Chapter 4

Mycology: the study of fungi

A *Trichophyton* species: they infect skin, hair and nails

KEY FACTS

- Fungi are eukaryotic cells and are mainly saprophytes (feeding on decaying organic material) and growing at temperatures below 25° C.
- They are responsible for spoilage of foods and medicines and so their growth needs to be controlled.
- Very few are pathogenic to humans, but those infections which do arise are usually persistent and difficult to treat.
- Some fungi are capable of producing materials which are extremely useful to us in the food, chemical and pharmaceutical industries.

Mycology is the name given to the study of fungi. This is a huge subject area but the vast majority of it is of no importance to those of us studying the pharmaceutical sciences. There are some areas which have an impact on our field and so these are the parts where we need to have a working knowledge of the subject. The parts we need to know something about are those where the fungi cause spoilage of products we make, where they cause disease and where the cells have some role in the production of pharmaceutically useful materials.

This chapter will comprise a general overview of the subject and introduce the main fungi of interest. More details on product spoilage and preservation, industrial aspects of mycology and the treatment of pathogenic fungi will be dealt with in other chapters.

4.1 Definitions

- **Fungus** is a general term and is used to describe both unicellular yeasts and multicellular moulds.
- **Moulds** are multicellular fungi usually having a branching, filamentous structure.

4.2 Main characteristics

Yeasts and moulds are eukaryotic organisms and thus they possess a nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, nucleolus and so forth. The fungal cell wall is composed of various polysaccharides, including chitin, but is markedly different from those

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found in bacteria. They are saprophytic organisms which are widespread in the environment and their principal role in nature is recycling organic matter. They prefer to grow at temperatures below 25° C and not at 37° C. Hence, only a few species are pathogenic to humans; most are opportunist and not obligate parasites. However, the infections they do cause are often persistent and difficult to treat. Since they are so abundant in the environment they frequently cause spoilage of pharmaceuticals and food. Their ability to break down a wide range of organic matter gives them a huge metabolic capability enabling them to synthesize useful products such as foodstuffs, vitamins, organic acids, steroids, enzymes and antibiotics.

4.2.1 Fungal morphology

The morphology of fungi is highly diverse and many species exist in different morphological forms. Most of this is not relevant to us and so for the purposes of this book we will consider fungi as falling into four broad morphological categories:

4.2.1.1 Yeasts

Yeasts are spherical or ovoid unicellular bodies typically 2–4 μm in diameter. Most reproduce by budding, but some like *Schizosaccharomyces rouxii* reproduce by binary fission. Budding is a process of reproduction where the offspring emerge as a bud on the side of the parent cell. This gradually increases in size and eventually pinches off, forming a daughter cell and leaving an area of scarring. A single parent cell can produce up to 24 offspring. A common example is *Saccharomyces cerevisiae* (baker's yeast, brewer's yeast) shown in Figure 4.1, and *Cryptococcus neoformans* is the only significant pathogen which causes the lung infection cryptococcosis.

4.2.1.2 Yeastlike fungi

These often behave like typical budding yeasts but under certain cultural conditions the buds become elongated to form pseudohyphae which are elongated filaments. The most important member of this group is *Candida albicans* (Figure 4.2), which is found as part of the normal microflora of the body. *C. albicans* can cause infections known collectively as candidiasis and involve the mouth, vagina, intestinal tract and lungs.

4.2.1.3 Dimorphic fungi

This morphological grouping contains a number of important human pathogens. As the name suggests these

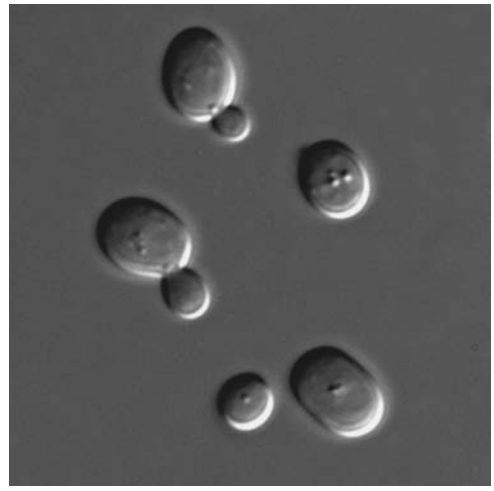


Figure 4.1 Budding *Saccharomyces cerevisiae*. *Source:* http://commons.wikimedia.org/wiki/File:S_cerevisiae_under_DIC_microscopy.jpg.

fungi exist as two distinct morphological forms. They grow as yeasts or filaments depending upon the cultural conditions. At temperatures below 22° C they grow in a filamentous form while at 37° C (body temperature) they grow in the yeast form. *Histoplasma capsulatum* (Figure 4.3) is the most important example and causes a disease known as histoplasmosis. This condition can manifest itself from a mild chest infection through to a fatal disseminated disease.

4.2.1.4 Filamentous fungi

Multicellular moulds grow as long slender branching filaments called hyphae, which are typically 2–10 μm in diameter. These hyphae may be nonseptate (coenocytic) or septate (with cross walls), growing over the surface of a food or medicine substrate and called the

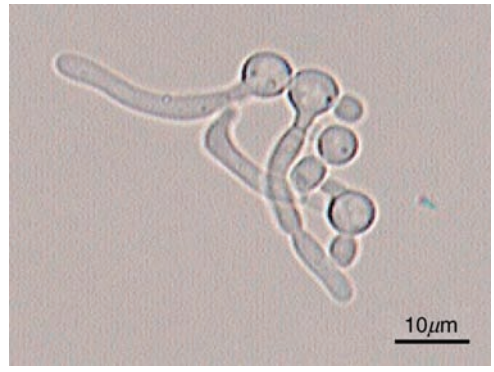


Figure 4.2 Pseudohyphae of *Candida albicans*. *Source:* http://commons.wikimedia.org/wiki/File:C_albicans_germ_tubes.jpg.

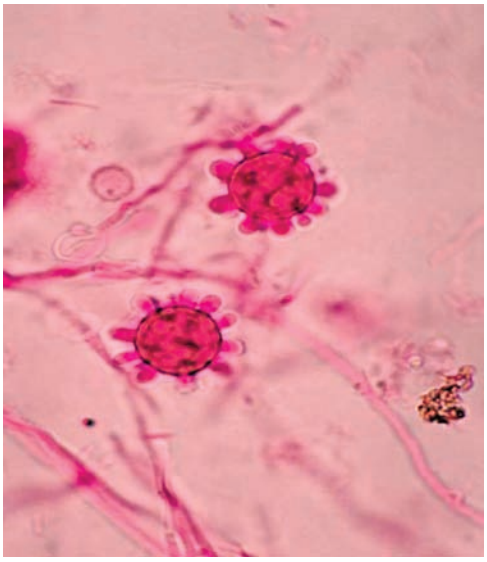


Figure 4.3 *Histoplasma capsulatum*. *Source:* PHIL ID #4023, Photo Credit: Libero Ajello, Centers for Disease Control and Prevention.

mycelium. Growth occurs by elongation at the hyphal tip. Because of the nature of their multicellular growth we cannot use cell number as a means of quantifying filamentous fungi. Instead it is necessary to monitor growth by measuring mass – for example, dry weight.

Lower fungi like *Mucor hiemalis* (Figure 4.4) and *Rhizopus stolonifer* produce nonseptate hyphae where the cytoplasm is freely diffusible along the filament. Higher fungi, such as *Penicillium* and *Aspergillus*, produce hyphae with septa but in this case the septa do not enclose individual cells. The presence of a pore in each septum allows the cytoplasm and even nuclei to diffuse along the filament. Adjacent filaments can fuse to allow exchange of contents.

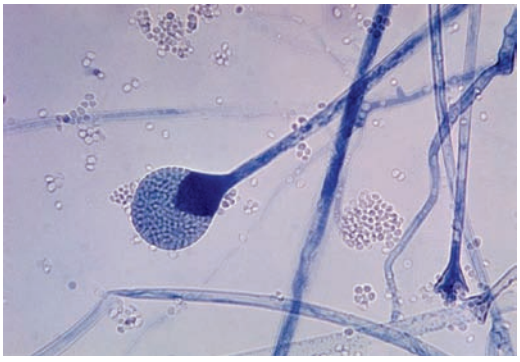


Figure 4.4 Sporangiophore of *Mucor hiemalis*. *Source:* PHIL ID #3961, Photo Credit: Dr. Lucille K. Georg, Centers for Disease Control and Prevention.

The branching filaments constitute the somatic structure of the fungus and are very similar from one species to another. Under correct environmental conditions the organism will switch from vegetative growth to a reproductive mode. This reproductive mode enables the fungus to propagate the species by moving to new substrates.

4.2.2 Reproduction in fungi

Fungi can exhibit both asexual and sexual reproduction:

4.2.2.1 Asexual reproduction

This is sometimes called somatic or vegetative reproduction. It does not involve the union of nuclei, sex cells or sex organs. In this process all progeny are identical to the parent cells and it is the process primarily responsible for propagating the species.

4.2.2.2 Sexual reproduction

Involves the union of two compatible nuclei and thus is primarily responsible for introducing genetic variation. The majority of fungi produce reproductive organs from a portion of the mycelium while the remainder continues its normal somatic activities.

Both of these processes are extremely diverse across the broad range of fungi and there is little value in discussing the individual reproductive mechanisms of many different species. Sexual reproduction, in particular, occurs less frequently than asexual reproduction and will only be dealt with superficially. However, it is necessary for us to have some grasp of asexual reproduction simply because it is such a common process and because it is the means by which many fungi infect their hosts and contaminate products. In addition, the asexual spore-bearing structures are often the major distinguishing features by which the organism is identified.

There are four common methods of asexual reproduction:

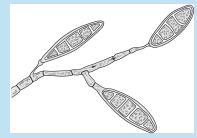
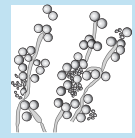
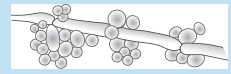
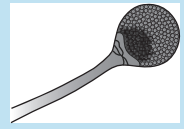
Binary fission

This is comparable to bacterial cell division where the parent cell divides to form two identical daughter cells. It occurs in some species of yeast cells.

Budding

This is the typical method of asexual reproduction for most yeast cells and was described earlier. Note that in the binary fission process the parent cell does not exist after the division has taken place. With budding there is a clear distinction between the parent cell and the daughter cell.

Table 4.1 Asexual spores of medically important fungi.

Asexual spore	Description	Morphology
Aleuriospore	Also called microconidia and macroconidia. Some occur singly or in groups on short lateral branches from the hyphae, others occur directly on hyphae, e.g. the Dermatophytes	
Arthrospore	Arise by fragmentation of the hyphae. Can be highly infectious, e.g. *Coccidioides immitis*	
Chlamydospore	Thick walled spores that form at the end of hyphae or in between hyphal segments. Very resistant to heat and drying, e.g. *Candida albicans*	
Blastospore	Formed by yeasts during budding. Can be formed by spherical yeasts and by elongated pseudohyphae, e.g. *Blastomyces spp.*	
Conidiospore	Occur singly or in groups at the end of specialized structures called conidiophores, e.g. *Penicillium spp.* and *Aspergillus spp.*	
Sporangiospore	Form within sac-like structures known as sporangia found at the end of specialized structures called sporangiophores, e.g. *Mucor spp.* and *Rhizopus spp.*	

Fragmentation

This can sometimes be thought of as a form of spore formation but there are no specialized structures involved (Table 4.1). Here the hyphae simply break up into component segments called arthroconidia which can be dispersed on the wind to other environments. After being formed they can aggregate together in a protective covering in order to survive harsh conditions perhaps imposed by winter weather.

Formation of spores

In this process the younger parts of the mycelium continue to grow across the substrate while in the older parts an abundance of spore-bearing structures arise. These structures are very varied in shape and size but are responsible for producing thousands of spores, each of which is genetically identical to the parent. When they are mature the spores are released and are light enough to be borne on the wind to find fresh food substrates. The most commonly found forms of asexual spores are shown in Table 4.1.

4.3 Commercially important fungi

Some fungi are important because of the damage they cause due to spoilage or because they can be exploited commercially. The following are just a few examples.

4.3.1 *Rhizopus stolonifer* and *Mucor hiemalis*

Both are members of the Zygomycetes (lower fungi) and reproduce asexually by means of sporangiospores produced within sporangia (see Figure 4.4). They are terrestrial saprophytic fungi which are widespread in the environment and are common contaminants. Both are important spoilage organisms and have the capacity to produce an abundance of enzymes. They are used commercially to produce a large number of organic acids – fumaric, lactic, citric and so forth, and are also used in the production of steroids (see Chapter 20).

4.3.2 *Claviceps purpurea*

This fungus is a contaminant of the cereal rye. It is mainly of historical interest from the perspective of infectious disease but the fungus is also an important source of pharmaceutical products. Spores penetrate the developing ears of the rye plant and establish themselves to form a hard resting stage termed a sclerotium (Figure 4.5). This falls to the ground during harvesting, where it overwinters and then germinates the following year, in order to infect the subsequent harvest. The presence of the sclerotium has been responsible for the disease called ergotism which has occurred throughout the last thousand years in central Europe and has killed hundreds of thousands of people.

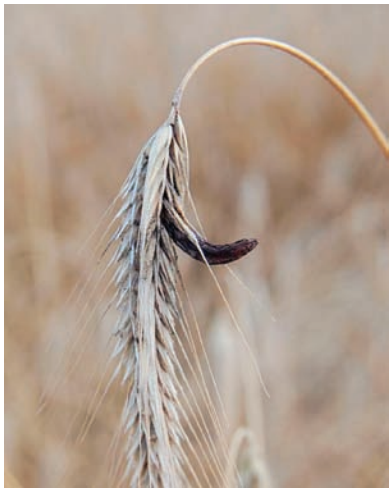


Figure 4.5 Ear of rye infected with *Claviceps purpurea* showing a sclerotium. *Source:* R. Altenkamp, Berlin; http://eo.wikipedia.org/wiki/Dosiero:Mutterkorn_090719.jpg.

4.3.2.1 Characteristics of ergots and ergotism

- During milling the ergots (sclerotia) are not separated from grain and the contents become incorporated into flour; this leads to slow poisoning of the population.
- First symptoms are coldness of extremities, followed by sensation of intense burning. Gangrene, necrosis and death may follow.
- Ergots contain a range of alkaloids having a wide spectrum of biological activity:
  - controlling haemorrhage;
  - induction of childbirth;
  - treatment of migraine.
- Now used commercially to produce ergotamine and ergometrine but cannot be cultivated in the laboratory.
- Last major outbreak in France in 1954; 200 people affected; four died.

4.3.3 *Aspergillus niger*

This is a very widely distributed fungus and is abundant in the environment. It is a member of the Deuteromycetes (higher fungi) which is an unusual group in that sexual reproduction has never been observed although the rest of their lifestyle suggests that it should. It is a spoilage organism contaminating crops like hay, nuts and grain. Contamination can lead to production of mycotoxins (aflatoxins), which can cause liver damage if ingested. A number of species of *Aspergillus* cause a disease called aspergillosis. These include a lung infection – 'farmer's lung' and also ear infections. They often grow as a solid mass within body cavities and these are termed aspergillomas.

While still young the mycelium produces an abundance of conidiophores (see Figure 4.6). These are not

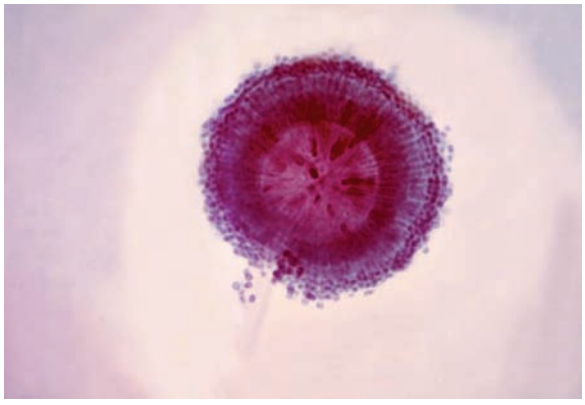


Figure 4.6 *Aspergillus* conidiophore. *Source:* PHIL ID #3965, Photo Credit: Dr. Lucille K. Georg, Centers for Disease Control and Prevention.

organized but arise individually from the somatic hyphae. Conidia are produced in their thousands and are pigmented, giving the colony its characteristic colour (see also Figure 1.6). The fungus has great enzymic activity and is used in the commercial production of a range of enzymes such as amylases, diastase and proteases. It is also used to produce organic acids like citric, gluconic etc. and also fermented products like sake and soy sauce.

4.3.4 *Penicillium chrysogenum*

This fungus is as common in the environment as the *Aspergillus* fungus. It is also a member of the Deuteromycetes (higher fungi) and again no sexual reproduction has been observed. The life history is similar to *Aspergillus* although the morphology of spore-bearing structures is different. The conidiophores have a characteristic brushlike appearance (Figure 4.7) and the colour of conidia is variable.

It is a frequent contaminant of foods and is often seen as a green or blue mould growing on food left around. It is widely used in the production of organic acids such as citric, fumaric, oxalic, gluconic acids and so forth. In the food industry it is also used in the production of veined cheeses like Roquefort, Stilton and Danish Blue. However, it is probably best known for its role in the production of penicillins. The natural product of the fungus is Penicillin G, and from that the whole range of other penicillins such as penicillin V, ampicillin, amoxicillin and others are produced semisynthetically (see Chapter 10).

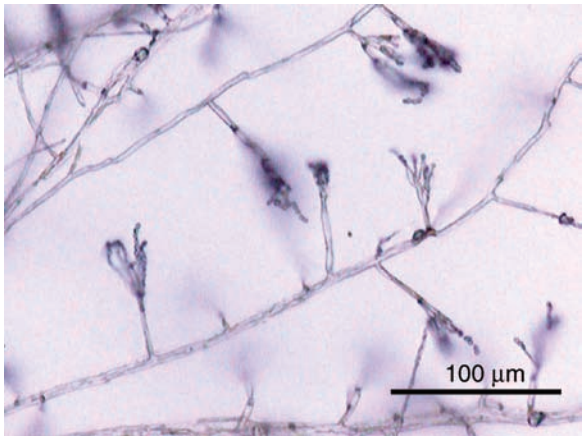


Figure 4.7 *Penicillium* showing hyphae and developing conidiophores. *Source:* http://commons.wikimedia.org/wiki/File:Penicillium.jpg.

4.4 Pathogenic fungi

It is not the intention to provide an exhaustive list of pathogenic fungi but merely to indicate the types of fungi and the breadth of infections produced. Table 4.2 shows the main pathogenic fungi and the diseases they cause.

As we previously grouped fungi according to their morphological characteristics it is also appropriate to use this scheme as a means of dividing the main pathogens into groups.

- **Yeasts**
  - *Cryptococcus neoformans*
- **Yeastlike fungi**
  - *Candida albicans*
- **Dimorphic fungi**
  - *Histoplasma capsulatum*
  - *Coccidioides immitis*
  - *Blastomyces dermatitidis*
  - *Paracoccidioides braziliensis*
- **Filamentous fungi**
  - Dermatophyte fungi
  - *Aspergillus*
  - *Mucor*

Some of these will be expanded below.

4.4.1 Yeasts

Cryptococcus neoformans is a single-celled yeast possessing a polysaccharide capsule. It reproduces by budding and is the only species of *Cryptococcus* capable of growing at 37° C. The fungus causes cryptococcosis, an infection that is contracted chiefly via inhalation although it can occur through the skin. Initial pulmonary infection may be mild but this can progress to systemic cryptococcosis where the liver, bones and skin may be infected. The most dangerous form is cryptococcal meningitis. The organism is often present in the soil and in the excrement of birds and bats where it can remain viable for months. Hence, poultry workers and cave explorers are potentially at risk.

4.4.2 Yeastlike fungi

Candida albicans is the main organism of interest and is part of the normal flora of the mouth, gut and vagina. Its numbers are kept in check by competition with the other microflora but under some circumstances it can overgrow. This may occur under conditions of antibiotic therapy,

Table 4.2 Some medically important fungi.

Disease type	Example	Microorganism
Systemic mycoses	Cryptococcosis	*Cryptococcus neoformans*
	Coccidioidomycosis	*Coccidioides immitis*
	Histoplasmosis	*Histoplasma capsulatum*
	Blastomycosis	*Blastomyces dermatitidis*
	Bronchpulmonary candidiasis	*Candida albicans*
	Gastrointestinal candidiasis	*Candida albicans*
	Endocarditis	*Candida albicans*
	Pneumonia (PCP)	*Pneumocystis jiroveci* (formerly *carinii)*
	Aspergillosis	*Aspergillus fumigatus*
	Phycomyosis	*Mucor sp., Rhizopus sp.*
Mucocutaneous mycoses	Oral candidiasis	*Candida albicans*
	Vulvovaginal candidiasis	*Candida albicans*
Subcutaneous mycoses	Sporotrichosis	*Sporothrix schenckii*
	Maduromycosis	*Madurella mycetomi*
	Chromomycosis	*Phialophora verrucosa*
Cutaneous mycoses	Ringworm	*Microsporum sp.*
		Trichophyton sp.
		Epidermophyton floccosum
	Intertriginous candidiasis	*Candida albicans*
Superficial mycoses	Pityriasis versicolor	*Melassezia furfur*
	Tinea nigra	*Exophiala werneckii*
	White piedra	*Trichosporon cutaneum*
	Black piedra	*Piedraia hortai*

diabetes, vitamin deficiency, long-term steroids, immunosuppressive therapy, alcoholism and inappropriate diet.

Candidiasis of the mucous membranes is termed 'thrush' and occurs both in the mouth (see also Figure 9.3) and the vagina. Cutaneous candidiasis is an infection of the skin, while of more concern is bronchopulmonary candidiasis (infection of the bronchi and lungs).

4.4.3 Dimorphic fungi

Histoplasma capsulatum causes a disease called histoplasmosis (Darling's disease), which is endemic in central areas of the United States (Kansas, Ohio) also Africa and the Far East. 95% of cases are subclinical and are only detected by a skin test. In Kansas 80–90% of the population show a positive skin test by the age of 20, while in the United States as a whole 40 million people are subclinically infected and an estimated 200 000 new cases emerge each year. Of these 5% may go on to develop chronic lung disease while 0.2% may develop disseminated disease. The organism is associated with bird excrement, particularly that of starlings, which pose a threat as they roost in flocks containing millions of birds.

A number of other dimorphic fungi are responsible for similar diseases to histoplasmosis (see Table 4.2) but they will not be dealt with here.

4.4.4 Filamentous fungi

4.4.4.1 Dermatophyte fungi

This is the term given to a range of different fungi which cause diseases of the skin, nails and hair in humans (see Table 4.3). They only affect the keratinized areas of the

Table 4.3 Dermatophyte infections of different sites.

Tinea	Area affected	Alternative name
Capitis	Scalp	Ringworm
Corporis	Body	Ringworm
Cruris	Groin	Jock Itch
Pedis	Feet	Athlete's foot
Barbae	Hair	Barber's itch
Unguium	Nails	Onychomycosis

body and the infections they cause are not life threatening but can be very persistent. The infections are referred to as Tinea infections, also dermatomycoses and ringworm. The name ringworm has arisen because the circular lesions on the skin resemble a worm under the skin (see Figure 4.8). More details on athlete's foot can be found in the text box on the right.

There are three main genera which are of importance:

Epidermophyton
- One of several dermatophytes causing athletes foot.
- Single species – *E. floccosum* attacks skin and nails.
- It is the commonest cause of ringworm of the groin.

Microsporum
- Attacks hair and skin but not nails.
- Infects skin of children – causes tinea capitis.
- May be spread by handling cats and dogs.

Trichophyton
- Attacks skin, nails and hair. *T. mentagrophytes* var interdigitale is the main cause of athlete's foot.

Figure 4.8 Ringworm lesions on the forearm. *Source:* http://commons.wikimedia.org/wiki/File:Herpes_circin%C3%A9_01.jpg.

Athlete's foot

- It is one of the most common infectious diseases.
- It affects males and females of every age and race.
- It is highly contagious; it may be spread by contaminated floors and towels.
- A single spore can initiate infection.
- The spore germinates and penetrates the stratum corneum.
- The fungus produces branching septate hyphae and forms arthrospores by fragmentation of hyphae.
- The infection begins as cracking or scaling between toes.
- Redness and itching.
- The condition can become chronic especially in humid conditions.
- The spores infect shoes and socks and are not readily killed.

4.4.4.2 *Aspergillus niger*

This organism causes systemic infections that occur more commonly in immunocompromised patients but they can arise in immunocompetent patients where there has been recent tissue damage. The main infections of interest include:

- Invasive aspergillosis.
  - Mainly affects immunocompromised.
  - Lungs affected in 80–90% of patients.
  - Dry cough, fever, chest pain and dyspnoea (shortness of breath).
- Aspergillus tracheobronchitis.
  - Mainly affects AIDS and lung transplant patients.
- Aspergillus sinusitis.
  - More common in bone marrow transplant patients.
- Cerebral aspergillosis.
  - Occurs in 10–20% of patients with invasive aspergillosis.
  - Usually only in immunocompromised.
- Aspergilloma.
  - Can colonize cavities within lungs or sinuses.
  - Ball of fungus develops in the cavity. May be no mucosal involvement. Surgical removal may be curative.
  - Patients may be asymptomatic, but most have persistent productive cough and weight loss.

4.4.4.3 Mucormycosis

This term covers a variety of infections caused by the Zygomycetes, for example *Mucor* and *Rhizopus*.

Spores enter the body via the respiratory tract but can be inoculated through the skin. Generally the diseases are limited to immunocompromised patients or those with diabetes, trauma or solid organ transplants. The infecting fungi are able to grow rapidly and produce abundant spores; hence disease spread can be rapid and is often fatal.

- Rhinocerebral mucormycosis is the most common form.
- Pulmonary mucormycosis is seen mostly in neutropenic patients.
- Cutaneous mucormycosis presents as a chronic non-healing ulcer with central necrosis – it often follows an insect bite or a gardening injury.

Acknowledgement

Chapter title image: PHIL ID #3053, Dr. Arvind A. Padhye, Centers for Disease Control and Prevention.