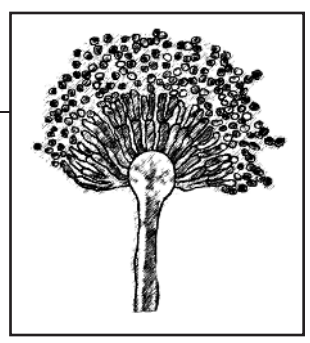
Chapter 1



Introduction to Fundamental Medical Mycology

1.1 TOPICS NOT COVERED, OR RECEIVING SECONDARY EMPHASIS

The Table of Contents is explicit but it is well to advise readers that some topics are either outside the scope of *Fundamental Medical Mycology* or receive secondary emphasis. Although caused by, or associated with, fungi it is not within the scope of this work to discuss mushroom poisoning (ingestion of toxins present in mushrooms), mycotoxicosis (ingestion of a fungal toxin), or allergies, except when encountered as a complication of one of the fungal diseases discussed.

- More information on mushroom poisoning (mycetismus) can be found in Benjamin (1995).
- Allergies caused by fungi are discussed in Kurup and Fink (1993) and Breitenbach et al. (2002). The health effects of exposure to molds, apart from infection, may be found in Storey et al. (2005) and U.S. Environmental Protection Agency publication 402K-01-001 (2001).
- Environmental mycology is discussed as it relates to the ecologic niche of the causative agents of mycoses.
- Veterinary medical mycology is covered in a concise section, "Veterinary Forms," in each disease-specific chapter.

1.2 BIOSAFETY CONSIDERATIONS: BEFORE YOU BEGIN WORK WITH PATHOGENIC FUNGI...

Safety in the laboratory is of prime importance. Clinical laboratory supervisors and principal investigators have the serious responsibility to train all technologists and students in the safe manipulation of clinical specimens and pathogenic fungi. Before working with pathogenic microbes, including fungi, microbiologists should participate in their organization's safety training program, be certified to work with pathogens, and, when questions about biosafety arise, consult the supervisor and the CDC/NIH biosafety manual: *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition (BMBL). The manual is available online at the URL http://www.cdc.gov/biosafety/publications/bmbl5/index.htm. This will ensure a safe work environment where the workers will not be afraid to work with fungi but instead will have confidence that they are observing prudent precautions.

Molds growing on Petri plates can produce far more infectious propagules (conidia or spores) than an environmental exposure! Therefore, mold cultures should be transferred to screw cap- or cotton-stoppered agar slants. Mold cultures on Petri plates should never be opened on the open laboratory bench. All cultures of unknown molds should be handled inside a biological safety cabinet (BSC). Petri plates should be sealed with shrink seals, which are colorless transparent cellulose bands. "Occupational Hazards from Deep Mycoses" is a useful and cautionary article summarizing laboratory infections (Schwarz and Kauffman, 1977; Padhye et al., 1998).

The BMBL should also be consulted for further information about selection of BSCs, and biosafety considerations of work with pathogenic fungi. If further questions arise on matters of fungal biosafety, please contact the State Department of Health in the United States of America or the CDC Mycotic Diseases Branch, which is the World Health Organization Center for Mycoses.

Fundamental Medical Mycology, First Edition. By Errol Reiss, H. Jean Shadomy and G. Marshall Lyon, III.

1.2.1 Biological Safety Cabinets (BSC)

What are the characteristics of Class II Biological Safety Cabinets? The Class II BSC is designed with inward airflow velocity (75–100 linear feet/min) and is fitted with high efficiency particulate air (*HEPA*) filters. This design ensures that the workspace in the cabinet receives filtered, downward, vertical *laminar airflow*. These characteristics protect personnel and the microbiologic work conducted in the BSC.

HEPA-filtered-exhaust air ensures protection of the laboratory and the outside environment. All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2, and 3.1. Fungi pathogenic for humans are classed in biosafety level 2 and work with them should be conducted in the BSC, and not on the open bench. Certain manipulations of fungal pathogens or environmental samples require biosafety level 3 (please see below).

Class II BSCs provide a microbe-free work environment. Class II BSCs are classified into two types (A and B) based on construction, airflow, and exhaust systems. Type A cabinets are suitable for microbiologic work *in the absence of* volatile or toxic chemicals and radionuclides, since air is recirculated within the cabinet. Type A cabinets may be exhausted into the laboratory or to the outdoors via a special connection to the building exhaust system. Type B cabinets are hard-ducted to the building exhaust system and contain negative pressure plenums to allow work to be done with toxic chemicals or radionuclides. A list of products that meet the standards for Class II BSCs are available from the National Sanitation Foundation International, Ann Arbor, Michigan. It is mission-critical that BSCs be tested and certified in situ at the time of installation, at any time the BSC is moved, and at least annually after that.

1.2.2 Precautions to Take in Handling Etiologic Agents that Cause Systemic Mycoses

The major known reasons for laboratory exposures to pathogenic fungi are dropped cultures, preparing soil suspensions and inoculating animals, opening Petri plates, and aerosols from needles and syringes (Padhye et al., 1998). The portals of entry for the fungi, resulting from the above exposures, are minor skin wounds or the inhalation of fungal conidia.

Pathologists and veterinarians should be mindful that autopsies and necropsies have caused accidental hand wounds, which have become infected. These infections are localized to the wound and have not disseminated. Needle stick injuries have also been the source of laboratory infections, and they too have remained localized. Localized infections require systemic antifungal therapy.

Laboratory exposures to aerosolized conidia (spores) have led to pulmonary infections and, in the case of *Coccidioides* species, to serious or even fatal infections. Some cases of coccidioidomycosis have occurred in laboratories beyond the endemic area and resulted when the laboratory did not suspect the mold they had isolated was *Coccidioides*.

Biosafety level 2 (BSL 2) practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues suspected of containing pathogenic fungi. BSL 2 is also sufficient for mold cultures identified as *Blastomyces dermatitidis, Cryptococcus neoformans*, dermatophytes, *Penicillium marneffei*, and *Sporothrix schenckii*. In addition to these agents, certain melanized molds have caused serious infection in immunocompetent hosts following inhalation or accidental penetrating injuries: *Bipolaris* species, *Cladophialophora bantiana, Wangiella* (*Exophiala*) *dermatitidis, Exserohilum* species, *Fonsecaea pedrosoi, Ochroconis gallopava, Ramichloridium mackenziei*, and *Scedosporium prolificans*.

All manipulations of clinical specimens and culture work are performed inside an annually inspected and certified, well-functioning, laminar flow biological safety cabinet (BSC), equipped with HEPA filtered exhaust. Workers should wear personal protective equipment (PPE).

- Clothing: laboratory coats with fronts fastened and shoes with closed fronts.
- Eye protection: safety glasses, goggles, as recommended by the supervisor.
- Gloves: latex or plastic.
- Respiratory protection: goggles, mask, face shield, or other splatter guards are used when the cultures must be handled outside the BSC. Surgical masks are not respirators and do not provide protection against aerosolized infectious agents. The N95 disposable respirator provides a level of protection. Supervisors should consult the National Institute of Occupational Safety and Health (NIOSH) Publication No. 99–143: TB Respiratory Protection Program in Health Care Facilities to match the respiratory protection to their risk assessment at the URL http://www.cdc.gov/niosh/docs/99-143.
- Sharps jars should be provided for disposal of needles and syringes.
- All waste should be autoclaved before disposal.

1.2.3 Additional Precautions at Biosafety Level 3 (BSL 3)

BSL 3 conditions should be observed when working with mold-form cultures identified as *Coccidioides* species and *Histoplasma capsulatum* according to the following specific situations.

- *Coccidioides immitis* and *C. posadasii*. Once *Coccidioides* species are identified in the clinical laboratory, biosafety level 3 practices, equipment, and facilities are required for manipulating sporulating cultures and for processing soil or other environmental materials known to contain infectious arthroconidia. *Coccidioides* species are subject to the regulations regarding Select Agents: biological agents and toxins that could pose a severe threat to public health and safety. These regulations are discussed in "Appendix F" of the BMBL.
- Clinical laboratory supervisors and principal investigators should be aware of what to do if there is a *Coccidioides* exposure in their laboratory (Stevens et al., 2009).
- BSL 3 practices, containment equipment, and facilities are recommended for propagating sporulating cultures of *H. capsulatum* in the mold form, as well as processing soil or other environmental materials known or likely to contain infectious conidia.

The criteria for BSL 3 practices are numerous and are detailed in the CDC/NIH manual, *Biosafety in Microbiological and Medical Laboratories*. BSL 3 conditions may require facility reconstruction: for example, two doors should separate the laboratory from a public area; the laboratory should be at negative pressure with respect to the entrance; and air that enters the laboratory should be vented through the biological safety cabinet HEPA filter and then vented outside the building. Waste is to be autoclaved before it leaves the BSL 3 laboratory, so that a room adjacent to the level 3 laboratory should be equipped with an autoclave.

1.2.4 Safety Training

The U.S. Centers for Disease Control and Prevention sponsor the International Symposium on Biosafety. Short courses at that conference cover biosafety practices in laboratories and in veterinary practice: for example, "Infection Control, Biosafety in Research and Clinical Settings" and "Moving from BSL 2 to BSL 3." Topics included are engineering controls, personal protective equipment (PPE), hand hygiene, environmental disinfection, and waste disposal.

1.2.5 Disinfectants and Waste Disposal

For information on these topics for the laboratory please see **Chapter 2, Section 2.3.1.1**, Disinfectants and Waste Disposal.

1.3 FUNGI DEFINED: THEIR ECOLOGIC NICHE

What are fungi? Where are they found? The kingdom Fungi is composed of unicellular or multicellular, *eukaryotic*, *heterotrophic* microbes. Each fungal cell contains a full array of organelles and is bound by a rigid cell wall containing chitin, glucan, and/or cellulose (Table 1.1). Please also see Section 1.12, General Composition of the Fungal Cell.

Of the thousands of fungal species that are free-living in nature or are pathogenic for plants, only a small group are known to be pathogenic for humans and animals. It is also true that *any fungus capable of growing at 37°C is a potential pathogen in a debilitated or immunocompromised host*.

Some fungi are primary pathogens (e.g., *Coccidioides* species) and can cause disease in immune-normal persons. Severity of a fungal disease is related to host factors (immune status, general health status) and the number of infectious propagules (conidia or spores) inhaled, ingested, or injected. Persons who are immunocompromised, or otherwise debilitated, are prone to develop more serious disease and to be susceptible to opportunistic fungi against which immune-normal persons have a high level of resistance.

Fungi are ubiquitous in nature, being found in the air, in soil, on plants, and in water, including the oceans, even as a part of lichens growing on rock. There is essentially no part of our earth where fungi are not found. A few fungal species are adapted to live as *commensals* in humans but for most fungal pathogens humans are accidental hosts. Of all the fungi with pathogenic potential most are opportunistic, whereas a select few are able to cause disease in otherwise healthy humans who have intact immune and endocrine systems.

1.4 MEDICAL MYCOLOGY

What is medical mycology? Medical mycology is a distinct discipline of medical microbiology concerned with all aspects of diseases in humans and lower animals caused by pathogenic fungi.

What are the mycoses? Mycoses are diseases of humans and lower animals caused by pathogenic fungi.

Table 1.1 Comparison of Structure/Function of Bacterial and Fungal Organelles

Organelles	Bacteria	Fungi
Cell wall	Murein = peptidoglycan	Glucan, mannan, chitin
Cell membrane	No sterols	Ergosterol
Metabolism	Aerobic or anaerobic	Aerobic
Energy transduction	Cell membrane	Mitochondria
Cytoplasm	Proteins	Glycoproteins, actin, tubulin, mitotic spindle, Golgi
Gene structure	Operons, no introns	Single genes, introns, repetitive DNA
Nuclear material	Prokaryotic: a single chromosome; no nuclear membrane.	Eukaryotic: multiple chromosomes contained in a nucleus bounded by a nuclear membrane; for example *Candida albicans* has 8 chromosomes
Ribosome	30S + 50S = 70S	40S + 60S = 80S
Extrachromosomal DNA	Plasmids	Mitochondrial DNA, dsRNA
Capsular polysaccharide	Several agents of meningitis	One agent of meningitis: *Cryptococcus neoformans*

There is a broad spectrum of mycoses ranging from superficial skin diseases to deep-seated, multisystem disseminated diseases. Please see Section 1.10, Classification of Mycoses Based on the Primary Site of Pathology.

1.5 A BRIEF HISTORY OF MEDICAL MYCOLOGY

Because the fruiting bodies of some fungi are large enough to see without the aid of a microscope, such as mushrooms, they were the first microorganisms known. Centuries later, it was discovered that mushrooms are only the obvious structures of complex fungi with a vast network of fungal cells found beneath the soil, tree bark, and so on.

1.5.1 Ancient Greece

Fungi have caused a variety of maladies affecting our quality of life for millennia. Hippocrates (460–377 B.C.E.), the father of medicine, recognized that persons with oral thrush (due to *Candida albicans*) were already debilitated by other diseases. This thought was echoed in our own time when Professor Graham S. Wilson, Director of the U.K's Public Health Laboratory Service said: "*Candida* is a much better clinician than we are, in its ability to detect abnormalities earlier in the course of development of such abnormalities than we can with all our chemical tests." This comment was made before the advent of AIDS but now it is well known that oral–esophageal candidiasis heralds the onset of that disease.

1.5.2 Middle Ages

In general, the vast majority of fungal infections are not spread from person to person (are not communicable). However, there are significant exceptions, for example, the *dermatophytes*. In the Middle Ages, children in Europe became infected with *favus*, a fungal disease of the scalp, smooth skin, even nails, due to *Trichophyton schoenleinii*. It was devastating to these individuals because they were considered unclean, separated from their peers, and sent to separate schools. There was no specific treatment at that time. Favus was so disfiguring that it was mistaken for leprosy by artists of the Renaissance (Goldman, 1968). A modern case of scalp ringworm is shown in Fig. 1.1.

1.5.3 Twentieth Century

Examples of outbreaks from a single source are not uncommon. In Witwatersrand, South Africa, from 1941 through 1944 nearly 3000 gold mine workers were infected with the subcutaneous fungal pathogen *Sporothrix schenckii*, which they acquired by brushing against mine timbers on which the fungus was growing (Du Toit, 1942). Figure 1.2 depicts the classic appearance of lymphocutaneous sporotrichosis.

1.5.4 Endemic Mycoses in the Americas

A review of thousands of induction center roentgenograms of young men inducted into the armed forces in World War II noted the "incidence of calcified lesions presumed to represent healed tuberculosis corresponded to the now well-known pattern of regional differences in the U.S." (reviewed by Iams, 1950). Mycologic investigations, including large scale skin testing with histoplasmin, established that delayed type hypersensitivity to *Histoplasma capsulatum* was widespread among residents of the major river valleys of the central United States, thus establishing the boundaries of the histoplasmosis endemic area.

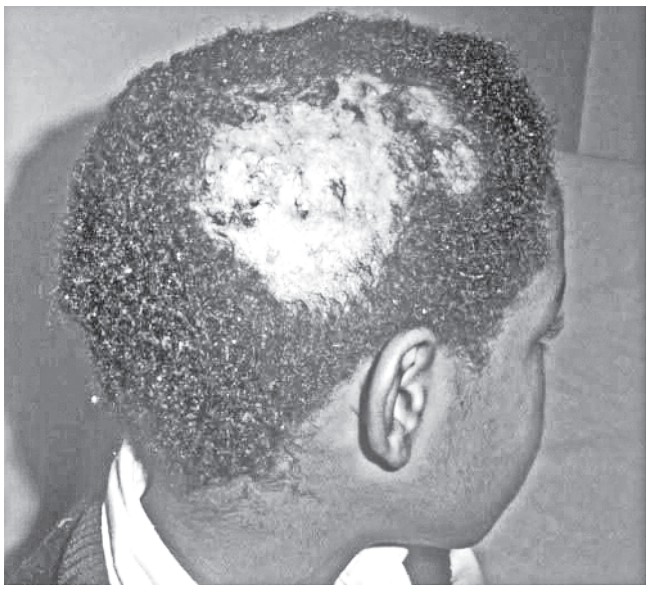


Figure 1.1 Head of a child with tinea capitis (scalp ringworm). A 9-year-old girl complained of cradle cap for over a year before developing itchy red patches on the right parietal scalp. She shed most of the hair in these areas. A culture was positive for *Trichophyton tonsurans*, and she was treated with oral griseofulvin with complete clearing of the tinea and regrowth of hair.
Source: Copyright Bernard A. Cohen, M.D. Used with permission from Dermatlas; www.dermatlas.org.

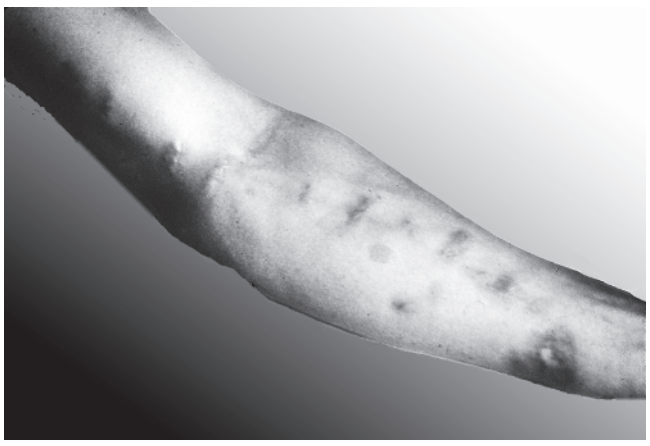


Figure 1.2 Sporotrichosis of the arm. Lesions draining each lymph node, from a primary lesion on the hand.
Source: Wilson and Plunkett (1965), used with permission from the University of California Press.

1.5.5 Era of Immunosuppression in the Treatment of Cancer, Maintenance of Organ Transplants, and Autoimmune Diseases

The era of cancer chemotherapy began when Sidney Farber synthesized a folic acid antagonist, now called methotrexate, and used it to treat childhood leukemia. His report in 1948 in the *New England Journal of Medicine* was greeted with ridicule because, at the time, the medical community held that childhood leukemia was incurable and children so afflicted should be allowed to die in peace. Since then, other researchers discovered drugs that blocked different functions involved in cell growth and replication ushering in the era of chemotherapy. The first cure of metastatic cancer was obtained in 1956 when methotrexate was used to treat a rare tumor called choriocarcinoma.

1.5.6 Opportunistic Mycoses

Cancer chemotherapy using cytotoxic drugs and systemic corticosteroids, by weakening the immune system, created opportunities for yeast and mold disease, principally candidiasis and aspergillosis, but also a long list of fungi formerly regarded as "saprophytes." Members of the genus *Aspergillus*, consisting of common environmental molds, have been known as pathogens since 1842, when one species was detected in the air sac of a bullfinch. Later, *Aspergillus fumigatus* was identified in other birds, and in humans where infections have increased proportional to the use of immunosuppressive therapy. Masses of the fungus have been found behind suspended ceilings in hospitals, in building materials, and outside windows of hospitals, especially during renovation or construction, and are the cause of single cases of aspergillosis as well as outbreaks in hospitalized patients.

1.5.7 HIV/AIDS

The AIDS epidemic in the United States, beginning in 1981, was brought to the attention of infectious disease specialists when two previously rare diseases, Kaposi's sarcoma and pneumocystosis, were encountered in men having sex with men (MMWR, 1981). An increase in *Pneumocystis* pneumonia (PCP) was noticed at the Centers for Disease Control (CDC) in April 1981, by Sandra Ford, a drug technician, who reported a high number of requests for the drug pentamidine, used to treat PCP. According to Ford, "A doctor was treating a gay man in his 20's who had pneumonia. Two weeks later, he called to ask for a refill of a rare drug that I handled. This was unusual, nobody ever asked for a refill. Patients usually were cured in one 10-day treatment or they died." As HIV, the cause of AIDS, and its role as a blood-borne and sexually transmitted pathogen was elucidated, AIDS brought to the forefront those opportunistic infections whose host defense was coupled to T-cell mediated immunity. In addition to pneumocystosis, other fungal opportunistic infections of AIDS were identified in quick succession as oro-esophageal candidiasis, cryptococcosis,

endemic mycoses in the United States and, in Southeast Asia, penicilliosis.

1.5.8 Twenty-first Century

1.5.8.1 Advances in Clinical Laboratory Mycology

Important milestones in clinical laboratory mycology include (i) more rapid tests to identify *Candida* species in blood cultures, (ii) the wider availability of antifungal susceptibility tests because of new commercial kits, and (iii) the transfer of technology into the clinical laboratory for sequence-based identification of fungi. These are state-of-the-art tests appropriate for well-resourced hospitals. In resource-limited countries, a lack of training, proper reagents, supplies, and equipment impacts their laboratories' ability to identify pathogens and to detect antimicrobial resistance. Beginning in 2005, the American Society for Microbiology (ASM) International Laboratory Capacity Building Program (URL www.labcap.org) began to strengthen and expand clinical microbiology services in those regions.

Rapid Results for Candidemia The *Candida albicans/Candida glabrata* peptide nucleic acid fluorescent in situ hybridization assay (PNA-FISH, AdvanDx, Inc. Woburn, MA) was described in 2002 and is approved by the U.S. Food and Drug Administration (FDA) as a kit to identify yeast directly from a newly positive blood culture (Shepard et al., 2008). Cost savings accrue because, by ruling out *C. glabrata*, unnecessary echinocandin therapy can be avoided. Please see Chapter 11, Section 11.12, Laboratory Detection, Recovery and Identification, for further information.

Wider Availability of Antifungal Susceptibility (AFS) Testing The availability of commercial AFS tests in good agreement with reference methods facilitates rapid test results, thus aiding in clinical treatment decisions. These methods are discussed in Chapter 3B and are referred to here as important milestones in advancing clinical laboratory mycology (Cantón et al., 2009). The tests listed below have good correlation with reference methods standardized by the U.S. Clinical Laboratory and Standards Institute (CLSI).

Microtitration Plates Precoated with Drugs Sensititre® YeastOne™ (Trek Diagnostic Systems Inc., Westlake, OH) is a broth microdilution method. Wells of microtitration plates come precoated with dried dilutions of antifungal agents. Because of that they are stable in storage for prolonged periods, even at room temperature.

YeastOne is approved by the U.S. FDA for testing fluconazole (FLC), itraconazole (ITC), and flucytosine against clinical yeast isolates, and is available for investigational use to test amphotericin B, ketoconazole, voriconazole (VRC), posaconazole (PSC), and caspofungin (CASF). The YeastOne method was also evaluated to test the susceptibility of molds to triazoles and to amphotericin B.

Automated Spectrophotometric Microdilution Susceptibility The VITEK® 2 (bioMérieux, Inc., France) is FDA approved for testing FLC against *Candida* species. It has also been evaluated for testing amphotericin B, VRC, and flucytosine.

Disk Diffusion Neo-Sensitabs® tablet (Rosco, Taastrup, Denmark) is an easy to perform disk diffusion method available in Europe for testing yeasts and molds against polyenes, azoles, and echinocandins.

Drug Gradient Strips Etest® (AB Biodisk, Solna, Sweden) is not new but accommodates newer antifungal agents so it can be viewed as an improved method for AFS testing of yeasts and molds. It is an agar diffusion method with each drug applied to a plastic strip in a gradient of concentrations and printed with a minimum inhibitory concentration scale. The FDA has approved the Etest for testing FLC, ITC, and flucytosine.

Sequence-Based Identification of Fungi Unusual yeasts and molds, fungi that are slow growing, or those that fail to sporulate pose challenges to morphologic identification. Sequence-based identification is moving from the research laboratory to the clinical microbiology laboratories of tertiary care medical centers, aided by the availablility of kits for DNA preparation and purification, and biotechnology core facilities.

As an indication of progress, the CLSI issued a guideline, "Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing" (CLSI, 2008) to address sequence analysis in clinical laboratory practice. The guideline provides a standardized approach to identify fungi by DNA sequencing using the most common target, the ITS region of rDNA. Topics covered include primer design, quality control of amplification and sequencing, and reference sequence databases. The progress-to-date and remaining challenges of DNA sequence-based identification of opportunistic molds are discussed by Balajee et al., (2009).

1.5.8.2 Advances in Antifungal Therapy

Licensing of extended spectrum azoles, and a new class of antifungal drugs, the echinocandins, in this century, expand the therapeutic choices to treat invasive mycoses. Extended spectrum triazole antifungal agents (VRC, PSC,

and the echinocandins CASF, micafungin, and anidulafungin) have become licensed for use and advance the therapy of serious mycoses (Fera et al., 2009). The echinocandins are the fourth class of antifungal agents available to treat systemic mycoses. The other classes are the polyenes (amphotericin B and its lipid formulations), azoles (ketoconazole, ITC, FLC, VRC, PSC), and pyrimidines (flucytosine). Please see Chapter 3A, for further information.

Echinocandins The echinocandins attack fungi via a novel mode of action by inhibiting ß-(1→3)-D-glucan synthase, a key enzyme in synthesis of ß-glucan,the fibrillar component of the fungal cell wall. CASF was approved by the FDA in 2001, followed by micafungin in 2005, and anidulafungin in 2006. In addition to approval for treating candidiasis, CASF is approved to treat invasive aspergillosis in patients intolerant of or refractory to other therapy.

Extended Spectrum Triazoles The spectrum of activity for VRC and PSC includes *Candida* species, molds, and dimorphic fungi. Their activity extends to both FLC- and ITC-resistant strains of *Candida*. VRC was approved by the U.S. FDA in May 2002 to treat invasive aspergillosis and infections caused by *Scedosporium apiospermum* and *Fusarium* species in cases of intolerance to or failure of other antifungal agents.

PSC was approved by the FDA in 2006 for the prophylaxis of invasive *Aspergillus* and *Candida* infections in adolescent and adult patients who are heavily immunosuppressed for a stem cell transplant or in transplant recipients with graft-versus-host disease or those with hematologic malignancies and prolonged neutropenia. Later, PSC was approved for oropharyngeal candidiasis.

1.5.8.3 Advances in the Molecular Basis of Pathogenesis and the Host Response

Fungal Genome Initiative This is an organized genome sequencing effort aimed at illuminating aspects of fungal biology which are fundamental to an understanding of fungal pathogenesis. To date, 110 assembled genomes for 81 fungi are available in public databases and more sequencing projects are underway. A list of fungal pathogens whose genomes have been sequenced is found in Cuomo and Birren (2010).

Genome-wide Expression Profiling During Pathogenesis Completion of the *Candida albicans* genome sequence, and those of other fungal pathogens, is being followed by annotation of the genes, which is a work in progress. Microarray technology[1] is used to capture a genome-wide portrait of the transcriptome expressed during infection in order to identify the genes, signaling pathways, and transcription factors involved in pathogenesis. The investigator is able to observe pathogen gene expression and to compare it, in the same system, with host gene expression to compile the time-sensitive events during pathogenesis and the host response (Wilson et al., 2009).

As an example, genes expressed by *C. albicans* growing on oral epithelial cells, and the level of gene expression, was revealed during hyphal formation and adherence, reflected at the molecular level with a number of genes encoding adhesins or other hyphal-associated functions (Wilson et al., 2009).

Proteomics Proteomics is defined as the set of proteins expressed in a given type of cell or by an organism at a given time under defined conditions. "Proteopathogen" (http://proteopathogen.dacya.ucm.es) is a protein database focused on the *Candida albicans* – macrophage interaction model (Vialás et al., 2009). There are 66 *C. albicans* proteins and 38 murine macrophage proteins identified in the model. Whereas two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the most widely used method to separate complex protein mixtures, there are newer methods that combine high pressure liquid chromatography with mass spectrometry to generate large data sets that have to be stored in a website to enable their retrieval for efficient data mining. The goal of proteomics is to elucidate the proteins of the fungus and of the host, which are produced during different stages of the pathogenic process. The objective of such analysis is to understand pathogenesis at the molecular and cellular levels, to develop better diagnostic tests, and to discover rational methods of antifungal therapy.

1.6 RATIONALE FOR FUNGAL IDENTIFICATION

Why do we need to identify fungi? When dealing with microbes causing disease in humans there are important reasons to identify the causal agent.

1.6.1 Developing the Treatment Plan

Knowledge of the pathogen will increase chances for successful therapy because the pathogenesis of most mycoses

[1]A microarray (or DNA chip) is a series of gene targets immobilized on glass. Hybridization of cDNA probes made from mRNA in the test sample to these targets allows analysis of relative amounts of gene expression (Bryant et al., 2004).

is well studied. That will influence the choice of diagnostic tests, medical and surgical procedures, and antifungal therapy.

1.6.2 Investigating Outbreaks

- *The Hospital Setting*. The source of infection may be in the environment, including construction or renovation near patient wards, faulty HVAC systems affecting airflow in operating rooms or patient wards, contaminated hospital injectable solutions, indwelling medical devices, substandard hand-washing procedures, or other factors implicated in the healthcare environment.
- *The Community Setting*. An outbreak of fungal disease in the community typically requires an investigation of the causal agent: for example, histoplasmosis during spring break in 2001 among college students in Acapulco, Mexico (MMWR, April 13, 2001).

1.6.3 Determining the Susceptibility to Antifungal Agents

Because different fungi are susceptible to different antifungal agents it is important to:

- Identify the causal agent in order to select the most appropriate antifungal agent.
- Determine its in vitro killing effectiveness. Susceptibility in vitro does not uniformly predict clinical success in vivo because host factors play a critical role in determining clinical outcome. Resistance in vitro, however, will often, but not always, correlate with treatment failure. Please see Chapters 3A and 3B.
- Monitor the therapeutic response of the patient. Additional diagnostic tests and/or surgical intervention may be necessary.

1.6.4 Estimating the Significance of Fungi Generally Considered to be Opportunists or Saprobes

The physician should consider the immunocompetence of the patient, among other risk factors, to assess whether a fungus generally considered a saprobe may be the cause of disease.

1.6.5 Types of Vegetative Growth

What are the major forms of microscopic fungi? The microscopic fungi are classified by the type of vegetative growth as either yeasts or molds.

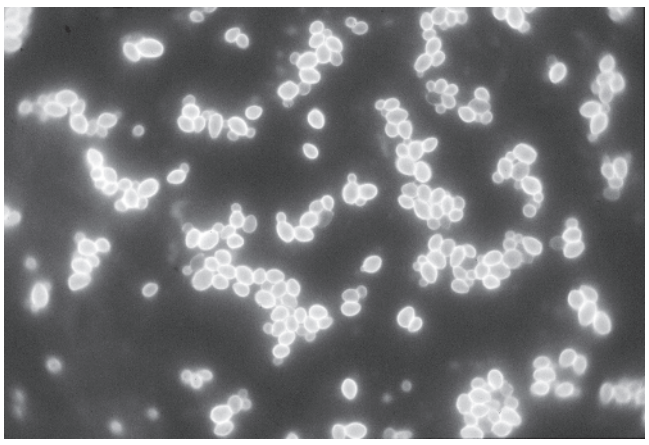


Figure 1.3 Budding yeast cells of *C. albicans*; immunofluorescence stained with an anti-mannan mAb. *Source:* PHIL, CDC, E. Reiss.

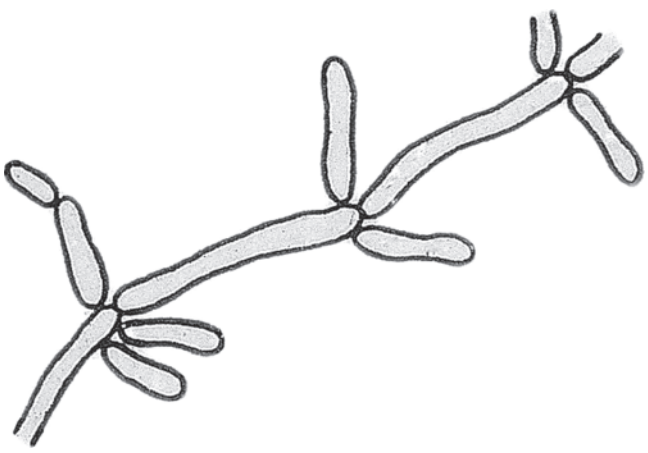


Figure 1.4 *Candida parapsilosis* pseudohyphae. *Source:* Adapted from Haley and Rice (1983).

1.6.5.1 Yeasts

The simplest fungi are the *yeasts* (Fig. 1.3). They are unicellular and reproduce by budding (e.g., *Candida albicans*) or fission (e.g., *Schizosaccharomyces pombe*). Yeasts causing disease in humans produce buds termed *blastoconidia*. An exception is *Trichosporon*, which, in addition to budding, produces hyphae that fragment into *arthroconidia*.

Pseudohyphae Pseudohyphae are seen in a wide variety of yeasts. Pseudohyphae are distinct from yeast forms and true hyphae. When blastoconidia remain attached in a chain of round to elongate cells, often resembling a string of pearls, the entire structure is called *pseudohyphae* (singular: pseudohypha). A mass of pseudohyphae is a pseudomycelium (Fig. 1.4).

The types of budding patterns in pseudohyphae are (i) unipolar, synchronous budding in which the first and later daughter buds are formed at the apex extending the length of the pseudohyphae; (ii) axial budding to form clusters of buds (whorls or *verticils*) behind pseudohyphal junctions; (iii) bipolar budding in which daughter cells are formed at both poles of a pseudohypha; and (iv) budding in which daughter cells are formed from both the distal and proximal ends of adjacent cells within a pseudohypha (Veses and Gow, 2009).

Nuclear division in pseudohyphae occurs at the point where the mother and daughter cells are most constricted. Septum formation also occurs at this point in the neck. Mitosis and septum formation in true hyphae of *C. albicans* are located at some distance within the true hypha. Cell divisions are near synchronous in pseudohyphae but in true hyphae, subapical cells are often arrested in G1 phase for several cell cycles (please see Section 1.12.1, yeast Cell Cycle.

1.6.5.2 Molds

The *molds* are formed by filamentous, cylindrical, often branching cells called *hyphae* (singular: *hypha*) (Fig. 1.5). A mass of hyphae is termed a *mycelium* (Fig. 1.6). The term *thallus* is sometimes used to refer to the entire body of a fungus (Fig. 1.7a). For a comparison, an agar plate with a yeast colony is shown in Fig. 1.7b. Hyphae occur in two different forms, depending on the phylum of fungi involved. The *Mucoromycotina* (please see Section 1.11.2.1) produce hyphae with sparse *crosswalls* or *septa* (singular: septum). Where septa occur, they are not perforated but serve to isolate reproductive structures or vacuolated regions in the mycelium (Fig. 1.8).

All other clinically encountered molds produce hyphae with crosswalls (septa) to separate the nuclei in different cells. Pores within the septa allow exchange

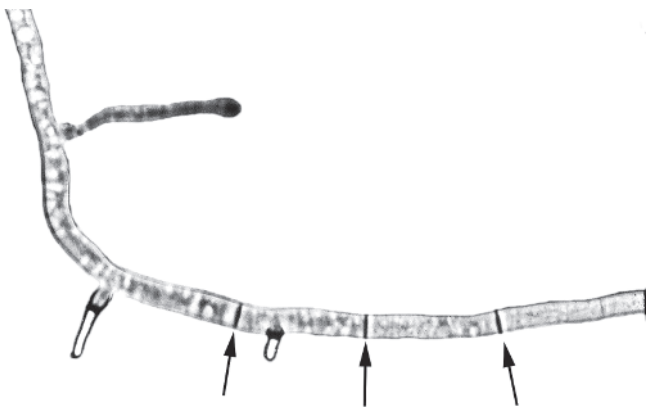


Figure 1.5 Microscopic view of septate hyphae: *arrows* point to septa.
Source: H. J. Shadomy.

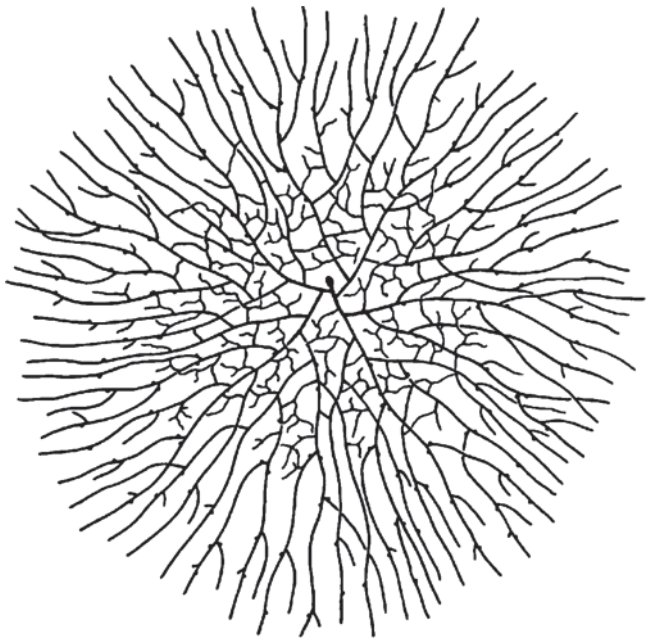


Figure 1.6 Radiating hyphae of a mycelium.
Source: Buller (1931).

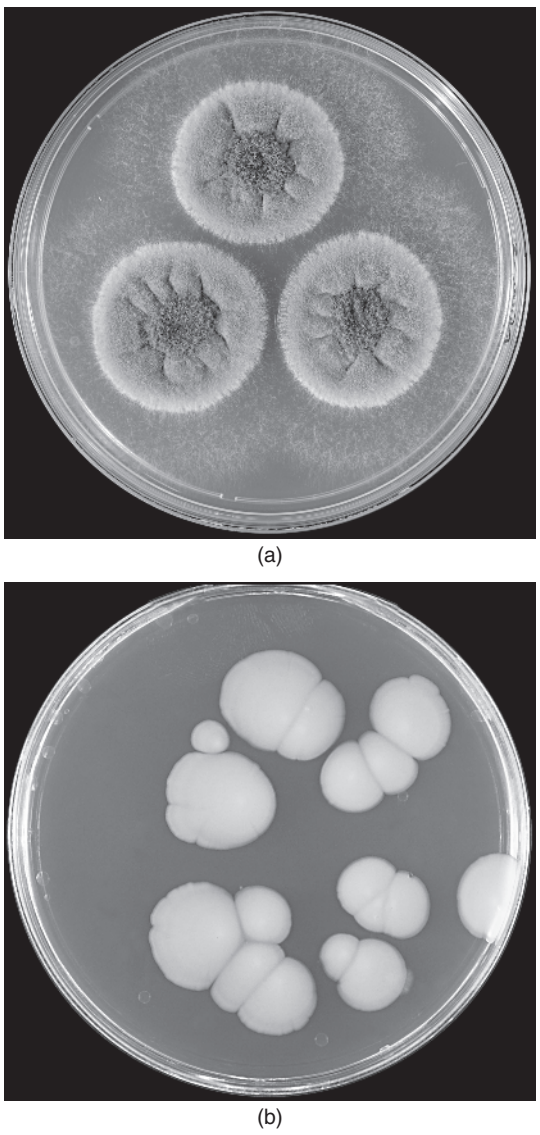
of cytoplasm and even nuclei. The types of septa differ physically depending on the phylum of fungi involved. This characteristic is not typically used in the mycology laboratory for identification purposes.

1.7 SPORULATION

What is fungal sporulation and how does it differ among species? Vegetative growth is necessary but is not sufficient to perpetuate fungi and a variety of reproductive propagules are formed for dispersal with the aid of air currents or in water. Fungal propagules are different types of spores, a means of asexual reproduction. The method of sporulation used by fungi is the major character with which clinical laboratory scientists use to identify fungi in the clinical laboratory and, as such, is discussed in Chapter 2, Section 2.3.8.7, Common Types of Asexual Sporulation Seen in the Clinical Laboratory and Generally Termed a Type of Conidium (or Spore).

1.8 DIMORPHISM

Dimorphism (*definition*: two forms) is an important characteristic of certain fungal pathogens. Dimorphism is morphogenesis that allows growth to occur in either the mycelial or yeast forms, (mycelium → yeast, or yeast → mycelium conversion); for example, *Histoplasma capsulatum* is a dimorphic fungus (Please see Chapter 6 for illustrations of this dimorphism.) Fungi causing



(a)

(b)

Figure 1.7 (a) Agar plate with mould colony: *Aspergillus fumigatus*. *Source:* Mr. Jim Gathany, CDC Creative Arts Branch. (b) Agar plate with yeast colony: *Candida albicans* growing on SABHI agar.
Source: Dr. William Kaplan, CDC.

primary systemic infections are typically filamentous soil-dwelling molds. The infectious propagules most frequently are conidia that are inhaled, along with hyphal fragments. Morphogenesis to the yeast form occurs during infection of tissues, usually in the lungs. This conversion is temperature sensitive, with the yeast form developing at 37°C. In the laboratory, growth at 35–37°C on an *enriched medium* may be used to help identify the fungus by this form change also known as "morphogenesis."

There are notable exceptions to the mold-to-yeast dimorphism. The primary systemic pathogens *Coccidioides immitis* and *C. posadasii* grow as a mold form

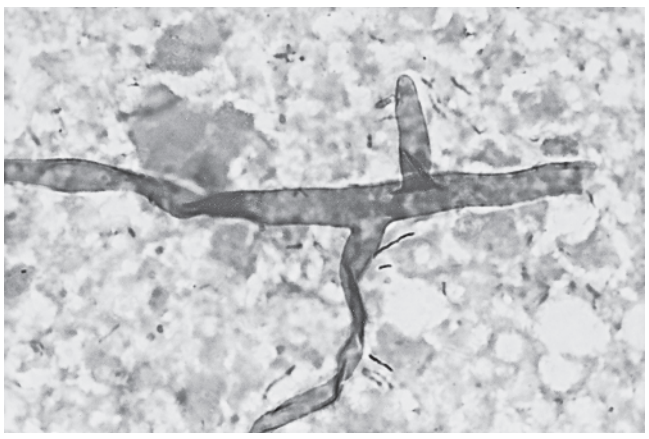


Figure 1.8 Broad aseptate hypha of *Rhizopus oryzae* in the histopathology section of a nasal mucosal biopsy from a case of rhinocerebral mucormycosis (GMS stain, 400×).
Source: Used with permission from Dr. Uma M. Tendolkar, LTM Medical College, Mumbai.

in the environment. The mold form fragments into *arthroconidia*, which are the infectious propagules. Once inhaled, arthroconidia convert to *spherules*, enlarge, and segment into endospores. *Melanized* molds (e.g., *Fonsecaea pedrosoi, Cladophialophora carrionii*), the causative agents of chromoblastomycosis, grow as molds in the environment but in the cutaneous and subcutaneous tissues convert to *muriform* cells—round cells that do not bud but enlarge and divide by internal septation. Growth by enlargement in all directions is called *isotropic*.

1.8.1 Dimorphism and Pathogenesis

How does dimorphism function in the pathogenesis of mycoses? As an adaptation to the host environment, dimorphism improves a fungus's ability as a pathogen; for example, *Histoplasma capsulatum* yeast forms survive after phagocytosis within alveolar macrophages and travel from the lungs via the bloodstream into the spleen and liver. Spherules produced by during infection by *Coccidioides* species produce many endospores, which spread the infection within the lung and to other body sites.

Although true of the primary systemic fungal pathogens, not all fungi that produce disease in humans are dimorphic. The opportunistic fungi may or may not be dimorphic. Monomorphic yeasts do not exhibit dimorphism and are seen as yeast in culture and in host tissues, for example, *Candida glabrata* and *Cryptococcus neoformans*. However, special studies can demonstrate dimorphism during the basidiomycetous sexual cycle of *C. neoformans* when that yeast produces a filamentous form with clamp connections. Many opportunistic pathogens are monomorphic molds, for example,

Aspergillus species or members of the *Mucorales*. They exhibit only the mold form in diseased tissue.

1.9 SEX IN FUNGI

Do fungi have sex and why is that important? Fungi, in addition to producing asexual spores or conidia, can undergo meiosis. Genetic coupling of nonidentical DNA occurs during meiosis, resulting in progeny with a new combination of the genes that were present in the parental haploid genomes. Diversity is produced by recombination of homologous chromosomes and crossing-over of chromosomal segments. This process results in a new and unique set of chromosomes, which, seen on a large scale, increases the level of genetic diversity in the entire population. With only mitosis, there would be no sharing of genetic information between compatible mating types; only division would be possible. The structures specialized to accomplish meiosis are the foundation used to classify fungi into Orders, Families, Genera, and Species. (Please see Section 1.11, Taxonomy/Classification: Kingdom Fungi.)

1.9.1 Anamorph and Teleomorph Nomenclature

The asexual state of fungi is termed the *anamorphic* state, while the sexual state is termed the *teleomorphic* state: for example, *Histoplasma capsulatum* (anamorph) and *Ajellomyces capsulatus* (teleomorph). Although the fungi are in a separate and unique kingdom, rules of nomenclature (naming genus and species) still follow the "International Code of Botanical Nomenclature." When the sexual state (teleomorph) of a fungus is identified, the genus and species of the teleomorph form takes priority and should be used thereafter. The asexual or anamorph name is subsidiary to the sexual state. In both medicine and clinical laboratory practice, however, the anamorph names persist so as to avoid confusion in understanding the actual causal fungus.

1.10 CLASSIFICATION OF MYCOSES BASED ON THE PRIMARY SITE OF PATHOLOGY

The thrust of medical mycology is to understand fungi as the causative agents of disease in humans and lower animals. This is the major difference between medical and general mycology. This section introduces the major fungal pathogens according to the organ system affected by fungal disease activity. In Section 1.11, Taxonomy/Classification: Kingdom Fungi, we will consider classification based strictly on cladistic analysis, method of sexual reproduction, and phenotypic characters. The following brief listing of the categories of fungal diseases is an opportunity to introduce the etiologic agents, which are covered in depth in the individual chapters.

1.10.1 Superficial Mycoses

Pityriasis versicolor is a mild infection of the nonliving keratinized outer layer of the epidermis caused by lipophilic yeasts, *Malassezia* species, and is mostly a cosmetic issue. More serious bloodstream infections caused by *Malassezia* species do occur, most often in neonates.

1.10.2 Cutaneous Mycoses

Dermatophytes or "ringworm" fungi cause disease of the skin, hair, and nails. These fungi are restricted to grow only on nonliving keratinized tissues. Dermatophytosis agents are, in order of prevalence, *Trichophyton tonsurans* > *T. rubrum* > *T. interdigitale* > *Microsporum canis*. Skin lesions may also be the cutaneous manifestations of deep-seated systemic mycoses: that is, the skin is a frequent site of dissemination for *Blastomyces dermatitidis*.

1.10.3 Systemic Opportunistic Mycoses

Systemic opportunistic mycoses cover a wide range of etiologic agents and clinical forms caused by molds and yeasts including environmental fungi and *endogenous commensal* fungi of the human microbiota (Table 1.2). Persons with normal immune and endocrine functions have normal levels of natural immunity sufficient to prevent these diseases. Factors affecting susceptibility to these fungi are presented in Section 1.15, Opportunistic Fungal Pathogens.

1.10.4 Subcutaneous Mycoses

These will be discussed in Part 5, Mycoses of Implantation. Usually initiated by a puncture with a thorn or splinter, this broad category of mycoses causes subcutaneous disease, in which *melanized* molds and their yeast-like relatives play an important role (Table 1.2).

1.10.5 Endemic Mycoses Caused by Dimorphic Environmental Molds

Endemic mycoses have a restricted geographic distribution as shown in Table 1.3. Most are primary pulmonary pathogens affecting immune-normal as well as immunocompromised persons. Exceptions are the dimorphic pathogens, *Penicillium marneffei* and *Sporothrix schenckii*. *Penicillium marneffei* is an

Table 1.2 Systemic Opportunistic Mycoses and Subcutaneous Mycoses

Category	Mycosis	Examples of etiologic agent(s)
Systemic mycoses caused by opportunistic yeasts	Candidiasis	*Candida albicans*, other *Candida* species
	Cryptococcosis	*Cryptococcus neoformans, Cr. gattii*
Systemic mycoses caused by opportunistic molds.	Aspergillosis	*Aspergillus fumigatus*, other *Aspergillus* species
	Mucormycosis, Entomophthoramycosis	*Mucorales, Entomophthorales*
	Fusarium mycosis	*Fusarium* species
	Pseudallescheria/Scedosporium Mycosis	*Pseudallescheria boydii, Scedosporium apiospermum*
Systemic mycoses caused by other opportunistic fungi	Pneumocystosis	*Pneumocystis jirovecii*
Subcutaneous mycoses of implantation.	Chromoblastomycosis	Dimorphic melanized molds (e.g., *Fonsecaea pedrosoi*)
	Phaeohyphomycosis	Melanized yeasts and their mold relatives (e.g., *Exophiala jeanselmei*)
	Sporotrichosis	*Sporothrix schenckii*
	Eumycetoma	*Pseudallescheria boydii, Madurella mycetomatis*

Table 1.3 Endemic Mycoses Caused by Dimorphic Environmental Molds

Endemic mycosis	Etiologic agent	Major endemic area
Histoplasmosis	*Histoplasma capsulatum*	States bordering major river valleys of central United States
Blastomycosis	*Blastomyces dermatitidis*	Overlaps with *Histoplasma* endemic area
Coccidioidomycosis	*Coccidioides immitis, C. posadasii*	Two major foci: Central Valley of California and Arizona endemic area
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*	Brazil, Colombia
Penicilliosis	*Penicillium marneffei*	Southeast Asia
Sporotrichosis	*Sporothrix schenckii*	Worldwide with areas of high endemicity

opportunistic endemic mycosis, causing pulmonary and disseminated disease in immunocompromised persons, especially in AIDS patients living in or traveling to Southeast Asia. Sporotrichosis is distributed worldwide but has regions of high endemicity. It is most often a subcutaneous mycosis caused by a penetrating injury with a thorn or splinter, but can spread by direct extension to joints and other organs.

1.11 TAXONOMY/ CLASSIFICATION: KINGDOM FUNGI

How are fungi organized in a taxonomic scheme? (See Fig. 1.9.) The taxonomic classification of fungi is based on sexual reproduction (meiosis). The mode of sexual reproduction is an important taxonomic criterion, if it can be demonstrated. In addition to the mode of reproduction, other characteristics useful in classification are morphology—including the structure of crosswalls or septa—life cycle, and physiology. If no sexual reproductive cycle has been observed, the fungi are referred to as *mitosporic* and are further classified by *cladistic* analysis. The ultimate determinant of relationships is a comparison of genetic sequences.

What is the value of knowing the classification? Clinical microbiologists may wonder about the value of studying the classification schemes of fungi. Understanding the "fungal tree of life" gives a holistic view of the subject

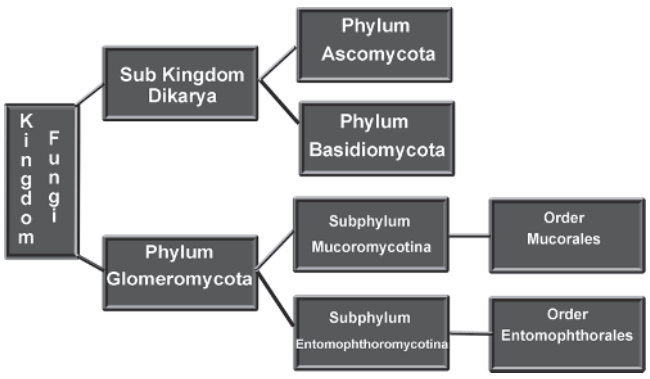


Figure 1.9 A higher level classification of the kingdom Fungi: phyla and subphyla containing pathogenic fungi (Hibbett et al., 2007).

that informs various important aspects, such as how individual species will respond to antifungal agents, the extent of their invasive potential, and their ecologic niche, which affects the mode of transmission.

1.11.1 The Phylogenetic Species Concept for Classification

Definition: A group of individuals with a shared genealogic relationship determined by phylogenetic analysis. Phylogenetic analysis may depend on phenotype but more reliably depends on genetic sequences. Another term for phylogenetic analysis is cladistics. Modern fungal taxonomic classification depends on *cladistic analysis*, which is the method of classifying organisms based on their phylogenetic relationships and evolutionary history. This method hypothesizes relationships among organisms determined through the construction of evolutionary trees. Organisms are classified exclusively on the basis of joint descent from a single ancestral species. The order of descent is represented in a branching diagram (a dendrogram or cladogram). Based on the phylogenetic classification, cladistic analysis produces a nested hierarchy where an organism is assigned a series of names to specifically locate it within the tree. A *monophyletic* group or clade is comprised of a single common ancestor and all the descendants of that ancestor. Another way to express monophyletic classification is that all groups within a phylum are descendants of one ancestor. The major gene targets for cladistic analysis are the DNA sequences in the ribosomal RNA genes (referred to here as rDNA) and also in selected somatic genes (e.g., translation-elongation factor 1α). *Multilocus sequence* analysis also plays a part in cladistic analysis.

The phylogenetic species concept stands in contrast to the older Linnaean system of classification, which depended on assigning an individual to a kingdom, phylum, class, order, family, genus, and species based on phenotype without taking into account the genotype. In that way birds and reptiles were placed in separate lineages, whereas we know from cladistics that birds are descended from reptiles.

Three assumptions of cladistic analysis are: (i) changes in characteristics in organisms occur in lineages over time; (ii) any group of organisms can be related by descent from a common ancestor; and (iii) there is a branching structure to lineage splitting. In this chapter we will rely on the phylogenetic species complex to make associations among fungal pathogens, especially when no sexual stage is known. The construction of phylogenetic trees is a subject in itself (Hall, 2007). A useful resource is TreeBASE at the URL www.treebase.org. Its main function is to store published phylogenetic trees and data matrices. The "how to" method for genetic identification of an unknown clinical isolate is discussed in Chapter 2, Section 2.4, Genetic Identification of Fungi.

Fungi are classified in the clinical microbiology laboratory by genus and species, and generally by the asexual state. The sexual state is rarely formed by cultures in the clinical laboratory. The identification is based on microscopic morphology and other phenotypic characters (e.g., enzymatic activities, presence of a capsule, temperature tolerance). Increasingly, the technology to conduct genetic identification is being used to supplement morphologic and other phenotypic characters.

1.11.2 The Higher Level Classification of Kingdom Fungi

The higher level classification of kingdom Fungi was revised by Hibbett et al. (2007). These revisions take into account *cladistic analysis*. The largest category of fungi pathogenic for humans is the subkingdom *Dikarya*, consisting of two phyla: *Ascomycota* and *Basidiomycota*. (The familiar phylum *Zygomycota* is not considered a valid taxon because it is not *monophyletic*.) Fungal pathogens previously classed in the *Zygomycota* are now found in the phylum *Glomeromycota*, subphylum *Mucoromycotina* and subphylum *Entomophthoromycotina*. In summary, the phyla and subphyla are constructed based on the the result of cladistic analysis and the mode of sexual reproduction: the *Mucoromycotina, Ascomycota*, and *Basidiomycota* (Fig. 1.9). The *Entomophthoromycotina* formerly classed with the *Zygomycota* are now considered separately. Other changes in taxonomy of fungi may be found in Boekhout et al. (2009). The mycotic disease agents in *Fundamental Medical Mycology* are aligned with these changes.

1.11.2.1 Mucoromycotina

The *Mucoromycotina* is considered the more primitive of these phyla and subphyla. Its members are identified by the production of sparsely septate or coenocytic hyphae, and sporangia with sporangiospores. The only septa in the *Mucoromycotina* isolate reproductive structures and wall-off vacuolated regions of the mycelium. Under special conditions a zygospore, the thick-walled sexual spore characteristic of the *Mucoromycotina*, is formed by the fusion of two gametangia (Fig. 1.10a, b). The *Mucoromycotina* are prolific producers of asexual spores formed inside the sporangia. Sporangiospores develop differently from the asexual spores termed conidia, of the *Ascomycota* and *Basidiomycota*. Sprangiospores form by internal cleavage of the sporangial cytoplasm. When mature, the sporangial wall deliquesces (dissolves) with resultant dispersal of the spores in air currents or water. Two orders of the subphyla *Mucoromycotina* and *Entomophthoromycotina*, which harbor species pathogenic for humans, are the *Mucorales* and the *Entomophthorales*.

The subkingdom *Dikarya* is so-named because of the feature held in common by the phyla *Ascomycota* and *Basidiomycota*: their hyphae contain pairs of genetically dissimilar unfused nuclei (dikaryons), which coexist and divide within hyphae before nuclear fusion (karyogamy) occurs. The *Ascomycota* and *Basidiomycota* are structurally similar, and it is believed that the *Ascomycota* probably gave rise to the *Basidiomycota*. The dikaryotic state in the *Basidiomycota* may be long-lasting. Furthermore, there is homology between structures that synchronize mitosis of the dikaryotic nuclei (*Ascomycota* croziers and *Basidiomycota clamp connections*). In the *Ascomycota*, the dikaryotic phase is limited to mycelium within the fruiting body (*ascoma*), but in the *Basidiomycota* growth in the dikaryotic stage lasts for some time before sexual reproduction occurs.

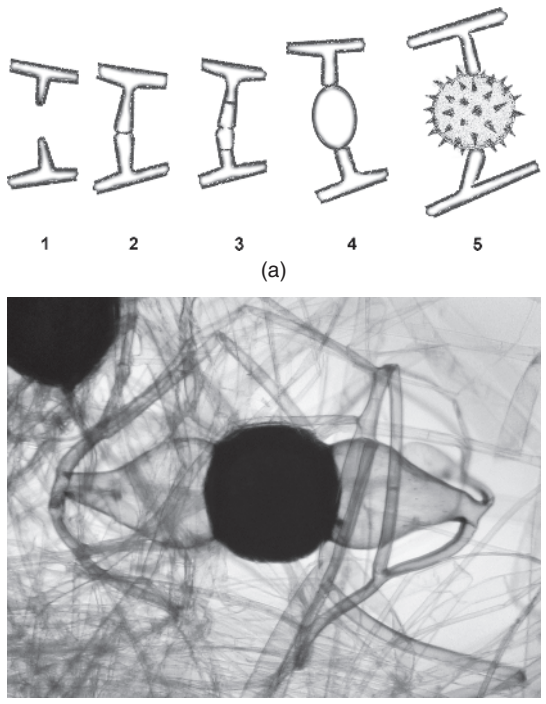


Figure 1.10 (a) Form development of zygospore production: 1, growth and attraction of hyphal branches of two compatible mating types; 2, progametangia—the branches touch and their tips swell; 3, gametangia—a septum forms between the gametangia and their vegetative hyphae; 4, fusion of gametangia occurs with formation of the zygospore; and 5, the mature ornamented zygospore. Genetic events that accompany sexual reproduction are displayed in Fig. 17A.2. (b) Zygospore with suspensor cells of *Syzgyites megalocarpus*.
Source: Used with permission from Dr. Gerald L. Benny, University of Florida, Gainesville. URL: www.zygomycetes.org.

1.11.2.2 Ascomycota

The *Ascomycota*, or sac fungi, are members of a *monophyletic* group that accounts for approximately 75% of all described fungi, including yeasts and molds. The *Ascomycota* reproduce sexually after plasmogamy, a *brief* dikaryotic stage, followed by karyogamy and meiosis within a sac or ascus (Fig. 1.11). One round of mitosis typically follows meiosis to leave 8 nuclei, packaged into 8 ascospores. The asci are formed within fruiting bodies, usually a cleistothecium or perithecium. A *cleistothecium* is a completely enclosed structure formed from specialized hyphae (Fig. 1.12a.). When mature, the cleistothecium ruptures, releasing the asci. A *perithecium* is similar but contains a pore or *osteole* from which the asci are extruded upon maturity (Fig. 1.12b) When ascospores are released, they germinate and develop as a haploid mycelium. Ascomycetous yeasts do not produce fruiting bodies; instead, "naked" asci are formed containing 4 ascospores. After germination, budding yeast forms develop. In that case identification depends on the texture, shape, and color of the ascospores (e.g., hat, walnut, spiny surface, etc.).

The *Ascomycota* also reproduce asexually by means of conidia produced by molds and blastoconidia or budding in yeast. The various genera and species are identified in the clinical laboratory by the method of conidiogenesis, or by genetic identification if they are slow-growing or fail to sporulate. Current updated classification of the phylum *Ascomycota* are published in electronic form (Lumbsch and Huhndorf, 2007) and may be viewed at the Myco-Net, URL http://www.fieldmuseum.org/myconet/outline.asp#Asco.

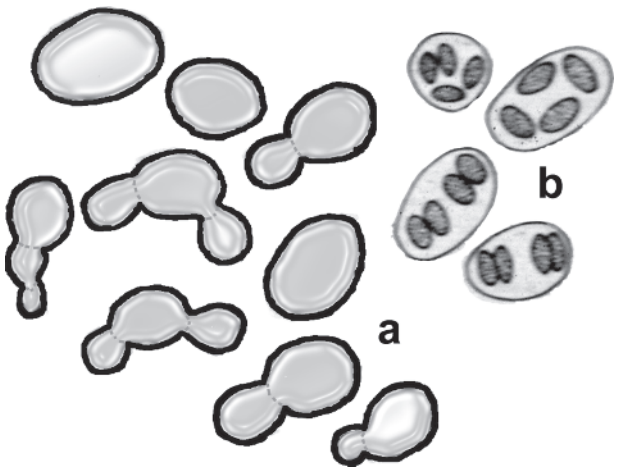
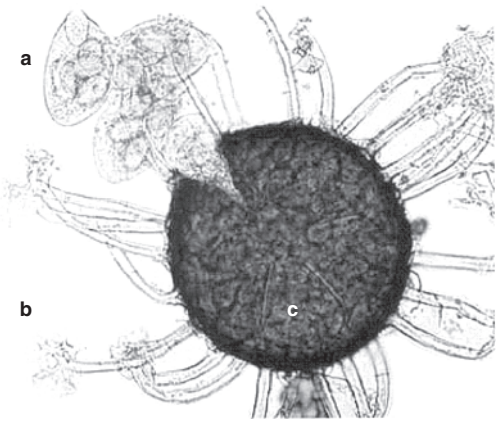


Figure 1.11 Asci with ascospores, yeast in the order *Saccharomycetales*.
Source: Adapted from Wilson and Plunkett (1965).

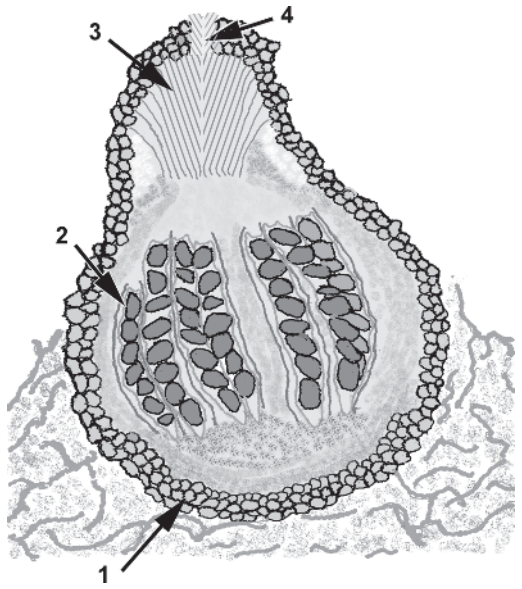
Subphyla of the *Ascomycota* containing fungi pathogenic for humans and animals are:

1. *Taphrinamycotina.* A basal lineage of *Ascomycota* consists of the class *Pneumocystidomycetes* containing the lung-dwelling opportunist fungus, *Pneumocystis jirovecii*.
2. *Saccharomycotina.* The class *Saccharomycetes* or "true yeasts" are composed of one large order, the *Saccharomycetales*. Pathogenic yeasts are found within three clades (Diezmann et al., 2004):
   - *Clade 1. Candida albicans, C. dubliniensis, C. tropicalis, C. viswanathii, C. parapsilosis, C. orthopsilosis, C. metapsilosis*.
   - *Clade 2. Candida guilliermondii*, and the teleomorph *Pichia guilliermondii, Clavispora* (*Candida*) *lusitaniae, Candida zeylanoides, Pichia* (*Candida*) *norvegensis*.
   - *Clade 3. Candida glabrata* and *Issatchenkia orientalis* (anamorph: *Candida krusei*). *Saccharomyces cerevisiae* is also in Clade 3.
3. *Pezizomycotina.* This subphylum contains over 90% of *Ascomycota*, as mold species, with sporocarp[2]-producing and mitosporic species. A feature characteristic of hyphal *Ascomycota* is the septal pore, which allows nuclei to migrate from cell to cell. The pore is at times blocked by a vesicle called the *Woronin body*, comprised of HEX-1 protein, which functions to respond to cell damage. Important orders in this subphylum are:
   - *Onygenales.* The *Onygenales* is a monophyletic lineage within the *Ascomycota* containing five families. Some members of this order are able to degrade keratin, the principal protein of the outer layer of the epidermis, hair, and nails. Keratinolytic activity defines the *Arthrodermataceae* and *Onygenaceae*, whereas the *Gymnoascaceae* and *Myxotrichaceae*, are nonkeratinolytic and cellulolytic. A fifth family, the *Ajellomycetaceae*, contains medically important genera.

[2]Sporocarp. *Definition*: A multicellular structure in which spores form; a fruiting body (e.g., perithecium, cleistothecium).



(a)



(b)

Figure 1.12 (a) Cleistothecium of *Erisiphe* (*Microsphaera*) species, an agent of powdery mildew. The cleistothecium is ruptured showing transparent asci containing oval ascospores: *a*, asci; *b*, appendages; *c*, cleistothecium. *Source:* Used with permission from Dr. Robert L. Wick, Plant, Soil and Insect Sciences Department, University of Massachusetts, Amherst. (b) Perithecium in longitudinal section, *Sordaria* species: *1*, the peridium is the perithecium wall; *2*, asci and paraphyseal hyphae are the fertile layer of the ascoma—the hymenium; *3*, periphyseal hyphae provide a channel for escape of the ascospores through *4*, the pore or ostiole. *Source:* E. Reiss.

- *Teleomorphs.* Members of the *Onygenales* produce ascospores in *gymnothecia* or *cleistothecia*. The ascomata contain round asci with unicelled, hyaline ascospores. The characteristic mode of conidiogenesis of their anamorphs is *thallic*, producing *aleurioconidia* or arthroconidia.

  The major lineages within the *Onygenales* are:

  - The *Ajellomycetaceae* consists of the *Ajellomyces-Paracoccidioides* clade (Untereiner et al., 2004). Important pathogens include *Histoplasma capsulatum* (teleomorph: *Ajellomyces capsulatus*) and *Blastomyces dermatitidis* (teleomorph: *Ajellomyces dermatitidis*). Here too is the anamorph species *Paracoccidioides brasiliensis* and the rodent and other small mammal pathogen *Emmonsia parva* and *E. crescens* (teleomorph: *Ajellomyces crescens*). Species of *Ajellomyces* form round cleistothecia with coiled appendages and small, finely ornamented ascospores. Anamorphs are the primary means of identification in the clinical setting. Conidia are smooth to slightly echinulate or are solitary, tuberculate *aleurioconidia*. Temperature-sensitive mold-to-yeast dimorphism is characteristic of this family, except for *Emmonsia* species, the tissue form of which are large, round, and nonbudding *adiaspores*. None of the members of this clade possess keratinolytic activity.
  - *Coccidioides*-containing clade. The remaining medically important anamorph genus, *Coccidioides*, remains in the *Onygenales* but is not closely related to the *Ajellomycetaceae*. This well-supported group also contains the look-alike arthroconidia-producing *Auxarthron* (teleomorph) and its anamorph, *Malbranchea aurantiaca*.
  - The *Arthrodermataceae* contains the keratin-degrading dermatophyte genera: *Trichophyton, Microsporum*, and their teleomorph *Arthroderma* species. Also present is the third and less common genus with a single species: *Epidermophyton floccosum*. The *Arthrodermataceae* also contains anamorphs assigned to *Chrysosporium*.

- *Hypocreales. Fusarium* species in this order are important plant pathogens and an emerging cause of deep-seated mycosis in immunocompromised hosts. The teleomorphs in the order are characterized by perithecia, which are brightly colored, yellow, orange, or red. Species pathogenic for humans are classed in the genus *Gibberella*.

  The characteristic mode of conidiogenesis is via phialides. Multilocus sequence typing found that Clade 3 of the *Fusarium solani* species complex (FSSC) contains at least 18 species isolated from mycoses of humans and animals (O'Donnell et al., 2008).

  Members of this complex are responsible for approximately two-thirds of *Fusarium* mycoses worldwide. The second most frequent causative agents of *Fusarium* mycosis are members of the *F. oxysporum* complex, where phylogenetic analyses has shown that a recently dispersed, geographically widespread clonal lineage is responsible for over 70% of all clinical isolates (O'Donnell et al., 2004).
- *Eurotiales.* This order is notable for the classification of *Aspergillus* species and their teleomorphs. The characteristic conidiogenous structure of the genus *Aspergillus* is the aspergillum, a stalk terminating in a bulbous vesicle on which a row of phialides are erected, either directly or upon another layer of basal cells or *metulae*. The conidia are produced within the phialides and released in an enteroblastic mode. (Please see Chapters 2 and 13.) Teleomorphs in the order form cleistothecia with *bitunicate*,[3] spherical asci and ornamented, unicellular ascospores (Table 1.4).

  The significant pathogen, *Penicillium marneffei*, like other penicillia, have rDNA sequences that cluster with the *Talaromyces* species. *Penicillium* species are now considered to be the asexual forms of *Talaromyces* within the *Eurotiales*.
- *Microascales.* Important pathogens are in this order, characterized by the cleistothecia-forming teleomorph *Pseudallescheria*, a rare example of a sexual stage that can be observed in the clinical laboratory, partly because the genus is homothallic. Anamorphs in the order include the genus *Scedosporium*, notably *S. apiospermum* and *S. prolificans*.

The following are three orders of melanized fungi:

- *Chaetothyriales.* The order contains the black yeasts and their melanized filamentous relatives and is related to the *Eurotiales* and the *Onygenales*. Melanized molds in this order, which are pathogenic for humans, are in a monophyletic clade, the family *Herpotrichiellaceae*. The teleomorph associated with this clade is *Capronia*. Genera of medical interest include *Exophiala* (yeast+filaments), *Cladophialophora* (filaments with or without yeast forms), *Fonsecaea* (filaments), *Phialophora* (filaments), *Ramichloridium* (filaments with or without yeast forms), and *Rhinocladiella* (filaments and yeast forms).

[3]Bitunicate. *Definition*: Ascus is enclosed in a double wall.

Table 1.4 Sexual Stages Associated with the Genus *Aspergillus*

Genus	Anamorphs of medical importance	Properties of the ascomata, cleistothecia
Neosartorya	*A. fumigatus, N. fischeri, A. lentulus*	Cleistothecial wall composed of flattened hyphae
Emericella	*E. nidulans*	Dark cleistothecia with walls composed of flattened cells; red ascospores
	A. ustus, A. calidoustus, A. versicolor	Cleistothecium surrounded by a stromatic layer of hülle cells
Fennellia	*A. terreus*	Cleistothecial wall composed of thick-walled cells
Petromyces	*A. flavus, A. niger (?)*	Multiple cleistothecia enclosed in a usually dark sclerotial stroma

Source: Adapted from Geiser (2008).

The following two orders are classed in the *Dothideomycetes*:

- *Dothidiales.* Black yeasts of medical importance in this order are *Aureobasidium* (yeast and filaments are produced) and *Hortaea* (yeast and filaments).
- *Pleosporales.* This order of melanized fungi is characterized by hairy, black, rapidly growing colonies. The anamorphs produce poroconidia: large multicelled conidia produced through a minute pore in the wall of the conidiophore or conidiogenous cell. The poroconidia are visible in the dissecting microscope. The pathogenic species are in a single family, the *Pleosporaceae*. Two groups are recognized, one group consisting of *Bipolaris, Curvularia*, and *Exserohilum* species have large-sized conidia, which can lodge in the nasal sinuses causing fungal sinusitis. Their teleomorphs are in the genera *Cochiobolus* and *Setosphaeria*. A second group contains *Alternaria* and *Ulocladium* species whose teleomorph genus is *Lewia*.

  Conidiogenesis in the *Pleosporales* is further characterized by the presence in some species of apical beaks on their poroconidia. Sometimes a new conidium is formed on top of the beak (e.g., *Alternaria*). The multicelled conidia are septate if the crosswall in the septum is continuous with the outer wall, or pseudoseptate if the septations are in the inner wall only, surrounded by an outer wall layer that forms a sac around the entire structure.

Further Reading About the *Ascomycota* Please consult "Myconet" an electronic and print journal specializing in fungal classification at the URL http://www.fieldmuseum.org/myconet/printed.asp. Monographs of fungal genomics are published in the journal *Fungal Biology*.

1.11.2.3 Basidiomycota

These fungi reproduce sexually by means of *basidiospores* produced on the outside of the spore-mother cell called a *basidium* (Fig. 1.13). A small number of species also produce conidia.

Characteristics of the *Basidiomycota*

Dolipore Septum (Fig. 1.14) The septum (crosswall) separates individual cells. Adjacent to the central pore in the septum each end flares out to produce the dolipore swelling. A septal plug is positioned on each side of the central pore, capable of blocking organelles from passing from one cell to the next. The septum is surrounded on each side by a dome shaped structure, which is perforated. This is the parenthesome, seen as crescent-shaped in cross section (Fig. 1.14). The dolipore septum of *Filobasidiella* species (including the perfect state of *Cryptococcus neoformans*) lacks the parenthesome. That structure is replaced by a simpler layer of porous striated material (Rhodes et al., 1981).

Clamp Connections in the Hyphae *Basidiomycota* occur as a binucleate cell type, containing two dissimilar but sexually compatible haploid nuclei that divide synchronously: for example, nuclei *a* and *b* both divide synchronously to give *a, a* and *b, b*. Nucleus *b* moves into the clamp and is sealed there by a new septum. Next, nuclear pair *a* and *b* migrate to the hyphal tip. The remaining nucleus *a* remains behind and a new septum is laid down separating it from the apical tip cell. Nucleus *b* in the clamp then joins nucleus *a* by dissolving the hyphal wall at that point. This process of synchronous binucleate cell division may continue for a long time. Eventually, karyogamy and meiosis occur to form a basidium and basidiospores.

Mushrooms are found here. The diverse human pathogens classed as *Basidiomycota* are illustrated:

- *Schizophyllum commune* is a bracket fungus classed in the order *Agaricales* with other gilled mushrooms.

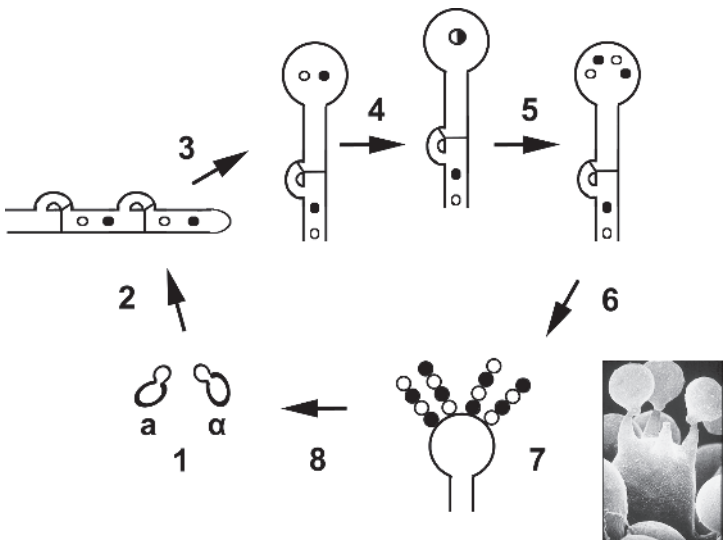


Figure 1.13 Sexual reproduction in a basidiomycete yeast: *Filobasidiella neoformans. Inset*: Scanning EM of a basidium with basidiospores. 1, Compatible mating type haploid yeast cells **a** and **α** secrete peptide pheromones that stimulate cell fusion (plasmogamy). 2, The resulting dikaryotic cell develops as a filamentous phase maintaining the dikaryon: the two parental nuclei migrate coordinately in the hyphae, divide, and septa separate the cells via clamp connections. 3, Later, the tip of the hypha enlarges into a round basidium. 4, Nuclear fusion (karyogamy) occurs in the basidium. 5, Meiosis occurs producing four haploid nuclei. 6, The haploid nuclei divide (mitosis) as they are packaged into basidiospores. 7, Basidiospores bud from the cell surface forming chains of basidiospores (these are infectious propagules). 8, Basidiospores disperse in air currents, germinate, and produce haploid yeast cells.

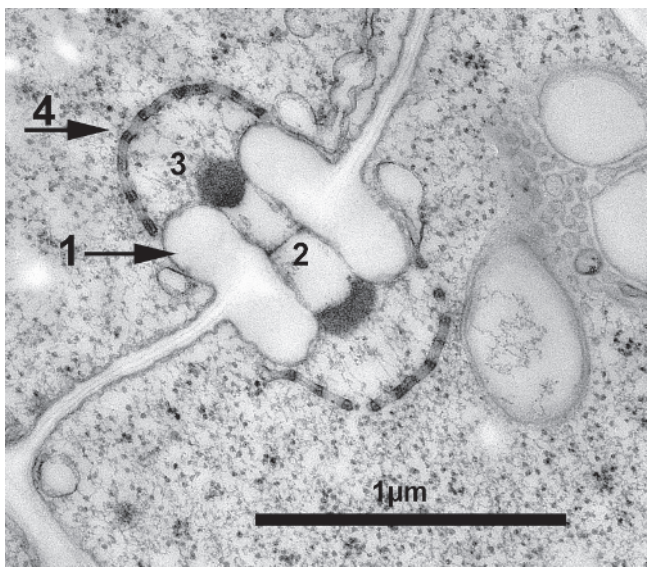


Figure 1.14 Dolipore septum of *Psilocybe cubensis*, present in all basidiomycetes. Transmission EM shows the following key features: 1, dolipore swelling; 2, septal pore; 3, pore plug; 4, dome-shaped perforated parenthesome.
Source: Used with permission from Dr. Robert Simmons, Director, Biological Imaging Core Facility, Department of Biology, Georgia State University, Atlanta, GA.

Schizophyllum commune, as a human pathogen, is found in chronic lung disease, meningitis, sinusitis, and onychomycosis (Sigler et al., 1999). *Schizophyllum commune* is identified in the clinical laboratory by the presence of the fruiting bodies, or by the occurrence of clamp connections in the hyphae, or, if neither is present, by genetic identification of the ITS region of rDNA. (Please see Chapter 2, Section 2.4, Genetic Identification of Fungi.)

- Order *Tremellales*. This order, notable for the jelly fungi found on rotting wood, contains important pathogenic yeasts and yeast-like fungi.
  - *Cryptococcus neoformans* and *C. gattii* are the etiologic agents of cryptococcal meningoencephalitis, an AIDS-associated opportunistic infection, also occurring in other immunosuppressed patients and, infrequently, in persons with no known underlying deficit. The haploid budding yeast has two mating types: α and a. In response to mating pheromones, the two mating partners produce conjugation tubes, and plasmogamy occurs, resulting in a heterokaryon filamentous form with clamp connections. Ultimately, meiosis occurs in a basidium. Spores are then produced on the surface of the basidium.
  - *Trichosporon* Species. Of 23 total species, the medically important species include *T. asahii*, recovered from superficial and deep-seated infections, *T. cutaneum* from superficial infections, and *T. inkin* from white piedra of the genital area (Diaz and Fell, 2004). The morphology of *T. asahii* consists of hyphae that disarticulate into cubic shaped arthroconidia. *Trichosporon inkin* also produces hyphae and arthroconidia, cylindric in shape. Budding is absent in these two species.
- Order *Malasseziales*. At least 14 species are known in the genus *Malassezia*, which are yeasts and members of the microbiota of human skin. *Malasseziales* are taxonomically related to the corn smut fungus, *Ustilago maydis*. No sexual stage for members of this order is known. These yeasts produce a distinct scar by repetitive bud formation from a single location on the mother cell, a feature most useful in their identification in direct examination of skin scrapings. Some

species are etiologic agents of pityriasis versicolor (please see **Chapter 21**) and others are implicated in seborrheic dermatitis. All of the species within the genus, with the exception of *Malassezia pachydermatis*, are lipid dependent due to an inability to synthesize C_{14} or C_{16} fatty acids (reviewed by Ashbee, 2007). Some species produce a mycelium as well as budding yeast cells.

Older texts refer to a form-phylum, the *Deuteromycota* (syn: *Fungi imperfecti*), composed of *mitosporic* fungi that have no known sexual reproductive cycle. This nomenclature allowed two broad categories: *Blastomycetes* containing the yeast *anamorphs*. Molds were described in a second category, *Hyphomycetes*, and were further classed in the form family *Dematiaceae*, with melanin pigment in their cell walls, or *Hyalohyphomycetes*, which lack melanin pigment. This nomenclature has become obsolete because cladistic analysis using multigene sequence comparisons can determine phylogenetic relationships even when the teleomorph has not been observed.

1.12 GENERAL COMPOSITION OF THE FUNGAL CELL

How is the fungal cell organized and how does it differ from the bacterial life form? (Table 1.4). (See Griffin, 1994 and Howard and Gow, 2007.)

Cells that do not have a nucleus are called prokaryotes (e.g., bacteria). Cells that have a nucleus are called *eukaryotes*. *Fungi* are simple eukaryotes whereas plants and animals are higher eukaryotes. Fungi are not plants—they lack chlorophyll and cannot undergo photosynthesis. Although most fungi are multicelled, some are single celled, the *yeasts*. Fungal cells possess a nucleus containing chromosomes and contain a full array of intracellular organelles that allow the cell to function (Fig. 1.15). The yeast cell cycle and hyphal morphogenesis introduce the topic of the organization of the fungal cell.

1.12.1 Yeast Cell Cycle

The cell cycle traces cell growth and division. A visual three-dimensional (3D) representation of the yeast cell cycle is found at the URL http://www.nformationdesign.com/portfolio/portfolio09.php. Each cell division requires the duplication of all essential components of the cell. The most important component is the DNA, organized in chromosomes. DNA must be accurately replicated into two copies and segregated to the mother and daughter cells. The processes of DNA replication and sister chromatid separation occur in distinct timed phases of the cell

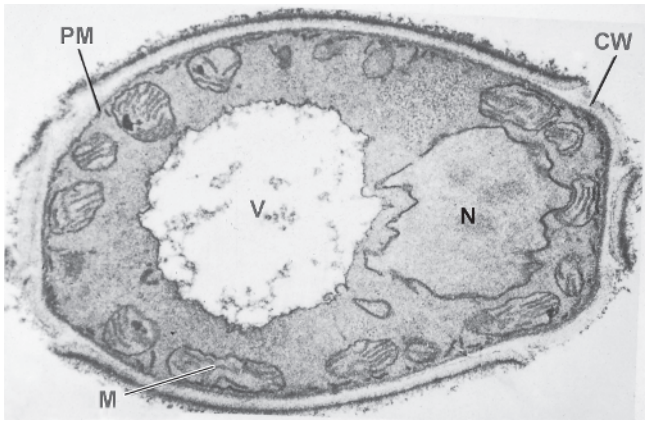


Figure 1.15 Ultrastructural features of the yeast cell visualized in a transmission EM depicting a cross section of yeast cell. CW, cell wall; PM, plasma membrane; N, nucleus; V, vacuole; M, mitochondria.

cycle. In phase G1 the yeast cell grows until it reaches a critical mass before DNA synthesis. Then, at START, the cell is committed to replicate its DNA and progresses into the S phase (DNA synthesis). The yeast bud is clearly visible. Following S phase, cells enter gap-2 (G2). The nucleus and mitochondria are oriented by microtubules. Mitochondria and vacuole inheritance into the daughter bud occurs. Next, M phase (metaphase) marks the stage of mitosis. Chromosomes align on the equator of the microtubule spindle, halfway between its two poles. The nuclear membrane remains intact.

Once the genome replicates, the spindle aligns parallel to the mother-bud axis and elongates. Anaphase is the stage of mitosis when two copies of each chromosome move to opposite poles of the spindle. The spindle elongates and provides the mother and daughter cell each with one nucleus. The nucleus moves through the bud neck, assuming an hourglass shape. Telophase is the last stage of mitosis when a complete set of chromosomes aligns at two poles of the mitotic spindle and the nuclei separate into mother and daughter cells Cytokinesis[4] occurs and the mother and daughter cells separate. Please consult Berman (2006) for the topic of cell cycle regulators including cyclins, cyclin-dependent kinases, and CDC proteins.

1.12.2 Hyphal Morphogenesis

Fungal hyphae originate from a germinating conidium or another hypha during branch formation (Fig. 1.16). An axis of polarity is set and cell surface expansion occurs along the axis to the hyphal tip and linear extension from it. Successive hyphal branching results in a tree-like

[4]Cytokinesis. *Definition*: Following the telophase of mitosis in cell division, the cytoplasm is divided between mother and daughter cells.

Figure 1.16 Organelles of the hyphal tip: *1*, septum with septal pore; 2, Woronin body; 3, nucleus with nucleolus; 4, rough endoplasmic reticulum; 5, mitochondrion; 6, cell membrane; 7, cell wall in three layers—inner layer (chitin), middle layer (glucan), and outer layer (mannan); 8, tubular vacuole; 9, Golgi; 10, *spitzenkörper*.
Source: E. Reiss.

mycelial network. Nutrients acquired at the colony periphery are distributed in the mycelium. As it grows older parts of the mycelium are recycled to support new growth. In the subkingdom *Dikarya* (including phyla *Ascomycota* and *Basidiomycota*) adjacent branches may fuse (anastomose), forming an interconnected network.

The establishment and maintenance of hyphal polarity require microtubule- and microfilament-based motor proteins, chitin deposition, and both cyclic AMP and mitogen-activated protein kinase signaling (Harris et al., 2005). *Spitzenkörper* are present in growing vegetative hyphal tips and branch points. They are located within the hyphal tip in the direction of hyphal growth. *Spitzenkörper* are complex, multicomponent structures that function to support directional growth by concentrating and delivering secretory vesicles to the hyphal tip. Some of these vesicles are chitosomes, containing chitin synthase. That enzyme is activated when the chitosome fuses with the plasmalemma, initiating new chitin synthesis and polymerization of chitin microfibrils.

The cytoskeleton is very important in hyphal morphogenesis. Microtubules are responsible for the transport of secretory vesicles to the *spitzenkörper*, while actin microfilaments control vesicle organization within the *spitzenkörper* and transport from there to the plasmalemma. The *spitzenkörper* may be viewed as a supply and distribution center for vesicles involved in apical growth.

Pseudohyphae are a feature of *Candida* species. Pseudohyphal cells bud in a unipolar manner, and the cells remain attached after cytokinesis. Pseudohyphae are described in detail in Section 1.6.5.1.

1.12.3 Cell Wall

All fungal cells are bounded by a rigid, laminated, cell wall that imparts protection and firmness to the internal structures. The outer layer consists of readily soluble mannan (or galactomannan) and inner layers of glucan fibrils. The cell walls of molds have an inner microcrystalline sleeve of chitin [poly ß-(1→4)-*N*-acetylglucosamine]. Chitin in yeast cells is also concentrated as disks in birth scars and bud scars. Some fungi have melanin in their cell walls.

1.12.3.1 Molecular Architecture of the Yeast (Candida) Cell Wall

Overview The outer surface layer of the *Candida* cell wall (Ruiz-Herrera et al., 2006) consists of mannoprotein, which is the major surface antigen, "mannan."[5] The fibrillar polysaccharides of the cell wall are ß-(1→6)-D-glucan (superficial layer), ß-(1→3)-D-glucan (deep layer), and the innermost layer is a microcrystalline sleeve of chitin: poly-ß-(1→4)-*N*-acetylglucosamine. Proteins are embedded in the cell wall either noncovalently or bound to fibrillar cell wall polysaccharides: to ß-(1→6)-D-glucan, to chitin via short chains of ß-(1→6)-D-glucan, or directly to chitin. The types of linkages between proteins and the glucans and chitin are:

1. Phosphodiester linked *g*lycosyl *p*hosphatidyl*i*nositol proteins ("GPI" binding motif).
2. *N*-glycosidic linkages between chitobiose and asparagine of the protein moiety (alkali stable). *This is the major linkage between the "inner core" mannan to protein* (please see "Mannan" for further explanation.
3. Reducing agent-extractable (RAE) proteins linked by disulfide bonds, which, in turn, are linked to ß-(1→3)-D-glucan by *O*-glycosidic bonds to threonine or serine. Several proteins can be extracted from the cell wall with sulfhydryl reagents such as 2-mercaptoethanol. This can occur because they are covalently linked to the cell wall via disulfide bridges or because they are released when the cell wall structure is loosened by reducing disulfide bridges. Among them are phospholipomannan and Pir proteins (please see below for definition and functions).

Mannan Mannoproteins comprise 38–40% of the cell wall mass. The largest mannoprotein constituent is mannan, the antigenic coat of *C. albicans*. Antigenic variations in mannan occur among the *Candida* species (Suzuki,

[5]Mannan is also referred to as "peptidophosphomannan," to distinguish it from glycoprotein enzymes that may also contain mannose-oligosaccharides.

1997). Mannan is of interest because it is the major surface antigen of *Candida* species, and tests to detect it in serum during candidemia and deep-seated candidiasis have been developed. Mannan is comprised of an inner core linked to protein and an antigenic outer chain region. The inner core consists of a linear α-(1→6)-mannan backbone substituted with 12–17 mannose residues and di-*N*-acetylglucosamine (chitobiose) units, which are linked via *N*-glycosidic bonds to asparagine of the protein moiety. Please see Fig. 11.11 for an illustration of mannan structure.

The outer chain region is disposed along the linear α-(1→6)-mannan backbone in two domains. An α-linked domain consists of oligomannosides up to 6 or 7 units linked α-(1→2) and α-(1→3). These are the antigenic epitopes. A separate domain in the outer chain is composed of ß-(1→2)-linked oligomannosides with a degree of polymerization of between 2 and 6. The ß-mannan is appended from the backbone via acid-labile phosphodiester bonds, the *O*-phosphonomannan. Both α- and ß-linked mannose oligosaccharide epitopes contribute to the antigenic mosaic of the *Candida* cell wall. Tests to detect mannan should incorporate antibodies to both the α- and ß-oligomannose epitopes.

The question of the relative importance of α- and ß-mannan domains to pathogenicity has been approached (Hobson et al., 2004). The *MNN4* gene is required for mannosylphosphate transfer, so that the Δ*Mnn4* mutant lacks the ß-mannan domain. The null mutant was unaffected in its growth, form development, mouse virulence, adherence to and uptake by macrophages. Other studies, however, implicated ß-(1→2)-oligomannosides in murine gastrointestinal colonization (reviewed by Masuoka, 2004). During infection mannan is sloughed from the cell wall and circulates in the plasma. It is rapidly cleared from the circulation so that its detection by sandwich EIA is difficult.

PLM PLM is a unique glycolipid shed from the cell when *C. albicans* comes in contact with host cells (Poulain et al., 2002). PLM secretion has been linked to survival of *C. albicans* in macrophages and in promoting macrophage apoptosis. PLM is a member of the mannose-inositol- phosphoceramide family.

PLM Structure: PLM is composed of a long linear chain of ß-(1→2)-mannose (average degree of polymerization of 14) linked via a ß-(1→2) glycosidic bond to the polyol, phosphatidyl inositol. Phosphatidyl inositol is then linked via phosphodiester bonds to ceramide. (*Definition*: Ceramide—a lipid composed of sphingosine linked to a fatty acid via an amide bond. Sphingosine is an 18-carbon amino alcohol with an unsaturated hydrocarbon chain.)

1.12.3.2 Considerations for the Fibrillar Polysaccharides of the Candida Cell Wall

Chitin The yeast form of *C. albicans* cell wall contains 2% chitin, whereas that of the mycelial form is higher, 6%. Twenty-four hundred chains of the poly-ß-(1→4)-*N*-acetylglucosamine polymer are united in antiparallel bundles to form highly insoluble crystalline chitin microfibrils. This microcrystalline sleeve is the base layer to which ß-(1→3)- and ß-(1→6)-D-glucan fibrils are linked. This chitin–glucan complex forms the scaffold of the cell wall onto which mannoproteins and ß-(1→3)-D-glucan are disposed. In addition to forming a microcrystalline sleeve in both yeast and mycelia forms, chitin is concentrated in the bud and birth scars and septa. At first chitin was thought to be a good target for antifungal therapy because it is absent in mammals, but up to now chitin synthase inhibitors, polyoxins and nikkomycins, have showed low in vivo activity.

Four genes, *CHS1, CHS2, CHS3*, and *CHS8*, encode chitin synthase isoenzymes with different biochemical properties and physiological functions (Lenardon et al., 2007). Specific chitin synthases produce chitin microfibrils of different lengths and act cooperatively to generate the pattern of chitin microfibrils in the cell wall. Electron microscopy in *chs8* mutants and *chs3* mutants showed that Chs8 synthesizes long-chitin microfibrils in the septum formed at cytokinesis. Chs3 synthesizes short-chitin rodlets in the septum and is responsible for the synthesis of the majority of chitin in the cell wall of yeast and hyphal cells. Chs1 is essential for growth and viability. Chs2 is the major chitin synthase activity measured in cell membrane preparations.

ß-(1→3)-D-Glucan, ß-(1→6)-D-Glucan The ß-(1→3)-D-glucan strands are helical and can form a tertiary structure of three strands united in a triple helix stabilized by interchain hydrogen bonds between C-2 hydroxyl groups. The glucan fraction comprises 58–60% of the cell wall dry weight. The relative proportions of ß-(1→3) and ß-(1→6) glucans in the cell wall alkali-insoluble fraction are 30–39% and 43–53%, respectively. Although there can be links between the two types of glucan, there are no polysaccharides in which the two glycosidic bond arrangements occur in the same strand.

The enzymes involved in ß-(1→3)-D-glucan synthesis are important because they are the target for the echinocandin class of antifungal agents (e.g., caspofungin, micafungin, and anidulafungin). The biosynthetic glucosyl transferases are encoded by the *FKS* genes. Mutations in *FKS1* result in echinocandin resistance, indicating it is the most important glucan synthase in *C. albicans*. Glucans are an important example of a "pathogen-associated molecular pattern" (PAMP)

functioning in the innate immune response by interacting with receptors such as dectin-1 on macrophages.

1.12.3.3 Cell Wall Associated Proteins

GPI Proteins The glycosyl–phosphatidylinositol linkage is the most prevalent means by which proteins are linked to ß-1→6-glucan (90%) or chitin (10%) in the cell wall. Function: Examples of GPI proteins are the adhesins, Hwp1, and the Als family in *C. albicans*. Other functions of GPI proteins are involved as enzymes in cell wall biosynthesis.

Pir Proteins These are a smaller group than GPI proteins and are so-named because they are *p*roteins with *i*nternal *r*epeats. They can be solubilized from the cell wall with dilute alkali. They contain a domain with 2–11 repetitive amino acid sequences and 4 cysteine residues arranged in the motif: (repeat(s)-Cys-6aa-Cys-16aa-Cys-12aa-Cys-COOH). They are attached to ß-(1→3)-D-glucan by alkali-labile bonds. Function: Pir proteins are believed to contribute to structural strength of the cell wall by crosslinking ß-(1→3)-D-glucan strands.

1.12.3.4 Cell Surface Carbohydrates Implicated in Pathogenicity

"Fuzzy Coat" Layer A fuzzy coat external to the cell wall and localized at the hyphal tips of *C. albicans* appears to be enriched in mannoprotein, possibly with *O*-phosphono-linked ß-(1→2)-mannan (Ruiz-Herrera et al., 2006). A role for this coat layer in adherence to and invasion of host tissue is speculated.

Surface Microfibrils and Fimbriae Various investigators have observed microfibrils or fimbriae on the surface of *C. albicans* blastoconidia and hyphae, also indicating they are composed of mannoproteins, and have proposed that these structures are involved in adherence to mammalian cells. The structures are delicate but are visible in freeze-fracture electron microscopy (Hazen and Hazen, 1993).

Extracellular Matrix Material (EMM) The EMM surrounds *Candida* species embedded in *biofilms* adherent to *biomaterials* and contains a high carbohydrate content, but the relative contribution of mannan and glucan to the EMM is not certain (Al-Fattani and Douglas, 2006). *Candida* embedded in biofilms are less susceptible to antifungal agents. Rats with central intravenous (IV) catheters were injected through the catheter with *C. albicans* and developed biofilms (Nett et al., 2007a). ß-(1→3)-D-glucan concentrations in plasma of rats with biofilm-embedded catheters were ten fold greater than rats infected IV without catheters. ß-(1→3)-D-glucan is a major component of *Candida* biofilms and is implicated as the constituent that binds azole antifungal agents, so that they are unable to enter the *Candida* embedded in biofilms (Nett et al., 2007b). Phospholipomannan, an antigenic wall component, secreted through the cell wall may blend with other constituents of the EMM (Poulain et al., 2002).

Hydrophobicity Hydrophobic *C. albicans* cells are more adherent than hydrophilic cells to host tissues and are also more resistant than hydrophilic cells to phagocytic killing. *Candida albicans* can regulate cell surface hydrophobicity by altering cell surface microfibrils composed of mannoproteins with ß-(1→2)-mannose linkages. *Candida albicans* can change the conformation of the microfibrils: on the surface of hydrophobic cells microfibrils are shorter while those of hydrophilic cells are longer and radiating (Masuoka and Hazen, 2004).

1.12.3.5 *Plasma Membrane* (Also Known as the Plasmalemma)

Just inside the cell wall lies the plasma membrane, a phospholipid bilayer. Importantly, ergosterol is a key membrane component. The plasma membrane controls cell semipermeability, resorption, excretion, and secretion, and is capable of enzymatic breakdown of substrates. Ergosterol is the target for amphotericin B and the azole class of antifungal drugs (please see Chapter 3).

1.12.3.6 Cytoplasm

The protoplasm of the fungal cell is organized as a cytoskeleton consisting of microfilaments (actin) and microtubules (tubulin) (Sudbery and Court, 2007).

Cytoskeleton in Yeast Actin cables, actin cortical patches, and a contractile acto-myosin ring occur at the site of septum formation. This contractile ring consists of the septins, a series of five proteins, which function to separate mother and daughter cells. This function is known as cytokinesis. Microtubules, the tubular network in fungi, are contiguous with the yeast vacuole.

Cytoskeleton in Molds Cortical patches of actin are clustered at the sites of hyphal tips in the molds. Long microtubules are oriented along the axis of hyphal growth and are contiguous with tubular lysosomes. Actin cables are oriented toward these sites. A tubular vacuole network is a normal function of the growing zone of the mycelium used for long distance transport of vesicles to the *spitzenkörper*.[6] Actin cables mediate short distance dispersal of vesicles to the cell surface.

[6] *Spitzenkörper*. Definition: Vesicles present at the hyphal tip involved in cell wall biosynthesis.

1.12.3.7 Genome

Genetic material (DNA) within the fungal cell is in the nucleus. It contains chromosomes and is bounded by the nuclear membrane; for example, *Candida albicans* has 8 chromosomes and a genome size of 16 Mb (haploid genome) (*Candida albicans* Physical Map website). The nucleolus is also found within the nucleus and is the locale where ribosomal RNA is transcribed and ribosomal subunits are assembled.

1.12.3.8 Golgi

The Golgi apparatus are a series of concentrically arranged double membranes that receive and often modify molecules made in the endoplasmic reticulum before exporting them to the exterior of the cell or to another location. It is the central conduit of the secretory pathway to deliver secretory products and membrane proteins to the cell surface or for transit into vacuoles.

1.12.3.9 Endoplasmic Reticulum (ER)

The ER is a system of tubules and vesicles within the cytoplasm where most membrane components and proteins are synthesized on ribosomes (polyribosomes). The ER is also the site of Ca^{2+} storage for release into the cytosol when induced by signaling cascades.

1.12.3.10 Mitochondria

Mitochondria are membrane-bound organelles, about the size of a bacterium, within the cytoplasm of eukaryotic cells. Mitochondria are where respiration and glucose catabolism occurs. They power cells by utilizing oxygen and glucose to generate energy (in the form of adenosine triphosphate, (ATP)) by a series of stepwise enzymatic reactions. Oxidative phosphorylation is the process for generating energy (ATP) via the electron transport sequence. The Krebs cycle (citric acid cycle) is the means by which glucose is converted to energy. Fatty acid oxidation also occurs in mitochondria. Mitochondria are self-replicating because they have their own DNA.

1.12.3.11 Nucleus

A compact sphere, the nucleus is the most prominent organelle and the control center of the eukaryotic cell. The nucleus is bounded by the nuclear envelope composed of two parallel membranes separated by a narrow space and perforated with pores. The nucleus contains chromosomes. The nucleolus is a dark area for rRNA synthesis and ribosome assembly (please see also Section 1.12.3.7, Genome).

1.12.3.12 Ribosomes

These cellular organelles are composed of ribosomal RNA and ribosomal proteins. They associate with messenger RNA and tRNA in order to translate the message into protein synthesis; 40S and 60S ribosomal subunits form the 80S ribosome.

1.12.3.13 Vacuoles

The largest organelles of a yeast cell also occur in the cytoplasm of molds. The vacuole is the key organelle involved in intracellular trafficking of proteins. It is part of the cell's intramembranous system analogous to lysosomes of mammalian cells. Vacuoles contain hydrolytic enzymes involved in digesting proteins: endopeptidases, aminopeptidases, and carboxypeptidases. Here are stored basic amino acids, polyphosphates, and certain metal ions. The vacuole regulates cytoplasmic ion concentrations and intracellular osmotic pressure. Vacuoles in yeast are inherited from the mother cell to the daughter cell as a single stream of vesicles that fuse into the vacuole.

Other smaller membrane-bound vesicles are present in the cytoplasm of fungi and perform a variety of tasks related to the transport of materials within the cell, for example, *spitzenkörper* located at the hyphal tips and involved in cell wall biosynthesis.

1.12.3.14 Intracellular Trafficking of Proteins

The ER is the site of protein synthesis and modification. After synthesis on polyribosomes on the surface of the ER membrane, precursor proteins are translocated into the lumen of the ER, where trimming, chaperone-assisted folding, and glycosylation occur. Proteins then are directed by vesicles to the Golgi apparatus where carbohydrate side chains may be added in a process of mannosylation. Proteins processed in the Golgi are transported via secretory vesicles to the vacuole, the plasma membrane, and the periplasmic space.

1.13 PRIMARY PATHOGENS

What are primary fungal pathogens? Where are they found? Primary dimorphic fungal pathogens are those found in specific geographic areas of the world, their endemic areas, and have the capacity to cause infection in any individual (i.e., immune-normal or compromised). They are *Coccidioides* species, *Blastomyces dermatitidis, Histoplasma capsulatum*, and *Paracoccidioides brasiliensis*. Infection is initiated after the infectious *propagules* (conidia) are inhaled when they are aerosolized by a

disturbance of the environment. Subcutaneous mycoses are also caused by primary pathogens (Table 1.1). In that case breaching the normally intact anatomic barrier of the skin by a puncture wound may be sufficient to initiate the pathogenic process.

1.13.1 Susceptibility to Primary Pathogens

Who is susceptible to primary pathogens? Immune normal persons are at risk to become exposed to primary pathogens and to develop disease along a spectrum from subclinical to moderate self-limited, to disseminated disease, the latter requiring timely therapeutic intervention. Whether exposure is benign, self-limited, or moderate to severe, and whether or not it will *disseminate* depends on a number of factors, among which are age, sex, race, physical health, immunologic status, and the number of infectious propagules inhaled. Diseases caused by these fungi are, with rare exceptions, not communicable. When an immunocompromised individual is exposed to a primary pathogen the clinical course may be severe.

Up to now, there are no vaccines available for these diseases, although a vaccine for coccidioidomycosis is under investigation (URL: www.valleyfever.com). Primary systemic fungi differ in whether recovery from an infection results in durable immunity. This will be discussed with each fungus and disease.

1.14 ENDEMIC *VERSUS* WORLDWIDE PRESENCE

Primary systemic fungal pathogens are found in geographically restricted (endemic) areas, whereas fungi producing opportunistic fungal disease are generally found worldwide. An exception is the fungal opportunist *Penicillium marneffei*, a dimorphic endemic pathogen restricted to Southeast Asia.

1.15 OPPORTUNISTIC FUNGAL PATHOGENS

What are opportunistic pathogens? Where are they found?

Opportunistic fungal pathogens (Table 1.5) may be common environmental molds (and some yeasts) whose cells and conidia circulate in the *aerospora* (e.g., *Aspergillus* species, *Cryptococcus* species). Otherwise, they may be endogenous commensal fungi such as *Candida albicans*, a yeast that has adapted to an ecologic niche on the oral, intestinal, and vaginal mucosae of warm-blooded animals and especially humans where it lives an inconspicuous existence, all the while probing the mucosal epithelium for signs of decreased immune surveillance or lack of anatomic integrity. Given the opportunity, any fungus with the ability to grow at 37°C may become an opportunistic pathogen. Physicians and laboratory personnel alike must be aware of this when diagnosis is difficult or unusual.

1.15.1 Susceptibility to Opportunistic Fungal Pathogens: Host Factors

1.15.1.1 Immunocompromised Status

Who is susceptible to opportunistic fungi? Persons may become susceptible to opportunistic fungal pathogens because of immunodeficiency disease (either inborn or acquired, e.g., HIV infection); deliberate immunosuppressive therapy to treat cancer, *collagen vascular disease*, or for maintenance of stem cell or solid organ transplants.

Table 1.5 Some Differences Between Systemic Mycoses Caused by Primary Dimorphic Environmental Molds and Opportunistic Fungal Pathogens

	Type of fungal pathogen	
	Primary systemic dimorphic	Opportunistic
Host resistance	Immunocompetent or immunocompromised	Immunocompetent but debilitated, or immunocompromised
Portal of entry	Lungs or subcutaneous penetrating injury	Lungs, gastrointestinal tract, intravascular catheter
Prognosis	±95% cases resolve spontaneously	Depends on severity of impairment of host defenses, timing of diagnosis
Morphology	Dimorphic, yeast forms or spherules in tissue	Either filamentous or yeast-like; no change between cultural and tissue forms generally seen
Distribution	Geographically restricted (endemic mycoses)	Some endogenous *microbiota*; others ubiquitous in nature; worldwide; one endemic mycosis: penicilliosis

1.15.1.2 Immune-Normal Host

Some of the host factors that allow immune-normal persons to become susceptible to systemic mycoses due to opportunistic pathogens are:

- Age (low birthweight–premature infants; the elderly)
- Burns
- Chronic respiratory disease
- Debilitating illness
- Dialysis, whether hemodialysis or peritoneal
- Endocrine disorders (e.g., diabetes mellitus)
- Intensive care requiring parenteral nutrition, high *APACHE II score*
- Surgery (e.g., cardiothoracic or abdominal)
- Traumatic injury

In many of the above situations the normal anatomic barriers are disrupted, enabling entry of the opportunist fungus, or, as in the case of diabetes mellitus, there is *functional neutropenia*.

1.16 DETERMINANTS OF PATHOGENICITY

Why are fungi pathogenic for humans? As eukaryotes, fungi use various stratagems to evade host defenses. The list below is a summary of microbial factors that have been shown to influence pathogenicity. Further information specific to each pathogen is discussed in the disease chapters under the section heading "Determinants of Pathogenicity."

- Thermotolerance. Fungi that can grow at 37°C are potential pathogens in a suitably susceptible host.
- Adaptation to a parasitic lifestyle, sometimes in an intracellular environment. The traditional assumption is that most primary and opportunistic fungal pathogens are free-living saprobes in nature.
  - Evidence is accumulating that the ecologic niche of *Cryptococcus neoformans* may include intracellular survival within soil amebae. In that case *C. neoformans* "learned how to become a pathogen." This theory may also apply to other environmental fungi and may help explain how some fungi have become adapted to intracellular survival within phagocytes (Steenbergen et al., 2001).
  - Environmental fungi can infect other mammalian hosts, including small rodents, and have adapted to a parasitic lifestyle.
- Adhesins. Pathogenesis of microbial disease proceeds via adherence to host tissues, a process of receptor–ligand interaction: for example, BAD-1 adhesin of *Blastomyces dermatitidis*, the Als family of surface adhesins of *Candida albicans*.
- Attack on host tissues using invasion promoting enzymes:
  - Secreted enzymes that damage host tissues: for example, aspartyl proteinases and phospholipases of *C. albicans*.
  - Production of catalase that decomposes hydrogen peroxide, thus interrupting the oxidative microbicidal pathway of polymorphonuclear neutrophilic granulocytes: for example, catalase of *Aspergillus fumigatus* and *Histoplasma capsulatum*.
- Dimorphism. Morphogenesis to distinct tissue forms confers an advantage to the pathogen. For example, *H. capsulatum* yeast forms are translocated intracellularly within monocytes from the lung to the spleen and liver; spherules and endospores of *Coccidioides* species spread the infection; yeast forms of *B. dermatitidis* are too large for endophagocytosis.
- Evasion of host immune defenses. For example, *Histoplasma capsulatum* survival in the phagosome is linked to preventing phago-lysosome fusion and by being a resourceful scavenger of iron from the host through secretion of siderophores, ferric reductase, and directly from host transferrin.
- Cell wall molecules are barriers that resist lysis by phagocytes and antifungal agents: for example, cell wall polymers, including α-(1→3)-D-glucan, melanin, and the glucuronoxylomannan polysaccharide capsule of *Cryptococcus neoformans*. To this list we add ß-(1→3)-D-glucan of *Candida* species, in shielding the yeast from antifungal agents by functioning as a major component of extracellular matrix material of biofilms embedded on intravascular catheters.

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Proteopathogen, a proteomics site. http://proteopathogen.dacya.ucm.es

TreeBASE: Its main function is to store published phylogenetic trees and data matrices. www.treebase.org

Valley Fever Vaccine Project. A vaccine for coccidioidomycosis is under investigation. www.valleyfever.com.

QUESTIONS

The Answer Key to multiple choice questions may be found at the end of the book.

1. Which of the following fungal diseases are communicable?
   A. Aspergillosis
   B. Dermatophytosis
   C. Histoplasmosis
   D. Sporotrichosis
   E. None of the above

2. Which of the following is not true of fungi?
   A. Eukaryotic
   B. Heterotrophic
   C. Mitochondria present
   D. Photosynthetic

3. Any fungus capable of growing at 37°C
   A. Could convert to the yeast form in tissue
   B. Has the capacity to cause disease in an immunocompromised or debilitated person
   C. Is a necessary but not sufficient property to cause disease in humans
   D. Is pathogenic for immune-normal persons

4. This soil dwelling mold produces conidia, which when inhaled germinate and grow in the lung as a budding yeast. The fungus is said to be
   A. Allergenic
   B. Dimorphic
   C. Opportunistic
   D. Thermophilic

5. The higher level classification of fungi pathogenic for humans is:
   A. *Ascomycota, Basidiomycota, Zygomycota, Pneumocystidales*
   B. Coelomycetes, Fungi imperfecti, Hyphomycetes, *Saccharomycetales*
   C. Dematiaceae, Hyalohyphomycetes, Dimorphics, Dermatophytes
   D. Subkingdom *Dikarya*, phylum *Ascomycota*, phylum *Basidiomycota*, subphylum *Mucoromycotina*, subphylum *Entomophthoromycotina*

6. Is vegetative growth sufficient to perpetuate fungi in the environment? What is meant by a fungal propagule? Besides direct extension, how do fungi spread in the environment?

7. Discuss the phylogenetic species concept. Compare it to the Linnean System. What role does cladistic analysis play in determining the relationship of species? Describe how a graphic method is used to illustrate a monophyletic group of related fungi.

8. To what taxonomic group (phylum, subphylum, order) do the *Candida* species belong? Describe the *Candida* species that assort into three clades.

9. Why is the *Onygenales* an important order for fungal pathogens? Indicate the major pathogens and how they associate within the *Onygenales*.

10. Compare the internal organization of the fungal cell with that of bacteria. How is the system of microtubules organized in the yeasts and in molds? What role does actin play in the cytoskeleton? What is the function of the *spitzenkörper*?