5 General Mycology

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General Characteristics of Fungi

Fungi are eukaryotic microorganisms (domain eucarya) that occur ubiquitously in nature. Only about 200 of the thousands of species have been identified as human pathogens, and among these known pathogenic species fewer than a dozen are responsible for more than 90% of all human fungal infections.

The basic morphological element of filamentous fungi is the hypha and a web of intertwined hyphae is called a mycelium. The basic form of a unicellular fungus is the yeast cell. Dimorphic fungi usually assume the form of yeasts in the parasitic stage and the form of mycelia in the saprophytic stage. The cell walls of fungi consist of nearly 90% carbohydrate (chitin, glucans, mannans) and fungal membranes are rich in sterol types not found in other biological membranes (e.g., ergosterol). Filamentous fungi reproduce either **asexually** (mitosis), by hyphal growth and tip extension, or with the help of asexual spores. Yeasts reproduce by a process of budding. **Sexual** reproduction (meiosis) on the other hand, produces sexual spores. **Fungi imperfecti** or deuteromycetes are the designation for a type of fungi in which the fructification forms are either unknown or missing entirely.

Definition and Taxonomy

Fungi are microorganisms in the domain eucarya (see. p. 5). They show less differentiation than plants, but a higher degree of organization than the prokaryotes bacteria (Table 5.**1**). The kingdom of the fungi (*Mycota*) comprises over 50 000 different species, only about 200 of which have been identified as human pathogens. Only about a dozen of these "pathogenic" species cause 90% of all human mycoses. Many mycotic infections are relatively harmless, for instance the dermatomycoses. In recent years, however, the increasing numbers of patients with various kinds of immune defects have resulted in more life-threatening mycoses.

Table 5.1 Some Differences between Fungi and Bacteria

Properties	Fungi	Bacteria
Nucleus	Eukaryotic; nuclear membrane; more than one chromosome; mitosis	Prokaryotic; no membrane; nucleoid; only one "chromosome"
Cytoplasm	Mitochondria; endoplasmic reticulum; 80S ribosomes	No mitochondria; no endoplasmic reticulum; 70S ribosomes
Cytoplasmic membrane	Sterols (ergosterol)	No sterols
Cell wall	Glucans, mannans, chitin, chitosan	Murein, teichoic acids (Gram-positive), proteins
Metabolism	Heterotrophic; mostly aerobes; no photosynthesis	Heterotrophic; obligate aerobes and anaerobes, facultative anaerobes
Size, mean diameter	Yeast cells: 3–5–10 µm. Molds: indefinable	1–5 µm
Dimorphism	In some species	None

The taxonomy of the fungi is essentially based on their morphology. In medical mycology, fungi are classified according to practical aspects as dermatophytes, yeasts, molds, and dimorphic fungi. Molds grow in filamentous structures, yeasts as single cells and dermatophytes cause infections of the keratinized tissues (skin, hair, nails, etc.). Dimorphic fungi can appear in both of the two forms, as yeast cells or as mycelia (see the following pages).

Fungi are carbon heterotrophs. The saprobic or saprophytic fungi take carbon compounds from dead organic material whereas biotrophic fungi (parasites or symbionts) require living host organisms. Some fungi can exist in both saprophytic and biotrophic forms.

Morphology

Two morphological forms of fungi are observed (Fig. 5.1):

- **Hypha:** this is the basic element of filamentous fungi with a branched, tubular structure, 2–10 µm in width.

Basic Morphological Elements of Fungi

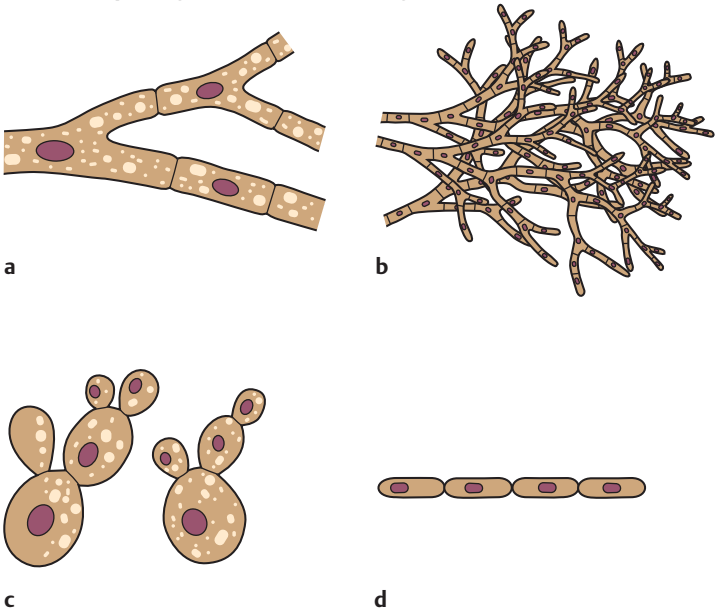

Fig. 5.**1** There are two basic morphological forms: hypha and yeast.
a Hypha, septate, or nonseptate.
b Mycelium: web of branched hyphae.
c Yeast form, budding (diameter of individual cell 3–5 µm).
d Pseudomycelium.

- **Mycelium:** this is the web or matlike structure of hyphae. Substrate mycelia (specialized for nutrition) penetrate into the nutrient substrate, whereas aerial mycelia (for asexual propagation) develop above the nutrient medium.
- **Fungal thallus:** this is the entirety of the mycelia and is also called the fungal body or colony.
- **Yeast:** the basic element of the unicellular fungi. It is round to oval and 3–10 µm in diameter. Several elongated yeast cells chained together and resembling true hyphae are called pseudohyphae.
- **Dimorphism:** some fungal species can develop either the yeast or the mycelium form depending on the environmental conditions, a property called dimorphism. Dimorphic pathogenic fungi take the form of yeast cells in the parasitic stage and appear as mycelia in the saprophytic stage.

Metabolism

All fungi are carbon heterotrophs, which means they are dependent on exogenous nutrient substrates as sources of organic carbon, and with a few exceptions, fungi are obligate aerobes. Many species are capable of maintaining metabolic activity in the most basic of nutrient mediums. The known metabolic types of fungi include thermophilic, psychrophilic, acidophilic, and halophilic species. The metabolic capabilities of fungi are exploited in the food industry (e.g., in the production of bread, wine, beer, cheese, or single-cell proteins) and in the pharmaceutical industry (e.g., in the production of antibiotic substances, enzymes, citric acid, etc.). The metabolic activity of fungi can also be a damaging factor. Fungal infestation can destroy foods, wooden structures, textiles, etc. Fungi also cause numerous plant diseases, in particular diseases of crops.

Reproduction in Fungi

Asexual reproduction. This category includes the vegetative propagation of hyphae and yeasts as well as vegetative fructification, i.e., formation of asexual spores.

- **Hyphae** elongate in a zone just short of the tip in which the cell wall is particularly elastic. This apical growth process can also include formation of swellings that develop into lateral hyphae, which can in turn also branch out.

- **Yeasts** reproduce by budding. This process begins with an outgrowth on the mother cell wall that develops into a daughter cell or blastoconidium. The isthmus between the two is finally cut off by formation of a septum. Some yeasts propagate in both the yeast and hypha forms (Fig. 6.**2**, p. 362).

- **Vegetative fructification.** A type of propagative form, the **asexual spores**, is formed in this process. These structures show considerable resistance to exogenous noxae and help fungi spread in the natural environment. Asexual spores come in a number of morphological types: **conidia, sporangiospores, arthrospores, and blastospores**. These forms rarely develop during the parasitic stages in hosts, but they are observed in cultures. The morphology of the asexual spores of fungi is an important identification characteristic.

Sexual fructification. Sexual reproduction in **fungi perfecti** (eumycetes) follows essentially the same patterns as in the higher eukaryotes. The nuclei of two haploid partners fuse to form a diploid zygote. The diploid nucleus then undergoes meiosis to form the haploid nuclei, finally resulting in the haploid

sexual spores: **zygospores, ascospores, and basidiospores**. Sexual spores are only rarely produced in the types of fungi that parasitize human tissues.

Sexual reproduction structures are either unknown or not present in many species of pathogenic fungi, known as **fungi imperfecti** (deuteromycetes).

General Aspects of Fungal Disease

■ Besides fungal allergies (e.g., extrinsic allergic alveolitis) and mycotoxicoses (aflatoxicosis), fungal infections are by far the most frequent fungal diseases. Mycoses are classified clinically as follows:

- **Primary mycoses** (coccidioidomycosis, histoplasmosis, blastomycoses).
- **Opportunistic mycoses** (surface and deep yeast mycoses, aspergillosis, mucormycoses, phaeohyphomycoses, hyalohyphomycoses, cryptococcoses; penicilliosis, pneumocystosis).
- **Subcutaneous mycoses** (sporotrichosis, chromoblastomycosis, Madura foot (mycetoma).
- **Cutaneous mycoses** (pityriasis versicolor, dermatomycoses).

Little is known about fungal pathogenicity factors. The natural resistance of the macroorganism to fungal infection is based mainly on effective phagocytosis whereas specific resistance is generally through cellular immunity. Opportunistic mycoses develop mainly in patients with immune deficiencies (e.g., in neutropenia). Laboratory diagnostic methods for fungal infections mostly include microscopy and culturing, in order to detect the pathogens directly, and identification of specific antibodies. Therapeutics for treatment of mycoses include polyenes (above all amphotericin B), azoles (e.g., itraconazole, fluconazole, voriconazole), allylamines, antimetabolites (e.g., 5-fluorocytosine), and echinocandins (e.g., caspofungin). Antimycotics are often administered in combination. ■

Fungal Allergies and Fungal Toxicoses

Mycogenic Allergies

The spores of ubiquitous fungi continuously enter the respiratory tract with inspirated air. These spores contain potent allergens to which susceptible individuals may manifest strong hypersensitivity reactions. Depending on the localization of the reaction, it may assume the form of allergic rhinitis, bron-

chial asthma, or allergic alveolitis. Many of these allergic reactions are certified occupational diseases, i.e., "farmer's lung," "woodworker's lung," and other types of extrinsic allergic alveolitis.

Mycotoxicoses

Some fungi produce mycotoxins, the best known of which are the aflatoxins produced by the *Aspergillus* species. These toxins are ingested with the food stuffs on which the fungi have been growing. Aflatoxin B1 may contribute to primary hepatic carcinoma, a disease observed frequently in Africa and Southeast Asia.

Mycoses

Data on the general incidence of mycotic infections can only be approximate, since there is no requirement that they be reported to the health authorities. It can be assumed that **cutaneous mycoses** are among the most frequent infections worldwide. **Primary** and **opportunistic mycoses** are, on the other hand, relatively rare. Opportunistic mycoses have been on the increase in recent years and decades, reflecting the fact that clinical manifestations are only observed in hosts whose immune disposition allows them to develop. Increasing numbers of patients with immune defects and a high frequency of invasive and aggressive medical therapies are the factors contributing to the increasing significance of mycoses. Table 5.**2** provides a summary view of the most important human mycoses. The categorization of the infections used here disregards taxonomic considerations to concentrate on practical clinical aspects.

Host-pathogen interactions

The factors that determine the onset, clinical picture, severity, and outcome of a mycosis include interactions between fungal pathogenicity factors and host immune defense mechanisms. Compared with the situation in the field of bacteriology, it must be said that we still know little about the underlying causes and mechanisms of fungal pathogenicity.

Humans show high levels of nonspecific resistance to most fungi based on mechanical, humoral, and cellular factors (see Table 1.**6**, p. 22). Among these factors, phagocytosis by neutrophilic granulocytes and macrophages is the most important. Intensive contact with fungi results in the acquisition of spe-

Table 5.2 Overview of the Most Important Mycoses in Humans

Disease	Etiology	Remarks
Primary mycoses (do not occur endemic in Europe)		
Coccidioidomycosis	*Coccidioides immitis*	Pulmonary mycosis. Inhalation of spores. Southwestern US and South America
Histoplasmosis	*Histoplasma capsulatum*	Pulmonary mycosis. Inhalation of spores. Dissemination into RES. America, Asia, Africa
North American Blastomycoses	*Blastomyces dermatitidis*	Primary pulmonary mycosis. Secondary dissemination (dermal). North America, Africa
South American Blastomycoses	*Paracoccidioides brasiliensis*	Primary pulmonary mycosis. Secondary dissemination
Opportunistic mycoses		
Candidiasis (soor)	*Candida albicans*, other *Candida* sp.	Endogenous infection. Primary infection of mucosa and skin with secondary dissemination
Aspergillosis	*Aspergillus fumigatus* (90%); other *Aspergillus* sp.	Aspergilloses of the respiratory tract, endophthalmitis; aspergillosis of CNS; septic aspergillosis
Cryptococcosis	*Cryptococcus neoformans* (yeast; thick capsule)	Aerogenic infection. Pulmonary cryptococcosis. Secondary dissemination into CNS
Mucormycoses (zygomycoses)	*Mucor* spp.; *Rhizopus* spp.; *Absidia* spp.; *Cuninghamella* spp., and others	Rhinocerebral, pulmonary, gastrointestinal, cutaneous mucormycosis
Phaeohyphomycoses (caused by "dematious" or "black" fungi)	Over 100 species discovered to date, e.g., *Curvularia* spp.; *Bipolaris* spp.; *Alternaria* spp. Melanin integrated in cell wall	Subcutaneous infections, paranasal sinus infections, infections of the CNS, sepsis also possible
Pneumocystosis	*Pneumocystis carinii*	Defective cellular immunity

Table 5.2 *Continued: Overview of the Most Important Mycoses in Humans*

Disease	Etiology	Remarks
Hyalohyphomycoses (caused by colorless [hyaline] molds)	More than 40 species discovered to date, e.g., *Fusarium* spp.; *Scedosporium* spp.; *Paecilomyces lilacinus*	Infections of cornea and eye, pneumonia, osteomyelitis, arthritis, soft tissue infections, sepsis also possible
Yeast mycoses (except candidiasis)	*Torulopsis glabrata; Trichosporon beigelii; Rhodotorula* spp.; *Malassezia furfur*, and others	Infections of various organs in immunosuppressed patients. Sepsis also possible. *Malassezia furfur* in catheter sepsis in neonates and in intravenous feeding with lipids
Penicilliosis	*Penicillium marneffei*	Most frequent opportunistic infection in AIDS patients in Southeast Asia. Primary infection focus in lungs
Subcutaneous mycoses		
Sporotrichosis	*Sporothrix schenckii*	Dimorphic fungus, ulcerous lesions on extremities
Chromoblastomycosis	*Phialophora verrucosa Fonsecea pedrosoi Cladosporium carrionii*, etc.	Black molds. Wartlike pigmented lesions on extremities. Tropical disease
Madura foot (mycetoma)	*Madurella mycetomi Scedosporium apiospermum*, etc.	Subcutaneous abscesses on feet or hands. Can also be caused by bacteria (see p. 273). In tropics and subtropics
Cutaneous mycoses		
Pityriasis (or tinea versicolor)	*Malassezia furfur*	Surface infection; relatively harmless; pathogen is dependent on an outside source of fatty acids
Dermatomycoses Tinea pedis, T. cruris, T. capitis, T. barbae, T. unguinum, T. corporis	*Trichophyton* spp. *Microsporum* spp. *Epidermophyton* spp.	All dermatophytes are filamentous fungi (hyphomycetes). Anthropophilic, zoophilic, geophilic species. Always transmitted by direct or indirect contact

cific immunity, especially the cellular type. The role of humoral immunity in specific immune defense is secondary.

Diagnosis

The primary concern here is identification of the pathogen.

- **Microscopy.** Native preparation: briefly heat material under coverslip with 10% KOH. Stained preparation: stain with methylene blue, lactophenol blue, periodic acid-Schiff (PAS), ink, etc.
- **Culturing.** This is possible on universal and selective mediums. Sabouraud dextrose agar can contain selective agents (e.g., chloramphenicol and cycloheximide), this medium has an acid pH of 5.6. The main identifying structures are morphological, in particular the asexual and, if present, sexual reproductive structures. Biochemical tests are used mainly to identify yeasts and are generally not as important in mycology as they are in bacteriology.
- **Serology.** By the identification of antibodies to special fungal antigens in patient's serum. The Interpretation of serological findings is quite difficult in fungal infections.
- **Antigen detection.** By finding of specific antigens in the diagnostic material by direct means using known antibodies, possible in some fungal infections (e.g., cryptococcosis).
- **Cutaneous test.** Cutaneous (allergy) tests with specific fungal antigens can be useful in diagnosing a number of fungal infections.
- **Nucleic acid detection.** Combined with amplification, such tests are useful for rapid detection of mycotic diseases in immunocompromised patients.

Therapy

A limited number of anti-infective agents are available for specific treatment of fungal infections:

- **Polyenes.** These agents bind to membrane sterols and destroy the membrane structure:
  - Amphotericin B. Used In systemic mycoses. Fungicidal activity with frequent side effects. There are conventional galenic form and (new) various lipid forms.
  - Nystatin, natamycin. Only for topical use in mucosal mycoses.

■ **Azoles.** These agents disrupt ergosterol biosynthesis. Their effect is mainly fungistatic with possible gastrointestinal side effects. Hepatic functional parameters should be monitored during therapy:
- Ketoconazole. One of the first azoles. No longer used because of side effects.
- Fluconazole. Oral or intravenous application. For the treatment of surface and systemic mycoses and cryptococcal meningitis in AIDS patients.
- Itraconazole. Oral and intravenous application. Use in systemic and cutaneous mycoses and also for the treatment of aspergillosis.
- Voriconazole. Oral and intravenous application. Good activity against *Candida* and *Aspergillus*. No acitivity against *Mucorales*.

■ **Antimetabolites.** 5-Fluorocytosine. Interferes with DNA synthesis (base analog). Given by oral application in candidiasis, aspergillosis, and cryptococcosis. It is necessary to monitor the course of therapy for the development of resistance. The toxicity of amphotericin B is reduced in combination with 5-fluorocytosine.

■ **Allylamines.** Terbinafine. By oral and topical application to treat dermatomycoses. Inhibition of ergosterol biosynthesis.

■ **Echinocandins. Caspofungin** has been approved as a salvage therapy in refractory aspergillosis. It is useful also in oropharyngeal and esophageal candidiasis. Inhibition of the biosynthesis of glucan of the cell wall.

■ **Griseofulvin.** This is an older antibiotic used in treatment of dermatomycoses. By oral application, therapy must often be continued for months.