Fungal Biology

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4th edition

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Preface

Fungal Biology (4th edition) is the successor to three previous editions of "Modern Mycology." The text has been fully updated and expanded to cover many new developments in fungal biology. Each of the 17 chapters is largely independent, with a clear theme and cross-referencing, so that the text can be used to focus on selected topics.

The early chapters deal with the unique structure and organization of fungi and fungus-like organisms, including modern experimental approaches in fungal biology, and the many ways in which fungi respond to environmental cues. These chapters also cover the diversity of fungi, and fungal products including immunosuppressants, antibiotics, and mycotoxins that contaminate food.

Recent developments in fungal genetics, molecular genetics, and genomics are discussed within the framework of a "biochemical and molecular toolbox," using in-depth examples such as the roles of virus-like double-stranded RNA for the control of chestnut blight, and the population dynamics of Dutch elm disease. Major sections of the text deal with the development of fungi as commercial biological control agents of plant pathogens and insect pests. In addition, one of the three new chapters deals with the symbiotic associations of fungi with plants and animals, and the biology of lichens. Plant pathogens and plant defense also are covered in depth, using selected examples of all the major pathosystems.

Two final chapters are devoted to the "moulds of man," covering the biology, pathogenicity, and virulence factors of the major fungal diseases of humans, and the antifungal drugs used to treat these conditions.

This text is designed to appeal to both undergraduates and postgraduates. The emphasis throughout is on the functional biology of fungi, with several examples from recent research, and many tables and illustrations. The text is supported by a comprehensive website (available via **www.blackwellpublishing.com/deacon**), with over 600 images, many in color, including "Special Focus Topics" and "Profiles of Significant or Interesting Fungi." My own images are identified, and can be used freely, without restriction. The website also has a large interactive (randomized) test bank of multiple-choice questions, designed to aid self-assessment and reinforcement of key learning outcomes.

I wish to thank many colleagues who have contributed to this book by providing images and resources. They include many of my doctorate students, and Nick Read's research group at the University of Edinburgh, who have been supportive throughout.

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Edinburgh

Chapter 1

Introduction: the fungi and fungal activities

This chapter is divided into the following major sections:

- the place of fungi in the "Tree of Life" – setting the scene
- the characteristic features of fungi: defining the fungal kingdom
- the major activities of fungi as parasites, symbionts and saprotrophs
- fungi in biotechnology

Fungi are a unique group of organisms, different from all others in their behavior and cellular organization. Fungi also have an enormous range of activities – as pathogens of crop plants or humans, as decomposer organisms, as experimental "model organisms" for investigating genetics and cell biology, and as producers of many important metabolites. The uniqueness of fungi is a prominent feature of this book, which adopts a functional approach, focusing on topics of inherent interest and broad significance in fungal biology.

The uniqueness of fungi is reflected in the fact that they have the status of a **kingdom**, equivalent to the plant and animal kingdoms. So, fungi represent one of the three major evolutionary branches of multicellular organisms.

In terms of biodiversity, there are estimated to be at least 1.5 million different species of fungi, but only about 75,000 species (5% of the total) have been described to date. For comparison, there are estimated to be 4.9 million arthropod species and about 420,000 seed plants (Hawksworth 2001, 2002).

If the estimate of the number of fungal species is even remotely accurate then we still have much to learn, because even the fungi that we know about play many important roles. To set the scene, we can mention just a few examples:

- Fungi are the most important causes of **crop diseases**, responsible for billions of dollars worth of damage each year, and for periodic devastating disease epidemics.
- Fungi are the main **decomposers** and recyclers of organic matter, including the degradation of cellulose and wood by the specialized enzyme systems unique to fungi.
- Fungi produce some of the most toxic known metabolites, including the **carcinogenic aflatoxins** and other **mycotoxins** in human foods and animal feedstuffs.
- With the advance of the acquired immune deficiency syndrome (AIDS) and the increasing role of transplant surgery, fungi are becoming one of the most significant causes of death of **immunocompromised** and **immunosuppressed patients**. Fungal diseases that were once extremely rare are now commonplace in this sector of the population.
- Fungi have an enormous range of **biochemical activities** that are exploited commercially – notably the production of antibiotics (e.g. **penicillins**), **steroids** (for contraceptives), **ciclosporins** (used as immunosuppressants in transplant surgery), and enzymes for food processing and for the soft drinks industry.
- Fungi are **major sources of food**. They are used for bread-making, for mushroom production, in several traditional fermented foods, for the production of Quorn™ mycoprotein – now widely available in supermarkets and the only survivor of the many

"single-cell protein" ventures of the late 1900s – and, of course, for the production of alcoholic drinks.
- Fungi can be used as "cellular factories" for producing **heterologous** (foreign) **gene products**. The first genetically engineered vaccine approved for human use was produced by engineering the gene for hepatitis B surface antigen into the yeast (*Saccharomyces cerevisiae*) genome. In this way the antigen can be produced and exported from the cells, then purified from the growth medium.
- The **genome sequences** of several fungi have now been determined, and in several cases the genes of fungi are found to be homologous (equivalent) to the genes of humans. So, fungi can be used to investigate many fundamental cell-biological processes, including the control of cell division and differentiation relevant to biomedical research.
- Fungi are increasingly being used as commercial **biological control agents**, providing alternatives to chemical pesticides for combating insect pests, nematodes, and plant-pathogenic fungi.

The first part of this book (Chapters 1–9) deals with the growth, physiology, behavior, genetics, and molecular genetics of fungi, including the roles of fungi in biotechnology. This part also includes an overview of the main fungal groups (Chapter 2). The second part (Chapters 10–16) covers the many ecological activities of fungi – as decomposers of organic matter, as spoilage agents, as plant pathogens, plant symbionts, and as pathogens of humans. A final chapter is devoted to the ways of preventing and controlling fungal growth, because this presents a major challenge in modern *Fungal Biology*."

The place of fungi in the "tree of life" – setting the scene

The **Tree of Life Web Project** is a major collaborative internet-based endeavor (see Online resources at the end of this chapter). Its aim is ultimately to link all the main types of organism on Earth according to their natural phylogenetic relationships. The hope is that this will lead us closer to the very root of life on earth, which is currently estimated to be some 3.6–3.8 billion years ago (1 billion = 1000 million years; 10^9 years). However, fungi arrived much later on the scene. The oldest known fossil fungi date to the Ordovician era, between 460 and 455 million years ago – a time when the largest land plants are likely to have been bryophytes (liverworts and mosses). This accords remarkably well with recent phylogenetic analyses based on comparisons of gene sequences, discussed below.

Carl Woese of the University of Illinois at Urbana-Champaign, USA, has championed the use of molecular phylogenetics. The basis of this is to identify genes that are present in all living organisms and that have an essential role, so they are likely to be highly conserved, accumulating only small changes (mutations and back mutations) over large spans of evolutionary time. Comparisons of these sequences can then indicate the relationships between different organisms. There are limitations and uncertainties in this approach, because of the potential for lateral gene transfer between species and because there are known to be variable rates of gene evolution between different groups of organisms. However several highly conserved genes and gene families can be used to provide comparative data.

Most phylogenetic analyses are based primarily on the genes that code for the production of **ribosomal RNA**. Ribosomes are essential components of all living organisms because they are the sites of protein synthesis. They occur in large numbers in all cells, and they are composed of a mixture of RNA molecules (which have a structural role in the ribosome) and proteins. In **prokaryotes** (non-nucleate cells) the ribosomes contain three different size bands of ribosomal RNA (**rRNA**), defined by their sedimentation rates (S values, also known as Svedberg units) during centrifugation in a sucrose solution. These three rRNAs are termed 23S, 16S, and 5S. In **eukaryotes** (nucleate cells) there are also three rRNAs (28S, 18S, and 5.8S). The genes encoding all of these rRNAs are found in multiple copies in the genome, and the different rRNA genes can be used to resolve differences between organisms at different levels.

For most phylogenetic analyses the genes that code for 16S rRNA (of prokaryotes) and the equivalent 18S rRNA (of eukaryotes) are used. These **small subunit rDNAs** contain enough information to distinguish between organisms across the phylogenetic spectrum. Using this approach, several different phylogenetic trees have been generated, but many of them are essentially similar, and one example is shown in Fig. 1.1.

Several points arise from Fig. 1.1, both in general terms and specifically relating to fungi.

- Ribosomal DNA sequence analysis clearly demonstrates that there are three evolutionarily distinct groups of organisms, above the level of kingdom. These three groups – the **Bacteria**, **Archaea**, and **Eucarya** (eukaryotes) – are termed **domains** and the differences between them are matched by many differences in cellular structure and physiology.
- Beneath the level of domains, there is still uncertainty about the taxonomic ranks that should be assigned to organisms. Plants, animals, and fungi are almost universally regarded as separate **kingdoms**

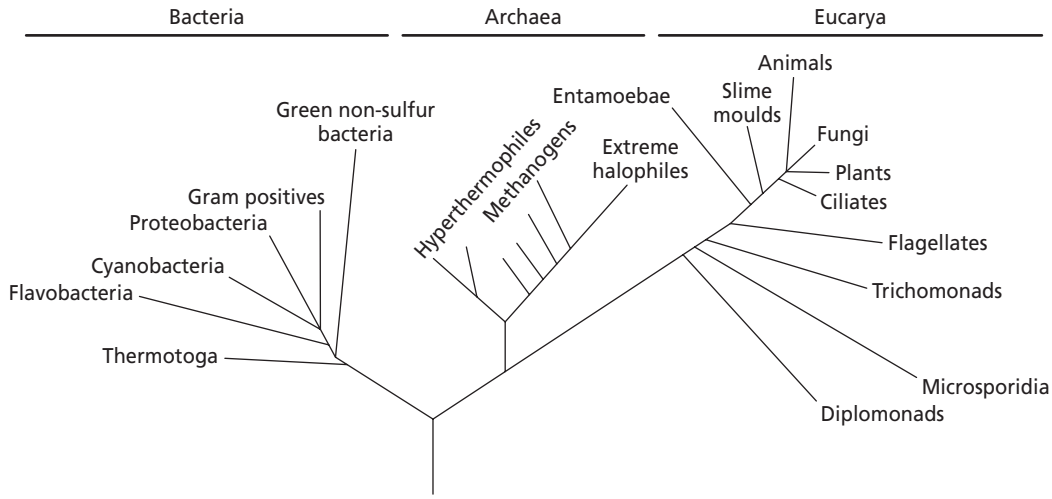


Fig. 1.1 A representation of the **Universal Phylogenetic Tree**, based on comparisons of the genes encoding small-subunit (16S or 18S) ribosomal RNA. The lengths of the lines linking organisms to their nearest branch point represent inferred evolutionary distances (rRNA gene sequence divergence). (Based on a diagram in Woese (2000) but showing only a few of the major groups of organisms.)

(Whittaker 1969). But, arguably, this status could also apply to the many "kingdoms" of bacteria, especially the enormous **Proteobacteria** kingdom which includes most Gram-negative bacteria. And, it could be argued that the many separate groups of unicellular eukaryotes (amoebae, slime moulds, flagellates, etc.) should also be regarded as kingdoms, based on their apparently long-term separation as judged by rDNA sequence divergence. However, many of these lower eukaryotes are still poorly studied, so they are often referred to collectively as "protists," pending further resolution of their relationships.

- The major multicellular organisms – the **animals**, **plants**, and **fungi** – form a cluster at the very top of the Eucarya Domain, so they are often termed the "**crown eukaryotes**". The interesting feature of these groups is that they seem to have diverged from one another at roughly the same time, and then underwent a major, rapid expansion and diversification. The time when this happened, **roughly half a billion years ago**, coincides with the period when the land surfaces were colonized by primitive plants such as bryophytes (mosses and liverworts) and when there were only three major continental land masses: (i) a land mass including present-day North America and Europe, located near the equator; (ii) part of modern Siberia, towards the north; (iii) a land mass consisting of present-day South America, Africa, Antarctica, India, and Australia in the southern hemisphere.
- Currently, the earliest **fossil evidence of fungi** dates to the Ordovician period, between 460 and 455 million years ago, but it is almost certain that aquatic fungi would have been present before that time, perhaps dating back to about 1 billion years ago. The Chytridiomycota are widely believed to be among the most ancient of the presently known fungi – not least because they have motile flagellate cells, indicating their dependence on free water. By contrast, in the Devonian period (417–354 million years ago) there is abundant evidence of fossil fungi associated with primitive land plants. For example, representatives of several major groups of fungi have been found in the Rhynie Chert deposits of Aberdeenshire, Scotland, representing the Devonian era. The early fossil fungi of the Rhynie deposits are very well preserved and, intriguingly, occur in close association with the underground organs of early land plants. These early terrestrial fungi, belonging to a newly defined group, the Glomeromycota (see Fig. 2.4), are remarkably similar to the arbuscular mycorrhizal fungi that colonize the roots of nearly 80% of present-day land plants (Fig. 1.2). So it seems that these fungi co-evolved with early land plants, and that their hyphae could have facilitated the uptake of mineral nutrients and water from soil, just as they do today (Lewis 1987; Chapter 13).
- Having made the case for a long-term association between fungi and land plants, we need to correct a widely held misconception: there is now **strong evidence that fungi are more closely related to animals than to plants** (Baldauf & Palmer 1993). The fungi evolved as an early branch from the animal

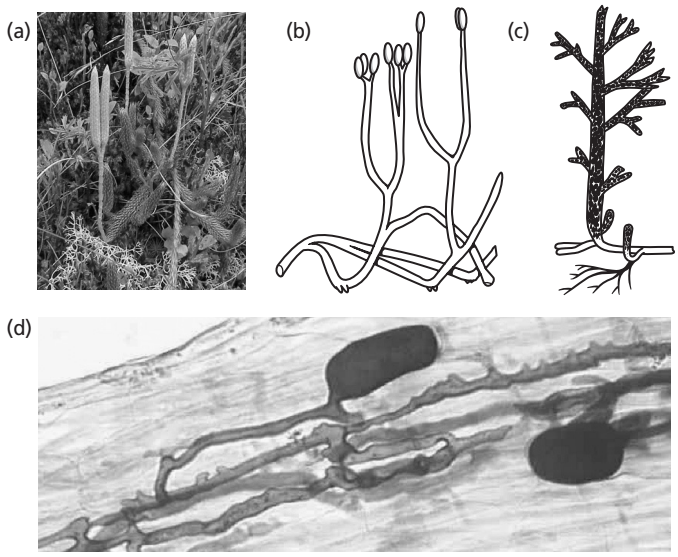


Fig. 1.2 (a) A present-day "club-moss," *Lycopodium*, which represents a primitive member of the ferns (pteridophytes), and (b,c) two fossil pteridophytes (*Asteroxylon mackiei*, and *Rhynia major*) from the Rhynie chert deposits. (d) Swollen vesicles of a present-day mycorrhizal fungus are remarkably similar to vesicles found in fossils from the Rhynie deposits (417–354 million years ago).

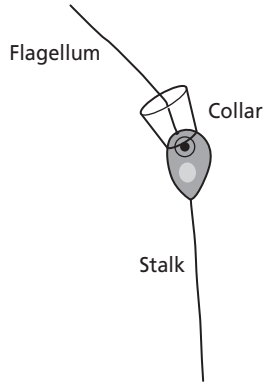


Fig. 1.3 *Codosiga gracilis*, a member of the choanoflagellates (organisms with a single flagellum and a collar), considered to be the common ancestors of both fungi and animals. (Based on a drawing from: http://microscope.mbl.edu/scripts/microscope.php?func=imgDetail&imageID=4575)

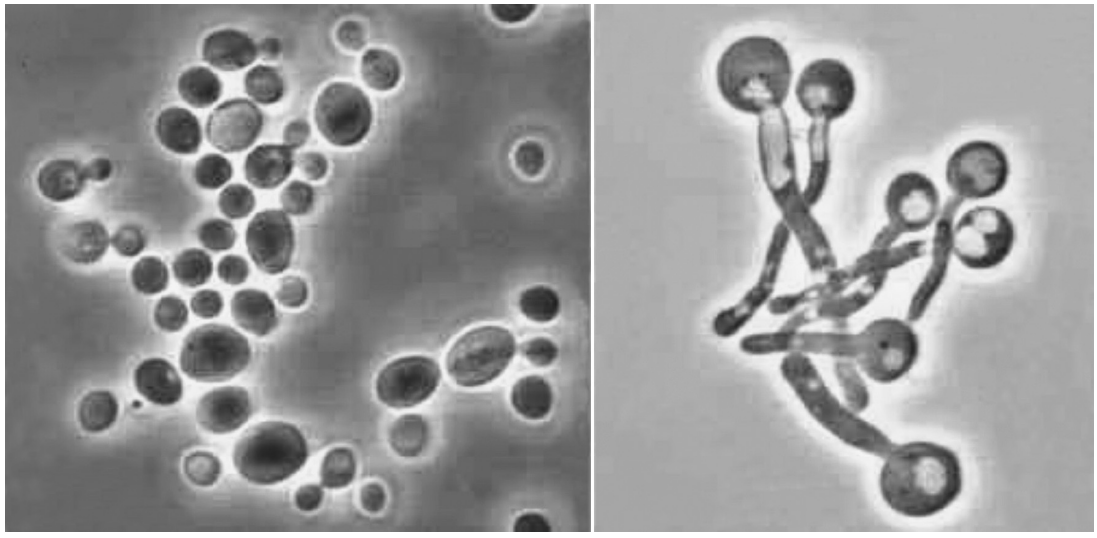
lineage, and both groups probably have a common origin in one of the simple unicellular eukaryotes. Currently it is believed that the most likely common ancestor of both the fungal and the animal kingdoms is a protozoan of the group termed **choanoflagellates**, also known as the collar-flagellates (Fig. 1.3). These resemble both the earliest branch of animals (the sponges) and the earliest branch of fungi (the chytrids). It is a humbling thought that humans should have evolved from something like this!

The characteristic features of fungi: defining the fungal kingdom

To begin this section we must make an important distinction between the **true fungi** and a range of **fungus-like organisms** that have traditionally been studied by mycologists, but are fundamentally different from fungi. Here we will focus on the true fungi, often termed the **Mycota** or **Eumycota**. We will discuss the fungus-like organisms in Chapter 2.

All true fungi have a range of features that clearly separate them from other organisms and that serve to define the fungal kingdom **(Mycota)**. These features are outlined below:

- All fungi are **eukaryotic**. In other words, they have membrane-bound nuclei containing several chromosomes, and they have a range of membrane-bound cytoplasmic organelles (mitochondria, vacuoles, etc.). Other characterisitics, shared by all eukaryotes, include: cytoplasmic streaming, DNA that contains noncoding regions termed introns, membranes that typically contain sterols, and ribosomes of the 80S type in contrast to the 70S ribosomes of bacteria ("S" refers to Svedberg units, as mentioned earlier).
- Fungi typically grow as filaments, termed **hyphae** (singular: hypha), which extend only at their extreme tips. So, fungi exhibit **apical growth** in contrast to many other filamentous organisms (e.g. filamentous green algae) which grow by repeated cell divisions within a chain of cells (intercalary growth). Fungal hyphae branch repeatedly behind their tips, giving



(a) (b)

Fig. 1.4 *Candida albicans*, a common dimorphic fungus that grows on the mucosal membranes of humans. Normally it is found as a budding yeast (a), but the yeast cells can produce hyphae (b) for invasion of the tissues.

rise to a network termed a **mycelium**. However, some fungi grow as single-celled yeasts (e.g. *Saccharomyces cerevisiae*) which reproduce by budding, and some can switch between a yeast phase and a hyphal phase in response to environmental conditions. These **dimorphic fungi** (with two shapes) include several species that are serious pathogens of humans (Chapter 16). They often grow as yeast-like cells for proliferation in the body fluids but convert to hyphae for invasion of the tissues (Fig. 1.4).

- Fungi are **heterotrophs** (chemo-organotrophs). In other words, they need preformed organic compounds as energy sources and also as carbon skeletons for cellular synthesis. The cell wall prevents fungi from engulfing food by phagocytosis, so fungi absorb simple, soluble nutrients through the wall and cell membrane. In many cases this is achieved by secreting enzymes at the hyphal tips to degrade complex polymers and then absorbing the simple, soluble nutrients released by the depolymerase (polymer-degrading) enzymes.
- Fungi have a distinctive range of **wall components**, which typically including **chitin** and **glucans** (polymers of glucose with predominantly β-1,3 and β-1,6 linkages). Short lengths of cellulose (a β-1,4-linked polymer of glucose) have been detected in some fungal walls, especially in some of the primitive fungi. However fungi differ from plants because they do not have cellulose-rich cell walls.
- Fungi have a characteristic range of soluble carbohydrates and storage compounds, including **mannitol** and other sugar alcohols, **trehalose** (a disaccharide of glucose), and **glycogen**. These compounds are similar to those of some animals – notably the arthropods – but are different from those of plants.
- Fungi typically have **haploid nuclei** – an important difference from almost all other eukaryotes. However, fungal hyphae often have several nuclei within each hyphal compartment, and many budding yeasts are diploid. These differences in nuclear status and nuclear arrangements have important implications for fungal genetics (Chapter 9).
- Fungi reproduce by both sexual and asexual means, and typically produce **spores**. Fungal spores vary enormously in shape, size and other properties, related to their various roles in dispersal or dormant survival (Chapter 10).

In summary, we can define fungi by the following characteristic features (Table 1.1):

- eukaryotic
- typically grow as hyphae, with apical growth, but sometimes as yeasts
- heterotrophic – they depend on pre-formed organic nutrients

Table 1.1 Comparison of some features of fungi with those of animals and plants.

Character	*Fungi (and chapter reference)*	*Animals*	*Plants*
Growth habit	Hyphal tip growth or budding yeasts (3, 4)	Not hyphal	Multicellular tissues
Nutrition	Heterotrophic, absorb soluble nutrients (6, 11)	Heterotrophic, ingest food	Photosynthetic
Cell wall	Typically contains chitin (3)	Absent, but chitin is found in insect exoskeletons	Mainly cellulose
Nuclei	Usually haploid; nuclear membrane persists during division (9)	Typically diploid; the membrane breaks down during nuclear division	Diploid; the membrane breaks down during nuclear division
Histones	Histone 2B	Histone 2B	Plant histones
Microtubules	Sensitive to benzimidazoles and griseofulvin (17)	Sensitive to colchicine	Sensitive to colchicine
Lysine synthesis	Synthesized by AAA pathway (7)	Not synthesized, must be supplied	Synthesized by DAP pathway
Golgi cisternae	Unstacked, tubular (3)	Stacked, plate-like	Stacked, plate-like
Mitochondria	Plate- or disk-like cisternae (3)	Plate- or disk-like cisternae	Tubular cisternae
Translocated carbohydrates	Polyols (mannitol, arabitol, etc.), trehalose (7)	Trehalose in insects	Glucose, fructose, sucrose
Storage compounds	Glycogen, lipids, trehalose (7)	Glycogen, lipids, trehalose in some	Starch
Mitochondrial codon usage	UGA codes for tryptophan	UGA codes for tryptophan	UGA codes for chain termination
Membrane sterols	Ergosterol (7, 17)	Cholesterol	Sitosterol and other plant sterols

AAA, alpha-amino adipic acid pathway; DAP, diamino-pimelic acid pathway.

- typically have a haploid genome
- have walls composed primarily of chitin and glucans
- absorb soluble nutrients through the cell wall and plasma membrane
- produce spores.

The major activities of fungi: pathogens, symbionts, and saprotrophs

As we have already seen, all fungi require organic nutrients for their energy source and as carbon nutrients for cellular synthesis. But a broad distinction can be made according to how these nutrients are obtained: (i) by growing as a **parasite** (or a **pathogen** – a disease-causing agent) of another living organism; (ii) by growing as a **symbiont** in association with another organism; or (iii) by growing as a **saprotroph** (saprophyte) on nonliving materials. These topics are covered in detail in Chapters 11–14.

Fungal parasites of plants

A large number of fungi are adapted to grow as parasites of plants, obtaining some or all of their nutrients from the living tissues of their host. Many of these associations are quite specific because the fungus infects only one type of host, and sometimes it is so specific that the fungus cannot grow at all in laboratory culture – it is an obligate parasite that can grow only in the host tissues. Many examples of this are found among the rust fungi and powdery mildew fungi (Chapter 14), while other examples are found in the fungus-like downy mildews (Chapter 2), and the plasmodiophorids (Chapter 2). These host-specific fungi are termed **biotrophic** parasites (*bios* = life; *trophy* = feeding) because they feed from living host cells without killing them, often by producing special nutrient-absorbing structures to tap the host's reserves. At the other end of the spectrum are many common fungi that

aggressively attack plant tissues. They are termed **necrotrophic** parasites (*necros* = death) because they kill the host tissues as part of the feeding process – for example by producing toxins or degradative enzymes. A common example is the fungus *Botryotinia fuckeliana* (more commonly known by its former name, *Botrytis cinerea*) which rapidly destroys soft fruits such as strawberries, raspberries, and grapes, covering the fruit surface with its gray sporing structures.

The fungal (or fungus-like) parasites of plants are enormously significant, accounting for more than 70% of all the major crop diseases, and for many devastating epidemics. To cite just a few examples:

- Potato blight caused by the fungus-like organism *Phytophthora infestans* destroyed the potato crops of Ireland in the 1840s, leading to the starvation of up to one million people, and large-scale emigration to the rest of Europe and the USA. Even today the control of *P. infestans* and its close relatives, the downy mildew fungi, accounts for about 15% of world fungicide sales. *The Advance of the Fungi* by E. C. Large (1940) provides a fascinating and highly readable account of potato blight and its legacy.
- Dutch elm disease, caused by *Ophiostoma novo-ulmi* and *O. ulmi* (Chapter 10), has destroyed most of the common elm (*Ulmus procera*) trees in Britain and Western Europe in the last 30 years, as it did in North America earlier in the 1900s. Similarly, chestnut blight caused by the fungus *Cryphonectria parasitica* (Chapter 9) has devastated the native American chestnut (*Castanea dentata*) population in the USA – an epidemic that can be traced to the first recorded diseased chestnut tree in the New York Zoological Garden in 1904 (Chapter 9). And, at the time of writing, a new species of *Phytophthora* (*P. ramorum*) is causing **sudden oak death** in southwestern USA and has already spread to several parts of Europe (Chapter 14).

Fungal symbionts of plants

Many fungi form symbiotic associations with plants, in which both of the partners are likely to benefit. The two most important examples are **lichens** and **mycorrhizas**. Lichens are intimate associations between two organisms – a photosynthetic partner (a green alga or a cyanobacterium) and a fungus – which together produce a thallus that can withstand some of the most inhospitable environments on Earth (Fig. 1.5). Typically, the fungus encases and protects the photosynthetic cells, and also absorbs mineral nutrients from trace levels in the environment, while the photosynthetic partner provides the fungus with carbon nutrients. There are about 13,500 lichen species across the globe, and they play essential roles as pioneer colonizers of habitats where no other organisms can grow, including rock surfaces and unstable, arid mineral soils (Chapter 13).

Mycorrhizas are intimate associations between fungi and the roots or other underground organs of plants. There are many types of mycorrhizal fungi, which have evolved independently of one another and which serve different roles. In almost all cases these fungi depend on the plant for a supply of carbon nutrients, while the plants depend on the fungi for a supply of mineral nutrients (phosphorus, nitrogen) from the soil. As we will see in Chapter 13, phosphorus is often the critical limiting factor for plant growth, because soil phosphates rapidly form insoluble complexes with organic matter or with divalent cations (Ca^{2+}, Mg^{2+}) and cannot easily diffuse to the plant roots. Mycorrhizal fungi help to alleviate this problem by providing an extensive hyphal network for capturing mineral nutrients and transporting them back to the roots. However, some other mycorrhizal fungi serve a quite different role. Orchids and some nonphotosynthetic plants are absolutely dependent on fungi for all or part of the plant's life, because the plant feeds on sugars supplied by a soil fungus.

Lichens and mycorrhizas are not the only examples of symbiosis. In recent years many plants have been found to harbor symptomless **endophytic fungi** within the plant walls or intercellular spaces. These fungi apparently do no harm to the plants. Instead they can be beneficial because they help to activate plant defense genes and produce insect anti-feedant compounds such as the ergot alkaloids. But this is a double-edged sword, because the toxins can cause serious damage to grazing animals such as horses, cattle, and sheep (Chapter 11).

Fungal pathogens of humans

In contrast to the many fungal parasites of plants, there are only some 200 fungi that infect humans or other warm-blooded animals. In fact, humans have a high degree of innate immunity to fungi, with the exception of the dermatophytic fungi which commonly cause infections of the skin, nails, and hair. However, the situation changes drastically when the immune system is compromised, and this is becoming common in patients with AIDS, transplant patients whose immune system is purposefully suppressed, patients suffering from cancer or advanced diabetes, and patients undergoing prolonged corticosteroid therapy. In any of these circumstances there is a significant chance of infection from fungi that pose no serious threat to healthy people. For example, the widespread and extremely common airborne fungus *Aspergillus fumigatus* normally grows on composts and in soil, but it has become one

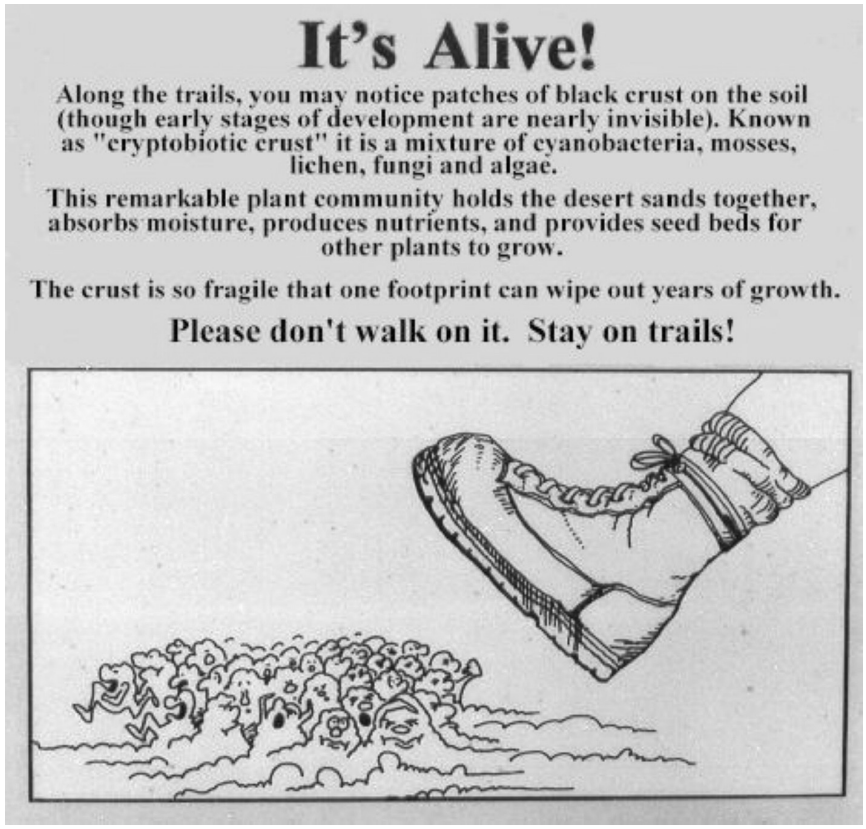


Fig. 1.5 A sign in Arches National Park, Utah, USA. ***Get your boots off our microbes!***

of the most significant invasive fungi in deep surgical procedures, and the survival rate can be as low as 30%. Many other fungi that can grow at 37°C have spores that are small enough to enter the lungs and reach the alveoli. These fungi were virtually unknown until recently but are now extremely common causes of infection in immunodeficient patients. For example, the fungus-like organism *Pneumocystis jiroveci* (previously named *P. carinii*) commonly causes pneumonia in patients infected with the human immunodeficiency virus (HIV). The onset of this disease in patients with HIV is regarded as one of the "AIDS-defining" symptoms.

Only a handful of antifungal drugs are available to treat the major human mycoses, and most need to be administered at low doses over a prolonged time to avoid excessive toxicity. Many of these drugs are expensive so they offer little hope to the poorest people in the developing world. Chapter 16 is devoted to the mycoses of humans, and Chapter 17 deals with the drugs available to treat these conditions.

Fungal parasites as biological control agents

Fungi parasitize many types of host, including other fungi (**mycoparasites**, Chapter 12), insects (**entomopathogens**, Chapter 15), and nematodes (**nematophagous fungi**, Chapter 15). In the past, such fungi might have been regarded as curiosities, but now they are recognized as being significant population regulators of their hosts and as potential **biological control (biocontrol) agents** of major pests or plant pathogens. We discuss biocontrol at many points in this book, notably in Chapters 12 and 17.

Fungal saprotrophs

Saprotrophs (saprophytes) are organisms that feed on dead organic matter (*sapros* = death; *trophy* = feeding). Fungi play a major role in this respect because they

produce a wide range of enzymes that degrade complex polymers such as starch, cellulose, proteins, chitin, aviation kerosene, keratin, and even the most complex lignified materials such as wood. In fact, there are few naturally occurring organic compounds that cannot be degraded by one fungus or another. One of the few exceptions is sporopollenin, the highly resistant polymer found in the walls of pollen grains.

Fungi are particularly important in the decomposition of cellulose, which represents about 40% of plant cell wall material and is the most abundant natural polymer on Earth. Grazing animals (ruminants) also consume significant amounts of cellulose, but this is broken down in the rumen (in effect, a large anaerobic fermentation vessel) and the rumen fungi are thought to play a significant role in the decomposition process. The breakdown of polymers by fungi is intimately linked to hyphal growth which provides both penetrating power and the coordinated release of extracellular enzymes and subsequent reabsorption of the enzymic breakdown products (Chapter 6). But different fungi are adept at degrading different types of polymer, so fungal saprotrophs often grow in complex, mixed communities reflecting their different enzymic capabilities (Chapter 11).

Although the decomposer fungi play vital roles in the recycling of major nutrients, they can also be significant spoilage agents. A well-known example is the dry-rot fungus, *Serpula lacrymans*, which is a major cause of timber decay in buildings (Chapter 5). Similarly the "sooty moulds" that commonly grow on kitchen and bathroom walls are extremely difficult to eradicate (Fig. 1.6). They utilize the soluble cellulose gels that are used as stabilizers in emulsion paints or as wallpaper pastes. These common fungi include species of *Alternaria*, *Cladosporium* and *Sydowia polyspora* (previously called *Aureobasidium pullulans*) which discolor the walls because of their darkly pigmented hyphae and spores. However, their natural habitat is the surface of leaves or the decaying stalk tissues of plants, and they occur in buildings only because they find similar conditions (and substrates) to those in their natural environment (Chapter 8). Public health authorities are now paying increasing attention to safety in the workplace, and particularly to the potential roles of fungi in "sick building syndrome," which has been linked (tenuously) to infant cot death. The conditions in underventilated buildings can certainly promote the growth of moulds, including *Stachybotrys chartarum*, another common sooty mould. But there is no definitive evidence to link these fungi to sick building syndrome.

Fig. 1.6 Part of a bathroom ceiling where the paint has flaked away, revealing extensive growth and sporulation of sooty moulds.

Some saprotrophic fungi pose a serious threat to human and animal welfare by growing on stored food products and producing **mycotoxins**. These are a diverse range of fungal secondary metabolites, often found in improperly stored materials. For example, **aflatoxins** are commonly produced in groundnuts and cottonseed meal. They are among the most potent known carcinogens and are strongly implicated in hepatomas. Similarly, the toxins produced by several *Fusarium* species on grain crops are implicated in esophageal cancer in Africa, and in kidney carcinomas. The pathways leading to the production of these compounds are discussed in Chapter 7; the maintenance of safe storage conditions is covered in Chapter 8.

Fungi in biotechnology

Fungi have many traditional roles in biotechnology, but also some novel roles, and there is major scope for their future commercial development (Wainwright 1992). Some of these roles are outlined below.

Foods and food flavorings

In 1994 the total world production of edible mushrooms was estimated to be over 5 million tonnes, with a value of US $14 billion. Much of the mushroom-growing industry is based on strains of the common cultivated mushroom *Agaricus bisporus* (or *A. brunnescens*) discussed in Chapters 5 and 11. But *Lentinula edodes* (the Shiitake mushroom, which is grown on logs; Fig. 1.7), *Volvariella volvacea* (the padi straw mushroom, which is grown on rice straw), and *Pleurotus ostreatus* (the oyster mushroom, Fig. 1.8) are traditionally grown in Japan and southeast Asia, and are now widely available in western supermarkets.

(a)

(b)

Fig. 1.7 (a,b) Commercial culture of the shiitake mushroom, *Lentinula edodes*, on inoculated logs. (Courtesy of Robert L. Anderson (photographer) and USDA Forest Service; www.forestryimages.org)

Fungi are used to produce several traditional foods and beverages, including alcoholic drinks (ethanol from the yeast *Saccharomyces cerevisiae*) and bread, where the yeast produces CO_2 for raising the dough. *Penicillium roqueforti* is used in the later stages of production of the blue-veined cheeses such as Stilton and Roquefort, to which it imparts a characteristic flavor. *P. camemberti* is used to produce the soft cheeses such as Camembert and bries; it grows on the cheese surface, forming a "crust," and produces proteases which progressively degrade the cheese to give the soft consistency. Less well known but equally significant is the role of fungi in the fermentation of traditional foods around the world. For example, *Rhizopus oligosporus* is used to convert cooked soybean "grits" to a nutritious staple food, called **tempeh** (Fig. 1.8). This involves only a short (24–36 hour) incubation time, during which the fungus degrades some of the fat and also degrades a trypsin inhibitor in soybeans, so that the naturally high protein content of this crop is more readily available in the diet, and a "flatulence factor" is broken down during this process. The food termed **gari** is part of the staple diet in southern Nigeria; it is produced from the high-yielding root crop, cassava, perhaps better known in its processed form, tapioca. Raw cassava contains a toxic cyanogenic glycoside termed linamarin, which is removed during a prolonged and largely uncontrolled fermentation in village communities. Much of this process involves bacteria, but the fungus *Galactomyces geotrichum* (asexual stage: *Geotrichum candidum*) gives the product its desired flavor. Details of the production of several traditional Asian fermented foods can be found in Nout & Aidoo (2002).

A major development in recent years has been the introduction of an entirely new type of food, termed **Quorn™ mycoprotein** (Fig. 1.9). This is produced commercially by growing a fungus (*Fusarium venenatum*) in large fermentation vessels, then harvesting the fungal hyphae and processing them into meat-like chunks and various oven-ready meals. ***Quorn*** (as it is now called) is widely available in British and European supermarkets. It has an almost ideal nutritional profile, with a high protein content, low fat content, and absence of cholesterol (Table 1.2). The production of Quorn is discussed in detail in Chapter 4.

Fungal metabolites

Metabolites can be grouped into two broad categories (Chapter 7):

- **Primary metabolites:** the intermediates or end products of the common metabolic pathways of all organisms (sugars, amino acids, organic acids, glycerol, etc.) and which are essential for the normal cellular functions of fungi.

(a) (b)

Fig. 1.8 (a) A tray of exotic mushrooms from a supermarket in the UK, including shiitake (centre) and the oyster mushroom, *Pleuotus ostreatus*, reputed to be an aphrodisiac. (b) An attempt to produce a homemade cake of tempeh, which tasted only marginally better than it looks.

Table 1.2 Nutritional composition of Quorn™ mycoprotein, compared with traditional protein sources. (Data from Trinci 1992.)

	Units	*Quorn*	*Cheddar cheese*	*Raw chicken*	*Raw lean beef*	*Fresh cod*
Protein	g $100g^{-1}$	12.2	26.0	20.5	20.3	17.4
Dietary fibre	g 100 g^{-1}	5.1	0	0	0	0
Total fats	g 100 g^{-1}	2.9	33.5	4.3	4.6	0.7
Fat ratio	Polyunsaturated: saturated	2.5	0.2	0.5	0.1	2.2
Cholesterol	mg $100g^{-1}$	0	70	69	59	50
Energy	kJ $100g^{-1}$	334	1697	506	514	318

(a) (b)

Fig. 1.9 Quorn: (a) the package and (b) one of several products: "Fillets in a Mediterranean marinade with tomato, red wine, and herbs."

- **Secondary metabolites:** a diverse range of compounds formed by specific pathways of particular organisms; they are not essential for growth, although they can confer an advantage to the organisms that produce them (e.g. antibiotics, fungal toxins, etc.).

Several metabolites of both groups are produced commercially from fungal cultures (Turner 1971; Turner & Aldridge 1983). One of the best examples of a fungal primary metabolite is **citric acid**, with an estimated global production of 900,000 tons in the year 2000 (Ruijter *et al*. 2002). Citric acid produced on this vast scale is the mainstay of the soft drinks industry (lemonade, etc.) because it has a tart taste and also enhances flavor, reduces sweetness, and has antioxidant and preservative qualities. Specially selected, overproducing strains of *Aspergillus niger* are used for the commercial production of citric acid, but several other conditions are necessary – the cultures must contain high levels of readily metabolizable sugars (up to 20% or more) and the concentration of either phosphate or nitrogen must be kept low, to limit the amount of fungal growth. In these conditions 80% or more of the sugar supplied to the cultures is converted into citric acid, which is then exported from the cells and accumulates in the culture medium. The effect of this is to lower the pH of the culture medium to 3.0 or less, which the fungus tolerates well. This secretion of the acid is a crucial feature, because fungal cells tightly regulate their internal pH. Recent evidence indicates that cells of *A. niger* maintain their intracellular pH at 7.7 when the cells are exposed to external pH levels ranging from 1.5 to 6.

Other organic acids are produced commercially by fungal fermentations. **Gluconic acid** (estimated annual global production of 50,000–100,000 tons) is used mainly as a food additive, and is produced by specific strains of *A. niger*, grown at normal pH. This acid is produced by the direct oxidation of glucose, catalyzed by the enzyme glucose oxidase. **Itaconic acid** (global production 70,000–80,000 tons) is produced by *Aspergillus terreus* and is used as a co-polymer in the manufacture of paints, adhesives, etc.

In some respects the production of citric acid and itaconic acid is similar to the **production of ethanol** by *Saccharomyces* spp. – the basis of the alcoholic drinks industry. Both types of product accumulate in the culture medium when **growth is restricted** by some factor but when the biochemical machinery continues to operate, like the engine of a car taken out of gear. For example, ethanol accumulates as a metabolic end-product when yeast is grown in a sugar-rich medium favoring metabolism, but in **anaerobic conditions** that limit cell growth.

In contrast to the bulk metabolites mentioned above, a vast range of **secondary metabolites** are produced by fungi, and they include several high-value products with pharmaceutical applications. A small selection of these is shown in Table 1.3. The best-known examples are the **penicillins** – a group of structurally related β-lactam antibiotics that are synthesized naturally from small peptides. As explained in Chapter 7, the naturally occurring penicillins such as penicillin G (produced by *Penicillium chrysogenum*) have a relatively narrow spectrum of activity. But a wide range of other penicillins can be produced by chemical modification of the natural penicillins. All modern penicillins are semisynthetic compounds; they are obtained initially from fermentation cultures but are then structurally modified for specific desirable properties. Schmidt (2002) reviewed the manufacture and therapeutic aspects of β-lactam antibiotics, including the **cephalosporins** which are structurally related to the penicillins. Remarkably, despite their age (the penicillins were first produced commercially in the late 1940s),

Table 1.3 Some valuable secondary metabolites produced commercially from fungi.

Metabolite	*Fungal source*	*Application*
Penicillins	*Penicillium chrysogenum*	Antibacterial
Cephalosporins	*Acremonium chrysogenum*	Antibacterial
Griseofulvin	*Penicillium griseofulvum*	Antifungal
Fusidin	*Fusidium coccineum*	Antibacterial
Ciclosporins	*Tolypocladium* spp.	Immunosuppressants
Zearalenone	*Gibberella zeae*	Cattle growth promoter
Gibberellins	*Gibberella fujikuroi*	Plant hormone
Ergot alkaloids and related compounds	*Claviceps purpurea* and related fungi	Many effects including: antimigraine, vasoconstriction, vasodilation, antihypertension, anti-Parkinson, psychiatric disorders

the β-lactam antibiotics still share 50% of the world market for systemic antibiotics, with sales in 1998 worth about US $4 billion for penicillins and about US $7 billion for the more recently developed cephalosporins.

Several non-β-lactam antibiotics are also produced by fungi. They include **griseofulvin** (from the fungus *P. griseofulvum*) which has been used for several years to treat dermatophyte infections of the skin, nails and hair of humans, although recently it has been replaced by less toxic drugs (Chapter 17). **Fusidic acid** (from various fungi) has been used to control staphylococci that have become resistant to penicillin, and there is renewed interest in a range of other natural fungal products for treating the systemic fungal infections of humans (Chapter 17). **Ciclosporins** from various fungi (but principally from species of *Tolypocladium*) are used as immunosuppressants to prevent organ rejection in transplant surgery. In fact, 17 different fungal taxa are reported to produce ciclosporins. Another powerful immunosuppressant is the antibiotic **gliotoxin** (from *Trichoderma virens*), which is better known for its role in biological control of plant pathogenic fungi (Chapter 12). The production and use of these immunosuppressants was reviewed by Kürnsteiner *et al*. (2002). As a final example, the **ergot alkaloids** and related toxins of the ergot fungus, *Claviceps purpurea* (Chapter 14), have many important pharmacological applications (Keller & Tudzynski 2002). The four-membered ring structure of the D-lysergic acid derivatives of ergot alkaloids mimic the ring structures of neurotransmitters (dopamine, epinephrine (adrenaline), and serotonin: Fig. 1.10). However, at present many of the ergot derivatives are too nonspecific in their modes of action to meet their true potential in treating human disorders.

Even these few examples raise fascinating questions about the roles of fungal secondary metabolites. What functions do they serve in fungi and what competitive advantage do they confer? In recent years many of the genes encoding the secondary biosynthetic pathways have been identified and sequenced. This should lead both to an understanding of their roles and to the potential construction of transgenic strains that overproduce valuable metabolites.

Some of the polysaccharides of fungi have potential commercial value. **Pullulan** is an α-1,4-glucan (polymer of glucose) produced as an extracellular sheath by *Sydowia polyspora* (formerly *Aureobasidium pullulans*), one of the sooty moulds. This polymer is used in Japan to make a film-wrap for foods. A potential new market could develop from the discovery that fungal wall polymers or their partial breakdown products can be powerful elicitors of plant defense responses (Chapter 14) so they might be used to "immunize" plants. For example, the β-glucan fractions from walls of the yeast *S. cerevisiae* have this effect. So too does **chitosan**, the de-acetylated form of chitin in fungal cell walls (Chapters 3 & 7). At present, chitosan is used on a large scale in Japan for clarifying sewage, but the source of this chitosan is crustacean shells. Fungi are an alternative, easily renewable source of this and other polymers.

Enzymes and enzymic conversions

Saprotrophic fungi and some plant-pathogenic fungi produce a range of extracellular enzymes with important commercial roles (Table 1.4). The **pectic enzymes** of fungi are used to clarify fruit juices, a fungal **amylase** is used to convert starch to maltose during breadmaking, and a fungal rennet is used to coagulate milk for cheese-making. A single fungus, *Aspergillus niger*, accounts for almost 95% of the commercial production of these and other bulk enzymes from fungi, although specific strains of the fungus have been selected for particular purposes. The methanol-utilizing yeasts (*Candida lipolytica*, *Hansenula polymorpha*, and *Pichia pastoris*) have potential commercial value because they produce large amounts of **alcohol oxidase**, which could be used as a bleaching agent in detergents. The wood-rotting fungus *Phanerochaete chrysosporium* is extremely active in degrading lignin; it has the

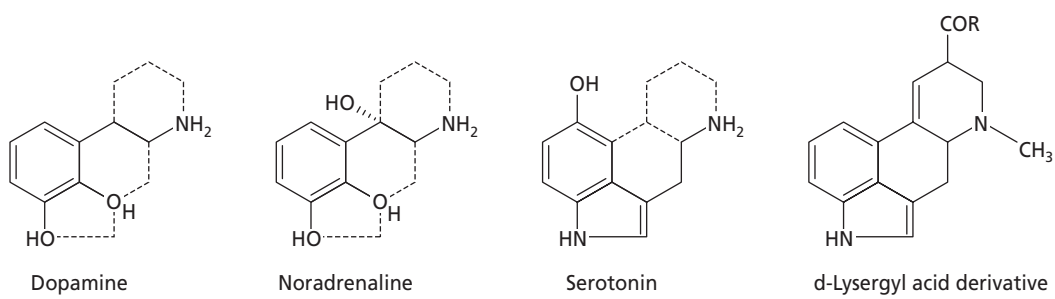


Fig. 1.10 Structural similarities between three neurotransmitters (dopamine, noradrenaline, and serotonin) and the D-lysergic acid derivatives of ergot alkaloids.

Table 1.4 Some fungal enzymes produced commercially. (Based on Wainwright 1992.)

Enzyme	*Fungal source*	*Application*
α-Amylase	*Aspergillus niger, A. oryzae*	Starch conversions
Amyloglucosidase	*A. niger*	Starch syrups, dextrose foods
Pullulanase	*Aureobasidium pullulans*	Debranching of starch
Glucose aerohydrogenase	*A. niger*	Production of gluconic acid
Proteases (acid, neutral, alkaline)	*Aspergillus* spp. etc.	Breakdown of proteins (baking, brewing, etc.)
Invertase	Yeasts	Sucrose conversions
Pectinases	*Aspergillus, Rhizopus*	Clarifying fruit juices
Rennet	*Mucor* spp.	Milk coagulation
Glucose isomerase	*Mucor, Aspergillus*	High fructose syrups
Lipases	*Mucor, Aspergillus, Penicillium*	Dairy industry, detergents
Hemicellulase	*A. niger*	Baking, gums
Glucose oxidase	*A. niger*	Food processing

potential to be developed for delignification of agricultural wastes and byproducts of the wood-pulping industry, so that the cellulose in these materials could be used as a cheap substrate for production of fuel alcohol by yeasts (Chapter 11).

In addition to these examples of "bulk" enzymes, fungi have many internal enzymes and enzymic pathways that can be exploited for the bioconversion of compounds such as pharmaceuticals. For example, fungi are used for the bioconversion of steroids, because fungal enzymes perform highly specific dehydrogenations, hydroxylations and other modifications of the complex aromatic ring systems of steroids. Precursor steroids are fed to a fungus, held at low nutrient level either in culture or attached to an inert bed, so that the steroid is absorbed, transformed and then released into the culture medium from which it can be retrieved.

Heterologous gene products

Genetic engineering of fungi, particularly *Saccharomyces cerevisiae*, has developed to the stage where the cells can be used as factories to produce pharmaceutical products, by the introduction of foreign (heterologous) genes, as we already noted for the hepatitis B vaccine. There are several advantages in using yeast to synthesize such products. *S. cerevisiae* is already grown on a large industrial scale, so companies are familiar with its culture. It is a **GRAS** organism, i.e. "**g**enerally **r**egarded **a**s **s**afe." Its genome was the first to be sequenced, and its genetics and molecular genetics are well-researched (Chapter 9). Furthermore, yeast has a well-characterized secretory system for exporting gene products into a culture medium. Examples of heterologous gene products that have been produced experimentally from yeast include **epidermal growth factor** (involved in wound healing), **atrial natriuretic factor** (for management of hypertension), **interferons** (with antiviral and antitumor activity), and **α-1-antitrypsin** (for potential relief from emphysema). There are, however, disadvantages in using *S. cerevisiae*. In particular, this fungus is genetically quite different from other fungi and other eukaryotes, including its use of different codons for some amino acids, so it does not always correctly read the introduced genes. For this reason attention has switched to some other fungi, such as the fission yeast *Schizosaccharomyces pombe* and the filamentous fungus *Emericella* (*Aspergillus*) *nidulans*, for both of which the genomes have now been sequenced.

Online resources

Forestry Images. http://www.forestryimages.org. [Many high-quality images of fungi, diseases, forestry practices, etc.]

Fungal Biology. http://www.helios.bto.ed.ac.uk/bto/FungalBiology/ [The website for this book.]

Tree of Life Web Project. http://tolweb.org/tree?group=life. [A major source of information on fungal systematics and phylogeny.]

General texts

Alexopoulos, C.J., Mims, C.W. & Blackwell, M. (1996) *Introductory Mycology*, 4th edn. John Wiley, New York.

Carlile, M.J., Watkinson, S.C. & Gooday, G.W. (2001) *The Fungi*, 2nd edn. Academic Press, London.

Jennings, D.H. & Lysek, G. (1999) *Fungal Biology: understanding the fungal lifestyle*, 2nd edn. Bios, Oxford.

Kendrick, B. (2001) *The Fifth Kingdom*, 3rd edn. Mycologue Publications, Sidney, Canada.
Turner, W.B. (1971) *Fungal Metabolites*. Academic Press, London.
Turner, W.B. & Aldridge, D.C. (1983) *Fungal Metabolites*. *II*. Academic Press, London.
Wainwright, M. (1992) *An Introduction to Fungal Biotechnology*. Wiley, Chichester.
Webster, J. (1980) *Introduction to Fungi*, 2nd edn. Cambridge University Press, Cambridge.

Cited references

Baldauf, S.L. & Palmer, J.D. (1993) Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins. *Proceedings of the National Academy of Sciences, USA* **90**, 11558–11562.
Hawksworth, D.L. (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* **105**, 1422–1432.
Hawksworth, D.L. (2002) Mycological Research News. *Mycological Research* **106**, 514.
Keller, U. & Tudzynski, P. (2002) Ergot alkaloids. In: *The Mycota X. Industrial Applications* (H.D. Osiewicz, ed.), pp. 157–181. Springer-Verlag, Berlin.
Kürnsteiner, H., Zinner, M. & Kück, U. (2002) Immunosuppressants. In: *The Mycota X. Industrial Applications* (H.D. Osiewicz, ed.), pp. 129–155. Springer-Verlag, Berlin.
Large, E.C. (1940) *The Advance of the Fungi*. Henry Holt, New York.
Lewis, D.H. (1987) Evolutionary aspects of mutualistic associations between fungi and photosynthetic organisms. In: *Evolutionary Biology of the Fungi* (eds Rayner, A.D.M., Brasier, C.M. & Moore, D.), pp. 161–178. Cambridge University Press, Cambridge.
Nout, M.J.R. & Aidoo, K.E. (2002) Asian fungal fermented food. In: *The Mycota X. Industrial Applications* (H.D. Osiewicz, ed.), pp. 23–47. Springer-Verlag, Berlin.
Ruijter, G.J.G., Kubicek, C.P. & Visser, J. (2002) Production of organic acids by fungi. In: *The Mycota X. Industrial Applications* (H.D. Osiewicz, ed.), pp. 213–230. Springer-Verlag, Berlin.
Schmidt, F.R. (2002) Beta-lactam antibiotics: aspects of manufacture and therapy. In: *The Mycota X. Industrial Applications* (H.D. Osiewicz, ed.), pp. 69–91. Springer-Verlag, Berlin.
Trinci, A.P.J. (1992) Myco-protein: a twenty-year overnight success story. *Mycological Research* **96**, 1–13.
Woese, C.R. (2000) Interpreting the universal phylogenetic tree. *Proceedings of the National Academy of Sciences, USA* **97**, 8392–8396.

Chapter 2

The diversity of fungi and fungus-like organisms

This chapter is divided into the following major sections:

- overview of the fungi and fungus-like organisms
- the true fungi (Kingdom Mycota): Chytridiomycota, Glomeromycota, Zygomycota, Ascomycota, Basidiomycota, mitosporic fungi
- the cellulose-walled fungus-like organisms (Kingdom Straminipila)
- other fungus-like organisms: slime moulds, cellular slime moulds (acrasids and dictyostelids), and plasmodiophorids

In this chapter we focus on the major groups of fungi and fungus-like organisms, covering the whole span of fungal diversity in its broadest sense. We will use selected examples to illustrate key features of the fungal groups, and their biological significance. There will be some surprises in store. For example, we will see that some of the most devastating plant pathogens are not fungi at all, but belong to an entirely separate kingdom. We will see that some of the organisms once considered to be among the most "primitive" – the microsporidia, trichomonads, and diplomonads (see Fig. 1.1) – are derived from fungi by the loss of features such as mitochondria, which they once possessed. We will also see how the development of molecular methods for determining the relationships between organisms has enhanced our understanding of fungi in many respects, but there is still no consensus on the best way to construct phylogenetic trees. In the words of Patterson & Sogin (Tree of Life Web Project, see Online Resources): "The consequence . . . has been to demolish the model of the 1990s, but not to replace it with something better."

Overview of the fungi and fungus-like organisms

Box 2.1 shows all the fungi and fungus-like organisms that are currently considered to be fungi in the broadest sense. The vast majority are true fungi, sometimes

Box 2.1 The several types of organism that constitute the fungi in a broad sense.

Kingdom: Fungi (Mycota)
Probably derived from a choanoflagellate ancestor
- Phylum **Chytridiomycota**
- Phylum **Zygomycota**
- Phylum **Glomeromycota**
- Phylum **Ascomycota**
- Phylum **Basidiomycota**

Kingdom: Straminipila
Probably derived from the protist group containing golden-brown algae, diatoms, etc.
- Phylum **Oomycota**
- Phylum **Hyphochytridiomycota**
- Phylum **Labyrinthulomycota**

Fungus-like organisms of uncertain affinity
- Phylum **Myxomycota** (plasmodial slime moulds)
- Phylum **Plasmodiophoromycota** (plasmodiophorids)
- Phylum **Dictyosteliomycota** (dictyostelid slime moulds)
- Phylum **Acrasiomycota** (acrasid slime moulds)

termed Eumycota (*eu* = "true"). Until recently, all the true fungi were assigned to four phyla – Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. But in 2001, a fifth phylum was erected – the Glomeromycota (arbuscular mycorrhizal fungi and their relatives). These had previously been included in the Zygomycota. It will be recalled from Chapter 1 that the Glomeromycota were associated with the earliest land plants (Schuessler *et al.* 2001) and are still associated with the vast majority of plants today.

Within the **Kingdom Fungi**, the Ascomycota and the Basidiomycota have many features in common, pointing clearly to a common ancestry. The phylum Chytridiomycota has traditionally been characterized on the basis of motile cells with a single posterior flagellum. This phylum was redefined recently, based on sequence analysis of the nuclear genes encoding small subunit (SSU) ribosomal RNA (18S rDNA). This has revealed that some nonmotile fungi, previously assigned to the Zygomycota, are closely related to the Chytridiomycota and must be reassigned. An example is the fungus *Basidiobolus ranarum* (Chapter 4), now transferred to the Chytridiomycota. The status of Zygomycota (as currently defined after excluding the Glomeromycota) is still unclear. Some of its members may need to be separated into new groups.

Nevertheless, the current view is that all organisms within the Kingdom Fungi constitute a monophyletic group (all derived from a common ancestor), sharing several features with animals (see Table 1.1). Gene sequence analyses provide the basis for a natural phylogeny, especially when data for the SSU rDNA are supported by sequence analysis of other gene families, such as the tubulin and actin genes.

The **Kingdom Straminipila** (straminipiles, or stramenopiles) is now universally recognized as being distinct from the true fungi. It consists of one large and extremely important phylum, the Oomycota, and two small phyla, the Hyphochytridiomycota (with about 25 species) and Labyrinthulomycota (with about 40 species). The Phylum Oomycota is remarkable in many ways. It includes some of the most devastating plant pathogens, including *Phytophthora infestans* (potato blight), *Phytophthora ramorum* (sudden oak death in California), *Phytophthora cinnamomi* (the scourge of large tracts of *Eucalyptus* forest in Australia), and many other important plant pathogens, including *Pythium* and *Aphanomyces* spp. But perhaps most remarkable of all is the fact that Oomycota have evolved a lifestyle that resembles that of the true fungi in almost every respect. We discuss this group in detail later in this chapter and at several points in this book.

The **fungus-like organisms of uncertain affinity** include four types of organism: the acrasid cellular slime moulds, the dictyostelid cellular slime moulds, the plasmodial slime moulds (Myxomycota), and the plasmodiophorids. For most of their life these organisms lack cell walls, and they grow as either a naked protoplasmic mass or as amoeboid cells, converting to a walled form at the onset of sporulation. There is no evidence that they are related to fungi, but they have traditionally been studied by mycologists, and they have several interesting features, which are discussed towards the end of this chapter.

Against this background, we now consider the individual phyla in more detail.

The true fungi (Kingdom Mycota)

Chitridiomycota

The Chytridiomycota, commonly termed chytrids, number about 1000 species (Barr 1990) and are considered to be the earliest branch of the true fungi, dating back to about 1 billion years ago. They have cell walls composed mainly of **chitin** and **glucans** (polymers of glucose) and many other features typical of fungi (see Table 1.1). But they are unique in one respect, because they are the only true fungi that produce **motile, flagellate zoospores**. Typically, the zoospore has a single, posterior whiplash flagellum, but some of the chytrids that grow in the rumen of animals have several flagella (Chapter 8), and some other chytrids (e.g. *Basidiobolus ranarum*, recently transferred to the Chytridiomycota based on SSU rDNA analysis), have no flagella. This provides a good example of the value of DNA sequencing in determining the true phylogenetic relationships of organisms.

Ecology and significance

Most chytrids are small, inconspicuous organisms that grow as single cells or primitively branched chains of cells on organic materials in moist soils or aquatic environments. They are considered to play significant roles as primary colonizers and degraders of organic matter in these environments. Two common examples are *Rhizophlyctis rosea* (a strongly cellulolytic fungus that is common in natural soils) and *Allomyces arbuscula*, both shown in Fig. 2.1.

Often, the Chytridiomycota are anchored to their substrates by narrow, tapering **rhizoids**, which function like hyphae in secreting enzymes and absorbing nutrients. Sometimes the rhizoidal system is extensive, or the **thallus** (the "body" of the fungus) resembles a string of beads, with inflated cells arising at intervals along a rhizoidal network. An example of this is the fungus *Catenaria anguillulae*, shown in Fig. 2.2. A few chytrids grow as obligate intracellular parasites of plants (e.g.

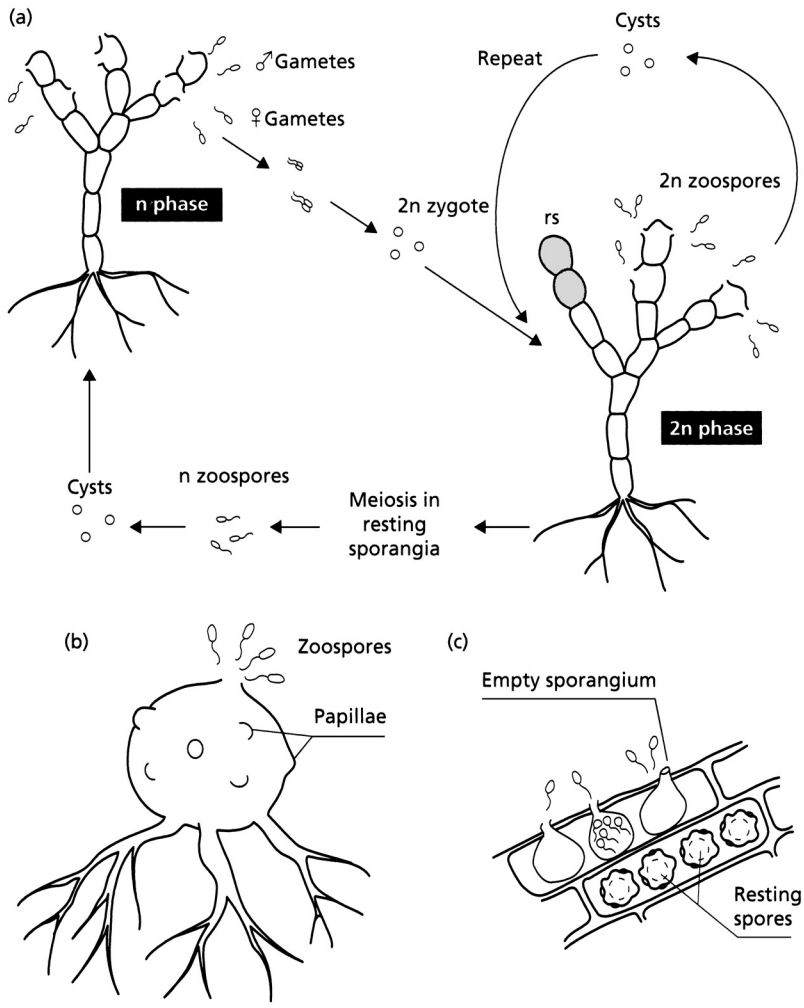


Fig. 2.1 Chytridiomycota. (a) Life cycle of *Allomyces*, which alternates between haploid (n) and diploid (2n) generations. The haploid thallus produces male and female gametangia that release motile gametes. These fuse in pairs and encyst to produce 2n zygotes, which germinate to produce a 2n thallus. The 2n sporangia release zoospores for recycling of the 2n phase. Thick-walled resting sporangia (rs) are formed in adverse conditions; after meiosis these germinate to release haploid zoospores. (b) *Rhizophlyctis rosea*, a common cellulolytic fungus in soil. It grows as a single large cell, up to 200 µm diameter, with tapering rhizoids. At maturity, the large, inflated cell converts into a sporangium, where the cytoplasm is cleaved around the individual haploid nuclei, and large numbers of zoospores are released through the exit papillae. (c) *Olpidium brassicae* grows as naked protoplasts in root cells of cabbages. At maturity, the protoplasts convert to sporangia, which release zoospores into the soil. These spores encyst on a host root, germinate, and release a protoplast into the host. Thick-walled resting spores are produced in adverse conditions and can persist in soil for many years (see Fig. 2.3).

Olpidium brassicae; Figs. 2.1c, Fig. 2.3), of small animals (e.g. *Coelomomyces*; see Fig. 15.5), of algae, or of fungal spores. But very few chytrids can be considered to be economically important – the most notable example is *Synchytrium endobioticum*, which causes potato wart disease, where the potato tubers develop unsightly galls that render them unmarketable.

Having said this, the significance of chytrids lies mainly in their fascinating biology. Anybody who has watched a chytrid zoospore crawling like an amoeba along the body of a nematode, searching for the best site to encyst, and then winding in its flagellum, encysting and penetrating the host will never forget the experience (Fig. 2.2). All these events can be

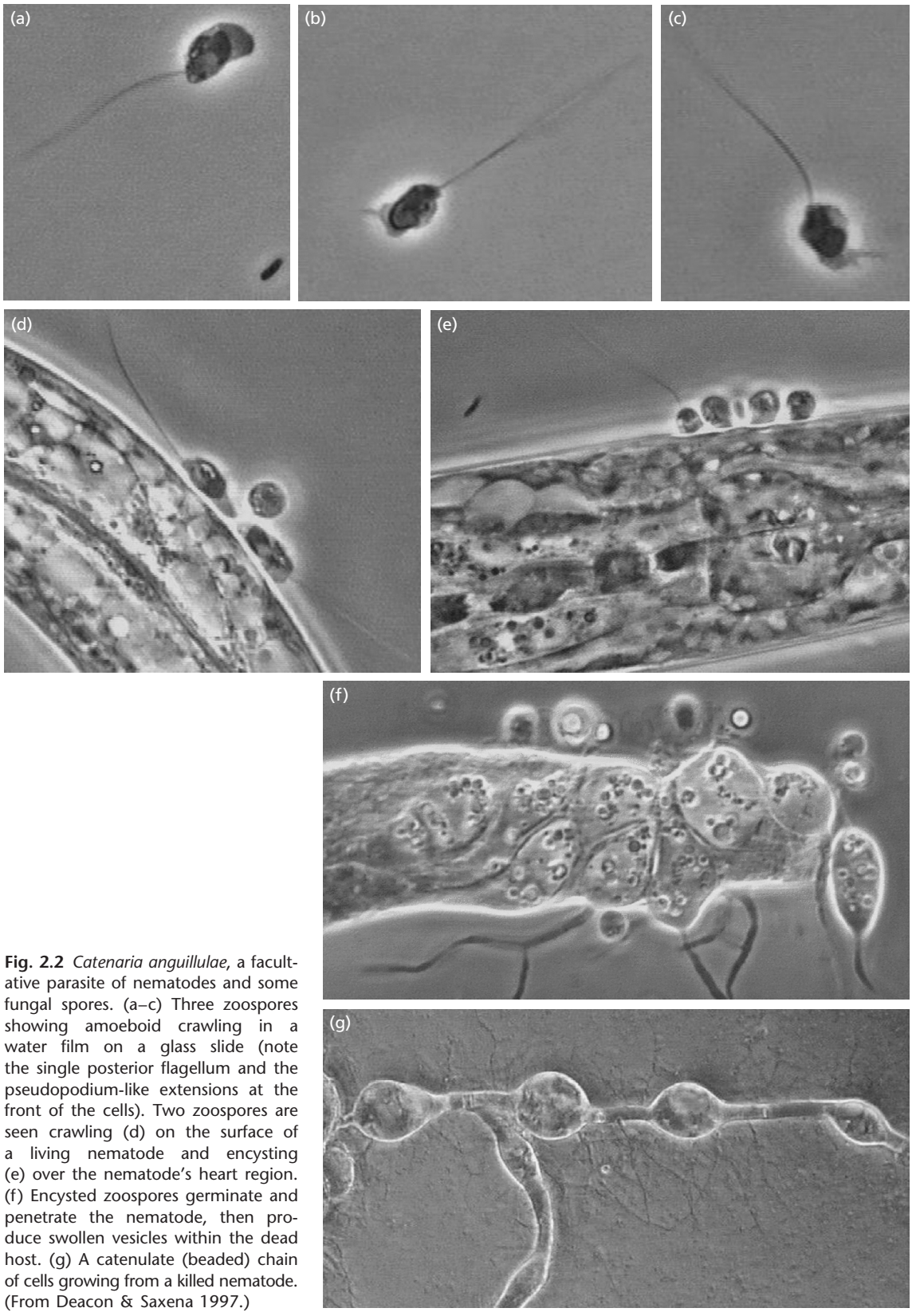


Fig. 2.2 *Catenaria anguillulae*, a facultative parasite of nematodes and some fungal spores. (a–c) Three zoospores showing amoeboid crawling in a water film on a glass slide (note the single posterior flagellum and the pseudopodium-like extensions at the front of the cells). Two zoospores are seen crawling (d) on the surface of a living nematode and encysting (e) over the nematode's heart region. (f) Encysted zoospores germinate and penetrate the nematode, then produce swollen vesicles within the dead host. (g) A catenulate (beaded) chain of cells growing from a killed nematode. (From Deacon & Saxena 1997.)

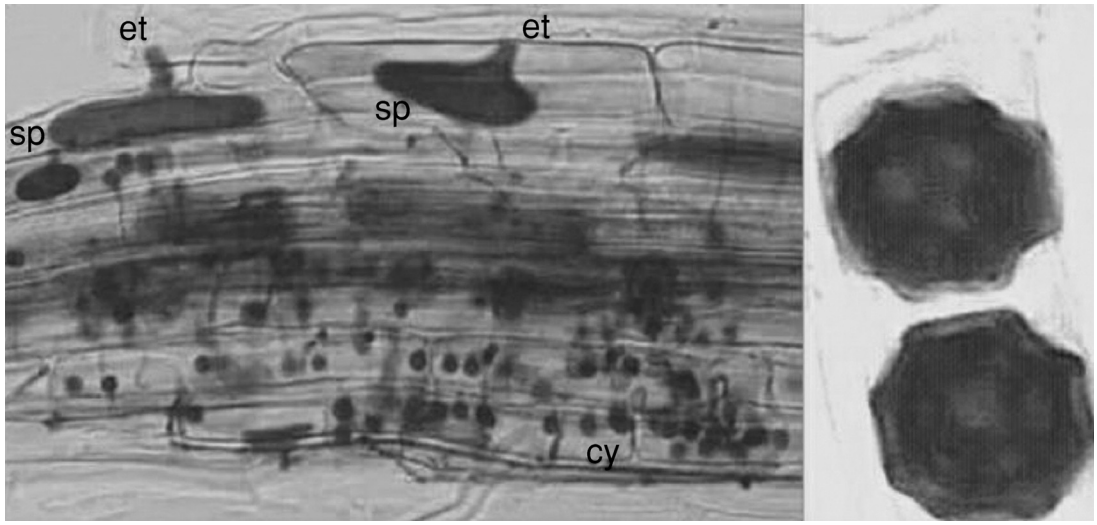


Fig. 2.3 *Olpidium brassicae*, a biotrophic (obligate) parasite commonly found in cabbage roots. The root cell contents were destroyed by treatment with hot KOH, then rinsed, acidified, and stained with trypan blue to reveal fungal structures within the roots. (a) Two sporangia (sp) about 30 μm long, with exit tubes (et) and many germinating zoospore cysts (cy). (b) Two thick-walled resting spores of *O. brassicae*, about 25 μm diameter, within a root cell.

followed in simple glass chambers on microscope slides (Deacon & Saxena 1997).

The Chytridiomycota are difficult to isolate by standard methods such as dilution plating of soil onto agar plates. But they can be found easily by suspending small "bait" particles on the surface of natural waters or in dishes of flooded soil. In these conditions, chytrid zoospores accumulate on baits such as cellulose, chitin, keratin, insect exoskeleton, or pollen grains. Then they encyst and produce rhizoids for anchorage to the substrate. There is strong experimental evidence that chytrid zoospores accumulate *selectively* on different types of bait (Mitchell & Deacon 1986). For example, when pieces of cellulose or purified crab-shell chitin were added to zoospore suspensions, the zoospores were seen to encounter the baits at random, but then changed their swimming pattern, making frequent random turns, and often encysted within 3–5 minutes. Zoospores of *Allomyces arbuscula* and *A. javanicus* accumulated and encysted on both cellulose and chitin, whereas zoospores of *Chytridium confervae* encysted preferentially on chitin, and zoospores of *Rhizophlyctis rosea* accumulated and encysted only on cellulose. The most likely explanation is that the zoospores have surface-located receptors that recognize different structural polymers – a phenomenon well known in zoospores of the fungus-like Oomycota (Chapter 10).

Taxonomy and relationships

Currently, the Phylum Chytridiomycota is subdivided into five orders (**Blastocladiales**, **Chytridiales**, **Monoblepharidales**, **Neocallimastigales**, and **Spizellomycetales**) based largely on ultrastructural features of the zoospores, which seem to indicate conserved patterns of evolution in the different lineages. For example, the zoospores of all members of the Order Blastocladiales (including *Catenaria anguillulae*) have a conspicuous nuclear cap that surrounds the nucleus and is filled with ribosomes (see Fig. 10.12), but different arrangements of the organelles are found in the other chytrid orders. Many of these differences relate to the arrangement of microtubular elements associated with anchorage of the flagellum, and the arrangements of mitochondria and lipid storage reserves that are essential for zoospore motility. We return to these points in Chapter 10, where we discuss zoospore ultrastructure. We should also note that the Order Neocallimastigales is unique in being obligately anaerobic. These organisms have recently been shown to have a **hydrogenosome**, equivalent to a mitochondrion, for generating energy (Chapter 8). But there is still doubt about whether the Neocallimastigales is a natural phylogenetic grouping. SSU rDNA analysis of a wider range of chytrids should help to clarify their relationships.

Reproduction and other features of Chytridiomycota

Most true fungi have a haploid genome but some species of *Allomyces* (Fig. 2.1) can alternate between haploid and diploid generations. This is also true for some species of *Blastocladiella*. There are different patterns of the life cycle within *Allomyces* spp. but one of these patterns, exemplified by *A. arbusculus* and *A. macrogynus* (Fig. 2.1), involves a predominantly diploid phase. Sporangia are produced on the diploid colonies, and cytoplasmic cleavage within the sporangium leads to the release of diploid zoospores. These encyst and then germinate to produce further diploid colonies. This process can continue as long as the environmental conditions are suitable, but at the onset of unfavorable conditions the fungus produces thick-walled resting sporangia. Meiosis then occurs within the resting sporangia, leading to the release of haploid zoospores, which encyst and then germinate to produce haploid colonies. These colonies produce male and female gametangia, which release male and female haploid gametes. The gametes of opposite mating type fuse to form a diploid zygote, which encysts and then germinates to repeat the diploid phase of the life cycle. In other *Allomyces* spp. there is no separate gametophyte generation; instead this is probably represented by a cyst, which germinates to produce a further asexual diploid colony.

One of the most intriguing features of chytrid zoospores is their ability to undergo prolonged amoeboid crawling, by pseudopodium-like extensions of the cell. This occurs both on glass surfaces and on the surfaces of potential hosts such as nematodes – a searching behavior for locating suitable sites for encystment (Fig. 2.2). Then the zoospores round-up and wind-in their flagellum by rotating most or all of the cell contents (Deacon & Saxena 1997).

Many details of the Chytridiomycota can be found in Fuller & Jaworski (1987).

Glomeromycota

As noted in Chapter 1 (see Fig. 1.2), fungal fossils resembling the common and economically important **arbuscular mycorrhizal (AM) fungi** were associated with plants in the Rhynie chert deposits of the Devonian era about 400 million years ago (mya). They probably played a vital role in the establishment of the land flora. Recent detailed analysis of these fungi, based on SSU rDNA sequences, has shown convincingly that they are distinct from all other major fungal groups, so they have been assigned to a new phylum, the **Glomeromycota** (Schuessler *et al.* 2001). The relationships between this group and other fungi are still unclear, but Fig. 2.4 shows that there is very strong bootstrap support for linking all of these AM fungi.

The arbuscular mycorrhizal fungi are considered in detail in Chapter 13, so we will discuss them only briefly here. They share several characteristic and quite remarkable features:

- They are found growing within the roots of the vast majority of plants, and yet they cannot be grown independently, in the absence of a plant.
- When they penetrate the roots they grow predominantly between the root cortical cells and often (but not always) produce large, swollen vesicles which are believed to function as food storage reserves.
- The AM fungal hyphae penetrate individual root cortical cells to form intricately branched **arbuscules** (tree-like branching systems). But they do not kill the root cells, and instead they establish an intimate feeding relationship, which seems to benefit both partners. Fig. 2.5 shows the extent of this type of relationship, in roots cleared of plant protoplasm, then stained with trypan blue.

Recently discovered fossil hyphae and spores from Wisconsin, USA, date back to the Ordovician, 460–455 mya (Redecker *et al.* 2000) and therefore pre-date the vascular plants (i.e. plants with water-conducting tissues). Examples of these early fossil fungi are shown in Fig. 2.6. They are remarkably similar to the AM fungal spores, although it is emphasized that they were not associated with plants. Nevertheless, they can provide calibration points in phylogenetic analyses, as shown in Fig. 2.7.

One final point – and perhaps the most remarkable – was the discovery of a novel type of symbiosis, first reported in 1996 and still known from only a few natural sites in Germany. In this symbiosis, AM fungi are attached to plant roots and grow up to the soil surface, where the AM fungus produces transparent bladders. These bladders engulf and internalize the cells of a cyanobacterium, *Nostoc punctiforme*, so that the fungus obtains sugars from the cyanobacterial partner. The resulting organism is called *Geosiphon pyriforme*, a member of the *Geosiphonaceae* (Glomeromycota) shown in Fig. 2.4. We return to this in Chapter 13.

Zygomycota

Five major features serve to characterize the phylum Zygomycota:

1 cell walls composed of a mixture of **chitin, chitosan** (a poorly- or non-acetylated form of chitin) and **polyglucuronic acid**;

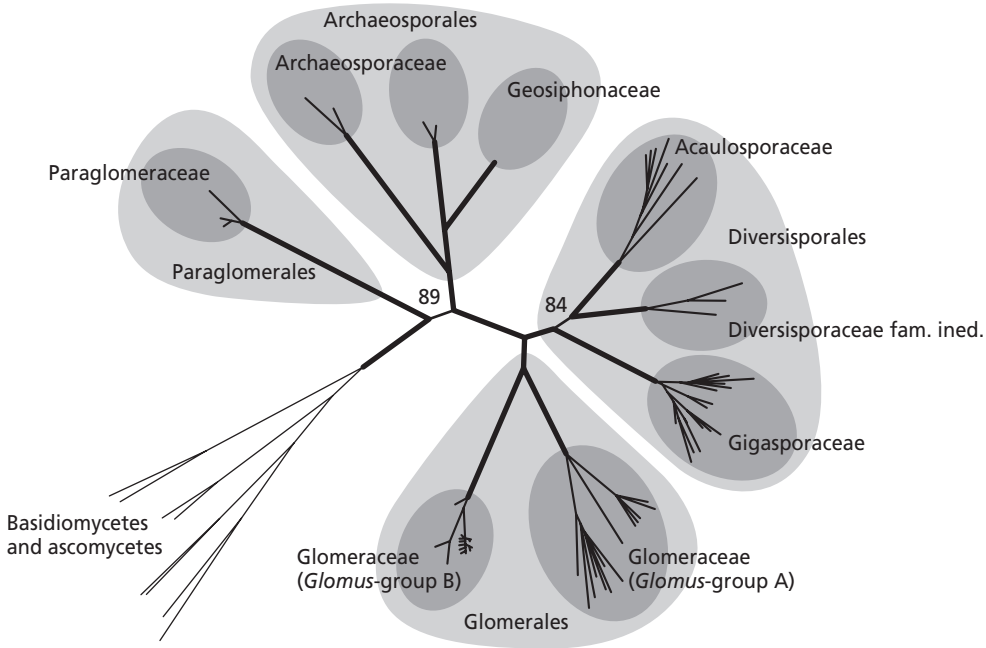


Fig. 2.4 Proposed generalized taxonomic structure of the AM fungi and related fungi (Glomeromycota). Thick lines delineate "bootstrap" values (indicating relatedness between the main branches) above 95%. Lower values (89% and 84%) are shown on two of the branches. (Reproduced by courtesy of Schuessler *et al.* 2001, and the British Mycological Society.)

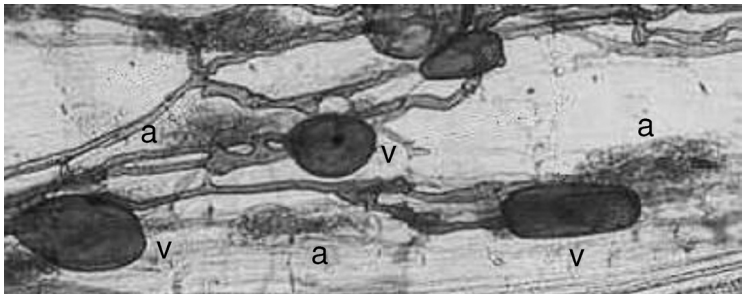


Fig. 2.5 Vesicles (v) and arbuscules (a) of present-day arbuscular mycorrhizal fungi in clover roots.

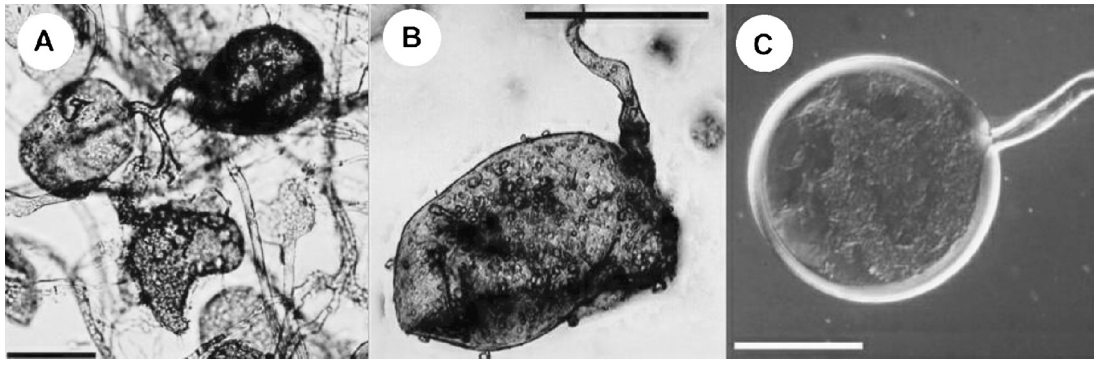


Fig. 2.6 (A,B) Fossil hyphae and spores from the Ordovician, about 460 mya, compared with a spore (C) of a present-day *Glomus* species (an arbuscular mycorrhizal fungus). All scale bars = 50 μm. (Images courtesy of Dirk Redecker; see Redecker *et al.* 2000.)