MYCOLOGY - CHAPTER ONE

INTRODUCTION TO MYCOLOGY

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INTRODUCTION

Classification

Fungi are eukaryotic organisms that do not contain chlorophyll, but have cell walls, filamentous structures, and produce spores. These organisms grow as saprophytes and decompose dead organic matter. There are between 100,000 to 200,000 species depending on how they are classified. About 300 species are presently known to be pathogenic for man.

There are five kingdoms of living things. The fungi are in the Kingdom Fungi.

KINGDOM	CHARACTERISTIC	EXAMPLE
Monera	Prokaryocyte	Bacteria Actinomycetes
Protista	Eukaryocyte	Protozoa
Fungi	Eukaryocyte *	Fungi
Plantae	Eukaryocyte	Plants, Moss
Animalia	Eukaryocyte *	Arthropods Mammals Man

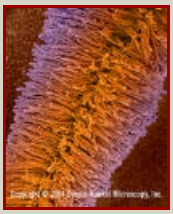
*This common characteristic is responsible for the therapeutic dilemma in anti-mycotic therapy.

The taxonomy of the Kingdom Fungi is evolving and is controversial. Formerly based on gross and light microscopic morphology, studies of ultra structure, biochemistry and molecular biology provide new evidence on which to base taxonomic positions. Medically important fungi are in four phyla:

- Ascomycota - Sexual reproduction in a sack called an ascus with the production of ascopspores (figure 1).


Figure 1. *Chaetomium globosum* spores. *Chaetomium* is an ascomycete, and in most species the spores are lemon-shaped, with a single germ pore


A


B
Figure 2. A. Bracket fungus basidiocarp (fruiting body). B. Lower surface showing generative hyphae. Reproductive spores are dispersed through pores in the surface of the brackets.

permission

- Basidiomycota - Sexual reproduction in a sack called a basidium with the production of basidiospores (figure 2).
- Zygomycota - sexual reproduction by gametes and asexual reproduction with the formation of zygospores (figure 3).
- Mitosporic Fungi (Fungi Imperfecti) - no recognizable form of sexual reproduction. Includes most pathogenic fungi.


Figure 3. *Mucor* spp. fruiting structure with spores. The fruiting structure (condiophore) has matured and its outer membrane is disintegrating allowing the spores (conidia) to be released. *Mucor* is a common fungus found in many environments. It is a Zygomycetes fungus which may be allergenic and is often found as saprobes in soils, dead plant material (such as hay), horse dung, and fruits. It is an opportunistic pathogen and may cause mucorosis in immuno-compromised individuals. The sites of infections are the lung, nasal sinus, brain, eye, and skin. Few species have been isolated from cases of zygomycosis, but the term mucormycosis has often been used. Zygomycosis includes mucocutaneous and rhinocerebral infections, as well as renal infections, gastritis, and pulmonary infections.


Figure 4. *Candida albicans* - yeast and hyphae stages. A yeast-like fungus commonly occuring on human skin, in the upper respiratory, alimentary & female genital tracts. This fungus has a dimorphic life cycle with yeast and hyphal stages. The yeast produces hyphae (strands) and pseudohyphae. The pseudohyphae can give rise to yeast cells by apical or lateral budding. Causes candidiasis which includes thrush (an infection of the mouth & vagina) and vulvo-vaginitis.

MORPHOLOGY

Pathogenic fungi can exist as yeasts or as hyphae (figure 4). A mass of hyphae is called mycelia. Yeasts are unicellular organisms and mycelia are multicellular filamentous structures, constituted by tubular cells with cell walls. The yeasts reproduce by budding. The mycelial forms branch and the pattern of branching is an aid to morphological identification. If the mycelia do not have septa, they are called coenocytic (non-septate). The terms "hypha" and "mycelium" are frequently used interchangeably. Some fungi occur in both the yeast and mycelial forms. These are called dimorphic fungi.

Dimorphic fungi

The dimorphic fungi have two forms (figure 5):

- YEAST - (parasitic or pathogenic form). This is the form usually seen in tissue, in exudates, or if cultured in an incubator at 37 degrees C.
- MYCELIUM - (saprophytic form). The form observed in nature or when cultured at 25 degrees C. Conversion to the yeast form appears to be essential for pathogenicity. Dimorphic fungi are identified by several morphological or biochemical characteristics, including the appearance of their fruiting bodies. The asexual spores may be large (macroconidia, chlamydospores) or small (microconidia, blastospores, arthroconidia).

MYCOTIC DISEASES

There are four types of mycotic diseases:

- Hypersensitivity - an allergic reaction to molds and spores
- Mycotoxicoses - poisoning of man and animals by food products contaminated by fungi which produce toxins from the grain substrate
- Mycetismus - the ingestion of toxin (mushroom poisoning)
- Infection - tissue invasion with a host response

We shall be concerned only with the last type: pathogenic fungi that cause infections. Most common pathogenic fungi do not produce toxins but they do cause physiologic modifications during a parasitic infection (e.g., increased metabolic rate, modified metabolic pathways and modified cell wall structure). The mechanisms that cause these modifications, as well as their significance as a pathogenic mechanism, are just being described.

Most pathogenic fungi are also thermotolerant, and can resist the effects of the active oxygen radicals released during the respiratory burst of phagocytes. Thus, fungi are able to

withstand many host defenses. Fungi are ubiquitous in nature and most people are exposed to them. The establishment of a mycotic infection usually depends on the size of the inoculum and on the resistance of the host. The severity of the infection seems to depend mostly on the immunologic status of the host. Thus, the demonstration of fungi, for example, in blood drawn from an intravenous catheter can correspond to colonization of the catheter, to transient fungemia (i.e., dissemination of fungi through the blood stream), or to a true infection. The physician must decide which is the clinical status of the patient based on clinical parameters, general status of the patient, laboratory results, etc. The decision is not trivial, since treatment of systemic fungal infections requires the aggressive use of drugs with considerable toxicity. Most mycotic agents are soil saprophytes and mycotic diseases are generally not communicable from person-to-person (occasional exceptions are: Candida and some dermatophytes). Outbreaks of disease may occur, but these are due to a common environmental exposure, not communicability. Most of the fungi which cause systemic infections have a peculiar, characteristic ecologic niche in nature. This habitat is specific for several fungi which will be discussed later. In this environment, the normally saprophytic organisms proliferate and develop. This habitat is also the source of fungal elements and/or spores, where man and animals, incidental hosts, are exposed to the infectious particles. It is important to be aware of these associations to diagnose mycotic diseases. The physician must be able to elicit a complete history from the patient including occupation, avocation and travel history. This information is frequently required to raise, or confirm, a differential diagnosis. The incidence of mycotic infections is currently increasing dramatically, due to an increased population of susceptibles. Examples are patients with AIDS, patients on immunosuppressive therapy, and the use of more invasive diagnostic and surgical procedures (prosthetic implants). Fungal diseases are non-contagious and non-reportable diseases in the national public health statistics.

VIDEO
Growth and Division of Budding Yeast (Saccharomyces cerevisiae)

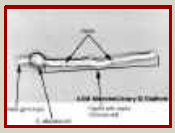
High Resolution
Low resolution


A

Candida albicans is a dimorphic fungus in that it grows as a unicellular yeast under some environmental conditions and as a filamentous fungus under other conditions.
Budding yeast cells. *C. albicans* was grown at 37°C with aeration for 3 h in yeast-peptone-dextrose (YPD) medium. In this image, unstained cells are magnified x400. The image was taken with phase-contrast microscopy.


B

Budding yeast with septum. The septum has formed between the daughter bud and the mother cell, but separation of the two has not occurred. This image is from a culture of cells grown at 37° C for 3 h in YPD medium. The unstained cell is magnified x1,000 using phase- contrast microscopy.


C

C. albicans cell at 3 h. Three hours after the appearance of the germ tube, the hypha has septa. A new germ tube at the distal pole of the
cell is also evident at this time. The unstained cells are magnified x1,000 using phase-contrast microscopy.

Figure 5 A-C

DIAGNOSIS

- Skin scrapings suspected to contain dermatophytes or pus from a lesion can be mounted in KOH on a slide (wet preparation) and examined directly under the microscope.

- Skin testing (dermal hypersensitivity) used to be popular as a diagnostic tool, but this is now discouraged because the skin test may interfere with serological studies, by causing false positive results. It may still be used to evaluate the patient's immunity, as well as a population exposure index in epidemiological studies.

- Serology may be helpful when it is applied to a specific fungal disease; there are no screening antigens for 'fungi' in general. Because fungi are poor antigens, the efficacy of serology varies with different fungal infections. The serologic tests will be discussed under each mycosis. The most common serological tests for fungi are based on double immunodiffusion, complement fixation and enzyme immunoassays (EIA). Double immunodiffusion and complement fixation usually detect IgG antibodies. Some EIA tests are being developed to detect both IgG and IgM antibodies. There are some tests that can detect specific fungal antigens, but they are just coming into general use.


Figure 6
Gomori staining technique, and under a relatively low magnification of 50X, this photomicrograph reveals histopathologic changes indicative of the presence of the dematiaceous fungal organism, Phialophora parasitica. Known to be a causative agent for chromoblastomycosis and phaeohyphomycosis, which affect the subcutaneous tissues, however, in the case of phaeohyphomycosis, many

organ systems may be affected, even becoming disseminated throughout the body.
CDC/ Dr. L. Ajello


Figure 7
A Sabouraud's dextrose agar plate culture growing a Mexican isolate of *T. rubrum var. rodhaini*. Dermatophytic members of the genus *Trichophyton* are some of the leading causes of hair, skin, and nail infections in humans, known as dermatophytoses. The genus includes anthropophilic, zoophilic, and geophilic species
CDC/Dr. Libero Ajello

- Direct fluorescence microscopy may be used for identification, even on non-viable cultures or on fixed tissue sections. The reagents for this test are difficult to obtain.

- Biopsy and histopathology. A biopsy may be very useful for the identification and as a source of the of tissue-invading fungi. Usually the Gomori methenamine silver (GMS) stain is used to reveal the organisms which stain black against a green background (figure 6). The H&E stain does not always tint the organism, but it will stain the inflammatory cells.

- Culture. A definitive diagnosis requires a culture and identification. Pathogenic fungi are usually grown on Sabouraud dextrose agar (figure 7). It has a slightly acidic pH (~5.6). Cycloheximide, penicillin, streptomycin or other inhibitory substances are often added to prevent bacterial contamination and overgrowth. Two cultures are inoculated and incubated separately at 25 degrees C and 37 degrees C to reveal dimorphism. The cultures are examined macroscopically and microscopically. They are not considered negative for growth until after 4 weeks of incubation.

- DNA probes. Ribosomal DNA is hybridized to a labeled DNA probe. This test is rapid (1 to 2 hours) and species-specific. It is not available for many organisms and it is expensive.

MOLECULAR STRUCTURE
Amphotericin B
Ketoconazole
Griseofulvin
5-fluorocytosine

MOLECULAR STRUCTURE

Ergosterol

Caspofungin

TREATMENT

Mammalian cells do not contain the enzymes that will degrade the cell wall polysaccharides of fungi. Therefore, these pathogens are difficult to eradicate by the animal host defense mechanisms. Because mammals and fungi are both eukaryotic, the cellular milieu is biochemically similar in both. The cell membranes of all eukaryotic cells contain sterols; ergosterol in the fungal cell membrane and cholesterol in the mammalian cell membrane. Thus, most substances which may impair the invading fungus will usually have serious side effects on the host. Although one of the first chemotherapeutic agents (oral iodides) was an anti-mycotic used in 1903, the further development of such agents has been left far behind the development of anti-bacterial agents. The selective toxicity necessary to inhibit the invading organism with minimal damage to the host has been difficult to establish within eukaryotic cells.

The primary antifungal agents are:

Amphotericin B

This is a polyene antimycotic. It is usually the drug of choice for most systemic fungal infections. It has a greater affinity for ergosterol in the cell membranes of fungi than for the cholesterol in the host's cells; once bound to ergosterol, it causes disruption of the cell membrane and death of the fungal cell. Amphotericin B is usually administered intravenously and patients are usually hospitalized. The drug is rather toxic; thrombo-phlebitis, nephrotoxicity, fever, chills and anemia frequently occur during administration. Lipid-based Amphotericin B is as effective less toxic and more expensive.

Azoles

The azoles (imidazoles and triazoles), including ketoconazole, fluconazole, itraconozole, voriconazole and posaconazole are being used for muco-cutaneous candidiasis, dermatophytosis, and for some systemic fungal infections. Fluconazole is presently essential for the maintenance of AIDS patients with cryptococcosis. The general mechanism of action of the azoles is the inhibition of ergosterol synthesis. Oral administration and reduced toxicity are distinct advantages.

Griseofulvin

Griseofulvin is a very slow-acting drug which is used for severe skin and nail infections. Its effect depends on its accumulation in the stratum corneum where it is incorporated into the tissue and forms a barrier which stops further fungal penetration and growth. It is administered orally. The exact mechanism of action is unknown.

5-fluorocytosine

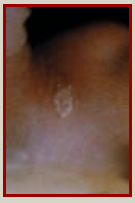


Figure 8.
Ringworm on the skin of the neck due to *Trichophyton rubrum*.
CDC/Lucille K. Georg

5-fluorocytosine (Flucytosine or 5-FC) inhibits RNA synthesis and has found its main application in cryptococcosis (to be discussed later). It is administered orally.

Terbinofine

(E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalenemethanamine hydrochloride (terbinafine hydrochloride).

This is an anti-fungal agent, also known as Lamisil, used to treat infections of fingernails and toenails. It is taken orally.

Caspofungin

1-[(4R,5S)-5-[(2-aminoethyl)amino]- N2-(10,12-dimethyl-1-oxotetradecyl)- 4-hydroxy-L-ornithine]-5-[(3R)- 3-hydroxy-L-ornithine] pneumocandin B0.

This anti-fungal works by inhibiting the enzyme β(1,3)-D-Glucan synthase and altering the integrity of the fungal cell wall. It is administered intravenously.

CLINICAL CLASSIFICATION OF THE MYCOSES

Fungal diseases may be discussed in a variety of ways. The most practical method for medical students is the clinical taxonomy which divides the fungi into:

- Superficial mycoses
- Subcutaneous mycoses
- Systemic mycoses
- Opportunistic mycoses

The superficial mycoses (or cutaneous mycoses) are fungal diseases that are confined to the outer layers of the skin, nail, or hair, (keratinized layers) rarely invading the deeper tissue or viscera (figure 8). The fungi involved are called dermatophytes. The subcutaneous mycoses are confined to the subcutaneous tissue and only rarely spread systemically. They usually form deep, ulcerated skin lesions or fungating masses, most commonly involving the lower extremities. The causative organisms are soil saprophytes which are introduced through trauma to the feet or legs. The systemic mycoses may involve deep viscera and become widely disseminated. Each fungus type has its own predilection for various organs which will be described as we discuss the individual diseases.

The opportunistic mycoses are infections due to fungi with low inherent virulence. The etiologic agents are organisms which are common in all environments.

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