

Introduction to Mycology



Mycology is the study of fungi. The name “fungi” is derived from “mykos” meaning mushroom. The fungi are eukaryotic organisms and they differ from the bacteria, which are prokaryotic organisms, in many ways (Table -1). The fungi possess rigid cell walls, which possess two characteristic cell structures: chitin and ergosterol.

Chitin: The fungi consist primarily of chitin, unlike peptidoglycan present in cell wall of bacteria. Hence, fungi are not sensitive to action of penicillin and other antibiotics that inhibit peptidoglycan synthesis. Chitin is a polysaccharide consisting of long chains of N-acetylglucosamine. In addition to chitin, the fungal cell wall also contains mannan and other polysaccharides. Of these, beta-glucan is most important, because it is the target of antifungal drug caspofungin.

Ergosterol: The cell membrane of fungus contains ergosterol, unlike human cell membrane which contains cholesterol. The antifungal agents, such as amphotericin B, fluconazole, and ketoconazole have selective action on the fungi due to this basic difference in membrane sterols.

Classification of Fungi

The fungi can be classified as follows:

Taxonomical Classification

The fungi are placed in the phylum Thallophyta. There are four classes of fungi: Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes or Fungi Imperfecti.

TABLE -1 Comparison of fungi and bacteria

Feature	Fungi	Bacteria
Diameter	Approximately 4m	Approximately 1m
Morphology	Yeast and mold	Cocci, bacilli, spirochete, branching filamentous
Staining property	Gram-positive, nonacid fast, stained with PAS and GMS	Gram-positive, Gram- negative, acid fast
Cell wall content	Chitin	Peptidoglycan
Cell membrane	Sterols present	Sterols absent except mycoplasma
Cytoplasm	Mitochondria and endoplasmic reticulum present	Mitochondria and endoplasmic reticulum absent
Nucleus	Eukaryotic	Prokaryotic
Spores	Sexual and asexual spores for reproduction	Endospores for survival, not for reproduction
Thermal dimorphism	Yes (seen in some fungi)	No

PAS , periodic acid –Schiff ; Gomori's methenamine silver .

Morphological Classification

The fungi can be classified into the following four main groups based upon the morphology: (a) yeast, (b) yeast-like form, (c) molds, and (d) dimorphic fungi.

Yeast: Yeasts are round or oval unicellular fungi that reproduce by asexual budding. On culture medium, such as Sabouraud's dextrose agar (SDA), they produce creamy mucoid colonies. Example: *Cryptococcus neoformans*.

Yeast-like fungi: These are the yeasts with pseudohyphae. Example: *Candida albicans*.

Molds: Molds grow as long filaments called hyphae. They usually measure 2–10 μ m in width. Some hyphae form transverse walls and hence they are called septate hyphae, whereas others do not produce walls, hence are called nonseptate hyphae. Nonseptate hyphae are multinucleated. The hyphae on their continuous growth form a mat known as mycelium. The part of the mycelium that projects above the surface in culture medium is called aerial mycelium. Examples include *Aspergillus*, *Penicillium*, *Rhizopus*, etc.

Dimorphic fungi: Many of medically important fungi are dimorphic. They exist as hyphal/mycelial forms in the soil and in the cultures at 22–25°C. They occur as yeasts or other structures in human tissue and in the culture at 37°C (Fig.-1). Examples include *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Sporothrix schenckii*.

Reproduction of Fungi

Fungi can reproduce sexually by forming sexual spores and asexually by forming conidia or asexual spores. Sexual spores are of three types: zygospores, ascospores, and basidiospores (Fig. 71-2). Ascospores are formed in a sac called ascus, whereas basidiospores are formed outside on the tip of a pedestal called a basidium. Zygospores are single, large spores with thick wall.

The fungi that do not produce sexual spores are called imperfect and are classified as Fungi imperfecti. Asexual spores are produced by mitosis. Fungi reproduce asexually by forming conidia. The shape, color, and arrangement of the conidia are helpful for identification of the fungi. Asexual spores can be vegetative or aerial spores as follows:

A. Vegetative spores: These include (a) arthrospores, (b) chlamydospores, and (c) blasstospores.

■ **Arthrospores** are formed by fragmentations of the ends of hyphae, resulting in rectangular thick-walled spores. The arthrospores are the infective stage of *C. immitis*.

■ **Chlamydospores** arise by rounding and thickening of hyphal segments. They are round and thick walled. The terminal chlamydospores help in the identification of *C. albicans*.

■ **Blastospores** are formed by budding process from parent cells, such as yeast. Some yeasts, such as *C. albicans* can form multiple buds that do not detach from the parent yeast, thus producing elongated structures called pseudohyphae.

B. Aerial spores: These include (a) sporangiospores, (b) conidiospores, (c) microconidia, and (d) macroconidia.

■ Sporangiospores are spores formed within a sac called sporangium, which develops at the ends of the hyphae called sporangiophores (e.g., *Mucor* and *Rhizopus*).

■ Conidiospores, or otherwise called conidia, are spores found externally on the sides or tips of hyphae. Conidia can be macroconidia or microconidia.

■ Macroconidia are large, aseptate, often multicellular conidia.

■ Microconidia are small and single.

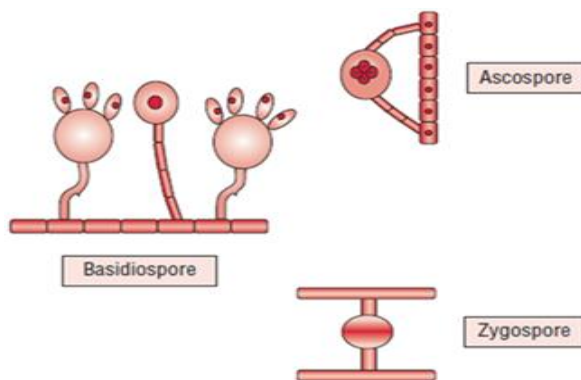
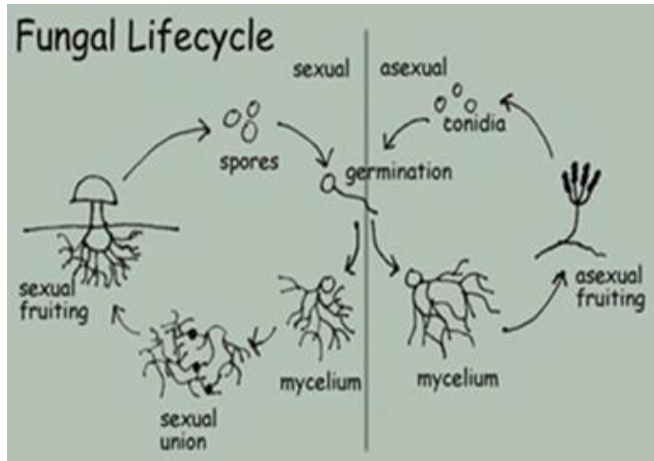


FIG. 71-2. Sexual spores.



Pathogenesis of Fungal Infection

Most fungi are obligate aerobes or facultative anaerobes, but none are obligate anaerobes. The natural habitat of most fungi is environment, because all these fungi require a preformed organic source of carbon, hence their constant association with decaying matter. *C. albicans* is exception and is an important fungus, which is a part of the normal human flora. Fungi are ubiquitous in nature, i.e., they occur as free-living saprobes, hence determining their role in human infection may sometimes be difficult. The effects of fungi on humans can be grouped in three major ways as follows: (a) colonization and disease, (b) hypersensitive diseases, and (c) diseases caused by mycotoxins or fungal toxins.

Colonization and Disease

Most fungal infections are mild and self-limited. Intact skin is an effective host defense against certain fungi. But if the skin is broken, organisms, the fungi enter through that broken skin and initiate the infection. Fatty acid content, pH, epithelial turnover, and normal bacterial flora of the skin contribute to host resistance against fungi. For example, the mucous membrane of the nasopharynx traps inhaled fungal spores.

Cell-mediated immunity is much important in conferring protection against fungi. Suppression of cell-mediated immunity can lead to reactivation and dissemination of asymptomatic fungal infection and to diseases caused by opportunistic fungi. The humoral immunity is mediated by production of IgG and IgM antibody. But their role in protection from fungal disease is uncertain.

Hypersensitivity Diseases

Humans are continually exposed to air-borne fungal spores and other fungal elements present in the environment. These spores can be antigenic stimulants and depending on individual's immunological status may induce a state of hypersensitivity by production of immunoglobulins or sensitized lymphocytes. Rhinitis, bronchial asthma, alveolitis, and various forms of atopy are the clinical manifestations of hypersensitive pneumonitis. The clinical manifestations of the hypersensitivity disease are seen only in sensitized person, after repeated exposure to the fungus, fungal metabolites, or other cross-reactive materials. Allergies to the fungal spores are manifested primarily by an asthmatic reaction including rapid bronchial constriction mediated by IgE , eosinophilia, and positive hypersensitivity skin test reaction. These are caused due to immediate hypersensitivity reactions of the host to fungal spores.

Diseases Caused by Fungal Toxins

Mycotoxicosis is caused by ingested fungal toxins. Mycotoxicosis caused by eating amanita mushroom is the best example of mycotoxicosis. This group of fungi produces five toxins. Of these, amanitine and phalloidin are the two most potent hepatotoxins. The toxicity of amanitine is due to its ability to inhibit cellular RNA polymerase, which prevents mRNA synthesis. Aflatoxin is another fungal toxin produced by *Aspergillus flavus* that causes disease in humans. Aflatoxin-B causes a mutation in the P53tumorsuppressor gene, resulting in a loss of P53 protein, thereby in a resultant loss of growth control in the hepatocytes. Hence, it causes damage to liver, and it induces tumor in liver in animals and is associated with hepatic carcinomas in humans. *Claviceps purpurea* is a mold that infects brain and produces alkaloids, such as ergotamine and lysergic acid diethylamide. These

compounds cause serious vascular and neurological effects. Yellow rice toxicosis in Japan and elementary toxic aleukia in the former Soviet Union are the examples of other mycotoxicoses.

Laboratory Diagnosis

Laboratory diagnosis of fungal infections depends on:

- (a) direct microscopy, (b) culture, (c) serological tests, (d) nonculture methods, and (e) molecular methods.

Direct Microscopy

Direct microscopic examination depends on demonstration of characteristic asexual spores, hyphae, or yeast in various clinical specimens by light microscopy. The commonly used clinical specimens are sputum, lung biopsy material, and skin scrapings. The specimen is either treated with 10% KOH or stained with special fungal stains. Use of 10% KOH dissolves tissue material, leaving the alkali-resistant fungi intact. The disadvantages of microscopy are that it shows low sensitivity and requires an experienced microscopist for specific identification.

Culture

Fungal culture is a frequently used method for confirming the diagnosis of fungal infection. SDA is the most commonly used medium for fungal culture. Other media include CHROM agar, blood agar, etc. The low pH of the medium and addition of chloramphenicol and cycloheximide to the medium inhibit the growth of bacteria in the specimen and thereby facilitate the appearance of slow-growing fungi. Fungal colony is identified by rapidity of growth, color, and morphology of the colony at the obverse and pigmentation at the reverse. Microscopy of the fungal colony is carried out in lactophenol cotton blue (LPCB) mount to study the morphology of hyphae, spores, and other structures. The appearance of the mycelium and the nature of the asexual spores are very much helpful to identify the fungus. Culture, however, is time-consuming in most cases and also the yield is not very good. Culture following lysis of the specimens, such as blood, obviates

this problem. Blood lysed by addition of certain substances, followed by centrifugation, increases yield of fungi by culture. Yield can be further increased with a shortening of time by combining with BACTEC systems.

Serological Tests

Demonstration of the antibodies in patient's serum or CSF is useful for diagnosis of fungal infections, especially in systemic fungal infections. A significant rise of antibody titer in a paired sera sample confirms the diagnosis. The complement fixation test was the earliest test used in fungal serology and is still used in the diagnosis of suspected cases of histoplasmosis, blastomycosis, or coccidiomycosis. Recently, newer tests like ELISA (enzyme-linked immunosorbent assay), Western blot, and radioimmunoassays are increasingly used for serodiagnosis of fungal infections. Nonculture Methods These methods include (a) detection of fungal antigen, (b) detection of fungal cell wall markers, and (c) detection of fungal metabolites.

Antigen detection: It is useful in immunocompromised hosts where antibody detection is not as sensitive. Detection of fungal antigen in serum, CSF, and urine is increasingly used for diagnosis of many fungal infections. Demonstration of antigen indicates recent or active infection. Latex agglutination test is a frequently used test to demonstrate polysaccharide capsular antigen of *C. neoformans* in CSF for diagnosis of *cryptococcal meningitis*. False-positive reactions due to *Trichosporon beigelli* and *Capnocytophaga canimorsus* are known.

Detection of fungal cell wall markers: Mannan is a highly immunogenic component of the candidal cell wall. Mannan antigen detection, therefore, is most widely used method in the diagnosis of candidiasis.

Galactomannan is a heat-stable heteropolysaccharide found in the cell walls of all *Aspergillus* species. Production of the galactomannan antigen is proportional to fungal load in tissue, hence is being used as the prognostic marker for diagnosis of invasive Aspergillosis. A sandwich ELISA using rat monoclonal antibody EB-A2

against galactomannan antigen is being currently used in Europe for diagnosis of invasive Aspergillosis.

Detection of fungal metabolites: Detection of distinctive fungal metabolites is another approach for the diagnosis of fungal infections. Gas liquid chromatography is being used to quantify arabinitol for diagnosis of *C. albicans* infections.

Molecular Diagnosis

DNA probes are the recent techniques, which are very useful to identify colonies growing in culture at an earlier stage of growth. These DNA probes are very useful for rapid diagnosis of these cultures in comparison to traditional methods of visual detection of colonies. DNA probes are now available for detection of *Cryptococcus*, *Histoplasma*, *Blastomyces*, and *Coccidioides*. Mitochondrial DNA has been used for the diagnosis of *C. albicans* and *Aspergillus* species.

Antifungal Drugs

A few drugs are available for therapy of systemic fungal infection, unlike a large number of antibiotics available to treat bacterial infections. The drugs used to treat bacterial disease have no effect on fungal diseases. Amphotericin B and various azoles are the most effective antifungal drugs. They act on the ergosterol of fungal cell membrane that is not found in bacterial or human cell membrane. Similarly, caspofungin inhibits synthesis of beta-glucan, which is found only in fungal membrane but not in bacterial or human cell membrane. Table -2 summarizes the common antifungal agents and their primary sites of activity.

Table -2 Antifungal agent Mechanism of action

Group of compounds	Antifungal agent	Mechanism of action
Polynes	Amphotericin B Nystatin	Bind to ergostero
Azole derivatives	Miconazole Ketoconazole Fluconazole Itraconazol	Inhibit cytochrome P-450 dependent enzymes
Nucleoside Analog	5-fluoro- cytosine	Inhibits DNA and RNA Synthesis
Grisans	Griseofulvin	Inhibits microtubular function
Allylamines	Naftifine Terbinafine	Squalene epoxidase inhibitors
Thiocarbamates	Tolnaftate Tolciclate	Squalene epoxidase inhibitors
Morpholines	Amorolfine	Inhibits ergosterol biosynthesis
Echinocandins	Caspofungin, Anidulafungin	-1, 3 glucan synthetase inhibitors