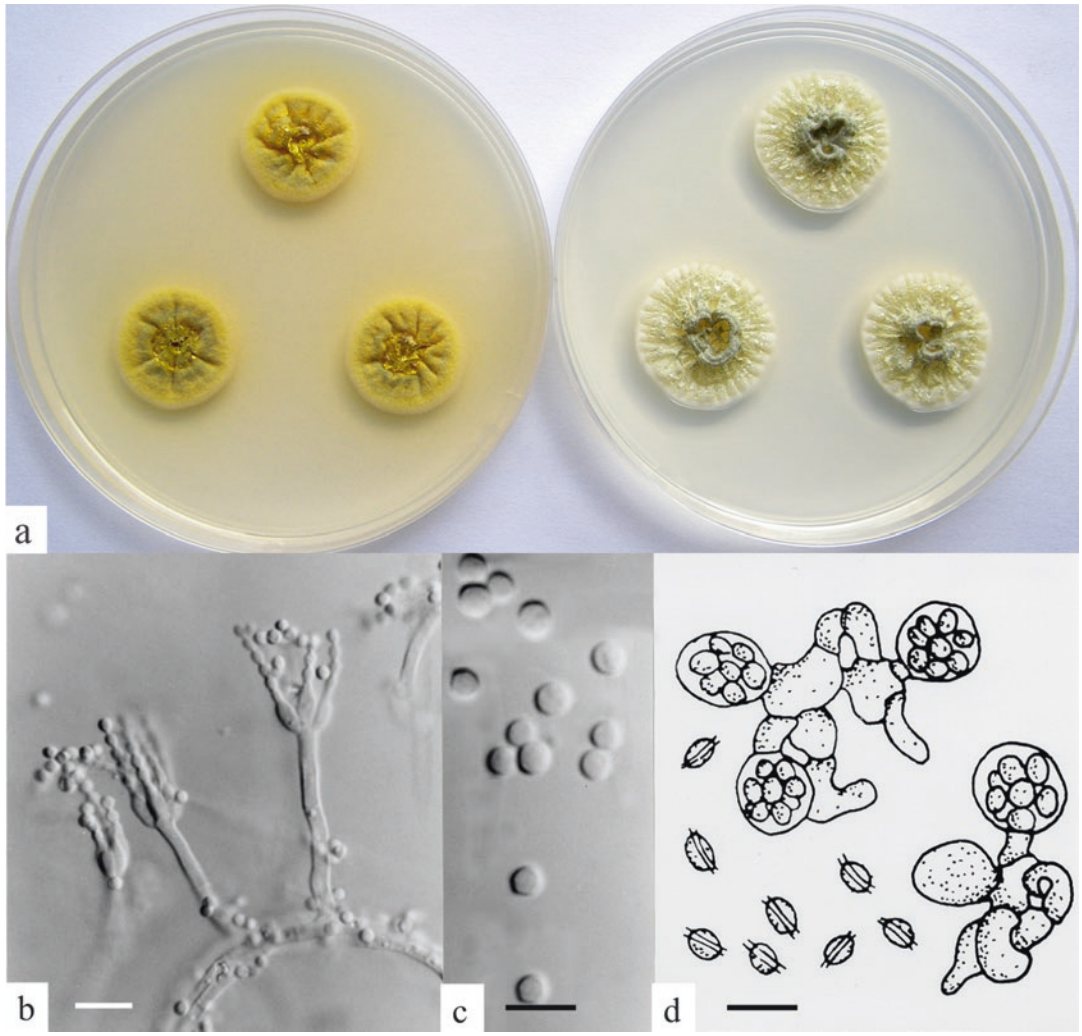

Fig. 7.7 *Penicillium hirayamae* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) penicilli, bar = 10 µm; (**c**) conidia, bar = 5 µm; (**d**) ascospores, bar = 5 µm

Penicillium implicatum Biourge

Fig. 7.8

Penicillium hispanicum Ramirez et al.

Colonies on CYA and MEA growing slowly, 15–20 mm diam or less, radially sulcate, strictly velutinous; mycelium low and dense, white or buff; conidial production light to heavy, Greyish Green to Dull Green (25-26D-E4-5); pale to deep brown exudate sometimes produced on CYA; brown soluble pigment typically produced; reverse yellow, brown or reddish. Colonies on G25N 8–12 mm diam, velutinous; colours similar to those on CYA except reverse pale, olive or brown. At 5 °C usually no germination. At 37 °C colonies of 5–10 mm diam usually produced, dense and velutinous; reverse brown to deep brown.

Conidiophores borne from subsurface or surface hyphae, stipes 30–100 µm long, with walls thin and smooth, monoverticillate, sometimes with two metulae, usually vesiculate up to 4–5 µm diam, but not exclusively so; phialides 8–11 µm long, slim, with short collula; conidia ellipsoidal

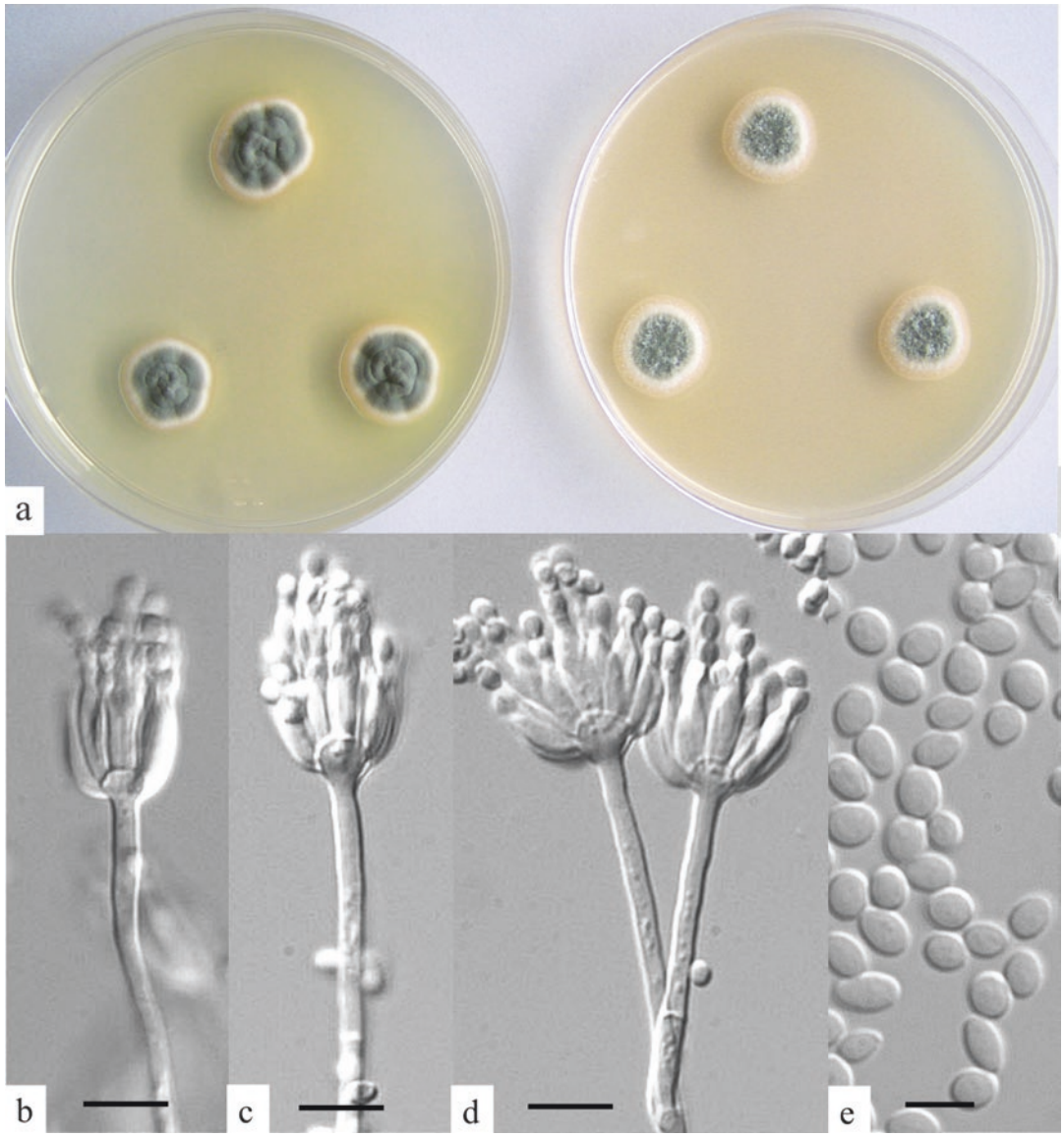

Fig. 7.8 *Penicillium implicatum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

to subspheroidal, 2.5–3.0 µm long, with thick, smooth or finely roughened walls, borne in loose columns.

Distinctive features *Penicillium implicatum* produces slowly growing, dense colonies on the standard media, often so dense that production of effective wet mounts can be difficult; typical isolates grow slowly at 37 °C. Conidiophores are relatively short and usually vesiculate, conidia are ellipsoidal and smooth or nearly so.

Taxonomy This species was synonymised with *Penicillium citrinum* by Frisvad et al. (1990b) on the basis that the "ex-type" strain of *P. implicatum* held at CBS (CBS 232.38) was a *P. citrinum* morphologically, molecularly and "is still a good producer of citrinin" (Frisvad et al. 1990c). However, the original protocol of Biourge describes a species remote from *P. citrinum*. *P. implicatum* was described by Biourge (1923) as having conidia "*rotundid vel oblongis*.

2 – 3.5 × 1.8 – 2.8", metulae "*binis vel singularis*" stipes "2 – 3.2 × 30 – 90", none of which fit descriptions of *P. citrinum* [which has stipes 100–300 µm long, metulae usually in groups of three, and conidia "spherical to subspheroidal, 2.2 – 3.0 µm diam" (Pitt 1979b)], but which closely fits the above description of *P. implicatum*. We conclude that the ex-type strain of *P. implicatum* held by CBS has been replaced by *P. citrinum*. *P. implicatum* is maintained here as a valid species.

Identifiers Mycobank MB267728; neotype IMI 190235 (Pitt 1979b); ex-type cultures CBS 184.81, IMI 190235, NRRL 2061, FRR 2061; ITS barcode AF033428; alternative markers 18S AY380456, 26S AF033428 (CBS 2016).

Physiology This species is one of the most xerophilic of the Penicillia, being able to germinate at 0.78 a_w in 10 days (Hocking and Pitt 1979). Conidiogenesis was unaffected by water activity (0.90 and 0.82) when grown on sterile maize grains, but fungal biomass increased at higher a_w (Cahagnier et al. 1993).

Mycotoxins Mycotoxin production has not been reported.

Ecology Perhaps because of its slow growth and xerophilic nature, *Penicillium implicatum* is readily overlooked in surveys. Pitt (1979b) regarded its basic habitat as soil; Nesci et al. (2006) reported this species in agricultural soils where maize was cultivated. While not of common occurrence, it is a significant biodeteriogen (Raper and Thom 1949: 203) and spoilage fungus in dried foods. It has been reported to be pathogenic on stored pomegranate fruit (Labuda et al. 2004; Khokhar et al. 2013). Doupnik and Bell (1971) reported *P. implicatum* from spoiled pecans; it was also common in Indonesian kemiri nuts (1% of all nuts examined; Pitt et al. 1998) and was found at lower levels in Thai cashews and Indonesian peanuts (Pitt et al. 1993, 1998). It has been isolated mainly from cereals: maize, rice, wheat and flour, but has also been reported from cashews and dried peas, meat products and frozen fruit pastries (see Pitt and Hocking 1997). It was relatively common in Australian flour and other milling fractions (Hocking, unpublished).

References Pitt (1979b, 2000).

Penicillium javanicum

J.F.H. Beyma **Fig. 7.9**

Eupenicillium javanicum (J.F.H. Beyma) Stolk & D.B. Scott

Penicillium indonesiae Pitt

Colonies on CYA 30–45 mm diam, radially sulcate, consisting of dense, velutinous, pale yellow mycelium; cleistothecia abundant, enveloped by the mycelium; conidia sparse; exudate copious, brown; reverse olive green, often also with deep reddish brown areas. Colonies on MEA 30–50 mm diam, similar to those on CYA but mycelium brighter yellow and reverse usually deep olive brown. Colonies on G25N 9–14 mm diam, of floccose, pale yellow mycelium; reverse olive brown. No germination at 5 °C. At 37 °C, colonies 25–50 mm diam, radially sulcate or irregularly wrinkled, mycelium usually white but sometimes deep brown; cleistothecia sometimes present but penicilli absent; clear to brown or reddish exudate and reddish brown soluble pigment usually produced; reverse pale, yellow, reddish brown or deep brown.

Cleistothecia dull yellow to brown, 80–200 µm diam, maturing in 2–3 weeks; ascospores ellipsoidal, 2.5–3.0 µm long, with slightly roughened walls and a faint longitudinal furrow. Conidiophores borne from aerial hyphae, stipes 50–100 µm long, smooth walled, nonvesiculate, bearing monoverticillate penicilli; phialides ampulliform, 8–11 µm long; conidia subspheroidal, ellipsoidal or pyriform, 2.5–3.0 µm long.

Distinctive features *Penicillium javanicum* grows rapidly at 37 °C, produces sparse strictly monoverticillate penicilli and produces bright colours in exudate and reverse on CYA at 25 °C. Mature ascospores are produced relatively rapidly, usually within 14 days at 25 °C.

Taxonomy Changes to the Botanical Code (McNeill et al. 2012) mean that the name *Eupenicillium javanicum* used in earlier editions of this work is now correctly *Penicillium javanicum* following synomymy of *Eupenicillium* (Houbraken and Samson 2011).

Identifiers Mycobank MB268394; neotype IMI 39733 (Pitt 1979b); ex-type cultures CBS 241.48, IMI 39733, ATCC 9099, NRRL 707,

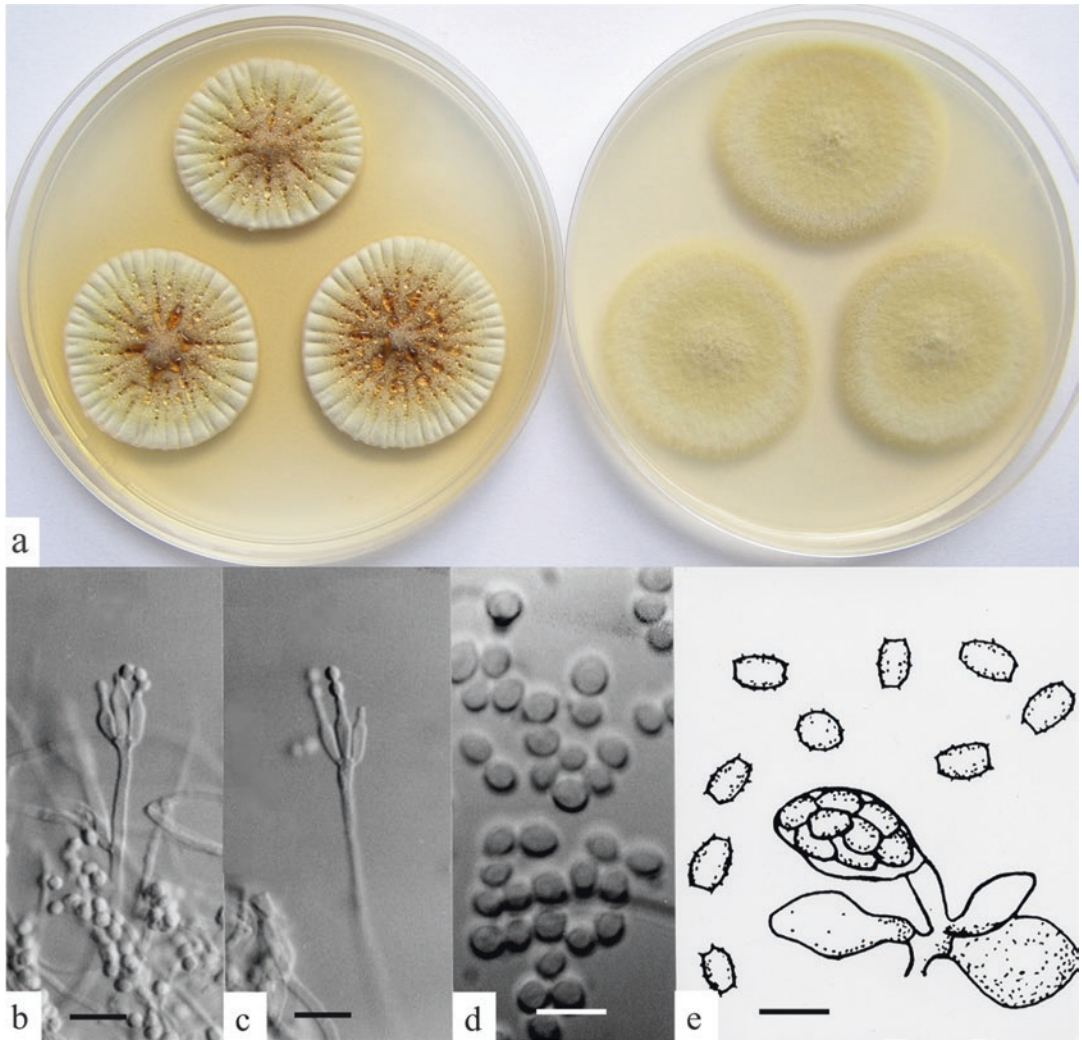

Fig. 7.9 *Penicillium javanicum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm; (**e**) ascospores, bar = 5 µm

FRR 707; ITS barcode GU981613; alternative markers *BenA* GU981657, *RPB2* JN121498, *CaM* 286387 (Visagie et al. 2014).

Physiology No relevant physiological studies are known.

Mycotoxins No significant mycotoxins are produced.

Ecology This is a common species in soils heated to select for those producing ascospores, but it occurs in foods rather rarely. It has been isolated from wheat and flour (Basu and Mehrotra 1976), fermented and cured meats (Leistner and Ayres 1968) and peanuts (Pitt et al. 1998).

Additional species *Penicillium brefeldianum* B.O. Dodge is closely related to *P. javanicum*; the two species produce colonies of similar sizes on the standard media. *P. brefeldianum* produces longer, sometimes metulate stipes, larger cleistothecia (150–250 µm long) and ascospores (3–4 µm long), and pale orange mycelium. It lacks green reverse colours.

This species has been reported to cause spoilage of apple juice in South Africa by surviving pasteurising treatments (Anon 1967; Van der Spuy et al. 1975). It has also been isolated from peanuts (Joffe 1969). Identifiers: Mycobank

MB258851; lectotype IMI 216896 (Pitt 1979b); ex-type cultures CBS 235.81, IMI 216896, NRRL 710, FRR 710; ITS barcode AF033435; alternative markers *BenA* GU981623, *RPB2* KF496421, *CaM* EU021683 (Visagie et al. 2014).

References Stolk and Scott (1967) and Pitt (1979b, 2000).

Penicillium ochrosalmoneum Udagawa

Fig. 7.10

Eupenicillium ochrosalmoneum D.B. Scott & Stolk

Colonies on CYA 18–28 mm diam, plane or sulcate, with a moderately deep, dense layer of mycelium enveloping abundant cleistothecia, overlaid by sparse to abundant penicilli; mycelium white to Yellow (2-4A7); conidia Greyish Green to Dull Green (27C-D3); clear to pale yellow exudate and pale to bright yellow soluble pigment sometimes produced; reverse Yellow to Cadmium Orange (3-5A8). Colonies on MEA 15–20 mm diam, plane, low and sparse to moderately deep and dense, velutinous; mycelium white at the margins, elsewhere bright yellow, as on

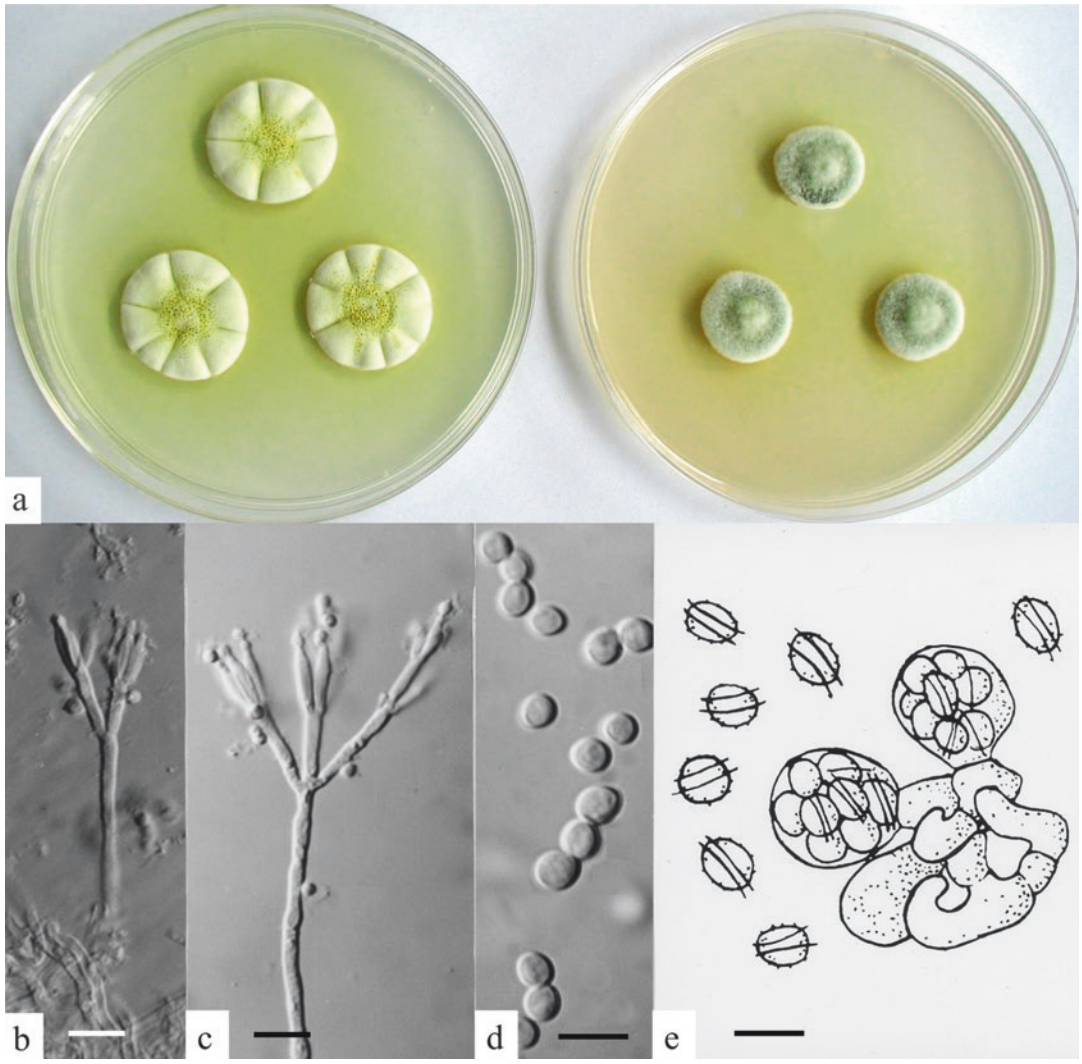

Fig. 7.10 *Penicillium ochrosalmoneum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm; (**e**) ascospores, bar = 5 µm

CYA; cleistothecia abundant, on the agar surface; conidial production sparse to moderate, coloured as on CYA; yellow soluble pigment usually produced; reverse bright yellow to orange. Colonies on G25N 1–7 mm diam, of deep, dense white mycelium; reverse pale or yellow. No germination at 5 °C. At 37 °C, colonies 10–30 mm diam, radially sulcate or wrinkled, closely resembling growth on CYA at 25 °C except for increased cleistothecial production by some isolates.

Cleistothecia pale to bright yellow or orange, 250–500 µm diam, sclerotioid, maturing in 4–6 weeks or more; ascospores ellipsoidal, 3.5–5.0 µm long, with walls smooth to roughened or spinulose, ornamented with two longitudinal flanges. Conidiophores borne from surface or aerial hyphae, stipes variable, (20–)40–200 µm long, smooth walled, bearing very irregular penicilli, basically biverticillate but with subterminal and intercalary metulae often present, the latter being indistinguishable from short monoverticillate conidiophores; phialides ampulliform-acerose 6–8(−10) µm long; conidia subspheroidal to apiculate, commonly 2.5–3.5 µm long, smooth walled, borne in disordered chains.

Distinctive features *Penicillium ochrosalmoneum* forms slowly growing, bright yellow colonies on both CYA and MEA at 25 °C. Growth on CYA at 37 °C is similar in size and colouration to that at 25 °C. These characteristics are shared with *P. hirayamae*: this latter species, however, produces strictly monoverticillate penicilli.

Taxonomy Changes to the Botanical Code (McNeill et al. 2012) mean that the name *Eupenicillium ochrosalmoneum* used in earlier editions of this work is now correctly *Penicillium ochrosalmoneum* following synomymy of *Eupenicillium* (Houbraken and Samson 2011).

Identifiers Mycobank MB302409; type NHL 6048; ex-type cultures CBS 489.66, IMI 116248, ATCC 18338, NRRL 196, FRR 196; ITS barcode EF626961; alternative markers *BenA* EF506212, *RPB2* JN121524, *CaM* EF506237 (Visagie et al. 2014).

Physiology *Penicillium ochrosalmoneum* is capable of growth down to 0.88 a_w (Hocking and Pitt 1979).

Mycotoxins This species is the second major producer of citreoviridin, a mycotoxin described in more detail in Chap. 13. Citreoviridin produced by *P. ochrosalmoneum* has been found naturally occurring in US maize, at levels up to 2.8 mg/kg (Wicklow et al. 1988).

Ecology This species has been reported from maize in North America (Wicklow and Cole 1984; Wicklow et al. 1988) and pig feed in Slovakia (Labuda and Taninová 2003). We have seen *P. ochrosalmoneum* in Australian wheat and wheat fractions during a survey of the microbiological status of Australian flour mills (Hocking, unpublished). Its importance lies in the potential to produce citreoviridin under natural conditions.

References Scott and Stolk (1967) and Pitt (1979b, 2000).

Penicillium restrictum

J.C. Gilman & E.V. Abbott **Fig. 7.11**

Colonies on CYA 18–25 mm diam, plane or lightly radially sulcate, typically deep and floccose; mycelium white; conidia absent or sparsely produced, pale grey to bluish grey; clear exudate occasionally present; reverse pale or light brown. Colonies on MEA 15–25 mm diam, plane or umbonate, texture variable, deeply floccose with sparse conidial production to low and funiculose with abundant conidia; mycelium white; conidia greenish grey; reverse pale to brown. On G25N, colonies 11–14 mm diam, plane, deeply floccose or less commonly mucoid; mycelium white; reverse pale. At 5 °C, typically no germination; rarely a proportion of conidia with germ tubes. At 37 °C, typically colonies 5–10 mm diam produced, sulcate or wrinkled, white or grey; rarely no growth.

Conidiophores borne from loose aerial hyphae or on MEA sometimes from rudimentary funicles, stipes very short, mostly 10–30 µm long, narrow, smooth walled, strictly monoverticillate, nonvesiculate; phialides ampulliform, short, (4–)6–7 µm; conidia spheroidal, ellipsoidal or less commonly pyriform, 2.0–3.0 µm long, finely to coarsely roughened, borne in short irregular chains.

Distinctive features *Penicillium restrictum* grows relatively slowly on CYA and MEA at 25 °C, and slowly at 37 °C. Colonies are usually floccose, with white mycelium and sparsely produced grey conidia. Penicilli are very small.

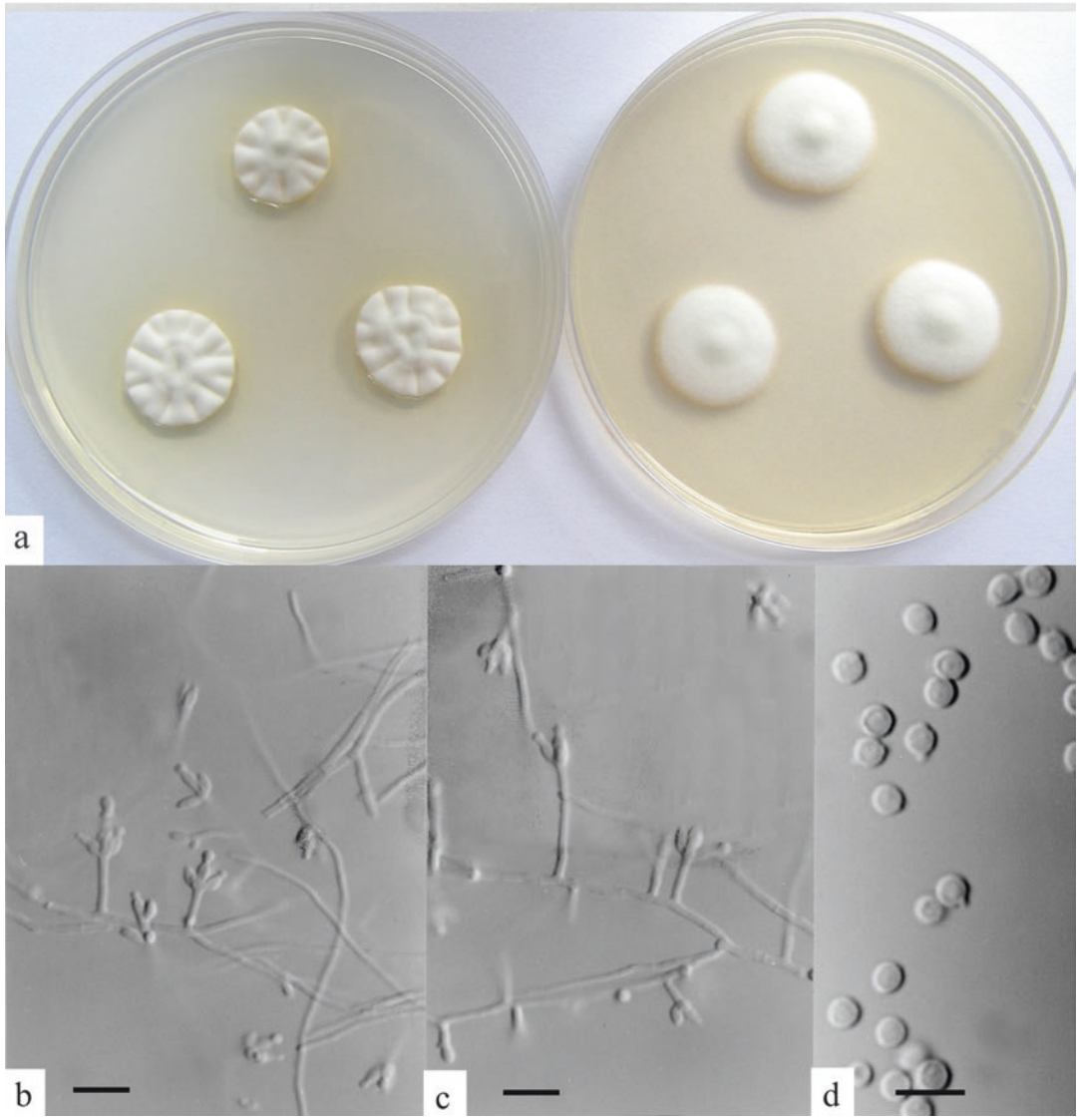

Fig. 7.11 *Penicillium restrictum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

Identifiers Mycobank MB276289; neotype IMI 40228 (Pitt 1979b); ex-type cultures CBS 367.48, IMI 40228, ATCC 11257, NRRL 1748, FRR 1748; ITS barcode AF033457; alternative markers *BenA* KJ834486, *RPB2* JN121506 (Visagie et al. 2014).

Physiology A minimum a_w for growth of 0.82 has been reported (Hocking and Pitt 1979).

Mycotoxins This species does not produce mycotoxins.

Ecology *Penicillium restrictum* is a soil fungus, and has not been reported to cause food spoilage. Wheat and flour are the foods from which it has been most frequently isolated (Kurata and Ichinoe 1967; Basu and Mehrotra 1976). Other reports have been from processed meats (Lofti et al. 1983), soybeans (Pitt et al. 1994) and poultry feeds (Magnoli et al. 1998).

References Pitt (1979b, 2000).

Penicillium sclerotiorum
J.F.H. Beyma **Fig. 7.12**

Colonies on CYA and MEA typically 30–40 mm diam, less commonly only 20–25 mm, wrinkled, low to moderately deep, dense, consisting of a layer of mycelium, white at the margins, becoming yellow to brilliant orange nearer the centres, usually enveloping abundant sclerotia and overlaid by scattered penicilli; conidia sparsely produced, Greyish Turquoise or Greenish Grey (24-26D2-3); exudate limited to abundant, pale, yellow, orange or orange red; yellow or brown soluble pigment usually produced; reverse orange yellow or orange to coffee coloured on CYA, on MEA similar or orange red. Colonies on G25N 13–18 mm diam, dense and wrinkled, generally in colours similar to those described above. Usually no germination at 5 °C; occasionally germination observed. No growth at 37 °C.

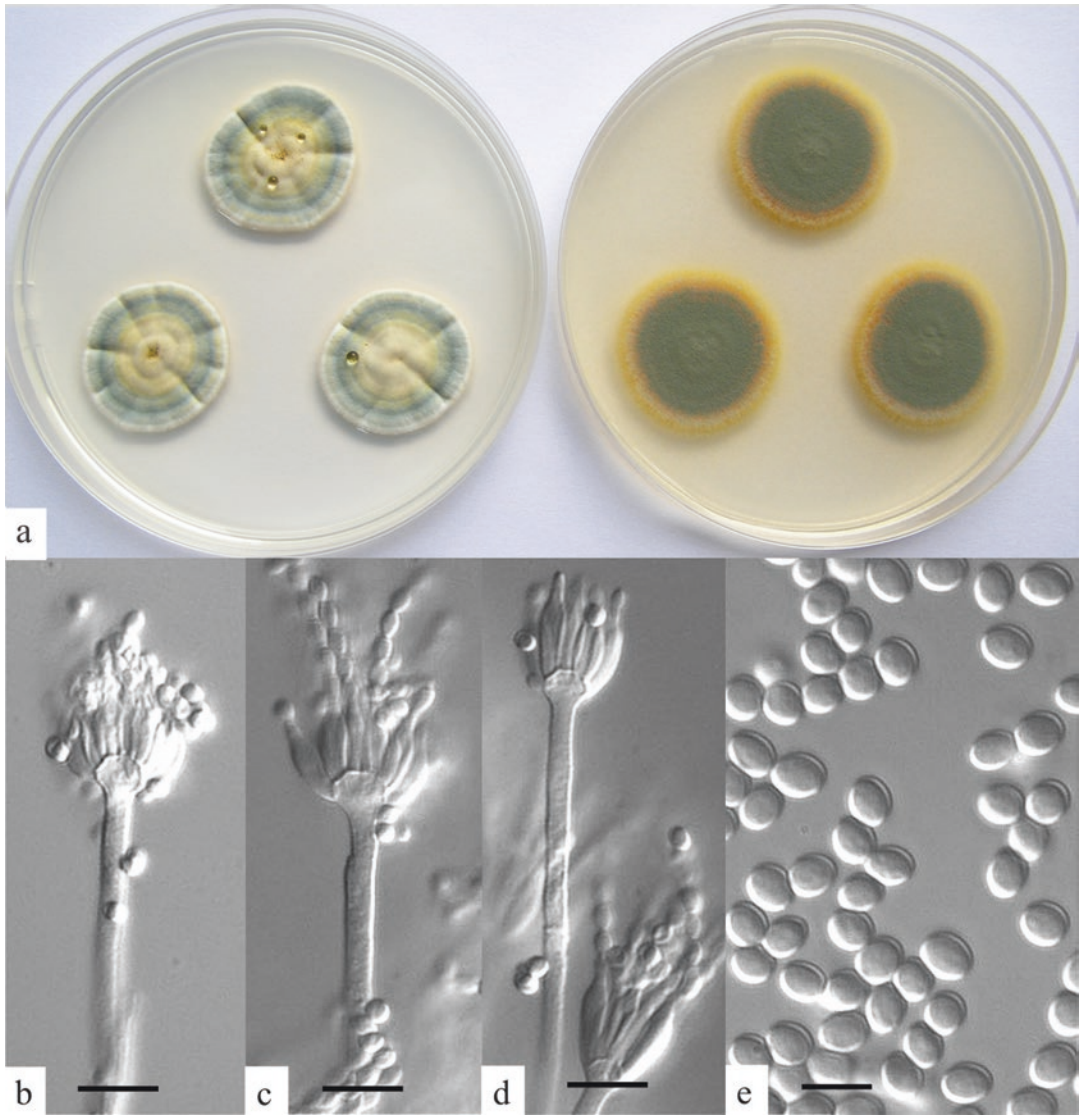

Fig. 7.12 *Penicillium sclerotiorum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Sclerotia usually present in fresh isolates, pale, spherical or irregular, 200–400 µm long. Conidiophores borne from surface or subsurface hyphae, stipes 100–300 µm long, slender, with thin, smooth to finely roughened walls, strictly monoverticillate, vesiculate, 4–6 µm diam; phialides numerous, ampulliform, 7–9(−11) µm long; conidia ellipsoidal, 2.5–3.0 µm long, with smooth to finely roughened walls, borne in long and well defined or irregular columns.

Distinctive features Vivid orange to red colony colours, in both obverse and reverse, are striking characteristics which distinguish *Penicillium sclerotiorum* from other monoverticillate *Penicillium* species. Rivera and Seifert et al. (2011) distinguished seven species in a clade which includes *P. sclerotiorum*. One, *P. cainii*, was isolated from a nut of the black walnut, *Juglans nigra*, but others were not associated with foods.

Identifiers Mycobank MB277708; lectotype IMI 40569 (Pitt 1979b); ex-type cultures CBS 28736, IMI 40569, ATCC 10494, NRRL 2074, FRR 2074; ITS barcode JN626132; alternative markers *BenA* JN626001, *RPB2* JN406585, *CaM* JN626044 (Visagie et al. 2014).

Physiology Judged from its occurrence on a wide range of dried foods, *Penicillium sclerotiorum* is probably a xerophile. No physiological studies have been reported, however.

Mycotoxins Mycotoxin production is not known.

Ecology With the exception of frozen fruit pastries (Kuehn and Gunderson 1963), this species has been isolated from dried foods: wheat and flour; rice, maize, soybeans and jam (see Pitt and Hocking 1997). It is not a common species in foods, but we isolated it from peanuts, mung beans and soybeans in Southeast Asia (Pitt et al. 1998). In the early literature *Penicillium sclerotiorum* is often known as *P. multicolor*, a name not accepted by Pitt (1979b) and now regarded as a synonym of *P. fellutanum* (Rivera and Seifert 2011).

References Pitt (1979b, 2000) and Rivera and Seifert (2011).

Penicillium thomii Maire **Fig. 7.13**

Colonies on CYA 40–60 mm diam, radially sulcate, often lightly floccose, with white mycelium usually surrounding pale to pinkish brown sclerotia, in central areas overlaid by penicilli borne on long stipes; conidial production moderate, Dull Green (27D-E3); exudate abundant, clear; reverse buff to yellow, or centrally brown in sclerotigenic isolates. Colonies on MEA 40–55 mm diam, plane or centrally wrinkled, usually floccose; sclerotia sometimes present, borne in a layer near the colony centre, coloured Apricot (5B6) or paler; other characteristics similar to those on CYA, except reverse orange brown. Colonies on G25N usually 20–24 mm diam, radially sulcate or wrinkled, low and dense; mycelium white; conidia dark green; reverse buff, olive or brown. Germination always occurring at 5 °C, usually colonies of 2–4 mm diam formed. No growth at 37 °C.

Sclerotia produced by most isolates, ellipsoidal to irregular in shape, usually 250–350 µm long, rapidly becoming hard, pale at first, then pinkish brown, becoming Apricot (5B6) on MEA. Conidiophores borne from surface or aerial hyphae, stipes 200–400 µm long, rough walled, strictly monoverticillate, vesiculate on CYA, less so on MEA; phialides large and crowded, 9–12 µm long, with long narrow collula; conidia ellipsoidal, commonly 3.5–4.0 µm long, finely to coarsely roughened, borne in long, rather irregular columns.

Distinctive features Most isolates of *Penicillium thomii* produce large sclerotia, coloured a distinctive apricot or salmon shade. Colonies grow very rapidly at 25 °C; stipes are long, vesiculate and rough walled; conidia are ellipsoidal and also rough walled.

Identifiers Mycobank MB202819; neotype IMI 189694 (Pitt 1979b); ex-type cultures CBS 225.81, IMI 189694, NRRL 2077, FRR 2077; ITS barcode KM189560; alternative markers *BenA* KM088799, *RPB2* KM089571, *CaM* KM089184 (Visagie et al. 2014).

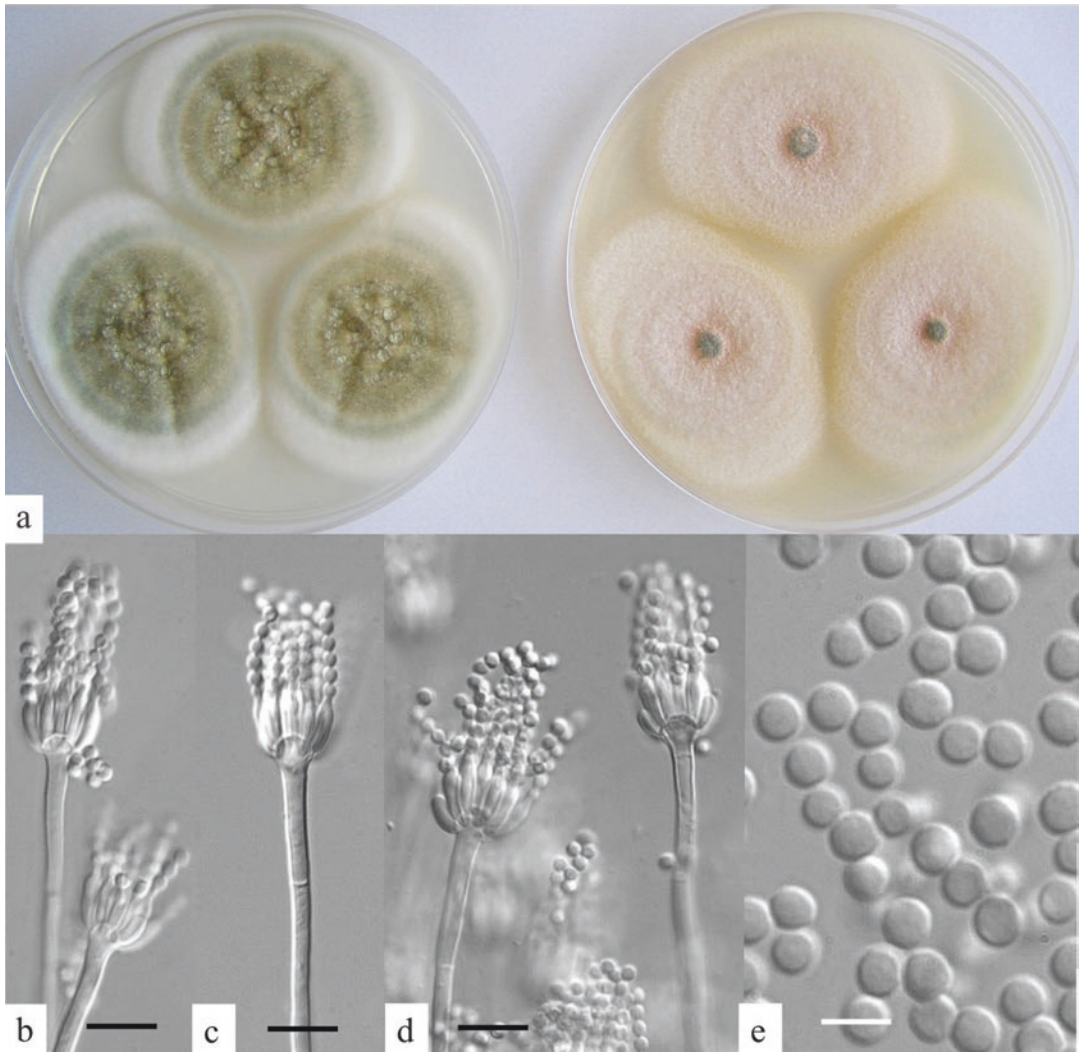

Fig. 7.13 *Penicillium thomii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Physiology Judged from quite common occurrence in dried foods, rapid growth on G25N, and affinity with *Penicillium glabrum*, *P. thomii* is probably a xerophile. It grows rapidly at 5 °C. Some isolates of *P. thomii* recovered from rotten grapes in French vineyards were able to synthesise 2-octen-1-ol and 2-methylisoborneol, compounds responsible for mushroom and earthy smells, respectively, on malt and grape juice agar (La Guerche et al. 2006).

Mycotoxins Mycotoxin production has not been reported for this species.

Ecology Pitt (1979b) reported that *Penicillium thomii* is a common fungus, widespread in decaying materials as well as soil. In foods, most isolations have been reported from cereals, especially wheat and barley (see Pitt and Hocking 1997). It occurred in almost 10% of Indonesian dried fish samples examined by Wheeler et al. (1986), and has also been isolated from meat products, peanuts, pistachios, miso (see Pitt and Hocking 1997) and grapes (Bau et al. 2005). Under the names *P. parallelosporum* and *P. yezoense*, Sasaki reported this species as the cause of spoilage of butter (Pitt 1979b: 186).

P. yezoense was recognised as a distinct species by Houbraken et al. (2014), but as all isolates they examined (apart from the extype strain) came from soil, its relevance to food spoilage appears marginal. We isolated *P. thomii* from spoiled Australian faba beans and at low levels from peanuts in Thailand and Indonesia (Pitt et al. 1993, 1998).

References Pitt (1979b, 2000).

7.4 *Penicillium* subgenus *Furcatum* Pitt

Penicillium subgenus *Furcatum* includes species which produce regularly or irregularly biverticillate penicilli, usually with 2–5 terminal metulae. Species in *Talaromyces* (= *Penicillium* subgenus *Biverticillium*) also produce biverticillate penicilli, but these two taxa are distinguishable by several features (Table 7.1). Species in subgenus *Furcatum* have phialides that are ampulliform, or at least have wide apical pores, and are distinctly shorter than their supporting metulae. Colonies on G25N always exceed 9 mm diam in 7 days at 25 °C. A few species in this subgenus produce metulae in verticils of 5–9 (like species in *Talaromyces*), but are readily recognised as members of subgenus *Furcatum* by their relatively rapid growth on G25N and by a ratio of metula to phialide length greater than one.

Two quite different types of penicillus occur in subgenus *Furcatum*. Some species produce penicilli in which metulae are almost exclusively borne terminally, i.e. the penicilli characteristically consist of verticils of metulae (Fig 7.2d, e). In contrast, a second group of species produces irregularly biverticillate penicilli, with metulae borne terminally, subterminally and lower down on the stipe (Fig. 7.2c). Most isolates classifiable in subgenus *Furcatum* can be readily assigned to one or other of these two groups, the recognition of which will greatly aid identification. In the key which follows, species have first been separated on penicillus morphology, and then on growth rates.

Most species in subgenus *Furcatum* are soil fungi. Some are included here because they are frequently isolated from foods enumerated by dilution plating. However, their occurrence in foods is usually only as ubiquitous contaminants. There are some important exceptions: of the species described here, *Penicillium citrinum*, *P. corylophilum*, *P. fellutanum* and *P. oxalicum* are important in spoilage of one kind of food or another.

Key to *Penicillium* subgenus *Furcatum* species included here

1	Penicilli predominantly terminal verticils of metulae	2
	Penicilli mostly irregular	7
2 (1)	Colonies on CYA at 25 °C exceeding 35 mm diam	3
	Colonies on CYA at 25 °C not exceeding 35 mm diam	5
3 (2)	Stipe walls usually rough	4
	Stipe walls smooth	*P. oxalicum*
4 (3)	No growth at 37 °C, conidia 3 µm or less in diameter, smooth walled	*P. raistrickii*
		P. novae-zeelandiae
	Usually growth at 37 °C, conidia up to 4 µm in diameter, rough walled	*P. simplicissimum*
5 (2)	Penicilli with verticils of 5–8 metulae	*P. paxilli*
	Penicilli with no more than 5 metulae	6
6 (5)	Colonies on MEA more than 25 mm diam; metulae often of unequal length	*P. corylophilum*
	Colonies on MEA less than 25 mm diam; metulae of equal length	*P. citrinum*
7 (1)	Growth at 37 °C	*P. janthinellum*
	No growth at 37 °C	8
8 (7)	Stipes and conidia smooth walled	*P. fellutanum*
		P. waksmanii
	Stipes or conidia rough walled	*P. janczewskii*
		P. canescens

Penicillium citrinum Thom Fig. 7.14

Colonies on CYA 25–30 mm diam, radially sulcate, marginal areas velutinous, sometimes floccose centrally; mycelium white in peripheral areas, at the centres white to Greyish Orange (6B5-6); conidial production moderate, Greyish Turquoise (24C2-3); exudate clear, pale yellow or pale brown to reddish brown, only rarely absent; soluble pigment bright yellow or absent; reverse yellow, yellow brown, reddish brown or olive. Colonies on MEA 14–18 mm diam, rarely 22 mm, plane or radially sulcate, mycelium white to Greyish Orange (6B5-6); conidial formation moderate to heavy, grey blue at the margins, elsewhere Dull Green (26-27E3); reverse pale brown to deep yellow brown. Colonies on G25N 13–18 mm diam, radially sulcate, velutinous or sometimes floccose centrally; mycelium white; conidia often abundant, dull green; reverse pale, dull brown, yellow brown or olive. No germination at 5 °C. At 37 °C colonies usually 8–10 mm diam, of wrinkled white mycelium only.

Conidiophores borne from subsurface or surface hyphae, stipes 100–300 µm long, smooth walled, characteristically terminating in well

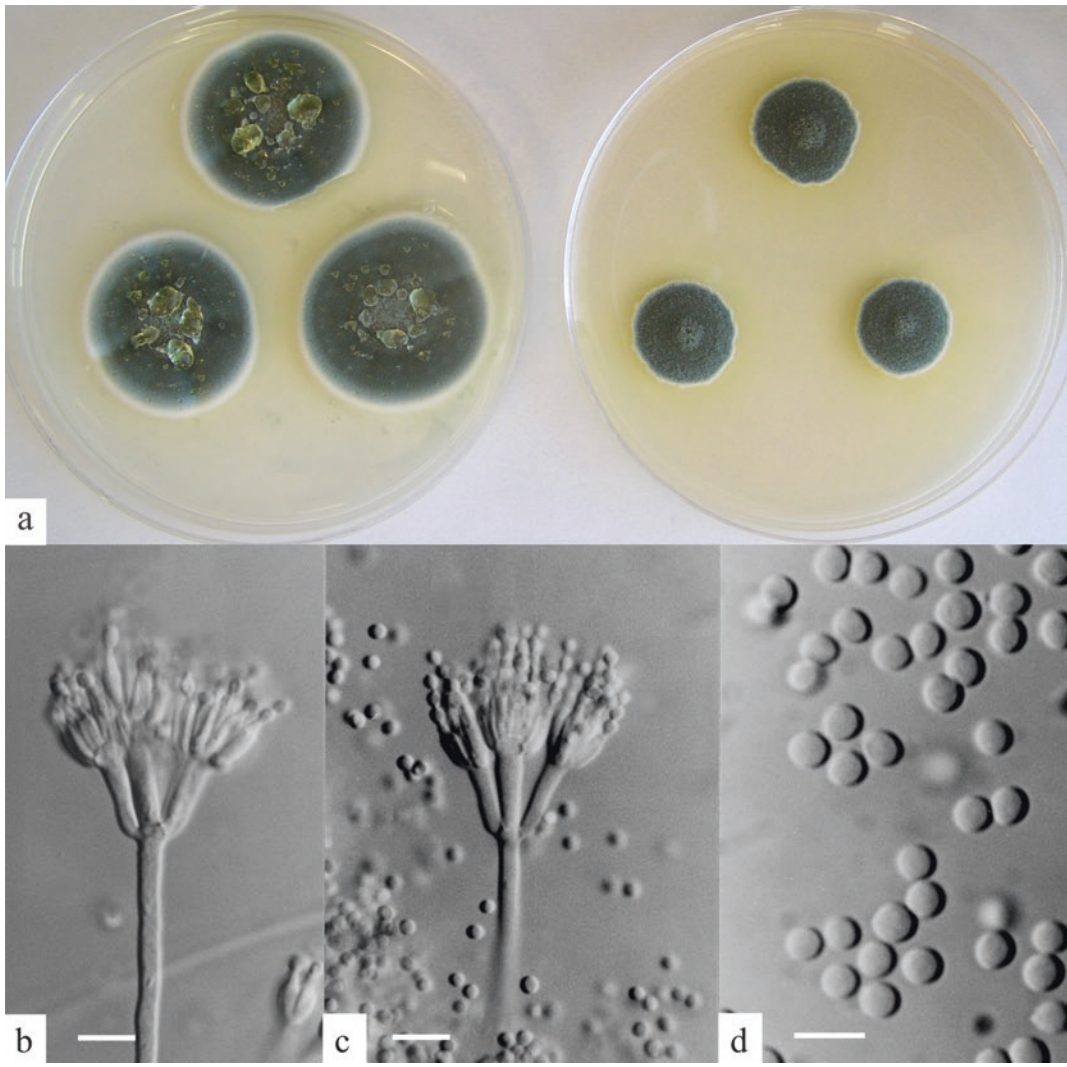

Fig. 7.14 *Penicillium citrinum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

defined verticils of 3–5 divergent metulae, less commonly with a divergent ramus, or metulae produced subterminally or along the stipe; metulae usually of uniform length, commonly apically enlarged or vesiculate, phialides ampulliform, 7–8(−12) µm long; conidia spherical to subspheroidal, 2.2–3.0 µm diam, with walls smooth or very finely roughened, typically borne in long well defined columns, one per metula, arranged in a characteristic whorl on each conidiophore.

Distinctive features *Penicillium citrinum* is readily recognised by its penicilli, which consist of 3–5 divergent and usually vesiculate metulae, bearing long well defined columns of conidia. Colonies on CYA at 25 °C are sometimes dominated by copious clear to yellow or brown exudate at the centres. On MEA growth is slower and usually dense, with heavy conidial production.

Taxonomy Houbraken et al. (2011a) have elevated "series *Citrina"* in Pitt (1979b) to "section *Citrina*" and included 37 species in it, almost half being newly described. Judged from the sources of the isolates examined, almost all are predominantly soil inhabitants and not found in foods.

Identifiers Mycobank MB165293; lectotype IMI 92196ii (Pitt 1979b); ex-type cultures CBS 139.45, IMI 92196, ATCC 36382, NRRL 1841, FRR 1841; ITS barcode AF033422; alternative markers *BenA* GU944545, *RPB2* JF417416, *CaM* GU944638 (Visagie et al. 2014). Whole genome sequencing of *P. citrinum* has been accomplished recently (Schmidt-Heydt et al. 2019).

Physiology This is a mesophilic species, with the minimum temperature for growth 5 °C or slightly above, the maximum just above 37 °C (Pitt 1973), and the optimum 26–30 °C (Domsch et al. 1980). At 25 °C, the minimum a_w for growth has been reported as 0.80–0.84 (Galloway 1935; Pitt and Christian 1968; Hocking and Pitt 1979). *Penicillium citrinum* grows over the pH range 2–10 (Wheeler et al. 1991).

Mycotoxins *Penicillium citrinum* is the main producer of citrinin, a mycotoxin of moderate toxicity. Citrinin is a significant renal toxin to monogastric domestic animals, but the effect of citrinin on humans, if any, remains undocumented (see Chap. 13).

P. citrinum produced citrinin in dry cured ham after 4 days incubation at 20 °C, compared with only 2 days on YES medium (Bailly et al. 2005). After 16 days, significantly higher amounts of citrinin were produced on ham (1330 mg/kg) than on YES (86.9 mg/kg). However, pure citrinin extract added to the surface of cured ham was quickly lost or degraded, with more than 50% lost after only 6 h of incubation at 20 °C, and less than 15% remaining after 192 h (Bailly et al. 2005).

Ecology *Penicillium citrinum* has been isolated from nearly every kind of food which has been surveyed for fungi. The most common sources are cereals, e.g. rice (Park et al. 2005; Aziz et al. 2006), wheat and barley (Aziz et al. 2006), maize (Mazzani et al. 2004; Aziz et al. 2006), and milled grains and flour (Ogundare and Adetuyi 2003; Hocking, unpublished; and also see Pitt and Hocking 1997). Among other reported sources are nuts, blackgram in India, amaranth seeds in Argentina, cinnamon and soy sauce (see Pitt and Hocking 1997). *Penicillium citrinum* has also been reported in fermented and cured meats (Tabuc et al. 2004), hams (Bailly et al. 2005), cocoa pulp (Ardhana and Fleet 2003), young coconuts (Waje et al. 2005), soybeans (Aziz et al. 2006), wine grapes (Bau et al. 2005) and dried vine fruits (Romero et al. 2005), curd and cheese (Kumaresan et al. 2003), bottled mineral water (Cabral and Fernandez Pinto 2002) as well as coffee beans, dried beans and peppercorns (see Pitt and Hocking 1997).

In Southeast Asia, *Penicillium citrinum* was isolated from maize, peanuts, copra, soybeans, sorghum and cashews (Pitt et al. 1993, 1994). *P. citrinum* was very widespread in Indonesian food commodities: we isolated it from dried fish, sorghum, peanuts and kemiri nuts, pepper, coriander, maize and mung beans (Pitt et al. 1998). Citrinin levels were never significant, however (see Pitt and Hocking 1997).

Instances of food spoilage caused by *Penicillium citrinum* are rare, but this species is much more than a mere contaminant. Because of

its mesophilic nature, distribution is world wide and, in addition, its ability to grow down to 0.80 a_w helps to secure this species a niche in a very wide range of habitats.

References Pitt (1979b, 2000) and Houbraken et al. (2011a).

Penicillium corylophilum Dierckx

Fig. 7.15

Colonies on CYA 25–35 mm diam, plane to deeply radially sulcate, low, moderately dense, strictly velutinous; mycelium white or rarely buff; conidial production light to moderate, Dull Green (25C-E3-4); clear exudate sometimes present; reverse pale, brownish or sometimes centrally dark grey. Colonies on MEA 30–45 mm diam, plane, low and relatively sparse, strictly velutinous; mycelium white or buff; conidia in

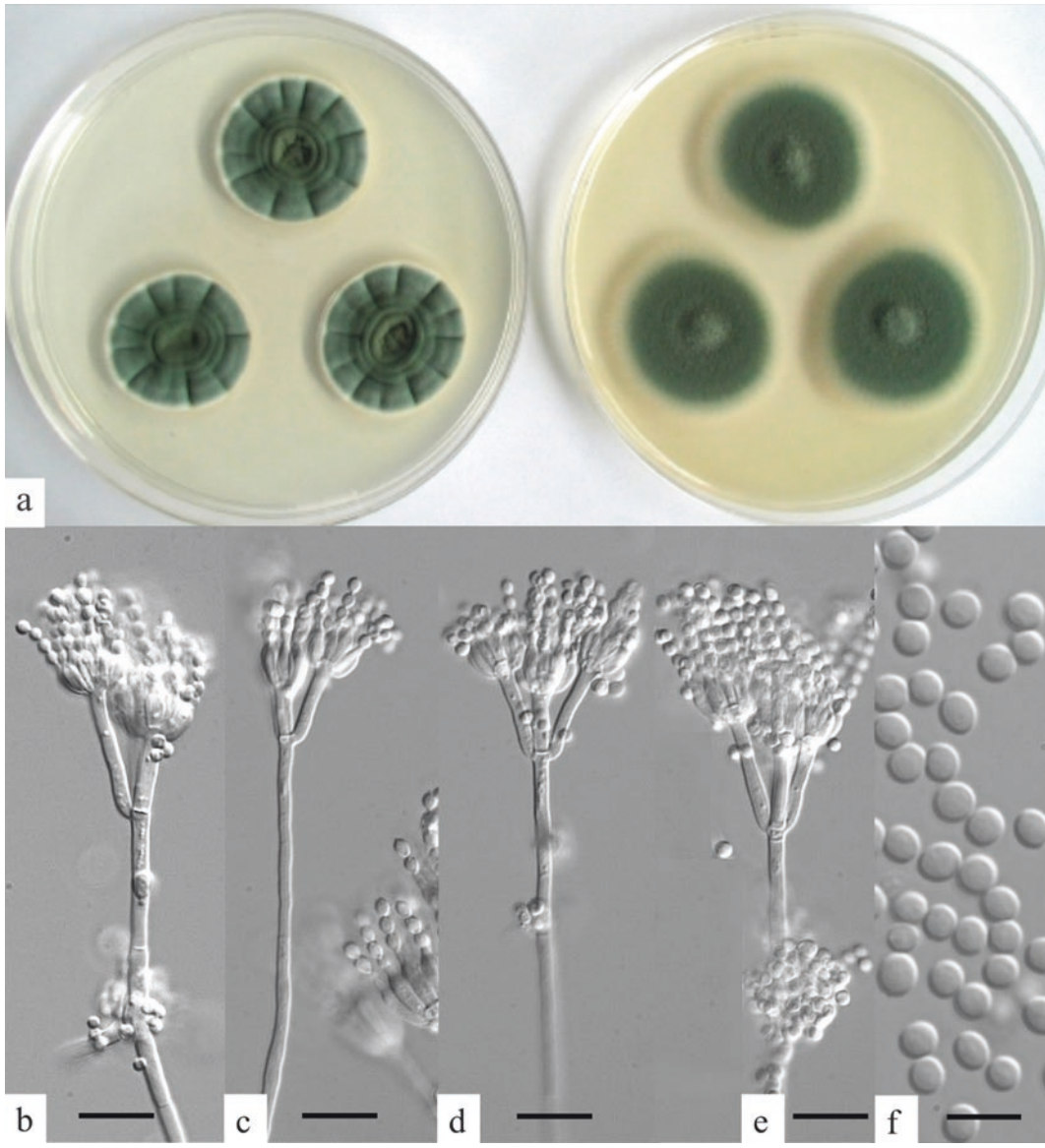

Fig. 7.15 *Penicillium corylophilum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**e**) penicilli, bars = 10 µm; (**f**) conidia, bar = 5 µm

moderate numbers, Dull Green (26-27D-E3); clear exudate occasionally present; reverse pale at the margins, but usually dull green to very dark green centrally. Colonies on G25N 10–16 mm diam, plane or centrally wrinkled, dense; mycelium white; colours similar to those on CYA; reverse pale. At 5 °C, usually germination of conidia to formation of microcolonies; occasionally small macroscopic colonies produced. No growth at 37 °C.

Conidiophores borne from subsurface hyphae, stipes 100–250 µm long, smooth walled; on CYA, penicilli usually verticils of 2–5 metulae, but sometimes with subterminal metulae or occasionally a ramus; on MEA usually less complex and frequently monoverticillate; in penicilli with two metulae the offset one often longer than the axial; phialides ampulliform, 7–11 µm long; conidia spherical to subspheroidal, commonly 2.5–3.0 µm diam, smooth walled, borne on CYA in disordered chains, on MEA sometimes in long columns.

Distinctive features Relatively long divergent metulae often of unequal length are characteristic of *Penicillium corylophilum*. This species produces strictly velutinous colonies; on MEA growth is rapid and usually dark green in reverse.

Identifiers Mycobank MB178294; holotype Herb K, Wright 666, Cuba (Pitt and Samson 1993); ex-type cultures CBS 312.48, IMI 39754, NRRL 802, FRR 802; ITS barcode AF033450; alternative marker *BenA* JX191042 (Visagie et al. 2014).

Physiology This is a xerophilic fungus: Hocking and Pitt (1979) reported germination at 0.80 a_w after 38 days at 25 °C. Growth was observed at 0.85 a_w (Guynot et al. 2005). The addition of sorbic acid to fermented bakery products completely inhibited the growth of this fungus regardless of water activity (Guynot et al. 2005). However, use of lower sorbic acid concentrations (<0.3%) enhanced growth of this species (Marin et al. 2002).

Mycotoxins Mycotoxins are not known to be produced. However, *P. corylophilum* is of common occurrence in damp buildings and produces secondary metabolites that may be important in human health (Bok et al. 2009; McMullin et al. 2014).

Ecology *Penicillium corylophilum* has been reported to cause spoilage of high fat foods, including rapeseed and rapeseed oil (Magan et al. 1993) and margarine (Hocking 1994). In our experience, *P. corylophilum* occasionally causes spoilage of low a_w foods such as jams. It has been reported quite frequently from cereals: barley, paddy rice, wheat and flour (see Pitt and Hocking 1997). It has also been isolated from salami (Cantoni et al. 2007), laban rayeb, a fermented dairy product (Ahmed and Abdel-Sater 2003), peanuts, pecans, hazelnuts, soybeans and frozen fruit pastries (see Pitt and Hocking 1997). In our laboratory we have isolated *P. coryophilum* from a range of substrates including bottled water, fruit juices, thickened cream, cosmetics and petroleum products. *P. corylophilum* occurred at low levels in mung beans and soybeans in Thailand (Pitt et al. 1994), maize in Indonesia (Pitt et al. 1998). and in peanuts in the Philippines (our unpublished data)

References Pitt (1979b, 2000) and Domsch et al. (1980).

Penicillium fellutanum Biourge — Fig. 7.16

Colonies on CYA 17–24 mm diam, very dense, radially sulcate, velutinous; mycelium white, usually visible only at the margins; formation of conidia light to heavy, coloured pale grey if sparse, but more commonly Dark Green (27-28F4); colourless exudate sometimes present; reverse pale. Colonies on MEA 14–18 mm diam, low and dense, radially sulcate, usually velutinous with a floccose central area, less commonly entirely floccose; conidia moderately abundant; wrinkled; colours similar to colonies on CYA. Colonies on G25N usually 12–16 mm diam, growth low and dense; reverse olive or yellow. No germination at 5 °C and no growth at 37 °C.

Conidiophores borne from aerial hyphae, characteristically of indeterminate form, sometimes terminating in well defined penicilli with 2–4 metulae, sometimes bearing metulae in a random manner, with or without solitary phialides as well, less commonly giving the impression of monoverticillate penicilli borne perpendicular to fertile hyphae, but always with at least two terminal metulae; stipes smooth walled, of irregular and often indetermi-

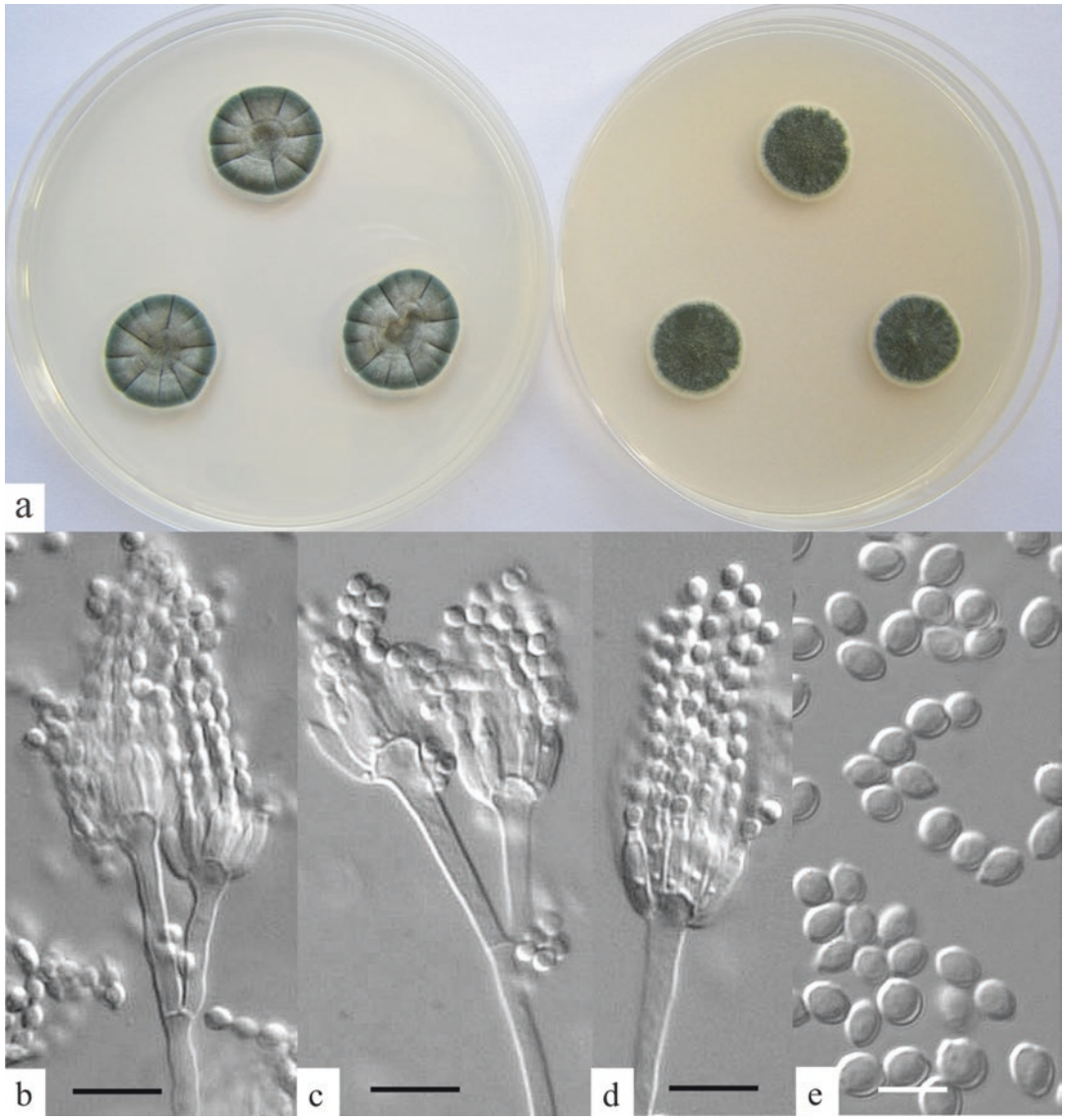

Fig. 7.16 *Penicillium fellutanum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

nate length; metulae usually long, terminating in well defined, thin walled vesicles; phialides ampulliform, 5–10 µm long; conidia ellipsoidal, 2.5–3.2 µm long, with surfaces finely to distinctly roughened, borne in long, irregular columns.

Distinctive features A well defined species, *Penicillium fellutanum* is distinguished microscopically by conidiophores with irregularly located metulae of variable length, terminating in definite vesicles, and macroscopically by closely textured colonies, dark green conidia and failure to grow at either 5 or 37 °C. Moreover growth on MEA is slower than on CYA, and little faster than on G25N.

Taxonomy Frisvad et al. (1990c) considered *Penicillium fellutanum* to be a synonym of *P. citrinum*, but that has not been accepted more recently (Pitt and Hocking 2009; Visagie et al. 2014).

Identifiers Mycobank MB264748; neotype IMI 39734 (Pitt 1979b); ex-type cultures CBS 229.48, IMI 39734, ATCC 10443, NRRL 746, FRR 746; ITS barcode AF033399; alternative markers *BenA* KJ834450, *RPB2* JN141460, *CaM* AY741753 (Visagie et al. 2014).

Physiology *Penicillium fellutanum* is a slowly growing xerophile. Snow (1949) reported a minimum a_w for germination of 0.80 at 25 °C and, in good agreement, Hocking and Pitt (1979) reported germination at 0.78 a_w after 89 days. As noted above, *P. fellutanum* has a relatively narrow temperature range for growth, within the limits of 5° and 37 °C.

Mycotoxins Mycotoxins are not known to be produced.

Ecology *Penicillium fellutanum* has been isolated from a wide variety of dried foods, which appear to be a major habitat, including barley, wheat and flour, nuts, beans and sultanas. It has been isolated also from frozen fruit pastries and miso (see Pitt and Hocking 1997). We isolated *P. fellutanum* at low levels from Thai cashews, and Indonesian maize, peanuts and milled rice (Pitt et al. 1993, 1998). It was the dominant species in stored Korean rice (Oh et al. 2011).

Additional species *Penicillium waksmanii* K.M. Zalessky has much in common with *P. fellutanum*, including the variable and irregular penicillus structure and closely textured colonies. However, Houbraken et al. (2011a) state that fresh isolates from Polish soil often produce terminal penicilli. The principal distinctions from *P. fellutanum* are that *P. waksmanii* often grows much faster on MEA (20–35 mm), germinates at 5 °C, and produces Greenish Grey (25C2-26E3) spherical conidia. Mycotoxins are not produced. Habitats in foods appear to be similar to those of *P. fellutanum*, but *P. waksmanii* has been reported less frequently in the literature. Reports include ham during ripening (Spotti et al. 1989), peanuts (Pitt et al. 1993) and pistachios (Heperkan et al. 1994). Identifiers: Mycobank MB121677; lectotype IMI 39746i (Pitt 1979b); ex-type cultures CBS 230.28, IMI 39746, ATCC 10516, NRRL 777, FRR 777; ITS barcode GU944602; alternative markers *BenA* JN606779, *RPB2* JN606627, *CaM* JN660431 (Visagie et al. 2014).

References Pitt (1979b, 2000) and Houbraken et al. (2011a).

Penicillium janczewskii K.M. Zalessky **Fig. 7.17**

Penicillium nigricans Bainier

Colonies on CYA 25–32 mm diam, radially sulcate, deep, dense to moderately floccose; mycelium usually white, in some isolates pale yellow; conidial formation variable, in lightly sporing isolates coloured Greenish Grey (25B2-3), but in those sporing more heavily much darker, Dull Green (28E3); clear exudate and brown soluble pigment sometimes produced; reverse coloured brown, dark brown, orange or deep reddish orange. Colonies on MEA 18–24 mm diam, less commonly 30 mm, plane or radially sulcate, moderately deep to deep, dense to floccose; mycelium white to pale yellow, occasionally pale orange or pinkish; conidial production moderate to heavy, marginal areas sometimes bluish but elsewhere Greenish Grey (25-26D-E2); orange or yellow soluble pigment sometimes produced; reverse typically Salmon (6A4-5) or, in the presence of soluble pigment, Dark Orange (5A8). Colonies on G25N 15–20 mm diam, occasionally only 12 mm, typically closely radially sulcate, velutinous to floccose; mycelium white to pale yellow; reverse usually pale yellow to dull brown. At 5 °C, typically germination by a proportion of conidia, less commonly general germination or microcolony formation. At 37 °C, typically no growth, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from aerial hyphae, stipes with thin smooth walls, commonly 50–200 µm long, but in some isolates much longer, and in the limit becoming indistinguishable from fertile hyphae bearing short conidiophores, characteristically bearing a terminal tetrad of divergent metulae, but frequently less regular in pattern, with intercalary rami and metulae commonly present, the latter appearing as short monoverticillate conidiophores; metulae 8–15 µm long, sometimes apically swollen; phialides ampulliform, 6–8 µm long, with short collula; conidia spherical, 2.5–3.5 µm diam, spinose, appearing olive brown, borne in short, poorly defined columns.

Distinctive features Despite considerable isolate to isolate variation, *Penicillium janczewskii* usually can be recognised by spherical spinose conidia and by metulae which characteristically

Fig. 7.17 *Penicillium janczewskii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

occur in tetrads and are often apically swollen. However in some isolates the most characteristic feature of the conidiophores may appear to be a total lack of order in their structure. Colonies are floccose to a greater or lesser extent; conidial production is light to moderate; when moderate, conidial colour is dark green.

Taxonomy Raper and Thom (1949: 325) used the name *Penicillium nigricans* for this taxon. However *P. janczewskii* is the earlier, valid name (Pitt 1979b; Pitt and Samson 1993), and it is now in general use.

Identifiers Mycobank MB120703; lectotype IMI 191499 (Pitt 1979b); ex-type cultures CBS 221.28, IMI 191499, NRRL 919, FRR 919; ITS barcode AY157486; alternative markers *BenA* KJ834460, *RPB2* JN406612, *CaM* KJ867001 (Visagie et al. 2014).

Physiology With an optimum temperature for growth near 25 °C, *Penicillium janczewskii* grows weakly at 5 °C and not above 33 °C (Domsch et al. 1980). This species is among the most xerophilic Penicillia, germinating down to 0.78 a_w at 25 °C (Hocking and Pitt 1979).

Mycotoxins *Penicillium janczewskii* has been reported to produce griseofulvin (El-Banna et al. 1987; Nicoletti et al. 2007) and penitrem A (Di Menna et al. 1986; Pitt and Leistner 1991; Frisvad et al. 2006). However as this species is rarely if ever involved in food spoilage, significant mycotoxin production in the human food supply is unlikely.

Ecology All the evidence indicates that *Penicillium janczewskii* is a soil fungus, present in foods only as a contaminant, as we have no record of it having caused food spoilage. It has been reported from barley, wheat, flour, peanuts, pecans, pistachios, soybeans, dried beans and meats (see Pitt and Hocking 1997).

Additional species *Penicillium canescens* Sopp is closely related to *P. janczewskii*, and in fact these species interface (Pitt 1979b: 253). Both species grow at similar rates under the standard conditions and produce similar pigmentation, although *P. canescens* is often more strongly coloured. Microscopically, typical isolates are easily distinguished: *P. canescens* produces rough walled stipes and smooth conidia, while *P. janczewskii* has smooth stipes and rough walled conidia. Like *P. janczewskii*, *P. canescens* has been reported to produce griseofulvin (Nicoletti et al. 2007). This is a soil fungus, present in foods as a contaminant. *P. canescens* has been isolated from similar substrates to *P. janczewskii*, though less frequently, and also from rice, field peas (our data) and from cheese (Leistner and Pitt 1977). Identifiers: Mycobank MB153765; neotype IMI 28260 (Pitt 1979b); ex-type cultures CBS 300.48, IMI 28260, ATCC 10419, NRRL 910, FRR 910; ITS barcode AF033493; alternative markers *BenA* JX140946, *RPB2* JN121485, *CaM* KJ867009 (Visagie et al. 2014).

References Pitt (1979b, 2000) and Domsch et al. (1980).

Penicillium janthinellum Biourge

Fig. 7.18

Colonies on CYA 35–50 mm diam, radially sulcate or irregularly wrinkled, floccose, mycelium dense, coloured white, greyish, buff, pale yellow or pale pink, and overlaid by conidiophores, varying from inconspicuous to abundant; conidial production very light to moderate, in the latter case coloured Greyish Green to Dull Green (25-27C-D3-4), sometimes appearing more yellow or olive because of the coloured mycelium; limited amounts of clear to brown exudate and reddish brown soluble pigment sometimes produced; reverse colours variable, pale yellow or yellow brown to reddish brown, or occasionally brilliant dark green. Colonies on MEA usually 35–45 mm diam, floccose, lacking the density of colonies on CYA; mycelium white or buff; conidial colours similar to those on CYA; yellow or brown soluble pigment occasionally produced; reverse pale, brownish, deep brown, dark green or quite commonly pink, centrally or in sectors, occasionally even bright red. Colonies on G25N 10–18 mm diam, typically plane, low or umbonate, velutinous to floccose; mycelium white or yellow; conidia grey green; reverse pale, yellow, brown or pinkish. Sometimes germination at 5 °C. At 37 °C, colonies 10–30 mm diam, dense and velutinous; clear exudate and brown or reddish soluble pigment sometimes produced; reverse pale, yellow, brown or reddish brown.

Conidiophores borne from surface or aerial hyphae, stipes smooth and slender, thin walled and easily bent, typically 200–400 µm long, but in some isolates also short, 30–70 µm, usually terminating in an irregular to regular verticil of 2–3 metulae, characteristically also with subterminal and intercalary metulae, the latter intergrading with short monoverticillate conidiophores, in some isolates long monoverticillate conidiophores present as well; phialides ampulliform, 7–11 µm long, typically with long slender collula; conidia most often spherical, but sometimes short pyriform to ellipsoidal,

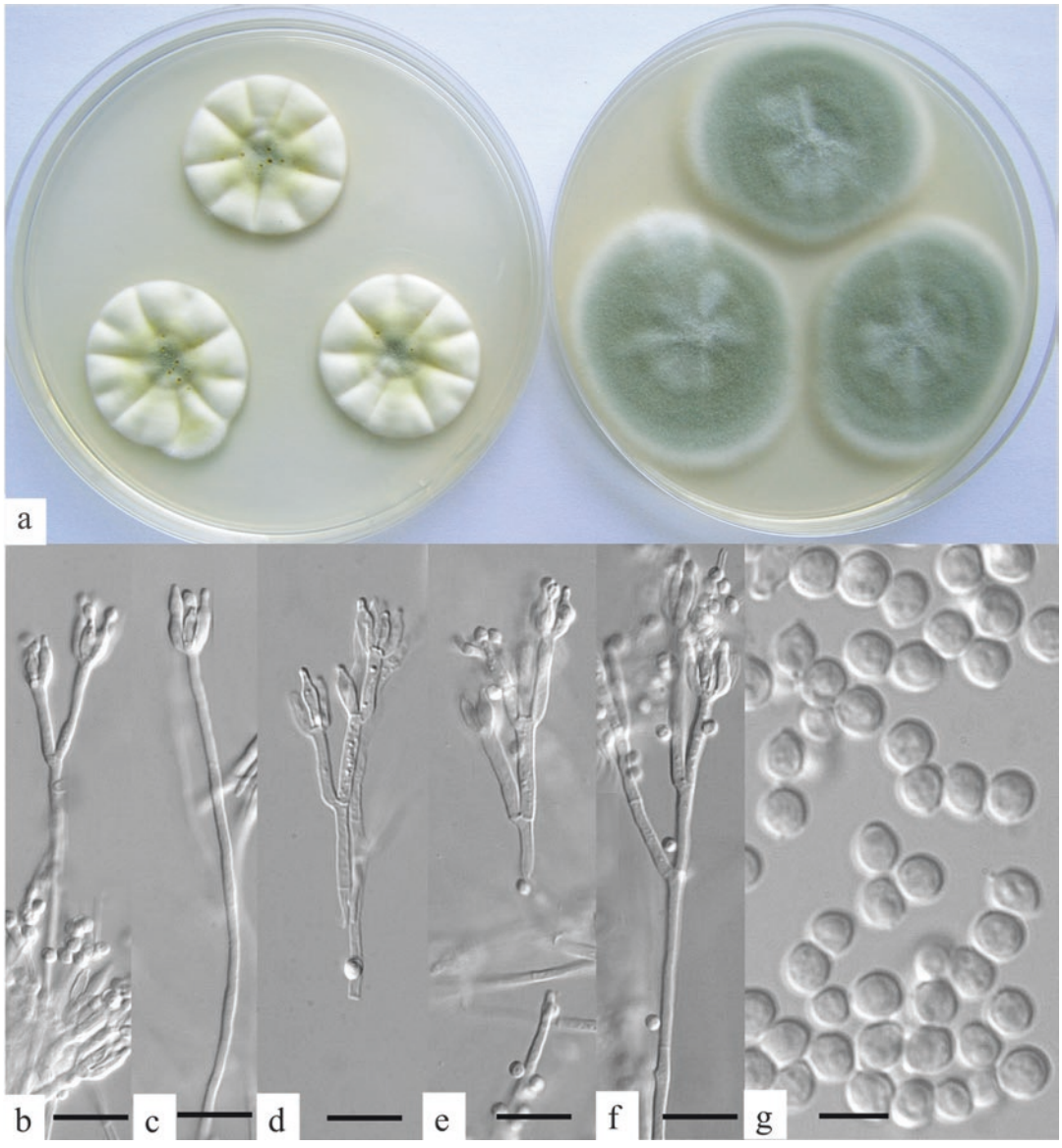

Fig. 7.18 *Penicillium janthinellum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–f**) penicilli, bars = 10 µm; (**g**) conidia, bar = 5 µm

2.2–3.0 µm diam or long, with smooth to finely roughened walls, borne in short to moderately long disordered chains.

Distinctive features *Penicillium janthinellum* is surely the most difficult of all Penicillia to define. Colonies on CYA and MEA at 25 °C grow rapidly; growth typically occurs at 37 °C; colonies are usually floccose and conidia rather sparsely produced. Penicilli, although usually biverticillate, are so irregular as to often appear monoverticillate. Stipes are delicate and smooth walled. Phialides characteristically have long slender necks and bear smooth to finely roughened conidia.

Identifiers Mycobank MB119134; neotype IMI 40238 (Pitt 1979b); ex-type cultures CBS 340.48, IMI 40238, ATCC 10455, NRRL 2016; ITS barcode GU981585; alternative markers *BenA* GU981625, *RPB2* JN121497, *CaM* KF296401 (Visagie et al. 2014).

Physiology Temperature requirements for this species are approximately known: minimum temperature for growth, above 5 °C, optimum 25–30 °C, good growth at 37 °C (Pitt 1973). No water relations studies have been reported, but this species is probably not a xerophile.

Mycotoxins *Penicillium janthinellum* produces a series of tremorgenic toxins known as janthitrems (Gallagher et al. 1980). Closely related in chemical structure to the penitrems (De Jesus et al. 1984), janthitrems might be expected to show similar toxicities, however, toxicological data are not available. As *P. janthinellum* is uncommon in foods, these toxins are unlikely to be important in human health.

Ecology As an ubiquitous soil fungus, the presence of *Penicillium janthinellum* on foods is adventitious. Isolations have been made occasionally from a wide variety of foods: dates and almonds, maize, dried beans, peanuts, pistachios, barley, fermented and cured meats, biltong and frozen fruit pastries (see Pitt and Hocking 1997). There appear to be no records of food spoilage by this species.

References Pitt (1979b, 2000) and Domsch et al. (1980).

Penicillium oxalicum Currie & Thom Fig. 7.19

Colonies on CYA 35–60 mm diam, plane or radially sulcate, velutinous or lightly floccose in central areas; mycelium usually inconspicuous, in floccose areas white or pale yellow, but the underlying surface growth coloured Salmon (6A4); conidial production typically very abundant, appearing as a continuous layer of long, closely packed chains under the low power microscope, and breaking off in masses if jarred, Greyish Green (25-26C3) at the margins, then Dull Green (27D3-28E4) or Olive (1E3-4) towards the centres; exudate limited, clear, or absent; reverse pale to yellow, brown, orange or pinkish. Colonies on MEA variable in size, 20–50 mm diam, plane or lightly radially sulcate, strictly velutinous; conidia very abundant, forming readily detached masses; colours similar to those on CYA except reverse sometimes greenish. Colonies on G25N 12–16 mm diam, plane or wrinkled, velutinous; mycelium white or salmon; reverse pale, greenish, olive or salmon. At 5 °C, germination by a proportion of conidia, or no germination. At 37 °C, colonies 10–40 mm diam, deeply radially sulcate and often centrally wrinkled, velutinous; mycelium white; reverse olive or brown.

Conidiophores borne from surface mycelium, stipes mostly 200–400 µm long, with thin, smooth walls, characteristically terminating in verticils of 2–4 closely appressed metulae; metulae 15–25(−30) µm long; phialides acerose, 10–15(−20) µm long, with short collula; conidia ellipsoidal, large, 3.5–5.0(−7) µm long, with walls smooth or rarely finely roughened, borne in long, closely packed columns.

Distinctive features Of all the cosmopolitan Penicillia, this species is perhaps the most obviously distinctive. Colonies usually grow rapidly on CYA at 25 and 37 °C, are strictly velutinous, and produce prodigious numbers of conidia. Under low magnifications, the conidia can be seen to lie in closely packed, readily fractured sheets, and to have a uniquely shiny, even silky, appearance. Under high magnification, the large penicilli and large smooth walled ellipsoidal conidia are also distinctive.

Identifiers Mycobank MB121033; lectotype IMI 192332 (Pitt 1979b); ex-type cultures CBS 219.30, IMI 192332, ATCC 1126, NRRL 787, FRR 787; ITS barcode AF033438; alternative markers *BenA* KF292462, *RPB2* JN121456, *CaM* KF296367 (Visagie et al. 2014).

Physiology Mislivec and Tuite (1970) reported 8 °C as the minimum growth temperature for *Penicillium oxalicum*, with an optimum near 30 °C. Judged from rapid growth at 37 °C, the maximum temperature for growth is in excess of 40 °C. The minimum a_w for germination has been reported as 0.86, both in glucose media at 23 and 30 °C (Mislivec and Tuite 1970) and in NaCl media at 25 °C (Hocking and Pitt 1979). *P. oxalicum* was one of several *Penicillium* species associated with production of volatiles in mouldy wheat (R.N. Sinha et al. 1988b).

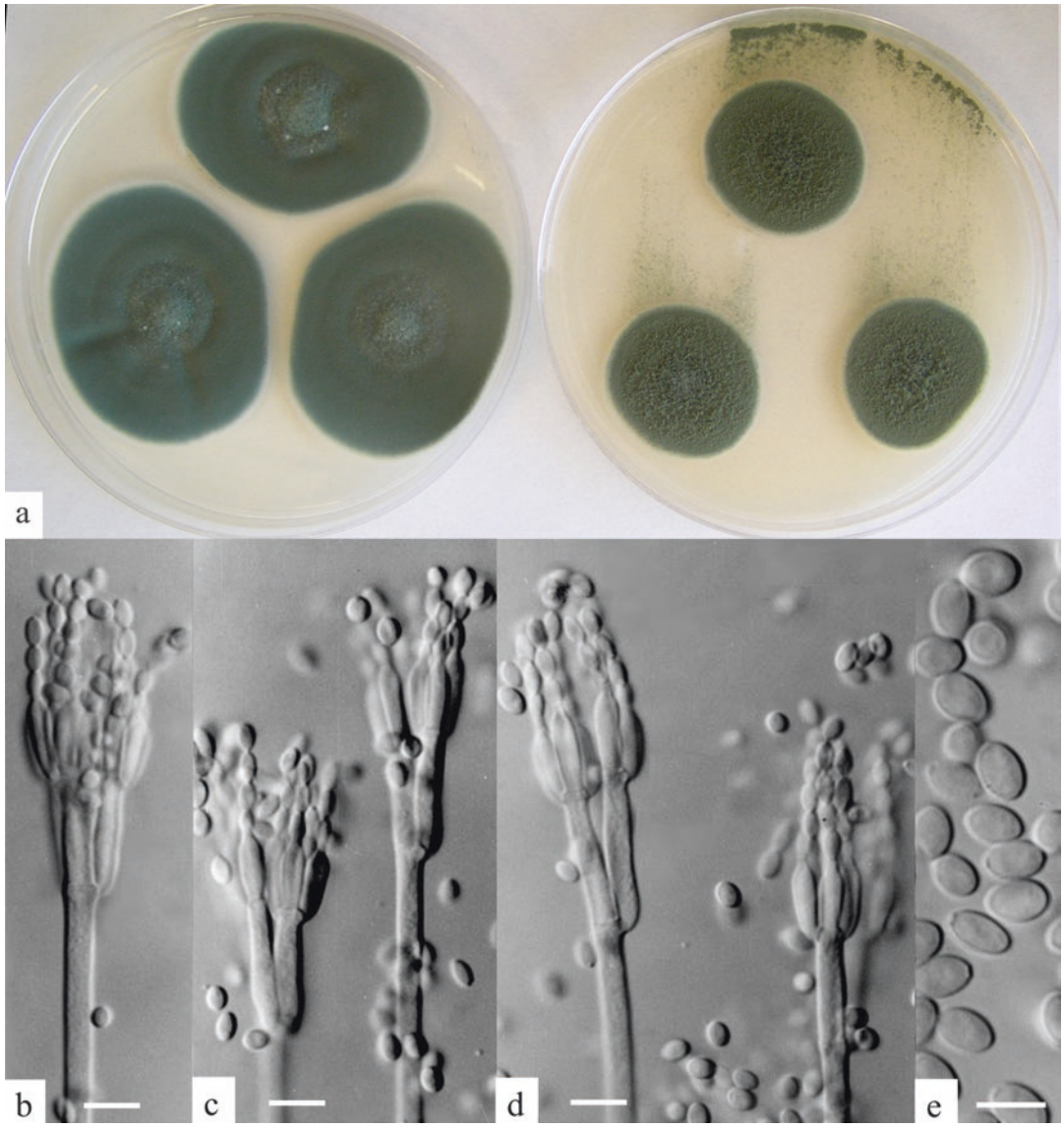

Fig. 7.19 *Penicillium oxalicum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Mycotoxins Secalonic acid D is produced as a major metabolite of *Penicillium oxalicum*. It has been found in nature, in freshly harvested maize (Palmgren and Fleischhacker 1987), grain dusts, at levels of up to 4.5 mg/kg (Ehrlich et al. 1982) and was also reported from grain dust collected in Belgian cereal storage facilities (Tangni and Pussemier 2007). The possibility that such levels can be toxic to grain handlers should not be ignored (see Chap. 13).

Ecology As a result of its rapid growth at 37 °C, *Penicillium oxalicum* is widespread in tropical commodities and foods. We isolated it from Indonesian maize, red rice, paddy rice, cowpeas and sorghum, infecting 1% of all grains examined in each case (Pitt et al. 1998) and at lower levels in peanuts, kemiri nuts, soybeans, mung beans, black pepper, coriander and milled rice in Thailand, Indonesia and/or the Philippines (Pitt et al. 1993, 1994, 1998).

A major niche for *Penicillium oxalicum* is preharvest maize (see Pitt and Hocking 1997). Koehler (1938) suggested that entry of this species to ripening maize ears was through insect damage or wounds. *P. oxalicum* has also been recorded in stored maize, though at lower incidence (Amusa et al. 2005; Askun 2006) This species is a pathogen on yams (Okigbo 2003) and greenhouse cucumbers (Menzies et al. 2005), is considered to be the dominant fungus on cassava (Adegoke et al. 1993) and is of common occurrence on copra (Zohri and Saber 1993) and cashew nuts (Freire and Kozakiewicz 2005). It has also been reported from a wide variety of other foods: barley, wheat, flour, pecans, hazelnuts, walnuts, peppercorns, soybeans, spices, fermented sausages and biltong (see Pitt and Hocking 1997).

The temperature and water relations of this species make it competitive with *Aspergillus flavus* except at very low a_w, and it occupies a similar range of habitats. It is not such a common cause of spoilage, however.

References Pitt (1979b, 2000) and Domsch et al. (1980).

Penicillium paxilli Bainier Fig. 7.20

Colonies on CYA 30–35 mm diam, occasionally only 25 mm, radially sulcate, velutinous to lightly floccose; mycelium white at the margins, sometimes centrally greyish orange; conidial production moderate, at the margins Greyish Turquoise (24D3-4), centrally Dull Green (25-26E3-4); clear to red brown exudate and red brown soluble pigment often produced; reverse pale to brownish orange. Colonies on MEA usually 25–30 mm diam, occasionally less, plane to deeply radially sulcate, velutinous or occasionally floccose centrally; mycelium white to buff; conidial production moderate to heavy, coloured as on CYA; exudate and soluble pigment produced occasionally, red brown; reverse pale to brownish orange. Colonies on G25N 16–20 mm diam, radially sulcate, deep, dense to floccose: mycelium white; exudate and soluble pigment occasionally produced, red brown; reverse pale to brownish orange or reddish brown. At 5 °C usually no germination; less commonly germination or formation of microcolonies. At 37 °C, typically no growth, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from subsurface or surface hyphae, stipes usually 200–400 µm long but sometimes much longer, walls smooth to finely roughened, typically bearing terminal verticils of 5–8 closely appressed metulae, but in some isolates subterminal metulae or even a subterminal ramus also occurring; metulae 10–15 µm long, sometimes enlarging at the apices; phialides ampulliform, 7–9 µm long, with short collula; conidia subspheroidal, 2.2–3.0 µm diam, smooth walled, borne in disordered chains.

Distinctive features *Penicillium paxilli* produces moderately sized, usually velutinous colonies, with terminal penicilli comprised of a crowded cluster of metulae. Stipes which are smooth or nearly so and the absence of sclerotia distinguish this species from *P. raistrickii* and *P. novae-zeelandiae.*

Identifiers Mycobank MB302838; neotype IMI 40226 (Pitt 1979b); ex-type cultures CBS 360.48, IMI 40226, ATCC 10480, NRRL 2008, FRR 2008; ITS barcode GU944577; alternative markers *BenA* JN606844, *RPB2* JN606610, *CaM* JN606566 (Visagie et al. 2014).

Physiology Little is known about the physiology of this species. It grows poorly, if at all, at 5 and 37 °C, and is probably not a xerophile.

Mycotoxins *Penicillium paxilli* has been reliably reported to produce two toxic compounds, verruculogen and paxilline. Verruculogen, a tremorgenic toxin with the same active indole moiety as penitrem A, but of quite different structure (Cole 1981), is almost equally toxic (Cole et al. 1972). However, the absence of reports of natural occurrence means it may be regarded as a mycotoxin of little consequence in foods and feeds, although not because it lacks toxicity. See Chap. 13, for further details.

Paxilline is also a tremorgen, but structurally quite different from the other tremorgens made by *Penicillium* species, and of lower toxicity. Again, this toxin has not been found from natural sources.

It is of interest that isolates of *P. paxilli* producing verruculogen appear to come from

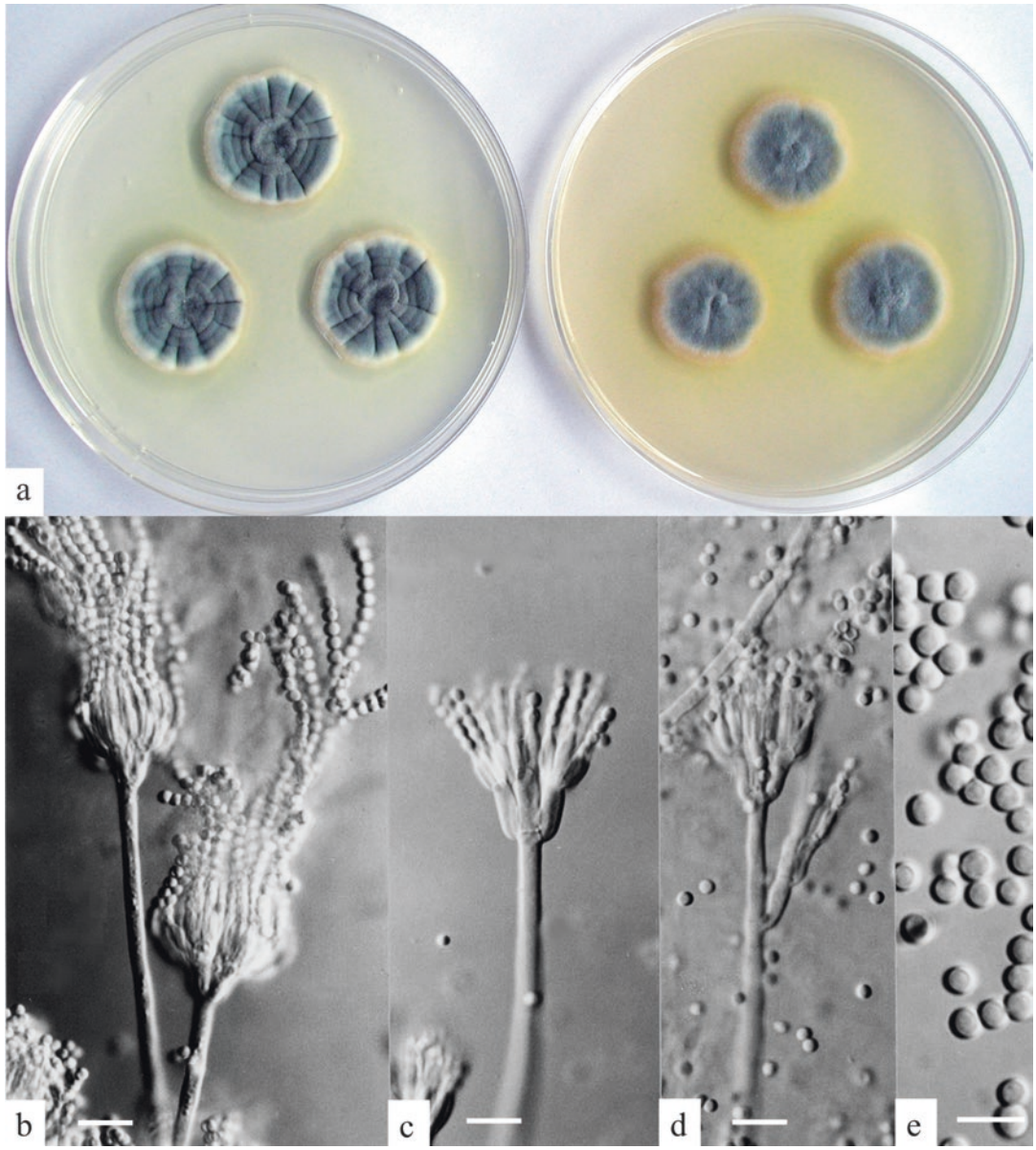

Fig. 7.20 *Penicillium paxilli* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Australia, while those producing paxilline are found in the United States (Cockrum et al. 1979).

Ecology Like most other species in subgenus *Furcatum*, *Penicillium paxilli* is a soil fungus and its occurrence in foods is usually adventitious. However, it has been reported to cause spoilage of garlic (Roy et al. 1977). Although there are few other references in the literature, it is our experience that this species has a widespread though sparse distribution in foods. It has caused visual spoilage of cheese (our observations), and we isolated it at low levels from a wide range of Southeast Asian foods: peanuts from Thailand and dried fish, maize, paddy rice, peanuts, sorghum and soybeans from Indonesia Wheeler et al. 1986; Pitt et al. 1993, 1998).

References Pitt (1979b, 2000) and Houbraken et al. (2011a).

Penicillium raistrickii G. Sm Fig. 7.21

Colonies on CYA 35–45 mm diam, plane or nearly so, moderately dense to deeply floccose; mycelium usually white, sometimes centrally Pale Yellow (1-3A3); sclerotia usually abundant, enveloped by the mycelium; conidial formation often sparse and confined to central areas, sometimes moderate and then coloured Dull Green (26-27D3); clear exudate usually present; reverse pale, yellow or yellow brown. Colonies on MEA usually 40–45 mm diam, occasionally as small as 30 mm, plane, sparse, velutinous to floccose; mycelium white to Pastel Yellow (3-4A3-4); sclerotia usually abundant, superficial or surrounded by wefts of mycelium; conidial production sparse, coloured as on CYA; exudate produced occasionally, red brown; reverse pale or yellow brown. Colonies on G25N 18–26 mm diam, plane or sulcate, often very deep and floccose at the centres; mycelium white; reverse pale. At 5 °C, at least germination; usually colonies of 2–4 mm diam produced, of white mycelium. No growth at 37 °C.

Sclerotia white or buff, rarely brown, 150–250 µm diam, texture firm to sclerotioid. Teleomorph not known. Conidiophores borne from subsurface mycelium and also from aerial hyphae adjacent to sclerotia, stipes usually 300–500(−800) µm long but from aerial hyphae sometimes much shorter, walls thick and roughened, bearing exclusively or predominantly terminal biverticillate penicilli, in some isolates accompanied by short subterminal rami; terminal metulae in divergent verticils of 3–5, apically inflated or clavate, of uniform appearance, 12–15 µm long, usually enlarging to 5–7 µm at the apices, sometimes rough walled; phialides acerose-ampulliform, 7–9 µm long, narrowing abruptly to short collula; conidia spherical to subspheroidal, 2.0–3.0 µm diam, smooth walled, borne in long, divergent columns.

Distinctive features *Penicillium raistrickii* grows rapidly and produces long, rough walled stipes bearing terminal biverticillate penicilli with apically inflated metulae. Almost all isolates produce abundant pale to brown sclerotia.

Identifiers Mycobank MB276069; lectotype IMI 40221 (Pitt 1979b); ex-type cultures CBS 261.33, IMI 40221, ATCC 10490, NRRL 1044, FRR 1044; ITS barcode AY373927; alternative markers *BenA* KJ834485, *RPB2* JN406952, *CaM* KJ867006 (Visagie et al. 2014).

Physiology Little is known about the physiology of this species. Isolates are just capable of growth at 5 °C, but not at 37 °C. It is probably not a xerophile.

Mycotoxins Griseofulvin is a valuable antibiotic, but is appreciably toxic, and must also be classed as a mycotoxin. *Penicillium raistrickii* is one species producing this compound. This species may also make other unknown tremorgenic toxins (Patterson et al. 1979).

Ecology Basically a soil fungus, *Penicillium raistrickii* has a widespread though sparse distribution in foods. This species was isolated from contaminated capsicums (Martín et al. 2005). We isolated it at low levels from Indonesian dried fish (Wheeler et al. 1986) and from Thai maize (Pitt et al. 1993). Other isolates in our culture collection have come from a mandarin fruit, from flour and from peanuts.

Additional species Perhaps a rarer species, *Penicillium novae-zeelandiae* J.F.H. Beyma appears to be very closely related to *P. raistrickii*. The major difference is that *P. novae-zeelandiae* characteristically produces irregular black sclerotia under the agar surface. What little is known of its physiology shows similarity to *P. raistrickii*. Isolates of *P. novae-zeelandiae* were found to produce an uncharacterised tremorgenic toxin (Di Menna and Mantle 1978; Di Menna et al. 1986). We have isolated *P. novae-zeelandiae* occasionally from a variety of foods, of note from "thread mould" spoilage of vacuum packed cheese blocks (Hocking and Faedo 1992), dried fruit and grape juice. Identifiers: Mycobank MB522253; lectotype IMI 40584ii (Pitt 1979b); ex-type cultures CBS 137.41, IMI 40584, ATCC 10473, NRRL 2128, FRR 2128; ITS barcode JN617688; alternative markers *BenA* KJ834477, *RPB2* JN406628, *CaM* KJ866996 (Visagie et al. 2014).

References Pitt (1979b, 2000).

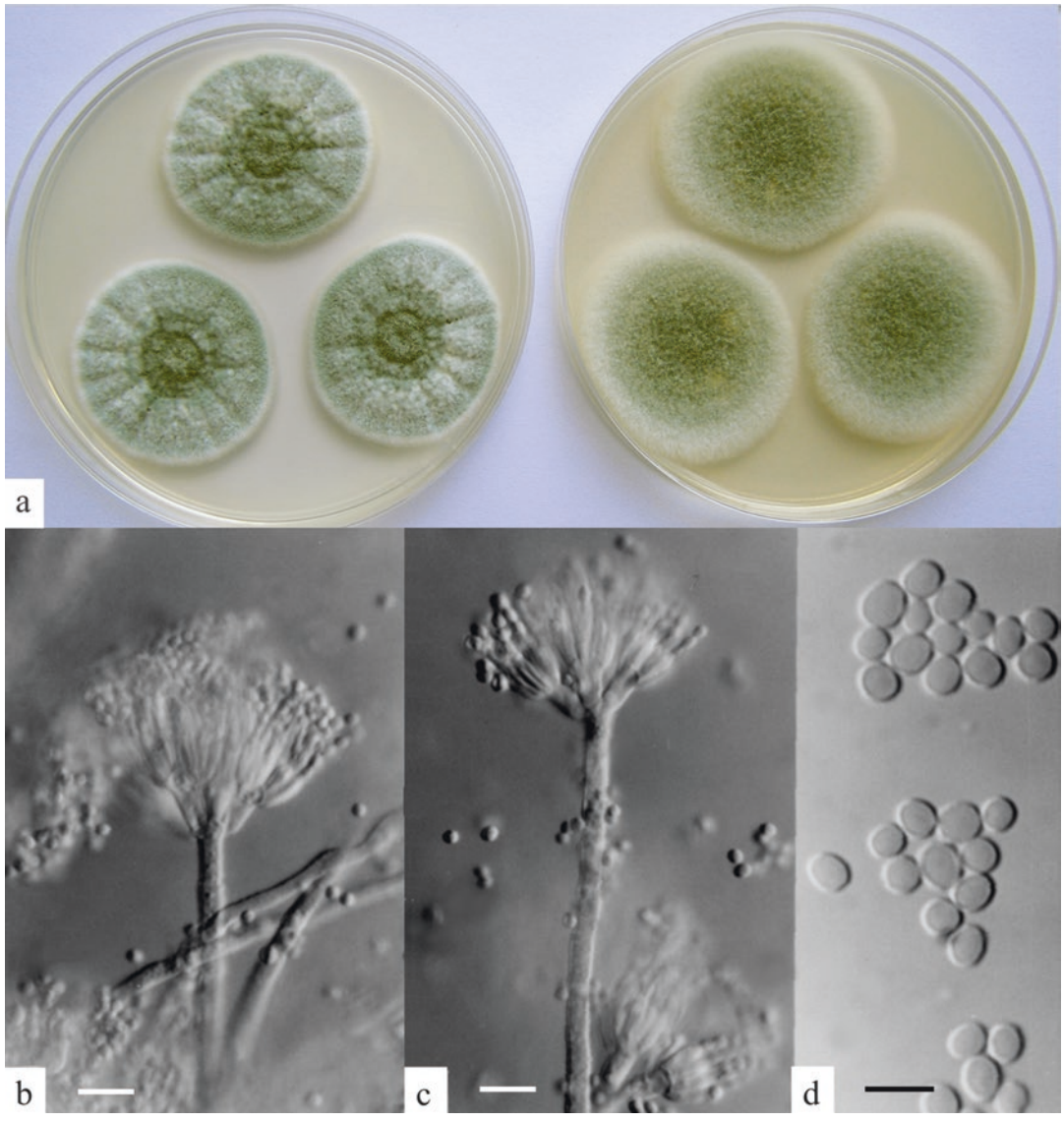

Fig. 7.21 *Penicillium raistrickii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

Penicillium simplicissimum (Oudem.) Thom Fig. 7.22

Colonies on CYA typically 40–50 mm diam, radially sulcate; mycelium dense, white or occasionally buff; conidial production absent in some isolates, light to moderate in others, typically coloured Greyish Green to Dull Green (25C-D4) but in some isolates centrally or predominantly Yellowish Grey (2-3C2); sometimes clear exudate and occasionally red brown soluble pigment present; reverse typically pale, but sometimes in Greyish Yellow shades such as Chamois or Khaki (4B-D5). Colonies on MEA typically 40–50 mm diam, plane, up to 2–3 mm deep, velutinous to floccose; mycelium white; conidial production moderate to heavy, Greyish Green to Dull Green (26-29C-E3-4); reverse pale

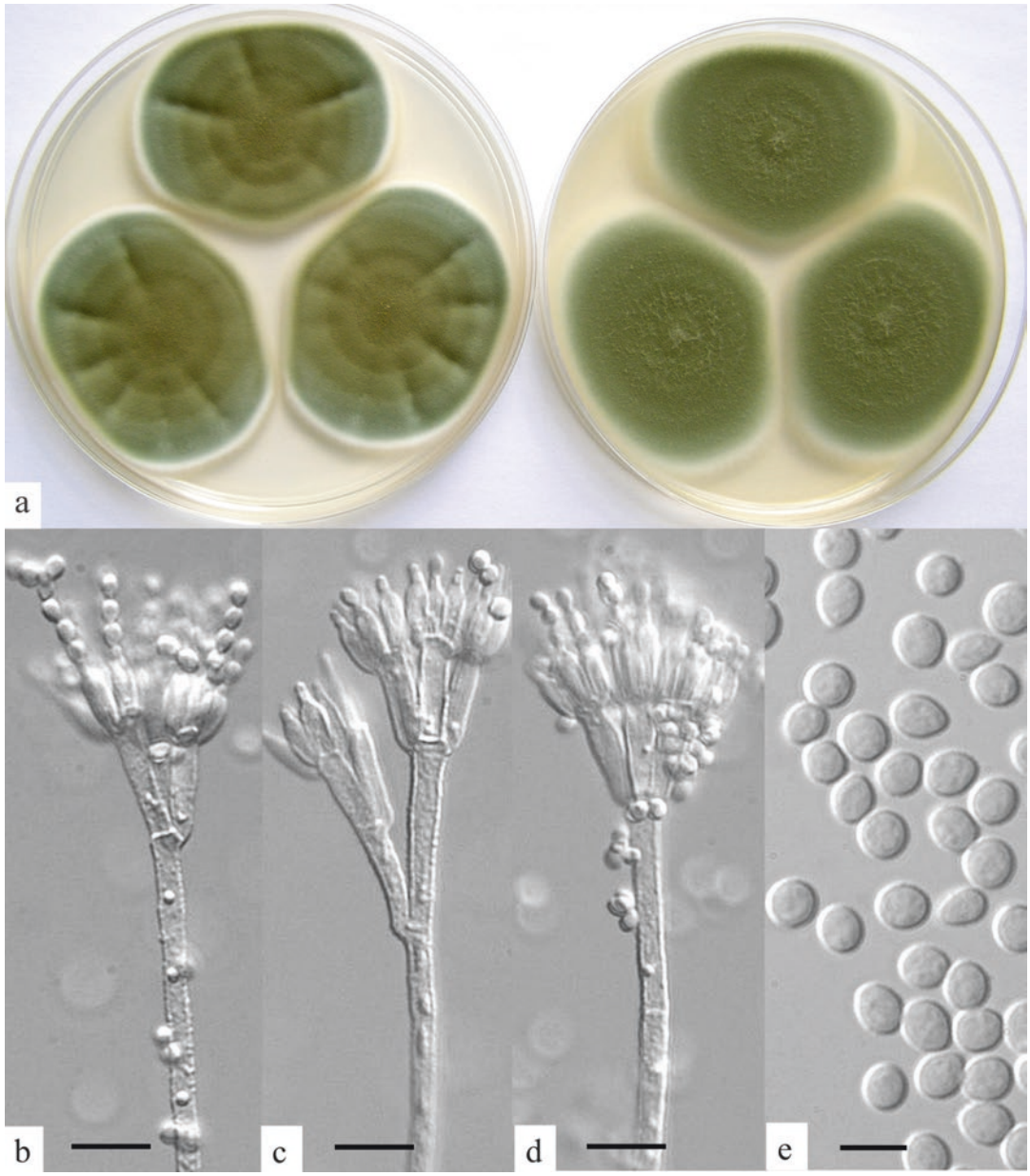

Fig. 7.22 *Penicillium simplicissimum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

or sometimes yellow brown. Colonies on G25N 12–18 mm diam, plane, sulcate or wrinkled, velutinous to floccose; mycelium white; reverse pale, yellowish or olive. At 5 °C, no germination to germination, occasional isolates forming microcolonies. At 37 °C, colonies usually 20–30 mm diam, occasional isolates weaker, 5–10 mm or even absent, sulcate to deeply wrinkled, of white to pinkish mycelium, rarely with grey or greenish grey conidia present; reverse pale or brownish.

Sclerotia occasionally produced on CYA or MEA at 25 °C, spheroidal or irregular, commonly 200–500 µm diam, brown, of soft texture.

Teleomorph not known. Conidiophores borne from surface or aerial hyphae, stipes commonly 400–800 µm long, usually rough walled, bearing regular to irregular verticils of metulae and occasionally a well defined ramus, but only rarely short conidiophores or metulae in other than terminal or subterminal positions; metulae in verticils of 2–5, 12–20 µm or more long, rough walled; phialides ampulliform, mostly 7–9 µm long, narrowing abruptly to long collula; conidia variable, commonly ellipsoidal, but at times spherical, subspheroidal or pyriform, 2.5–4.0 µm long, with walls roughened or spinulose, borne in disordered chains.

Distinctive features Isolates of *Penicillium simplicissimum* grow rapidly at 25 °C, forming comparatively deep colonies due to long conidiophores. The conidiophores are characteristic: stipes are long and coarse with rough walls, and bear terminal or subterminal metulae and occasionally rami. Short conidiophores or intercalary metulae are uncommon.

Taxonomy Controversy over the correct name of this species (Stolk and Samson 1983; Frisvad et al. 1990c; Pitt 2000) has been resolved by maintaining the neotypification by Pitt (1979b), based on a herbarium specimen of Jensen's from Cornell University (Pitt and Samson 1993). More recent authors (Tuthill et al. 2001; Visagie et al. 2014) have concluded that *P. simplicissimum* encompasses several distinct species (*P. brasilianum, P. ochrochloron, P. piscarium, P. pulvillorum* and others unnamed) so that the name should be applied more narrowly. However, all of these species are believed to have very similar physiology, so present purposes are served adequately by identification of these species as *P. simplicissimum*.

Identifiers Mycobank MB278201; neotype CUP Jensen 1912: no 5921; representative cultures CBS 372.48, ATCC 10495, NRRL 902, FRR 902; ITS barcode GU981588; alternative markers *BenA* GU981632, *RPB2* JN121507, *CaM* KF296388 (Visagie et al. 2014). Note that no cultures derived from the neotype exist: NRRL 902 was considered to be representative of *P. simplicissimum* by Tuthill et al. (2001).

Physiology Isolates of *Penicillium simplicissimum* from different parts of the world show variation in growth at higher temperatures. Tropical isolates grow strongly at 37 °C; some from cooler regions of Australia grow at most weakly at this temperature. This probably reflects the fact that *P. simplicissimum* as circumscribed here includes several species (Tuthill et al. 2001). This species is not a xerophile, germinating down to 0.86 a_w only (Hocking and Pitt 1979).

This species appears to be resistant to formaldehyde. Isolates in our collection and at the International Mycological Institute, Egham, Surrey, UK, have come from 10% formalin solution or preserved specimens. Several IMI isolates have also come from polyvinyl chloride or polyester plastics.

Mycotoxins *Penicillium simplicissimum* has been reported to produce the mycotoxins verruculogen (Pitt 1979c; see Chap. 13), fumitremorgen B (El-Banna et al. 1987), penicillic acid and viridicatumtoxin (El-Banna et al., 1987; Pitt and Leistner 1991). Fumitremorgen B is closely related to verruculogen, and can be expected to have a similar toxicity. However, natural production of these toxins by this species has not been shown, and their significance in human or animal health is doubtful. The importance of viridicatumtoxin is unknown (see *P. aethiopicum*).

In splitting *Penicillium simplicissimum* into several species, Tuthill et al. (2001) showed that fumitremorgens, verruculogen and viridicatumtoxin are produced by *P. brasilianum*, not *P. simplicissimum*. However, it is not clear which of these species occurs in foods.

Ecology *Penicillium simplicissimum* appears to have its primary habitat in decaying vegetation, and perhaps soil. Its occurrence on foods is therefore not as a spoilage fungus, but as a contaminant. It has been reported from country cured hams (Monte et al. 1986), cashews, peanuts and mung beans (Pitt et al. 1993, 1994). Isolates in our collection have also come from spring water, sorghum malt and fermented sausage.

References Pitt (1979b, c, 2000) and Tuthill et al. (2001).

7.5 *Penicillium* subgenus *Penicillium*

Penicillium Sect. *Asymmetrica* Raper & Thom

In *Penicillium* subgenus *Penicillium*, penicilli are predominantly terminal terverticillate structures, i.e. phialides are always borne on metulae and usually metulae are borne on well defined terminal rami. Biverticillate and quaterverticillate penicilli are also produced by some species. Phialides are typically ampulliform (flask shaped), but are cylindroidal to acerose (needle shaped) in a few species.

Colonies on G25N normally exceed 18 mm diam, although they are consistently less in one species treated here, *Penicillium digitatum*. Only *P. aethiopicum* and *P. chrysogenum* sometimes grow at 37 °C. Growth at 5 °C is always positive, and usually strong: most species produce macroscopic colonies in 7 days.

By far the most important *Penicillium* subgenus with respect to food spoilage, subgenus *Penicillium* is also by far the most difficult taxonomically, both because there are numerous species, and because apparent differences between species are small. For a number of years, the taxonomy of this subgenus remained controversial, with the systems of Raper and Thom (1949), Samson et al. (1976) and Pitt (1979b) all being commonly used. During the 1980s, students of *Penicillium* sought consensus on the classification of this subgenus. Secondary metabolite profiles (Frisvad 1981; Frisvad and Filtenborg 1983) and isoenzyme patterns (Cruickshank and Pitt 1987) provided independent evidence about species concepts. Integration of classical morphology with these new approaches led to consensus for most species (Samson and Pitt 1985). Later, a list of "Names in Current Use" (NCU) was established for *Penicillium* species (Pitt and Samson 1993; Pitt et al. 2000). This provided much needed stability, although some recent studies have ignored this provision. Howver, all names used here for species in this subgenus are protected by this NCU.

Samson and Frisvad 2004 revised this subgenus and increased the number of included species from 23 to 58. The number was further increased to 72 in the next comprehensive review (Visagie et al. 2014). Where these additions affect species included here they are discussed under the relevant species.

Identifications It is usually relatively easy to decide whether an isolate belongs in subgenus *Penicillium*, but accurate identifications to species level require care and often acquired skill. In the keys used here, the roughness or smoothness of stipes and small differences in colour will affect the disposition of an isolate. Perhaps the most important point to note in the keys which follow is that species which have perfectly smooth stipe walls under the light microscope at 400–600x magnification are distinguished from those in which some stipes, though not necessarily all, are finely to distinctly roughened. Roughening of stipe walls is often more readily seen in wet mounts made from cultures grown on MEA.

A second point to note is that judgment of colour should be made in daylight or artificial daylight conditions, i.e. in the latter case, under daylight type fluorescent tubes, not incandescent lamps. The use of the Methuen "Handbook of Colour" (Kornerup and Wanscher 1978) will greatly assist colour differentiation of species in this subgenus. Some of the species accepted here are undoubtedly closely related, and intermediate isolates will be encountered from time to time. Nevertheless, with care it should be possible to key out and recognise the majority of isolates from foods using the classical techniques described here.

Neutral creatine sucrose agar Creatine sucrose agar (CREA) was introduced as a new approach to the problem of identifying species in subgenus *Penicillium* (Frisvad 1981, 1985). He pointed out that species in this subgenus fall into two groups: those with an affinity for proteinaceous foods and those which will grow vigorously on foods rich in carbohydrate. CREA was designed to permit differentiation between these two groups. It contained the organic base creatine as a sole source of nitrogen, sucrose as a source of carbon, and an indicator, bromocresol purple, to detect pH changes related to differential acid production (from sucrose) or base production by liberation of ammonium ions (from creatine).

The concept was innovative and sound, but CREA was not easy to use in practice. A number of variations were introduced by Frisvad (1993), but without firm recommendations.

A medium based on CREA, but of initial pH neutral to bromocresol purple and with a change in sucrose to creatine ratio to provide more positive responses by some species, was developed by Pitt (1993) and termed Neutral Creatine Sucrose agar (CSN). The formula is given in Appendix 1.

CSN has proved to be very useful in identification of species in *Penicillium* subgenus *Penicillium*, as eight different types of responses have been observed among common species (Table 7.2; modified from Pitt 1993). Reaction to CSN has been included in the descriptions and keys which follow. If only occasional isolates from this subgenus are seen, use of CSN is not essential. However, for the laboratory which frequently isolates species from this subgenus, CSN can be of great assistance. CSN is of limited value outside this subgenus.

Use of Ehrlich reagent In the quest for more rapid and reliable methods for identifying species in subgenus *Penicillium*, Lund (1995a) developed a simple test to distinguish among several species common in cheese and bread factories

Table 7.2 Responses of common species of *Penicillium* subgenus *Penicillium* to Neutral Creatine Sucrose agar (CSN) after incubation for 7 days at 25 °C[a]

Species	Colony growth	Colony diam mm	Medium reaction	Colony reverse
Penicillium aethiopicum	Moderate	15–20	Acid, margins some times neutral	Acid + brown
P. allii	Weak	15–20	Acid	Acid
P. aurantiogriseum	Moderate	15–25	Acid + brown	Acid + brown
P. brevicompactum	Weak	8–14(−20)	Neutral to weakly acid	Neutral (occasion ally weakly acid)
P. camemberti	Strong	15–20	Alkaline, margins neutral	Alkaline
P. chrysogenum	Moderate to strong	12–18	Neutral or weakly acid	Neutral to weakly acid
P. commune	Strong	20–26	Alkaline, sometimes weakly acid	Alkaline
P. crustosum	Strong	25–30	Acid, occasionally alkaline	Akaline, occasionally we
P. digitatum	Weak	4–10	Neutral	Neutral
P. echinulatum	Strong	22–25	Alkaline	Alkaline
P. expansum	Strong	24–30	Acid	Acid + brown, occasionally alkaline
P. glandicola	Moderate	12–18	Weakly acid to acid	Acid + brown
P. griseofulvum	Weak	18–24	Neutral	Neutral
P. hirsutum	Strong	24–30	Acid	Acid (+ brown)
P. hordei	Strong	20–24	Acid	Acid + brown
P. italicum	Weak	10–20	Neutral	Neutral
P. nalgiovense	Weak	10–18	Neutral to weakly acid	Neutral to weakly acid
P. olsonii	Weak	6–14	Neutral	Neutral
P. roqueforti	Strong	25–40	Variable	Alkaline, occasionally neutral
P. solitum	Strong	18–22	Usually alkaline	Alkaline
P. ulaiense	Weak	4–8	Neutral	Neutral
P. verrucosum	Weak	10–15	Neutral	Neutral
P. viridicatum	Moderate to strong	15–22	Acid	Acid (+brown)

[a]Acid, yellow; weakly acid, pale yellow; neutral, grey, sometimes with a reddish cast; weakly alkaline, grey violet; alkaline, violet or reddish violet. Brown responses are from naturally produced pigments

in Denmark. The test used Ehrlich reagent to visualise production of indole compounds as secondary metabolites. Ehrlich reagent is made by dissolving 4-dimethylaminobenzaldehyde (2 g) in 96% ethanol (85 ml) and adding 10 N HCl (15 ml).

To carry out the test, a cork borer (4 mm diameter) is used to cut an agar plug from a culture on CYA and then the plug is placed, mycelium side up, on a Petri dish lid. A strip (12 × 6 mm) of Whatman No. 1 filter paper is dipped in the Ehrlich reagent and then placed across the agar plug "like a propeller", with the agar plug in the centre of the strip, so the strip does not touch the Petri dish lid. The arrangement is then placed in an air stream in a fume hood and allowed to dry. A positive response is indicated by a violet ring in the filter paper, appearance after 2–6 minutes being a strong response, and after 7–10 minutes a weak one. Observation must be made within 10 minutes, as the ring fades after that time. Most isolates produce the colour on CYA after incubation for 7 days, however, a few require 14 days incubation. The test is of more value when positive than negative, therefore.

Species for which this test is positive include *Penicillium camemberti*, *P. commune, P. expansum* and *P. roqueforti* (Lund 1995a). A few other species produce yellow colours with this test, including *P. crustosum* (Lund 1995a).

Physiology As noted above, species in subgenus *Penicillium* are very important in food spoilage. They are able to grow at low temperatures and quite low water activities, and are of universal occurrence in cereals, refrigerated foods and many other environments. Controlling these fungi in many types of bulk stored food commodities relies on a combination of low water activity and low temperature: even marginal errors in these controls may sometimes lead to high losses.

Mycotoxins With the possible exception of the fruit rotting species *Penicillium digitatum*, *P. italicum*, *P. solitum* and *P. ulaiense*, all of the species under consideration here produce mycotoxins. Moreover, mycotoxin production appears to occur more consistently than in most other genera: a large majority of the isolates encountered from subgenus *Penicillium* will be mycotoxigenic. Fortunately, most of the toxins are believed to have relatively low potency or are not produced under conditions occurring in foods.

The question of species–mycotoxin associations for species in *Penicillium* subgenus *Penicillium*, considered a major problem when the first edition of this book was published, has been clarified. A comprehensive collaborative study, in which more than 1500 *Penicillium* isolates were critically examined both taxonomically and for mycotoxin production, greatly assisted this process (El-Banna et al. 1987; Pitt and Leistner 1991). When combined with data from Frisvad and Filtenborg (1989) and Samson and Frisvad 2004, this information has enabled recognition of the fact that species–mycotoxin associations in this genus are mostly quite specific. These data have been incorporated below.

Key to *Penicillium* subgenus *Penicillium* species included here

1	Conidia white or pale grey green	2
	Conidia more strongly coloured, blue, green or grey	3
2 (1)	Stipes often rough walled, conidia up to 5 µm diam; on CSN, reaction alkaline	*P. camemberti*
	Stipes consistently smooth walled, conidia not usually exceeding 3.5 µm diam; on CSN, reaction neutral to weakly acid	*P. nalgiovense*
3 (1)	Stipes on CYA and MEA smooth walled, or at most very finely roughened	4
	Stipes commonly rough walled, especially on MEA	16
4 (3)	Conidia borne as cylinders, with at least a proportion remaining so at maturity	5
	Conidia borne as ellipsoids or spheres and remaining so at maturity	7
5 (4)	Conidia olive, longer than 6 µm	*P. digitatum*
	Conidia green, shorter than 6 µm	6

(Continue)

6 (5)	Colonies on CYA and MEA exceeding 30 mm diam	*P. italicum*
	Colonies on CYA and MEA not exceeding 30 mm diam	*P. ulaiense* (see *P. italicum*)
7 (4)	Penicilli with 3 or more rami in large, compact terminal penicilli	*P. olsonii*
	Penicilli with 1–2 rami, penicillus structure variable	8
8 (7)	Penicilli complex or irregular, reverse on MEA strongly coloured, deep orange brown to brown, phialides with short, broad collula; on CSN, reaction neutral to weakly acid	*P. nalgiovense*
	Penicilli well defined, mostly terverticillate, reverse on MEA pale or dull yellow or brown, phialides with relatively long, narrow, collula; CSN response variable	9
9 (8)	Colonies on CYA exceeding 30 mm diam	10
	Colonies on CYA not exceeding 30 mm diam	14
10 (9)	Conidia grey blue	11
	Conidia grey green to green	12
11 (10)	Colonies on CYA 35–45 mm diam, yellow exudate and soluble pigment often present, conidia ellipsoidal to subspheroidal; on CSN, medium response neutral to weakly acid	*P. chrysogenum*
	Colonies on CYA 30–38 mm diam, exudate clear to pale brown, soluble pigment sometimes present, brown to reddish brown, conidia spherical to subspheroidal; on CSN, medium response acid, reverse acid plus brown	*P. aurantiogriseum*
12 (10)	Reverse on CYA pale to deep brown; colonies on CSN usually exceeding 24 mm diam	*P. expansum*
	Reverse on CYA pale, yellow or orange; colonies on CSN less than 24 mm diam	13
13 (12)	Colonies dull green, conidia broadly ellipsoidal, reverse on CYA bright golden yellow; on CSN reverse acid plus brown	*P. aethiopicum*
	Colonies dark green, conidia mostly spherical to subspheroidal, reverse on CYA usually pale, sometimes orange or pale yellow; CSN reverse alkaline	*P. solitum*
14 (9)	Phialides commonly 4.5–6 µm long; on CSN, response neutral	*P. griseofulvum*
	Phialides exceeding 6 µm long; CSN response variable	15
15 (14)	On CYA colonies dull green, penicilli very broad, metulae often apically inflated, conidia ellipsoidal; on CSN, reverse neutral to weakly acid	*P. brevicompactum*
	On CYA, colonies dark green, penicilli not very broad, metulae not usually apically inflated, conidia mostly spherical to subspheroidal; on CSN, reverse alkaline	*P. solitum*
16 (3)	Colonies on CYA exceeding 30 mm diam	17
	Colonies on CYA not exceeding 30 mm diam	24
17 (16)	Conidia with walls finely roughened to rough or spinose	18
	Conidia smooth walled	19
18 (17)	Mycelium inconspicuous, white, conidia dark green; growth on CSN strong, medium and reverse alkaline	*P. echinulatum* (see *P. crustosum*)
	Mycelium yellow, conidia dull green; on CSN, medium and reverse acid	*P. hordei* (see *P.hirsutum*)
19 (17)	Growth on CSN strong, colonies more than 20 mm diam, medium and reverse alkaline	20
	Growth on CSN variable, medium and reverse acid, reverse sometimes also brown	22
20 (19)	Colonies on CYA and MEA exceeding 40 mm diam, reverse on CYA and/or MEA often green to deep blue green; conidia up to 6 µm diam	*P. roqueforti*
	Colonies on CYA or MEA less than 40 mm diam, reverse pale or yellow to orange brown, conidia mostly 4 µm or less diam	21

(Continue)

21 (20)	Colonies on CYA exceeding 35 mm diam and on MEA usually exceeding 30 mm diam; colonies on MEA often shedding masses of conidia when jarred	*P. crustosum*
	Colonies on CYA and MEA not exceeding 35 and 30 mm diam respectively; conidia adhering to colonies on MEA	*P. commune*
22 (19)	Colonies on CYA often exceeding 35 mm diam	23
	Colonies on CYA not exceeding 35 mm diam	*P. viridicatum*
23 (22)	Exudate on CYA near maroon; mycelium often yellow; penicilli sometimes quaterverticillate	*P. hirsutum*
	Exudate on CYA pale yellow; mycelium white, penicilli terverticillate or somewhat irregular	*P. allii* (see *P. hirsutum*)
24 (16)	Conidia mostly ellipsoidal, dark green	*P. glandicola* (see *P. brevicompactum*)
	Conidia near spherical, yellow green	25
25 (24)	Colonies on CYA and MEA exceeding 25 mm diam	*P. viridicatum*
	Colonies on CYA and MEA not exceeding 25 mm diam	*P. verrucosum*

Penicillium aethiopicum Frisvad — Fig. 7.23

Colonies on CYA 32–40 mm diam, finely radially sulcate, deeply fasciculate; mycelium white, visible only at the margins; conidial production moderate to heavy, Dull Green (25-26E-F4); copious exudate usually present, clear to pale brown; reverse closely sulcate, golden yellow near Mustard Yellow or Maize Yellow (3-4B-C5-6). Colonies on MEA 32–40 mm diam, plane, low and dense, surface velutinous to granular; mycelium inconspicuous, white; conidial production moderate, Dull Green (26D-E4); reverse uncoloured to dull yellow brown. Colonies on G25N 22–24 mm diam, plane or sulcate, low to moderately deep, dense, usually fasciculate; mycelium white; sporulation moderate, dull green; soluble pigment sometimes produced, Pale Orange (5A3); reverse pale, bright yellow or pale orange. At 5 °C, germination to colonies 3 mm diam. At 37 °C, no growth to colonies 10 mm diam, of dense white mycelium.

On CSN, colonies 15–20 mm diam, growth moderate, medium reaction acid (pale yellow), sometimes neutral at the margins; reverse acid (yellow), plus brown.

Conidiophores borne singly or more commonly in fascicles, mostly from subsurface hyphae, stipes 200–400 µm long, or of indeterminate length in fascicles, with smooth or at most very finely roughened walls, typically bearing appressed, terminal terverticillate penicilli; phialides ampulliform, 7–9 µm long, with short collula; conidia broadly ellipsoidal, 3.2–3.8 µm long, smooth walled, borne in long, sometimes well defined, columns.

Distinctive features *Penicillium aethiopicum* resembles *P. chrysogenum* in growth rates, including the ability to grow weakly at 37 °C; *P. expansum*, in producing fasciculate colonies; and both species, in producing conidiophores with smooth walled stipes. The most obvious feature distinguishing *P. aethiopicum* from these other species is the formation of a golden yellow reverse on CYA in the absence of yellow soluble pigment. Penicilli are more compact and robust than those of *P. chrysogenum*, while brown pigmentation is much less than occurs in colonies of *P. expansum* on CYA. Growth on CSN resembles that of *P. chrysogenum*.

Taxonomy Using molecular methods, this species has been shown to be identical to *P. lanosocoeruleum* Thom 1930 (Houbraken et al. 2012) and, as the earlier name, has priority. However, the use of the name *P. aethiopicum* is

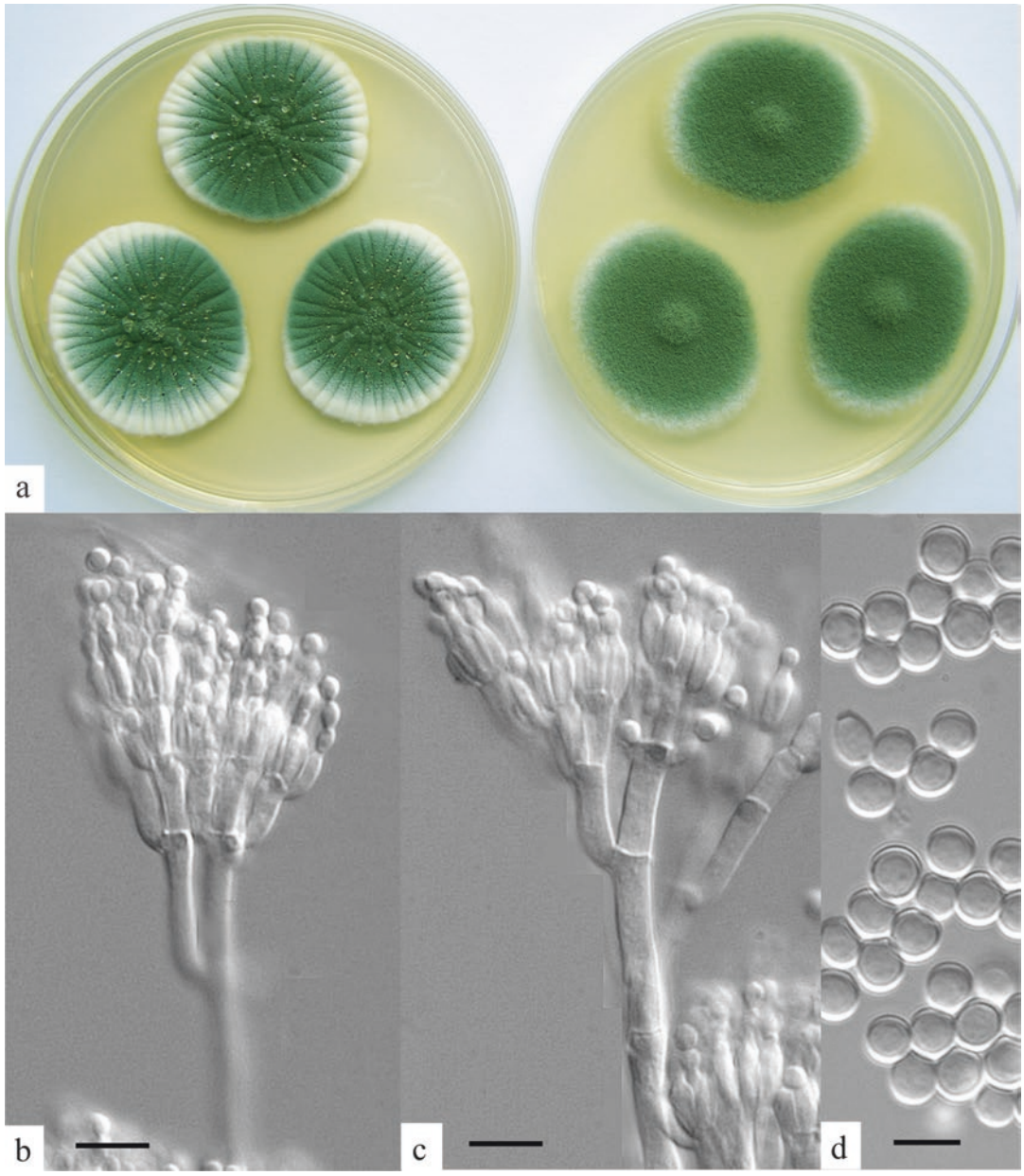

Fig. 7.23 *Penicillium aethiopicum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

maintained here for two reasons. First, the type culture of *P. lanosocoeruleum* was of poor quality even when examined by Raper and Thom (1949), who placed it in their illdefined "*Asymmetrica-Lanata*" group, while (Pitt 1979b) noted that it had been found as a floccose variant in a culture of *P. cyclopium* and considered it to be a synonym of that species (= *P. aurantiogriseum* in that work). That deteriorated culture is unsuitable as a representative of the species under discussion here. Second, and more important, *P. lanosocoeruleum* was not included in the list of

"Names in Current Use" (Pitt and Samson 1993), a list which eminent nomenclaturalists believe provides priority over old neglected names. Taking up old names such as *P. lanosocoruleum* only causes confusion, especially as the *P. aethiopicum* genome has been sequenced (Chooi et al. 2010), and it is being used as a model organism in antibiotic research and production.

Identifiers Mycobank MB126403; holotype IMI 285524; ex-type cultures IMI 285524, FRR 3624; ITS barcode KC411740; alternative markers *BenA* JX996843, *RPB2* JX996723, *CaM* JX996967 (Visagie et al. 2014).

Physiology No studies on physiology are known to us. The ability to grow at 37 °C suggests a physiology similar to *Penicillium chrysogenum*.

Mycotoxins *Penicillium aethiopicum* produces griseofulvin (see *P. griseofulvum*) and viridicatumtoxin (Frisvad and Filtenborg 1989). The latter is a moderately toxic compound, with an LD_{50} of 70 mg/kg when injected into mice. Its oral toxicity is unknown and its practical significance unclear.

Ecology *Penicillium aethiopicum* was originally described from barley from Ethiopia (Frisvad and Filtenborg 1989). We found this species frequently in Southeast Asian commodities: in 16% of Indonesian kemiri nut samples, with a 4% infection rate in all nuts examined, in 18% of Indonesian cow pea samples, 9% of Indonesian peanut samples and 4% of Philippine maize samples. Incidence in some individual samples was high, up to 30–40% of all particles examined, providing a 1% infection level overall in each of these commodities. It was also present, at low levels, in Thai peanuts and cashews, Philippine soybeans and mung beans, and Indonesian milled rice, soybeans and mung beans (Pitt et al. 1993, 1994, 1998).

References Frisvad and Filtenborg (1989) and Samson and Frisvad 2004.

Penicillium aurantiogriseum Dierckx **Fig. 7.24**

Penicillium puberulum Bainier
Penicillium aurantiovirens Biourge
Penicillium martensii Biourge

Colonies on CYA 30–37 mm diam, radially sulcate, moderately deep, texture smooth to granular; mycelium white, usually inconspicuous; conidial production moderate to heavy, Greyish Turquoise to Dull Green (24-25D-E3-4); exudate usually conspicuous, clear or pale brown; soluble pigment produced by some isolates, brown to reddish brown; reverse pale, light to brilliant orange, or reddish to violet brown. Colonies on MEA 24–35 mm diam, plane or rarely radially sulcate, low and relatively sparse, surface texture finely granular; mycelium usually subsurface, occasionally conspicuous and then bright yellow; conidial production usually moderate to heavy, Greyish Turquoise to Dull Green (24-25D-E4-5); soluble pigment sometimes produced, yellow brown to reddish brown; reverse pale, orange, or reddish brown. Colonies on G25N 18–24 mm diam, usually radially sulcate, moderately deep, dense, texture granular; reverse pale, yellow or brown. At 5 °C, colonies 2–5 mm diam, of white mycelium. No growth at 37 °C.

On CSN, colonies usually 15–25 mm diam, with moderately dense to dense growth, medium reaction acid (yellow); reverse acid (yellow) plus brown soluble pigment.

Conidiophores borne singly or in fascicles, mostly from subsurface hyphae, stipes 200–400 µm long, or of indeterminate length in fascicles, with walls smooth to finely roughened, bearing terminal terverticillate or less commonly biverticillate penicilli; phialides slender, ampulliform, mostly 7–10 µm long; conidia spherical to subspheroidal, less commonly ellipsoidal, 3.0–4.0 µm long, with smooth walls, mostly borne in long, well defined columns.

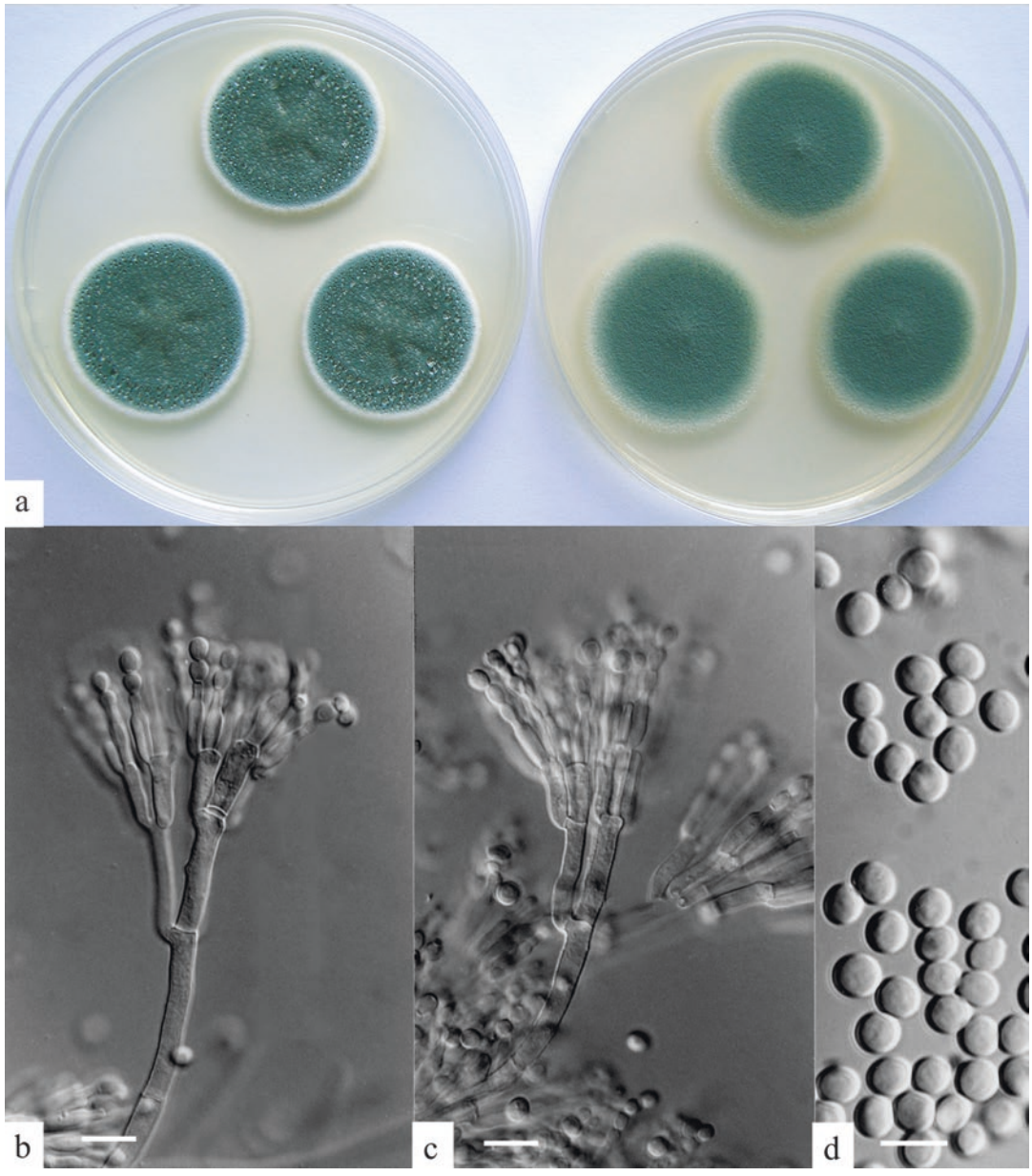

Fig. 7.24 *Penicillium aurantiogriseum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

Distinctive features *Penicillium aurantiogriseum* produces blue grey conidia on both CYA and MEA. Colonies on CYA grow relatively slowly (37 mm diam at most) and are dense and of granular texture. Colonies on MEA are relatively low and sparse, with distinctly granular margins. Stipes are usually smooth or nearly so. The response on CSN is distinctive: quite rapid growth, an acid reaction in medium and colony reverse, characteristically with brown colours also in the medium and beneath the colony.

Taxonomy *Penicillium aurantiogriseum* as described here is a relatively broad species. It has essentially the same circumscription as given by

Pitt et al. (1986, 2000), but narrower than that described by Samson et al. (1976) or Pitt (1979b). More recently, several other species were segregated from *P. aurantiogriseum* on the basis of secondary metabolite, morphological and ecological characters (Lund and Frisvad 1994; Samson and Frisvad 2004).

We have maintained the concept of *Penicillium aurantiogriseum* described by Pitt et al. (1986, 2000) as we believe that, with some practice, it can be distinguished purely on morphological criteria from other species described in this book. If further differentiation is required, see Lund and Frisvad (1994) or Samson and Frisvad 2004.

Identifiers Mycobank 247,956; neotype IMI 195050; ex-type cultures CBS 249.89, IMI 195050, ATCC 48920, NRRL 971, FRR 971; ITS barcode AF033476; alternative markers *BenA* AY674296, *RPB2* JN406573 (Visagie et al. 2014).

Physiology *Penicillium aurantiogriseum* has a minimum temperature for growth near −2 °C, an optimum near 23 °C, and a maximum near 30 °C (Armolik and Dickson 1956; Mislivec and Tuite 1970). The minimum a_w for growth is 0.81 (Mislivec and Tuite 1970). Growth at reduced a_w is little affected by pH or substrate (Hocking and Pitt 1979). Growth was stimulated by carbon dioxide levels above 10% (Magan and Lacey 1984). Growth was observed in 30% and 50% CO_2 but not in atmospheres containing 70% CO_2 (Zardetto 2005).

Considerable work has been carried out on the production of volatiles in stored grains by *Penicillium aurantiogriseum*. Major compounds reported include 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone on barley, wheat and synthetic media (Sinha et al. 1988a; Wilkins and Scholl 1989; Börjesson et al. 1990; Larsen and Frisvad 1995).

The lipolytic activity of *Penicillium aurantiogriseum* was high, as judged by action on a medium containing 1% rapeseed oil (Magan et al. 1993). Lipase production was enhanced when a medium containing 0.5% yeast extract and 1% olive oil was supplemented with ammonium sulphate (Lima et al. 2003).

Mycotoxins Isolates of *Penicillium aurantiogriseum* commonly produce penicillic acid (see Chap. 13), verrucosidin and nephrotoxic glyopeptides (Frisvad and Samson 2004). Accumulation of penicillic acid by *P. aurantiogriseum* is favoured by low temperatures, in the range 1–10 °C; production is faster at higher temperatures, but so is degradation (Ciegler and Kurtzman 1970).

Reports that *P. aurantiogriseum* isolates may also produce roquefortine C, xanthomegnin or viomellein (El-Banna et al. 1987; Frisvad and Filtenborg 1989) are now believed to be incorrect (Samson and Frisvad 2004)

Ecology Along with some other species in this subgenus, *Penicillium aurantiogriseum* is among the most commonly encountered fungi on earth. It is ubiquitous in maturing or drying crops, especially cereals and cereal products (see Pitt and Hocking 1997). This association with cereals is very important: reports of *P. aurantiogriseum* from proteinaceous foods including meat and cheese are almost always in error (Frisvad and Filtenborg 1989; Lund et al. 1995). It has been isolated frequently from nuts (see Pitt and Hocking 1997).

Penicillium aurantiogriseum has been reported to cause spoilage of a variety of stored fruits and vegetables, including apples, pears, strawberries, grapes, melons, tomatoes, cassava and potatoes (see Pitt and Hocking 1997). Not all such reports have been authenticated: it is likely that related species are responsible for some of these cases. Other reported sources include cold stored eggs, frozen fruit pastries, spices, dried beans and peas, soybeans, dried fruit, health foods (see Pitt and Hocking 1997) and luncheon meats (Ismail and Zaky 1999).

We isolated *Penicillium aurantiogriseum* from a variety of Southeast Asian food commodities, including maize, peanuts, cashews, soybeans, mung beans and rice, but only at low levels (Pitt et al. 1993, 1994, 1998).

References Pitt (1979b, 2000) and Samson and Frisvad (2004).

Penicillium brevicompactum Dierckx

Fig. 7.25

Penicillium stoloniferum Thom

Colonies on CYA 20–30 mm diam, radially sulcate, moderately deep, dense, texture typically velutinous; mycelium white; conidial formation light to moderate, Dull Green (25-28D-E3); exudate usually present in minute droplets, often deeply embedded, but sometimes copious, pale to deep reddish brown; soluble pigment usually produced, reddish brown; reverse sometimes pale but more usually yellowish to reddish brown. Colonies on MEA 12–22 mm diam, plane or less commonly radially sulcate, usually velutinous; mycelium white; conidial production moderate to heavy, Dull Green to Dark Green (27-29E-F4), rarely paler or more bluish; exudate occasionally present, clear to reddish brown; reverse pale or brown. Colonies on G25N 14–22 mm diam,

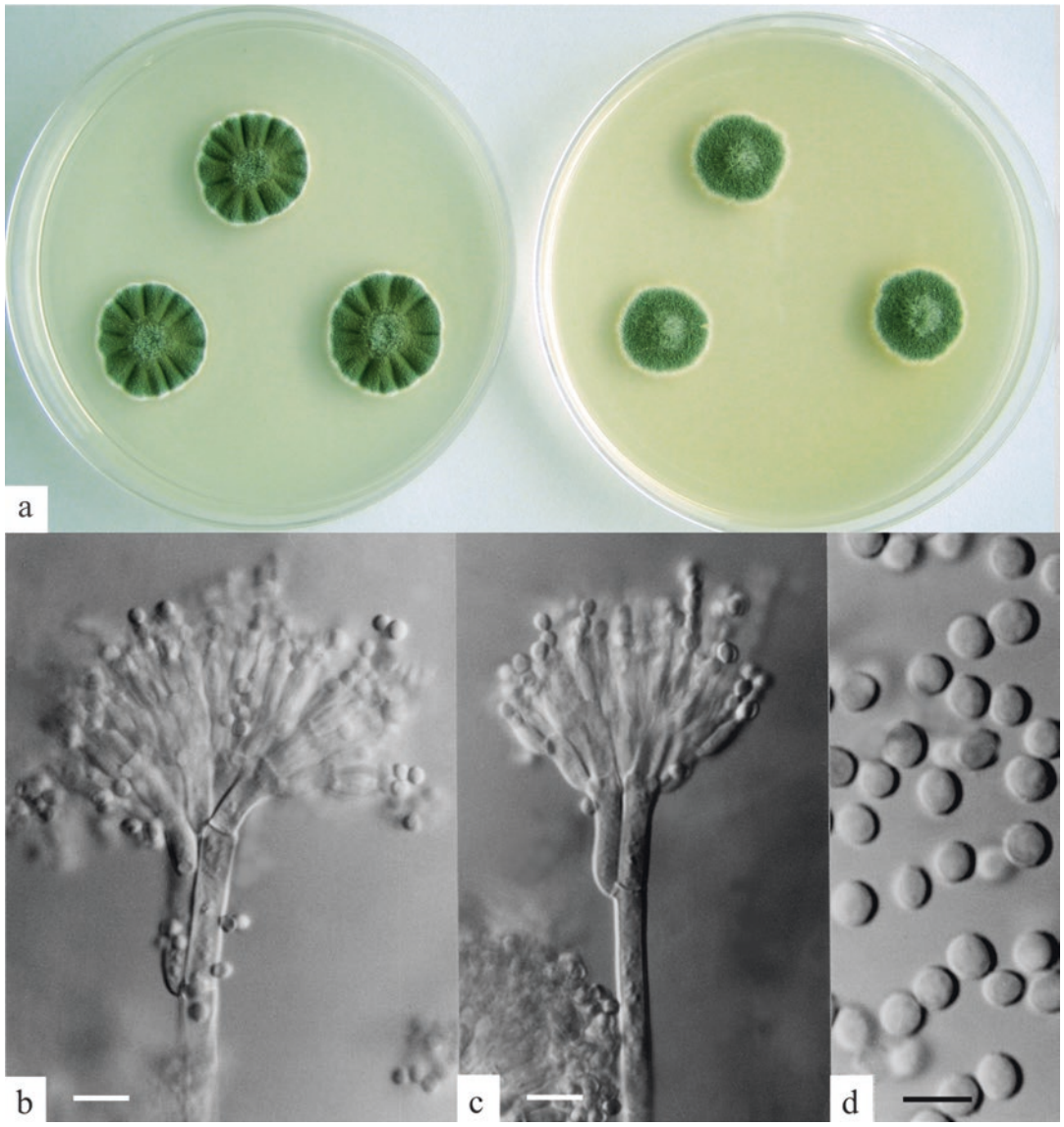

Fig. 7.25 *Penicillium brevicompactum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

plane or radially sulcate, texture granular; clear exudate and red brown soluble pigment sometimes produced; reverse pale, yellow or reddish brown. At 5 °C, microcolonies to colonies up to 4 mm diam produced. No growth at 37 °C.

Colonies on CSN 8–14 mm diam, occasionally larger, growth weak and sparse, medium response neutral to weakly acid (yellow); reverse neutral, occasionally weakly acid.

Conidiophores borne from surface mycelium, stipes usually broad, 500–800 µm long, smooth walled, characteristically bearing compact, broad terverticillate penicilli, usually less than 40 µm long and 40–50 µm across the phialide tips, with quaterverticillate and biverticillate penicilli usually evident also; rami short and broad, often bent away from the axis; metulae in divergent clusters, short and broad, 9–15 µm long, typically inflated apically; phialides in divergent verticils, ampulliform, 6–9 µm long; conidia ellipsoidal, 2.5–3.5 µm long, with walls smooth to very finely roughened, borne in divergent and disordered chains.

Distinctive features *Penicillium brevicompactum* produces compact (though not small) penicilli, often as wide as long. Metulae are short and broad, often apically inflated, fanning out so that the outermost phialides may point in almost diametrically opposed directions. As a result, conidial chains are borne over almost a hemisphere, in a shape suggesting *Aspergillus*.

Identifiers Mycobank MB149773; neotype IMI 40225 (Pitt 1979b); ex-type cultures CBS 257.29, IMI 40225, ATCC 9056, NRRL 862, FRR 862; ITS barcode AY484912; alternative markers *BenA* AY674437, *RPB2* JN406594, *CaM* AY484813 (Visagie et al. 2014).

Physiology The minimum and maximum temperatures for growth of *Penicillium brevicompactum* are −2 and 30 °C, respectively (Mislivec and Tuite 1970), with an optimum near 23 °C. The minimum a_w for germination and growth is 0.78 at 25 °C (Hocking and Pitt 1979), categorising *P. brevicompactum* as one of the most xerophilic Penicillia.

Mycotoxins This species produces mycophenolic acid (Frisvad and Filtenborg 1989; Frisvad and Samson 2004), a weakly toxic compound with an oral LD_{50} of 700 mg/kg in rats (Cole and Cox 1981). It is now used as an immunosuppressant in heart and kidney transplant patients (Bentley 2000). The presence of mycophenolic acid in ginger due to infection by *P. brevicompactum* has been reported (Overy and Frisvad 2005) but is unlikely to be of practical concern.

Ecology Although less common than some other species in this subgenus, *Penicillium brevicompactum* is nevertheless of widespread occurrence especially, because of its xerophilic nature, in dried foods: beans, soybeans, pecans, pistachios and peanuts, health foods and peppercorns (see Pitt and Hocking 1997). It has also been isolated from Brazilian cashew nuts (Freire et al. 1999), brazil nuts and black and white pepper (Freire et al. 2000). It commonly occurs in European meat products, hams, biltong (see Pitt and Hocking 1997) and salami (Cantoni et al. 2007). *P. brevicompactum* can also spoil refrigerated products such as cheese (Kure and Skaar 2000; Kure et al. 2001; Hayaloglu and Kirbag 2007). It has also been reported in tap water in Portugal (Gonçalves et al. 2006). In our laboratory *P. brevicompactum* has been isolated from a range of substrates including spoiled margarine, dairy products, fruit purée, curry paste, sumac, bakery products and bottled water.

Penicillium brevicompactum can also behave as a weak pathogen, having caused spoilage of apples, mushrooms, cassava, potato, pumpkin (see Pitt and Hocking 1997), grapes (Bau et al. 2005, 2006; Patiño et al. 2007) yams (Aboagye-Nuamah et al. 2005) ginger (Overy and Frisvad 2005) and lychees (our observations). This species was present at low levels in a variety of Southeast Asian commodities: rice, mung beans, soybeans, maize and peanuts (Pitt et al. 1993, 1994, 1998).

Additional species *Penicillium glandicola* (Oudem.) Seifert & Samson (synonym *P. granulatum* Bainier) grows at similar rates to *P. brevicompactum* under all standard conditions. *P. glandicola* is distinguished by a granular texture on CYA and MEA, with small coremia apparent at the margins; stipes, rami and metulae with rough walls; and dark green conidia. On CSN, colonies are 12–18 mm diam, and growth is moderate, with an acid (yellow) reaction in both medium and colony reverse; brown soluble pig-

ment is usually also produced. Hocking and Pitt (1979) reported 0.86 as the minimum a_w for germination and growth of *Penicillium glandicola*. This species has been reported to be one cause of taint in wine corks (Daly et al. 1984) and as one species producing 2,4,6 trichloroanisole, a cause of off flavour in coffee (Liardon et al. 1992). *Penicillium glandicola* is one potential source of penitrem A, patulin and roquefortine C, which may be produced in silage (Frisvad and Samson 2004). Natural occurrence of these toxins in foods due to this species has not been reported. This is not a commonly occurring fungus, but it has been isolated from cereals sufficiently frequently to warrant mention here: from wheat, barley, maize and rice. Other sources include peanuts and meat products (see Pitt and Hocking 1997). Identifiers: Mycobank MB114761; holotype Valkenburg, Netherlands, *Rick* in herb. Oudemans (L), July 1901; ex-type cultures CBS 496.75, IMI 154241; ITS barcode AB479308; alternative marker *BenA* AY674415 (Visagie et al. 2014).

References Pitt (1979b, 2000), Domsch et al. (1980), and Samson and Frisvad (2004).

Penicillium camemberti Thom Fig. 7.26

Penicillium candidum Roger
Penicillium caseicola Bainier

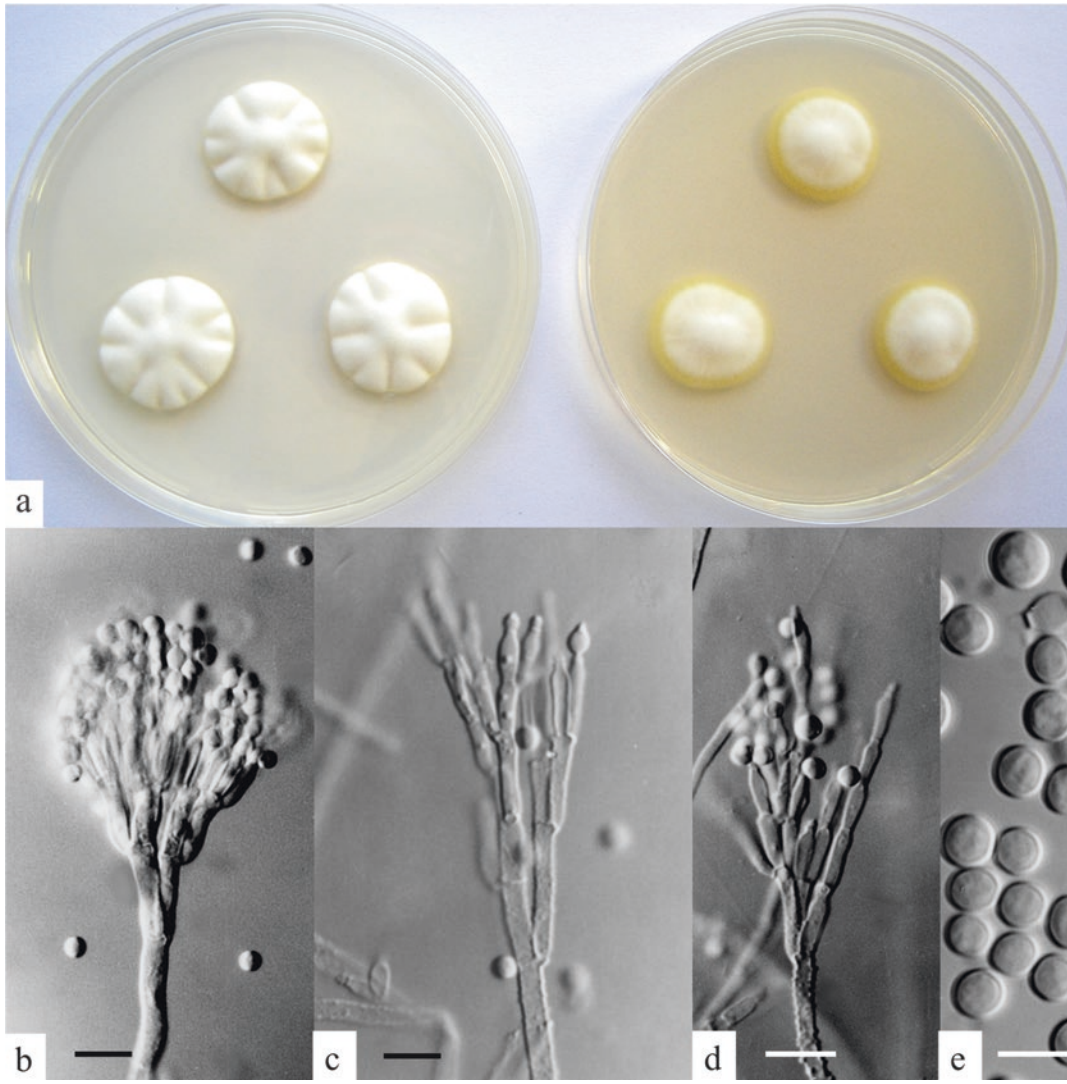

Fig. 7.26 *Penicillium camemberti* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Colonies on CYA 25–35 mm diam, occasionally smaller, plane or lightly radially sulcate, convex, floccose; mycelium white; conidial production usually absent to light, pale grey green or in some isolates persistently white, occasionally heavier, Greyish Green (25-26C3); clear exudate sometimes present; reverse pale, yellow or weakly reddish brown. Colonies on MEA 25–40 mm diam, plane, similar to those on CYA, but without exudate. Colonies on G25N 18–22 mm diam, plane or lightly radially sulcate, similar to those on MEA. At 5 °C, colonies commonly 3–6 mm diam. No growth at 37 °C.

On CSN, colonies 15–20 mm diam, growing strongly, with medium reaction alkaline (purple), though often neutral near the margins; reverse alkaline.

Conidiophores borne from aerial hyphae, stipes 200–400 µm long, with smooth or roughened walls, typically bearing terminal terverticillate or quaterverticillate penicilli, sometimes irregular; phialides ampulliform, 10–12(−15) µm long, with long, wide collula; conidia subspheroidal to spherical, smooth walled, 3.5–5.0 µm long, borne in short, disordered chains.

Distinctive features Apart from its unique habitat in cheeses and cheese factories, *Penicillium camemberti* is readily distinguished by its white, floccose colonies, sometimes coloured pale grey in age by tardily produced conidia. Penicilli are large and often irregular; conidia are white or grey, large and smooth walled. Strong growth occurs on CSN, with an alkaline response in medium and reverse. A violet reaction is produced with Ehrlich reagent (Lund 1995a).

Taxonomy Raper and Thom (1949) recognised two species used for the manufacture of white cheeses, *Penicillium camemberti* and *P. caseicola*. The two species were distinguished by conidial colour, as conidia of *P. caseicola* remained white in age. Later taxonomists concluded that the strains with white conidia are mutants of the grey green parent, which have been selected for properties desirable in cheese manufacture. *P. camemberti* is the earliest valid name for this species (Samson et al. 1977; Pitt 1979b). *P. camemberti* is correctly described as a domesticated species, derived from *P. commune* as the wild type (Pitt et al. 1986; Polonelli et al. 1987).

Identifiers Mycobank MB175171; lectotype IMI 27831 (Pitt 1979b); ex-type cultures CBS 299.48, IMI 27831, ATCC 1105, NRRL 878, FRR 878; ITS barcode AB479314; alternative markers *BenA* FJ930956, *RPB2* JN121484 (Visagie et al. 2014).

Physiology *Penicillium camemberti* grows strongly at refrigeration temperatures. No other information is available.

Mycotoxins *Penicillium camemberti* produces cyclopiazonic acid on synthetic media (Frisvad and Samson 2004). Although most studies have reported that the toxin does not occur in cheese, not all agree. Cyclopiazonic acid is quite toxic (Purchase 1971). Although no direct evidence of toxicity to humans has been reported, absence of toxicity in cheeses cannot be taken for granted, as searches for naturally nontoxigenic stains have been unsuccessful (Leistner 1990).

Ecology *Penicillium camemberti* and its white mutant derivatives are used in the manufacture of soft cheeses such as Camembert, Brie and Neufchatel, and are rarely found away from the local environment surrounding the manufacture of such cheeses. *P. camemberti* has occasionally been isolated from other sources: meats and pecans (see Pitt and Hocking 1997).

References Samson et al. (1977), Pitt (1979b, 2000), Pitt et al. (1986), and Samson and Frisvad (2004).

Penicillium chrysogenum Thom **Fig. 7.27**

Penicillium griseoroseum Dierckx (rejected name)
Penicillium notatum Westling
Penicillium meleagrinum Biourge

Colonies on CYA 35–45 mm diam, occasionally less, radially sulcate, usually low and velutinous; mycelium white to yellowish; conidial production light to moderate, Greyish Turquoise to Dull Green (24-25D-E3-4), in some isolates appearing more yellow green because of the presence of exudate; pale to brilliant yellow or yellow brown exudate and bright yellow soluble pigment usually produced; reverse usually brilliant yellow or

Fig. 7.27 *Penicillium chrysogenum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

yellow brown, but pale or red brown in the absence of soluble pigment. Colonies on MEA 25–40 mm diam, usually plane, low and velutinous, occasionally floccose centrally or somewhat granular; mycelium inconspicuous; conidial production moderate to heavy, Greyish Turquoise to Dull Green (24-26D3, 26-27E3-4); reverse pale, yellowish, yellow brown or reddish brown. Colonies on G25N 18–22 mm diam, usually radially sulcate and dense; reverse pale to bright yellow brown or reddish brown. At 5 °C, at least microcolony formation; sometimes visible colonies up to 4 mm diam produced. At 37 °C, response varying from no growth to colonies up to 5 mm diam.

On CSN, colonies mostly 12–18 mm diam, growth moderate to strong, medium neutral or weakly acid (yellow); reverse similar.

Conidiophores borne from surface or subsurface hyphae, stipes commonly 200–300 µm long, with thin smooth walls, penicilli typically terverticillate, with 1–2 rami, either terminal and appressed or sometimes subterminal and divergent, in that case appearing biverticillate; phialides ampulliform, 7–8(−10) µm long; conidia ellipsoidal to subspheroidal, 2.5–4.0 µm long, smooth walled, borne in long, irregular columns.

Distinctive features *Penicillium chrysogenum* is usually a readily recognisable species: colonies grow rapidly on the standard media at 25 °C, and on CYA produce blue-green conidia, and yellow exudate, soluble pigment and reverse; microscopically penicilli are terverticillate, smooth walled and rather delicate by comparison with those of *P. expansum* or *P. brevicompactum*. However, some isolates lack the yellow pigmentation. Growth on CSN is moderate to strong, with a neutral or weakly acid reaction in both medium and reverse.

Taxonomy The name *Penicillium griseoroseum* predates *P. chrysogenum* (Cruickshank and Pitt 1987; Frisvad and Filtenborg 1989). To overcome this problem, *P. chrysogenum* was conserved under the provisions of the International Code of Botanical Nomenclature (Frisvad et al. 1990; Kozakiewicz et al. 1992). As a matter of interest, it has been shown that *P. chrysogenum* as commonly understood includes a second species, *P. rubens* Biourge, which is the penicillin producer (Houbraken et al. 2011b). Note that the name *P. chrysogenum* has not been replaced by *P. rubens*, as indicated in the otherwise comprehensive account of the use of *P. chrysogenum* as a cell factory (Guzmán-Chávez et al. 2018). Several new species have been segregated from *P. chrysogenum,* but are very similar to it (Houbraken et al. 2012). A sexual state of *P. chrysogenum* (in fact *P. rubens*) has been produced in the laboratory (Böhm et al. 2013). This state has not been found in nature.

Identifiers Mycobank MB165757; lectotype IMI 24314; ex-type cultures CBS 306.48, IMI 24314, ATCC 10106, NRRL 807, FRR 807; ITS barcode AF033465; alternative markers *BenA* AT495981, *RPB2* JN121487, *CaM* JX996273 (Visagie et al. 2014).

Physiology A mesophilic species, *Penicillium chrysogenum* has a minimum temperature for growth of 4 °C, an optimum at 23 °C and a maximum at 37 °C (Mislivec and Tuite 1970; Pitt 1979b). Among the most xerophilic Penicillia, this species has been observed to germinate at 0.78 a_w by Hocking and Pitt (1979), at 0.79 a_w by Armolik and Dickson (1956) and 0.81 a_w by Mislivec and Tuite (1970). The minimum inhibitory concentration of sorbic acid effective against *P. chrysogenum* was reported to be 1–2 mMol/L of undissociated acid across the pH range 4–6 (Skirdal and Eklund 1993). Ethanol (4%, w/w) inhibited the germination of *P. chrysogenum* conidia on PDA (Dantigny et al. 2005).

Penicillium chrysogenum is a major cause of tainting in foods transported in shipping containers. The taint is due to chloroanisole production from chlorinated phenols used to preserve timber in containers. *P. chrysogenum* was isolated from several such timber samples, and shown to carry out this biochemical conversion (Hill et al. 1995).

Mycotoxins *Penicillium chrysogenum* produces roquefortine C, PR toxin and secalonic acids (Frisvad and Samson 2004). Roquefortine C induced *in vivo* inflammatory responses in mouse lungs, following instillation with purified toxin. Mice subjected to high doses (12.5 nMol/g of body weight of animal) of roquefortine C showed signs of trembling and lethargy until 24 h post instillation (Rand et al. 2005). Given by intaperitoneal injection, PR toxin was highly toxic to mice (LD_{50} 5.8 mg/kg; Chen et al. 1982). However, as *P. chrysogenum* rarely causes food spoilage, production of toxic compounds does not appear to be a serious practical issue.

Ecology *Penicillium chrysogenum* is a ubiquitous fungus, and occupies a very wide range of habitats. As a contaminant of foods, it is probably more common even than *P. aurantiogriseum*. The original high penicillin producing strain of *P. chrysogenum* (now known to be *P. rubens*) was isolated from a spoiled cantaloupe (Raper and Thom 1949), and it has occasionally caused spoilage in stored grapes (Barkai-Golan 1974) and carrots (Snowdon 1991). Apart from these records, *P. chrysogenum* is not known as a pathogen. Few

records report spoilage: that of margarine in Australia is an exception (Hocking 1994).

This species has been reported very commonly from cereals: for example rice, wheat, barley, maize, flour (see Pitt and Hocking 1997; Lugauskas et al. 2006) and maize based snack foods (see Pitt and Hocking 1997). Other major sources have been luncheon meats (Ismail and Zaky 1999; Mohamed and Hussein 2004), dry-cured Spanish ham (Rodríguez et al. 1998; Ockerman et al. 2001; Alapont et al. 2014), cheese (Hayaloglu and Kirbag 2007), dried fish, nuts and spices (see Pitt and Hocking 1997). In our laboratory we have isolated *P. chrysogenum* from spoiled bakery products, flavoured dairy products, cheese, margarine, lactose powder and pharmaceutical products.

Several authors have looked at the possibility of using *Penicillium chrysogenum* as a starter culture for fermented European meat production (e.g. aEl-Banna et al. 1987; Philipp and Pedersen 1988; Krotje 1992) and a nontoxigenic strain of *P. chrysogenum* (Pg222) has been used as a starter culture in the ripening of dry-cured ham (Martín et al. 2004, 2006).

References Pitt (1979b, 2000), Samson and Frisvad (2004), and Houbraken et al. 2012).

Penicillium commune Thom Fig. 7.28

Penicillium lanosogriseum Thom

Colonies on CYA 30–37 mm diam, radially sulcate, velutinous to floccose; mycelium white, usually inconspicuous; conidial production moderate, of variable colour, Greyish Turquoise to Dull Green (24-27D-F3-5); exudate usually present, clear to pale brown; reverse usually pale, occasionally yellow, brown or purple. Colonies on MEA 23–30 mm diam, plane or lightly sulcate, low and dense, surface velutinous or fasciculate; mycelium inconspicuous, white; conidial production moderate, Dull Green (26-27D-E3-4); reverse usually uncoloured. Colonies on G25N 18–22 mm diam, plane, sulcate or wrinkled, low to moderately deep, dense, usually fasciculate; mycelium white to yellowish; reverse pale to orange brown. At 5 °C, at least microcolony formation; typically colonies of 2–4 mm diam formed. No growth at 37 °C.

On CSN, colonies 20–26 mm diam, growth strong, medium reaction neutral to alkaline, less commonly weakly acid (pale yellow); reverse alkaline (purple).

Conidiophores borne singly or in fascicles, mostly from subsurface hyphae, stipes 200–400 µm long, or of indeterminate length in fascicles, with walls finely to conspicuously roughened, typically bearing terminal terverticillate penicilli; rami 1–2 per penicillus, phialides ampulliform, 9–11 µm long; conidia spherical, less commonly subspheroidal, 3.5–4.0(−5.0) µm diam, smooth walled, borne in disordered chains.

Distinctive features *Penicillium commune* is similar to *P. aurantiogriseum* in many characteristics. However, *P. commune* produces grey green rather than grey blue conidia on MEA and growth of *P. commune* is often slower on that medium. Stipes are finely roughened to rough, only rarely smooth. *P. commune* is also distinguished by the ability to grow strongly on CSN and show a positive reaction to the Ehrlich test (Lund 1995a). Techniques for rapidly identifying this species in cheese factories were described by Lund et al. (1996).

Immunological procedures suitable for detecting *Penicillium commune* in *P. camemberti* starter cultures were developed by Polonelli et al. (1987). Differentiation of *P. commune* from the closely related species *P. palitans* and *P. solitum* by secondary metabolite analysis was described by Lund (1995b).

Taxonomy Regarded as a rare floccose species by Raper and Thom (1949), *Penicillium commune* was placed in synonymy with *P. puberulum* by Pitt (1979b). Pitt et al. (1986) showed that *P. puberulum* was a synonym of *P. aurantiogriseum*, so *P. commune* was revived as the earliest valid name for the isolates in question. *P. commune* is the wild type ancestor of *P. camemberti* (Pitt et al. 1986; Polonelli et al. 1987).

Identifiers Mycobank MB164241; neotype IMI 39812 (Pitt and Samson 1993); ex-type cultures CBS 311.48, IMI 39812, ATCC 10428, NRRL 890, FRR 890; ITS barcode AY213672; alternative marker *BenA* AY674366 (Visagie et al. 2014).

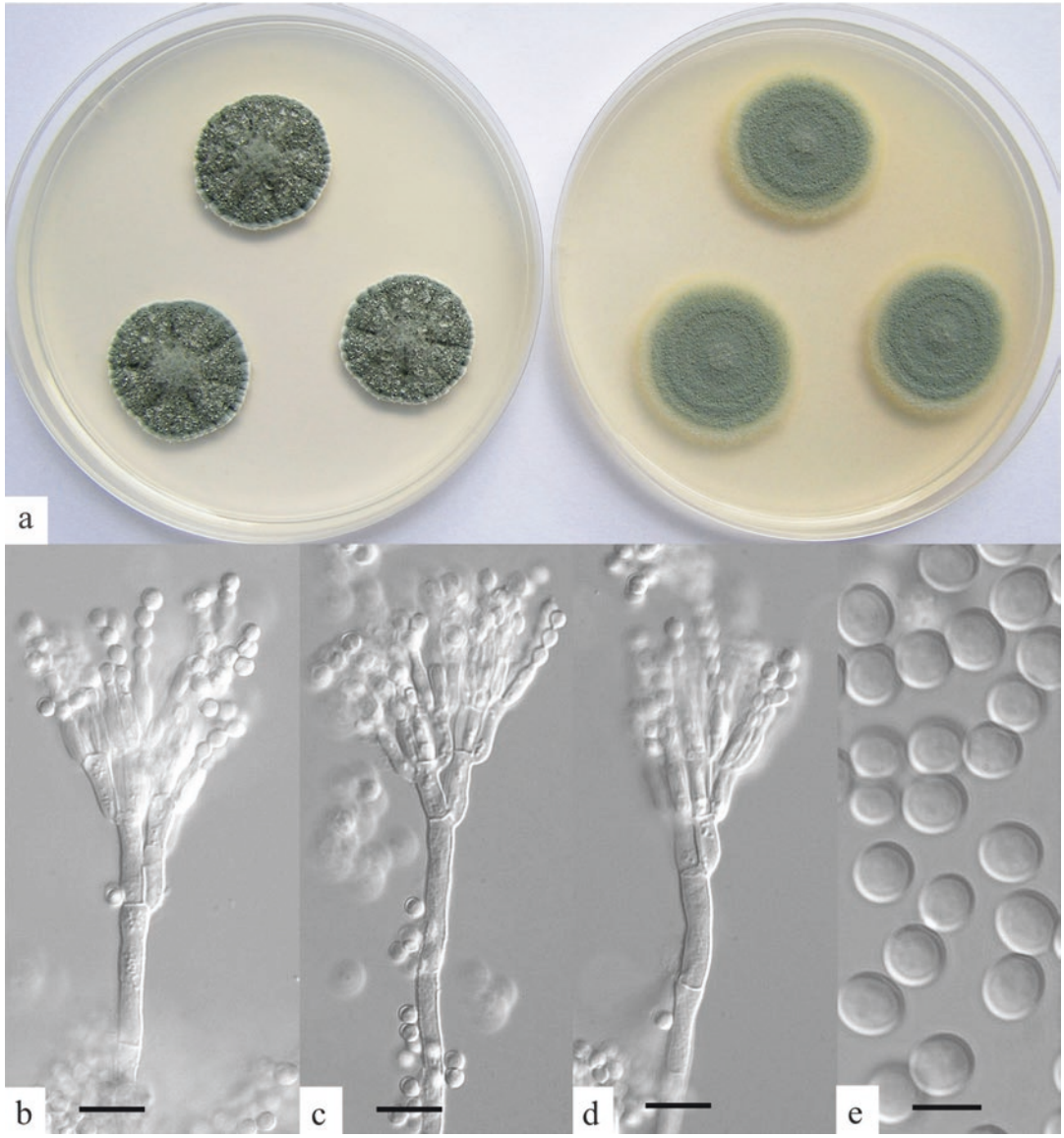

Fig. 7.28 *Penicillium commune* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Physiology Like most other species in *Penicillium* subgenus *Penicillium*, *P. commune* grows rapidly at refrigeration temperatures, has an optimum near 25 °C and a maximum near 35 °C. It is probably capable of growth below 0.85 a_w. *P. commune* failed to grow in an atmosphere of 20% CO_2 and less than 0.5% O_2. However, it grew slowly in an atmosphere of 80% CO_2 and 20% O_2 (Taniwaki 1995).

Mycotoxins Most isolates of *Penicillium commune* produce cyclopiazonic acid (El-Banna et al. 1987; Polonelli et al. 1987; Frisvad and Filtenborg 1989), a toxin described in more detail in Chap. 13. A variety of other possibly toxic compounds may also be produced, including cyclopaldic acid, cyclopolic acid, cyclopiamine, palitantin and rugulovasines (Frisvad and Filtenborg 1989; Lund 1995b). *P. commune* pro-

duced cyclopiazonic acid, rugulovasines and viridicatin on cheese agar (Larsen et al. 2002).

Ecology The primary habitat for *Penicillium commune* in foods is cheese, of which it is the principal cause of spoilage (Lund et al. 1995; Kure et al. 2001, 2004; Hayaloglu and Kirbag 2007). We have isolated it frequently from spoiled soft and hard cheeses in our laboratory, as well as from yoghurt, margarine, sour cream, lactose powder and cakes containing high-fat fillings. It was a major cause of "thread mould" spoilage of vacuum packed cheese blocks in Australia (Hocking and Faedo 1992). The domesticated species *P. camemberti* likely had its origins from wild *P. commune* during centuries of cheese manufacture.

Penicillium commune has been implicated as a cause of "phenol defect" in Italian hams during ripening (Spotti et al. 1988) and it was commonly isolated from dry-cured Spanish hams (Alapont et al. 2014). Other sources include apples (Amiri and Bompeix 2005), pears and associated processing machinery (Sanderson and Spotts 1995), flour (Dragoni et al. 1980) and European sausages (López-Díaz et al. 2001; Papagianni et al. 2007). We isolated *P. commune* from maize, peanuts, soybeans and cowpeas in Southeast Asia, but always at low levels (Pitt et al. 1993, 1994, 1998).

References Pitt (1979b, 2000) and Samson and Frisvad 2004.

Penicillium crustosum Thom Fig. 7.29

Colonies on CYA 30–40 mm diam, plane or less commonly radially sulcate, typically low with a velutinous or granular texture and surface appearing powdery, sometimes with small coremia at the margins or centres; mycelium inconspicuous, white; conidial production heavy over the entire colony area, coloured predominantly Dull Green (26-27D-E3-4) or slightly greyer (26D2), often Greyish Turquoise (24D3) in marginal areas; exudate clear to pale brown or occasionally deep brown; soluble pigment sometimes present, brown; reverse pale or more commonly yellow to orange brown, often intensely coloured at the margins. Colonies on MEA 25–40 mm diam, plane, usually low and velutinous; mycelium subsurface; conidia produced very abundantly, characteristically forming masses with a dry powdery appearance, breaking off in large numbers or in crusts when jarred, coloured Dull Green (26-27C-D3); reverse pale or yellow brown. Colonies on G25N 20–26 mm diam, finely radially sulcate, deep but dense; yellow or brown soluble pigment occasionally produced; reverse pale, yellow brown or brown. At 5 °C, typically macroscopic colonies formed, 2–6 mm diam. No growth at 37 °C.

On CSN, colonies 25–30 mm diam, showing strong growth, medium reaction acid (yellow) or occasionally alkaline (purple); colony reverse alkaline, occasionally weakly so.

Conidiophores mostly borne from subsurface hyphae, stipes commonly 200–400 µm long, with rough walls, bearing terminal penicilli, terverticillate to quaterverticillate; phialides ampulliform, 9–11 µm long; conidia spherical, less commonly subspheroidal or ellipsoidal, 3.0–4.0 µm diam, with smooth walls, borne in long parallel columns, on MEA adhering in masses.

Distinctive features Colonies of *Penicillium crustosum* on CYA often show blue green margins, but mature conidia *en masse* are definitely grey green, not bright yellow green, blue green or blue. All conidiophore elements are large, and stipe walls are definitely rough. When grown on MEA for 7–10 days, nearly all isolates of *P. crustosum* produce enormous numbers of conidia which readily break loose when the Petri dish is jarred. This is a remarkably consistent and useful diagnostic character.

Isolates of *Penicillium crustosum* produced a yellow colour with the Ehrlich test (Lund 1995a). Growth on CSN is strong, usually with an acid medium reaction and an alkaline colony reverse.

Identifiers Mycobank MB262401; lectotype IMI 91917 (Pitt 1979b); ex-type cultures CBS 115503, IMI 91917, ATCC 52044, FRR 1669; ITS barcode AF033472; alternative markers *BenA* AY674353, *CaM* DQ911132 (Visagie et al. 2014).

Physiology Perhaps because of poor recognition, physiological studies on *Penicillium crustosum* are meagre. From our data obtained during growth studies, it can be inferred that it will grow down to ca −2 °C, has an optimum near 25 °C,

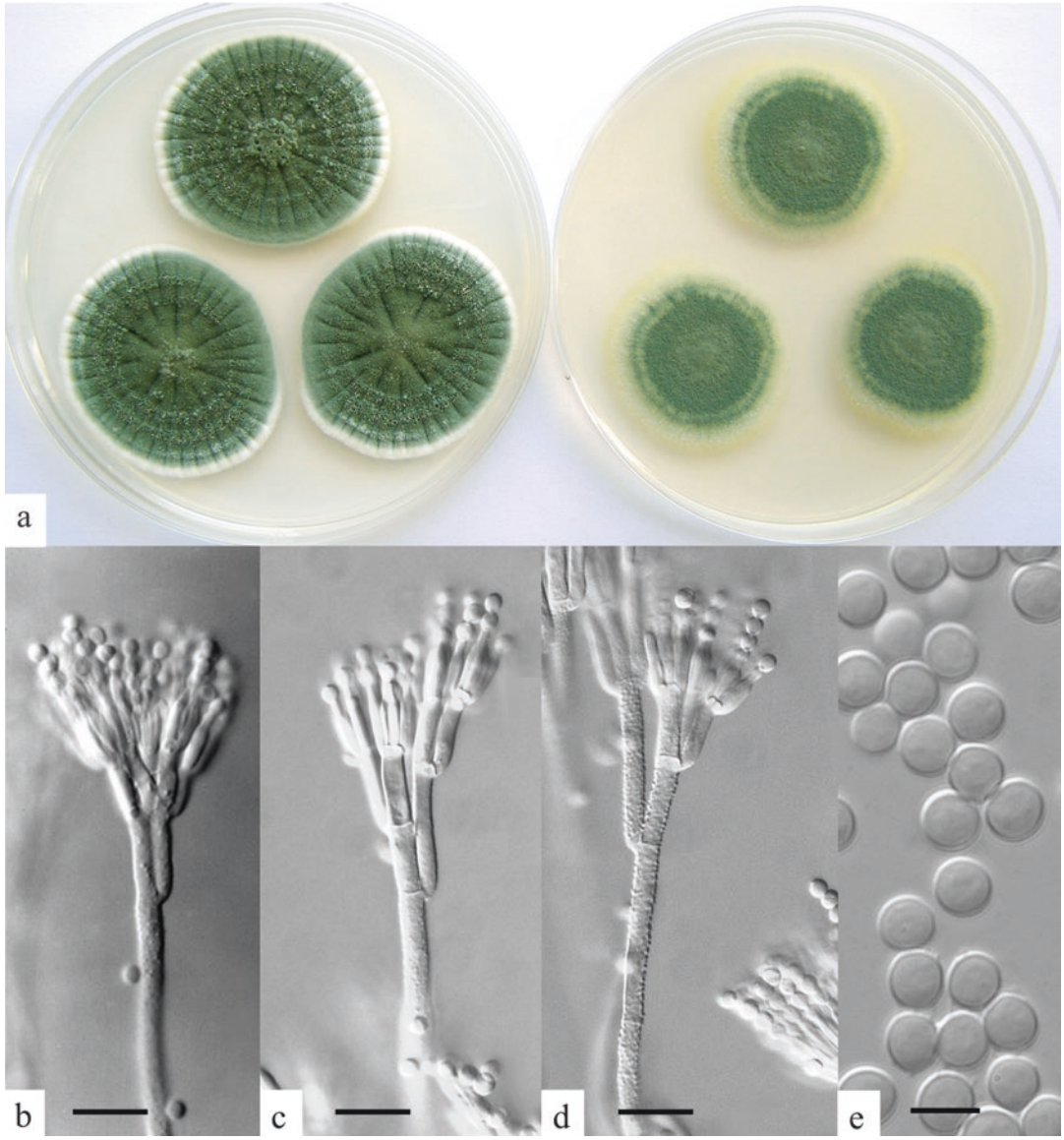

Fig. 7.29 *Penicillium crustosum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

and a maximum about 30 °C. The pH limits for growth are from less than 2.2, optimally 4.5–9.0, to above 10.0 (Wheeler et al. 1991).

This species is an efficient degrader of sorbic acid, producing 1,3-pentadiene, which results in off-odours in many kinds of preserved foods (Kinderlerer and Hatton 1990). Rancidity in oils due to ketone formation is also caused by growth of fungi including *Penicillium crustosum* (Kinderlerer and Hatton 1991). It is also capable of producing chloroanisoles from chlorophenols used as preservatives, leading to taints in chickens from mouldy litter (Curtis et al. 1974), in goods packed in fibreboard cartons, and in paper or jute sacks (Tindale et al. 1989).

Mycotoxins *Penicillium crustosum* is the major producer of penitrem A, a powerful neurotoxin (Pitt 1979c; El-Banna et al. 1987; Frisvad et al. 2006; see Chap. 13). All isolates of *P. crustosum* produce penitrem A at high levels (Sonjak et al. 2005; Frisvad et al. 2006), so the presence of this species in foods (or feeds) is a warning signal (Pitt 1979c; aEl-Banna et al. 1987).

Penitrem A is produced only at high moisture levels, above about 0.92 a_w, with an optimum around 0.995 a_w (ICMSF 1996). This probably explains the relatively low number of reports of poisoning from a very toxic compound produced by a very common fungus.

Penicillium crustosum also produces roquefortine C and the cardiotoxin terrestric acid (Frisvad and Samson 2004). All 120 strains of *P. crustosum* tested were able to produce roquefortine C, viridicatols, and terrestric acid as well as a range of other secondary metabolites (Sonjak et al. 2005). *P. crustosum* also produces thomitrems A and E (Rundberget et al. 2004).

Ecology *Penicillium crustosum* is a ubiquitous spoilage fungus. It was isolated from the majority of cereal and animal feed samples examined by us over three decades. Because of confusion over species names, it was seldom mentioned in the earlier literature, but our examination of isolates published under a range of names indicates that *P. crustosum* has been responsible for spoilage of maize, processed meats, cheese, biscuits, cakes and fruit juices. It has been isolated as a weak pathogen from citrus fruits and melons (Snowdon 1990) as well as apples and pears (Hee et al. 2002; Yun et al. 2006) and onions (in our laboratory). We have also isolated *P. crustosum* from spoiled dairy and jelly desserts, bread, rice product, fresh pistachios and from a soup can implicated in a human mycotoxicosis (Lewis et al. 2005). Other published sources include fresh cabbage, processed meats, hazelnuts, pistachios, peanuts, dried peas and amaranth grain (see Pitt and Hocking 1997), cheese (Lund et al. 1995; Minervini et al. 2002; Hayaloglu and Kirbag 2007) and chestnuts (Overy et al. 2003).

While not commonly isolated from Southeast Asian commodities, *Penicillium crustosum* occasionally occurred in high numbers. Ten per cent of Philippine peanut samples contained this species, with up to 40% of individual nuts infected, resulting in infection of 1% of all kernels examined. Only one of 82 maize samples from Indonesia contained *P. crustosum,* but more than 50% of kernels in that sample were infected. Other sources were sorghum, rice, soybeans, mung beans and pepper, at levels of 1% or less total infected particles (Pitt et al. 1993, 1994, 1998).

Additional species *Penicillium echinulatum* Fassat. differs from *P. crustosum* by producing distinctly roughened conidia which are dark green *en masse*. Growth rates on the standard media are similar, but colonies of *P. echinulatum* usually produce copious clear to pale brown exudate, while conidia on MEA do not usually form crusts. Penicilli of the two species are similar. *P. echinulatum* grows strongly on CSN (22–25 mm diam). Colony reverses are usually alkaline, similar to *P. crustosum*, but unlike *P. crustosum*, an alkaline (violet) reaction is normally produced in the medium.

Penicillium echinulatum has been reported to produce territrems (Frisvad and Samson 2004). It is not commonly isolated from foods but has been reported from pecans, processed meats, rice, katsuobushi (see Pitt and Hocking 1997) and margarine (in our laboratory). Identifiers: Mycobank MB319269; holotype PRM 778523; ex-type cultures CBS 317.48, IMI 40028, ATCC 10434, NRRL 1151, FRR 1151; ITS barcode AF033473; alternative markers *BenA* AY674341, *CaM* DQ911133 (Visagie et al. 2014).

References Pitt (1979b, 2000) and Samson and Frisvad (2004).

Penicillium digitatum (Pers.: Fr.) Sacc

Fig. 7.30

Colonies on CYA 35–55 mm diam, plane, surface texture velutinous to deeply floccose; mycelium white; conidial production moderate to heavy, Greyish Green to Olive (1D-E3); reverse pale or brownish. Colonies on MEA of variable diameter, from 35 mm to greater than 70 mm, plane, relatively sparse, strictly velutinous; conidial production moderate, greenish olive or Dull Yellow Green (30D4); reverse pale or brownish. Colonies on G25N 6–12 mm diam, plane, sparse, often mucoid; reverse pale or olive. At 5 °C, at least germination, sometimes colonies up to 3 mm diam. No growth at 37 °C.

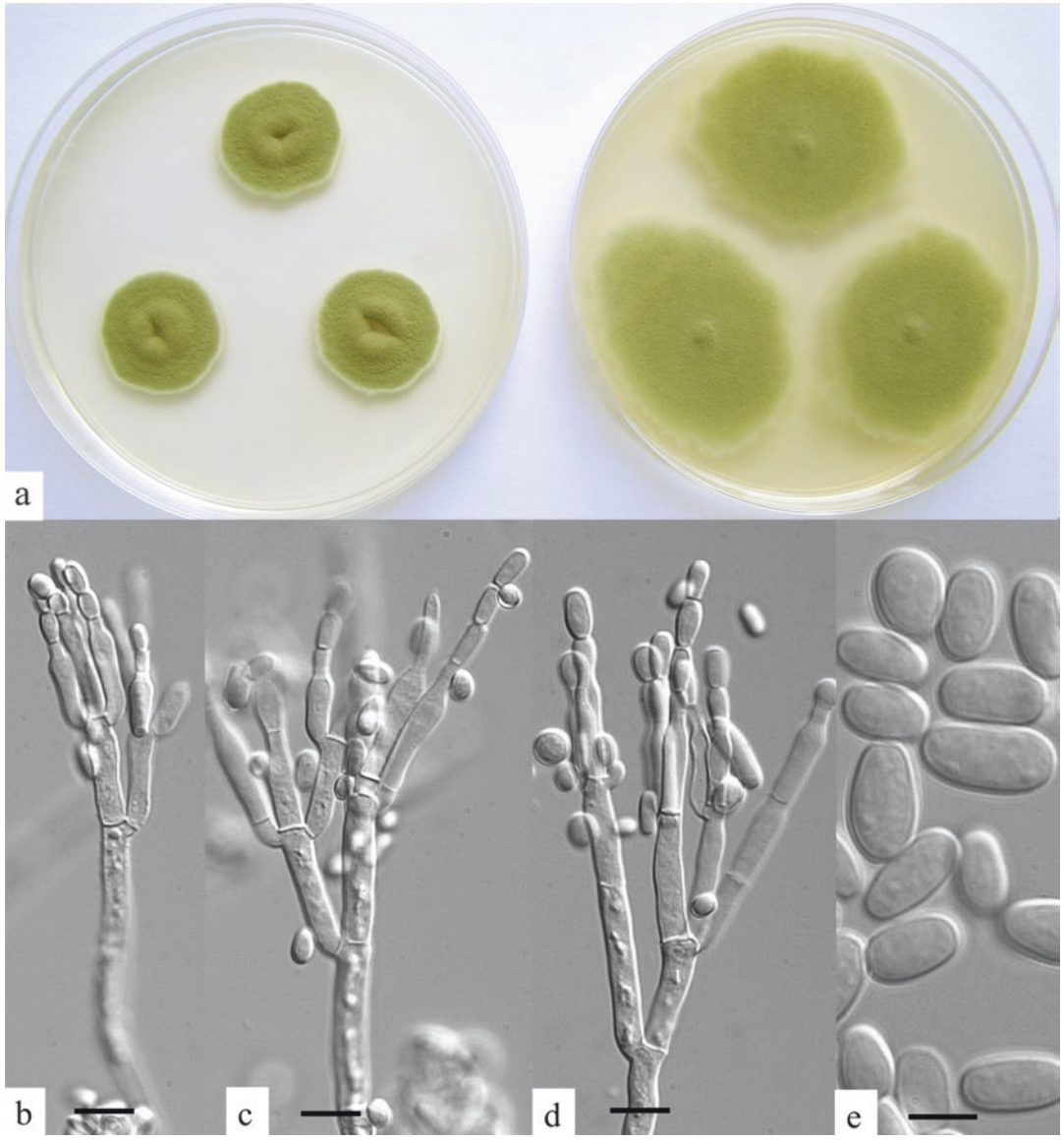

Fig. 7.30 *Penicillium digitatum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

On CSN, colonies 4–10 mm diam, growth weak, with medium pH remaining neutral and with no change in reverse colour.

Conidiophores borne from surface or aerial hyphae, stipes 70–150 µm long, with thin, smooth walls, bearing terminal penicilli, when best developed terverticillate but frequently biverticillate or irregular; phialides broadly ampulliform to cylindroidal, 10–15(−20) µm long, narrowing abruptly to large cylindroidal collula; conidia very large, ellipsoidal to cylindroidal, 6–8(−15) µm long, smooth walled, borne in disordered chains.

Distinctive features The production of conidia coloured yellow green to olive on all substrates, and the close association with rotting fruit of *Citrus* species distinguish *Penicillium digitatum*. It is also distinctive microscopically: no other species of *Penicillium* consistently produces such large phialides or conidia.

Identifiers Mycobank MB169502; lectotype icon in Saccardo, Fung. Ital: Table 894, July 1881 (Pitt 1979b); ex-type culture CBS 112082; ITS barcode KJ834506; alternative markers *BenA* KJ834447, *RPB2* JN121426 (Visagie et al. 2014).

Physiology *Penicillium digitatum* can grow between 6–7 °C and 37 °C (Domsch et al. 1980). The minimum a_w for growth at 25 °C is 0.90 (Hocking and Pitt 1979), but 0.95 a_w at 30 °C and 0.99 a_w at 5 °C (Plaza et al. 2003). No germination was recorded at 0.87 a_w or at 37 °C (Plaza et al. 2003). The minimum inhibitory concentration of sorbic acid preventing growth of *P. digitatum* was 0.02–0.025% at pH 4.7 and 0.06–0.08% at pH 5.5 (Ray-Schroeder 1983).

Mycotoxins This species has not been reported to produce significant mycotoxins. However, about 70% of 24 isolates of *Penicillium digitatum* were toxic to brine shrimp or chick embryos (Faid and Tantaoui-Elaraki 1989).

Ecology The cause of a destructive rot of *Citrus* fruits (Snowdon 1990), *Penicillium digitatum* is universally distributed, with a preference for warmer climates (Domsch et al. 1980). Initial control involves reducing spore build up, in the orchard by removing fallen fruit and in the factory by removing culled fruit (Snowdon 1990). Fungicidal sprays or dips provide the main control measure, but resistance to thiabendazole, benomyl and imazalil has developed in most countries to a greater or lesser degree (see Pitt and Hocking 1997). Control of imazalil-resistant *P. digitatum* by pyrimethanil has been reported (Smilanick et al. 2006). Other control techniques include the use of essential oil vapours (Szczerbanik et al. 2007), UV light in conjunction with either a biocontrol agent or heating (D'Hallewin et al. 2005; Ben-Yehoshua et al. 2005), curing at elevated temperatures, which induces tissue healing (Plaza et al. 2004; Nunes et al. 2007) and vapour heat (Brown et al. 1991). Bacterial and yeast sprays are being considered as biocontrol agents (Torres et al. 2007; Zamani et al. 2009; Pimenta et al. 2010; Chen et al. 2016; Wang et al. 2018).

It has been proposed that the acidification of host tissue by *P. digitatum* enhances the pathogen's virulence and that pH may be an important regulator of gene expression in pathogenesis (Prusky et al. 2004).

Death of an immunocompromised patient from a lung infection due to *P. digitatum* has been reported (Oshikata et al. 2013) but such an occurrence would be very rare.

Penicillium digitatum has occasionally been isolated from other food sources: hazelnuts and pistachio nuts (see Pitt and Hocking 1997), kola nuts (Adebajo 2000), black olives (Heperkan et al. 2006), rice, maize and meats (see Pitt and Hocking 1997). We isolated this species at low levels from Southeast Asian peanuts, soybeans and sorghum (Pitt et al. 1993, 1994).

References Pitt (1979b, 2000), Domsch et al. (1980), and Samson and Frisvad (2004).

Penicillium expansum Thom Fig. 7.31

Colonies on CYA 30–40 mm diam, lightly radially sulcate, moderately deep to very deep, with surface typically tufted (coremial) in one or more annular bands, with adjacent areas velutinous to floccose; mycelium white; conidia produced in moderate numbers, Dull Green (27E3-4); exudate clear to pale orange brown; soluble pigment Brownish Orange near Caramel (6C6); reverse pale to deep brown, often with areas of Brownish Orange (7C-E7-8). Colonies on MEA variable, ranging from 20 to 40 mm diam, plane, some isolates persistently velutinous, others at least partly coremial; mycelium often entirely subsurface; conidial production usually heavy, coloured as on CYA or slightly greyer (27C-D3-4); soluble pigment sometimes produced, coloured as on CYA; reverse pale or, in the presence of soluble pigment, orange

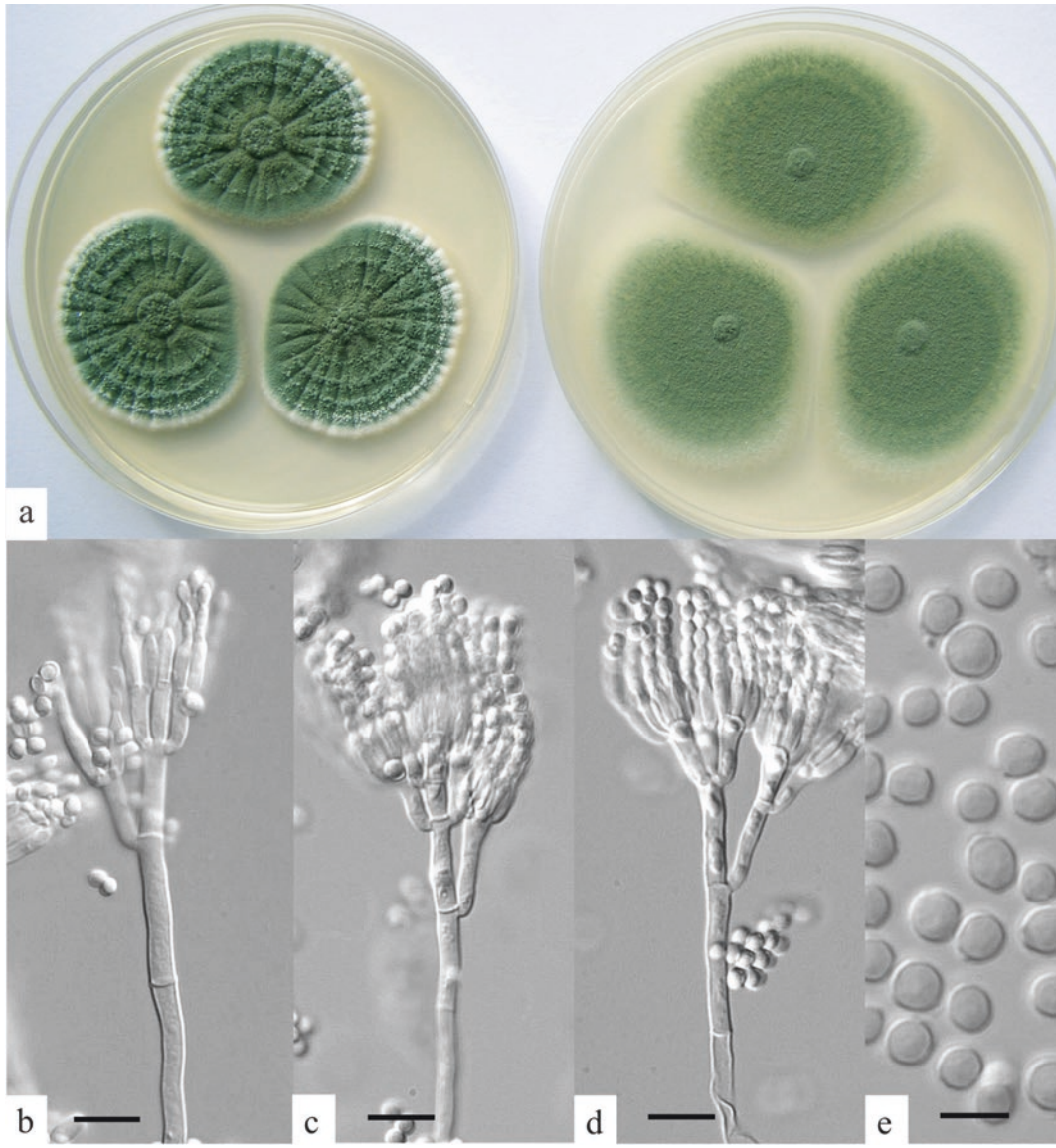

Fig. 7.31 *Penicillium expansum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

brown. Colonies on G25N 17–22 mm diam, radially sulcate, dense, surface texture velutinous to granular; reddish brown soluble pigment sometimes produced; reverse pale, dull brown or reddish brown. At 5 °C, typically colonies of 2–4 mm diam formed, occasionally only microcolonies. No growth at 37 °C.

Growth on CSN strong, 24–30 mm diam, medium acid (yellow); reverse usually acid plus brown soluble pigment, occasionally alkaline (purple).

Conidiophores borne from surface or subsurface hyphae, singly, in fascicles, or in definite coremia, sometimes visible with the unaided eye, stipes 200–500 µm long, with smooth walls, bearing terminal penicilli, typically terverticillate, less commonly biverticillate; rami borne singly, phialides closely packed, ampulliform to almost cylindroidal, 8–11 µm long, with short collula; conidia ellipsoidal, 3.0–3.5 µm long, smooth walled, borne in long, densely packed, irregular chains.

Distinctive features The most important features distinguishing *Penicillium expansum* are dull green conidia, often borne in coremia, smooth walled stipes on both CYA and MEA and, frequently, the presence of orange brown to brown exudate, soluble pigment and reverse colours. Isolates of *P. expansum* inoculated into apples or pears typically produce destructive rots. Growth on CSN is strong; medium and reverse colours are typically yellow. *P. expansum* produces a violet colour with Ehrlich reagent (Lund 1995a).

Identifiers Mycobank MB159382; neotype CBS 325.48 (Samson et al. 1976); ex-type cultures CBS 325.48, IMI 39761, ATCC 7861, NRRL 796, FRR 796; ITS barcode AY373912; alternative markers *BenA* AY674400, *RPB2* JF417427 (Visagie et al. 2014).

Physiology Like most other species in this subgenus, *Penicillium expansum* is a psychrophile: minimum temperatures for growth have been reported as −6 °C (Brooks and Hansford 1923), −3 °C (Panasenko 1967) and at most −2 °C (Mislivec and Tuite 1970). Growth is quite strong at 0 °C (Kuehn and Gunderson 1963). The maximum temperature for growth is near 35 °C (Panasenko 1967). The effect of temperature on the germination time and growth rate of *Penicillium expansum* was modeled by Baert et al. (2007), who provided excellent data indicating that the optimum temperature for growth is 25 °C. The minimum a_w for germination is 0.82–0.83 (Mislivec and Tuite 1970; Hocking and Pitt 1979). Sodium chloride was more inihibitory than glucose, glycerol or sorbitol at a_w values between 0.98 and 0.89, with germination and growth at 15 °C only slightly slower than at 25 °C (Lahlali et al. 2005). At 5 °C, growth ceased at 0.91 a_w (NaCl and glucose) or 0.89 a_w (glycerol, sorbitol) but the incubation period studied was only 25 days (Lahlali et al. 2005).

Penicillium expansum has a very low requirement for oxygen. Golding (1940a, 1945) showed that growth was virtually unaffected by levels of oxygen as low as 2.1%. When reduction in rates of growth did occur, it was at higher temperatures. Growth of *P. expansum* and some other fungi was stimulated by carbon dioxide concentrations up to 15% in air, but growth rates declined at higher CO_2 levels. Spores of *P. expansum* were inactivated by holding in an atmosphere of 13% CO_2 for 21 days, assisting the fumigation of fruit boxes (Cossentine et al. 2004).

Mycotoxins *Penicillium expansum* is an important producer of patulin and citrinin (Harwig et al. 1973; Ciegler et al. 1977). Indeed 98% of 260 isolates of *P. expansum* studied by Andersen et al. (2004) produced patulin and roquefortine C, and 85% produced citrinin.

Penicillium expansum produces patulin as it rots apples and pears. Poor quality control, i.e. the use of rotting fruit in juice or cider manufacture can result in high concentrations of patulin in juice (Brackett and Marth 1979; Watkins et al. 1990). Levels of patulin ranging from 538 to 1822 µg/mL were detected in apple cider stored at 25 °C and up to 396 µg/mL in cider stored at 4 °C (McCallum et al. 2002). Aggressively pathogenic strains of *P. expansum* produced more patulin than less aggressive strains, and patulin synthesis seemed correlated with an ability to increase the acidity of cider, a characteristic of the virulent strains (McCallum et al. 2002). Patulin is important as an indicator

of the use of poor quality raw materials in juice manufacture.

Patulin can be produced by *P. expansum* over the range 0–25 °C at least, the optimum being at 25 °C (Paster et al. 1995), but not at 31 °C (Northolt et al. 1978). The minimum a_w for patulin production by *P. expansum* is 0.95 at 25 °C (Patterson and Damoglou 1986). Patulin is produced over the narrow pH range of 3.2–3.8 in apple juice (Damoglou and Campbell 1986). A modified atmosphere of 3% CO_2 and 2% O_2 completely inhibited patulin production at 25 °C, but production occurred in atmospheres of 2% CO_2 and 10% or 20% O_2 (Paster et al. 1995). Patulin in quite stable in apple juice during storage (Damoglou and Campbell 1986), and pasteurisation at 90 °C for 10 seconds caused less than 20% reduction (Wheeler et al. 1987). No patulin was produced in apples stored under controlled atmospheres of either 2.5% O_2/ 3.9% CO_2 or 1.5% O_2/ 2.5% CO_2 at 1 °C. Patulin was only detected in apples after further storage at 20 °C (Morales et al. 2006, 2007). It has been shown that *P. expansum* can invade apples through the stems during storage (Rosenberger et al. 2006).

Ecology One of the oldest described *Penicillium* species, *P. expansum* has been established as the principal cause of spoilage of pome fruits (Raper and Thom 1949). Isolates of *P. expansum* come predominantly from rotting apples and pears (Snowdon 1990).

Control of the growth of *P. expansum* in fresh and stored fruit relies on several measures, including sprays to prevent *Colletotrichum* infections (which permit entry by *P. expansum*), prevention of damage to fruit, cool storage and rapid processing (Snowdon 1990). A wide variety of other techniques have been advocated, including use of deoxyglucose as a control agent (Janisiewicz 1994; El-Ghaouth et al. 1995), chlorine dioxide in wash water (Roberts and Reymond 1994; Okull et al. 2006) or wash water filtration (Spotts and Cervantes 1993), and calcium chloride infiltration under increased temperature and pressure (Conway et al. 1988; Sams et al. 1993). Biological control using bacteria or yeasts has also been studied, and two commercial products, one based on *Candida oleophila* and the other on *Cryptococcus albidus* have been registered in the United States and South Africa (Janisiewicz and Korsten 2002; Fravel 2005). However, the efficacy of these products still depends on supplementation with low doses of synthetic fungicides to achieve large scale control (Droby et al. 2003). Other compounds including salicylic acid and cytokinin have been examined in conjunction with biocontrol yeasts to enhance the control of *P. expansum* in pears (Yu et al. 2007; Zheng et al. 2007). Two biocontrol strains of *Rhodotorula* significantly reduced disease incidence due to *P. expansum* but at the same time caused increased patulin production in individual lesions (Zheng et al. 2017). Nevertheless, total patulin contamination was reduced. *Metschnikowia* yeast strains have also been tested as biocontrol agents (Spadaro et al. 2013).

Penicillium expansum has been isolated from a wide range of other fruits, including tomatoes, strawberries, avocados, mangoes and grapes (see Pitt and Hocking 1997; Snowdon 1990) indicating that it is a broad spectrum pathogen on fruits. Isolation from fresh vegetables has been uncommon: onions, carrots and cabbages have been reported (Lugauskas et al. 2005).

Isolations from stored foods have been less frequent: in particular, *Penicillium expansum* appears to be much less common on cereals than some other species in this subgenus. Isolations have been reported from maize, wheat, rice and barley (Aziz et al. 2006) and a variety of retail cereal products (Aran and Eke 1987). It is more widespread in other foods, especially meat and meat products (see Pitt and Hocking 1997; Cantoni et al. 2007). Other records include pecans and pistachios (see Pitt and Hocking 1997), peanuts, (Aziz et al. 2006), olives (Kivanç and Akguel 1990), dried beans (see Pitt and Hocking 1997), cheese (Hayaloglu and Kirbag 2007) and margarine (Hocking 1994), health foods, rapeseed, dried fish and frozen fruit pastries (see Pitt and Hocking 1997). We have isolated *P. expansum* from spoiled cheese, fruit yoghurt, jellied fruit desserts, apple sauce and apple juice. Only low levels were found in Southeast Asian commodities (Pitt et al. 1993, 1994).

References Pitt (1979b, 2000), Domsch et al. (1980), and Samson and Frisvad (2004).

Penicillium griseofulvum Dierckx

Fig. 7.32

Penicillium patulum Bainier
Penicillium urticae Bainier

Colonies on CYA 20–25 mm diam, occasionally 30 mm, finely radially sulcate, moderately deep, dense, surface texture granular; mycelium white; conidial production moderate to heavy, at the margins Greyish Green (26-27C3), centrally Greenish Grey (26-27C2); exudate usually present, clear to pale yellow; soluble pigment sometimes produced, reddish brown; reverse pale, dull yellow or brown. Colonies on MEA 15–25 mm diam, plane or rarely radially sulcate, moderately deep, of granular texture; mycelium usually inconspicuous, white; conidial production moderate to heavy, coloured as on CYA; reverse pale to brown.

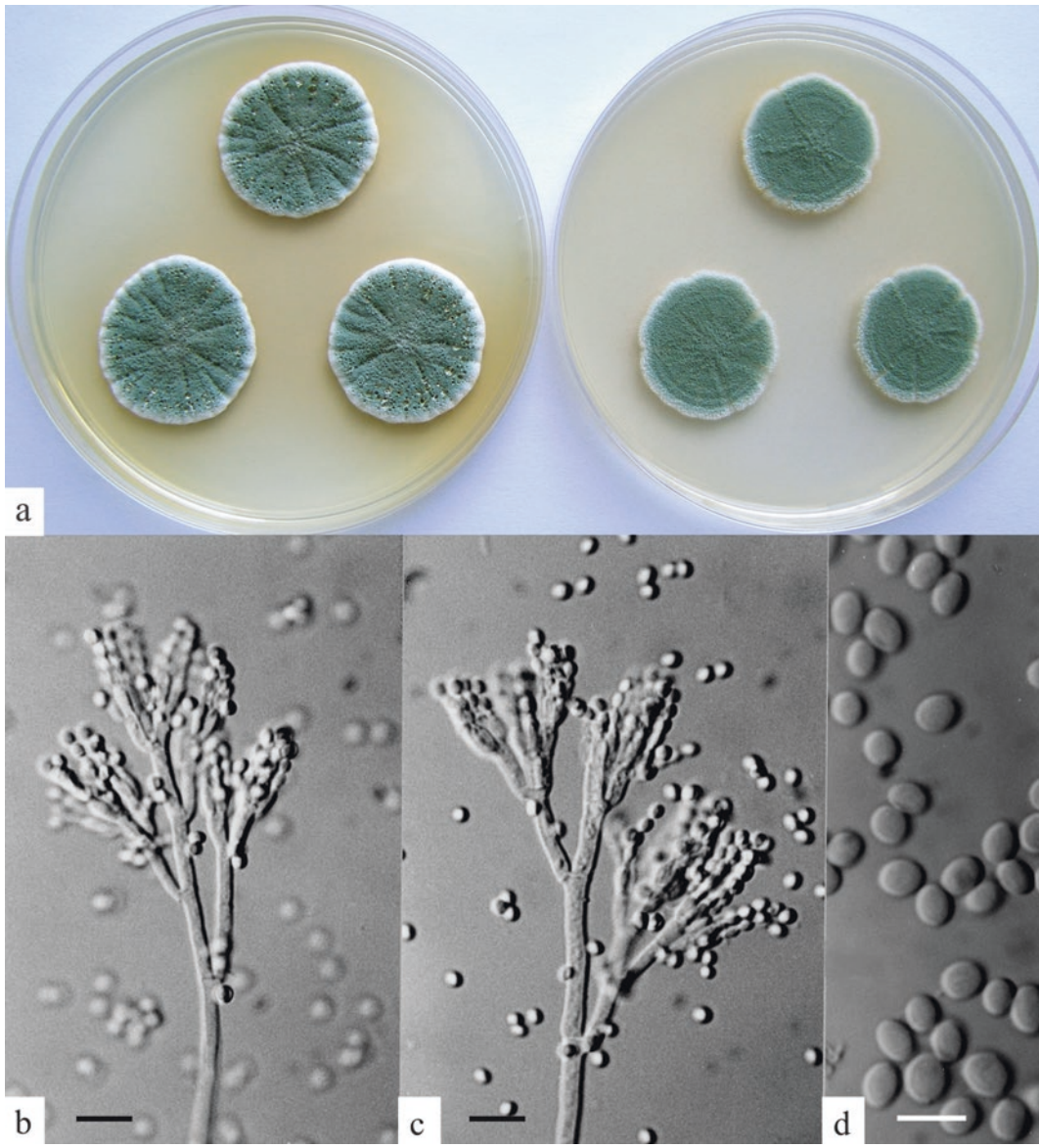

Fig. 7.32 *Penicillium griseofulvum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

Colonies on G25N 16–22 mm diam, plane, low and velutinous at the margins, often floccose centrally; reverse pale. At 5 °C, colonies up to 4 mm diam usually formed. No growth at 37 °C.

On CSN, colonies 18–24 mm diam, growth moderate; medium and reverse reaction neutral.

Conidiophores borne in fascicles, with stipes of indeterminate length, often sinuous, smooth walled, brownish, terminating in distinctive penicilli, sometimes terverticillate, more commonly quaterverticillate and not infrequently with five or even more branch points between stipe and phialide; phialides closely packed, exceptionally short, 4.5–6.0 µm, abruptly tapering to short collula; conidia ellipsoidal, 3.0–3.5 µm long, smooth walled, borne in closely packed, disordered chains.

Distinctive features *Penicillium griseofulvum* is unique in two respects: it produces very short phialides and it bears them on highly branched conidiophores. Other features include the production of grey colonies on CYA and MEA, with only weak greenish overtones, and a neutral reaction on CSN.

Identifiers Mycobank MB120566; neotype IMI 75832 (Pitt 1979b); ex-type cultures CBS 185.27, IMI 75832, ATCC 11885, NRRL 2040, FRR 2040; ITS barcode AF033468; alternative markers *BenA* AY674432, *RPB2* JN121449 (Visagie et al. 2014).

Physiology Growth temperatures for *Penicillium griseofulvum* range from 4 to 35 °C, with an optimum near 23 °C (Mislivec and Tuite 1970). The minimum a_w for germination is 0.81 at 23 °C, and 0.83 at 16 or 30 °C (Mislivec and Tuite 1970).

Mycotoxins *Penicillium griseofulvum*, sometimes reported under its synonyms *P. patulum* and *P. urticae*, produces four mycotoxins, patulin, cyclopiazonic acid, roquefortine C and griseofulvin (Samson and Frisvad 2004). Patulin and cyclopiazonic acid are moderately toxic compounds (see Chap. 13). Griseofulvin, with an intravenous LD_{50} in rats of only 500 mg/kg, is sufficiently nontoxic to permit use as an antifungal antibiotic. Griseofulvin has been of great value in the treatment of cutaneous infections by dermatophytic fungi in both man and animals. However, as well as inhibiting invasive fungi, it also shows mammalian toxicity, acting as a mitotic blocker in the host's dividing cells, such as those of bone marrow, the intestinal lining, and tumours (see Vanden Bossche et al. 2003 and Woodward 2005 for reviews).

Although one outbreak of cattle poisoning in Japan was attributed to *Penicillium griseofulvum* (= *P. maltum* M. Hori & T. Yamamoto) (Hori et al. 1954), recent literature provides no other example of animal toxicity due to this species. It is unlikely that *P. griseofulvum* is a significant contributor to human illness.

Patulin was produced by *Penicillium griseofulvum* down to 0.88 a_w (Lötzsch and Trapper 1978). Production occurred over the range 4–31 °C at 0.99 a_w, and 8–31 °C at 0.95 a_w (Northolt et al. 1978). Potato dextrose broth supplemented with manganese optimised patulin synthesis (Dombrink-Kurtzman and Blackburn 2005).

Ecology Although *Penicillium griseofulvum* can produce lesions when inoculated into apples and pears, it is not a common cause of spoilage of these fruits (Sanderson and Spotts 1995). *P. griseofulvum* occurs quite commonly on cereals and nuts. Records from cereals include barley, maize, rice and wheat (Aziz et al. 2006), flour and bakery products (see Pitt and Hocking 1997) and from nuts: peanuts (Aziz et al. 2006), pecans and pistachios (see Pitt and Hocking 1997). Other sources include dried peas and beans, meats, rapeseed, health foods and frozen fruit pastries (see Pitt and Hocking 1997). *P. griseofulvum* occurs only rarely in tropical foods: we isolated it only from Thai and Philippine peanuts at low levels (Pitt et al. 1993, and our unpublished data).

References Samson et al. (1976), Pitt (1979b, 2000), Domsch et al. 1980), and Samson and Frisvad (2004).

***Penicillium hirsutum* Dierckx** **Fig. 7.33**

Penicillium corymbiferum Westling

Penicillium verrucosum var. *corymbiferum* (Westling) Samson et al.

Colonies on CYA 30–40 mm diam, radially sulcate or less commonly plane, surface texture typically granular to coremial; mycelium white at the margins, white to bright yellow elsewhere; conidial production moderate, Greyish Green to Dull Green near Spanish Green or Jade Green (26-27D-E4-5); exudate usually Violet Brown near Maroon (11E-F8) but occasionally lighter brown; soluble pigment typically produced, deep yellow to orange brown, occasionally reddish brown; reverse in similar colours. Colonies on MEA 25–35 mm diam, plane or rarely sulcate, surface texture usually granular to coremiform, less commonly velutinous to fasciculate;

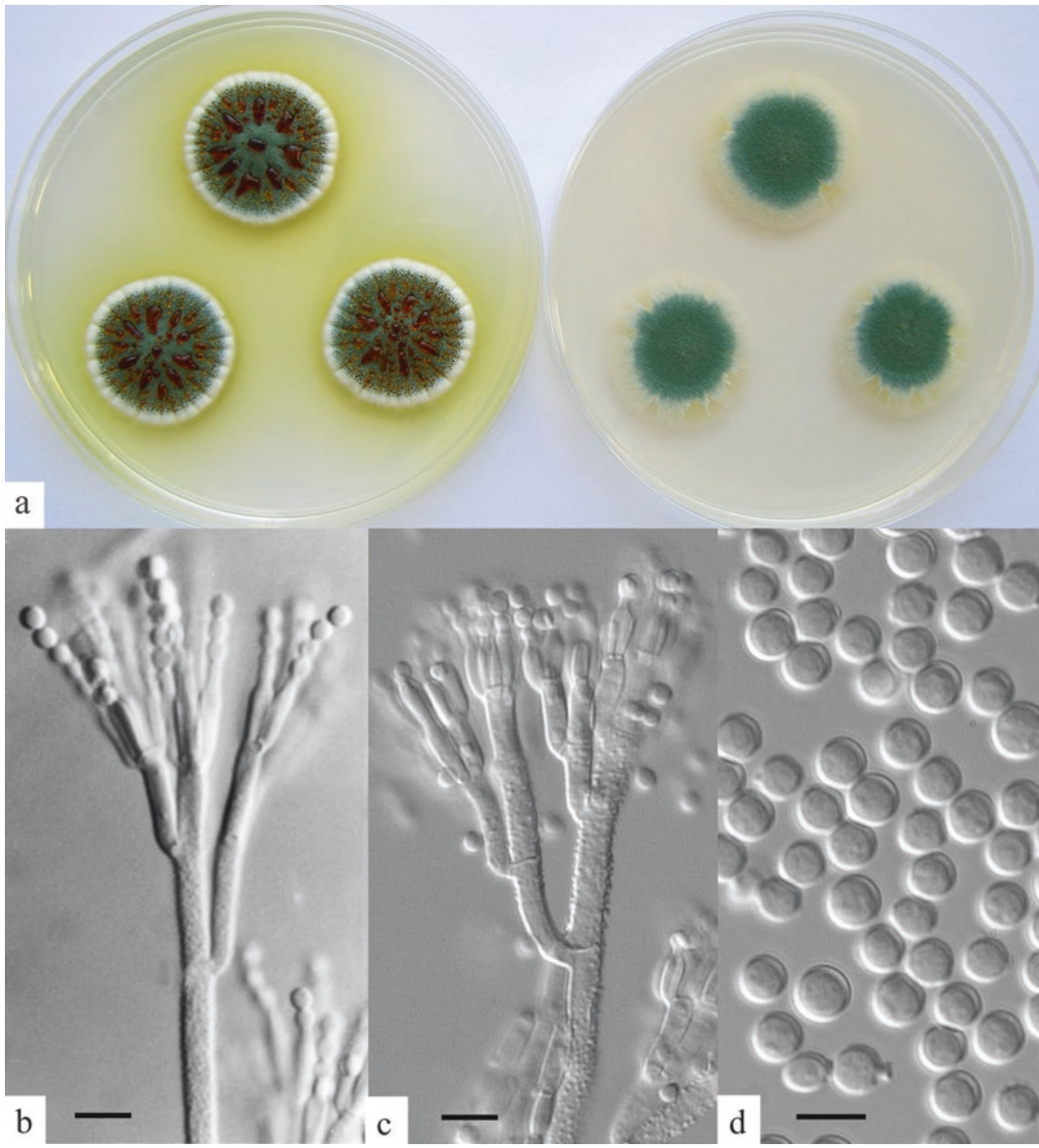

Fig. 7.33 *Penicillium hirsutum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

mycelium white to deep yellow; conidial production moderate, in colours similar to those on CYA; exudate produced by some isolates, deep red; reverse pale to deep yellow, green or orange brown. Colonies on G25N 18–22 mm diam, plane or sulcate, low to deeply floccose; mycelium white to yellow; yellow soluble pigment often produced; reverse bright yellow green or orange to brown. At 5 °C, colonies of 2–5 mm diam usually produced. No growth at 37 °C.

On CSN, colonies 24–30 mm diam, growth strong, medium reaction acid (yellow); reverse acid, sometimes with brown pigment also.

Conidiophores commonly borne from surface hyphae, with stipes 200–500 µm long, but also borne in fascicles and then of indeterminate length, typically with conspicuously roughened walls, bearing large terminal terverticillate to quaterverticillate penicilli; rami 1–2 per penicillus, typically with walls roughened; metulae usually rough walled; phialides ampulliform, smooth walled, 9–12 µm long, abruptly tapering to generally short collula; conidia spherical, 3.0–3.5 µm diam, less commonly ellipsoidal, 3.0–3.5 µm long, with walls smooth to very finely roughened, borne in disordered chains or irregular columns.

Distinctive features On CYA, colonies of *Penicillium hirsutum* are usually deep and fasciculate to coremiform, exudate is reddish or violet brown, soluble pigment is deep yellow to orange brown and conidia are usually pure green, without blue or yellow tones. Conidiophores are frequently borne in fascicles, stipes are usually rough walled and penicilli are large. *P. hirsutum* grows strongly on CSN, and normally produces an acid reaction in both medium and colony reverse.

Taxonomy Raper and Thom (1949: 544) called this taxon *Penicillium corymbiferum*. Samson et al. (1976) reduced this species to varietal status under *P. verrucosum*, but this change in rank was not accepted by Pitt (1979b), who took up the earlier name *P. hirsutum*. This has been accepted (Frisvad and Filtenborg 1989; Pitt and Samson 1993; Visagie et al. 2014).

Identifiers Mycobank MB152720; neotype IMI 40213 (Pitt 1979b); ex-type cultures CBS 135.41, IMI 40213, ATCC 10429, NRRL 2032, FRR 2032; ITS barcode AY373918; alternative markers *BenA* AF003243, *RPB2* JN406629 (Visagie et al. 2014).

Physiology *Penicillium hirsutum* is capable of growth at particularly low temperatures, with a minimum of −4 to −5 °C and relatively rapid growth at −1 °C (Lowry and Gill 1982; Bertolini and Tian 1996). Growth at 0 °C with an optimum temperature of 20 °C was reported by Overy et al. (2005a). It appears to be moderately xerophilic (Gill and Lowry 1982).

Mycotoxins This species produces roquefortine C (Frisvad and Filtenborg 1989; Frisvad and Samson 2004). The production of cyclopiazonic acid was reported by El-Banna et al. (1987), but this was not confirmed by later studies (Frisvad 1989; Frisvad and Filtenborg 1989; Frisvad and Samson 2004).

Ecology Although an infrequently encountered species, *Penicillium hirsutum* is important as a cause of black spot in refrigerated meat (Gill et al. 1981). It has also caused spoilage of fresh asparagus (Saito et al. 2003), onion and garlic bulbs (Overy et al. 2005b), even when stored at −2 °C (Overy et al. 2005a) and refrigerated pear puree (Pitt 1979b: 351). It has been reported from wheat, flour, rice and peanuts (see Pitt and Hocking 1997). We isolated it infrequently from Indonesian soybeans (Pitt et al. 1998).

Additional species *Penicillium allii* Vincent & Pitt [synonym *P. hirsutum* var. *allii* (Vincent & Pitt) Frisvad] is similar to *P. hirsutum* in many respects. However, colonies are larger at 25 °C (35–45 mm diam on both CYA and MEA), and growth does not occur at 5 °C. Colonies are velutinous or minutely fasciculate. Stipes are very rough. Growth on CSN is weak, 15–20 mm diam, with an acid reaction in both medium and colony reverse. Like *P. hirsutum, P. allii* produces roquefortine C (Overy et al. 2005b). This species causes a destructive rot in garlic (Vincent and Pitt 1989; Snowdon 1991, as *P. corymbiferum*), which is invaded in the field (Valdez et al. 2006). In an extensive study, it was found to be the main cause of garlic spoilage in Argentina, a major producing country (Valdez et al. 2009). We isolated it from 2% of all kemiri nuts examined from

Indonesia (Pitt et al. 1998), and at low levels from Philippine maize (our unpublished data). Identifiers: Mycobank 125498; holotype MU Vincent 114; ex-type cultures CBS 131.89, IMI 321505, ATCC 64636, NRRL MB13630, FRR 3184; ITS barcode AJ005484; alternative markers *BenA* AY67433 (Visagie et al. 2014). This species should not be confused with *P. allii-sativi* Frisvad et al., also a pathogen on garlic, which is closely related to to *P. chrysogenum* (Houbraken et al. 2012).

Additional species *Penicillium hordei* Stolk is distinguished from *P. hirsutum* by the production of yellow coremia on MEA, by stipes with walls less conspicuously roughened, and by smaller, rough walled conidia (Stolk 1969). Pitt (1979b) considered these taxa to be a single species, while *P. hordei* was treated as *P. hirsutum* var. *hordei* (Stolk) Frisvad by Frisvad and Filtenborg (1989). Ecological evidence (Stolk 1969; Frisvad and Filtenborg 1989) and differences in isoenzyme patterns (Cruickshank and Pitt 1987) indicated that *P. hirsutum* and *P. hordei* are separate species, and this is now accepted (Pitt and Samson 1993; Frisvad and Samson 2004; Visagie et al. 2014). *P. hordei* isolates always produce the minor toxins roquefortine C and terrestric acid (Frisvad and Filtenborg 1989; Overy et al. 2005b). On CSN, growth is similar to that of *P. hirsutum*, colonies 20–24 mm diam, growth strong, medium reaction acid, reverse acid with deep brown pigments also. This species is of quite common occurrence in barley in Europe (Stolk 1969) and also occurs on tomatoes and in spices (Frisvad and Filtenborg 1989). *P. hordei* also causes blue rot in tulip bulbs (Overy et al. 2005b). Identifiers: Mycobank MB335734; holotype CBS 701.68; ex-type cultures CBS 701.68, IMI 151748, ATCC 22053, NRRL 3700, FRR 815; ITS barcode not known; alternative marker *BenA* AY674347 (Visagie et al. 2014).

References *P. hirsutum*: Pitt (1979b, 2000), Samson and Frisvad 2004. *P. allii*: Vincent and Pitt (1989), Samson and Frisvad (2004). *P. hordei*: Stolk (1969), and Samson and Frisvad (2004).

Penicillium italicum Wehmer Fig. 7.34

Colonies on CYA 30–40 mm diam, plane or radially sulcate, usually low and dense, velutinous to granular, some isolates with minute coremia at the margins; mycelium white; conidia abundant, Greyish Green (25-26C2-3); clear exudate and brown soluble pigment produced by some isolates; reverse usually Brownish Orange to Greyish Brown (7C7-F3). Colonies on MEA 35–55 mm diam, plane and sparse, usually strictly velutinous, sometimes with minute coremia at the margins; conidial formation moderate to heavy, coloured as on CYA; reverse typically Chocolate Brown (6E-F4). Colonies on G25N 12–17 mm diam, plane or sulcate; brown soluble pigment sometimes produced; reverse yellow brown to deep brown. At 5 °C, microcolonies to colonies of 4 mm diam produced. No growth at 37 °C.

On CSN, colonies 10–20 mm diam, growth weak, medium and colony reverse reaction neutral.

Sclerotia produced by some isolates, up to 300 µm diam, brown and soft. Teleomorph unknown. Conidiophores borne from surface or subsurface hyphae, stipes commonly 200–400 µm long, with thin, smooth walls, bearing large regular to irregular terminal terverticillate penicilli; rami 1–2 per penicillus, metulae often apically inflated; phialides 10–14 µm long, roughly cylindroidal in shape, then tapering abruptly to long cylindroidal collula; conidia borne as cylinders, enlarging and rounding with maturation, ellipsoidal to short cylindroidal, 3.0–5.0 µm long, with smooth walls, borne in long, disordered chains.

Distinctive features *Penicillium italicum* is readily recognised in nature as the cause of a destructive bluish grey rot on lemons or other *Citrus* fruit. In culture it forms relatively broad, grey green colonies with deep brown reverse colours, with conidia borne as cylinders, enlarging and rounding during maturation. It grows poorly on CSN, with little or no change in the medium.

Identifiers Mycobank MB62660; neotype CBS 339.48; ex-type cultures CBS 339.48, IMI 39760, ATCC 10454, NRRL 983, FRR 983; ITS

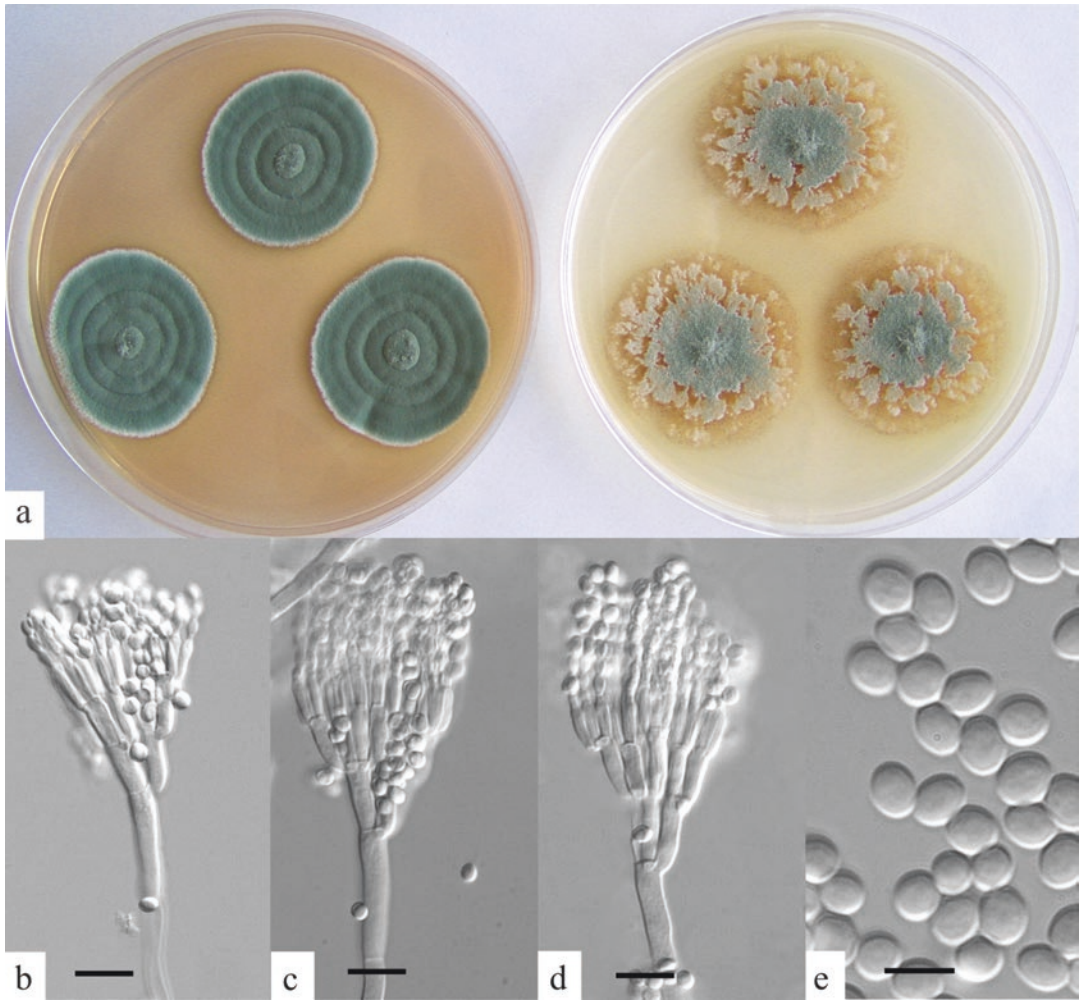

Fig. 7.34 *Penicillium italicum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

barcode KJ834509; alternative markers *BenA* AY674398, *RPB2* JN121496 (Visagie et al. 2014).

Physiology According to Panasenko (1967), *Penicillium italicum* grows between −3 °C and 32–34 °C, with an optimum at 22–24 °C; a minimum of 0 °C was reported by Wyatt et al. (1995). The minimum a_w for germination at 10° and 25 °C is 0.87 (Panasenko 1967; Plaza et al. 2003). The pH range for growth is 1.6 to 9.8 (Panasenko 1967).

Mycotoxins Although *Penicillium italicum* has been shown to exhibit toxicity in some biological assays (Faid and Tantaoui-Elaraki 1989) and an isolate was somewhat toxic to ducklings (Kriek and Wehner 1981), mammalian toxicity has not been shown.

Ecology The primary habitat for *Penicillium italicum* is fruit of *Citrus* species, on which it produces a destructive rot of considerable economic importance (Snowdon 1990). Control measures are essentially similar to those used against *P. digitatum*. *P. italicum* has also shown resistance to commonly used fungicides (Wild 1983; Diaz-Borras et al. 1987; Davé et al. 1989). It has been reported only rarely from other foods: from avocados, tomatoes, sapodillas and rice (see Pitt and Hocking 1997), meat (Papagianni et al. 2007), sausages, salami and cheese (Guillet et al. 2003) and fruit juices (Wyatt et al. 1995).

Additional species *Penicillium ulaiense* H.M. Hsieh & al. is closely related genetically to *P. italicum* (Holmes et al. 1994; Frisvad and Samson 2004). It differs by much slower growth on CYA (15–20 mm in 7 days at 25 °C) and MEA (15–17 mm). Colony appearance is very similar, though reverse colours on CYA and MEA are yellowish orange, and coremium production more marked (Holmes et al. 1994; Frisvad and Samson 2004). Colonies on CSN are 4–8 mm diam, growth weak and sparse, medium and reverse neutral. Like *P. italicum*, *P. ulaiense* is pathogenic on *Citrus* fruits, especially oranges and lemons. It has been isolated from most citrus growing areas around the world (Holmes et al. 1994; Carrillo 1995; Frisvad and Samson 2004). Most isolates are resistant to imazalil, the chemical of choice for control of *P. italicum*. *P. ulaiense* is a weaker pathogen than *P. italicum* (Holmes et al. 1994): they postulated that *P. ulaiense* is a rare species, but has developed imazalil resistance and became significant once *P. italicum* was controlled. *Penicillium ulaiense* grows quite strongly at 5 °C and reaches its optimum near 25 °C; growth is weak at 30 °C and absent at 33 °C (Holmes et al. 1994). It is not known to produce mycotoxins, and has not been found away from *Citrus* fruits or citrus packing houses. Identifiers: Mycobank MB126489; holotype PPEH 29001.87; ex-type cultures CBS 210.92, ATCC 90828, FRR 3647; ITS barcode KC411695; alternative markers *BenA* AY674408 (Visagie et al. 2014).

References *Penicillium italicum*: Samson et al. (1976), Pitt (1979b, 2000), Domsch et al. (1980), Samson and Frisvad (2004). *P. ulaiense*: Holmes et al. (1994), and Samson and Frisvad (2004).

Penicillium nalgiovense Laxa Fig. 7.35

Colonies on CYA usually 28–35 mm diam, radially sulcate, dense and velutinous to quite floccose, conidial production light to heavy, white to Dull Green (25-26B-E3-5); clear exudate produced by most isolates; reverse pale to Golden or Blonde (4-5B-E3-6). Colonies on MEA variable, 10–30 mm diam, plane, dense to somewhat floccose, usually heavily sporing, white to Dull Green (26-27D-E3-5); exudate and soluble pigment absent; reverse usually strongly coloured, in deep orange brown to brown shades (6C-E6-8). Colonies on G25N 14–22 mm diam, plane, dense to floccose, conidial production moderate to heavy, white to dull green, as on CYA; exudate and soluble pigment absent; reverse pale, yellow or golden brown. At 5 °C, germination. No growth at 37 °C.

Colonies on CSN usually 10–18 mm diam, of neutral to weakly acid reaction in both medium and colony reverse.

Conidiophores borne from surface hyphae, stipes commonly 150–400 µm long, or of indeterminate length, often branching irregularly from basal hyphae, smooth walled, bearing irregular penicilli; penicilli very variable within a single preparation, in the most characteristic form a compact terminal terverticillate structure with a divergent subterminal ramus, occasionally quaterverticillate or more complex, less commonly biverticillate, sometimes with intercalary irregular metulae or rami; phialides ampulliform to acerose or nearly cylindrical, 7–10 µm long, with short, broad collula; conidia spherical to subspheroidal, 2.8–3.5 µm diam, smooth walled, borne in short columns.

Distinctive features Closely related to *Penicillium chrysogenum*, this species is distinguished by moderate growth, white to pale green sporulation, usually deep orange reverse colours on MEA, and complex, often irregularly branched penicilli. Growth on CSN is weak.

Taxonomy For a long time, the name *Penicillium nalgiovense* has been applied to a mould used in the manufacture of fermented meats in Europe (Incze and Mihalyi 1976; Dragoni and Marino 1979). Based on the type isolate, from cheese, this species was synonymised with *P. jensenii* by Pitt (1979b). However, comparative studies of isolates from meats with the type of *P. nalgiovense* have shown that all belong to a single species (R.H. Cruickshank and J.I. Pitt, unpublished; Houbraken et al. 2012). While it appeared likely that *P. nalgiovense* was a domesticated form of *P. chrysogenum* (Pitt and Hocking 2009), more recent molecular work has indicated that it is a

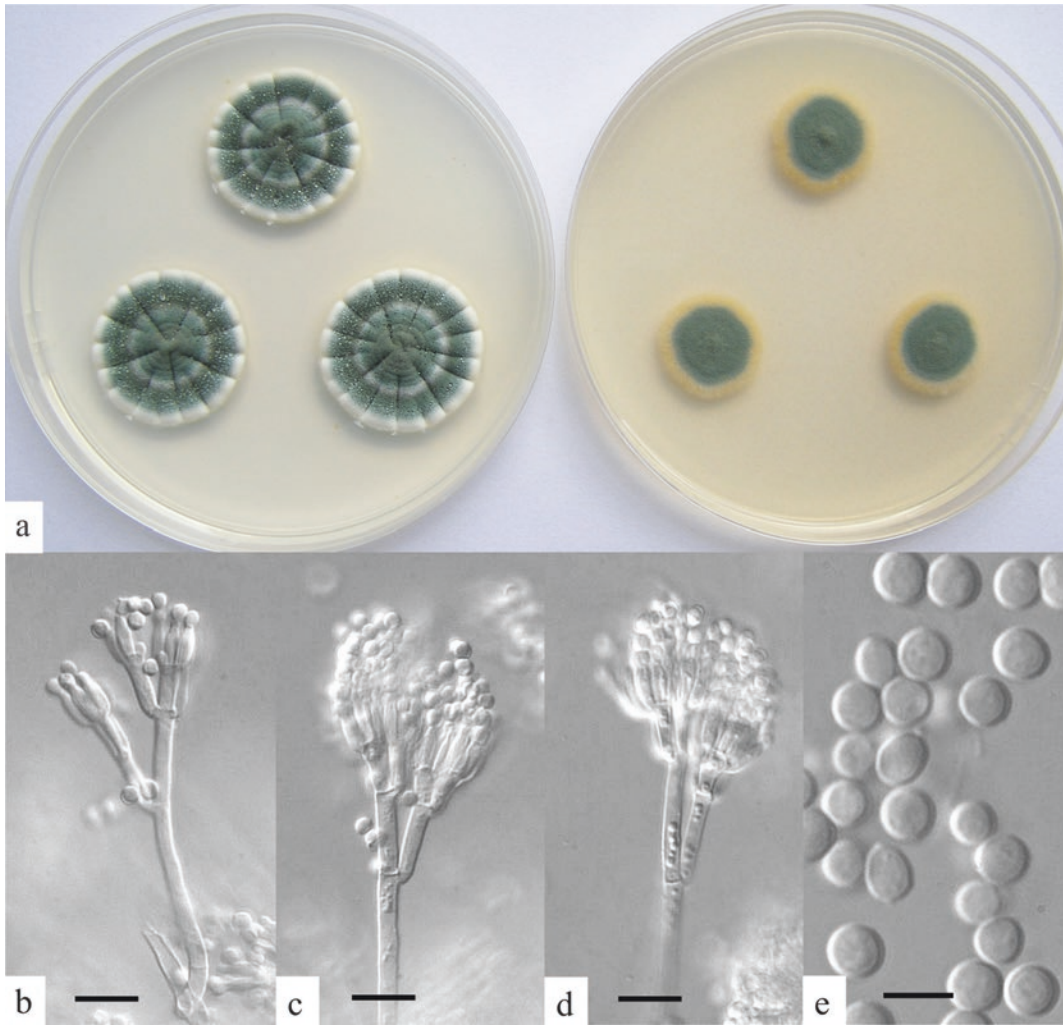

Fig. 7.35 *Penicillium nalgiovense* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

distinct species, related to, but distinct from, *P. chrysogenum* (Houbraken et al. 2012).

Isolates of *Penicillium nalgiovense* show substantial variation in morphology and the species can be divided into six biotypes. These vary in rates of growth on MEA and colour and degree of sporulation on CYA and MEA (Fink-Gremmels et al. 1988).

Identifiers MycoBank MB114239; lectotype CBS 352.48; ex-type culture CBS 352.48, IMI 39804, ATCC 10472, NRRL 911. ITS barcose AY371617, alternative markers *BenA* AY495999, *RPB2* JX996719, *CaM* JX996974 (Visagie et al. 2014).

Physiology As with cheese starter cultures, much is known about the enzymatic and functional properties of strains of this species for use as starter cultures for fermented meat production. Little has been published on the gross physiological properties of interest here. A close similarity to *Penicillium chrysogenum* is to be expected.

Mycotoxins The possibility of mycotoxin production by *Penicillium nalgiovense* has been widely investigated. Most isolates show very low toxicity, and selection of nontoxigenic strains has been successful (Leistner and Pitt 1977; Fink-Gremmels et al. 1988; Hwang et al. 1993; Andersen 1995). Some isolates produce penicillin

(Andersen and Frisvad 1994; Papagianni et al. 2007) and nalgiovensin, not reported to be toxic. Diaportins have been produced by isolates of *P. nalgiovense* on cheese agar (Larsen et al. 2002).

Ecology The main ecological niche occupied by *Penicillium nalgiovense* is as a starter culture for fermented meat production in Europe. Consult the review by Sunesen and Stahnke (2003) for the supply, use and safety of such starter cultures, now widely available commercially.

Other sources are rare: they include nuts (Sahin and Kalyoncuoglu 1994) and cheese (Lund et al. 1995).

References Fink-Gremmels et al. (1988), Samson et al. (1995), Samson and Frisvad (2004), and Houbraken et al. 2012).

Penicillium olsonii Bainier & Sartory Fig. 7.36

Fig. 7.36 *Penicillium olsonii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Colonies on CYA 30–40 mm diam, plane or lightly sulcate, deep but velutinous; mycelium inconspicuous, white to pale brown; conidial production moderate to heavy over the whole colony area, Greyish Green to Dull Green (26-27C-E3); limited amounts of clear exudate sometimes produced; reverse pale yellow or yellow brown. Colonies on MEA 25–35 mm diam, plane and velutinous; mycelium inconspicuous except sometimes centrally, white to buff; conidial production moderate to heavy, Greenish Grey to Greyish Green (26-28C2-3); reverse pale or dull yellow brown. Colonies on G25N 22–28 mm diam, similar in morphology and colouration to colonies on CYA; reverse pale to yellow. At 5 °C, germination of some conidia to formation of microcolonies. No growth at 37 °C.

On CSN, colonies 6–14 mm diam, growth weak and sparse, no reaction in medium or colony reverse.

Conidiophores borne from subsurface hyphae, very large, stipes typically 500–2000 µm long, and 4.0–6.0 µm wide, but occasionally even larger, smooth walled, terminating in characteristic closely appressed multiramulate penicilli, usually terverticillate but sometimes quaterverticillate; rami commonly 2–3 but up to 5–6 per stipe; phialides ampulliform, 9–10 µm long, with short collula; conidia ellipsoidal, 3.0–4.0 µm long, with walls smooth or finely roughened, borne in disordered chains.

Distinctive features Exceptionally large multiramulate terverticillate penicilli borne on long, wide stipes set *Penicillium olsonii* apart from other *Penicillium* species. Average penicilli probably contain some 200 phialides and numbers in excess of 300 are not impossible.

Taxonomy *Penicillium olsonii* is closely related to *P. brevicompactum* (Stolk and Samson 1985; Cruickshank and Pitt 1987; Samson et al. 2004), *P. astrolabium* described from grapes in Portugal (Serra and Peterson 2007) and *P. salamii* which was found to colonise cured meat during seasoning (Perrone et al. 2015).

Identifiers Mycobank MB121021; neotype IMI 192502 (Pitt 1979b); ex-type cultures CBS 232.60, IMI 192502, NRRL 13058, FRR 432; ITS barcode EU587341; alternative markers *BenA* AY674445, *RPB2* JN121464, *CaM* DQ658165 (Visagie et al. 2014).

Physiology Growth was reported down to 0.86 a_w (the minimum tested) by López-Díaz et al. 2002).

Mycotoxins No mycotoxins are produced (Frisvad and Filtenborg 1989) but penicillin production has been reported (Papagianni et al. 2007).

Ecology This was a very poorly recognised species until recently, so reports of its occurrence in foods are limited. It is not a rare species, however. It was reported to cause postharvest rots in tomatoes in Pakistan (Anjum et al. 2018). It comprised 8–15% of isolates from a large number of mould ripened sausages (Andersen 1995; Papagianni et al. 2007). We have seen it occasionally from spoiled margarine in our laboratory. We isolated it from 20% of soybean samples from the Philippines; it was present on up to 34% of individual kernels in a sample, and on 3% of all seeds examined (our unpublished data). It occurred at low levels in maize, peanuts, cashews, sorghum, mung beans and copra from Thailand, from maize and paddy rice in the Philippines (our unpublished data) and from peanuts and mung beans in Indonesia (Pitt et al. 1993, 1994, 1998).

Additional species *Penicillium salamii* Perrone et al. was recently found to be a frequent coloniser of salamis in Europe (Perrone et al. 2015). Phenotypically and molecularly it is close to *P. olsonii*, distinguised by slower growth on CYA and, frequently, the production of brown sclerotia. Physiological properties are likely to be similar to those of *P. olsonii. P. salamii* has a world-wide distribution on cured meats (Perrone et al. 2015). Identifiers: MycoBamk MB909605; holotype CBS H-21341; ex-type culture CBS 135391.

References Pitt (1979b, 2000), Samson and Frisvad (2004), and Samson et al. (2004).

Penicillium roqueforti Thom Fig. 7.37

Colonies on CYA and MEA growing rapidly, 40–70 mm diam, plane or lightly radially sulcate, low, strictly velutinous; mycelium inconspicuous, white; conidial production moderate to heavy, at the margins Greyish Turquoise (24C3-4), predominantly Dull Green (25-26E4), and sometimes centrally Olive Brown (4D3-4); reverse pale, brown, or green to deep blue green, almost black. Colonies on G25N usually 20–22 mm diam, but sometimes with spreading

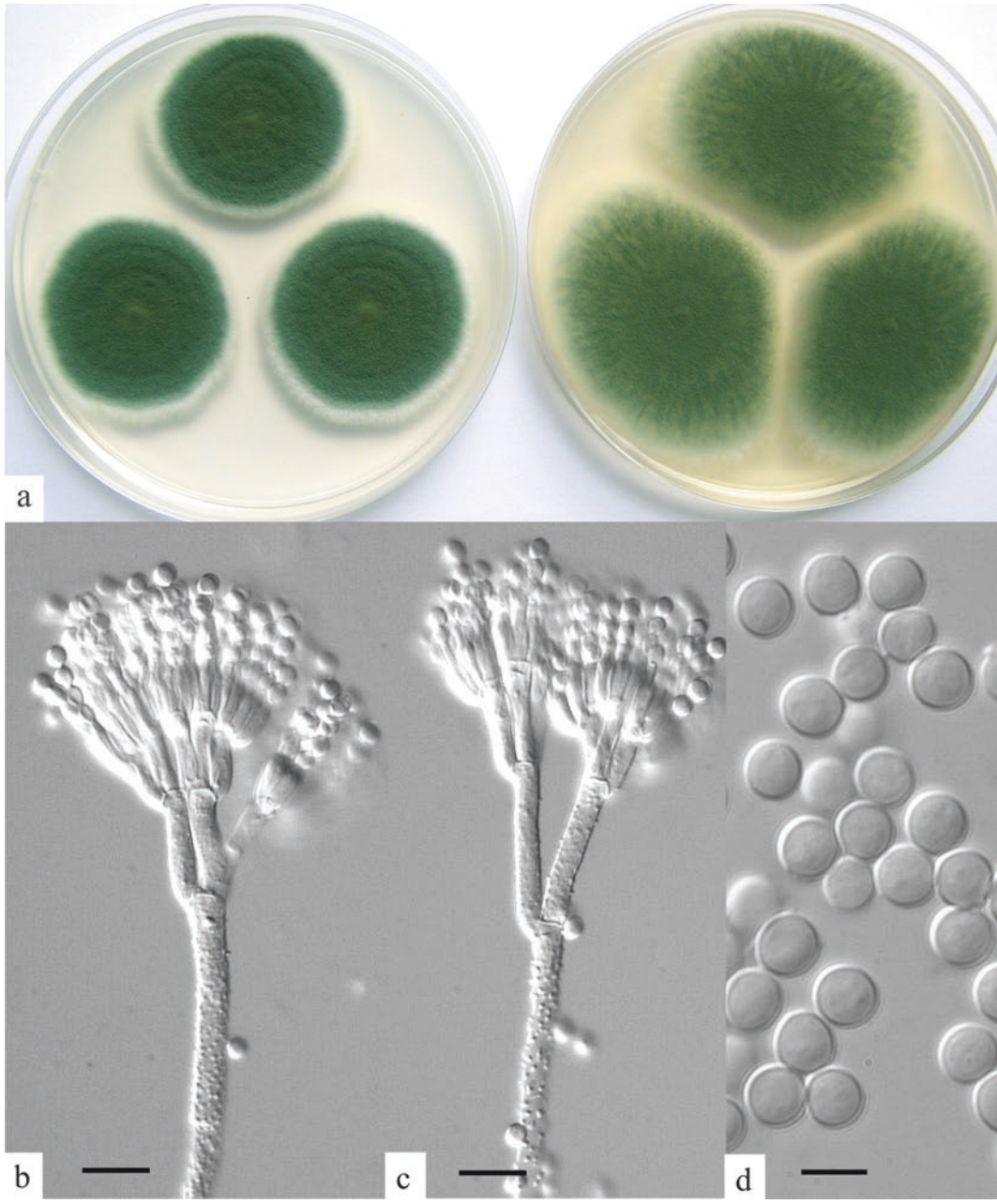

Fig. 7.37 *Penicillium roqueforti* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

submerged margins and then up to 28 mm diam, plane or lightly radially sulcate, colours similar to those on CYA. At 5 °C, colonies usually 2–5 mm diam. No growth at 37 °C.

On CSN, colonies 25–40 mm diam, growth strong (though not deep), medium reaction variable, from acid (yellow) to alkaline (violet); reverse usually alkaline.

Conidiophores borne from subsurface hyphae, stipes 100–200 µm long, with walls characteristically very rough, bearing large terminal penicilli, typically terverticillate, occasionally quaterverticillate, or rarely biverticillate, with elements appressed; rami rough walled; metulae occasionally rough walled; phialides ampulliform, commonly 8–10 µm long, with short collula; conidia spherical, 3.5–4.0(−6) µm diam, with thin, perfectly smooth walls, dark green, borne in long, closely packed, irregular to disordered columns.

Distinctive features *Penicillium roqueforti* grows very rapidly and produces low and velutinous dark green colonies; stipes have very rough walls and conidia are large, spherical and smooth walled. Growth on CSN is rapid and colony reverse is usually alkaline.

Taxonomy The species described as *Penicillium roqueforti* by Pitt (1979b, 2000) and Pitt and Hocking (1997) comprised more than one taxon (Frisvad and Samson 2004). Two varieties were recognised by Frisvad and Filtenborg (1989): *P. roqueforti* var. *roqueforti*, used in cheese manufacture, and *P. roqueforti* var. *carneum*, a spoilage fungus. These varieties differed in mycotoxin production and to some extent in ecology. On the basis of substantial differences in ITS DNA, Boysen et al. (1996) separated *P. roqueforti* into three species: *P. roqueforti*, *P. carneum* and *P. paneum*. Subsequent metabolic analyses have confirmed the existence of three separate species (Karlshøj and Larsen 2005). These three species are difficult to distinguish morphologically. However, *P. roqueforti* isolates produce a deep green colour in the reverse on CYA, whereas the reverses of the other two species are yellow brown. *Penicillium roqueforti* and *P. carneum* produce a violet colour with the Ehrlich reaction, whereas *P. paneum* does not (Frisvad and Samson 2004). *P. carneum* colonies on CYA produce a strong earthy smell, whereas those of *P. paneum* do not (Frisvad and Samson 2004).

During a survey of fungi occurring on cold stores apples in the Netherlands, one isolate of a species related to *P. roqueforti* produced a sexual stage in laboratory culture. It was described as a new species, *Penicillium psychrosexualis* (Houbraken et al. 2010). A sexual stage was reported to have been induced in *P. roqueforti* by Ropars et al. (2014), a discovery of great importance in assisting the development of improved cheese making strains. Ropars et al. (2014) also reported great genetic diversity within this species. However, in a comprehensive study of 164 isolates of *P. roqueforti* from 120 cheeses from around the world, plus 21 strains from other substrates, it was concluded that all belonged to a single species. A wide range of morphological and molecular methods were used. No evidence for cryptic species was found, though small observed differences often related to the different substrates and types of cheeses (Gillot et al. 2015).

Identifiers Mycobank MB213525; lectotype IMI 24313 (Pitt 1979b); ex-type cultures CBS 221.30, IMI 24313, ATCC 10110, NRRL 849, FRR 849; ITS barcode EU427296; alternative markers *BenA* AF000303, *RPB2* JN406611, *CaM* HQ442332 (Visagie et al. 2014).

Physiology *Penicillium roqueforti* appears to have the lowest oxygen requirement for growth of any *Penicillium* species. Growth is unaffected by reduction in pressure down to 75 mm Hg, i.e. only 10% of standard atmospheric pressure (Golding 1940a). Growth of *P. roqueforti* is little affected by oxygen concentrations down to 4.2%, and is stimulated by carbon dioxide concentrations up to 15% in air (Golding 1940b, 1945). In an atmosphere with less than 0.5% O_2, *P. roqueforti* grew in the presence of 20% but not 40% CO_2 at 25 °C, and growth and sporulation also occurred in an atmosphere containing 20% O_2 and 80% CO_2 (Taniwaki 1995). *P. roqueforti* grew on rye bread in a modified atmosphere comprising 0.03% O_2 and almost 100% CO_2 (Suhr and Nielsen 2005). These properties are undoubtedly a major reason for the dominant growth of this species in ripening cheeses.

Like other species in this subgenus, *Penicillium roqueforti* is a psychrophile. It grows vigorously at refrigeration temperatures, but not above 35 °C (Moreau 1980). At a_w 0.89 and 0.92, conidia of *P. roqueforti* germinated at 25° and 30 °C but not at 37 °C or at lower a_w values (Gock et al. 2003). It is tolerant of alkali, the pH range for growth being 3–10 (Moreau 1980). Furthermore it is highly tolerant of weak acid preservatives, being able to grow in the presence of 0.5% acetic acid, a property used as the basis for a selective medium (Engel and Teuber 1978). Growth was stimulated in the presence of 0.3% propionic acid at 0.97 a_w (Suhr and Nielsen 2004). Tolerance to sorbic acid has also been observed (Liewen and Marth 1984; Bullerman 1984 and our unpublished data), accompanied by degradation of the preservative (Finol et al. 1982) and development of a "kerosene" taint due to 1,3-pentadiene formation (Liewen and Marth 1985; Daley et al. 1986). *P. roqueforti* also causes taints in bread due to production of a mixture of 2-methylisoborneol and various 8-carbon alcohols and ketones (Harris et al. 1986).

Mycotoxins *Penicillium roqueforti* produces PR toxin in pure culture (Frisvad et al. 2006; Nielsen et al. 2006), and sometimes in cheese at very low levels (Scott 1981; Teuber and Engel 1983; Schoch et al. 1984), where it is degraded over time (Chang et al. 1993; Siemens and Zawistowski 1993). It was not detected in Tulum cheese, a traditional Turkish cheese (Erdogan and Sert 2004) or blue cheese in Europe (Finoli et al. 2001). *P. roqueforti* also produces roquefortine C and mycophenolic acid (Frisvad and Samson 2004; Nielsen et al. 2006), sometimes in cheese (Finoli et al. 2001) but these compounds have very low toxicity (Scott 1981).

Ecology Although best known for its role in the manufacture of Roquefort and related cheese types, *Penicillium roqueforti* is also a widely distributed spoilage fungus (though some reports may actually be *P. carneum* or *P. paneum*). Its ability to grow rapidly at refrigeration temperatures makes it a common cause of spoilage in cool stored foods, both commercial and domestic. Like *P. commune*, it is a common cause of cheese spoilage (see Pitt and Hocking 1997; Finoli et al. 2001; Erdogan et al. 2003; Erdogan and Sert 2004; Kure et al. 2004; Hayaloglu and Kirbag 2007). One source can be raw milk (Frevel et al. 1985; Engel 1986).

Penicillium roqueforti has caused a particular problem with spoilage of packaged rye bread in Europe (see Pitt and Hocking 1997), due to preservative resistance mentioned above (though *P. paneum* may also be responsible). *P. roqueforti* is less frequently isolated from cereals than some other species in this subgenus, but has been reported from barley, rice, flour, baked goods, and refrigerated dough products (see Pitt and Hocking 1997). Frequent reports of *P. roqueforti* from meats and meat products (dried beef, salami and cured meats, see Pitt and Hocking 1997) are probably due to *P. carneum*. *P. roqueforti* has also been reported from peanuts, pecans, hazel nuts and walnuts, almonds and dried peas and fresh vegetables (see Pitt and Hocking 1997). *P. roqueforti* is uncommon in the tropics: we isolated it at low levels in peanuts from the Philippines and Indonesia (Pitt et al. 1998 and our unpublished data).

Additional species *Penicillium carneum* (Frisvad) Frisvad is almost indistinguishable from *P. roqueforti* using morphological criteria (see Taxonomy). *P. carneum* is associated with spoilage of meat products, but has also been isolated from silage, rye bread, water, beer, cheese, mouldy bakers yeast and cork (Frisvad and Samson 2004). It has been reported to be a pathogen on stored apples (Peter et al. 2012; Yin et al. 2017). *P. carneum* produces patulin, penicillic acid (Frisvad and Filtenborg 1989), penitrem A, mycophenolic acid and roquefortines (Frisvad and Samson 2004; Nielsen et al. 2006). An isolate identified as *P. crustosum* involved in mycotoxicosis of a man who drank mouldy beer (Cole et al. 1983) was reidentified by Frisvad and Samson (2004) as *P. carneum*. Identifiers: Mycobank MB415652; holotype IMI 293204; ex-type cultures CBS 112297, IMI 293204, ATCC 58627, NRRL 25170, FRR 6121; ITS barcode HQ442338; alternative markers *BenA* AY674386, *RPB2* JN406642, *CaM* HQ442322 (Visagie et al. 2014).

Additional species *Penicillium paneum* Frisvad is very similar to *P. carneum* (see Taxonomy). It has been reported from mouldy rye bread, baker's yeast, silage and cassava chips (Frisvad and Samson 2004). It has also been

reported to be a pathogen on stored apples (Yin et al. 2017). *P. paneum* produces patulin and roquefortine C (Frisvad and Samson 2004; Nielsen et al. 2006). Identifiers: Mycobank MB415570; holotype C 25000; ex-type cultures CBS 101032, FRR 6122; ITS barcode HQ442346; alternative markers *BenA* AY674387, *CaM* HQ442331 (Visagie et al. 2014).

References *P. roqueforti*: Samson et al. (1976), Pitt (1979b, 2000), Moreau (1980), Frisvad and Samson (2004). *P. carneum* and *P. paneum*: Boysen et al. (1996), Frisvad and Samson (2004), and Karlshøj and Larsen (2005).

Penicillium solitum Westling Fig. 7.38

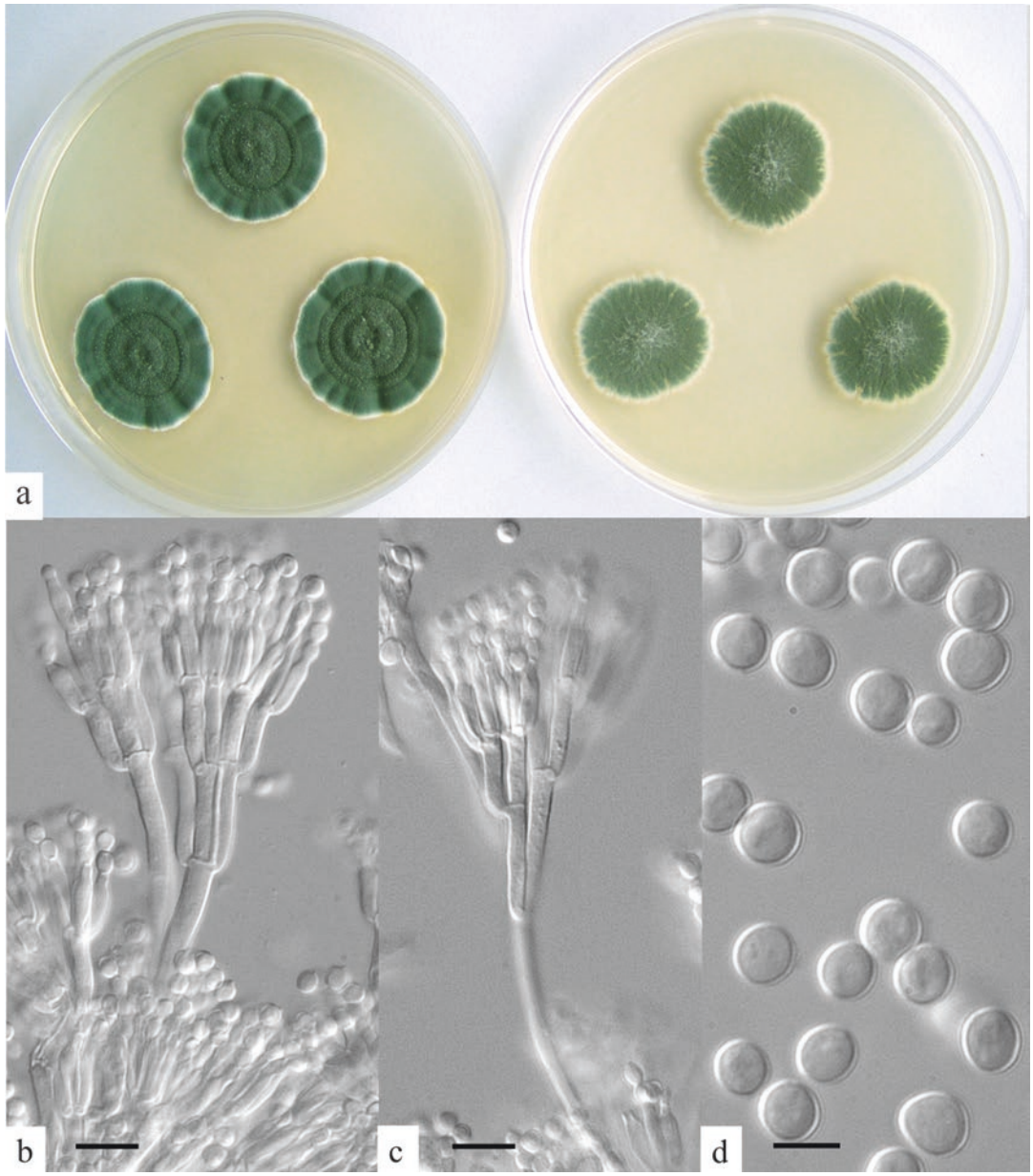

Fig. 7.38 *Penicillium solitum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

Colonies on CYA 22–28 mm diam, usually lightly radially sulcate, less commonly plicate or plane; low to moderately deep, very dense, with surface texture velutinous or less commonly granular or fasciculate; mycelium white, usually visible only at the margins; conidia usually produced abundantly, dark bluish green or Dark Green (25-26E-F4-7); exudate usually absent or inconspicuous, occasionally abundant or even dominating colony appearance, clear; reverse usually pale or uncoloured, uncommonly yellow or brown, or light salmon to orange as on MEA. Colonies on MEA 20–28 mm diam, plane, low with velutinous to distinctly granular texture, or less commonly floccose; mycelium white or occasionally yellow, usually inconspicuous, but uncommonly visible over much of the colony surface; conidia abundant in most isolates, coloured as on CYA, rarely confined to central areas or absent; reverse characteristically Greyish Orange to Brownish Orange (5-6B-C4-6). Colonies on G25N usually 16–22 mm diam, closely resembling colonies on CYA; conidia dark green; reverse uncoloured to pale yellow. At 5 °C, microcolonies or colonies up to 4 mm diam formed. No growth at 37 °C.

On CSN, colonies 18–22 mm diam, growth strong, though not rapid; medium reaction usually alkaline (violet), but sometimes neutral or weakly acid (yellow); reverse alkaline.

Conidiophores borne singly or in definite fascicles, usually from subsurface hyphae, stipes commonly 150–250 µm long, but of indeterminate length when in fascicles, with walls smooth to finely roughened or, rarely, rough; penicilli predominantly terverticillate, but appreciable numbers biverticillate or quaterverticillate in some isolates; rami usually borne singly, sometimes rough walled; phialides ampulliform but tending to acerose or cylindroidal in some isolates, commonly 9–10 µm long, with short collula; conidia spherical to subspheroidal, uncommonly broadly ellipsoidal, 3.0–4.0(−4.5) µm diam or in length, with walls smooth to very finely roughened, borne in disordered chains.

Distinctive features Conidial colour, dark bluish green to dark green, is the most useful feature distinguishing *Penicillium solitum* from other closely related species. On MEA, greyish to brownish orange reverse colours are a distinctive characteristic. Conidia are quite large. Growth on CSN is strong, though slow, and the colony reverse is alkaline. *P. solitum* can also be distinguished from *P. commune* by lack of reaction with the Ehrlich test (Lund 1995a) and patterns of secondary metabolites (Lund 1995b).

Taxonomy Accepted by Raper and Thom (1949) as a poorly defined, floccose species, *Penicillium solitum* was ignored for nearly 40 years. Samson et al. (1976) included it as a synonym of *P. verrucosum* var. *cyclopium*, while Pitt (1979b) considered it to be a synonym of *P. aurantiogriseum*. Cruickshank and Pitt (1987) revived *P. solitum* on the basis of distinctive isoenzyme patterns, and morphological examination of a number of fresh isolates. It has been accepted without question since. Complete descriptions were provided by Pitt et al. (1991, 2000).

Identifiers Mycobank MB206172; neotype CBS 424.89 (Frisvad et al. 1990c); ex-type cultures CBS 424.89, IMI 92225, ATCC 9923, NRRL 937, FRR 937; ITS barcode AY373932; alternative marker *BenA* AY674354 (Visagie et al. 2014).

Physiology Lack of recognition of this species until relatively recently has meant that little information exists on its physiology. However, it is a typical member of *Penicillium* subgenus *Penicillium* in showing ability to grow at low temperatures and a_w, and absence of growth at 37 °C.

Mycotoxins This species does not produce mycotoxins (Frisvad and Samson 2004).

Ecology *Penicillium solitum* is a significant pathogen of pomaceous fruit (Frisvad 1981; Pitt et al. 1991; Sanderson and Spotts 1995; Amiri and Bompeix 2005). It is resistant to the fungicides used to control growth of *P. expansum* and so its role in apple spoilage has increased (Pitt et al. 1991; Sanderson and Spotts 1995). This species also causes spoilage of cheese (Hocking and Faedo 1992; Lund et al. 1995; Kure et al. 2004), and has been reported from European sausages during manufacture (Andersen 1995; Papagianni et al. 2007). Absence of other literature reports probably reflects lack of recognition, not rarity in foods. We isolated it, at a low frequency, from Thai cashews (Pitt et al. 1993), Indonesian peanuts (Pitt et al. 1998), and Philippine peanuts, maize and mung beans (our unpublished data).

References Pitt et al. (1991, 2000) and Frisvad and Samson (2004).

Penicillium verrucosum Dierckx Fig. 7.39

Colonies on CYA 15–25 mm diam, usually closely sulcate, varying from low and velutinous to deep and fasciculate or floccose; mycelium white; conidial formation light to moderate, Greyish Green to Dull Green (26-27D-E4-5); clear to pale yellow exudate produced, copiously by some isolates; reverse yellow brown to deep brown. Colonies on MEA 12–15(−20) mm diam, sulcate, dense and velutinous or centrally floccose; mycelium white; conidial production moderate, coloured as on CYA; clear exudate occasionally produced; reverse dull brown or olive. Colonies on G25N 16–20 mm diam, plane or more commonly sulcate, velutinous to somewhat floccose; mycelium white; reverse pale to yellow. At 5 °C, microcolony formation at least;

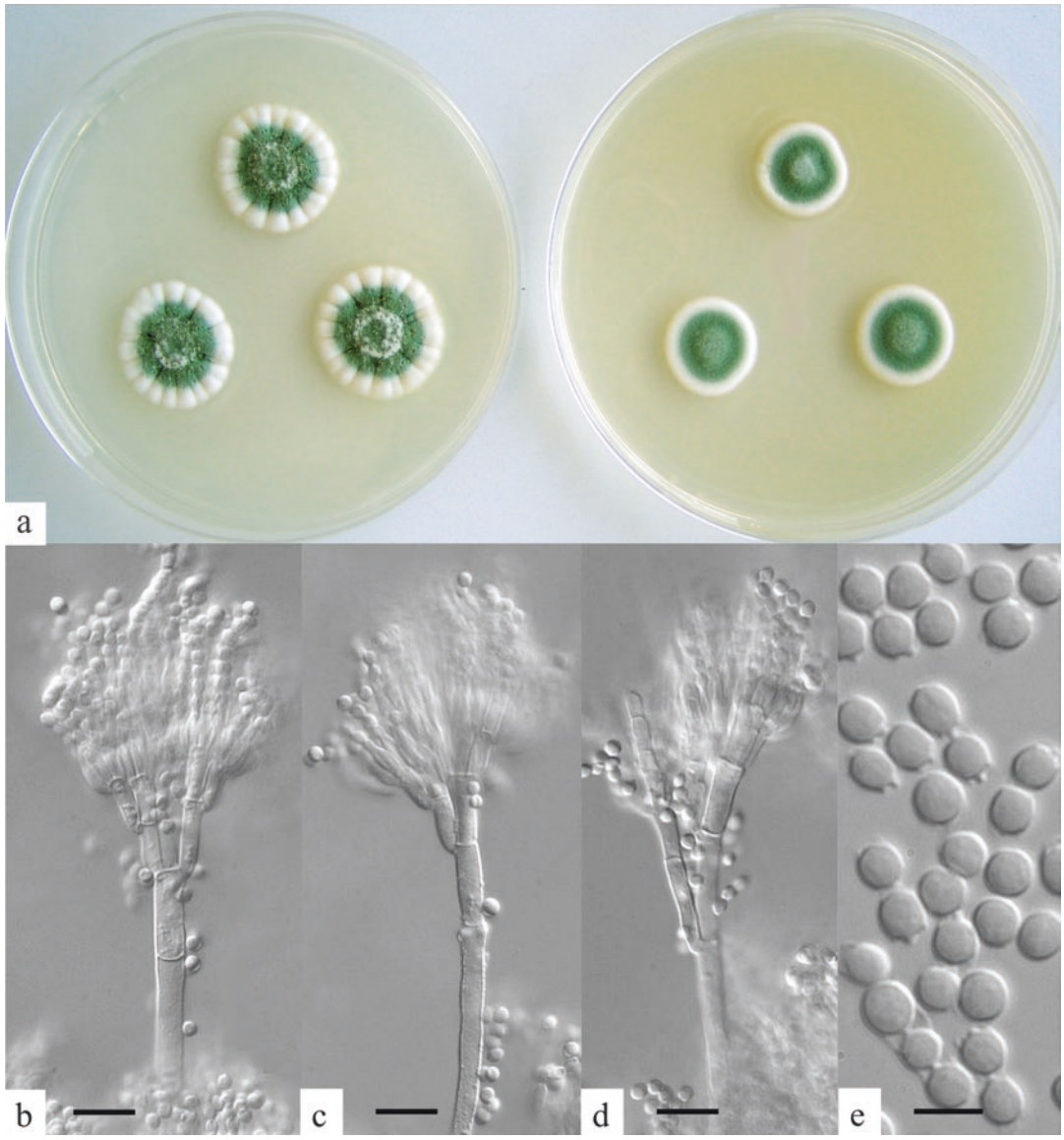

Fig. 7.39 *Penicillium verrucosum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

often colonies of 2–4 mm diam produced. No growth at 37 °C.

On CSN, colonies usually 10–15 mm diam, growth weak, mostly subsurface; reaction neutral in medium and reverse.

Conidiophores borne from subsurface or surface hyphae, stipes robust, 200–500 µm long, with walls finely to conspicuously roughened, bearing terminal penicilli; penicilli variable, some isolates producing compact terverticillate and quaterverticillate forms almost exclusively, others predominantly terverticillate and biverticillate, often with elements irregularly disposed; rami 1–2 per stipe, sometimes rough walled; phialides ampulliform, 7–9 µm long, narrowing abruptly to short collula; conidia usually spherical, 2.5–3.0 µm diam, less commonly subspheroidal to ellipsoidal, 3.0–3.5 µm long, with smooth walls, borne in disordered chains.

Distinctive features *Penicillium verrucosum* is characterised by slow growth on CYA and especially on MEA, by conidia coloured relatively bright green, and by the absence of other conspicuous pigmentation. Growth on CSN is weak, with no change in medium colour. *P. verrucosum* produces a red brown to terracotta reverse on YES agar (Frisvad and Samson 2004).

Taxonomy Raper and Thom (1949: 486) placed *Penicillium verrucosum* "in the series with" *P. viridicatum*, while neither recognising the species nor placing it in synonymy. Samson et al. (1976) considered *P. viridicatum* and *P. verrucosum* as synonyms, under the name *P. verrucosum* var. *verrucosum*. Pitt (1987) separated the two species and showed that *P. verrucosum* is the source of ochratoxin A. Frisvad and Samson (2004) placed *P. verrucosum* in Section *Viridicata*, series *Verrucosa*, along with *P. nordicum* (see Additional species, below), while placing *P. viridicatum* in a separate series, *Viridicata*.

Identifiers Mycobank MB212252; neotype IMI 200310 (Pitt 1979b); ex-type cultures CBS 603.74, IMI 200310, ATCC 48957, NRRL 965, FRR 965; ITS barcode AY373938; alternative markers *BenA* AY674323, *RPB2* JN121539, *CaM* DQ911138 (Visagie et al. 2014).

Physiology *Penicillium verrucosum* grows from 0 to 31 °C, with the optimum at 20 °C. The minimum a_w for germination and growth is *ca* 0.80 (Northolt et al. 1979; Cairns-Fuller et al. 2005; Pardo et al. 2006). Growth and ochratoxin A production were significantly inhibited by 50% CO_2 at 0.90–0.995 a_w at 25 °C (Cairns-Fuller et al. 2005). Growth can occur over the pH range 2.1–10.0 at least (Wheeler et al. 1991).

Mycotoxins *Penicillium verrucosum* is the major source of ochratoxin A in cool temperate zone commodities, especially cereals. Ochratoxin A is also produced by several *Aspergillus* species, notably *A. carbonarius, A. westerdijkiae* and *A. steynii*. Those species are responsible for ochratoxin A production in warmer climates. For further details about ochratoxin A, see Chap. 13.

It has been known for nearly 50 years that *P. verrucosum* (originally identified as *P. viridicatum*) is endemic in cereals in northern Europe, and so commonly occurs in foods, especially breads and other cereal products. It also occurs in animal feeds, and in animal tissue (Krogh et al. 1973, 1976; Olsen et al. 2006). Relatively high amounts are found in kidney and liver in comparison with muscle or fat. In high doses it also appears in eggs (JECFA 2001). After studying over 70 samples of European wheat, barley and rye contaminated with ochratoxin A and 17 samples with no detectable level, it was concluded that *P. verrucosum* was the sole source of ochratoxin A in European cereals (Lund and Frisvad 2003). Cabañas et al. (2008) isolated 100 *Aspergillus* strains from retail samples of wheat flour in Spain and found that none produced ochratoxin A in culture. However 17 strains, probably of *P. verrucosum,* were also isolated and 14 produced ochratoxin A. They concluded that *P. verrucosum* is the source of ochratoxin A even in Southern Europe, and not *Aspergillus* species. The majority of *P. verrucosum* isolates are ochratoxin A producers. Some *P. verrucosum* isolates also produce citrinin (Frisvad and Samson 2004).

Ochratoxin A has recently been found to be present in stored wheat and barley in Canada, due to poor silo design and moisture migration

(Limay-Rios et al. 2017). These authors identified several risk factors for ochratoxin A contamination, including grain clumps accumulated around or under manholes; debris remaining on storage floor and augers; and grain clumps around side doors. Hotspots can occur even when grain enters storage below the acceptanced limit of 14.5% due to inadequate aeration and exposure to moisture from precipitation or condensation (Limay-Rios et al. 2017).

Growth and ochratoxin A production by *P. verrucosum* were studied on various autoclaved grains (wheat, triticale, rye, barley, maize and rice) stored at 10 °C, 20 °C and 30 °C for 40 days. It was found that while maximum growth occurred at 30 °C, maximum ochratoxin production occurred at 20 °C (Wawrzyniak and Waśkiewicz 2014). Although maximum ochratoxin A production occurred on rice, this commodity is unlikely to be contaminated from this source, as rice grows under environmental conditions where *P. verrucosum* is not known to occur.

Ochratoxin A is produced over the whole temperature range for growth of *Penicillium verrucosum* (0–31 °C), and down to 0.86 a_w (Northolt et al. 1979). The optimum conditions on a bread dough were reported to be 0.92 a_w at pH 5.6 (Patterson and Damoglou 1986). Higher amounts of ochratoxin A were produced on wheat than other substrates including maize, peanuts, rapeseed and soybeans. Citrinin production was supported by both wheat and maize (Madhyastha et al. 1990). High concentrations of ochratoxin A were detected in exudate from *P. verrucosum* colonies on CYA (Gareis and Gareis 2007). Ochratoxin A has been found in spoiled cheeses, at up to 7 mg/kg (Jarvis 1983) and mouldy cheesecake at up to 1 mg/kg (Piskorska-Pliszczynska and Borkowska-Opacka 1984).

A study by Biffi et al. (2004) on the ochratoxin A levels in a range of cereal products, including commercial flours and other bakery products, revealed that although all samples analysed contained ochratoxin A, most were below the legal limits set by the Italian Ministry of Health of 3 μg/kg for cereal derivatives and 0.5 μg/kg for baby foods. In another investigation of rice and rice products in Europe, González et al. (2006) similarly found low levels of ochratoxin A in rice and other products from both organic and traditional sources.

Ecology *Penicillium verrucosum* is endemic in European and Canadian wheat, barley, rye and oats (Pohland et al. 1992; Lund and Frisvad 2003; Samson and Frisvad 2004; Lugauskas et al. 2006). It is a cold climate fungus, and is rare outside cool temperate zones, and away from cereals. However, it has been isolated from cheese in Europe (Lund and Frisvad 2003).

Additional species *Penicllium nordicum* Dragoni and Canatoni ex Ramirez is very similar in appearance to *P. verrucosum* but its ecology is quite different. *P. nordicum* is generally associated with high protein substrates such as meat products (Frisvad and Samson 2004). This species (including reports as *P. viridicatum* or *P. verrucosum*) has been isolated quite frequently from meat products in Europe (Spotti et al. 1989, 1994; Andersen 1995; Battilani et al. 2007; Berni et al. 2011), from Australian salami and prosciutto in our laboratory, and from cheese (Hocking and Faedo 1992; Lund et al. 1995). *P. nordicum* produces a cream yellow reverse on YES medium, in contrast to the red brown reverse produced by *P. verrucosum* (Frisvad and Samson 2004). All tested strains of *P. nordicum* produced ochratoxin A (Frisvad and Samson 2004), and this toxin has been found in meat products (Spotti et al. 2001; Pietri et al. 2006; and our unpublished observations). Identifiers: Mycobank MB114762; neotype ATCC 44219; ex-type cultures ATCC 44219; ITS barcode KJ834513; alternative marker *BenA* KJ834476 (Visagie et al. 2014).

References Pitt (1987, 2000) and Frisvad and Samson (2004).

Penicillium viridicatum Westling

Fig. 7.40

Penicillium olivinoviride Biourge
Penicillium aurantiogriseum var. *viridicatum* (Westling) Frisvad & Filtenborg

Colonies on CYA 28–32 mm diam, radially sulcate, dense, typically relatively low, velutinous, granular or less commonly floccose; mycelium usually inconspicuous, white; conidial production moderate to heavy, yellow green, usually near Cactus Green (27-29E4-5), but sometimes brighter (27D4-6) or greyer (26-27D-E2-3); exudate present, clear, pale yellow, pale brown or pale pink; soluble pigment sometimes produced, orange or reddish brown; reverse in shades of orange brown, from pale, near Orange White (6A2) to bright or deep, near Brownish Orange (6B-D7). Colonies on MEA 25–30 mm diam, plane or occasionally radially sulcate, velutinous to granular or centrally floccose; mycelium white

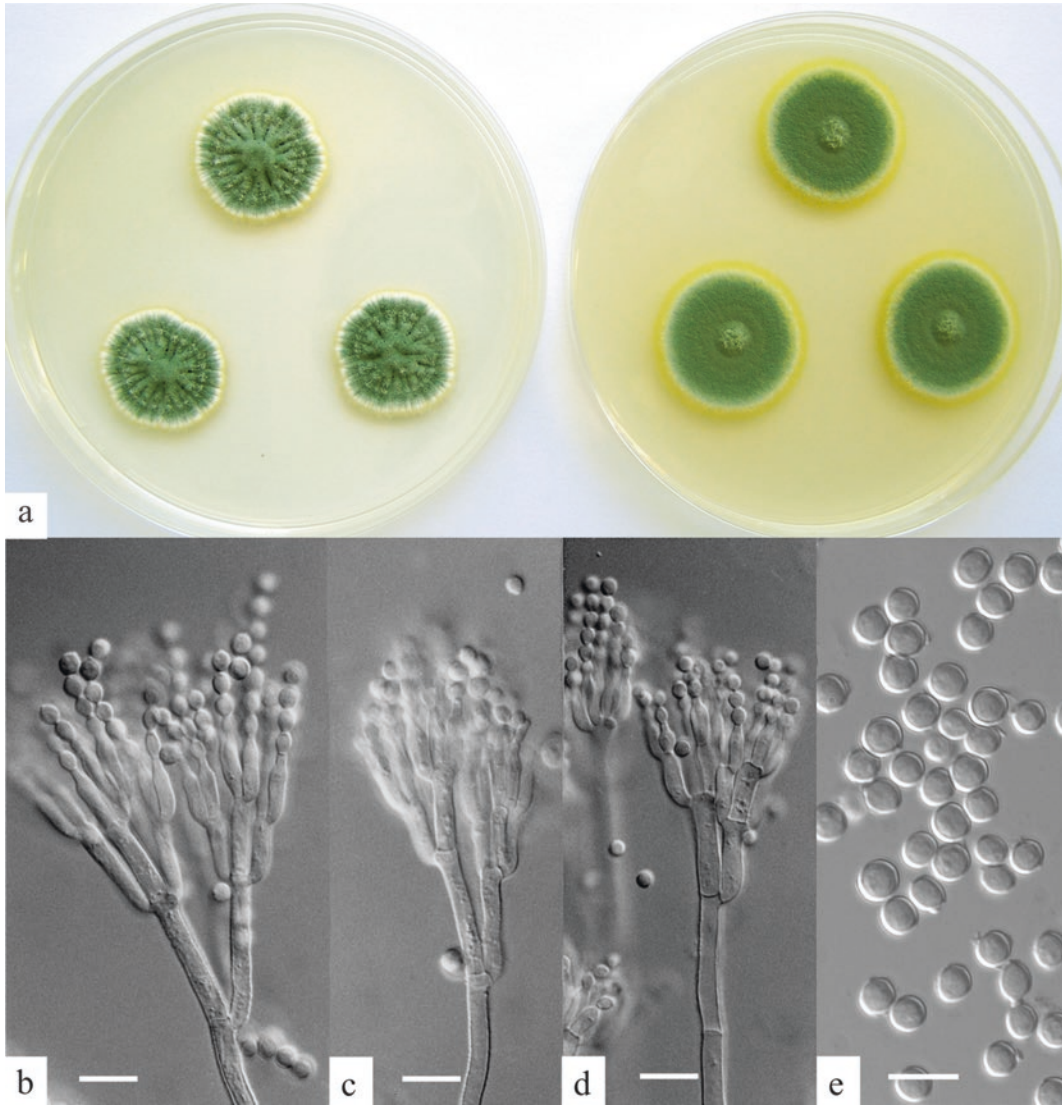

Fig. 7.40 *Penicillium viridicatum* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

or brown; conidial formation moderate, in yellow green colours similar to those on CYA; reverse orange brown to yellow brown. Colonies on G25N 18–22 mm diam, plane or finely radially sulcate, dense, surface granular; conidial production blue grey, yellow green or brown; reverse pale, yellow or orange. At 5 °C, macroscopic colonies formed, usually 2–5 mm diam. No growth at 37 °C.

On CSN, colonies usually 15–22 mm diam, growth moderate to strong; medium and colony reverse usually acid (yellow), the reverse sometimes brown also.

Conidiophores borne from surface or subsurface hyphae, stipes commonly 200–300 µm long, usually with walls roughened, conspicuously so on MEA, bearing large, appressed penicilli, usually terverticillate but occasionally quaterverticillate; phialides ampulliform, 7–8(−10) µm long, usually with short collula; conidia subspheroidal to ellipsoidal, 3.0–4.0 µm long, with smooth or finely roughened walls, borne in disordered chains.

Distinctive features On CYA, *Penicillium viridicatum* produces dense and compact colonies which grow moderately slowly; conidia are yellow green; stipes are rough walled. Unlike *P. verrucosum*, *P. viridicatum* grows moderately well on CSN, with an acid reaction in both medium and reverse. Like *P. verrucosum*, this species produces a distinctive violet brown reverse on DRYS (Frisvad 1983) but a bright yellow reverse on YES, compared with red-brown for *P. verrucosum* (Frisvad and Samson 2004).

Taxonomy *Penicillium viridicatum* was confused with *P. verrucosum* for a long time (Raper and Thom 1949; Samson et al. 1976). Ciegler et al. (1973, 1981) reported that *P. viridicatum* isolates produced two distinct classes of mycotoxins. The situation was clarified by Pitt (1987) who showed that anthraquinone toxins alone were produced by *P. viridicatum*. Frisvad and Filtenborg (1989) considered *P. viridicatum* a variety of *P. aurantiogriseum*, but these are separate species (Pitt and Samson 1993; Frisvad and Samson 2004).

Identifiers Mycobank MB163349; neotype IMI 39785ii (Pitt 1979b); ex-type cultures CBS 390.48, IMI 39785, ATCC 1990, NRRL 963, FRR 963; ITS barcode AY373939; alternative markers *BenA* AY674295, *RPB2* JN121511 (Visagie et al. 2014).

Physiology A psychrophile capable of growth down to at least −2 °C, *Penicillium viridicatum* grows optimally near 23 °C and has its maximum at 36 °C (Mislivec and Tuite 1970). The minimum a_w for growth is 0.80–0.81 at 23–25 °C (Mislivec and Tuite 1970; Hocking and Pitt 1979).

Mycotoxins *Penicillium viridicatum* produces several naphthoquinones, notably xanthomegnin and viomellein, capable of causing liver and kidney damage in mice, rats and pigs. Both compounds caused illness in young mice when administered at 450 mg/kg body weight (Carlton et al. 1973, 1976). Viomellein has been found in nature, in a Danish barley sample, at a level of 1 mg/kg (Hald et al. 1983). This level appears to be too low to be considered hazardous. Eleven of 19 samples of poor quality cereals and compounded feeds examined in the U.K. contained xanthomegnin and viomellein and a third related compound, vioxanthin. Levels of each up to 2 mg/kg were found, again a significant finding of naturally occurring mycotoxins, but below a clearly hazardous level (Scudamore et al. 1986). The role, if any, of these toxins in natural disease of animals remains unknown.

Ecology The reported association of *Penicillium viridicatum* with European barley (Krogh et al. 1973) and Canadian wheat (Wallace et al. 1976) was incorrect: *P. verrucosum* is the major species involved. *P. viridicatum* is much less common, though its occurrence in Canadian grain has been verified (Mills et al. 1995). This species has also been reported from meats, dried fruit, spices, pasta, health foods, peanuts, pistachio nuts, almonds (see Pitt and Hocking 1997) and cheese (Hayaloglu and Kirbag 2007). We isolated *P. viridicatum* from Philippine maize, peanuts, milled rice, soybeans and black pepper and also from dehydrated infant food in Australia (our unpublished data).

References Pitt (1979b, 2000), Samson et al. (1976) and Domsch et al. (1980), both under the name *Penicillium verrucosum* var. *verrucosum*, and Frisvad and Samson (2004).

7.6 Genus *Talaromyces* C.R. Benj

As discussed in the chapter preamble, *Talaromyces* was described by Benjamin (1955) as an ascomycete genus with a gymnothecial sexual state usually associated with a *Penicillium* asexual state. When genetic studies showed that *Penicillium* as then understood was polyphyletic (LoBuglio et al. 1993; Berbee et al. 1995), *Penicillium* subgenus *Biverticillium* was transferred to *Talaromyces* by Samson et al. (2011). The greatly expanded genus *Talaromyces* was monographed by Yilmaz et al. 2014) with some amplification by Yilmaz et al. (2016). Their taxonomy has been followed here. However, the division of *Talaromyces* into seven sections by Yilmaz et al. (2014) has not been adhered to here, mainly because so few species are treated here. The monographs by Yilmaz et al. (2014, 2016) are comprehensive and include morphological descriptions. They should be consulted for further information about *Talaromyces* taxonomy.

If an unknown isolate produces gymnothecia (Fig. 3.3) in association with a *Penicillium*-like asexual state, it is clearly classifiable in *Talaromyces*. In the absence of a sexual state, care is required. See Sect. 7.1.

After some species not closely related were transferred elsewhere, and the description of a number of new species, Yilmaz et al. (2014) included 88 species in *Talaromyces*, and Yilmaz et al. (2016) added four more. Most species are soil inhabiting and rarely if ever occur in foods. A few are of more common occurrence and are described below. All species have relatively high water requirements, and appear to be associated primarily with soil or moist, decaying vegetation. Some produce ascospores, which are heat resistant and, consequently, have occasionally been isolated from pasteurised fruit juices and fruit based products (Hocking and Pitt 1984). The most commonly isolated heat resistant species is *T. macrosporus*, but we have also seen *T. trachyspermus* a number of times.

In *Talaromyces*, penicilli are characteristically biverticillate, though with sometimes a proportion terverticillate, and always terminal; metulae are numerous, in symmetrical appressed or divergent verticils, and of approximately equal length to phialides; phialides are typically acerose (shaped like a pine needle), with conical collula, tapering to narrow apical pores; conidia in species considered here are ellipsoidal to fusiform. Colonies on CYA at 25 °C commonly show yellow or red colours in mycelium, exudate, soluble pigment and/or reverse. Growth at 37 °C commonly occurs. Growth at 5 °C is confined to a single species considered here, *Penicillium rugulosum*. Growth on G25N is slow; colonies are not more than 10 mm diam in 7 days at 25 °C.

Molecular studies have shown that some asexual species in *Talaromyces* have sexual species as their nearest neighbours, indicating multiple losses of the teleomorph. In particular, *T. macrosporus* was shown to be closely associated with *T. minioluteus* and *T. stipitatus* with *T. funiculosus*. However, in only one case was synonymy proposed. *P. variabile* was placed in synonymy with *T. wortmannii* (Yilmaz et al. 2016) and that has been followed here, with some reservations.

Key to *Talaromyces* species included here

1	Gymnothecia produced; mature ascospores evident within 2 weeks	2
	No evidence of a sexual state	5
2 (1)	Colonies exceeding 25 mm diam on MEA in 7 days	3
	Colonies not exceeding 25 mm diam on MEA in 7 days	4
3 (2)	Ascospores more than 5 µm long	*T. macrosporus*
	Ascospores less than 5 µm long	*T. flavus*
4 (2)	Colonies yellow	*T. wortmannii*
	Colonies white	*T. trachyspermus*

(continue)

5 (1)	Colonies on MEA exceeding 25 mm diam	6
	Colonies on MEA not exceeding 25 mm diam	8
6 (5)	Red soluble pigment produced by colonies on CYA	*T. purpureogenus*
	Soluble pigment on CYA pink or absent	7
7 (6)	Mycelium on CYA and MEA bright yellow; stipes more than 100 µm long	*T. pinophilus*
	Mycelium on CYA and MEA not bright yellow; stipes less than 100 µm long	*T. funiculosus*
8 (5)	Colonies on CYA deep and convex; the dominant colony colour bright yellow or orange mycelium	*T. islandicus*
	Colonies on CYA low and velutinous; the dominant colony colour green conidia	9
9 (8)	Colonies on CYA at 25 °C not exceeding 12 mm diam; sometimes germination at 5 °C	*T. rugulosus*
	Colonies on CYA at 25 °C exceeding 12 mm diam; no germination at 5 °C	*T. wortmannii*

Talaromyces flavus (Klöcker) Stolk & Samson — Fig. 7.41

Penicillium vermiculatum P.A. Dang
Talaromyces vermiculatus (P.A. Dang.) C.R. Benj
Penicillium dangeardii Pitt

Colonies on CYA 18–30 mm diam, occasionally larger, plane, low and quite sparse to moderately deep and floccose; mycelium bright yellow, less commonly buff or reddish brown, in most isolates concealing developing gymnothecia; conidial production usually sparse and inconspicuous, but if more profuse, greenish grey; clear to reddish exudate present occasionally; reverse sometimes yellow, more usually orange, reddish or brown. Colonies on MEA 30–50 mm diam, generally similar to those on CYA but gymnothecia more abundant; reverse usually dull orange or brown, but sometimes deep brown or deep red. Colonies on G25N 2–7 mm diam, low, of sparse white mycelium; occasionally microcolonies or no growth. No germination at 5 °C. At 37 °C, colonies 20–45 mm diam, usually similar to those on CYA, but sometimes with white or brown mycelium or overlaid with grey conidia; gymnothecia absent; reverse yellow, orange or brown.

Gymnothecia of tightly interwoven mycelium, bright yellow, about 200–500 µm diam, closely packed, maturing within 2 weeks; ascospores yellow, ellipsoidal, 4.0–5.0 µm long, with spinose walls. Conidiophores borne from aerial hyphae, stipes 20–80 µm long, bearing terminal biverticillate or less commonly monoverticillate penicilli; phialides acerose, 10–16 µm long; conidia ellipsoidal to fusiform, 2.5–4.0 µm long, with smooth to spinulose walls.

Distinctive features *Talaromyces flavus* and the morphologically similar *T. macrosporus* grow relatively rapidly on MEA, produce bright yellow colonies at both 25 and 37 °C, and usually produce abundant yellow gymnothecia, especially on MEA. *T. flavus* produces ascospores less than 5 µm long, smaller than those of *T. macrosporus*. The species are molecularly distinct. *T. wortmannii* has a very similar morphology to *T. flavus*, indeed Raper and Thom (1949) used differences in gymnothecial initials to separate them, but growth rates on the standard media differ widely and they are distinct using molecular methods.

Identifiers Mycobank MB324416; neotype CBS 310.38 (Stolk and Samson 1972); ex-type cultures CBS 310.38, IMI 197477, NRRL 2098, FRR 2098; ITS barcode JN899360; alternative markers *BenA* JX494302, *RPB2* JF417426, *CaM* KF741949 (Yilmaz et al. 2014).

Physiology Beuchat et al. (1988) and Quintavalla and Spotti (1993) reported that the heat resistance of *Talaromyces flavus* ascospores was substantially lower than that of isolates with larger ascospores now known to belong to *T. macrosporus*. Secondary metabolite production by *T. flavus* has been comprehensively reviewed by Proksa (2010). Seed treatment with *T. flavus* has been shown to benefit growth of cotton and potato plants (Naraghi et al. 2012).

Mycotoxins This species is not known to produce mycotoxins.

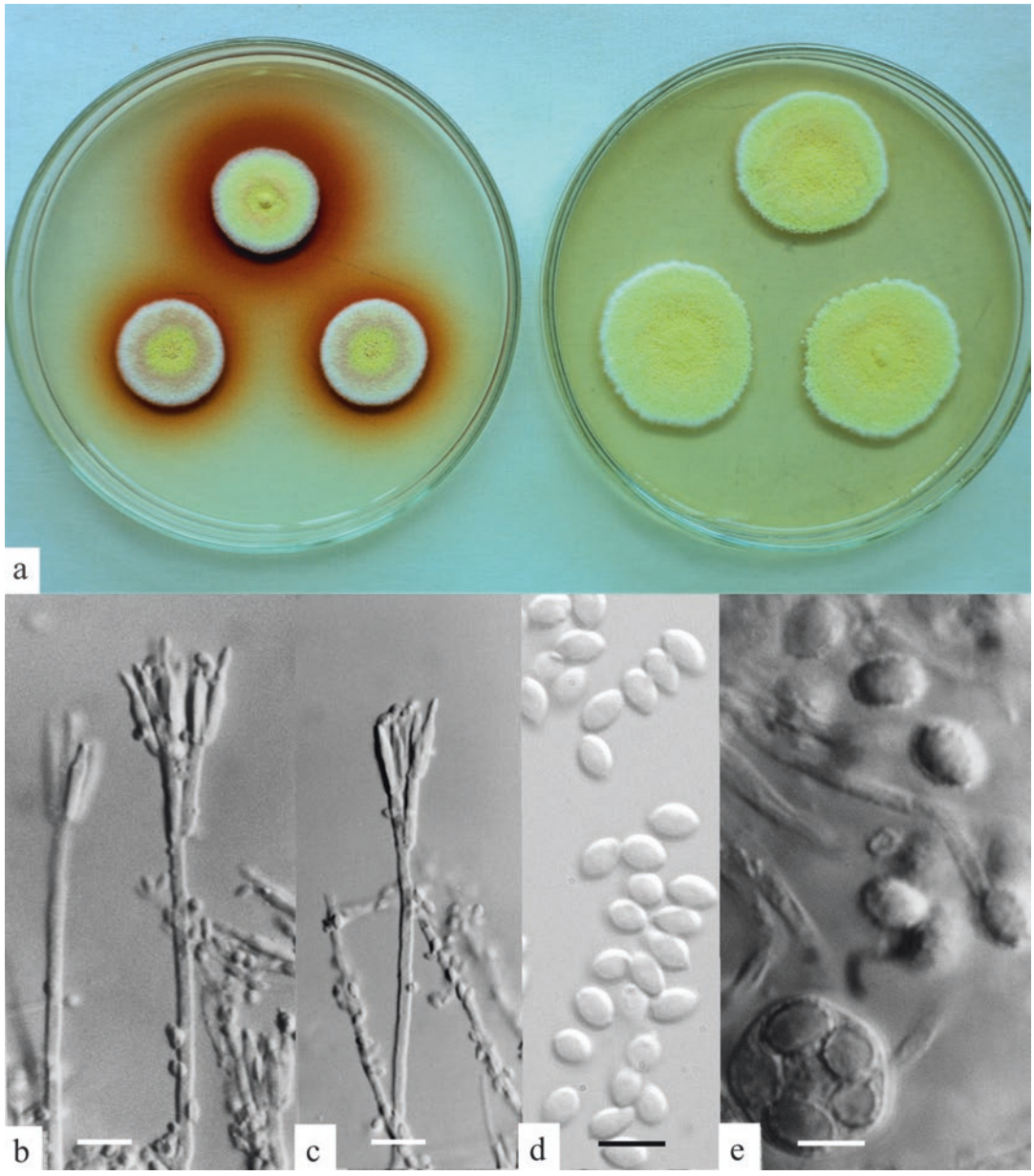

Fig. 7.41 *Talaromyces flavus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm; (**e**) ascospores, bar = 5 µm

Ecology This is the most common species of *Talaromyces* that consistently produces a sexual state. It is found universally in soils in warmer climates. Consequently it occurs in foods from time to time, most probably as a contaminant rather than as a spoilage fungus. Reports of *T. flavus* as a heat resistant fungus have always referred to *T. macrosporus* so far as can be established. Panek and Frąc (2019) used loop-mediated isothermal amplification for detection of *T. flavus* but the limit of detection (64 ascospores/g of soil) was too high to have practical application.

References Stolk and Samson (1972), Pitt (1979b), and Yilmaz et al. (2014).

Talaromyces funiculosus (Thom) Samson et al. Fig. 7.42

Penicillium funiculosum Thom

Colonies on CYA 25–40 mm diam, plane, usually 2–5 mm deep, with conspicuous ropes of aerial hyphae (funicles), occasionally almost velutinous; mycelium Salmon to Peach (6-7A4-5) or Brownish Orange to Brownish Red (7-8C5-6); conidia moderately abundant, Dull Green (26-27C-D3-4); clear exudate and pink soluble pigment produced by some isolates; reverse pale, brown, or more commonly deeply pigmented, Brownish Red to Red (8-10B-C8). Colonies on MEA 25–45 mm diam, usually conspicuously funiculose; mycelium usually white, less commonly Salmon (6A4) to Brownish Red (8-9C6-7); conidial production moderate to heavy,

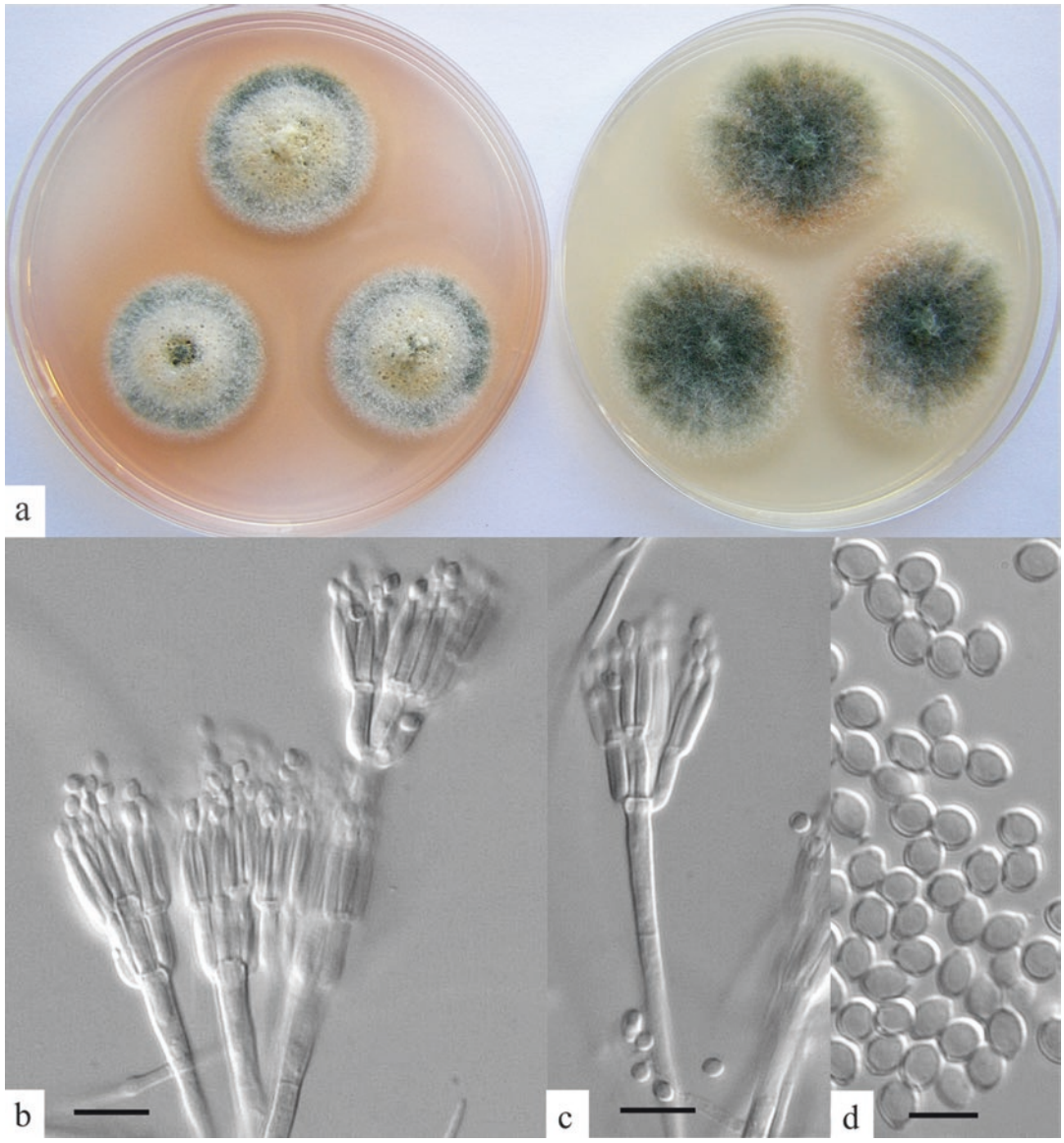

Fig. 7.42 *Talaromyces funiculosus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

coloured as on CYA; reverse pale, brown or orange to reddish brown. Colonies on G25N 3–8 mm diam, plane, usually funiculose; reverse pale to olive. No germination at 5 °C. At 37 °C, colonies 20–45 mm diam, usually similar to those on CYA at 25 °C or more floccose, with reverse pale, brown or reddish brown. No sexual state known.

Conidiophores borne from aerial hyphae, usually from well defined funicles, stipes short, commonly 25–40(−60) µm long, in uncommon isolates shorter or longer, with walls smooth to finely roughened, bearing terminal biverticillate penicilli, or occasionally more complex or irregular forms; metulae and phialides closely appressed, acerose, 9–11 µm long, phialides with gradually tapering collula; conidia small, cylindroidal to ellipsoidal, 2.2–3.0 µm long, smooth walled, borne in short columns. Sexual state not known.

Distinctive features Colonies of *Talaromyces funiculosus* often produce well defined ropes of hyphae (funicles) bearing short conidiophores at right angles. Growth at 37 °C is rapid, similar to that on CYA at 25 °C or more floccose; penicilli are closely appressed and conidia are pale greyish green.

Taxonomy Molecular studies have shown that *T. funiculosus* is closely related to the sexual species *T. stipitatus* (Thom) C.R. Benjamin, though the two species appear to be distinct.

Identifiers Mycobank MB560653; neotype IMI 193019 (Pitt 1979b); ex-type cultures CBS 272.86, IMI 193019, FRR 1630; ITS barcode JN899377; alternative markers *BenA* JX091383, *RPB2* KM023293, *CaM* KF741945 (Yilmaz et al. 2014).

Physiology Mislivec and Tuite (1970) reported that *Talaromyces funiculosus* grows optimally at ca 30 °C, with a minimum near 8 °C; the maximum growth temperature is near 42 °C (Domsch et al. 1980). The minimum a_w for germination and growth is 0.90 at 23, 25 and 30 °C (Mislivec and Tuite 1970; Hocking and Pitt 1979). This species is very acid tolerant; we have isolated it from soils on media of pH 2.0.

Mycotoxins *Talaromyces funiculosus* strains toxic to day old cockerels were isolated from chestnuts (Wells and Payne 1975) and pecans (Wells 1980). The toxic compounds have not been characterised.

Ecology *Talaromyces funiculosus* has been found quite frequently in foods, including fruit, nuts and cereals. It is responsible for fruitlet core rot and other diseases in pineapple fruit (Oxenham 1962; Lim and Rohrbach 1980; Matos et al. 2005). It is one cause of core rot in apples (Combrink et al. 1985; Vismer et al. 1996) and peaches (Mukhtar et al. 2019) and of diseases in onions (Hussein et al. 1977). It was reported as causing spoilage in pecans, rots in kola nuts and as being common in peanuts both before and after harvest (see Pitt and Hocking 1997). This species also has a close association with maize (see Pitt and Hocking 1997) and maize products (Ribeiro et al. 2003). It was present in 42% of maize samples we examined from Thailand, at levels up to 56% of kernels in a sample, and in 4% of all kernels examined. Levels in Philippine and Indonesian maize were lower (Pitt et al. 1993, 1998).

Isolations of *T. funiculosus* have also been made from walnuts, wheat and flour, pasta, barley, soybeans, rice (see Pitt and Hocking 1997), fresh cabbages (Lugauskas et al. 2005) and parboiled rice (Jayaraman and Kalyanasundaram 1994). Other sources include dried peas, miso, biltong, a Nigerian spice, yam chips and carbonated soft drinks (see Pitt and Hocking 1997).

As well as peanuts and maize, mentioned above, we isolated *T. funiculosus* at low levels from cashews and black rice in Thailand (Pitt et al. 1994).

References Pitt (1979b, 2000) and Yilmaz et al. (2014).

Talaromyces islandicus (Sopp) Samson et al. Fig. 7.43

Penicillium islandicum Sopp

Colonies on CYA 17–22 mm diam, plane or centrally raised, velutinous to lightly floccose; mycelium intensely coloured, dominating the colony appearance, Deep Orange to Brownish Orange (6A-C8) or Copper (7C8); conidia produced in moderate numbers, usually enveloped by the mycelium, but if conspicuous coloured Greyish Turquoise to Greyish Green (24-25D4); clear to pale yellow exudate sometimes produced; reverse very strongly coloured, Orange to Rust Brown or Burnt Sienna (6-7B-E8), sometimes Reddish Brown (9D-E8). Colonies on MEA 17–22 mm diam, similar to those on CYA but with mycelium usually less dominant; conidial production heavy, Greyish Turquoise (24D5); reverse commonly with central areas Brown to Reddish Brown (7-8B-E8). Colonies on G25N 4–9 mm diam, similar in

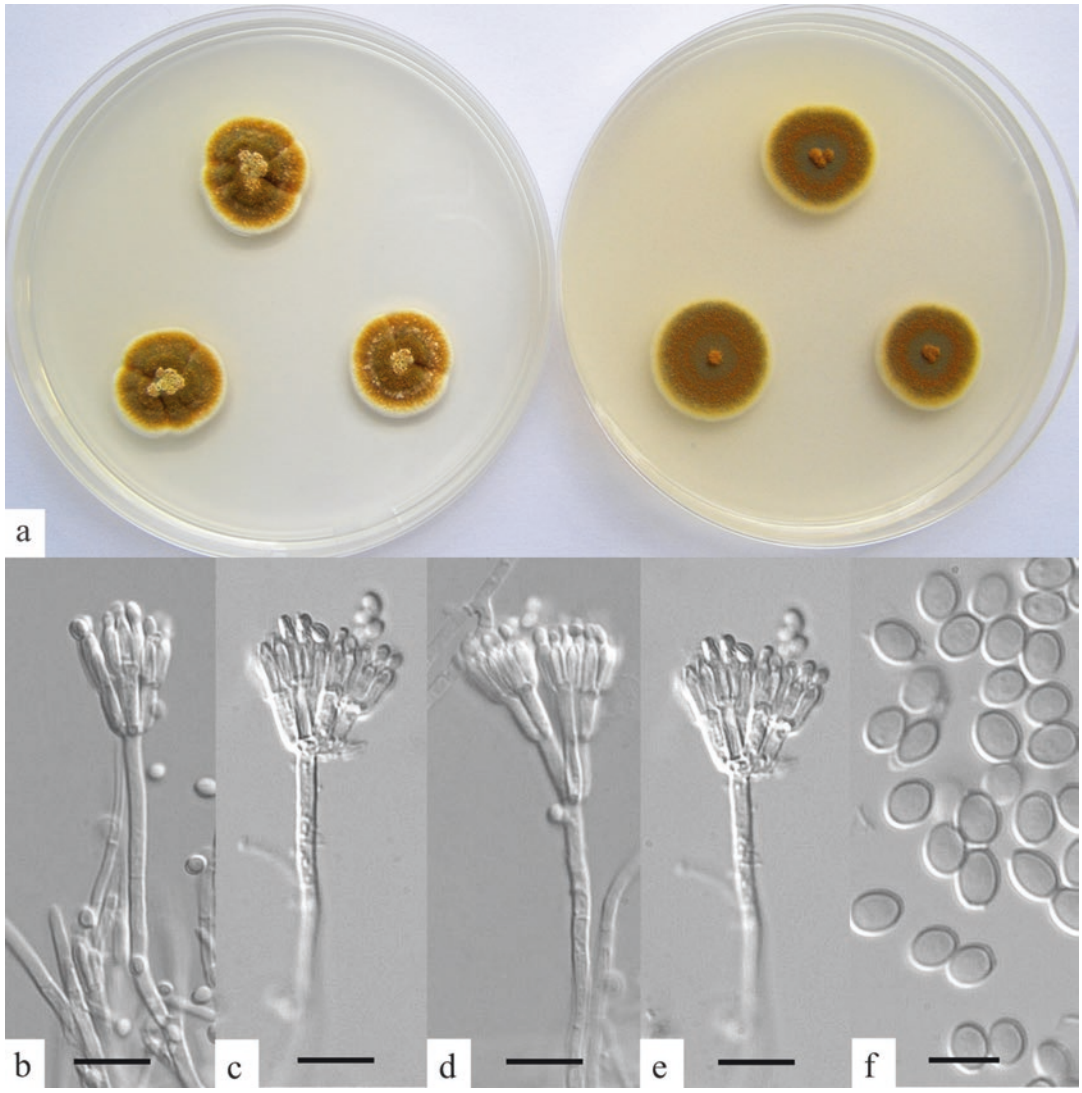

Fig. 7.43 *Talaromyces islandicus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**e**) penicilli, bars = 10 µm; (**f**) conidia, bar = 5 µm

appearance and colouration to those on CYA and MEA. No germination at 5 °C. At 37 °C, colonies 10–20 mm diam, deep and floccose; mycelium white or coloured as at 25 °C; conidia absent to abundant, Dark Green near Bottle Green (26F5); reverse pale or as at 25 °C. No sexual state known.

Conidiophores borne from aerial hyphae, stipes 30–60 µm long, usually smooth walled, bearing terminal penicilli, typically biverticillate but not uncommonly bearing an appressed ramus; phialides acerose, 7–8 µm long, with abruptly narrowing collula; conidia broadly ellipsoidal to subspheroidal, mostly 3.0–3.5 µm long, with smooth heavy walls, borne in short, irregular to well defined columns.

Distinctive features *Talaromyces islandicus* differs from other species in the genus by the production of compact colonies with brilliant orange to brown mycelium and reverse on CYA. Conidia are usually blue, and are smooth walled. Gymnothecia and ascospores are not produced.

Identifiers Mycobank MB560654; neotype IMI 40042 (Pitt 1979b); ex-type cultures CBS 338.48, IMI 40042, ATCC 10127, NRRL 1036, FRR 1036; ITS barcode KF984885; alternative markers *BenA* 984,655, *RPB2* KF985018, *CaM* KF984780 (Yilmaz et al. 2014).

Physiology The optimum temperature for growth of *Talaromyces islandicus* has been reported as 31 °C, with a minimum and maximum of 10 and 38 °C respectively (Domsch et al. 1980). However, a consistent ability to grow nearly as rapidly at 37 °C as at 25 °C indicates the maximum growth temperature may be nearer 42 °C. The minimum a_w for growth at 31 °C is 0.83 (Ayerst 1969), and at 25 °C, 0.86 (Hocking and Pitt 1979). Growth occurs over the pH range 2.1–9.2 at least (Wheeler et al. 1991).

Mycotoxins *Talaromyces islandicus* produces at least four mycotoxins, unique to the species. Cyclochlorotine and islanditoxin are chlorine containing cyclic peptides which have the same toxic moiety, a pyrrolidine ring with two attached chlorine atoms, and share a number of other physical and chemical properties (Scott 1977). Both compounds are very toxic: cyclochlorotine has an oral LD_{50} in mice of 6.5 mg/kg, while that of islanditoxin by subcutaneous injection was 3 mg/kg. Fed to mice at the rate of 40 µg per day, cyclochlorotine caused liver cirrhosis, fibrosis and tumours (Uraguchi et al. 1972).

Luteoskyrin is a dimeric anthraquinone and erythroskyrin a heterocyclic red pigment. Both are liver and kidney toxins, though less acutely toxic than cyclochlorotine. Luteoskyrin is also carcinogenic.

The toxic "yellow rice" syndrome, described in Japan last century, has resulted in Japanese scientists taking a particular interest in *T. islandicus*, a species which causes yellowing of rice (Saito et al. 1971a). However, the practical importance of the toxins produced by this species remains unclear. *T. islandicus* has not been commonly found growing in nor causing spoilage of foods.

Ecology A marginal xerophile, *Talaromyces islandicus* is more tolerant of low a_w than most other species in the genus. *T. islandicus* is an active agent of spoilage in cereals stored a little above safe moisture contents. It occurs in soil, though not abundantly, and perhaps also is a weak animal pathogen (Pitt 1979b; p 447). It appears to be more widespread in tropical than temperate regions.

Talaromyces islandicus is often associated with rice and has been reported from Japan (Sakai et al. 2005) and South America (Tonon et al. 1997). Other reports include flour, peanuts, pecans and soybeans (see Pitt and Hocking 1997), without mention of spoilage. We isolated *T. islandicus* from Southeast Asian commodities including maize from the Philippines (our unpublished data), peanuts from Indonesia and milled rice and mung beans from both countries (Pitt et al. 1998). Levels were always low, without evidence of spoilage or toxin production.

References Pitt (1979b, 2000) and Yilmaz et al. (2014).

Talaromyces macrosporus (Stolk & Samson) Frisvad et al. Fig. 7.44

Talaromyces flavus var. *macrosporus* Stolk & Samson

Penicillium macrosporum Frisvad et al.

Colonies on CYA of variable size, usually 20–40 mm diam, plane, floccose; mycelium pale to bright yellow (3A2-4); immature gymnothecia sometimes present, bright yellow (2-3A6); conidial production sparse and inconspicuous; exudate clear or more commonly red; soluble pigment usually present, orange red (8A6-8); reverse brown to dark brown, or, in the presence of soluble pigment, dark red approaching black. Colonies on MEA 45–55 mm diam, sometimes smaller, plane, usually floccose, mycelium pale yellow (3A2-4), usually enveloping abundant

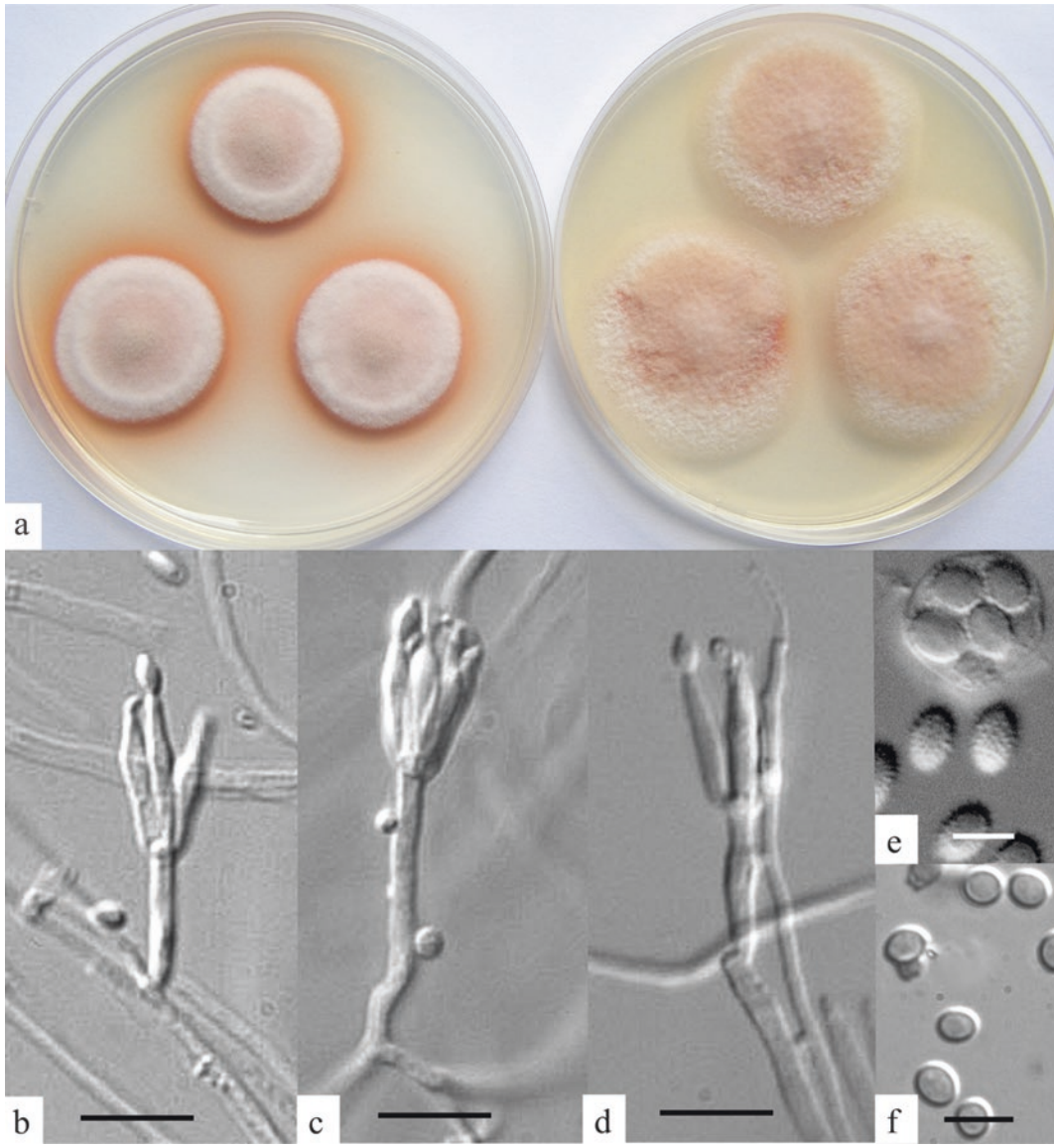

Fig. 7.44 *Talaromyces macrosporus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) ascospores, bar = 5 µm; (**f**) conidia, bar = 5 µm

developing gymnothecia, bright yellow (2-3A6); exudate and soluble pigment absent; reverse often strongly coloured, orange, brown, red or olive. On G25N, microcolonies to colonies 7 mm diam formed. No germination at 5 °C. At 37 °C, colonies usually 40–50 mm diam, occasionally less, plane, floccose, mycelium white to pale yellow; gymnothecia not produced; red exudate and soluble pigment sometimes present; reverse pale, brown, or in the presence of soluble pigment, dark red.

Gymnothecia borne as a contiguous layer on the agar surface, or sometimes discrete, 1.0–1.5 mm diam, composed of fine, bright yellow hyphae, maturing within 2 weeks; ascospores ellipsoidal, commonly 5.0–6.5 µm long, with spinose walls. Conidiophores borne from aerial hyphae, sparsely produced, stipes 20–80 µm long, bearing terminal biverticillate or less commonly monoverticillate penicilli; phialides acerose, 10–16 µm long; conidia ellipsoidal to fusiform, 2.5–4.0 µm long, with smooth to spinulose walls.

Distinctive features Like morphologically similar *Talaromyces flavus*, *T. macrosporus* grows relatively rapidly on MEA, produces bright yellow colonies at both 25 and 37 °C, and usually forms abundant yellow gymnothecia. *T. macrosporus* produces ascospores that are more than 5 µm long.

Taxonomy Established for strains of *T. flavus* with large ascospores (Stolk and Samson 1972), *Talaromyces flavus* var. *macrosporus* was not recognised by Pitt (1979b). On the basis of differences in secondary metabolite production, Frisvad et al. (1990b) raised the variety to species status. Molecularly, these species are not closely related (Samson et al. 2011; Yilmaz et al. 2014). Molecular studies have shown that *T. macrosporus* is closely related to the asexual species *T. mineoluteus,* though the two species appear to be distinct (Yilmaz et al. 2014).

Identifiers Mycobank MB126704; neotype CBS 713.63 (Pitt and Samson 1993); ex-type cultures CBS 317.63, IMI 197478, FRR 404; ITS barcode JN899333; alternative markers *BenA* JX091382, *RPB2* KM023292, *CaM* KF741952 (Yilmaz et al. 2014).

Physiology From the food industry viewpoint, the main attribute of *Talaromyces macrosporus* is the very high heat resistance of its ascospores. Published decimal reduction times and *z* values vary: a D_{88} of 7–22 min, a D_{91} of 2.9–5.4 min (Beuchat 1986); a D_{90} of 2–7 min and a *z* value of 10.3 C° (King and Halbrook 1987); a D_{90} of 2.2 min and a *z* value of 5.2 C° (Scott and Bernard 1987); a D_{80} of 190 min, a D_{90} of 6 min and a *z* value of 6.7 C° (King and Whitehand 1990). Dijksterhuis and Teunissen (2004) demonstrated a D_{85} of 30–100 min. These values are considerably higher than reported for *Byssochlamys* species (Beuchat 1986). The influence of organic acids, including preservatives, on the heat resistance of *T. flavus* was reported by Beuchat et al. (1988).

Ascospores of *T. macrosporus* contain trehalose, which is degraded to glucose following heat activation. Upon germination, glucose is released from the ascospores. The presence of trehalose in ascospores is thought to act as a stress protectant against dehydration and heat (Dijksterhuis et al. 2002). The effect of sugars such as trehalose in protecting vital enzymes from heat has been shown in other organisms such as *Neurospora crassa* (Dijksterhuis et al. 2002).

Dijksterhuis and Teunissen (2004) showed that high pressure treatments at 400–800 MPa activated ascospores to germinate. The authors explained that high pressure constituted a stress which induced germination, in a situation akin to heat (Dijksterhuis et al. 2002). Ascospore age also affects heat resistance, with mature ascospores being more resistant to heat. This phenomenon has also been shown in ascospore suspensions, where young ascospores stored in buffer developed increased heat resistance over time (Reyns et al. 2003; Dijksterhuis and Teunissen 2004).

A PCR based assay using hydrophobin as the target molecule has been developed for detection of low levels of *T. macrosporus* and *T. trachyspermus* in food products (Yamashita et al. 2019). Many other species of *Talaromyces* and other foodborne ascosporic species were tested and not detected, but no mention is made of exclusion of

T. flavus, closely related to *T. macrosporus* but lacking similar heat resistance.

Inactivation of ascospores of *T. macrosporus* in clarified apple juice by a combination of UV-C light and high pressure processing (200 MPa) has been reported (Sauceda-Gálvez et al. 2019).

Mycotoxins This species has not been reported to produce significant mycotoxins.

Ecology *Talaromyces macrosporus* (sometimes reported as *T. flavus*) has been reported fairly frequently from heat processed juices (see Pitt and Hocking 1997), often as a cause of spoilage. *T. macrosporus* has rarely been reported away from heat processed juices. It has been found in low levels in pasteurised dairy products (Aydin et al. 2005).

References Stolk and Samson (1972) and Yilmaz et al. (2014).

Talaromyces pinophilus (Hedgcock) Samson et al. Fig. 7.45

Penicillium pinophilum Hedgcock

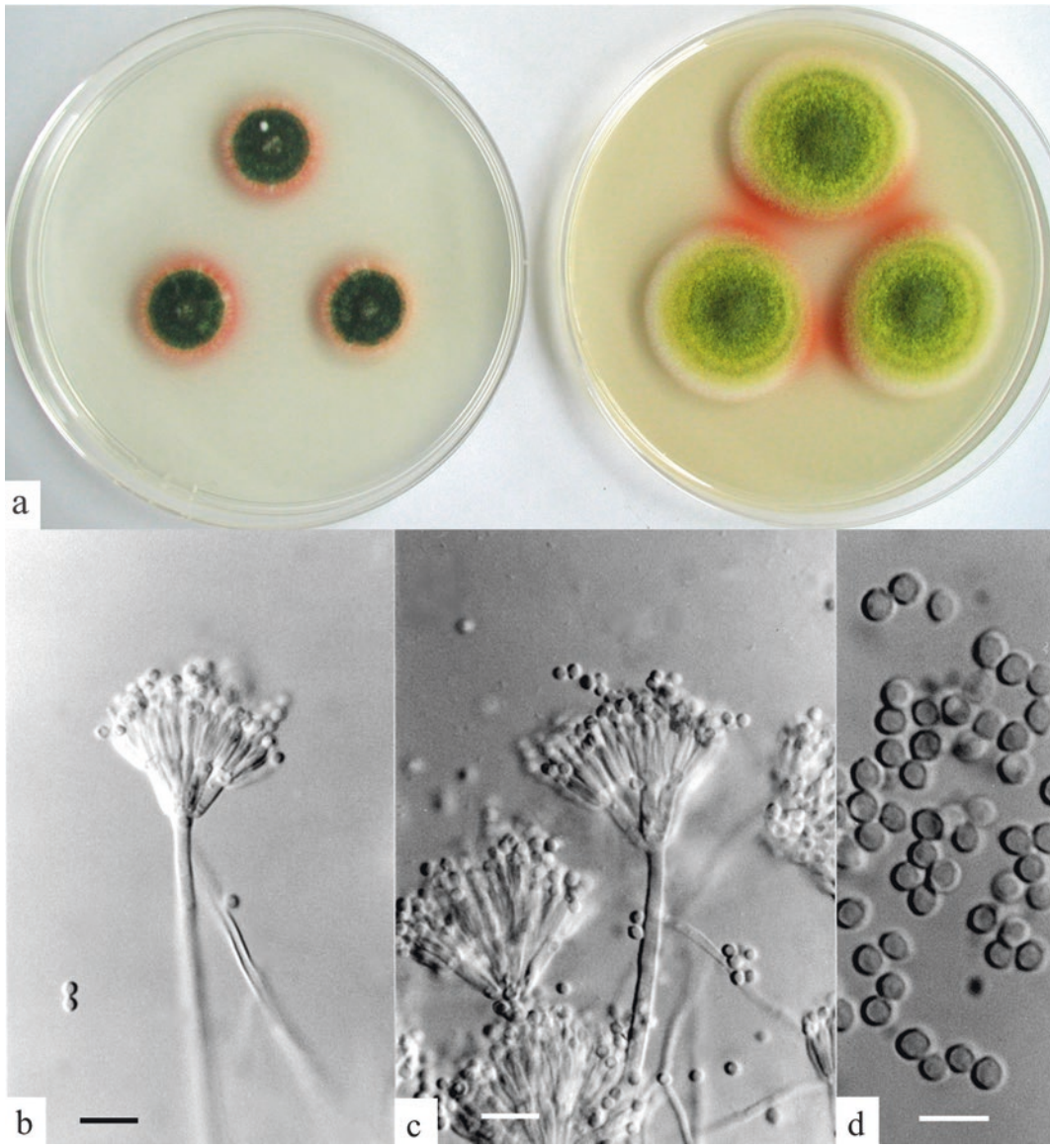

Fig. 7.45 *Talaromyces pinophilus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**d**) conidia, bar = 5 µm

Colonies on CYA 20–30 mm diam, plane or less commonly sulcate, texture rather loose and floccose, often with tufts of rudimentary funicles also; mycelium white at the margins and also sometimes in superficial overlays, but predominantly slightly brighter than Sulphur Yellow (1A5), usually produced in a relatively dense overlay over most or all of the colony area, dominating colony appearance and masking conidial colour; conidial formation moderate, usually Dark Green (28F5) but often appearing lighter due to the yellow mycelium; exudate sometimes present in small amounts, clear to red; reverse usually strongly coloured, Golden Brown (5C-D7), sometimes masked by or mixed with deep red shades as well. Colonies on MEA 25–35 mm diam, plane, deep and rather floccose, characteristics similar to colonies on CYA, except for paler reverse colouration and absence of white mycelial overlays. Colonies on G25N 2–5 mm diam, rarely as large as 8 mm, low and sparse; mycelium white; reverse pale, brown or olive. No germination at 5 °C. At 37 °C colonies 20–40 mm diam, plane or lightly sulcate, sometimes low and velutinous and coloured yellow green, more commonly deeply floccose with white mycelium predominant; conidial production absent to moderate, bluish to greenish grey; clear exudate usually present; reverse usually yellowish or reddish brown.

Sclerotia produced by some isolates, dark red to reddish brown, of variable size, composed of soft compacted hyphae, not of sclerotioid tissue. Ascospore formation not known. Conidiophores borne from aerial hyphae, stipes 150–180 µm long, smooth walled, often with vesiculate apices, bearing terminal biverticillate penicilli; metulae in verticils of 8–12 or even 16, 9–12 µm long, forming a 60–90° angle and in the latter case 12–15 µm across the apices; phialides acerose, 8–10 µm long, with gradually tapering collula; conidia subspheroidal, rarely ellipsoidal, commonly 2.5–2.8 µm long, with walls relatively heavy and smooth to finely roughened, borne in short disordered chains or loose columns.

Distinctive features *Talaromyces pinophilus* is characterised by the production of bright yellow mycelium which dominates colony appearance on CYA and MEA. Conidia are subspheroidal to ellipsoidal, less than 3 µm in long axis, and with smooth to finely roughened walls.

Taxonomy Using molecular methods Peterson and Jurjević (2019) divided the *"Talaromyces pinophilus"* complex into 10 species, including five that were newly described. All were very closely related: some came from peanuts or maize.

Identifiers Mycobank MB560662; neotype IMI 114933 (Pitt 1979b); ex-type cultures CBS 631.66, IMI 114933, ATCC 36839; ITS barcode JN899382; alternative markers *BenA* JX091381, *RPB2* KM023291, *CaM* KF741964 (Yilmaz et al. 2014).

Physiology Little is known about the physiology of this species. It grows rather strongly at 37 °C. From its taxonomic position we can infer that it not a xerophile and is similar in most properties to *Talaromyces funiculosus*.

Mycotoxins Mycotoxin production has not been reported.

Ecology Although *Talaromyces pinophilus* has been reported from foods only occasionally, in our experience it is not rare. It was reported from fresh meat by Nassar and Ismail (1994) and it was found in low levels on Spanish grapes (Bau et al. 2005). We isolated it from Philippine peanuts, and in one sample 85% of kernels were infected: this was exceptional, however (our unpublished data). We also found it in maize from Thailand and the Philippines and peanuts from Indonesia (Pitt et al. 1993, 1998).

References Pitt (1979b, 2000) and Yilmaz et al. (2014).

Talaromyces purpureogenus (Stoll) Samson et al. Fig. 7.46

Penicillium purpurogenum Stoll

Colonies on CYA 15–30 mm diam, plane or radially sulcate, dense, usually velutinous; mycelium bright yellow or red due to encrusted hyphae; conidial production moderate to heavy, Dark Green (25-27E-F5); exudate orange to red; soluble pigment Vivid Red (10A8); reverse dark red or purple, approaching black. Colonies on MEA 22–35 mm diam, plane, dense, velutinous; mycelium white to bright yellow; conidia abundantly produced, Dark Green (26-27E-G4-7); reverse usually pale, often brown or dull red centrally. Colonies on G25N microscopic to 6 mm diam, coloured as on CYA; reverse pale to deep brown. No germination at 5 °C. At 37 °C, colonies commonly 12–22 mm diam, usually similar to those on CYA at 25 °C, occasionally lacking soluble red pigment. Sexual state not known.

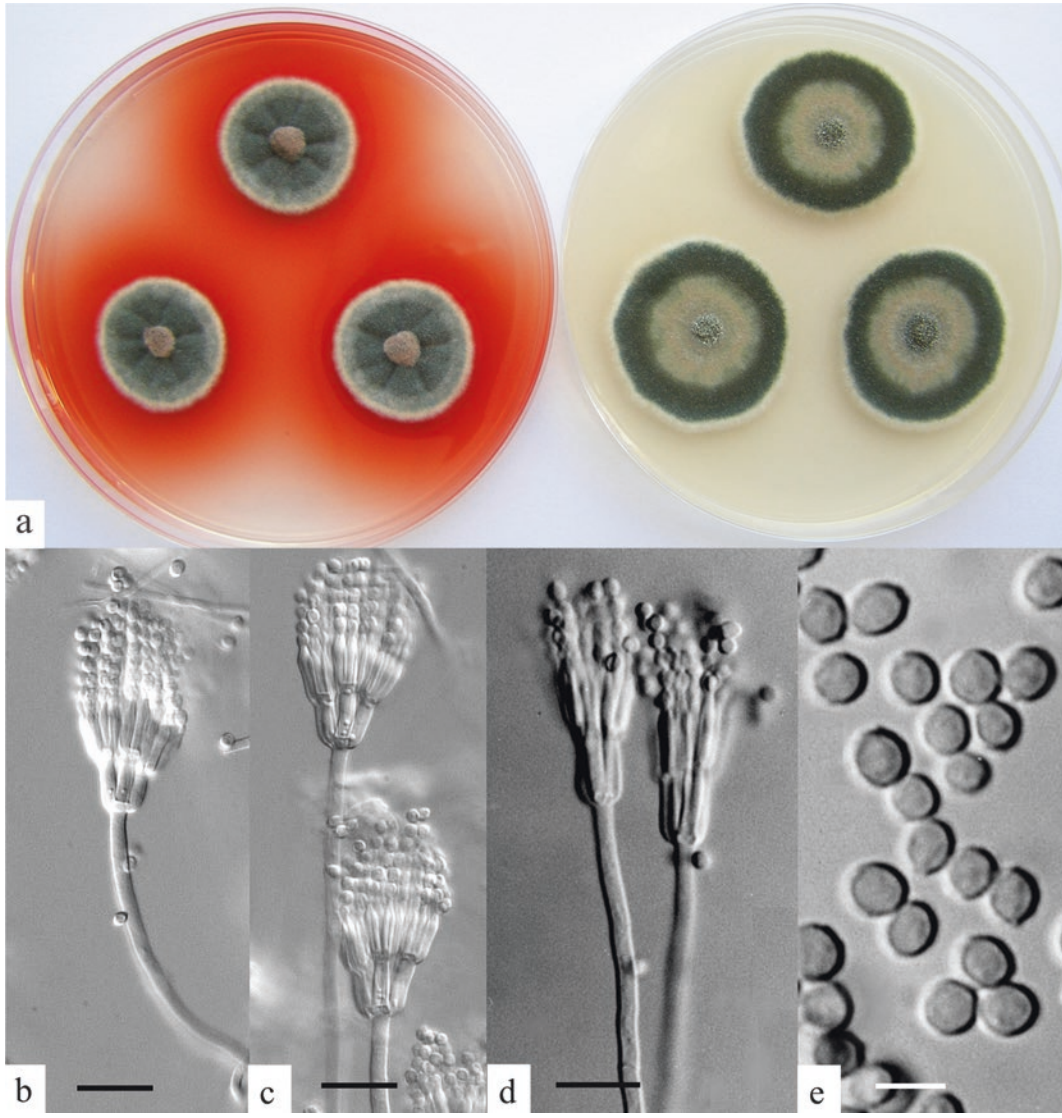

Fig. 7.46 *Talaromyces purpureogenus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Conidiophores borne from surface or aerial mycelium, stipes 70–300 µm long, smooth walled, bearing terminal biverticillate penicilli; penicilli narrow, metulae and phialides appressed, 10–14 µm long; conidia ellipsoidal, sometimes becoming subspheroidal at maturity, 3.0–3.5 µm long, with walls smooth, finely roughened or warty, borne in short irregular chains.

Distinctive features An intense, rapidly diffusing red pigmentation on CYA at both 25 °C and 37 °C is the most striking characteristic of *Talaromyces purpureogenus*. In the (rare) absence of such pigmentation, other diagnostic features include: conidia very dark green on both CYA and MEA; moderate growth at 37 °C (15–22 mm in 7 days); penicilli narrow and conidia ellipsoidal to subspheroidal and heavy walled, .

Identifiers Mycobank MB560667; lectotype IMI 91926 (Pitt 1979b); ex-type cultures CBS 286.36, IMI 91926; ITS barcode JN899372; alternative markers *BenA* JX315639, *RPB2* JX315709, *CaM* KF741947 (Yilmaz et al. 2014).

Physiology Minimum and optimum temperatures for growth by *Talaromyces purpureogenus* are reported as 12 °C and 30 °C (Mislivec and Tuite 1970); from our growth data, a maximum growth temperature near 40 °C would be expected. Hocking and Pitt (1979) reported 0.84 as the minimum a_w for germination and growth of this species. Like *T. funiculosus*, *T. purpureogenus* is highly tolerant of acid and can be readily isolated on media of pH 2.0 (Pitt, unpublished).

Mycotoxins The rubratoxins were originally reported from *Penicillium rubrum*, a species synonymised with *P. purpurogenum* by Pitt (1979b). Rubratoxins were originally suggested as causal agents of mouldy corn toxicosis, or haemorrhagic anaemia in chickens (Burnside et al. 1957; Forgacs et al. 1958). However, later studies (Wyatt and Hamilton 1972) concluded that rubratoxins were not the cause of these syndromes. Recent taxonomic studies (International Commission on *Penicillium* and *Aspergillus*, unpublished) indicate that rubratoxins are produced by only three known fungal isolates which represent an undescribed species.

Ecology A marginal xerophile and a recognised biodeteriogen (Pitt 1981), *Talaromyces purpureogenus* has been reported as a cause of spoilage in pineapples in India and pears and *Averrhoa bilimbi* fruit in Sri Lanka (see Pitt and Hocking 1997). It has been isolated from a wide variety of other foodstuffs, but seldom as a cause of spoilage: from cassava (C.J. Rabie, unpublished), maize, rice, wheat, barley, peanuts, soya beans and kidney beans (Aziz et al. 2006), pecans and betel nuts (see Pitt and Hocking 1997). It has also been found in processed meats (see Pitt and Hocking 1997).

References Pitt (1979b, 2000) and Yilmaz et al. (2014).

Talaromyces rugulosus (Thom) Samson et al. Fig. 7.47

Penicillium rugulosum Thom
Penicillium tardum Thom

Colonies on CYA 4–8 mm or occasionally 12 mm diam, plane, low and dense, velutinous; mycelium usually inconspicuous, mainly white but yellow or red encrusted hyphae often visible under magnification; conidia abundant, Greenish Grey to Dark Green (26-27E-F2-4); exudate and soluble pigment usually absent; reverse pale, dull olive or brown. Colonies on MEA 10–20 mm diam, similar to those on CYA except for more numerous and conspicuous yellow encrusted hyphae. Colonies on G25N 2–8 mm diam, velutinous; conidial production moderate, coloured as on CYA, other pigmentation absent. At 5 °C, in some isolates germination of conidia, in others germination limited or absent. At 37 °C, typically no growth, rarely colonies up to 4 mm diam formed.

Conidiophores borne from surface or aerial hyphae, stipes 70–100 µm long, with thin, smooth walls; penicilli basically biverticillate, but more complex structures often present, for example rami in verticils of up to 4, or rami and metulae from a common origin; metulae appressed; phialides acerose, tending towards ampulliform or less commonly cylindroidal, 8–11 µm long, typically with collula tapering to narrow pores, but sometimes untapered or even enlarging at the apices; conidia ellipsoidal, 3.0–3.5 µm long, with heavy, smooth to rough walls, borne in short to quite long, irregular columns. No sexual state known.

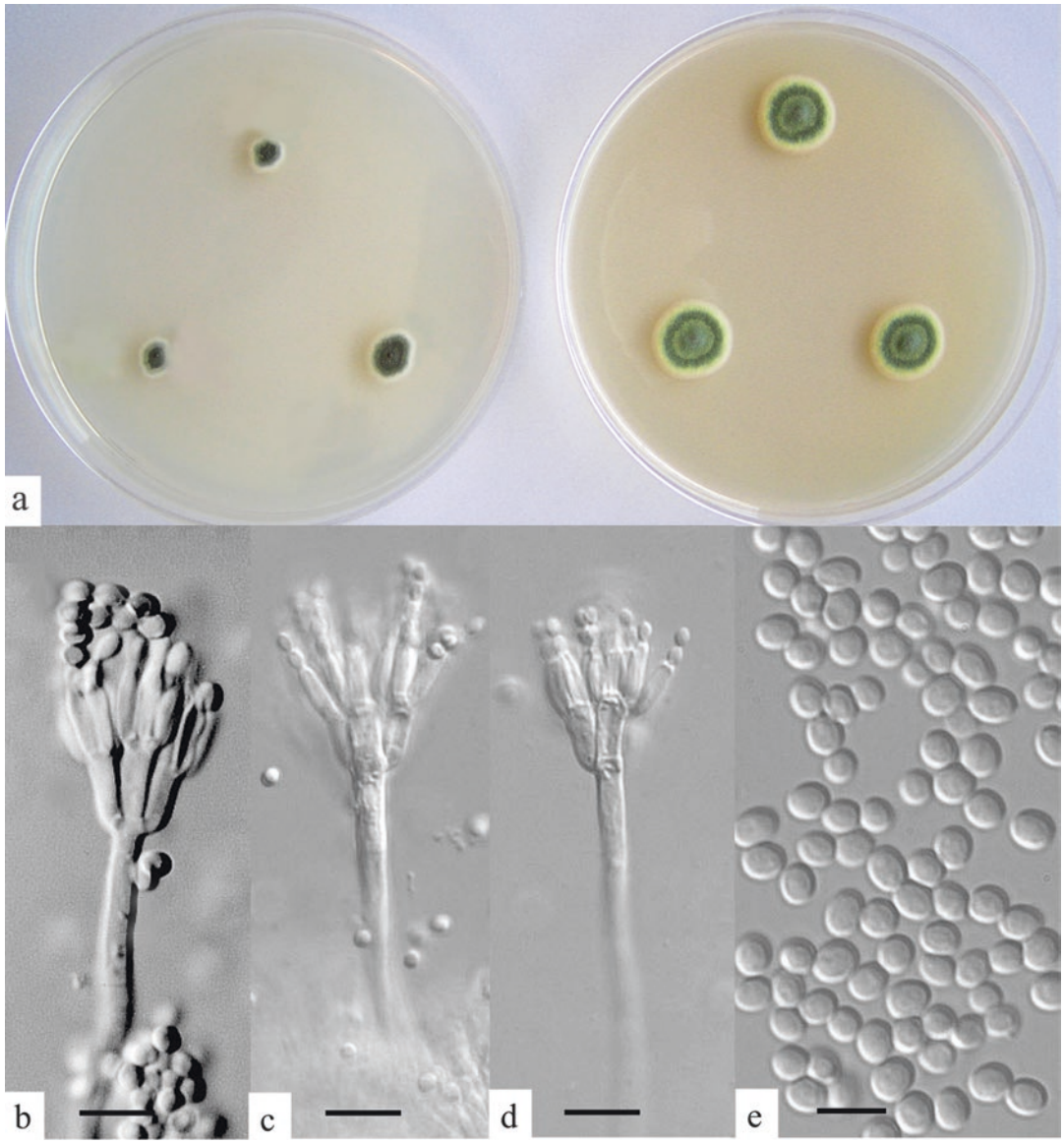

Fig. 7.47 *Talaromyces rugulosus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) penicilli, bars = 10 µm; (**e**) conidia, bar = 5 µm

Distinctive features *Talaromyces rugulosus* is characterised by very slow growth, velutinous colonies, and usually by germination at 5 °C. Although this species clearly belongs to this genus, penicilli may be of atypical structure at times, and may also bear ampulliform to cylindroidal phialides.

Identifiers Mycobank MB560672; lectotype IMI 40041 (Pitt 1979b); ex-type cultures CBS 371.48, IMI 40041, ATCC 10128, NRRL 1045, FRR 1045; ITS barcode KF984834; alternative markers *BenA* KF984575, *RPB2* KF984925, *CaM* KF984702 (Yilmaz et al. 2014).

Physiology From growth data (Pitt 1973), this species grows between 5 °C and 37 °C; the optimum would be expected to be near 25 °C. Snow (1949) reported 0.86 a_w to be its minimum for germination.

Mycotoxins Production of rugulosin by *Talaromyces rugulosus* was reported by Yamazaki

et al. (1971) and El-Banna et al. (1987). Although clearly a toxic compound when given intraperitoneally, with an LD_{50} of 83 mg/kg in mice (Cole and Cox 1981), rugulosin may be only a laboratory toxin. Disease due to rugulosin has not been reported.

Ecology Because of its very slow growth, *Talaromyces rugulosus* is easily overlooked in isolation procedures. It is probably a more commonly occurring species than records would suggest, and it appears to be widely distributed. This species can be a plant pathogen: it was described by Charles Thom in 1910 from rotting potato tubers, and was isolated by Barkai-Golan (1974) from decaying cold stored apples and the surface of fresh, healthy apples (Amiri and Bompeix 2005). *T. rugulosus* has been reported quite frequently from dried and processed meats (see Pitt and Hocking 1997). It has also been found in cheese (Hocking and Faedo 1992; Lund et al. 1995), rice, maize, barley, wheat, peanuts, soybeans and kidney beans (Aziz et al. 2006), flour and pecans (see Pitt and Hocking 1997). We isolated it at low levels from Thai cashews and Philippine soybeans (Pitt et al. 1994, 1998).

References Pitt (1979b, 2000) and Yilmaz et al. (2014, 2016).

Talaromyces trachyspermus (Shear) Stolk & Samson — Fig. 7.48

Arachniotus trachyspermus Shear
Talaromyces spiculosporus (Lehman) C. R. Benj
Penicillium lehmannii Pitt

Colonies on CYA 15–20 mm diam, occasionally smaller, plane, sparse at the margins, otherwise floccose; mycelium white; gymnothecia not produced; conidial production sparse; reverse pale to dull yellow brown. Colonies on MEA 20–25 mm diam, sparse at the margins but floccose and more dense centrally; mycelium white to creamish; gymnothecia tardily produced, few if any conidial structures, reverse pale to deep orange brown. On G25N, no growth or microcolonies. No germination at 5 °C. At 37 °C, colonies 25–40 mm diam, sparse at the margins, but deeper and floccose centrally; mycelium white, usually enveloping developing gymnothecia; conidial formation sparse or absent; reverse pale to dull brown.

Gymnothecia of densely woven hyphae, cream to pale yellow when mature (2–3 weeks); 300–500 µm diam; ascospores uncoloured, ellipsoidal, 3.5–4.5 µm long, with spinose walls. Conidiophores borne from aerial hyphae, short stipes (5–20 µm long) bearing terminal penicilli, biverticillate or monoverticillate, sometimes with subterminal elements; phialides acerose, 8–15 µm long, conidia variable, ellipsoidal to fusiform, 2.5–4.5 µm long, with smooth walls.

Distinctive features *Talaromyces trachyspermus* colonies are slow-growing, pale, with straw-coloured gymnothecia produced readily at 30–37 °C after 7–14 days.

Taxonomy Shear designated BPI 5798 as type of his species *Arachniotus trachyspermus,* transferred to *Talaromyces* by Stolk and Samson (1972). No living cultures ex type are known. Pitt (1979b) designated IMI 40043 as type of his species *Penicillium lehmannii* (set up under the then current Code of Nomenclature) and cultures now considered representative of *T. trachyspermus* are derived from that culture. The listing by Yilmaz et al. (2014) of IMI 40043 as type of *T. trachyspermus* and of ex-type cultures of *T. trachyspermus* is therefore in error. The correct citations are given below. Given that it is accepted that *P. lehmannii* and *T. trachyspermus* represent the same species, the ITS barcode and alternative markers are considered to be correct for *T. trachyspermus*.

Identifiers Mycobank MB324421; holotype BPI 5798; representative cultures (of *Penicillium lehmannii*) CBS 373.48, IMI 40043, ATCC 10497, NRRL 1028, FRR 1028 (Pitt 1979b); ITS barcode JN899354; alternative markers *BenA* KF114803, *RPB2* JF417432, *CaM* KJ885281 (Yilmaz et al. 2014).

Physiology Ascospores of *Talaromyces trachyspermus* are heat resistant. D values ranged between 50.0 and 90.9 min at 75 °C; 13.6 and 20.8 min at 78 °C; 5.1 and 12.4 min at 80 °C; 1.6 and 2.6 min at 82 °C depending on the medium (Tranquillini et al. 2017). While lower than values for *T. macrosporus,* those quoted above are high enough to withstand pasteurisation

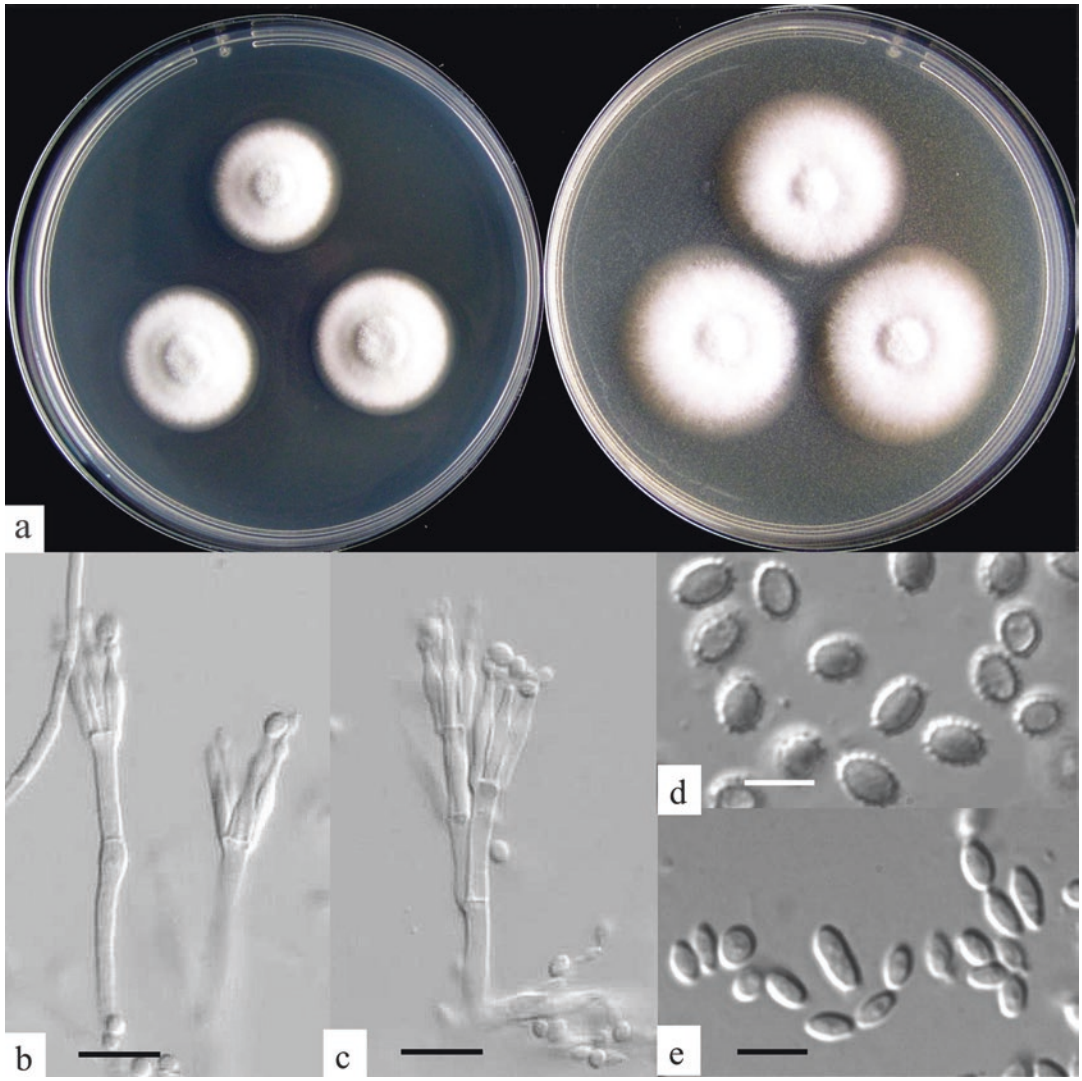

Fig. 7.48 *Talaromyces trachyspermus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) penicilli, bars = 10 µm; (**e**) ascospores, bar = 5 µm; (**f**) conidia, bar = 5 µm

(Tranquillini et al. 2017). *T. trachyspermus* was isolated from pineapple concentrate heated at 80 °C for 30 min (Enigl et al. 1993) and we have isolated it from a tea-based beverage that had received a heat treatment of 94 °C for 30 sec. Jesenská et al. (1992) isolated *T. trachyspermus* from soil that had been heated at 70 °C for 60 min, but not from samples that were heated at 80 °C for 60 min.

A PCR based assay using hydrophobin as the target molecule has been developed for detection of low levels of *T. macrosporus* and *T. trachyspermus* in food products (Yamashita et al. 2019).

Mycotoxins No mycotoxins have been reported from *T. trachyspermus*.

Ecology The natural habitat for *Talaromyces trachyspermus* is soil, reported from Nepal (Minoura et al. 1975), India (Rajak et al. 1991) and the Slovak Republic (Jesenská et al. 1992). Isolates in the CBS catalogue have come from soil in Morocco, Nigeria and Germany and bird dung in Venezuela. Food sources include pine-

apple juice and concentrate (Enigl et al. 1993), canned strawberries, pasteurised drinking yoghurt and honey tea with ginsing (CBS catalogue). In our laboratory all isolates of *T. trachyspermus* have come from heat processed products: fruit juice jelly, pineapple slices, canned strawberries and an iced-tea beverage.

References Stolk and Samson (1972), Pitt (1979a), and Yilmaz et al. (2014).

Talaromyces wortmannii (Klöcker) Stolk & Samson **Fig. 7.49**

Penicillium wortmannii Klöcker
Talaromyces variabilis (Sopp) Samson et al.
Penicillium variabile Sopp

Colonies on CYA and MEA 10–20 mm diam, plane or irregularly furrowed, usually low, dense and velutinous (Fig. 7.49b), sometimes floccose

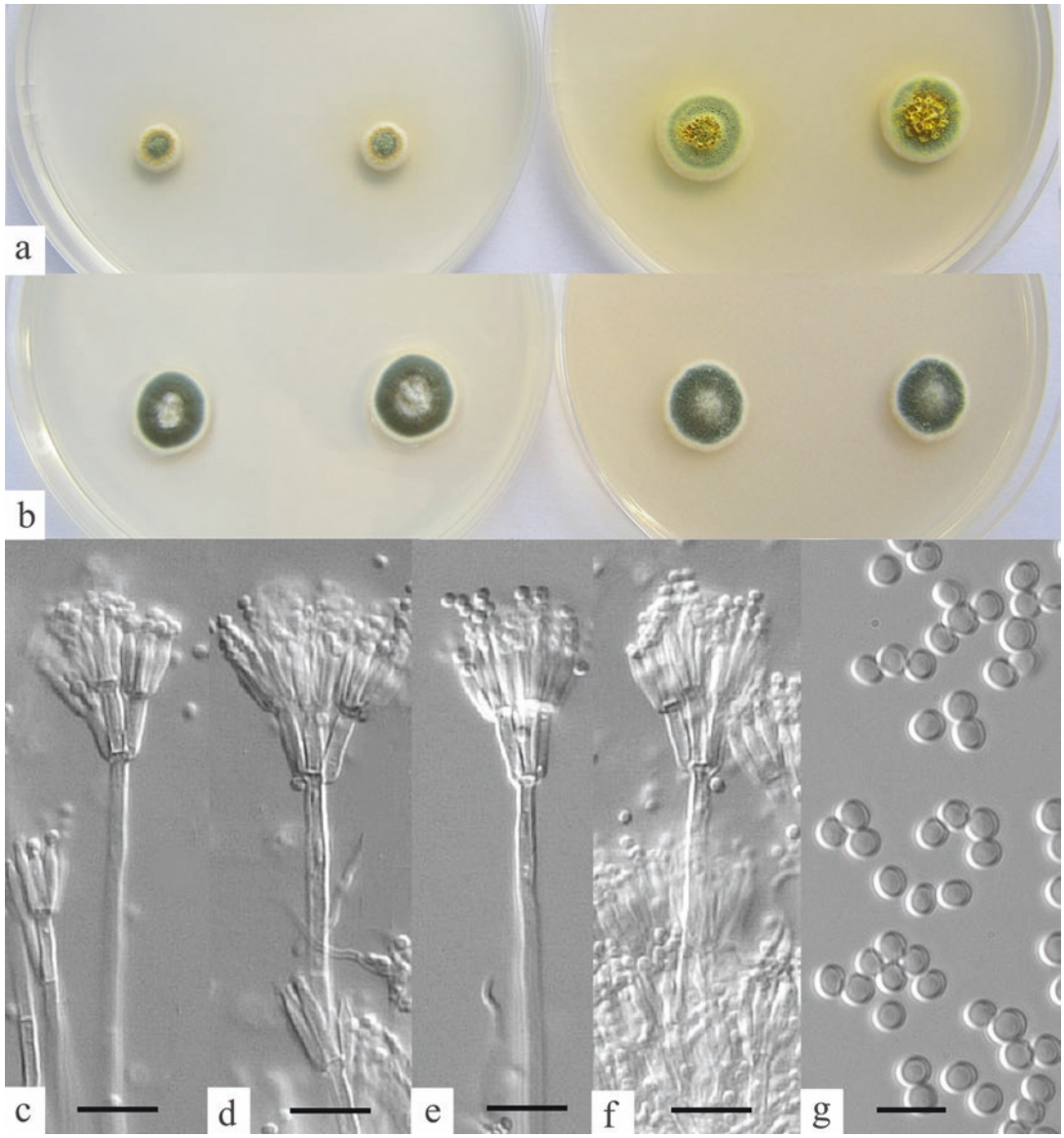

Fig. 7.49 *Talaromyces wortmannii. C*olonies exhibiting (**a**) the sexual state and (**b**) no sexual state on CYA and MEA, 7 d, 25 °C; (**c**–**f**) penicilli, bars = 10 µm; (**g**) conidia, bar = 5 µm

in isolates with a sexual state (Fig. 7.49a); mycelium commonly Sulphur Yellow (1A4-5), usually conspicuous only at the margins and near colony centres, more obvious in isolates with a sexual state; conidia moderately to abundantly produced, Greenish Grey (25-27D2); clear exudate and pale yellow soluble pigment occasionally produced; reverse on CYA Deep Orange to Raw Umber (5A-F8), on MEA more muted, usually Deep Orange to Brownish Yellow (5A-C8). Colonies on G25N microscopic to 9 mm diam, velutinous, sometimes heavily sporing; other colouration absent. No germination at 5 °C. At 37 °C, response variable, commonly no growth but sometimes colonies up to 5 mm diam produced, of white to pale yellow mycelium.

Sexual state produced by some isolates on MEA, gymnothecia of loosely woven yellow hyphae, maturing in 10–15 days, 100–200 µm diam; ascospores ellipsoidal, pale yellow, 3–5–5.0 µm long, with spinose walls.

Conidiophores borne mostly from surface hyphae, stipes commonly 100–200 µm long, but if from aerial growth much shorter, 10–30 µm long, smooth walled; penicilli usually biverticillate, sometimes with a subterminal ramus or with concurrent metulae and phialides, 8–12 µm long, appressed to quite divergent; conidia narrowly ellipsoidal, 3.0–4.0(−6.0) µm long, with walls smooth or faintly roughened, borne in closely packed but disordered chains.

Distinctive features *Talaromyces wortmannii* usually produces velutinous colonies with abundant greenish grey conidia, and with sulphur yellow mycelium conspicuous only in peripheral and central areas (Fig. 7.49b). However, occcasional isolates produce a sexual state, when colonies are usually more floccose with less conidial production (Fig. 7.49a). Growth is poor to negative at 37 °C. As a distinction from *T. rugulosus*, penicilli of *T. wortmannii* are typical of the genus and conidia never germinate in 7 days at 5 °C.

Taxonomy In transferring species from *Penicillium* subgenus *Biverticillium* to *Talaromyces*, Samson et al. (2011) maintained *T. wortmannii* and *T. variabilis* as distinct species. However in a more detailed study, Yilmaz et al. (2016) placed *T. variabilis* in synonymy and this decision has been followed here.

Identifiers Mycobank MB344294; lectotype IMI 40047 (Pitt 1979b); ex-type cultures CBS 391.48, IMI 40047, ATCC 10517, NRRL 1017, FRR 1017; ITS barcode KF984829; alternative markers *BenA* KF984648, *RPB2* KF984977, *CaM* 984,756 (Yilmaz et al. 2014).

Physiology Mislivec and Tuite (1970) reported 12 and 30 °C to be the minimum and optimal growth temperatures for *Talaromyces wortmannii* (as *Penicillium variabile*); the maximum is near 37 °C (Pitt 1973). The minimum a_w for germination and growth is 0.86 (Hocking and Pitt 1979).

Mycotoxins Like *Talaromyces rugulosus*, *T. wortmannii* produces rugulosin (Yamazaki et al. 1972; El-Banna et al. 1987, as *P. variabile*). This is probably not an important toxin, with no records of implication in human or animal disease.

Ecology Although the primary habitats of *Talaromyces wortmannii* would be expected to be soil and decaying vegetation, it has quite commonly been found in foods. Isolations have come principally from cereals: wheat and flour, maize, rice and barley (see Pitt and Hocking 1997). Some other reported sources have been processed meats (Cantoni et al. 2007), peanuts (Pitt et al. 1998), biltong, cheese, pecans, walnuts and betel nuts (see Pitt and Hocking 1997). Isolates producing the sexual state have been isolated less commonly, from wheat, pecans and salami (see Pitt and Hocking 1997). Theere have been no reports of spoilage or occurrence in pasteurised products.

References Pitt (1979b, 2000) and Yilmaz et al. (2014, 2016).

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Aspergillus and Related Teleomorphs

8

Thriving in, or at least tolerant of, elevated temperatures and reduced water activities, many species of *Aspergillus* and its teleomorphs are the epitome of spoilage fungi. There are few kinds of foods, commodities and raw materials from which Aspergilli cannot be isolated consistently.

Aspergillus species compete with *Penicillium* (including *Talaromyces*) and *Fusarium* species for dominance among the world's fungal flora. *Aspergillus* lacks the sheer numbers and diversity of species of *Penicillium,* but compensates by the ability to grow at higher temperatures or lower water activities or both. Aspergilli usually grow more rapidly than Penicillia, although they take longer to sporulate, and produce spores which are often more resistant to light and/or chemicals. It is fair to say that *Aspergillus* species dominate spoilage in the tropics in the way *Penicillium* species do in temperate zones.

Aspergillus is the asexual state of a group of genera characterised by the formation of conidiophores with large, often heavy walled stipes and swollen apices, termed **vesicles**. Vesicles are usually roughly spherical, but are elongated or less conspicuously swollen in a few species. Vesicles bear crowded phialides, or metulae and phialides, which are characteristically all borne simultaneously (Fig. 8.1a). This character unequivocally distinguishes *Aspergillus* from *Penicillium* and *Talaromyces* (Pitt and Hocking 1985). Phialide production in *Penicillium* and *Talaromyces* is always successive, not simultaneous (Fig. 8.1b). Ready differentiation between these two types of genera may be obtained by microscopic examination of developing conidiophores picked from near the colony margins. The presence of immature metulae or phialides all at the same stage of development indicates *Aspergillus*; structures with some phialides producing conidia while one or more others are still developing indicates *Penicillium* or *Talaromyces*.

Two other useful features are characteristic of most, though not all, species with *Aspergillus* asexual states. First, stipes are usually formed from a short cell termed a **footcell** within a fertile hypha. Second, stipes are usually nonseptate, so that the vesicle, stipe and footcell all form a very large single cell. Stipes of *Penicillium* and *Talaromyces* species are usually septate and footcells are exceptional.

For many years, the authoritative text on *Aspergillus* was "The Genus *Aspergillus*" by Raper and Fennell (1965). However, contrary to the then current edition of the Botanical Code, Raper and Fennell (1965) declined to give status to teleomorph names, failed to typifiy species and used some terminology already known to be misleading. These issues were subsequently addressed by various taxonomists. Subramanian (1971) and Malloch and Cain (1972) provided names for *Aspergillus* teleomorphs. Gams et al. (1985) developed a new classification of *Aspergillus* based on subgenera and series to replace the incorrect term "group". Gams and

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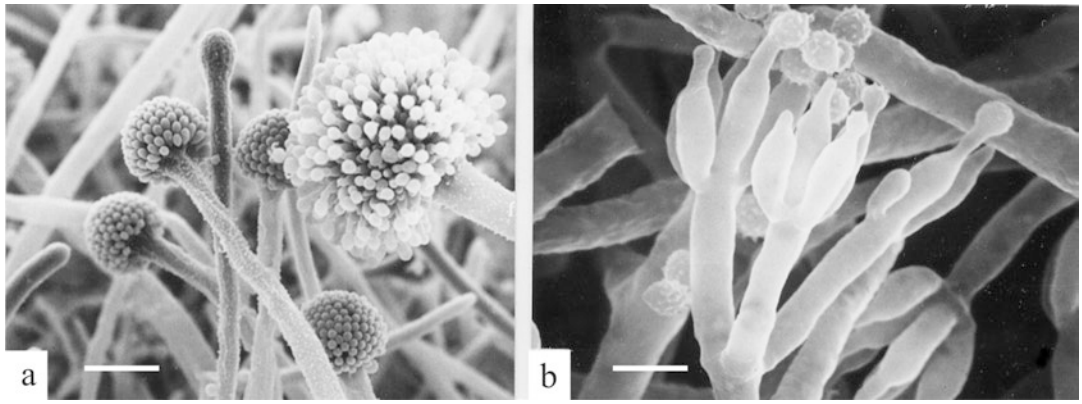

Fig. 8.1 Scanning electron micrographs: *Aspergillus oryzae* showing simultaneous production of phialides, bar = 50 µm; *Penicillium janczewskii* showing successive production of phialides, bar = 10 µm

Samson (1985) provided typifications of *Aspergillus* and its associated teleomorph genera, while Samson and Gams (1985) typified the species of *Aspergillus* accepted by Raper and Fennell (1965). Samson (1979) produced a useful compendium and taxonomic outline of the 90 species described between 1965 and that date. A guide to common *Aspergillus* species and *Aspergillus* teleomorphs which incorporated all of these advances was published by Klich (2002). Pitt and Samson (1993) produced a list of Names in Current Use (NCU) for the family *Trichocomaceae*, which includes *Aspergillus* and related teleomorphs. By this means the names used by Raper and Fennell (1965) were given *de facto* protection against earlier valid names. This was especially significant in *Aspergillus*, where a number of common names cannot be justified under the Botanical Code. The names used here are in all cases those given protected status by the NCU list. One particularly important name, *Aspergillus niger*, was conserved under the provisions of the Botanical Code (Frisvad et al. 1990; Kozakiewicz et al. 1992) because this name was known to be predated by *A. phoenicis* (Corda) Thom & Currie and *A. ficuum* (Reichardt) Hennings.

Students of food mycology soon recognised that teleomorphs of *Aspergillus* were associated with particular physiological properties. Those given the name *Eurotium* signified species with exceptional xerophilic properties, particularly studied by Pitt (1966, 1973), while *Neosartorya* species often produced highly heat resistant ascospores troublesome to manufacturers of pasteurised foods, especially juices (Hocking and Pitt 1984). Taxonomic books on food spoilage (Samson et al. 1981; Pitt and Hocking 1985) used these teleomorph names for sexual *Aspergillus* species and that continued over subsequent editions. In consequence food bacteriologists and food manufacturers came to rely on those teleomorph names for information about specific types of food spoilage throughout the next half century.

The asexual fruiting structure that characterises *Aspergillus* is associated with about 10 teleomorph genera, with widely varied types of cleistothecia and distinct differences in the *Aspergillus* states as well. The more important of these are shown in Fig. 8.2. The figure shows an astonishingly large and extremely diverse group of genera, unified only by the characteristics of the *Aspergillus* state, as delineated by Pitt and Hocking (1985). (A well known phylogenist has suggested that *Aspergillus* as shown in Fig. 8.2 contains as much genetic variability as all genera of fish combined).

Under the "dual nomenclature" system that prevailed under successive Botanical Codes, when the teleomorph name was known, the sexual generic name and the asexual *Aspergillus* name were used interchangeably. That presented no nomenclatural or taxonomic problem until the

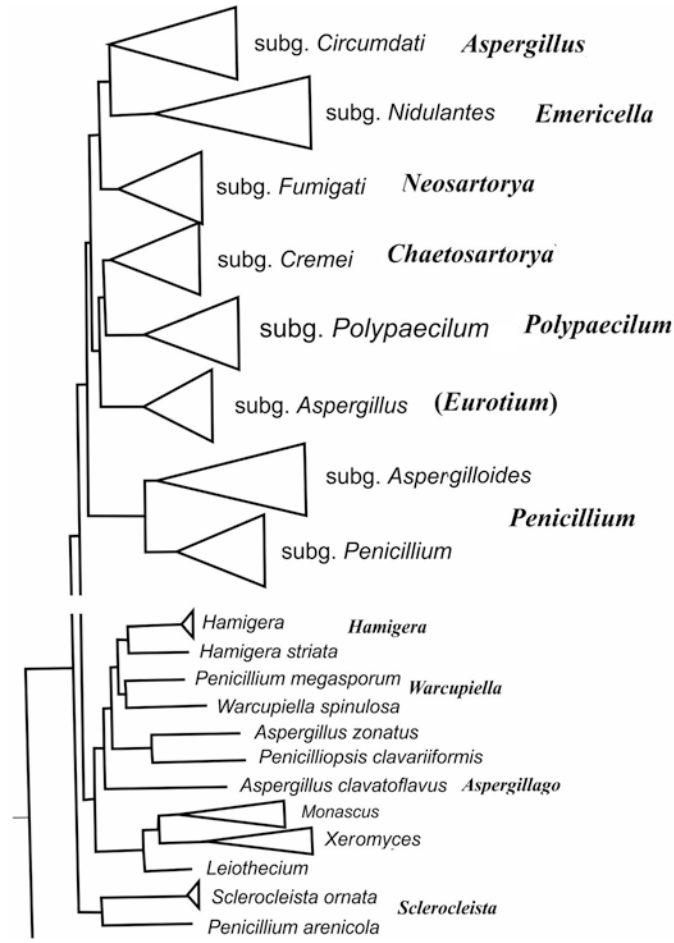

Fig. 8.2 Phylogeny of *Aspergillus* in the classical sense. (Redrawn from Fig. 3A and a partial reproduction of Fig. 2A of Kocsubé et al. (2016), without omission or distortion of elements. "Monophyletic groups are collapsed and shown as triangles" Kocsubé et al. (2016). Names of teleomorph genera correlating to clades have been added)

new International Code of Nomenclature for algae, fungi and plants (ICN) was approved by the Botanical Congress in Melbourne in 2012. The ICN decreed that henceforth each fungus could have only one name (McNeill et al. 2012). That caused one immediate issue, still unresolved: by sheer chance the types of *Aspergillus* P. Micheli 1729 and *Eurotium* Link 1809 are the same species. *Eurotium herbariorum* (Weber ex F.H. Wigg.) Link ex Nees was shown to be the same species as *Aspergillus glaucus* (L.) Link by Anton de Bary in 1854. This means that the moment that the decision by the Botanical Congress in 2011 took effect, the name *Eurotium* could no longer be validly used because it had become an obligate synonym of *Aspergillus*. (It was unthinkable that *Eurotium* could be given priority over *Aspergillus)*.

This has created a very unfortunate situation. As noted above, the name *Eurotium* is very important to the food industry as *Eurotium* species are major spoilers of foodstuffs. However, as well as causing food spoilage, *Eurotium* species are capable of causing spoilage of almost any organic material stored above a safe water activity, including textiles, leather goods, paper products, watercolour paintings, museum artefacts and even photographic slides and microscope lenses. Everyone associated with all of these areas is now forced by the ICN to use the name *Aspergillus* in place of *Eurotium*. Using the name "*Aspergillus*" for what food mycologists have come to know as *Eurotium* species over the past 40 years loses clarity. Indeed "*Aspergillus*" is associated in the minds of food manufacturers with the very important mycotoxin producing

fungi *A. flavus*, *A. ochraceus*, *A. niger* and their relatives. This is not a trivial matter. Identification of a common *Eurotium* species as "*Aspergillus*" is capable of ringing alarm bells for thousands of small scale food processors around the world.

A sensible, scientifically based answer to this problem is to move the type of *Aspergillus* from *A. glaucus* (= *Eurotium herbariorum*) to a more logical place, *A. niger*, as proposed by Pitt and Taylor (2016) and Taylor et al. (2000). Those papers placed a formal proposal, known as Proposal 2441, to make that change before the Nomenclature Committee for Fungi (NCF) of the International Botanical Congress. A decision on that Proposal has not yet been formally reached. In the meantime, or indeed if the NCF and then the next Botanical Congress decide that the type of *Aspergillus* will remain where it is, *Eurotium* must be known as "*Aspergillus* subgenus *Aspergillus"* and *Eurotium* species must be known by their corresponding *Aspergillus* names.

Molecular taxonomy soon showed that the entire clade encompassing all species with *Aspergillus* asexual states was monophyletic – provided that it included *Penicillium* and one or two minor genera which also lacked *Aspergillus* asexual states. To consider *Penicillium* a synonym of *Aspergillus* was unthinkable, so Houbraken et al. (2012) chose instead to exclude from *Aspergillus* all clades with *Aspergillus* asexual states that lay more distant than *Penicillium* from a hypothetical central species in *Aspergillus* subgen. *Circumdati* or subgen. *Nidulantes*. They used existing sexual state names where possible and erected new genera where necessary. They then considered that all species remaining closer to that hypothetical centre be called *Aspergillus*. That included those formally in *Eurotium* as well as those with sexual states in *Neosartorya*, *Chaetosartorya* Subram. and *Emericella* and those in a clade allied with *Polypaecilum*, which lacks the *Aspergillus* state (Houbraken et al. 2012). These concepts are shown in Fig. 8.2, above the scale break.

This *Aspergillus* concept has been maintained by several groups of authors anchored in the Westerdijk Institute, Utrecht, over the succeeding years. However, such a concept ignores several issues. First, *Aspergillus* subgenus *Aspergillus* (*Eurotium*), *Neosartorya*, *Emericella* and *Petromyces* (the unused sexual name for species in *Aspergillus* subgenus *Circumdati*) have sexual states markedly different from each other. These are sufficiently different, both morphologically and phylogenetically, to warrant use of the sexual state names. Second, the physiological properties of these genera are greatly different, leading to totally different problems for the food microbiologist or technologist (Table 8.1). Third, these sexual state names have been used by food mycologists, bacteriologists and technologists for 40 years, so that the identification of an isolate using a specific name provides instant awareness of the food spoilage or toxicity problem that has been encountered. *"Eurotium"* to a food manufacturer means spoilage of foods held above a safe moisture content. "*Neosartorya*" indicates spoilage by a heat resistant fungus. "*Emericella*" includes mostly soilborne contaminants of lower importance. The use of "*Aspergillus"* loses all of those connotations. Third, "*Aspergillus"* suggests a possible mycotoxin problem – which the other sexual state names do not. Fourth, *Polypaecilum,* and related genera of no consequence here, lacks the characteristic *Aspergillus* fruiting structure and looks much less like an *Aspergillus* than do species of *Penicillium* – which has been deliberately kept separate.

For these reasons, the generic names *Neosartorya*, *Emericella* and *Polypaecilum* have been retained here. Names in these genera are valid and will remain so into the foreseeable future. Molecular studies show that species within these genera occupy very well delimited clades, with no overlap, whether or not the particular species is known to have a sexual state. The morphologies of the *Aspergillus* states also correlate completely with molecular data. No intermediate or uncertain species lie between these clades or genera (Houbraken et al. 2012; Kocsubé et al. 2016). Because these clades are so strongly supported, we believe it is important to introduce new combinations for four common species that lie within those clades, but which have no known sexual state and have always been known by their *Aspergillus* names. These are

Table 8.1 Characteristics of major genera considered to be *Aspergillus* by Houbraken et al. (2011a, b)

Property	*Aspergillus* subgenus *Aspergillus* [*Eurotium*]	*Neosartorya*	*Emericella*	*Polypaecilum*	*Petromyces*
Vesicles	Spherical	Spathulate	Pyriform	Absent	Spherical
Conidiogenous cells	Phialides only	Phialides only	Metulae and phialides	Single or polyphialdes	Usually metulae and phialides
Conidiogenous cells borne over	3/4 of vesicle	2/3 of vesicle	Half of vesicle	Absent	Completely around vesicle
Water relations	Xerophilic	Mesophilic	Mesophilic	Halophilic	Moderately xerophilic
Temperature relations	Mesophilic	Thermophilic	Moderately thermophilic	Mesophilic	Moderately thermophilic
Ecology	Dried seeds, foods and artefacts	Soil fungi	Soil fungi	Salty habitats	Mostly in fresh plants and fruit
Mycotoxins	Unimportant	Gliotoxin, others unimportant	Unimportant	None	Aflatoxins, ochratoxin fumonisins

Emericella sydowii, *Emericella usta*, *Emericella versicolor* and *Neosartorya clavata.* Such new combinations are valid under the ICN.

Classification Classification of *Aspergillus* and the sexual genera of interest here relies primarily on three features: the presence of a sexual state and its characteristics; in the absence of a sexual state, morphology of fruiting structures; and colony colours. Cleistothecia are distinctively coloured; in species lacking cleistothecia, colony colours are dominated by conidial colour. Colony diameters are a valuable additional character. The key which follows includes all *Neosartorya, Emericella* and *Aspergillus* subgenus *Circumdati* species of relevance here, but a second key is provided to *Aspergillus* subgenus *Aspergillus* (*Eurotium*) because of its complexity.

In keeping with standardisation of media and growth conditions throughout this book, colony diameters at 5 °C are incorporated in the descriptions which follow. However it should be noted that very few *Aspergillus* or *Aspergillus* teleomorph species germinate at 5 °C in 7 days, so if the user knows in advance that an isolate is an *Aspergillus* or related teleomorph, the 5 °C plate is logically omitted.

Key to *Aspergillus* Species and Teleomorphs Included Here

1	Colonies on CYA at 25 and 37 °C both exceeding 35 mm diam	2
	Colonies on CYA at 25 °C or at 37 °C not exceeding 35 mm diam	12
2(1)	Colonies black or grey	3
	Colonies white or coloured	6
3(2)	Colonies black, exceeding 50 mm diam at 25 °C	4
	Colonies grey, not exceeding 50 mm diam at 25 °C	*Aspergillus calidoustus* See *Emericella usta*
4(3)	Vesicles bearing metulae and phialides	5
	Vesicles bearing phialides alone	*A. aculeatus* *A. japonicus*
5(4)	Conidia less than 6 µm diam	*A. niger*
	Conidia more than 6 µm diam	*A. carbonarius*
6(2)	Colonies white	*Neosartorya spinosa* *Neosartory glabra*
	Colonies coloured	7

(continued)

7(6)	Colonies blue	*Neosartorya fumigata*
	Colonies yellow, green or brown	8
8(7)	Conidia dark green; developing cleistothecia present, surrounded by cells like chlamydoconidia (Hülle cells)	*Emericella nidulans*
	Conidia yellow, yellow green or brown; developing cleistothecia not present	9
9(8)	Conidia yellow green or yellow	10
	Conidia brown or olive brown	11
10(9)	Conidia with relatively thin walls, smooth or finely roughened, spherical to broadly ellipsoidal; vesicles up to 50 µm diam, usually metulae present	*A. flavus* *A. oryzae* *A. nomius*
	Conidia with consistently rough, thick walls, spherical; vesicles not usually exceeding 30 µm diam; usually only a low proportion of heads with metulae	*A. parasiticus*
11(9)	Colonies olive brown on CYA and MEA at 25 °C; conidia 5–8 µm diam, rough walled; heads radiate	*A. tamarii*
	Colonies brown on all media; conidia less than 3 µm diam, smooth walled; heads developing into long columns	*A. terreus*
12(1)	Colonies grey	*Emericella usta*
	Colonies white or brightly coloured	13
13(12)	Colonies white, off-white or cream	14
	Colonies coloured	16
14(13)	Vesicles fertile over the entire area; metulae more than 10 µm long	15
	Vesicles fertile over the upper half to two thirds; metulae less than 10 µm long	*A. niveus*
15(14)	Vesicles spherical, 30–40 µm diam, no growth at 37 °C	*A. candidus*
	Vesicles somewhat elongate, <20 µm diam on long axis, colonies CYA at 37 °C > 10 mm	*A. tritici*
	See *A. candidus*	
16(13)	Developing yellow cleistothecia present, particularly in colonies on G25N	*Aspergillus* subgenus *Aspergillus* (genus *Eurotium)*
	Developing cleistothecia not present	17
17(16)	Conidia in shades of yellow, orange or brown	18
	Conidia green or blue	20
18(17)	Colonies on CYA exceeding 40 mm diam; conidia pale brown (ochre)	*A. ochraceus*
	Colonies on CYA not exceeding 40 mm diam; conidia in yellow or orange shades	19
19(18)	Colonies greyish orange, conidia 2–3 µm diam, growth on G25N less than 15 mm diam	*A. flavipes*
	Colonies greyish yellow to olive, conidia 4–5 µm diam, growth on G25N exceeding 25 mm diam	*A. wentii*
20(17)	Colonies on CYA exceeding 30 mm diam; vesicles more than 50 µm diam	*Neosartorya clavata*
	Colonies on CYA not exceeding 30 mm diam; vesicles less than 25 µm diam	21
21(20)	Colonies on CYA exceeding 15 mm diam; heads with metulae	22
	Colonies on CYA not exceeding 15 mm diam; heads with phialides only	23
22(21)	Conidia green	*Emericella versicolor*
	Conidia blue	*Emericella sydowii*
23(21)	Colonies on CYA and MEA 6 mm or more diam; conidia cylindrical to barrel shaped, borne in columns	*A. restrictus* See *Aspergillus* subgen. *Aspergillus*
	Colonies on CYA and MEA not exceeding 6 mm diam; conidia subspheroidal to ellipsoidal, borne in radiate heads	*A. penicillioides* See *Aspergillus* subgen. *Aspergillus*

8.1 Genus *Emericella* Berk

Emericella was originally described by Berkeley in 1857, but the name was little used until revived by Benjamin (1955). The genus includes species with *Aspergillus* conidiophores but also characterised by the formation of white cleistothecia which mature within two weeks and contain red or purple ascospores. The cleistothecia are usually surrounded by Hülle cells, which are thick walled roughly spherical cells resembling chlamydoconidia. All such species produce distinctive *Aspergillus* conidiophores, mostly with short, slightly sinuous, brown walled stipes which bear both metulae and phialides from small heads. The conidia are always green. Most species grow well at 37 °C and are not xerophilic.

Christensen and States (1982) accepted 29 species within that circumscription and provided keys and descriptions. Many of those species are known primarily or solely from desert soils in the western United States and other parts of the world.

The concept of *Emericella* was broadened by Gams et al. (1985) to include species with no known sexual state. They divided the genus (as *Aspergillus* subgenus *Nidulantes* W. Gams et al.) into five sections, with three of interest to us here: *Nidulantes, Versicolores* and *Usti.* Molecular taxonomy (Houbraken et al. 2000, 2011a, b) resulted in sections *Nidulantes* and *Versicolores* being combined, a decision confirmed by Chen et al. (2016), who provided a more complete taxonomy. Chen et al. (2016) also provided excellent colony photographs, photomicrographs of the *Aspergillus* states and scanning electron micrographs of ascospores of the *Emericella* states. Many of the sexual species are very closely related and the monograph of Chen et al. (2016) should be consulted for detailed information on them. Four species are of interest to us here: *E. versicolor*, *E. sydowii* and *E. usta*, all of which are transferred from *Aspergillus* herein; and *E. nidulans*, representative of the species with sexual states. These species have all been keyed in the general key above.

Emericella nidulans (Eidam) Vuill. Fig. 8.3

Aspergillus nidulans (Eidam) G. Winter

Colonies on CYA 40–50 mm diam, plane, low, moderately dense to dense, sometimes with a floccose overlay; mycelium white; cleistothecia white, surrounded by white to buff or dull yellow Hülle cells; conidial heads sparse to quite dense, radiate when young, later forming long, well defined, columns, conidia coloured pale green or when dense dark green; exudate sometimes present, dull red to brown; violet soluble pigment sometimes produced; reverse sometimes pale, usually brightly coloured in shades of orange, orange brown, deep brown or violet brown. Colonies on MEA 35–60 mm diam, occasionally only 25 mm, sometimes low, plane and velutinous with heavy conidial formation and few cleistothecia, sometimes deeper and with abundant cleistothecia surrounded by dull yellow or buff Hülle cells; mycelium white; cleistothecia sometimes abundant, surrounded by Hülle cells, at other times sparsely produced; conidia dark green; reverse pale, brown or violet brown. Colonies on G25N 10–15 mm diam, low and dense; conidia pale green; reverse pale. No growth at 5 °C. At 37 °C, colonies 50–70 mm diam, low and sparse, usually predominantly cleistothecial, sometimes with areas of dark green conidia; reverse usually orange or brown.

Cleistothecia mostly 100–250 µm diam, white at first but at maturity dark red, maturing on CYA in 8–10 days, surrounded by heavy walled Hülle cells, 15–25 µm diam; ascospores red to purple, ellipsoidal, 4–6 µm long, smooth walled, usually ornamented with two conspicuous longitudinal flanges. Conidiophores borne from aerial hyphae, stipes 60–150 µm long, often sinuous, with smooth, brown walls and with conspicuous footcells; vesicles spathulate to pyriform, 8–12 µm wide, bearing metulae and phialides over the upper half or less, of similar size, 6–8 µm long; conidia spherical, green, 3.0–3.5 µm diam, with roughened walls, borne in radiate to loosely columnar heads.

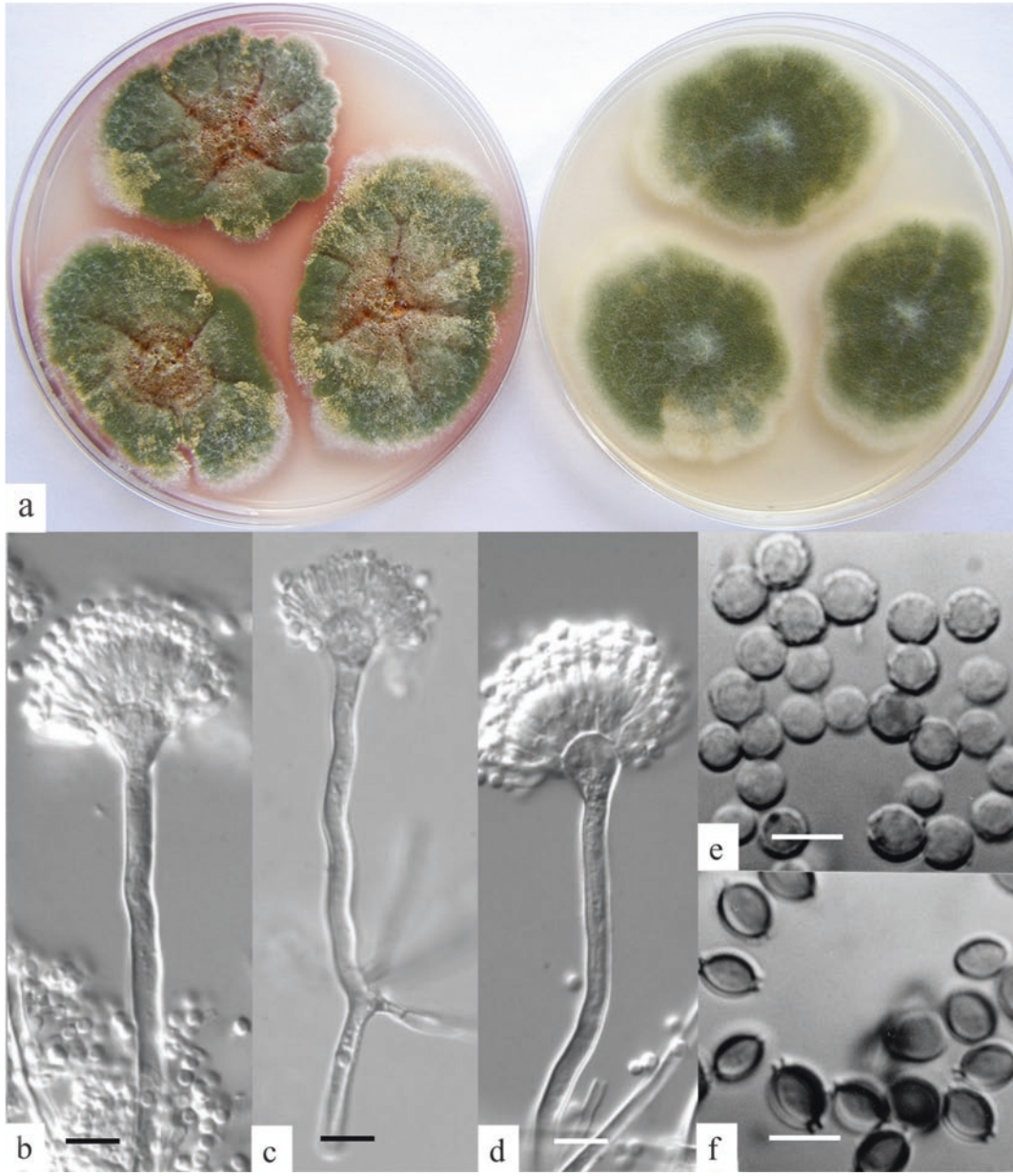

Fig. 8.3 *Emericella nidulans* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) heads, bars = 10 µm; (**e**) conidia and (**f**) ascospores, bars = 5 µm

Distinctive features *Emericella nidulans* is distinguished by growth on CYA at least as fast at 37 °C as at 25 °C, developing cleistothecia surrounded by Hülle cells and sparse to abundant green conidia borne on metulae and phialides from diminutive brown walled stipes. Mature ascospores are red, and have either two longitudinal flanges or no ornamentation. Other cleistothecial *Emericella* species show different ascospore ornamentation (see Chen et al. 2016).

Taxonomy In the absence of a decision by a Botanical Congress on whether the sexual or asexual state of this species (and all related species) has priority, both names given above are valid and can be used.

Identifiers Mycobank MB182069; neotype IMI 86806 (Samson and Gams 1985); ex-type cultures CBS 589.65 = IMI 86806 = NRRL 187 = ATCC 10074 = FRR 187; ITS barcode EF652427; alternative markers *BenA* EF652251, *CaM* EF652339, *RPB2* EF652163 (Houbraken et al. 2007).

Physiology According to Panasenko (1967), *Emericella nidulans* is able to grow from 6–8 °C to 46–48 °C, with an optimum at 35–37 °C. Lacey (1980) gave a range of 10–51 °C, so this species is a marginal thermophile. *E. nidulans* germinated at 0.82 a_w at 20 °C (Snow 1949), at 0.80 a_w at 37 °C, 0.81 a_w at 25 °C, 0.83 at 20 °C and 0.90 a_w at 15 °C (Ayerst 1969), so this species is also a xerophile, a most unusual combination. The heat resistance of *E. nidulans* ascospores does not appear to have been studied: because of its thermophilic nature, that property might be expected to be high.

Mycotoxins *Emericella nidulans* has been reported to produce sterigmatocystin, a mycotoxin more commonly associated with *E. versicolor* (Frisvad et al. 2006b) and emestrin (Terao et al. 1988, 1990). Emestrin is highly toxic, with an LD_{50} of 13 mg/kg when given to mice intraperitoneally (Terao et al. 1988). This species produces a number of other well characterised metabolites, some of pharmaceutical value (Chen et al. 2016). Toxicity due to the growth of *E. nidulans* has not been reported under practical conditions.

Ecology Although not especially common in foods, *Emericella nidulans* has been isolated from a wide variety of sources. Cereals and cereal products have been the most common, including wheat, flour and bread, barley, rice and maize and sorghum (see Pitt and Hocking 1997). Other sources include wine grapes (Bau et al. 2005) peanuts, hazelnuts, meat, soybeans, dried beans, peppercorns, chocolate and spices (see Pitt and Hocking 1997). We isolated *E. nidulans* at low levels for Thai soybeans, sorghum and cassava (Pitt et al. 1994).

References Christensen and States (1982); Klich (2002); Chen et al. 2016).

Emericella sydowii (Bainier & Sartory) Pitt & A.D. Hocking comb. nov. Fig. 8.4

Aspergillus sydowii (Bainier & Sartory) Thom & Church

Colonies on CYA 18–30 mm diam, plane or lightly sulcate, low to moderately deep, dense and velutinous to somewhat floccose; mycelium white; conidial heads sparse to quite dense, dark turquoise to dark green, especially in marginal areas, centrally white, less commonly buff to orange brown; dark brown exudate and/or soluble pigment sometimes produced; reverse pale to orange brown. Colonies on MEA 16–25 mm diam, plane, dense, velutinous to lightly floccose; mycelium inconspicuous, white; conidial heads numerous, coloured like those on CYA; reverse pale. Colonies on G25N 15–20 mm diam, plane, dense; mycelium white; often heavily sporing, dull green, blue or brown; reverse pale or yellowish. Usually no growth at 5 °C, occasionally germination. At 37 °C, no growth or colonies up to 10 mm diam formed.

Conidiophores borne from surface or aerial hyphae, stipes 250–500 µm long, often sinuous, with heavy, pale brown, smooth walls; vesicles only slightly swollen, club-shaped, 10–20 µm diam, bearing metulae and phialides over the upper two-thirds to three-quarters; smaller conidiophores also produced from aerial hyphae, ranging down to tiny monoverticillate penicilli; metulae 4–7 µm long; phialides 7–10 µm long; conidia spherical, 2.5–3.5 µm diam, with spinose walls, borne in radiate heads from the larger conidiophores.

Distinctive features *Emericella sydowii* grows slowly at 25 °C and often not at all at 37 °C, produces heads with both metulae and phialides, and blue conidia. Vesicles on the larger stipes are small and club-shaped, and diminutive penicilli are also formed.

Taxonomy The taxonomy of *Emericella versicolor* and related species, including *E. sydowii*, was reviewed and revised by Klich (1993) and Jurjevic et al. (2007), both under the name

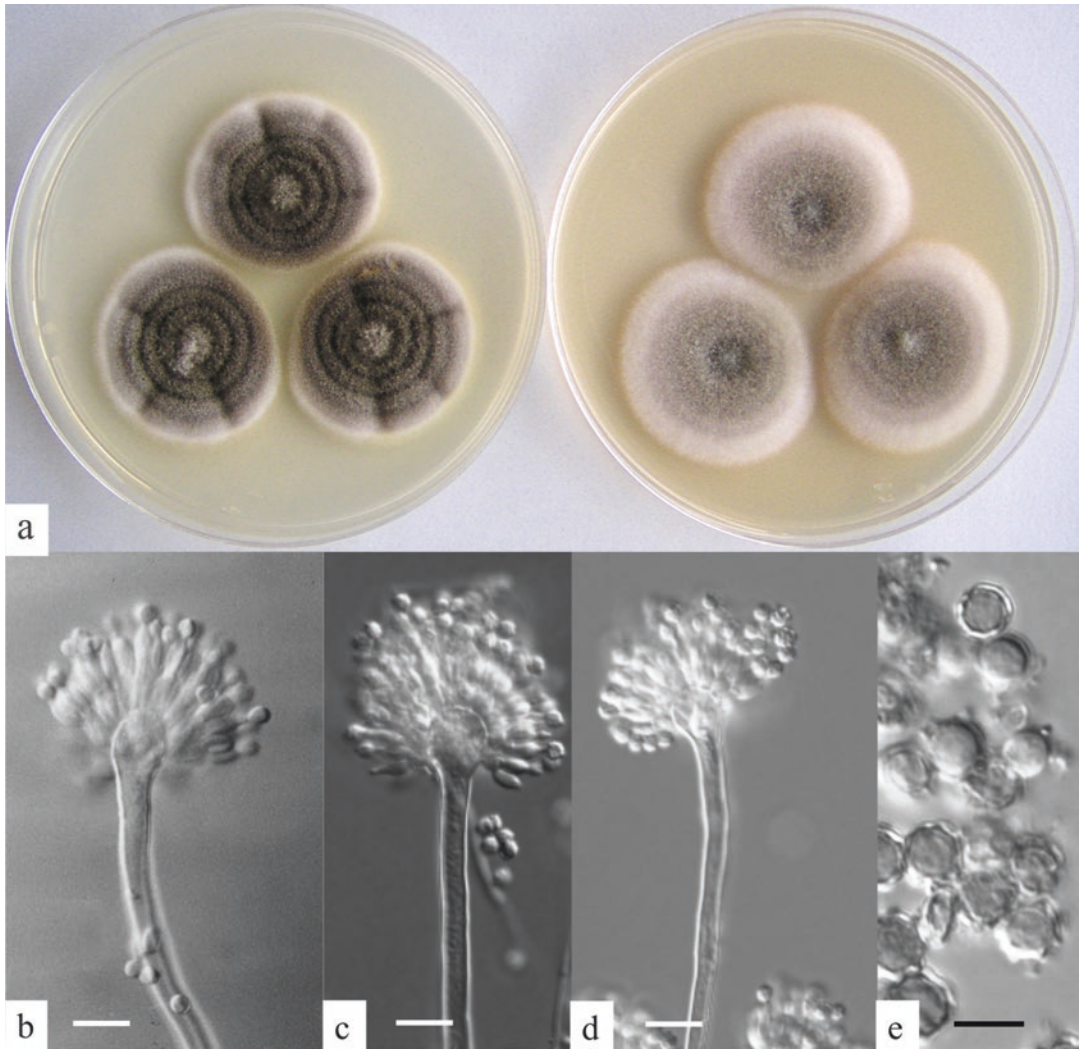

Fig. 8.4 *Emericella sydowii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) conidia, bar = 5 µm

Aspergillus sydowii. As noted in the genus preamble, this species has been described as the new combination *Emericella sydowii* in this work.

Identifiers Mycobank MB838069; neotype IMI 211384 (Samson and Gams 1985); ex-type cultures CBS 593.65 = IMI 211384 = NRRL 250; ITS barcode EF652450; alternative markers *BenA* EF652274, *CaM* EF652362, *RPB2* EF652186 (Houbraken et al. 2007).

Physiology Closely related to *Emericella versicolor*, *E. sydowii* can be expected to have similar physiological properties. Snow (1949) reported 0.78 a_w to be the minimum for growth on

a natural substrate; Wheeler and Hocking (1988) gave the same figure for growth on a glucose/fructose medium and 0.81 for growth on NaCl based media. Only 0.7% of *E. sydowii* conidia survived heating at 50 °C for 10 min (Pitt and Christian 1970).

Mycotoxins No significant mycotoxins have been reported from this species.

Ecology Widely distributed, *Emericella sydowii* is a storage fungus. Most isolations have come from dried foods, including various nuts: peanuts, pistachios, coconut, hazelnuts, walnuts and pecans (see Pitt and Hocking 1997); fermented milk (Ahmed and Abdel-Sater 2003), coffee beans (Silva et al. 2003), dried fish, paddy rice, stored rice, soybeans, cured meats and biltong, dried beans, health foods, red pepper powder (see Pitt and Hocking 1997) and spices (Ramaswamy et al. 2004). It appears to be relatively uncommon in cereals, but has been isolated from barley, wheat and flour (see Pitt and Hocking 1997), maize and soy beans (Sepulveda and Piontelli 2005). It has been reported as one cause of spoilage in fresh breadfruit (Omobuwajo and Wilcox 1989). An isolate from a type of Chinese tea was found to be an effective caffeine degrader (Zhou et al. 2018).

Emericella sydowii was found to be quite common in Southeast Asian commodities. We isolated it from 80% of pepper samples from Indonesia and 31% of such samples from the Philippines. Levels in Indonesian coriander were also high. It was significant (1–2% overall) in cashews and copra from Thailand, soybeans from the Philippines, and kemiri nuts, sorghum and black soybeans from Indonesia. It was also present at low levels in maize, peanuts, mung beans, paddy rice and milled rice from one country or more (Pitt et al. 1993, 1994, 1998 and our unpublished data).

References Klich (1993, 2002); Jurjevic et al. (2007), both as *Aspergillus sydowii*.

Emericella usta (Bainier) Pitt & A.D. Hocking comb. nov. Fig. 8.5

Aspergillus ustus (Bainier) Thom & Church

Colonies on CYA 30–40 mm diam, plane or lightly sulcate, dense, sometimes with a floccose overlay; mycelium white to greyish; conidial production sparse to quite heavy, pure grey to brownish grey; bright yellow soluble pigment usually produced; reverse greyish brown and often dull to bright yellow from soluble pigments as well. Colonies on MEA 40–50 mm diam, low, plane, dense and velutinous, or lightly floccose; mycelium white; conidial production moderate, olive brown or greyer; reverse pale green or dull brown. Colonies on G25N 10–14 mm diam, low and dense; reverse greenish or brown. At 5 °C, sometimes germination by a proportion of conidia. No growth at 37 °C.

Conidiophores borne from surface or aerial hyphae, stipes 100–300 µm long, sometimes curved or sinuous, with brown walls; vesicles spherical to pyriform, 10–16 µm diam, fertile over the upper two thirds, bearing metulae and phialides, both 5–7 µm long; conidia spherical, 3.5–4.5 µm diam, brown, with rough walls, borne in small, densely packed radiate heads.

Distinctive features *Emericella usta* is distinguished by grey conidia, small heads and vesicles bearing metulae and phialides. *E. usta* does not grow at 37 °C: if colonies on CYA at 25 and 37 °C have similar growth rates, the species is *Aspergillus calidoustus* Varga et al. 2007a

Taxonomy Gams et al. (1985) introduced *Aspergillus* subgenus *Nidulantes* section *Usti* W. Gams et al. as a correct name for the "*Aspergillus ustus* Group" of Thom and Raper (1941). Using molecular methods, *Aspergillus* section *Usti* was revised and expanded by Houbraken et al. (2007) and Samson et al. (2011) to its present size, which includes 21 species. The most important of these are *E. usta* and *A. calidoustis*. *E. usta* is described below as the most common species in foods, while

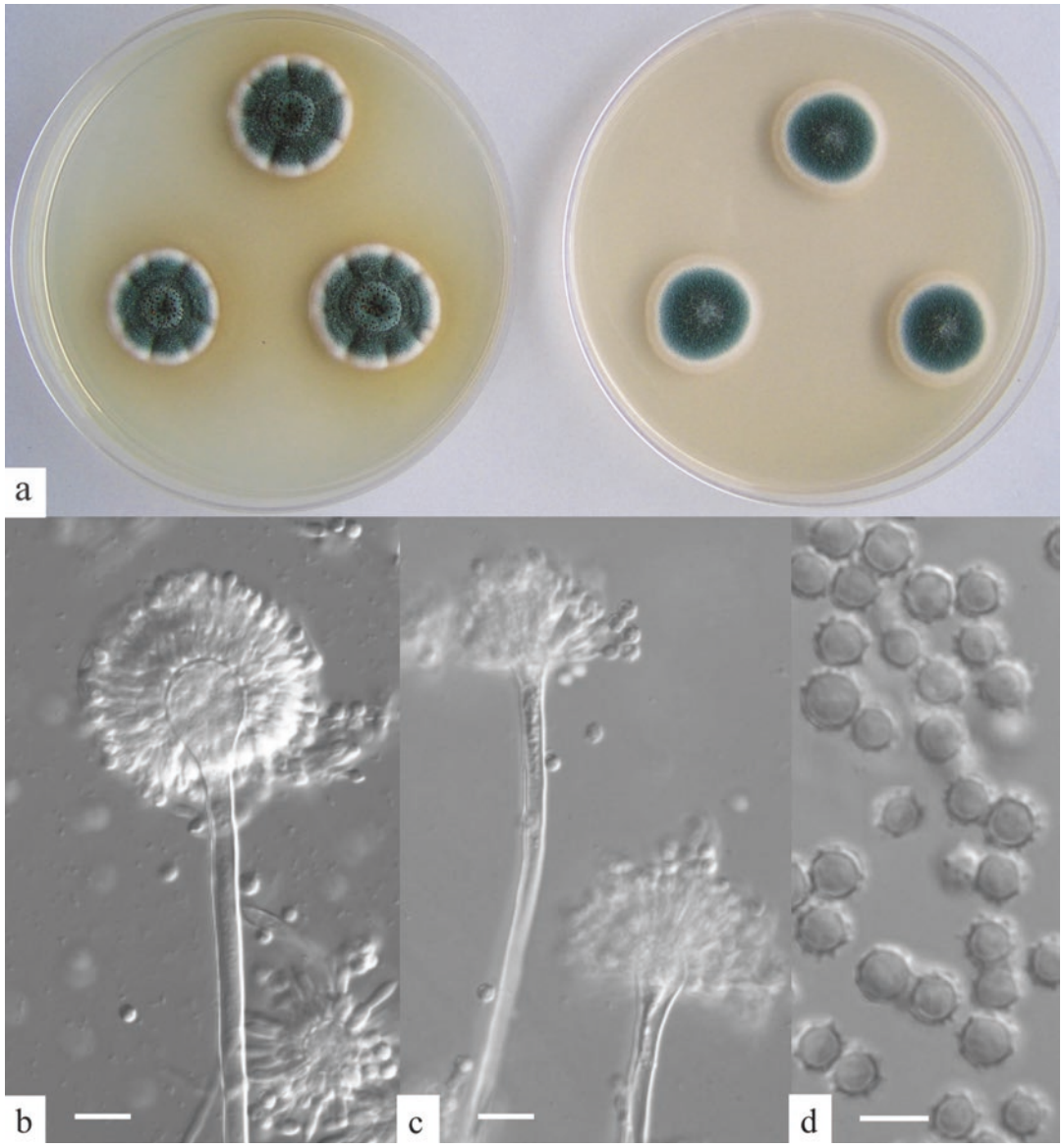

Fig. 8.5 *Emericella usta* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) heads, bars = 10 µm; (**e**) conidia, bar = 5 µm

A. calidoustis, introduced by Varga et al. (2008) to accommodate isolates from medical sources previously placed in *A. ustus*, is very similar but grows strongly at 37 °C. As noted in the genus preamble, *A. ustus* has been described as the new combination *Emericella usta* in this work.

Identifiers MycoBank MB838032; neotype: IMI 211805 (Samson and Gams 1985); ex-type cultures CBS 261.67 = IMI 211805 = ATCC 1041 = NRRL 275; ITS barcode EF652455; alternative markers *BenA* EF652279, *CaM* EF652367, *RPB2* EF652191 (Houbraken et al. 2007).

Physiology *Emericella usta* is one of the very few *Aspergillus* species (in the broad sense) we have encountered that can grow at temperatures much below 10 °C. It is not xerophilic.

Mycotoxins This species produces a number of secondary metabolites (Frisvad and Samson 1991), but has not been reliably reported to produce significant mycotoxins.

Ecology Although *Emericella usta* has been reported from a wide range of foods, occurrence is rather infrequent. Recent records include

soybeans (Tariq et al. 2005), cashews (Freire and Kozakiewicz 2005), almonds (Giridhar and Reddy 2001) and grapes (Bau et al. 2006). *E. usta* has also been reported in wheat, barley, flour, peanuts, walnuts, pecans, betel nuts, sago, frozen meat, biltong and cheese (see Pitt and Hocking 1997). It has not been reported to cause food spoilage.

References Houbraken et al. (2007); Varga et al. (2008); Samson et al. (2011).

Emericella versicolor (Vuill.) Pitt & A.D. Hocking comb. nov. Fig. 8.6

Aspergillus versicolor (Vuill.) Tirab.

Colonies on CYA 16–25 mm diam, plane or lightly sulcate, low to moderately deep, dense; mycelium white to buff or orange; conidial heads sparse to quite densely packed, greyish green; pink to wine red exudate sometimes produced; reverse brownish orange or reddish brown.

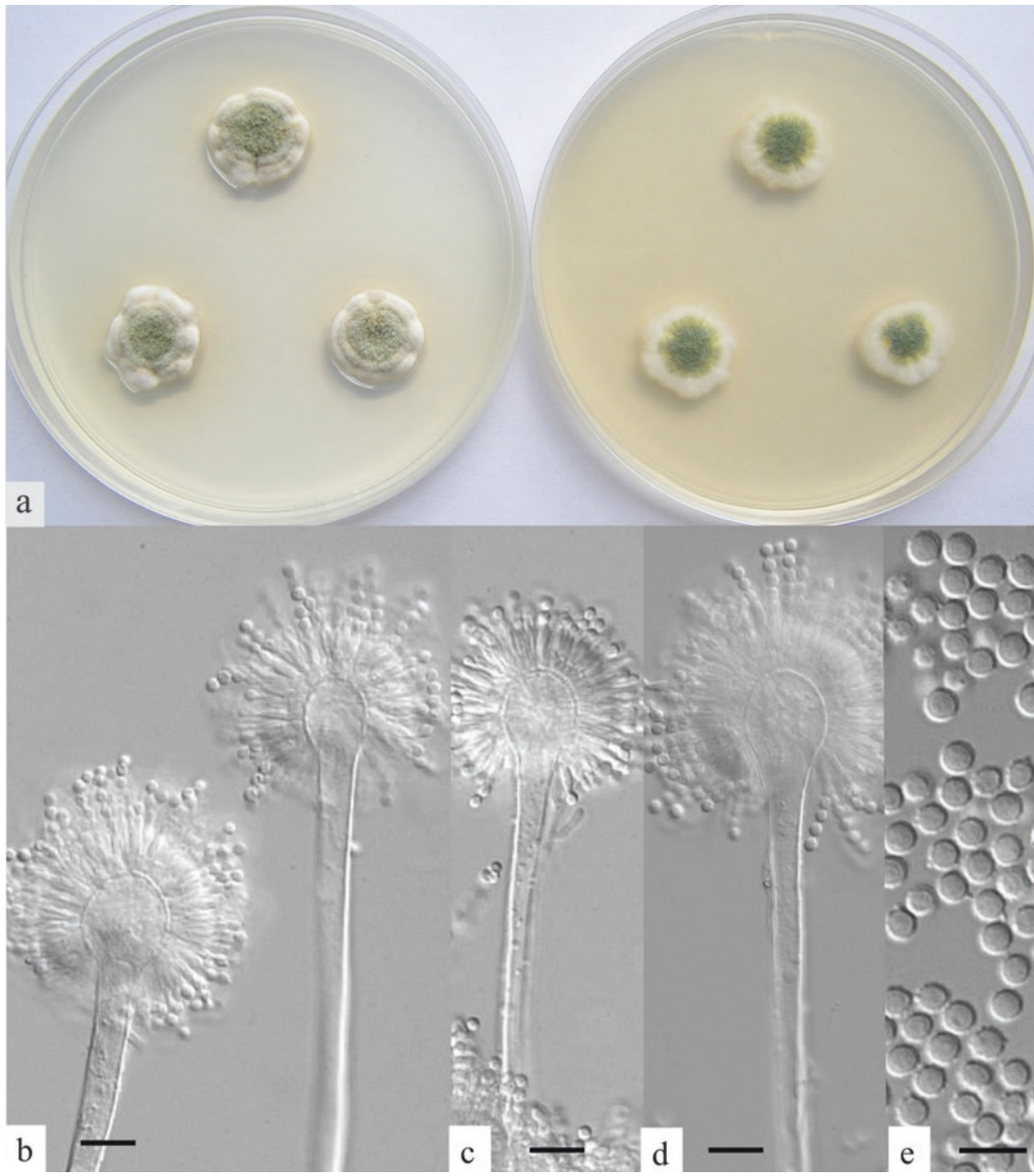

Fig. 8.6 *Emericella versicolor* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) heads, bars = 10 µm; (**e**) conidia, bar = 5 µm

Colonies on MEA 12–25 mm diam, low, plane, and dense, usually velutinous; mycelium white to buff; conidial heads numerous, radiate, dull green; reverse yellow brown to orange brown. Colonies on G25N 10–18 mm diam, plane or umbonate, dense, of white, buff or yellow mycelium; reverse pale, yellow brown or orange brown. No growth at 5 °C. Usually no growth at 37 °C, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from surface or aerial hyphae, stipes 300–600 µm long, with heavy yellow walls; vesicles variable, the largest nearly spherical, 12–16 µm diam, fertile over the upper half to two-thirds, the smallest scarcely swollen at all and fertile only at the tips, bearing closely packed metulae and phialides, both 5–8 µm long; conidia mostly spherical, very small, 2.0–2.5 µm diam, with walls finely to distinctly roughened or spinose, borne in radiate heads.

Distinctive features *Emericella versicolor* grows slowly, produces both metulae and phialides from small vesicles, and green conidia. Growth at 37 °C is weak or absent. This species is remarkable for the wide range of mycelial and reverse pigmentation it may produce, especially if cultures are incubated for 14 days or so.

Taxonomy Gams et al. (1985) introduced *Aspergillus* subgenus *Nidulantes* section *Versicolores* W. Gams et al. as a correct name of the "*Aspergillus versicolor* Group" of Thom and Church (1926). Klich (1993) reviewed and revised the taxonomy of *Emericella versicolor* and related species. As noted in the genus preamble, *A. versicolor* has been described as the new combination *Emericella versicolor* in this work.

Identifiers MycoBank MB838068; neotype CBS 583.65 (Samson and Gams 1985); ex-type cultures CBS 583.65 = IMI 229970 = NRRL 238 = ATCC 9577; ITS barcode EF652442; alternative markers *BenA* EF652266, *CaM* EF652354, *RPB2* EF652178 (Houbraken et al. 2007).

Physiology *Emericella versicolor* is a mesophile. Its minimum temperature for growth was reported to be 9 °C at 0.97 a_w, its maximum 39 °C at 0.87 a_w, and optimum 27 °C at 0.98 a_w (Smith and Hill 1982). Fomicheva et al. (2006) reported minimum growth on CYA at 5 °C, maximum growth between 35 and 37° and optimal growth between 25 and 27 °C. The minimum a_w for growth was reported to be 0.78 (Snow 1949) or 0.80 (Pitt and Christian 1968) and optimal growth at 0.96 a_w (Leggieri et al. 2017) all at 25 °C. Growth rates at various water activities were little affected by the use of NaCl or glycerol as controlling solute, or adjusting pH to 4.0 or 6.5 (Avari and Allsopp 1983). Addition of NaCl to CYA to a concentration of 3.5% (0.98 a_w) favoured the growth of *E. versicolor* (Fomicheva et al. 2006). Low pH (below 3.1) prevented growth, while at 25 and 30 °C growth at pH 10.2 was little different from that at pH 7.4 (Wheeler et al. 1991).

Growth of *Emericella versicolor* is greatly affected by modified atmospheres, growth being halved by reduction of O_2 concentration to 5%, or by addition of 12–15% CO_2, over the range 0.98–0.90 a_w (Magan and Lacey 1984b). It is a major source of volatile compounds produced by mouldy grain (see Pitt and Hocking 1997).

The decimal reduction time (D_{10}) for *Emericella versicolor* subjected to low dose gamma irradiation was given as 0.45 kGy on grain (O'Neill et al. 1991).

Mycotoxins *Emericella versicolor* is the major producer of sterigmatocystin, a precursor of the aflatoxins. Sterigmatocystin has the potential to cause human liver cancer (see Chap. 13, Mycotoxins) and its occurrence in foods should not be taken lightly. However, unlike aflatoxins and ochratoxin, which occur in the field or during crop drying, sterigmatocystin is produced only after harvest. If sterigmatocystin is formed by growth of *Emericella versicolor* in rice, most of the toxin is removed at the milling stage (Takahashi et al. 1984). In Canada, the risk of sterigmatocystin formation in stored grains is considered to be very low (Mills 1990). Sterigmatocystin was found in all of 48 paddy rice samples from Italy, with a range of 0.3–16 µg/kg, but the highest level in polished rice was 1.1 µg/kg (Bertuzzi et al. 2017).

Sterigmatocystin is also found in cheese, again due to the growth of *E. versicolor*. However, cheese is a manufactured product, over which control of contamination can be achieved (Engel and Teuber 1980; Veringa et al. 1989). Moreover, the optimal temperature for sterigmatocystin production by *E. versicolor* is about 30 °C, well above the normal ripening temperatures for cheeses (Leggieri et al. 2017). Only two of 21 Belgian and Latvian cheeses tested positive for sterigmatocystin, at levels below 1.5 μg/kg (Veršilovskis and De Saeger 2010).

E. versicolor is commonly reported in indoor air, and its presence in damp buildings has been linked to complaints of mouldy odour and eye, nose and throat irritation (Samson et al. 1994). A single dose of 10^8 *E. versicolor* spores inhaled by mice induced acute inflammation of the lungs (Jussila et al. 2002). Aerosols of *E. versicolor* extract administered to mice also caused upper respiratory tract irritation (Korpi et al. 2003). Continuous exposure of baby rats to *E. versicolor* spores caused pituitary tumors (Sumi et al. 1990, 1994).

A high proportion of *Emericella versicolor* isolates produce sterigmatocystin, e.g. 18 of 58 tested (Miyaki et al. 1970), or 30 of 32 (Mills and Abramson 1986). However, *E. versicolor* is a slowly growing invader of dried foods, and rarely grows on the large scale necessary for significant toxin production.

Ecology A very widely distributed fungus, *Emericella versicolor* has been reported from most kinds of foods. Although it occurs at harvest in crops such as wheat and barley (see Pitt and Hocking 1997), it is of much more common occurrence in stored products. Major sources include cereals and oilseeds: wheat, cereal flakes, stored barley, milled rice, oats, rapeseed, sunflower seeds, amaranth seed and vegetable oil (see Pitt and Hocking 1997). More recent reports include maize (Sepulveda and Piontelli 2005), flour (Halt et al. 2004), rice (Sakai et al. 2005; Bertuzzi et al. 2017), soybeans (Sepulveda and Piontelli 2005), sorghum (Sreenivasa et al. 2010; Garba et al. 2017) and dried medicinal herbs, especially lemon balm (Janda and Ulfig 2005). Other sources include peanuts, hazelnuts, walnuts, pistachios, pecans, green coffee beans, frozen meat, biltong, peppercorns, spices and health foods (see Pitt and Hocking 1997). *E. versicolor* has also been reported in sugar used in the manufacture of soft drinks (Ancasi et al. 2006), fermented and cured meats (Cantoni et al. 2007) and smoked sardines (Nketsia-Tabiri et al. 2003).

Emericella versicolor has been reported from cheese quite frequently (Minervini et al. 2002 and see also Pitt and Hocking 1997), and occasionally causes spoilage (Northolt et al. 1980). It is one fungus responsible for decay in fresh breadfruit (Omobuwajo and Wilcox 1989), and is also one cause of the "Rio" off-flavour in coffee due to the formation of trichloroanisoles (Liardon et al. 1992).

Occurrence of *Emericella versicolor* in Southeast Asian commodities we examined was variable, being quite high in foodstuffs from Indonesia, but low in those from Thailand and the Philippines. This presumably reflected differences in storage times and practice in these countries. Levels in Indonesian pepper (in 12% of all peppercorns examined), kemiri nuts (11%), red beans (10%), cowpeas (5%), coriander and mung beans (2%), peanuts and paddy and milled rice (1%) are probably indicative of growth in storage rather than association with a particular kind of food substrate. In commodities from Thailand, low levels of *E. versicolor* were found in peanuts, copra, soybeans, paddy and milled rice, wheat and sorghum, while from the Philippines, incidence was limited to low levels in maize, soybeans, mung beans, paddy and milled rice, pepper and sesame seeds (Pitt et al. 1993, 1994, and our unpublished data).

References Raper and Fennell (1965); Domsch et al. (1980); Klich (1993, 2002).

8.2 Genus *Neosartorya* Malloch & Cain

The genus *Neosartorya* was erected by Malloch and Cain (1972) to accommodate *Aspergillus* species that grew rapidly, produced cleistothecia and which had previously sometimes been included in an illegitimate genus, *Sartoya* Vuill. *Neosartorya* produces cleistothecia with cellular walls like those in *Aspergillus* subgen. *Aspergillus* (*Eurotium*), however, the walls and ascospores are colourless or white, not yellow. *Neosartorya* also produces vesicles bearing phialides only, but vesicles are small and pyriform, enlarging towards the apices.

Twelve species and varieties were accepted by Kirk et al. (2001). The advent of molecular taxonomy saw the incorporation of 10 asexual species into the *Neosartorya* clade, which at that time was expanded to include 23 species (Samson et al. 2007). Four new species were added by Hong et al. (2008). The study of the Family *Trichocomaceae* by Houbraken et al. (2011a, b) resulted in the addition of *Aspergillus* subgen. *Clavati* (Samson and Gams 1985) to the *Neosartorya* clade.

Most *Neosartorya* species inhabit soils and decaying vegetation, especially when heaped up and heating. As many species are thermoduric or thermophilic, all *Neosartorya* isolates should be handled with care. No *Neosartorya* species is xerophilic.

The species treated here are *Neosartorya spinosa* and the closely related *N. glabra,* of interest to the food technologist because of the very high heat resistance of their ascospores, *N. fumigatus*, an important human pathogen that sometimes occurs in foods, and *N. clavata*, transferred from *Aspergillus* herein. These species are keyed in the general key above.

Neosartorya clavata (Desm.) Pitt & A.D. Hocking Fig. 8.7

Aspergillus clavatus Desm.

Colonies on CYA and MEA 30–45 mm diam, plane, of sparse surface mycelium surmounted by regular or irregular clusters of positively phototropic conidiophores up to 3 mm long, growing vertically if incubated in darkness; mycelium white; conidiophores readily visible under the stereomicroscope, with stout stipes and heads like match heads, with spore chains coloured greyish turquoise; clear exudate sometimes present in minute droplets; sometimes faint brown soluble pigment produced; reverse pale. Colonies on G25N 8–12 mm diam, of sparse, floccose, white mycelium and small, scattered conidiophores; reverse pale. No growth at 5 °C. At 37 °C, colonies 10–30 mm diam, of white mycelium, with small blue grey heads; sometimes yellow brown soluble pigment produced; reverse yellow green.

Conidiophores borne from subsurface or surface hyphae, stipes 1.5–3.0 mm long, with thick, smooth walls; vesicles narrow ellipsoids up to 250 × 70 µm, fertile over the whole area, bearing phialides only; phialides very closely packed, mostly 7–8 µm long; conidia ellipsoidal, 3.0–4.5 µm long, smooth walled, borne in radiate heads, each splitting into two or more ordered columns in age.

Distinctive features Long ellipsoidal vesicles, heads with phialides only and grey blue conidia set *Neosartorya clavata* apart from other species.

Taxonomy Gams et al. (1985) introduced *Aspergillus* subgenus *Clavati* W. Gams et al. as a correct name for the "*Aspergillus clavatus* Group" of Thom and Raper (1941). Using molecular methods, the relationship of *Neosartorya clavata* with closely related species

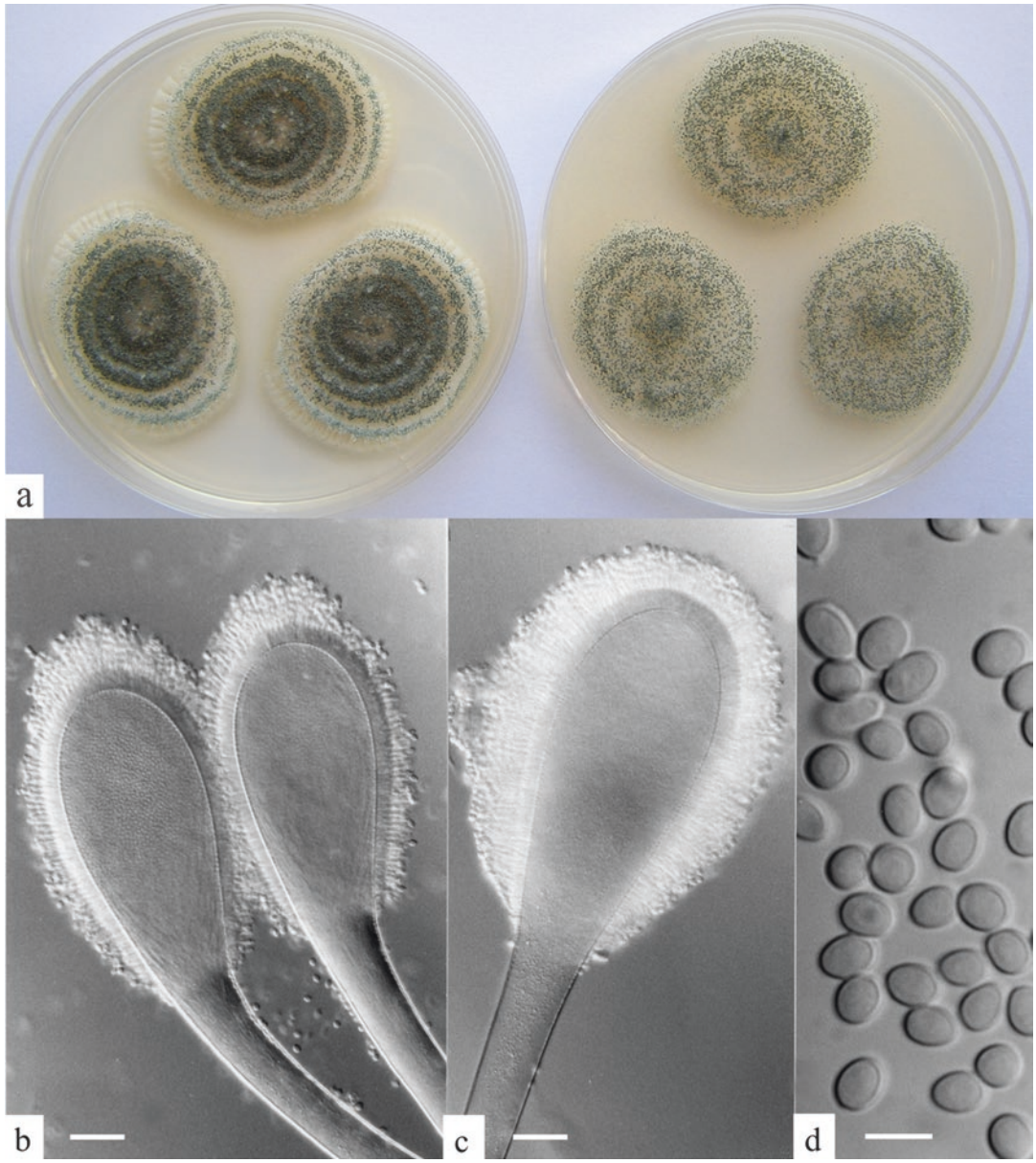

Fig. 8.7 *Neosartorya clavata* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 25 µm; (**d**) conidia, bar = 5 µm

was studied by Varga et al. (2003, 2007a). The comprehensive study of the family *Trichocomaceae* by Houbraken and Samson et al. (2011) showed that *Aspergillus* subgenus *Clavati* lay within the broader *Aspergillus* subgenus *Fumigati*.

It was shown by Ojeda-López et al. (2018) that *A. clavatus* is heterothallic and that a viable sexual state could be induced by mating suitable strains. Cleistothecia and ascospores are similar to those in *A. acanthosporus*, a closely related species (Varga et al. 2007a). In agreement with that placement and finding, *A. clavatus* is described here as the new combination *Neosartorya clavata*.

Identifiers MycoBank MB838031; neotype IMI 15949 (Samson and Gams 1985); ex-type cultures CBS 513.65 = IMI 15949 = ATCC 9602 = NRRL 1 = FRR 1; ITS barcode EF669942; alternative markers *BenA* EF669802, *CaM* EF669871, *RPB2* EF669730 (Houbraken et al. 2020).

Physiology Panasenko (1967) reported that *Neosartorya clavata* had an optimal growth temperature near 25 °C, a minimum of 5–6 °C, and a maximum of 42 °C. Similar figures were reported by Northolt et al. (1978). The minimum a_w permitting growth was reported as 0.88 a_w by Panasenko (1967), and graphed as near 0.87 a_w by Northolt et al. (1978).

Mycotoxins This species produces patulin which has the potential to cause ill thrift in animals and may have harmful effects in man. Patulin is discussed further in Chap. 13, Mycotoxins. *Neosartorya clavata* produces a number of other mycotoxins of marginal importance including kojic acid, cytochalasin E, tremorgenic tryptoquivalines and glyantrypine (Varga et al. 2003; Sabater-Vilar et al. 2004).

A tremorgenic neurotoxicosis in cattle has resulted on several occasions from feeding sprouted barley (McKenzie et al. 2004; Botha et al. 2014) or a similar maize product (Kellerman et al. 1984) that was heavily mouldy with predominantly *Neosartorya clavata*. The causal mycotoxins remain uncertain.

Patulin was produced by *N. clavata* at 0.99 a_w but not 0.95 a_w on laboratory media (Northolt et al. 1978).

Ecology *Neosartorya clavata* is of particularly common occurrence in barley during malting, and can build to unacceptably high levels if malting temperatures are elevated or spontaneous heating occurs (Flannigan et al. 1984; Flannigan 1986; Sabater-Vilar et al. 2004). In extreme cases blue green mats of *N. clavata* may form on grain during malting (Shlosberg et al. 1991). Under these conditions it can be allergenic and is reported to be the cause of "malt workers' lung" (Riddle et al. 1968; Flannigan 1986; Sabater-Vilar et al. 2004).

Neosartorya clavata is mostly associated with cereals, and has been reported from wheat, flour, bread, rice and maize (see Pitt and Hocking 1997). Other sources include red peppers, pecan nuts, health foods, biltong and salted dried fish (see Pitt and Hocking 1997).

In Southeast Asian food commodities, *Neosartorya clavata* occurred at low levels in maize from Thailand and the Philippines, peanuts, kemiri nuts and pepper from Indonesia and milled rice, soybeans and mung beans from the Philippines. Levels were slightly higher in copra from Thailand (Pitt et al. 1993, 1994, 1998 and unpublished).

References Klich (2002); Varga et al. (2003, 2007a); Ojeda-López et al. (2018).

Neosartorya fumigata O'Gorman et al. **Fig. 8.8**

Aspergillus fumigatus Fresen.

Colonies on CYA 40–60 mm diam, plane or lightly wrinkled, low, dense and velutinous or with a sparse, floccose overgrowth; mycelium inconspicuous, white; conidial heads borne in a continuous, densely packed layer, greyish turquoise to dark turquoise (blue-green); clear exudate sometimes produced in small amounts; reverse pale or greenish. Colonies on MEA 40–60 mm diam, similar to those on CYA but less dense and with conidia in duller colours; reverse uncoloured or greyish. Colonies on G25N less than 10 mm diam, sometimes only germination, of white mycelium. No growth at 5 °C. At 37 °C, colonies covering the available area, i.e. a whole Petri dish in 2 days from a single point inoculum, of similar appearance to those on CYA at 25 °C, but with conidial columns longer and conidia darker, greenish grey to pure grey.

Conidiophores borne from surface hyphae, stipes 200–400 µm long, sometimes sinuous, with colourless, thin, smooth walls, enlarging gradually into pyriform vesicles; vesicles 20–30 µm diam, fertile over half or more of the enlarged area, bearing phialides only, the lateral ones characteristically bent so that the tips are approximately parallel to the stipe axis; phialides crowded, 6–8 µm long; conidia spherical to subspheroidal, 2.5–3.0 µm diam, with finely roughened or spinose walls, forming radiate heads at first, then well defined columns of conidia.

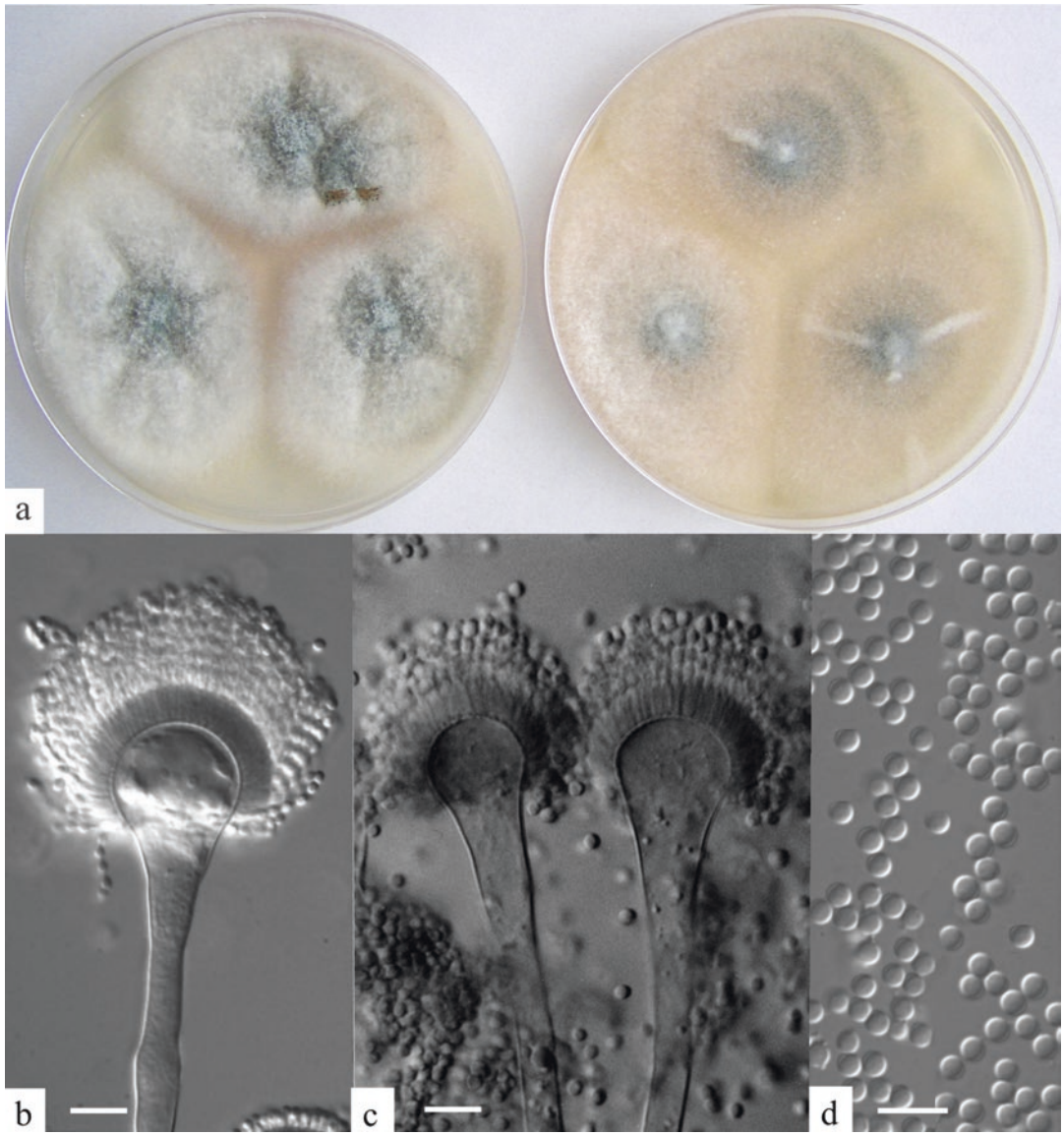

Fig. 8.8 *Neosartorya fumigata* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) conidia, bar = 5 µm

Distinctive features This distinctive species can be recognised in the unopened Petri dish by its broad, velutinous, bluish colonies bearing characteristic, well defined columns of conidia. Growth at 37 °C is exceptionally rapid. Conidial heads are also diagnostic: pyriform vesicles bear crowded phialides which bend to be roughly parallel to the stipe axis. Care should be exercised in handling cultures of this species (see Chap. 4).

Taxonomy *Aspergillus fumigatus* was designated as the type species of *Aspergillus* subgenus *Fumigati* W. Gams et al. (1985) and this status was maintained in the molecular analyses of Samson et al. (2007) and Houbraken et al. (2011a, b). It was shown by O'Gorman et al. (2009) that this species, like several other *Neosartorya* species, is heterothallic. Mating of two appropriate strains induced the production of cleistothecia and ascospores typical of *Neosartorya*.

Identifiers MycoBank MB512563; neotype IMI 16152 (Samson and Gams 1985); ex-type cultures CBS 133.61 = IMI 16152 = ATCC 1022 = NRRL 163 = FRR 163; ITS barcode EF669931; alternative markers *BenA* EF669791, *CaM* EF669860, *RPB2* EF669719 (Houbraken et al. 2020).

Physiology Undoubtedly the most important physiological character of *Neosartorya fumigata* is its thermophilic nature: its minimum growth temperature is near 12 °C, the optimum 40–42 °C and maximum near 55 °C (Panasenko 1967; Ayerst 1969: Evans 1971; Domsch et al. 1980). *N. fumigata* is a marginal xerophile, Ayerst (1969) recording 0.82 a_w as the minimum for growth, near 40 °C. It required more than 15% CO_2 to reduce growth rates by half at 0.98 a_w, but only 2.5% at 0.90 a_w (Magan and Lacey 1984b).

Mycotoxins *Nesartorya fumigata* produces fumitremorgens, verruculogen and gliotoxin and a role in animal disease seems likely (Cole 1981a, b; Dorner et al. 1984; Moreau 1980; Frisvad et al. 2006a). Evidence suggests gliotoxin is important in the invasion of animal and bird lungs by *N. fumigata* (Richard et al. 1994). The presence of *N. fumigata* in pepper used in foods for hospital patients is undesirable (De Bock et al. 1989; Bouakline et al. 2000).

The fumigaclavines, produced by *N. fumigata*, are related to ergot alkaloids (Panaccione and Coyle 2005). *N. fumigata* also produces fumagillin, a compound with some antibacterial and antifungal activity, which is also an angiogenesis inhibitor (Yang et al. 2003).

Ecology The prime habitat for *Neosartorya fumigata* is decaying vegetation, particularly grass clippings, in which it causes spontaneous heating (Cooney and Emerson 1964) and compost, where it dominates spore numbers (Kwon-Chung and Sugui 2013). Major food habitats are in cocoa beans during and after fermentation and in spices (see Pitt and Hocking 1997). It has been recorded frequently from stored commodities in the tropics, where its ability to grow simultaneously at low a_w and at high temperatures provides an ecological advantage, e.g. stored oilseeds, stored eggs, copra, soybeans and vegetables (see Pitt and Hocking 1997). It has been reported as one cause of "Rio" off-flavours in coffee (Liardon et al. 1992), but it is not usually an important spoilage fungus. Cereals are a common source, e.g. wheat (Soldevilla et al. 2005; Lugauskas et al. 2006), rice, parboiled rice and barley (see Pitt and Hocking 1997). It is quite common on cured and processed meats, especially in the tropics (Sabater-Vilar et al. 2003a, b) and see also Pitt and Hocking 1997). *Neosartorya fumigata* also occurs in nuts, e.g. hazelnuts, walnuts, peanuts (see Pitt and Hocking 1997) and cashews (Adebajo and Diyaolu 2003). Other sources include dried fish, baladi bread, corn snacks, rootstock snacks, melon seeds, mango pickles, dried onion, low fat buffalo milk cottage cheese, processed cheese (see Pitt and Hocking 1997) and kuflu cheese (Hayaloglu and Kirbag 2007). *N. fumigata* isolated from marine sediments produced gliotoxin under hypersaline conditions. After 6 days in gliotoxin-contaminated seawater, fresh mussels were able to accumulate gliotoxin at levels up to 2.9 µm/mg in the meat (Grovel et al. 2003).

Pathogenicity *N. fumigata* is the main agent of pulmonary aspergillosis in man and birds. However, disseminated infections in patients without apparent immune disorders are extremely rare (De Hoog et al. 2000).

References Klich (2002); Samson et al. (2007); O'Gorman et al. (2009).

Neosartorya spinosa (Raper & Fennell) Kozak. **Fig. 8.9**

Aspergillus fischeri var. *spinosa* Raper & Fennell

Colonies on CYA 50–60 mm diam, occasionally larger, plane, sparse to moderately dense, surface texture floccose; mycelium white to pale yellow, enveloping abundant developing cleistothecia; conidial heads usually small and sparsely produced, above the layer of cleistothecia, grey green; reverse pale to pinkish brown, sometimes yellow centrally. Colonies on MEA 60 mm or more diam, usually covering the whole Petri dish, low and sparse; mycelium white to pale yellow, surrounding layers of developing white cleistothecia; conidial heads small and sparse, grey

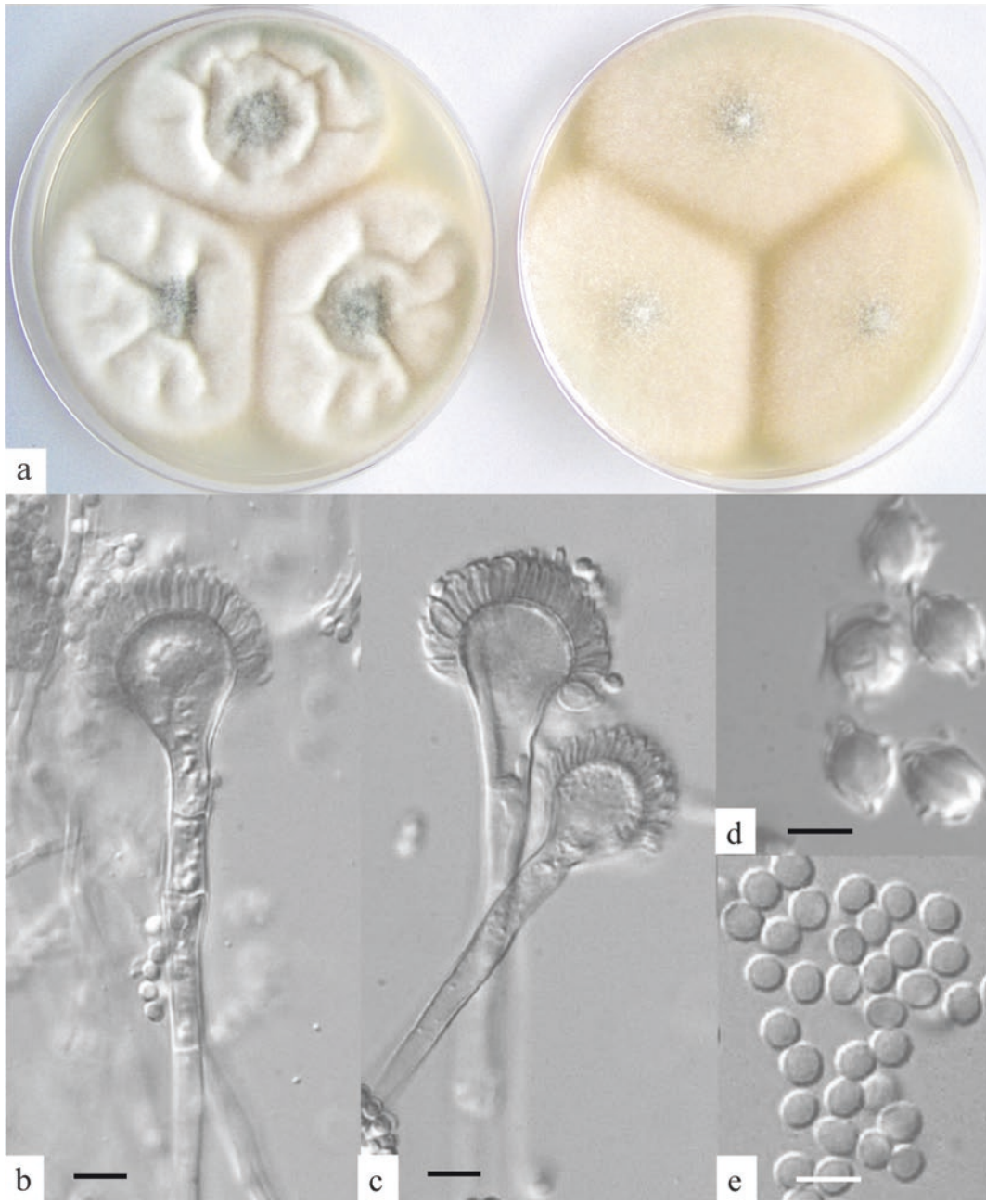

Fig. 8.9 *Neosartorya spinosa* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) ascospores and (**e**) conidia, bars = 5 µm

green; reverse pale, pale brown or dull yellow. Colonies on G25N 8–12 mm diam, of sparse to dense white mycelium; reverse pale to dull yellow. No growth at 5 °C. At 37 °C, colonies covering the whole Petri dish, plane or sulcate, of white or grey mycelium and abundant conspicuous white cleistothecia; reverse pale to pinkish brown.

Cleistothecia 200–400 µm diam, with a definite wall of flattened cells with hyphae attached,

white to cream, maturing in 9–12 days at 25 °C, slightly faster at 37 °C; ascospores ellipsoidal, 7–8 µm long overall, including two prominent, sinuous longitudinal flanges, other irregular ridges sometimes present as well. Conidiophores borne from aerial mycelium, stipes usually 300–500 µm long, with thin, colourless, smooth walls, enlarging terminally to pyriform vesicles; vesicles 12–18 µm diam, fertile over the upper half, bearing phialides only; phialides 5–6 µm long, with short necks; conidia subspheroidal to ellipsoidal, 2.5–3.0 µm long, smooth walled, borne in radiate to columnar heads.

Distinctive features Colonies of *Neosartorya spinosa* spread rapidly at both 25 and 37 °C, and are white; white cleistothecia and inconspicuous grey green *Aspergillus* heads are produced.

Taxonomy Earlier literature discussed this species under the name *Neosartorya fischeri* (Wehmer) Malloch & Cain and more commonly its anamorph name *Aspergillus fischeri* Wehmer (which was invalid under the existing Code). Two varieties of this species, *A. spinosus* and *A. glaber,* were accepted by Raper and Fennell (1965), distinguished by differences in ascospore ornamentation. These varieties were raised to species status by Kozakiewicz (1989) as *N. spinosa* (Fennell & Raper) Kozak. and *N. glabra* (Fennell & Raper) Kozak. The teleomorphs are produced under most growth conditions and, from the food technologist's point of view, are the states responsible for these species' most important property, high heat resistance, so it is appropriate to use the *Neosartorya* names here. Differences apart from ascospore ornamentation appear to be very small. However, the molecular taxonomy of Samson et al. (2007) indicated that these two species are not especially closely related, while *N. fischeri* and *Neosartora fumigata* are.

A PCR method using specific primer sets for detecting and distinguishing several heat resistant species of *Neosartorya* was developed by Yaguchi et al. (2012). As this methodology does not distinguish living or dead cells, it is only of use on raw materials, not finished product.

Identifiers MycoBank MB127755; neotype IMI 211390 (Samson and Gams 1985); ex-type cultures CBS 483.65 = IMI 211390 = ATCC 16898 = NRRL 5034; ITS barcode EF669988; alternative markers *BenA* EF669844, *CaM* EF669914, *RPB2* EF669775 (Houbraken et al. 2020).

Physiology Ascospores of these species rank with those of *Byssochlamys* as the most heat resistant fungal spores known. Kavanagh et al. (1963) reported that ascospores of an isolate more recently identified as *Neosartorya fischeri* withstood boiling in distilled water for 60 min. They reported that spore age, pH and sugar concentration affected heat resistance. McEvoy and Stuart (1970) heated ascospores of *N. fischeri* in distilled water: they reported 100% survival after 20 min at 80 °C, and 0.002% survival after 5 min at 100 °C. This degree of heat resistance is comparable with that of many bacterial spores, and is higher than that of *Byssochlamys fulva* ascospores. More detailed studies of the heat resistance of ascospores of *Neosartorya fischeri* (or a closely related species) indicate D_{88} values of 1.2–7.5 min (Quintavalla and Spotti 1993) or of 4.2–16.2 min (Beuchat 1986) or a D_{88} value of 1.4 min and a *z* value of 5.6 C° (Scott and Bernard 1987). A mathematical model for the combined effect of a_w, pH and redox potential on the heat resistance of *N. fischeri* has been published (Reichart and Mohacsi-Farkas 1994). Salomão et al. (2007) reported that citric acid had a greater influence than malic acid on the thermal resistance of ascospores of *N. fisheri*. Ninety day old ascospores, isolated from tomatoes, were successfully killed by a heat treatment at 115 °C for 30 sec (Pacheco and De Massaguer 2004). D values of the strains tested ranged between 3.7 and 13.5 min at 87 °C; 1.5 and 3.5 min at 90 °C; and 0.3 and 0.4 min at 95 °C in a glucose solution. Similarly, D values ranged between 3.3 and 15.4 min at 87 °C; 1.3 and 4.3 min at 90 °C; and 0.3 and 0.6 min at 95 °C in a strawberry based formulation. For all strains, the corresponding z values ranged between 5.7 and 8.3 C° in glucose solution and from 5.7 to 8.4 C° in a strawberry formulation (Berni et al. 2017).

High pressure treatments (600–900 MPa) for 20 min at 20 °C in apricot nectar reduced counts of *Neosartorya fischeri* 100 fold. At 50 °C, inactivation required less than 4 min at 800 MPa, and

at 60 °C, 1–2 min at 700 MPa. Pressure resistance in distilled water was lower (Maggi et al. 1994). Pressure resistance of ascospores increased with age: ascospores that were three weeks old were relatively sensitive to pressure treatment (600 MPa for 10 min), with pressure resistance increasing as ascospores matured, at least up to 15 weeks (the oldest tested). Ascospores of *N. spinosa* that were 9–15 weeks old were activated by a pressure treatment of 600 MPa for 10 min (Chapman et al. 2007). High pressure (600 Mpa) and temperature (75 °C) produced a 3.3 log reduction in 4 week old ascospores of *N. fischeri* (Evelyn et al. 2016). At a maximum holding temperature of 85 °C and 600 MPa pressure, *N. fischeri* ascospores were inactivated by >5.0 log reduction after 7 min (Timmermans et al. 2020).

The minimum a_w for growth for *N. glabra* was reported as 0.87–0.88 a_w for media based on strawberries or blueberries with added sucrose (Berni et al. 2017). Growth of *N. fisheri* (or a related species) did not occur at 0.86 a_w under a range of environmental and substrate conditions, including strawberry purees. However, growth occurred at 0.87 a_w at O_2 levels above 0.15% (Dos Santos et al. 2020b). Growth did not occur at temperatures below 10 °C under any conditions studied. Growth has been reported in O_2 levels as low as 0.1% at 25 °C (Nielsen et al. 1989).

A quantitative spoilage risk assessment was carried out on pasteurised strawberry purees by Dos Santos et al. (2020a). They concluded that normal pasteurising treatments and storage at room temperature carried a high risk, only reduced by pasteurising at temperatures of 95 °C or above. As a result, a set of specifications for heat resistant fungi in raw materials was suggested.

Mycotoxins The production of fumitremorgens A and C and verruculogen by this species has been confirmed (Beuchat et al. 1988; Nielsen et al. 1988; Frisvad and Samson 1991). However, although these compounds are highly toxic, this is unlikely to have practical significance, as these species have only rarely been reported as growing in foods.

Ecology *Neosartorya fischeri* ascospores have been isolated from canned strawberries, which had been processed at 100 °C for 12 min (Kavanagh et al. 1963). Isolation of *N. fischeri* from pasteurised fruit juices and fruit powders (e.g. Jesenska and Petrikova, 1985; Scott and Bernard 1987; dos Santos et al. 2018) has been reported several times but only occasionally from spoiled product (Splittstoesser and Splittstoesser, 1977).

On several occasions we have isolated *Neosartorya fischeri* from pasteurised fruit juices, strawberry purees and syrups, and a sports drink, but have only seen actual spoilage in canned strawberry puree. Heat treated fresh strawberries and other fruit prone to soil contamination from rain splash frequently yield isolates of this species (Spotti et al. 1992; and our observations).

Neosartorya fischeri has been reported only rarely from foods which have not been heat treated or processed: from pistachios (Mojtahedi et al. 1979), soybeans (El-Kady and Youssef, 1993) and fresh mangoes (Ugwuanyi and Obeta, 1991). Contamination from soil rather than fungal growth appears probable.

References Raper and Fennell (1965); Hocking and Pitt (1984); Samson et al. (2007).

8.3 *Aspergillus* Subgenus *Aspergillus* P. Micheli ex Haller [Genus *Eurotium* Link: Fr]

Nomenclatural note As explained in the chapter preamble, the fact that the types of *Aspergillus* and *Eurotium* belong to the same species means that under the ICN the name *Eurotium* can no longer be used. All species previously known as *Eurotium* must be named under the equivalent *Aspergillus* name (not always the same epithet). A proposal to move the type of *Aspergillus* from *A. glaucus* to *A. niger* (Pitt and Taylor 2016) is still unresolved. If that proposal is in due course accepted by the Nomenclatural Committe for Fungi, and the General Committee, and is ratified by a Botanical Congress, the name *Eurotium* will again be valid, and the names in *Eurotium*

given in square brackets in this section will again be valid. Until such a change is made, the *Aspergillus* names given below are the correct and valid ones.

Aspergillus subgen. *Aspergillus* [*Eurotium*] is a very well defined and very well known subgenus [genus] of ascomycetes, characterised by the formation of barely macroscopic yellow cleistothecia with smooth, cellular walls. The *Aspergillus* anamorphs are also characteristic: vesicles bear uncrowded phialides and the phialides produce dull green, spinose conidia in loosely radiate heads.

All species of *Aspergillus* subgen. *Aspergillus* [*Eurotium*] are xerophilic, and often do not develop characteristic fruiting structures on high water activity media such as CYA and MEA. Incorporation of 20% sucrose into Czapek agar greatly assisted identification of *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species (Thom and Raper 1941). This medium, of about 0.98 a_w, produces suboptimal growth of all such species but permits development of both anamorphs and teleomorphs (where they occur) plus mycelial colours, which also aid species identification. Media of lower a_w, such as G25N, usually produce rampant growth which is of less value in determinative taxonomy. For compatibility with CYA, we introduced Czapek yeast extract agar with 20% sucrose (CY20S) for taxonomy of this set of taxa (Pitt and Hocking 1985). This medium has proved to be of considerable value in general *Aspergillus* taxonomy (Klich 2002), but its use here is confined to *Aspergillus* subgen. *Aspergillus* [*Eurotium*] identification.

When an isolate has been identified as belonging here by using the standard media and incubation conditions, and growth data recorded as usual, the culture should be inoculated onto CY20S agar. After incubation for 7 days at 25 °C, diameters and colours should be recorded and the cultures returned to the incubator. At intervals, wet mounts of cleistothecia from the central area of a colony should be made, stained with lactofuchsin and examined with the 40x objective. Identification can be completed when mature ascospores are produced, which will be within 14 days for the majority of isolates. Ascospores are mature when they are readily liberated from asci, and do not take up stain immediately. Conidia are usually present in wet mounts also, and may be distinguished from ascospores by their uniformly spinose walls and the fact that lactofuchsin stains them quite readily. Unstained conidia are green under the microscope while mature ascospores are faintly yellow and are more refractile. Ascospore dimensions should be measured under the 100× objective, and ornamentation noted, i.e. smooth or rough walls, and presence or absence of a longitudinal furrow, ridges or flanges.

It is logical for the teleomorph names to be used for these species, as the teleomorphs are normally present in Petri dish cultures on CY20S and are often essential for identification. Differences do exist among the *Aspergillus* states of these species but, ironically, these are not usually considered in species identification procedures. However, as noted above, in the current circumstances it is invalid to use *Eurotium* names. Hubka et al. (2013) revised this genus and selected the correct *Aspergillus* names, as used below. They accepted 17 species. The genus was again revised and monographed by Chen et al. (2016) who accepted 31 species. Four of those are exceedingly common in all kinds of environments where just sufficient moisture exists to support fungal growth and two others are described below.

It has been shown that two old and well established names, *A. amstelodami* and *A. repens*, used in previous editions of this book, are invalid, and have been replaced by *A. montevidensis* and *A. pseudoglaucus* (Hubka et al. 2013). In addition, *E. herbariorum* has been replaced by its anamorph name, *A. glaucus*.

It has long been known, e.g. Raper and Fennell (1965), that species in the "*Aspergillus restrictus* Group", validated as *Aspergillus* section *Restricti,* are closely related to those in *Aspergillus* subgen. *Aspergillus* [*Eurotium*]. Molecular studies confirmed this (Houbraken et al. 2011a, b). This section was revised and greatly expanded by Sklenář et al. (2017) and now includes 21 species. Only two of these, *A. penicillioides* and *A. restrictus*, are important. They are described below.

Aspergillus subgenus *Circumdati* section *Wentii* was established by Gams et al. (1985) to include *Aspergillus* species related to *Aspergillus wentii*. From a phylogenetic study, Peterson (1995) synonymised this section with *Aspergillus* subgenus *Circumdati* section *Cremei*, associated with the sexual genus *Chaetosartorya* Subram., and concluded that section *Cremei* did not belong in subgenus *Circumdati*, but was more closely related to subgenus *Aspergillus* [*Eurotium*]. That placement was accepted by later authors, i.e. Peterson (2000), Houbraken et al. (2011a, b), Kocsubé et al. (2016) and Steenwyk et al. (2019). It is unclear at this time whether *Chaetosartorya* should be recognised as a distinct genus or considered to be a synonym of *Aspergillus*. The second alternative is chosen here: *A. wentii* is considered to belong in *Aspergillus* subgenus *Aspergillus* and is included in this section.

Key to *Aspergillus* Subgenus *Aspergillus* [*Eurotium*] Species Included Here

1	No growth on CYA, MEA or CY20S; on MY50G cleistothecia white	*A. halophilicus* (see *A. ruber*)
	Growth on CY20S; usually growth on CYA and MEA	2
2(1)	Colonies on CY20S not exceeding 25 mm, developing cleistothecia absent	3
	Colonies on CY20S exceeding 25 mm, developing cleistothecia evident	4
3(2)	Colonies on CYA and MEA 6 mm or more diam; conidia cylindrical to barrel shaped, borne in columns	*A. restrictus*
	Colonies on CYA and MEA not exceeding 6 mm diam; conidia subspheroidal to ellipsoidal, borne in radiate heads	*A. penicillioides*
4(2)	Ascospores with conspicuous ridges or flanges	5
	Ascospores without conspicuous ridges or flanges	7
5(4)	Colonies coloured only yellow, from cleistothecia, and green, from conidial heads; ascospores with two prominent, irregular, broad, longitudinal ridges	6
	Colonies with conspicuous yellow to orange sterile hyphae; ascospores like pulley wheels, with two prominent, narrow, longitudinal flanges	*A. chevalieri*
6(5)	Ascospores with rough walls, not exceeding 5 µm long overall	*A. montevidensis*
	Ascospores with walls smooth or very finely spiky, up to 6 µm long overall	*A. cristatus* (see *A. montevidensis*)
7(4)	Colonies with yellow or orange sterile hyphae; ascospores smooth walled and with just a trace of a longitudinal furrow	*A. pseudoglaucus*
	Colonies with orange to reddish hyphae, in age becoming red brown; ascospores with a fine but distinct longitudinal furrow flanked by two low, minutely roughened ridges	8
8(7)	Ascospores up to 6 µm long, asci maturing within 14 days	*A. ruber*
	Ascospores frequently exceeding 6 µm long, ascospores usually not maturing within 14 days	*A. glaucus* (see *A. ruber*)

Aspergillus chevalieri L. Mangin **Fig. 8.10**

[*Eurotium chevalieri* L. Mangin]

Colonies on CYA 16–25 mm diam, low and dense, plane or lightly sulcate; mycelium bright yellow, often darker centrally, enveloping abundant abortive yellow cleistothecia and overlaid by sparse to abundant greyish green conidial heads; yellow brown soluble pigment sometimes produced; reverse pale to orange or deep brown. Colonies on MEA 16–25 mm diam, plane, dense to floccose; mycelium white to yellow, overall colours and characteristics as on CYA; yellow soluble pigment sometimes produced; reverse pale, olive, orange or brown. Colonies on G25N

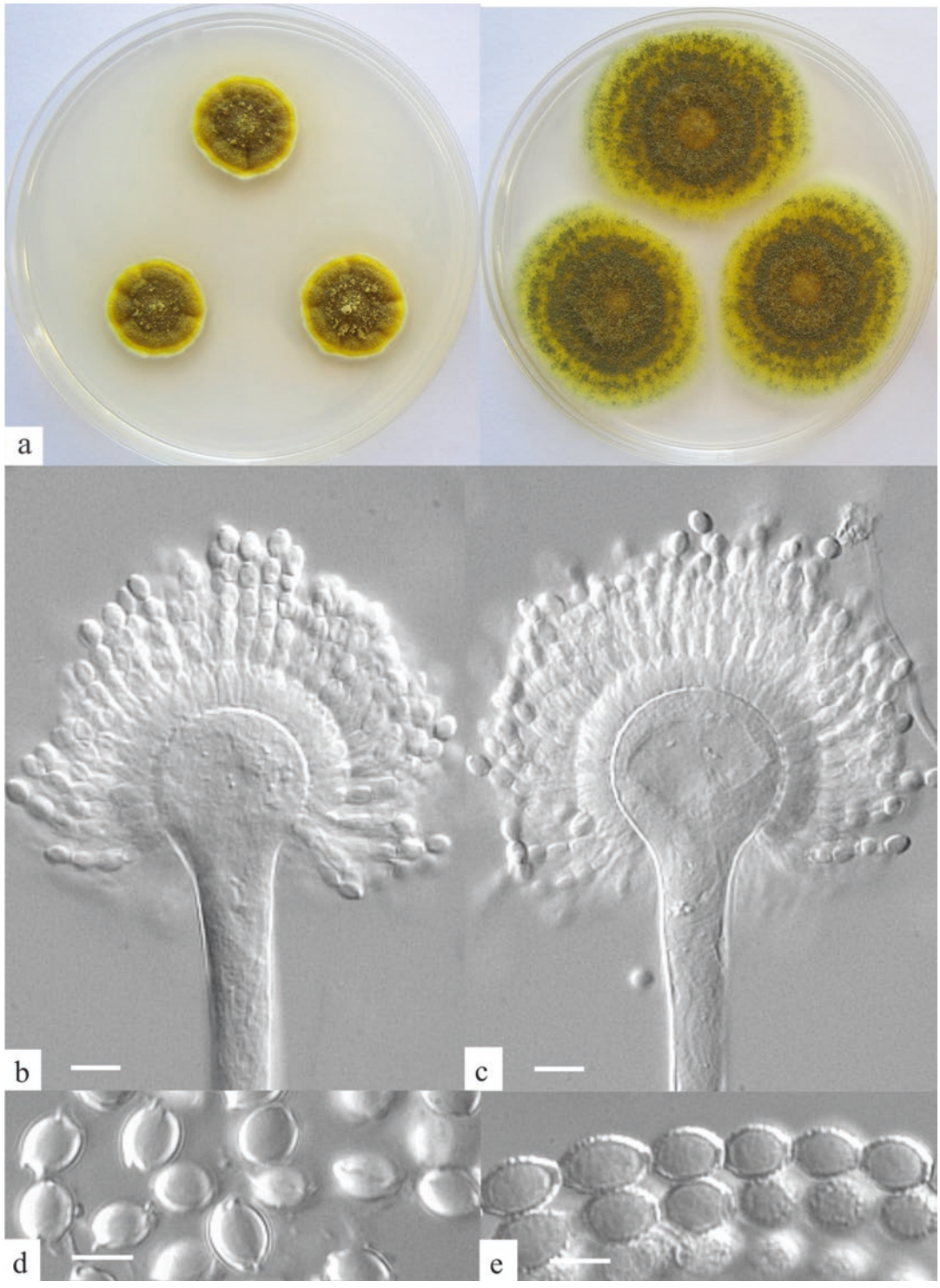

Fig. 8.10 *Aspergillus chevalieri* (**a**) colonies on CYA and CY20S, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) ascospores and (**e**) conidia, bars = 5 µm

20–30 mm diam, usually very deep and floccose, with pale to bright yellow mycelium enveloping abundant developing cleistothecia and overlaid by sparse to abundant greyish green conidial heads, occasionally development more sparse and producing few cleistothecia; reverse usually pale, sometimes yellow or dull green. No growth on CYA at 5 °C. Usually no growth on CYA at 37 °C, occasionally colonies up to 10 mm diam formed.

Colonies on CY20S 45–65 mm diam, plane, low and dense; colony appearance varying with production of conidial heads, in isolates with sparse heads, mycelium white at the margins, then bright to deep yellow or orange centrally, enveloping abundant cleistothecia, in isolates with heavy conidial production these elements more or less obscured and colony appearance dominated by dull green conidia; reverse pale, yellow, orange brown or dull green.

Cleistothecia bright yellow, spherical, 100–140 µm diam, enveloped in yellow to orange vegetative hyphae, maturing at colony centres in 8–10 days on CY20S and 12–14 days on G25N; ascospores yellow, ellipsoidal, shaped like pulley wheels, 4.5–5.0 µm long, smooth walled, with two prominent, parallel, narrow, sometimes sinuous, longitudinal flanges. Conidiophores borne from aerial hyphae, stipes mostly 400–700 µm long, thin walled, already collapsing in 7 days, broadening to vesicles 25–35 µm diam, fertile over the whole area, bearing phialides only; phialides ampulliform, 5–8 µm long; conidia on CY20S and G25N ellipsoidal or doliiform (barrel-shaped), 4.0–5.5 µm long, with spinose walls.

Distinctive features As with other *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species, the ascospore is the prime distinguishing feature: in *A. chevalieri* these are characteristically shaped like minute pulley wheels. Colonies grow quite well on CYA and MEA, but sporulate poorly; colonies on CY20S produce conspicuous yellow to orange hyphae, while on CYA and MEA they can be brown.

Taxonomy This taxon was originally described as a holomorph species, so the name "*chevalieri*" is valid for both the sexual and asexual morphs (Pitt and Samson 1993). The taxonomy of this species has been reviewed by Hubka et al. (2013) and Chen et al. (2016).

Identifiers MycoBank MB292839; epitype IMI 211383 (Hubka et al. 2013); ex-epitype cultures CBS 522.65 = IMI 22383 = ATCC 16443 = NRRL 78 = FRR 2795 (Hubka et al. 2013)); ITS barcode EF652068; alternative markers *BenA* EF651911, *CaM* EF652002, *RPB2* EF651954 (Samson et al. 2014).

Physiology The optimum temperature for growth of *A. chevalieri* is 30–35 °C (Domsch et al. 1980), with a maximum at 40–43 °C (Blaser 1975). Ayerst (1969) obtained growth down to 0.71 a_w at 33 °C; Pitt and Christian (1968) reported a minimum of 0.74 a_w at 25 °C on a medium of pH 3.8. Germination of ascospores was little affected by glucose or glycerol as solute, but was slower in NaCl. Media of pH 4 or 6.5 did not affect germination. Maximum growth rates were much higher on glucose/fructose or NaCl media than in the presence of glycerol; again pH had little effect. The optimum a_w for growth was 0.94–0.95 (Pitt and Hocking 1977). *A. chevalieri* was able to grow in the presence of 2000 ppm potassium sorbate, following a 20-day lag period, at 0.85 a_w and pH 6.0. No growth was recorded at lower pH and water activity values (eg: 0.80 a_w and pH 5.5) in the presence of preservative (Char et al. 2006). A model for the influence of a_w and temperature on germination and growth of *A. chevalieri* was developed by Greco et al. (2018).

Aspergillus chevalieri was the most heat resistant xerophilic fungus studied by Pitt and Christian (1970): 18–25% of ascospores survived heating at 70 °C for 10 min in a medium of 0.98 a_w and pH 3.8, and up to 0.5% a similar treatment at 80 °C. The decimal reduction line had an F_{80} of 3.3 min with a high *z* value, 12.8 C°, under these conditions. However, conidia were not especially heat resistant: only 0.1% survived heating for 10 min at 60 °C.

This species has been reported as one source of isoprene, a cause of off-odour in a bakery product (Berenguer et al. 1991). It is also a cause of ketonic rancidity in coconut (Kinderlerer 1984; Kinderlerer and Kellard 1984). High pro-

teolytic activity on meat (Binzel 1980) and moderate lipolytic activity on vegetable oils (Kuku 1980) have been reported.

Mycotoxins *Aspergillus chevalieri* has been reported to produce toxic compounds, identified as echinulin and neoechinulin, which were reported to cause feed refusal in swine (Vesonder et al. 1988). However, other tests for toxicity of *A. chevalieri* have been negative (Frisvad and Samson 1991; Adebajo and Oyesiku 1994).

Ecology *Aspergillus chevalieri* must rank as one of the most common spoilage fungi on earth, especially in warmer regions. It has been reported to cause spoilage of high moisture prunes, pecans, Japanese traditional noodles, a semisoft baked cookie and, in our laboratory, cheese spread, chick peas and faba beans (see Pitt and Hocking 1997). It has been shown to be the most important species in the production of katsuobushi, a Japanese fermented fish product (Kadooka et al. 2020).

Like the other common species in this genus, *A. chevalieri* has been isolated from a great variety of foods, especially cereals, including wheat and flour, rice, rice flour and rice bran, maize and cereal flakes. It is also common in processed and dried meats and nuts: peanuts, pecan nuts, hazelnuts and walnuts and coconut. Other sources include filled chocolates, dried beans and peas, soybeans, sunflower seeds, cocoa beans, spices, dried salt and cured fish (see Pitt and Hocking 1997). It has been isolated from evaporated milk products in Argentina (Char et al. 2005) and black pepper in Brazil (Gatti et al. 2003). Many other habitats no doubt exist.

This species was commonly isolated in our study of Southeast Asian commodities, particularly from peanuts and maize from Thailand, Indonesia and the Philippines. *Aspergillus chevalieri* infections in other nuts and oilseeds were also heavy (Pitt et al. 1993, 1994, 1998). Infections in beans were usually much lower, except for soybean samples from the Philippines (Pitt et al. 1993, 1994, 1998). Rice was less affected: no more than 20% of samples were infected, and overall infection rates in milled rice were only 2–4% (Pitt et al. 1993, 1994, 1998).

References Raper and Fennell (1965); Blaser (1975); Pitt (1985); Klich (2002); Hubka et al. (2013); Chen et al. (2016).

Aspergillus montevidensis Talice & J.A. Mackinnon **Fig. 8.11**

[*Eurotium montevidens*is (Talice & J.A. Mackinnon) Malloch & Cain]

Aspergillus amstelodami sensu Raper & Fennell, Manuel Aspergilli, 166, 1965

Colonies on CYA 14–18 mm diam and on MEA 16–25 mm diam, low and dense, plane; mycelum inconspicuous, white or yellow; abortive yellow cleistothecia conspicuous centrally in patches or sectors, surrounded by well formed bright to dark green conidial heads; reverse pale or occasionally dark green. Colonies on G25N 25–35 mm diam, plane, deep and floccose, with appearance usually uniformly dull green from layers of conidial heads; mycelium inconspicuous, white; yellow cleistothecia sometimes visible; conidial heads abundant, dull green; reverse yellow under cleistothecia, or uncoloured. No growth on CYA at 5 °C. On CYA at 37 °C, colonies up to 15 mm diam sometimes formed.

Colonies on CY20S usually 30–45 mm diam, plane, low and velutinous, usually with a layer of yellow cleistothecia near the agar surface surmounted by a dense layer of radiate dull green conidial heads; reverse uncoloured, i.e. yellow beneath cleistothecia, and pale grey green under conidial areas.

Cleistothecia bright yellow, mostly spherical, 75–150 µm diam, usually without surrounding sterile hyphae; maturing in 9–12 days on CY20S and 12–14 days on G25N; ascospores yellow, ellipsoidal, 4.5–5.0 µm long, with rough walls, and with two conspicuous, often sinuous, longitudinal ridges of low, irregular height and spacing. Conidiophores borne from aerial hyphae, stipes relatively short, 250–400 µm long, vesicles spherical to spathulate, 18–30 µm diam on CY20S, 35–40 µm on G25N, fertile over the upper two-thirds to three quarters, bearing phialides only; phialides 5–8 µm long; conidia spherical to subspheroidal, 4.0–5.0 µm diam, with densely spinulose walls, borne in radiate to loosely columnar heads.

Distinctive features The principal diagnostic feature of *Aspergillus montevidensis* is its conspicuous ascospore ornamentation: wide,

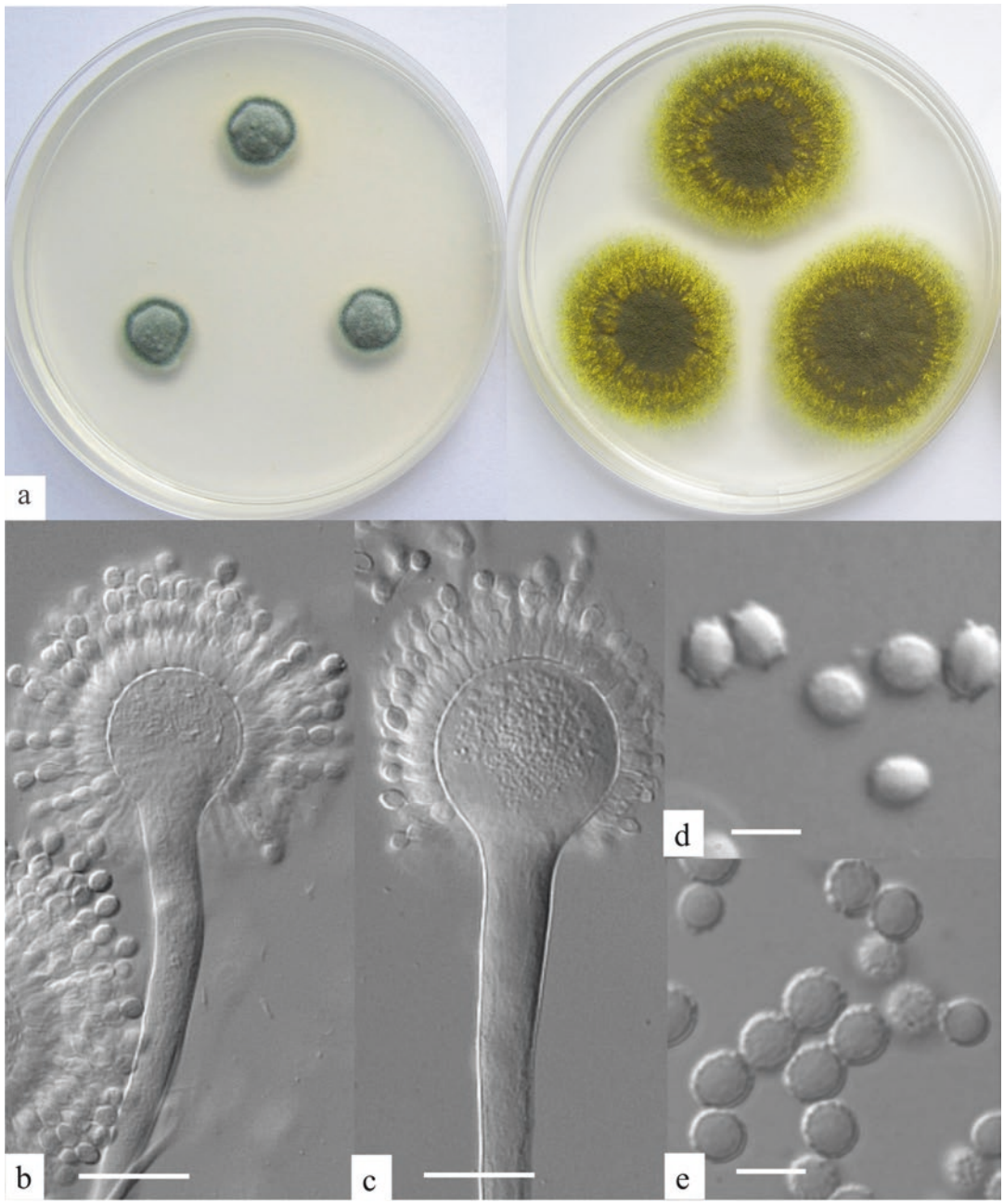

Fig. 8.11 *Aspergillus montevidensis* (**a**) colonies on CYA and CY20S, 7 d, 25 °C; (**b**, **c**) heads, bars = 20 µm; (**d**) ascospores and (**e**) conidia, bars = 5 µm

irregular ridges or flanges and rough walls. Mycelium remains inconspicuous; colony colours consist only of yellow from the cleistothecia and dull green from the conidia, in both obverse and reverse.

Taxonomy Pitt (1985) pointed out that Mangin's original concept of *Aspergillus amstelodami* differed from later usage (Thom and Raper 1941; Raper and Fennell 1965), including the neotypification by Blaser (1975), and that *A.*

montevidensis was probably the correct name. In monographing this genus and transferring all species to *Aspergillus*, Hubka et al. (2013) abandoned the name *amstelodami*, considering the original useage to be synonymous with *A. repens*, and accepted *A. montevidensis* as the correct name for this species.

Identifiers MycoBank MB309231; neotype BPI 884202; ex-type cultures CBS 491.65 = IMI 172290 = ATCC 10077 = NRRL 108 = FRR 108 (Hubka et al. (2013); ITS barcode EF652077; alternative markers *BenA* EF651898, *CaM* EF652020, *RPB2* EF651964 (Samson et al. 2014).

Physiology The optimal temperature for growth of *Aspergillus montevidensis* is 33–35 °C (Domsch et al. 1980), with a maximum at 43–46 °C (Blaser 1975). It was reported to grow down to 0.70 a_w at 25 °C by Armolik and Dickson (1956). Spore germination was noted after 120 days, but not below 0.75 a_w within 100 days by Wheeler and Hocking (1988). Scott (1957) showed that the optimal a_w for growth was near 0.96 regardless of controlling solute, but the maximum growth rate in sucrose media was twice as fast as in glycerol. Faster growth was observed on media controlled with NaCl than on glycerol based media, and maximum growth rates occurred at 0.90 to 0.93 a_w (Avari and Allsopp 1983). A model for the influence of a_w and temperature on germination and growth of *A. montevidensis* was developed by Greco et al. (2018).

In a medium of pH 3.8 and 0.98 a_w, 80–85% of ascospores of *Aspergillus montevidensis* survived heating at 60 °C for 10 min; 1–3% survived a similar treatment at 70 °C; and 0.2% at 75 °C. Only 0.3% of conidia survived heating for 10 min at 60 °C (Pitt and Christian 1970). Tolerance of sorbate was developed after 21 transfers through increasing concentrations, rising to 0.07% at pH 5.5 (Viñas et al. 1990).

In an atmosphere of 1% O_2, *Aspergillus montevidensis* grew at 50% of the rate in air (Hocking 1990). This species has been reported as one source of chloroanisoles which may cause off-odours in food carried in shipping containers (Hill et al. 1995), and of isoprene, a cause of off–odour in a bakery product (Berenguer et al. 1991). Ketonic rancidity in coconut has also been caused by *A. montevidensis* (Kinderlerer 1984; Kinderlerer and Kellard 1984). It showed the highest proteolytic activity on meat of any species in *Aspergillus* subgen. *Aspergillus* (Binzel 1980) and was relatively resistant to propionic acid (Müller et al. 1981), but lipase activity was low (Magan et al. 1993). Marín et al. (2003) reported N-acetyl-β-D-glucosaminidase activity at 0.90 a_w at pH 6 and at 0.85 a_w at pH 7.5. Conidia of *A. montevidensis* were found to survive in 30% (w/v) NaCl solution over a period of 14 weeks (Butinar et al. 2005).

Mycotoxins A variety of metabolites are produced by this species, but in common with other species in this subgenus, none are of importance in public health (Chen et al. 2016).

Ecology In Australia, *Aspergillus montevidensis* is less common than the other species in *Aspergillus* subgen. *Aspergillus* described here; however literature reports indicate that elsewhere it is a ubiquitous foodborne species, usually associated with stored goods. This species has been reported to spoil Japanese traditional noodles and cheese and rapeseed due to heat damage (see Pitt and Hocking 1997). *A. montevidensis* has been reported as one cause of gushing in beer (Gyllang and Martinson 1976). It has been shown to be the second most important species (after *A. cheval-ieri*) in the production of katsuobushi, a Japanese fermented fish product (Kadooka et al. 2020).

As with other similar species, major substrates for *A. montevidensis* are cereals, including wheat, flour, refrigerated dough, bread and bakery products, rice and rice bran, barley, maize, cereal flakes and corn snacks (see Pitt and Hocking 1997). Other sources include grapes and raisins (Valero et al. 2007a), mixed feeds, cereals and legume feeds (Accensi et al. 2004), pasta (Halt et al. 2004), peanuts, hazelnuts and walnuts, pistachios, soybeans, sunflower seeds, meat products, smoked bacon, biltong, dried salt fish and jam (see Pitt and Hocking 1997). It is frequently isolated from meju (dried fermented soybeans) (Chen et al. 2016).

Aspergillus montevidensis was less common in Southeast Asian commodities than *A. cheval-*

ieri or *A. ruber*. Nevertheless we isolated it at 1% or more of all particles examined in maize, peanuts, soybeans, cashews, copra, paddy rice, mung beans, sorghum and peppercorns. Percentages of samples infected were often relatively low, usually 8–15%, but rates of infection in infected samples were often high, up to 40–60%. This indicates some samples becoming infected during storage (Pitt et al. 1993, 1994, 1998).

Additional species *Aspergillus cristatus* Raper & Fennell in culture resembles *A. montevidensis* and is often mistaken for it. However, ascospores are larger, up to 6 µm long including crests, and have walls ornamented with discrete, very fine spikes. Unlike *A. montevidensis,* colonies have bright yellow hyphae surrounding the cleistothecia. No physiological studies are known, and mycotoxin production is also unknown.

A. cristatus, known locally as the "golden flower fungus" is the species used in the fermentation of "Fuzhuan" brick tea produced in Guizhou Province, China (Tan et al. 2017). We have isolated this species uncommonly, from heated strawberry puree and Philippine peanuts. Identifiers: MycoBank MB326622; neotype IMI 172280; ex-type cultures CBS 123.53 = IMI 172280 = ATCC 16468 = NRRL 4222 = FRR 1167 (Hubka et al. 2013).

References *Aspergillus montevidensis:* Raper and Fennell (1965), Pitt (1985), Klich (2002) as *Aspergillus amstelodami*; Hubka et al. (2013); Chen et al. (2017). *A. cristatus*: Hubka et al. (2013); Chen et al. (2016).

Aspergillus penicillioides Speg. Fig. 8.12

Colonies on CYA up to 5 mm diam, sometimes only microcolonies, of white mycelium only. Growth on MEA usually limited to microcolonies, occasionally colonies up to 5 mm diam, similar to those on CYA. Colonies on G25N 8–15 mm diam, plane or centrally raised, sometimes sulcate or irregularly wrinkled, texture velutinous or lightly floccose; mycelium usually inconspicuous, white; conidial production moderate, coloured dull green to dark green; reverse pale to dark green. No growth at 5 or 37 °C on CYA.

Colonies on CY20S varying from microcolonies up to 10 mm diam, similar to those on CYA, occasionally some dull green conidial production but conidiophores poorly formed; reverse pale. Colonies on MY50G 10–16 mm diam, plane or umbonate, relatively sparse, velutinous to floccose; conidial production moderate, greyish green to dull green; reverse pale.

No teleomorph known. Conidiophores borne from surface or aerial hyphae, showing optimal development on G25N, stipes (150–)300–500 µm long, sometimes sinuous, with colourless, thin, smooth walls, enlarging gradually from the base, then rather abruptly to pyriform or spathulate vesicles; vesicles mostly 10–20 µm diam, usually fertile over two thirds of the area, bearing phialides only; phialides 8–11 µm long; conidia borne as ellipsoids, at maturity ellipsoidal to subspheroidal, 4.0–5.0 µm diam, with spinose walls, typically borne in radiate heads, uncommonly in loose columns also.

Distinctive features In common with *Aspergillus restrictus, A. penicillioides* grows very slowly under all standard conditions, and produces green conidia. It differs from *A. restrictus* by very weak growth on CYA and MEA, by forming radiate conidial heads from spathulate vesicles, fertile over more than the upper half; and by bearing conidia as ellipsoids, which usually separate in liquid mounts. No sexual state is known.

Identifiers MycoBank MB309234; neotype IMI 211342 (Samson and Gams 1985); ex-type cultures CBS 540.65 = IMI 211432 = ATCC 16910 = NRRL 4548; ITS barcode EF652306; alternative markers *BenA* EF651928, *CaM* EF652024, *RPB2* EF651930 (Samson et al. (2014).

Physiology *Aspergillus penicillioides* is an extreme xerophile, now recorded as the most drought tolerant organism known, with a limit for growth below 0.6 a_w (Stevenson et al. 2017). As is clear from the description above, *Aspergillus penicillioides* grows very poorly at high a_w. The optimal a_w for growth is 0.89 at 30 °C in glucose-fructose media of pH 5.5 (Gock et al. 2003) or 0.91–0.93 at 25 °C on media containing either glucose/fructose or NaCl, at pH 6.5 (Andrews and Pitt 1987; Stevenson et al. 2017). At 25 °C,

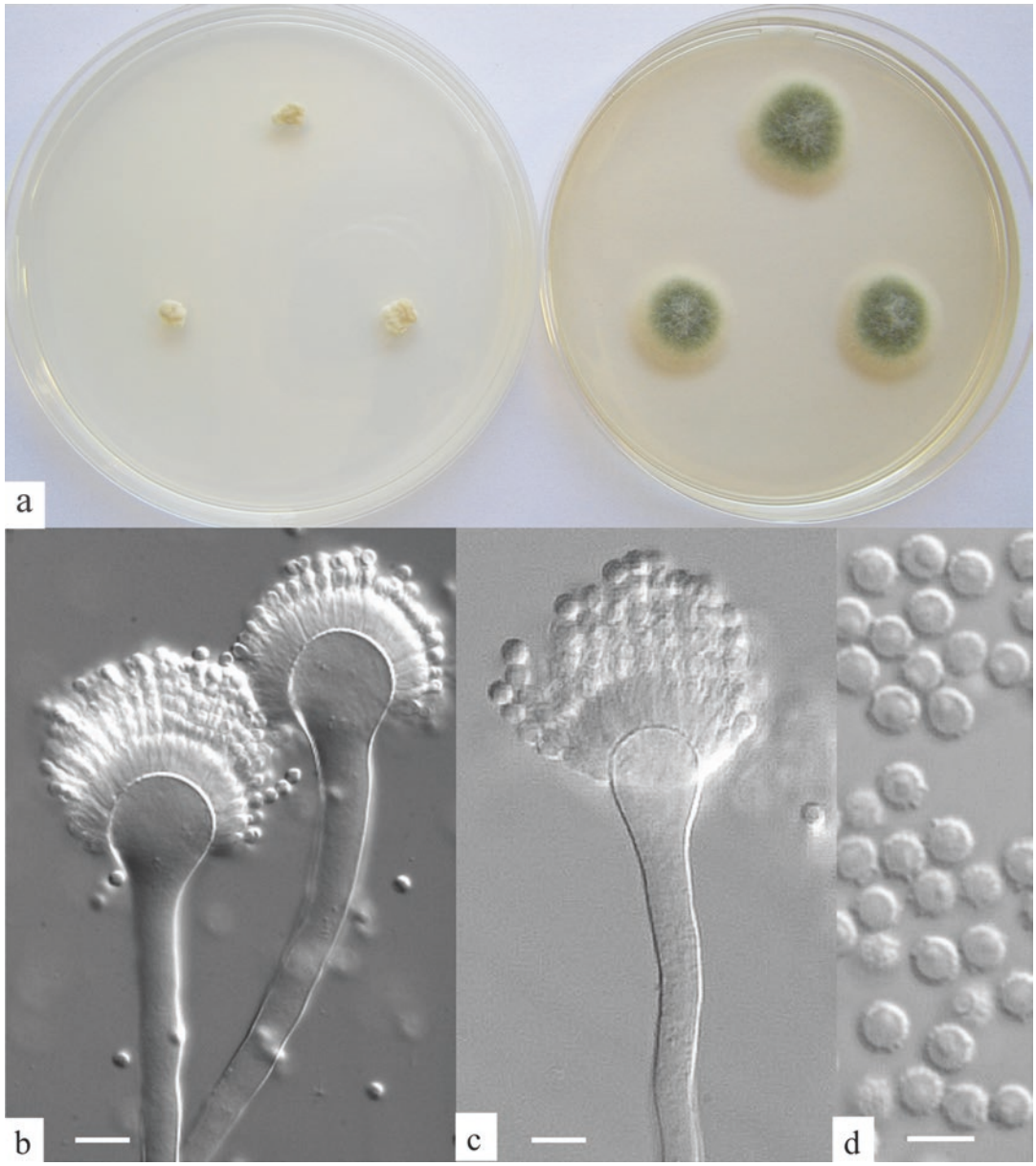

Fig. 8.12 *Aspergillus penicillioides* (**a**) colonies on CYA and MY50G, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) conidia, bar = 5 µm

A. penicillioides was reported to be capable of germination but not growth at 0.70 a_w and pH 4.5–5.5 in media containing glucose/fructose (Gock et al. 2003). On a medium with NaCl as sole a_w controller, the limit germination limit observed was 0.75 a_w, the minimum obtainable with NaCl alone (Andrews and Pitt 1987). However, on a glycerol-NaCl medium, germination was observed down to 0.64 a_w, the lowest examined (Stevenson et al. 2017). Subsequently, Stevenson et al. (2017) observed germination below 0.59 a_w at 24 °C on a glycerol substrate under stringently controlled conditions. Even in natural substrates, *A. penicillioides* is capable of growth at very low water activities: we have reproducible evidence of its ability to multiply at

0.68 a_w in wheat and pet food within 6 months (our unpublished observations).

The minimum a_w previously recorded for growth at 20, 30 and 34 °C was higher than at 25 °C, 0.76–0.79 a_w on glucose/fructose and 0.80–0.84 on NaCl. At 37 °C, minimum figures were 0.79 and 0.88 a_w, respectively (Wheeler et al. 1988).

Taking into account the effect of a_w, growth temperatures are not exceptional. Growth does not occur at 37 °C on CYA, but occurs readily at this temperature at lower a_w (Wheeler et al. 1988; Gock et al. 2003; Stevenson et al. 2017). Growth occurs down to 15 °C or lower, at 0.95–0.90 a_w at least (Wheeler et al. 1988; Stevenson et al. 2017).

Mycotoxins This species has not been reported to produce mycotoxins.

Ecology Reports of *Aspergillus penicillioides* in foods are quite rare, primarily because it grows poorly or not at all on media commonly used for fungal isolation and enumeration. Greatly improved results can be obtained if a low a_w medium such as DG18 is used (Hocking 1981). It is also true that this species has frequently been misidentified as *A. restrictus.* For example, *A. restrictus* was reported to be a major cause of loss of germinability in wheat stored just above the safe moisture content (Tuite and Christensen 1957; Christensen 1963): in fact the species responsible is *A. penicillioides.*

In this laboratory, we have isolated *Aspergillus penicillioides*, often in very high numbers, from a wide variety of foods, including flour, dried fruit and dried fish, and from spices, including pepper and dried chillies. Other reported isolations have come from milled rice (Taligoola et al. 2004), fermented and cured meats, cocoa and peanuts (see Pitt and Hocking 1997) and salted dried fish (Nketsia-Tabiri et al. 2003; Gräu et al. 2007). This species was quite competitive at 30 °C on glucose media, but was less so on NaCl media or salt fish, or at other temperatures (Wheeler and Hocking 1993).

From Southeast Asian commodities, we isolated *Aspergillus penicillioides* quite frequently. Although the proportion of samples infected was low, individual samples often showed high infection rates, no doubt reflecting prolonged storage at water activities just above safe limits. *A. penicillioides* was commonly isolated from soybeans in Thailand and Indonesia, where 6% and 17% of samples were contaminated respectively. Black pepper from the Philippines showed up to 60% infection in contaminated samples, and 2% infection in peppercorns overall. Lower levels of infection were found in paddy and milled rice, various other kinds of beans including mung beans, and peanuts (Pitt et al. 1993, 1994, 1998 and our unpublished data).

References Raper and Fennell (1965); Pitt and Samson (1993); Klich (2002); Sklenář et al. 2017).

Aspergillus pseudoglaucus Blochwitz **Fig. 8.13**

[*Eurotium repens* de Bary]
Eurotium pseudoglaucum (Blochwitz) Malloch & Cain
Aspergillus glaber Blaser

Colonies on CYA and MEA 10–20 mm diam, plane, deep and dense, mycelium white, yellow or orange, either enmeshing abortive yellow cleistothecia or surmounted by dull green to dull blue conidial heads, or both, depending on isolate; reverse pale, dull yellow, green or orange, less commonly bright yellow or orange. Colonies on G25N 25–45 mm diam, plane, deep and floccose, sometimes reaching the Petri dish lid; mycelium white to bright yellow or orange, usually enmeshing many layers of developing bright yellow cleistothecia and overlaid by sparse dull green conidial heads, in uncommon isolates cleistothecia and yellow hyphae inconspicuous and dull green conidial heads dominant; reverse pale, brilliant yellow to orange, or orange brown. No growth on CYA at 5 or 37 °C.

Colonies on CY20S 45–65 mm diam, plane, low or somewhat floccose, broader but much less luxuriant than on G25N; mycelium white to yellow or orange, overall colour varying from yellow with scattered dull green areas in predominantly cleistothecial isolates to dull green or bluish green in those with conidial heads dominant; reverse dull green or bright yellow to orange or both, at maturity dull yellow brown.

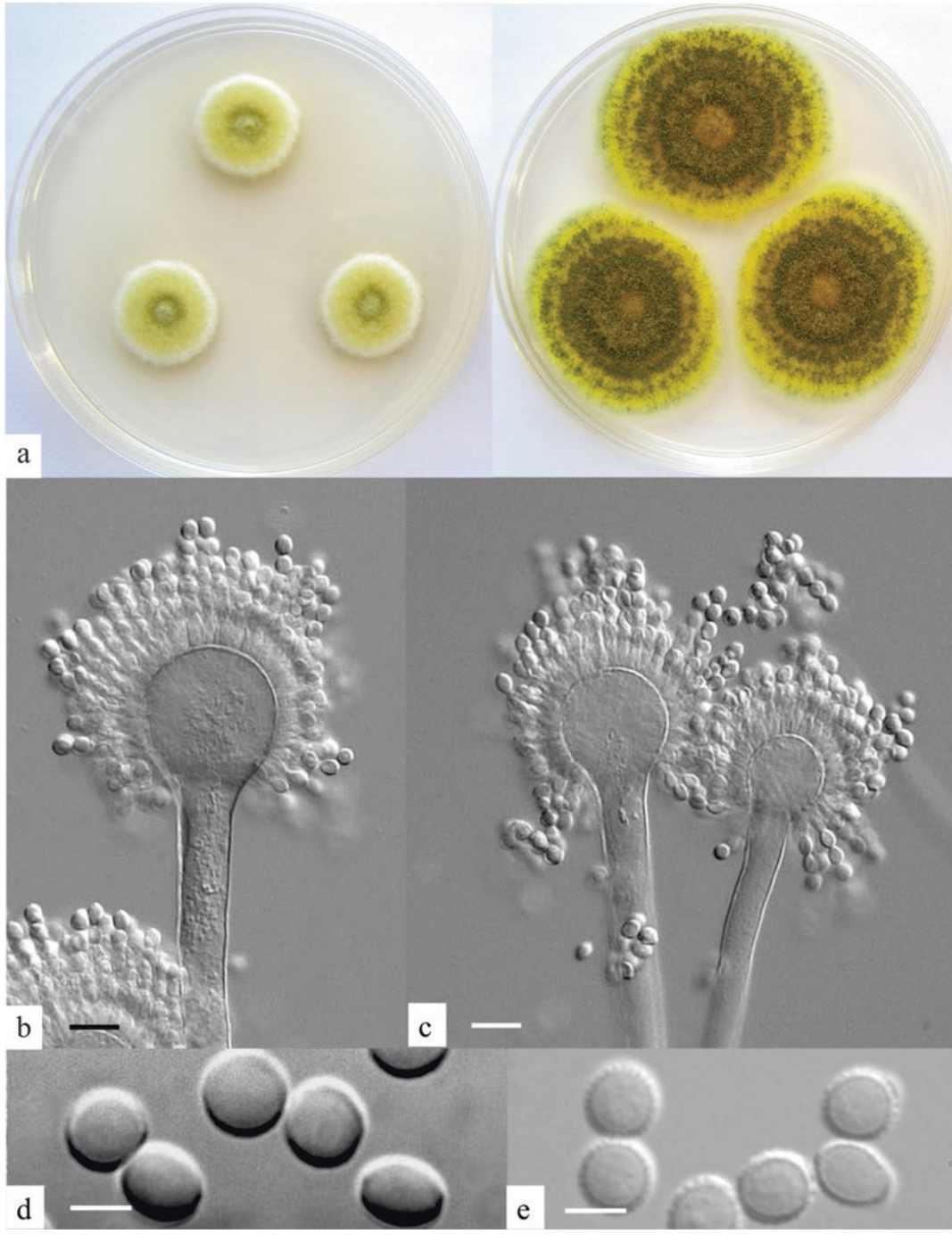

Fig. 8.13 *Aspergillus pseudoglaucus* (**a**) colonies on CYA and CY20S, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) ascospores and (**e**) conidia, bars = 5 µm

Cleistothecia on CY20S or G25N borne from and enveloped in sterile yellow to orange hyphae, bright yellow, spherical, 75–100(−125) µm diam, maturing at colony centres in 7–10 days; ascospores yellow, ellipsoidal, 5.0–5.5 µm long, without ridges or flanges and with no more than a trace of a longitudinal furrow, smooth walled, borne in loosely radiate heads. Conidiophores borne from aerial hyphae, stipes mostly 500–1000 µm long, broadening to vesicles 15–30 µm diam, fertile over the whole area, bearing phialides only; phialides ampulliform, 7–10 µm long; conidia on CY20S spherical to subspheroidal, 5–7 µm diam, on G25N ellipsoidal to pyriform, 7–10 µm long, with densely spinulose to spinose walls.

Distinctive features The prime distinction of *Aspergillus pseudoglaucus* from other species in this subgenus is the production of ascospores without ridges or flanges, usually without a longitudinal furrow, and smooth walls. Hyphal and reverse colours on CYA and MEA are yellow to orange, never red.

Taxonomy De Bary described *Eurotium repens* in 1870, but stated that his species was different from *Aspergillus glaucus* var. *repens* Corda 1842, so the combination *Aspergillus repens* (Corda) Sacc., used by many authors, cannot be used for this species (Hubka et al. 2013). According to those authors, the earliest valid name in *Aspergillus* for this species is *A. pseudoglaucus* Blockwitz 1929. That disposition is accepted here.

Identifiers MycoBank MB275429; neotype IMI 16122ii (Hubka et al. 2013); ex-type cultures CBS 123.28 = IMI 16122 = ATCC 10066 = NRR 40 = FRR 42 (Hubka et al. 2013); ITS barcode EF652050; alternative markers *BenA* EF651917, *CaM* EF652007, *PRB2* EF651952 (Samson et al. 2014).

Physiology *Aspergillus pseudoglaucus* grows between 4–5 °C and 38–40 °C, with an optimum at 25–27 °C (Panasenko 1967; Gonzalez et al. 1988). The minimum a_w for germination of *E. repens* was reported as 0.72 on media of neutral pH, at temperatures of 20 to 25 °C (Snow 1949; Armolik and Dickson 1956; Magan and Lacey 1984a). Andrews and Pitt (1987) reported the minimum a_w for germination in NaCl and glycerol media were 0.83 and 0.72 a_w, with optima at 0.95 and 0.91 a_w respectively. In media controlled by glucose/fructose, the minimum a_w for germination was much lower, 0.69 a_w (Andrews and Pitt 1987). The influence of pH from 4.0–6.5 was slight (Avari and Allsopp 1983), with pH 4.5–5.5 being optimal (Gock et al. 2003). Models for the influence of a_w and temperature on germination and growth of *A. pseudoglaucus* were developed by Dagnas et al. (2014) and Greco et al. (2018).

Aspergillus pseudoglaucus ascospores are tolerant of elevated temperatures, and survived heating to 60 °C and 70 °C for 10 min. However, they did not recover after heating at 75 °C for 10 min (Pitt and Christian 1970).

Aspergillus pseudoglaucus has been reported as one source of chloroanisoles which may cause off-odours in food carried in shipping containers (Hill et al. 1995). It is one of a group of species producing odorous compounds, including 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone in grain, useful in quality monitoring systems (Sinha et al. 1985).

Mycotoxins Mycotoxins are not known to be produced by this species (Frisvad and Samson 1991).

Ecology *Aspergillus pseudoglaucus* is a very common species. It has been reported to cause spoilage of cheese, cheesecake, pecans, maize and traditional Japanese noodles. It is very common in stored grains, for example wheat, barley, maize and rice. *A. pseudoglaucus* is of common occurrence on processed and dried meat or fish, for example salami, meat products, ripening and ripened hams, biltong, dried salted fish and katsuobushi. Other sources include nuts, sunflower seed and red pepper powder (see Pitt and Hocking 1997).

In our laboratory, *Aspergillus pseudoglaucus* has been isolated from spoiled prunes, salami, strawberry puree, bread, cake, nuts, cheese and other products. It was relatively uncommon in Southeast Asian commodities by comparison with *A. chevalieri* or *A. montevidensis*, exceeding 1% infection overall only in copra from Thailand (3%) and kemiri nuts from Indonesia (2%) (Pitt et al. 1993, 1994, 1998).

Aspergillus pseudoglaucus has found use in food manufacture, as a starter culture in the manufacture of katsuobushi from bonito (Dimici and Wada 1994) and of fish sauce from fish meal (Hayakawa et al. 1993).

References Raper and Fennell (1965) as *Aspergillus repens*; Blaser (1975); Pitt (1985); Klich (2002); Hubka et al. (2013); Chen et al. (2016).

Aspergillus restrictus G. Sm. Fig. 8.14

Colonies on CYA 6–12 mm diam, sulcate or wrinkled, low, dense and velutinous; mycelium inconspicuous, white; conidial heads often poorly formed, sparse to numerous, in the latter case dull green; reverse pale to very dark green. Colonies on MEA 6–12 mm diam, occasionally smaller, similar to those on CYA or centrally raised,

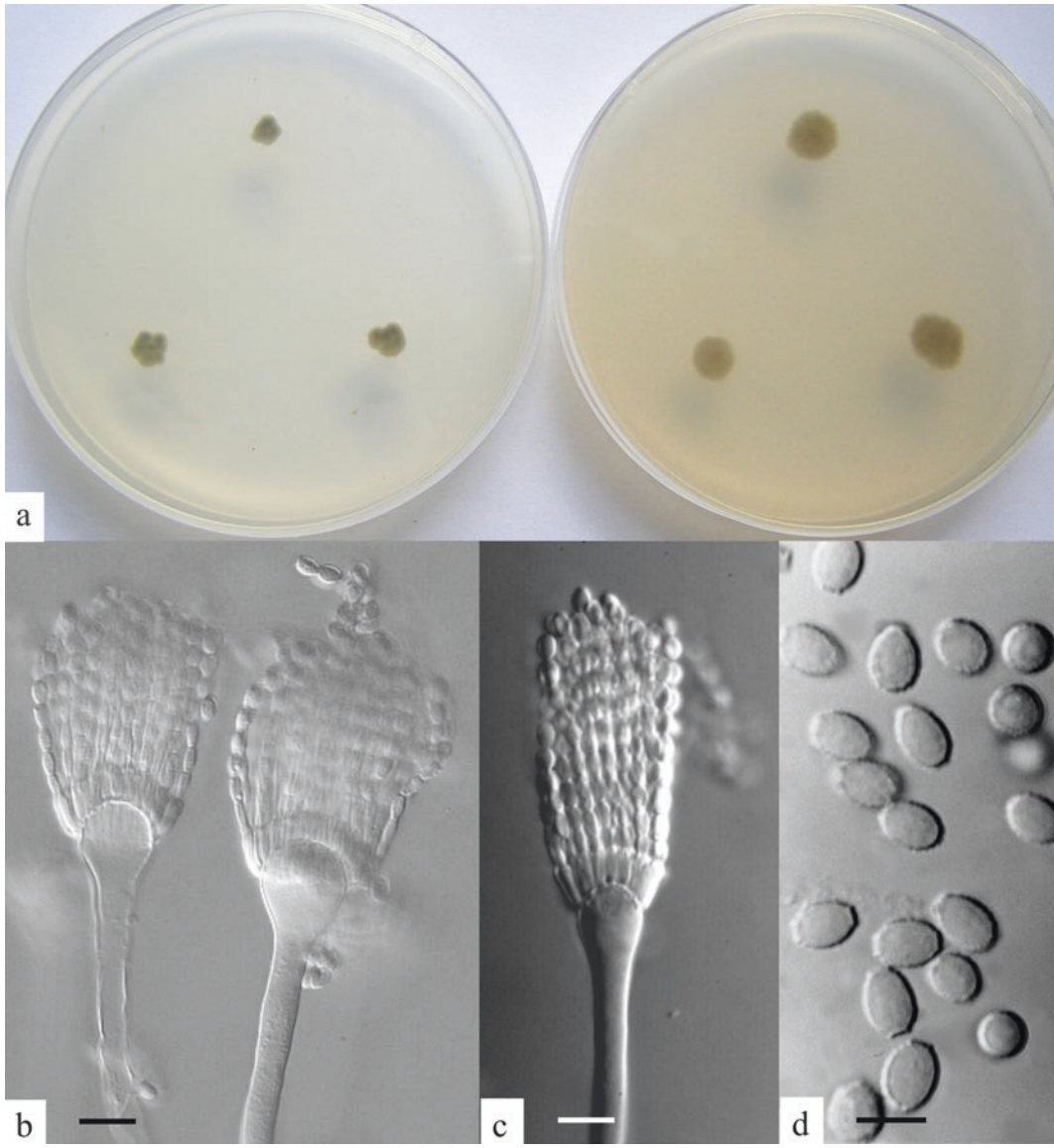

Fig. 8.14 *Aspergillus restrictus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) conidia, bar = 5 µm

conidial production heavy but heads poorly formed, coloured dull green to dark green; reverse usually pale. Colonies on G25N 10–15 mm diam, plane or umbonate, usually similar to those on MEA, but heads well formed; reverse sometimes dark green. No growth at 5 or 37 °C on CYA.

Colonies on CY20S 16–20 mm diam, generally similar to those on G25N apart from slightly more rapid growth. Colonies on MY50G 12–16 mm diam, plane or umbonate, with aerial growth and conidial production usually sparse, coloured greyish green to dull green; reverse pale.

No teleomorph known. Conidiophores borne from surface hyphae, developing optimally on CY20S, stipes 75–200 µm long, sometimes sinuous, with colourless, thin, smooth walls, enlarging from the base gradually then abruptly to pyriform vesicles; vesicles 10–18 µm diam, fertile over the upper hemisphere or less, bearing phialides only; phialides crowded, 8–10 µm long; conidia borne as cylinders, in long, appressed columns, adhering in liquid mounts, when mature nearly cylindrical to doliiform (barrel-shaped), 4.0–6.0 µm long, with rough walls, borne in long columns under favourable growth conditions.

Distinctive features In common with *Aspergillus penicillioides, A. restrictus* grows very slowly under all conditions, and produces green conidia. It differs from *A. penicillioides* by vesicles which are fertile over the upper half or less and by conidia borne as cylinders and adhering in long columns, usually persisting in liquid mounts. *A. restrictus* grows more rapidly than *A. penicillioides* on CY20S.

Identifiers Mycobank MB276290; neotype IMI 16267 (Samson and Gams 1985); ex-type cultures CBS 117.33 = IMI 16267 = ATCC 16912 = NRRL 154 = FRR 3689; ITS barcode EF652042; alternative markers *BenA* EF651880, *CaM* EF652029, *RPB2* EF651978 (Samson et al. 2014).

Physiology Smith and Hill (1982) reported the temperature range for growth of *Aspergillus restrictus* was minimum, 9 °C, optimum, 30 °C and maximum, 40 °C. Growth of this species has been observed down to 0.75 a_w (Snow 1949; Pelhate 1968). This species grows strongly in 1% O_2 but not at all in 0.1% O_2 (Hocking 1990).

Mycotoxins This species has not been reported to produce mycotoxins.

Ecology Considering the slow growth rate of this species, and its inconspicuous habit, *A. restrictus* has been isolated from foods quite frequently. Most records have come from dried foods: wheat, rice, maize, dried beans, pecans, peppercorns, salted dried fish, cured meats, Japanese traditional noodles, spices, pepper, carriers for dough during breadmaking, dried prunes and health foods (see Pitt and Hocking 1997). *Aspergillus restrictus* has been reported recently in cashew nuts (Adebajo and Diyaolu 2003), coffee beans (Ahmad and Magan 2003), cocoa beans (Wojcik-Stopczynska 2006) and cured fish (Nketsia-Tabiri et al. 2003).

We isolated *Aspergillus restrictus* quite frequently from Southeast Asian commodities. As with *A. penicillioides,* the proportion of samples infected was often low, but with individual samples showing high infection rates, reflecting prolonged storage. The highest levels of *A. restrictus* found were in samples of red rice from Indonesia. Soybeans from the Philippines, Indonesia and Thailand showed low levels of infection (see Pitt and Hocking 1997). Mung beans, red beans and cowpeas showed up to 24% infection in individual samples. Black pepper from the Philippines showed up to 100% infection in contaminated samples. Only one sample of kemiri nuts (of 20) from Indonesia was infected, but this species infected 30% of nuts in that sample. Low levels of infection occurred in paddy and milled rice, maize and peanuts (Pitt et al. 1993, 1994, 1998 and and our unpulished data).

References Raper and Fennell (1965); Klich (2002); Sklenář et al. 2017).

Aspergillus ruber (Jos. König et al.) Thom & Church Fig. 8.15

[*Eurotium rubrum* Jos. König et al.]

Colonies on CYA 10–20 mm diam, plane, deep, usually dense and velutinous, sometimes floccose; mycelium yellow to bright orange; conidiophores and developing cleistothecia usually present, but often poorly formed; reverse pale yellow to orange brown. Colonies on MEA usually 10–20 mm diam, sometimes only 5–8 mm, similar to those on CYA, but sometimes less deep and with more conspicuous orange hyphae. Colonies on G25N 30–45 mm diam, plane, often floccose and with hyphal strands sometimes reaching the Petri dish lid, consisting of cleistothecia in layers supported and surrounded by relatively sparse orange to dark orange hyphae;

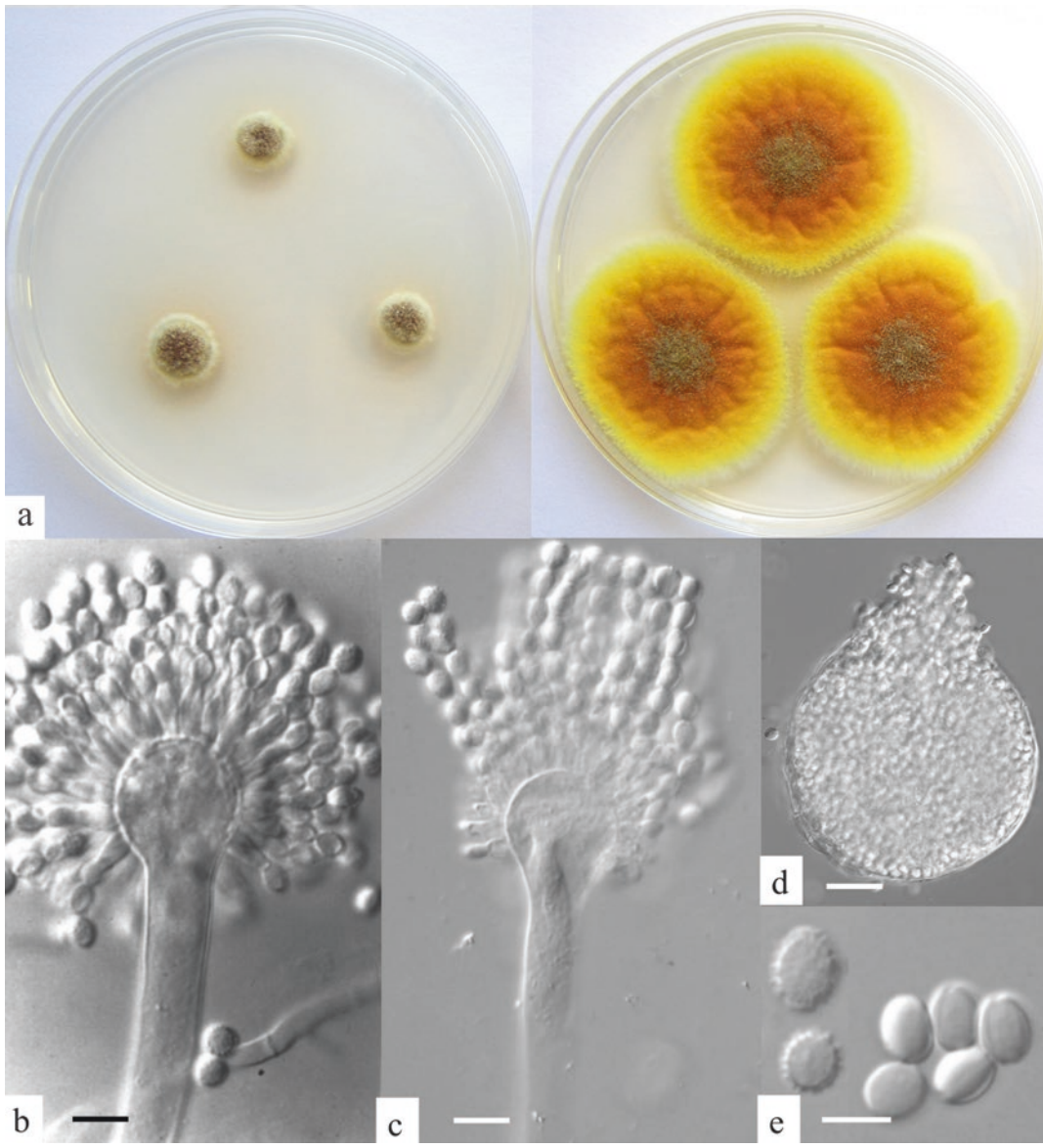

Fig. 8.15 *Aspergillus ruber* (**a**) colonies on CYA and CY20S, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) mature cleistothecium releasing ascospores, bar = 25 µm; and (**e**) conidia (spiny spores) and ascospores, bar = 5 µm

conidial heads usually rare, above or within the cleistothecial layer, dull green; reverse yellow, brown or reddish orange . No growth on CYA at 5 °C or 37 °C.

Colonies on CY20S at 7 days 35–60 mm diam, plane or lightly sulcate, usually low, dense and velutinous; mycelium conspicuous, at the margins yellow, becoming orange or more reddish elsewhere, enveloping abundant yellow cleistothecia and surmounted by sparse to abundant dull green heads; reverse in colours similar to the mycelium, though rarely so bright, or deep yellow brown; in age, 10 days or more, developing bright to brick red brown hyphal colours over the whole plate or in patches, not under densely conidial areas or, rarely, remaining orange; reverse darkening over time to deep brown or almost black.

Cleistothecia on CY20S or G25N borne from and enveloped in sterile orange to red hyphae, spherical, yellow, 80–150 µm diam, maturing in 9–12 days; ascospores ellipsoidal, yellow, 5.0–6.0 µm long, with a shallow longitudinal furrow flanked by low, usually minutely roughened ridges, otherwise with smooth walls. Conidiophores borne from aerial hyphae, stipes mostly 300–700 µm long, terminating in spherical vesicles, 20–35 µm wide, fertile over the upper two-thirds, bearing phialides only; phialides 7–9 µm long; conidia subspheroidal to ellipsoidal, less commonly spherical or pyriform, generally 6–7 µm long, larger in occasional isolates, with spinose walls, borne in loosely radiate heads.

Distinctive features Colonies of *Aspergillus ruber* on CY20S usually show areas of brilliant red or rusty colours after 10 days or more incubation. Ascospores are 5.0–6.0 µm long, rarely otherwise, and show a definite longitudinal furrow and low, minutely roughened ridges.

Identifiers MycoBank MB276893; neotype CBS 530.65 (Samson and Gams, 1965; designated for *Eurotium rubrum*, as the *Aspergillus* state is also present, accepted here); ex-type cultures CBS 530.65 = IMI 211380 = ATCC 16441 = NRRL 52 (Hubka et al. 2013); ITS barcode EF652066; alternative markers *BenA* EF651920, *CaM* EF652009, *RPB2* EF651947 (Samson et al. 2014).

Physiology Growth temperatures for *Aspergillus ruber* are probably similar to those reported for *Aspergillus pseudoglaucus*: minimum ca 5 °C, optimum 25–27 °C, and maximum near 40 °C (Panasenko 1967). The minimum a_w for germination has been reported as 0.70 a_w (Snow 1949; Gock et al. 2003), 0.72 a_w (Armolik and Dickson 1956), at 25 °C, or 0.72 at 20 °C and 0.73 at 25 °C (Wheeler et al. 1988). On a medium of pH 3.8, germination occurred down to 0.75 a_w after 98 days at 25 °C (Pitt and Christian 1968). Maximum growth was achieved at 30 °C at 0.92 a_w (Gock et al. 2003). *E. rubrum* was able to grow on media with up to 20% (w/v) NaCl (0.87 a_w) and germination occurred at 25% (w/v) NaCl (0.82 a_w), although mycelial growth did not follow (Butinar et al. 2005). Growth rates were similar in glycerol at pH 4.0 and 6.5, and NaCl at pH 6.5, over a wide a_w range; growth in NaCl at pH 4.0 was much slower (Wheeler et al. 1988). The optimum a_w for growth was ca 0.94, and varied little over all conditions tested (Avari and Allsopp 1983). At higher temperatures on glucose/fructose media of pH 6.5, the minimum a_w was 0.76 at 30 °C and 0.79 at 37 °C (Wheeler et al. 1988). On NaCl based media, the minimum a_w for germination was 0.86–0.88 a_w from 20–34 °C; at 37 °C it was 0.91 a_w, indicating temperature had little effect (Wheeler et al. 1988). *A. ruber* was a highly competitive species over a wide range of a_w and temperature on glucose based media, but not in NaCl based media (Wheeler and Hocking 1993). A model for the influence of a_w and temperature on germination and growth of *A. ruber* was developed by Greco et al. (2018).

A. ruber was relatively tolerant of modified atmospheres, producing visible colonies in 65% CO_2 + 1% O_2 at 0.90 a_w in 15 days. At lower a_w values, growth was inhibited: at 0.82 a_w and 50% CO_2 + 1% O_2, visible growth occurred after 35 days, and at 0.78 a_w, growth occurred in 20% CO_2 + 1% O_2 after 25 days. Growth did not occur in higher concentrations of CO_2 at these a_w values (our unpublished data).

A high percentage (80–100%) of ascospores of *Aspergillus ruber* survived heating at 60 °C for 10 min, at a_w 0.98 and pH 3.8; 0.5% or less survived 10 min at 70 °C and there were no survivors after 10 min at 75 °C. Conidia were much less heat resistant: only 8% survived 10 min at 50 °C, 3% 10 min at 60 °C and none at 70 °C under the same conditions (Pitt and Christian 1970).

This species developed tolerance to sorbate after 21 transfers through increasing concentrations, rising to 0.07% at pH 5.5 (Viñas et al. 1990).

Mycotoxins Several reports have been published which indicate that *Aspergillus ruber* produces a range of toxic compounds. However well documented confirmation of toxicity is lacking (Frisvad and Samson 1991). Production of echinulin and flavoglaucin by *E. rubrum* was not correlated with pH nor water activity. Further, metabolite production was enhanced on media containing 0.3% propionate (Suhr and Nielsen 2004).

Ecology A very widely distributed xerophilic fungus, *Aspergillus ruber* appears to be somewhat more common in warmer regions. It has caused spoilage of dried and high moisture prunes, coconut and pancake syrup. It has been reported from a wide range of cereals (Lugauskas et al. 2006), especially wheat, maize and rice. It is also common on nuts, meat products, and dried salt fish. Other reports have been from sunflower seeds, sesame seeds, cocoa beans, palm kernels, dried vegetables and jam (see Pitt and Hocking 1997). It has frequently spoiled fruit cakes in Australia (our observations). *A. ruber* has been isolated from salterns in the Adriatic (Butinar et al. 2005).

Along with *Aspergillus pseudoglaucus, A. ruber* has found use in food manufacture, as a starter culture in the manufacture of katsuobushi from bonito (Dimici and Wada 1994).

A. ruber was readily isolated from most food commodities we examined from Southeast Asia. It was particularly common in peanuts, kemiri nuts, cow peas and red kidney beans, mung beans, soybeans and maize kernels, with high levels in some soybeans. Levels in milled rice were low, however (Pitt et al. 1993, 1994, 1998).

Additional species *Aspergillus glaucus* (L.) Link [teleomorph, *Eurotium herbariorum* (Weber ex F.H. Wigg.) Link ex Nees, synonym *Aspergillus manginii* Raper & Thom (invalid name) resembles *A. ruber* in many features. It is distinguished by the following: (i) colonies on CYA and MEA at 25 °C do not exceed 10 mm diam in 7 days, and are sometimes absent; (ii) colonies on CY20S and G25N rarely exceed 35 mm diam in 7 days; (iii) cleistothecia on CY20S and G25N develop slowly, with ascospores usually taking more than 14 days to mature; and (iv) while ascospores are similar in appearance to those of *A. ruber*, they are larger, commonly 6–7(−8) µm long.

Taxonomic confusion exists because some authors have synonymised *Aspergillus glaucus* with *Aspergillus ruber* or *A. pseudoglaucus*. These three species are distinct, although closely related.

Aspergillus glaucus is a vigorous xerophile. When grown on media of pH 3.8 containing glucose/fructose as the controlling solute, ascospores of *A. glaucus* germinated at 0.74 a_w after 19 days, the shortest lag time of any of the common species. Conidia germinated at 0.75 a_w in 14 days (Pitt and Christian 1968). When heated in 5°Brix grape juice, *A. glaucus* ascospores showed a D_{70} of 2.5 min and a z value of 9.1 C°; in 65°Brix concentrate, the D_{70} was 5.2 min and z value 7.1 C° (Splittstoesser et al. 1989).

Aspergillus glaucus is able to grow in low oxygen concentrations, producing visible colonies at 25 °C in 0.5% O_2 at 0.82 a_w within 60 days, and at 0.78 a_w within 75 days. It also tolerated elevated CO_2 concentrations at reduced a_w: at 0.82 a_w producing visible colonies in 20 days in 35% CO_2 + 1% O_2 and 34 days in 50% CO_2 + 1% O_2. At 0.78 a_w, visible growth occurred in 35% CO_2 + 1% O_2 only after 95 days, and no growth occurred in 50% CO_2 + 1% O_2 (our unpublished data).

Although a far less common species than *Aspergillus ruber*, *Aspergillus glaucus* is nevertheless widespread. Spoilage by *A. glaucus* has occurred in French and Australian prunes, and European cheese (see Pitt and Hocking 1997). It has also been recorded from maize, meat products, rice, sardines, miso and spices (see Pitt and

Hocking 1997). We isolated it from samples of sorghum and peanuts from Indonesia, and from one of velvet beans (30% infection). Low levels were present in coriander from Indonesia and soybeans and mung beans from Thailand (Pitt et al. 1993, 1994, 1998). Identifiers: MycoBank MB161735; neotype DAOM 137960 (Malloch and Cain 1972; not IMI 211383, as stated by Samson et al. 2014); ex-type cultures CBS 516.65 = IMI 211383 = ATCC 15469 = NRRL 116 = FRR 116 (Hubka et al. 2013); ITS barcode EF652052; alternative markers *BenA* EF651887, *CaM* EF651989, *RPB2* EF691934 (Samson et al. 2014).

Additional species *Aspergillus halophilicus* C.M. Chr. et al. is distinguished by the production of white cleistothecia, and by inability to grow on CYA, MEA or CY20S, even after 14 days incubation. On MY50G, after 14 days, colonies are 15–20 mm diam, consisting almost entirely of cleistothecia surrounded by a weft of white mycelium (Hocking and Pitt 1988). *Aspergillus* heads, characteristic of those from this subgenus, are formed only on concentrated media, below 0.85 a_w, and bear pyriform, rough walled conidia 8–11 µm long. This species was originally described as a halophile (Christensen et al. 1959), but in fact is an extreme xerophile, failing to germinate above 0.94 a_w, with growth occurring optimally between 0.85 and 0.80 a_w. The minimum recorded germination occurred at 0.68 a_w after only 38 days incubation at 25 °C (Andrews and Pitt 1987). Germination occurred on saturated NaCl based media (0.75 a_w) in a similar time.

This is undoubtedly a rare species, but is of interest because it will not be isolated on any medium designed for the recovery of *Eurotium* species. It is also very difficult to recognise because its cleistothecia are white and it produces its anamorph only at very low a_w. In our laboratory we isolated it from long stored cardamom seeds on MY50G, the medium usually reserved for *Xeromyces bisporus* and xerophilic *Xerochrysium* species (Hocking and Pitt 1988). Identifiers: MycoBank MB326633; lectotype BPI 566153 (Hubka et al. 2013); ex-type cultures CBS 122.62 = IMI 211802 = ATCC 16401 = NRRL 2739 = FRR 2739 (Hubka et al. 2013); ITS barcode EF652088; alternative markers *BenA* EF651926, *CaM* EF652034, *RPB2* EF651982 (Samson et al. 2014).

References *Aspergillus ruber:* Raper and Fennell (1965), Pitt (1985), Klich (2002), Hubka et al. (2013), Chen et al. (2016); *Aspergillus glaucus:* Raper and Fennell (1965), under *A. manginii*; Pitt (1985), Klich (2002) under *E. herbariorum*, Hubka et al. (2013), Chen et al. (2016); *A. halophilicus*, Hocking and Pitt (1988), Klich (2002), Sklenář et al. (2017).

Aspergillus wentii Wehmer **Fig. 8.16**

Colonies on CYA 25–35 mm diam, plane or lightly wrinkled, moderately deep to deep, floccose, mycelium white to pale yellow; conidial production moderate, coloured greyish yellow; clear exudate sometimes produced; reverse pale. Colonies on MEA 22–30 mm diam, plane, relatively dense, velutinous; mycelium white; conidial production abundant, orange yellow; reverse pale. Colonies on G25N 30–45 mm diam, plane, deep, with areas of floccose white mycelium, heavily sporing on long stipes; conidia brownish yellow; reverse pale brown. No growth at 5 °C or 37 °C.

No sexual state known. Conidiophores borne from aerial hyphae, on CYA 500–1200 µm long, on G25N up to 5 mm long, with thin, smooth walls; vesicles nearly spherical, on CYA 25–35 µm diam, on G25N 70–100 µm diam, with metulae and phialides densely packed over the entire surface; metulae 10–18 µm long; phialides ampulliform, 7–12 µm long; conidia spherical to broadly ellipsoidal, 3.5–5.0 µm diam, smooth walled, borne in loose, radiate heads.

Distinctive features Golden brown colony colour, faster growth usually on G25N than CYA, and absence of growth at 37 °C are the principal characters which set *Aspergillus wentii* apart. Long stipes on G25N, production of metulae and phialides and large smooth walled conidia are also distinctive.

Identifiers MycoBank MB172623; neotype IMI 17295 (Samson and Gams 1985); ex-type cultures CBS 104.07 = IMI 17295 = ATCC 1023 = NRRL 375; ITS barcode EF652151; alternative markers *BenA* EF652106, *CaM* EF652131, *RPB2* EF652092 (Samson et al. 2014).

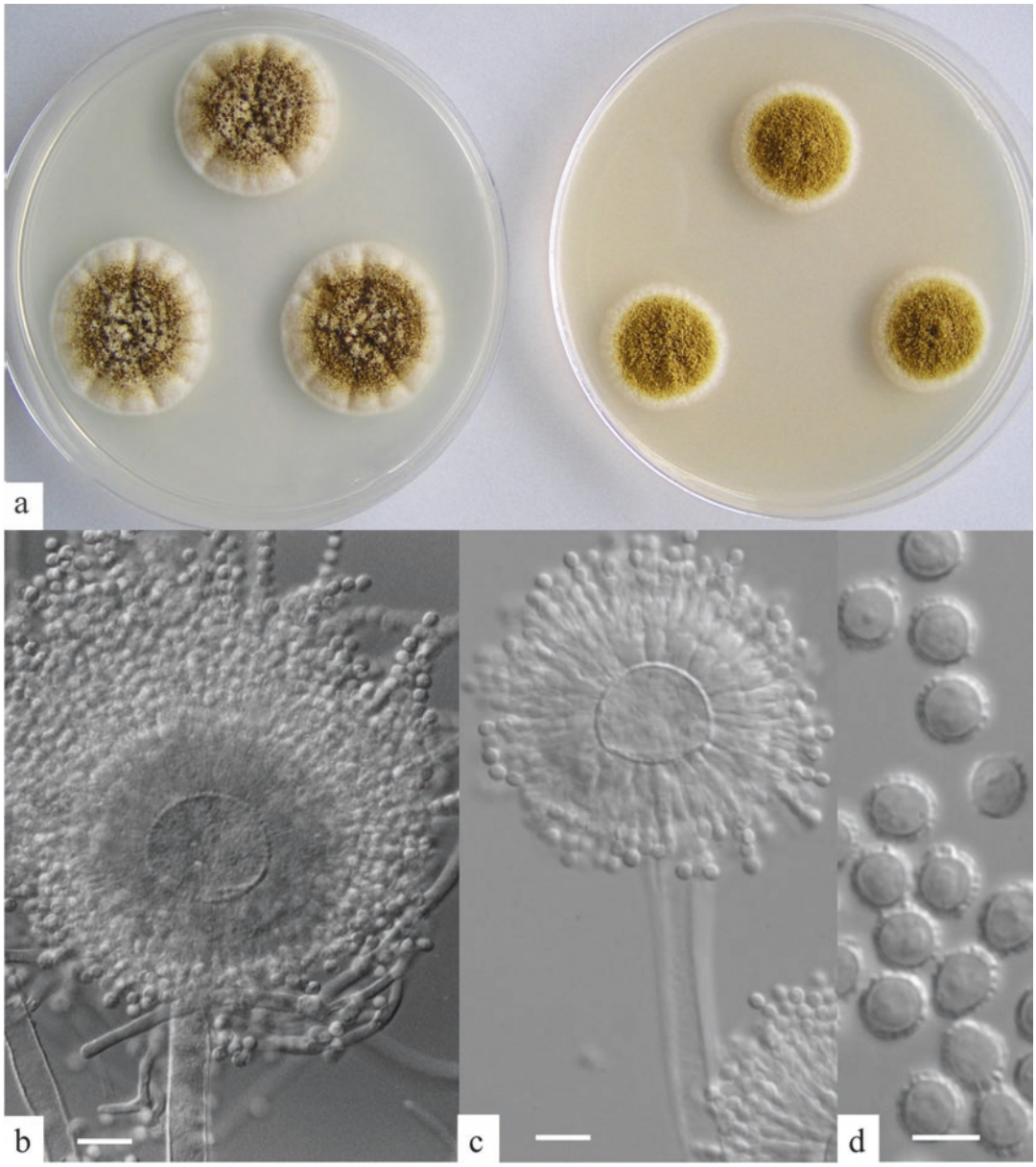

Fig. 8.16 *Aspergillus wentii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 20 µm; (**d**) conidia, bar = 5 µm

Physiology *Aspergillus wentii* is a xerophile which exhibits strong growth in both sugar and salt environments. The optimum a_w for growth is near 0.94 in both glucose/fructose and NaCl based media (Andrews and Pitt 1987). The minimum a_w for germination at 25 °C was reported to be 0.73 a_w in glucose/fructose, 0.75 in glycerol and 0.79 a_w in NaCl based media (Andrews and Pitt 1987). A slightly higher limit (0.76 a_w in glucose/fructose) was reported by Wheeler et al. (1988). The minimum a_w for germination in glucose/fructose was little affected by temperature: 0.77 at 20 and 30 °C, 0.79 at 34 °C and 0.83 at 37 °C (Wheeler et al. 1988).

Mycotoxins Emodin is the only mycotoxin of significance produced by *Aspergillus wentii* (Wells et al. 1975). Administered to day old cockerels, the oral LD_{50} was 3.7 mg/kg, indicating high toxicity (Wells et al. 1975). It has also been reported to be a mutagen (Masuda and Ueno 1984; Müller et al. 1996). There are no reports of toxicoses associated with *A. wentii*, perhaps reflecting the lack of reports of substantial growth, i.e. spoilage, in foods.

Ecology This species has only rarely been reported as a spoilage fungus, and is considered to be among the less common major *Aspergillus* species in foods. Nevertheless in our experience it is widely distributed. We isolated it in high percentages from many of the Southeast Asian commodities we examined. Twenty three per cent of nearly 500 peanut samples examined contained *Aspergillus wentii*, at up to 100% infection in infected samples and in 6% of all kernels examined. Thirty seven per cent of kemiri nut samples from Indonesia were infected, but the maximum infection level was only 35%, with 6% of nuts infected overall. A wide range of other commodities, maize from all three countries, cashews from Thailand, pepper and soybeans from the Philippines, and mung beans, pepper, coriander and cowpeas from Indonesia all showed 1–3% overall infection in the particles examined. Low levels of infection were seen in mung beans, soybeans, paddy rice, sorghum, red beans, cassava and copra from Thailand, and cashews, soybeans, paddy and milled rice from Indonesia (Pitt et al. 1993, 1994, and 1998a). It was also quite common in dried fish (Wheeler et al. 1986).

Other records of *Aspergillus wentii* from foods include peanuts, pistachios, pecans, walnuts, various cereal products, wheat, barley, bread, green coffee beans, dried beans, ripened raw hams and biltong (see Pitt and Hocking 1997) and also rice (Taligoola et al. 2004), maize and soybeans (Sepulveda and Piontelli 2005) and grapes (Pollastro et al. 2005).

References Raper and Fennell (1965); Domsch et al. (1980); Klich (2002).

8.4 *Aspergillus* Subgenus *Circumdati* Gams et al.

Aspergillus subgenus *Circumdati* includes *Aspergillus* species which produce large, robust, straight, single celled stipes, bearing more or less spherical vesicles, usually, though not exclusively, bearing both metulae and phialides around almost the entire circumference. Conidia are often strongly coloured and may be black, grey, yellow brown (ochre), white or green. Colonies usually lack any other colouration. This subgenus includes some of the best known and most frequently recognised fungal species on earth. They are among the most economically important species, on the positive side being very widely used for synthesis of chemicals, for biosynthetic transformations, and enzyme production. On the negative side, they are of importance in food spoilage, but more particularly for production of the aflatoxins, the most important mycotoxins, and ochratoxin A, also a very widespread mycotoxin.

Gams et al. (1985) included several sections within *Aspergillus* subgen. *Circumdati*, including sections *Flavi, Nigri, Wentii* and *Candidi* of relevance here. Based on molecular methodology, the latest taxonomy (Houbraken et al. 2020) has greatly expanded the numbers of sections and series within this subgenus, without materially affecting the overall picture. This subgenus includes a only few species with known sexual states, classified in the genera *Chaetosartorya* Subram., *Cristaspora* Fort & Guarro, *Fennellia* B.J. Wiley & E.G. Simmons, *Neopetromyces* Frisvad & Samson and *Petromyces* Malloch & Cain. These are all now considered to be synonyms of *Aspergillus.* They are unimportant, except that it is worth mentioning that all are distinct from the other major *Aspergillus* teleomorphs, *Neosartorya*, *Emericella* and the sexual state of *Aspergillus* subgenus *Aspergillus* [*Eurotium*].

Current taxonomies recognise about 140 species in *Aspergillus* subgen. *Circumdati* (Houbraken et al. 2020), distinguished by molecular differences. Perhaps 20 of these are well defined and usually readily distinguished.

Peripheral to these central, common species are a large number of variations on each theme, often with the status of species, but which are seldom encountered and are often little more than ephemeral variants. Placement of an unknown isolate in the central species is sufficient identification in all but the most detailed investigations.

Twelve species are described below. Together with the species included in *Neosartorya*, *Emericella* and the sexual state of *Aspergillus* subgenus *Aspergillus* [*Eurotium*] described earlier in this chapter, these 12 represent the vast majority of Aspergilli which will be isolated from foods. These species are all keyed out in the general key at the beginning of this chapter and are described in alphabetical order below.

Aspergillus aculeatus Iizuka **Fig. 8.17**

Aspergillus japonicus var. *aculeatus* (Iizuka) Al-Musallam

Colonies on CYA 65–70 mm diam or more, plane, velutinous, sometimes with a central floccose overlay; mycelium white; conidial heads red brown to black; pinkish sclerotia sometimes present; exudate and soluble pigment absent; reverse drab yellow to brown. Colonies on MEA 45–70 mm diam, plane, velutinous, mycelium white; conidial heads rather sparse, reddish brown or black; reverse uncoloured or yellowish. Colonies on G25N 15–20 mm diam, plane, sparse, velutinous, similar to those on CYA; reverse pale. No growth at 5 °C. At 37 °C, colonies 5–30 mm diam, consistently less than half of the diameter on CYA at 25 °C.

Conidiophores borne from subsurface or surface hyphae, stipes 500–2000 µm long, smooth-walled, sometimes brown; vesicles 25–60 µm diam, bearing phialides only, covering three quarters of the vesicle surface or more, 5–9 µm long; conidia ellipsoidal, sometimes subspheroidal, 4–5 µm long, spinose, borne in radiate heads, in age splitting into columns.

Distinctive features Red brown to black colonies indicate a close relationship with *Aspergillus niger*; however, vesicles of *A. aculeatus* bear phialides only, unlike those of *A. niger*. *A. japonicus* also produces phialides only, but *A. aculeatus* is distinguished from it by the production of larger vesicles and ellipsoidal conidia.

Taxonomy *A. aculeatus* and *A. japonicus* are very closely related species, and *A. aculeatus* has sometimes been considered to be a variety of *A. japonicus* (Al-Musallam 1980). These two species have morphological differences (Raper and Fennell 1965), and differences in secondary metabolites and RFLP profiles that correlate with each other (Pařenicová et al. 2001). A more recent study has indicated that these two species are well separated molecularly (Varga et al. 2011b).

Morphological identifications based on the work of Raper and Fennell (1965) indicate general agreement that *A. aculeatus* is the species commonly found in foods (Pitt and Hocking 1997; Leong et al. 2004; Bau et al. 2005), so *A. aculeatus* is given priority here.

This species does not appear to have been typified formally as both Samson and Gams et al. (1985) and Pitt and Samson (1993) considered it a synonym of *A. japonicus* and neither Samson et al. (2014) nor Houbraken et al. (2020) have considered this issue. IMI 211388 is formally designated as neotype of *A. aculeatus* here. This isolate was derived from WB 5094, considered to be typical of the species by Raper and Fennell (1965).

Identifiers MycoBank MB292381; neotype IMI 211388 (designated here); ex-type cultures CBS 172.66 = IMI 211388 = ATCC 16872; ITS barcode EF661221; alternative markers *BenA* HE577806, *CaM* EF661148, *RPB2* EF661046 (Samson et al. 2014).

Physiology At 0.97 a_w *A. aculeatus* grew at 20, 30 and 40 °C. At a lower water activity (0.92), it grew better at 30 °C than at 20 °C and no growth was recorded at water activity values below 0.92 (0.87 or 0.82 a_w) (Valero et al. 2007a). It has a high pectinolytic and cellulolytic enzyme activity (Adisa 1989) and a fructosyltransferase with thermal and pH stability has recently been characterised (Ghazi et al. 2007).

Mycotoxins This species produces secalonic acid D (Frisvad et al. 2006a) and some other minor compounds (Frisvad and Samson 1991). Secalonic acid D has significant animal toxicity

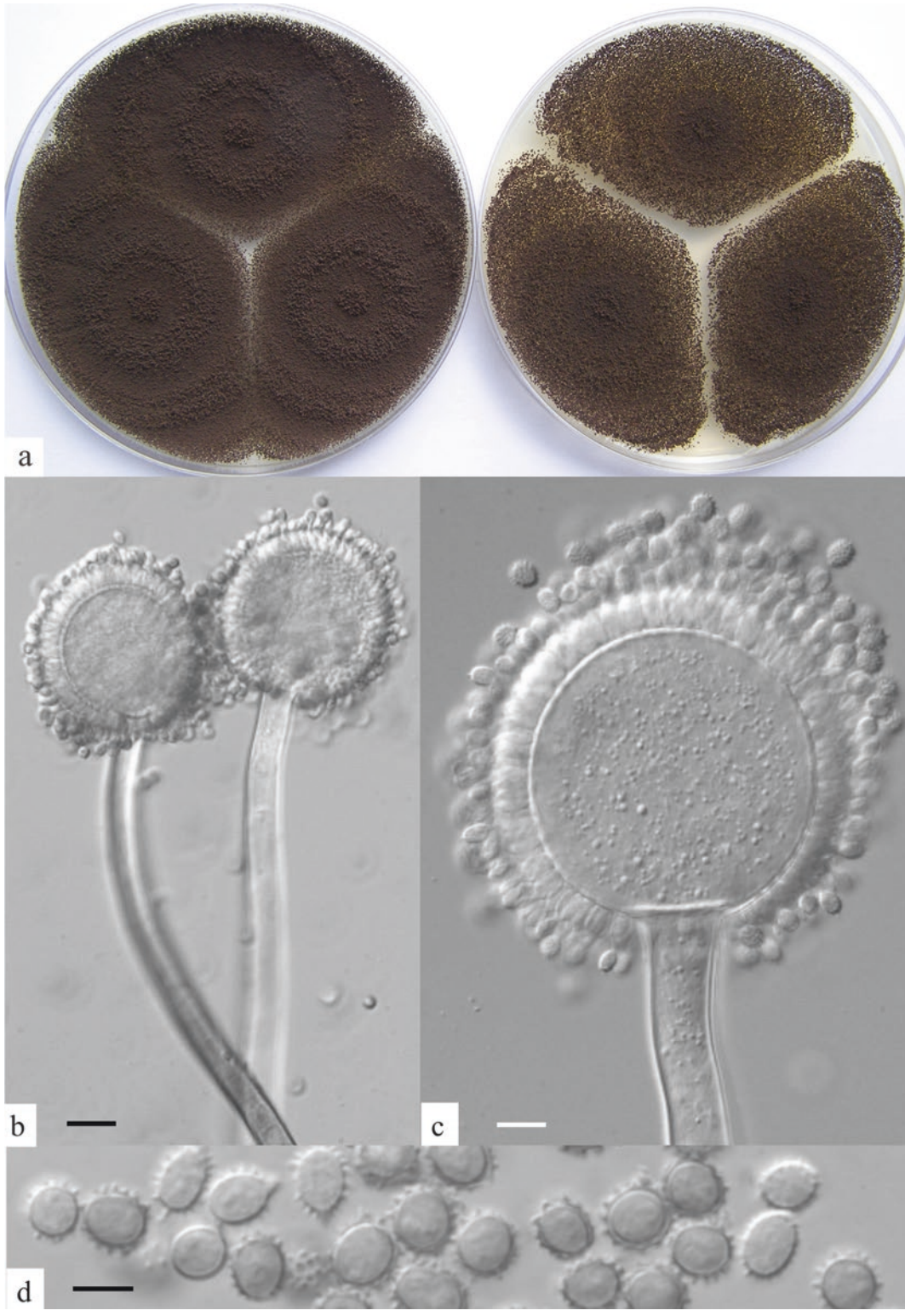

Fig. 8.17 *Aspergillus aculeatus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) heads, bar = 20 µm; (**c**) head showing the presence of phialides only, bar = 10 µm; (**d**) conidia, bar = 5 µm

(See Chap. 13) but a role in human or animal disease has not been shown from this species.

Ecology *Aspergillus aculeatus* causes a post-harvest dry rot of tomatoes (Fajola 1979). It is commonly associated with grapes (Leong et al. 2004; Bau et al. 2005; Martínez-Culebras and Ramón 2007; Valero et al. 2007a) and is one of the causes of bunch rot (Jarvis and Traquair 1984). It has been isolated from maize (Nyongesa et al. 2015). We isolated it in low numbers from Philippine and Indonesian peanuts, and soybeans from the Philippines (Pitt et al. 1998 and unpublished).

Additional species *Aspergillus japonicus* Saito closely resembles *A. aculeatus* (see Taxonomy above). *A. japonicus* sometimes produces larger colonies on CYA at 37 °C, up to 50 mm diam. Vesicles are smaller, usually less than 30 µm diam, and conidia are spherical. It does not produce secalonic acid D (Frisvad and Samson 1991). The two species would be expected to have a similar physiology. *Aspergillus japonicus* has been isolated from Nigerian maize (Aja-Nwachukwu and Emejuaiwe 1994), but as noted above, this species does not appear to be as common in foods as *A. aculeatus*. Identifiers: MycoBank MB160656; lectotype CBS 114.51 (Samson and Gams 1985); ex-type culture CBS 114.51; ITS barcode AJ279985; alternative markers *BenA* HE577804, *CaM* FN594551 (Samson et al. 2014), *RPB2* MN969079 (Houbraken et al. 2020).

References Raper and Fennell (1965); Klich (2002); Varga et al. (2011b).

Aspergillus candidus Link Fig. 8.18

Colonies on CYA 15–20 mm diam, plane, low to moderately deep, dense, surface texture granular to floccose; mycelium white; conidial heads densely packed, persistently pure white to off-white; sometimes small amounts of clear exudate produced; reverse pale or yellow orange. Colonies on MEA 10–25 mm diam, similar to those on CYA except reverse dull brown. Colonies on G25N 10–16 mm diam, similar to those on CYA but conidial production often sparse and reverse pale or sometimes yellow. No growth at 5 °C or 37 °C.

Conidiophores borne from surface or aerial hyphae, stipes usually 500–1000 µm long, with colourless, smooth to quite roughened walls; vesicles varying with isolate, 30–40 µm diam, bearing metulae and phialides over the entire surface; metulae variable, mostly 15–20 µm long; phialides 5–9 µm long; conidia mostly spherical, 2.5–3.5 µm diam, with smooth walls, borne in radiate heads.

Distinctive features *Aspergillus candidus* and the closely related *A. tritici* are the only species of *Aspergillus* with persistently white conidia. *A. niveus* is similar but usually produces some yellow to brown colouration. *A. candidus* differs from *A. niveus* by producing vesicles fertile over the entire area and metulae usually more than 10 µm long.

Taxonomy For a long time *Aspergillus candidus* was regarded as a species with rather variable characteristics, the only species in *Aspergillus* subgenus *Circumdati* section *Candidi* (Gams and Samson 1985). In a recent taxonomic revision based on molecular methods, Varga et al. (2007b) accepted four species in this section. Two are of importance in foods: *A. candidus* and *A. tritici*. *A. tritici* has much smaller vesicles than *A. candidus* and grows at 37 °C. Much of the literature on occurrence of these species in foods predates that paper, so it is unclear whether either species has a more specialist role.

Identifiers MycoBank MB204868; neotype CBS 567.65 (Samson and Gams 1985); ex-neotype cultures CBS 567.65 = IMI 230752 = ATCC 16871 = NRRL 312; ITS barcode EF669592; alternative markers *BenA* EU014089, *CaM* EF669550, *RPB2* EF660634 (Houbraken et al. 2020). Note: Houbraken et al. (2020) incorrectly selected a new neotype. As the existing neotype has not been shown to be different taxonomically from the original description (ICN, Articles 9.18, 9.19; Turland et al. 2018), it cannot be changed, despite having been based on a different NRRL strain than Samson and Gams et al. (1985) had believed.

Physiology As noted by Pitt and Hocking (1997), growth rates reported for this species vary considerably, with optimum temperatures ranging from of 20–24 °C to 45–50 °C, minima from 3–4 °C to 11–13 °C and maxima of

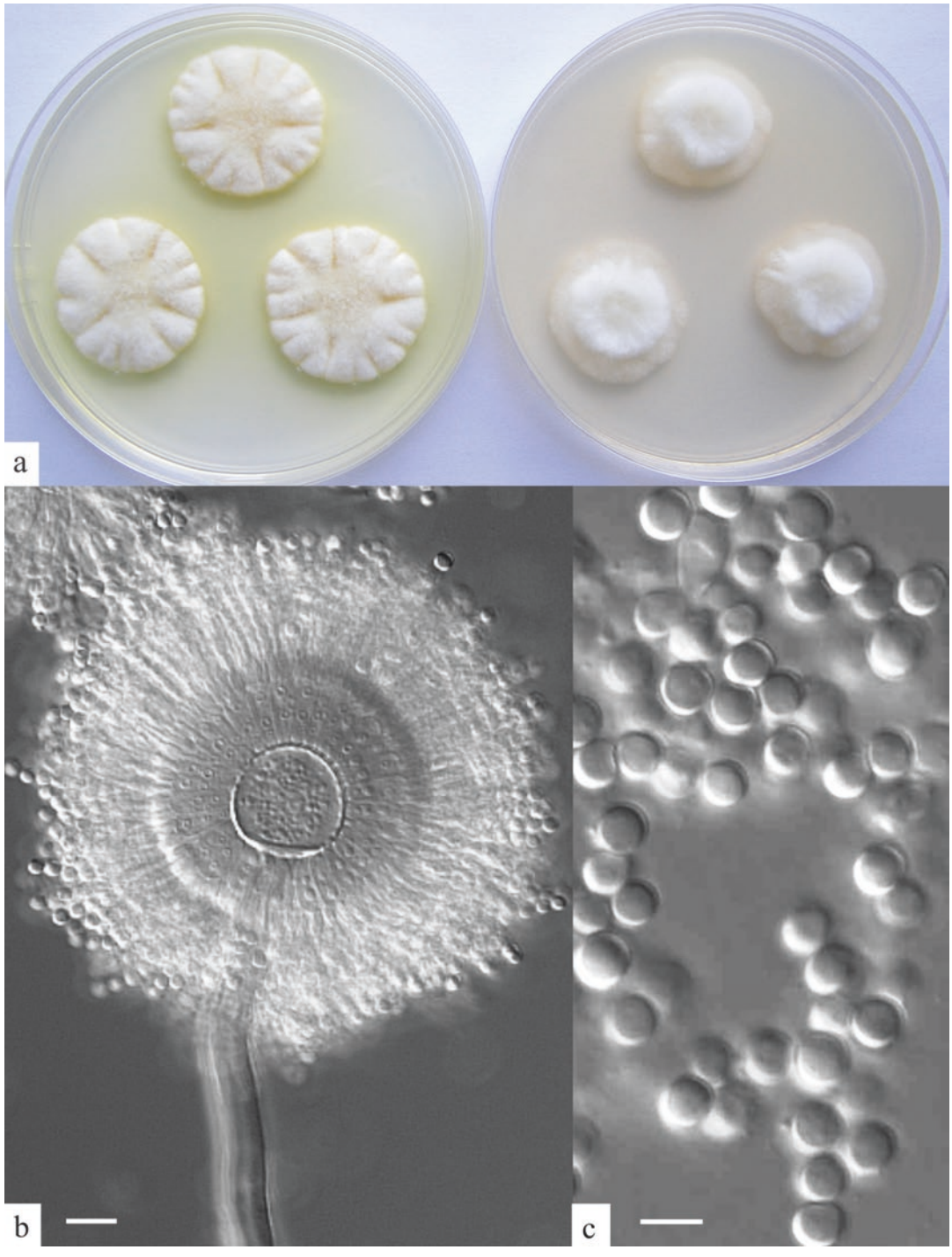

Fig. 8.18 *Aspergillus candidus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) head, bar = 10 µm; (**c**) conidia, bar = 5 µm

41–42 °C to 50–55 °C. Resolution of this species into two species had solved this issue: without definitive data, it can reasonably be assumed that the lower figures are typical of *A. candidus*, while the higher figures are likely to be representative of *A. tritici* isolates.

The minimum a_w for growth of *Aspergillus candidus* was reported as 0.75, by both Galloway (1935) and Ayerst (1969), after 14 days incubation at 25 °C and *ca* 30 days at 30 °C, respectively. The optimum a_w for growth was greater than 0.98 (Ayerst 1969). It is likely that *A. tritici* is also a strong xerophile, as it was originally isolated from stored wheat. Conidia of *A. candidus* showed 100% survival after heating for 10 min at 50 °C, but no survival after 10 min at 60 °C (Pitt and Christian 1970).

This species, or *A. tritici*, is more tolerant of low O_2 than most *Aspergillus* species, being capable of growth in 0.45% O_2. More than 15% CO_2 was required to halve the growth rate in air (Magan and Lacey 1984b). Tolerance to propionic acid used as a grain preservative is also high (Müller et al. 1981).

Mycotoxins *Aspergillus candidus* and *A. tritici* produce only a small range of secondary metabolites (Varga et al. 2007b) and none has known toxicity.

Ecology *Aspergillus candidus* and *A. tritici* are widespread in foods, but distribution between the two species is unclear. Although *A. tritici* was published by Mehrotra and Besu (1976), the name was little used until it was recognised by Varga et al. (2007b). Strong xerophiles, these species are of common occurrence in stored cereals, including wheat – freshly harvested, during drying and in storage – flour and refrigerated dough, bran, bread, stored and mouldy maize (see Pitt and Hocking 1997; Lugauskas et al. 2006), milled rice (see Pitt and Hocking 1997; Taligoola et al. 2004), polished rice (Park et al. 2005a, b) and yellow rice (Phillips et al. 1988), barley (see Pitt and Hocking 1997; Lugauskas et al. 2006) and cereal flakes (Weidenbörner and Kunz 1994).

A. candidus is also a major species infecting nuts, including peanuts, hazelnuts, walnuts and pecans. It occurs frequently on salamis and other processed meats. Other sources include dried fish, cheesecake, soybeans, faba beans, sorghum, rapeseed and sunflower seed, mango powder, and health foods (see Pitt and Hocking 1997). It is unclear whether some of these infections are due to *A. tritici*, but is seems likely, as many have come from the tropics.

Aspergillus candidus was present in many samples of commodities we examined from Southeast Asia, but usually at low levels (Pitt et al. 1993, 1994). The exception was Indonesian milled rice, where *A. candidus* was present in high levels (occasionally up to 100% infection) in 56% of samples (Pitt et al. 1998). On the positive side, *Aspergillus candidus* has been recommended as a starter culture of low toxicity for processed meat manufacture (Grazia et al. 1986; Spotti et al. 1994).

A. candidus has been reported from a wide range of human infections, including invasive aspergillosis and ear infections (De Hoog et al. 2000). However, as *A. candidus* does not grow at 37 °C, it is reasonable to assume that reports of pathogenicity actually involve *A. tritici.*

Additional species *A. tritici* B.S. Mehrotra & M. Basu closely resembles *A. candidus*, however it produces colonies usually 20–25 mm diam at 37 °C, typically centrally umbonate and radially sulcate, velutinous or centrally floccose. Further information is provided above. Identifiers: MycoBank MB309248; lectotype CBS H-24289; ex-epitype culture CBS 266.81; ITS barcode MN431381; alternative markers *BenA* MN969368, *CaM* MN969233, *RPB2* MN969098 (Houbraken et al. 2020).

References Klich (2002); Varga et al. (2007b).

Aspergillus carbonarius (Bainier) Thom

Fig. 8.19

Colonies on CYA 60 mm or more diam, consisting of surface or subsurface white mycelium, surmounted by a layer of conidiophores; near margins individual conidial heads visible with the naked eye, more or less covering central areas, jet black; reverse colourless to pale yellow. Colonies on MEA 60 mm or more diam, similar to on CYA but less dense; mycelium subsurface and inconspicuous; conidial heads usually individually visible over the entire colony, jet black; reverse uncoloured. Colonies on G25N 8–25 mm

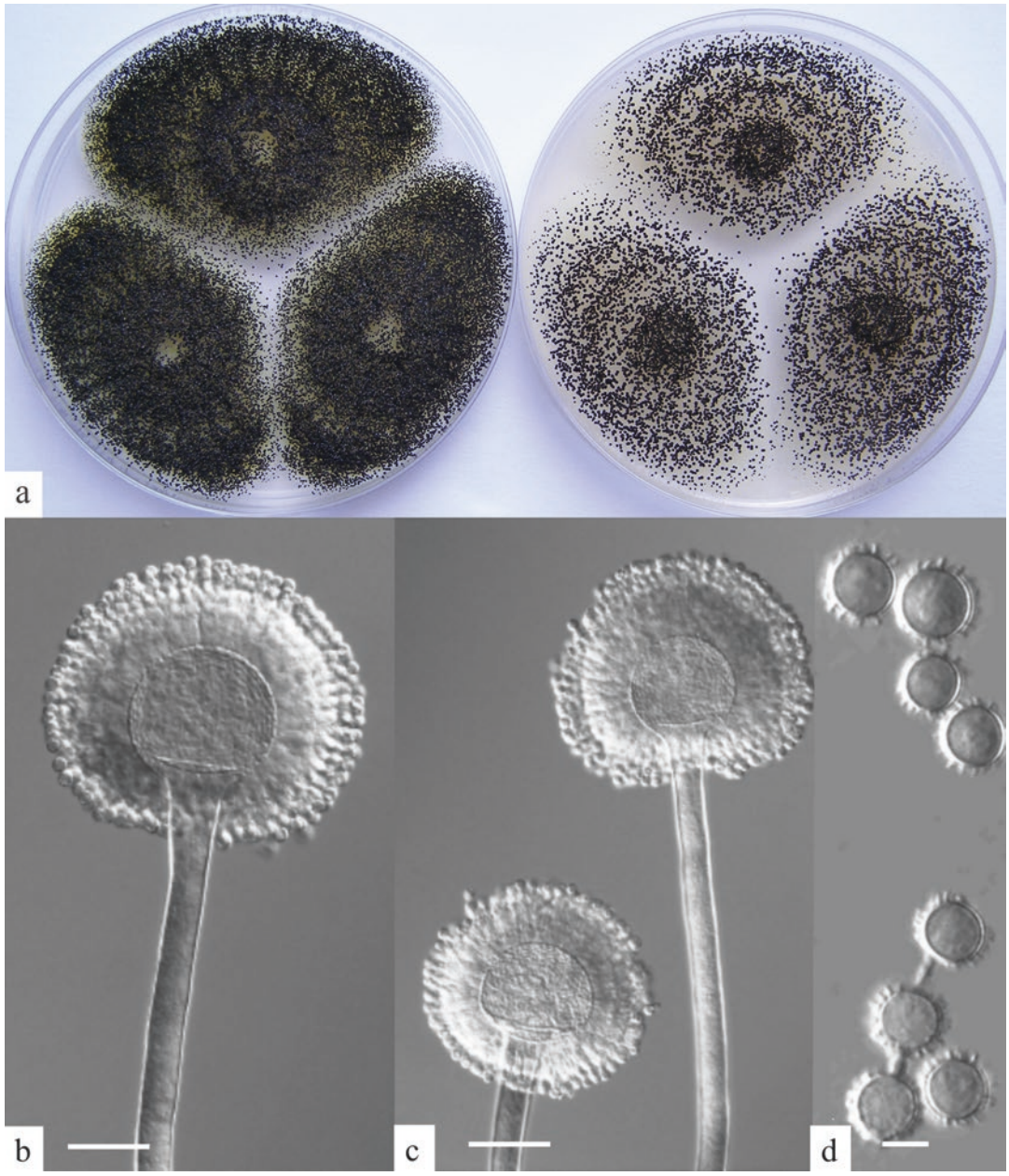

Fig. 8.19 *Aspergillus carbonarius* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 40 µm; (**c**) conidia, bar = 5 µm

diam, of sparse white surface or subsurface mycelium surmounted by sparse black conidial heads; reverse uncoloured. No germination at 5 °C. At 37 °C, colonies 15–35 mm diam, of dense white to yellow mycelium; margins irregular; sporulation sparse, with visible individual heads, to very dense, jet black; reverse pale, pale brown, or grey to black.

Conidiophores borne from surface or subsurface hyphae, 2.0–3.0 mm long, with heavy, smooth walls, uncoloured or grey; vesicles spherical or oblate, usually 60–90 µm diam, bearing closely packed metulae and phialides over the entire surface; metulae 12–18 µm long; phialides 9–12(−15) µm long; conidia spherical or near, 6–7(−8) µm diam, black, with walls conspicuously roughened or sometimes spiky, borne in large, radiate heads.

Distinctive features *Aspergillus carbonarius* is distinguished from related black *Aspergillus* species by producing conidia more than 6 µm in diameter. Colonies are jet black, and all heads produce metulae.

Taxonomy Molecular taxonomy has shown that *Aspergillus carbonarius* is a distinct species in *Aspergillus* subgenus *Circumdati* section *Nigri* (Varga et al. 2011b).

Identifiers MycoBank MB100545; neotype CBS 556.65 (Al-Musallam 1980); ex-type cultures CBS 111.26 = IMI 16136 = ATCC 1025 = NRRL 369 = FRR 369; ITS barcode EF661204; alternative markers *BenA* EF661099, *CaM* EF661167, *RPB2* EF661068 (Samson et al. 2014).

Physiology *Aspergillus carbonarius* can grow at 10 °C but not 7–8 °C, has an optimum near 30 °C and a maximum near 41 °C (Bellí et al. 2004; Mitchell et al. 2004; Leong et al. 2006b; Marín et al. 2006; Romero et al. 2007). The optimum a_w for growth is 0.96–0.98 (Mitchell et al. 2004; Leong et al. 2006b), with a minimum near 0.85 a_w at 25–30 °C (Romero et al. 2007). No growth was observed at 0.82 a_w in a glycerol based medium over the temperature range 15–35 °C (Romero et al. 2007). *A. carbonarius* grows over a wide pH range (2–10) (Esteban et al. 2005). Low levels (100 mg/L) of sodium metabisulphite, which is widely used in the wine industry to treat grape juice before fermentation, stimulated growth of *A. carbonarius,* and high levels (720–1000 mg/L) were required to inhibit growth by 90% (Pateraki et al. 2007).

A. carbonarius is very tolerant of elevated levels of CO_2. Compared with growth in air, 25% CO_2 + 1% O_2 stimulated growth, and an atmosphere of 50% CO_2 + 1% O_2 resulted in growth rates only slightly slower than the controls in air on grape juice medium at 0.985–0.93 a_w (Pateraki et al. 2007). Conidia of *A. carbonarius* were more resistant to ultraviolet radiation than those of *A. niger*, but more sensitive than those of *Alternaria alternata* (Valero et al. 2007b). *A. carbonarius* conidia dried onto filter papers survived well at low a_w (0.4 a_w) and low temperature (1 °C) with little loss of viability after 618 d storage (Leong et al. 2006e).

Mycotoxins Ochratoxin A production by *A. carbonarius was* first reported by Horie (1995), Téren et al. (1996) and Wicklow et al. (1996). Since then, *A. carbonarius* has been recognised as the primary source of ochratoxin A contamination in grapes and grape products throughout the world (Battilani and Pietri 2002; Cabañes et al. 2002; Sage et al. 2002; Abarca et al. 2003) and many subsequent papers. Frisvad et al. (2011) reported that 30/30 (100%) of *A. carbonarius* isolates studied were able to produce ochratoxin A. To determine whether an *A. carbonarius* strain can produce ochratoxin A, grow it on coconut cream agar (Dyer and McCammon 1994) at 25 °C.

Where grapes are further processed to make dried vine fruits, the black Aspergilli continue to grow, as in most producing countries grapes are dried in the sun without preservatives. Dried grapes (raisins, sultanas) can therefore readily contain unacceptable levels of ochratoxin A (Lombaert et al. 2004; Iamanaka et al. 2005; Jørgensen 2005; Meyvaci et al. 2005). *A. carbonarius* is also a potential source of ochratoxin A in coffee. Infection occurs postharvest, and ochratoxin A may be formed if beans are not dried efficiently (Taniwaki et al. 2003).

A. carbonarius produces ochratoxin A optimally at cooler temperatures: 15 °C and 0.95–0.97 a_w or 20 °C and 0.98–0.99 a_w. Little or no ochratoxin A is formed at temperatures above 35 °C. The lower a_w limit for ochratoxin A pro-

duction is near 0.92 (Mitchell et al. 2004; Esteban et al. 2004; Bellí et al. 2005; Marín et al. 2006; Leong et al. 2006b). Ochratoxin A is produced over the pH range 2–10 at 15 and 30 °C on CYA and YES media (Esteban et al. 2005).

Sodium metabisulphite inhibits ochratoxin A production, but at high a_w (0.985), at least 650–700 mg/l is required for 90% inhibition (Pateraki et al. 2007). Modified atmosphere storage (25% or 50% CO_2) has little effect on ochratoxin formation, with 25% CO_2 resulting in stimulation of toxin production (Pateraki et al. 2007). Application of fungicides to grapes can reduce fungal growth and ochratoxin production, but some fungicides appear to stimulate ochratoxin production (Bellí et al. 2006).

Ochratoxin A is mostly produced in wine within the first 48 hr. of fermentation, after which both viability of *A. carbonarius* and ochratoxin A formation declined (Jiang et al. 2014). The ochratoxin A is largely removed during the wine-making process as it is bound to solid fractions such as wine lees and sediment. Use of some fining agents can also reduce ochratoxin levels in wine. Red wines retain slightly more ochratoxin A than white wines but, overall, the carryover from grapes into finished wine is between 1% and 8% (Leong et al. 2006c, 2006d; Fernandes et al. 2007).

Isolates of *A. carbonarius* or related species can be screened for production of ochratoxin A by growing them on coconut cream agar (Dyer and McCammon 1994; Heenan et al. 1998). These authors incubated coconut cream agar plates at 30 °C, but 25 °C is more appropriate for this species.

Ecology The primary food related habitat for *Aspergillus carbonarius* is grapes and the vineyard environment. *A. carbonarius* was first reported as an agent of Aspergillus bunch rot in India by Gupta (1956), well before the problem of ochratoxin A contamination of grape products was recognised. Since the late 1990s, there has been a concerted effort to understand the ecology of *A. carbonarius* and ochratoxin A formation in both wine grapes and dried grapes. *A. carbonarius* has been reported from grapes in Australia, South America and countries around the Mediterranean rim including Italy, Spain, Portugal, France, Greece, Turkey, Tunisia, Morocco, Lebanon and Israel (see Battilani et al. 2006 and references therein; El-Khoury et al. 2006). *A. carbonarius* can be isolated from vineyard soil and vine remnants (dried berries, bunch stems, dead canes, etc.). It occurs on grapes from berry set to harvest, but populations increase from veraison onwards. *A carbonarius* is a saprophyte rather than a pathogen, so predisposing factors such as insect damage, infections with powdery mildew or *Botrytis*, or berry splitting due to rain or storm damage are needed to initiate Aspergillus bunch rot, which generally occurs near harvest when sugar content of berries is highest (Leong et al. 2006a). *A. carbonarius* occurs on grapes during drying, and can develop to high numbers on fruit that has been rain affected before harvest (Leong et al. 2004, 2006a). *A. carbonarius* has been reported from dried vine fruits from many countries including Spain (Abarca et al. 2003), Greece (Tjamos et al. 2004), Australia (Leong et al. 2004), Brazil (Iamanaka et al. 2005) and Argentina (Romero et al. 2005).

Populations of *A. carbonarius* in vineyards can be reduced by vineyard management practices such as irrigation, pruning to improve air flow thorugh vines, use of cover crops between rows and appropriate fungicide applications (Pollastro et al. 2005; Bellí et al. 2006; Leong et al. 2006a). Biocontrol of black Aspergilli on grapes using epiphytic yeasts has been proposed (Bleve et al. 2006).

A. carbonarius has been reported from coffee beans in Brazil (Taniwaki et al. 2003; Magnani et al. 2005), Ivory Coast (Kouadio et al. 2006), Uganda (Ngabirano et al. 2001), Thailand (Joosten et al. 2001) and Vietnam (Leong et al. 2007). Other reported sources of *A. carbonarius* are figs (Doster et al. 1996), peanuts and maize (Magnoli et al. 2006a, b), bee pollen (Gonzalez et al. 2005), paprika (Almela et al. 2007) red bay berries (Li et al. 2002) and fermenting cocoa beans (Mounjouenpou et al. 2008). In our laboratory we have isolated *A. carbonarius* from dried vine fruits, walnuts and margarine. *A. carbonarius* isolated from Greek tannery wastes reduced tannins by 78%, significantly reducing the BOD of the effluent (Marakis 1995).

References Raper and Fennell (1965); Klich (2002); Varga et al. (2011b).

Aspergillus flavipes (Bainier & Sartory) Thom & Church Fig. 8.20

Colonies on CYA 15–30 mm diam, deep but velutinous, lightly radially sulcate, occasionally floccose centrally; mycelium white to yellow; conidial heads borne uniformly over the colony surface, pale greyish orange; clear exudate sometimes present; reverse pale, pale yellow, or light to yellowish brown. Colonies on MEA 20–35 mm diam, plane, velutinous or sometimes slightly granular; mycelium white, inconspicuous; conidial production light, pale orange brown; reverse greyish yellow to golden brown. Colonies on G25N 8–12 mm diam, plane, dense to floccose, white to greyish orange; reverse brown. No growth at 5 °C. At 37 °C, colonies 5–20 mm diam.

Conidiophores borne from surface or aerial hyphae, stipes 150–600 µm long, uncoloured to pale brown, walls smooth to slightly roughened,

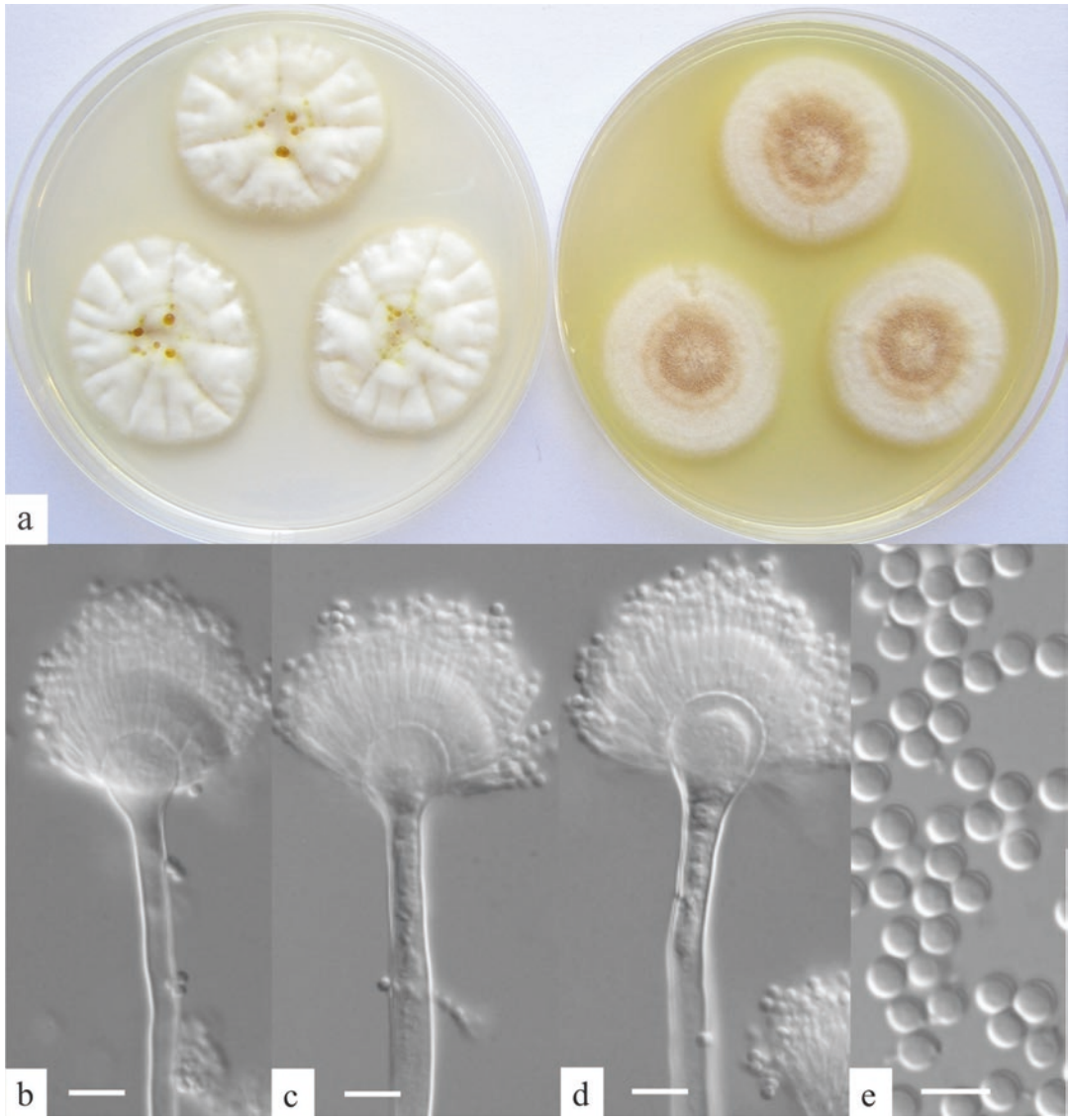

Fig. 8.20 *Aspergillus flavipes* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b–d**) heads, bars = 10 µm; (**e**) conidia, bar = 5 µm

broadening gradually into spathulate to subspheroidal vesicles mostly 10–20 µm diam, bearing metulae and phialides over the upper half to two thirds; metulae and phialides 5–7 µm long; conidia spherical, 2.0–3.0 µm diam, smooth walled, borne in radiate to loosely columnar heads.

Distinctive features This species is distinguished by pale greyish orange colonies with conidiophores bearing both metulae and phialides and small, smooth walled, spherical conidia.

Taxonomy The taxonomy of *Aspergillus flavipes* and related species was revised by Hubka et al. (2015). Using molecular methods, they showed that the teleomorph described by Wiley and Fennell (1973), transferred to *Fennellia* by Wiley and Simmons (1973), and previously associated with this species, is actually the sexual stage of the related species *A. neoflavipes* Hubka et al. As Hubka et al. (2015) regarded *A. flavipes* as a rare species, some doubt exists as to whether the species described here is correctly classified as *A. flavipes*. The most similar species accepted by Hubka et al. (2015) is *A. neoflavipes*, which differs from *A. flavipes* by producing the sexual state, never seen in the isolates from food on which this species description is based. Moreover, only one of the 50 isolates examined in the Hubka et al. (2015) study was associated with food, so it cannot be concluded that *A. flavipes* is rare in foods. It is considered here that *A. flavipes* is likely to be the correct name for this species isolated from foods.

Identifiers MycoBank MB265045; epitype IMI 171885; ex-type cultures IMI 171885 = ATCC 24487 = NRRL 302 = FRR302; ITS barcode EF669591; alternative markers *BenA* EU014085, *CaM* EF669549, *RPB2* EF669633 (Hubka et al. 2015).

Physiology This species grows from 6–7 °C to 38–40 °C, with an optimum near 26–28 °C (Domsch et al. 1980). It grows at 37 °C but not 40 °C (Hubka et al. 2015). It is capable of growth in at least 25% (w/v) NaCl, corresponding to 0.82 a_w (Tresner and Hayes 1971).

Mycotoxins This species produces a variety of secondary metabolites (Frisvad and Samson 1991) including lovastatin (Valera et al. 2005), but no known mycotoxins.

Ecology *Aspergillus flavipes* is common in tropical soils (Domsch et al. 1980; Hubka et al. 2015), but uncommon in foods. It has been reported to cause spoilage of a variety of tropical fruit and cassava. It has been reported from wheat from France and we isolated it at low levels from cassava, peanuts and coriander in Southeast Asia (Pitt and Hocking 1997).

References Raper and Fennell (1965); Klich (2002); Hubka et al. (2015).

Aspergillus flavus Link **Fig. 8.21**

Petromyces flavus Horn et al. (teleomorph)

Colonies on CYA 60–70 mm diam, plane, sparse to moderately dense, velutinous in marginal areas at least, often floccose centrally, sometimes deeply so; mycelium only conspicuous in floccose areas, white; conidial heads usually borne uniformly over the whole colony, but sparse or absent in areas of floccose growth or sclerotial production, characteristically greyish green, yellow green or olive yellow, but sometimes pure yellow, then becoming greenish in age; sclerotia produced by about 50% of isolates, at first white, becoming deep reddish brown, density varying from inconspicuous to dominating colony appearance and then almost entirely suppressing conidial production; exudate sometimes produced, clear, or reddish brown near sclerotia; reverse uncoloured or brown to reddish brown beneath sclerotia. Colonies on MEA 50–70 mm diam, similar to those on CYA although usually less dense. Colonies on G25N 25–40 mm diam, similar to those on CYA or more deeply floccose and with little conidial production, reverse pale to orange or salmon. No growth at 5 °C. At 37 °C, colonies usually 55–65 mm diam, similar to those on CYA at 25 °C, but more velutinous, with olive conidia, and sometimes with more abundant sclerotia.

Sclerotia produced by some isolates, at first white, rapidly becoming hard and reddish brown to black, spherical, usually 400–800 µm diam, but less than 400 µm in 'S' strains (Cotty 1989). Species heterothallic: mature sexual state formed after mating of opposite mating type strains and prolonged incubation, ascospores *fide* Horn et al. (2009a) "8.0–12.5 × 7.5–12.0 µm, finely tuberculate with a thin equatorial ridge, hyaline to pale

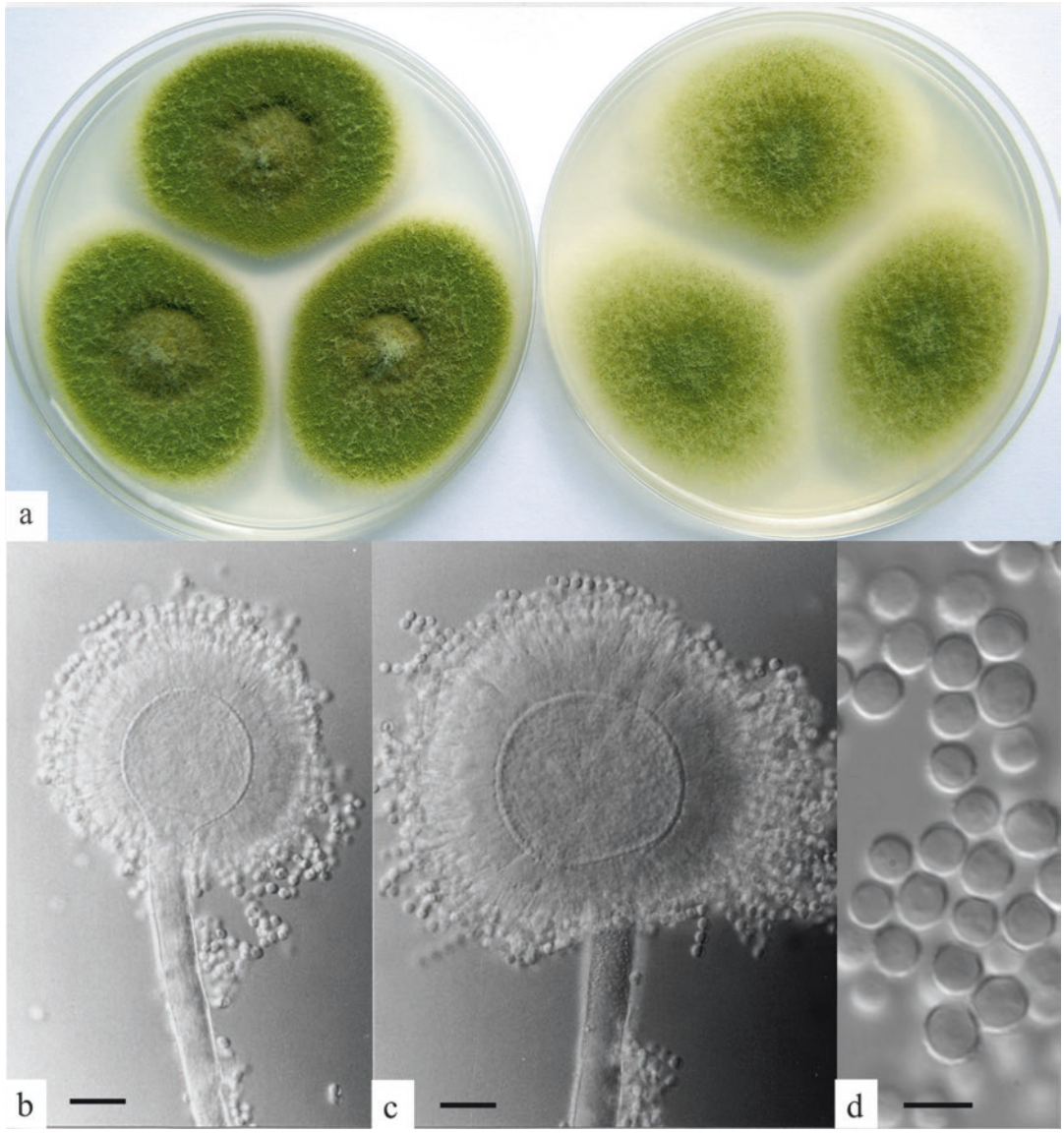

Fig. 8.21 *Aspergillus flavus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 20 µm; (**d**) conidia, bar = 5 µm

brown". Conidiophores borne from subsurface or surface hyphae, stipes 400 µm to 1 mm or more long, colourless or pale brown, rough walled; vesicles spherical, 20–45 µm diam, fertile over three quarters of the surface, typically bearing both metulae and phialides, but in some isolates a proportion or even a majority of heads with phialides alone; metulae and phialides of similar size, 7–10 µm long; conidia spherical to subspheroidal, usually 3.5–5.0 µm diam, with relatively thin walls, finely roughened or, rarely, smooth.

Distinctive features *Aspergillus flavus* and *A. parasiticus* are distinguished by their rapid growth at both 25 °C and 37 °C, and their bright yellow green (or less commonly yellow) conidial colours. The definitive difference between the two species is that *A. flavus* produces conidia

which are rather variable in shape and size, have relatively thin walls, and range from smooth to moderately rough, the majority being finely rough. In contrast, conidia of *A. parasiticus* are spherical and have relatively thick, rough walls. In addition, vesicles of *A. flavus* are larger, up to 50 µm in diameter, and usually bear metulae, while vesicles of *A. parasiticus* rarely exceed 30 µm in diameter and metulae are uncommon.

Taxonomy Klich and Pitt (1985, 1988) showed that metula production, the principal character used by Raper and Fennell (1965) for distinguishing *Aspergillus flavus* from *A. parasiticus*, was not a satisfactory criterion. Some *A. flavus* isolates produce very few metulae. Careful study showed several differences between the two species, the most useful of which are mentioned above.

Recently, a great deal of attention has been given to species that produce aflatoxins, most of which are closely related to *A. flavus*. *Aspergillus* subgenus *Circumdati* section *Flavi*, which is built around *A. flavus,* was reviewed and revised by Varga et al. (2011a) and most recently by Frisvad et al. (2019). This section now includes 33 species. Frisvad et al. (2019) provide a very comprehensive account of the taxonomy of this section as well as details of mycotoxin and other major secondary metabolite production. Eight new species are also described. It is recommended that Frisvad et al. (2019) be consulted for further information.

Identifiers MycoBank MB209842; neotype IMI 124930 (Kozakiewicz 1982); ex-type cultures CBS 569.65 = IMI 124930 = ATCC 16883 = NRRL 1957; ITS barcode AF027863; alternative markers *BenA* EF661485, *CaM* EF661508, *RPB2* EF661440 (Frisvad et al. 2019).

Physiology A problem in assessing physiological data for *Aspergillus flavus* and related species has been inaccurate identification. Most physiological studies reported on *A. flavus* up to 1990 in fact dealt with *A. parasiticus* or *A. nomius* (Pitt 1993). Fortunately, differences among these species do not appear to be great, but some data below cannot with certainty be identified as relating to *A. flavus*. Reported growth temperatures for *A. flavus* show some variation: a minimum near 10–12 °C, a maximum near 43–48 °C, and an optimum near 33 °C appear to be most frequently mentioned (see Pitt and Hocking 1997). Differences in water activities permitting growth have also been rather variable: from a low of 0.78 a_w at 33 °C (Ayerst 1969) to 0.84 at 25 °C (Pitt and Hocking 1977). Pitt and Miscamble (1995) reported a minimum of 0.82 at 25 °C, 0.81 at 30 °C and 0.80 at 37 °C. Data from that paper were used to provide a predictive model for *A. flavus* growth in relation to a_w and temperature (Gibson et al. 1994). High temperatures (41 °C) decreased germination rates of *A. flavus* by 45% compared with germination at 30 °C (Araujo and Rodriges 2004). Growth of *A. flavus* occurred over the pH range 2.1 to 11.2 (the entire range examined) at 25 °C, 30 °C and 37 °C, with optimal growth over a broad range from pH 3.4–10 (Wheeler et al. 1991) and a peak near 7.5 (Olutiola 1976). The use of acetic acid to adjust the pH of growth media to 4.5 reduced the germination of *A. flavus* conidia, whereas lactic and hydrochloric acids had no effect on germination (Araujo and Rodriges 2004).

The heat resistance of *Aspergillus flavus* has been studied under various conditions by several authors. The most reliable figures indicate a D_{45} value of more than 160 hr., a D_{50} of 16 hr., a D_{52} of 40–45 min and a D_{60} of 1 min, at neutral pH and high a_w, with z values from 3.3 to 4.1 C° (Beuchat 1981a, c; Doyle and Marth 1975a; ICMSF 1996).

The addition of phosphine, used to control insects, to grain at 0.80 or 0.86 a_w reduced growth of *Aspergillus flavus* while having little effect on the survival of conidia (Hocking and Banks 1991).

After 42–48 hr. incubation on AFPA (Pitt et al. 1983), colonies of *Aspergillus flavus* and *A. parasiticus* exhibit a brilliant orange yellow reverse colouration (see Chap. 4)

Mycotoxins *Aspergillus flavus* is the main source of aflatoxins, the most important mycotoxins in the world's food supplies. Aflatoxins are produced in nature by *A. flavus, A. parasiticus* and a number of other less important species. These include *A. nomius* (see below), *A. bertholletius* Taniwaki et al. which is found in brazil nuts

(Taniwaki et al. 2012), *A. minisclerotigenes* Vaamonde et al. and *A. arachidicola* Pildain et al. both of which occur in peanuts (Pildain et al. 2008). Other aflatoxin producing species listed in Frisvad et al. (2019) are of little or no importance in foods.

To determine whether particular isolates of *A. flavus* (or *A. parasiticus*) can produce aflatoxins, grow them on coconut cream agar (Dyer and McCammon 1994; Chap. 4).

A strong synergy is observed between aflatoxin and hepatitis B (HBV) virus in the aetiology of liver cancer (Williams et al. 2004). The relative risk of cancer for HBV antigen-positive patients rises from about 5 to about 60 when combined with aflatoxin exposure. In some areas where HBV and aflatoxin co-occur, hepatomas are the predominant cancer and may be a predominant cause of death (Williams et al. 2004). This known co-carcinogenicity is so high that every effort must be made to monitor levels in human foods and to reduce them as far as possible. For further information on aflatoxins, see Chap. 13.

Cyclopiazonic acid is produced by some strains of *Aspergillus flavus.* Its role in human health remains unclear, but evidence for its involvement in animal disease is strong (Cole 1986). See Chap. 13.

From the few studies of the effects of physical factors on aflatoxin production, it appears that *Aspergillus flavus* can produce aflatoxins over the range 13–37 °C, and above 0.82 a_w, with optima perhaps 16–31 °C and 0.95–0.99 a_w (ICMSF 1996). Aflatoxin production by *A. flavus* in agricultural soils appears to be correlated with latitude, aflatoxigenic strains being more prevalent in the southern peanut growing regions of the United States. It appears that dry, hot conditions favour the presence of toxigenic strains of *A. flavus* (Horn 2003). We have made similar observations: *A. flavus* strains from New Zealand produce aflatoxins less frequently and at lower concentrations than strains from Australia or SE Asia (our unpublished data). The influence of crop species has also been raised as a factor in selecting for toxigenic *A. flavus* in agricultural soils (Horn 2003). Additional studies are still needed to provide a more accurate picture.

Ecology The quest for knowledge about potential aflatoxin problems means that *Aspergillus flavus* has been sought in every conceivable kind of foodstuff. *A. flavus* has become the most widely reported foodborne fungus, reflecting its economic importance and relative ease of recognition as much as its ubiquity. It is especially abundant in the tropics, and it has a particular affinity for nuts and oilseeds as substrates.

A. flavus was prevalent in 97% of peanuts examined in Southeast Asia. Maize samples from Indonesia and the Philippines also contained high levels of *A. flavus* contamination. Other Southeast Asian commodities found to harbour *A. flavus* included kemiri nuts, peppercorns, velvet and talo beans, copra, cassava, sorghum, paddy rice and soybeans (see Pitt and Hocking 1997).

A. flavus occurs in most types of nuts from time to time, and is capable of causing spoilage and/or producing aflatoxins (see Pitt and Hocking 1997). Reports include tree nuts, pistachios (Bayman et al. 2002) and halva containing pistachios (Var et al. 2007), hazelnuts and walnuts (Bayman et al. 2002; Gürses 2006), coconut (Onifade and Jeff-Agboola 2003), copra (Srinivasulu et al. 2003), pecans, and kola nuts (see Pitt and Hocking 1997).

Cereals are also a common source of *Aspergillus flavus.* Maize and maize products are a particular problem (see Pitt and Hocking 1997; Sekiyama et al. 2005; García and Heredia 2006; Giorni et al. 2007). Insects may be involved in infection (García and Heredia 2006) and a number of practices including good storage conditions (Saleemullah et al. 2006) and planting genetically modified crops (Marasas and Vismer 2003) are now employed to control aflatoxin levels. Contamination has also been reported commonly in other cereals and cereal products: wheat (Lugauskas et al. 2006; Giray et al. 2007); wheat flour (Kumar et al. 2002; Ogundare and Adetuyi 2003) and flour products including bread (Lugauskas et al. 2006), pasta (Halt et al. 2004) and bran; barley (Lugauskas et al. 2006; Medina et al. 2006; see also Pitt and Hocking 1997); paddy, milled and parboiled rice (see Pitt and Hocking

1997); rice and rice bran (Sales and Yoshizawa 2005a); sorghum (da Silva et al. 2004; Lugauskas et al. 2006) and pearl millet (Wilson et al. 2006). However, unlike the situation with crops high in oil, spoilage by *A. flavus* or aflatoxin production in small grain cereals is almost always the result of poor handling. Aflatoxin levels in properly handled small grains are usually negligible (Stoloff 1977; Pohland and Wood 1987).

Spices of many kinds frequently contain *Aspergillus flavus* (Elshafie et al. 2002; Gatti et al. 2003; Mandeel 2005 and also see Pitt and Hocking 1997). Ionising radiation (see Pitt and Hocking 1997) and treatment of fresh plant materials before grinding or immediately after processing can reduce fungal and bacterial loads and aflatoxin levels in spices (Schweiggert et al. 2005).

Aspergillus flavus is common in green coffee beans (Martins et al. 2003; Magnani et al. 2005) and herbal drugs (Rizzo et al. 2004). Other sources of *Aspergillus flavus* include chickpeas (Singh et al. 2005), pigeon peas (Reddy et al. 2006), soybeans (Ahammed et al. 2006), olives, rapeseed, amaranth seeds (see Pitt and Hocking 1997), mustard seeds (Ahmad and Sinha 2006), sesame seeds (Singh et al. 2003; Pillai et al. 2003); freshly harvested and stored blackgram (Goyal and Jain 1998), betel nuts (see Pitt and Hocking 1997) and sunflower seeds (Narasimhan and Muthumary 2005). Processed and smoked meats, bacon (see Pitt and Hocking 1997), milk (Garrido et al. 2003; Ruggia and Galiero 2005) and cheese (Hayaloglu and Kirbag 2007) often contain *A. flavus* and sometimes aflatoxins. Some processed fish products from Nigeria and Sierra Leone supported growth of *A. flavus* but it was relatively uncommon in salted dried fish from Southeast Asia or dry cured hams in Spain (see Pitt and Hocking 1997).

Aspergillus flavus is capable of causing spoilage of some kinds of fresh fruit and vegetables, including citrus, tomatoes, peppers, litchis, pineapples and pomegranates, but it is not usually of great importance (Snowdon 1990, 1991). It has recently been reported to cause rot of peaches in Greece (Michailides and Thomidis 2007).

It is still often assumed that *Aspergillus flavus* is a soil fungus (e.g. Drott et al. 2019). This is erroneous: *A. flavus* is very uncommon in undisturbed soils, even in forest soil 50 m from a peanut crop where *A. flavus* counts exceeded 10^4/g (J.I. Pitt, unpublished data). *A. flavus* thrives on high energy substrates such as nuts and grains, or in drought stressed soils, in both cases where a_w is reduced below about 0.95, conditions under which soil fungi and bacteria are largely inhibited.

Additional species *Aspergillus oryzae* (Ahlburg) Cohn is very similar molecularly to *A. flavus* and is widely considered to be the domesticated form of *A. flavus,* adapted by centuries of use in fermented food manufacture (Wicklow 1983). *A. oryzae* produces colonies of similar or slightly smaller size to *A. flavus* on the standard media, and is usually distinguishable from *A. flavus* only by a more floccose and lightly sporing appearance, and sometimes a tendency towards pale brown conidial colours. Colonies of *A. oryzae* change in conidial colour from green towards olive brown with continued incubation at 25 °C for 7–14 days. Colonies of *A. flavus* and *A. parasiticus* remain yellow green or become greyish green under these conditions. Conidial heads of *A. oryzae* usually bear metulae and phialides, and conidia are usually larger than those of *A. flavus*, with thin, smooth to finely roughened walls. *A. oryzae* can be distinguished from *A. flavus* by amplified fragment length polymorphism (AFLP) (Monteil et al. 2003; Lee et al. 2004).

Aspergillus oryzae is of great economic importance, as it forms the basis for much of the fermented food industry in Japan and other parts of Asia (see Pitt and Hocking 1997). Tane koji, prepared by growing *A. oryzae* on cooked rice, provides a source of enzymes used in the production of shoyu (soy sauce), miso, hamanatto and other important Oriental products, which are mostly used as food flavourings (Tanaka et al. 2006).

Aspergillus oryzae is rarely isolated from sources other than fermented foods. Unlike *A. flavus, A. oryzae* is not known to produce aflatoxins. However, fermented foods may not be as free from the hazards of mycotoxins as is popularly believed as strains of *A. oryzae* can produce

cyclopiazonic acid and kojic acid (Tanaka et al. 2006; Frisvad et al. 2019). Identifiers: MycoBank MB184394; neotype IMI 16266 (Samson and Gams 1985); ex-ype cultures CBS 10207 = IMI 16266 = ATCC 1011 = NRRL 447; ITS barcode EF661560; alternative markers *BenA* EF661483, *CaM* EF661506, *RPB2* EF661438 (Frisvad et al. 2019).

Additional species *Aspergillus nomius* Kurtzman et al. (Kurtzman et al. 1986a) is also closely related to *A. flavus*. In the absence of sclerotia, the two species are morphologically indistinguishable. However, *A. nomius* produces both B and G aflatoxins and *A. nomius* sclerotia are bullet shaped, not spherical like those of *A. flavus*. Physiologically, *A. nomius* resembles *A. flavus*, but does not grow at quite such low water activities, with 0.83 a_w the minimum at 25 and 30 °C and 0.81 at 37 °C (Pitt and Miscamble 1995). Its genome has been studied in detail (Moore et al. 2015). Methods designed to separate this species from *A. flavus* and *A. tamarii* in clinical specimens have been described (Tam et al. 2014).

Aspergillus nomius was considered to a comparatively rare species, originally isolated from alkali bees in the United States (Kurtzman et al. 1986a). We isolated it from a range of Thai commodities: maize, peanuts, soybeans, cassava and black beans, but always at low levels (Pitt et al. 1993, 1994). It was reported subsequently from agricultural and native forest soils in Thailand where it was found to produce greater amounts of aflatoxins than *A. flavus* (Ehrlich et al. 2007), and then found to be of common occurrence in brazil nuts (Olsen et al. 2008; Gonçalves et al. 2012; Baquião et al. 2013), where it produced both B and G aflatoxins. It was subsequently shown that tthe presence of *A. nomius* in brazil nuts was the result of several species of local bees visiting flowering brazil nut trees (Massi et al. 2015). Identifiers: MycoBank MB133392; holotype BPI NRRL 13137 (Kurtzman et al. 1986a); cultures ex-type CBS 260.88 = IMI 331920 = ATCC 15546 = NRRL 13137 = FRR 3339; ITS barcode AF027860; alternative markers *BenA* AF255067, *CaM* AY017588, *RPB2* EF661456 (Frisvad et al. 2019).

References Raper and Fennell (1965); Varga et al. (2011a); Frisvad et al. (2019).

Aspergillus niger Tiegh. Fig. 8.22

Colonies on CYA 60 mm or more diam, usually covering the whole Petri dish, plane, velutinous, of low, usually subsurface, white mycelium, surmounted by a layer of closely packed, dark brown to black conidial heads, ca 2–3 mm high; reverse usually pale, sometimes pale to bright yellow. Colonies on MEA varying from 30–60 mm diam, usually smaller than those on CYA and often quite sparse by comparison, otherwise similar. Colonies on G25N 18–30 mm diam, plane, velutinous, with white or pale yellow mycelium visible at the margins, otherwise similar to those on CYA; reverse pale or occasionally with areas of deep brown. No growth at 5 °C. At 37 °C, colonies 60 mm or more diam, covering the available space, sometimes sulcate, otherwise similar to those on CYA at 25 °C.

Conidiophores borne from surface hyphae, 1.0–3.0 mm long, with heavy, hyaline, smooth walls; vesicles spherical, usually 50–75 µm diam, bearing closely packed metulae and phialides over the whole surface; metulae 10–15 µm long, or sometimes more; phialides 7–10 µm long; conidia spherical, 4–5 µm diam, brown, with walls conspicuously roughened or sometimes striate, borne in large, radiate heads.

Distinctive features One of the best known of all fungal species, *Aspergillus niger* is distinguished by its spherical black conidia, produced on colonies which show little or no other colouring. *A. carbonarius* (Bainier) Thom, which produces conidia 7–10 µm in diameter and *A. awamori* Nakaz., which produces finely roughened conidia, are closely related. *A. awamori* (= *A. welwitschiae*) is used in food fermentations and is perhaps a domesticated form of *A. niger*.

Taxonomy The name *Aspergillus niger*, in use for most of this century, was predated by other valid names which had priority under the ICN. *A. niger* Teigh. was conserved by the International Botanical Congress in 1993 (Greuter et al. 1994) following application by Kozakiewicz et al. (1992).

The advent of molecular taxonomy has shown that *Aspergillus* subgen *Circumdati* section *Nigri*, centred on *A. niger*, consists of about 25 species (Ismail 2001), four of which are included in this

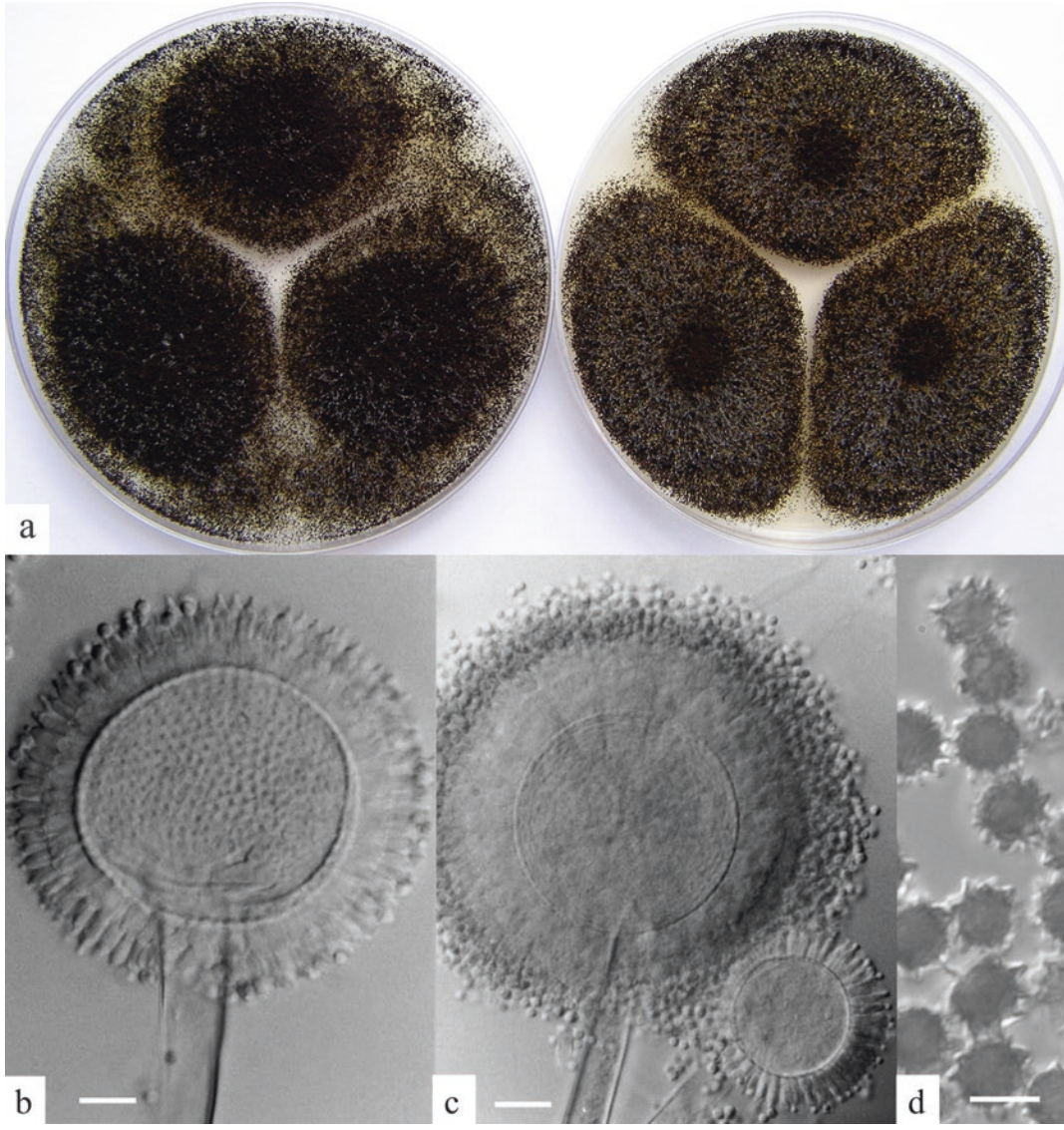

Fig. 8.22 *Aspergillus niger* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) head, bar = 15 µm; (**c**) heads, bar = 10 µm; (**d**) conidia, bar = 5 µm

work. The *A. niger* clade consists of some 10 species, several of which have been split from *A. niger* in the past few years. The differences are small and all are likely to have similar physiological properties to *A. niger*.

Identifiers MycoBank MB284309; type CBS 554.65; ex-type cultures CBS 554.65 = IMI 50566 = ATC 16888 = NRRL 326; ITS barcode EF661186; alternative markers *BenA* EF661089, *CaM* EF661154, *RPB2* EF661058 (Samson et al. 2014).

Physiology Growth temperatures for *Aspergillus niger* are minimum, 6–8 °C, maximum, 45–47 °C, and optimum 35–37 °C (Panasenko 1967; Leong et al. 2006b). However, Palacios-Cabrera et al. (2005) reported no growth at 8 °C on three different media. *A. niger* is a xerophile: Ayerst (1966) reported germination at 0.77 a_w at 35 °C. Only slight differences in growth rates were observed on media based on NaCl or glycerol, or of pH 4.0 and 6.5, at various water activities (Avari and Allsopp 1983). *A. niger* is

able to grow down to pH 2.0 at high a_w (Pitt 1981). Microwaves were found to have no effect on conidia of *A. niger*, apart from that from substrate heat (Ishitani et al. 1981). *A. niger* was relatively resistant to UV-C radiation but more sensitive than *A. carbonarius* or *Alternaria alternata* (Valero et al. 2007b).

Mycotoxins *Aspergillus niger* has been regarded as a benign fungus, widely used in enzyme production and ingredients for food processing. It holds GRAS (Generally Regarded as Safe) status from the U.S. Food and Drug Administration. However, two of 19 *A. niger* isolates were reported to produce ochratoxin A by Abarca et al. (1994) and further research has shown than many more isolates of *A. niger* aggregate can produce ochratoxin A under various conditions (Bellí et al. 2004; Esteban et al. 2004; Esteban et al. 2006a, b; Leong et al. 2006b). Usually only a low percentage of *A. niger* isolates have been reported to produce ochratoxin A; however 20% of 150 *A. niger* strains studied by Frisvad et al. (2011), produced ochratoxin A, including 12 used in industrial processes. Apart from *A. carbonarius* and one strain of *A. sclerotiniger*, no other species in series *Nigri* produced ochratoxin A. Although Medina et al. (2006) and Perrone et al. (2006) reported the production of ochratoxin A by some isolates of *A. tubingensis*, a study of 83 isolates by Frisvad et al. (2011) indicated no production. To determine whether an *A. niger* strain can produce ochratoxin A, grow it on coconut cream agar (Dyer and McCammon 1994) at 30 °C.

Unexpectedly, genes for fumonisin B_2 were detected in genome sequences of three *A. niger* strains, then cultures of those three, including the ex type culture, were shown to produce fumonisin B_2 (Frisvad et al. 2007). Subsequently, *A. niger* was shown to produce fumonisin B_2 and fumonisin B_4 in grapes and raisins (Mogensen et al. 2010), and fumonisin B_2 in coffee (Noonim et al. 2009). It has also been shown that the very closely related species *A. welwitschiae* (also known as *A. awamori*) produces fumonisin B_2 (Mogensen et al. 2010). About 50% of 175 Brazilian isolates collected from various food sources and previously identified as *A. niger* were reidentified as *A. welwitschiae*. Seventy four per cent of *A. niger* isolates produced fumonisin B_2, compared to 34% of *A. welwitschiae* isolates (Massi et al. 2016). The importance, if any, of fumonisin production by these species remains unclear.

Ecology Among the fungi most commonly reported from foods, *Aspergillus niger* is more prevalent in warmer climates, both in field situations and stored foods. The black spores provide protection from sunlight and UV light, providing a competitive advantage in such habitats (Valero et al. 2007b). *A. niger* is very frequently isolated from sun dried products, such as vine fruits (Abarca et al. 2003; Leong et al. 2004; Magnoli et al. 2004; Iamanaka et al. 2005), dried, smoked and cured fish, biltong, cocoa beans (see Pitt and Hocking 1997) and spices (Elshafie et al. 2002; Mandeel 2005). A similar physiology to *A. flavus* means that the two species compete in many situations. Various suggestions have been made for using *A. niger* as a competitor to *A. flavus* in the field (e.g. Wicklow et al. 1987; Shantha et al. 1990; Paster et al. 1992), but in our opinion *A. niger* is too destructive for this to be a practical proposition.

Aspergillus niger is by far the most common *Aspergillus* species responsible for postharvest decay of fresh fruit, including apples, pears, peaches, citrus, grapes, figs, strawberries, mangoes and melons (Snowdon 1990 and also see Pitt and Hocking 1997). Most of these diseases are sporadic and of minor significance. *A niger* has also been reported to cause serious losses in tomatoes (Muhammad et al. 2004) and breadfruit (Omobuwajo and Wilcox 1989), and in some vegetables, especially in onions and garlic (Rath and Mohanty 1985; Snowdon 1991; Sinha et al. 1994), stored cabbages (Lugauskas et al. 2005) and yams (Adeniji 1970; Ogundana 1972). It is frequently isolated from fresh grapes where it may be involved in Aspergillus bunch rot (Valero et al. 2005; Lasram et al. 2007; Fredj et al. 2007; Ponsone et al. 2007; Iamanaka et al. 2005). It is one species causing thread-mould spoilage of cheese (Hocking and Faedo 1992).

Aspergillus niger is among the most common fungi isolated from nuts, especially peanuts, and

also pecans (see Pitt and Hocking 1997), pistachios, almonds and walnuts (Bayman et al. 2002). It has been isolated from cashews (Adebajo and Diyaolu 2003), kola nuts (Adebajo 1994), coconut (Zohri and Saber 1993) and copra (Srinivasulu et al. 2003). Cereals and oilseeds are also frequent sources, especially maize (see Pitt and Hocking 1997; also Magnoli et al. 2007 for a review), corn snacks (Caldas et al. 2002) and also barley (see Pitt and Hocking 1997; Soldevilla et al. 2005; Lugauskas et al. 2006; Viswanath et al. 2006), soybeans (Aziz et al. 2006), rapeseed (Magan et al. 1993; Ahamad et al. 2003) and rape oil (Mondal and Nandi 1984), sorghum (Navi et al. 2002 and see Pitt and Hocking 1997), stored and parboiled rice (Aziz et al. 2006; Taligoola et al. 2004 and see Pitt and Hocking 1997), blackgram (Ahmad 1993), sunflower seeds (Begum et al. 2003), chickpeas (Ahmad and Singh 1991) and pigeon peas (Maximay et al. 1992).

Meat products are another common source (Farghaly et al. 2004; Hammad et al. 2006 and see Pitt and Hocking 1997). Other records include cheese (Sinigaglia et al. 2004; Hayaloglu and Kirbag 2007 and see Pitt and Hocking 1997), olives (Roussos et al. 2006), fresh vegetables (Lugauskas et al. 2005) and strawberries, rootstock snacks and a variety of tropical products (see Pitt and Hocking 1997).

Aspergillus niger was commonly isolated during our study of Southeast Asian commodities. The highest contamination occurred in peanuts, maize and kemiri nuts. From Thailand, sorghum (58% of samples), cashews (53%) and copra (43%) contained *A. niger*, with overall infection rates of 7%, 5% and 18% respectively. Infections in pepper and spice from Indonesia and and the Philipines were also quite high. Seventy eight per cent of coriander samples from Indonesia were infected with *A. niger*, with 18% infection overall.

Infection rates with *Aspergillus niger* were lower in commodities other than the nuts and oilseeds mentioned above. Philippine soybeans were exceptional, with an infection in 47% of samples. Otherwise, soybeans from the other countries, mung beans, black beans, cowpeas, paddy rice and milled rice all were infected at no more than 2% of all particles examined (Pitt et al. 1993, 1994, 1998 and our unpublished data).

Additional species *Aspergillus luchuensis* Inui, a long neglected name, was taken up by Hong et al. (2015) as the correct name for the black fungus involved in the production of koji. Hong et al. (2015) showed that the name *A. awamori* Nakazawa used for this species since Raper and Fennell (1965) revived it, was a synonym of *A. welwitschiae*, also a long neglected name (see below). Morphologically this species is very similar to *A. niger* and *A. tubingensis*, but is distinct molecularly. Isolates have variable morphology according to Hong et al. (2015), presumably becuase of long domestication. it is unclear whether it is to be found outside koji production. Its physiological properties are likely to be very similar to those of *A. niger.* Identifiers: MycoBank MB151291; lectotype MBT392280, epitype MBT392281, CBS H-24280; ex-epitype culture CBS 205.80; ITS barcode JX500081; alternative markers *BenA* JX500062, *CaM* JX500071, *RPB2* MN969081 (Houbraken et al. 2020).

Additional species *Aspergillus tubingensis* Mosseray belongs in the *A, niger* clade and is very similar to *A. niger* morphologically. It is most readily distinguished from it by molecular differences (Varga et al. 2011b). It is quite common in foodstuffs. It may be expected to resemble *A. niger* physiologically, but is notable because it does not produce fumonisins or ochratoxin A (Frisvad et al. 2011). Identifiers: MycoBank MB255209; lectotype MBT392362, epitype MBT393363, CBS H-24288 (Houbraken et al. 2020); ex-epitype cultures CBS 133056 = NRRL 4875; ITS barcode EF661193; alternative markers *BenA* EF661086, *CaM* EF661151m *RPB2* EF661055 (Houbraken et al. 2020).

Additional species *Aspergillus welwitschiae* (Bres.) Henn. was revived by Hong et al. (2015) for an isolate taken from the desert plant *Welwitschia mirabilis* in Namibia. Distinguished from *A. niger* only be molecular differences, *A. welwitschiae* has been shown to be very common in foods – grapes, dried fruit, coffee, cocoa and other sources according to Hong et al. (2015). Of 175 Brazilian *A. niger* isolates collected from dried fruits, brazil nuts, coffee beans, grapes, cocoa and onions, 50% were reidentified as

A. welwitschiae (Massi et al. 2016). It was reported by Varga et al. (2011b) that most isolates of this species (designated *A. awamori* by them) cannot grow on a medium using 2-deoxy-D-glucose as sole carbon source. This species (designated *A. awamori* by them) produces fumonisins and occasionally ochratoxin A (Frisvad et al. 2011). Thirty four per cent of the *A. welwitschiae* isolates identified by Massi et al. (2016) produced fumonisin B_2. Identifiers: MycoBank MB176748; epitype CBS 139.54; culture ex-epitype CBS 139.54; ITS barcode FJ629340; alternative markers *BenA* MN969369, *CaM* KC480196, *RPB2* MN969100 (Houbraken et al. 2020).

References Raper and Fennell (1965); Varga et al. (2011b); Hong et al. (2015); Houbraken et al. (2020).

Aspergillus niveus Blochwitz Fig. 8.23

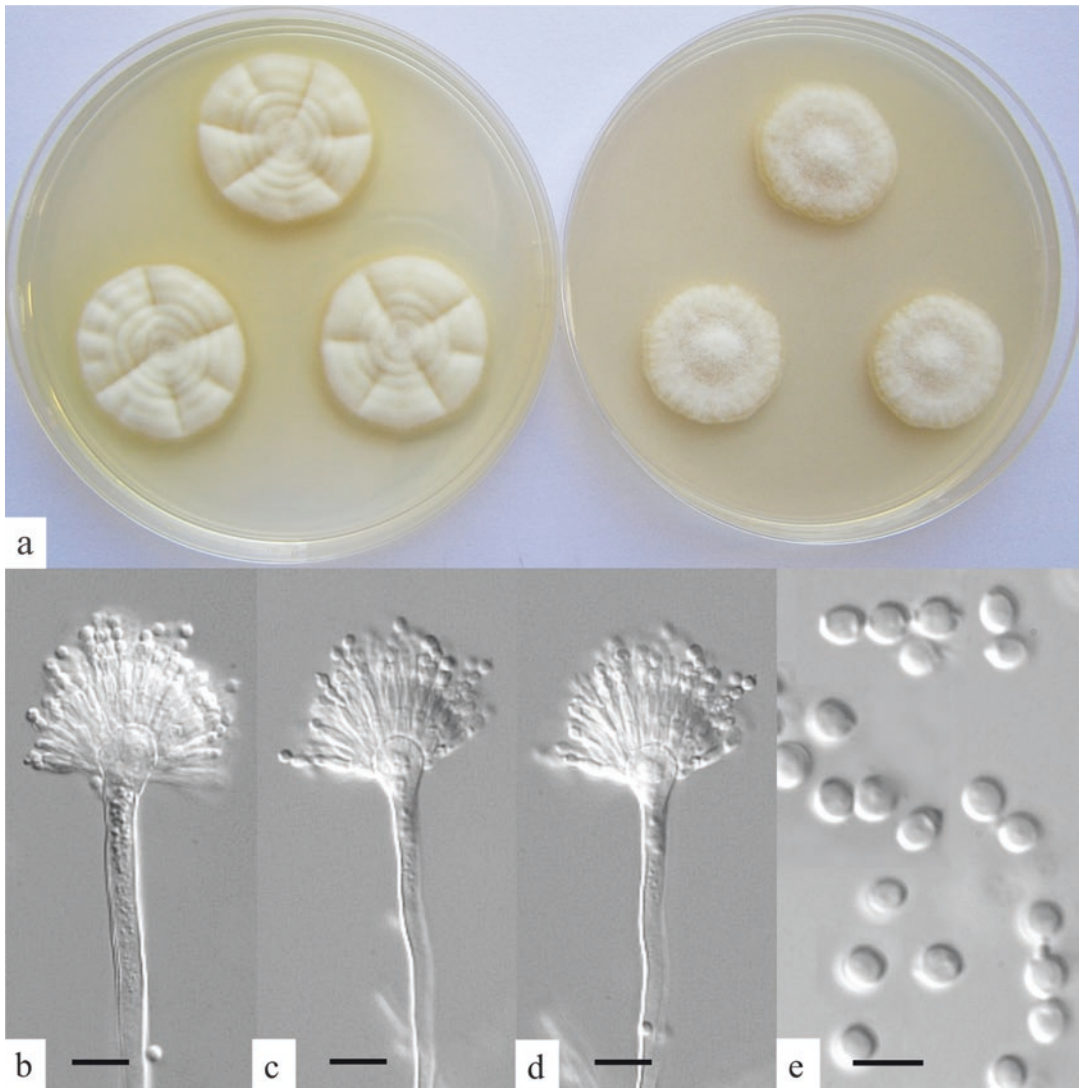

Fig. 8.23 *Aspergillus niveus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**–**d**) heads, bars = 10 µm; (**e**) conidia, bar = 5 µm

Colonies on CYA 18–30 mm diam, plane or lightly sulcate, dense and velutinous; mycelium white, occasionally with bright yellow sectors; conidial heads closely packed, white to pale yellow; exudate clear, yellow brown or sometimes dull red; yellow soluble pigment sometimes present; reverse yellow brown to dark green. Colonies on MEA 18–30 mm diam, plane, similar to those on CYA but texture granular to floccose, exudate clear; reverse pale to dull yellow. Colonies on G25N 10–16 mm diam, dense to floccose, white; reverse pale to dull yellow. No growth at 5 °C. At 37 °C, colonies usually 30–45 mm diam, similar to those on CYA at 25 °C, occasionally much smaller.

Conidiophores borne from surface hyphae, stipes usually 100–500 µm long, with uncoloured, smooth walls; vesicles hemispherical to spathulate, 8–15 µm diam, bearing crowded metulae and phialides over the upper half to two thirds; metulae and phialides 5–9 µm long; conidia spherical to subspheroidal, 2.5–3.5 µm diam, with smooth to finely roughened walls, borne in radiate to loosely columnar heads.

Distinctive features *Aspergillus niveus* is one of only two *Aspergillus* species with white or off white conidia. It is distinguished from *A. candidus* by yellow conidial colours in age, and more readily by vesicles which are fertile only over the upper half to two thirds, while those of *A. candidus* are fertile over the entire area of the vesicle.

Taxonomy Originally located in *Aspergillus* subgenus *Nidulantes* section *Flavipes* by Samson and Gams et al. (1985), *A. niveus* was transferred to subgenus *Circumdati* section *Terrei* by Peterson (2008). This species was believed to occasionally produce a teleomorph, which was described as *Aspergillus niveus* by Wiley and Fennell (1973), was provided with the teleomorph name *Emericella nivea* by Wiley and Simmons (1973), and transferred to *Fennellia* by Samson (1979). However, Samson et al. (2011) showed using molecular methods that this teleomorph represented a separate species which they named *A. neoniveus* Samson et al. *A. niveus* is the species occasionally found in foods.

Identifiers MycoBank MB272402; neotype IMI 171878 (Samson and Gams 1985); cultures ex-neotype CBS 115.27 = IMI 171878 = NRRL 5505; ITS barcode EF669615; alternative markers *BenA* EF669528, *CaM* EF669573, *RPB2* EF669657 (Samson et al. 2014).

Physiology *Aspergillus niveus* grows strongly at higher temperatures, with a reported minimum of 11–13 °C, an optimum near 36–42 °C and a maximum at 47–48 °C (Domsch et al. 1980). Water relations have not been studied.

Mycotoxins A culture of *Aspergillus niveus* was toxic to ducklings (Scott 1965). The toxicity was probably due to citrin production (Frisvad and Samson 1991; Samson et al. 2011). As *A. niveus* is not known to spoil foods, this is not likely to have commercial significance.

Ecology The main habitat of *Aspergillus niveus* is soil in the tropics (Domsch et al. 1980) and reports from foods are rare. However, we isolated this species from peanuts in Thailand, the Philippines and Indonesia, from soybeans, sorghum and black rice in Thailand and milled rice in Indonesia (Pitt et al. 1993, 1994, 1998). Pectinase enzymes isolated from *A. niveus* were reported to improve the fermentation of tea leaves when compared with a commercial enzyme preparation (Angayarkanni et al. 2002).

References Raper and Fennell (1965); Klich (2002); Samson et al. (2011).

Aspergillus ochraceus K. Wilh. Fig. 8.24

Colonies on CYA 40–55 mm diam, plane or sulcate, low and velutinous or lightly floccose; mycelium white; conidial heads closely packed, light yellow to golden yellow; sclerotia sometimes produced, white when young, later pink to purple; clear exudate sometimes present, some exuded from stipe walls; reverse greyish orange to brown. Colonies on MEA 40–55 mm diam, plane, similar to those on CYA but quite sparse; reverse pale yellow brown or slightly darker. Colonies on G25N 20–30 mm diam, plane, low and dense to deep and floccose, conidial production light to moderate, coloured as on CYA; reverse pale yellow or brown. No growth at 5 °C. At 37 °C, colonies up to 30 mm diam produced.

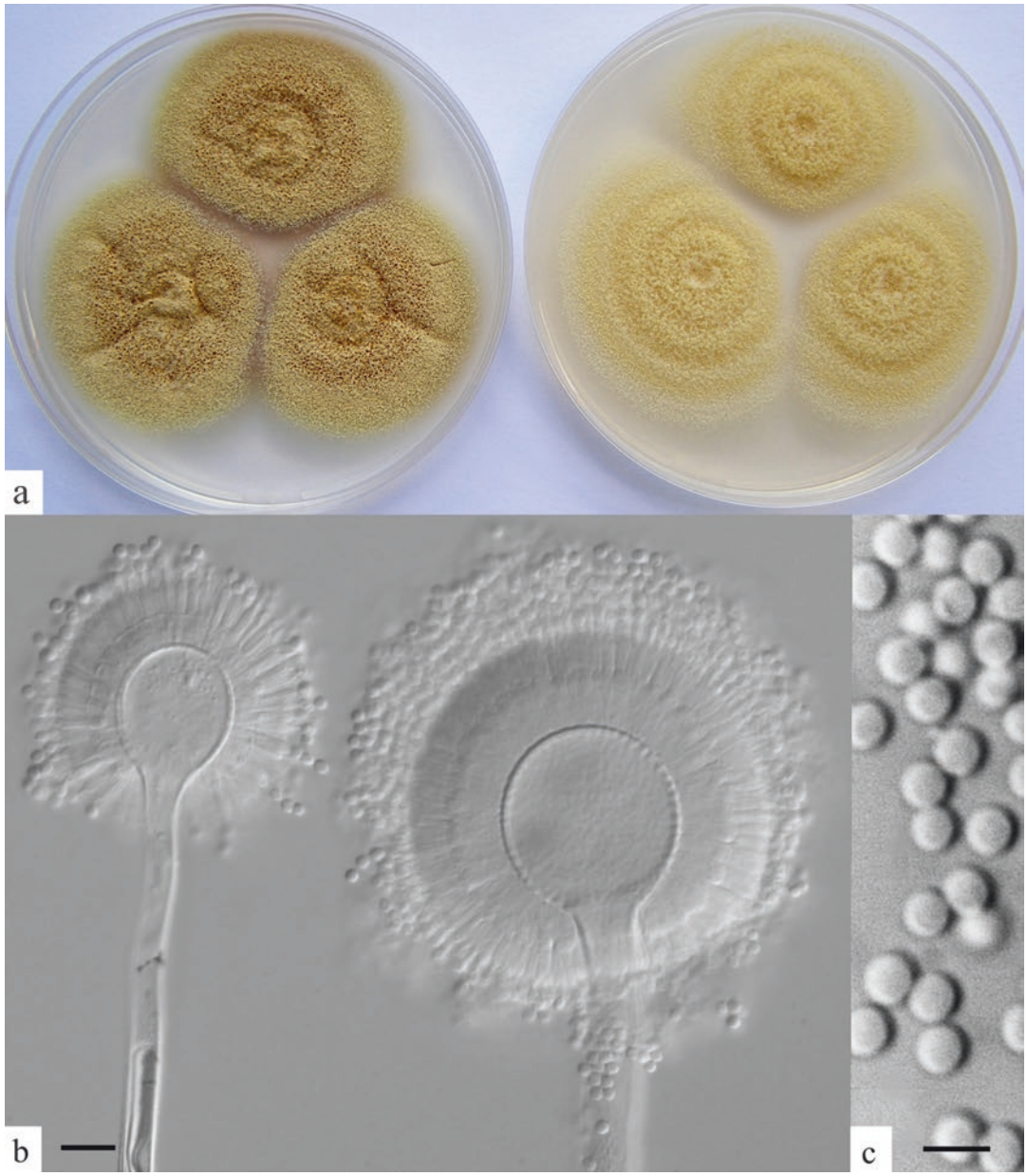

Fig. 8.24 *Aspergillus ochraceus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**) heads, bar = 20 µm; (**c**) conidia, bar = 5 µm

Conidiophores borne from surface hyphae, stipes 1.0–1.5 mm long, with yellowish to pale brown walls, finely to conspicuously roughened; vesicles spherical, 25–50 µm diam, bearing tightly packed metulae and phialides over the entire surface; metulae 15–20 µm long; phialides 9–12 µm long; conidia spherical to subspheroidal, 2.5–3.5 µm diam, with smooth to finely roughened walls, borne in radiate heads when young, splitting into two or more broad columns with maturity.

Distinctive features *Aspergillus ochraceus* produces yellow brown (ochre) conidia, borne on long stipes; vesicles bear metulae and phialides over the entire surface. *A. ochraceus* grows at 37 °C, unlike the closely related species

A. westerdjikiae and *A. steynii* (see Additional species below).

Taxonomy Domsch et al. (1980) and Samson and Gams (1985) took up the name *Aspergillus alutaceus* Berk. & Curtis for this species as it predates *A. ochraceus*. However this change did not meet with broad acceptance, as doubt existed as to whether these two species were synonymous. *A. ochraceus* was accepted in the list of Names in Current Use (Pitt and Samson 1993) and is the currently accepted name (Visagie et al. 2014). In searches, the name *A. alutaceus* should also be checked in literature from about 1990–2000. *A. ochraceus* is the type species of *Aspergillus* subgenus *Circumdati* section *Circumdati* (Gams et al. 1985). The latest taxonomy of this section includes 27 species, of which *A. ochraceus* and two closely related species, *A. westerdijkiae* Frisvad & Samson and *A. steynii* Frisvad & Samson (Frisvad et al. 2004) are considered to be the most important (Visagie et al. 2014). See Additional species below.

Identifiers Mycobank MB190223; type IMI 16247iv (Visagie et al. 2014); ex-type cultures CBS 108.08 = IMI 16247 = ATCC 1008 = NRRL 398; ITS barcode ES116414; alternative markers *BenA* EF661322, *CaM* EF661381, *RPB2* EF661302 (Visagie et al. 2014); Houbraken et al. 2020).

Physiology As *A. ochraceus* was confused with *A. westerdjikiae* and *A. steynii* until recently, the physiological data reported for *A. ochraceus* sometimes refers to one or both of these other two species. In particular, *A. ochraceus* isolates grow quite strongly at 37 °C, indicating a maximum for growth of at least 40 °C. The other two species do not grow at 37 °C. It is not clear which species were studied when Palacios-Cabrera et al. (2005) reported no growth by *A. ochraceus* at either 8 °C or 41 °C on a selection of media.

It seems likely that all three species will have similar water relations, though it is unknown which species was studied in the reports that follow. Growth was reported down to 0.79 a_w on glucose/fructose media and down to 0.81 a_w on media based on NaCl (Pitt and Hocking 1977). Pardo et al. (2006a) indicated germination occurred down to 0.80 a_w at 20 or 30 °C and 0.85 a_w at 10 °C. No germination occurred at 0.75 a_w on barley meal extract medium (Pardo et al. 2004a) or on green coffee extract agar (Pardo et al. 2005a). The optimum a_w for growth is 0.95–0.99 a_w (ICMSF 1996; Pardo et al. 2006a). *A. ochraceus* grew well between pH 3 and pH 10, and slowly at pH 2.2 (Wheeler et al. 1991).

The decimal reduction time (D_{10}) for *Aspergillus ochraceus* subjected to low dose gamma irradiation was given as 0.44 kGy on grain (O'Neill et al. 1991). Up to 4 kGy of gamma irradiation was necessary to prevent growth of *A. ochraceus* on barley (Szekely et al. 1991). 1 Kgy of gamma radiation inactivated 10^3 spores/g of *A. ochraceus* in green coffee beans, while approximately 2.5 kGy was necessary to inactivate 10^7 spores/g (Kumar et al. 2002). Irradiation was reported to have no intrinsic effect on ochratoxin A production (Chelack et al. 1991), but a high dose (10KGy) greatly reduced preformed or in vitro added OTA (50 µg/kg) in coffee beans (Kumar et al. 2002).

In the presence of 20% O_2, *Aspergillus ochraceus* was able to grow in 60% but not 80% CO_2 (Paster et al. 1983). Germ tube elongation was significantly inhibited by 50% CO_2 at 0.90–0.95 a_w and both growth and ochratoxin A production were affected by 25 and 50% CO_2, regardless of the water activity (Cairns-Fuller 2004).

Like *Aspergillus flavus* and *A. parasiticus*, *A. ochraceus* and related species produce a bright orange reverse on AFPA, though not the same pigment (Assante et al. 1981). Growth is much slower: reading results from AFPA plates before five days will avoid false positives from *A. ochraceus* and related species (Pitt et al. 1983). *Aspergillus niger* has been reported to inhibit growth of *A. ochraceus* (Paster et al. 1992), but this does not appear to have practical applications.

Mycotoxins The toxigenic nature of *Aspergillus ochraceus* was discovered not as the result of a natural toxicosis, but in laboratory studies on toxigenic fungi (Van der Merwe et al. 1965). Three toxins were found: the major one was named ochratoxin A, while minor components of lower toxicity were named ochratoxins B and C. Subsequent work has shown that only a minority of *A. ochraceus* isolates are toxigenic (see Miyaki et al. 1970; Ciegler 1972; Cvetnic

1994; Chourasia 1995), and that other species closely related to *A. ochraceus,* i.e. *A. westerdjikiae* and *A. steynii*, are the main producers of ochratoxin A (Frisvad et al. 2004). To determine whether isolates of *A. ochraceus* or related species can produce ochratoxin A, grow them on coconut cream agar (Dyer and McCammon 1994; Heenan et al. 1998). These authors incubated coconut cream agar plates at 30 °C, but 25 °C is more appropriate for these species.

It is now believed that the well documented occurrence of ochratoxin A in temperate climate foods results from growth of *Penicillium verrucosum*. See Chap. 13. *Aspergillus ochraceus* and related species produce most of the ochratoxin A found in coffee, but it is likely that most of the ochratoxin A produced in other tropical commodities is due to the growth of *A. carbonarius* (see Ecology below). Possible involvement of *Aspergillus ochraceus* in the production of ochratoxin under tropical conditions should not be overlooked, however.

Pardo et al. (2004b) found optimum ochratoxin A production on barley grains by *Aspergillus ochraceus* or a related species occurred at 0.98–0.99 a_w and 25–30 °C. Similar results were observed for maximum ochratoxin A production on irradiated green coffee beans and the highest yields occurred at 20 °C (Pardo et al. 2005b). The minimum water activity allowing ochratoxin A formation was reported to be 0.85 a_w on sterilised coffee beans (Taniwaki et al. 1999; Pardo et al. 2005b), however, no ochratoxin A was produced at this water activity on irradiated barley grains or on grapes (Pardo et al. 2004b, 2005c and see Pardo et al. 2006a for a review).

The effect of pH and trace metals on the production of ochratoxin A by *A. ochraceus* was examined in liquid media (Mühlencoert et al. 2004). At 25 °C, *A. ochraceus* synthesised ochratoxin A (up to 2 mg/L) at pH 5.5–8.5. At pH 6.5, variations in trace metal concentrations such as Zn and Fe affected ochratoxin A production (Mühlencoert et al. 2004).

From a study of the growth of *Aspergillus ochraceus* on cereals (maize and wheat) and oilseeds (peanuts, rapeseed and soybeans), it was concluded that ochratoxin production was favoured more by peanuts and soybeans than by the other substrates studied (Madhyastha et al. 1990). Pardo et al. (2006b) also reported that the nature of the substrate influenced toxin production by *A. ochraceus*. The highest ochratoxin A yields were produced on green coffee beans (>2 g/kg), barley grains (*ca* 1 g/kg), Yeast Extract Sucrose medium (13.9 mg/kg) and grape berries (*ca* 3 µg/kg). The toxigenic potential of the strains studied was independent of their origin (Pardo et al. 2006b).

Ochratoxin A production by *Aspergillus ochraceus* was similar in atmospheres with 5% or 1% O_2 as in air. In the presence of 20% O_2, low levels of CO_2 had no influence on ochratoxin A production, but it was completely inhibited by 30% CO_2 in the presence of 20% O_2 (Paster et al. 1983). Sorbic acid had little effect on ochratoxin A production at 25 °C and pH 5.0 (Bullerman 1985).

The effect of heat on ochratoxin A produced in wheat by a strain of *Aspergillus ochraceus* was reported by Boudra et al. (1995). For dry wheat, 50% decomposition occurred in 700, 200, 12 and 6 min at 100, 150, 200 and 250 °C, respectively. The addition of 50% water increased decomposition rates at 100 and 150 °C, but not at higher temperatures (Boudra et al. 1995).

Aspergillus ochraceus also produces penicillic acid (Frisvad et al. 2004), a mycotoxin of lesser importance (see Chap. 13). It is produced by *A. ochraceus* between about 10 and 35 °C and down to 0.81 a_w (ICMSF 1996). Sorbic acid (500 mg/kg) had little effect on penicillic acid production at 15–35 °C, but 1000 mg/kg markedly reduced production at 15 and 25 °C (Gourama and Bullerman 1988).

Emodin is also produced by *A. ochraceus* or a sibling species and was found at low levels in chestnuts on the Canadian market (Overy et al. 2003). See *Aspergillus wentii* for details of emodin.

Ecology Green coffee beans are an important source of *Aspergillus ochraceus* and the closely related *A. westerdjikiae* and *A. steynii* (Mislivec et al. 1983; Tsubouchi et al. 1985; Téren et al. 1997; Urbano et al. 2001; Bucheli and Taniwaki

2002; Martins et al. 2003; Taniwaki et al. 2003; Suárez-Quiroz et al. 2005) which may result in ochratoxin A contamination of roasted and instant coffee (Jørgensen 2005; Clark and Snedeker 2006).

Aspergillus ochraceus and related species have also been isolated from a wide range of other foods, but are more common in dried and stored foods than elsewhere. Stored foods from which they have been isolated frequently include soybeans (Pacin et al. 2002; Aziz et al. 2006), pepper (Gatti et al. 2003) and dried fruit (Iamanaka et al. 2005). Other foods include smoked dried fish, salted dried fish, dried beans, biltong, chickpeas, rapeseed, sesame seeds before and after fermentation, blackgram seeds and rootstock snacks (see Pitt and Hocking 1997). Nuts are also a major source, including peanuts (Aziz et al. 2006), pecans, pistachios, hazelnuts, walnuts and betel nuts (see Pitt and Hocking 1997).

Aspergillus ochraceus and related species have been reported from cereals and cereal products, but rather infrequently. Recent records include rice (Pacin et al. 2002) and barley (Medina et al. 2006), though levels in Europe are lower than for some other fungal species (Sepitkova and Jesenska 1986), maize (Sepulveda and Piontelli 2005; Magnoli et al. 2006b and see Magnoli et al. 2007 for a review) and maize meal (Souza et al. 2003), corn snacks (Adebajo et al. 1994), wheat (Aziz et al. 2006; Hajjaji et al. 2006), flour (Halt et al. 2004) and bran (Dragoni et al. 1979).

Aspergillus ochraceus and related species were the major contaminant in Bhutanese cheese (Sinha and Ranjan 1991). They have also been reported from cheese in the temperate zone (see Pitt and Hocking 1997). Electron beam irradiation of 0.42 kGy enabled a greatly increased shelf life for refrigerated cheddar cheese surface inoculated with *A. ochraceus* (Blank et al. 1992). Other less common sources include spices (Almela et al. 2007), cassava (Souza et al. 2003), black olives and processed meats (see Pitt and Hocking 1997).

We isolated *Aspergillus ochraceus* and related species from a variety of Southeast Asian commodities, including maize, peanuts, soybeans and other beans, cashews and sorghum. Its presence or absence in any sample probably related to length of storage rather than geographical location or other factors (Pitt et al. 1993, 1994, 1998).

Rots in garlic have been reported to be caused by *Aspergillus ochraceus*, but the species responsible is *A. alliaceus* Thom & Church (Snowdon 1991).

Additional species *Aspergillus westerdijkiae* Frisvad et al. (2004) is closely related to *A. ochraceus* with perhaps slightly faster growth rates on CYA at 25 °C. No growth occurs at 37 °C and sclerotia, which are sparsely produced, remain white to cream with age. Conidia of *A. westerdijkiae* are consistently finely roughened, compared with smooth conidia in *A. ochraceus*. *A. westerdijkiae* produces large amounts of ochratoxin A. It also produces penicillic acid, xanthomegnin, viomellein and vioxanthin. Some physiological studies conducted on isolates identified as *A. ochraceus* undoubtedly relate to *A. westerdijkiae* (Frisvad et al. 2004). Because of its ability to synthesise high levels of ochratoxin A, *A. westerdijkiae* is of importance in commodities such as coffee, cereals and beverages. Identifiers: MycoBank MB500000; holotype CBS H-13444; ex-holotype cultures CBS 112803 = ATCC 22947 = NRRL 3174; ITS barcode EF661427; alternative markers *BenA* EF661329, *CaM* EF661360, *RPB2* EF661307 (Samson et al. 2014).

Additional species *Aspergillus steynii* Frisvad & Samson (Frisvad et al. 2004) is also closely related to *A. ochraceus*. The absence of growth at 37 °C, ellipsoidal conidia and pale yellow conidia distinguish this species from *A. ochraceus*. Like *A. westerdijkiae*, *A.steynii* produces copious amounts of ochratoxin A, along with penicillic acid and xanthomegnins. Its broad distribution in comodities such as coffee, soybeans and rice makes *A. steynii* an important species (Frisvad et al. 2004). Identifiers: MycoBank MB500006; holotye CBS H-13445; ex-holotype cultures CBS 112812 = NRRL 35675; ITS barcode EF661416; alternative markers *BenA* EF661347, *CaM* EF661378, *RPB2* JN121428 (Samson et al. 2014).

References Raper and Fennell (1965); Klich (2002); Frisvad et al. (2004); Visagie et al. (2014).

Aspergillus parasiticus Speare Fig. 8.25

Colonies on CYA 50–70 mm diam, plane, low, dense and velutinous; mycelium inconspicuous, white; conidial heads in a uniform, dense layer, dark yellowish green (29-30D-F6–8); sclerotia occasionally produced; reverse uncoloured or brown. Colonies on MEA 50–65 mm diam, generally similar to those on CYA but usually less dense and with reverse uncoloured. Colonies on G25N 20–40 mm diam, plane, low and velutinous, generally similar to those on CYA; reverse uncoloured, yellow or brown. No growth at 5 °C. At 37 °C, colonies covering the available area, similar to those on CYA at 25 °C, or with conidia deeper green or brownish; reverse pale.

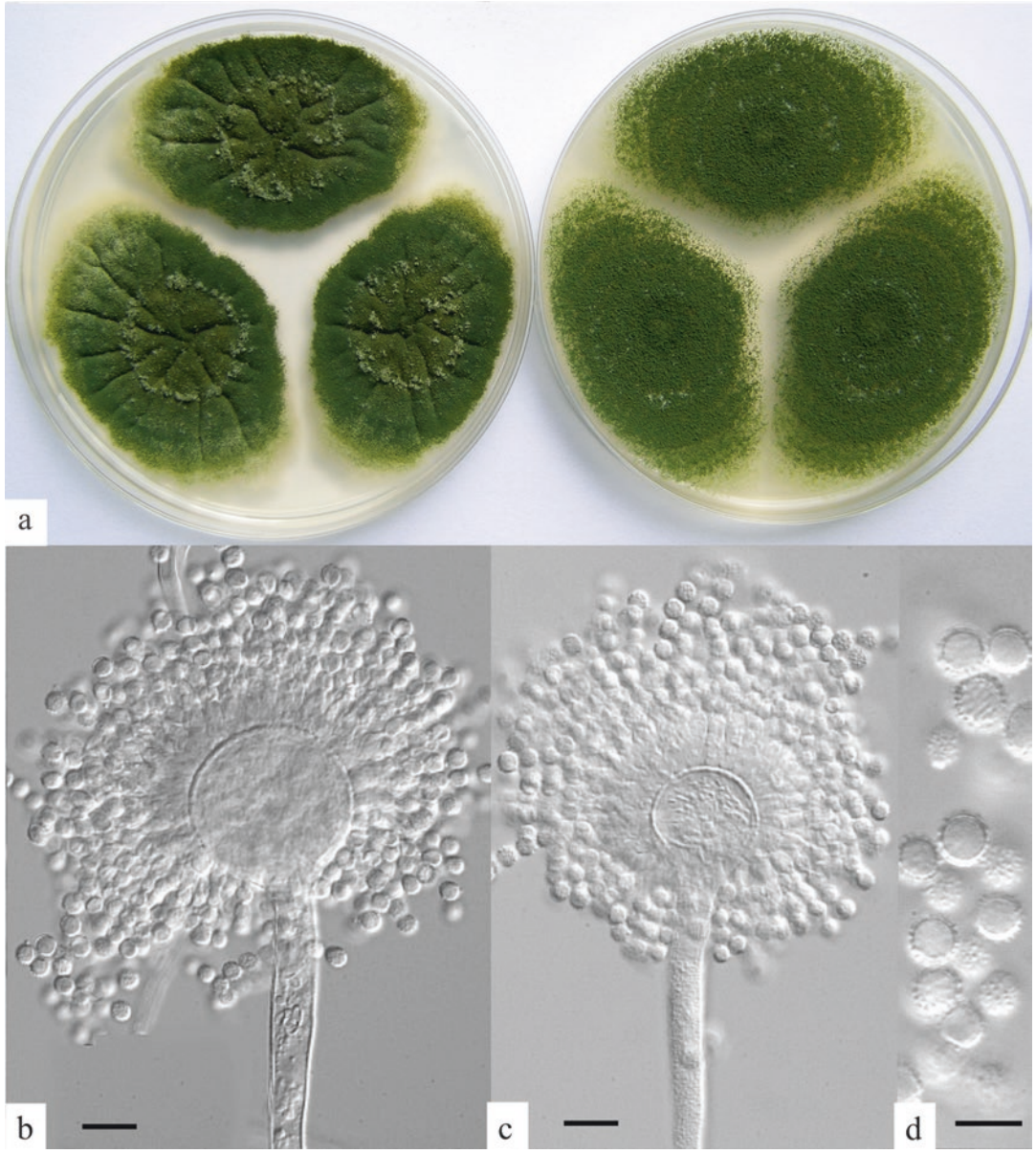

Fig. 8.25 *Aspergillus parasiticus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) conidia, bar = 5 µm

Sclerotia occasionally produced, white at first, becoming black, spherical, 400–800 µm diam. Species heterothallic: mature sexual state formed after mating of opposite mating type strains and prolonged incubation, ascospores *fide* Horn et al. (2009b) "sub-globose, 9.7 × 9.0 µm, finely roughened with a thin equatorial ridge, hyaline to pale brown. Conidiophores borne from subsurface or surface hyphae, stipes 250–500 µm long, with colourless or pale brown, smooth walls; vesicles spherical, 20–35 µm diam, fertile over three quarters of the surface, mostly bearing phialides only, but in some isolates up to 20% of heads bearing metulae as well; phialides 7–11 µm long; conidia spherical, mostly 4.0–6.0 µm diam, with distinctly roughened walls, usually borne in radiate heads.

Distinctive features *Aspergillus parasiticus* shares with *A. flavus* fast growth at both 25 and 37 °C, and distinctive yellow green conidial colours, a little darker in *A. parasiticus*. *A. parasiticus* differs from *A. flavus* by the production of spherical conidia with heavy, rough walls, while *A. flavus* conidia are more variable in shape and have relatively thin, usually finely roughened walls. Vesicles in *Aspergillus parasiticus* isolates rarely exceed 30 µm diam, while those of *A. flavus* are up to 50 µm diam.

Taxonomy Production of phialides only was the principal character used by Raper and Fennell (1965) to distinguish *Aspergillus parasiticus* from *A. flavus*. Klich and Pitt (1985, 1988) showed that this criterion was unsatisfactory, as some *A. flavus* isolates produce very few metulae. Careful study showed several differences between the two species, the most useful of which are mentioned above. *Aspergillus parasiticus* belongs in subgenus *Circumdati* section *Flavi* (Gams et al. 1985), which was reviewed and revised by Varga et al. (2011a) and most recently by Frisvad et al. (2019). This section now includes 33 species. Frisvad et al. (2019) provided a very comprehensive account of the taxonomy of this section as well as details of mycotoxin and other major secondary metabolite production. Eight new species were also described. It is recommended that Frisvad et al. (2019) be consulted for further information.

Kurtzman et al. (1986b) reduced *Aspergillus parasiticus* to the status of subspecies, as *A. flavus* subspecies *parasiticus* (Speare) Kurtzman et al. but as discussed here under *A. flavus,* compelling reasons exist for maintaining distinct species names for these taxa, as has been done here. *A. sojae*, reduced to the status of variety by Kurtzman et al. (1986b), appears to be a domesticated form of *A. parasiticus*. As with *A. oryzae, A. sojae* is widely used in food fermentations and recognition of it as a separate species is a practical necessity. Random amplification of polymeric DNA (RAPD) has been used to distinguish between *A. parasiticus* and *A. sojae* (Yuan et al. 1995).

Identifiers MycoBank MB191085; neotype IMI 15958ix (Kozakiewicz 1982); ex-neotype cultures CBS 103.13 = IMI 15957 = ATCC 1018 = NRRL 502; ITS barcode AY373859; alternative markers *BenA* EF661481, *CaM* AY017584, *RPB2* EF661449 (Samson et al. 2014).

Physiology A number of earlier studies reported to be on the physiology of *Aspergillus flavus* have used misidentified strains, and have really dealt with *A. parasiticus* (Pitt 1993). These two species are physiologically very similar, so that the studies reported under *A. flavus* are probably equally applicable to *A. parasiticus*. Growth of *A. parasiticus* has been reported over the range 12–42 °C, with an optimum at 32 °C (ICMSF 1996). A minimum a_w for growth of 0.82 at 25 °C, 0.81 at 30 °C and 0.80 at 37 °C, similar to *A. flavus*, has been reported (Pitt and Miscamble 1995). Data from that paper were used to provide a predictive model for *A. parasiticus* growth in relation to a_w and temperature (Gibson et al. 1994). Growth of *A. parasiticus* occurred over the pH range 2.4–10.5 at 25, 30 and 37 °C, but growth did not occur at pH 2.2 at 25 °C. Optimal growth occurred over a broad range from pH 3.5–8, with growth at pH 10.5 still more than half that under optimal conditions (Wheeler et al. 1991). High levels of sorbic acid (1000 mg/kg) were needed to inhibit growth of *A. parasiticus* on synthetic media at pH 5 (Bullerman 1983).

Conidia of *Aspergillus parasiticus* have a low heat resistance: a D_{55} up to 9 min at pH 7 in

phosphate buffer (Doyle and Marth 1975a). However values were much higher if a_w was reduced: a D_{55} of 230 min in 16% NaCl (0.90 a_w), 210 min in 60% glucose (0.85 a_w) and 200 min in 60% sucrose (0.90 a_w) (Doyle and Marth 1975b; ICMSF 1996). D values for the effect of gamma irradiation on conidia of *A. parasiticus* ranged from 0.3–0.6 kGy (ICMSF 1996).

Like *Aspergillus flavus*, *A. parasiticus* colonies exhibit a brilliant orange yellow reverse colouration after 42–48 hr. incubation on AFPA (Pitt et al. 1983).

Mycotoxins. The important differences in mycotoxin production between *Aspergillus parasiticus* and *A. flavus* are that *A. parasiticus* produces G as well as B aflatoxins, that *A. parasiticus* isolates often produce aflatoxins in much higher concentrations (Pitt 1993) and that nontoxigenic *A. parasiticus* strains are rare (Horn et al. 1994; Tran-Dinh et al. 1999). *A. parasiticus* does not produce cyclopiazonic acid (Vaamonde et al. 2003; Horn 2003; Frisvad et al. 2005) but does produce kojic acid, a metabolite of low toxicity now finding wide use in medicine and cometics (El-Aasar 2006).

The conditions under which *Aspergillus parasiticus* produces aflatoxins are similar to those reported for *A. flavus*. The best available figures indicate production over the temperature range 12–40 °C, down to 0.86 a_w, and over the pH range 3–8 or higher (ICMSF 1996). The ratio of aflatoxin G_1 production relative to B_1 on a rice substrate was higher at lower temperatures (at 15–18 °C, G_1: B_1 was 1.3–1.5) and much reduced at high temperatures (28 °C, 0.24 and 32 °C, 0.06) (Sorenson et al. 1966; ICMSF 1996). The G_1: B_1 ratio was up to 2.5 at 15 °C on peanuts (Diener and Davis 1967).

In the presence of 20% O_2, at least 60% CO_2 was needed to prevent production of aflatoxins on a moist peanut substrate at 30 °C, though the production in 60% CO_2 was only 10% of that in air (Landers et al. 1967). In reduced levels of O_2 in N_2, 5% O_2 caused aflatoxin production to be reduced to 30% of that in air, while in 1% O_2 production was only 1% of that in air (Landers et al. 1967).

Other aspects of aflatoxins, including toxicity and control, are discussed in Chap. 13.

Ecology Records of *Aspergillus parasiticus* from foods are relatively rare. Although *A. parasiticus* is certainly widely distributed in soils and foodstuffs in the United States, Latin America, South Africa, India and Australia, is it essentially unknown in Southeast Asia (Pitt et al. 1993, 1994, 1998).

Like *Aspergillus flavus*, *A. parasiticus* is a tropical and subtropical species, less prevalent in warm temperate zones, and rare in the cool temperate regions of the world. Its absence from Southeast Asia indicates a limited distribution. In soils, populations of *A. parasiticus* are generally associated with peanut cultivation in the USA and Australia (Carter 1998; Horn 2003). This species has only been reported in low densities in corn fields (Horn 2003). The most important food source is peanuts, in which we believe *A. parasiticus* is endemic, but about which reliable information is surprisingly limited. Klich and Pitt (1988) reported studying isolates from peanuts from the USA, Australia and Uganda. This species has been reported in peanuts in Argentina (Vaamonde et al. 2003) and Botswana (Mphande et al. 2004). Isolations of *A. parasiticus* from Australian peanuts are now more numerous than those of *A. flavus* (our data). Other types of nuts may also be infected: hazelnuts and walnuts, pistachios and pecans (see Pitt and Hocking 1997).

Maize is perhaps a source (Aja-Nwachukwu and Emejuaiwe 1994), but we believe that *A. parasiticus* is much less common on this commodity than *A. flavus*. A recent survey of Italian maize revealed that most contamination was due to *Aspergillus flavus* and *A. parasiticus* occurred in low frequency (Giorni et al. 2007). *A. parasiticus* has been reported at low frequency in rice and other rice derivatives (Sales and Yoshizawa 2005b). Other reported sources include soybeans (Vaamonde et al. 2003), processed meats (El-Tabiy 2006), black pepper (Gatti et al. 2003; Mandeel 2005) as well as rootstock snacks, herbal drugs, amaranth seeds and pearl millet (see Pitt and Hocking 1997).

References Raper and Fennell (1965); Klich and Pitt (1988); Klich (2002); Frisvad et al. (2019).

Aspergillus tamarii Kita **Fig. 8.26**

Colonies on CYA 50–65 mm diam, plane, velutinous to lightly floccose; mycelium inconspicuous, white; conidial heads abundant, olive brown; reverse uncoloured. Colonies on MEA 55–65 mm diam, similar to those on CYA but relatively sparse, and with olive conidia; reverse uncoloured. Colonies on G25N 35–40 mm diam, similar to those on CYA but coloured deep olive brown; reverse uncoloured. No growth at 5 °C. At 37 °C, colonies 50–65 mm diam, much more dense than on CYA at 25 °C, low and velutinous, with conidia coloured coffee brown; reverse pale.

Conidiophores borne from subsurface or surface hyphae, stipes 300–1500 µm long, colourless, usually with rough, thin walls; vesicles spherical to subspheroidal, 25–50 µm diam, fer-

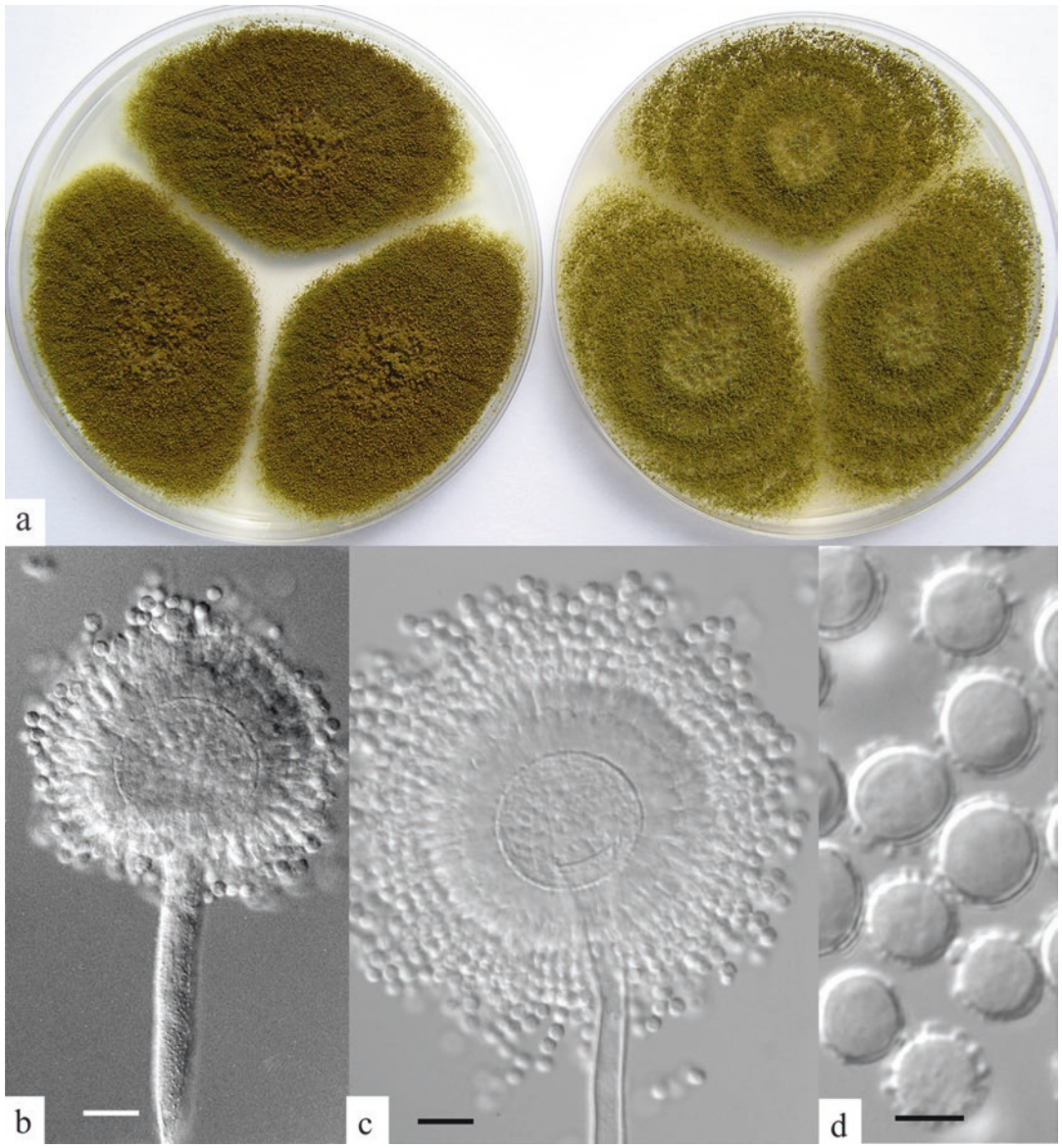

Fig. 8.26 *Aspergillus tamarii* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 20 µm; (**d**) conidia, bar = 5 µm

tile virtually all over, bearing both metulae and phialides or less commonly phialides alone; metulae 12–20 µm long; phialides 9–12 µm long; conidia spherical to subspheroidal, 5–8 µm diam, brown, with characteristic thick, rough to spiny walls, borne in radiate heads.

Distinctive features *Aspergillus tamarii* shows an unmistakable resemblance to *A. flavus* and *A. parasiticus*. The main distinguishing features are that conidia of *A. tamarii* are coloured olive to brown on CYA and MEA, and are larger, with thick, conspicuously roughened walls. On AFPA, *A. tamarii* produces a deep brown reverse colouration, in contrast to the orange yellow of *A. flavus* and *A. parasiticus*. This is a useful diagnostic aid.

Taxonomy *Aspergillus tamarii* belongs in subgenus *Circumdati* section *Flavi* (Gams et al. 1985), which was reviewed and revised by Varga et al. (2011a) and most recently by Frisvad et al. (2019). This section now includes 33 species. Frisvad et al. (2019) provide a very comprehensive account of the taxonomy of this section as well as details of mycotoxin and other major secondary metabolite production. Eight new species were also described. It is recommended that Frisvad et al. (2019) be consulted for further information.

Identifiers MycoBank MB191425; lectotype CBS 104.13 (Samson and Gams 1985); ex-lectotype cutures CBS 104.13 = NRRL 20818; ITS barcode AF004929; alternative markers *BenA* EF661474, *CaM* EF661526, *RPB2* EU012629 (Samson et al. 2014).

Physiology Although less has been published on the physiology of *Aspergillus tamarii* than on *A. flavus*, gross physiological behaviour of the two species is likely to be similar. Ayerst (1969) reported that *A. tamarii* was capable of growth down to 0.78 a_w at 33 °C, a figure identical with that which he obtained for *A. flavus*. Fastest growth of *A. tamarii* on high a_w media occurs between 30 and 35 °C, while on media controlled by glucose/fructose or NaCl fastest growth was at 0.95–0.92 a_w and 0.95 a_w, respectively (Mohamed et al. 2012). Germination on NaCl based media occurred down to 0.82 a_w at 25 °C and 30 °C and 0.85 a_w at 35 °C and 40 °C. *A. tamarii* germinated at 0.79 a_w on glucose/fructose media, the lowest tested (Mohamed et al. 2012).

Mycotoxins Despite some reports to the contrary (e.g. Goto et al. 1996), *Aspergillus tamarii* does not produce aflatoxins, but does produce cyclopiazonic acid (Dorner 1983; Frisdav et al. 2019). See Chap. 13. "Kodua poisoning" from kodo millet seed (*Paspalum scrobiculatum*) in India was attributed to cyclopiazonic acid produced by *A. tamarii* (Rao and Husain 1985), and perhaps to the alkaloid fumiclavine A, also found in kodo millet seed from that outbreak (Janardhanan et al. 1984). Antony et al. (2003) demonstrated that exposure to cyclopiazonic acid through ingestion of contaminated kodo millet may cause acute hepatotoxicity in men and animals.

Ecology While not as universally encountered in foods as *Aspergillus flavus*, *A. tamarii* is nevertheless of widespread occurrence in tropical and subtropical regions. Like *A. flavus*, it occurs commonly in nuts and oilseeds (see Pitt and Hocking 1997), including almonds (Rodrigues et al. 2011). Isolations from cereals have been infrequent. *A. tamarii* has been recorded from wheat, barley and sorghum (see Pitt and Hocking 1997). Other sources include soybeans and maize (Amusa et al. 2005; Sepulveda and Piontelli 2005), green coffee beans (Silva et al. 2003; Tharappan and Ahmad 2006), cocoa beans (Sánchez-Hervás et al. 2008), meat products (Mohamed and Hussein 2004), spices, peppercorns, salted, smoked and dried fish and a variety of tropical products including cocoa, palm kernels, maize, yams and melon ball snacks (see Pitt and Hocking 1997).

Adeniji (1970) reported isolation of *Aspergillus tamarii* from rotting yams, and it was reported as one cause of kernel rot in cashews (Esuruoso 1974). Amusa et al. (2003) isolated this species from African star apple.

Southeast Asian food commodities we examined were commonly infected with *Aspergillus tamarii*. Indonesian pepper was severely contaminated, although peppercorns harboured slightly lower rates of infection. Levels in peanuts were high, but lower in maize. *A. tamarii* was also found on kemiri nuts from Indonesia, peppercorns and soybeans from the Philippines, copra and black beans from Thailand and paddy rice from Indonesia. Other commodities, including cashews, mung beans and soybeans from Thailand and Indonesia, mung beans and paddy

and milled rice from the Philippines, and cowpeas from Indonesia showed low levels of infection (Pitt et al. 1993, 1994, 1998 and our unpublished data).

References Raper and Fennell (1965); Klich (2002); Frisvad et al. (2019).

Aspergillus terreus Thom Fig. 8.27

Colonies on CYA 40–50 mm diam, plane, low and velutinous, usually quite dense; mycelium white; conidial production heavy, pale pinkish brown to blonde; reverse pale to dull brown or

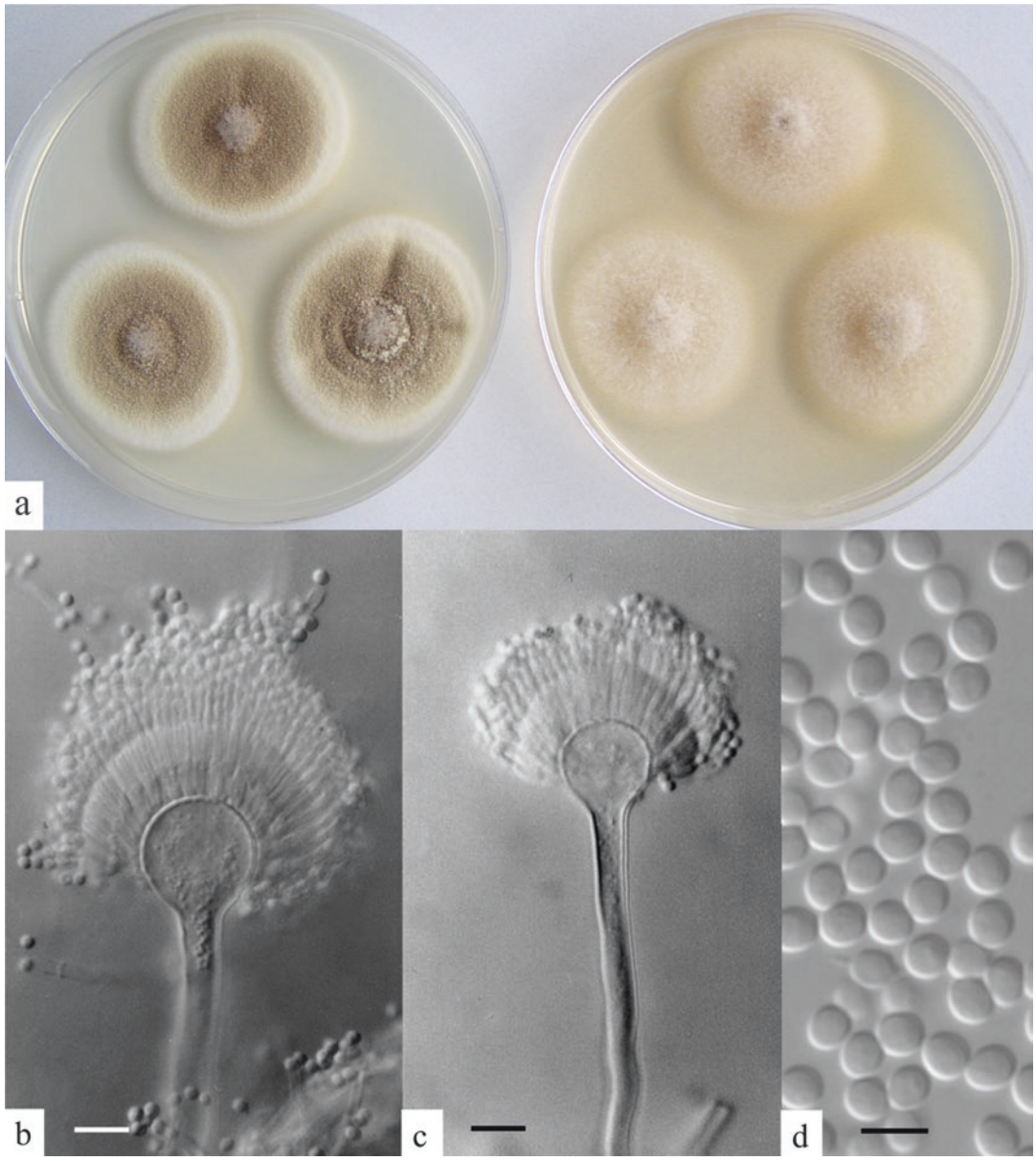

Fig. 8.27 *Aspergillus terreus* (**a**) colonies on CYA and MEA, 7 d, 25 °C; (**b**, **c**) heads, bars = 10 µm; (**d**) conidia, bar = 5 µm

yellow brown. Colonies on MEA 40–60 mm diam, similar to those on CYA or less dense. Colonies on G25N 18–22 mm diam, plane or irregularly wrinkled, low and sparse; conidial production light, pale brown; brown soluble pigment sometimes produced; reverse brown. No growth at 5 °C. Colonies at 37 °C growing very rapidly, 50 mm or more diam, of similar appearance to those on CYA at 25 °C.

Conidiophores borne from surface hyphae, stipes 100–250 µm long, smooth walled; vesicles 15–20 µm diam, fertile over the upper hemisphere, with densely packed, short, narrow metulae and phialides, both 5–8 µm long; conidia spherical, very small, 1.8–2.5 µm diam, smooth walled, at maturity borne in long, well defined columns.

Distinctive features Velutinous colonies formed at both 25 and 37 °C, uniformly brown, with no other colouration, and minute conidia borne in long columns, make *Aspergillus terreus* a distinctive species.

Taxonomy *Aspergillus terreus* was placed in *Aspergillus* subgenus *Nidulantes* section *Terrei* by Gams et al. (1985) but more recent molecular information indicated that this section was more appropriately placed in Subgenus *Circumdati* (Houbraken and Samson 2011). The section was extensively studied by Samson et al. (2011), who raised some varieties of *A. terreus* to species level and transferred some species previously placed in *Aspergillus* subgenus *Nidulantes* sections *Flavipedes* and *Versicolores* to section *Terrei*. Samson et al. (2011) provided descriptions of new species but not *A. terreus*.

A sexual state for *Aspergillus terreus* was described by Arabatzis and Velegraki (2013). They crossed strains determined to have either MAT1–1 or MAT1–2 genes and in a few cases found well defined bodies which contained 3–5 cleistothecia. Cleistothecia were 70–250 × 55–160 µm and contained subspherical to pyriform asci which contained eight ascospores, spherical to broadly ellipsoidal, approximately 4.5–9 µm in length, smooth walled, hyaline to pale yellow and with a single equitorial ridge. The teleomorph of *A. terreus* is most similar to that of *A. flavipes* (Arabatzis and Velegraki 2013).

Identifiers MycoBank MB191719; lectotype IMI 17294 (Samson and Gams 1985); ex-lectotype cultures CBS 601.65 = IMI 17294 = ATCC 1012 = NRRL 255 = FRR 266; ITS barcode EF669586; alternative markers *BenA* EF669519, *CaM* EF669544, *RPB2* EF669628 (Samson et al. 2014).

Physiology Growth data reported here indicate that *Aspergillus terreus* is thermophilic, but there appear to be no published data on this aspect of its physiology. A minimum a_w for growth of 0.78 at 37 °C has been reported (Ayerst 1969).

Mycotoxins *Aspergillus terreus* produces a wide range of metabolites (Frisvad and Samson 1991; Samson and Frisvad 2004), but of these only territrems appear to have significant toxicity. Territrems are tremorgenic toxins which, unlike all other tremorgens, lack a moiety containing nitrogen. Maximum toxin production occurred at 28 °C following 12 days' incubation in potato dextrose liquid medium (Fang and Peng 2003). Territrem B synthesis increased at alkaline pH. Immunoelectron microscopy revealed that the toxin was localised in the cytoplasm of conidia after a minimum of four days growth on potato dextrose medium. No toxin was detected in the fungal hyphae (Peng et al. 2004). These toxins have not been implicated in disease (Cole and Dorner 1986; Ling 1994) and their importance is a matter for conjecture.

Ecology Although not usually a cause of spoilage in foods, *Aspergillus terreus* is of common occurrence. It is well adapted to growth in stored foods in tropical and subtropical climates. It commonly occurs in nuts, e.g. pistachios (Heidarian et al. 2006), peanuts, hazelnuts, walnuts and pecans (see Pitt and Hocking 1997). Reports from cereals include maize (Bhattacharya and Raha 2002; Sepulveda and Piontelli 2005) and maize meal (Souza et al. 2003), wheat (Hajjaji et al. 2006), barley, paddy rice and parboiled rice (see Pitt and Hocking 1997). *A. terreus* has also been reported from animal feeds containing a mixture of cereals (Magnoli et al. 2005) or from silage (El-Shanawany et al. 2005). This species has been found in cassava flour (Souza et al. 2003), flour and refrigerated dough products, pasta, miso and soy sauce (see Pitt and

Hocking 1997). Other records include soybeans (Sepulveda and Piontelli 2005), chickpeas and mungbeans (Javaid et al. 2005), rapeseed, blackgram seeds, dried beans and peas, meat and meat products, biltong (see Pitt and Hocking 1997), spices (Mandeel 2005), stored coffee beans (Ahmad and Magan 2003), dry salted fish (Gräu et al. 2007), cheese and curds (Kumaresan et al. 2003).

Pathogenicity *A. terreus* is the third most important pathogenic *Aspergillus* species, responsible for about 10% of cases of human pulmonary aspergillosis, and it is regularly implicated in a wide range of other conditions including cutaneous and ophthalmic infections (Arabatzis and Velegraki 2013). German Shepherd dogs appears to be particularly susceptible to *A. terreus* infections (De Hoog et al. 2000).

References Raper and Fennell (1965); Klich (2002); Samson et al. (2011).

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Xerophiles

9

Xerophilic fungi are distinguished by their ability to grow under conditions of reduced water activity, i.e. to complete their life cycles on substrates that have been dried or concentrated, in the presence of high levels of soluble solids such as salts or sugars. Early usage (Scott 1957) confined the word "xerophile" to filamentous fungi and used the term "osmophile" for yeasts; the term "halophile" was used rather indiscriminately for moulds, yeasts and bacteria with the ability to grow on concentrated salt solutions.

The terminology used for fungi which grow at low a_w was discussed by Pitt (1975). He pointed out that osmophile was an inappropriate term, because high osmotic pressures were not involved in the growth of these fungi, as they balance the outside environment with internal solutes, maintaining just sufficient osmotic pressure to enable growth. The crucial point was that they preferred to grow at reduced water activities. In the absence of a suitable term for "lovers of low water activity", xerophile seemed the most suitable appellation, for both moulds and yeasts.

Some other authors have used the term "xerotolerant" in place of xerophile, arguing that these fungi exhibit "tolerance of" not "love for" reduced a_w. However, xerotolerant is an inappropriate term for extremely xerophilic fungi such as *Xeromyces bisporus, Bettsia alvei* and *Xerochrysium dermatitidis*, which have an absolute requirement for, not tolerance of, reduced a_w. Secondly, although many xerophiles grow optimally at quite high a_w, most are basically slowly growing fungi and cannot compete in mixed cultures at high a_w levels such as prevail in soils or fresh foods. Survival of xerophiles in nature depends on access to environments of reduced a_w where competition is restricted or eliminated. In this vital sense they are lovers of lowered water activity, i.e., xerophiles.

Xerophilic fungi may be defined in a variety of ways, usually relating to minimal or optimal a_w for growth. The definition of Pitt (1975) is used here: a xerophile is a fungus capable of growth, under at least one set of conditions, at a water activity below 0.85. This has proved to be a practical working definition.

In discussing the use of the word halophile for fungi, Pitt (1975) wrote that it was inappropriate because there were no known fungi with a preference for salt environments. More recently, however, such fungi have been shown unequivocally to exist, exhibiting quite superior growth on media with NaCl as controlling solute. *Scopulariopsis halophilica* Tubaki (now *Phialosimplex halophilus*) was the first; Pitt and Hocking (1985) added *Polypaecilum pisce*. Perhaps there are others. Such fungi are still classed as xerophiles: if there is a need to differentiate them from other xerophiles, the term "halophilic xerophile" is appropriate (Andrews and Pitt 1987).

For practical reasons, "xerophile" as used in this book has a different, much narrower

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circumscription than the definition given above. Here the definition relates solely to responses when grown on the standard media. A species has been included, or at least keyed, in this chapter if colony diameters on G25N after 7 days at 25 °C exceed those on CYA and MEA. Many marginally xerophilic fungi which meet the definition of Pitt (1975) do not meet this criterion, and have simply been keyed in other appropriate chapters.

In this chapter, *Aspergillus* subgenus *Aspergillus* (*Eurotium*) species and a few other Aspergilli are keyed out with a miscellaneous group of other fungi. To maintain an orderly presentation, those *Aspergillus* species have been described in Chap. 8. The miscellaneous fungi are described below, in alphabetical order.

Note that in the key which follows, couplets 1 to 3 are based on growth on G25N and sort out species which are placed elsewhere. It is not possible satisfactorily to differentiate the remaining xerophiles on this medium. The subsequent couplets, 4 to 8, are based on colonies grown on MY50G agar (see Chap. 4). So before entering the key at couplet 4 it will be necessary to inoculate the unknown isolate onto MY50G agar and incubate at 25 °C for 14 days, and perhaps longer, until fruiting structures differentiate and mature.

Key to Xerophilic Fungi

1	Bright yellow, barely macroscopic spherical bodies (cleistothecia) visible in the aerial mycelium of colonies on G25N (grow on CY20S agar and use key in Chap. 8)	*Aspergillus* subgenus *Aspergillus* (*Eurotium*)
	Cleistothecia not visible in colonies on G25N	2
2(1)	Colonies on G25N showing yellow or green conidial colours (use key in Chap. 8)	*Aspergillus*
	Colonies on G25N white, brown or black	3
3(2)	Colonies on G25N chocolate brown	*Wallemia*
	Colonies on G25N white, pale brown or black (grow on MY50G)	4
4(3)	Colonies on MY50G white or pale brown	5
	Colonies on MY50G black or with black areas	*Bettsia*
5(4)	Colonies on CYA and MEA 10 mm diam or more in 7 days; conidia on MY50G lemon shaped	*Polypaecilum*
	Colonies on CYA and MEA not exceeding 10 mm diam in 7 days; conidia on all media spherical of ellipsoidal	6
6(5)	On MY50G, solitary asci produced, containing or releasing mature ascospores in 14 days	*Eremascus, Skoua*
	Mature asci and ascospores not evident in cultures on MY50G in 14 days	7
7(6)	Colonies on MY50G producing spherical to cylindroidal aleurioconidia (solitary conidia on short pedicels, Fig. 9.1) or similar conidia in 14 days	8
	Colonies on MY50G not producing aleurioconidia in 14 days; 3–celled cleistothecial initials or developing cleistothecia may or may not be evident	*Xeromyces*
8(7)	Intercalary chlamydoconidia and arthroconidia present; aleurioconidia on tiny pedicels or solitary	9
	Intercalary chlamydoconidia and arthroconidia absent; aleurioconidia on short conidiophores, sometimes in short chains	*Phialosimplex*
9(8)	Colonies on MY50G exceeding 15 mm diam in 7 days; dominant conidial type solitary aleurioconidia	*Bettsia*
	Colonies on MY50G not exceeding 15 mm diam in 7 days, dominant conidial types intercalary chlamydoconidia and arthroconidia (Fig. 9.7)	*Xerochrysium*

9.1 Genus *Bettsia* Skou

The genus *Bettsia* was erected by Skou (1975) as a new name for *Pericystis* Betts 1912 (*non Pericystis* J. Agardh 1848). *Bettsia* is a relict genus of Ascomycetes characterised by the formation of small black cleistothecia with a distinctive three celled appendage (Fig. 9.1). Asci are evanescent, and ascospores have smooth, dark walls. The type species, *B. alvei* (Betts) Skou ex Pitt et al., is the only species known to produce this sexual state. It also produces a hyphomycete anamorph, with aerial hyphae bearing solitary aleurioconidia and intercalary chlamydoconidia. Skou (1975) provided a name for this state in the asexual genus *Chrysosporium, C. farinicola* (Burnside) Skou. That name was used by Pitt and Hocking (2009). However, more recent molecular analysis (Pitt et al. 2013) showed that *Chrysosporium* as currently circumscribed belonged to six different clades. The species placed in *Chrysosporium* by Pitt and Hocking (2009), all of which are xerophiles and belong in two clades, are unrelated to the other four clades, which are not xerophiles. One clade of xerophiles, including *Bettsia*, lies in the Order Leotiales, with a possible relationship to the genus *Pseudeurotium* J.F.H. Beyma (Pitt et al. 2013). This clade is considered here. The other xerophilic clade, with the new name *Xerochrysium,* lies in the *Trichocomaceae* (Pettersson et al. 2011) and is considered separately below.

One other species, related morphologically, physiologically and molecularly to *B. alvei*, but with no known sexual state, also belongs here: *B. fastidia* (Pitt) Pitt.

Key to *Bettsia* Species

1.	Colonies on MY50G pale yellow or brown with a yellow brown reverse, sexual state unknown	*B. fastidia*
	Colonies on MY50G often persistently white with a pale reverse; but a sexual state sometimes produced, then showing dark grey to black areas	*B. alvei*

Bettsia alvei (Betts) Skou ex Pitt et al. Fig. 9.1

Bettsia alvei (Betts) Skou (invalid name)
Ascosphaera alvei (Betts) L.S. Olive & Spiltoir
Pericystis alvei Betts
Chrysosporium farinicola (Burnside) Skou
Ovularia farinicola Burnside

No growth on CYA at 5, 25 or 37 °C or on MEA. Colonies on G25N 10–20 mm diam, of low, white mycelium, or if the teleomorph is present, centrally grey from the production of immature cleistothecia; margins fimbriate; reverse pale, but grey centrally if the *Bettsia* state is being produced. Colonies on MY50G growing relatively rapidly, at 7 days 20–30 mm diam, low, plane, persistently white, or showing sectors becoming translucent or greyish if the *Bettsia* state is present, reverse pale beneath white areas, but darker beneath grey sectors; at 14 days, 40–65 mm diam, colonies remaining white if only the anamorph is present, but with dark grey to black areas in the presence of the teleomorph; reverse pale, or dark under the teleomorph.

Reproduction on MY50G predominantly solitary aleurioconidia, broadly ellipsoidal to pyriform, thick walled, highly refractile, 7–11 µm long. Fertile hyphae mostly undifferentiated, dissolving in age. In isolates producing the teleomorph, areas or sectors of translucent growth developing. At maturity such areas becoming grey and then black as small cleistothecia formed. Cleistothecia formed on MY50G at 25 °C, dark brown to black, usually maturing only after several weeks at 15–25 °C, 25–60 µm diam, with walls thin and smooth, and without internal structure; initials a row of three short cells, 12–18 × 6–8 µm overall, adhering to the cleistothecial wall as a distinctive appendage; ascospores not liberated readily, spherical, 5–6 µm diam, with dark walls, smooth to minutely roughened.

Distinctive features *Bettsia alvei* is distinguished from *B. fastidia* by the following features: (1) colonies on MY50G grow more rapidly; (2) colonies on G25N and MY50G remain pure white, with reverses virtually uncoloured; and (3) terminal chlamydoconidia are often larger, up to

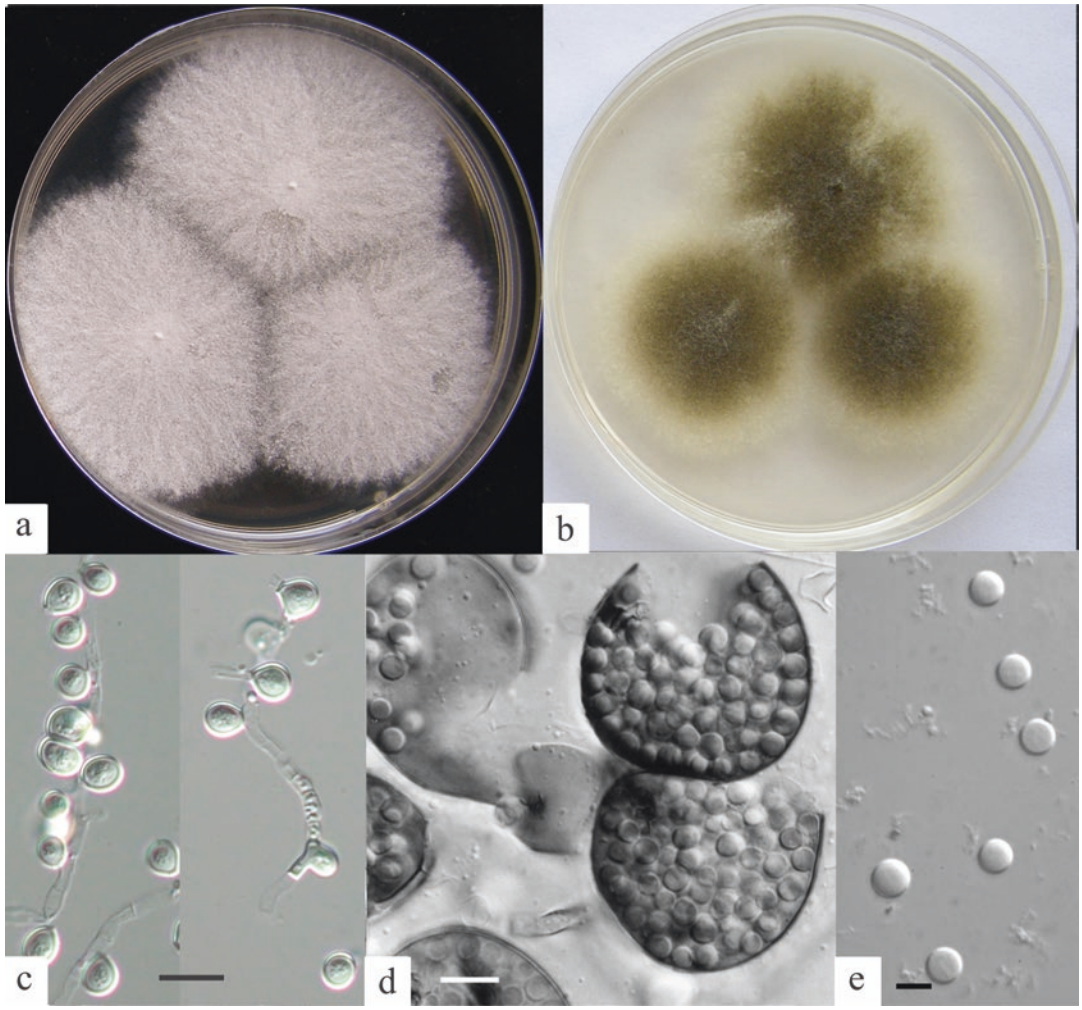

Fig. 9.1 *Bettsia alvei* (**a**) colonies of the asexual state alone, and (**b**) with the sexual state also present, both on MY50G, 14 d, 25 °C; (**c**) aleurioconidia, bar = 10 µm; (**d**) mature cleistothecia containing ascospores, bar = 25 µm; (**e**) ascospores, bar = 5 µm

13–18 µm in diameter. In addition, some isolates of *B. alvei* produce the sexual state. No teleomorph is known for *B. fastidia*.

Taxonomy In previous editions of this book, this species was known as *Chrysosporium farinicola*. As mentioned in the genus preamble, molecular studies showed that this species was unrelated to the type of that genus (Pitt et al. 2013). With the change to one fungus, one name, this species is logically known by its teleomorph name, *Bettsia alvei*. That combination was described by Skou (1975) and accepted in previous editions of this book. However, it has been pointed out that the combination was invalid, as the basionym (basal name) was not correctly indicated in the protologue (K. Bensch, pers. comm.). A valid combination was provided by Pitt et al. (2013) and that is used here. Note that in formal publications this species is correctly called *Bettsia alvei* (Betts) Skou ex Pitt et al. or *Bettsia alvei* (Betts) Pitt et al. but not *Bettsia alvei* (Betts) Skou.

Identifiers Mycobank MB631273; neotype CBS 688.71 (Skou 1975); ex-type cultures CBS 688.71, IMI 160840; marker 28S (partial) KR139933.1.

Mycotoxins No mycotoxins are known from this species.

Physiology *Bettsia alvei* was able to germinate at 0.73 a_w at 25 °C and to grow down to at least 0.78 a_w at 20–30 °C, with an optimum near 25 °C (Pitt et al. 2013). Studies on recovery of heat stressed *B. alvei* aleurioconidia showed that older cultures were less heat sensitive than 14 day cultures (Beuchat and Pitt 1990b). Twenty day old aleurioconidia showed less than one log reduction after heating at 56 °C for 10 min. Aleurioconidia survived well in maize starch at 0.43 and 0.71 a_w at 1 °C and 25 °C, showing no reduction in viable numbers after 45 days storage. Heat stressed *B. alvei* aleurioconidia grew better on glucose based media than on media containing sorbitol, glycerol or NaCl (Beuchat and Pitt 1990b). Isolates of *B. alvei* were able to grow in the presence of 1.05–1.76% (w/v) ethanol and at temperatures ranging from 4–25 °C (Kinderlerer 1997). Growth was recorded in a controlled atmosphere including 6% O_2 or 10% CO_2 in 20% O_2 (Kinderlerer 1997). Weak protease activity and moderate lipolytic activity were reported for *B. alvei* from Kenyan coconut (Ismail 2001).

Ecology Recent studies have shown that the natural habitat for *Bettsia* is in association with bees, beehives and honey (Wynns 2015). *B. alvei* (usually as *Chrysosporium*) has been isolated from a range of low a_w substrates, including honeycomb, prunes and prune processing equipment, sultanas, mixed dried fruit, chocolate, jelly crystals and coconut from Australia, the United Kingdom, Sri Lanka, Czechoslovakia, Denmark and Kenya (Kinderlerer 1997; Ismail 2001; FRR and CBS culture catalogues). This species can cause cheesy butyric spoilage in coconut (Kinderlerer 1984).

References Skou (1975); van Oorschot (1980); Pitt et al. (2013); Wynns (2015).

Bettsia fastidia (Pitt) Pitt Fig. 9.2

No growth on CYA at 5, 25 or 37 °C, or on MEA. Colonies on G25N 1–5 mm diam, of low, dense, white mycelium. Colonies on MY50G at 7 days 15–22 mm diam, low, plane and sparse, pale yellow or brown, reverse yellow brown; at 14 days, 35–42 mm diam, low and plane, margins sparse and fimbriate, white, centres more dense, dull yellow; reverse yellow to pale brown.

Reproduction on MY50G predominantly by smooth walled aleurioconidia borne singly on short pedicels or less commonly sessile, spheroidal (oblate or prolate) to broadly ellipsoidal, 6–9 × 5–8 µm, in age released by dissolution of the pedicels; terminal chlamydoconidia, spherical to pyriform or pedunculate, 8–12 × 6–10 µm, also produced, but intercalary chlamydoconidia and arthroconidia rare. No teleomorph known.

Distinctive features *Bettsia fastidia* forms dull yellow to yellow brown colonies; conidia are predominantly aleurioconidia with few intercalary chlamydoconidia or arthroconidia produced on G25N or MY50G. It closely resembles *B. alvei* from which it can be distinguished by colony colour and other features listed under *B. alvei.*

Taxonomy Van Oorschot (1980) placed this species in synonymy with *Chryosporium farinicola* (= *B. alvei*), however the culture she used was incorrectly labelled (Pitt et al. 2013). Pitt et al. (2013) recombined this species into *Bettsia* in accordance with McNeill et al. (2012).

Identifiers Mycobank MB806122; holotype UAMH 2368 (Pitt 1966); ex-type cultures CBS 154.67, IMI 126288, ATCC 18053, FRR 77; ITS barcode CBS 154.67_ex39243_72187_ITS (CBS 2007).

Physiology A mesophilic obligate xerophile, *Bettsia fastidia* has a maximum a_w for growth of 0.98 and a minimum of 0.69 (Pitt and Christian 1968). It does not utilise nitrate and appears to require accessory factors for growth. Conidia have an unexceptional heat resistance (Pitt and Christian 1970).

Mycotoxins No mycotoxins are known from this species.

Ecology This species has been repeatedly isolated from prunes (dried and high moisture) and prune processing machinery in N.S.W., Australia. There are no records of isolation from other substrates or other locations, indicating that *B. fastidia* is a rare species with a restricted habitat.

References Pitt (1966); Pitt et al. (2013).

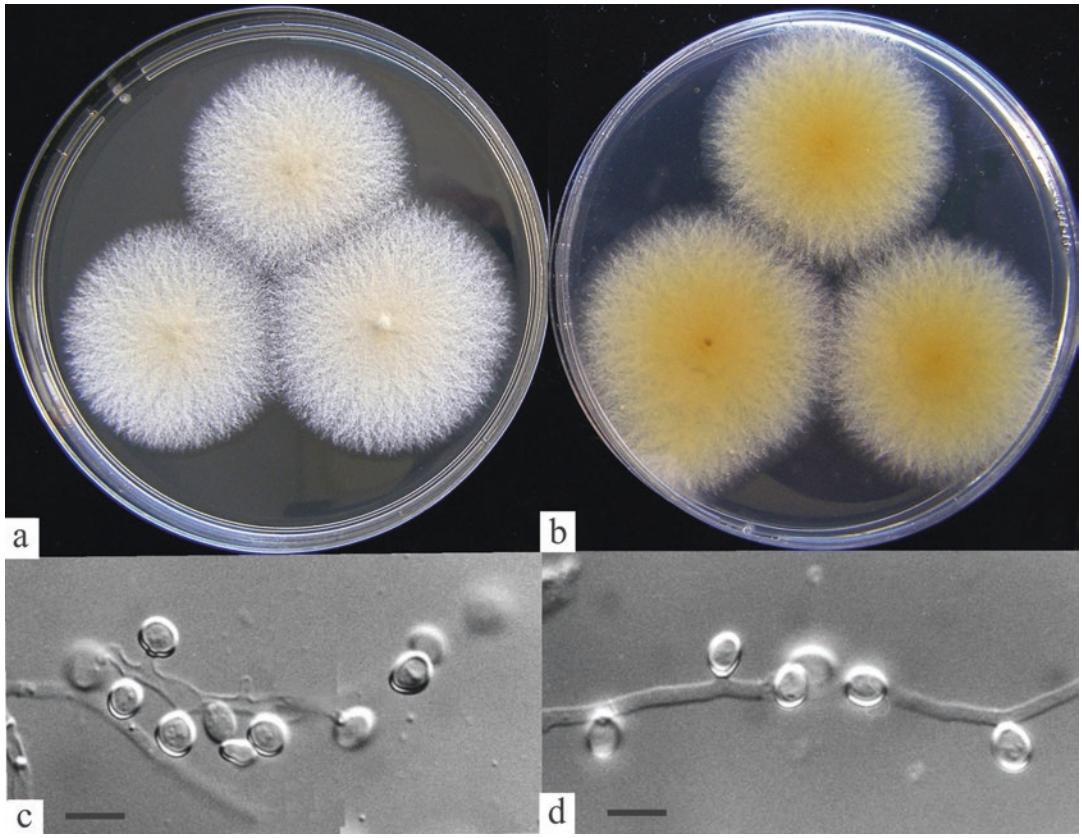

Fig. 9.2 *Bettsia fastidia* (**a**) colonies on MY50G, 14 d, 25 °C; (**b**, **c**) aleurioconidia, borne on pedicels, bar = 10 µm

9.2 Genera *Eremascus* Eidam and *Skoua* Wynns

As traditionally circumscribed, *Eremascus* was a small genus with only two known species distinguished by the formation of asci borne singly from undifferentiated hyphae without any surrounding wall or hyphal network. Molecular analysis of the ITS region placed one species, *E. albus,* in the Eurotiomycetidae along with *Aspergillus, Penicillium* and other genera described here (Berbee and Taylor 1992). A more recent analysis (Wynns 2015) confirmed that placement, and showed that the bee pathogen *Ascosphaera apis* is a near neighbour. This analysis indicated that the other *Eremascus* species, *E. fertilis,* is quite distant, belonging in the Leotiomycetes, near *Bettsia* (Wynns 2015). This is surprising, as the only clear morphological difference between the two species is the difference in coiling of suspensor cells supporting the ascus. Wynns (2015) suggested this has resulted from convergent evolution, as both species are associated with bees in nature – as are both *Ascosphaera* and *Bettsia*, their respective near neighbours phylogenetically. The new genus *Skoua* was erected by Wynns (2015) to accommodate *E. fertilis.*

Both *Eremascus albus* and *Skoua fertilis* are strict xerophiles, growing only at reduced a_w. Colonies are floccose and remain persistently white, and apparently sterile. Under the compound microscope, abundant asci can be seen. *E. albus* is distinguished from *S. fertilis* by differences in the cells supporting the asci: in *E. albus* these cells are coiled, while in *S. fertilis* they are uncoiled. Both have been reported extremely rarely, but are of sufficient interest to be included here, partly in the hope that this will lead to further isolations of these unusual xerophilic fungi, whose primary habitat is likely to be associated with bees (Wynns 2015).

Eremascus albus Eidam **Fig. 9.3**

No growth on CYA at 5, 25 or 37 °C, or on MEA. Colonies on G25N at 7 days 2–3 mm diam, convex, of white mycelium; at 14 days, 11–12 mm diam, convex, centrally 3–4 mm deep, of floccose white mycelium; reverse uncoloured; occasionally no growth at all. Colonies on MY50G at 7 days 4–5 mm diam, of low, sparse white mycelium; at 14 days, 14–17 mm diam, deep and floccose, similar to those on G25N.

Reproductive structures solitary asci borne laterally from vegetative hyphae on a pair of spiral suspensors (ascus initials) coiled 2–3 turns, and originating from adjacent cells; asci maturing within 14 days on G25N and MY50G, 12–14 µm diam; ascospores broadly ellipsoidal, 6–8 × 5.0–6.5 µm, hyaline and smooth walled. Terminal chlamydoconidia, measuring 7–15 µm diam, occasionally produced.

Distinctive features See genus preamble.

Identifiers Mycobank MB221322; type unknown; representative cultures CBS 239.50, ATCC 11665, FRR 92; marker LSU CBS 239.50_ex29507_47135_LSU (CBS 2007).

Physiology *Eremascus albus* is a mesophilic obligate xerophile, with a maximum a_w for growth between 0.98 and 0.997 and a minimum of 0.70 a_w (Pitt 1975). Heat resistance of the ascospores is not known, but may be expected to be quite high.

Mycotoxins No mycotoxins are known from this species.

Ecology This is a rare fungus: described in 1881 by Eidam, it was not reported again until nearly 70 years later, following its discovery in several samples of English mustard stored for long periods (Harrold 1950). In this laboratory, it has been isolated once from high moisture prunes, and once from spoiled mustard. Occasional further isolations from foods have been recorded in culture collection catalogues.

Additional species As stated above, *Skoua fertilis* (Stoppel) A.A. Wynns is not closely related to *E. albus* but is so similar morphologically, and equally so rare, that it is best treated here. In culture it is distinguished from *E. albus* primarily by forming asci in which the suspensors are not coiled around each other (Fig. 9.3). Other differences exist: *S. fertilis* will sometimes grow slightly on CYA or MEA, and asci and

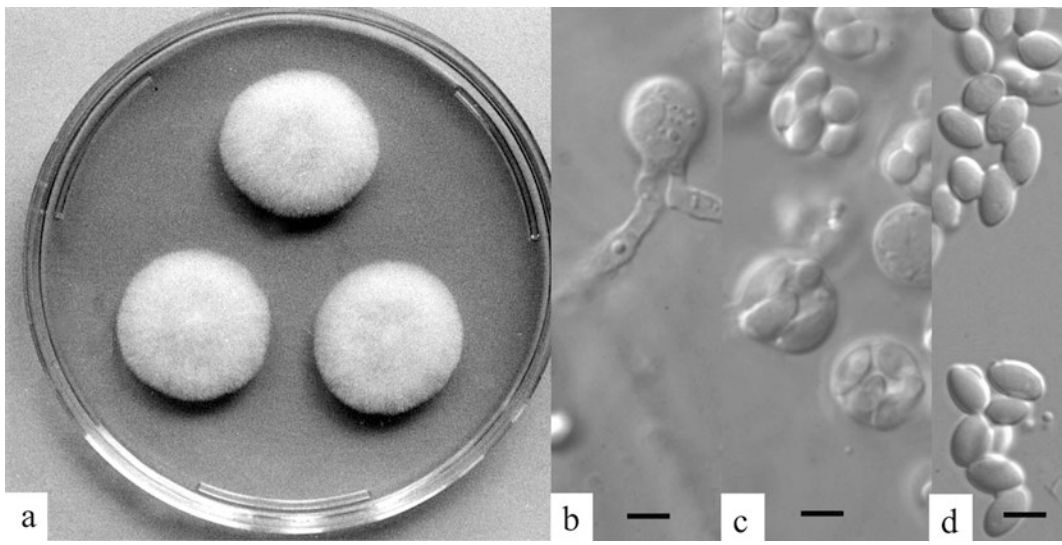

Fig. 9.3 *Eremascus* and *Skoua* species (**a**) colonies of *Eremascus albus* on MY50G, 7 d, 25 °C; (**b–d**) *Skoua fertilis*, (**b**) conjugating hyphal elements forming an ascus, bar = 5 µm; (**c**) asci containing ascospores, bar = 5 µm; (**d**) ascospores, bar = 5 µm

ascospores are smaller, 7–10 µm diam and 5–7 × 2.5–3.5 µm, respectively. Identifiers: MB551199; holotype Herb C, Wynns 5159 (Wynns 2015). Although it seems likely that CBS 103.09 is type material, as it was received from Germany only two years after Stoppel described this species, and no other isolates were known for many years, no proof exists. Therefore the type designated by Wynns (2015) stands. Representative cultures: CBS 175.71; ATCC 22794; FRR 87.

Nothing is known of this species' physiology and little of its ecology**.** It has been reported only rarely, one occasion being from high moisture prunes in this laboratory (Pitt and Christian 1968), other isolations being from preserved fruit, honeycomb and cake (CBS 2007).

Reference Harrold (1950); Wynns (2015).

9.3 Genus *Phialosimplex* Sigler et al.

In *Phialosimplex*, conidia are borne in short chains on single phialides borne from simple, short conidiophores, cut off successively from the tip of the conidiophore so that it shortens as the conidia form. Conidia often separate at maturity. This provides a distinction from *Bettsia* and *Xerochrysium,* as in these two genera aleurioconidia are formed solitarily. Moreover *Phialosimplex* species do not produce conidia borne along the lengths of the vegetative hyphae, i.e. chlamydoconidia and arthroconidia. The genus includes several species, but only one has been recorded from foods, *Phialosimplex halophilus.*

Phialosimplex halophilus was originally described as *Oospora halophila* by van Beyma, was transferred to *Scopulariopsis* by Tubaki, then to *Basipetospora*, the anamorph of *Monascus* species, by Hocking and Pitt (1984). Molecular studies (Houbraken and Samson 2011) indicated that this species was closely related to species in *Phialosimplex* Sigler et al. This was confirmed by Greiner et al. (2014) who made the transfer.

Samson et al. (2014) transferred the genus *Phialosimplex* to *Aspergillus*. That transfer is not accepted here, for reasons discussed under *Aspergillus* in Chap. 8.

Phialosimplex halophilus (J.F.H. Beyma) Greiner et al. Fig. 9.4

Oospora halophila J.F.H. Beyma
Scopulariopsis halophilica Tubaki
Basipetospora halophila (J.F.H. Beyma) Pitt & A.D. Hocking
Aspergillus baarnensis Samson et al.

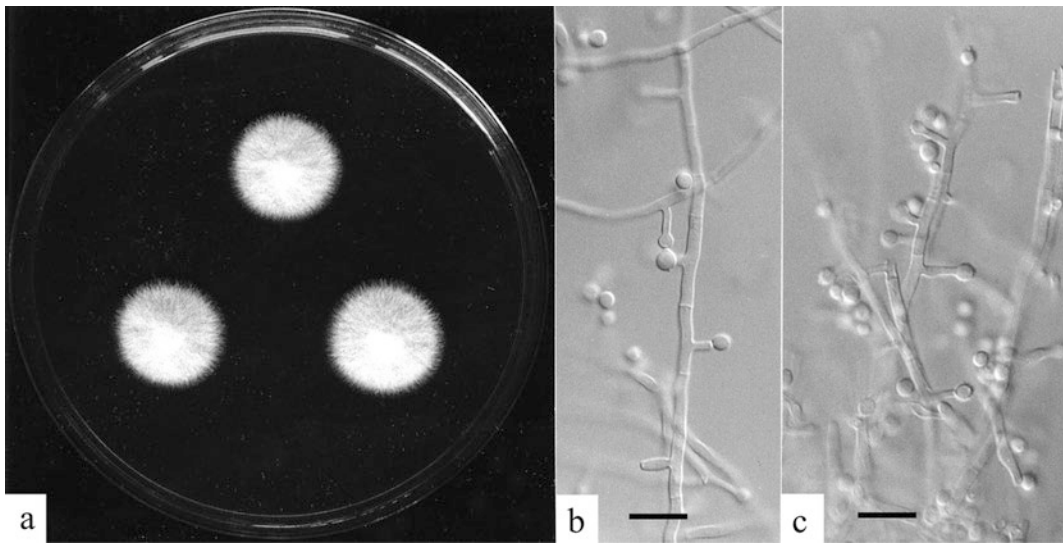

Fig. 9.4 *Phialosimplex halophila* (**a**) colonies on MY5-12, 14 d, 25 °C; (**b**, **c**) conidiophores and aleurioconidia, bars = 20 µm

No growth on CYA at 5, 25 or 37 °C or on MEA. Colonies on G25N at 14 days 4–8 mm diam, occasionally 12 mm, of dense and tough mycelium, centrally raised, sulcate or irregularly wrinkled; mycelium persistently white, with little or no sporulation; reverse pale. Colonies on MY50G at 14 days 4–8 mm diam, similar to those on G25N. Colonies on MY5-12 at 14 days 10–16 mm diam, low or umbonate, plane or irregularly wrinkled, of dense mycelium overlaid by floccose to funiculose aerial hyphae; mycelium persistently white, sporulation light to moderate, in lower layers of the mycelium; reverse pale to yellow brown. Colonies on MY10-12 at 14 days 18–22 mm diam, similar to those on MY5-12, but growth more rapid and vigorous; sporulation in surface mycelial layers moderate to heavy; mycelium and conidia persistently white; reverse pale to yellow brown.

Reproductive structures short, solitary conidiophores borne at irregular intervals along vegetative hyphae, sometimes bearing a short chain of conidia, but more commonly a single developing conidium, shed at maturity and succeeded by another blown out terminally from the conidiophore, the conidiophore shortening a little with each successive conidium; conidiophores often curved, usually cylindrical, 2.0–3.0 µm diam, but sometimes narrowing towards the apex; when young 8–20 µm long, in age down to 3–4 µm long, smooth walled; mature conidia spherical to broadly ellipsoidal or pyriform with a truncate base, 3.5–6.0 µm diam, with heavy walls, smooth to finely roughened, in wet mounts usually solitary, but sometimes in chains of 3 or 4. No teleomorph known.

Distinctive features Although it superficially resembles *Xerochrysium* species, *Phialosimplex halophilus* is readily distinguished by (1) very slow growth on MY50G; (2) aleurioconidia sometimes in short chains; and (3) the absence of intercalary conidia in the vegetative hyphae.

Taxonomy This distinctive species was described as *Oospora halophila* by van Beyma (1933). The name was not used by later authors, however. Tubaki (1973) described *Scopulariopsis halophilica* in terms which did not lead to association with van Beyma's species. Pitt and Hocking (1985) established that *O. halophila* and *S. halophilica* were a single species and transferred it to *Basipetospora*, the anamorph of *Monascus*. However, as noted in the genus preamble, molecular studies indicated that this species is not closely related to *Monascus*, but belongs in *Phialophora*, close to *P. salinarus* Greiner et al.

Samson et al. (2014) transferred the genus *Phialosimplex* to *Aspergillus*. As the name *Aspergillus halophilicus* was already occupied, *P. halophilicus* was given the new name *Aspergillus baarnesis*, thus losing all connection of this species with its salty habitats. That transfer is not accepted here, for reasons discussed under *Aspergillus* in Chap. 8.

Identifiers Mycobank MB810710; lectotype CBS 232.32 (Pitt and Hocking 1985); ex-type cultures CBS 232.32, ATCC 42877, FRR 2184, CBS 129.65_ex39287_76466_ITS; LSU CBS 129.65_ex39287_76658_LSU (CBS 2007). ITS barcode

Physiology The differences in rates of growth of this fungus on MY50G and MY5-12, media of similar water activity and nutritional status, show clearly that *Phialosimplex halophilus* is a true halophilic xerophile. Detailed studies on the water relations of this species confirmed this observation (Andrews and Pitt 1987; Wheeler et al. 1988b). Three isolates of *P. halophilus* germinated in 6–11 days on a saturated NaCl medium (0.747 a_w). Germination was slower on media containing glucose/fructose or glycerol as controlling solute; however one isolate germinated at 0.73 a_w in 40 days on glucose/fructose. The lowest a_w for germination on glycerol media was 0.78. Growth rates of three isolates were similar, but showed marked differences on the different media: the radial growth rate on NaCl at 0.75 a_w (10 µm/hr) was comparable with the maximum rate observed on glucose/fructose, at 0.90 a_w. Maximum growth rates on NaCl, *ca* 30 µm/hr., occurred at 0.87–0.88 a_w. Strong growth of *P. halophilus* on NaCl based media was also demonstrated by Diyaolu and Adebajo et al. (1994).

The optimum temperature for growth of *Phialosimplex halophilus* is near 30 °C, although it was more tolerant of high a_w conditions at

lower temperatures, growing at 0.99 a_w on NaCl based media at 25 °C. At 37 °C, *P. halophilus* demonstrated slow growth on NaCl based media, and over only a limited a_w range, 0.86–0.77 a_w (Wheeler et al. 1988b). The maximum temperature for growth is probably not much higher than 37 °C. The minimum temperature for growth is not known.

Phialosimplex halophilus is well adapted to its ecological niche – dried, salted seafoods and similar substrates. Studies on competition between common xerophiles such as *Aspergillus* subgenus *Aspergillus* (*Eurotium)* species and halophilic xerophiles growing together on salted fish have clearly shown that *P. halophilus* and the other halophilic xerophile, *Polypaecilum pisce,* can outgrow *Aspergillus* species below about 0.87 a_w under these conditions (Wheeler and Hocking 1993).

Mycotoxins No mycotoxins are known from this species.

Ecology *Phialosimplex halophilus* is a halophilic xerophile which is restricted to salty environments. Most isolates of *P. halophilus* have come from salted, dried fish: it has been reported from dried or cured fish from Japan, Indonesia, the Philippines, Sri Lanka and Nigeria (see Pitt and Hocking 1997). Other sources include dried food grade seaweed in Japan, a gelatine hydrolysate (Tubaki 1973) and sea salt (as *Scopulariopsis halophilica*; CBS 2007).

References Pitt and Hocking (1985); Greiner et al. (2014).

9.4 Genus *Polypaecilum* G. Sm

The genus *Polypaecilum* was established by Smith (1961) to accommodate species characterised by the production of cells which resemble phialides but from which conidia are produced at more than one aperture. These structures are known as polyphialides. (Some species of *Fusarium* produce structures of this kind). An unnamed halophilic xerophile found on salt fish in tropical regions showed a similar characteristic, so it was named *Polypaecilum pisce* (Pitt and Hocking 1985). This species showed little other resemblance to the type species and addition to the genus was based purely on polyphialide production. It was surprising to find that molecular studies showed the two species to be closely related (Houbraken and Samson 2011). *Polypaecilum* was transferred to *Aspergillus* by Samson et al. (2014). That transfer is not accepted here, for reasons discussed under *Aspergillus* in Chap. 8.

In *Polypaecilum pisce* the polyphialides are very large, up to 60 µm long, sometimes with a distinct resemblance to a human forearm, hand and fingers. Other species have not been isolated from foods.

Polypaecilum pisce A.D. Hocking & Pitt **Fig. 9.5**

Aspergillus pisce (as "pisci")
(A.D. Hocking & Pitt) Houbraken et al.

On CYA and MEA at 14 days, colonies 15–20 mm diam, usually low and sparse, sometimes centrally umbonate or irregularly wrinkled, with a dense, velutinous or weakly funiculose texture; sporulation light, mycelium and conidia persistently white; on CYA, sclerotia formed by a few isolates, white to buff, 250–400 µm diam, with walls of pseudoparenchymatous cells, becoming firm at maturity; reverse pale to yellow brown. Colonies on G25N at 14 days 22–26 mm diam, low and sparse, often deeply and irregularly wrinkled centrally, texture usually velutinous, sometimes floccose or funiculose centrally; mycelium persistently white; conidia sparsely produced, uncoloured; exudate and soluble pigment not produced; reverse pale. Colonies on MY50G at 14 days 10–18 mm diam, plane and sparse, margins entire, velutinous to floccose, heavily sporing; mycelium and conidia persistently white; exudate and soluble pigment absent; reverse pale. Colonies on MY5-12 at 14 days 35–45 mm diam, radially sulcate or irregularly wrinkled and often centrally raised; some isolates with growth low, dense and velutinous, others with rudimentary fascicles bearing conidial structures terminally, or sometimes with quite well developed funicles, up to 1 mm high, with scattered conidial structures; conidial structures

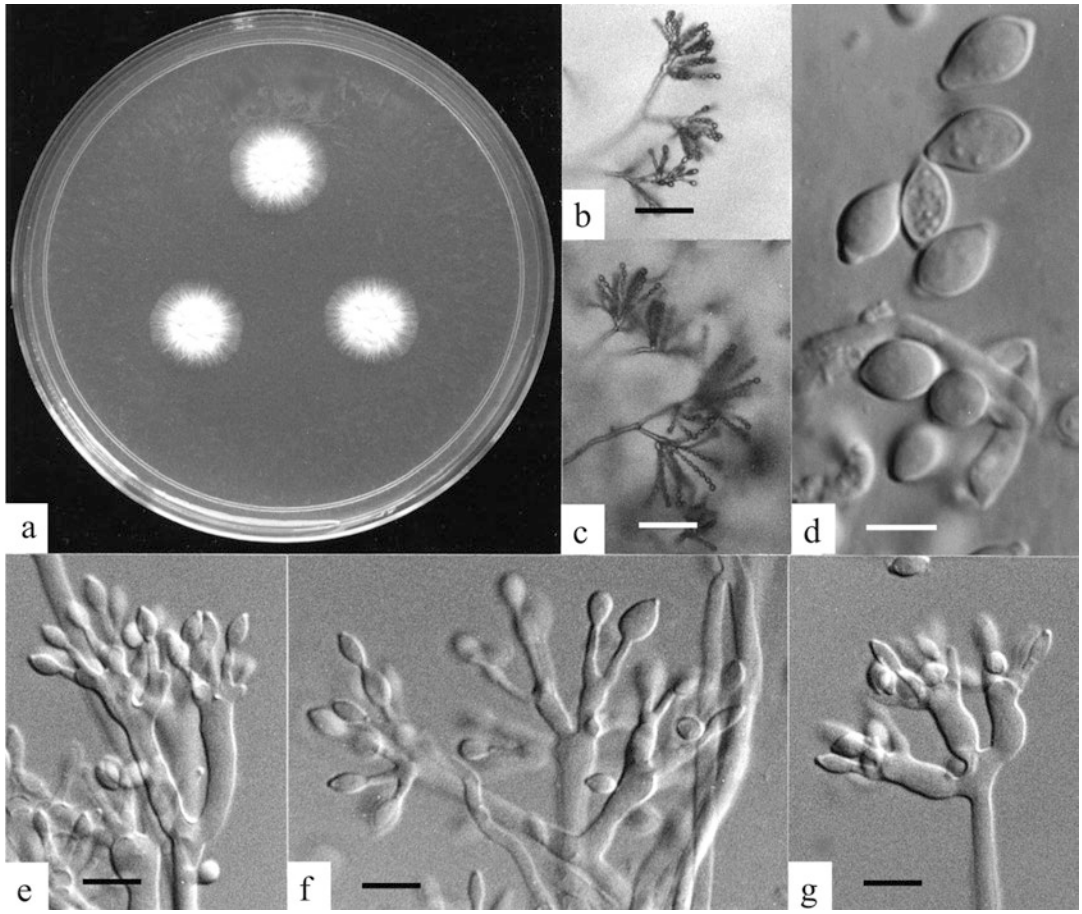

Fig. 9.5 *Polypaecilum pisce* (**a**) colonies on MY5-12, 7 d, 25 °C; (**b**, **c**) conidiophores with polyphialides and conidia *in situ,* bar = 50 µm; (**d**) conidia, bar = 5 µm; (**e**–**g**) polyphialides and developing conidia, bar = 10 µm

borne in profusion, mostly terminal on ascending or trailing hyphae, each bearing several short chains of conidia clearly visible under the low power microscope; mycelium and conidia persistently white; exudate and soluble pigment not produced; reverse pale to buff. No growth on CYA at 5 or 37 °C.

Reproductive structures polyphialides, borne solitarily on short conidiophores from vegetative hyphae; polyphialides large and complex, with a body 15–60 µm long and of varying width, usually 3–5 µm, with thin, smooth walls, unbranched or more commonly dichotomously or irregularly branched near the apex, each branch terminating in 2–5 necks, 3.0–5.0 µm long, each bearing conidia; conidia ellipsoidal to lemon shaped, 5–8 µm long, hyaline, smooth walled, borne in long chains, breaking up in wet mounts.

Distinctive features See the genus preamble. Other species in this genus, *Polypaecilum insolitum* G. Sm. and *P. capsici* (J.F.H. Beyma) G. Sm., have not been reported to grow on media of reduced water activity.

Taxonomy As noted above, Samson et al. (2014) transferred this species to *Aspergillus*, as *Aspergillus pisci.* However, "pisce" is the correct epithet (Latin, fish, *piscis*, third declension, Group 11, Stearn, 1966; *pisce,* ablative, from a fish), so "pisci" is correctly treated as an orthographic variant.

Identifiers Mycobank MB105705; holotype FRR 2732; ex-type cultures CBS 101166, IMI 288726, ATCC 56982, FRR 2732.

Physiology *Polypaecilum pisce* is a tropical halophilic xerophile with an optimum a_w range for growth between 0.96 and 0.87 a_w. Growth is faster and the growth optimum occurs over a broader a_w range on NaCl based media than on glucose/fructose or glycerol based media (Andrews and Pitt 1987; Wheeler et al. 1988b). The optimum temperature for growth is near 30 °C although *P. pisce* also grows strongly at 37 °C over a narrower a_w range (Wheeler et al. 1988b). At 30 °C, the minimum a_w for germination in NaCl media was 0.75 (saturated NaCl) after 17 days, and in glucose/fructose 0.71 a_w after 7 days (Wheeler et al. 1988b). At 25 °C, *P. pisce* germinated at 0.83 a_w in 7.5 days in NaCl media, in glucose/fructose 0.77 a_w after 4 days and in glycerol 0.75 a_w after 7 days (Andrews and Pitt 1987). In NaCl media, the maximum radial growth rate of 85–90 μm/hr. was achieved over the temperature range 30–37 °C and a_w range of 0.95–0.88 a_w (Andrews and Pitt 1987). This rate of growth was also achieved in glucose/fructose at 37 °C but only at 0.92 a_w (Wheeler et al. 1988b). *P. pisce* accumulates glycerol as an internal solute when growing under conditions of reduced a_w on NaCl based media (Hocking 1986), not different from the other xerophiles examined in that work.

Polypaecilum pisce is very well adapted to its habitat of dried salted fish. Studies on the interactions of five xerophilic fungi isolated from salt fish [*P. pisce*, *Phialosimplex* (*Basipetospora*) *halophilus, Aspergillus* (*Eurotium*) *rubrum, Aspergillus wentii* and *A. penicillioides*] on NaCl based media and on salt fish at 30 °C showed that below about 0.88 a_w *P. pisce* will outgrow the other fungi, despite relatively slow growth rates at higher a_w values (Wheeler and Hocking 1993).

Mycotoxins No mycotoxins have been reported from this species.

Ecology This species was initially isolated in our laboratory from imported Asian dried fish in 1979. It appeared to be a curiosity. During a much more extensive study on dried fish, however, it became apparent that *Polypaecilum pisce* is a major cause of spoilage of dried fish in Indonesia. This species was isolated from 42% of 74 samples of Indonesian fish; 20% showed profuse growth of this fungus (Wheeler et al. 1986). *P. pisce* appears to be of restricted distribution: all cultures in the FRR collection have come from Indonesian dried seafood with the exception of the original isolate, which came from the Philippines.

Reference Pitt and Hocking (1985).

9.5 Genus *Wallemia* Johan-Olsen

The tiny brown fungus *Wallemia* is a fungal curiosity, a relict genus. It is a xerophilic basidiomycete, rare indeed, and molecular analysis has shown that it occupies an ancestral position in the Basidiomycota, likely to be a sister group to the Agaricomycotina (mushrooms). Often reported as a halophile because salterns are a common "natural" source, *Wallemia* species are mostly indifferent to the type of solute that lowers water activity, again an unusual property for any organism. Once regarded as having a single species, *W. sebi*, molecular analysis first showed that it included three species, *W. sebi, W. muriae* and *W. ichthyofaga* (Zalar et al. 2005). Then *W. sebi* was divided into four clades, with the description of three more species, *W. mellicola, W. tropicalis* and *W. canadensis* (Jančič et al. 2015a). Further species, *W. hederae* (Jančič et al. 2015b) and *W. peruviensis* (Díaz-Valderrama et al. 2017) have been added subsequently, with new physiological information. It is impossible to know which of the numerous isolations described as *W. sebi* in the pre 2005 literature relates to a particular one of these species. All grow over an exceptionally wide range of a_w and are indifferent to solute type as well. From Jančič et al. (2015a) it seems likely that the common foodborne species is *W. sebi*, described below.

Wallemia sebi (Fr.) Arx **Fig. 9.6**

Sporendonema sebi Fr.
Sporendonema epizoum (Corda) Cif. & Redaelli

Colonies on CYA and MEA 1–6 mm diam, plane or crateriform, velutinous, margins narrow, coloured uniformly brown; reverse deep brown. Colonies on G25N at 7 days 2–6 mm diam, as on CYA. No growth on CYA at 5 or 37 °C. Colonies on MY50G at 7 days 2–5 mm diam, paler and less dense than on CYA.

Reproductive structures short fertile hyphae, septating into segments during elongation, then segments subdividing into four cylindrical cells, subsequently rounding up into conidia; conidia 1.5–3.0(−4.0) µm diam, with walls finely roughened or spinose, adhering in short chains.

Distinctive features *Wallemia sebi* is unique among foodborne fungi, readily

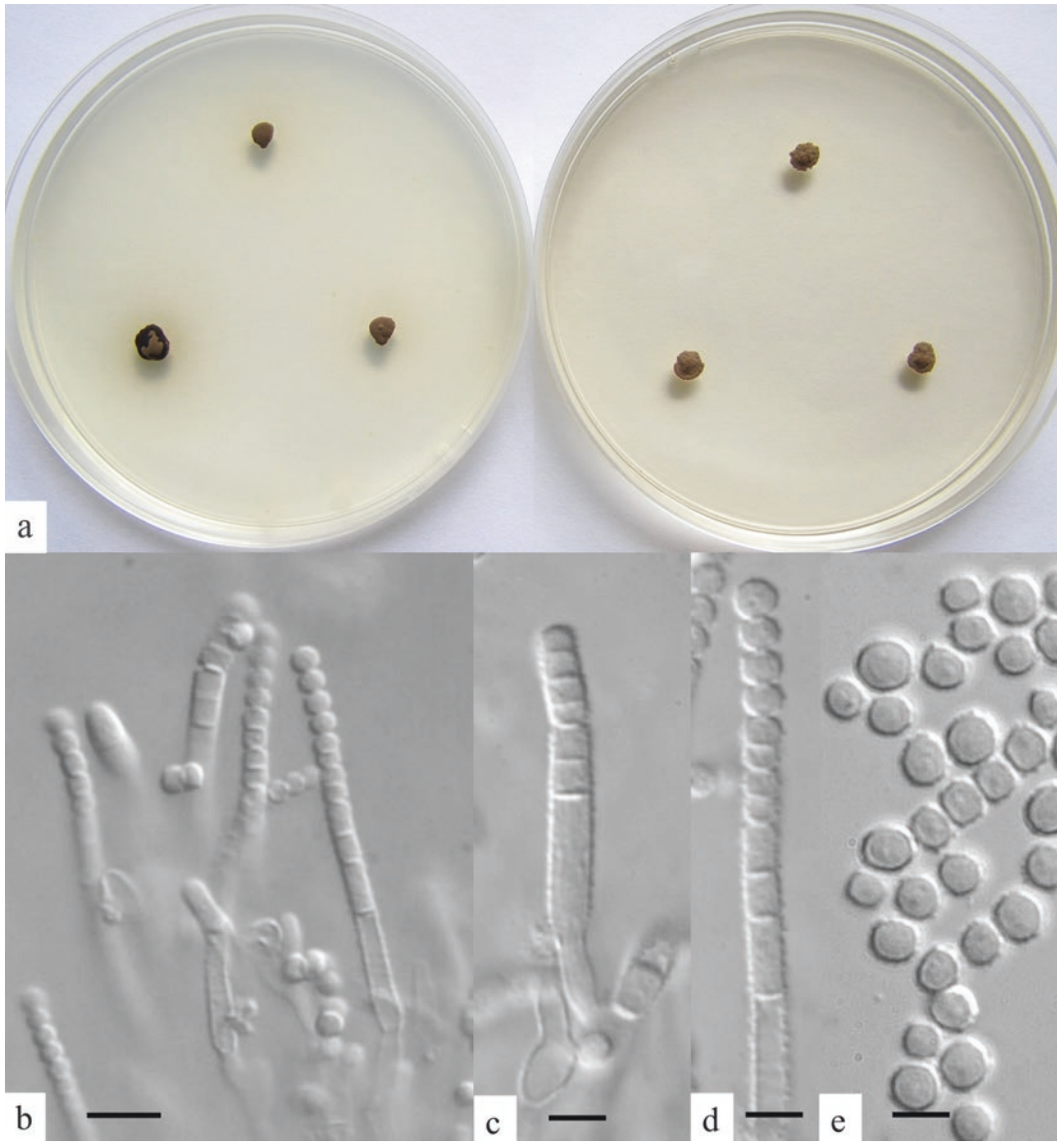

Fig. 9.6 *Wallemia sebi* (**a**) colonies on CYA and MY50G, 7 d, 25 °C; (**b**–**d**) conidiophores with developing conidia, (**b**) bar = 10 µm; (**c**, **d**) bars = 5 µm; (**e**) conidia, bar = 5 µm

distinguished by the formation of small, brown colonies on many types of foods and also on the standard media at 25 °C. The method of reproduction, forming conidia in groups of four meristamatically (Hill 1974), results in the rapid and virtually complete differentiation of aerial hyphae into small spherical brown conidia. If mature colonies are jarred, the aerial growth, consisting almost entirely of conidia, is released as a powdery mass.

Taxonomy For many years, this fungus was known as *Sporendonema sebi* or *S. epizoum*, but the type species of *Sporendonema* is unrelated. Barron (1968) revived the little known name *Wallemia* and von Arx (1970) made the combination which gave priority to the earliest epithet. On the basis of ultrastructure studies, Moore (1986) suggested that *Wallemia* could be a basidiomycete. This was confirmed by Zalar et al. (2005) and Matheny et al. (2006), who used broadly based molecular analyses. Zalar et al. (2005) proposed a new basidiomycete class Wallemiomycetes, covering an order Wallemiales. The only genus within this order is *Wallemia*, which now includes eight species.

W. sebi, W. muriae and *W. ichthyofaga* are distinguishable by differences in conidial size, water relations and molecular sequence data (Zalar et al. 2005). Jančič et al. (2015a) provided a key to all species except *W. hederae*, for which see Jančič et al. (2015b). Unfortunately the key in Jančič et al. (2015a) provides no entry to the couplet that includes *W. sebi*. However, if the leads "5" and "6" are replaced by "4" and "5" respectively, the key will function. A key has also been provided by Díaz-Valderrama et al. (2017).

Identifiers Mycobank MB325537; neotype CBS 818.96 (Zalar et al. 2005); ex-type culture CBS 103.10; ITS barcode AY328915; alternative markers *MCM7* KM034937, *RPB1* KM035115, *TSR1 CaM* KM035026 (Jančič et al. 2015a).

Physiology Conidia of *Wallemia sebi* are produced in vast numbers, and extremely rapidly. *W. sebi* can sporulate within 1–2 days of germination on media between 0.997 and 0.92 a_w and within 5 days at 0.85 a_w (Hocking 1986). However, conidia are short lived on both media and natural substrates. They are relatively sensitive to low pH (Ormerod 1967) and are of low heat resistance (Pitt and Christian 1970; Beuchat and Pitt 1990a). A $D_{57.5}$ value of 1 min, and a *z* value of 8.1 C° for conidia heated in phosphate buffer, pH 5.5, with 400 g/l sucrose has been reported (Baggerman and Samson 1988).

Wallemia sebi is capable of growth over a very wide a_w range, about 0.997–0.69 at 25 °C in glucose/fructose media at pH 4.0 (Pitt and Hocking 1977) and 0.92–0.74 at pH values 4.5–7.5 (Gock et al. 2003). In media with NaCl as the major solute, the lower limit for growth at 25 °C has been reported as 0.80 a_w at pH 4 and 0.75 a_w at pH 6.5 (Pitt and Hocking 1977). At 20 °C, *W. sebi* germinated at 0.75 a_w (saturated NaCl) in 15 days and in glucose/fructose at 0.69 a_w in 46 days, but its minimum a_w for growth at this temperature was 0.78 a_w in NaCl and 0.80 in glucose/fructose based media (Wheeler et al. 1988a). *W. ichthyophaga* was found to be the most halophilic of these three species (Zalar et al. 2005). In a liquid medium, this species showed optimal growth at a concentration between 15% and 20% NaCl (w/v; ca 0.88 a_w) and grew in saturated NaCl (0.75 a_w) (Zajc et al. 2014).

In glucose/fructose based media at 20 °C, optimum growth occurred between 0.98 and 0.93 a_w, whereas at 30 °C, optimum growth occurred over a lower a_w range, 0.93–0.85 a_w. This temperature effect was not observed on NaCl based media. The fastest growth rate for *W. sebi* (18–20 μm/hr) was observed on glucose/fructose media at 20 and 30 °C over the a_w ranges discussed above. The lowest a_w at which growth occurred at 20 °C (0.78 a_w) was on NaCl based media (Wheeler et al. 1988a). Vindelov and Arneborg (2002) reported growth at 10 °C in crystalline sucrose at 0.82 a_w but not at 0.76 a_w.

Wallemia sebi accumulates glycerol as its main compatible solute when growing under conditions of reduced a_w. In media containing glucose and fructose, these two solutes were accumulated along with glycerol and traces of mannitol and arabitol (Hocking and Norton 1983). On NaCl based media, glycerol was the major compatible solute (Hocking 1986).

Although Zajc et al. (2014) claimed that *Wallemia ichthyophaga* is obligately halophilic, i.e. has an absolute requirement for NaCl for growth, no evidence was presented. This seems to be unlikely, as its sister species *W. sebi* grows equally well in media in which water activity has been adjusted with NaCl or glucose/fructose. These fungi have an obligate requirement for reduced water activity for growth, but appear unique in being quite indifferent to the solute responsible for this state.

Mycotoxins Two related, but distinct toxic compounds have been reported from *Wallemia sebi*, walleminol A and B (Wood et al. 1990). Walleminol A was reported to be a tricyclic dihydroxysesquiterpene with an LD_{50} of 40 µg/ml for brine shrimp and an MIC of 50 µg/ml for rat liver cells. There is no indication of toxicity *in vivo* in commonly used animal systems. Although some recent papers (Biango-Daniels and Hodge 2018; Zajc and Gunde-Cimerman 2018) have discussed health threats from *Wallemia* species, no evidence has been presented to indicate that compounds produced by *Wallemia* species can be considered to be "mycotoxins" as we define them.

Ecology Its ability to grow at almost any a_w supporting microbial growth, its rapid sporulation and small, easily dispersed conidia ensure that *Wallemia sebi* is common in nature. It has long been considered to be the principal fungus spoiling dried and salt fish (Frank and Hess 1941), on which it is known as "dun" mould. It is rare on tropical fish in our experience, however (Wheeler et al. 1986).

It has been isolated in our laboratory from a very wide range of foods, especially dried commodities, including dried prunes, dried peas, maple syrup, sultanas, jams and rice (see Pitt and Hocking 1997). A straw hat imported from the Philippines became completely covered in *W. sebi* during a particularly humid Sydney summer. Counts in dried chillies and pepper have exceeded 10^8 and 10^9 conidia per gram respectively (Hocking and Pitt 1980; Hocking 1981). Almost any sample of Australian rice, wheat or bread, suitably moistened and incubated, will yield this fungus. During storage trials, we have observed populations of *W. sebi* develop in wheat stored at 20 °C at a_w values between 0.71 and 0.75 over a period of one year. Data sheets of the International Mycological Institute, Egham, Surrey, record isolations from bread, milk, condensed milk, jams, jellies, dates, marzipan cakes, suet, gingerbread, etc.

Wallemia spp. are commonly isolated from hypersaline waters of salterns (Zalar et al. 2005), however, other records of *Wallemia sebi* in the literature are relatively rare. This must be due to oversight or inadequate isolation techniques. *W. sebi* is ubiquitous: it has been reported from Australia, Japan, Southeast Asia, UK, Europe, Scandinavia, Canada, the USA and more recently from the Shaanxi Province, China (Sun et al. 2006). Isolations have been reported from rice, jam, pecans, meat products including Italian salami (Cantoni et al. 2007), rapeseed and Japanese noodles (see Pitt and Hocking 1997). We have found *W. sebi* in maize, peanuts, cashews and soybeans from Thailand (Pitt et al. 1993, 1994), peanuts, maize, paddy and milled rice, soybeans and mung beans from Indonesia and peanuts, maize, soybeans, mung beans and black peppercorns from the Philippines (Pitt et al. 1998 and our unpublished data). It is interesting that C.M. Christensen who with his coworkers examined the fungi on North American grains for many years (for a summary see Christensen and Kaufmann 1965), first mentioned *W. sebi* in grains (Christensen 1978a) after Pitt (1975) drew attention to this omission.

Wallemia sebi is significant in indoor environments (Samson and Hoekstra 1994), but is often not reported because high a_w media are used in air sampling equipment. The use of DG18 in air sampling equipment is recommended for detection of xerophilic fungi in indoor air (Samson and Hoekstra 1994). Since that recommendation, *W. sebi* has been commonly reported from indoor environments worldwide (e.g. Desroches et al. 2014), and has been implicated as one cause of farmer's lung disease (Reboux et al. 2001). Using DG18, we have isolated *W. sebi* in high numbers from mould affected buildings.

References Barron (1968); Zalar et al. (2005); (Jančič et al. 2015a).

9.6 Genus *Xerochrysium* Pitt

The genus *Xerochrysium* was erected for two slowly growing xerophilic species of *Chrysosporium* when molecular studies established that these species were neither related to that genus nor to rapidly growing species now accommodated in *Bettsia* (Pettersson et al. 2011; Pitt et al. 2013). Molecularly, *Xerochrysium* species belong in Eurotiales near *Monascus* and *Xeromyces*. Morphologically, they produce white colonies which grow slowly on media of all water activities, and are characterised by production of abundant chlamydoconidia and arthroconidia. Two species are known: *Xerochrysium dermatitidis* (formerly *Chrysosporium inops*) and *X. xerophilum*.

Key to *Xerochrysium* Species

1	Colonies on MY50G typically 15 mm or less (occasionally up to 20 mm), no growth at 37 °C	*X. dermatitidis*
	Colonies on MY50G 20–38 mm diam after 14 days, growth at 37 °C	*X. xerophilum*

Xerochrysium dermatitidis (A. Agostini) Pitt — Fig. 9.7

Chrysosporium inops J.W. Carmich.

Colonies on CYA and MEA microscopic or up to 3 mm diam. Colonies on G25N 2–9 mm diam, varying from low and translucent to deep and floccose, white; reverse pale to amber or duller yellow brown. No growth on CYA at 5 or 37 °C. Colonies on MY50G at 7 days 6–10 mm diam, at 14 days 12–20 mm diam, varying from low, sparse and translucent to moderately deep, dense and with a floccose surface, white or if translucent, uncoloured; reverse uncoloured to pale yellow brown.

Reproductive structures on G25N or MY50G at 7 days primarily short chains of chlamydoconidia and arthroconidia, borne by retrogressive differentiation from hyphal tips and as intercalary chains; some terminal chlamydoconidia also present; at maturity on MY50G (2–4 weeks), clusters of chlamydoconidia and arthroconidia also present, formed by retrogressive differentiation of groups of short branching hyphae with a lateral stipe as their common origin. Chlamydoconidia spherical, 4–7(−10) µm diam; arthroconidia cylindroidal or doliiform (barrel shaped), 3–8 × 3–6 µm, those of greater width intergrading with chlamydoconidia; aleurioconidia, the conidial type characteristic of *Bettsia*, uncommon, ellipsoidal to pyriform, 5–8 µm diam or longer. Large chlamydoconidia, up to 25 µm diam, with walls up to 2 µm thick, produced by some isolates. Teleomorph unknown.

Distinctive features In culture, *Xerochrysium dermatitidis* and *X. xerophilum* are very similar: *X. dermatitidis* grows more slowly on standard media. In young colonies (7 days), terminal chlamydoconidia are often the dominant conidium type in *X. dermatitidis*. Aleurioconidia are rare.

Taxonomy In a major study of species that could be considered to belong to the genus *Chrysosporium*, Carmichael (1962) transferred a culture (CBS 132.31) that he had received as *Glenosporella dermatitidis* A. Agostini to this genus. However, as he had already used the epithet "dermatitidis" for *C. dermatitidis* (Gilchrist & W.R Stokes) J.W. Carmich., he transferred *G. dermatitidis* as *C. inops nom. nov.* On transfer to the new genus *Xerochrysium*, this epithet is again available, so the new combination is correctly *X. dermatitidis* (A. Agostini) Pitt. This is unfortunate: although Agostini's isolate came from human skin, this species is not a dermatophyte, so the epithet is misleading.

Identifiers Mycobank MB807005; lectotype CBS 132.31; ex-type cultures CBS 132.31, IMI 96729, FRR 2376; ITS barcode CBS 132.31_ex62110_194700_ITS; 26S, CBS_132.31-14389_lr0r__D02_010_14389_lr5__D02_010 (CBS 2007).

Physiology Van Oorschot (1980) reported that *Xerochrysium dermatitidis* (as *Chrysosporium inops*) had a minimum growth temperature of 20 °C, an optimum of 25 °C, and a maximum of 30 °C. However it is unclear that the isolate she studied was correctly named. Contrary to earlier reports (Pitt and Christian 1968; Van Oorschot 1980), it is clear that

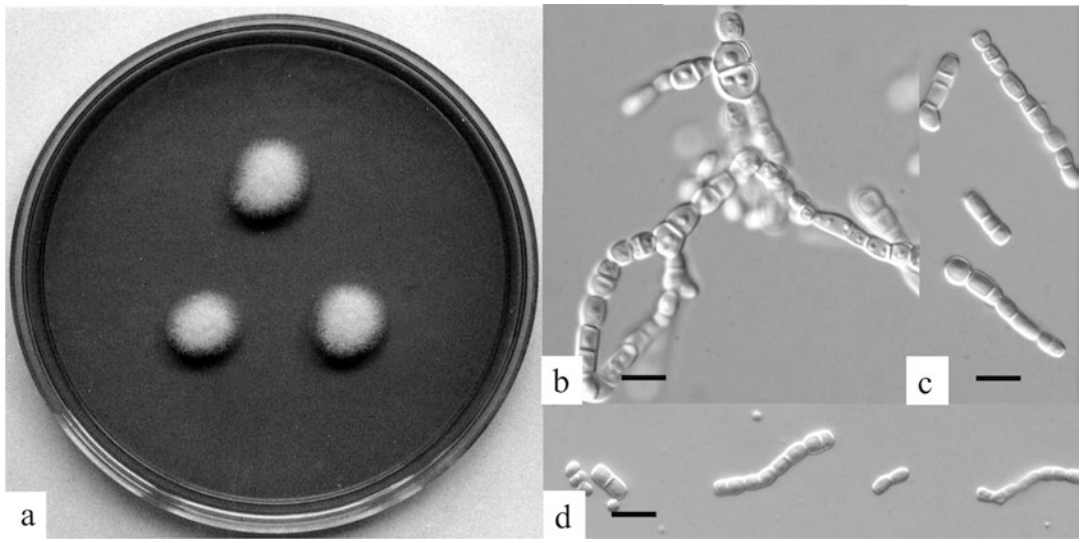

Fig. 9.7 *Xerochrysium dermatitidis* (**a**) colonies on MY50G, 14 d, 25 °C; (**b–d**) chains of arthroconidia, borne by hyphal transformation, bar = 10 µm

X. dermatitidis is an extreme xerophile. The water relations of this species have not been studied in detail, but isolations in our laboratory have come from growth under controlled a_w levels as low as 0.72. However, it is also able to grow (very slowly) at the high a_w of CYA and MEA.

Arthroconidia of *Xerochrysium dermatitidis* are moderately heat resistant, and their heat resistance increases with maturity. The decimal reduction time at 66 °C (D_{66}) was 1.67 min for 3 week old spores, 1.95 min for 4 week old spores, and 5.49 min for 6 week old spores (Kinderlerer 1996). A small population of heat resistant arthroconidia survived heating at 66 °C for 1 hr., representing approximately 0.001% of the population (Kinderlerer 1996). The heating menstruum in these experiments was 0.1% peptone. A higher heat resistance could be expected in high sugar environments.

Xerochrysium dermatitidis can produce ethanol from glucose (Kinderlerer 1987). Its ability to utilise the Embden-Meyerhof-Parnas pathway may explain why this species and the closely related *X. xerophilum* may become dominant in low a_w foods stored at low oxygen tension (Kinderlerer 1987).

Mycotoxins No mycotoxins are known from this species.

Ecology This appears to be an uncommon species, but as with other extreme xerophiles, this may only reflect the need for well chosen isolation techniques and identification procedures. We have isolated *Xerochrysium dermatitidis* from a variety of spoiled products: nutmeg powder, Chinese five spice powder, mixed spice powder, chopped Chinese dates and a gelatine confection made in a starch mould. In the latter case, 30 tonnes of starch were heavily contaminated with this fungus, causing a serious loss of confectionery products. There was no evidence that the starch had ever been more than marginally above a safe a_w level. *X. dermatitidis* has also been isolated from table jelly (Kinderlerer 1987) and spoiled chocolate and hazelnuts in the UK (Kinderlerer 1997). Christensen (1978b) reported *X. dermatitidis* (as *Chryosporium inops*) from samples of maize stored for 12 months or more at low moisture in tightly closed containers. His identification was in error (see below), however we have recovered this species from a sample of safflower seeds stored in a similar way in our laboratory.

Additional species *Xerochrysium xerophilum* (Pitt) Pitt (syn. *Chrysosporium xerophilum* Pitt) is closely related to *X. dermatitidis*. It differs by (1) faster growth, especially on MY50G,

where colonies at 7 days are 10–15 mm diam and at 14 days 25–32 mm diam; (2) higher numbers of aleurioconidia, which measure 7–8 × 5–7 µm; (3) larger terminal chlamydoconidia, 10–12(−15) µm diam; and (4) at maturity, the almost complete differentiation of vegetative hyphae into intercalary chlamydoconidia and arthroconidia, even aleurioconidium pedicels often becoming thick walled spores. CZC50G medium may be useful in differentiating these two species (Kinderlerer 1995).

A mesophilic xerophile, *Xerochrysium xerophilum* has a minimum a_w for growth of 0.71 (Pitt and Christian 1968) and, like *X. dermatitidis*, a high maximum limit. Although most conidia of this species have a low heat resistance, surviving less than 10 min at 60 °C, a small proportion of conidia appear to be quite resistant, surviving at least 70 °C for 10 min (Pitt and Christian 1970). A similar effect was observed for conidia of *X. dermatitidis* (Kinderlerer 1996).

For a long time this species was known only from the type isolate, from moist Australian prunes. Christensen (1978b) isolated it (reported as *Chrysosporium inops*) from U.S. maize and other oilseed samples which had been stored at moisture contents of 15–16% in sealed containers for periods of 1–10 years. Other isolates have come from coconut and chocolate (Kinderlerer 1997) in the UK and Australia (our laboratory). In spoiled coconut, *C. xerophilum* formed a wide range of volatile compounds including aliphatic methyl ketones, esters and secondary alcohols as well as free medium chain length fatty acids (Kinderlerer et al. 1988). Identifiers: Mycobank MB807006; holotype UAMH 2368; ex-type cultures CBS 153.67, ATCC 18053, FRR 503.

Reference Pitt et al. (2013).

9.7 Genus *Xeromyces* L.R. Fraser

The genus *Xeromyces* has a single species, *X. bisporus*, which is distinctive. It was for a long time considered to be the most xerophilic organism known, but now is considered second to *Aspergillus penicillioides* (Stevenson et al. 2017). Growth will not occur on media of high a_w, usually not even on G25N. On more favourable media, and carbohydrate rich substrates at lower a_w, it grows quite rapidly and produces colourless cleistothecia, with evanescent asci containing two "D"-shaped ascospores.

Xeromyces bisporus L.R. Fraser **Fig. 9.8**

Monascus bisporus (L.R. Fraser) Arx

No growth on CYA at 5, 25 or 37 °C, or on MEA. On G25N, at 7 days, no germination to microcolony formation; at 14 days no germination to dense colonies up to 4 mm diam. Colonies on MY50G at 7 days, 3–6 mm diam, low and sparse; at 14 days, 15–20 mm diam, low and dense, translucent with a glistening surface, colourless or very pale red brown; reverse uncoloured; at 4 weeks, 50–70+ mm diam, low, translucent and sometimes glistening, colourless or faintly red brown, with contiguous layers of colourless cleistothecia visible under the low power microscope; reverse uncoloured.

Cleistothecial initials evident on MY50G at 2 weeks, commencing as three short cells, then developing distinctive finger-like processes from the bottom cell, enveloping the second, the latter then enlarging to form the cleistothecium; cleistothecia maturing in 4–6 weeks, 40–120 µm diam, with walls thin and structureless; asci inconspicuous and evanescent, containing 2 ascospores only; ascospores ellipsoidal, flattened on one side ("D"-shaped), 10–12 × 4.0–5.0 µm, smooth walled. Aleurioconidia developing below 0.90 a_w, solitary, usually measuring 15–20 × 12–15 µm.

Distinctive features In culture, *Xeromyces bisporus* is distinguished by its inability to grow on CYA, MEA or (usually) G25N; by relatively fast but, in young cultures, strictly mycelial growth on MY50G, with cleistothecial formation occurring only after about 2 weeks incubation at 25 °C; and, later, by its distinctive D-shaped ascospores.

On carbohydrate rich foods of 0.75 a_w or less, the presence of *Xeromyces* may be inferred from luxuriant but low white or translucent growth. The observation of D-shaped ascospores under

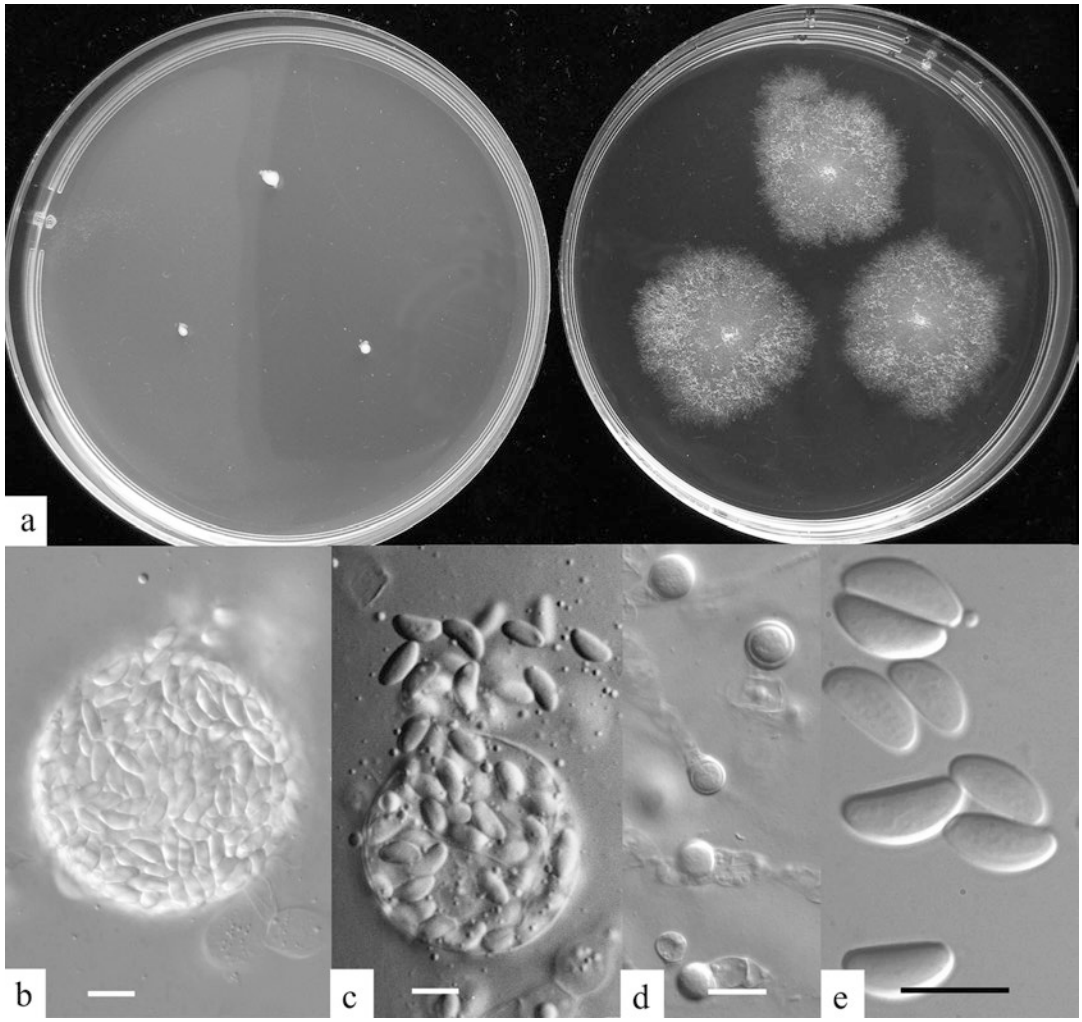

Fig. 9.8 *Xeromyces bisporus* (**a**) colonies on G25N and MY50G, 14 d, 25 °C; (**b**) developing cleistothecium, bar = 25 µm; (**c**) mature cleistothecium releasing ascospores, bar = 25 µm; (**d**) aleurioconidia, bar = 10 µm; (**e**) ascospores, bar = 10 µm

the microscope is diagnostic for *X. bisporus*. *Bettsia* and *Xerochrysium* species may produce superficially similar growth, but colonies appear powdery and, under the microscope, mostly consist of aleurioconidia or chlamydoconidia.

Taxonomy Von Arx (1970) transferred *Xeromyces bisporus* to *Monascus*. However, other taxonomists have maintained *Xeromyces* as a distinct genus, related to *Monascus*. Analysis of the D1/D2 region of the large subunit rDNA suggested that *Xeromyces* and *Monascus* are monophyletic, justifying placement of the two genera in the same family within the *Eurotiales* (Park and Jong 2003). Using nrDNA and ß-tubulin genes, Pettersson et al. (2011) placed *Xeromyces* closer to xerophilic *Chrysosporium* species (=*Xerochrysium*) than to *Monascus*.

Identifiers Mycobank MB307870; type unknown; ex-type cultures CBS 236.71, IMI 63718; FRR 525; ITS barcode CBS 236.71_ex39279_76465_ITS; alternative marker 18S AB024049 (CBS 2007).

Physiology *Xeromyces bisporus* is unable to grow above 0.96 a_w, and it for a long time it had the lowest known requirement for available water (0.61 a_w) of any organism (Pitt and Christian 1968), a record now eclipsed by *Aspergillus penicillioides* (Stevenson et al. 2017). Germination at 0.61 a_w required 120 days. Its optimum a_w for growth is 0.85 at 25 °C (Pitt and Hocking 1977) or 0.84 a_w at 30 °C (Leong et al. 2011). Germination and growth at low a_w are extraordinarily rapid in comparison with other xerophiles, its radial growth rate on glucose/fructose media at 0.75 a_w (ca 25 μm/hr) still being nearly half that of its optimum. Germination was recorded at 0.70 a_w at 25, 30 and 37 °C and the maximum growth rate occurred at 37 °C (Gock et al. 2003).

The ability to complete a sexual life cycle at water activities lower than almost any other life form can grow is remarkable. Ascospores were observed in cultures on glucose/fructose media at 0.67 a_w in 80 days; aleurioconidia were formed at 0.66 a_w in a similar time period (Pitt 1975). Only *Zygosaccharomyces rouxii* has a comparable ability.

Growth of *Xeromyces bisporus* is much more rapid on media containing glucose/fructose as controlling solute than on other media. Maximum growth rates on glycerol based media are less than one third of those on glucose/fructose at the optimal a_w. On both types of media, pH 4.0 or 6.5 had little effect on growth rates. Growth on NaCl media occurred only at pH 4.0 and only over the range 0.96–0.87 a_w (Pitt and Hocking 1977).

Xeromyces bisporus appears to have a high tolerance of CO_2. Dallyn and Everton (1969) reported growth in an atmosphere of 95% CO_2, in the presence of 1% O_2. However, in work carried out in our laboratory, *X. bisporus* failed to grow on MY50G agar in atmospheres of 80% CO_2 with 20% O_2 or 20% CO_2 with <0.5% O_2 (Taniwaki 1995).

Ascospores of *Xeromyces bisporus* are quite heat resistant. Pitt and Christian (1970) reported that a small proportion (0.1%) survived 10 min heating at 80 °C, while Dallyn and Everton (1969) observed that to kill 2000 ascospores in a medium of 0.9 a_w and pH 5.4 required more than 2 min at 90 °C, 4 min at 85 °C and 9 min at 80 °C. Using these data, Pitt and Hocking (1982) constructed a thermal death time curve, which was defined by a z value of 16.0 C° and a $D_{82.2}$ of 2.3 min.

Mycotoxins No mycotoxins are known from this species, indeed secondary matabolites do not appear to be produced (Leong et al. 2015).

Ecology *Xeromyces bisporus* is probably a much less rare fungus than the literature would indicate. The original isolation (Fraser 1953) was from liquorice, and it has been seen in our laboratory causing spoilage of this product twice since. Dallyn and Everton (1969) reported it from British table jelly, dried prunes, tobacco, currants (of 0.67 a_w) and chocolate sauce. It was the most common spoilage mould on Australian prunes in the study reported by Pitt and Christian (1968), but its dominance on that substrate has been less marked in recent years. We have isolated *Xeromyces* from spice powders, nutmegs, imported Chinese dates of 0.72 a_w, fruit cakes of 0.75–0.76 a_w, gelatine confectionery, mixed dried fruit and cookies containing a high proportion of dried fruit pieces, liquorice allsorts, fudge and strawberry fruit snacks. Growth on the dates and fruit cakes was luxuriant and commercial losses high.

Xeromyces bisporus can become established in plants producing low a_w products such as fruit cakes and cookies, providing a continuous low level inoculum. Ascospores appear capable of surviving the baking process, leading to sporadic spoilage which is often not manifested until many months after production. Tracing the source of the contamination can be difficult, if not impossible, particularly in view of the elapsed time between production and visual spoilage. Careful attention to cleaning of all product residues from production lines and equipment before sanitation is essential for eliminating this type of spoilage.

Xeromyces bisporus has been reported from the Australian and UK sources discussed above, and in date honey from Israel, tobacco in the Netherlands, and chocolate in the UK (CBS 2007).

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10 Yeasts

Yeasts are single celled fungi which reproduce vegetatively by budding, or less commonly, divide by fission. This property enables yeasts to increase rapidly in numbers in liquid environments, which favour the dispersal of unicellular microorganisms. Many yeasts grow readily under strictly anaerobic conditions, again favouring their growth in liquids. On the other hand, reproduction as single cells restricts spreading on, or penetration into, solid surfaces, where filamentous fungi have an advantage. Being eukaryotic organisms, yeasts reproduce more slowly than do most bacteria, and hence do not compete in environments which favour bacteria, i.e. at pH values near neutral or at very high temperatures. In common with filamentous fungi, many yeasts are tolerant of acid conditions. In broad terms, then, yeasts are more likely to be active in acidic, liquid environments than elsewhere. However, many yeasts also appear to be highly resistant to sunlight and desiccation and so occur widely in nature on the surfaces of leaves, fruits and vegetables.

When defined as above, i.e. as budding, non-photosynthetic eukaryotes, yeasts are a heterogeneous assembly of often quite unrelated fungi. Kurtzman et al. (2011) recognised over 1400 species of yeasts in all, divided into about 100 genera: 60% were classified as ascomycetes and 40% as basidiomycetes. Most of these genera lie outside our interests here. The issue of whether teleomorph or anamorph names should be recognised has been more or less resolved. Our choice in the genera that follow has been guided by the relevant literature and especially by the catalogue at the Westerdijk Biodiversity Centre (CBS 2016). It is emphasised that, the absence of any approved list of names, in all cases either teleomorph or anamorph name can be validly used.

As with other fungi, yeasts are classified into genera primarily on the type and appearance of spores – ascospores, basidiospores or, in the absence of either, vegetative cells. Yeasts possess limited morphological variability, so traditional classification at species level has relied on biochemical tests, principally the utilisation of various carbon or nitrogen sources, and vitamin requirements. Some physiological properties, i.e. growth at high temperature and reduced water activity, are used in a secondary role. The 2000 edition of "Yeasts: characteristics and identification" (Barnett et al. 2000) listed 90 separate tests, including utilisation of 47 carbon sources, 10 nitrogen sources, vitamin requirements, fermentation patterns, growth at various temperatures and in the presence of elevated glucose and NaCl levels, and morphological characteristics. Clearly, taxonomy of that kind is beyond the scope of the present work.

In recent years, DNA sequencing has become a major classification tool and is now commonly used for yeast identification. Molecular methods have clarified some of the bases for taxonomy of yeasts, but at the same time have led to increasing

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complexity, with a seemingly random reassortment of species into genera with each new monograph. However, as such techniques have now become quite refined, changes seem less likely in the future.

In the synonymy given with each species, a valid asexual name is often provided. It is emphasised that the use of these names will continue to be valid until such time as a Botanical Congress ratifies a list of yeast names based on a single nomenclature for each species. That is unlikely to happen soon.

Yeasts in foods By comparison with many strictly filamentous fungi, yeasts possess limited biochemical pathways and quite fastidious nutritional requirements. Foodstuffs are generally rich in the hexose sugars, minerals and vitamins which many yeasts require for growth, so are an ideal substrate. Their association with the phyllosphere of many crops ensures their presence on fruit and vegetables, and their entry into food processing plants.

Relatively few yeast species cause significant spoilage in processed foods, most being adventitious contaminants from natural sources. Deak and Beuchat (1996) listed 99 yeast species which occur in foods including fruit, beverages, wine, beer, meat, dairy products, low a_w products and low pH products. Many of these yeasts naturally occurring yeasts are intolerant of reduced water activity, heat processing or preservatives, so grow poorly if at all in properly formulated processed foods. Even if limited metabolism by such yeasts does occur, it is usually of little consequence unless significant gas production occurs. It is doubtful whether any yeasts produce mycotoxins, and few produce even marginally unacceptable off-odours. There are, of course, exceptions. Certain species must be classified as spoilage yeasts because they possess one or more undesirable properties. These commonly occurring spoilage species form the basis for most of the subject matter of this chapter.

Spoilage yeasts In our experience, only about ten species of yeasts are responsible for spoilage of foods which have been processed and packaged according to normal standards of good manufacturing practice. These species are responsible for major losses of processed foods around the world every year. We believe that relatively simple tests can be used to identify them when taken in conjunction with their spoilage habitats. These species are listed in Table 10.1, with their principal undesirable properties. Two species which do not cause spoilage, but are of widespread occurrence in foods, are also included in Table 10.1 and are described in this chapter. It is important to note that many other adventitious yeast contaminants can develop in a product if good manufacturing practice is neglected, i.e. if factory hygiene is poor, if preservatives are omitted, either deliberately or unintentionally, if pasteurising temperatures are inadequate, or filling machinery or factory premises are unsanitary, raw materials are of poor quality, brining or syruping procedures are poorly controlled, etc. In such cases the following text will be of little value, and neither will identification of the yeasts concerned. The correct approach, and often the only recourse in such cases, is to pay attention to manufacturing guidelines, so that this kind of problem is positively eliminated.

Identification of spoilage yeasts Considerable work has been carried out to develop simplified systems for identification of foodborne yeasts, as yeast identification procedures based on biochemical reactions are too complex and take too long to be to be of value in the food or industrial microbiology laboratory. Several automated systems are now commonly used, as well as simpler laboratory based systems, and these are discussed in some detail in Chap. 4. Identification using DNA sequencing is increasingly becoming the method of choice, as extensive databases such as GenBank are freely available for identification purposes. The 600–650 nucleotide D1/D2 region of the large subunit (26S) ribosomal DNA is the most widely targeted section of the genome, and sometimes the ITS region may also be used (Kurtzman et al. 2008, 2011). However, a word of caution. Results presented by a computer identification system or DNA sequence database still need to be interpreted with care: computers are not infallible, and sequences in DNA databases do not always have the correct species name! Does the result make sense? Keep in mind the source of the yeast isolate and the spoilage problem being investigated. Is the yeast reported a relatively common

Table 10.1 Spoilage yeasts

Yeast	Important properties
Candida parapsilosis	Lipolysis, fermentative; causes spoilage in a wide range of foods including cheese, margarines, dairy and fruit products
Debaryomyces hansenii	Growth at low water activities in foods preserved with NaCl, especially salt meat
Dekkera bruxellensis	Production of off-odours in beer, cider and soft drinks
Hanseniaspora uvarum	Spoilage of fresh and processed fruit
Kazachstania exigua	Moderately preservative resistant; of common occurrence in olive brines, relevantly rarely the cause of spoilage of sauerkraut or of juices, dairy products or soft drinks
Pichia kudriavzevii	Preservative resistant; film formation on olives, pickles and sauces
Pichia membranaefaciens	Preservative resistant; film formation on olives, pickles and sauces
Rhodotorula mucilaginosa *R. glutinis*	Common food contaminants; spoilage of dairy products; occasional spoilage of fresh fruits
Saccharomyces cerevisiae	Common food contaminant; sometimes fermentative spoilage of soft drinks; some strains preservative resistant
Schizosaccharomyces pombe	Preservative resistant; relatively rare spoilage yeast
Wickerhamomyces anomalus	Spoilage of processed fruit and yoghurts containing fruit
Zygosaccharomyces bailii	Preservative resistant; fermentative spoilage of acid, liquid preserved products such as juices, sauces, ciders and wines
Zygosaccharomyces bisporus	Preservative resistant; properties intermediate between *Z. bailii* and *Z. rouxii*
Zygosaccharomyces rouxii	Growth at extremely low water activities; fermentative spoilage of juice concentrates, honey, jams, confectionery, packaged dried fruits, etc

foodborne yeast, or a rare yeast only ever isolated once or twice from some obscure source? If a key gives a list of yeasts differing by one result (one discrepant test), check this list along with the discrepant test to see if your yeast is really more likely to be one of those listed as a second option. A weak reaction may have been incorrectly interpreted as negative. Perhaps carrying out one or two other tests can give better results.

A simplified approach has been used here which differentiates the species of yeasts most commonly responsible for actual spoilage of processed foods and beverages. Table 10.2 lists a series of media and conditions which will enable differentiation of the species listed in Table 10.1. To simplify and expedite identification as far as possible most media and conditions specified here have been used in the identification of filamentous fungi elsewhere in this book. The exceptions are the use of malt acetic agar, Czapek agar and growth on MEA at 37 °C. The first of these media distinguishes preservative resistant yeasts from others; on the second only yeasts which utilise nitrate as a sole carbon source can grow. MEA is used at 37 °C rather than the customary CYA because some yeasts grow poorly on CYA. Czapek agar is made with the same ingredients and procedures as CYA but yeast extract is omitted.

It is emphasised that the procedures used here will work only for yeasts causing actual spoilage: the system is not designed to cope with the many species of yeasts than can occur adventitiously in foods. Even so, the procedures are not rigorous and may on occasion misidentify an excluded yeast as being one of those discussed here. Provided all tests are carried out as specified and morphological observations made such occasions will be infrequent. In particular preservative resistant yeasts, the most important yeasts in food spoilage, will usually be readily recognised by the techniques described below.

Procedures for yeast identification Pour Petri dishes with the media listed in Table 10.2. From a 3–7 day old slant or plate culture of the yeast, preferably growing on MEA, disperse a small loopful of cells in 3–5 mL sterile water or 0.1% peptone. Streak each plate with a loopful of cells from this inoculum. A suitable streaking technique is described in Chap. 4. Incubate plates for 3 days, then examine each for presence or

Table 10.2 Media and conditions for identification of spoilage yeasts[a]

Medium	Purpose
Czapek agar	Assessing ability to utilise nitrate as a sole nitrogen source
Malt Extract Agar (MEA)	Colony and cell morphology
Malt Acetic Agar (MAA)	Assessing preservative resistance
MEA at 37 °C	Assessing growth at elevated temperatures
Malt Yeast 50% Glucose Agar (MY50G)	Assessing growth at reduced water activities in the presence of high carbohydrate levels
Malt Yeast 10% Salt 12% Glucose Agar (MY10-12)	Assessing growth at reduced water activities in the presence of high sodium chloride

[a]Incubation is at 25 °C unless specified. Inspection should be at 3 and 7 days after inoculation

absence of growth. Note also colony colour, and the size and shape (regular or irregular) of well separated colonies. Make a wet microscopic mount in water, lactic acid or lactofuchsin (see Chap. 4) from the MEA plate grown at 25 °C and from malt acetic agar. Record approximate cell size and shape, position of budding, and the presence or absence, type and number of ascospores (see the following figures for a guide). Reincubate plates and repeat observations at 7 days.

Salient properties on these media of yeasts included here are listed in Table 10.3. The key which follows is based on growth for 7 days on the media in Table 10.2. Cell sizes are from colonies on MEA at 25 °C, aged between 3 and 7 days. The species are described and discussed below in alphabetical order.

Identifiers The CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands, has carried out a monumental study on the molecular taxonomy of all yeast species (and strains) in the CBS culture collection (Vu et al. 2016). This has provided publically available, authentic information on molecular characteristics of most accepted yeast species. Selected information is included here, mostly taken directly from the CBS-KNAW culture collection database for yeasts and referenced as CBS (2016).

Key to spoilage yeasts

1	Colonies on MEA white, off-white or brownish	2
	Colonies on MEA pink or red	*Rhodotorula mucilaginosa* *Rhodotorula glutinis*
2 (1)	Cells dividing by transverse fission	*Schizosaccharomyces pombe*
	Cells dividing by budding	3
3 (2)	Growth on MY10-12	4
	No growth on MY10-12	6
4 (3)	Cells nearly spherical	*Debaryomyces hansenii*
	Cells ellipsoidal to elongate	5
5 (4)	Growth on Czapek agar, ascospores hat shaped	*Wickerhamomyces anomalus*
	No growth on Czapek agar, ascospores smooth walled	*Zygosaccharomyces rouxii*
6 (3)	Growth on malt acetic agar	7
	No growth on malt acetic agar	11
7 (6)	Growth on MY50G	8
	No growth on MY50G	9
8 (7)	Strong growth at 37 °C	*Candida parapsilosis*
	No growth at 37 °C	*Zygosaccharomyces bailii*
9 (7)	Cells mostly 4–6 μm long; growth at 37 °C weak at most	*Pichia membranifaciens*
	Cells often exceeding 6 μm long; growth at 37 °C vigorous	10

(continued)

10 (9)	Larger cells cylinders, up to 25 µm long; isolated colonies on MEA at 25 °C often exceeding 5 mm diam	*Pichia kudriavzevii*
	Larger cells ellipsoids, rarely exceeding 12 µm long; isolated colonies on MEA at 25 °C not exceeding 5 mm diam	*Saccharomyces cerevisiae*
11 (6)	Growth at 37 °C	12
	No growth at 37 °C	14
12 (11)	Colonies white, cells narrow ellipsoids 4–7 µm long; isolated colonies on MEA at 25 °C not exceeding 2.5 mm diam	*Dekkera bruxellensis*
	Colonies off white to cream, cells narrow to broadly ellipsoidal, often exceeding 7 µm long; colonies on MEA at 25 °C exceeding 2.5 mm diam	13
13 (12)	Cells narrow ellipsoids, long (>10 µm) cells often present	*Candida parapsilosis*
	Cells broad ellipsoids, 5–12 µm long	*Saccharomyces cerevisiae*
14 (11)	Growth on MY50G	*Zygosaccharomyces bisporus* (See *Z. bailii*)
	No growth on MY50G	15
15 (14)	Larger cells 7–9 µm long, budding terminally only	*Hanseniaspora uvarum*
	Larger cells 4–5 µm long, budding irregularly	*Kazachstania exigua*

Table 10.3 Salient properties of yeasts at 7 days[a]

Species	Cell length MEA µm	Colony diam MEA mm	Colony colour MEA	Growth on				
				Czapek	MEA 37 °C	MAA	MY50G	MY10-12
C. parapsilosis	3–20	3–4	White	w	+	v	+	+
D. hansenii	2.5–4	2.5–4	White	w	0	0	w	+
D. bruxellensis	4.5–7	1.5–2	White	0	+	0	w	0
H. uvarum	3.5–6	2–4	White	+/–	0	0	0	0
K. exigua	4–5	1–2	White	0	0	0	0	0
P. kudriavzevii	3–25	5–8	White	w	+	+	0	0
P. membranaefaciens	4–6	3–4	White	w	vw	+	0	0
R. mucilaginosa *R. glutinis*	4.5–5	5–10	Red	+/–	0	0	0	0
S. cerevisiae	5–12	2.5–4	White	w	+	w	0	0
Sch. pombe	5–7	1–2	White	w	+	+	w	0
W. anomalus	3–7	3–4	White	+	0-vw	0	vw	+
Z. bailii	5–8	2–3	White	0	0	+	+	0
Z. bisporus	3.5–7	2–3	White	0	0	0	+	0
Z. rouxii	5–7	2–3	White	0	0	0	+	+

[a]0, no growth; w, weak; vw, very weak; +, 1 mm diam or more in 7 days

Candida parapsilosis (Ashford) Langeron & Talice Fig. 10.1

Monilia parapsilosis Ashford

Colonies on MEA at 3 days 1.5–2.5 mm diam, off white; convex, margins circular, surface matt; at 7 days 3–4 mm diam, off white to cream coloured, often centrally umbonate, margins appearing entire, but showing thin filamentous growth when viewed with the stereomicroscope. Cells on MEA varying widely in size and shape, from short ellipsoids 3.5–4.0 × 2.5–3.5 µm to long, irregularly cylindroidal cells, straight or curved, 10–15(−20) × 1.5–3.0 µm; reproducing by irregular budding, occurring singly or in short chains. Ascospores not produced. Strong growth

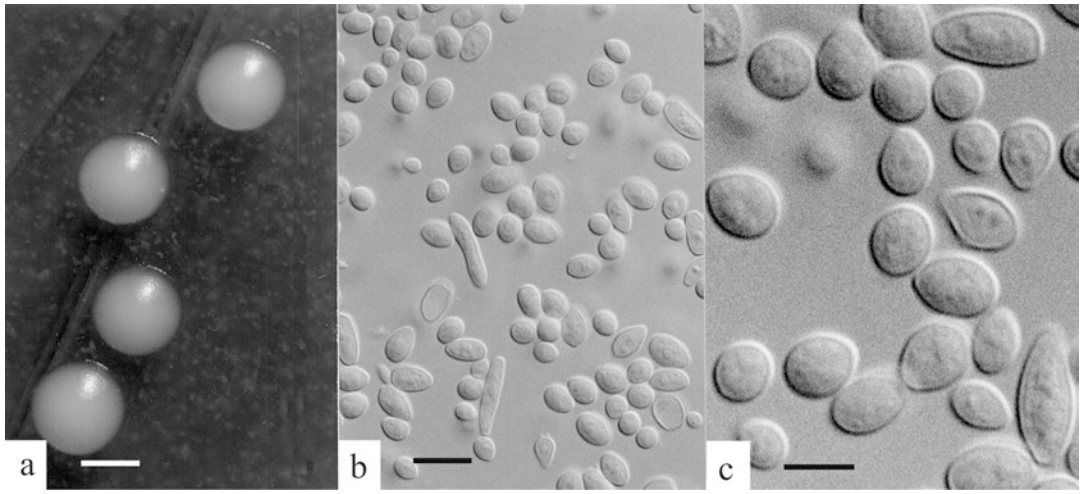

Fig. 10.1 *Candida parapsilosis* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

at 37 °C; weak growth on Czapek agar; sometimes growth on malt acetic agar; growth on MY50G and MY10-12.

Distinctive features The production of elongated cells and pseudohyphae is a feature of *Candida parapsilosis*. Smaller colonies on MEA and the ability to grow on MY50G distinguish it from *Pichia kudriavzevii*.

Identifiers MycoBank MB253819; holotype CBS 604; marker D1/D2 LSU rRNA 5U45754 (CBS 2016).

Physiology *Candida parapsilosis* grows between 8 and 42 °C, with an optimum temperature near 35 °C (Suzzi et al. 2003). It is lipolytic (Bours and Mossel 1973) and is strongly proteolytic at 37–42 °C (Kobatake et al. 1992; Suzzi et al. 2003). It has average heat resistance, with a D_{62} of 2.2 min (Hur et al. 1993), is tolerant of NaCl (5 to 10%) and actidione (100 and 1000 mg/l) (Suzzi et al. 2003).

Ecology *Candida parapsilosis is* often associated with high fat foods such as butter, cheese, margarine, salad dressing and yoghurt (Deak and Beuchat 1996; see also Pitt and Hocking 1997; Suzzi et al. 2003) and has also been isolated from human breast milk (Rosa et al. 1990). *C. parapsilosis* has been reported from raw meat and meat products (Deak and Beuchat 1996; see also Pitt and Hocking 1997; Hammad, et al. 2006), raw seafood and shellfish, a fermented fish product in the Philippines, vegetables, fruit products, fruit juice concentrates and soft drinks, chalky bread (Deak and Beuchat 1996; see also Pitt and Hocking 1997), pickles and olive brines (Deak and Beuchat 1996; Mourad and Nour-Eddine 2006).

Pathogenicity *Candida parapsilosis* is an opportunistic pathogen that can cause infections in burns patients and those with impaired immunity. It can also form biofilms, colonising tubing and prostheses (De Hoog et al. 2000; Trofa et al. 2008; Wang et al. 2016; Tóth et al. 2019).

References Kurtzman and Fell (1998); Barnett et al. (2000); Kurtzman et al. (2011).

Debaryomyces hansenii (Zopf) Lodder & Kreger **Fig. 10.2**

Debaryomyces membranaefaciens H. Nagan.
Candida famata (F.C. Harrison) S.A. Mey. & Yarrow (valid anamorph name)
Torulopsis candida Saito
Torulopsis famata (F.C. Harrison) Lodder & Kreger

Colonies on MEA at 3 days 1–2 mm diam, off-white, becoming brown when ascospores produced, convex to hemispherical with circular margins, surface glistening; at 7 days colonies

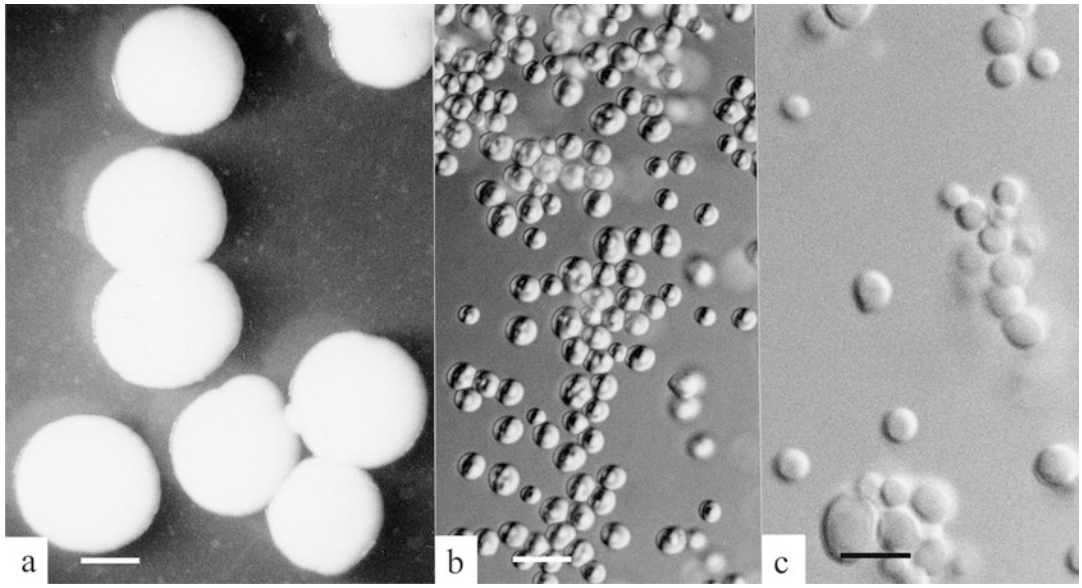

Fig. 10.2 *Debaryomyces hansenii* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

2–4 mm diam, similar to at 3 days, but relatively less deep and with surface sometimes matt. Cells on MEA at 3 days spherical to subspheroidal, 2.5–4.0 µm diam, with some larger ellipsoidal cells, up to 8 µm long; reproduction by irregular budding, sometimes simultaneously at more than one site on the mother cell; occurring singly, in pairs or in small clusters. Ascospores sometimes observed in older cultures; ascus formation occurring in the mother cell after conjugation between mother and daughter cells; ascospores 1, rarely 2, per ascus, spherical, with finely roughened walls. Weak growth on Czapek agar; rarely growth at 37 °C; no growth on malt acetic agar; slow growth on MY50G (up to 1 mm diam in 7 days); rapid growth on MY10-12 (1–4 mm in 7 days).

Distinctive features Spherical cells and rapid growth on MY10-12 agar distinguish *Debaryomyces hansenii* from other species considered here, and, indeed, from almost all other yeasts.

Taxonomy *Debaryomyces hansenii* can be identified using Debaryomyces Differential Medium, on which this yeast produces a violet colony colouration (Quirós et al. 2005), although a few isolates identified by molecular methods tested negative (Ramos et al. 2017). This medium was effective in naturally contaminated food products (Quirós et al. 2005).

Identifiers MycoBank MB296478; lectotype CBS 767; ex-type cultures CBS 767, NRRL Y-7426, JCM 1990; markers D1/D2 LSU rRNA 5U45808, ITS5 AB053101, SSU rRNA 5AB013590 (CBS 2016).

Physiology The most important physiological feature of *Debaryomyces hansenii* is its ability to grow in salt concentrations as high as 24% (w/v; 0.84 a_w; Mrak and Bonar 1939; Prista et al. 2005; Corte et al. 2006; review by Breuer and Harms 2006). Tilbury (1980) reported growth of one isolate of *Torulopsis famata* (= *Candida famata*) at 0.65 a_w in sucrose/glycerol syrups. This species is capable of utilising a wider range of carbon sources than most other spoilage yeasts (Barnett et al. 2000; Breuer and Harms 2006) and is lipolytic (Marquina et al. 1992; Baruzzi et al. 2006). Pectinase activity has been reported (Deak and Beuchat 1996).

Debaryomyces hansenii grows between 2 and 33 °C in YM broth (Kobatake et al. 1992) but its T_{max} increases to around 38 °C in the

presence of 60% (w/w) glucose (Jermini and Schmidt-Lorenz 1987b). Its optimum temperature range for growth is 24–25 °C in 10% (w/w) glucose increasing to 27–30 °C in the presence of 60% (w/w) glucose (Jermini and Schmidt-Lorenz 1987b). The pH range for growth at 25 °C is 2.0–2.5 up to at least pH 8.0 (the highest pH tested), with one strain able to grow in 50% glucose at pH 2.0 (Praphailong and Fleet 1997). Sørensen and Jakobsen (1997) used flow cytometry to study the combined effects of temperature (10–30 °C), pH (4.7–6.0) and NaCl concentration (1–12% w/v) on growth of *D. hansenii,* and used the data to construct a predictive model. *D. hansenii* was unable to grow in 250 mg/L sorbic or benzoic acid at pH 3.0, but some strains grew in 500 mg/L benzoic acid at pH 5.0 (Praphailong and Fleet 1997). Approximately 10^5 vegetative cells/mL were found to survive 20 min at 55° and 10 min at 60 °C; they did not survive 20 min at 60° or 10 min at 62.5 °C (Put et al. 1976).

Ecology The high salt tolerance of *Debaryomyces hansenii* accounts for its frequent occurrence in salt brines including those used for olives (Deak and Beuchat 1996; Quintana et al. 2005), cheeses (see Pitt and Hocking 1997), fermented milk (Kebede et al. 2007) and in soy sauce koji (Lee and Lee 1970). *D. hansenii* has been isolated from fermented and cured meats on many occasions (see Pitt and Hocking 1997; Samelis and Sofos 2003; Simoncini et al. 2007; Ramos et al. 2017) and has been shown to cause dark spots on the casing of ripening salamis (Papa et al. 1995). *D. hansenii* has also been isolated from fresh meat (Dalton et al. 1984; Deak and Beuchat 1996; Samelis and Sofos 2003).

Debaryomyces hansenii is a common component of the microbiota of cheeses (see Pitt and Hocking 1997) where it may have a role in ripening (Roostita and Fleet 1996). *D. hansenii* is sometimes added as part of a combined lactic acid bacteria-yeast starter culture mixture in certain types of cheeses including Pecorino Romano (Deiana et al. 1984), soft cheeses (Kang et al. 1980), Cheddar cheese (Ferreira and Viljoen 2003) and other varieties (Fleet 1990).

Debaryomyces hansenii has caused spoilage of fruit juice, marzipan, and canned figs (Deak and Beuchat 1996; Pitt and Hocking 1997). It has been isolated from a variety of fruit (Deak and Beuchat 1996), candied pumpkin (Martorell et al. 2005a), soft drinks (Ancasi et al. 2006), raw tomatoes (Wade et al. 2003), sugar cane (Azeredo et al. 1998) and been reported in high numbers in yoghurt (Suriyarachchi and Fleet 1981; Yamani and Abu-Jaber 1994). It has also been isolated from malting barley (Petters et al. 1988). Barnett et al. (2000) listed milk, rancid butter, miso, mushrooms, fruit and berries, wine, beer and salt beans as other sources. In our laboratory *D. hansenii* has been isolated from chilli sauce, tomato paste, fruit purée, ham, blue-vein cheese, pickling brine, boiled peanuts, chalky bread and kangaroo biltong. *D. hansenii* has been evaluated as a biological control agent against fungal pathogens on fruits and vegetables including *Penicillium digitatum* postharvest rots in citrus, *Botrytis cinerea* on apples (Droby et al. 1989; Wilson and Chalutz 1989; Santos et al. 2004) and *Rhizopus macrosporus* on peaches (Singh 2004). Optimal conditions for production of toxin by this species have been established (Çorbacı and Uçar 2017).

References Kurtzman and Fell (1998); Barnett et al. (2000); Kurtzman et al. (2011).

Dekkera bruxellensis van der Walt **Fig. 10.3**

Brettanomyces bruxellensis Kuff. & van Laer (valid anamorph name)
*Brettanomyces intermedius (*Krumbholz & Tauschan.) van der Walt & Keuken
Dekkera intermedia van der Walt

Colonies after 3 days on MEA at 25 °C, minute, white; at 7 days 1.5–2.0 mm diam, white, convex, margins circular, surface glistening. Cells on MEA at 3 days mostly ellipsoidal to ogival (pointed at one end, rounded at the other), less commonly spherical or cylindrical, 4.5–7 × 3.0–4.0 µm, reproducing by budding, terminally or subterminally, but not laterally; occurring singly, in pairs, short chains or clusters. Teleomorph not produced under conditions used here. Under

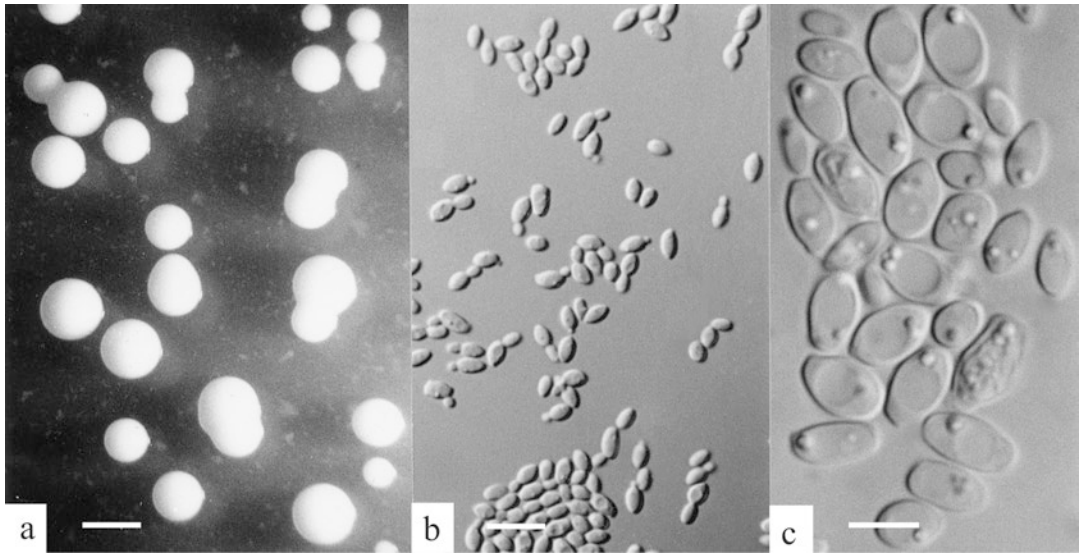

Fig. 10.3 *Dekkera bruxellensis* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

appropriate conditions, vegetative cells can be directly transformed into asci containing 1–4 ascospores, hat-shaped or spherical with a single flange. An acetic acid or other sharp or fruity off-odour usually produced.

No growth on Czapek agar; growth on MEA at 37 °C more rapid than at 25 °C; no growth on malt acetic agar; slow growth on MY50G; no growth on MY10-12.

Distinctive features Species of *Dekkera* are distinguished by the formation of ogival cells, exclusively terminal budding and the production of acetic acid from glucose under aerobic conditions. Cultures are usually short lived unless 2% calcium carbonate is incorporated in the growth medium to neutralise the acid produced.

Most *Dekkera* species have similar properties so identification of an isolate to genus is usually sufficient. *D. bruxellensis,* in our experience and that of others, is the species most commonly isolated from foods.

Taxonomy Both the sexual state name *Dekkera* and the asexual name *Brettanomyces* are in common use and both are valid. *Dekkera* is used here, though the sexual state is not present under the conditions described above. A method for molecular identification of *D. bruxellensis* and its sister species *D. anomola* using specific primers targetting the D1-D2 loop of the 26S rRNA gene has been reported (Cocolin et al. 2004).

Identifiers Mycobank MB329741; holotype CBS 74; ex-type cultures CBS 74, ATCC 36234, NRRL 12961; markers D1/D2 LSU rRNA 5U45738, SSU rRNA 5X83815 (CBS 2016).

Physiology As noted above, the most important characteristic of *Dekkera bruxellensis* and other *Dekkera* species is the ability to produce acetic acid from glucose. Pitt (1974) reported growth of this species down to pH 1.8 in a medium acidified with HCl, and to pH 2.3 in citric acid. *Dekkera* species are resistant to acetic acid and SO_2 (Stratford and James 2003), although Loureiro and Malfeito-Ferreira (2006) reported 70–75 mg/l to be the maximum SO_2 level tolerated at pH 3.5. *D. bruxellensis* can grow in 15.5% ethanol at pH 3.5 (Loureiro and Malfeito-Ferreira 2006). Growth temperatures have been reported as betwwen 19 and 35 °C, with some strains to 42 °C (Schifferdecker et al. 2014).

The biochemical activities of *Dekkera* species contribute to off flavours in beverages spoiled by

these yeasts: extracellular enzymes such as pectinesterases and proteases are formed (Rosi 1993; Panon et al. 1995) and various volatile compounds detrimental to wine aroma may be produced (Ciolfi 1991). Production of phenolic compounds in fermenting grape juice is a characteristic of sporulating cultures (Heresztyn 1986; see Suárez et al. 2005 for a review). However, growth of *Dekkera* species also makes a positive contribution to the acetic flavour of lambic beers (Deak and Beuchat 1996; Stratford and James 2003). *Dekkera* species sometimes survived heating for 10 min, but not 20 min, at 60 °C, but did not survive 10 min at 62.5 °C (Put et al. 1976). Couto et al. (2005) demonstrated that heat inactivation of *D. bruxellensis* begins at 50 °C in tartrate buffer at pH 4.0. However, in wine, significant inactivation was apparent at 35 °C due to the presence of ethanol, phenolic compounds, low pH and other wine parameters (Couto et al. 2005).

Ecology *Dekkera bruxellensis* and other *Dekkera* species have been isolated almost exclusively from beer and similar beverages (Fleet 1992; and see Pitt and Hocking 1997), wines (Kalathenos et al. 1995; Deak and Beuchat 1996; Renouf et al. 2007), and soft drinks (see Pitt and Hocking 1997; Stratford and James 2003; and in our laboratory). Spoilage is often due to undesirable odours. *Dekkera* species have also been reported from spoiled fruit yoghurt (Comi et al. 1982) and from sour dough (Haznedari 1976).

References Kurtzman and Fell (1998); Loureiro and Malfeito-Ferreira (2006); Kurtzman et al. (2011); Schifferdecker et al. (2014).

Hanseniaspora uvarum (Niehaus) Shehata et al. ex M.T. Sm. Fig. 10.4

Kloeckera apiculata (Reess) Janke (valid anamorph name)

Colonies on MEA at 3 days 1–2 mm diam, off-white, almost hemispherical, margins circular, surface glistening; at 7 days 2–4 mm diam, pale brown, low to convex, margins circular, surface glistening. Cells on MEA at 3 days ranging from small narrow ellipsoids, 3.0 × 1.5 µm, and larger, broader ellipsoids, 5–6 × 3.0–4.0 µm, to characteristic apiculate cells, 7–9 × 3.0–4.0 µm, budding terminally only, occurring singly or in pairs. Ascospores sometimes produced in old cultures

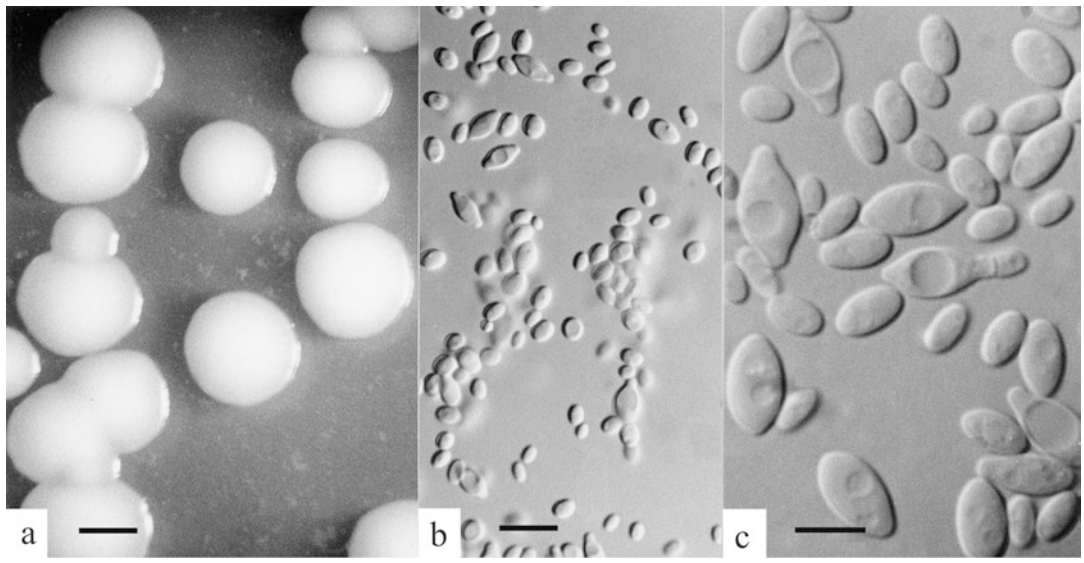

Fig. 10.4 *Hanseniaspora uvarum* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

on MEA, under a coverslip; asci formed from single cells; ascospores 1–2 per ascus, not released in age, spherical, finely roughened or with a minute equatorial ledge. No growth on Czapek agar; sometimes growth at 37 °C; no growth on malt acetic agar, on MY50G or on MY10-12.

Distinctive features The sexual genus *Hanseniaspora* and its asexual name *Kloeckera,* which is still often used, are distinguished by the formation of cells which bud only terminally. *H. uvarum* is the most common species in foods. It is distinguished from other species by minor differences in the utilisation of carbon sources and in ascospore ornamentation.

Identifiers Mycobank MB530461; neotype CBS 314; ex-type cultures CBS 314, NRRL Y-1614. ATCC 32369 (CBS 2016).

Physiology Miller and Mrak (1953) reported a minimum growth temperature for *Hanseniaspora uvarum* near 8 °C and a maximum near 40 °C. It is capable of growth in 9% ethanol and tolerates 12.5% ethanol at 15 °C, but becomes more sensitive at 10 and 30 °C (Gao and Fleet 1988). Pina et al. (2004) reported tolerance to 22.5% v/v ethanol when grown aerobically before exposure to ethanol. *H. uvarum* is extremely acid tolerant, growing at pH 1.5 in inorganic buffer, and pH 2.0 in citrate-phosphate buffer at 25 °C in yeast nitrogen base with 0.5% or 5.0% glucose. However, it was unable to grow at pH 8.0 under these conditions (Praphailong and Fleet 1997). *H. uvarum* is quite sensitive to preservatives: the MIC of benzoic acid was 188 mg/L at pH 3.5 and 25 °C (Warth 1989c). However, it was reported to tolerate levels of SO_2 up to 100 mg/L in red wine (Heard and Fleet 1988b). Praphailong and Fleet (1997) reported no growth in 250 mg/L benzoate or sorbate at pH 3.0, but at pH 5.0, *H. uvarum* grew in 750 mg/L benzoate or 250 mg/L sorbate. *H. uvarum* grew in 12.5% (w/v) NaCl and 50% (w/v) sucrose from pH 2.0–7.0 (Praphailong and Fleet 1997). Heat resistance is average, with cells (10^5/mL) of this species surviving for 20 min at 55 °C but not 10 min at 60 °C (Put et al. 1976). Later work reported that 10^4–10^5 cells when heated at 56 °C in either milk or Ringers solution did not survive 30 seconds (Engel et al. 1994).

Hanseniaspora uvarum is a strongly fermentative yeast which exhibits a wide range of biochemical activities, producing extracellular proteases (Rosi 1993), polygalacturonase (Masoud and Jespersen 2006), α-glucosidase (Rosi et al. 1994), and also acetoin (Romano et al. 1993), erythritol and sorbitol (Sponholz et al. 1986) and ethanol (Heard and Fleet 1988a; Duenas et al. 1994). It can be isolated from mixed cultures of yeasts in natural fermentations using lysine agar (Heard and Fleet 1986b).

Ecology The natural habitat of *Hanseniaspora uvarum* (often under its asexual name *Kloeckera apiculata*) is fruit, particularly grapes, and it may play an active role during the early stages of fermentation of grape juice (Fleet et al. 1984; Heard and Fleet 1986a,b; Suarez-Lepe 1991; Deak and Beuchat 1996). It is also associated with cocoa fermentation (Ravelomanana et al. 1984; Mazigh 1994; Masoud and Jespersen 2006), malting barley (Petters et al. 1988) and cider fermentation (Morrissey et al. 2004). *H. uvarum* has been reported to spoil figs, tomatoes, canned black cherries and in our laboratory, strawberry topping and fruit-flavoured yoghurt (see Pitt and Hocking 1997). *H. uvarum* has been isolated from fresh strawberries, blackcurrants and wine grapes and is associated with sour rot of grapes (see Pitt and Hocking 1997) and fermenting mango (Suresh et al. 1982). Other sources include citrus, orange concentrate, fruit juices and fruit syrups (see Pitt and Hocking 1997; Las Heras-Vazquez et al. 2003).

References Barnett et al. (2000); Kurtzman et al. (2011).

Kazachstania exigua (Reess ex E.C. Hansen) Kurtzman Fig. 10.5

Saccharomyces exiguus Reess
Candida holmii (A. Jörg.) S.A. Mey. & Yarrow (valid anamorph name)
Torulopsis holmii (A. Jörg.) Lodder
Torula holmii A. Jörg.

Colonies on MEA at 3 days 4–5 mm diam, white, circular with a smooth margin, and a low, convex, glistening surface; at 7 days 6–8 mm diam, appearance as at 3 days. Cells on MEA small, ellipsoidal, 4.0–5.0 × 2.5–3.5 µm, reproducing by irregular budding, occurring singly or in pairs. Ascospores not usually formed under the conditions used here; asci formed directly from vegetative cells; ascospores 1–4 per ascus, spherical to ellipsoidal and smooth walled. No growth on Czapek agar or at 37 °C; slow growth on malt acetic agar; no growth on MY50G or MY10-12.

Distinctive features *Kazachstania exigua* is of similar appearance to *S. cerevisiae,* but does not grow on Czapek agar or at 37 °C.

Taxonomy After extensive studies involving multigene sequencing and phylogenetic analysis (Kurtzman and Robnett 2003), *Saccharomyces exiguus* was transferred to *Kazachstania* as *K. exigua* by Kurtzman (2003). The two genera (*Saccharomyces* and *Kazachstania*) are difficult to distinguish using morphological criteria (but see above). The sexual name *Saccharomyces exiguus* and asexual names *Candida holmii* and *Torulopsis holmii* have been widely used for *K. exigua* (Kurtzman and Fell 1998; Barnett et al. 2000).

Identifiers MycoBank MB486276; neotype CBS 379. Ex-type cultures CBS 379, NRRL Y-6695, ATCC 22034 (CBS 2016).

Physiology *Kazachstania exigua* is preservative resistant. Pitt (1974) reported growth in 400 mg/kg benzoic or sorbic acid at pH 4.0, and Frohlich-Wyder (2003) reported growth in 500 mg/kg and 800 mg/kg sorbic and benzoic acid, respectively, at the same pH, but with considerable strain to strain variation. *K. exigua* is also capable of growth under very acid conditions, pH 1.5 in HCl, pH 1.7 in H_3PO_4, and pH 1.9–2.1 in organic acids (Pitt 1974). Suihko and Makinen (1984) demonstrated that *K. exigua* was relatively resistant to acetate, propionate and sorbate at pH 4.5, and had better dough raising power than *S. cerevisiae* when these compounds were present. Growth at 4–7 °C within 1–3 weeks has been reported (Fleet 2006). This species vigorously ferments a wide range of sugars.

Ecology *Kazachstania exigua* is of common occurrence in brines during the early stages of pickle fermentation (Etchells et al. 1952, 1953). Steinbuch (1965, 1966) reported that *K. exigua*

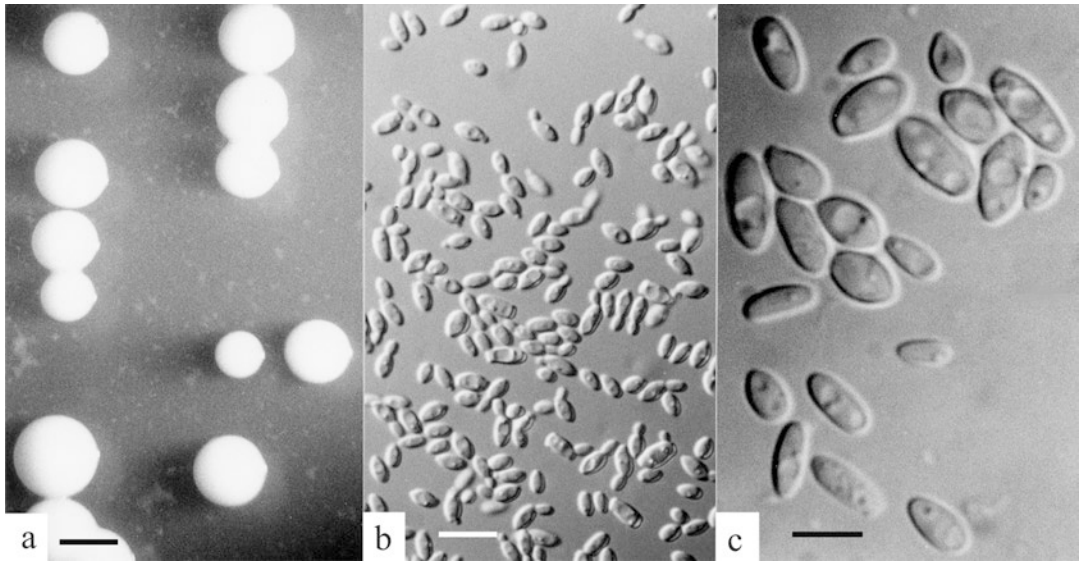

Fig. 10.5 *Kazachstania exigua* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

and *C. holmii,* regarded at that time as distinct species, were the cause of pink or grey discolouration in sauerkraut. *K. exigua* has been identified as an important component of the microflora of sour dough in France (Infantes and Schmidt 1992), Italy (Galli et al. 1988; Gobbetti et al. 1994; Pulvirenti et al. 2004); Finland (Salovaara and Savolainen 1984), Germany (Spicher et al. 1979; Spicher 1986a), in San Francisco sour dough (Sugihara et al. 1971) and in Italian panettone dough (Galli and Ottogalli 1973). It has also been identified as an important component of the microflora of silage made from maize (Middelhoven 1998) and grass (O'Brien et al. 2007).

Kazachstania exigua has been isolated from spoiled soft drinks in Australia (Pitt and Richardson 1973) and elsewhere (Fröhlich-Wyder 2003), and it is a common spoilage yeast in delicatessen salads (Baumgart et al. 1983; Fleet 1992; Bonestroo et al. 1993; Hunter et al. 1994). Other recorded sources include green olives (Mrak et al. 1956), citrus products (Recca and Mrak 1952), kefir (Iwasawa et al. 1982; Fröhlich-Wyder 2003; Latorre-Garcia et al. 2007), fresh cheese (quarg) (Engel 1988), yoghurt (Viljoen, et al. 2003b) and occasionally from other dairy and meat products (Fleet 2006).

References Kurtzman (2003); Kurtzman et al. (2011).

Pichia kudriavzevii Boidin et al. Fig. 10.6

Candida krusei (Castell.) Berkhout
(valid anamorph name)
Issatchenkia orientalis Kudryavtsev

Colonies on MEA at 3 days 2–4 mm diam, white, convex, with margins irregularly lobate or fimbriate and surface matt; at 7 days colonies large, 5–8 mm diam, white, often centrally umbonate, margins characteristically filamentous. Cells on MEA at 3 days varying from short ellipsoids (3.0–4.0 × 2.0–10 µm) to long cylinders (10–25 × 3.0–4.0 µm), occasionally with larger ellipsoids (10–15 × 5–7 µm) also; reproducing by irregular budding, occurring singly and in chains. Ascospores rarely observed, one per ascus, smooth walled. Weak growth on Czapek agar; strong growth at 37 °C (3–4 mm diam in 7 days); growth on malt acetic agar; no growth on MY50G or on MY10-12.

Distinctive features *Pichia kudriavzevii* grows strongly at 37 °C, and grows on malt acetic agar, although slowly. Large cylindrical cells are produced in cultures on MEA.

Taxonomy This species was known for many years as *Candida krusei* (and that remains a valid name at this time). *Issatchenkia orientalis* was described by Kudryavtsev in 1960 as a sexual yeast and transferred to *Pichia* as *P. orientalis* by Kreger-Van Rij in 1964; as that name was occupied Biodin and coworkers provided the new

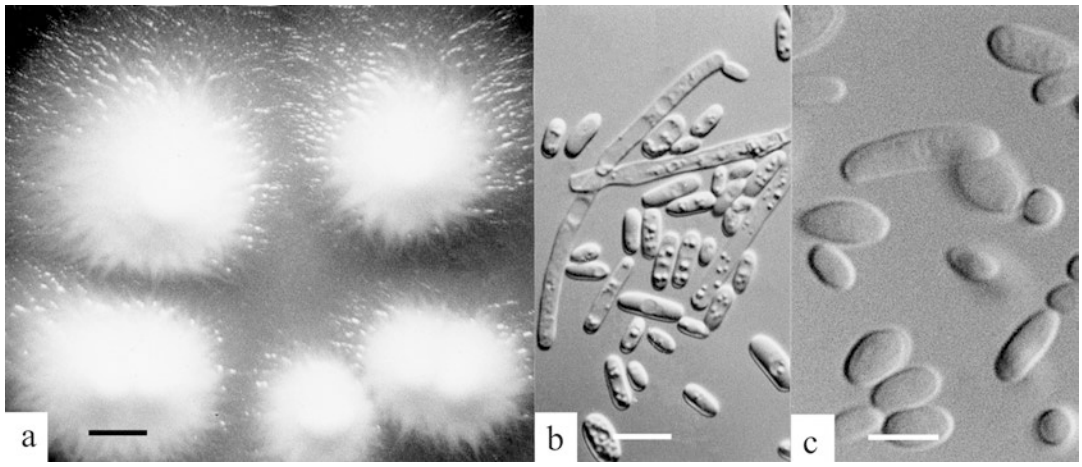

Fig. 10.6 *Pichia kudriavzevii* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

name *Pichia kudriavzevii*. The synonymy with *Candida krusei* was shown later by molecular methods (Douglass et al. 2018).

Identifiers Mycobank MB337013; holotype CBS 5147; ex-type cultures CBS 5147, NRRL Y-5396, NRRL Y-755. Markers LSU (incl. D1/D2) rRNA 5EF550222, SSU rRNA 5EF550360, *TEF-1α* 5EF552446 (CBS 2016).

Physiology In the present context, the most important physiological characteristic of *Pichia kudriavzevii* (always reported as *Candida krusei*) is its ability to grow in the presence of preservatives (Pitt and Richardson 1973). *P. kudriavzevii* was able to grow anaerobically at pH 3.5 in the presence of 3 mM (335 ppm) sorbic acid, 3 mM (360 ppm) benzoic acid or 0.48 mM (30 ppm) free SO_2 (Warth 1985). For *P. kudriavzevii* cells adapted to benzoic acid the minimum inhibitory concentrations (MICs) of various preservatives were: acetic acid 13.5 g/L; propanoic acid 8.0 g/L; benzoic acid 440 mg/L and methyl paraben 1.0 g/L (Warth 1989c). The ability of this yeast to grow in the presence of acetic acid has been linked to the citrate synthase gene *cs1* (Casey and Dobson 2003). *P. kudriavzevii* also grows at exceptionally low pH: in a medium acidified with HCl, at pH 1.3, equivalent to 0.05N HCl; and at pH 1.7–1.9 in media acidified with H_3PO_4 and organic acids, respectively (Pitt 1974). *P. kudriavzevii* has a minimum growth temperature near 8 °C and a maximum near 47 °C (Miller and Mrak 1953). It has been reported as relatively heat resistant, surviving heating at 56 °C for 80 minutes, though inactivation took less than 2 minutes at 65 °C (Engel et al. 1994).

Ecology Although it ferments glucose, *Pichia kudriavzevii* (almost always reported as *Candida krusei*) is usually a surface-growing, film forming yeast on foods. It has caused spoilage of African cocoa beans (Maravalhas 1966), United States figs and Australian tomato sauce (see Pitt and Hocking 1997). It has also been isolated from citrus and other fruit products, fermenting mango, olives, soft drinks (Deak and Beuchat 1996; see also Pitt and Hocking 1997; Stratford and James 2003), grapes with sour rot (Fleet 2003), low-salt bacon (Samelis and Sofos 2003), cassava flour (Okagbue 1990), spoiled wine (Deak and Beuchat 1996), fresh and matured cheeses and fermented milk products (see Pitt and Hocking 1997; Frölich-Wyder 2003). *P. kudriavzevii* is associated with a number of fermentations: cocoa beans (Schwan and Wheals 2003; Jespersen et al. 2005; Nielsen et al. 2005; Pereira et al. 2017); sour dough (Spicher et al. 1979; Spicher 1986a; Gobbetti et al. 1994), idli batter in India (Venkatasubbaiah et al. 1985), maize dough in West Africa (Hounhouigan et al. 1994; Jespersen et al. 1994; Obiri-Danso 1994), kamu, a fermented millet cake from Nigeria (Oyeyiola 1991), fermented cassava flour (Nigerian fufu; Oyewole 2001) and various other fermented foods such as cheese (Bockelmann et al. 2005; Shuangquan et al. 2006) and beverages (Batra and Millner 1976; Deak and Beuchat 1996).

Pathogenicity Although *Pichia kudriavzevii* is of common foodborne origin, has been used for centuries to make a wide range of food products, and has a growing role in biotechnology, warnings have recently been sounded that it is also an important human pathogen (under the name *Candida krusei*), responsible for significant levels of morbidity and mortality in immunocompromised patients (Douglass et al. 2018).

References Kurtzman and Fell (1998); Kurtzman et al. (2011); Douglass et al. (2018).

Pichia membranifaciens (E.C. Hansen) E.C. Hansen Fig. 10.7

Candida valida (Leberle) Uden & H.R. Buckley (valid anamorph name)

Colonies on MEA at 3 days 1–3 mm diam, off-white, convex but not hemispherical, margins irregular, surface usually matt; at 7 days 3–4 mm diam, white, margins circular, centrally heaped up or wrinkled, surface dull and granular. Cells on MEA at 3 days small and ellipsoidal to cylindrical, 4.0–6 × 2.0–4.0 µm, reproducing by irregular budding. Ascospores regularly formed on malt acetic agar after 7 days at 25 °C; asci formed from single cells; ascospores usually 4 per ascus, tiny, shaped like a bowler hat, quickly liberated from the ascus but adherent to each other in clumps. Very weak growth on Czapek agar; usually no growth at 37 °C; growth on malt acetic

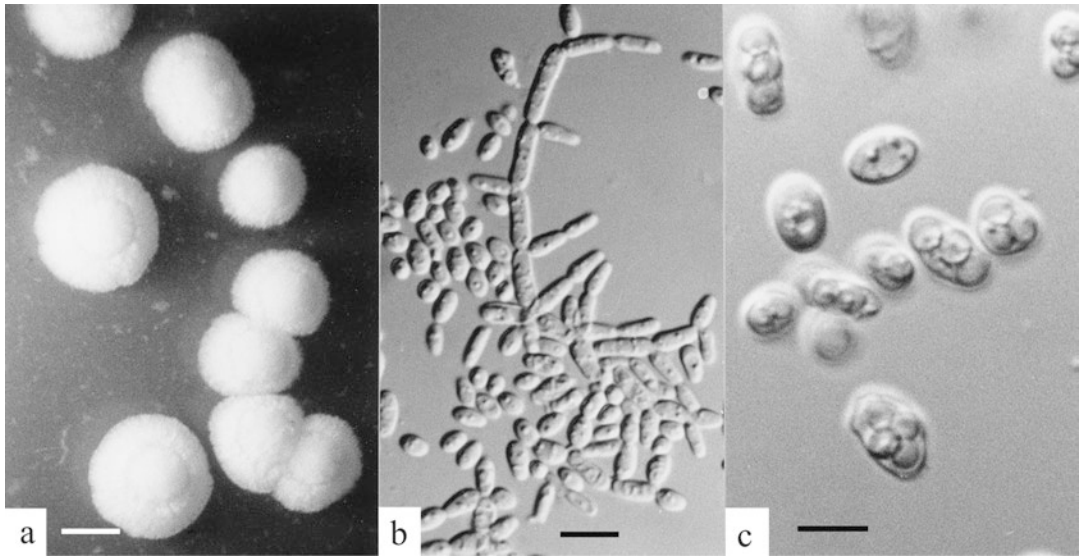

Fig. 10.7 *Pichia membranaefaciens* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**) vegetative cells, bar = 10 µm; (**c**) asci and ascospores, bar = 5 µm

agar (1 mm diam in 3 days); no growth on MY50G or on MY10-12.

Distinctive features A diagnostic feature for *Pichia membranifaciens* is the formation of tiny bowler hat shaped ascospores on malt acetic agar within 7 days. Spores usually number 4 per ascus but are quickly liberated.

Identifiers MycoBank MB227217; lectotype CBS 10; ex-type cultures CBS107, NRRL Y-2026; ATTC 26288; ITS barcode DQ104710, alternative markers *RPB1* GQ327958, *RPB2* AY497599, *EF1* AY497634 (CBS 2016).

Physiology Miller and Mrak (1953) reported a minimum growth temperature near 5 °C for *Pichia membranifaciens,* and a maximum near 37 °C. Mrak et al. (1956) reported poor but positive growth of this species in the presence of 15.2% NaCl: assuming the percentage is weight in volume, this is equivalent to 0.90 a_w. Praphailong and Fleet (1997) reported growth of *P. membranifaciens,* in 12.5% (w/v) NaCl at pH 3, but at pH 2.0 only 2.5% (w/v) NaCl was tolerated, and at pH 5.0, 5.0% NaCl. *P. membranifaciens* was able to grow in 50% (w/v) sucrose from pH 2.0–7.0 (Praphailong and Fleet 1997). Pitt and Richardson (1973) reported growth in the presence of 1% acetic acid; Pitt (1974) observed growth in the presence of 400 mg/kg benzoic acid at pH 4.0; Ethiraj and Suresh (1988) isolated a strain from spoiled mango pulp that grew in the presence of 1500 mg/kg sodium benzoate at pH 4.0, and in 3000 mg/kg benzoate at pH 4.5. Praphailong and Fleet (1997) reported growth in 750 mg/L benzoate or 250 mg/L sorbate at pH 5.0, but no growth was observed with 250 mg/L of either preservative at pH 3.0. Growth occurred down to pH 1.9 in media acidified with HCl and pH 2.1–2.2 in media acidified with organic acids (Pitt 1974). Praphailong and Fleet (1997) reported similar results, with growth between pH 2.0–7.0 in inorganic buffer and between 2.5–7.5 in citrate-phosphate buffer. No growth occurred at pH 8.0.

Pichia membranifaciens is sensitive to heat: Put et al. (1976) reported survival of vegetative cells (10^5/mL) for 10 min at 55 °C, but not 20 min at 55 °C or 10 min at 60 °C. Engel et al. (1994) reported survival of *P. membranifaciens* (10^4–10^5 cells) heated in Ringers solution or milk for 30 min at 56 °C.

Enzymic activity of *Pichia membranifaciens* includes production of pectin methyl-esterase (Gauthier et al. 1977; Panon et al. 1995) and extracellular proteases (Rosi 1993). *P. membranifaciens* produced 2-propanol and 2-hexanone when inoculated into grape must (Mamede and Pastore 2006).

Ecology The most common food-associated source of *Pichia membranifaciens* has been olive brines (Mrak and Bonar 1939; Mrak et al. 1956; Marquina et al. 1992; Duran et al. 1994; Oliveira et al. 2004), but it also occurs in cheese brines (Kaminarides and Laskos 1993) and appears to be an important component of cocoa fermentations (Gauthier et al. 1977; Nielsen et al. 2005, 2007). Vaughn et al. (1943) associated this and other yeasts with "stuck" olive fermentations, in which carbohydrate is depleted without the desired build up in lactic acid concentration. Dakin and Day (1958) reported the isolation of *P. membranifaciens* from a variety of acetic acid preserves including onions, gherkins, pickles, beetroot and sauerkraut. Muys et al. (1966) and Pitt and Richardson (1973) reported spoilage of tomato sauce due to film formation by this yeast. It is also relatively common in delicatessen salads (Bonestroo et al. 1993; Hunter et al. 1994) and salad dressings and mayonnaise (our observations).

Pichia membranifaciens has been isolated from "chalk mould" spoilage of bread (Spicher 1986b), fermented milk products (Rohm et al. 1992), fresh and ripened cheeses (see Pitt and Hocking 1997;Westall and Filtenborg 1998; Lioliou et al. 2001), fresh sausages (Dalton et al. 1984), Brazilian guava fruit (Abranches et al. 2000) and in our laboratory from cherries in brine. Other recorded sources of *P. membranifaciens* include cereal grains and flours (Spicher and Mellenthin 1983), citrus and citrus products including orange concentrate, fermenting mango, grape must and soft drink processing lines (see Pitt and Hocking 1997).

P. membranifaciens shows promise as a biocontrol agent against *Monilia fructicola* on cherries (Qin et al. 2006), *Botrytis cinerea* on grapes (Masih and Paul 2002; Santos et al. 2004; Santos and Marquina 2004), *Rhizopus* rot of nectarines (Fan and Tian 2000), postharvest pathogens of apples (Chan and Tian 2005) and undesirable yeasts in wines including *Brettanomyces bruxellensis* (for review see Belda et al. 2017). Activity appears to be due to production of killer toxins (Santos and Marquina 2004) which bind to (1→6)-β-d-glucans (Belda et al. 2017).

References Kurtzman and Fell (1998); Barnett et al. (2000); Kurtzman et al. (2011).

Rhodotorula mucilaginosa (A. Jörg.) F.C. Harrison **Fig. 10.8**

Rhodotorula rubra (Demme) Lodder

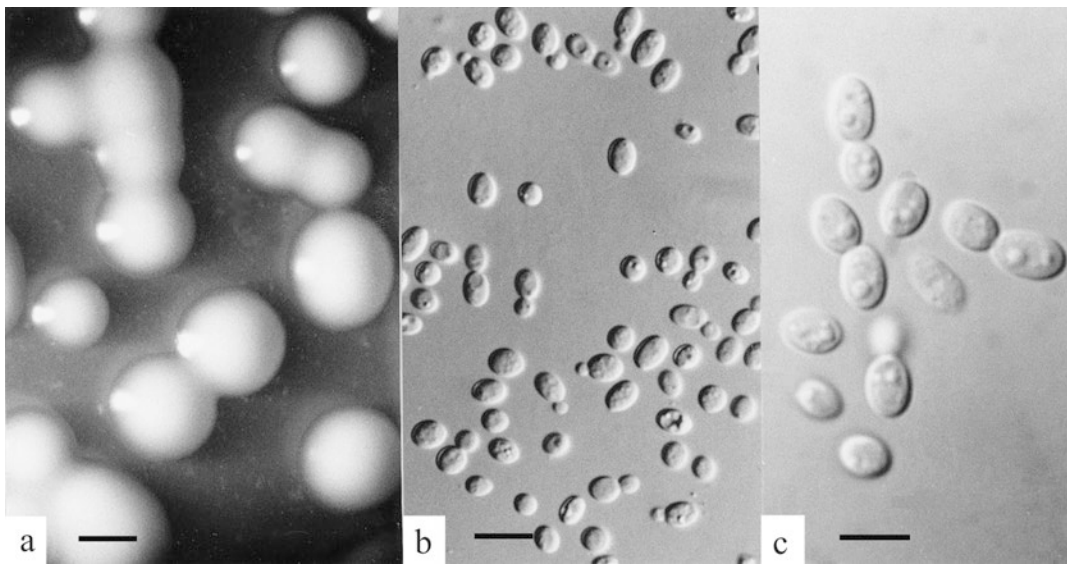

Fig. 10.8 *Rhodotorula mucilaginosa* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm

Colonies on MEA at 3 days 1.5–3 mm diam, coloured dull to bright pink to red, margins circular or spreading, convex, surface glistening or appearing mucoid; at 7 days 5–10 mm diam, with appearance as at 3 days. Cells on MEA at 3 days mostly ellipsoidal, 4.0–5.5 × 3.0–3.5 µm; reproduction by irregular budding; occurring singly or in pairs. Ascospores not produced. Growth on Czapek agar weak to quite strong; sometimes weak growth at 37 °C; no growth on malt acetic agar, MY50G, or MY10-12.

Distinctive features *Rhodotorula mucilaginosa* and the closely related *R. glutinis* are distinguished from other species described here by pink to red, often mucoid colonies and their inability to grow on malt acetic agar or MY50G.

Taxonomy The name *Rhodotorula rubra* (Demme) Lodder used for this species by Kreger-van Rij (1984) is a later homonym of *Rhodotorula rubra* (Saito) F.C. Harrison, so it is illegitimate. However, as *R. rubra* (Saito) F.C. Harrison is a synonym of *R. glutinis* (Barnett et al. 2000, see below), the correct name for this species is *R. mucilaginosa* (Barnett et al. 2000).

Identifiers MycoBank MB271749; lectotype CBS 316; ex-type culture CBS 316 (CBS 2016).

Physiology *Rhodotorula mucilaginosa* has a minimum growth temperature between 0.5 and 5 °C and a maximum near 35 °C, according to Miller and Mrak (1953). Other reports suggest that some *Rhodotorula* species can grow well below 0 °C (Walker 1977; Table 10.1), but these reports are often unsubstantiated. Collins and Buick (1989) reported good growth of *R. glutinis* on blanched frozen peas stored at 0 °C for 8 weeks, and a significant increase in yeast numbers after 24 weeks at −18 °C. A minimum a_w for growth near 0.92 was reported by Bem and Leistner (1970). pH 2.2 was the minimum for growth in the presence of HCl or organic acids; growth was inhibited by 100 mg/kg or less of benzoic or sorbic acid at pH 4 or below (Pitt 1974). Cultures of R. *mucilaginosa* and *R. glutinis* (10^5 cells/mL) sometimes survived heating at 62.5 °C for 10 min (Put et al. 1976). This is a high heat resistance for an asporogenous yeast. However heat resistance is lower in orange juice. *R. mucilaginosa* had a D_{50} value of 2.6 min and a z value of 9.8 C° (Barreiro et al. 1981) and Shearer et al. (2002) reported D_{60} values between 0.12 min at pH 3 and 0.16 min at pH 4.0 with z values of 4.5–4.7 C° in 0.1 M citrate buffer.

Rhodotorula species produce extracellular lipases and proteases (Rapp and Backhaus 1992; Rosi 1993).

Ecology Both *Rhodotorula mucilaginosa* and *R. glutinis* are of widespread occurrence on fresh fruits and vegetables (e.g. Recca and Mrak 1952; Buhagiar and Barnett 1971). Reports of spoilage are rare. Leaves and plant stems are major habitats, and as a result these species sometimes occur in cereals and flours, dough, malting barley, olives, soaking soybeans, citrus products and fruit juice concentrates (see Deak and Buechat 1996; Pitt and Hocking 1997).

The ability to grow rapidly at refrigeration temperatures means that *Rhodotorula* species are commonly associated with and may cause spoilage in dairy products such as yoghurts, cream, butter and cheeses (Fleet and Mian 1987; see also Deak and Beuchat 1996; Pitt and Hocking 1997; Frölich-Wyder 2003; Viljoen et al. 2003a). In our laboratory we have frequently encountered *Rhodotorula* species causing spoilage of cream, sour cream and dairy desserts. They are also common in seafoods, including fish, shellfish and crustaceans, and in marine waters (Hood 1983; Comi et al. 1984; Kobatake et al. 1992). *Rhodotorula* species are also associated with fresh and processed meats (Dillon and Board 1991; see also Deak and Beuchat 1996; Pitt and Hocking 1997; Samelis and Sofos 2003).

Spoilage by *Rhodotorula* species has been reported in heat treated apple sauce and strawberries (Put et al. 1976) and cut potato chips packaged in 97–99.8% carbon dioxide (Cerny and Granzer 1984). *Rhodotorula* species have been isolated from ready to eat airline meals (Saudi and Mansour 1990). We have isolated *R. mucilaginosa* from apple pie filling and *R. glutinis* from soft cheeses.

Rhodotorula species produce ß-carotene and other carotenoids (Martin et al. 1993; Frengova et al. 1994) and can be cultivated to provide a good source of these compounds and protein for aquaculture fish (Hari et al. 1992).

The potential for *R. mucilaginosa* as a biocontrol agent to reduce growth of *Penicillium expansum* in apples and reduction of patulin formation was reported by Li et al. (2019). Efficacy was reported to be increased by addition of phytic acid (Yang et al.. 2015).

Pathogenicity Since the first edition of this book (Pitt and Hocking 1985), *Rhodotorula* species, especially *R. mucilaginosa*, have emerged as significant human pathogens. Most commonly that has been associated with central venous catheter use, although other reported infection sites include skin, eye, peritoneum and prosthetic joints (Tuon and Costa 2008; Wirth and Goldani 2012).

Additional species *Rhodotorula glutinis* (Fresen.) F.C. Harrison [synonym *Rhodotorula rubra* (Saito) F.C. Harrison] differs from *R. mucilaginosa* by the ability to use nitrate as a nitrogen source. It occurs in a similar range of habitats. but is less likely to cause human disease (Tuon and Costa 2008; Wirth and Goldani 2012). Identifiers: MycoBank MB266169, lectotype CBS 20, ex-type cultures ATCC 2527; NRRL Y-2502; ITS barcode AF444539, 26S rDNA AF070430, 18S rDNA AB016291 (CBS 2016).

References Kurtzman and Fell (1998); Barnett et al. (2000); Kurtzman et al. (2011).

Saccharomyces cerevisiae Meyen ex E.C. Hansen **Fig. 10.9**

Colonies on MEA at 3 days 1–2 mm diam, off-white, convex, margins circular, surface glistening; at 7 days 2–3 mm diam, as at 3 days except margins sometimes becoming fimbriate. Cells usually spherical to subspheroidal, 5–12 × 5–10 µm, occasionally also ellipsoidal to cylindrical, 5–20(−30) × 3–9 µm, reproducing by irregular budding, occurring singly, in pairs or in chains. Ascospores sometimes formed on MEA after prolonged incubation; asci formed directly from vegetative cells without conjugation; ascospores 1–4 per ascus, spherical to subspheroidal and smooth walled. Weak growth on Czapek agar; growth at 37 °C usually as fast as, or faster than, that at 25 °C; growth on malt acetic agar very weak or absent; no growth on MY50G or MY10-12.

Distinctive features *Saccharomyces cerevisiae* is included here as an example of a strongly fermentative yeast which commonly occurs on foods but only infrequently causes spoilage. Colony and cell characteristics together with sporulation of the kind described above are reasonably diagnostic. In the absence of sporulation, identification remains a matter of conjecture unless the full identification systems of Kurtzman et al. (2011) or molecular methods are applied.

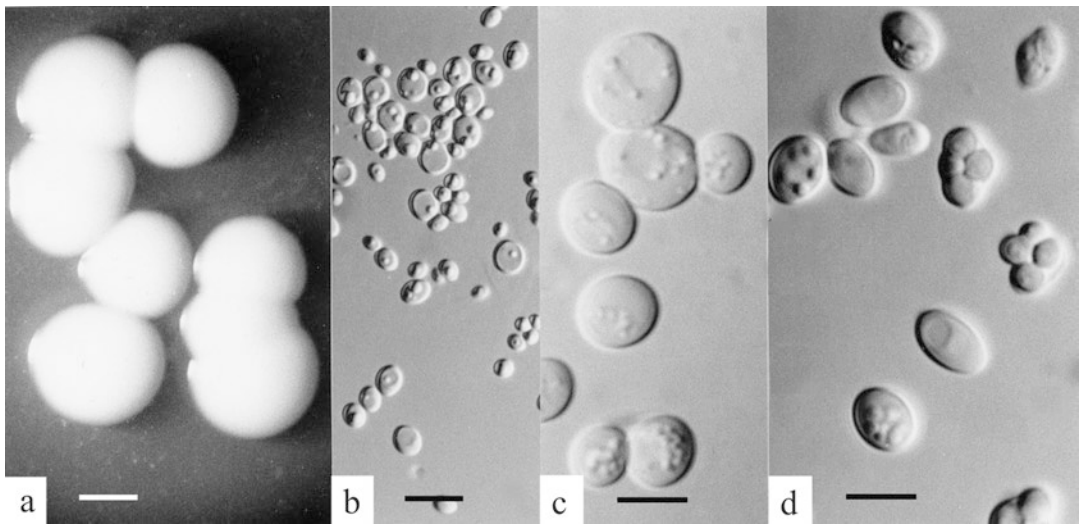

Fig. 10.9 *Saccharomyces cerevisiae* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm; (**d**) asci and ascospores, bar = 5 µm

Taxonomy Kurtzman and Robnett (2003) used multigene sequence analysis to examine *Saccharomyces* and related genera. After extensive phylogenetic analysis they concluded that the genus should be split into a number of genera, with *Saccharomyces* reserved for *S. cerevisiae* and closely related species, whilst the remaining *Saccharomyces* species were assigned to several other genera, some of which were newly described (Kurtzman 2003). *Saccharomyces exiguus* was included in the genus *Kazachstania.* Fleet (2006) provided a useful summary of the changes in taxonomic treatment of these yeasts.

Barnett et al. (2000) listed almost 120 synonyms for *Saccharomyces cerevisiae,* including many subspecies and varieties encompassing bread-making, brewing, wine and cider yeasts, as well as yeasts from the natural environment. Consequently, the biochemical reactions given for *S. cerevisiae* are highly variable.

Identifiers MycoBank MB163963; neotype CBS 1171; ex-type cultures CBS 1171; ATCC 18824; NRRL Y-12632. ITS barcode AB018043; alternative markers 26S rDNA AF005702, 18S rDNA AB182465, *RPB1* AF527884, *RPB2* AF527884, *EF1* AF402004 (CBS 2016).

Physiology *Saccharomyces cerevisiae* grows optimally between 33 and 35 °C in 10–30% glucose. Its minimum growth temperature is reported as 4 °C in 10% glucose and 13 °C in 50% glucose, with a maximum growth temperature of 38–39 °C (Jermini and Schmidt-Lorenz 1987b). Juven et al. (1978) demonstrated growth of *Saccharomyces cerevisiae* down to 0.89 a_w in glucose media at neutral pH. Praphailong and Fleet (1997) reported growth in 7.5% (w/v) NaCl (0.95 a_w) at pH 5 and pH 7, but at pH 3, only 5% (w/v) NaCl was tolerated. The lowest pH at which Praphailong and Fleet (1997) recorded growth was pH 2.5 in yeast nitrogen base with 5% glucose in citrate-phosphate buffer. Over the pH range 3.0–7.0, *S. cerevisiae* grew in 50% but not 60% (w/v/) sucrose. *S. cerevisiae* was capable of growth down to pH 1.6 in HCl, 1.7 in H_3PO_4, and 1.8–2.0 in organic acids with a maximum tolerance to benzoic acid of 100 mg/kg at pH 2.5–4.0, and to sorbic acid of 200 mg/kg at pH 4.0 (Pitt 1974). *S. cerevisiae* can degrade sorbic acid to 1,3-pentadiene (Stratford et al. 2007).

It has been known for over 100 years that *Saccharomyces cerevisiae* is one of the yeast species most resistant to sulphur dioxide (Cruess 1912) and that is important in controlling undesirable yeasts in winemaking. However, inaccurate information on the dissociation constants of free SO_2, H_2SO_3, HSO_3^- and $SO3^=$ caused much misleading information in the literature, as only the undissociated molecule is the toxic moiety (King et al. 1981). They calculated the first dissociation constant as $1.7 \times I0^{-2}$ and the second as 6.31×10^{-8}. They corrected the long standing error that the proportion of the undissociated molecule is low at the pH levels of interest (pH 2–pH 4–5), and that in fact <2 μg/kg of H_2SO_3 is sufficient to reduce viable counts of *S. cerevisiae* by four log cycles (King et al. 1981). As the fraction of SO_2 in the form of undissociated H_2SO_3 is higher at lower pH, so the effectiveness of SO_2 treatment is higher at lower pH. Thus 100 μg/kg of SO_2 was required to be lethal to *S. cerevisiae* at pH 3.5, but at pH 2.5 only 13 μg/kg was required (King et al. 1981).

Using about 20 isolates, Put and de Jong (1982) determined that vegetative cells of *Saccharomyces cerevisiae* had a D_{60} of 0.1–0.3 min, while ascospores were more resistant, with a D_{60} of 5.1–17.5 min. Splittstoesser et al. (1986) reported that S. *cerevisiae* ascospores were over 100-fold more heat resistant than vegetative cells of the same strain, with a D_{60} of 6.1 min for ascospores in apple juice, and a z value of 3.8 C°. Juven et al. (1978) found that heating cells of *S. cerevisiae* in media of reduced a_w greatly enhanced their heat resistance. In a medium based on fruit juice (pH 3.1, 0.99 a_w, 12° Brix), D_{60} was 0.3–2 min, but at 0.93 a_w D_{60} was 5 min or more. Using a 0.5% glucose heating medium, Török and King (1991) obtained a value for D_{60} of 1.05–2.67 min, and a *z* value of 4.0–6.2 C°, while Hur et al. (1993) reported a D_{62} of 3.5 min, and a *z* value of 7.2 C° for ascospores in 0.05 M phosphate citrate buffer. Shearer et al. (2002) assessed the heat resistance of *S. cerevisiae* in a range of buffers and juices. The highest D_{60} they recorded was 6.9 min in calcium fortified apple juice at pH 3.9. Dry heat resistance was much greater. When 10^8 *S. cerevisiae* cells were dried in wheat flour or skim milk powder to a_w values between 0.10 and 0.70, then heated at

150° or 200 °C for 5–30 s, survival was greatest in skim milk powder at 0.40 a_w, with less than 1 $\log_{10}$ reduction at 200 °C for 5 sec, and 5 log reduction after 30 sec at 200 °C, indicating the difficulty of sterilising dry powders (Laroche et al. 2005).

High pressure inactivation of 4×10^5 *Saccharomyces cerevisiae* cells in apricot nectar required 700 MPa for 5 min (Gola et al. 1994), although Hocking et al. (2006) reported a 4–5 $\log_{10}$ reduction in *S. cerevisiae* cells suspended in 20°Brix sucrose at pH 4.2 after treatment at 400 MPa for 2 min. Concentrated solutions provide protection against high pressure processing: a treatment of 600 MPa for 30 s resulted in less than 1 log reduction in viability of *S. cerevisiae* cells in glycerol, sucrose or NaCl solutions at 0.86 a_w (Goh et al. 2007). High intensity pulsed electric field treatment has also been used to inactivate *S. cerevisiae* in orange juice (Elez-Martinez et al. 2004).

Saccharomyces cerevisiae produces polygalacturonase (Panon et al. 1995; Djokoto et al. 2006) and extracellular proteases (Rosi 1993).

Ecology Best known for its domesticated role in the manufacture of breads and alcoholic beverages, *Saccharomyces cerevisiae* is also of widespread natural occurrence, in nectars and exudates, and on leaves and fruits. Not surprisingly, then, it occurs widely in foods, and can be a source of spoilage. Soft drinks commonly contain S. *cerevisiae* (see Deak and Beuchat 1996; Pitt and Hocking 1997). Sand and van Grinsven (1976) and Put et al. (1976) reported spoilage of some cold sterilised products. The other major source of *S. cerevisiae* is fruit juices and concentrates (see Deak and Beuchat 1996; Pitt and Hocking 1997; Boekhout and Robert 2003; Fleet 2006). Back and Anthes (1979) reported spoilage of fruit juice drinks and Put et al. (1976) of heat processed cherries. We have also isolated *S. cerevisiae* from spoiled fruit juices, tomato paste, fruit purees and bottling machinery and preservative resistant strains from spoiled sports drinks, mineral water with added *Aloe vera* and orange fruit juice cordial.

Saccharomyces cerevisiae has been isolated from a variety of dairy products. It was the dominant yeast in Egyptian Kareish cheese (Zein et al. 1983), and was relatively common in labaneh, a strained, concentrated yoghurt (Yamani and Abu-Jaber 1994) and in some other yoghurts (Green and Ibe 1987; Hur et al. 1992). Rohm et al. (1992) found *S. cerevisiae* in fermented milk products, kefir and cheeses. It has also been isolated from cocoa fermentation (Mazigh 1994; Schwan and Wheals 2003).

Rapid methods for identification of *Saccharomyces cerevisiae* have been reported based on genetic techniques such as multiplex PCR of plasmid DNA (Pearson and McKee 1992), electrophoretic karyotyping, DNA-DNA hybridisation (Török et al.1993) and real time PCR (Martorell et al. 2005b).

Pathogenicity *Saccharomyces cerevisiae* has always been regarded as a benign organism, indeed has been used as a health supplement and probiotic. However in the last two decades, *S. cerevisiae* has increasingly been reported from infections, probably because of increasing numbers of immunocompromised patients and advances in hospital diagnoses such as the use of molecular techniques. A wide variety of infections has been reported, from vaginitis and cutaneous infections in healthy patients to systemic infections in immunocompromised and critically ill patients. *S. cerevisiae* must now considered as an emerging opportunistic pathogen (Pérez-Torrado and Querol 2016; Algazaq et al. 2017).

References Fleet (2006) for an overview of *Saccharomyces* in foods; Boekhout and Robert (2003) for industrial and food uses, and application of molecular methods; Kurtzman et al. (2011) for taxonomy.

Schizosaccharomyces pombe Lindner **Fig. 10.10**

Colonies on MEA at 3 days very small, up to 0.5 mm diam, white, circular with a smooth margin, surface convex, glistening; at 7 days 1–2 mm diam, of similar appearance. Cells on MEA dividing by lateral fission, at 3 days short cylinders with rounded ends, 5–7 × 3.0–5.0 µm, longer before division, at maturity becoming ellipsoidal or becoming wider at one end than the other, and sometimes showing fission scars. Ascospores often formed on MEA at 25 or

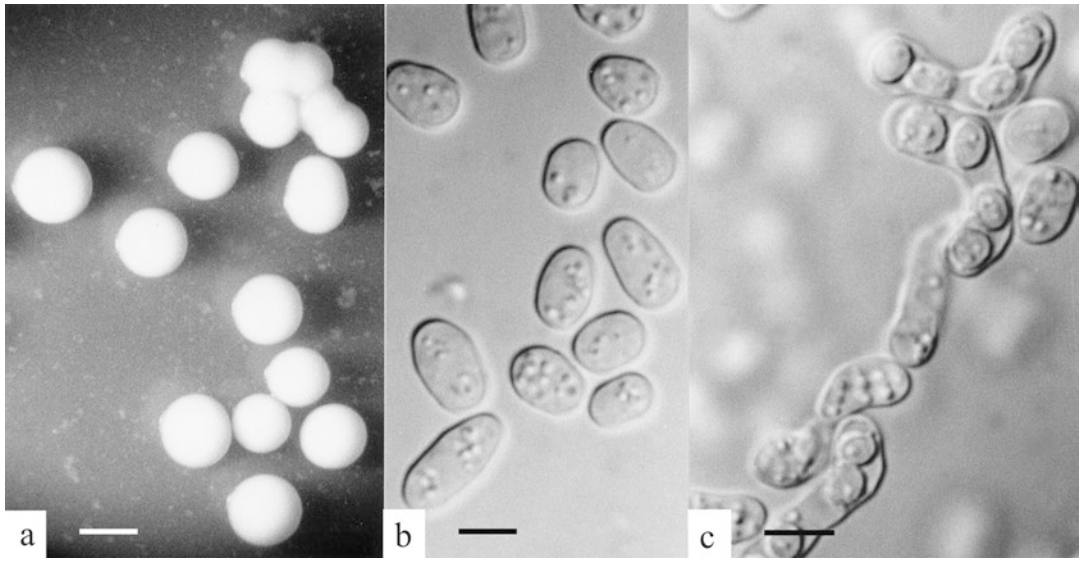

Fig. 10.10 *Schizosaccharomyces pombe* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**) vegetative cells, bar = 10 µm; (**c**) asci and ascospores, bar = 5 µm

37 °C within one week, asci of irregular shape, formed by fusion of two cells, usually more or less end to end, dehiscing at maturity, usually containing 4 ascospores; ascospores ellipsoidal, 3.0–4.0 µm long, with rough walls. Growth on Czapek agar very weak or absent; growth at 37 °C often more rapid than at 25 °C; growth on malt acetic agar equivalent to on MEA at 25 °C; weak growth on MY50G; no growth on MY10-12.

Distinctive features The genus *Schizosaccharomyces* is characterised by reproducing vegetatively by lateral fission. *S. pombe* forms asci with four ascospores, and grows well at 37 °C and on malt acetic agar.

Identifiers MycoBank MB212377; lectotype CBS 356. Ex-type cultures CBS 356, NRRL Y-12796. ITS barcode AY046223, additional markers *RPB1* DQ471170*, EF1* AF402093*, 26S* rDNA AY048171, 18S rDNA AF442355 (CBS 2016).

Physiology *Schizosaccharomyces pombe* grows at least as vigorously at 37 °C as at 25 °C. It is xerophilic, growing at 0.81 a_w in glucose based media (van Eck et al. 1993). However in media based on NaCl the minimum a_w is only 0.97–0.95 (van Eck et al. 1993; Deak and Beuchat 1996). Papouskova and Sychrova (2007) reported that the presence of 600 mM NaCl or KCl, or 15% sorbitol, enhanced the ability of *S. pombe* to grow at 34 and 37 °C, but at 25 and 30 °C, the opposite was the case. Corry (1976) studied the heat resistance of vegetative cells of *S. pombe* at 0.95 a_w in various solutes. Sucrose was the most protective, and gave a D_{65} value of 1.48 min. Glucose, fructose and glycerol were much less protective, with D_{65} values of 0.41, 0.27 and 0.21, respectively. *S. pombe* is resistant to the common food preservatives, as shown by its ability to grow on malt acetic agar. It is resistant to free SO_2 at levels up to 120 mg/kg (Warth 1985), and up to 600 mg/L benzoic acid (Warth 1988, 1989c). *S. pombe* may produce high levels of H_2S off-flavours if sulphite is present (Rankine 1964).

Ecology *Schizosaccharomyces pombe* is a relatively uncommon spoilage yeast, However, its ability to grow at reduced water activities and at 37 °C in the presence of preservatives, particularly SO_2, gives it great potential to cause spoilage in warmer regions. It has been isolated on several occasions in our laboratory from sugar syrups undergoing fermentative spoilage with the production of H_2S. The syrups, used in the manufacture of glace fruits, had

been preserved with substantial levels of SO_2. We have also isolated *S. pombe* from raspberry cordial concentrate, of 45° Brix and pH 3.0, containing 250 mg/kg SO_2.

In the wine industry, *Schizosaccharomyces pombe* may be used to deacidify wine because of its ability to metabolise L-malic acid (Sousa et al. 1993, 1995; Gao and Fleet 1995), but it may also be responsible for off flavours under some circumstances (Unterholzner et al. 1988). *S. pombe* may also be used in fermentation of sugar cane juice in rum production (Pech et al. 1984; Fahrasmane et al. 1988) and in the production of palm wine (Chrystopher and Theivendirarajah 1988; Sanni and Loenner 1993). It is associated with cocoa fermentation (Ravelomanana et al. 1984; Mazigh 1994; Schwan and Wheals 2003) and has also been reported from coffee fermentation (Silva et al. 2000).

References Kurtzman and Fell (1998); Barnett et al. (2000); Kurtzman et al. (2011).

Wickerhamomyces anomalus (E.C. Hansen) Kurtzman et al. Fig. 10.11

Pichia anomala (E.C. Hansen) Kurtzman
Hansenula anomala (E.C. Hansen) Syd. & P. Syd.
Candida pelliculosa Redaelli (valid anamorph name)

Colonies on MEA at 3 days 1.5–2.0 mm diam, off-white, convex, margins circular, surface matt; at 7 days 3–4 mm diam, off-white, convex, smooth, margins entire. Cells on MEA at 3 days very variable in size and shape, from small and subspheroidal to large and ellipsoidal, 3.0–7 × 2.5–5.0 µm, with smaller numbers elongate to cylindroidal, up to 12 × 3.0 µm, reproducing by multilateral budding. Ascospores produced without conjugation in some of the larger ellipsoidal cells, bowler hat shaped, 2–4 per ascus. Moderate growth on Czapek agar, colonies 1.0–2.0 mm diam at 7 days, white and convex, margins entire; growth on MY10-12 only a little slower than on MEA, 2–3 mm diam at 7 days, colonies of similar appearance to those on MEA. Very slow growth on MY50G (pinpoint colonies at 7 days). Usually no growth at 37 °C, no growth on malt acetic agar.

Distinctive features W*ickerhamomyces anomalus* utilises nitrate as a sole nitrogen source, and most isolates of this species readily produce characteristic bowler hat shaped ascospores.

Taxonomy The genera *Hansenula* and *Pichia* were originally separated on the basis of

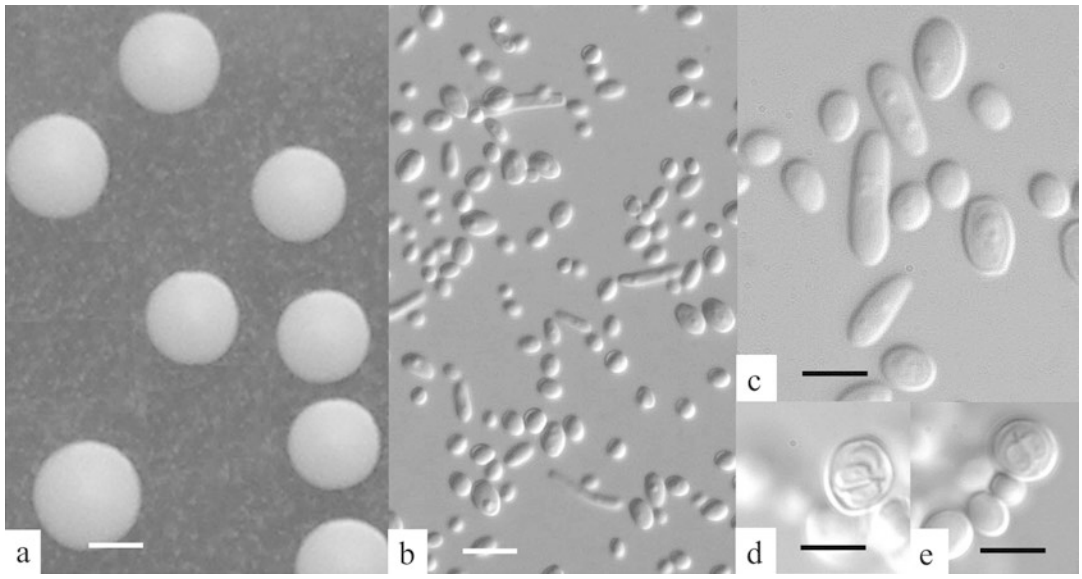

Fig. 10.11 *Wickerhamomyces anomalus* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm; (**d**,**e**) asci and ascospores, bar = 5 µm

the ability of the former to utilize nitrate as a sole nitrogen source. Later taxonomists did not consider this difference sufficient to justify two genera, and species of *Hansenula* with hat shaped ascospores were transferred to *Pichia* (Kurtzman 1984). However a phylogenetic study found that most members of the former genus *Hansenula* formed a separate clade from *Pichia* (Kurtzman and Robnett 1998). From further molecular studies, Kurtzman et al. (2008) reclassified this species as *Wickerhamomyces anomalus*.

Identifiers MycoBank MB508390; type unknown.

Physiology W*ickerhamomyces anomalus* grows between 3 °C and 37 °C, and from pH 2.0 to 12.4 at 25 °C, but not at pH 1.9 (Fredlund et al. 2002). *W. anomalus* was able to grow at 0.85 a_w with glycerol as the controlling solute, but when NaCl was used, the lowest a_w at which growth was observed was 0.92 a_w (Fredlund et al. 2002). Praphailong and Fleet (1997) reported growth of *W. anomalus* in 15% (w/v) NaCl (*ca* 0.91 a_w) at pH 3–5 or 70% (w/v) sucrose (*ca* 0.89 a_w) at pH 2–7. Quintana et al. (2003) recorded growth of *W. anomalus* at 7 °C in 8% NaCl during fermentation of table olives.

Wickerhamomyces anomalus is moderately resistant to preservatives. At pH 5, *W. anomalus* grew in 500 mg/L benzoic acid or 250 mg/l sorbic acid, but at pH 3, was unable to grow in 250 mg/L of either preservative (Praphailong and Fleet 1997). Fredlund et al. (2002) reported MICs for *W. anomalus*: for propionic acid, 1000–5000 mg/L at pH 3.6, for potassium sorbate, 100–500 mg/L at pH 5.3 and for sodium benzoate, 100–500 mg/L at pH 5.2. Stratford et al. (2007) reported that *W. anomalus* has the gene (PAD1) that enables decarboxylation of sorbic acid to 1,3-pentadiene. *W. anomalus* is able to produce ethyl acetate from glucose (Passoth et al. 2006), and this compound may be formed during growth on bread, resulting in spoilage taints. *W. anomalus* produces a killer toxin that is effective against other yeasts and possibly some moulds (Passoth et al. 2006).

Ecology Deák and Beuchat (1996) listed W*ickerhamomyces anomalus* (as *Pichia anomala*) as the third most frequently reported foodborne yeast, after *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. It is most commonly associated with low pH products, but also fruits, beverages, wine and beer, meat and dairy products, and low a_w products. However, it is not always associated with spoilage. *W. anomalus* appears to play an important role in olive fermentation (Marquina et al. 1992; Quintana et al. 2003; Coton et al. 2006; Hernandez et al. 2007), but may also be implicated in deterioration of olives at the end of fermentation (Faid et al. 1994; Asehraou et al. 2000). *W. anomalus* has been reported from coffee (Schwann and Wheals 2003; Masoud et al. 2004; Masoud and Kaltoft 2006) and cocoa fermentations (Schwann and Wheals 2003). It is involved in a number of indigenous fermentations including brem, a Balinese rice wine (Sujaya et al. 2004), gowe (fermented sorghum) in Benin (Vieira-Dalode et al. 2007), murcha cakes in India (Tamang and Sarkar 1995) and idli (fermented lentils) in the Indian subcontinent (Nout 2003).

The antifungal activity of *W. anomalus* makes it a potentially useful biocontrol organism. It has been shown to be active against *Botrytis cinerea* on apples (Jijakli and Lepoivre 1998; Santos et al. 2004; Kwasiborski et al. 2014) and grapes (Masih et al. 2000) and against the growth of *Penicillium roqueforti* and other moulds in grain silage (e.g. Björnberg and Schnürer 1993; Druvefors et al. 2005; Passoth et al. 2006).

W*ickerhamomyces anomalus* has been reported to cause spoilage in a wide range of foods. It can produce ethyl acetate in 'chalky' bread (Legan and Voysey 1991; Bonjean and Guillaume 2003, and in our laboratory). It has been reported from butter (Mushtaq et al. 2007), wine (Deák and Beuchat 1996; Menke et al. 2007), fruit juices and soft drinks (Deák and Beuchat 1996), fruit juice concentrates (Deák and Beuchat 1993), yoghurt (Kosse et al. 1997; Caggia et al. 2001) and cream-filled cakes (Lanciotti et al. 1998). In our laboratory we have

isolated it consistently from fruit purees and flavourings used as ingredients for the dairy industry. We have also isolated it from spoiled yoghurt, sugar syrup, chocolate syrup, tomato dip and bakery products.

Pathogenicity W*ickerhamomyces anomalus* can cause infections in immunocomprised and paediatric patients (de Hoog et al. 2000; Paula et al. 2006; Bhardwaj et al. 2007). An identification and strain typing method has been developed based on the ribosomal intergenic spacer region IGS1 (Bhardwaj et al. 2007).

References Kurtzman and Fell (1998); Barnett et al. (2000); Kurtzman et al. (2011).

Zygosaccharomyces bailii (Lindner) Guillierm. Fig. 10.12

Saccharomyces bailii Lindner
Zygosaccharomyces acidifaciens W.J. Nick.
Saccharomyces acidifaciens (W.J. Nick.) Lodder & Kreger

Colonies on MEA at 3 days small, less than 2 mm diam, white, almost hemispherical, margins circular, surface glistening; at 7 days colonies up to 3 mm diam, of similar appearance as at 3 days. Cells large, ellipsoidal, usually 5–8 × 3.0–5.0 µm, reproducing by budding, characteristically subapically, or at an acute angle to the cell longitudinal axis, leaving a flat subapical "shoulder" on one side only of both mother and daughter cells; occurring singly or in pairs, rarely in short chains. Ascospores formed by most isolates in 7 days on MEA or malt acetic agar or both; asci formed by union of two cells to give characteristic "dumbbell" or less regular shapes; ascospores 1–4 per ascus, spheroidal to ellipsoidal, smooth walled and refractile, not readily liberated from the ascus. No growth on Czapek agar; rarely growth at 37 °C; growth on malt acetic agar, only slightly slower than on MEA; slow growth on MY50G; no growth on MY10-12.

Distinctive features Using the methods and media described here, *Zygosaccharomyces bailii* is a readily recognised species. Growth on MEA is slow and colonies are hemispherical; growth on malt acetic agar is only slightly slower. Asci are produced on one or the other of these media within 7 days by conjugation of two cells, which will contain 1–4 smooth walled ascospores.

Taxonomy For a long time, controversy existed over whether highly fermentative yeasts related to *Saccharomyces* but undergoing cell conjugation before sporulation should be classified in *Saccharomyces* or as a separate genus, *Zygosaccharomyces*. *Zygosaccharomyces* is now accepted as the appropriate name for those taxa where cell conjugation precedes ascospore formation (von Arx et al. 1977).

Genetic techniques have been used to examine the relationships between species in the genus *Zygosaccharomyces* (James et al. 1994) and now offer the most reliable means of species identification (James and Stratford 2003). PCR targeting a short region of the 18S rRNA gene was able to discriminate to species level (Stubbs et al. 1994). Plasmid DNA was used as the target for multiplex PCR to distinguish between *Z. bailii, Z. rouxii* and *Saccharomyces cerevisiae* as the basis for a rapid identification system (Pearson and McKee 1992), microsatellite PCR fingerprinting analysis was able to discriminate between the closely related species *Z. bailii* and *Z. bisporus* (Baleiras Couto et al. 1996) and RFLP of the ITS-5.8S rRNA gene was able to distinguish between 10 species within the genus (Esteve-Zaroso et al. 2003). The gene encoding the D1/D2 region of the 26S ribosomal RNA has been used as a target for sequencing and primer design to enable real-time PCR detection of *Z. bailii* in beverages (Rawsthorne and Phister 2006) with discrimination of living from dead cells (Rawsthorne and Phister 2009). Isoenzyme patterns have also been used to differentiate *Z. bailii* from other closely related *Zygosaccharomyces* species (Duarte et al. 2004).

Identifiers MycoBank MB258102, lectotype CBS 680. Ex-type cultures CBS 680, ATCC 58445; NRRL Y-2227. ITS barcode AY046191. Additional markers *EF1* AF402059, 26S rDNA AF399789, 18S rDNA AB182473 (CBS 2016).

Physiology Physiologically, the most outstanding feature of *Zygosaccharomyces bailii* is its resistance to weak acid preservatives including sorbic, benzoic, acetic and propionic acids and also SO_2. The mechanisms of this

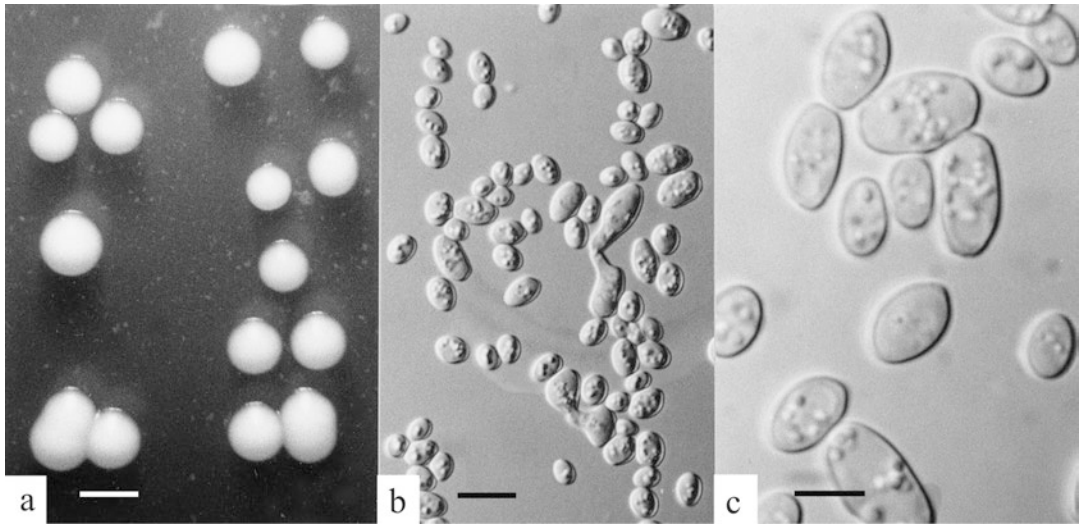

Fig. 10.12 *Zygosaccharomyces bailii* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**) vegetative cells, asci and ascospores, bar = 10 µm; (**c**) vegetative cells, bar = 5 µm

resistance, and effects of other parameters such as solute type, pH and a_w, have been extensively studied by Warth (1977, 1985, 1986, 1989a,b,c, 1991), Lenovich et al. (1988, as *Z. rouxii*) and Jermini and Schmidt-Lorenz (1987d). *Z. bailii* is remarkable in another characteristic: exposure to low levels of preservative, such as may occur in imperfectly cleaned filling lines, causes adaptation to preservative, and the ability to survive and grow in much higher concentrations than before adaptation (J.I. Pitt, unpublished; Warth 1977, 1986, 1988). Warth (1986) reported an MIC for benzoic acid of 1000–1400 mg/L at pH 3.5 in adapted cells of *Z. bailii.* Growth in commercially packed fruit based cordials – of pH 2.8–3.0, 40–45° Brix, preserved with 800 mg/L benzoic acid – has been observed by us on several occasions. A similar level of resistance to sorbic acid was reported by Neves et al. (1994) in strains of *Z. bailii* from spoiled foods and alcoholic beverages. *Z. bailii* is also resistant to combinations of alcohol and acids present in wine (Kalathenos et al. 1995). Sousa et al. (1996) reported that ethanol inhibits uptake of acetic acid by *Z. bailii* cells, which could explain this protective effect.

Like *Saccharomyces cerevisiae, Zygosaccharomyces bailii* vigorously ferments glucose solutions to CO_2, and the reaction is not inhibited until at least 80 psig (= 560 kPa) overpressure is reached; unlike S. *cerevisiae, Z. bailii* can carry out this fermentation in the presence of 400 mg/kg or more of benzoic or sorbic acid. In the absence of high CO_2 pressure, *Z. bailii* can tolerate relatively low pH and low a_w as well. Tolerance of low pH is not as great as for some other yeasts: according to Pitt (1974), pH 2.2–2.5 is the minimum for growth.

Jermini and Schmidt-Lorenz (1987b), reported that the optimum temperature for growth of *Zygosaccharomyces bailii* ranged from 30–32 °C in 10% (w/w) glucose to 34–36 °C in 60% (w/w) glucose. The minimum temperature for growth of *Z. bailii* was 6.5 °C in 10% and 30% (w/w) glucose, rising to 13 °C in 60% glucose. *Z. bailii* grew at 37 °C in the lower glucose concentrations, while its maximum growth temperature in 60% (w/w) glucose was 40 °C. López-Malo and Palou (2000) reported growth of *Z. bailii* at 5 °C in mango puree (0.97 a_w, pH 3.5) containing 1000 mg/L benzoate after 29 days, but no growth under equivalent conditions with sorbate instead of benzoate. *Z. bailii* is a xerophile, capable of

growth down to at least 0.80 a_w at 25 °C (Pitt 1975), although at 30 °C, Jermini and Schmidt-Lorenz (1987a) reported a minimum a_w for growth in fructose of 0.86.

Mathematical modelling has been used to predict growth/no-growth boundaries and time to visible growth of *Z. bailii* under various combinations of conditions. López-Malo and Palou (2000) based their model on mango puree, containing either 1000 mg/L potassium sorbate or sodium benzoate, pH 3.5, at 0.97–0.99 a_w over the temperature range 5–25 °C. Jenkins et al. (2000) based their model on yeast nitrogen broth, incubated at 30 °C with combinations of acetic acid (1.0–2.8% v/v), fructose (7.0–32.0% w/v) and NaCl (2.6–4.2% w/v).

Put and de Jong (1982) reported that vegetative cells of *Zygosaccharomyces bailii* showed a low heat resistance, with a D_{50} value of 0.1–0.3 min. Ascospores were more resistant; for three isolates the D_{60} value for ascospores ranged from 8–14 min. Data of Jermini and Schmidt-Lorenz (1987c) confirmed this, reporting a D_{60} of 14.9 min for *Z. bailii* ascospores heated in yeast extract glucose broth with 60% (w/w) glucose, 0.86 a_w, at pH 4.5. *Z. bailii* can also be inactivated by high hydrostatic pressure (Palou et al. 1997a), although ascospores are more resistant than vegetative cells (Raso et al. 1998) and high concentrations of sugars can provide some protection (Palou et al. 1997b). Pandya et al. (1995) reported a reduction of 10^7 *Z. bailii* cells with 3000 atm for 10 min at 25 °C in spaghetti sauce with meat at pH 3.0, 4,0 or 5.0, and at 2500 atm for 20 min at pH 5.0.

Zygosaccharomyces bailii is sensitive to ozone, with 10^4 cells killed instantaneously in ozonated water in a recirculating concurrent reactor (Restaino et al. 1995).

Strategies to look at the entire genome have been studied, to providing information on the molecular targets, signalling pathways and mechanisms of the tolerance of *Z. bailii* to acetic acid (Palma et al. 2018).

Ecology Outside the food and beverage industries, *Zygosaccharomyces bailii* is a virtually unknown yeast, although reports of spoilage caused by it date back to the beginning of the century (Thomas and Davenport 1985). Only within the past 20 years has *Z. bailii* been isolated from "natural" sources, from fermenting fruit in vineyards and orchards (see Pitt and Hocking 1997; Nisiotou et al. 2007).

Within the food and beverage industries, in contrast, *Zygosaccharomyces bailii* has become notorious. The preservation of acid, liquid products against fermentative yeasts has traditionally relied on chemical preservatives; i.e. sorbic and benzoic acids, and sulphur dioxide, and the "natural" preservatives acetic acid and ethanol. The resistance of *Z. bailii* to all of these compounds at permitted (and against other yeasts, effective) levels means that such products must really be packed sterile, or be pasteurised in the final sealed container. Products at risk and in which fermentation or explosive spoilage has been observed are listed by Thomas and Davenport (1985) and include tomato sauce, mayonnaise, salad dressing, soft drinks including sports drinks, fruit juices and concentrates, ciders and wines (see Pitt and Hocking 1997; James and Stratford 2003; Kurtzman and James 2006). We and others (Put et al. 1976; R.R. Davenport, unpublished) have also seen spoilage in cordial concentrates, fruit syrups intended for cake and confectionery manufacture, and a variety of other products. *Z. bailii* has been reported from cough mixture (Charnock et al. 2005), mustard (Buchta et al. 1996), balsamic vinegar (Solieri et al. 2006), Haipao and Kombucha tea fermentation (Liu et al. 1996; Teoh et al. 2004), fermenting cassava (fufu; Oyewole 2001) and black olives (Kotzeidou 1997). Losses to *Z. bailii* around the world run to many millions of dollars per annum.

Our experiments with adapted and unadapted *Zygosaccharomyces bailii* inoculated into canned carbonated soft drinks (J.1.Pitt, unpublished) have shown that only 5 cells per can of adapted *Z. bailii* are sufficient to cause spoilage of a high percentage of containers. Noncarbonated products will certainly fare no better. It seems probable that a single healthy, adapted cell of *Z. bailii* per container of any size will ultimately lead to spoilage in a high percentage of cases. Prevention of spoilage, then, must usually rely on the total exclusion of living *Z. bailii* cells

from the final product. Pasteurisation in the final, sealed container is the method of choice, though not always practical. Centre temperatures of 65 to 68 °C for an appreciable number of seconds is an adequate pasteurisation treatment. Heat treatments of this kind are insufficient to kill ascospores (Put and de Jong 1982), so it appears that ascospores of *Z. bailii* are not a common problem. If pasteurisation precedes filling, as is often the case with a wide range of products, rigorous daily cleaning of filling machinery is essential if spoilage by *Z. bailii* is to be avoided. Common sites for yeast proliferation include filler heads, diaphragm valves, pressure gauges and dead ends in pipes. Yeasts can also survive in lubricating oils and can infect product from aerosols generated by moving machinery (Thomas and Davenport 1985). Membrane filtration immediately before filling is an effective treatment, but of course is only practicable for clear products such as ciders and white wines. Membrane filtration is widely practiced by the Australian wine and cider industries.

Where possible, synthetic products such as soft drinks and water ices should be manufactured without a utilisable nitrogen or carbon source. *Zygosaccharomyces bailii* is unable to ferment sucrose, and the use of sucrose not glucose as a sweetener in such products is highly recommended. Manufacturers should be aware that the commercially desirable practice of adding fruit juices to soft drinks or mineral waters is microbiologically hazardous. Far more stringent cleaning procedures are essential with such products than with purely synthetic or mineral products.

Additional species *Zygosaccharomyces bisporus* (H. Nagan.) Lodder & Kreger shares many characters with *Z. bailii* including the formation of similar colonies on MEA and malt acetic agar. *Z. bisporus* is distinguished by smaller cells, 3.5–7 × 2.5–4.5 µm, which often adhere in short chains and which do not produce ascospores on either of the above media in 7 days. Relatively little has been published about this species, which appears to be less common than *Z. bailii*, but it has a similar capability to cause food spoilage. It can be safely assumed to have similar physiological properties to *Z. bailii.* It is more xerophilic, however. Tilbury (1980) reported growth down to 0.70 a_w in sucrose/glycerol syrups. Put et al. (1976) reported survival of ascospores (5×10^4/mL) after 10 min at 60 °C, but not 20 min.

Identifiers: MycoBank MB433575, lectotype CBS 702, ex-type cultures ATCC 52405; NRRL Y-7558; ITS barcode AJ229176, 26S rDNA AF399790, 18S rDNA AF442325, *EF1* AF402061 (CBS 2016).

References James and Stratford (2003); Kurtzman and James (2006); Kurtzman et al. (2011).

Zygosaccharomyces rouxii (Boutroux) Yarrow **Fig. 10.13**

Saccharomyces rouxii Boutroux
Candida mogii Vidal-Leir. (valid anamorph name)

Colonies on MEA at 3 days 0.2–0.5 mm diam, white, margins circular, almost hemispherical, surface glistening; at 7 days 2–3 mm diam, appearance as at 3 days. Cells on MEA at 3 days subspheroidal to ellipsoidal, 4.0–9 × 2.5–7 µm, mostly 5–7 × 4.0–5 µm, budding irregularly, occurring singly, in pairs or in small groups. Ascospores rarely seen on artificial media, but in our experience frequently observed on such low a_w, high sugar substrates as prunes. Asci of irregular shape, usually formed by conjugation of two cells; ascospores 1–4 per ascus, spherical to subspheroidal, with walls smooth or finely roughened. No growth on Czapek agar; sometimes weak growth at 37 °C; no growth on malt acetic agar; growth on MY50G and on MY10-12, with macroscopic colonies in 3 days.

Distinctive features *Zygosaccharomyces rouxii* shares with *Debaryomyces hansenii* the ability to grow on both MY50G and MY10-12. Unlike *D. hansenii, Z. rouxii* does not grow on Czapek agar; also *Z. rouxii* does not produce the small (2.5–4.0 µm) spherical cells characteristic of young cultures of *D. hansenii.*

Taxonomy *Zygosaccharomyces* is differentiated from *Saccharomyces* by undergoing cell conjugation before ascospore formation. However, Yarrow (1984) noted that *Z. rouxii*

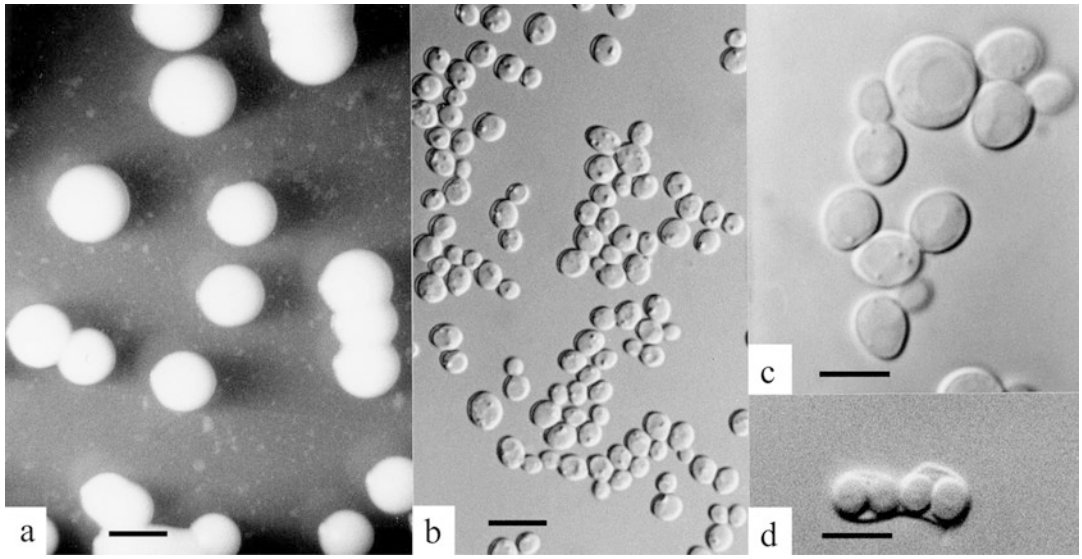

Fig. 10.13 *Zygosaccharomyces rouxii* (**a**) colonies on MEA, 7 d, 25 °C, bar = 2 mm; (**b**, **c**) vegetative cells (**b**) bar = 10 µm, (**c**) bar = 5 µm; (**d**) ascospores, bar = 5 µm

sometimes does not conjugate before ascospore formation. As with *Z. bailii* (see above) genetic techniques are now being used to investigate relationships within the genus *Zygosaccharomyces,* and applied to detection and identification systems for *Z. rouxii* (see Kurtzman and James 2006).

Identifiers MycoBank MB325702, neotype CBS 732, ex-type cultures ATCC 2623, NRRL Y-229. ITS barcode AB302827, other markers 26S rDNA AB302806, 18S rDNA AB182458, *EF1* AF402054 (CBS 2016).

Physiology *Zygosaccharomyces rouxii* is one of the most xerophilic organism known (Pitt 1975), being able to grow down to 0.62 a_w in fructose solutions (von Schelhorn 1950) and to 0.65 a_w in sucrose/glycerol (Tilbury 1980). Comi et al. (1992) reported growth down to 0.63 a_w at 30 °C but only down to 0.76 a_w at 4 °C in ice cream mix. In our laboratory, formation of ascospores has been observed down to 0.70 a_w on a favourable substrate (prunes). The optimum temperature for growth varies with a_w, from 24 °C in 10% (w/w) glucose (0.99 a_w) to about 33 °C in 60% (w/w) glucose (0.87 a_w) (Jermini and Schmidt-Lorenz 1987b). In 10% glucose, Z. *rouxii* grew at 4 °C, but its minimum temperature for growth in 60% glucose was 7 °C. The maximum temperature for growth was 37 °C in 10% glucose, but 42 °C in 60% glucose (Jermini and Schmidt-Lorenz 1987b). Martorell et al. (2007) reported that *Z. rouxii* was unable to grow at 4 °C or 37 °C at pH 4.0 in a medium containing only 2% glucose. English (1954) reported growth of Z. *rouxii* over the range pH 1.8 to 8.0 in a medium containing 46% glucose. Onishi (1963) showed that, while the pH range for growth in 1 M NaCl solutions was very broad, in 2–3 M NaCl it was greatly restricted, in the latter case to within the range pH 3.0 to 6.0. *Z. rouxii* was able to grow in 15% (w/v) NaCl at pH 3 and pH 5, but at pH 7, only 7.5% (w/v/) was tolerated. No growth occurred at pH 2.0 (Praphailong and Fleet 1997). The pH range for *Z. rouxii* in 5% glucose medium reported by Praphailong and Fleet (1997) was pH 2.5–7.5 in citrate phosphate buffer, but only pH 3.0–7.0 in inorganic buffer. Growth/no growth models for *Z. rouxii* associated with acidic, sweet intermediate moisture food products were published by Marvig et al. (2015). Ultrasound at different temperatures, pH and water activity conditions was used to reduce levels of *Z. rouxii* in juice concentrates (Kirimli and Kunduhoglu 2016).

Heat resistance studies for this species have been reported under both the teleomorph and anamorph names. As ascospores are usually not formed on media of high a_w (our observations), it seems likely that all of these reports relate only to the resistance of vegetative cells, not ascospores. Put et al. (1976) reported that 10^5 cells/mL of *Candida mogii* barely survived heating at 62.5 °C for 10 min. The calculated D_{60} value for *Zygosaccharomyces rouxii* in 0.1 M citrate buffer was reported by Shearer et al. (2002) to be 0.039 min at pH 3.5 and 0.008 min at pH 4.0, with *z* values of 3.3 and 2.1C° respectively. Marked differences were observed in the influence of reduced water activities as generated by glucose and sucrose solutions on the heat resistance of *Z. rouxii* (probably actually *C. mogii*) (Gibson 1973). At 55 °C, D values ranged from less than 0.1 min at 0.995 and 0.98 a_w through 0.6 min at 0.94 a_w and 7 min at 0.90 a_w to 55 min at 0.85 a_w. At 60 °C, the D value at 0.94 a_w or above was less than 0.1 min, but at 0.85 a_w was 10 min. At 65.5 °C, D values were all less than 0.1 min except at 0.85 a_w, where it was 0.4 min. These very large effects may not occur with less xerophilic yeasts. At 0.90 and 0.85 a_w, *z* values were about 8 C°. Török and King (1991) obtained a similar D_{55} value to that reported by Gibson (1973): 0.16 min in 0.5% glucose, with a *z* value of 5.5 C°. Jermini and Schmidt-Lorenz (1987c) reported a D_{60} value for *Z. rouxii* asci of 3.5 min at 0.86 a_w, with D values for asci being 20–50 fold higher than for vegetative cells at 0.96 a_w. Corry (1976) compared the effect of different solutes on the heat resistance of *Z. rouxii* (again, probably only vegetative cells) at a single a_w, 0.95. Sucrose was the most protective solute, D_{65} being 1.9 min in that substrate. Glucose, fructose and glycerol produced rather variable results, with D_{65} values ranging from 0.2–0.6 min.

Z. rouxii is moderately resistant to preservatives. Martorell et al. (2007) reported MIC values for *Z. rouxii* of a range of antimicrobial compounds in a basal medium containing 2% glucose, pH 4.0: sorbic acid 2.8–2.85 mM (approx, 310–320 ppm), benzoic acid 3.05 – >3.6 mM (*ca* 370 – >440 ppm) and acetic acid 106–110 mM (6360–6600 ppm). Praphailong and Fleet (1997) reported growth in YNB + 5% glucose at pH 5 with 750 ppm benzoic acid or 500 ppm sorbic acid. At pH 3.0, *Z. rouxii* was unable to grow in the presence of 250 ppm of either preservative. In yeast extract glucose broth at pH 4.0, *Z. rouxii* was able to grow in 400 ppm potassium sorbate or 600 ppm sodium benzoate (El Halouat and Debevre 1996). However, these authors reported that preservative tolerance was reduced when the yeast was grown in 80% CO_2 compared with growth in air. CO_2 concentration (20–80%, balance N_2) compared with air had little effect on growth rate of *Z. rouxii* at 30 °C at 0.94 or 0.90 a_w. However, at 0.83 a_w, elevated CO_2 levels reduced initial (4 days) growth rates, but by 12 days at 30 °C there was no significant difference in final yeast cell numbers (El Halouat and Debevre 1996). *Z. rouxii* is able to metabolise sorbate to 1,3-pentadiene (Casas et al. 1999, 2004) and has also be reported to degrade the fungicide iprodione (Zadra et al. 2006).

Ecology The combined ability to grow at exceptionally low water activities and to vigorously ferment hexose sugars makes *Zygosaccharomyces rouxii* second only to *Z. bailii* as a cause of fermentative food spoilage. Tilbury (1980) reported isolations by himself and others from raw cane sugar, malt extract, fruit juice concentrates, ginger and glace cherries. *Z. rouxii* has also been reported from wine musts (Delfini et al. 1990), traditional balsamic vinegar (Giudici 1990; Solieri et al. 2006), honey (Poncini and Timmer 1986; Schneider et al. 2003; Beckh et al. 2005), hydrated prunes (El-Halouat and Debevere 1997) and soy sauce koji (Lee and Lee 1970; O'Toole 1997). Jermini et al. (1987) isolated *Z. rouxii* from honey, apple, orange and other fruit juice concentrates, marzipan, sugar syrup and candied pineapple. The unpublished list of sources in our laboratory includes jams, ginger, glucose syrup, liquid malt extract, fruit juice concentrates, cake icings, chocolate sauce, dried and high moisture prunes, sultanas, candied citrus peel and flavouring syrups. The list of sources in Barnett et al. (2000) is equally wide.

Concentrated liquid foods and ingredients, which rely on their low a_w for microbial stability, simply cannot be made concentrated enough to inhibit growth and fermentation by *Zygosaccharomyces rouxii*. As is the case with *Z. bailii* in preserved foods, concentrated foods must be free of *Z. rouxii* to be stable. Raw materials such as orange and apple concentrates are usually stored in large (200 litre) drums. Even very low initial contamination rates with *Z. rouxii* cells can eventually cause huge losses. The short term cure for 200 litre drums with swelling ends is immediate refrigeration and, if possible, rapid utilisation. The only satisfactory long term solution, other than continued refrigeration near 0 °C, has been dilution, pasteurisation and reconcentration. However, Rojo et al. (2019) have reported that the use of a combined heat and high pressure process can reduce numbers in concentrated juices to acceptable levels. Refrigeration temperatures must eventually kill the yeast, but no figures appear to exist on the length of time necessary. Preservatives are effective against *Z. rouxii,* but are rarely permitted in concentrated or dried foods. In any case, dispersal of a preservative through large masses of a viscous concentrate would be difficult to achieve.

Confectionery fillings and marzipan are also highly susceptible to infection by *Zygosaccharomyces rouxii* and leakage in soft centred chocolates almost always is indicative of *Z. rouxii* spoilage (see Pitt and Hocking 1997). Losses are often high because the inoculum per chocolate block need be only a few cells. *Z. rouxii* may grow continuously in lines and feed into the manufactured product for a long period before spoiled product is detected.

Generally speaking, the growth of *Zygosaccharomyces rouxii* on dried, loosely packaged foods is not a problem, although *Z. rouxii* may be isolated from dried foods such as dried condiments (Ekundayo 1987). "Sugaring" on dried prunes or dates often contains cells of *Z. rouxii* which act as crystallisation nuclei for glucose, but most Pure Food Acts do not regard this as spoilage.

Once such commodities are packaged, however, usually at somewhat higher a_w levels, CO_2 production by *Zygosaccharomyces rouxii* can rapidly lead to spoilage (Pitt 1963; Bolin et al. 1972).

Jams do not usually spoil due to this yeast because the hot fill process is sufficient for inactivation. Entry of a *Zygosaccharomyces rouxii* cell after a jam is opened will often lead to fermentative spoilage, but such occurrences are rare and outside the manufacturer's control. We have seen marmalade spoil due to *Z. rouxii;* Zaake (1979) has reported a similar occurrence.

References Onishi (1963); Barnett et al. (2000); James and Stratford (2003); Kurtzman and James (2006); Kurtzman et al. (2011).

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Fresh and Perishable Foods

11

In relation to spoilage by fungi, foods can be divided onto two broad classes: fresh or perishable foods and stored or processed foods. Spoilage of these two classes of foods occurs in quite different ways and is caused by separate types of fungi, so is treated in two separate chapters. Many kinds of foods, however, can undergo spoilage both when fresh and after processing, and so have been included in both chapters.

In considering spoilage, fresh foods can again be divided into two types: foods composed of living cells, including fruit, vegetables, nuts and cereals, and nonliving foods, such as meat, milk or fruit juice. Again the kind of spoilage which may occur and the types of fungi which cause spoilage are quite different.

11.1 Spoilage of Living, Fresh Foods

Living foods are biologically active to a greater or less degree, and plants have developed extremely effective defence mechanisms against invasion by their natural predators, the fungi. The fungi in turn have sought evolutionary pathways to enable them to invade living plant tissue. Plant tissues are invariably of high a_w, of neutral or acid pH, and grow at mesophilic temperatures so that invasion is much less related to physical factors than in other types of food spoilage. It is a contest between plant defence mechanisms and the ability of the fungi to overcome them. Here we are dealing with plant-parasite relationships, not with the physical parameters which govern spoilage of other kinds of foods.

The interaction between plant and parasite is very complex and often poorly understood; hence here as in other similar publications we can do little more than catalogue the common forms of spoilage and provide general references to methods for reducing losses. Just as food mycology is a relatively neglected part of food microbiology, so postharvest spoilage of fruits and vegetables is a relatively unexplored area both of food mycology and plant pathology.

Spoilage of living fresh foods can again be usefully subdivided into two categories: foods such as fruit and vegetables which are perishable unless rapidly processed, and foods such as nuts and cereals which tend to dry naturally and become stable in the field.

The basic difference in the spoilage of fresh fruits and vegetables lies in the pH of the living tissue. Fruit are usually quite acid, in the range pH 1.8–2.2 (passionfruit, lemons) to 4.5–5.0 (tomatoes, figs; for a list see Splittstoesser 1987), and are quite resistant to invasion by bacteria. Microbial spoilage of fruit and fruit products is almost always caused by fungi. Vegetables on the other hand are of near neutral pH, and are susceptible to bacterial invasion as well. Bacterial and fungal spoilage of vegetables are of roughly equal importance, with *Erwinia, Pseudomonas*

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and *Xanthomonas* the most commonly implicated bacterial genera (Brackett 1987; Snowdon 1991).

Control of postharvest spoilage of these perishable foods relies on various treatment combinations including chemical fungicides, volatiles derived from plants, cool storage and modified atmospheres, including ozone. For reviews on the application of ozone to postharvest fruit and vegetable processing and storage see Karaca and Velioglu (2007) and Tzortzakis and Chrysargyris (2017).

11.2 Fruits

Defence mechanisms in fruit appear to be highly effective against nearly all fungi, as only a relatively few genera and species are able to invade and cause serious losses. Some of these are highly specialised pathogens, attacking only one of two kinds of fruit, others have a more general ability to invade fruit tissue.

Fruits become increasingly susceptible to fungal invasion during ripening, as the pH of the tissue increases, skin layers soften, soluble carbohydrates build up and defence barriers weaken. The storage of fresh fruit postharvest is a branch of science in its own right, with the need to balance desirable maturity against storage life and transportability, ripening against overripening, balanced maturation against breakdown of desirable qualities, all with the ever present problems of controlling fungal invasion and spoilage as well. The postharvest diseases and disorders of fruit have been comprehensively documented by Snowdon (1990) and Barkai-Golan (2001), and the reader is referred to these texts for a more complete guide. The most important fungal diseases of fruits are briefly described below.

11.2.1 Citrus Fruits

By far the most common causes of citrus fruit decay throughout the world are the *Penicillium* rots due to *P. italicum* and *P. digitatum,* termed blue rot and green rot respectively. Fruit can be attacked by these species at any stage after harvest. Invasion initially requires damage to skin tissue, which readily occurs in modern bulk handling systems. Decay spreads by contact from fruit to fruit. As would be expected from their physiology, growth of these species in citrus is rapid at 20–25 °C but very slow below 5 °C or above 30 °C (Hall and Scott 1977).

Control relies primarily on careful handling of the fruit. Postharvest treatments are based on washes heated to 40–50 °C and containing detergents, weak alkali, and/or fungicides such as benomyl, thiabendazole, imazalil, guazatine, fludioxonil or sodium *o*-phenylphenate (SOPP) (Talibi et al. 2014). Pyrimethanil has been approved for postharvest use on citrus fruit to control *P. digitatum* and other species, which have developed a resistance to common fungicides (Smilanick et al. 2006). In Australia, fungicides containing guazatine are currently restricted to domestic markets. Therefore, the use of generally regarded as safe (GRAS) compounds such as sodium bicarbonate or sodium carbonate and gibberellic acid in combination with heat treatments has been investigated to control *P. digitatum* and *Galactomyces candidus* in citrus fruit destined for export markets (Cunningham and Taverner 2007). In a search for alternatives to chemical treatment, biological control of postharvest rots is an active research area, reviewed by Talibi et al. (2014). Bacteria such as *Bacillus subtilis, Pseudomonas cepacia* and *P. syringae* and the yeasts *Debaryomyces hansenii* and *Candida guillermondii* [= *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki] have been shown to be active against the main postharvest pathogen *Penicillium digitatum* (Droby et al. 1989; Wilson and Chalutz 1989; Arras 1993; Huang et al. 1993; McGuire 1994). *Candida oleophila* [*Yarrowia lipolytica* (Wick et al.) van der Walt & Arx] and *Pichia anomala* (= *Wickerhamomyces anomalus*) at inoculum levels of 10^6–10^8 cfu/ml were effective in protecting Clementine and Valencia oranges against *P. italicum* and *P. digitatum* (Lahlali et al. 2004). A wide range of plant extracts, natural compounds and approved food grade salts and preservatives have also been investigated as potential alternative

treatments, some in conjunction with microbial biocontrol agents (Tabili et al. 2014).

Penicillium ulaiense is a more recently emerged pathogen of citrus (Holmes et al. 1993, 1994). This fungus is resistant to the commonly used preservatives and is posing problems in citrus packing plants in many countries. Rots are distinguished from those of *P. italicum* by the formation of coremia on the fruit (Holmes et al. 1994). Originally described from decaying oranges in Taiwan (Hsieh et al. 1987), *P. ulaiense* has been isolated from citrus fruit in the United States (Holmes et al. 1993, 1994), Australia (Hocking and Pitt 1996), Argentina (Carrillo 1995), Egypt (Youssef et al. 2000), Pakistan (Khan et al. 2017), Korea (Park et al. 2018) and Spain (Palou and Taberner 2018). *Penicillium ulaiense* is effectively controlled with fungicides based on pyrimethanil (Smilanick et al. 2006).

Geotrichum candidum var. *citri-aurantii* (= *Galactomyces citri-aurantii* E.E. Butler) causes sour rot of citrus, primarily in lemons and limes, in all parts of the world (Butler et al. 1965; Snowdon 1990; Talibi et al. 2014). The rot is a pale, soft area of decay which later develops into a creamy, slimy surface growth. At favourable temperatures of 25–30 °C, fruit will rot completely in 4 or 5 days, and the disease can spread by contact (Hall and Scott 1977; Ryall and Pentzer 1982; Snowdon 1990). The characteristic cylindrical conidia of *Galactomyces* are readily seen in mounts made from advanced, slimy rots. Infection usually occurs in overmature fruit after long, high temperature storage. Control relies on storage at temperatures below 5 °C.

Black centre rot of oranges, caused by an *Alternaria* species generally known as *A. citri* M.B. Ellis & N. Pierce, appears as an internal blackening of the fruit. Peever et al. (2005) showed that *Alternaria* species associated with black rot of fruits belong to a single *A. alternata* clade and that the name *A. citri* to describe species associated with citrus black rot is not justified. Culturing of blackened areas on DCMA will lead to growth of dark colonies bearing characteristic *Alternaria* conidia. Simmons (2007) listed 22 *Alternaria* species that may be found on *Citrus* plants and fruit. *Cladosporium sphaerospermum* has been reported to produce a soft rot of oranges in India, causing postharvest losses (Gaur and Chenulu 1981). Anthracnose of tangerines, caused by *Colletotrichum gloeosporioides* (Pent.) Penz. & Sacc. has also been reported (Timmer et al. 1998; Teixeira et al. 2001).

Less common and usually less serious spoilage of citrus can be produced by a variety of fungi not described in this book. They include *Guignardia citricarpa* [=*Phyllosticta citricarpa* (McAlpine) Aa], which produces black spots; *Septoria depressa* McAlpine, the cause of *Septoria* spot; *Sphaceloma fawcettii* (= *Elsinoe fawcettii* Bitan. & Jenkins), causing scab on lemons; and stem end rots caused by *Diaporthe citri* (H.S. Fawc.) F.A. Wolf (anamorph: *Phomopsis citri*) and *Lasiodiplodia theobromae* (Hall and Scott 1977; Splittstoesser 1987; Ryall and Pentzer 1982; Brown et al. 1988; Snowdon 1990).

11.2.2 Pome Fruits

The most common and destructive fungal spoilage agent in pome fruits (apples and pears) is again a *Penicillium* causing a blue rot, in this case *P. expansum* (Fox 1994). Decay commences as a soft, light coloured spot which rapidly spreads across the surface and also deeply into the fruit tissue. As growth spreads, blue green coremial fruiting structures appear on the surface. *Penicillium expansum* grows at low temperatures, so cold storage retards, rather than prevents, spoilage (Hall and Scott 1977). Damaged and overmature fruit are the most susceptible.

Diagnosis may be made by inspection in advanced cases, or by culturing on DRBC and then CYA and CSN. Infected fruit held for a few days at room temperature will develop blue green coremia of *Penicillium expansum.*

Control measures include careful handling and the use of fungicides such as benomyl or SOPP. Because the rot spreads by contact from fruit to fruit, it is also common practice to pack fruit on fibreboard trays which reduce physical contact between individual fruits. Biological control using yeasts and bacterial species have

been investigated in an effort to reduce the dependence on fungicides. *Aureobasidium pullulans* (Bencheqroun et al. 2007), *Pseudomonas syringae* (Nunes et al. 2007), *Metschnikowia pulcherrima* Pitt & M.W. Mill. and *Cryptococcus laurentii* [=*Papillotrema laurentii* (Kuff.) Xin Zhan Liu et al.] (Conway et al. 2007) have all been reported to reduce *P. expansum* infection on apples.

Penicillium solitum is another *Penicillium* causing apple decay (Pitt et al. 1991; Sanderson and Spotts 1995). Like *P. ulaiense, P. solitum* is resistant to the commonly used fungicides. *T. funiculosus* has also been reported from stored apples in South Africa (Vismer et al. 1996). Postharvest infection with *P. expansum* and occasionally other species of *Penicillium* may lead to significant levels of patulin contamination in stored apples (Vismer et al. 1996; Baert et al. 2007; Morales et al. 2007). Louw and Korsten (2014) reported that *P. crustosum* and *P. digitatum* could cause significant rots when inoculated into apples and pears, but spontaneous spoilage by these Penicillia has not been recorded.

Another spoilage fungus in pome fruits is *Botrytis cinerea,* which causes grey mould rot in cold stored pears (Hall and Scott 1977) and, less commonly, apples. The rot is firmer than blue mould rot, and becomes covered in ash-grey spore masses. The mould invades through wounds or abrasions and can spread rapidly in packed fruit. Control measures are similar to those used for *Penicillium expansum.* As well as benomyl, dichloran (Allisan, Botran) is an effective fungicide against *B. cinerea.* Storage of fruit at −0.5 °C to 0 °C also provides good control.

Other fungi which can cause rots of pome fruits include *Gloeosporium album* [= *Neofabreae vagabunda* (Desm.) Rossman], which causes "bulls eye rot"; *Phytophthora* sp., which produces brown rots; and *Spilocaea pomi* Fr. [=*Venturia inequaelis* (Cooke) G. Winter] and *Fusicladium pyrorum* (=*Venturia pirina* Aderh.) which produce black spots on apples and pears respectively (Hall and Scott 1977; Splittstoesser 1987; Wicks and Blumbieris 1981; Snowdon 1990). *Neocosmospora solani* and *F. equiseti* have been reported causing soft or dry rots of apples and pears in India (Tandon et al. 1975; Geeta et al. 1979; Jain et al. 1982). *Talaromyces purpureogenus* and *Paecilomyces variotii* [= *Byssochlamys spectabilis*] have also been found from postharvest rots of pears (Tandon et al. 1975). *Alternaria alternata* and the *Stemphylium* state of *Pleospora herbarum* (Pers.: Fr.) Rabenh have been reported causing postharvest rots of apples in Spain and Taiwan (Viñas et al. 1992; Ko et al. 1994), however, Simmons (2007) lists *A. mali* and *A. pomicola* as species associated with apples. Postharvest fungal diseases of pears have been reviewed by Sardella et al. (2016).

As with citrus fruit, the search for effective biological control agents for the two major postharvest pathogens of pome fruit is ongoing. Activity against *Penicillium expansum* has been demonstrated by cultures of the bacteria *Bacillus subtilis* (Sholberg et al. 1995), *Pseudomonas cepacia* (Smilanick and Denis-Arrue 1992), *P. syringae* and the pink yeast *Sporobolomyces roseus* Kluyver & C.B. Neil (Janisiewicz 1994; Janisiewicz and Bors 1995) and against *Botrytis cinerea* by *S. roseus* and *Candida oleophila* [=*Yarrowia* lipolytica (Wick et al.) Van der Walt & Arx] (Janisiewicz and Bors 1995; Mercier and Wilson 1995). Nunes et al. (2002) reported that a 50:50 mixture of *Candida sake* (Saito & M. Ota) Uden & H.R. Buckley ex S.A. Mey. & Ahearn and the bacterium *Pantoea agglomerans* reduced grey mould incidence in pears in cold storage by 95%. and reduced blue and grey mould infection on apples at room temperature by 90%. *Pseudomonas syringae* was shown to be effective in controlling *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* on apples and pears (Nunes et al. 2007). *Pseudomonas fluorescens* was tested as a biological control agent against *Penicillium expansum* and *P. solitum* and found to reduce blue mould in apples (Etebarian et al. 2005). However, none of these treatments is yet in commercial application, and the food safety aspects of these strategies still need to be fully investigated. For reviews of biological control of postharvest diseases in fruits and the challenges associated with commercial development, see Janisiewicz and Korsten (2002), Upsall et al. (2016) and Droby et al. (2016).

11.2.3 Stone Fruits

Stone fruits (peaches, plums, apricots, nectarines and cherries) are all susceptible to brown rot caused by *Monilinia fructicola* (G. Winter) Honey (asexual state *Monilia fructicola*), *Monilinia fructigena* (Aderh. & Rühle) Honey (= *Monilia fructigena*) and *Monilinia laxa* (Aderh. & Rühle) Honey (= *Monilia laxa*) (Snowdon 1990). In each case, the asexual *Monilia* state is the only one found occurring on commercial fruit or in Petri dish culture. Brown rot is the most important market disease of apricots, peaches and nectarines. Early symptoms of this rot are water soaked spots on the fruit, which within 24 h become brown, enlarging and deepening rapidly, then producing a dusting of pale brown conidia. The whole fruit may rot in 3–4 days (Hall and Scott 1977). Infection commences in the orchard. Rigorous preharvest spray programmes with benomyl or similar benzimidazole fungicides are necessary to achieve control, but postharvest dips with dichloran, iprodione or triforine may assist (Fourie and Holz 1987; Sharma et al. 1989; Adaskaveg and Ogawa 1994). Hot water (40 °C) treatment has shown promise also (Liu et al. 2012). Storage temperatures below 5 °C are recommended.

A second major rot in all kinds of stone fruits is transit rot, so named because it usually develops in boxed fruit during transport. It is caused by *Rhizopus stolonifer* and *R. oryzae* (Heaton 1980; Snowdon 1990). Soft rot commences in a single fruit, which then becomes surrounded by a coarse, loose "nest" of mycelium. Growth spreads rapidly, engulfing several fruit adjacent to the originally infected one, and sometimes all the fruit in a box, in only 2–3 days. This characteristic growth form, together with sporangia which are white when young but darken as they mature, is diagnostic. *Mucor* species, particularly *M. piriformis,* and also *M. racemosus* and *M. plumbeus,* can also cause postharvest rots in stone fruits (Sholberg 1990; Michailides 1991).

Dichloran is an effective fungicide against *Rhizopus* (Sharma et al. 1989). A combined benomyl and dichloran preharvest spray programme for the control of both *Monilinia* and *Rhizopus* is recommended for peaches, apricots and nectarines (Hall and Scott 1977; Ryall and Pentzer 1982). Wade and Gipps (1971) reported almost complete control of *Monilinia* and greatly reduced losses from *Rhizopus* in fruit dipped in a mixture of benomyl and dichloran.

Penicillium expansum causes blue mould rot in cherries and plums, but is uncommon in other types of stone fruits (Ryall and Pentzer 1982). *Alternaria* species and *Botrytis cinerea* can cause spoilage of stone fruits (Splittstoesser 1987; Fourie and Holz 1985, 1995), but are generally of lesser economic importance. Infection of peaches with *Lichthemia (Absidia) corymbifera* and *Botryosphaeria ribis* H.L. Gross & Duggar has been reported from India (Singh and Prashar 1988). Alternative postharvest measures to control brown rot and blue mould in stone fruits include exposure to ozone (0.3 ppm, v/v; Palou et al. 2002), hot water brushing (Karabulut et al. 2002) and biological control (*Cryptococcus laurentii*) in combination with a salicylic acid spray (Yu et al. 2007).

11.2.4 Tomatoes and Other Solanaceous Fruit

Tomatoes, with an internal pH of 4.2–4.5, and other solanaceous fruit (capsicums and eggplant) can be affected by both fungal and bacterial diseases. Several of those produced by fungi are important.

All solanaceous fruit are susceptible to rots caused by *Alternaria* species. Alternaria rots appear as dark brown to black, smooth, only slightly sunken lesions, which are of firm texture and can become several centimetres in diameter. Infection can occur at the stem end of the fruit, or through mechanical injury, cracking from excessive moisture during growth, or chilling (Snowdon 1991). *Alternaria solani* Sorauer causes early blight rot in tomatoes (Snowdon 1991) but not in other solanaceous fruit.

Alternaria rots develop at all acceptable handling temperatures, and can be avoided only by rapid marketing. As several other diseases have a similar appearance, diagnosis is best carried out by culturing on a medium such as DCMA.

Chilling injury allows the entry of other fungi also. Cladosporium rot caused by *Cladosporium herbarum* and grey mould rot due to *Botrytis cinerea* can both be potentiated by chilling injury. *B. cinerea* can also affect mechanically damaged green fruit, on which it forms "ghost spots", small whitish rings, often with darker centres. Rots can spread rapidly at higher temperatures during packing and transport (Ryall and Lipton 1979). Diagnosis of both types of rot is best done by culturing the fungus.

Rhizopus species appear to be able to attack almost any kind of fruit or vegetable, and the tomato is no exception. In severe cases of Rhizopus rot, and there seem to be no mild ones, the fruit resembles "a red, water filled balloon" (Ryall and Lipton 1979). When the fruit collapses, grey mycelium, a fermented odour and white to black spore masses become visible. The disease starts in cracked or injured fruit but may spread by contact thereafter. Mucor rot of tomato caused by *M. hiemalis* (Sonoda et al. 1981) and of tomato, capsicum and eggplant caused by *M. mucedo* Fresen. (Reyes 1990) has been reported.

Sour rot in tomatoes is caused by *Galactomyces candidus* (asexual state *Geotrichum candidum*). Lesions are a light greenish grey and may extend as a sector from end to end of the fruit. Tissue remains firm at first, but later weakens and emits a sour odour. White mycelium may become visible, and in wet mounts can be seen to consist mainly of arthroconidia, with their characteristic microscopic appearance. This disease invades only damaged or cracked fruit and is disseminated by *Drosophila* flies (Ryall and Lipton 1979).

Tomatoes grown without stakes or trellises can develop soil rot caused by *Rhizoctonia solani* J.G. Kahn. Small brown spots of this disease develop concentric rings when they become 5 mm or more in diameter (Snowdon 1991). Injury is not necessary for the development of this rot, but soil contact is.

Other postharvest pathogens reported from solanaceous fruits include *Fusarium* species, *F. oxysporum* (Onwuzulu et al. 1995), *F. semitectum* (= *F. incarnatum*), *F. acuminatum* (Kusum 1992), *F. avenaceum* and *F. equiseti* (Snowdon 1991), *Trichothecium roseum* (Takahashi et al. 1995), *Scelerotinia, Phytophthora, Pythium,* and *Diaporthe* species (Ryall and Lipton 1979; Snowdon 1991).

11.2.5 Melons and Other Cucurbits

Water melons sometimes develop anthracnose from *Colletotrichum lagenaria* (Pass.) Ellis & Halst. [= *Gloeosporium orbiculare* (Berk.) Berk.] This disease forms circular or elongate welts which are initially dark green and later become brown, disfiguring the melon surface. Pink conidia may be produced in acervuli if humidities remain high. Rock melons (cantaloupes) may be affected by several diseases, the most important being Alternaria rot due to *A. alternata.* Mould invasion usually takes place at the stem scar, producing dark brown to black lesions and eventually invading the flesh, forming firm, adherent areas. Diagnosis can be made from microscopic examination of cultures on DCMA.

Cladosporium species can also invade melons through the stem scar, forming a rot similar to that caused by *Alternaria.* In both cases prompt shipping and cool storage will limit the losses from these diseases.

Several *Fusarium* species, particularly *F. oxysporum, F. solani, F. semitectum* (= *F. incarnatum*) and *F. culmorum* can invade melons, especially when storage temperatures are high or storage periods become excessive. *Fusarium acuminatum* has been reported to cause rot in pumpkins (Elmer 1996). *Penicillium* species may also occasionally cause problems under these conditions (Ryall and Lipton 1979; Hawthorn 1988; Snowdon 1991). On muskmelons and watermelons, pink rot caused by *Trichothecium roseum* has been reported from Japan, USA, South America, India and UK (Snowdon 1991; Takahashi et al. 1995). Silicon oxide and sodium silicate significantly reduced the severity of pink rot in rock melons (Guo et al. 2007). Infection with *Myrothecium roridum* [= *Paramyrothecium roridum* (Tode) L. Lombard & Crous] occurs on various melons and cucumbers in USA, Mexico and India (Snowdon 1991; Kuti and Boehm 1994). *Mucor mucedo* Fresen. and

Botrytis cinerea can cause rots of stored cucumbers (Reyes 1990).

Other diseases of cucurbits are caused by *Macrophomina phaseolina* (Tassi) Guid. (charcoal rot), *Galactomyces candidus* (sour rot), *Epicoccum nigrum* (red rot), *Pythium* spp. (cottony leak of cucumbers and melons), *Verticillium dahliae* Kleb. (cavity rot of wintermelons), *Phytophthora* spp., *Rhizopus* spp., *Sclerotium rolfsii* Sacc. [= *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] and *Rhizoctonia solani* J.H. Kuhn (soil rot) (Wade and Morris 1982; Snowdon 1991; Gubler and Bernhardt 1992; Bruton et al. 1993; Smith et al. 1993).

11.2.6 Grapes

Botrytis cinerea is regarded as the highly desirable "noble rot" in certain wine grapes (Coley-Smith et al. 1980), but it is by far the most serious cause of spoilage in table grapes (Ryall and Pentzer 1982; Nair et al. 1987; Snowdon 1990). In the early stages of invasion, the fungus develops on stems and inside the berry; later growth erupts at the surface and produces grey conidia. Growth then expands rapidly through tight bunches where humidity is high, and large "nests" of rot may develop rapidly. Under the microscope the characteristic conidiophores of *Botrytis* are readily seen.

Control involves the use of preharvest sprays with benomyl and rapid transfer of fruit to cool stores after picking. Postharvest treatments with sulphur dioxide or benomyl are also effective (Hall and Scott 1977). The use of pads releasing sulphur dioxide in boxes of table grapes is a common storage practice (Lichter et al. 2008). Esterio et al. (2007) reported the first incidence of fenhexamid resistance in *Botrytis cinerea* in Chilean grapes, prompting research into alternative control measures. Recent alternatives to control postharvest infection of table grapes include the use of natural antimicrobial compounds such as carvacrol (Martínez-Romero et al. 2007), essential oils of culinary herbs under hypobaric conditions (Sevili et al. 2017), peptides from *Bacillus subtilis* (Zhang et al. 2017) and ozone (Tzortzakis et al. 2007) Spray application of calcium chloride, sodium bicarbonate or sodium carbonate (1%) 90 and 30 days before harvest significantly reduced postharvest grey mould (Nigro et al. 2006).

Yeasts are a major component of the natural flora on the surface of fresh grapes. The apiculate yeast *Hanseniaspora uvarum* is usually the dominant species, comprising 50–75% of isolations. *Metschnikowia pulcherrima* Pitt & M.W. Mill., *Candida stellata* [= *Starmerella stellata* (Kroemer & Krumbholz) C.A. Rosa & Lachance and various species of *Cryptococcus, Rhodotorula, Pichia* and *Kluyveromyces* also are common. Fermentative species such as *Saccharomyces cerevisiae* are usually present in low numbers (Fleet and Heard 1992; Fleet et al. 2002; Fleet 2003).

Penicillia do not usually attack grapes before harvest, but are prevalent in stored grapes, with *P. expansum* the most commonly encountered species (Hall and Scott 1977; Ryall and Pentzer 1982; Snowdon 1990). *P. aurantiogriseum, P. brevicompactum, P. chrysogenum, P. citrinum, P. crustosum, P. decumbens* and *P. glabrum* have also been isolated from spoiled grapes in Israel, Morocco and Italy (Barkai-Golan 1974; Benkhemmar et al. 1993; Pollastro et al. 2005). As with *Botrytis,* postharvest control relies on treatments with benomyl or sulphur dioxide.

Black *Aspergillus* species such as *A. niger* can cause Aspergillus bunch rot in grapes, particularly in warmer climates (Barkai-Golan 1980; Nair 1985; Snowdon 1990; Pollastro et al. 2005; Leong et al. 2006; Hocking et al. 2007). *Aspergillus carbonarius* also occurs on grapes and is the primary producer of ochratoxin A in that commodity (Leong et al. 2006). Other diseases of grapes include *Alternaria alternata* rot in cold stored grapes in South Africa (Swart and Holz 1994), black rot due to *Guignardia bidwellii* [= *Phyllosticta ampellicida* (Engelm.) Aa] in Europe and eastern USA, Cladosporium rot in some U.S. varieties, and Rhizopus rot in market fruit stored at elevated temperatures (Barbetti 1980; Snowdon 1990).

11.2.7 Berries

Because of their shape and proximity to the ground during growth, berries are readily contaminated with soil and fungal spores. They are also readily damaged during picking and handling, and are vulnerable to fungal invasion. Most kinds of berries have similar susceptibilities to disease fungi and can be considered as a group.

The principal fungal rots in most berry crops are caused by *Botrytis cinerea, Rhizopus stolonifer* and *Mucor piriformis* (Snowdon 1990; Tournas and Katsoudas 2005). *Botrytis* causes soft rots in cane berries such as raspberries and loganberries, but a firm, dry rot in strawberries. In both cases the fruit become covered with a growth of grey mould. Losses in strawberries can be high as the fungus spreads by contact and forms "nests" of rotting fruit. Preharvest spraying programmes are important for control, as is refrigerated storage. Postharvest antifungal treatments are of little benefit, however Park et al. (2005) reported that chitosan (2%) based coatings supplemented with potassium sorbate proved useful in reducing *Cladosporium* sp. and *Rhizopus* sp. infection on strawberries. Chitosan has been shown to have three different activities that contribute to reduction of postharvest rots on berries and other fruits. Chitosan can elicit defence responses in fruit, has intrinsic antimicrobial activity against postharvest pathogens and forms protective, edible films on treated fruits (Romanazzi et al. 2018).

Rhizopus stolonifer and *Mucor piriformis* cause a large proportion of marketing losses on all berry fruits. Rotting fruit collapse completely, exuding juice, and at favourable temperatures (above 20 °C) the fungi spread rapidly. Lower temperatures reduce growth markedly, so control is mainly based on low temperature storage and handling (Ryall and Pentzer 1982; Snowdon 1990).

Strawberries can also be invaded by *Rhizoctonia solani* J.H. Kuhn, which causes a dry, spongy, black rot and by *Phytophthora cactorum* (Lebert & Cohn) J. Schröt., which causes dry, tough "leather rot". Overripe or damaged berries can be invaded by *Penicillium* and *Cladosporium* species. Greenhouse grown strawberries are susceptible to anthracnose caused by *Colletotrichum acutatum* J.H. Simmonds (Smith and Gupton 1993). Various other field disorders occur in berries, but they are usually not of major significance (Ryall and Pentzer 1982; Snowdon 1990).

Yeasts are normal colonisers of strawberries, being present at up to 10^5/g in macerates of mature berries (Buhagiar and Barnett 1971; Fleet 2003). A wide variety of yeast species were isolated by these authors, and although spoilage of strawberries by yeasts is uncommon (Dennis 1983), it can occur in tissues damaged by modified atmosphere storage (Snowdon 1990). Lowings (1956) reported spoilage of English strawberries by *Hanseniaspora uvarum* (= *Kloeckera apiculata*).

Botrytis cinerea is the major postharvest pathogen of kiwifruit, causing stem end rots. The presence of this disease may predispose the fruit to invasion by other fungi such as *Botryosphaeria, Fusicoccum, Diaporthe, Fusarium, Phoma, Glomerella, Colletotrichum* and *Cryptosporiopsis* (Brook 1992). *Phialophora* species have been reported causing lesions on and below the skin of stored kiwifruit in Italy (Marchi et al. 1994).

11.2.8 Figs

There are few fungal diseases of figs. However, smut caused by *Aspergillus niger* and endosepsis caused by growth and sporulation of *Fusarium verticillioides* in Calimyrna figs (Michailides and Morgan 1998) have been investigated with a view to using *Paecilomyces lilacinus* [= *Purpureocillium lilacinum*] as a biocontrol agent (Subbarao et al. 1993). Doster and Michailides (2007) reported high incidences of *Aspergillus niger*, *Alternaria alternata* and *A. atra* (Preuss) Woudenb. & Crous) in two cultivars of Californian figs. Other reported diseases of figs are due to *Alternaria alternata, Botrytis cinerea, Cladosporium, Penicillium* and *Rhizopus* species (Snowdon 1990).

Miller and Phaff (1962) documented the invasion of Smyrna figs by yeasts. This type of fig is pollinated by the fig wasp which introduces the

yeast *Candida guilliermondii* [= *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki] and a bacterium, *Serratia* species. These microorganisms do not cause spoilage, but at maturity attract *Drosophila* flies which carry spoilage yeasts. The spoilage yeasts are *Hanseniaspora* species and *Torulopsis stellata* [=*Starmerella stellata* (Kroemer & Krombholz) C.A. Rosa & Lachance], which produce "souring" of the figs by acid production.

11.2.9 Tropical Fruit

Fruit from tropical areas are susceptible to an array of diseases quite different from those grown in subtropical or temperate climates. Study of such diseases is still a developing science with many pressing problems, not the least being that tropical fruit are injured by low temperatures, so disease control cannot be assisted by refrigeration.

Bananas are the most important tropical fruits in international trade. Most postharvest diseases of bananas are due to fungal rots in the stalks and crowns, less commonly on the sides of the fruit (Eckert et al. 1975; Snowdon 1990). A comprehensive study of bananas shipped from the Windward Is. to England (Wallbridge 1981) showed that nearly 20 fungal species can cause crown rots. The most important were *Colletotrichum musae* (Berk. & M.A. Curtis) and *Fusarium semitectum* (= *F. incarnatum*), with several other *Fusarium* species also significant. *Verticillium theobromae*, [= *Musicillium theobromae* (Turconi) Zare & W. Gams], *Lasiodiplodia theobromae, Alternaria alternata* and *Nigrospora sphaerica* are less common (Wallbridge 1981; Wade et al. 1993). Other studies have reported *Fusarium verticillioides, F. subglutinans, F. solani , [= Neocosmospora solani] F oxysporum* and *F. proliferatum* to be important in postharvest disease of bananas (Chakrabarti et al. 1977; Dharam 1977; Jimenez et al. 1993*;* Wade et al. 1993*;* Vesonder et al. 1995). A survey of bananas in Brazil identified *Colletotrichum musae* (Berk. & M.A. Curtis) Arx, *Trichoderma harzianum*, *Fusarium equiseti, Penicillium* sp., *Aspergillus parasiticus*, *Trichothecium roseum*, *Colletotrichum acutatum* J.H. Simmonds, *Alternaria* sp., *Cladosporium musae* [= *Metulocladosporiella musae* (E.W. Mason) Crous et al.] and *Curvularia lunata* as spoilage agents (Moraes et al. 2006). Kamel et al. (2016) reported that in the Dominican Republic, etiological agents of crown rot could be ranked based on presence and pathogenicity as follows: *Fusarium incarnatum, Colletotrichum musae, F. verticillioides, F. sacchari* (E.J. Butler & Hafiz Khan) W. Gams and *Lasiodiplodia theobromae*. Benomyl, thiabendazole and prochloaz, chlorine and hot water have all been quite successful in controlling banana rots (Eckert et al. 1975; Wade et al. 1993).

Anthracnose and stem end rots are the most destructive postharvest diseases of avocados and mangoes (Galsurker et al. 2018). Anthracnose is manifested as brown or black spots on the skin which at best reduce crop value and may eventually destroy the fruit. Anthracnose is usually caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (referred to under a variety of species names in *Gloeosporium* and *Colletotrichum* in the literature), *C. acutatum* J.H. Simmonds, *Botryosphaeria parva* [= *Neofusicoccum parvum* (Pennycook & Samuels) Crous et al.], *B. dothidea* (Moug.) Ces. & De Not. and *Phomopsis* sp. (Everett and Timudo-Torrevilla 2007) and stem end rots by *Lasiodiplodia theobromae* and *Dothiorella* species (Snowdon 1990; Johnson et al. 1991). *Colletotrichum gloeosporioides* can also cause pepper spot of avocados (Willingham et al. 2000). The use of boscalid/pyraclostrobin and dithianon fungicides proved effective in controlling spore germination and may be an alternative to copper sprays (Everett and Timudo-Torrevilla 2007). Mangoes are also susceptible to black spot disease, the agent of which is *Alternaria alternata* (Johnson et al. 1990; Prusky et al. 1993). Control may rely on benomyl, oprochloraz or a variety of other fungicides. Hot water dips with or without fungicides for 2–5 min at 52–55 °C have been quite effective in control of anthracnose and stem end rots in mangoes (Smoot and Segall 1963; Johnson et al. 1990) and other fruit. The posthar-

vest quality of mangoes was improved by UV-C treatment (González-Aguilar et al. 2007).

Papayas (pawpaws) are affected by the same types of anthracnose and stem end rots as mangoes, by black rot caused by *Mycosphaerella caricae* [= *Stagonosporopsis caricae* (Syd. & P. Syd.) Aveskamp et al.], Phytophthora rots, Fusarium rots caused by *Fusarium solani [Neocosmospera solani] F. equiseti* and *F. oxysporum* and a variety of other rots (Simmonds 1965; Quimio et al. 1975; Snowdon 1990; Persley and Ploetz 2003).

Pineapples are susceptible to a number of diseases of fungal origin, including core rots (black rot) caused by *Ceratocystis paradoxa* (Dade) C. Moreau, fruitlet core rots (*Talaromyces funiculosus*) and skin blemishes such as leathery spot caused by *T. purpureogenus* and *Fusarium moniliforme* or *F. verticillioides* (Lim 1983; Mourichon 1983; Snowdon 1990; Damayanti et al. 1992; Rohrbach and Schmitt 2003). A new species, *Fusarium ananatum* A. Jacobs et al., was found to be responsible for internal and external dark lesions of pineapple fruit in South Africa (Jacobs et al. 2010). Postharvest control of black rot may be achieved by a hot (54 °C) water dip for 3 min (Wijeratnam et al. 2005) or by biological antagonism using *Pichia guilliermondii* [=*Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki](Reyes et al. 2004).

In passionfruit, *Alternaria passiflorae* J.H. Simmonds causes brown spot (Inch 1978; Fullerton 1982), and a number of other fungi are associated with more generalised rots (Snowdon 1990; Manicom et al. 2003). In guava fruit, the most significant postharvest pathogens are *Rhizopus oryzae, Lasiodiplodia theobromae* and *Colletotrichum acutatum* J.H. Simmonds (Snowdon 1990; Mukta and Bora 1993; Patel and Pathak 1993).

11.3 Vegetables

As noted earlier, the near neutral pH of vegetables increases their susceptibility to bacterial invasion, and reduces the dominant role of fungal pathogens to near equality. However, vegetables are susceptible to attack from a wide range of fungi, and these have been extensively documented and illustrated by Snowdon (1991). Bacterial rots are usually distinguishable from those of fungal origin by a watery or slimy appearance, lack of visible mycelium, and disagreeable odour. Wet mounts stained with lactofuchsin can be a useful aid.

11.3.1 Peas

The most common fungal rot of peas is caused by *Botrytis cinerea.* Water soaked spots enlarge and develop grey mycelium and spores (Ryall and Lipton 1979; Snowdon 1991). Peas are also susceptible to a number of other postharvest diseases including anthracnose (*Colletotrichum*), Ascochyta pod spot due to *Ascochyta pisi* (= *Didymella pisi* Chilvers et al.) and Alternaria blight caused by *A. alternata* (Snowdon 1991). Control is by refrigerated storage.

11.3.2 Beans

Beans are susceptible to several pathogens, the most important being anthracnose due to *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, "cottony leak" caused by *Pythium butleri* Subraman. and "soil rot" (small rusty brown lesions) from *Rhizoctonia solani* J.G. Kühn (Snowdon 1991). Beans are also susceptible to *Botrytis cinerea* (Reyes 1990). Careful sorting, rapid cooling and low temperature transport provide control.

11.3.3 Onions and Garlic

As a hypogean vegetable, the onion is enveloped in fungi during growth and maturation. However onions are highly resistant to invasion, and some diseases only develop after harvest. Few *Aspergillus* species cause plant diseases, but *A. niger* is a well known pathogen of onions, producing unsightly deposits of black conidia between the outer scales. Lesions may also be

produced (Ryall and Lipton 1979; Morris and Ward 1986; Snowdon 1991). *Aspergillus alliaceus* Thom & Church has also been reported to be capable of causing rots in onions (Filtenborg et al. 1996). *Fusarium* species including *F. solani* and *F. oxysporum* and *Botrytis* species (*B. allii* Munn, *B. aclada* Fresen. and *B. cinerea*) may invade in the field and develop in storage (Hussein et al. 1977; Dang and Singh 1982; Ji et al. 2018). A number of *Penicillium* species have been reported to cause blue rot of onions around the world (Snowdon 1991; Dugan et al. 2007). *P. glabrum* causes spoilage of Australian onions (our unpublished observations) and has been reported elsewhere (Filtenborg et al. 1996). A survey of fungal pathogens of onions in Puerto Rico found that *A. niger*, *Phoma sorghina* (= *Epicoccum sorghinum*), *Talaromyces purpureogenus*, and *Sclerotium rolfsii* [=*Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] were pathogenic to mature bulbs (Velez-Rodriguez and Rivera-Vargas 2007). Prompt drying and curing, reduced temperatures and humidities are the most effective control for onion diseases (Ryall and Lipton 1979; Snowdon 1991).

Garlic bulbs are susceptible to *Fusarium, Penicillium* and *Botrytis* rots, particularly if bulbs are damaged (Böttcher and Pohle 1993). Many post-harvest pathogens of garlic may be carried in seed cloves (Dugan et al. 2007). *Fusarium* species reported include *F. oxysporum, F. verticillioides, F. solani [= Neocosmospora solani]* and *F. camptoceras* Wollenw. and Reinking (Roy et al. 1977; Rath and Mohanty 1986; Gargi and Roy 1988; Koch and Taanami 1995; Mahmoody 1998; Dugan et al. 2007), and *F. proliferatum* and *F. acuminatum* in our laboratory. *Fusarium proliferatum* was the most common pathogen of onions and garlic in Serbia (Stankovic et al. 2007) and Spain (Galvez et al. 2017). *Aspergillus niger* and *A. ochraceus* have also been isolated from garlic cloves (Dugan et al. 2007). *Penicillium allii* causes rots in garlic (Vincent and Pitt 1989; Bertolini and Tian 1996; Dugan et al. 2007, as *P. hirsutum*) and has also been reported as a field pathogen (Valdez et al. 2006).

Dugan et al. (2007) demonstrated pathogenicity of North American isolates of *Penicillium albocoremium* (Frisvad) Frisvad (= *P. hirsutum*), *P. crustosum, P. expansum, P. glabrum, P. paraherquei, P. polonicum* and *P. radicicola* Overy & Frisvad in both garlic and onions.

11.3.4 Potatoes

Potatoes are mostly affected by bacterial rots. Fungal diseases are usually caused by *Fusarium* species, particularly *F. solani* var. *caeruleum* (=*F. caeruleum* Lib. ex Sacc.), and *F. sulphureum* Schltdl. (responsible for dry rot). Potatoes are also susceptible to diseases such as silver scurf (*Helminthosporium solani* Durieu & Mont.) and skin spot [*Polyscytalum pustulans* (M.N. Owen & Wakef.) M.B. Ellis]. *Phytophthora, Pythium, Phoma* and a number of other genera can also cause diseases (Hide et al. 1994). Infection often occurs through wounds: lesions are brown and tissues shrink and become wrinkled as the decay progresses (Snowdon 1991). Control relies on careful handling and sorting, surface drying and refrigerated storage. A hot water treatment of 60 °C was effective in controlling postharvest pathogens such as *Fusarium oxysporum*, *Botryodiplodia theobromae* (=*Lasiodiplodia theobromae*) and *Rhizopus oryzae* (Salami and Popoola 2007).

11.3.5 Roots and Tubers

Carrots may be invaded by *Stemphylium radicinum* (= *Alternaria radicina* Meier et al.), by *Rhizopus* species, by *Botrytis cinerea* and by *Sclerotinia sclerotiorum* (Lib.) de Bary (Ryall and Lipton 1979; Snowdon 1991). Root rot caused by *Fusarium avenaceum* and *F. solani* and sour rot (*Galactomyces candidus*) have also been described (El-Tobshy et al. 1979; Marziano et al. 1992; Arjona et al. 1996). None of these diseases causes large commercial losses as a rule.

Sweet potatoes are susceptible to several diseases, one of the most serious being black rot caused by *Ceratocystis fimbriata* Ellis & Halst. *Ceratocystis* is an ascomycete genus forming perithecia with long necks and long narrow asco-

spores. It invades sweet potatoes in the field, but causes losses only after storage. Lesions start as small, round spots which may enlarge to 20–50 mm in diameter. Perithecia may often be seen at this stage. Chemical treatments are ineffective, but heat treatments may reduce the severity of this rot with little effect on the tubers (Snowdon 1991).

Macrophomina phaseolina, *Diaporthe batatas* [=*Phomopsis phaseoli (Desm.) Sacc.*] and *Lasiodiplodia theobromae* all cause firm or dry, brown to black rots of sweet potatoes (Snowdon 1991; Tournas 2005). In contrast, *Rhizopus stolonifer* and *R. oryzae* produce soft, watery rots with little colour change. Under moist conditions, the characteristic coarse mycelium of these fungi envelops the decaying tubers. This serious disease affects sweet potatoes in most areas of the world (Ryall and Lipton 1979; Snowdon 1991; Clark 1992). Control relies on careful handling and culling of damaged tubers. El Nashawy (2016) reported that tuber exposure to UV-C for 1–3 h significantly reduced *Rhizopus* rot during cold storage for 3 months and throughout the transport and distribution chain. Other diseases include Java black rot (*Lasiodiplodia theobromae*) and Fusarium rots (*F. oxysporum F. semitectum* (= *F. incarnatum*) and *Neocosmospora solani* (Clark 1980, 1992; Snowdon 1991; Ray et al. 1994; Ray and Misra 1995; Ray and Ravi 2005).

Ginger is affected mainly by Fusarium rot caused by various species especially *Fusarium oxysporum* (Teakle 1965). *Pythium* (Snowdon 1991) and *Sclerotium rolfsii* [=*Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.](Pegg et al. 1974) also cause postharvest diseases of ginger.

11.3.6 Yams

Yams are a very important crop in many parts of Africa. Decay of yams in storage has been intensively studied in Nigeria, where losses may be as high as 10% of the crop (Ogundana 1972). The principal fungi causing decay in yams are *Lasiodiplodia theobromae, Fusarium verticillioides, Penicillium sclerotigenum* W. Yamam., and *Aspergillus niger* (Adeniji 1970; Ogundana et al. 1970; Obi and Moneke 1986; Snowdon 1991; Aboagye-Nuamah et al. 2005). A number of other fungi, including other *Penicillium* species and *Rhizopus stolonifer,* occur as secondary invaders (Snowdon 1991). Benomyl and thiabendazole are the most effective fungicides for prevention of storage rots of yams (Ogundana 1972; Ogundana and Dennis 1981). Reducing insect damage in storage barns may also be an effective way of minimising postharvest fungal attacks (Nweke and Enujeke 2006).

11.3.7 Cassava

Cassava is an important staple food in many parts of three continents: Africa, South America and Asia. The two major postharvest spoilage rots of cassava are caused by *Lasiodiplodia theobromae* and *Neocosmospora solani* (Snowdon 1991). *Rhizopus* (*R. oryzae* and *R. stolonifer*) and *Aspergillus* species are also important (Clerk and Caurie 1968; Ray et al. 1990; Snowdon 1991; Obadina et al. 2007). Aflatoxins were detected in dried roots of cassava and cassava flour from Burundi and Eastern Democratic Republic of Congo, although all samples were below 10 μg/kg (Udomkun et al. 2018).

11.3.8 Leafy and Other Green Vegetables

The most generally damaging postharvest fungal diseases of leafy vegetables such as lettuces, celery and fennel are caused by *Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani* J.H. Kühn and *Alternaria* species. Overall, *Botrytis* is the most destructive fungal pathogen on these vegetables. It is readily recognised microscopically once sporulation commences. Control is difficult, low temperature storage being recommended (Ryall and Lipton 1979; Snowdon 1991; Tournas 2005). Storage under modified CO_2/O_2 atmosphere may also retard fungal growth at low temperatures (Tournas 2005).

Cabbages and broccoli may be attacked by *Botrytis cinerea, Alternaria* species, including *A. alternata* and *A. brassicicola,* which cause dark spots, and also *Phytophthora* and *Fusarium* species during cool storage (Mercier et al. 1991; Snowdon 1991; Leifert et al. 1993; Heimann 1994; Tournas 2005).

Stored asparagus spears are susceptible to rots of the bracts caused by *Fusarium verticillioides, Phytophthora* rots predominantly at the base of the spears, and basal zone rots caused by *Penicillium hirsutum* (Snowdon 1991; Montealegre and Palma 1994). Elmer (2001) reported *Fusarium oxysporum* and *F. proliferatum* causing spear rots in asparagus. In China, *F. asiaticum* O'Donnell et al. was reported as an important pathogen of cool stored asparagus. The isolates produced low levels of 3-acetyldeoxynivalenol in the asparagus spears (Zhu et al. 2013). *Galactomyces candidus* and *Botrytis cinerea* can also cause damage in stored asparagus (Tournas 2005).

11.4 Dairy Foods

Fresh milk, a liquid of neutral pH, is highly susceptible to bacterial spoilage and hence fungi are rarely a problem. In milk processed to cream, cottage cheese or butter, the growth of lactic acid bacteria will cause the pH to fall, favouring the growth of spoilage yeasts and, less commonly, fungi. Garnier et al. (2017) have published a comprehensive review of spoilage of dairy products by yeasts and filamentous fungi, including sources of contamination, types of spoilage and preventative methods.

Yeasts may cause gas and off flavour production in cream and cottage cheese and rancidity or other flavour defects in butter (Walker and Ayres 1970; Frölich-Wyder 2003; Garnier et al. 2017). *Galactomyces candidus* can cause spoilage of cream (Marth 1978; Craven et al. 2001) as a result of unclean machinery on farms. We have isolated several *Penicillium* species from spoiled sour cream, including *P. glabrum, P. commune* and *P. chrysogenum*. *Penicillium* species, particularly *P. chrysogenum* and *P. glabrum,* as well as *Emericella sydowii*, have been isolated in our laboratory from spoiled dairy desserts containing light sour cream and yoghurt.

Fungal spoilage of UHT dairy products sometimes occurs, for example with *Galactomyces candidus,* usually as a result of postprocessing contamination. We have isolated *Fusarium oxysporum* on several occasions from UHT flavoured milk drinks. *F. oxysporum* is not recognised as a heat resistant species, but its thick walled chlamydoconidia appear to permit survival of some pasteurising processes, and its ability to grow at very low oxygen tensions then enables this species to cause fermentative spoilage.

Heat resistant fungi rarely cause spoilage in UHT dairy products, but as milk can be contaminated with soil, such occurrences are not unknown. We have seen *Hamigera avellanea* Stolk & Samson [= *Talaromyces avellaneus* (Thom & Turesson) C.R. Benj.] and *Talaromyces macrosporus* causing spoilage of a UHT custard product and a strawberry yoghurt respectively. We have isolated heat resistant fungi on three separate occasions from cream cheese. The species involved were *Byssochlamys nivea, H. avellanea, Neosartorya spinosa* and *Penicillium brefeldianum. Byssochlamys nivea, Talaromyces macrosporus* and *Neosartorya fischeri* have also been isolated from heat treated dairy beverages made from reconstituted powdered ingredients.

Yeasts are very common in yoghurts and can sometimes cause spoilage, particularly in products containing fruit. Species of *Candida,* especially *C. parapsilosis, C. famata* (= *Debaryomyces hansenii*) and *C. diffluens* [= *Colacogloea diffluens* (Ruinen) Q.M. Wang et al.], *Rhodotorula* species, particularly *R. mucilaginosa*, *Kluyveromyces marxianus* (E.C. Hansen) van der Walt and *Yarrowia lipolytica* (Wick et al.) van der Walt & Arx are most common (Suriyarachchi and Fleet 1981; Fleet and Mian 1987; Green and Ibe 1987; Rohm et al. 1990; Fleet 1990; Rohm et al. 1992; Frölich-Wyder 2003; Viljoen et al. 2003; Garnier et al. 2017). We have repeatedly isolated *Pichia anomala* (= *Wickerhamomyces anomalous*) from fermenting yoghurts containing fruit or flavouring syrups. The characteristics which enable

yeasts to cause spoilage in yoghurt are: (1) growth at low temperatures (<10 °C); (2) production of proteolytic and lipolytic enzymes to hydrolyse milk proteins and fats; (3) ability to ferment or utilise lactose and sucrose, the main sugars of plain and flavoured yoghurts; and (4) ability to assimilate lactic and citric acids which are the main organic acids in yoghurt (Fleet and Mian 1987). Yoghurts containing fruit can also be spoiled by fungi introduced with the fruit preparation. We have seen fermentative spoilage of fruit flavoured yoghurt caused by *Mucor circinelloides* which grows strongly at refrigeration temperatures, and can grow under extremely low oxygen tensions. *Mucor* species (*M. racemosus* and *M. hiemalis*) and *Penicillium aurantiogriseum* have also been reported from spoiled yoghurt in Italy (Foschino et al. 1993).

Solid perishable dairy foods and substitutes such as butter and margarine are susceptible to the growth of spoilage fungi. Muys et al. (1966) studied the fungal flora of margarine and concluded that *Galactomyces candidus, Moniliella suaveolens*, *Cladosporium herbarum* and *Yarrowia lipolytica* could cause spoilage by their lipolytic action. As few as 500 cells of *Y. lipolytica* may produce perceptible off flavours. *Cladosporium butyri* C.N. Jensen is a particularly undesirable contaminant in milk, cream, butter or margarine, because it causes rancidity as a result of the production of ketones, detectable in very low concentrations. Muys et al. (1966) also outlined specific methods for the detection of undesirable fungi in butter and margarine.

Low salt margarines are more susceptible to fungal spoilage than those containing the normal amount of salt. In Australia, we have observed that the most common spoilage fungi are *Penicillium* species, particularly *P. glabrum, P. expansum* and *P. chrysogenum* and *Cladosporium* species, with *C. cladosporioides* by far the most common (Hocking 1994). As well as being lipolytic, these fungi can cause spoilage due to off flavours, particularly earthy taints from the production of 2-methyl-isoborneol and geosmin.

11.5 Meats

"There has been little study during the last 50 years of mould spoilage of meats, although it is still of importance … moulds … only spoil meat if the spoilage conditions prevent bacterial growth, but there are few firm data on the time and temperature requirements for visible mould growth to develop …". This quotation from Gill and Lowry (1982) summed up the situation then and little has changed since. Moulds only compete with bacteria on meat when storage temperatures are lowered to 0 °C or below, or when the meat surface dries to an a_w that enables fungi to compete. In earlier literature, spoilage of chilled or frozen meat by fungi was usually attributed to Mucorales, especially *Thamnidium elegans* and *Mucor* species which grew as "whiskers" on cold stored meat (Brooks and Hansford 1923; Empey and Scott 1939). Campano et al. (1985) reported that the most commonly isolated fungi from beef aging at refrigeration temperature were *Thamnidium elegans, Mucor mucedo* and *Helicostylum pulchrum* (Preuss) Pidopl. & Milko (= *Chaetostylum fresenii* Tiegh. & G. Le Monn.). Hadlok and Schipper (1974) reported very infrequent isolation of Mucorales from meat and questioned their significance, but it seems more probable that techniques for meat storage have changed rather than that the prewar meat technologists were wrong.

Dry-aged beef is considered a gourmet product. Meat is aged at refrigeration temperature with good airflow at a relative humidity of 75–80%. The meat undergoes a series of microbiological changes, and by the end of the aging period may develop some fungal growth. Ryu et al. (2017) reported *Penicillium camemberti* and *Debaryomyces hansenii* present at the end of the aging period and concluded that they may play an important role in flavour development of the dry-aged beef.

Michener and Elliott (1964) cited several reports of bacteria and fungi growing on meats at −5 °C, with fungi predominating as temperatures were further lowered, to a limit at about

−12 °C. Schmidt-Lorenz and Gutschmidt (1969) reported that fungi grew on chickens stored at −7.5 and −10 ± 0.2 °C for 1 year. Spoilage of chilled meats in postwar years has principally been the result of "black spot", traditionally believed to be due to *Cladosporium herbarum* (Brooks and Hansford 1923). Gill et al. (1981) cultured such black spots and identified *Cladosporium cladosporioides, Penicillium hirsutum* and *Aureobasidium pullulans* as well as C. *herbarum.* All were capable of producing black spots on meat at −1 °C. Gill and Lowry (1982) showed that C. *herbarum* would take 4 months to produce a visible colony 1 mm in diameter at −5 °C, and concluded that this temperature was near the practical limit for spoilage of meat by black spot fungi.

Yeasts occur in low numbers on freshly slaughtered cuts of red and poultry meats, but can proliferate in minced or ground meats. Yeasts may reach 10^6–10^7 cells per gram, but effects are insignificant compared with those of bacteria. The yeasts most frequently isolated from comminuted meats are *Candida zeylanoides* (Castell.) Langeron & Guerra, *Debaryomyces hansenii, C. sake* (Saito & M. Ota) Uden & H.R. Buckley ex S.A. Mey. & Ahearn, *Yarrowia lipolytica* and *Cryptococcus laurentii* [= *Papiliotrema laurentii* (Kuff.) Xin Zhan Liu et al.] (Fleet 1992). Dipping fresh turkey breast fillets in 0.5% fumaric acid solution or 5% sodium tripolyphosphate solution reduced yeast counts immediately by 0.4 and 0.7 log units respectively and extended shelf life by 4 days (Baysal and Unluturk 2007).

11.6 Cereals, Nuts and Oilseeds

The fungi growing on crops which will subsequently be dried have been divided traditionally into "field" and "storage" fungi. Although this distinction has become blurred in recent years with the discovery that certain species, especially *Aspergillus flavus,* are equally at home in both situations, it is still a useful concept.

Field fungi are plant pathogens, which invade the growing seed or nut before harvest. Deterioration or spoilage of a particular crop usually results from invasion by a specific fungus, because climatic conditions, plant variety or agricultural practice produce circumstances where invasion by that species can occur on a large scale. Control, if it exists, is by refinements in agricultural practice. Field fungi rarely play a significant role in further deterioration of the crop postharvest. Here storage fungi become dominant, as will be discussed in Chap. 12.

All cereal, nut and oilseed crops are subject to growth of field fungi. Only the most important will be described here.

11.6.1 Wheat, Barley and Oats

From a comprehensive 2 year survey of the mycobiota of Scottish wheat, barley and oats, Flannigan (1970) concluded that field contamination of these cereal crops was similar. The most commonly occurring fungus was *Alternaria alternata,* which was present on more than 85% of kernels examined. *A. alternata* causes downgrading of cereals due to grey discolouration, and the production of mycotoxins (Watson 1984; Webley et al. 1997). *Cladosporium* species were also very common in barley and oats (85% and 95% of grains respectively) but rather less so in wheat (77%). Grey discolouration can result from growth of these species also. Other commonly occurring fungi were *Epicoccum nigrum* and *Penicillium* species. *A. alternata* was also the dominant species on English barley, occurring on more than 75% of a large number of experimental plots sampled over three seasons (Hill and Lacey 1983). These authors reported large numbers of *Penicillium* species, principally *P. verrucosum, P. aurantiogriseum, P. hordei*, *P. piceum* Raper & Fennell and *P. roqueforti* in barley, with *Cladosporium cladosporioides, Aureobasidium pullulans* and *Epicoccum nigrum* also very common (Hill and Lacey 1984).

A 2 year study of malting barley grains in Slovakia confirmed that *Alternaria* species were the most prevalent fungi. Other species isolated included *Cochliobolus sativus* [= *Bipolaris sorokiniana* (Sacc.) Shoemaker] and *Fusarium avenaceum* (Hudec 2007). Similarly, *Alternaria*

alternata was the most common species found in Argentinean grains (Broggi et al. 2007).

From freshly harvested wheat grains in Egypt, 77 fungal species from 26 genera were isolated, including 16 species of *Aspergillus* and 21 of *Penicillium* (Moubasher et al. 1972). Other genera of importance were *Alternaria, Cladosporium* and *Fusarium.* No indication was given that any of these species were causing spoilage or unacceptable deterioration. The dominant species were *A. niger* and *P. chrysogenum*.

In a study of freshly harvested barley in Egypt, Abdel-Kader et al. (1979) isolated 37 genera and 109 species. The dominant genera were *Aspergillus,* represented by 25 species, *Penicillium* (32 species), *Rhizopus, Alternaria, Fusarium* and *Drechslera*.

The principal invasive field fungi in U.S. wheat and barley have been reported to be *Alternaria, Fusarium, Drechslera* and *Cladosporium. Alternaria* was present in nearly 100% of grains (under the pericarp) and of barley (under the hull) (Christensen 1965, 1978, 1987). *Fusarium graminearum* and *F. poae* were the most common Fusaria found in North American wheat (Miller 1994). A more recent survey of *Fusarium* species causing head blight in wheat, barley and oats in Canada found that *F. graminearum* was predominant in spring wheat, whereas *F. graminearum* and *F. poae* were equally common in barley. In oats, *F. poae* was the dominant species, with *F. avenaceum, F. equiseti* and *F. sporotrichioides* also being common (Xue et al. 2019). A Swedish survey of *Fusarium* in wheat identified twelve *Fusarium* species, the most important being *F. graminearum* and *F. avenaceum*. *Fusarium poae, F. tricinctum, F. culmorum* and *F. sporotrichioides* were also common (Karlsson et al. 2017). No detectable difference in *Fusarium* communities was observed between organic and conventionally grown crops.

In Japan, an important disease of wheat and barley, termed red mould disease, is caused predominantly by *Fusarium graminearum* producing trichothecene mycotoxins (Yoshizawa et al. 1979). Korean barley also showed heavy infection with *Fusarium* species (Park and Lee 1990). In contrast, Thai wheat showed little invasion by *Fusarium* species (Pitt et al. 1994). Principal fungi found were *Alternaria alternata, Cladosporium cladosporioides* and *Penicillium aurantiogriseum.*

Alternaria alternata was found to be the most prevalent species in Australian wheat from Northern NSW and Southern Queensland affected by black point (Webley et al. 1997). In a survey of Australian wheat carried out in our laboratory, the most common species of field fungi were *Alternaria infectoria, A. alternata* and *Epicoccum nigrum. Nigrospora oryzae* and *Cladosporium cladosporioides* were also common (our unpublished data). *F. graminearum* was an uncommon component of the postharvest mycobiota, usually invading less than 1% of grains (our unpublished data). Other *Fusarium* species isolated were *F. acuminatum, F. avenaceum* and *F. equiseti,* also at very low levels. Similar results were reported by Barkat et al. (2016) who surveyed stored wheat from four Australian states. They found that *Alternaria infectoria* was common in wheat from Western and South Australia, less common in Victoria and rare in New South Wales. Overall, the most frequently encountered genera were *Alternaria* (*A. infectoria, A. alternata*), *Rhizopus, Aureobasidium, Penicillium* and *Cladosporium*. *Fusarium* species were rarely isolated. The low incidence of *Fusarium* species in Australian wheat is due mainly to the much drier and hotter growing conditions in the main wheat belts of Australia compared with North America and Europe (Burgess et al. 1981). The distribution of *F. graminearum* is restricted to warm temperate to subtropical regions where rainfall is moderate to high in summer (Backhouse and Burgess 2002). Southwell et al. (2003) reported an outbreak of Fusarium head blight in durum wheat grown in the Liverpool Plains in NSW in 1999. The disease incidence ranged from 2% to 100% of crops and *Fusarium graminearum* was the dominant pathogen. In a further head blight outbreak in 2010–11, *F. graminearum* and *F. pseudograminearum* O'Donnell & T Aoki were found to be responsible, resulting in deoxynivalenol levels in immature grains of more than 1 mg/kg

in some samples (Obanor et al. 2013). However, *F. pseudograminearum* is principally a causal agent of crown rot rather than head blight in wheat (Obanor and Chakraborty 2014).

11.6.2 Rice

Southeast Asian rice carries a wide variety of fungi up to the point of husk removal. Most common are *Trichoconiella padwickii, Curvularia* species, *Fusarium incarnatum, Bipolaris oryzae* (Breda de Haan) Shoemaker, *Nigrospora oryzae, Chaetomium* species, *Phoma* species and *Diplodia maydis* [= *Stenocarpella maydis* (Berk.) B. Sutton] (Iizuka 1957, 1958; Majumar 1974; Kuthubutheen 1979; Pitt et al. 1994). The same genera were reported as the dominant biota of freshly harvested Indian rice (Mallick and Nandi 1981). On Egyptian paddy rice, a number of *Aspergillus* species (*A. flavus, A. sydowii, A, terreus*, *A. ochraceus* and *Neosartoria fumigata*) and *Penicillium* species (*Penicillium chrysogenum* and *P. corylophilum*), were isolated, along with *Fusarium oxysporum, Alternaria alternata, Cladosporium cladosporioides, Trichoderma viride* and *Mucor racemosus.* In a survey of Spanish cereals the most common fungi isolated from rice were *Penicillium* and *Rhizopus* species, while *Fusarium* species were less common (Cantalejo et al. 1997). In paddy rice from Northeastern Argentina and Southern Paraguay, Tonon et al. (1997) found that the major invaders were *P. citrinum* and *Talaromyces islandicus*. In a study of potentially mycotoxigenic fungi and mycotoxins in Brazilian rice, a very high incidence (52%) of *F. graminearum* occurred preharvest, while other *Fusarium* species were uncommon. *Aspergillus flavus* was present in 5–9% of samples postharvest. Aflatoxin B_1 was detected in only one sample of parboiled rice (4.9 μg/kg) whereas zearalenone was detected in 60% of samples (91–126 μg/kg). No deoxynivalenol was detected (Savi et al. 2018). In Italy, rice was found to be highly contaminated with fungi before harvest. *Fusarium* spp. were found most commonly (20% of total fungal contamination) but isolates were not identified to species. *A. flavus* occurred in less than 1% of samples, but *E. versicolor* was consistently isolated (incidence 0–5%). Sterigmatocystin was the most widespread mycotoxin, detected in all paddy rice samples collected post-flowering, at levels <10 μg/kg. Aflatoxin B_1 and and deoxynivalenol were detected at low levels in only a few samples (Bertuzzi et al. 2019).

When cellulose agar was used, the most prevalent fungi found in rice were *Stachybotrys chartarum* (Ehrenb.) S. Hughes, *S. bisbyi* (Sriniv.) G.L. Barron, *Aspergillus niger, A. flavus, Fusarium oxysporum, Alternaria alternata, Bipolaris sorokiniana* Shoemaker and *Acremonium strictum* [=*Sarocladium strictum* (W. Gams) Summerb.] (Abdel-Hafez et al. 1987). In Australia, information is limited: weather damaged rice examined in our laboratory contained *Fusarium proliferatum, F. equiseti* and *F. anthophilum* (A. Braun) Wollenw. Mycobiota of freshly harvested rice may be similar to that found in Thailand (Pitt et al. 1994).

11.6.3 Maize

Developing ears of maize are encased in a strong, protective husk which greatly reduces invasion by fungi. *Fusarium* is the principal pathogen causing spoilage of maize in the ear, the most commonly occurring species being *F. graminearum, F. verticillioides* and *F. subglutinans* (Burgess et al. 1981; Marasas et al. 1984). *F. graminearum* usually causes a generalised rot, with a pronounced reddish discolouration of grains and husk, and with pinkish to red mycelium also visible on the grain surface. Most *Fusarium* species invade through the sites of insect damage, and perhaps also through the silks (Miller et al. 2007). However, *Fusarium verticillioides* and related species including *F. proliferatum* appear to be commensals and are endemic in maize in most parts of the world (Desjardins and Busman 2006; Leslie and Summerell 2006): in the U.S.A. (Cole et al. 1973; Bullerman and Tsai 1994), Europe (Visconti and Doko 1994), South Africa (Marasas et al. 1979), Zambia (Marasas et al. 1978), China (Yoshizawa et al. 1994), Thailand (Pitt et al. 1993; Yoshizawa et al. 1996), other parts of Asia

(Pitt et al. 1998) and Australia (Leslie and Summerell 2006). Control of Fusaria in maize is very difficult (Burgess et al. 1981).

The economic importance of *Fusarium* diseases in maize is exacerbated by the fact that all produce potent mycotoxins, of considerable, even devastating, significance to the health of man and domestic animals. For further information see Chap. 13.

Of no less importance than the *Fusarium* diseases is the fact that *Aspergillus flavus* also invades maize. In the early literature, *A. flavus* was regarded only as a storage fungus, but in the mid 1970s the realisation came that freshly harvested maize in the southeastern United States was sometimes infected by *A. flavus* with the consequent production of aflatoxins (Lillehoj et al. 1976a, 1976b; Shotwell 1977). Maize from the cooler areas in the midwestern maize belt, however, showed little if any preharvest invasion. Insect damage to cobs is probably the major means of entry for the fungus (Lillehoj et al. 1980; Hesseltine et al. 1981), but it has also been shown that *A. flavus* can invade maize cobs down the silks without an insect vector (Williams et al. 2006; Windham and Williams 2007). Invasion is favoured by high growing temperatures, above 30 °C, and plant stress also appears to be important, at least under laboratory conditions (Lillehoj 1983). In contrast with *A. flavus, A. parasiticus* appears to be an infrequent invader of maize. For a review see Horn (2007).

Maize from Southeast Asia is also heavily invaded by *Aspergillus flavus.* It was present in more than 85% of 150 samples of Thai maize, at up to 100% of grains in some infected samples (Pitt et al. 1993). Similar figures were obtained from Indonesia and the Philippines (Pitt et al. 1998). The first step towards aflatoxin control in countries such as these relies on rapid drying immediately after harvest (Siriacha et al. 1988).

Aspergillus flavus and *A. parasiticus* used to be uncommon in Europe, being first reported from Italian maize in 2003 when *A. flavus* was found on 93% of samples examined and 70% produced aflatoxins (Giorni et al. 2007). As average temperatures in many European countries are rising, incidence of *A. flavus* and aflatoxins in maize appears to be increasing. Since the first report from Italy, *A. flavus* has been reported from maize and soils in Hungary, and approximately half were aflatoxin producers. *A. parasiticus* was much less common but all isolates were toxigenic (Sebök et al. 2016). Aflatoxins have been found in maize in Serbia (Janić Hajnal et al. 2017) and France when exceptionally hot and dry climatic conditions in 2015 favoured aflatoxin production (Bailly et al. 2018). Of 67 strains of *Aspergillus* section *Flavi* isolated, most (69%) were *A. flavus*, but 28% were *A. parasiticus* (Bailly et al. 2018). Such a high incidence of *A. parasiticus* can be considered unusual.

Maize is also invaded preharvest by *Penicillium* species. Mislivec and Tuite (1970) found 6.4% of some hundreds of samples of midwestern U.S. maize were infected with Penicillia, the most common species being *P. oxalicum* and *T. funiculosus. Penicillium ochrosalmoneum* has been isolated from US maize, and shown to produce significant levels of citreoviridin in naturally infected kernels (Wicklow et al. 1988). Moubasher et al. (1972) found a much less extensive mycobiota in maize than in wheat, with numbers of types of both genera and species being only 50% of those in the latter crop. *Penicillium chrysogenum* and *Aspergillus niger* were dominant. In Australia, *T. funiculosus* and *T. pinophilus* have been isolated from maize (Burgess and Hocking, unpublished). The most commonly encountered *Penicillium* species in Thai maize were *P. citrinum,* found in 64%, and *T. funiculosus* in 42% of 154 samples (Pitt et al. 1993).

Other commonly isolated fungi were *Lasiodiplodia theobromae* and *Fusarium semitectum* (= *F. incarnatum*), with significant levels of *Rhizoctonia solani* J.H. Kühn, *Rhizopus oryzae* and *Trichoderma harzianum* encountered in some samples (Pitt et al. 1993). The role of the Penicillia and these other species in subsequent spoilage is uncertain.

11.6.4 Soybeans and Mung Beans

In soybeans and mung beans from Thailand, the most commonly isolated field fungi were

Fusarium semitectum (= *F. incarnatum*), *Lasiodiplodia theobromae, Macrophomina phaseolina* (Tassi) Guid., *Chaetomium* (*C. brasiliense, C. globosum* and *C. funicola*) and *Cladosporium* species (*C. cladosporioides* and *C. sphaerospermum*) (Pitt et al. 1994). *Aspergillus flavus* was detected in 67% of the 49 samples of soybeans examined, but was less common in mung beans (45% of samples). Infection levels were usually low. *Alternaria alternata* was the most commonly isolated fungus on freshly harvested soybeans in Argentina (Broggi et al. 2007). Weather damaged soybeans from midwestern U.S.A. were heavily infected with *Alternaria alternata, Fusarium graminearum* and *Phomopsis* species. Soybean seedlings in Ohio were also heavily infected with *F. graminearum* (Broders et al. 2007). Zearalenone, zearalenol, deoxynivalenol and diacetoxyscirpenol were detected in damaged soybeans (Jacobsen et al. 1995). *Fusarium* species were present in only 5% of Argentinian soybeans surveyed, the most common species being *F. equiseti, F. semitectum* (=*F. incarnatum*) and *F. verticillioides* (Vaamonde et al. 1987). Freshly harvested soybeans in Ecuador were the source of *F. verticillioides*, *F. semitectum*, *Aspergillus flavus* and *A. ochraceus* (Pacin et al. 2002). *F. semitectum* (= *F. incarnatum*) was also common in weather damaged Australian soybeans (Hocking and Pitt 1996).

In Argentina, the most commonly encountered genera from 89 samples of freshly harvested soybeans were *Alternaria, Sclerotinia, Chaetomium, Cladosporium, Aspergillus, Penicillium, Phomopsis* and *Fusarium. Alternaria alternata, A. tenuissima, Aspergillus flavus, Penicillium citrinum, Fusarium verticillioides* and *F. semitectum* (=*F. incarnatum*) were the dominant toxigenic species (Garrido et al. 2003). When high throughput rDNA sequencing was applied to soybeans from two growing regions in China, 69 taxa were recovered by ITS sequencing. The four dominant species were *Aspergillus flavus, A. niger, Fusarium moniliforme* and *Penicillium chrysogenum* (Liu et al. 2007).

11.6.5 Other Beans and Pulses

Chickpeas are an important crop in many tropical countries. In India, a survey of chickpea pods from their earliest stages of development revealed a prevalence of *Alternaria alternata, Cladosporium herbarum* and *Fusarium* spp. in the standing crop. After harvesting, *Aspergillus flavus* dominated (Singh and Ahmed 1989). Australian weather damaged chickpeas contained *Alternaria alternata, Botrytis cinerea, Stemphylium botryosum, Fusarium acuminatum* and *Chaetomium* and *Cladosporium* species, with *Aspergillus flavus, Eurotium amstelodami* (= *Aspergillus montevidensis*), *A. chevalieri, Penicillium brevicompactum* and *Rhizopus oryzae* also present. Weather damaged faba beans examined at the same time contained a similar range of fungi (Hocking and Pitt 1996). A comprehensive review of the occurrence of toxigenic fungi and mycotoxins in chickpeas and derived products has been published (Ramirez et al. 2018).

11.6.6 Sunflower Seed

Little information exists in the literature on the mycobiota of smaller oilseeds such as sunflower. One study from Pakistan reported field fungi: *Rhizoctonia solani* J.G. Kühn, *Macrophomina phaseolina* (Tassi) Guid., *Fusarium verticillioides, F. solani, F. semitectum* (=*F. incarnatum*), *F. equiseti, Drechslera* species, *Alternaria alternata* and *A. tenuissima,* along with a high incidence of *Aspergillus flavus* and *A. niger* infection (Shahnaz and Ghaffar 1991). From India, *Macrophomina phaseolina, Alternaria alternata* and *Aspergillus flavus* were reported from sunflower by Vijayalakshmi and Rao (1993). Gowdar et al. (2007) found similar fungi, but reported *Alternaria helianthi* (Hansf.) Tubaki & Nishih. as the most commonly occurring species. Weather damaged Australian sunflower seeds examined in our laboratory were heavily invaded by *Botrytis,* with *Alternaria alternata, Cladosporium,* and *Stemphylium* species also isolated.

11.6.7 Sorghum

The mycobiota detected in Southeast Asian sorghum was rather different from that found in other commodities. *Aspergillus flavus* was present in very high levels, comparable to those found in maize. The presence of high numbers of *Curvularia lunata, C. pallescens,* other *Curvularia* species, *Alternaria alternata, Alternaria longissima* Dreighton & MacGarvie, *Fusarium moniliforme, F. semitectum* (= *F. incarnatum*), *Lasiodiplodia theobromae, Nigrospora oryzae* and *Phoma* species indicated that sorghum is host to a wide range of field fungi (Pitt et al. 1994). A similar range of fungi have been reported from Indian sorghum (see Pitt and Hocking 1997; Raj et al. 2007) and sorghum in Egypt (Osman et al. 1988). *Aspergillus flavus*, *Fusarium verticillioides* and *F. proliferatum* were isolated from freshly harvested and stored sorghum in Brazil (da Silva et al. 2004).

The dominant species in Australian sorghum samples examined in our laboratory was *Alternaria alternata.* Other fungi commonly isolated were *Alternaria infectoria, Phoma sorghina* (= *Epicoccum sorghinum*), *Bipolaris sorghicola* [=*Bipolaris cookie* (Sacc.) Shoemaker], *Exserohilum rostratum* (Drechsler) K.J. Leonard & Suggs and *Cladosporium* species.

11.6.8 Peanuts

Freshly harvested peanuts and peanut shells are colonised by a diversity of fungi. The intimate contact of soil with the shells of developing nuts is an ideal situation for fungal colonisation. Although the shell represents a physical barrier and protects the developing kernels from most fungal invasion, fungi may still enter via cracks in the shells or via the pegs.

In peanut kernels, *Aspergillus* species, particularly *A. niger* and *A. flavus,* have been reported frequently (see Pitt and Hocking 1997). In Thai peanuts, 95% of 109 samples contained *A. flavus,* with invasion of 41% of all kernels examined. Figures for peanuts from the Philippines were similar, while from Indonesia, 98% of 256 samples contained *A. flavus,* with invasion of 61% of all kernels examined (Pitt et al. 1993, 1998). *A. parasiticus* is also very common in peanuts in the United States (Horn et al. 1994; Horn 2007) and Australia (Pitt and Hocking 2006) but rare in Southeast Asia (Pitt et al. 1993). *Aspergillus niger*, *A. glaucus* and *A. ochraceus* were found in Spanish peanuts (Jiménez and Mateo 2001). In Brazilian peanuts, the most commonly occurring fungi in kernels sampled 2 weeks before harvest and immediately after harvest were *Fusarium* species (not identified) followed by *A. flavus*. Other fungi reported included *A. parasiticus, A. niger, Penicillium* species (not identified) and *Rhizopus* (Atayade et al. 2012).

Other commonly occurring fungi in peanuts are *Fusarium* species [*F. solani,* (= *Neocosmospora solani*) *F. semitectum* (= *F. incarnatum*) and *F. oxysporum*], *Macrophomina phaseolina* (Tassi) Guid., *Rhizoctonia solani* J.G. Kühn, *Rhizopus* species (*R. stolonifer* and *R. oryzae*), *Chaetomium* and *Curvularia* species. *Penicillium* species are also relatively common, with *Talaromyces funiculosus* most frequently reported, but also *P. citrinum, P. aurantiogriseum, P. glabrum, P. chrysogenum, P. janthinellum* and *Talaromyces pinophilus* (see Pitt and Hocking 1997).

11.6.9 Cashews and Brazil Nuts

Similar fungi occur in cashews as in peanuts, but at much lower levels. The dominant species detected on cashews from Thailand were *Aspergillus* species (*A. flavus, A. niger* and *A. sydowii*), *Chaetomium* species (*C. globosum*, and *C. funicola*), *Nigrospora oryzae*, *Penicillium citrinum* and *Cladosporium cladosporioides* (Pitt et al. 1993). In Brazil, dwarf cashew nuts were contaminated with species of *Aspergillus* (*A. niger* and *A. flavus*), *Penicillium* (*P. brevicompactum* and *P. glabrum*), *Chaetomium globosum*, *Aspergillus chevalieri* and *A. montevidensis* (=*Eurotium amstelodami*), *Nigrospora oryzae* and *Rhizopus oryzae* (Freire et al. 1999). In Nigeria, *A. flavus, A. tamarii, Rhizopus stolonifer, Fusarium* and *Gliocladium* species were

associated with dry rot disease of cashew kernels (Esuruoso 1974).

Freire et al. (2000) isolated 17 fungal species from brazil nuts. *Aspergillus flavus* was dominant followed by *A. niger*. *P. citrinum* and *P. glabrum* were the only *Penicillium* species found. Other fungi reported included *Acremonium curvulum* [= *Kiflimonium curvulum* (W. Gams) Summerb. et al.], *Cunninghamella elegans, Exophiala* sp., *Fusarium oxysporum, Rhizopus oryzae, Scopulariopsis* sp., *Thielavia terricola* [= *Pseudothielavia terricola* (J.C. Gilman & E.V. Abbott) X. Wei Wang & Houbraken] and *Trichoderma citrinoviride* Bissett. Arrus et al. (2005) sampled brazil nuts from several forest areas, and found that yeasts, *Penicillium, Acremonium* and an unidentified Coelomycete species were dominant on the whole pods, while nuts in shell yielded a similar suite of fungi. From aseptically shelled nuts, the unidentified Coelomyces was most common, followed by *Penicillium* spp. and a few colonies of *Aspergillus wentii*. *Aspergillus flavus* was isolated from intact pods, but *A. parasiticus* was not detected. The *Penicillium* species identified included *P. glabrum,, P. citrinum, P. sclerotiorum, Talaromyces funiculosus* and *T. wortmanii* (Arrus et al. 2005). A comprehensive study of fungi throughout the brazil nut supply chain was published by Taniwaki et al. (2017). *A. flavus* and a number of other species from *Aspergillus* section *Flavi* (but not *A. parasiticus*) were present in more than 50% of brazil nut kernels collected from the rainforest. *A. niger* and related species were also commonly isolated, while *P. citrinum* was the most commonly encountered *Penicillium* species. *Syncephalastrum racemosum* was the most frequently isolated Zygomycete, occurring in 25% of rainforest samples (Taniwaki et al. 2017).

11.6.10 Almonds, Walnuts, Hazelnuts and Pecans

Tree nuts are protected by thick shells which, while they remain intact, protect the nuts against fungal invasion. In almonds from California, *Aspergillus niger* and *Penicillium glabrum* were reported most frequently, with late harvested nuts showing lower fungal counts than those harvested earlier (King and Schade 1986). In almonds from California which had not been surface disinfected, Bayman et al. (2002a, b) found that *Penicillium* spp. and *A. niger* were most prevalent, followed by *Rhizopus, A. ochraceus* group, *A. melleus* Yukawa, *A. flavus* and *Neosartorya. fumigata.* From a survey of 149 samples of hazelnuts, Senser (1979) reported *Rhizopus stolonifer* and *Penicillium aurantiogriseum* as the most common of 33 species of fungi isolated. In hazelnuts and walnuts from Egypt, *Aspergillus* (*A. flavus, N. fumigata, A. niger*), *Cladosporium* (*C. cladosporioides, C. herbarum*), *Penicillium* (*P. chrysogenum, P. citrinum, P. oxalicum*) and *Fusarium* species (*F. equiseti, F. moniliforme* (= *F. verticillioides*), *F. oxysporum*) were reported. After incubation at 45 °C, *N. fumigata* and *Rhizomucor pusillus* were commonly isolated (Abdel-Hafez and Saber 1993). Fungal development in Californian almonds was followed from May to August, 2015 by Ortega-Beltran et al. (2018). They reported the most commonly identified genera were *Acremonium* (52.9%), *Cladosporium* (5.4%), *Aureobasidium* (4.1%), *Aspergillus* (3.1%) and *Fusarium* (1.0%), with *Alternaria* and *Penicillium* both 0.3%. During August, *Aspergillus* counts increased markedly with almond maturity, from 0–6 cfu to 1200 cfu/g.

In freshly harvested Californian walnuts, *Penicillium* species were most common, then *Rhizopus, Aspergillus niger, A. tamarii, A. nidulans, A. flavus* and *Emericella nidulans* (Bayman et al. 2002a, b). Tannins in walnut skin have been shown to inhibit aflatoxin production and are the probable reason why walnuts are less susceptible to aflatoxin contamination than other tree nuts (Molyneux et al. 2007). However, the toxicity of mouldy walnuts to dogs caused by the production of penitrem A by *P. crustosum* has been well documented (Richard et al. 1981). Indeed, the frequency of cases of tremorgenic mycotoxicosis in dogs that have eaten mouldy walnuts in France (76 cases from 2008 to 2015; Adamczyk et al. 2016) and the USA (65 cases from 2001 to 2012; Coleman and Merola 2016) indicate that

Penicillium crustosum is a regular contaminant of fallen walnuts.

In pecans, most published information relates to stored nuts. However, dominant field fungi recovered from pecans include *Cladosporium, Penicillium, Alternaria* and *Epicoccum,* with *Alternaria alternata, Trichothecium roseum, Pestalotia* (= *Pestalotiopsis*) species and *Fusarium* species also reported (Wells 1980). If weather conditions are conducive (wet and warm), pecan kernels may be affected by pink mould disease caused by *Trichothecium roseum*, as occurred in California in 2002 (Sparks 2007). A Brazilian study of 52 samples of pecans from producers from four regions of Rio Grande del Sul State, collected at the point of entry to a processing plant, reported that *Aspergillus flavus* was absent although other species of *Aspergillus* (particularly *A. wentii, A. chevalieri, A. ruber* and *A. pseudoglaucus* were commonly encountered. Of 10 *Penicillium* species listed, the most common were *P. citrinum* and *P. brevicompactum. Fusarium* species were found in 23–75% of samples, *Cladosporium* species in 29–33% and dematiacious fungi in 29–75% of samples (Garcia et al. 2019).

11.6.11 Pistachios

Aspergillus species have been the most frequently reported fungi from pistachios, but this may reflect the greater interest in this genus because of the potential for aflatoxin production rather than the real frequency of occurrence. In Californian pistachios, Doster and Michailides (1994) reported 14 *Aspergillus* species, with *A. niger* common in early splitting nuts. *A. flavus, A. parasiticus, A. ochraceus* and *A. melleus* Yukawa were also reported. In freshly harvested nuts, Bayman et al. (2002a, b) also found that *A. niger* was the most prevalent species, followed by *Penicillium* species, *Emericella. nidulans, A. ochraceus* group and *A. flavus.* In California, pistachios are often planted in close proximity to almonds, so were included in a comprehensive study of fungal communities in developing almonds (Ortega-Beltran et al. 2018). They sampled pistachio nuts during the period April–September 2015 and reported the most common genera as *Aureobasidium* (25.4%), *Cladosporium* (24.6%), *Alternaria* (19.2%), *Penicillium* (7.6%), *Aspergillus* (2.3%), *Botrytis* (1.7%) and yeasts (15.2%). As observed in almonds, *Aspergillus* counts increased from 200 cfu/g to an average of 1500 cfu/g at the approach of harvest. *A. flavus* was present in a significant proportion of 143 samples of freshly harvested Turkish pistachios (Heperkan et al. 1994). In Iran, *Aspergillus* and *Penicillium* species were common in pistachios during various stages of development. Of the Aspergilli, *A. niger, A. flavus* and *Neosartorya spinosa* occurred most frequently (Mojtahedi et al. 1979). Early split nuts and cracked seedless shells contributed most to *A. flavus* and aflatoxin contamination of pistachio nuts in Iran (Bonjar 2004), while insect damage was also reported to be a contributing factor in Californian pistachios (Campbell et al. 2003).

In freshly harvested Australian pistachios, *Fusarium* species, particularly *F. equiseti* and *F. acuminatum,* were found to be common, with *Aspergillus flavus*, *A. niger* and *A. terreus, Alternaria* species, Coelomycetes and basidiomycetous yeasts also important components of the postharvest mycobiota (Hocking and Pitt 1996). *Rhizopus* species quickly developed on freshly harvested, unhulled nuts that were left unbrined.

11.6.12 Copra

Coconut meat is probably sterile before the fruit is opened, but because it is then dried on the ground, it rapidly becomes contaminated. In 21 samples of Thai copra, *Aspergillus flavus* was the dominant species encountered. Other fungi present included other Aspergilli (*A. niger, A. tamarii, A. sydowii, A. versicolor, Neosartorya. clavata*), *Rhizopus oryzae, Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not., *Penicillium citrinum, Mucor* species, *Nigrospora oryzae* and *Eurotium* (= *Aspergillus* subgenus *Aspergillus*) species (Pitt et al. 1993). In India and the Philippines, fungi associated with deterioration of copra

included *Rhizopus oryzae, Mucor hiemalis, Penicillium citrinum, Lasiodiplodia theobromae* and species of *Curvularia, Paecilomyces* and *Aspergillus* (*A. niger, A. flavus, A. tamarii*) (Susamma and Menon 1981; Susamma et al. 1981; Morantte et al. 1986). *Rhizopus, Aspergillus niger and Penicillium italicum* were reported from Nigerian copra (Chuku et al. 2007). In Brazil, *Lapsiodiplodia theobromae* has been reported causing postharvest blackening and splitting of coconuts (Viana et al. 2002).

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12 Spoilage of Stored, Processed and Preserved Foods

It is trite to say that dried foods must be kept dry, heat processed foods must be heated enough to inactivate all relevant spores, and preservative concentrations must be high enough to inhibit all fungi. However, the science of preserving foods, like so many other disciplines, requires compromise. Really dry foods, i.e. of a safe a_w, may be impossible to obtain for climatic or economic reasons, or be unacceptable to the consumer; a sufficient heat process may destroy desirable flavours; and permitted preservative levels are set by law. Some fungi, by virtue of specific attributes, simply cannot be processed out of certain types of foods. Of particular importance are *Xeromyces bisporus* and *Zygosaccharomyces rouxii* – extreme xerophiles which grow in concentrated foods; *Byssochlamys* spp., *Talaromyces* spp. and *Neosartorya fischeri* with ascospores of very high heat resistance which can survive heat processing and may grow in heat processed acid foods; and *Zygosaccharomyces bailii,* a preservative resistant yeast. Making foods safe from these fungi requires that they be absent from raw materials or destroyed by pasteurisation, and then excluded from the processing and packing lines.

Some other fungi, especially *Aspergillus* and *Penicillium* species, cause less specific problems than the species mentioned above. However, these genera are ubiquitous and often rapid colonisers, and so will cause spoilage whenever processing is inadequate, formulation incorrect, or moisture content too high. Still others are opportunists, capable of explosive growth if storage conditions break down as a result of water leakage, moisture migration in shipping containers, etc. This chapter briefly discusses commodities and processed foods at greatest risk from such spoilage fungi. Details of the fungi themselves, their physiology and methods for isolation have been given in earlier chapters.

12.1 Low Water Activity Foods: Dried Foods

Dried foods are categorised here as solid foods, low in moisture and soluble carbohydrate, and include cereals, nuts, dried meat (biltong and jerky), dried milk and spices. Spoilage of such foods is due to the normal range of xerophilic fungi which are capable of rapid growth above about 0.77 a_w, and of slow growth at 0.75 a_w, and below – down to about 0.68 a_w. Of particular importance are *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species, which have no apparent preference for substrate; *Wallemia sebi,* which is especially common in cereals and spices; and *Aspergillus penicillioides,* an extreme xerophile which is often overlooked because of limited growth on high a_w media. Nuts are very susceptible to invasion by other *Aspergillus* species, especially *A. flavus, A. niger, A. candidus* and *A. ochraceus.* Cereals always become contaminated with *Penicillium* species. Control of these fungi

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in foods normally relies on keeping the a_w sufficiently low to prevent growth. A good rule of thumb is that for long term storage (1 year or more) foods must be held at or below 0.68 a_w; for 6 months shelf life 0.72 a_w, is adequate; and a_w levels above 0.77 are unsafe except in the short term. These a_w figures apply to normal ambient temperatures, i.e. 20–30 °C. Refrigerated storage will prolong shelf life at any a_w, provided that the air is effectively dehumidified. The moisture contents corresponding to these water activities vary widely. For humidity isotherms for particular products see Iglesias and Chirife (1982).

12.1.1 Cereals

Many studies have been carried out on the mycobiota of dried cereals and flours. Dried cereals often show high levels of field fungi, especially *Alternaria* and *Fusarium* species, which are incapable of growth or toxin production in dried crops, as well as the more xerophilic fungi capable of causing spoilage. For wheat, see for example Christensen and Kaufmann (1965, 1969), Pelhate (1968), Moubasher et al. (1972), Wallace et al. (1976), Sauer et al. (1984), Mills et al. (1995), Riba et al. (2008), Barkat et al. (2017), Kumari et al. (2019), and Solanki et al. (2019); for barley, Flannigan (1969), the review by Apinis (1972), which has more than 60 references, Abdel-Kader et al. (1979) and Stenwig and Liven (1988); for rice, Tsuruta and Saito (1980), Mallick and Nandi (1981), Mheen et al. (1982), and Pitt et al. (1994, 1998).

Reported results are strongly influenced by the kinds of media used. Dilute media such as DRBC, DCPA, CZID or PDA (see Chap. 4) will often produce quite different results from media of reduced a_w, such as DG18. PDA and CZID will be biased towards the field fungi, and will give a picture of the history of samples preharvest. DRBC will give a comprehensive picture of the common Aspergilli, Penicillia and other ubiquitous flora, as will DG18. DG18 will also provide information about *Aspergillus* subgen. *Aspergillus* species, especially *A. penicillioides,* and *Wallemia.*

The most common causes of spoilage of dried cereals are *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species, particularly *A. chevalieri, A. pseudoglaucus, A. ruber, A. montevidensis* and *A. penicillioides.* The latter, often misidentified as *A. restrictus,* is a major cause of loss of germinability in cereal grains (Christensen 1978). *A. penicillioides* is probably the pioneer species in the development of fungal populations in stored grains (Wicklow 1995; Hocking 2003). In wheat stored at 0.68 a_w for 12 months, we observed development of *A. penicillioides,* followed later by *Aspergillus* subgen. *Aspergillus* species and *Wallemia sebi*. *Wallemia* and other *Aspergillus* species are commonly present in stored cereals, but with the possible exception of *A. candidus,* relatively rarely cause spoilage.

Counts of Penicillia are often high in cereal grains, probably reflecting growth during the drying period. However some species may have specific associations with particular cereals. The most common species in wheat, barley and oats are from *Penicillium* subgen. *Penicillium,* especially *P. aurantiogriseum, P. chrysogenum, P. brevicompactum* and *P. crustosum* (Scudamore et al. 1993; Mills et al. 1995; Filtenborg et al. 1996). *P. verrucosum* appears to have a specific association with wheat, barley, oats and rye in Europe (Olsen et al. 2006). *P. verrucosum* is the source of ochratoxin A in these cereal grains in Europe and other cool climates (Lund and Frisvad 2003; Frisvad et al. 2005). *P. verrucosum* and ochratoxin A have recently been found in stored Canadian wheat and barley (Limay-Rios et al. 2017), and in oat grains from South Dakota, USA (Dhungana et al. 2018). Levels of *P. verrucosum* were consistent, but low, only 1–4% infection in positive samples. Ochratoxin A was detected in <2% of samples analysed, at levels below 1.0 ug/kg. *P. verrucosum* contaminates grain postharvest, the most common sources of infection being harvesting and grain handling equipment and silos. Rapid drying is a key to prevention of ochratoxin formation (Olsen et al. 2006). Of species from other *Penicillium* subgenera, *P. citrinum* and *P. glabrum* are ubiquitous.

Aspergillus species are significant components of the rice storage mycobiota in tropical

conditions. Important are the four common *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species, plus *A. flavus, A. candidus, A. niger, E. versicolor, A. wentii* and *Neosartorya fumigata* (see Pitt and Hocking 1997). In 139 Indonesian milled rice samples, the most frequently encountered species was *A. candidus,* present in 56% of samples, sometimes infecting up to 100% of grains, followed by *A. flavus, A. chevalieri, A. ruber, A. niger and E. fumigata* (Pitt et al. 1998). In 73 samples of milled rice from the Philippines, *A. flavus* was the most common species (present in 53% of samples), followed by *A. candidus, A. chevalieri, A. niger* and *E. fumigata* (Pitt et al. 1998). *Aspergillus flavus, A. candidus, A. niger* and *A. ochraceus* were the most common *Aspergillus* species in rice from Vietnam (Trung et al. 2001), Korea (J.W. Park et al. 2005a; S.-I. Park et al. 2005b; Oh et al. 2008), northern areas of South America (Tonon et al. 1997) and Nigeria (Makun et al. 2007a, b).

Penicillium citrinum was the most common *Penicillium* species encountered in milled rice samples from Thailand, Indonesia and the Philippines (Pitt et al. 1994, 1998). *Penicillium citrinum* was reported to be common in rice from northern regions of South America (Tonon et al. 1997), Vietnam (Trung et al. 2001) and Korea (J.W. Park et al. 2005a; S.-I. Park et al. 2005b). Yellowing of rice during storage has been attributed to fungal activity, especially to *Talaromyces islandicus*. Tonon et al. (1997) found high levels of *T. islandicus* in rice from northeastern Argentina and southern Paraguay. However, *T. islandicus* was present in only 5% of Indonesian rice samples, was rarely detected in samples from the Philippines, and not found at all in rice from Thailand (Pitt et al. 1994, 1998). Although yellowing can occur in dry grain, it is more likely to be due to mould growth before and during drying than growth during storage. Although *Talaromyces islandicus* has been implicated, the exact species of fungi responsible and their role in rice yellowing is yet to be determined (Miyaki et al. 1970; Phillips et al. 1988).

12.1.2 Flour

The kinds of fungi found in wheat are reflected in those found in flour and in goods baked from it (Graves and Hesseltine 1966; Kurata and Ichinoe 1967; Dragoni et al. 1980a; Eyles et al. 1989; Berghofer et al. 2003). However it is evident that the numbers of field fungi that can be isolated from flour are much lower than those present in the wheat or rice before milling, and that the numbers of Penicillia and Aspergilli are markedly increased. Both these changes reflect the degree of sporulation which has taken place. Field fungi produce relatively few spores; Penicillia and Aspergilli relatively many. *Aspergillus candidus* was the most common species encountered in two types of flour in Germany, with *Penicillium aurantiogrisum* the next most common (Weidenbörner et al. 2000). In wholemeal flour for breadmaking in Brazil, Santos et al. (2016) reported the most common genera as *Penicillium* (38.2%), *Aspergillus* (23.6%) and *Aspergillus* subgen. *Aspergillus* [*Eurotium*] (19.1%). The most commonly encountered species were *Penicillium polonicum* K.W. Zaleski (16.8%), *Aspergillus candidus* (15.2%), *P. commune* (8.8%) and *Aspergillus pseudoglaucus* (8%). *A. candidus* was also the most common species in flour used for cake making (Morassi et al. 2018) and in bread production (Garcia et al. 2019a) in Brazil. Both of these studies found *Aspergillus* subgen. *Aspergillus* species to be the second most commonly encountered group, along with *Emericella versicolor*. The most prevalent *Penicillium* species were *P. aurantiogriseum, P. roqueforti* and *P. citrinum* (Morassi et al. 2018; Garcia et al. 2019a). Similar results were reported by Tournas and Niazi (2018) for a limited number of retail flour samples in the USA. They found *A. tritici* B.S. Mehrotra & M. Basu commonly, and also *A. terreus*. In an extensive survey of Australian flour and mill products, *Penicillium citrinum* was the most common species, followed by *P. aurantiogriseum*; with *Emericella versicolor* and *A. penicillioides*.

Wallemia sebi, Aspergillus subgen. *Aspergillus* [*Eurotium*] and *Cladosporium* species have also been frequently isolated from flour (Eyles et al. 1989; Weidenbörner et al. 2000; Berghofer et al. 2003; Morassi et al. 2018; Garcia et al. 2019a). The rare xerophile *Geomyces pulvereus* A.D. Hocking & Pitt has been reported from Australian flour (Hocking and Pitt 1988). Yeasts are common (Kurtzman et al. 1970; Spicher and Mellenthin 1983; Eyles et al. 1989; Berghofer et al. 2003). Mucoraceous fungi such as *Rhizopus, Mucor* and *Lichtheimia* species have also been reported in flour (see Pitt and Hocking 1997; Weidenbörner et al. 2000; Berghofer et al. 2003).

12.1.3 Pasta

Dry pasta products are usually safe from mould spoilage. However, improperly dried pasta can be spoiled by xerophilic fungi. We have encountered *Aspergillus* subgen. *Aspergillus* [*Eurotium*] species, *Aspergillus candidus, Wallemia sebi,* Penicillia and yeasts in such products. Halt et al. (2004) reported *Aspergillus candidus* as the most common species in 132 samples of dried pasta, followed by *A. flavus*. *Penicillium* and *Mucor* species were also frequently isolated. *Epicoccum nigrum* has been reported causing red spots on the surface of gnocchi (a fresh pasta dumpling containing potato). *Mucor racemosus* was isolated from the same product (Dragoni and Cantoni 1979). *Paecilomyces variotii* (= *Byssochlamys spectabilis*), *Emericella versicolor*, *Aspergillus pseudoglaucus*, *Geotrichum candidum* (= *Galactomyces candidus*), *Penicillium crustosum* and *P. solitum* were reported from fresh, modified atmosphere packaged pasta products (Colavita et al. 1999).

12.1.4 Bakery Products

Spoilage of baked goods is very much dependent on water activity. High a_w products such as bread and some pastries spoil rapidly from Penicillia (e.g. *P. roqueforti, P. brevicompactum* and *P. chrysogenum*), *Wallemia, Aspergillus* subgen. *Aspergillus* species and other common moulds including the red bread mould, *Neurospora sitophila, Rhizopus* and *Mucor* species and *Hyphopichia burtonii* (Pitt and Hocking 1997; Abellana et al. 1997; Vytřasová et al. 2002; Garcia et al. 2019a and our unpublished observations). *Monascus ruber,* which is resistant to propionic acid, can produce red spots on white and multigrain breads (Spicher and Isfort 1988). Growth of white yeast-like fungi (*Endomyces fibuliger* and *Hyphopichia burtonii*) and yeasts [*Zygosaccharomyces bailii, Saccharomyces cerevisiae* and *Pichia anomala* (= *Wickerhamomyces anomalus*)] can cause "chalk mould" defects on bread (Seiler 1980; Spicher 1984b; Legan and Voysey 1991). Flavour defects such as the production of ethyl acetate by *Wickerhamomyces anomalus* can also occur (Legan and Voysey 1991) and we have seen several such spoilage incidents in white and mixed grain bread. We have also seen spoilage of Lebanese bread due to *Geosmithia putterillii* on more than one occasion, and even spoilage caused by *Aspergillus flavus,* probably due to severe build up of these particular kinds of fungi in inaccessible parts of bakeries or bakery equipment. Garcia et al. (2019a) analysed 90 samples of mouldy bread (linseed, wholemeal, rye, multigrain and white bread) returned from supermarkets in Brazil. The most commonly encountered species were *Penicillium roqueforti* and *Hyphopichia burtonii*, followed by the xerophilic *Aspergillus* species *A. pseudoglaucus, A. chevalieri* and *A. restrictus.* Other reported species were *Penicillium* (*P. brevicompactum, P. corylophilum, P. crustosum, P. citrinum* and *P. lividum* Westling), *Aspergillus* (*A. tamarii*) and *Emericella* (*E. versicolor*), yeasts, *Saccharomycopsis fibuliger* and *Wallemi sebi* (Garcia et al. 2019a). Santos et al. (2016) reported *Penicillium paneum* Frisvad and *P. polonicum* K.W. Zaleski as dominant spoilage species in multigrain and wholemeal bread stored at 25 and 30 °C for up to 29 days beyond shelf life.

European rye breads are susceptible to spoilage by *Penicillium* species, particularly *P. roqueforti, P. commune* and related species (Spicher 1984, 1985; Filtenborg et al. 1996; Lund et al. 1996; Garcia et al. 2019a). Yeasts and yeast-like

fungi can also be a problem in sliced rye bread (Filtenborg et al. 1996).

Penicillium species can cause spoilage in cakes (Morassi et al. 2018) including sponge and Madeira cakes. Sorbate or propionate may be added to these products to extent the shelf life, but as the pH is near 6.0, the preservatives are largely ineffective (Marín et al. 2004). Some Penicillia are capable of decarboxylating sorbate to 1,3 pentadiene (Daley et al. 1986), which produces a kerosene-like odour causing the cake to spoil even before mould growth is apparent. In one such spoilage incident investigated in our laboratory, *P. crustosum, P. glabrum, P. commune, P. chrysogenum* and *P. brevicompactum* were isolated from the spoiled cakes, but the species responsible for the production of the 1,3 pentadiene was not identified.

We have also seen spoilage of icing on cakes from a xerophilic yeast which was causing liquefaction of small areas of the icing. Presumably *Zygosaccharomyces rouxii* was the cause, and cleaning of the factory, especially the equipment used in manufacturing the icing, the answer. Seiler (1980) and Legan and Voysey (1991) have discussed in detail the problems caused by yeasts in baked goods.

Modified atmosphere packaging and the use of oxygen absorbing sachets can extend the shelf life of bakery products (Guynot et al. 2003a, b), but this technology is ineffective against yeast and chalk mould spoilage. Mould-free shelf life can also be extended by the use of ethanol vapour generators (Powers and Berkowitz 1990; Legan and Voysey 1991; Smith 1994; Franke et al. 2002).

Spoilage in fruit cakes is discussed under concentrated foods.

12.1.5 Maize

Maize cobs and kernels are relatively large. Moist conditions at harvest often result in slow drying. In consequence both preharvest and postharvest fungi may become well established. Lichtwardt et al. (1958) and Barron and Lichtwardt (1959) carried out a very thorough study of the mycobiota of dried and stored maize in Iowa. Lichtwardt et al. (1958) identified the internal flora of surface disinfected maize grains both in sterile moist chambers and on malt salt agar (6% NaCl). In addition, samples were ground and dilution plated. Approximately 50 genera were recognised. A combination of isolation methods enabled Barron and Lichtwardt (1959) to estimate the relative importance of the isolated genera in the spoilage of stored maize. Xerophilic *Aspergillus* species, especially *A. ruber, A. pseudoglaucus* and *A. chevalieri* were the most significant, together with *A. restrictus* and *Penicillium* species, especially *P. aurantiogriseum, P. viridicatum* and closely related species. In maize samples from Thailand, the most commonly encountered storage fungi were again xerophilic *Aspergillus* species (*A. chevalieri, A. ruber* and *A. pseudoglaucus*) but *Wallemia sebi, A. flavus*, *A. wentii, A. tamarii* and *A. niger* were also present in a significant number of samples (Pitt et al. 1993). Similar results were obtained from 148 samples of maize from the Philippines, and 82 samples of maize from Indonesia, except that *W. sebi* was not isolated from the Indonesian samples (Pitt et al. 1998). *Fusarium* species, particularly *F. verticillioides, F. semitectum* and *F. proliferatum* persisted in high numbers in stored maize from all three countries (Pitt et al. 1993, 1998). Wicklow et al. (1998) also noted dominance of *A. chevalieri* and *A. flavus,* and the persistence of *F. verticillioides* in maize stored for up to 2 years under a range of temperature (10–40 °C) and moisture (40–88% r.h.) conditions. A similar range of fungi was reported from maize in Venezuela (Mazzani et al. 2004). In stored Zambian maize, the most commonly encountered species were *F. verticillioides, A. flavus, A. niger,* as well as other *Fusarium, Aspergillus* and *Penicillium* species (Kankolongo et al. 2009). Mendoza et al. (2017) examined fungi in maize from six farms in the Guatemalan highlands, from harvest through 90 days storage, using molecular identification techniques. Yeasts and *Cladosporium* species were common in freshly harvested maize, but by 90 days storage, these had been replaced by *Fusarium* species, including *F. graminearum* and *F. verticillioides*, and

other species including *Acremonium* sp. and *Stenocarpella maydis* (Berk.) B. Sutton. *Aspergillus flavus* was detected in some samples, usually early in the storage period.

Penicillium and *Talaromyces* species occurring in maize both preharvest and in storage, and the factors which influenced their role as spoilage fungi, were investigated by Mislivec and Tuite (1970a, b). Some common preharvest species, such as *Talaromyces funiculosus,* were rarely isolated later; species such as *P. citrinum* and *P. oxalicum* were commonly present at all times; others again, such as *P. aurantiogriseum* and *P. viridicatum*, were almost exclusively associated with the stored grain. In Thai, Philippine and Indonesian maize, *P. citrinum* was the most frequently encountered *Penicillium* species, with *T. funiculosus, T. pinophilus, P. oxalicum* and *P. raistrickii* also commonly detected (Pitt et al. 1993, 1998). *P. citrinum* was also the most commonly occurring *Penicillium* species in the storage study of Wicklow et al. (1998), who reported that *T. funiculosus* declined during storage.

Recent studies have emphasised the importance of *Aspergillus flavus* as a preharvest invader (see Chap. 11) and the consequent spoilage of maize, not from fungal growth *per se*, but from the production of unacceptable levels of aflatoxins. Consequently, maize should always be checked for aflatoxins at marketing. AFPA can also be used as a monitor for *A. flavus*.

Difficulties in field drying of maize in the midwestern United States due to the onset of winter rains has led to attempts to store moist maize under refrigeration. However, many *Penicillium* species grow well below 5 °C, and such maize may develop "blue eye" disease, with the production of high levels of penicillic acid (Ciegler and Kurtzman 1970). High moisture maize (16.4%) stored in underground silos in Brazil developed populations of *Aspergillus candidus* and film-forming yeasts in the upper layers, with *Fusarium* species persisting during the storage period of 8 months (Sartori et al. 1991). Auerbach et al. (1998) reported development of *P. roqueforti* in whole-crop maize silage, with roquefortine detected in some visibly mouldy samples. Mansfield et al. (2008) reported roquefortine, patulin, mycophenolic acid and cyclopiazonic acid in fresh and ensiled maize.

Fungi acquired in the field, particularly *Fusarium verticillioides, F. proliferatum, F. oxysporum* and *Aspergillus flavus,* can persist in maize and the moulds and their toxins may be carried through to maize products such as flour, grits, corn chips, tortillas, breakfast cereals etc. (Pitt and Hocking 1997). Other fungi reported in maize products include *T. funiculosus, T. duclauxii* (Delacr.) Samson et al. and *P. brevicompactum* in maize meal and maize starch in Brazil (Ribeiro et al. 2003), *A. parasiticus, P. citrinum, T. funiculosus* and *Alternaria alternata* in milled maize in Argentina (Broggi et al. 2002), and *A. flavus, A. sulphureus, P. brevicompactum* and *P. aurantiogriseum* in maize flour in Kenya (Muriuki and Siboe 1995). The presence of aflatoxins and fumonisins is of particular concern for maize products, as both mycotoxins are very heat stable and survive the temperatures used for drying and processing maize (Mann et al. 1967; Stoloff and Trucksess 1981; Dupuy et al. 1993). Ochratoxin A has also been reported in maize products (Muriuki and Siboe 1995; Juan et al. 2007).

Fungi detected in processed maize products such as maize meal and popcorn include *Aspergillus flavus, A. niger, A. pseudoglaucus, Neosartorya fumigata, Cladosporium sphaerospermum, Penicillium chrysogenum, P. oxalicum, Talaromyces purpureogenus, T. verruculosus, Fusarium* species including *F. verticillioides* as well as other *Aspergillus* and *Penicillium* species (Kamphuis et al. 1992; Zohri et al. 1995; Touras and Niazi 2017).

12.1.6 Soybeans, Mung Beans, Other Beans, Seeds and Chickpeas

Although soybeans have a strongly protective seed coat, they are still subject to fungal deterioration in storage. Mislivec and Bruce (1977) found the mycobiota of stored soybeans dominated by xerophilic *Aspergillus* [*Eurotium*] species, *A. flavus, A. candidus, Penicillium*

aurantiogriseum, Alternaria and *Cladosporium* species. In 49 samples of soybeans from Thailand, Pitt et al. (1994) observed that after *A. flavus* (present in 67% of samples) the most common storage fungi were *Aspergillus* subgen. *Aspergillus* species, particularly *A. ruber,* which was present in 51% of the samples. Other storage species were *A. chevalieri* (in 33% of samples), *A. montevidensis* (16%), *A. pseudoglaucus* (4%), *A. restrictus* (16%), *A. penicillioides* (6%) and *Penicillium cinnamopurpureum* (8%). In 30 samples of soybeans from the Philippines, *A. flavus* was the most commonly encountered species, present in 90% of the 30 samples examined, followed in frequency by *A. chevalieri* (80%), *A. ruber* (50%), *A. niger* (47%), *P. citrinum* (33%), *A. restrictus* (27%) and *P. olsonii* (20%). In soybeans from Indonesia, a similar range of fungi was encountered, but the dominant xerophilic *Aspergillus* species was *A. ruber* (present in 60% of 48 samples), there were less Penicillia, and an unusual species, *Scopulariopsis gracilis* Samson, was isolated from 17% of samples (Pitt et al. 1998).

The storage mycobiota of mung beans in Thailand, Indonesia and the Philippines was very similar to that detected in soybeans. *Aspergillus flavus, A. niger, A. ruber* and *A. chevalieri* were the most common Aspergilli, and *P. citrinum* the most common *Penicillium* species. *P. olsonii* was present in some samples from Thailand and Indonesia (Pitt et al. 1994, 1998). Barua et al. (2007) reported *A. flavus, A. niger, Fusarium oxysporum*, *F. verticillioides*, *F. semitectum* and *Penicillium* spp. in stored mung beans in Bangladesh.

The storage fungi of kidney beans (*Phaseolus vulgaris*) in India included *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Fusarium, Penicillium, Rhizoctonia, Stemphylium* and *Trichoderma* (Sud et al. 2005). A comparison of the mycobiota of similar beans from Canada and Taiwan found that *Aspergillus* and *Penicillium* spp. were most common in Taiwanese beans, but in Canadian beans, the most prevalent fungi were *Alternaria, Fusarium* and *Rhizoctonia* (Tseng et al. 1995). On velvet beans, dominant fungi were *A. ruber, A. montevidensis, A. flavus, A. niger, Fusarium, Rhizopus, Mucor* and *Cladosporium* spp. and yeasts (Bhat et al. 2007).

Seeds of melon and sesame collected in markets in Nigeria in wet and dry seasons contained *Aspergillus* sections *Circumdati, Flavi* and *Nigri, Cladosporium, Fusarium fujikuroi* species group, *Penicillium* and Pleosporales/Didymellaceae (Esan et al. 2020). Aflatoxins, sterigmatocystin and citrinin were the most prevalent mycotoxins reported (Esan et al. 2020).

Aspergillus flavus was found to dominate the mycobiota of Indian chickpeas after harvest (Singh and Ahmad 1989), and storage fungi comprised mainly *Aspergillus flavus, A. niger, A. ochraceus, Emericella nidulans* and *Penicillium* species (Ahmad and Singh 1991). In Australian chick peas, Sarantinos et al. (1996) found that the dominant fungi were *Stemphylium botryosum* and *Botrytis cinerea*, followed by *A. flavus, Alternaria alternata, A. chevalieri* and *A. montevidensis*.

12.1.7 Nuts: General Considerations

As noted in the previous chapter, some nuts, such as macadamias, are well protected by a heavy shell during development and rarely suffer from mould invasion; others such as peanuts may be invaded preharvest by a wide range of fungi.

Dried nuts are very susceptible to spoilage because their soluble carbohydrate content is low, so any increase in moisture content causes an appreciable rise in a_w. Such a rise can readily be caused by moisture movement due to uneven storage temperatures, as may happen in shipping containers. Also if refrigerated storage, widely used to retard the development of rancidity, is not efficiently dehumidified, increases in moisture can rapidly result. If moisture does increase marginally, spoilage will result from growth of xerophilic *Aspergillus* species. Nuts shipped in containers across the tropics can become a total loss from moisture movement due to unsuitable stowage, for example on deck or near engine rooms. Under these conditions we have seen ram-

pant growth of *Aspergillus flavus* and very high aflatoxin levels, resulting in the complete loss of container loads of peanuts. We have also examined samples from a container load of hazelnuts lost to *A. pseudoglaucus.*

12.1.8 Peanuts

The most significant papers on the mycobiota of stored peanuts are those by Joffe (1969), McDonald (1970), and Pitt et al. (1993). During a 5 year period, Joffe (1969) isolated fungi from freshly harvested peanuts and samples stored for up to 6 months. Over 400 samples of stored peanuts were examined. By far the most common species encountered was *Aspergillus niger,* isolated from a low of 8.4% of kernels in 1 year to a high of 71%. The other dominant members of the biota were *A. flavus* (0.2–8.4%); *Talaromyces funiculosus* (2.6–16.2%); *T. purpureogenus* (1.6–7.8%); and *Neocosmopora solani* (0–9.1%). From our observations on Australian peanuts, and those of Joffe (1972) on peanuts in Israel, *A. niger* is a competitor of *A. flavus,* and the relative abundance of these two species is dependent on poorly understood climatic and agricultural factors.

In Thai peanuts, Pitt et al. (1993) found that after *Aspergillus flavus* (present in 95% of 109 samples), the most frequently isolated species was *A. niger* (in 86% of samples, at up to 100% infection rate within a sample). Other Aspergilli were also common: *A. tamarii* in 31% of samples, *A. wentii* (20%) and *A. candidus* (4%). *Aspergillus* subgen. *Aspergillus* species were an important component of the storage mycobiota, with *A. ruber* most common (in 51% of samples), followed by *A. chevalieri* (46%), *A. montevidensis* (9%) and *A. pseudoglaucus* (6%). *Wallemia sebi* was present in 12% of samples, with an infection rate of 98% observed in one sample. The most common *Penicillium* or *Talaromyces* species was *P. citrinum* (in 46% of samples, at infection rates up to 60% within a sample), with *T. funiculosus,* (14% of samples, up to 92% infection within a sample), *P. olsonii* (in 8% of samples), *T. pinophilus* (4%), *P. glabrum* and *P. janthinellum* (3% each). Other Aspergilli and Penicillia were encountered at lower frequencies (Pitt et al. 1993).

In peanuts from Indonesia (256 samples) and the Philippines (132 samples), a similar range of fungi was encountered. The most common Aspergilli were *A. flavus* (present in 98% of Indonesian and 97% of Philippine samples), *A. niger* (80% and 90% respectively) and *A. tamarii* (38% and 19%). The most common xerophilic *Aspergillus* species were *A. ruber* and *A. chevalieri,* and of the Penicillia, *P. citrinum* was most common, present in 55% of Indonesian and 41% of Philippine peanuts, followed by *T. funiculosus* (4% and 12% respectively) (Pitt et al. 1998).

In an extensive study of peanuts from Uganda and Kenya, Ismail (2001) reported that the most frequently isolated species was *A. niger,* followed by *A. flavus*, *Macrophomina phaseolina* (Tassi) Goid. and the common xerophilic *Aspergillus* species. *Rhizopus stolonifer, A. parasiticus, Neocosmospora solani, Lasiodiplodia theobromae* and *Penicillium chrysogenum* were also common. Ihejirika et al. (2005) identified *A. flavus, A. niger* and *Emericella versicolor* as the major fungi responsible for storage rots of peanuts in Nigeria. Other studies have reported a similar range of fungi in stored peanuts (Oluma and Nwankiti 2003; Gachomo et al. 2004; Mphande et al. 2004). Recent studies on the mycobiota of peanuts have concentrated on the presence of mycotoxigenic fungi, including those capable of producing ochratoxin A (Magnoli et al. 2006, b, 2007a, b; Nakai et al. 2008).

The great majority of the fungi present in peanuts are not capable of causing spoilage due to visible growth, but may cause discolouration, which from both the processor's and consumers' points of view is a type of spoilage. At least in developed countries, discoloured peanuts are sorted out by colour sorting machines. Colour sorting, introduced to eliminate discoloured nuts, has proved to be an effective way of removing nuts which contain aflatoxins also. Reject nuts may be used in the manufacture of peanut oil, where refining processes remove both fungi and aflatoxins. Xerophilic *Aspergillus* species sometimes cause pitting and erosion in peanuts, rather

than discolouration, but the conditions responsible have not been established (our observations).

12.1.9 Hazelnuts, Walnuts, Pecans and Almonds

Several studies on fungi in hazelnuts and walnuts have been published (Senser 1979; Abdel-Hafez and Saber 1993; Sahin and Kalyoncuoglu 1994) and on pecans (Schindler et al. 1974; Huang and Hanlin 1975; Wells and Payne 1976; Wells 1980). Senser (1979) isolated 33 fungal species from 149 samples of hazelnuts; the most commonly occurring were *Rhizopus stolonifer* and *Penicillium aurantiogriseum.* In Egyptian hazelnuts and walnuts, Abdel-Hafez and Saber (1993) detected 51 fungal species: the most commonly occurring species were *Aspergillus flavus, Neosartorya fumigata, A. niger, Cladosporium cladosporioides, C. herbarum, Penicillium chrysogenum, P. citrinum* and *P. oxalicum. Fusarium* species (*F. equiseti, F. moniliforme* (= *F. verticillioides*) and *F. oxysporum*) were recovered from walnuts at a moderate frequency. Xerophilic *Aspergillus* species were commonly isolated when a reduced a_w medium was used. Aflatoxins were detected in 90% of hazelnut samples, at levels of 25–175 μg/kg, and in 75% of walnut samples, with similar levels of aflatoxin. Zearalenone was detected in one walnut sample at a level of 125 μg/kg. In Turkish hazelnuts, *Penicillium* and *Aspergillus* species dominated the mycobiota, but some Zygomycetes (*Rhizopus stolonifer, Lichtheimia corymbifera* and *Syncephalastrum racemosum* were also common (Sahin and Kalyoncuoglu 1994). Potentially aflatoxigenic fungi were detected in 47–79% of Turkish hazelnuts at harvest. Of the 5564 strains isolated, 89% were *A. flavus* and 11% were *A. parasiticus.* It is notable that aflatoxins were only detected in nuts that had been in contact with the ground during drying (Ozay et al. 2008).

In walnuts in the USA, Bayman et al. (2002) reported that the most commonly occurring fungi were *Aspergillus niger, Rhizopus* spp. and *Penicillium* spp., with *A. flavus* uncommon in surface disinfected nuts. In Indian walnuts, Singh and Shukla (2008) found highest levels of fungi in nuts stored during the rainy season (July–October). *Aspergillus flavus* was present in 64% of kernels, with *Penicillium citrinum* in 32%, and *A. niger* and *Rhizopus* present in 26% of kernels. *Chaetomium globosum, Alternaria alternata, Cladosporium herbarum, Syncephalastrum racemosum* and *Trichothecium roseum* were detected in 2–5% of kernels stored in both summer (April–June) and the rainy season, but were rare in the nuts stored over the winter months (November–February) (Singh and Shukla 2008). Molyneux et al. (2007) reported that phenolic compounds present in walnuts, particularly tannic acid, reduced the probability of aflatoxin contamination. Mouldy walnuts were reported to have caused a tremorgenic mycotoxicosis in a dog in New Zealand. *Penicillium crustosum* was identified from mouldy walnuts collected from the ground where the dog had been, and penitrem A and roquefortine were isolated from the nuts (Munday et al. 2008). Mouldy walnuts from north-western China contained species of *Fusarium, Alternaria, Penicillium, Aspergillus, Mucor, Trichoderma* and *Cladosporium.* Only a limited number of isolates were identified to species (Wei et al. 2020).

From 37 samples of pecans, Huang and Hanlin (1975) isolated 119 species from 44 genera. As in other studies on stored dried foods, *Aspergillus*, especially xerophilic species, and *Penicillium* were dominant. *Aspergillus* species, excluding those in *Aspergillus* subgen. *Aspergillus*, accounted for 48% of the more than 1300 isolates obtained; next came *Penicillium* (19%), *Aspergillus* subgen. *Aspergillus* (18%) and *Rhizopus* (8%). The dominant species were *A. niger* and *A. flavus,* followed by *A. pseudoglaucus, A. ruber, A. parasiticus, Rhizopus oryzae* and *Penicillium expansum.* Garcia et al. (2019b) examined 52 samples of freshly harvested Brazilian pecans from Rio Grande do Sul State. *Aspergillus* was the most common genus. Xerophilic species were dominant: *A.wentii, A. ruber, A. pseudoglaucus* and *A. chevalieri.* No aflatoxigenic fungi were isolated. *Penicillium* spp. were also present in high numbers, with *P. paxilli, P. citrinum* and *P. olsonii* dominant.

Fusarium was the third most prevalent genus, followed by *Cladosporium.* Other genera reported were *Verticillium, Nigrospora, Rhizopus, Alternaria, Colletotrichum* and *Trichoderma.*

Wells and Payne (1976) obtained an unusual distribution of genera from pecans which had been invaded by weevils in the field. Nearly half of 2300 isolates from several hundred mouldy nuts were *Alternaria* or *Epicoccum* species. *Penicillium* species made up 25% of the total, and *Aspergillus* only 1.0%. In pecans taken from drying bins 4 months after harvest, Wells (1980) found that the mycobiota was dominated by *Cladosporium* (39% of isolates), *Penicillium* (21%), *Alternaria* and *Epicoccum* species (9% each).

Aspergillus niger and *Penicillium glabrum* were the most prevalent of 12 genera and 51 species identified during a survey of the mycobiota of almonds during harvest and storage (King and Schade 1986). Species isolated after 1 month of storage at 25 °C were xerophilic *Aspergillus* species, *A. flavus, A. wentii, P. implicatum* and *Talaromyces rugulosus. Rhizopus stolonifer* and *R. oryzae,* which were common on freshly harvested nuts, persisted during storage. In almonds collected in the field, Bayman et al. (2002) found that *Penicillium* spp. were the most commonly occurring fungi, followed by *A. niger, A. ochraceus* and *Rhizopus* spp., whereas in retail almonds, the dominant fungi were *Rhizopus* spp., then *A. niger* and *A. flavus*.

Kenjo et al. (2007) examined 30 samples of almond powder imported into Japan, the majority from the USA. They reported that the dominant fungi were *A. niger, A. flavus* and related species, *Penicillium, Cladosporium* and *Rhizopus*. *A. parasiticus* and *A. nomius* were also isolated.

12.1.10 Pistachio Nuts

The storage mycobiota of pistachios appears to comprise mainly *Aspergillus* and related species, although, as observed in Chap. 11, this reporting may be biased by the predisposition of this commodity to aflatoxin contamination, rather than the true composition of the storage mycobiota. In Iranian pistachios, *A. niger, A. flavus* and *Neosartorya spinosa* were most prevalent, followed by *A. terreus, A. tamarii* and *Emericella nidulans* (Mojtahedi et al. 1979). *Penicillium* species were also common, but the species detected were not specified. In pistachios from California, *A. niger, A. flavus, A. parasiticus, A. ochraceus* and *A. melleus* Yukawa were reported (Doster and Michailides 1994). Bayman et al. (2002) found that *A. niger* was dominant in field collected and retail Californian pistachios, with *Emericella nidulans, A. ochraceus, A. melleus, Neosartorya fumigata, A. tamarii* and *A. flavus* also present. *Penicillium* and *Rhizopus* spp. were also common. The mycobiota of stored Turkish pistachios was dominated by *Aspergillus, Penicillium, Cladosporium* and *Rhizopus* species, with *Ulocladium, Trichothecium, Aureobasidium* and *Aspergillus* subgen. *Aspergillus* species present in lower numbers (Heperkan et al. 1994). In 50 samples of retail pistachios purchased in Spain, the most commonly encountered species reported by Fernane et al. (2010) were *Aspergillus* Section *Nigri, A. flavus* and *Penicillium* spp., with *P. verrucosum* the only named *Penicillium* species. Xerophilic *Aspergillus* spp. and *N. fumigata* were also present, as were a number of other genera in low numbers. Aflatoxins above the legal limit were detected in 5 samples, with one sample exceeding 1100 µg/kg.

12.1.11 Brazil Nuts

Brazil nuts are harvested from the Amazon rainforest. Many studies have been published on the presence of fungi and mycotoxins, particularly aflatoxins. From four samples of brazil nuts, the most common fungi reported by Freire et al. (2000) were *Aspergillus flavus, Penicillium glabrum, Cunninghamella elegans, P. citrinum, Rhizopus oryzae, A. niger* and *Fusarium oxysporum*. Bayman et al. (2002) [reported that *Rhizopus* was the most prevalent fungus in the brazil nuts they purchased in California. *A. flavus* was the next most common, followed by *Penicillium* spp., *Aspergillus tamari, A. niger, E. nidulans* and

N. fumigata. Olsen et al. (2008) isolated *A. nomius* as well as *A. flavus* from brazil nuts and suggested that *A. nomius* may be an important source of aflatoxin. Reis et al. (2012) assessed fungi and mycotoxins in 200 brazil nut samples and 200 samples of husk collected in four Amazon regions. The genera detected in the nuts were, in order of decreasing frequency, *Phialomonium* W. Gams & McGinnis*, Penicillium, Fusarium, Phaeoacremonium* W. Gams et al. and *Aspergillus.* The 254 *Aspergillus* isolates were identified to species, with *A. nomius* dominant, followed by *A. flavus*, with a lower number of *A. parasiticus* isolates. All *A. nomius* and *A. parasiticus* isolates produced B and G aflatoxins, and 52% of *A. flavus* isolates produced B aflatoxins. Aflatoxins and cyclopiazonic acid were detected in some nut and husk samples. Baquião et al. (2013), who examined 55 samples of brazil nuts and 55 samples of shell, reported the common occurrence of *A. flavus*, along with *A. nomius* and *A. parasiticus.* Other fungi (*Fusarium, Penicillium, Cladosporium* and *Acremonium*) were also commonly isolated but were not identified to species level. Despite high aflatoxin production by some of the isolated fungi, aflatoxins were not detected in the nut samples.

A comprehensive assessment of the biodiversity of fungi throughout the brazil nut supply chain has been published, with emphasis on fungi potentially producing aflatoxins (Olsen et al. 2008; Gonçalves et al. 2012; Calderari et al. 2013; Massi et al. 2014; Taniwaki et al. 2017). Overall, *Aspergillus nomius* was the most prevalent species and all isolates were able to produce both B and G aflatoxins. *A. flavus* was also common, with about 40% able to produce B aflatoxins. Smaller numbers of other potentially aflatoxigenic species, *A. arachidicola, A. bombycis, A. pseudocaelatus* and *A. pseudotamarii,* were also isolated, along with the closely related species *A. caelatus* and *A. tamarii*, which did not produce aflatoxin. Highest fungal infection levels were detected in nuts from the rainforest and lowest in nuts from supermarkets.

A high diversity of other *Aspergillus* and *Penicillium* species, as well as Zygomycetes and dematiaceous fungi were also documented by Taniwaki et al. (2017). Important toxigenic species in *Aspergillus* section *Flavi* and section *Nigri* were distinguished. Ochratoxin A production was found in 3% of *A. niger* and 100% of *A. carbonarius* isolates. Cyclopiazonic acid was found to be produced by *A. bertholletius* (94%), *A. tamarii* (100%), *A. caelatus* (54%) and *A. flavus* (41%). Tenuazonic acid production was detected in several *Aspergillus* species. *Penicillium* species were also prevalent, with the most common being *P. citrinum* and *P. excelsum* Taniwaki et al. Other genera encountered included *Paecilomyces, Absidia, Mucor, Rhizopus, Syncephalastrum, Cladosporium, Trichoderma* and *Fusarium*. Dematiaceous hyphomycetes and yeasts were commonly encountered but not further identified (Taniwaki et al. 2017).

12.1.12 Other Nuts

Storage fungi encountered in 45 samples of cashews from Thailand included several *Aspergillus* species: *A. flavus* (in 60% of samples), *A. niger* (53%), *A. chevalieri* (40%), *A. ruber* (31%) and *A. montevidensis* (16%), *A. wentii* (9%) and *A. tamarii* (7%), as well as *Emericella sydowii* (11%). Some *Penicillium* species were also common: *P. citrinum* (in 29% of samples), *P. olsonii* (7%), *P. implicatum* and *P. solitum* (both in 4% of samples). *Wallemia sebi* was detected in 9% of samples (Pitt et al. 1993). Adebajo and Diyaolu (2003) reported *A. niger*, *A. flavus*, *A. restrictus* and *Neosartorya fumigata* from retail cashews in Nigeria. From Brazilian cashews, *A. niger* was the most frequently isolated species, followed by *A. flavus*. *Penicillium brevicompactum* and *P. glabrum* were the most common Penicillia, and *Chaetomium globosum* was also recorded at a high level (Freire et al. 1999).

In Indonesian kemiri nuts (19 samples), the storage mycobiota was dominated by the same range of fungi encountered in other tropical stored commodities: *Aspergillus flavus, A. niger, A. wentii, A. tamarii, A. ruber, A. chevalieri* and *Penicillium citrinum.* Two less common species,

P. aethiopicum and *P. allii,* were detected in 16% and 10% of samples respectively, comprising a significant proportion of the total mycobiota (Pitt et al. 1998)

Overy et al. (2003) isolated fungi from chestnuts collected from seven Canadian provinces. The mycobiota of the chestnuts was dominated by *Penicillium* spp., the most frequently isolated being *P. crustosum* (76%), *P. glabrum/spinulosum* (19%) and *P. discolor* (18%). Aspergilli were rarely encountered, the most common being *A. ochraceus* (2%) (Overy et al. 2003).

12.1.13 Coconut

A wide range of fungi can be found on copra, as detailed in Chap. 11. Desiccated coconut is produced from copra, and is also subject to fungal spoilage. Zohri and Saber (1993) investigated the mycobiota of coconut and reported a wide range of fungi, including *Aspergillus flavus, A. niger, A. chevalieri, Emericella sydowii, Penicillium chrysogenum, P. oxalicum, Cladosporium cladosporioides* and *Alternaria alternata.* Aflatoxin B_1 was detected in 5 of 25 samples (15–25 µg/kg) and 3 samples were contaminated with ochratoxin A (50–205 µg/kg). Kinderlerer (1984b) isolated 28 species from coconut. Xerophilic *Aspergillus* species were the principle group, but *Penicillium, Cladosporium, Aspergillus flavus* and *A. niger* were also common. *Bettsia alvei* was also isolated. Ismail (2001) reported over 60 different fungal species from desiccated coconut samples collected in Kenyan and Ugandan markets. The mycobiota was dominated by *A. niger, A. flavus* and *P. chrysogenum*. On DRBC, *A. ochraceus, Penicillium waksmanii, Paecilomyces variotii* (= *Byssochlamys spectabilis*), *Talaromyces islandicus* and *Rhodotorula mucilaginosa* were common, whereas on DG18, species of *Cladosporium* and xerophilic *Aspergillius* species were more frequent. *Xerochrysium fastidium*, *X. dermatitidis* and *B. alvei* were also detected (Ismail 2001).

Two distinct types of spoilage in coconut due to the action of xerophilic fungi have been reported: ketonic rancidity due to production of aliphatic methyl ketones and secondary alcohols by *Aspergillus* subgen. *Aspergillus* species, and cheesy butyric spoilage brought about by growth and metabolism of *Bettsia alvei* (Kinderlerer 1984a; Kinderlerer and Kellard 1984). Fermentation of coconut by *Xerochrysium xerophilum*) resulted in the production, after 9 months, of a complex mixture of aliphatic methyl ketones, esters, secondary alcohols and medium chain length free fatty acids (Kinderlerer et al. 1988).

12.1.14 Spices

Because of their tropical origin, and the methods used in their production, spices are frequently heavily contaminated with xerophilic fungi. Counts on enumeration media may be very high, figures up to 10^9/g having been recorded in our laboratory (Hocking 1981). *Aspergillus,* including xerophilic species, and *Penicillium* species are often the dominant flora of dried, whole or ground spices (see Pitt and Hocking 1997), with *A. halophilicus* M. Chr. et al., a rare species, reported from cardamom seeds (Hocking and Pitt 1988). Pepper is often cited as a source of fungal contamination for foods, and may carry high fungal loads of up to 10^7 cfu/g (King et al. 1981; Muhamad et al. 1986; Atanda et al. 1990). The most common species in 42 samples of black peppercorns from the Philippines were *A. flavus* (present in 95% of samples), *A. niger* (76%), *A. ruber* (74%), *Chaetomium globosum* (45%), *A. chevalieri* (38%) and *Emericella sydowii* (31%). Overall, 60 species of fungi from 24 genera were isolated from the peppercorns, including 16 species of *Aspergillus* and 9 species of *Penicillium*. Freire et al. (2000) reported 42 species of fungi from Brazilian black and white pepper. *Aspergillus flavus* was the dominant species, followed by *A. niger, Chaetomium globosum, Penicillium brevicompactum, Emericella nidulans,* xerophilic *Aspergillus* species and *P. glabrum*. Gatti et al. (2003) reported that xerophilic *Aspergillus* species [*A. chevalieri, A. ruber* and

A. montevidensis] were the most common fungi in 115 samples of Brazilian black pepper. *Aspergillus flavus* and *A. niger* were the next most common. *Aspergillus tamari, A. carbonarius, A. restrictus, A. ochraceus, A. parasiticus* and *Neosartorya fumigata* were also isolated. Other genera included *Rhizopus, Penicillium, Curvularia, Cladosporium, Emericella* and *Paecilomyces*. Despite the prevalence of potentially mycotoxigenic fungi, no aflatoxins or ochratoxin A were detected in the pepper samples (Gatti et al. 2003).

It is difficult to know what constitutes spoilage of a spice. So little is eaten that slight off flavours may be irrelevant, Some concern has been expressed about the potential for mycotoxin contamination of foods from spices (Llewellyn et al. 1992), but the levels of aflatoxin contamination reported (40–160 µg/kg) are not high enough to represent a real health threat, given the dilution factors involved. The important point for food use is that a mouldy spice may contaminate other ingredients, and hence the final product. Where a heat process is not used, such as in processed meat manufacture, the spices should be sterilised before use (Hadlok 1969). Gamma irradiation of spices is effective in reducing microbial contamination, and may be used in some countries (Muhamad et al. 1986; Llewellyn et al. 1992).

12.1.15 Coffee Beans

The possibility of ochratoxin A contamination in coffee beans has been of concern for many years (Levi et al. 1974; Tsubouchi et al. 1984; Micco et al. 1989; Studer-Rohr et al. 1995). Most recent studies on the mycobiota of green coffee beans have concentrated on occurrence of potentially ochratoxigenic fungi. The revelation that *Aspergillus westerdijkiae,* and not *A. ochraceus,* is the main producer of ochratoxin in *Aspergillus* section *Circumdati* (Frisvad et al. 2004) has caused uncertainty about the identity of isolates from coffee reported as *A. ochraceus,* as many of these may actually have been *A. westerdijkiae.* Morello et al. (2007) published a method for detection and quantification of *A. westerdijkiae* in coffee beans, based on selective amplification of the ß-tubulin gene by real-time PCR.

Mislivec et al. (1983) reported that *Aspergillus* species dominated the mycobiota of 944 coffee bean samples, and *A. ochraceus* was the most common toxigenic species isolated. In an investigation of the source of ochratoxin A in Brazilian coffee beans, Taniwaki et al. (2003) found that the mycobiota was dominated by *Aspergillus niger* (63% of isolates), with *A. ochraceus* also common (31% of isolates), and *A. carbonarius* also detected (6%). Most of the *A niger* isolates (93%) were nontoxigenic, however most of the *A. ochraceus* (75%) and *A carbonarius* isolates (77%) produced ochratoxin A when grown in pure culture. These results were similar to those reported by Urbano et al. (2001), who found that although *A. ochraceus* represented only 10% of isolates from green coffee, 88% of them were capable of producing ochratoxin. Martins et al. (2003) examined Brazilian coffee beans and reported the dominant fungal genus was *Aspergillus*, including *A. niger* (83% of isolates), *A. ochraceus* (53%) and *A. flavus* (25%). The occurrence of *Cladosporium* (17%) and *Penicillium* (10%) was substantially lower than *Aspergillus.* Taniwaki et al. (2003) concluded that *A. ochraceus* (possibly = *A. westerdijkiae*) and *A. carbonarius* were responsible for most of the ochratoxin A in coffee, and that better harvesting, drying and processing could minimise mycotoxin contamination. Batista et al. (2003) also found that species from *Aspergillus* section *Circumdati* were the most prevalent in Brazilian coffee beans, and that 75% of these isolates produced ochratoxin A.

In coffee beans from Vietnam, Ilic et al. (2007) did not detect *A. ochraceus* or *A. carbonarius,* but found that 93% of samples contained *A. niger,* of which 8.7% produce ochratoxin. Leong et al. (2007) examined Robusta (65 samples) and Arabica (11 samples) beans from southern and central Vietnam, and found that Robusta beans were much more heavily infected than Arabica beans. They reported that *Aspergillus niger* infected 89% of Robusta beans, whereas *A. carbonarius* and *Aspergillus* section *Circumdati* isolates each infected 12–14% of beans. Ochratoxin

was not produced by *A. niger* (98 isolates) nor *A. ochraceus* (77 isolates), but was detected in 110 of 113 isolates of *A. carbonarius*, 10 isolates of *A. westerdijkiae* and one isolate of *A. steynii*. These results indicate that species from *Aspergillus* section *Nigri* may be largely responsible of ochratoxin contamination of coffee beans in Vietnam.

Ochratoxin A and toxigenic fungi were examined in Ethiopian coffee beans by Geremew et al. (2016). *Aspergillus* (79%), *Fusarium* (8%) and *Penicillium* (5%) were the dominant toxigenic genera. *Aspergillus ochraceus, A. westerdijkiae* and *A. steynii* were all present, with *A. ochraceus* being most common, but *A. westerdijkiae* isolates produced by far the highest levels of ochratoxin A.

In India, coffee may undergo a process known as monsooning, a solid-state fermentation, resulting in flavour changes that impart characteristic aroma, flavour and cup quality. Tharappan and Ahmed (2006) followed mycological changes over 9 weeks of monsooning. The highest fungal populations occurred between 4 and 7 weeks, and comprised mainly *A. niger, A. ochraceus, A. tamari, A. candidus*, *Emericella versicolor*, *Penicillium chermesinum*, *Talaromyces rugulosus* and *Absidia heterospora*. *Wallemia sebi* was also detected in Arabica beans between 3 and 7 weeks fermentation. Taniwaki et al. (2005) reported distinct flavour changes in green coffee beans, some of which were positive and some negative, depending on the fungi present. The most common fungi isolated from green coffee beans which still produced a good, clean beverage were *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., black moulds and yeasts. The most common fungi isolated from coffee beans which had a wood and 'Rio' taste were *Aspergillus niger*, *A. ochraceus*, xerophilic *Aspergillus* species, *Fusarium* species and dematiaceous fungi. In a further study on Brazilian coffee beans, Iamanaka et al. (2014) examined fungal species in mature and immature cherries, dry floaters, cherries from the ground and stored beans. They reported the most common isolates were *Penicillium brevicompactum*, a new *Penicillium* species related to *P. crustosum*, species from *Aspergillus* section *Nigri, A. westerdijkiae* and *Fusarium lateritium*. In some cases, fungal contamination appeared to enhance flavour, but a high percentage of *Aspergillus* species produced a negative impact.

Severe fungal infection of coffee beans can lead to a defect known as "Rio flavour", which is caused by the formation in the beans of trichloroanisoles by fungal metabolism (Liardon et al. 1992). A number of species of *Aspergillus, Penicillium,* and also *Paecilomyces variotii* (= *Byssochlamys spectabilis*) have been shown to be capable of methylation of chlorophenols to chloroanisoles (Hill et al. 1995).

12.1.16 Cocoa Beans

Cocoa beans are subject to a microbial fermentation as the first stage of chocolate production. The first 2–3 days of fermentation are characterised by a succession of filamentous fungi, yeasts, lactic acid bacteria and acetic acid bacteria, with the latter stages of the fermentation dominated by *Bacillus* species (Ardhana and Fleet 2003; Schwan and Wheals 2003; Nielsen et al. 2007). Filamentous fungi may develop during the fermentation, or later during storage of the beans, leading to deleterious changes. Guehi et al. (2007) examined cocoa beans from Ivory Coast with high free fatty acid content. They isolated *Lichtheimia corymbifera*, *Rhizopus oryzae* and *Penicillium chrysogenum* from 80% of the beans, while *Aspergillus niger, A. flavus* and *A. tamarii* were present in 40–50% of samples. Dharmaputra et al. (1999) reported *A. flavus, A. montevidensis*, *A. chevalieri* and *Penicillium citrinum* from insect damaged cocoa beans in Indonesia. Thermophilic fungi (*Neosartorya fumigata, Rhizomucor pusillus* and *Thermoascus aurantiacus* Miehe) were isolated from fermenting cocoa beans with a temperature of 45–50 °C (Ogundero 1983).

The effect of various stages of cocoa production on fungal growth and toxin formation in Brazilian cocoa beans was investigated by Copetti et al. (2010, 2011b). Relevant to the formation of ochratoxins, ochratoxigenic fungi were most prevalent in stored cocoa beans, with *Aspergillus carbonarius* and species of the *A.*

niger aggregate commonly encountered, though *A. ochraceus, A. melleus* and *A. westerdijkiae* were also isolated. Ochratoxin A levels were highest in stored beans, though the majority of samples were less than 2 μg/kg (Copetti et al. 2010). Relevant to aflatoxins, the most commonly occurring aflatoxigenic species were *A. flavus* and *A. parasiticus,* with a few isolates of *A. nomius.* Despite high fungal numbers, particularly during storage, only low levels of aflatoxins were found in the cocoa bean samples (Copetti et al. 2011b). Copetti et al. (2011a) examined the overall mycobiota of cocoa from farm to finished product. They reported that the largest numbers and diversity of fungi occurred during drying and storage on the farm, with the most commonly encountered species being *Lichtheimia corymbifera*, a novel *Aspergillus* species, *A. flavus, Penicillium paneum* Frisvad and yeasts. Potentially mycotoxigenic *Aspergillus* species were also present, but in lesser numbers (Copetti et al. 2011a). Akinfala et al. (2020) looked at secondary metabolite production during cocoa bean fermentation and storage, and reported the presence of six genera (*Aspergillus, Paecilomyces* (= *Byssochlamys*), *Talaromyces, Penicillium, Pseudopithomyces* Ariyaw. & K.D. Hyde and *Simplicillium* W. Gams & Zare, but species were not identified.

12.1.17 Dried Meat

Van der Riet (1976) studied the mycobiota of 20 samples of South African biltong. Xerophilic *Aspergillus* species were dominant, followed by other *Aspergillus* and *Penicillium* species. A surprising number of other filamentous fungi were also isolated. Van der Riet (1976) reported that yeasts were also isolated from all of the samples and at least some were identified as lipolytic species. None were known xerophiles, and presumably all had grown during the drying period. If drying were prolonged, rancid spoilage might be expected. As the samples were not surface disinfected, it is a matter of conjecture which of the filamentous fungi had grown in the meat during drying and which were merely aerial contaminants. In samples of dried beef jerky and kangaroo biltong examined in our laboratory, again xerophilic *Aspergillus* species were the most common spoilage fungi, but *Aspergillus candidus* and *Debaryomyces hansenii* were also isolated. As with other dried foods, the shelf life of such products is dictated by their water activity, but in this case fat rancidity is an added factor, where spoilage may be induced by yeast or filamentous fungal growth during drying and may continue in storage.

12.2 Low Water Activity Foods: Concentrated Foods

Concentrated foods are defined here as including both evaporated products and those to which sugars have been added. The list includes jams, dried fruit, fruit cakes, confectionery and fruit concentrates. Such foods are as susceptible to spoilage by the common xerophiles as are dried foods, and in addition provide ideal habitats for two of the most xerophilic fungi known – *Xeromyces bisporus* and *Zygosaccharomyces rouxii.* As noted earlier in this chapter, the important point about these two fungi is that processing and handling systems must positively exclude them from concentrated foods, as commercial product cannot be manufactured of an a_w sufficiently low to prevent them from growing.

12.2.1 Jams

Traditional jams and conserves, made almost entirely from fruit and sucrose, are boiled or evaporated down to 0.75 a_w or below and hot filled into jars before closing. Consequently they very rarely spoil. The answer to any spoilage problem with a traditional type of jam must rely on that basic premise: to produce jams which will not spoil, the water content of the product must be reduced to a safe a_w. However, commercially prepared products are made by a much reduced cooking process and are heated for a shorter time, and their a_w is often closer to 0.80–0.82. At this a_w they are much more likely to support mould

growth, and mould-free shelf life depends on hot-filling into jars with a reliable, airtight seal. Jams which have much lower levels of added sugars and consequently much higher water activities are manufactured for diabetics. Such products are usually stabilised by preservatives, but the precise recipes permitting stable products have rarely been published.

If jams spoil, *Aspergillus* subgen. *Aspergillus* species are usually responsible, although we have seen xerophilic Penicillia, especially *P. coryloph-ilum,* from time to time. Because jams are hot filled, *Zygosaccharomyces rouxii* is unlikely to be a problem, but we have seen the occasional jar of jam bubbling over from an infection with that yeast, presumably from contamination after opening. The black yeast-like fungus *Trichosporonoides nigrescens* A.D. Hocking & Pitt (= *Moniliella nigrescens* (A.D. Hocking & Pitt) C.A. Rosa & Lachance) has also been reported from spoiled, fermenting jam (Hocking and Pitt 1981).

12.2.2 Dried Fruit

Some fruit, including apricots, peaches, pears and bananas, are dried after preservation with SO_2, which is essential to prevent browning from the Maillard reaction. The high levels of SO_2 also completely eliminate the microflora, even during prolonged storage. If the SO_2 levels decline to less than 0.1 g/kg during storage, these products may be spoiled by the xerophilic yeast *Zygosaccharomyces rouxii,* which is slightly resistant to preservatives, and xerophilic fungi such as *Xeromyces bisporus* and *Aspergillus* subgen. *Aspergillus* species.

Dried prunes, and most dried vine fruits, however, are not processed with SO_2, and are susceptible to spoilage by xerophiles. Pitt and Christian (1968) reported the isolation of nearly every known xerophilic fungus from Australian dried and high moisture prunes, which at that time relied on hot filling for microbial stability. The most common fungi isolated were *Aspergillus* subgen. *Aspergillus* species, especially *A. glaucus, Xeromyces bisporus,* and xerophilic *Chrysosporium* (= *Bettsia* or *Xerochrysium*) species. Prunes are still a rich source of xerophiles: the rare xerophile, *Monascus eremophilus* A.D. Hocking & Pitt was isolated from Australian prunes (Hocking and Pitt 1988). Most countries now permit the addition of preservatives to high moisture prunes.

Australian vine fruits which are sun dried and not preserved with SO_2 are in our experience always contaminated with *Aspergillus niger* and related species, which undoubtedly grow to some extent during drying and are presumably highly resistant to the very strong sunlight in the growing areas in inland Australia. Other fungi, including *P. citrinum,* other *Penicillium* species, *Alternaria, Epicoccum, Trichoderma, Rhizopus, Cladosporium* and yeasts also occur but are much less common (King et al. 1981). We have observed spoilage of dried grapes (sultanas) by *Xeromyces bisporus* after prolonged storage (2 years).

The seed cavities of mature figs are always contaminated by yeasts (Miller and Phaff 1962; see Chap. 11). Spoilage of dried figs sometimes occurs if these contaminant yeasts include xerophilic species. *Aspergillus* species can occur in figs and may cause spoilage and form mycotoxins postharvest (Doster et al. 1996). Although *A. niger* and related species are most common, infection by members of *Aspergillus* section *Flavi* has more serious consequences, with aflatoxin production a significant problem in figs from some countries (Sharman et al. 1991; Doster et al. 1996). Aflatoxin contamination is much more likely if *A. parasiticus* rather than *A. flavus* is present. Ochratoxin A has been detected in figs naturally contaminated with *A. ochraceus* and related species (Doster et al. 1996).

Glace fruits are preserved by SO_2 which is added in the syrup with which the fruit are infused in a series of increasing concentrations. On several occasions we have seen samples of partially prepared glace pineapple spoiling from the yeast *Schizosaccharomyces pombe,* which apparently possesses a unique combination of resistance to SO_2 and tolerance to reduced a_w, enabling it to grow at a particular point in the infusion process.

Mixed dried fruits usually contain sultanas, currants, raisins, glace citrus peel and glace cherries. The high price of glace cherries has led to the substitution by imitation cherries made from gelatine and sugar. These have a higher a_w and usually contain a preservative such as sorbic or benzoic acid. If insufficient preservative is added, imitation cherries may act as a focus for infection by xerophilic fungi such as *Xeromyces bisporus.*

12.2.3 Fruit Cakes

Fruit cakes and similar puddings are concentrated foods because, as well as the fruit, the cake or pudding mix itself is high in sugar. Such cakes and puddings are expected to have quite a long shelf life, often 6 months, and therefore must be prepared and baked to give a final a_w of 0.75 or below. Under these conditions spoilage is not usually a problem. However we have seen very severe cases of spoilage in fruit cakes of 0.75 a_w caused by *Xeromyces bisporus.* In one instance, cakes showed patches of mould several centimetres across only a few weeks after manufacture, with mycelium penetrating deeply into the interior (Pitt and Hocking 1982). The number of cakes undergoing spoilage was large, indicating a systemic contamination of the cakes in the factory, and in all probability the survival of ascospores of *Xeromyces* through the baking process. Because of this, and the ability of *X. bisporus* to thrive at 0.75 a_w, fruit cakes cannot be made which will be resistant to spoilage by this fungus. Fortunately, *X. bisporus* is a rare species, and once eliminated from the factory by thorough cleaning, is unlikely to appear again. Tracing the source of *X. bisporus* contamination in the factory environment can be extremely difficult, if not impossible, as the more common xerophiles such as *Aspergillus* subgen. *Aspergillus* species are usually present also, and will overgrow *X. bisporus* on MY5OG isolation plates. The time between production date and visible spoilage can often be quite long – from as little as 6 weeks up to 9 months. This delay can add to the difficulty of tracing the contamination source.

Fruit cakes are also subject to spoilage by *Aspergillus* subgen. *Aspergillus* species and *Wallemia sebi.* Control relies on good hygienic practices in the manufacture and packaging of these products. Preservatives (sorbate or propionate) may be added to some higher a_w cakes such as light fruit cakes to extent shelf life, but the pH of these products (5.5–6.5) renders the preservatives only marginally effective.

12.2.4 Confectionery

Most confectionery including chocolates, jubes, gelatine confections and licorice have high sugar contents, and rely for stability on their low a_w. Formulations are usually traditional and are often prepared in small factories unaware of water activity and its implications, but usually well versed in the control of soluble solids by refractometry. Correctly made, such products are stable for long periods against normal xerophiles such as *Aspergillus* subgen. *Aspergillus* species, but are at risk from the extreme xerophiles.

For example, *Zygosaccharomyces rouxii* can cause spoilage of filled chocolates, such as caramel filled Easter eggs, chocolate coated marshmallow biscuits and strawberry cream chocolates (Hocking and Pitt 1996). An infection of this yeast in a chocolate filling line can be impossible to detect, but will provide low level contamination of the final product. Even a few cells will eventually grow, produce gas and cause spoilage by splitting the chocolate casing. The characteristic symptom of this kind of spoilage is wet wrappers due to leaking fillings. Microscopic examination is usually sufficient to confirm the presence of this yeast. The problem is readily cured by cleaning of the filling lines, but losses may be high.

De Clercq et al. (2015) examined the mycobiota of air and ingredients in Belgian chocolate confectionery factories for potential xerophilic spoilage fungi. The xerophilic spore load, as assessed by air sampling onto MY50G medium, was only 50 CFU/m^3. Penicillia were common and *P. brevicompactum* was the most prevalent species. Contamination of nuts by *A.*

pseudoglaucus was assessed as the most likely source of potential contamination. In Denmark, Marvig et al. (2014) surveyed the microbiota of chocolate pralines from nine manufacturers and sugar syrups used in their production. *Zygosaccharomyces rouxii* was the most commonly isolated yeast and *Aspergillus terreus* and *A. montevidensis* the most commonly isolated fungi. Isolates were assessed for their ability to grow at low a_w, low pH, in ethanol, sorbic acid and various temperatures. *Z. rouxii* was overall the most tolerant organism, followed by *A. montevidensis*.

Xerochrysium and *Bettsia* species have been isolated from a number of different types of confections in our laboratory. In one case, *X. dermatitidis* caused spoilage of a range of Australian gelatine confectioneries, of the type made in a dry starch mould. The a_w of the 30 tonne batch of starch in use in that particular factory was maintained at what was believed to be an acceptably low level, but was sufficiently high to permit growth of *X. dermatitidis.* This species differentiates into large numbers of aleurioconidia and arthroconidia, even at very low a_w. The confectionery was thus systematically contaminated by spores in the starch during manufacture, and rapidly spoiled despite having an a_w of 0.72. The diagnosis of the problem involved culture of both confectionery and starch on MY50G agar. The cure involved a long and very careful heat treatment of the starch, which eventually destroyed the fungus without generating an explosion in the starch.

Chocolate can also be spoiled by *Bettsia* species. We have isolated *Bettsia alvei* from spoiled hazelnut chocolate and this species has also been isolated from spoiled compounded chocolate in the UK (J. Kinderlerer, pers. comm.). Spoilage of an Argentinian caramel-jam filled chocolate confection by *A. chevalieri* has been reported (Larumbe et al. 1991).

Xeromyces bisporus was originally isolated from licorice in a CSIRO laboratory (Fraser 1953). We have seen this mould on that product on two occasions since. Licorice appears to be safe from spoilage by any other fungus. *X. bisporus* has also been isolated from spoiled gelatine confections in our laboratory.

12.2.5 Fruit Concentrates

Fruit juices are shipped around the world as concentrates, of 65 to 80° Brix, in 200 litre drums and one tonne pallecons. Pasteurised, evaporated and hot filled, such concentrates are of low pH and low a_w, and are as a rule microbiologically stable. The pasteurising step removes all but the most heat resistant fungal ascospores, i.e. *Byssochlamys* and *Neosartorya,* but these cannot grow at the reduced a_w of the product. Occasionally, however, the xerophilic yeast *Zygosaccharomyces rouxii* will enter the filling system downstream from the pasteuriser. *Z. rouxii* can grow and produce CO_2 down to 0.62 a_w, and so is capable of spoiling any liquid, concentrated food. Growing slowly in the lines, it will contaminate the product with sufficient cells to eventually cause the containers to become swollen, and even to explode. Spoilage is insidious, because the time to visible swelling may be many months. Losses can be very high. Concentrates undergoing spoilage can be recovered by dilution, pasteurisation and reconcentration, then refilling into drums through carefully cleaned lines. However, a useful short term expedient is to refrigerate the drums, which will stop growth and fermentation by the yeast, and permit use or reprocessing.

Monitoring product for this yeast is difficult. The most effective technique of which we are aware is to aseptically collect ca 500 ml samples in sterile bottles, preferably 2 litre and made of plastic, add 500 ml sterile water, mix gently, and incubate at ca 25 °C. If gas is not produced when containers are shaken after a 7 day incubation period, the product is probably sound.

12.2.6 Honey and Syrups

Walker and Ayres (1970) discussed at length the various reports of spoilage in honey, generally due to *Zygosaccharomyces rouxii.* Honey can also be contaminated with the ascospores of *Ascosphaera apis* (Claussen) L.S. Olive & Spiltoir, the cause of chalkbrood disease of honey bees (Hale and Menapace 1980). Modern technology for handling honey

includes a heat treatment to prevent crystallisation of glucose, and this treatment also effectively sterilises it. Although species of xerophilic yeasts can often be isolated from unprocessed honey (Poncini and Wimmer 1986), spoilage of commercially processed honey is now a very rare problem.

Glucose syrups and liquid malt extracts are susceptible to spoilage by xerophilic yeasts. Spoilage may be manifested by slow fermentation in the product itself, or through foaming when the viscosity of the syrup is reduced by heating to allow easier handling. *Zygosaccharomyces rouxii* is usually the cause, but other xerophilic yeasts including *Zygosaccharomyces bailii, Torulaspora delbrueckii* (Lindner) Lindner, *Candida lusitanae* Uden & Carmo Souza and *Schizosaccharomyces* spp. may also cause spoilage (Tilbury 1980; Jermini et al. 1987; Fleet 1992). In maple syrup, which has a higher a_w, we have seen spoilage by surface growth of *Wallemia sebi.*

12.3 Low Water Activity Foods: Salt Foods

The principal salted food which is susceptible to fungal spoilage is salt fish. This is a very widely distributed product, as salting is the most common way of preserving fresh fish in most tropical regions, and in some temperate zone countries as well. In temperate climates, *Wallemia sebi* is regarded as the principal spoilage fungus (Frank and Hess 1941). In Brazil, Mok et al. (1981) reported the human pathogen *Hortaea werneckii* (Horta) Nishim. & Miyaji to be one fungus growing on salt fish.

In tropical countries, *Aspergillus* species often dominate the mycobiota of dried and salted fish. Townsend et al. (1971) reported that Vietnamese dried fish were contaminated by a wide variety of Aspergilli, especially *A. clavatus, A. flavus* and *A. niger.* On Malaysian dried fish, *A. niger* and related species were most common, with *A. flavus, Neosartorya fumigata* and *A. ochraceus* also present. *Penicillium chrysogenum* was reported as common (Ito and Abu 1985). The principal fungus isolated during a study of fungi on salted and dried fish in Indonesia was *Polypaecilum pisce* (Pitt and Hocking 1985). It was isolated from nearly 50% of the 60 samples of mouldy fish examined. In some cases growth was apparent over most of the fish surface. *Aspergillus* subgen. *Aspergillus* species were also common, being found on 25–30% of the fish. However, growth was usually less extensive than that of *Polypaecilum pisce.* Other *Aspergillus* species were also quite frequently isolated. *A. niger* (20%), *A. flavus* (18%), *A. wentii* (10%) and *Emericella sydowii* (16%) were of most common occurrence. Apart from *Aspergillus* subgen. *Aspergillus* species, it is doubtful whether the other *Aspergillus* species had actually grown on the fish. *Penicillium* species were less common, the most frequently isolated being *P. citrinum* and *P. thomii,* each of which was isolated from 18% of the fish examined. *Cladosporium cladosporioides* was also present on 18% of the fish. Of interest also was the isolation *of Basipetospora halophila* (= *Phialosimplex halophilus*). Although encountered infrequently, the water relations of this fungus will clearly allow it to grow on fully dried fish. This was also the second most common species occurring on Sri Lankan salt fish (Atapattu and Samarajeewa 1990). They isolated ten other species, of which *Aspergillus niger* was the most common, followed by *A. flavus, Neosartorya fumigata and Aspergillus* subgen. *Aspergillus* species. An unusual *Penicillium* species, *P. chalybeum* Pitt & A.D. Hocking, originally described from Indonesian salt fish (Pitt and Hocking 1985), was encountered frequently on the Sri Lankan salt fish (Atapattu and Samarajeewa 1990). *Phialosimplex halophilus* was also isolated frequently from cured fish in Nigeria, but as with other studies, *Aspergillus* species were reported more commonly. *Scopulariopsis* species, including S. *brevicaulis,* were also present (Diyaolu and Adebajo 1994).

In smoke-dried fish from Sierra Leone, the only fungi isolated were four species of *Aspergillus: A. flavus, A. ochraceus, A. tamarii* and *A. niger.* The plating medium was MEA, so if halophilic fungi were present, they would not

have been detected (Jonsyn and Lahai 1992). In a similar study, Bassey and Effiong (2016) used PDA to isolate fungi from dried freshwater catfish (*Clarias gariepinus*) in Nigeria. They reported the most commonly isolated fungi were *Aspergillus flavus, A. niger, Fusarium* sp. and *Rhizopus*. Other fungi recorded were *Epicoccum, Absidia, Penicillium, Trichoderma, Mucor, Cladosporium* and *Alternaria. A. glaucus* was isolated occasionally. Results from these studies emphasise the importance of using appropriate media when examining the mycobiota of low a_w foods.

12.4 Intermediate Moisture Foods: Processed Meats

A wide variety of meat products of reduced a_w are manufactured around the world. They include bacon, hams and many types of salamis. These products are more or less shelf stable, depending on the ingredients and process used, but are frequently contaminated by fungi. At first, interest in these fungi stemmed from consideration of possible beneficial effects on flavour, etc., of the cured products (e.g. Leistner and Ayres 1968), however, it was soon realised that these fungi may produce mycotoxins, stimulating further interest. A number of studies were subsequently carried out, twelve of which were summarised by Leistner and Eckardt (1981). The dominant mycobiota on cured meats are *Penicillium* and *Talaromyces* species. Leistner and Eckardt (1981) listed 50 species, the most frequently isolated being *P. chrysogenum, P. expansum, P. roqueforti, T. rugulosus, T. variabile* and *P. viridicatum. Aspergillus* subgen. *Aspergillus* species were also quite common. With the exception of *A. flavus* and *Emericella versicolor*, other *Aspergillus* and related species were relatively uncommon. Potentially mycotoxigenic species of both *Aspergillus* and *Penicillium* were isolated quite frequently by Leistner and Pitt (1977). These results have been confirmed by later studies (Grazia et al. 1986; Mutti et al. 1988; Andersen 1995) where *Penicillium* species were found to be dominant on fermented sausages. On Italian goose sausages spoiled by a flavour defect (ammonia), the dominant fungal species were *Penicillium nalgiovense, P. chrysogenum* and *P. viridicatum* (Iacumin et al. 2016). Black spot spoilage of Spanish dry-cured fermented sausages was attributed to *Cladosporium oxysporum* Berk. & M.A. Curt. (Lozano-Ojalvo et al. 2015).

The *Penicillium* species now most frequently isolated from fermented sausages is a starter culture, *P. nalgiovense* (Andersen 1995). This was developed by the Bundesanstalt für Fleishforschung, Kulmbach, Germany, who sought a desirable *Penicillium,* nontoxigenic and with a persistently white appearance (Mintzlaff and Christ 1973). Use of a starter culture is a satisfactory way to avoid overgrowth by undesirable moulds during the drying and curing of salamis and other semi-preserved meats. A new species related to *P. olsonii*, described as *Penicillium salamii* Perrone et al. (Perrone et al. 2015), was found to be widespread on surfaces of salami and capocollo in Italy, and was also isolated in Denmark and Slovenia from cured meats. Perrone et al. (2015) suggested that this species could also be used as a starter culture for cured meat.

Yeasts also form a considerable component of the mycobiota of fermented sausages, dominating during the early stages of fermentation (Andersen 1995; Filtenborg et al. 1996). *Debaryomyces hansenii* (often reported as its anamorph synonym *Candida famata*), is by far the most frequently reported species (Comi and Cantoni 1980; Grazia et al. 1986; Fleet 1992).

Both yeasts and moulds can cause spoilage of ham during maturation. As with fermented sausages, yeasts occur mainly on the surface of ripening hams, where they may cause rancidity due to their lipolytic activity. *Debaryomyces hansenii* and *Candida saitoana* Nakase & M. Suzuki are the species most commonly reported, with *Hansenula* species, *Galactomyces candidus, Rhodotorula glutinis, Cryptococcus albidus* (Saito) C.E. Skinner and *Trichosporon* species

also present (Comi and Cantoni 1983; Comi et al. 1983; Monte et al. 1986; Molina et al. 1990). The mould flora of raw hams during ripening is dominated by *Aspergillus* and *Penicillium* species. The most commonly reported are *P. verrucosum, P. viridicatum*, *P. expansum*, P. *aurantiogriseum, P. chrysogenum* and *P. brevicompactum* (Monte et al. 1986; Spotti et al. 1989). *P. commune* has been found to be responsible for "phenol defect" (production of a phenol-like odour) of ham during ripening (Spotti et al. 1988). Comi et al. (2014) described a flavour defect (ammoniac and vinegar odour) in dry cured ham, attributing it to a combination of *P. chrysogenum, P. viridicatum, P. aurantiogriseum, P. nalgiovense* and *Aspergillus candidus.* At lower a_w values (less than 0.85 a_w) *Aspergillus* species, including *A. flavus, A. candidus, A. wentii, A. niger* and *Neosartorya fumigata* are generally dominant (Dragoni et al. 1980b; Monte et al. 1986; Spotti et al. 1989; Rojas et al. 1991). *A. halophilicus* has been reported from the surface of dry-salted Spanish ham in the latter stages (6–12 months) of the ripening period (Huerta et al. 1987). Alía et al. (2016) suggested that a particular strain of *P. chrysogenum* (CECT 20922) inoculated onto the surface of dry cured hams could be useful in controlling black spot spoilage caused by *Cladosporium oxysporum*.

Scopulariopsis species have been isolated from cured meats. *S. brevicaulis* was isolated from the surface of maturing smoked bacon (Dragoni et al. 1988) while *S. candida* (Gueg.) Vuill. and *Penicillium chrysogenum* provided an optimum combination as a starter for the manufacture of rohwurst, a raw ripened German sausage (Hwang et al. 1993).

12.5 Heat Processed Acid Foods

Because bacterial spores are not a problem, heat processes for acid foods such as fruits and fruit products have traditionally been light. For most processes, pasteurisation at temperatures of about 70–75 °C is effective, as it inactivates most enzymes, yeasts and the conidia of common contaminant fungi. However, fungi producing ascospores are capable of surviving such processes and causing spoilage.

In practice, only a few ascosporogenous species have been isolated from fruit products after a heat process, and still fewer have been recorded as causing spoilage. The list of such species is headed by *Byssochlamys fulva* and *B. nivea*, which have been recorded as causing spoilage in strawberries in cans or bottles (Hull 1939; Put and Kruiswijk 1964; Richardson 1965), blended juices with a passionfruit content, and fruit gel baby foods (Hocking and Pitt 1984). *Neosartorya fischeri* has also been repeatedly isolated from strawberries (Kavanagh et al. 1963; McEvoy and Stuart 1970, and in our laboratory) and other products, but has not been reported to cause spoilage. *Talaromyces trachyspermus* (Shear) Stolk & Samson has been reported from retail packaged canned and frozen pineapple juice (Enigl et al. 1993), and from apple juice in our laboratory. *Talaromyces macrosporus, T. bacillisporus* and ascosporic *Penicillium* species are also potential causes of spoilage in heat processed products (Hocking and Pitt 1984).

Techniques for detection of heat resistant moulds have been outlined in Chap. 4. Raw materials which should be screened routinely for heat resistant moulds include grapes, passionfruit, pineapple and mango juices and pulps, strawberries and other berries, indeed any raw material which may have come in contact with soil directly or as a result of rain splash.

12.6 Preserved Foods

Acid liquid foods are frequently stabilised by the use of preservatives. Benzoic acid, sorbic acid and/or sulphur dioxide are usually added to fruit juices, soft drinks, cordials and a variety of other products. The natural preservative acetic acid is used in products such as tomato sauce, mayonnaises and salad dressings. Ciders and wines are preserved by alcohol.

All of these products are susceptible to spoilage by preservative resistant yeasts, yeasts which are capable of growth in the presence of maxi-

mum levels of preservatives permitted in such products (Pitt and Richardson 1973). By far the most significant of these is *Zygosaccharomyces bailii* which is capable of spoiling all of the products listed above. Like *Xeromyces* and *Z. rouxii, Z. bailii* cannot be excluded from products with normal food technological processes. If present in a final product, *Z. bailii* will cause spoilage of most preserved foods. There are exceptions: safe products are synthetic products such as soft drinks and water ices which lack a nitrogen source, or are made with sucrose, which *Z. bailii* usually cannot assimilate.

Other species of yeast are also capable of causing spoilage in acid liquid foods. In products preserved with a combination of SO_2 and sorbate or benzoate, *Schizosaccharomyces pombe* Lindner may cause spoilage, as this yeast appears to be more resistant to SO_2 than does *Zygosaccharomyces bailii. Candida parapsilosis, Pichia anomala, P. membranaefaciens* and *Torulaspora delbrueckii* (Lindner) Kudriavsev have been isolated from salad dressings and mayonnaise, all of which rely on the preservative effects of acetic acid. We have seen several spoilage incidents caused by preservative resistant *Saccharomyces cerevisiae* in beverages containing sorbate or benzoate as preservatives.

Almost every factory in Australia which produces acid liquid products has had problems at some time or another with preservative resistant yeasts. Other products, too, have been spoiled from time to time: the list in Australia includes cherries for cake manufacture, chocolate topping, mineral water with added fruit juice, and water ices made with glucose as a proportion of the sugar.

Pasteurisation is an effective method for eliminating *Zygosaccharomyces bailii* and other preservative resistant yeasts from liquid products. The temperature required depends on the product – pH, sugar content and preservative level, in particular. The precise heat treatments which will be effective should be worked out for each product type. Temperatures around 65–70 °C for more than a few seconds should eliminate low numbers of cells. Pasteurisation within the final closed container is to be preferred: if this is not possible, then scrupulous attention to cleaning the lines and fillers downstream from the pasteuriser is essential.

Filter sterilisation is also an effective technique for ridding products of *Zygosaocharomyces bailii,* but its use is confined to clear products such as ciders and wines.

Like *Zygosaccharomyces rouxii, Z. bailii* can cause spoilage from very low inocula, which makes detection in the plant very difficult. The most effective quality control technique is to test for the presence of the yeast in the final product itself, using the techniques outlined in Chap. 4. For detection of preservative resistant yeasts in the factory or in raw materials, the use of TGY + 0.5% acetic acid or malt acetic agar is recommended.

12.7 Cheese

Cheese is very susceptible to mould growth and is normally kept under refrigeration. Many retail packs are vacuum packaged or gas flushed. Thus, spoilage is generally confined to moulds which are psychrotolerant and can grow under conditions of relatively low oxygen.

In Australasian Cheddar cheese, fungal growth may occur during maturation, causing a defect known as “thread mould”. Cheeses manufactured by continuous forming systems such as the Wincanton tower, designed for the rapid processing of curd into 20 kg blocks, are particularly susceptible to this type of spoilage. The blocks are vacuum packaged into polyethylene-nylon bags, and matured for up to 9–12 months at 8–12 °C. Of 195 fungi isolated from thread mould defects, 44% were *Cladosporium,* with *C. cladosporioides* and *C. herbarum* most common, and 27% were *Penicillium* species, with *P. commune* and *P. glabrum* dominant. A Coelomycete, tentatively identified as a *Phoma* species, comprised 15% of isolates (Hocking and Faedo 1992). Yeasts were also frequently encountered (19% of isolates), with *Candida intermedia* (Cif. & Ashford) Langeron & Guerra, *C. parapsilosis, C. multisgemmis* (Buhagiar) S.A. Mey. & Yarrow and *Yarrowia lipolytica* (Wick. et al.) van der Walt & Arx most common.

The range of fungi causing spoilage in Australian and New Zealand cheeses in the retail sector differs from that found on maturing Cheddar blocks. *Penicillium commune* and *P. roqueforti* were the most common spoilage species, but other species from *Penicillium* subgen. *Penicillium* (*P. chrysogenum, P. expansum, P. solitum, P. viridicatum* and *P. brevicompactum*) were a significant proportion of the spoilage mycobiota (Hocking 1994). *P. roqueforti* and *P. commune* (from which *P. camemberti* is derived) are particularly well suited to growth in the cheese environment, having been associated with cheese manufacture for centuries.

In packaged cheeses in Europe, *Penicillium commune* is also the most common spoilage species (Lund et al. 1995), with *P. verrucosum, P. solitum, P. roqueforti* and *P. nalgiovense* also significant. Species other than *Penicillium* were rarely encountered in that study. In a study of the diversity of spoilage fungi associated with French dairy products, Garnier et al. (2017) also found *P. commune* to be the most prevalent spoilage species in hard cheese. Other Penicillia (*P. bialowiezense* K.W. Zalesky*, P. discolour* Frisvad & Samson*, P. antarcticum* A.D. Hocking & C.F. McRae*, P. nalgiovense, P. solitum, P. nordicum, P. brevicompactum, P. palitans* Westling and *P. roqueforti*), *Mucor* species (*Mucor racemosus, M. circinelloides*, and *M. spinosus*), *Thamnidium elegans* and *Cladosporium sphaerospermum* were also present. In spoiled and unspoiled Spanish semi-hard ripened cheeses, Ramos-Pereira et al. (2019) reported that 66% of isolates were *P. commune,* but *P. solitum, P. chrysogenum, P. nordicum, P. expansum* and *P. cvjetkovicii* S.W. Peterson et al. were also present. Jurado and Vicente (2020) found the presence of *P. commune* changed the surface of extra hard cheeses, making them drier and more difficult to penetrate. In Italian grana cheeses (Grana Padano and Parmigiano Reggiano) Decontardi et al. (2018) found two *Aspergillus* species and 14 *Penicillium* species, with *P. solitum* dominant. Other species isolated included *P. verrucosum, P. crustosum, A. flavus, Aspergillus puulaauensis* Jurjevic et al.*, P. commune, P. chrysogenum* and *P. paneum.*

Traditional Italian artisan cave cheeses in the Puglia region are matured using autochthonous fungi. Anelli et al. (2019) found that *Aspergillus westerdijkiae* and *P. biforme* (= *P. camemberti*) were the most prevalent species, followed by *P. roqueforti* and *P. solitum. A. westerdijkiae* and *A. steynii*, both potential sources of ochratoxin A, were present on 45% of samples. They also described a new species of *Penicillium, P. gravinicasei,* which is close to *P. cinnamopurpureum*, which was present on many of the cave cheeses (Anelli et al. 2018). By contrast, in northern Sweden where goat's cheeses were stored in a cellar at 4–6 °C, the mycobiota comprised *Debaryomyces hansenii* and *Penicillium* species, with *Mucor mucedo* forming the rind (Bath et al. 2012).

Yeasts and yeast-like fungi are often present in cheese, and are important in the surface smears used as starters for soft-ripened cheeses because of their lipolytic and proteolytic activities. *Geotrichum candidum* (= *Galactomyces candidus*) is a normal part of the mycobiota of the smear of surface ripened cheeses (Marcellino and Benson 1992; Lefortier-Medvey 1993; Lund et al. 1995), although it can also be present as a spoilage organism in other types of cheese (Gueguen 1988). *Debaryomyces hansenii, Kluyveromyees* species, *Saccharomyces cerevisiae* and *Candida* species are common on the surface of St Nectaire, Camembert and blue-veined cheeses, and may play an important role in the development of texture and flavour of these products (Vergeade et al. 1976; Schmidt et al. 1979; Schmidt and Lenoir 1980; Nunez et al. 1981; Roostita and Fleet 1996). However, when present in Cheddar and Gouda cheeses, such yeasts can cause taints and off flavours due to their lipolytic and proteolytic activities, and the production of bitter compounds from lactose fermentation (Viljoen and Greying 1995). Geronikou et al (2020) reviewed the occurrence of yeasts in white-brined (Feta-type) cheeses. The most commonly encountered species were *D. hansenii, G. candidus, Kluyveromyces marxianus* (E.C. Hansen) van der Walt*, K. lactis* (Stell.-Dekk.) van der Walt*, Rhodotorula mucilaginosa*

and *Trichosporon* spp. It is often unclear whether these yeasts play a part in the development of the flavour profiles of these cheeses, or occur as contaminants with potential for spoilage.

Sporendonema casei Desm. is an uncommon species that can occur on the surface of some types of cheese. *S. casei* and *Aspergillus candidus* were the dominant species on Provolone cheese after 4–5 months of ripening at 15 °C and 85% ERH (Galli and Zambrini 1978). On Rodez cheese (a Toscanello variety), *S. casei* is responsible for the production of reddish orange surface spots, giving the cheese its so-called 'sunburnt' colour at the end of the ripening period. *S. casei* grows optimally at 0.97 a_w, at 18 °C and pH 7.0 (Ratomahenina et al. 1994). A brown stain found on the cut surface of a Brie cheese was caused by *Helicostylum pulchrum* (Preuss.) Pidopl. & Milko and *Scopulariopsis flava* F.J. Morton & G. Sm., two uncommon species not usually associated with cheese spoilage. *Debaryomyces hansenii* was also isolated from the affected area (Sato et al. 2013).

Heat resistant fungi can occasionally cause spoilage in heat processed cheeses such as cream cheese. Ascospores of *Byssochlamys nivea* and other heat resistant species may be present in raw milk, and can readily survive the pasteurisation processes applied to milk and cream (Engel and Teuber 1991). Prolonged storage and inadequate cooling (>12 °C) can allow growth of heat resistant moulds in dairy products, and we have seen several such cases in Australia. Three separate spoilage incidents involving cream cheese were caused by *Byssochlamys nivea, Talaromyces avellaneus* (Thom & Turesson) C.R. Benj., *Neosartorya spinosa* and *Penicillium brefeldianum. Talaromyces avellaneus* also caused spoilage of UHT custard.

Although most cheeses are not permitted to contain preservatives, some types of cheese, and products such as cheese spreads, may contain sorbate or other mould inhibitors. A number of fungal species, including *Penicillium roqueforti*, are capable of decarboxylating sorbate to *trans-1,3*-pentadiene, causing a flavour defect described as a "kerosene" flavour, a problem not restricted to cheese (Liewen and Marth 1985; Daley et al. 1986; Kinderlerer and Hatton 1990; Sensidoni et al. 1994). Some fungi (particularly *Penicillium* species) can also reduce sorbic acid to 4-hexanol and 4-hexanoic acid (Kinderlerer and Hatton 1990)

The factors enabling fungi to cause spoilage in cheese are the ability to grow at refrigeration temperatures, to grow in low oxygen concentrations, lipolytic activity, resistance to the preservative action of free fatty acids, and growth at reduced a_w. *Penicillium roqueforti* and *P. commune* meet all these criteria, and are thus the most successful spoilage moulds on cheese. Toxin production (roquefortine and PR toxin from *P. roqueforti* and cyclopiazonic acid from *P. commune*) is a definite, though probably small, hazard. PR-imine was detected in 50 of 60 samples of blue-vein cheese, but PR toxin was not (Siemens and Zawistowski 1993). Sterigmatocystin produced by *Emericella versicolor* has been detected in the surface layer of hard cheeses in the Netherlands (Northolt et al. 1980),

Mouldy cheese is unsuitable for sale, and for manufacturing purposes. Protection from the Penicillia relies on low temperature storage, low oxygen atmospheres, integrity of packaging materials, intact rinds, preservative impregnated wrappers, and rapid turnover of stock.

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Mycotoxins

13

13.1 Background

Mycotoxins are fungal metabolites which when ingested, inhaled or absorbed through the skin can cause sickness or death in man or domestic animals, including birds (Pitt 1966). Toxins ingested when fungi are eaten as food (mushrooms) are excluded by common consent, as are compounds that show toxicity only to lower animals, such as insects, plants or microorganisms.

Mycotoxins have been responsible for major epidemics in man and animals throughout history (Matossian 1989). The most important epidemic was ergotism, due to growth of the fungus *Claviceps purpurea* in rye grains, which killed and maimed many thousands of people in Europe over the last millennium (Fuller 1969 and also occurred in the United States (Matossian 1982). Also of high importance have been alimentary toxic aleukia (ATA), caused by T-2 toxin produced by *Fusarium sporotrichioides* in rye grain, which was responsible for the death of at least 100,000 Russian people between 1942 and 1948 (Mirocha and Pathre 1973; Joffe 1978); and stachybotryotoxicosis, caused by growth of *Stachybotrys chartarum* in hay, which killed tens of thousands of horses in the USSR in the 1930s (Moreau 1980). These diseases are causing little problem at the present time.

A few excellent studies of disease due to fungi in food or feed were reported in the first half of the 20th century, but understanding of the significance of mycotoxins in human and animal health occurred only more recently. The term **mycotoxicosis** was first used by Forgacs and Carll (1952). The modern era of mycotoxin investigation was ushered in by the discovery of the aflatoxins, which killed 100,000 young turkeys in the United Kingdom in 1960 and which resulted from the growth of *Aspergillus parasiticus* in peanut meal (Austwick and Ayerst 1963; Pitt and Miller 2017). Laboratory experiments and the investigation of field outbreaks of disease rapidly led to the discovery that many commonly occurring fungi – both spoilage fungi and plant pathogens – are able to produce a staggering array of toxic metabolites, many with chemical structures of types never before encountered in natural products chemistry.

Mycotoxins are secondary metabolites, i.e. they appear to have no role in the normal metabolism concerned with growth of the fungus. They are frequently bizarre molecules, with structures ranging from single heterocyclic rings with molecular weights of scarcely 50, to groups of irregularly arranged 6 or 8 membered rings with total molecular weights greater than 500. Some, such as patulin, appear to be isolated compounds, whereas others, most notably the trichothecenes, exist as a family of 100 or more compounds, all fungal metabolites. No particular group of atoms, or moiety, can be considered to define "mycotoxin", as the biologically active groups vary widely between classes of toxin.

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Hazards from mycotoxins Mycotoxins seldom elicit the rapid responses associated with bacterial toxins. Unlike the bacterial toxins, most mycotoxins are not proteinaceous in character and, being relatively small molecules, are not recognised by the immune systems of man or animals. A major potential danger of mycotoxins in the human diet therefore resides in our inability to detect them biologically. Some mycotoxins cause acute effects, the most common being deterioration of liver function, which in extreme cases may lead to death. However, the more common influence of mycotoxins in the diet of humans or domestic animals is to cause chronic disease, usually without obvious symptoms. Attempts to reduce the harmful effects of mycotoxins therefore mostly rely on detection in foods or commodities before sale to consumers.

The most widespread chronic effect of a mycotoxin is the induction of liver cancer. However, some mycotoxins act primarily by interfering with protein synthesis, causing effects ranging from skin sensitivity or necrosis to extreme immunodeficiency, which can lead to death from infectious agents. Others are neurotoxins, which in low doses may cause sustained trembling in animals, but at only slightly higher levels can cause permanent brain damage or death.

The symptoms of mycotoxin poisoning are almost as diverse as the chemical structures of the compounds themselves. Some compounds may elicit few symptoms until death results, while others may induce severe effects including skin necrosis, leucopoenia, and immunosuppression. Doses producing chronic disease are usually far below those responsible for acute effects, so consequences such as cancer induction are undetected at the time of ingestion, and indeed may remain so until disease is quite advanced.

Fungal sources of mycotoxins Many mycotoxigenic fungi are of widespread occurrence, and indeed in some cases have a strong ecological link with major crops used for human food supplies. The natural fungal flora existing in conjunction with food production is dominated by three genera: *Fusarium*, *Aspergillus* and *Penicillium* (Pitt et al. 2012). *Fusarium* species are destructive pathogens on cereal crops and other commodities, and generally produce mycotoxins before harvest. Certain species of *Aspergillus* and *Penicillium* are also plant pathogens or commensals, but these genera are more commonly associated with commodities after harvest, i.e. during drying, storage and transport.

13.2 Categorising Mycotoxins

Mycotoxins can be divided into three categories on the basis of their toxicity and their occurrence in foods and feeds. For ease of discussion, mycotoxins are divided into those considered to be of major, minor or low importance.

Major mycotoxins The major mycotoxins are compounds that have been definitely established to cause sickness in humans or domestic animals and in consequence result in health or economic losses associated with their presence in food commodities. Five mycotoxins are considered today to fit into this category: aflatoxins, ochratoxin A, fumonisins, deoxynivalenol (and the related trichothecene nivalenol) and zearalenone (Miller 1995; Pitt et al. 2012). Some authorities also include patulin in this category. These compounds are dealt with in detail in separate entries below.

Minor mycotoxins Minor mycotoxins are considered here to be compounds with demonstrated toxicity, known to occur naturally in toxic concentrations, and which from time to time may cause sickness or economic loss on a limited scale. Included here are a number of compounds which occur in pasture or field crops, together with some found in grains and/or which occur in processed foods. These include some field toxins, T-2 toxin, cyclopiazonic acid, citreoviridin, penitrem A and citrinin.

Mycotoxins of low importance This category includes two classes of compounds. The first are compounds with demonstrated toxicity, but no known disease syndrome, which is usually due to uncommon occurrence in foods or feeds. Included here are sterigmatocystin, janthitrems, territrem and others. The second includes compounds of lower toxicity, which might produce an

appreciable effect in humans or domestic animals if ingested at levels much higher than are normally known to occur in foods.

13.3 Aflatoxins

Aflatoxins are produced in foods primarily by *Aspergillus flavus* and the closely related species *A. parasiticus*. A number of other *Aspergillus* species make aflatoxins, but only two of these are of some importance in foods: *A. nomius* and *A. minisclerotigenes*. Both of these species resemble *A. flavus* in culture but *A. nomius* produces bullet shaped sclerotia, as distinct from the spherical sclerotia produced by many *A. flavus* isolates, while *A. minisclerotigenes* produces small spherical sclerotia. The four major naturally produced aflatoxins are known as aflatoxins B_1, B_2, G_1 and G_2. 'B' and 'G' refer to the blue and green fluorescent colours produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds respectively. These are historical names from early research, but they remain in common use. Almost all *A. flavus* isolates produce only B aflatoxins, whereas those of *A. parasiticus, A. nomius* and *A. minisclerotigenes* produce both B and G aflatoxins. Other species that produce aflatoxins are rare and none is of practical consequence in foods or feeds.

13.3.1 History

Aflatoxins were discovered in 1960 as the result of a mysterious disease outbreak that killed 100,000 turkey poults in England. It became known as "Turkey X" disease. It was soon established that peanut meal from Brazil was the direct cause, and soon afterwards that the toxin was produced by the common spoilage fungi *Aspergillus flavus* and *A. parasiticus.* In the same year, it was discovered that cottonseed meal was the cause of tumours in hatchery reared trout in California, a problem first reported in 1934. Within two years, the toxins had been characterised, named aflatoxins after their major fungal source, and assays developed using the new technique called thin layer chromatography (Pitt and Miller 2017). TLC showed that four major aflatoxins are naturally produced, known as aflatoxins B_1, B_2, G_1 and G_2. 'B' and 'G' refer to the blue and green fluorescent colours produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds respectively. By 1970, it was established that aflatoxins were widespread in tropical and warm temperate commodities, especially peanuts, maize and cottonseed, and that aflatoxins were potent liver carcinogens in animals. Indeed aflatoxin B_1 is now considered to be the most potent liver carcinogen known, either natural or chemical. During the 1970s, outbreaks of human illness and death showed that aflatoxins can be acutely toxic to humans, and carcinogenicity to humans was postulated. In the 1980s, it became clear that hepatitis B virus was a cause of liver cancer and the validity of aflatoxin as a cause was questioned. However, during the 1990s, it was established that both hepatitis B and aflatoxin caused liver cancer and were synergistic. Equations were developed relating aflatoxin intake to the likelihood of developing liver cancer, in the presence or absence of the hepatitis B virus. In the past decade, it has become apparent that aflatoxins are likely to play an even wider role in human health as they are immunosuppressive and may be involved in childhood stunting (Pitt et al. 2012; Pitt and Miller 2017).

13.3.2 Chemical Characteristics

Aflatoxins are highly substituted coumarins, with a fused dihydrofurofuran moiety. There are four naturally occurring aflatoxins of importance. Aflatoxin B_1 (CAS 1162-65-8) and aflatoxin B_2, which is dihydroaflatoxin B_1 (CAS 7720-81-7), are characterized by fusion of a cyclopentenone ring to the lactone ring of the coumarin. Aflatoxin G_1 (CAS 1165-39-5) and aflatoxin G_2, which is dihydroaflatoxin G_1 (CAS 7241-98-7), have an additional lactone ring (Fig. 13.1).

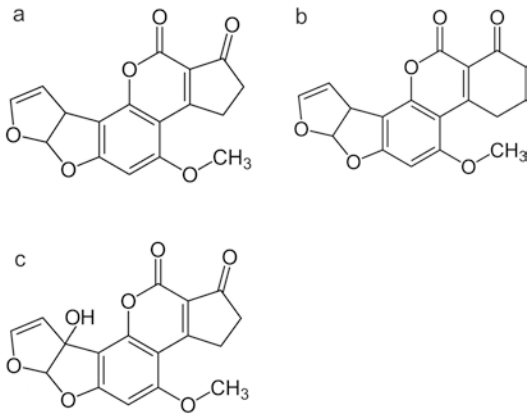

Fig. 13.1 Structures of major aflatoxins: (**a**) aflatoxin B_1; (**b**) aflatoxin G_1; (**c**) aflatoxin M_1

When aflatoxins B_1 and G_1 are ingested by lactating animals, small proportions (1–2%; Frobish et al. 1986) are excreted in milk as aflatoxins M_1 (4-hydroxyaflatoxin B_1; CAS 6795-23-9; Fig. 13.1) and M_2 (4-hydroxyaflatoxin G_1; CAS 6885-57-0), hydroxylated derivatives of the parent compounds.

13.3.3 Hazard Identification

As noted above, aflatoxins have been known to cause both acute and chronic toxicity for many years. However these compounds now appear to have even more diverse effects on human and animal health. Aflatoxins have a likely involvement in five toxic effects: acute toxicity, liver carcinogenicity, growth retardation in children, immunosuppression and liver cirrhosis (Pitt et al. 2012). Environmental exposure in the handling of grains or nuts contaminated with aflatoxin is also of concern.

Acute toxicity – aflatoxicosis An outbreak of hepatitis due to aflatoxin ingestion from maize occurred in India in 1974. Almost 400 cases were identified, and 106 deaths were reported. Clinical features were jaundice preceded by fever, vomiting and anorexia, with ascites and oedema in the lower limbs in extreme cases. Levels in analysed foods were often extremely high, up to 15 mg/kg (Krishnamachari et al. 1975). Given that the average adult consumption of maize is >300 g/day, estimated daily ingestion was 2–6 mg of aflatoxins. Based on data from this and two other outbreaks in Kenya reviewed by Wild and Gong (2010), it was concluded by JECFA (2018) that doses of 20–100 μg/kg bw/day would be fatal.

In 2004, a large, severe outbreak of aflatoxicosis occurred in eastern Kenya, where 317 people fell ill and 125 died. This was followed by a second outbreak in 2005 during which a further 25 people died (Strosnider et al. 2006). A case study conducted soon after the first outbreak revealed that mouldy maize was the source of the aflatoxin (Azziz-Baumgartner et al. 2005; Lewis et al. 2005). A smaller outbreak occurred in Malaysia in 1988, where 13 children died from acute hepatic encephalopathy due to consumption of commercially prepared noodles contaminated with aflatoxin (Lye et al. 1995).

With the exception of the Malaysian incident, aflatoxicosis has been known to occur only when drought or famine causes exceptionally high levels of aflatoxins in stored staple foods, especially maize, and forces the eating of substandard food. However, constant exposure to aflatoxins through the consumption of less contaminated foods leads to chronic diseases which have a much greater impact on human health (Williams et al. 2004).

Liver carcinogenicity Studies involving a variety of animal species have demonstrated that aflatoxin B_1 is the most potent liver carcinogen known. Aflatoxin B_1 is classified as a Category I human carcinogen by the International Agency for Research on Cancer (IARC 1993; Rastogi et al. 2006). Kuiper-Goodman (1991) concluded that the 50% probability of increasing the incidence of primary liver cancer in a population by a factor of 1 case per 10,000 population was between 5 and 9 μg/kg body weight/day aflatoxin intake. Based on those figures and a world wide survey of aflatoxin exposure, Liu and Wu (2010) estimated that between 25,000 and 155,000 cases of liver cancer due to aflatoxin alone occur across the world each year. Using different methodology, Liu et al. (2012) revised this estimate to between 105,000 and 142,000 new cases due to aflatoxin alone per annum. Liver cancer is almost always a terminal illness.

A strong synergy has been observed between aflatoxins and hepatitis B virus in the aetiology of liver cancer (JECFA 2018). In its risk assessment of aflatoxins, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) derived two potency factors for cancer formation by aflatoxins: for aflatoxin alone, 0.01 cases per 100,000 people per annum per ng per kg body weight per day and for individuals carrying hepatitis B infection, 0.30 cases (JECFA 2018). Thus the two agents together are about 30 times as potent as aflatoxin alone. In some areas where HBV and aflatoxin co-occur, hepatomas are the major cancer and may be a dominant cause of death. This known carcinogenicity is so high that every effort must be made to monitor levels in human foods and to reduce them as far as possible.

In the liver, aflatoxin B_1 is converted by cytochrome P450 enzymes to the 8–9 epoxide. This is capable of binding to liver proteins, leading to liver failure and potentially to aflatoxicosis. This epoxide is also able to bind to DNA, a precursor step to the development of liver cancer (Pitt et al. 2012).

Stunting in children Stunting is defined by WHO as height for age being more than two standard deviations below average in a given population. Studies have shown an inverse association between levels of aflatoxin–albumin adducts (an aflatoxin biomarker) and childhood growth (Gong et al. 2002, 2004) and an association between aflatoxin exposure and impaired growth during the first year of life (Turner et al. 2007; Shirima et al. 2015; JECFA 2018). Given the high numbers of people consuming uncontrolled levels of aflatoxin, it was believed that stunting from aflatoxin consumption by young children was an important disease burden, only recently recognised (Khlangwiset et al. 2011). However, recent studies have suggested that fumonisins may have an equal or larger role in stunting than aflatoxins (see Fumonisins).

Kwashiorkor, also known as protein energy malnutrition, is a leading cause of disease and death in children under 5 years of age in Northern Africa and elsewhere. Hendrickse et al. (1982) proposed a link with aflatoxin intake and that has recently been confirmed (McMillan et al. 2018).

Immunosuppression Aflatoxins have been shown to suppress the cell-mediated immune response in cell lines, domestic animals and, in limited studies, humans. Effects include the impairment of delayed type hypersensitivity, decreases in the phagocytic activity of macrophages, increased susceptibility to infection and reduced response to vaccines. If the effects in humans mirror those in animals even approximately then the immunosuppressive effects of aflatoxins probably have very wide implications for human health (Bondy and Pestka 2000).

13.3.4 Exposure Assessment

Accurate exposure assessment of aflatoxins in foods and feeds has been very difficult. Measuring consumption levels of foods, accompanied by aflatoxin assays, is unreliable because of wide variation in aflatoxin levels in individual food samples. It is especially difficult in subsistence economies, where locally produced and consumed grain is more likely to contain excessive levels of aflatoxin. However, the development of biomarkers which integrate aflatoxin consumption over time have been a major step forward (Groopman et al. 2008). The most reliable biomarker of chronic aflatoxin exposure is the aflatoxin-albumin adduct, as measured by ELISA or isotope dilution mass spectrometry (McMillan et al. 2018). Significant exposure comes from just a few foodstuffs, in particular maize and peanuts, to a lesser extent other tree nuts and figs. Cottonseed is also a problem in feeds for milking cows. Maize, peanuts and cotton are crops with which *A. flavus* appears to have an affinity, invading the crop before harvest. Small grains – wheat, barley and rice – do not appear to have such an affinity, and will contain significant aflatoxin levels only as the result of poor storage. When aflatoxin is fed to domestic animals, residues in animal products are generally low. In particular, ruminant animals detoxify aflatoxins quite efficiently. Levels in eggs and meat from monogastric animals may be higher, but in general toxicity to the animal limits feeding of excessively contaminated feed, so minimising residues (Stoloff 1977).

Counts of *Aspergillus flavus* in spices are often high, and concern has been expressed over the possible occurrence of aflatoxins in these commodities. Although the quantities of spices used in most foods are small, limiting any direct hazard, spices are used in the preparation of many types of foods, so high levels of *A. flavus* in spices are undesirable. Sterilisation of spices by chemicals or irradiation is now widely practised. The presence of *A. flavus* in spices such as pepper may result in infections in hospital patients (de Bock et al. 1989).

In developed countries, children often consume large quantities of milk, so the possible presence of aflatoxin M_1 in milk has been of concern. Stringent limits have been imposed on aflatoxin levels in feeds for dairy animals, and this has been an effective control measure.

People in developed economies, with effective regulatory controls, are seldom exposed to levels of aflatoxins that might lead to disease consequences. However, in less developed tropical countries, maize and peanuts are such an important food source that it has been reliably estimated that more than 5 billion people worldwide suffer from uncontrolled exposure to aflatoxins (Strosnider et al. 2006). Aflatoxins in staple foods constitute a huge public health problem, the magnitude of which has only recently been recognised (Wild and Gong 2010; Liu et al. 2012).

Environmental exposure to aflatoxins also occurs. High aflatoxin concentrations, >600 μg/kg, have been reported in airborne dust arising during the handling of contaminated maize and peanuts (Miller 1997 Sorenson 1999). A substantial fraction of the aflatoxin is contained in the conidia of *A. flavus* and *A. parasiticus* (Miller 1994). Aflatoxin from spores is readily absorbed into the lungs, with potentially detrimental effects on alveolar macrophage function (Miller 1994; Williams et al. 2004). Some evidence of elevated aflatoxin biomarkers in handlers of contaminated feed has been reported (Autrup et al. 1993). Aflatoxin can also be absorbed through the skin, thus people working in contact with materials infected with aflatoxins, e.g. peanuts or other crops, may be at risk of exposure.

Risk assessment modelling by Liao and Chen (2005) suggested little risk exists in maize harvest and handling, but relatively higher risk in feeding pigs or cleaning silos.

13.3.5 Risk Characterisation

In general terms, the risk from aflatoxin in developed economies is low (Liu et al. 2012). Commodities, especially peanuts, maize and cottonseed, are closely monitored and sorted to ensure the safety of foods and animal feeds. Ruminant microorganisms detoxify aflatoxins, so feeds for ruminant animals for meat production can contain quite high levels of aflatoxins, up to at least 300 μg/kg, without effect on the animal or leaving aflatoxin residues in the meat (Rodricks and Stoloff 1977). However, feeds for dairy cows must contain very low levels of aflatoxins to limit the risk of aflatoxin M_1 in milk. Nonruminants, such as poultry and fish, are sensitive to aflatoxins, so feeds must contain only low levels to prevent ill thrift. As a consequence, residues in poultry meat, eggs or aquaculture products are normally very low (Rodricks and Stoloff 1977).

In many tropical and subtropical areas of the world, peanuts and maize are grown and stored under suboptimal conditions of moisture and insect control, frequently leading to excessive aflatoxin levels. Concentrations of 100–1000 μg/kg are not uncommon. Control measures, such as good agricultural practice or sorting of defective grains or kernels are of value. However these are often neglected and, in any case, cannot prevent aflatoxin production in these commodities before harvest or during drying (Pitt et al. 2013).

13.3.6 Chemical Analysis

Analysis of aflatoxin levels in the main commodities in which these compounds occur has become standardised. Methods set out by AOAC International (AOAC 2019) are used throughout the world. These are briefly summarized below.

Updates on methodology are published frequently, for example Shephard et al. (2013) and Berthiller et al. (2014).

Sampling Aflatoxins are usually very unevenly distributed in commodities. Peanuts are a particular problem, as it is common for not more than 1 nut in 1000 to be contaminated, but contamination levels in individual nuts are often high, up to 1 mg/kg. Sampling is often the dominant source of error in aflatoxin assays. Sampling plans have been developed for continuous lines, for 10 tonne lots and for bag stacks. For peanuts, recommended methods generally use samples sizes of 8 kg or more.

The Food and Agriculture Organisation of the United Nations (FAO) has published an online document called the "FAO Mycotoxin Sampling Tool" (FAO 2015). This is a readily understood, hands on system for assessing the plan needed to provide a required level of confidence for sampling any major commodity. It is highly recommended.

Sample preparation Entire samples of 8 kg or more should be comminuted in a vertical chopper or similar mill. Subsamples are then further processed.

Extraction Extraction of subsamples, ideally of 500 g or more, employs a variety of mixed polar and nonpolar solvents, depending on the food matrix being analysed. Methanol: water (80:20) is recommended for commodities such as maize, peanuts and cottonseed.

Assays The traditional methodology of thin layer chromatography is still in use, and is recommended for less developed economies as it is inexpensive and reliable. For acceptable/not acceptable testing, immunochemical methods are most frequently used. For advanced users such as high volume analytical laboratories or regulatory authorities, liquid chromatography, sometimes coupled with mass spectroscopy has become normal practice. Limits of detection are now below 1 μg/kg.

13.3.7 Levels in Foods

The universal occurrence of *Aspergillus flavus* and aflatoxins in commodities such as peanuts, maize and cottonseed has prompted a great deal of work on causes, formation and prevention; work too voluminous to report in detail here (for reviews see Diener et al. 1987; Payne 1992; Sinha and Bhatnagar 1998; Leslie et al. 2010). Early studies assumed that invasion was primarily a function of inadequate drying or improper storage and this is still the case in many regions. However more recent work has shown that invasion before harvest is also important and is dependent primarily on plant stress induced by drought and/or high soil temperatures (Pitt et al. 2013). This is especially true in developed countries. This particular problem can be overcome by irrigation, but this is not a practical solution in most regions, where peanuts are grown under dry culture.

Other closely watched commodities are tree nuts, especially pistachios and brazil nuts, figs and spices. However, although levels in these latter commodities may exceed regulatory limits from time to time, such levels seldom pose a long term risk to human health. Under inadequate storage conditions, other grains including sorghum and rice may also permit growth of *Aspergillus flavus* and aflatoxin production. These commodities have very high consumption levels in many communities, so adequate storage is of great importance.

Good agricultural practice has been the standard method in developed countries for attempting to control aflatoxin levels in commodities, particularly maize and peanuts. Good agricultural practice involves careful farm management, including weed control, optimal row and plant spacing, insect control, adequate water supplies, rapid and complete drying, removal of defects and effective control of storage conditions. By themselves, these approaches are inadequate: too

often drought stress and/or insect damage results in aflatoxin formation before harvest, out of farmer control, even in developed countries. The only effective HACCP for aflatoxins remains end product testing (Pitt et al. 2012, Chap. 9).

13.3.8 Management of Aflatoxins in Foods

Regulatory limits Regulation of aflatoxin levels in foods commenced in 1971, using the then limit of detection, 5 μg/kg, as the permitted limit. It soon became clear that peanut producers could not reach that limit, even in developed economies, so higher limits were set up in peanut exporting countries including the USA and Australia. As analytical techniques improved with the introduction of liquid chromatography, lower limits were frequently set by importing countries, especially in Europe. Many countries have set limits for aflatoxins in maize and peanuts of 20 μg/kg, sufficiently low to protect populations at the level of less than 1/10,000 additional lifetime cases of liver cancer (Wu et al. 2013).

Reducing aflatoxin levels Reduction in aflatoxin levels in peanuts is accomplished by colour sorting of individual kernels after shelling. The process was developed originally to reject commercially unacceptable discoloured nuts, regardless of cause, but as fungal growth is a prime cause of discolouration, the process is also an effective nondestructive means of removing nuts containing aflatoxins. Maize and fig samples are screened for the presence of aflatoxin by the examination of cracked kernels or fruit by ultraviolet light, although the technique has limited reliability, especially in the tropics. Effective nonchemical testing techniques are still being sought for cottonseed or pistachios which, like peanuts, are naturally fluorescent. Accurate nondestructive assays are not available for any commodity.

It is normal practice to assay aflatoxin levels in all consignments of peanuts and maize in major developed producing countries, often repeatedly, from intake to shellers to final product. Such controls rarely exist in less developed countries. Other commodities are similarly screened according to needs and markets.

Interventions In recent years, a variety of approaches have been put forward that aim to reduce aflatoxin levels in foods and feeds before harvest, or to reduce the effects in human or animal consumers. These approaches are summarised in Pitt et al. (2012, Chap. 9) and the economics of the various processes have been discussed by Khlangwiset and Wu (2010).

The most advanced of these interventions is biocontrol by competitive exclusion, for peanut and cotton crops, and more recently, maize. The technique was developed independently in the USA and Australia. As only about 40% of *Aspergillus flavus* strains produce aflatoxins, it is not difficult to select nontoxigenic strains that are both competitive in the field and unlikely to revert to toxicity. High numbers of spores of such strains are introduced into soils in fields where peanut or cotton crops are being grown (Dorner et al. 1992, 1998; Garber and Cotty 1997; Pitt 2004; Pitt and Hocking 2006; Dorner and Horn 2007). The nontoxigenic spores compete with the existing toxin producing spores in the soil for infection sites on developing nuts. When sufficiently high numbers are produced, control can be very effective. However, dry soil or heavy rain can limit effectiveness (Pitt et al. 2015). Biocontrol by competitive exclusion is used commercially in the USA for peanut and cotton crops and under investigation for some tree nuts (Doster et al. 2014). Biocontrol is being introduced on a large scale on maize crops in Africa using the process known as “Aflasafe” (Atehnkeng et al. 2008, 2014) Bandyopadhyay et al. 2019). Claims of high efficacy should be viewed with caution. Sampling systems used to evaluate efficacy in that work have not been shown to have statistical validity: indeed it is not clear that any feasible sampling plan can prove the efficacy of biocontrol on small scale farms (Pitt 2019).

Nixtamalization, an alkaline cooking process which has been used for centuries in Mexico and other Central American countries, is very effective in reducing aflatoxin levels in maize (Torres et al. 2001; Méndez-Albores et al. 2004).

Another approach has aimed to improve farm efficiency, by providing advice and improved management for farmers in less developed countries. Some success has been achieved (Turner et al. 2005). Forecasting of weather patterns conducive to preharvest aflatoxin formation in peanuts, enabling advice to farmers on optimal harvest dates, has been effective in Queensland, Australia (Chauhan et al. 2010).

Following the discovery that a particular smectite clay, known as NovaSil, binds aflatoxins in the gut, the addition of low levels of it to animal feeds has been shown to reduce aflatoxin toxicity very effectively (Pimpudkee et al. 2004). Some human trials have also yielded promising results (Phillips et al. 2008). It is unclear whether this approach will be of value in practice.

Some naturally occurring compounds are believed to be active against chemically induced cancers. Polyphenols from green tea and chlorophyllin, a derivative of chlorophyll, may have application here as dietary supplements (Groopman et al. 2008).

13.4 Ochratoxin A

Ochratoxin A is produced by three well defined groups of fungi. The first group, which may be termed the ochre coloured Aspergilli, comprises *Aspergillus ochraceus, A. westerdijkiae, A. steynii* and a few other (much less important) related species. This group is largely responsible for ochratoxin A production in coffee, and also in long stored grains. The second comprises two of the black spored Aspergilli, *A. carbonarius* and (much less frequently) the closely related and much more common *A. niger*. These species are responsible for most ochratoxin A formation in grapes and grape products and, in some regions, in coffee. The third includes two *Penicillium* species, *P. verrucosum* plus the closely related *P. nordicum*. The well documented occurrence of ochratoxin A in temperate climate cereals results from growth of *P. verrucosum*. *P. nordicum* has been reported to produce ochratoxin A in cool stored processed meats.

13.4.1 History

Ochratoxin A was originally described as a metabolite of *Aspergillus ochraceus* from laboratory experiments on fungal toxicity (van der Merwe et al. 1965) and also from *Penicillium viridicatum* soon afterwards (van Walbeek et al. 1969). Production was then detected from several other *Aspergillus* species (Ciegler 1972). Natural occurrence was first reported in 1969 (Shotwell et al. 1969). Practical importance, in the aetiology of kidney disease in Scandinavian pigs, was first linked with *P. viridicatum* (Krogh et al. 1973), later correctly identified as *P. verrucosum* (Pitt 1987). Ochratoxin A was soon characterised as a potent kidney toxin (Krogh et al. 1973). The serum adduct of ochratoxin A was found to be common in the blood of pigs (Hult et al. 1980) and then humans (Golinski and Grabarkiewicz-Szczesna 1985).

Similarities between symptoms resulted in suggestions that ochratoxin A was a likely cause of Balkan endemic nephropathy, a kidney disease which had a high mortality rate in post World War II years in certain areas of Bulgaria, Yugoslavia, and Romania (Barnes et al. 1977; Krogh et al. 1976; Austwick 1981; Mead 2007). However, recent work indicates that this syndrome is likely to result from contamination of wheat grains with seeds of the endemic weed *Aristolochia clematitis*, which commingle with the wheat before harvest (Grollman et al. 2007; Bui-Klimke and Wu 2014). The postulated causal compound, aristolochic acid, is a potent renal toxin and a Class I human carcinogen (IARC 2002). For further details on the history of ochratoxin A research see Pitt and Miller (2017).

13.4.2 Chemical Characteristics

Ochratoxins are derived from a dihydroisocoumarin ring linked to phenylalanine through a 7-carboxy group (Fig. 13.2). Several ochratoxins are known: ochratoxin A, its ethyl ester ochratoxin C and some dechlorinated analogues,

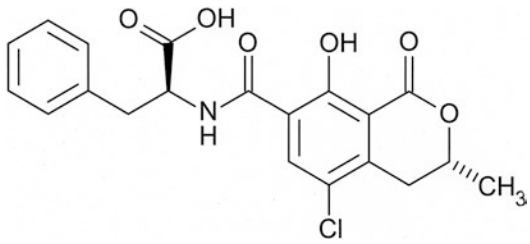

Fig. 13.2 Structure of ochratoxin A

specifically ochratoxin B and its methyl and ethyl esters. Only ochratoxin A is of importance. It has the CAS no. 303-47-9.

13.4.3 Hazard Identification

Ochratoxin A is an acute and chronic nephrotoxin, affecting kidney function in all animal species tested, causing damage to a variety of kidney tissues. Ochratoxin A also has immunosuppressive, teratogenic, and genotoxic effects in animals. Its effects in humans, if any, remain elusive. For reviews see EFSA (2006, 2020), WHO (2008) or Bui-Klimke and Wu (2015). Ochratoxin A is readily absorbed through the intestines, and once it enters the blood stream, has a long half life, up to 3 weeks in monkeys and 4 weeks or more in human volunteers (Malir et al. 2012). As a consequence, the blood of healthy humans may contain detectable levels of ochratoxin A in areas where it is frequently ingested in the diet (JECFA 2001). This issue is confined to cool temperate regions because *P. verrucosum* has a restricted distribution. Recent indications are that levels in Europe have been declining in recent years due to increased regulation (EFSA 2020). Ochratoxin A in human blood from other parts of the world is almost certainly due to the presence of ochratoxigenic *Aspergillus* species in foods.

Ochratoxin A also has carcinogenic properties, and has been classified by the International Agency for Research on Cancer as a possible human carcinogen (IARC 1997). However, the mechanism of carcinogenicity remains unknown.

13.4.4 Exposure Assessment

Ochratoxin A occurs in beer, wines, coffee, cocoa, chocolate and dried vine fruits, so people everywhere are exposed to low levels of this toxin. Populations in Europe have been exposed to higher levels in barley and wheat and their products, especially bread, and also from meat, especially pork, derived from animals fed contaminated feed (Krogh et al. 1973 1976; Lund and Frisvad 2003; Olsen et al. 2006; Duarte et al. 2010; Wu et al. 2014). Stringent regulations governing levels of ochratoxin A in European foods and feeds has led to a sharp reduction in this issue. As wheat and barley crops from warmer climates such as the USA, South Africa and Australia are not infected by *P. verrucosum*, ochratoxin A intake is lower in regions outside Europe. A recent study has shown that the presence of ochratoxin A in Canadian wheat is due to poor silo construction and a minor issue on the commercial scale (Limay-Rios et al. 2017).

A large number of studies have now been published on ochratoxin A levels in human blood (for a review see Malir et al. 2012), but the correlation between blood level and measured food intake is uncertain. Several studies have shown that urinary biomarkers correlated well with measured dietary intakes, while no correlation was observed with serum assays (Gilbert et al. 2001); Ali et al. 2017; Collins et al. 2021).

13.4.5 Risk Characterisation

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended, then maintained, a Provisional Tolerable Weekly Intake (PTWI) of ochratoxin A at 100 ng/kg bw (JECFA 2001). This is based on the lowest observed level of chronic toxicity in pigs, as risk assessments have indicated that chronic toxicity occurs at lower intake levels than any carcinogenic effect (WHO 2008). A more recent study found that ochratoxin A was negative in highly specific genotoxicity assays (Haighton et al. 2012). The European Food Safety Authority has

recommended a PTWI of 120 ng/kg bw (EFSA 2006).

JECFA has assessed the dietary exposure to ochratoxin A in Europe from processed cereals as 8–17 and EFSA as 7–10 ng/kg bw per week, both well below the PTWI. Even with some additional intake from wines and coffee, the hazard from intake of ochratoxin A in Europe appears to be acceptable. Intake in other parts of the world are lower in the absence of appreciable levels in cereals. A review of the evidence concluded that no human health risk from ochraroxin A was evident, except perhaps for sufferers from nephritic syndrome (Bui-Klimke and Wu 2015). A recent risk assessment found that oats (consumed as oatmeal porridge) was the highest source of ochratoxin A in the diets of both adults and children in the USA, but in all cases intake was well below JECFA guidelines (Mitchell et al. 2017). Chen and Wu (2017) have warned of the possibility that ochratoxin A may be synergistic with other human kidney disorders such as diabetes and obesity, although little evidence was presented to support this.

13.4.6 Chemical Analysis

Analysis of ochratoxin A from foods is complicated by the facts that (a) it is unstable in alkali, which causes lactone ring opening, so any subsequent purification on an immunoaffinity column will be inaccurate and (b) ochratoxin A binds to protein, so purification by protein precipitation will also lead to serious inaccuracy.

Extraction The AOAC International standard method uses phosphoric acid for acidification before extraction with chloroform (Chung and Kwong 2007; AOAC 2019). Acetonitrile: water is also used.

Purification In the AOAC method, purification is carried out by absorption on a sodium bicarbonate/diatomaceous earth column, washing with hexane then chloroform, followed by elution using acetic acid/benzene. Immunoaffinity columns are also recommended.

Assays The traditional methodology of thin layer chromatography is still in use, and is recommended for less developed economies as it is inexpensive and reliable. A suitable developing solvent is benzene: methanol: acetic acid (18: 1: 1), with visualisation under UV light. Confirmation is blue fluorescence after exposure to ammonia vapour or spraying with ethanolic sodium bicarbonate.

More modern methods for analysis are based on high performance liquid chromatography, with or without mass spectrometry.

13.4.7 Levels in Foods

The crops and foods affected by fungi producing ochratoxin A are quite specific. Ochratoxin A is produced in cool temperate climate cereals (wheat, barley and oats) by *Penicillium verrucosum* and more rarely at low levels in refrigerated meat products by the closely related *P. nordicum.* It is produced by *Aspergillus ochraceus* and related species, and by *A. carbonarius* and (to a much lesser extent) by *A. niger*, during drying or processing of coffee and cocoa, where the dominant causative fungus varies from region to region. Grapes are susceptible to infection by *A. carbonarius* just before or after harvest, due to skin splitting from rain, mechanical damage or infection by pathogenic fungi, especially *Rhizopus stolonifer* or powdery mildews. Ochratoxin A may be produced just before harvest, during drying of raisins and other dried fruits, or in grape juice before crushing in wine making. For details see individual fungal species.

Levels of ochratoxin A in foods on a worldwide basis have been reviewed by Duarte et al. (2010) and Wu et al. (2014). Lee and Ryu (2015) analysed over 450 breakfast cereal samples from the USA and found that 40% were contaminated with ochratoxin A. However, only 16 samples, all oat cereals, exceeded 3 μg/kg and none exceeded 10 μg/kg. The impact on human health would be very low. A more recent study of over 500,000 samples of cereals and nuts obtained world wide

showed that although ochratoxin A was present in >25% of samples, levels were mostly very low (Eskola et al. 2020).

13.4.8 Management of Ochratoxin A in Foods

Regulatory limits The Codex Alimentarius Commission recommended a limit of 5 μg/kg of ochratoxin A in cereals and cereal products (CAC 1998), and a number of countries have accepted this limit, though it is still under discussion by the Commission. The European Union has set a limit of 5 μg/kg of ochratoxin A in unprocessed cereals and roasted coffee beans, 3 μg/kg in processed cereals and 10 μg/kg in dried vine fruits and instant coffee. In wines and grape juice, the limit has been set at 2 μg/kg, while infant foods must not contain more than 0.5 μg/kg (EFSA 2006).

Preharvest control Control of ochratoxin A levels prior to processing relies on good agricultural practice (Pitt et al. 2013). As none of the fungi that produce ochratoxin A are known to be systemic invaders or pathogens, the key to low levels in foods is rapid drying followed by maintenance of low moisture, regardless of the crop. The problem with cereals in cool European and Canadian climates is the accomplishment of rapid drying and then prevention of moisture migration in silos (Olsen et al. 2006; Limay-Rios et al. 2017). Coffee is grown under conditions often conducive to mist or rain at harvest, and again effective drying is the key to minimising ochratoxin A levels (Taniwaki and Pitt 2013). For dried grapes and wines, good agricultural practice is key to ochratoxin A control. This includes disease and insect control, avoidance of mechanical damage before drying, rapid harvest and crushing for wines, and selection of cultivars resistant to splitting in late preharvest rain (Leong et al. 2006).

Processing Processing of wheat to make flour reduces ochratoxin A levels, though the process used greatly influences final levels in bread. Milling hard wheat to produce white flour produced ca 65% reduction in ochratoxin A, and a further 10% decrease occurred during baking. Wholemeal flour and bread showed much less reduction in ochratoxin A during processing, as might be expected, because less of the grain is discarded (Osborne et al. 1996).

Like most mycotoxins, ochratoxin A is relatively heat stable. In dry wheat, 50% inactivation at 100, 153, 200 and 250 °C required 707, 201, 12 and 6 min, respectively. In moist wheat, the corresponding inactivation times at 100, 150 and 200 °C were 145, 64 and 19 min, respectively. Complete destruction did not occur even at 250 °C (Boudra et al. 1995).

Processing coffee beans, including roasting, drink preparation and instant coffee production all reduce ochratoxin A levels in coffee. Dark roasting may remove up to 98% of ochratoxin A, but light roasts have much less effect (Ferraz et al. 2010).

Ochratoxin A is largely removed during the wine making process as it is bound to solid fractions and sediment. Some fining agents can also reduce ochratoxin A levels (Taniwaki and Pitt 2013). Red wines retain slightly more ochratoxin A than white wines. Overall, the carryover from grapes into finished wine is between 1% and 8% (Leong et al. 2006).

13.5 Fumonisins

Fumonisins are produced by *Fusarium verticillioides* (formerly known as *F. moniliforme*), by the closely related species *F. proliferatum,* uncommonly by *F. subglutinans* and *F. oxysporum*, and about 10 other species not treated here. The first two named species are by far the most important. *F. proliferatum* is one cause of maize ear rot in Europe, and is a pathogen on a variety of other crops. *F. verticillioides* is endemic in maize, and found wherever that crop is grown. This species grows well at higher temperatures. Ear rot and fumonisin accumulation are associated with drought, insect stress and growing hybrids outside their areas of adaptation (Miller 2001, 2008). All aspects of fumonisin occurrence, production and management have been comprehensively reviewed by Braun and Wink (2018) and Kamle et al. (2019).

It has been shown relatively recently that fumonisins can also be produced by *Aspergillus niger*, a totally unexpected discovery (Frisvad et al. 2007). The practical significance of this remains unclear. See Chap. 8, *Aspergillus niger*, for further information.

13.5.1 History

A lethal and widespread neurological disease in horses, mules and donkeys in the USA was reported from at least 1850 and by 1910 it was suspected to be associated with the feeding of maize. The disease was named leucoencephalomalacia, and was subsequently found to occur very widely, in countries as far apart as the USA, Mexico, South Africa, New Caledonia and China (Pitt and Miller 2017). However the cause remained elusive. In the 1970s, South African scientists recognised that it was due to a mycotoxin and, after 20 years of work, were able to isolate and characterise a new class of toxins, the fumonisins (Wilson and Maronpot 1971; Gelderblom et al. 1988). This was published independently (as macrofusin) by scientists in New Caledonia the following year (Laurent et al. 1989). Both groups of workers were able to demonstrate that fumonisins were directly responsible for the horse deaths by administering or feeding the pure compound to horses (Marasas et al. 1988).

About 1990, a large scale, often fatal, outbreak of pulmonary disease in southeastern USA pigs occurred. South African experiments in the 1980s had indicated that maize containing fumonisins could cause such a syndrome. Experimental work soon confirmed that fumonisins were responsible.

For many years, the species primarily responsible for fumonisin production was known as *Fusarium moniliforme*. However, due to confusion over the application of that name to a number of distinct species, in 2003 taxonomists agreed to solve the problem by taking up the older name *F. verticillioides* for the major fumonisin producer (see Chap. 5, *Fusarium*).

13.5.2 Chemical Characteristics

Fumonisins are a family of compounds made up of a 19 or 20 carbon chain aliphatic acid backbone with amino or polyhydroxy side groups, two of which are esterified with propane tricarboxylic acid. This results in a hydrophobic/hydrophilic dichotomy unique among the mycotoxins. Fumonisins are analogues of sphingosines, essential components of cell membranes. Fumonisins have been reported to interfere specifically with dihydroceramide, an essential enzyme in the biosynthesis of sphingosine and related compounds (Wang et al. 1991).

The most important fumonisin is known as fumonisin B_1 (CAS 116355-30-0), and has a molecular formula of $C_{34}H_{59}NO_{15}$, with a molecular weight of 721 (Fig. 13.3). Most naturally occurring fumonisins belong to the B series, but some members of the C series, identical to the B series but with the terminal methyl group absent, have also been found in maize. Other much less common fumonisins, designated variously as A and P series, have been isolated from pure fungal cultures.

13.5.3 Hazard Identification

Fumonisins are remarkable for the wide range of effects caused in animals and possibly in man. Fumonisins act by inhibiting the enzyme ceramide synthase, which causes accumulation of intermediates in the sphingolipid metabolism pathway, and also causes depletion of complex sphingolipids. This inhibition interferes with the

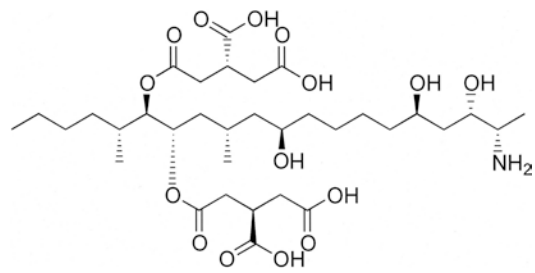

Fig. 13.3 Structure of fumonisin B_1

binding of folate and some other proteins in cell membranes. The most dramatic effect occurs in horses and other equines, where the disease called equine leucoencephalomalacia occurs. This is a rapidly progressing disease that causes equine brains to liquefy. For horses, consumption of feed containing >10 mg/kg fumonisin B_1 in the diet (equivalent to 0.2 mg/kg body weight per day) was associated with increased risk of developing this disease (JECFA 2018).

In pigs, fumonisins cause pulmonary oedema, due to left ventricle heart failure (JECFA 2018), while in rats the primary effect is to cause liver cancer (JECFA 2018). Fumonisins can also cause programmed cell death (apoptosis; Tolleson et al. 1996). It is unusual for a single toxin to have such diverse effects in different animal species, but careful investigations have confirmed that. The reasons for such diversity have recently been clarified to a large extent (JECFA 2018).

In humans, fumonisins and *Fusarium verticillioides* are associated with oesophageal cancer. Extensive studies in areas of low and high maize consumption in South Africa have established this connection (Sydenham et al. 1990; Rheeder et al. 1992; JECFA 2001). This disease is also prevalent in areas of China and occurs at significantly higher levels than background also in parts of Iran, northern Italy, Kenya, and a small area of the southern USA (JECFA 2001). In all of those areas consumption of maize and maize products is very high (JECFA 2001). Evidence has been increasing that fumonisins are associated with neural tube defects such as spinal bifida in areas of Guatamala, South Africa and China, in a population along the Texas – Mexican border (Desjardins et al. 2006), and in childhood stunting in Sub-Saharan Africa and Central America (Chen et al. 2018).

13.5.4 Exposure Assessment

The primary source of fumonisins is maize. *Fusarium verticillioides* is endemic in maize, so fumonisins are of universal occurrence wherever maize is grown. Unacceptable levels occur only in plants that are drought stressed near harvest dates or where cobs are extensively damaged by insects. Intake can be high in countries where maize is a dietary staple, notably in Africa, some South and Central American countries and parts of China. Urinary biomarkers have been shown to be very effective for assessing fumonisin intake (Riley et al. 2012; van der Westhuizen et al. 2013).

The discovery of fumonisin formation by *Aspergillus niger* means that fumonisins occur in many other food commodities, including grapes, wine, dried vine fruits and probably coffee and cocoa. However, it seems likely that levels in these foods do not add significantly to exposure. For a detailed overview of the incidence of fumonisins in foods world wide see JECFA (2018).

13.5.5 Risk Characterisation

For the human population, a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight per day has been established by JECFA (2001). This PTMDI was maintained by JECFA at a meeting in 2011. The European Commission has established the same tolerable daily intake for fumonisins B_1, B_2 and B_3, alone or in combination.

13.5.6 Chemical Analysis

Analytical techniques have concentrated on the estimation of fumonisin B_1, as it is the most commonly occurring form. Screening methods in foods were based on thin layer chromatography, but ELISA techniques are now more commonly used for fumonisin analysis. Although antibodies are raised against fumonisin B_1, cross reactivity usually occurs with fumonisins B_2 and B_3 as well (Bird et al. 2002). Commercial kits use a range of methods and are available from several manufacturers.

For analysis by HPLC, fumonisins lack suitable chromophores, so must be derivatised and detected by fluorescence. The most common derivatisation is with an ortho-phthalaldehyde precolumn. Liquid chromatography–mass spectrometry is increasingly being used, as derivatisation is not necessary (Silva et al. 2009).

13.5.7 Levels in Foods

The occurrence of fumonisins in foods due to growth of *Fusarium verticillioides* and *F. proliferatum* is of significance only in maize. Fumonisins have been detected in a variety of cereals due to other *Fusarium* species, and due to *Aspergillus niger* in grapes, wines and other commodities, but levels are usually low. Excessive fumonisin levels occur when maize plants are drought stressed or suffer extensive insect damage. Maize is a widely consumed cereal and a dietary staple in many regions of the world, i.e. in parts of Africa, South and Central America and China. Drought stress occurs quite commonly in all of these regions, hence levels in foods frequently exceed regulatory limits. Maize consumption has increased greatly in some parts of Africa over the past 50 years, replacing traditional crops such as sorghum and millet which had much lower susceptibility to fumonisin (and aflatoxin) contamination (Pitt and Miller 2017). Consumption of undesirable levels of fumonisins occurs quite widely in those regions (Shirima et al. 2013; Schatzmayr and Streit 2013).

13.5.8 Management of Fumonisins in Foods

Regulatory limits For unprocessed maize, the European Commission has set maximum permitted limits of 4 mg/kg total fumonisins and for maize based foods and baby foods after processing, the limit is 200 μg/kg fumonisins (EC 2007). The US Food and Drug Administration has set guidelines for fumonisin levels in processed foods: degermed dry milled maize products, 2 mg/kg total fumonisins; dry milled maize bran, 4 mg/kg and cleaned maize for popcorn, 3 mg/kg.

Preharvest control Fumonisins are produced in maize preharvest. Drought stress is the major factor causing production, though insect damage may also be important. Good agricultural practice, irrigation and the use of Bt cultivars are all important in limiting fumonisin formation (Pitt et al. 2013). Some progress has been made in breeding cultivars resistant to ear rot due to *F. verticillioides*. Biological methods for decreasing fumonisins have been reviewed by Alberts et al. (2016).

Freshly harvested maize should be rapidly dried to a stable moisture level. In practice fumonisin accumulation ceases after the initial stages of drying because *Fusarium* species grow only very slowly below 0.9 a_w, which point normally occurs during field drying before harvest. For the same reason, fumonisins will not be produced in storage. Even if very high moisture occurs due to water ingress, competition with other microorganisms at high water activities will prevent any significant increase in fumonisin levels.

Processing In most geographical areas, the main methods for controlling fumonisin levels are visual inspection of lots for fungal damage, followed by fumonisin analyses and rejection of lots that do not meet specifications.

In Central America, the process of nixtamalization is commonly used in preparation of meals based on maize. Nixtamalization is a centuries old process in which maize is soaked then cooked with ash or lime high in alkali. It removes almost all fumonisins (and aflatoxins), resulting in tortillas and other maize based foods being substantially free of these mycotoxins (de la Campa et al. 2004).

Maize is wet milled to obtain maize starch, germ and fibre, whereas dry milling produces bran, germ, and fractions of decreasing particle size – grits, corn meal and flour. Fumonisins are not destroyed during these processes and are found in all fractions, with higher concentrations in bran and germ (Brera et al. 2003; Pietri et al. 2009).

Processing at temperatures above 150 °C reduces fumonisin levels. Maize meal production, frying, baking, roasting and alkaline cooking all have effects, dependent on the temperature actually attained. Extrusion processing is used extensively in the production of breakfast cereal, snack and textured foods based on maize meal. Extrusion temperatures of 160 °C or higher have a significant effect, especially if glucose is included (Bullerman and Bianchini 2007).

13.6 Deoxynivalenol and Nivalenol

Deoxynivalenol (DON) and nivalenol are trichothecene toxins produced by *Fusarium graminearum* (often reported as *Gibberella zeae*, its sexual stage), *F. culmorum* and less commonly other related species. *Fusarium graminearum* occurs in maize, and both *F. graminearum* and *F. culmorum* in small grains, especially wheat and barley. These species are rank pathogens, invading plants and grains by causing diseases, known as Gibberella ear rot in maize and Fusarium head blight in wheat, barley and triticale. Epidemics of Gibberella ear rot require the congruence of three factors: airborne or insect-borne spores, inoculation at the susceptible time, and appropriate moisture and temperature. This disease is prevalent in northern temperate climates, especially in wet years, but is much less common in the tropics. Fusarium head blight affects all commercial cultivars of wheat and barley. *F. culmorum* always produces DON, but whether DON or nivalenol is produced by *F. graminearum* depends on the geographical origin of the fungal strain. Indeed the situation concerning toxin production by *Fusarium graminearum* is even more complex (see Chap. 5, *Fusarium graminearum*). Like all trichothecene mycotoxins, DON and nivalenol are inhibitors of protein synthesis (Feinberg and MacLaughlin 1989). Nivalenol is much more toxic than DON, but is produced in much lower quantities in grains, and is considered a less significant mycotoxin (Miller et al. 2001).

13.6.1 History

The consumption of grain damaged by what is now known as Fusarium head blight has been known to cause human toxicosis, called Akakabi-byo, in southern Japan since about 1890. Symptoms include nausea, vomiting, diarrhoea, abdominal pain, fever and throat irritation. Similar diseases have been reported from China and Korea also (Yoshizawa 1983, 2013). Issues with Fusarium head blight in the United States were known from the same period and by 1920 feed refusal by pigs had become a major concern. Feeding trials soon established the source as blighted wheat grains and the source of toxicity as *Fusarium graminearum.* This was confirmed by experimental inoculation and feeding (Pitt and Miller 2017).

Identification of the toxin or toxins involved had to wait for improvements in chemical techniques. In the 1970s the isolation and structural characterisation of several trichothecene toxins took place in the USA and Japan. In the USA, DON, then termed vomitoxin, was shown unequivocably to be the cause of the feed refusal by pigs (Vesonder and Hesseltine 1981), while in Japan both DON and nivalenol were seen as the likely causes of Akakabi-byo (Yoshizawa and Morooka 1973). In the 1990s, Canadian scientists discovered the full range of toxins produced by *F. graminearum* and that DON was produced by two distinct pathways in different species (Miller et al. 1991, 2001).

By the turn of the 21st century, more than 100 trichothecene molecules were known to occur naturally, produced by a number of plant pathogenic fungal genera. Of these, *Fusarium* is by far the most important trichothecene source. Production of DON and sometimes nivalenol by *F. graminearum* and *F. culmorum* are the main sources of trichothecenes in foods and feeds, and are the main causes for concern.

13.6.2 Chemical Characteristics

DON belongs to the family of chemicals known as the trichothecenes, sesquiterpenoid compounds that are characterized by a 12,13 epoxy ring. At least 100 trichothecene molecules are known, differentiated by hydroxy or acetyl groups and side chains. DON, still sometimes known as vomitoxin in the USA, is 12,13-epoxy-3,7,15-trihydroxy-trichothec-9-en-8-one, CAS number 51481-10-8 (Fig. 13.4a). It is the most commonly produced trichothecene in foodstuffs. Nivalenol, less commonly produced but more toxic, differs from deoxynivalenol by the substitution of a hydroxy group for the hydrogen atom

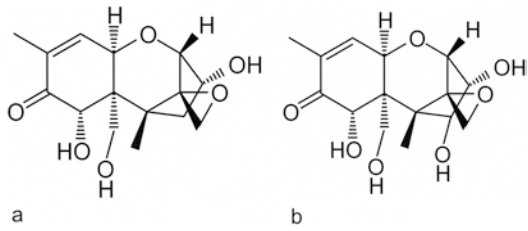

Fig. 13.4 Structures of trichothecenes: (**a**) deoxynivalenol; (**b**) nivalenol

at the C-4 position (Fig. 13.4b). The most toxic trichothecene is known as T-2 toxin. It differs from deoxynivalenol in several positions: at C-4, an acetyl ester in place of H; at C-7, H in place of OH; at C-8 an isovalerate ester in place of O; and at C-15, an acetyl ester in place of OH.

13.6.3 Hazard Identification

Trichothecenes are potent inhibitors of protein synthesis. DON and other trichothecenes bind to ribosomes, interfering with normal ribosomal function by causing dysregulation of various proteins related to immune function and sometimes apoptosis. Toxicity of the particular molecule varies with conformation, and that depends on the particular side groups on the molecule (Feinberg and MacLaughlin 1989; Pestka 2008).

DON is a neurotoxin (Prelusky et al. 1992) whih can cause inflammation in the brain (Bonnet et al. 2012) and anorexia (Flannery et al. 2012). It can also cause growth retardation (Amuzie and Pestka 2010). DON is especially toxic to pigs, as at quite low intake levels (<5 mg/kg in feed) it causes vomiting, feed refusal and reduced weight gain. Cattle and poultry are resistant to reasonable levels of DON (>5 mg/kg in feed) (Miller et al. 2001). In humans, high doses of DON may cause abdominal pains, dizziness, headache, nausea, vomiting and other effects. Cases of such acute poisoning are rare, but have been recorded from India (Bhat et al. 1989), Japan (Udagawa 1988) and several outbreaks in China (JECFA 2011).

The most comprehensive study of DON including occurrence, toxicity, analytical methods and intake levels is that by JECFA (2011), who concluded that DON is not carcinogenic, mutagenic or teratogenic. Based on the neurotoxic effects in pigs, JECFA set a provisional tolerable daily intake of 1 μg/kg weight per day, but were unable to estimate the level of DON in foods below which any acute effect in humans could occur.

13.6.4 Exposure Assessment

DON occurs world wide in maize, wheat and sometimes other small grains due to growth of *Fusarium graminearum* and related species (Bianchini et al. 2015; Mishra et al. 2020). Consumption of maize containing excessive levels of DON has been associated with numerous incidents of poisoning in China and India. Tens of thousands of people have sometimes been affected. Recovery is usually complete.

Wheat tolerant to Fusarium head blight conjugates DON with a sugar to form a glucoside which helps to protect the plant (Miller and Arnison 1986; Boutigny et al. 2008), but is not analysed by the usual methods. Levels of deoxynivalenol glucoside in both European and Canadian grains have now become very high (Zhang et al. 2019). DON glucoside is much less toxic than DON in model systems, but the sugar is cleaved off by the gut microbiome in both humans and pigs (Gratz et al. 2018). For this reason, 3-deoxynivalenol-glucoside has been added to the TDI that applies in Europe (EFSA 2017). However, where the *F. graminearum* genotype 15-acetyldeoxynivalenol dominates, in much of the USA and Canada, the major glucoside is not measured (Renaud et al. 2019).

13.6.5 Risk Characterisation

The main risks from DON for humans are a range of neurological effects: anorexia, brain inflammation, headaches, nausea, etc. In one episode in India, DON levels in wheat ranged from 0.4 to 8.4 mg/kg, while in China, poisoning was linked to wheat contaminated with 0.3–100 mg/kg DON. In consequence, it has been suggested that

acute toxicity may occur from exposures in the low mg/kg range.

A potential risk to farm workers exists from DON inhalation. Grain dusts may contain quite high concentrations of DON. Air samples from Canadian grain elevators contained up to 2.6 μg/m^3 of DON, and airborne dust from the same sources up to 5.8 mg/kg of DON, plus smaller amounts of T-2 toxin. Some evidence has been reported that grain farming may be associated with midterm pregnancy deliveries in northern Europe.

13.6.6 Chemical Analysis

ELISA techniques are commonly used for analyses for DON, but the situation with commonly used ELISA kits is problematic. The available kits do not respond, or respond variably, to some naturally formed derivatives of DON (Nguyen et al. 2019). Effective assay requires gas chromatography and mass spectroscopy (Mirocha et al. 1999; Romagnoli et al. 2010). Extraction may use chloroform–ethyl acetate or 70% methanol, and clean up is accomplished by filtering through a C18 or silica gel column (AOAC 2019).

13.6.7 Levels in Foods

DON is found in maize and small grains in all areas where these crops are grown. It is especially prevalent in cooler areas where rainfall is higher, such as Canada and Argentina (Bianchini et al. 2015) and occurs less commonly in drier, hotter areas such as Australia (Pacin et al. 2011). Levels of DON and other related trichothecenes are usually lower than 1 mg/kg in foods (Bianchini et al. 2015), but can be much higher, potentially causing poisoning episodes (see Sect. 13.6.5). A number of studies have shown that fungal infection rates are higher in crops planted in fields previously planted with maize, particularly when residues from those crops were left in the field. Once grains are dried, increases in levels of *Fusarium* mycotoxins rarely occur (Pitt et al. 2013).

Favourable weather conditions are critical for infection to occur in wheat heads. Field observations has confirmed that lower temperatures and moist conditions during heading and anthesis are the major factors of importance (Schaafsma et al. 2001; Bianchini et al. 2015). Wheat cultivars also show variable susceptibility (Bianchini et al. 2015).

13.6.8 Management of Trichothecenes in Foods

The US Food and Drug Administration has issued guidelines for DON in foods and feeds as follows: for finished wheat products that may be consumed by humans, 1 mg/kg; for grains and byproducts for feedlot and dairy cattle, 10 mg/kg, except that for dairy cattle the total DON content in feed should not exceed 5 mg/kg; in feed for pigs, 5 mg/kg, but not exceeding 20% of the total diet; and for all other animals, 5 mg/kg, not exceeding 40% of the total diet. Based on that guideline and European consumption of grains and grain based foods, EFSA (2017) concluded that the risk from DON and it derivatives pose little human or animal health risk in Europe. However, based on a review of global analyses of DON in wheat, Chen et al. (2019) have cautioned that some populations in countries with very high wheat consumption may exceed that guideline. In a study using human volunteers, Vidal et al. (2018) showed that the majority of ingested DON or its masked glucoside are excreted by humans within 24 h.

Some success has been achieved in controlling DON formation in wheat by the use of azole fungicides at anthesis (Paul et al. 2008). Forecasting systems to advise farmers of the likelihood of DON formation have been developed in Canada and Europe (Hooker et al. 2002; Gourdain et al. 2011). Otherwise, control relies on reducing levels of *Fusarium* species in the field by good management and crop rotation (Blandino et al. 2012; Pitt et al. 2013; McKee et al 2019).

Torres et al. (2019) have comprehensively reviewed strategies for controlling *F. graminearum* growth and DON formation in wheat. Recently, Wang et al. (2020) reported the horizontal transfer of a gene produced by an *Epichloe* species into a wild wheat relative (*Thinopyrum*), then bred into a domestic wheat cultivar. The encoded glutathione S-transferase detoxifies DON, in theory also conferring resistance to Fusarium head blight.

13.7 Zearalenone

Zearalenone, one of the five most significant mycotoxins (Miller 1995), is not a true mycotoxin, being a nonsteroidal oestrogen produced by a fungus. It is not acutely toxic and has not been associated with any fatal disorder in animals or humans. However it has caused oestrogenic syndromes in pigs, and perhaps in human adolescents as well. Zearalenone is produced by the same *Fusarium* species as produce DON and nivalenol, i.e. *Fusarium graminearum* and *F. culmorum* (*see* Sect. 13.6) and also by *F. crookwellense* and some strains of *F. equiseti* and *F. semitectum* (Desjardins 2006). Generally speaking, formation occurs under the same conditions as trichothecene production, and the main sources are maize and small grains. So far as is known, zearalenone production in these commodities is similar to trichothecene production, i.e. before harvest, mostly at temperatures between 10 °C and 30 °C, and above 0.90 water activity. The association of these fungi with cereal crops is worldwide, as is the production of zearalenone (Marasas et al. 1984). All aspects of the occurrence and toxicology of zearalenone were reviewed comprehensively by Zinedine et al. (2007).

13.7.1 History

Oestrogenic effects in pigs in the USA were first reported in the 1920s (McNutt et al. 1928). In females, symptoms included enlargement of mammary glands and genital organs, ovary atrophy, infertility, reduced litter size and reduced piglet weight. In males, contaminated feed was associated with enlargement of mammary glands and atrophy of testes. The oestrogenic syndrome was later reproduced in pigs by feeding maize on which a pure culture of *Fusarium graminearum* had been grown (Stob et al. 1962) and the pure compound was characterised subsequently (Urry et al. 1966). Zearalenone was a major problem in maize in the USA and Canada, but the problem had largely disappeared by the early 1980s. A combination of warmer temperatures and maize hybrids that matured earlier reduced the accumulation of zearalenone (Pitt and Miller 2017).

13.7.2 Chemical Characteristics

Zearalenone is one of a large family of fungal metabolites described as belonging to the resorcyclic acid lactone group. It has the formula 6-[*10*-hydroxy-6-oxo-*trans*-1-undecenyl]-ß-resorcyclic acid lactone, with a molecular formula of $C_{18}H_{22}O_5$ and a molecular weight of 318 (Fig. 13.5). Its CAS registry number is 17924-92-4.

13.7.3 Hazard Identification

Pigs are especially sensitive to zearalenone. Signs of hyperoestrogenism generally appear at dietary levels >1 mg/kg, but can occur at lower levels. In prepubertal gilts, clinical signs include vulval swelling, uterine enlargement and mammary development. Mature sows can develop ovarian atrophy, constant oestrus and pseudopregnancy (Hagler et al. 2001). Prepubertal male pigs can undergo a feminising effect with

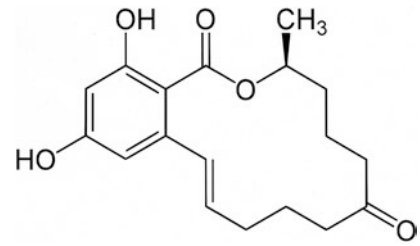

Fig. 13.5 Structure of zearalenone

mammary development, decreased testicular size and loss of libido, but mature boars are resistant (Hagler et al. 2001). Non-human primates are also very sensitive (Hobson et al. 1977). Zearalenone exposure may result in precocious pubertal changes in girls in some parts of Europe (Massart et al. 2008; Massart and Saggese 2010) and China (Deng et al. 2012).

Ruminants are quite resistant to zearalenone, likely due to rumen microorganisms metabolising it to compounds of lower toxicity. Chickens are also comparatively resistant, tolerating up to 30 mg/kg of zearalenone in feed.

Ingestion of zearalenone by mice in one experimental study resulted in an increased incidence of liver and pituitary tumours, consistent with a hormonal carcinogenic action. However, no effects were seen in rats, and the International Agency for Research on Cancer considered the evidence for animal carcinogenicity was limited (IARC 1993). No studies on human carcinogenicity have been reported. The nature of its toxicity is not completely understood.

The metabolism of zearalenone involves reduction of the 6-keto group, resulting in the formation of α- and β-zearalenol (Fink-Gremmels and Malekinejad 2007). α-zearalenol has greater estrogenic activity that zearalenone, so that transformation results in increased toxicity.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA 2000) has established a provisional maximum tolerable daily intake for zearalenone and its metabolites of 0.5 μg/kg bw per day.

13.7.4 Exposure Assessment

Zearalenone occurs in the same situations as deoxynivalenol, i.e. as the result of growth of certain *Fusarium* species in maize and small grains. It occurs in all regions of the world, but is less commonly found than deoxynivalenol. Levels in foods are usually less than 1 mg/kg in good quality grain, but in mouldy material concentrations of 10 mg/kg or more may occur. As with deoxynivalenol, levels are higher in crops grown in cooler, damper climates.

13.7.5 Risk Characterisation

The potential risks to humans from zearalenone appear to be limited to precocious pubertal changes in girls and perhaps female reproduction, as mentioned above. Zearalenone does not appear to be carcinogenic.

13.7.6 Chemical Analysis

Analyses for zearalenone can be carried out effectively by thin layer chromatography, but high performance liquid chromatography with fluorescence detection is now widely used (Scott 1995). Rapid screening tests based on zearalenone antibodies are commercially available also (Pestka 1994).

13.7.7 Management of Zearalenone in Foods

No internationally recognised limits exist for zearalenone, with country regulations varying from 20 to 1000 μg/kg of food or animal feed, sometimes even higher. Control of zearalenone in crops is similar to that for deoxynivalenol. However, attempts to control zearalenone as such are unlikely, because control measures will be aimed at deoxynivalenol, the more potent compound.

13.8 Patulin

Patulin was once considered to be a possible cure for the common cold, but is now regarded as a mycotoxin. It is important because the main producer of patulin is *Penicillium expansum*, a very common fungus responsible for rotting of apples and pears. Although consumers will always avoid rotten fruit, commercial juice manufacture may involve delays after picking that enable rot development, so apple juice is liable to contain patulin.

Patulin is produced by 10 or more *Penicillium* species, including *P. carneum, P. paneum, P. glandicola* and *P. griseofulvum*, all of which occur in foods, and also by the heat resistant spoilage

fungus *Byssochlamys fulva*. However, the only significant soure in foods is *P. expansum.*

Patulin is also produced by *Aspergillus clavatus* (= *Neosartorya clavata*), a common contaminant in malting barley. Maltworkers' lung was an important disease in malthouse workers, but the role of patulin in that disease remains uncertain.

13.8.1 History

In the heady days after the discovery of penicillin, extensive searches were conducted for other fungal compounds that might be of therapeutic value. Many metabolites produced by *Penicillium* species were discovered and evaluated. For a short time, patulin was considered to be a cure for the common cold, but like most *Penicillium* metabolites apart from penicillin, it turned out to be too toxic to be useful as a drug. We now know patulin as a mycotoxin.

13.8.2 Chemical Characteristics

Patulin (CAS 149-29-1) is a furopyranone, *4*-hydroxy-4*H*-furo[3,2-*o*]pyran-2(6*H*)-one, molecular formula $C_7H_6O_4$, molecular weight 154.12 (Fig. 13.6).

13.8.3 Hazard Identification

Patulin has been reported to affect renal cells (Heussner et al. 2006) and decrease sperm count in rats (Selmanoğlu 2006). Patulin damages the DNA of mammalian cells, but the effect can be negated by ascorbic acid (Alves et al. 2000). Patulin also causes oxidative stress response in mammalian cell lines, which can lead to cell changes during transformation and differentiation (Liu et al. 2007a). However, patulin is still classified as a Group 3 compound by the International Agency for Research on Cancer (IARC) on the basis that cytotoxicity in animals does not constitute adequate evidence of human toxicity (IARC 1993).

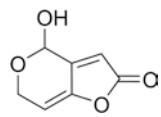

Fig. 13.6 Structure of patulin

13.8.4 Exposure Assessment and Risk Characterisation

From the results of US food surveys in 1994–1996, the US Food and Drug Administration examined results from nearly 3000 samples of apple juice, both for sale as fresh juice and as ingredients for other foods. It was found that the mean patulin exposure for all age groups, 1–2 year old children, and children under 1 year was 0.14, 0.80 and 0.21 μg/kg bw per day respectively. At the 90th percentile of exposure, the figures were 0.26, 1.7 and 0.40 μg/kg bw per day respectively. Figures for 1–2 year old children exceeded the PTDI. However, if a limit of 50 μg/l of patulin in apple juice was enforced, the figures for the 90th percentile of exposure were 0.10, 0.67 and 0.27 μg/kg bw per day respectively. The figure for young children still exceeded the PTDI, but because of safety factors in that figure, intake levels would still be many times lower than the no effect level calculated by JECFA.

In consequence, a regulatory limit of 50 μg/l for patulin in apple juice and other apple products was recommended to the Codex Alimentarius Commission, and has since been set by a number of countries (FAO 2003), with a limit of 10 μg/L for apple products destined for consumption by children (EU 2004). Many others, including Canada and Australia, have not set limits.

Two points are worth noting. First, unlike the other major mycotoxins reviewed in this book, no human or animal health problem has ever been associated with patulin. Second, patulin intake is highest in temperate zone developed countries. Apples are a cool climate crop, and consumption in less developed countries in tropical regions is very low by comparison.

13.8.5 Chemical Analysis

Although determination of patulin levels in juices can be carried out by TLC, it is recommended that HPLC be used where possible. TLC methods are imprecise because the recommended extraction by ethyl acetate also extracts other compounds such as 5-hydroxymethylfurfural, and the visualisation methods are also relatively insensitive (Kubacki and Goszcz 1988).

13.8.6 Management of Patulin in Foods

Penicillium expansum produces patulin as it rots apples and pears. However, as consumers of fresh fruit will always avoid rotting fruit, patulin is not an issue in fresh fruit. More importantly, harvesting for commercial juice manufacture often causes fruit damage, while delays after picking enable rot development, so apple juice is liable to contain patulin. Poor quality control, i.e. the use of rotting fruit in juice or cider manufacture, can result in high concentrations of patulin in juice: levels of up to 350 µg/L, 630 µg/L 1770 µg/L and even 3990 µg/L have been reported in the literature. The worst results were obtained from apple juice sold at roadside stalls (Brackett and Marth 1979; Lindroth and Niskanen 1978; Watkins et al. 1990).

In good commercial practice, rots, which contain nearly all the patulin, are removed by water flumes or, better, by high pressure water jets, before apples are crushed. These measures provide effective control (Sydenham et al. 1990). Some patulin migrates from rots to sound tissue, but residual levels are low (Taniwaki et al. 1992). Patulin has frequently been used as an indicator of the use of poor quality raw materials in juice manufacture.

Patulin is stable in many kinds of foods, including apple and grape juices and dry maize, but not in wet maize, orange juice, flour, fermented juices or cheese. Disappearance appears to be due to reaction with sulphhydryl groups of amino acids or proteins, but that does not necessarily lead to a complete loss in biological activity. Patulin is moderately heat stable, surviving pasteurisation processes.

13.9 Minor Mycotoxins

A variety of compounds can be considered to be mycotoxins of lesser importance, either because health effects are less pronounced, or because they occur in a limited geographical area, or because their fungal sources are uncommon in foods. They can be divided between those that occur in the field in pasture or fodder crops for domestic animals and those produced in human foods, grains or manufactured foods.

13.9.1 Field Toxins

Toxins from *Claviceps* species Fungi from the genus *Claviceps* grow on grasses, including cultivated cereals, throughout temperate zones. *Claviceps* species infect the flowers of susceptible hosts, replacing the ovaries with a specialised mass of fungal tissue, a sclerotium, more commonly known as an ergot. The ergots produced by the most important species, *Claviceps purpurea*, are dark purple to black and are found mostly in rye, but can also occur in barley, oats and wheat. Ergots of *C. purpurea* contain toxic alkaloids, of which ergotamine is the best known. Ergotism, the disease caused by consumption of ergots from rye, may cause convulsions or gangrene. The convulsive symptoms are often accompanied by hallucinations, while gangrene, caused by constriction of blood vessels, may result in loss of limbs. This disease syndrome was well known in the Middle Ages, when it was known as “St Anthony’s Fire” (Fuller 1969). Ergotism has declined in importance with the decrease in use of rye and with increased knowledge of the problem, but outbreaks have occurred in Africa in recent years. Ergotamine has found utility in treating some diseases, including HIV,

so modern medicine is now concerned with ergotism as a side effect of such treatment (Ayarragaray 2014).

Facial eczema Facial eczema is a disease primarily of sheep in New Zealand, but also known from Australia and South Africa. It is caused by sporidesmin, produced by *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis [= *Pseudopithomyces chartarum* (Berk. & M.A. Curtis) Jun F. Li, Ariyaw. & K.D. Hyde] as it sporulates on pasture debris in late summer and autumn. Sporidesmin causes obstruction of the bile duct, liver damage and photosensitisation (Di Menna et al. 2009).

Ryegrass staggers The disease known as perennial ryegrass staggers occurs in New Zealand and less commonly in South Africa and Australia. *Acremonium lolii* Latch, M.J. Chr. & Samuels (= *Epichloe festucae* Leuchtm., Schardl & M.R. Siege), an endophytic fungus in ryegrass (*Lolium* spp.) interacts with the plant to produce lolitrem, a potent tremorgenic toxin, which causes trembling, staggers and lack of muscle coordination in sheep, cattle and horses grazing affected pasture. Death may result in severe cases (Prestidge 1993).

Lupinosis Lupinosis was important in Western Australia, where sheep and cattle are often grazed on lupin stubble after harvest. This disease has also been reported from South Africa and Germany. It is caused by phomopsin A, produced in lupins by the pathogenic fungus *Phomopsis leptostromiformis* (J.G. Kühn) Bubák ([= *Diaporthe leptostromiformis* (J.G. Kühn) Rossman & Udayang]. Lupinosis in animals is characterised by jaundice and the development of yellow, fatty livers. In severe cases death may occur within a few days. Phomopsin was also produced in lupin seed, and is of concern as lupin seed is increasingly being sold for human consumption. Breeding of varieties of lupin resistant to the fungus has greatly reduced the problem in the past 20 years (Hepworth 2018).

Diplodiomycosis An important field mycotoxicosis in southern Africa is diplodiomycosis, due to grazing cattle on maize plants that become infected by *Diplodia maydis* (Berk.) Sacc. [= *Stenocarpella maydis* (Berk.) B. Sutton] after grain harvest. This disease is a neurotoxicosis, characterised in cattle by ataxia, a peculiar walk, incoordination and often paralysis and death. The chemical cause of diplodiomycosis remains obscure.

Stachybotryotoxicosis In eastern Europe, stachybotryotoxicosis is a well known disease of domestic animals. It was responsible for the deaths of tens of thousands of horses in the Ukraine in the 1930s, and also affected farm workers when the problem was severe. Symptoms varied, but in horses the most common was necrosis of membranes in the mouth, the result of direct contact with straw infected by *Stachybotrys chartarum* (Ehrenb.) S. Hughes. A general toxicosis followed, consistent with modern knowledge that the cause was a group of macrocyclic trichothecenes. The same fungus and toxins are now considered to be responsible for this disease in indoor environments, especially in Eastern Europe and North America, where water damage has occurred (Hossain et al. 2004; Pestka et al. 2008), though opinion is not unanimous (Miller et al. 2003).

13.9.2 Toxins in Grains and Processed Foods

T-2 toxin T-2 toxin was the cause of Alimentary Toxic Aleukia (ATA) a devastating disease which occurred in the USSR during and after World War II, in times of extreme food shortage that resulted in consumption of overwintered cereals (Joffe 1978; Beardall and Miller 1994). Many people, probably hundreds of thousands, died as a result (Marasas et al. 1984). ATA was characterised by leucopoenia, bleeding from nose, throat and gums, haemorrhagic rash, exhaustion of the bone marrow, and fever. Vomiting, nausea, diarrhoea and abdominal pain also usually occurred. Decrease in immunological functions led to susceptibility to bacterial and viral diseases and often death (Joffe 1978; Beardall and Miller 1994). Haemorrhagic syndrome in cattle, pigs and poultry in the USA in the 1960s was also probably due to T-2 toxin (Desjardins 2006).

T-2 toxin is produced by *Fusarium sporotrichioides* and, less commonly, *F. poae* (Desjardins 2006). It appears to be produced only under cold conditions, and fortunately is now uncommon. The occurrence, toxicity and biology of T-2 toxin were examined in detail by JECFA (2001). They established that T-2 (and its metabolite HT-2) were immunotoxic and haemotoxic compounds in several animal species after short term intake. Long term effects could not be evaluated. T-2 was at most weakly genotoxic. In the absence of long term studies, T-2 was not classifiable as to carcinogenicity (JECFA 2001).

T-2 toxin is the most toxic trichothecene when tested in cell lines, and equal to nivalenol by intraperitoneal injection. T-2 toxin in more than 10 times as toxic as DON by injection, and 70 times as toxic to a cell line.

Tenuazonic acid Tenuazonic acid is produced by several fungal species and has been found to occur in a wide range of foods including cereals, bakery products, dried figs, tomato products, sunflower seeds, wine and beer (López et al. 2016; Oliveira et al. 2018). The principal sources are *Alternaria* species, especially *A. alternata*, except in sorghum, where tenuazonic acid is produced by *Epicoccum sorghinum* (= *Phoma sorghina*). The toxicity of tenuazonic acid was investigated by EFSA (2011) who concluded that although data were limited, this toxin was probably not a cause for concern for human health. Our observations indicate that it is a major cause of ill-thrift in chickens.

Penitrem A Penitrem A is a potent neurotoxin (Pitt 1979; El-Banna et al. 1987; Frisvad et al. 2006). Toxicity of penitrem A to animals, especially dogs, is well documented (see Pitt and Hocking 1997; Naude et al. 2002; Minervini et al. 2002; Richard et al. 2012). Dogs that eat mouldy food, discarded hamburger buns, cream cheese and especially fallen walnuts may exhibit sustained trembling and other adverse effects (Richard et al. 2012). Sometimes effects last for months (Eriksen et al. 2010). Fortunately penitrem A appears to have an emetic effect in humans, limiting its toxicity. Only occasional occurrences of human toxicity have been reported (Pitt and Hocking 1997; Lewis et al. 2005; Botha et al. 2019). In the latter reference, it is suggested that high amounts of roquefortine C may have influenced the syndrome observed. *Penicillium janczewskii* has been reported to produce penitrem A (di Menna et al. 1986; Pitt and Leistner 1991; Frisvad et al. 2006), however this species is rarely if ever involved in food spoilage.

Citreoviridin Produced by *Penicillium citreonigrum* when it grows in rice, citreoviridin was the cause of acute cardiac beri beri, a disease which sporadically affected young healthy Japanese people 100 years and more ago. As the result of pioneering toxicological work by Sakaki before 1900, Japan banned the sale of yellow rice in 1910, and the disease disappeared (Uraguchi 1969; Ueno and Ueno 1972; Pitt and Miller 2017). *P. citreonigrum* appears to be a rare species now. However, this disease reappeared in northern Brazil in 2006, specifically among subsistence farmers cropping rice on recently cleared land. It was more common in young healthy males. More that 1000 cases occurred and resulted in at least 40 deaths (Rosa et al. 2010). Although other mycotoxins were found, the presence of citreoviridin was confirmed and symptoms of the disease indicated this toxin was the cause (Almeida et al. 2012).

Citreoviridin may also be the cause of Keshan disease, a disease in Northern China characterised by pulmonary oedema and heart failure (Yang 2012; Carvajal-Merino 2015).

Citreoviridin is an unusual molecule consisting of a lactone ring conjugated to a furan ring, with a molecular weight of 402 (Cole and Cox 1981). It is a neurotoxin, acutely toxic to mice, with intraperitoneal and oral LD_{50}s of 7.5 mg/kg and 20 mg/kg respectively (Ueno and Ueno 1972).

Citreoviridin can also be produced by *Penicillium ochrosalmoneum* when it infects maize kernels (Wicklow et al. 1988). The significance of this finding is unknown, as no further work has been carried out. Other mycotoxins that occur in maize are much more important.

Citrinin Citrinin is produced by several *Penicillium* species, notably *P. citrinum*. It is a significant renal toxin to monogastric domestic animals, including pigs (Keblys et al. 2004) and dogs (Kogika et al. 1993; Krejci et al. 1996). The

oral LD_{50} in mice is about 110 mg/kg (Scott 1977). Domestic birds are susceptible: citrinin causes watery diarrhoea, increased food consumption and reduced weight gain due to kidney degeneration in chickens (Mehdi et al. 1981; Glahn et al. 1989), ducklings and turkeys (Mehdi et al. 1984). Citrinin has also been reported to cause teratogenic effects in rats (Singh et al. 2007). An impact of citrinin on human T cells has been demonstrated (Wichmann et al. 2002, Tammer et al. 2007), however no link between the ingestion of citrinin and any human disease syndrome has been shown.

Secalonic acid D Secalonic acids are dimeric xanthones produced by a range of taxonomically distant fungi (Cole and Cox 1981; Frisvad and Thrane 2004). Secalonic acid D is a major metabolite of *Penicillium oxalicum*. It is known to induce cleft palate in animals and potentially also in humans (Dhulipala et al. 2005; Reddy 2005). It can also induce apoptosis in some cell lines (Zhang et al. 2019). As *P. oxalicum* is a common invader of maize, secalonic acid D has been found in freshly harvested maize (Palmgren and Fleischhacker 1987), in grain dusts in the USA, at levels of up to 4.5 mg/kg (Ehrlich et al. 1982) and from grain dust collected in Belgian cereal storage facilities (Tangni and Pussemier 2007). The possibility that such levels can be toxic to grain handlers should not be ignored. Secalonic acid D is also produced by *Aspergillus aculeatus* (Frisvad et al. 2006) but a role in disease has not been postulated.

Cyclopiazonic acid Cyclopiazonic acid is produced by several *Aspergillus* and *Penicillium* species, the most important being *A. flavus*, *P. commune* and *P. camemberti*. Cyclopiazonic acid is quite toxic, with an oral LD_{50} value in male mice of 36 mg/kg (Purchase 1971), is immunosuppressive in low doses (Hill et al. 1986), and has produced a mycotoxicosis in pigs (Lomax et al. 1984; Keblys et al. 2004). A role in "Turkey X" disease along with aflatoxin appears likely (Cole 1986; Bradburn et al. 1994). It is one cause of ill thrift in chickens (Dorner 1983). When fed to chickens, appreciable amounts of this compound were also detected in eggs (Dorner et al. 1994).

When fed to lactating ewes, most was excreted in milk within 10 days, the maximum concentration in milk (570 µg/kg) being ca 10% of the ingested dose (5 mg/kg live weight; Dorner et al. 1994).

Cyclopiazonic acid was reported to be nonmutagenic to human liver cells and to reduce the mutagenicity of aflatoxin B_1 (Sabater-Vilar et al. 2003b).

Verruculogen Verruculogen is a potent neurotoxin produced by some isolates of *P. simplicissimum* and *P. paxilli* (not by *Talaromyces verruculosus*, the species used to name the toxin, but misidentified; Pitt 1979). Its structure is quite different from that of penitrem A, but includes one similar moiety (Cole 1981), and it is almost equally toxic (Cole et al. 1972, reported as *P. verruculosum*). Verruculogen tested positive in a mammalian microsome assay (Sabater-Vilar et al. 2003a). However this compound has not been reported from foods or feeds.

It was reported by Tuthill et al. (2001) that verruculogen is produced by *P. brasilianum*, not *P. simplicissimum*. It is not clear which of these species actually occurs in foods.

Penicillic acid Penicillic acid was isolated and characterised by Alsberg and Black (1913) from a toxic sample of mouldy maize infected with *Penicillium puberulum* (=*P. aurantiogriseum*). It was later found to be produced in "blue-eye" maize in midwesterrn USA where the principal infecting species was *P. martensii* (= *P. aurantiogriseum*) (Kurtzman and Ciegler 1970). When fed to mice, maize on which *P. martensii* had been grown was lethal in 3–5 days (Kurtzman and Ciegler 1970). It is unclear whether this toxin is of practical importance although blue eye disease of maize occurs in the USA from time to time.

Toxins from *Talaromyces islandicus* (=*Penicillium islandicum*)

Like *P. citreonigrum*, *T. islandicus* was believed to be common in rice in Asia in the 19th and early 20th centuries. It produces four toxic compounds, the skyrins erythroskyrin and leuteoskyrin, and the cyclic peptides islanditoxin and cyclochlorotine (Saito et al. 1971; Uraguchi et al. 1972). It is now an uncommonly isolated fungus.

Rubratoxins Rubratoxins A and B are highly toxic, and were studied quite intensively 20 years ago. However, they are produced by only three known isolates of *Penicillium ruber* (Stoll) Yilmaz. Rubratoxin was implicated in one poisoning outbreak involving two people, from home made rhubarb wine (Sigler et al. 1996).

Toxins from *Penicillium viridicatum*. *Penicillium viridicatum* produces several naphthoquinones, notably xanthomegnin and viomellein, capable of causing liver and kidney damage in mice, rats and pigs. Both compounds caused illness in young mice when administered at 450 mg/kg body weight (Carlton et al. 1973, 1976). Viomellein has been found in nature, in a Danish barley sample, at a level of 1 mg/kg (Hald et al. 1983), a level too low to be considered hazardous. *P. viridicatum* was erroneously believed to be a major source of ochratoxin A until the 1980s.

Janthitrems Janthitrems are potent neurotoxins produced by *Penicillium janthinellum* isolates taken from New Zealand pastures involved in ryegrass staggers outbreaks (Gallagher et al. 1980; Babu et al. 2018). However, as this species has not been reported to cause food spoilage, their influence over human health appears to be low.

Sterigmatocystin Sterigmatocystin is produced by *Emericella versicolor* and a range of less important fungi. It comprises a xanthene ring fused to a dihydrofuranofuran moiety, lies in the aflatoxin biosynthesis pathway and is a toxic precursor to the aflatoxins. Like aflatoxins, sterigmatocystin affects primarily the liver and kidney, though both acute toxicity and carcinogenicity are lower. Acute oral toxicity is rats was 120 μg/kg or higher, 10 fold or less than that of aflatoxin B_1 (Purchase and van der Watt 1969), perhaps due to very low solubility in water or gastric juices (Terao 1983). Due to this insolubility, experimental doses given to animals are only adsorbed to a small extent. As absorption is dependent on the method of administration, LD_{50} figures lack accuracy: literature values vary from 60–800 mg/kg body weight. However, even low doses can cause tumours in mice (Fujii et al. 1976) and pathological changes to the livers of rats (Terao 1983). Rats orally administrated sterigmatocystin at the rate of 8.3–16.6 mg per kg body weight per day for 10 days showed severe fatty acid changes and necrosis of hepatocytes (Aal et al. 1997).

A review by JECFA (2017) concluded that although sterigmatocystin has demonstrated carcinogenicity, levels reported in foods worldwide are too low for it to be a threat to human health. The possible exception was in Africa in regions where sorghum is the staple diet. A major study of the presence of mycotoxins in four sub-Saharan countries found 15% were contaminated with sterigmatocystin (Ssepuuya et al. 2018). Information on the occurrence of sterigmatocystin in foods was reviewed by Nieto et al. 2018), who also provided details of methods of analysis.

As a liver carcinogen, sterigmatocystin appears to be only about 1/150th as potent as aflatoxin B_1, but this is still much more potent than most other liver carcinogens. Levels as low as 15 mg/day fed continuously, or a single 10 mg dose, caused liver cancer in 30% or more of male Wistar rats (Terao 1983). Sterigmatocystin also increased the development of intestinal cancer in Mongolian gerbils infected with a known carcinogenic *Helicobacter pylori* toxin (Ma et al. 2003).

Gliotoxin. Gliotoxin, produced by *Neosartorya fumigata*, assists this highly pathogenic fungus to invade lung tissue (Knowles et al. 2020). As *N. fumigata* primarily occurs in decaying vegetation, gliotoxin is of little relevance as a potential cause of foodborne disease.

Other toxins from *Fusarium* species *Fusarium* species make a wide range of metabolites with demonstrable toxicity, but which rarely if ever are produced at problematic levels in foods or feeds. None of these toxins has been reliably associated with disease of man or animals.

Moniliformin is a very unusual molecule with a four membered carbon ring as its central moiety. It is produced by at least 20 *Fusarium* species and is of widespread occurrence in grain crops and to a lesser extent in grain based products. It is of low or negligible importance in foods or animal feeds (EFSA 2018). It was found to be relatively common in Italian maize and was

associated with fumonisin formation (Bertuzzi et al. 2020).

Butenolide, 4-acetamido-4-hydroxy-2--butenoic acid γ-lactone, is produced by several *Fusarium* species, and is moderately toxic to mice (Burmeister et al. 1980). A possible role in Keshan disease, a heart disease endemic in some regions of China, has been postulated (Liu et al. 2007b).

Fusarochromanone, produced by *Fusarium equiseti*, can cause bone deformities in chickens (Lee et al. 1985, Desjardins 2006). It has been reported more recently to be a potent anticancer agent (Mahdavian et al. 2014; Miriyala et al. 2018).

Sambutoxin, a metabolite of *Fusarium oxysporum* and *F. sambucinum,* was found to cause haemorrhaging of the digestive tract in rats (Kim and Lee 1994; Kim et al. 1995). It was found to interfere with transport between cytochromes (Kawai et al. 1997).

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Media Appendix

Aspergillus flavus and parasiticus agar (AFPA)

Peptone, bacteriological	10 g
Yeast extract	20 g
Ferric ammonium citrate	0.5 g
Chloramphenicol	100 mg
Agar	15 g
Dichloran (0.2% in ethanol, 1.0 ml)	2 mg
Water, distilled	1 litre

After addition of all ingredients, sterilise by autoclaving at 121 °C for 15 min. The final pH of this medium is 6.0–6.5.

Creatine sucrose neutral agar (CSN)

CS concentrate	10 ml
Sucrose	10 g
Creatine	5.0 g
KH_2PO_4	1.0 g
Bromocresol purple	0.05 g
Agar	15 g
Water, distilled to	1 litre

Creatine sucrose (CS) concentrate

KCl	5 g
$MgSO_4.7H_2$	5 g
$FeSO_4.7H_2O$	0.1 g
$ZnSO_4.7H_2O$	0.1 g
$CuSO_4.5H_2O$	0.05 g
Water, distilled	to 100 ml

Sterilise by autoclaving at 121 °C for 15 min. Final unadjusted pH is approximately 6.8. A pH between 5.5 and 6.8 is satisfactory. For identification of *Penicillium* subgenus *Penicillium* species.

Czapek concentrate

$NaNO_3$	30 g
KCl	5 g
$MgSO_4.7H_2O$	5 g
$FeSO_4.7H_2O$	0.1 g
$ZnSO_4.7H_2O$	0.1 g
$CuSO_4.5H_2O$	0.05 g
Water, distilled	100 ml

Czapek concentrate will keep indefinitely without sterilisation. The precipitate of $Fe(OH)_3$ which forms in time can be resuspended by shaking before use.

Czapek iprodione dichloran agar (CZID)

Sucrose	30 g
Yeast extract	5 g
Chloramphenicol	100 mg
Dichloran (0.2% in ethanol, 1 ml)	2 mg
Czapek concentrate	10 ml
Trace metal solution	1 ml
Agar	15 g
Water, distilled	1 litre
Iprodione (suspension)	1 ml

Add iprodione suspension [0.3 g Roval 50WP (Rhone-Poulenc Agro-Chemie, Lyon, France) in 50 ml sterile water, shaken before addition to medium] after autoclaving. Sterilise by autoclaving at 121 °C for 15 min. This formulation is an adaptation of the original published formulation (Abildgren et al. 1987), made from basic ingredients rather than using commercial Czapek-Dox broth. Chloramphenicol (100 mg/l) replaces the original combination of chlortetracycline (50 mg) and chloramphenicol (50 mg).

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Czapek yeast extract agar (CYA)

K_2HPO_4	1 g
Czapek concentrate	10 ml
Yeast extract, powdered	5 g
Sucrose	30 g
Agar	15 g
Water, distilled	1 litre

Refined table grade sucrose is satisfactory for use in CYA provided it is free from sulphur dioxide. Sterilise by autoclaving at 121 °C for 15 min. Final pH 6.7.

Czapek yeast extract agar with 20% sucrose (CY20S)

K_2HPO_4	1 g
Czapek concentrate	10 ml
Yeast extract	5 g
Sucrose	200 g
Agar	15 g
Water, distilled	1 litre

Sterilise by autoclaving at 121 °C for 15 min. Final pH 5.2.

Dichloran chloramphenicol malt extract agar (DCMA)

Malt extract	10 g
Dichloran (0.2% w/v in ethanol, 1 ml)	2 mg
Chloramphenicol	0.1 g
Agar	15 g
Distilled water	to 1 litre

Sterilise by autoclaving at 121 °C for 15 min. Recommended for identification of *Alternaria* species and some other dematiaceous Hyphomycetes. Final pH is 5.5–6.0.

Dichloran chloramphenicol peptone agar (DCPA)

Peptone	15 g
KH_2PO_4	1 g
$MgSO_4.7H_2O$	0.5 g
Chloramphenicol	0.1 g
Dichloran (0.2% in ethanol, 1 ml)	2 mg
Agar	15 g
Water, distilled	1 litre

After addition of all ingredients, sterilise by autoclaving at 121 °C for 15 min. The final pH of this medium is 5.5–6.0.

Dichloran 18% glycerol agar (DG18)

Glucose	10 g
Peptone	5 g
KH_2PO_4	1 g
$MgSO_4.7H_2O$	0.5 g
Glycerol, A.R.	220 g
Agar	15 g
Dichloran (0.2% w/v in ethanol, 1 ml)	2 mg
Chloramphenicol	100 mg
Water, distilled	1 litre

To produce this medium, add minor ingredients and agar to *ca* 800 ml distilled water. Steam to dissolve agar, then make to 1 litre with distilled water. Add glycerol: note that the final concentration is 18% weight in weight, not weight in volume. Sterilise by autoclaving at 121 °C for 15 min. The final a_w of this medium is 0.955 and pH is in the range 5.5–5.8.

Dichloran rose bengal chloramphenicol agar (DRBC)

Glucose	10 g
Peptone, bacteriological	5 g
KH_2PO_4	1 g
$MgSO_4.7H_2O$	0.5 g
Agar	15 g
Rose bengal (5% w/v in water, 0.5 ml)	25 mg
Dichloran (0.2% w/v in ethanol, 1 ml)	2 mg
Chloramphenicol	100 mg
Water, distilled	1 litre

After the addition of all ingredients, sterilise by autoclaving at 121 °C for 15 min. The final pH is in the range 5.5–5.8. Store prepared media away from light; photoproducts of rose bengal are highly inhibitory to some fungi, especially yeasts. In the dark, the medium is stable for at least 1 month at 1–4 °C. The stock solutions of rose bengal and dichloran need no sterilisation, and are also stable for very long periods. The chlortetracycline in the original formulation of King et al. (1979) has been replaced with chloramphenicol, an effective antibiotic originally recommended for mycological media by Put (1974). Media containing chloramphenicol are easier to prepare, are not affected by autoclaving, and have greater long term stability.

Dichloran rose bengal yeast extract sucrose agar (DRYS)

Yeast extract	20 g
Sucrose	150 g
Dichloran (0.2% in ethanol, 1 ml)	2 mg
Rose bengal (5% w/v in water, 0.5 ml)	25 mg
Chloramphenicol	50 mg
Agar	20 g
Water, distilled	to 1 litre
Chlortetracycline (l% in water, filter sterilised, 5 ml)	50 mg

Sterilise all ingredients except chlortetracycline by autoclaving at 121 °C for 15 min. Add chlortetracycline after tempering to 50 °C. In our experience, chloramphenicol at twice the concentration specified (i.e. 100 mg/l), adequately controls bacteria in most situations, and this avoids the need for a second antibiotic which must be filter sterilised.

25% Glycerol nitrate agar (G25N)

K_2HPO_4	0.75 g
Czapek concentrate	7.5 ml
Yeast extract	3.7 g
Glycerol, analytical grade	250 g
Agar	12 g
Water, distilled	750 ml

Glycerol for G25N should be of high quality, with a low (1%) water content. If a lower grade is used, allowance should be made for the additional water. Sterilised by autoclaving at 121 °C for 15 min. Final pH 7.0.

Malt acetic agar (MAA)

To 100 ml sterile tempered Malt Extract Agar, aseptically add 0.5 ml glacial acetic acid, giving a final concentration of 0.5% acetic acid. Mix well before pouring. Note that MAA cannot be autoclaved or reheated as the low pH (approx. 3.2) causes the agar gel to break down if the medium is subjected to any further heat treatment after the addition of the acetic acid. There is no need to sterilise the glacial acetic acid.

Malt extract agar (MEA)

Malt extract, powdered	20 g
Peptone	1 g
Glucose	20 g
Agar	20 g
Water, distilled	1 litre

Commercial malt extract used for home brewing is satisfactory for use in MEA, as is bacteriological peptone. Sterilise by autoclaving at 121 °C for 15 min. Do not sterilise for longer, as this medium will become soft on prolonged or repeated heating. Final pH 5.6.

Malt extract yeast extract 50% glucose agar (MY50G)

Malt extract	10 g
Yeast extract	2.5 g
Agar	10 g
Water, distilled	to 500 g
Glucose, A.R.	500 g

Add the minor constituents and agar to *ca* 450 ml distilled water and steam to dissolve the agar. Immediately make up to 500 g with distilled water. While the solution is still hot, add the glucose all at once and stir rapidly to prevent the formation of hard lumps of glucose monohydrate. If lumps do form, dissolve them by steaming for a few minutes. Sterilise by steaming for 30 min; note that this medium is of a sufficiently low a_w not to require autoclaving. Food grade glucose monohydrate (dextrose) may be used in this medium instead of analytical reagent grade glucose, but allowance must be made for the additional water present. Use 550 g of $C_6H_{12}O_6.H_2O$, and 450 g of the basal medium. As the concentration of water is unaffected by this procedure, the quantities of the minor ingredients are unaltered. The final a_w of this medium is 0.89. Final pH is 5.3.

Malt extract yeast extract 70% glucose fructose agar (MY70GF)

Malt extract	6 g
Yeast extract	1.5 g
Agar	6 g
Water, distilled	to 300 g
Glucose, A.R.	350 g
Fructose, A.R.	350 g

After steaming to dissolve agar, make the solution accurately to 300 g with water and, while it

is still hot, add both sugars. Steam gently for up to 30 min to completely dissolve the sugars. Further sterilisation is unnecessary: contaminant microorganisms of any kind are unable to grow on this medium, which is about 0.76 a_w. MY70GF will take some hours to gel, because of the low proportion of water and agar. If possible, allow 24 h after pouring for the medium to attain gel strength before use.

Malt extract yeast extract 5% (or 10%) salt 12% glucose agar (MY5–12 and MY10–12)

Malt extract	20 g
Yeast extract	5 g
NaCl (100 g for MY10–12)	50 g
Glucose	120 g
Agar	20 g
Water, distilled	to 1 litre

Sterilise MY5–12 by autoclaving at 121 °C for 10 min, and MY10–12 by steaming for 30 min. Overheating of these media will cause softening. The final a_w of MY5–12 is 0.93 and of MY10–12 is 0.88.

Oxytetracycline glucose yeast extract agar (OGY)

Glucose	20 g
Yeast extract	5 g
Agar	15 g
Water, distilled	1 litre
Oxytetracycline	100 mg

Sterilise by autoclaving at 121 °C for 15 min. After tempering to 50 °C, add 10 ml of filter sterilised oxytetracycline (Terramycin, Pfizer; 0.1% aqueous) per 100 ml of medium. The final pH is 6.8–7.2.

Potato dextrose agar (PDA)

Potatoes	250 g
Glucose	20 g
Agar	15 g
Water, distilled	to 1 litre

PDA prepared from raw ingredients is more satisfactory than commercially prepared media. Wash the potatoes, which should not be of a red skinned variety, and dice or slice, unpeeled, into 500 ml of water. Steam or boil for 30–45 min. At the same time, melt the agar in 500 ml of water. Strain the potato through several layers of cheese cloth into the flask containing the melted agar. Squeeze some potato pulp through also. Add the glucose, mix thoroughly, and make up to 1 litre with water if necessary. Sterilise by autoclaving at 121 °C for 15 min.

Tap water agar (TWA)

Agar	15 g
Tap water	to 1 litre

Sterilise by autoclaving at 121 °C for 15 min. Natural substrates such as carnation leaf pieces, wheat straw, wheat or millet grains may be added to TWA plates after the agar is poured and before it sets. These can provide a substrate for growth and sporulation of plant pathogenic fungi like *Fusarium*, *Drechslera, Bipolaris* and some other dematicaeous Hyphomycetes.

Tryptone glucose yeast extract agar (TGY)

Glucose	100 g
Tryptone	5 g
Yeast extract	5 g
Chloramphenicol	0.1 g
Agar	15 g
Distilled water	to 1 litre

Sterilise by autoclaving at 121 °C for 10 min. Prolonged heating will cause browning of the medium. Chloramphenicol may be omitted if suppression of growth of bacteria is not required. Final pH 5.5–6.0.

Tryptone glucose yeast extract acetic agar (TGYA)

Make as for Malt Acetic Agar (MAA), but use TGY agar without chloramphenicol as the base rather than MEA. As with MAA, TGYA should not be reheated. The final pH is 3.8.

Tryptone glucose yeast extract broth (TGY broth)

Make as for TGY agar, but omit the agar from the formulation.

Tryptone glucose yeast extract acetic broth (TGYA broth)

Make as for TGY broth with the addition of glacial acetic acid to give a final concentration of 0.5%. Sterilise by steaming for 30 min. Final pH 3.8.

V-8 Juice Agar (V-8J)

V-8 Juice, clarified	200 ml
$CaCO_3$	23 g
Agar	20 g
Water, distilled	800 ml

Clarify V-8 juice by straining through cheese cloth. Mix ingredients well and sterilise by autoclaving at 121 °C for 15 min.

Taxonomic Novelties and Corrections

The following new combinations are introduced here:

Emericella sydowii (Bainier & Sartory) Pitt & A.D. Hocking comb. nov.
MycoBank number MB838069.
Basionym: *Sterigmatocystis sydowii* Bainier & Sartory [as "*sydowi*"], Annls mycol. 11(1): 25 (1913).
Synonym: Aspergillus sydowii (Bainier & Sartory) Thom & Church, The Aspergilli: 147 (1926).
Neotype: IMI 211384, a culture in a metabolically inactive state, designated here.

Emericella versicolor (Vuill.) Pitt & A.D. Hocking comb. nov.
MycoBank number MB838068.
Basionym: *Sterigmatocystis versicolor* Vuill., in Mirsky, Sur Quelques Causes d'Erreur dans la Détermination des Aspergillées Parasites de l'Homme: 15, 1903.
Synonym: Aspergillus versicolor (Vuill.) Tirab, Ann. Bot., Roma 7: 9, 1908.
Neotype: CBS 583.65, a culture in a metabolically inactive state, designated here.

Emericella usta (Bainier) Pitt & A.D. Hocking comb. nov.
MycoBank number MB 838032.
Basionym: *Sterigmatocystis usta* Bainier, Bull. Soc. Bot. Fr. 28: 78, 1881.
Synonym: *Aspergillus ustus* (Bainier) Thom & Church, Aspergilli: 152, 1926.
Neotype: IMI 211805, a culture in a metabolically inactive state, designated here.

Neosartorya clavata (Desm.) Pitt & A.D. Hocking comb. nov.
MycoBank number MB 838031.
Basionym: *Aspergillus clavatus* Desm., Ann. Sci. Nat., Bot., Sér. 2, 2: 71.
Neotype: IMI 15949, a culture in a metabolically inactive state, designated here.

The following neotype is designated here:

Penicillium cinnamopurpureum Abe ex Udagawa, J. Agric. Sci., Tokyo 5: 1, 1950. Neotype IFO 6032, a culture in a metabolically inactive state, designated here. From milled US rice, Japan, 1956, *S. Abe*. Isoneotype cultures CBS 429.65, ATCC 18489, NRRL 162, FRR 162.
MycoBank number MBT 10001329.

The following corrections are made to neotypes listed in recent publications:

Aspergillus glaucus (MycoBank MB161735). The neotype is DAOM 137960 (Malloch and Cain 1972), not IMI 211383 as stated by Samson et al. (2014).

Aspergillus candidus (MycoBank MB204868). Houbraken, J et al. (2020) incorrectly selected a new neotype. As the existing neotype has not been shown to be different taxonomically from the original description (ICN, Articles 9.18, 9.19, Turland et al. 2018), it cannot be changed, despite having been based on a different NRRL strain than Samson and Gams (1985) had believed.

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Glossary

A

Acerose needle-like; shaped like a pine needle.
Acervulus a flat or cup-shaped fruiting body, usually embedded in the agar, containing conidiophores and conidia.
Aleurioconidium a terminal conidium, usually thick-walled, blown out from the end of a sporogenous cell.
Ampulliform flask-shaped.
Anamorph the asexual or conidial form of a fungus.
Annelide a conidiogenous cell which produces conidia in succession, each conidium being produced through the scar of the previous one, leaving a ring-like scar at the apex of the spore-bearing cell.
Apical at the apex, e.g. of a hypha or phialide.
Apiculate having a short projection at one end.
Arthroconidium (pl. arthroconidia) conidia, often cylindrical, produced by fragmentation of hyphae into separate cells.
Ascocarp a fruiting body in Ascomycetes containing asci and ascospores.
Ascospore a sexual spore formed in an ascus.
Ascus (pl. asci) a thin walled sac containing ascospores, usually eight, but in some cases one, two or four.
Aseptate without any crosswalls; usually refers to hyphae.
Asporogenous not having any spores.

B

Basipetal describes the succession of conidia in which the youngest conidium is at the base of the chain.
Biverticillate having two branching points; usually referring to a penicillus or similar spore-bearing structure with metulae and phialides.

C

Chlamydoconidium (pl. chlamydoconidia) a thick-walled resting spore formed by the swelling and thickening of a single cell, usually within a hypha.
Clavate club-shaped.
Cleistothecium an ascocarp with a well defined wall, but without a special opening (ostiole).
Coelomycete a fungus forming conidia in a closed body such as an acervulus.
Collula the necks of phialides or annelides.
Columella in some Mucorales species, the swollen tip of the sporangiophore formed within the sporangium.
Conidioma (pl. conidiomata) any structure which bears conidia, including conidiophores, acervulae, pycnidia and sporodochia.
Conidiophore a specialised hypha, either simple or branched, bearing conidiogenous cells and conidia.

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Conidium (pl. conidia) an asexually produced spore.
Coremium (pl. coremia) an erect, compact, sometimes fused cluster of conidiophores, bearing conidia at the apex only, or on both apex and sides.
Culture ex-type see "ex-type culture".

D

Dendritic irregularly branched; tree-like.
Denticle a smooth tooth-like projection, especially one on which a spore is borne.
Doliiform barrel-shaped.

E

Ellipsoidal elliptical in optical section.
Epitype a specimen or (for fungi) a metabolically inactive culture chosen to best represent a species when no holotype or neotype exists.
Ex-type culture a culture derived from a type.
Exudate drops of liquid on the surface of fungal colonies; sometimes minute droplets adhering to hyphae.

F

Fascicle a little group or bundle, especially of hyphae.
Fimbriate fringed; delicately toothed; referring to colony margins.
Floccose cottony, fluffy.
Footcell in *Aspergillus*, the basal cell from which a stipe forms.
Funicle a fine rope of hyphae.
Funiculose aggregated into rope-like strands.
Fusiform spindle-like; narrowing towards the ends.

G

Geniculate bent like a knee.
Gymnothecium an ascocarp having walls composed of hyphae.

H

Holomorph referring to the whole fungus; both anamorph and teleomorph.
Holotype the specimen or illustration or (for fungi) the culture preserved in a metabolically inert state (by lyophisation or deep freezing) designated by the describer as representing his new species.
Hülle cells thick walled cells surrounding ascocarps in e.g. *Emericella nidulans*.
Hyaline transparent or nearly so; colourless.
Hyphomycete a fungus bearing conidia on an aerial fruiting structure.

I

Intercalary between the apex and the base.

M

Macroconidium in *Fusarium*, the larger type of conidium, multicelled and more or less curved.
Merosporangium in Mucorales, a cylindrical outgrowth from the swollen end of the sporangiophore, in which sporangiospores are produced.
Metula (pl. metulae) apical branch of a stipe bearing phialides, especially in *Penicillium* and *Aspergillus*.
Microconidium in *Fusarium* species, a small, usually one-celled conidium, distinction from macroconidium.

N

Neotype a specimen or (for fungi) a culture preserved in a metabolically inert state (by lyophisation or deep freezing) designated as a replacement when a holotype was not designated by the original describer or has been lost or destroyed.
Nonseptate without any crosswalls; usually referring to hyphae.

O

Oblate flattened at the poles.
Ogival pointed at one end, rounded at the other.
Ontogeny development (of fruiting structures or conidia).
Ostiole a pore by which spores are freed from an ascocarp or other enveloping fruiting body.

P

Papilla a small, rounded process.
Pedicel a small stalk.
Penicillus the structure which bears conidia in *Penicillium* and similar genera; consisting of phialides alone or in combination with metulae or other supportive elements, borne on a stipe.
Perithecium (pl. perithecia) a subglobose or flask-shaped ascocarp, closed at maturity except for a narrow passage (ostiole) through which the ascospores are liberated.
Phialide a conidiogenous cell which produces conidia in basipetal succession, without an increase in the length of the phialide itself.
Pionnotes a spore mass with a mucoid or grease-like appearance (in *Fusarium*).
Polyphialide conidiogenous cell with more than one opening, through which conidia are produced in basipetal succession.
Pycnidium flask-shaped or spherical fruiting body superficially resembling a perithecium, but lined with conidiophores and conidia.
Pyriform pear-shaped.

R

Ramus (pl. rami) a specialised cell giving rise to a whorl of metulae and phialides.
Reniform kidney-shaped.
Representative culture a culture believed to be typical of a species, but not derived from a type.
Rhizoid a root-like structure, usually acting as a holdfast or feeding organ for hyphae (in *Rhizopus*).
Rugose with surface roughened.

S

Sclerotioid hard, like a sclerotium.
Sclerotium (pl. sclerotia) a resting body, usually globose, consisting of a compacted mass of mycelium, often very hard.
Septum (pl. septa) a crosswall in a cell.
Spinose spiny.
Sporangiole a small sporangium.
Sporangiophore a specialised hyphal branch which supports one or more sporangia.
Sporangiospore an asexual spore borne within a sporangium.
Sporangium (pl. sporangia) a closed unicellular structure, usually round, in which asexual spores are produced (e.g. in Mucorales).
Sporodochium (pl. sporodochia) a cushion-like mass of conidiogenous cells producing conidia (e.g. macroconidia in *Fusarium*).
Stipe a hypha supporting a fruiting structure, the whole forming a conidiophore.
Stolon a "runner", as in *Rhizopus*.
Striate marked with ridges, grooves or lines.
Stroma (pl. stromata) a layer or matrix of vegetative hyphae bearing spores on very short conidiophores, or having perithecia or pycnidia embedded in it.
Sulcate furrowed or grooved.
Sympodial describes a mechanism of conidiogenous cell proliferation in which each new growing point appears just behind and to one side of the previous apex, producing a succession of fruiting structures.

T

Taxon (pl. taxa) taxonomic groups of any rank.
Teleomorph the ascosporic state of a fungus.
Terverticillate refers to a penicillus with three branch points, i.e., bearing rami, metulae and phialides.
Truncate ending abruptly, as if cut straight across.
Type the specimen, illustration of (for fungi) the culture preserved in a metabolically inert state that has been chosen to represent the description on which a species was based. It is not necessarily typical of the species as it becomes circumscribed in the future.

U

Umbonate having the central portion of the colony raised.

V

Velutinous with a surface texture like velvet.
Verticil a cluster of metulae or phialides with a common origin.
Vesicle a swelling; the apical swelling of a stipe.
Vesiculate terminating in a vesicle.

X

Xerophile a fungus which is able to grow at or below a water activity of 0.85.

Z

Zygospore a thick-walled sexual spore produced by Zygomycetes.

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