Fungi and Food Spoilage

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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 updating – 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 basic 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 are 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 *A. 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 *A. 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 Anne-Laure 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 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 the 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 Nomarski 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.

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 the 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 five-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 of support, the book would not have been completed.

Contents

Chapter 1
Introduction

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

Until 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 200 years ago, and the acute toxicity of macrofungi has long been known. Japanese scientists recognised the toxic nature of yellow rice 100 years ago, but 70 years elapsed before its fungal cause was confirmed. Alimentary toxic aleukia killed many thousands of people in the USSR in 1944–1947; although fungal toxicity was suspected by 1950, the causal agent, T-2 toxin, was not clearly recognised for another 25 years.

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 disasters followed in rapid succession, that the Western world became aware that common spoilage moulds could produce significant toxins. Since 1960 a seemingly endless stream of toxigenic fungi and potentially toxic compounds has been discovered. On these grounds alone, the statement "It's only a mould" is 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 flurry 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 of 5–10% of all food production 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 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 often 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

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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, *Eurotium chevalieri* on hazel nuts, *Penicillium roqueforti* 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 the young discipline, 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 isolated fungi from foods? What are the best media to use? How do we go about identifying food spoilage fungi? Third, the commodity: what fungi are usually associated with a particular food? Here ecological factors interact to produce a more or less specific habitat. Major classes of foods and their associated spoilage fungi are described. Finally, the fungus: what fungus is that? 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. Accurate information on mycotoxin production 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, 1979b) and extended here to other genera relevant to the food industry. Under this regimen, cultures are incubated for 1 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 7 days. For a few kinds of fungi, notably 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?"

Chapter 2
The Ecology of Fungal Food Spoilage

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 fruiting 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 substrate for a range of fungi. Humans have aided and abetted the development of food spoilage fungi through their vast and varied food stores. It can be argued, indeed, some rapidly evolving organisms, such as haploid asexual fungi, are moving into niches created by man's exploitation of certain 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 Chapter 11 and under particular species.

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

(l) water activity;
(2) hydrogen ion concentration;
(3) temperature – of both processing and storage;
(4) gas tension, specifically of oxygen and carbon dioxide;
(5) consistency;
(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 microbiologists by Scott (1957), who showed that a_w effectively quantified 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.

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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 relation 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); Rockland and Beuchat (1987).

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 all other organisms, fungi are 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). 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 and bacteria can grow in saturated sodium chloride (0.75 a_w), 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 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 Chapter 9.

Information about the water relations of many fungi remains fragmentary, 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

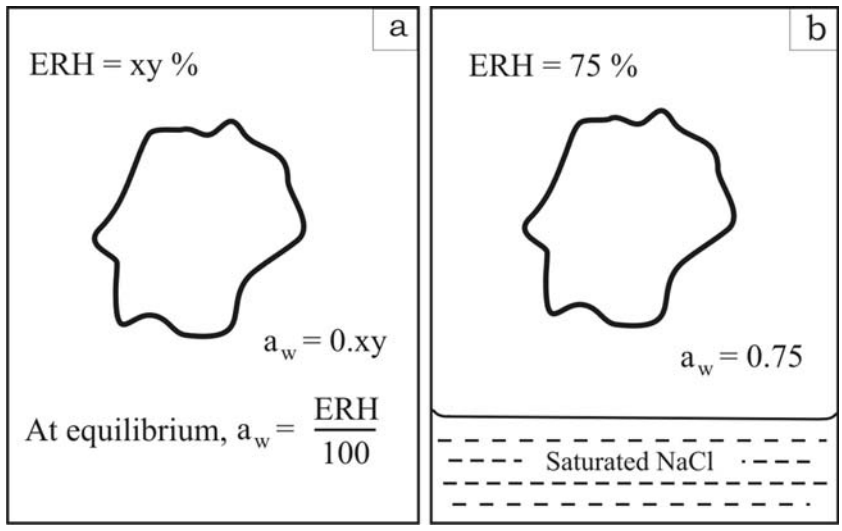

Fig. 2.1 The concept of water activity (a_w) **(a)** the relationship between a_w and equilibrium relative humidity (ERH); **(b)** one method of controlling a_w by means of a saturated salt solution, which generates a specific ERH at a specific constant temperature

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	*Chrysosporium* *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.

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. 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: few accurate data points exist and the diagram is schematic.

For heat-processed foods, pH 4.5 is of course critical: heat processing 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 (Section 2.3).

2.3 Temperature

The influence of temperature in food preservation and spoilage has two separate facets: temperatures 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 6–7C° (King et al., 1969) are practical working figures. The heat resistance of

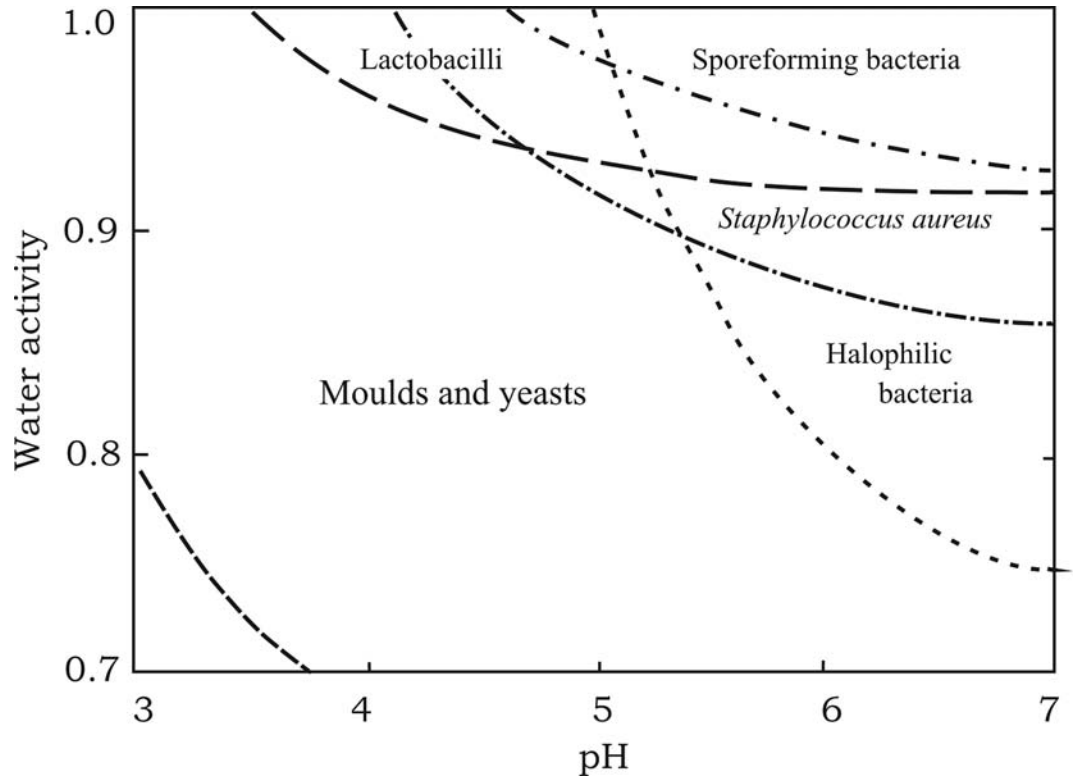

Fig. 2.2 A schematic diagram showing the combined influence of water activity and pH on microbial growth

B. nivea ascospores is marginally lower (Beuchat and Rice, 1979; Kotzekidou, 1997a).

Ascospores of *Neosartorya fischeri* have a similar heat resistance to those of *Byssochlamys fulva*, but have been reported less frequently as a cause of food spoilage. Heat resistant fungi are discussed further in Chapter 4.

Food products may be stored at ambient temperatures, in which case prevention of spoilage relies on other parameters, or under refrigeration, where temperature is expected to play a preservative role. Food frozen to –10°C or below appears to be microbiologically stable, despite some reports of fungal growth at lower temperatures. The lowest 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).

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

			Survivors (%)		
Fungus	Spore type	Initial viable count/ml	50°C	60°C	70°C
Eurotium amstelodami	Ascospores	5.0×10^2	93	85	3
	Conidia	7.3×10^2	107	0.3	0
Eurotium chevalieri	Ascospores	1.0×10^3	103	62	21
	Conidia	8.9×10^2	128	0.1	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).

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 moulds known.

2.4 Gas Tension

Food spoilage moulds, like almost all other filamentous fungi, have an absolute requirement for oxygen. However, many 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* grows virtually normally in 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* produced normal colonies at 25°C under 650 mm of vacuum (Pitt, unpublished).

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. 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* has been reported to grow in similar levels of carbon dioxide (Dallyn and Everton, 1969).

Byssochlamys species appear to be particularly tolerant of conditions of reduced oxygen and/or elevated carbon dioxide. Growth of *Byssochlamys 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 *Byssochlamys nivea* and *B. fulva* were capable of growth in atmospheres containing 20, 40 or 60% carbon dioxide with less than 0.5% oxygen, but inhibition increased with increasing carbon dioxide concentration (Taniwaki et al., 2001a). *Byssochlamys 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 such fungi as *Mucor* species, *Absidia spinosa, Geotrichum candidum*, *Fusarium oxysporum* and *F. solani* (Stotzky and Goos, 1965; Curtis, 1969; Taniwaki, 1995). 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 most food spoilage problems due to filamentous fungi 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 *Zygosaccharomyces 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 moulds 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.

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.

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 sources 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* are unable to utilise nitrate (Pitt, 1979b).

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 and *Xeromyces bisporus* also 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.

A further point on nutrients in foods is worth making here. Certain foods (or nonfoods) lack nutrients essential for the growth of spoilage fungi. Addition of nutrient, for whatever reason, can turn a safe product into a costly failure.

Two cases from our own experience illustrate this point, both involving spoilage by the preservative-resistant yeast *Zygosaccharomyces bailii*. 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 *Zygosaccharomyces 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 *Eurotium* (*Aspergillus*) *amstelodami* 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 *Eurotium chevalieri* and reported that the extreme xerophiles *Chrysosporium fastidium* 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* was 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. *Basipetospora halophila* and *Polypaecilum pisce* grow more rapidly in media containing NaCl as controlling solute (Andrews and Pitt, 1987; Wheeler et al., 1988c). 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, because to be effective they must be present as the undissociated acid. For studies of the mechanism of action of weak acid preservatives see Warth (1977, 1991); Brul and Coote (1999); Stratford and Anslow (1998) 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, 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 and this property 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.

Chapter 3
Naming and Classifying Fungi

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 or underlined. The classification of organisms in genera and species was a concept introduced by Linneaus in 1753 and it is the keystone of biological science. It 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: Biosystematics

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 fungi, plants and algae, is governed by the International Code of Botanical Nomenclature (ICBN).

The ICBN is a relatively complex document of about 70 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 17th version of the ICBN (the Vienna code) is the most recently published (McNeill et al., 2006). The ICBN 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 ICBN 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 ICBN; 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 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 ICBN 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

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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 ICBN arise from this example. *P. 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, they retained the name *P. frequentans*, which was nomenclaturally incorrect, causing confusion when Subramanian (1971) and Pitt (1979b) 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 ICBN, not from that of later taxonomists who correctly interpreted it.

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 subkingdoms. Similarly a species can be divided into smaller entities: subspecies, varieties and *formae speciales* (a term usually reserved for plant pathogens).

In most modern classifications, the fungi are ranked, like plants and animals, as a separate kingdom. Traditionally, fungi have been divided into several subkingdoms, based on spore type and some environmental considerations. Modern molecular methods have revolutionised this. Fungi have been shown to be more closely related to animals than plants, where traditional taxonomy has always placed them. Some of the so-called "lower fungi" have been shown not to be fungi of all (though mycologists will no doubt continue to study them).

The most important change from the point of view of the food mycologist is the demise of the subkingdom Deuteromycotina, and its absorption (almost entirely) into the Ascomycotina. The connection between the Ascomycetes, the fungi that produce sexual spores in sacks, and the Deuteromycetes, where spores are always asexual, has been known for a long time. However, molecular taxonomy has provided the fundamental assurance needed to make this change. From the point of view of the food mycologist, this is a mixed blessing. The demands of the molecular systematists may yet make the taxonomy of foodborne fungi even more complicated. The taxonomic system used here is believed to be both practical and in line with the current "best practice" of the nomenclaturalists.

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:

Kingdom	Fungi	Fungi	Fungi
Subkingdom	Zygomycotina	Ascomycotina	Basidiomycotina
Class	Zygomycetes	Plectomycetes	Wallemiomycetes
Order	Mucorales	Eurotiales	Wallemiales
Family	Mucoraceae	Trichocomaceae	Wallemiaceae
Genus	*Rhizopus*	*Eurotium*	*Wallemia*
Species	*stolonifer*	*chevalieri*	*sebi*
Variety		*intermedius*	

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

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

3.3 Zygomycotina

Most fungi within the subkingdom Zygomycotina belong to 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 food spoilage point of view, 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 food safety point of view, Zygomycetes have rarely been reported to produce mycotoxins.

3.4 Ascomycotina

The subkingdom Ascomycotina is distinguished from Zygomycotina 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 subkingdom is usually slower than that of Zygomycetes, although there are some exceptions.

Fungi in the subkingdom Ascomycotina, loosely 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 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

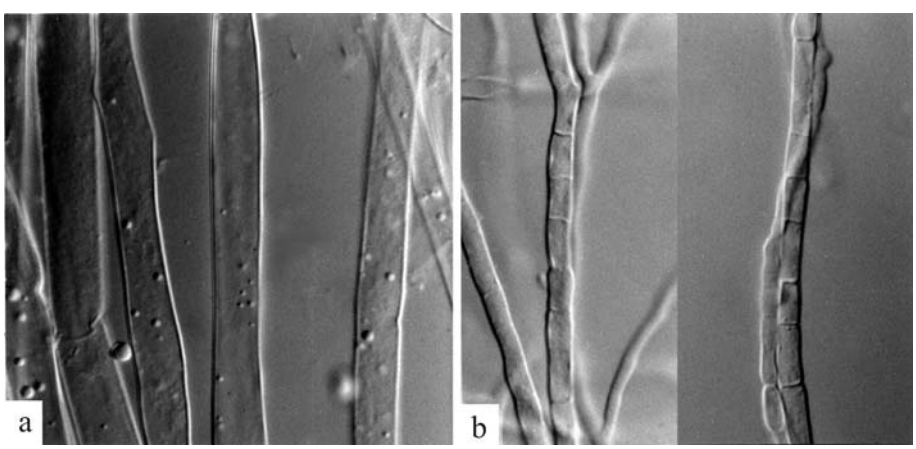

Fig. 3.1 **(a)** Nonseptate mycelium of *Syncephalastrum racemosum*; **(b)** septate mycelium of *Fusarium equiseti*

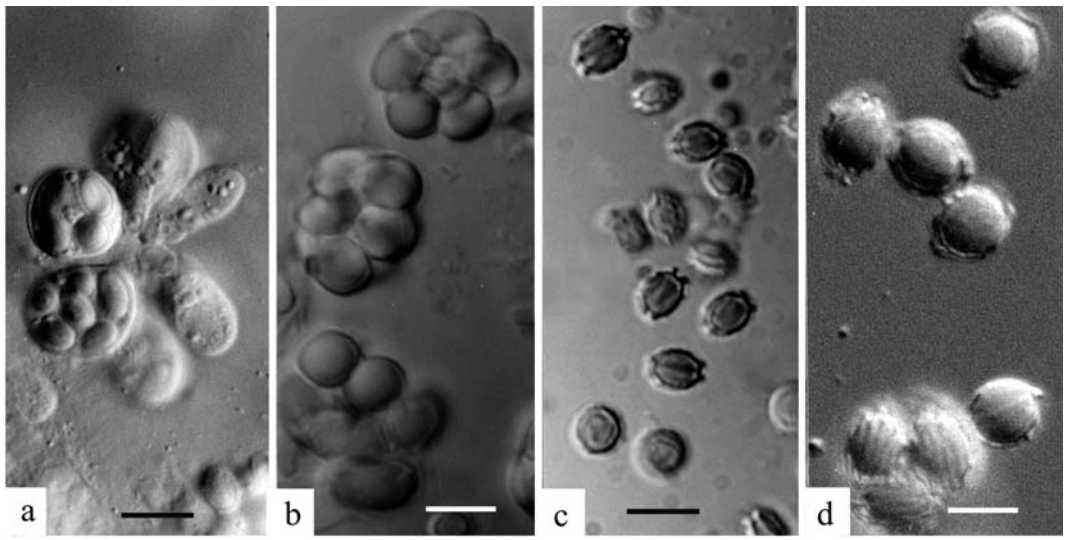

Fig. 3.2 Asci and ascocarps: **(a)** asci of *Talaromyces* species; **(b)** asci of *Byssochlamys fulva*; **(c)** ascospores of *Eupenicillium alutaceum*; **(d)** ascospores of *Neosartorya quadricincta*. Bars = 5 μm

and chemicals. Almost all xerophilic fungi are ascomycetes.

Besides their sexual spores, ascospores, ascomycetes commonly produce 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. Along the evolutionary process, some Ascomycetes with well-developed asexual stages lost the ability to produce ascospores, and rely entirely on conidia for dispersal.

Conidia, and the specialised hyphae from which they are borne, are astonishingly diverse in appearance. The size, shape and ornamentation of conidia and the complexity of the structures producing them

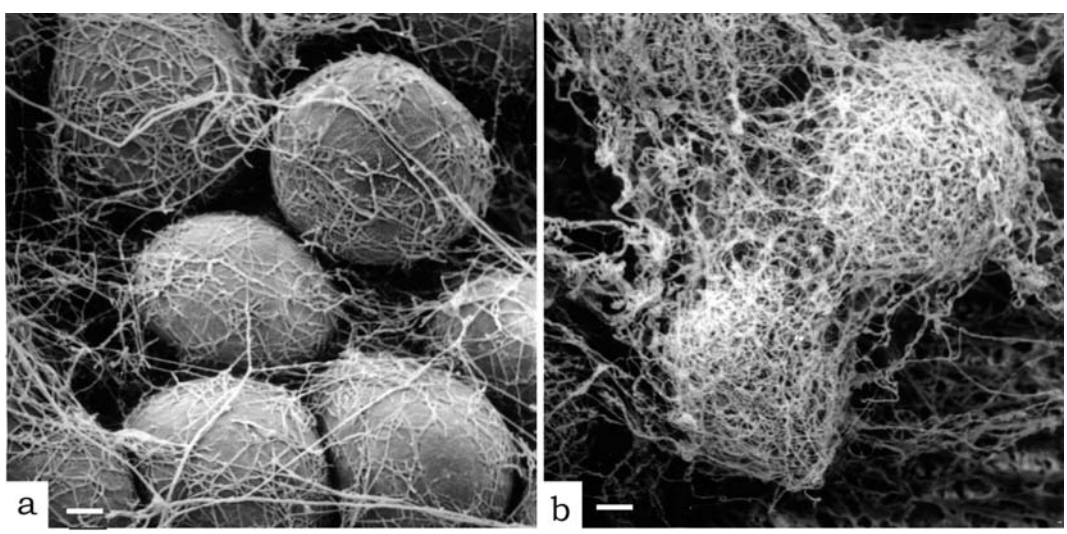

Fig. 3.3 **(a)** Cleistothecia of *Eupenicillium*; **(b)** gymnothecia of *Talaromyces*. SEM. Bars = 50 μm

provide the basis for classification of Ascomycetes that no longer produce the ascosporic (sexual) stage.

Lacking ascospores, conidial fungi are not usually heat resistant, but conidia may be quite resistant to chemicals. Some conidial fungi are xerophilic.

3.5 Basidiomycotina

The Subkingdom Basidiomycotina includes mushrooms, puffballs and the plant pathogenic rusts and smuts. Until recently it was not considered of any interest to the food mycologist. However, molecular studies indicate that the small brown species *Wallemia sebi*, long a curiosity because of its lack of resemblance to any other fungus, 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 of foodborne fungi, *Trichosporonoides nigrescens* Hocking and Pitt (1981), has a known affinity with this subkingdom.

3.6 The Ascomycete – Conidial Fungus Connection

It was established more than a century ago that many fungal species carry the genetic information to produce both ascospores and conidia. These two kinds of spores are produced by different mechanisms and have different functions, so they are not always formed simultaneously. Not surprisingly, mycologists sometimes have given different generic and species names to a single fungus producing both an ascosporic and a conidial state. The usage of these names under the ICBN depends on the circumstances under which they were originally given. Some of these circumstances are discussed briefly below.

The ascomycete state, now usually referred to as the *teleomorph*, is regarded by nomenclaturalists as the more important reproductive state, and the name applied to the teleomorph should be used when the ascomycete state is present. If the conidial state is also in evidence, the fungus is now a *holomorph* and is still correctly known by the teleomorph name. If the conidial state, known as the *anamorph*, has a separate name, this strictly speaking applies to the conidial state. It should be used only when the ascomycete state is absent, or to refer specifically to the conidial state if the ascomycete is present. However, the reader is warned that some anamorphic names are, and will continue to be, in common use for holomorphic fungi.

Under the Articles of the ICBN, a generic name originally given to an anamorphic or conidial fungus cannot be used for a teleomorphic or ascomycetous fungus. For example, the name *Penicillium*, originally given to an anamorphic fungus with no known teleomorph, cannot be used for the teleomorphs later found to be produced by other *Penicillium* species. Such teleomorphs are classified in the genera *Eupenicillium* or *Talaromyces*, depending on whether ascospores are produced in cleistothecia or gymnothecia.

Correct species names for the ascomycetous and conidial states of a single holomorphic fungus may or may not be the same, depending both on the circumstance in which the names were originally given, and on later synonymy. For example, *Eupenicillium ochrosalmoneum* Scott and Stolk and *Penicillium ochrosalmoneum* Udagawa refer to the teleomorph and anamorph of a single fungus. Udagawa (1959) described the anamorph; the teleomorph was later found, in the same isolate, by Scott and Stolk (1967).

On the other hand, the anamorph of *Eupenicillium cinnamopurpureum* Scott and Stolk (1967) is *Penicillium phoeniceum* van Beyma (1933), with *P. cinnamopurpureum* Abe ex Udagawa (1959) as a synonym. Scott and Stolk (1967) found a teleomorph in Udagawa's *P. cinnamopurpureum*; Pitt (1979b) later showed that this species was a synonym of the earlier *P. phoeniceum*. *E. cinnamopurpureum*, the first name applied to the teleomorph, is unaffected by this change in the anamorph name. In passing, note that "Abe ex Udagawa" indicates invalid (incomplete) publication of this species by Abe, with validation later by Udagawa. The species dates from the year of validation.

3.7 Dual Nomenclature

An important point here is that some isolates of *Penicillium phoeniceum* regularly produce the teleomorphic state *Eupenicillium cinnamopurpureum*, while

others, taxonomically indistinguishable, fail to produce a teleomorph at all. Because of this, it is essential to have a separate name for teleomorph and anamorph. The system of two names for a single fungus, known as dual nomenclature, has a place in the classification of fungi despite its apparent complexity. In the descriptions in later chapters, fungi for which both teleomorphs and anamorphs are known have both names listed. As noted above, if both states are found in a particular isolate, the teleomorph name is the more appropriate: to use that given to the anamorph is not incorrect, but this name is more sensibly applied to the conidial state only.

Dual nomenclature would be relatively simple if the relationship between anamorph and teleomorph was always one to one. This is not the case. As has already been mentioned, species classified in *Penicillium* may produce teleomorphs in two genera, *Eupenicillium* and *Talaromyces*. On the other hand *Talaromyces* produces anamorphs in two genera, *Penicillium* and *Paecilomyces*. *Aspergillus* is the anamorph of eight or ten teleomorphic genera. Most teleomorph–anamorph relationships encountered in food mycology belong to the genera mentioned here. These relationships will be described where necessary under these particular genera.

3.8 Practical Classification of Fungi

Fungi are classified in a vast array of orders, families, genera and species. Among natural organisms, the numbers of taxa of fungi are rivalled only by those of the 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 only in association with plant roots. From the point of view of the food microbiologist, these kinds of fungi are irrelevant. In one sense, most 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 Chapter 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 or of similar appearance.
- 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 nearly all species included in this work is based entirely on inoculation of a single series of Petri dishes, incubation under carefully 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.

The use of cultural characters has long been implicit in the study of fungi in pure culture on artificial substrates, especially in such genera as *Aspergillus* and *Penicillium*, genera of paramount importance in food spoilage. In *Penicillium*, cultural characters have been used as taxonomic criteria since the turn of the 20th century, but have assumed greater importance through the work of Pitt (1973, 1979b), who used the measurement of colony diameters, following incubation under standardised conditions, as a taxonomic criterion. The use of

pure culture techniques and growth data in fungal taxonomy is now widespread.

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 in the present work. This approach is a logical extension of the system used by the first author in *Penicillium* taxonomy and which has been found to have a much broader applicability.

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.

The approach used here has been to examine 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 first the xerophiles, many of which grow poorly if at all on the standard media, and, second, 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 Chapter 4.

Chapter 4
Methods for Isolation, Enumeration and Identification

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 were held in Copenhagen, Denmark (1994), Uppsala, Sweden (1998), Samsø, Denmark (2003) and Key West, Florida (2007). Papers from the third and fourth workshops were published in the *International Journal of Food Microbiology* and the proceedings of the fifth (Samsø) workshop were published as "Advances in Food Mycology" (Hocking et al., 2006a).

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.

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,

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