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# Chapter 5. General Mycology

## Table of Contents

GENERAL MYCOLOGY .....	1
CLASSIFICATION OF FUNGI .....	2
Morphological Classification .....	2
Clinical Classification of Fungal Diseases .....	3
LABORATORY DIAGNOSIS OF FUNGAL INFECTIONS .....	3
Specimen Collection .....	3
Microscopy .....	3
Culture .....	5
Immunological Methods .....	5
Automation .....	6
Molecular Methods .....	6
TREATMENT OF FUNGAL INFECTIONS .....	6

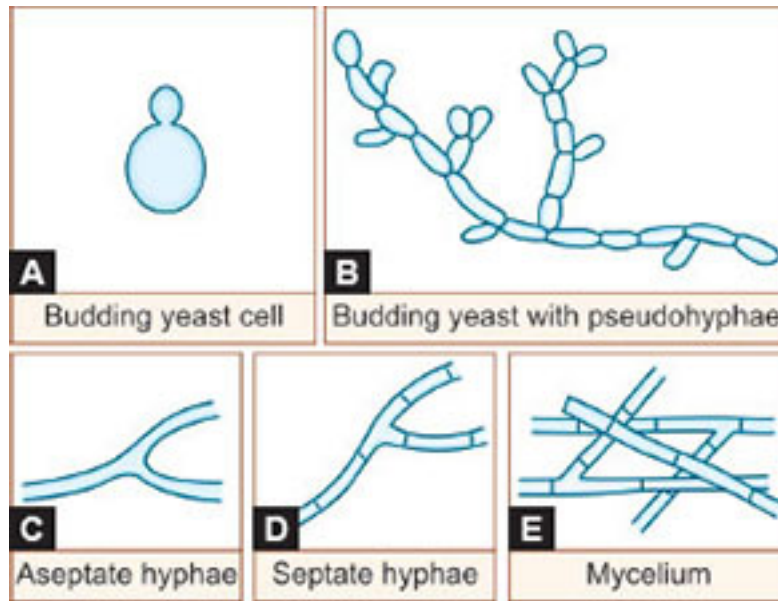
### CHAPTER PREVIEW

- General Mycology
- Classification of Fungi
- Laboratory Diagnosis of Fungal Infections
- Treatment of Fungal Infections

## GENERAL MYCOLOGY

Medical mycology is the branch of medical science that deals with the study of medically important fungi. The name 'fungus' is derived from Greek '*mykes*' meaning mushroom (a type of edible fungus). Some of the important properties of fungi are:

- Fungi are eukaryotic and they possess all the eukaryotic cell organelles
- They possess a rigid cell wall, composed of chitin,  $\beta$ -glucans, and other polysaccharides
- The fungal cell membrane contains ergosterol instead of the cholesterol
- They divide by asexual and/or sexual means by producing spores.

**Figs. 5.1A to E. Morphological forms of fungi.**

## CLASSIFICATION OF FUNGI

### Morphological Classification

Based on the morphological appearance, there are four main groups of fungi as follows (Figs. 5.1A to E):

1. **Yeast:** They grow as round to oval cells that reproduce by an asexual process called *budding* in which cells form protuberances that enlarge and eventually separate from the parent cells. An example includes —*Cryptococcus neoformans*
2. **Yeast-like:** In some yeasts (e.g. *Candida*), the bud remains attached to the mother cell, elongates, and undergoes repeated budding to form chains of elongated cells known as *pseudohyphae*.
3. **Molds:** They grow as long branching filaments of 2–10  $\mu\text{m}$  width called *hyphae*.
  - Hyphae are either septate (i.e. form transverse walls) or nonseptate (there are no transverse walls)
  - Hyphae grow continuously and form a branching tangled mass of growth called *mycelium*
  - Molds reproduce by formation of different types of sexual and asexual spores
  - Examples of true molds include—Dermatophytes, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, etc.
4. **Dimorphic fungi:** They exist as molds (hyphal form) at 25°C and as yeasts in human tissues at body temperature (37°C). Several medically important fungi are thermally dimorphic such as:
  - *Histoplasma capsulatum*
  - *Blastomyces dermatitidis*
  - *Coccidioides immitis*
  - *Paracoccidioides brasiliensis*

- *Penicillium marneffei*
- *Sporothrix schenckii*.

## Clinical Classification of Fungal Diseases

Fungal infections (or mycoses) can be categorized into the following clinical types (Table 5.1).

# LABORATORY DIAGNOSIS OF FUNGAL INFECTIONS

The laboratory diagnosis of fungal diseases comprises the following:

## Specimen Collection

It depends on the site of infection such as skin scraping, hair, nail, sputum, etc. For systemic mycoses, blood sample may also be collected. Cerebrospinal fluid (CSF) is collected for cryptococcal meningitis.

## Microscopy

Microscopy is useful to demonstrate fungal elements in clinical specimens. Following microscopy techniques are used:

- **Potassium hydroxide (KOH) preparation:** Keratinized tissue specimens such as skin scrapings and plucked hair samples are treated with 10% KOH which digests the keratin material so that the fungal hyphae will be seen under the microscope (Fig. 5.2A)
- **Gram stain:** It is useful in identifying the yeasts (e.g. *Cryptococcus*) and yeast-like fungi (e.g. *Candida*). They appear as gram-positive budding yeast cells
- **India ink and nigrosin stains:** They are used as negative stains for demonstration of capsule of *Cryptococcus neoformans* (Fig. 35.8A, of Chapter 35)

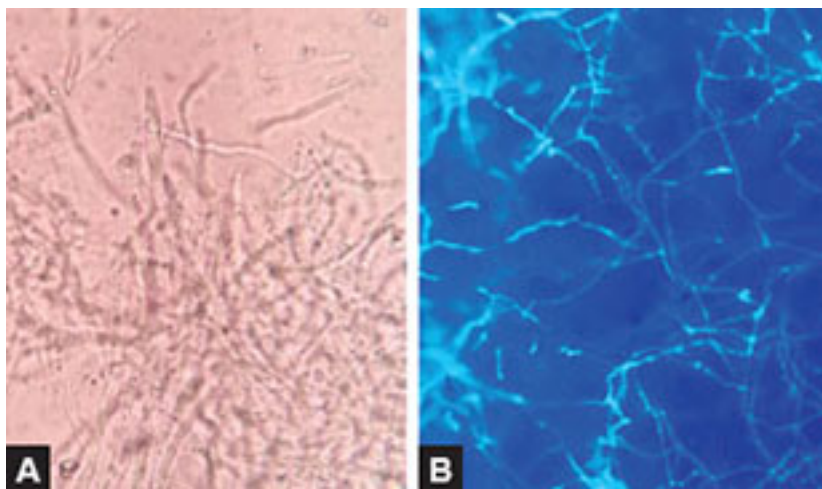
**Table 5.1. Classification of fungal diseases.**

<i>Fungal disease</i>	<i>Agents</i>
<b>Superficial mycoses</b>	
Tinea versicolor	<i>Malassezia furfur</i>
Tinea nigra	<i>Hortaea werneckii</i>
Piedra	<i>Trichosporon beigelii</i> , <i>Piedraia hortae</i>
Dermatophytosis	<i>Trichophyton</i> , <i>Microsporum</i> , <i>Epidermophyton</i>
<b>Subcutaneous mycoses</b>	
Mycetoma	<i>Madurella mycetomatis</i> , and others
Sporotrichosis	<i>Sporothrix schenckii</i>
Chromoblastomycosis	<i>Phialophora</i> and others
Phaeohyphomycosis	<i>Exophiala</i> and others
Rhinosporidiosis	<i>Rhinosporidium seeberi</i>

<i>Fungal disease</i>	<i>Agents</i>
<b>Systemic mycoses</b>	
Histoplasmosis	<i>Histoplasma capsulatum</i>
Blastomycosis	<i>Blastomyces dermatitidis</i>
Coccidioidomycosis	<i>Coccidioides immitis</i>
Paracoccidioidomycosis	<i>Paracoccidioides brasiliensis</i>
<b>Opportunistic mycoses</b>	
Candidiasis	<i>Candida albicans</i> and other species
Cryptococcosis	<i>Cryptococcus neoformans</i>
Zygomycosis	<i>Rhizopus, Mucor</i>
Aspergillosis	<i>Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger</i>
Penicilliosis	<i>Penicillium marneffeii</i>
Pneumocystosis	<i>Pneumocystis jirovecii</i>
Fusariosis	<i>Fusarium species</i>

- **Calcofluor white stain:** It is more sensitive than other stains; fungal elements fluoresce under UV light (*Fig. 5.2B*)
- **Histopathological stains:** They are useful for demonstrating fungal elements from biopsy tissues. This is useful for detecting invasive fungal infection
  - Periodic acid Schiff (PAS) stain
  - Gomori methenamine silver (GMS) stain
  - Hematoxylin and Eosin (H and E) stain.
- **Lactophenol cotton blue (LPCB):** It is used to study the microscopic appearance of the fungal isolates grown in culture.

**Figs. 5.2A and B. Fungal hyphae in: A. KOH mount; B. Calcofluor white stain mount.**



*Source: A. Dr Sherly Antony, Pushpagiri Medical College, Thiruvalla, Kerala; B. Department of Microbiology, JIPMER, Puducherry (with permission).*

Fungal elements are stained blue colored.

## Culture

A fungal culture is frequently performed for isolation and correct identification of the fungi.

### Culture Media

- **Sabouraud's dextrose agar (SDA):** It is the most commonly used medium in diagnostic mycology
- **Brain heart infusion (BHI) agar and blood agar:** They are the enriched media, used for growing fastidious fungi like *Cryptococcus* and *Histoplasma*
- **Niger seed agar** and *bird seed agar*: They are used for the selective growth of *Cryptococcus*
- **CHROMagar Candida medium:** It is used for isolation as well as a differential medium for speciation of *Candida*.

### Culture Condition

- **Temperature:** Most of the fungi grow well at 25–30°C except the dimorphic fungi that grow at both 25°C and 37°C
- **Incubation:** A special incubator called as *BOD incubators* (biological oxygen demand) are used for fungal culture and culture plates should be incubated for 2–3 weeks
- **Antibiotics** such as cycloheximide, and chloramphenicol can be added to the culture media to inhibit bacterial growth.

### Culture Identification

The correct identification of the fungus is based on the macroscopic appearance of the colonies grown on culture and microscopic appearance (LPCB mount of colonies).

- **Macroscopic appearance of the colony:** Following growth characters are noted, such as—rate of growth (rapid/slow), pigmentation, texture and colony surface appearance.
- **Microscopic appearance of fungi:** Microscopic examination of the fungi can be done by:
  - **LPCB teased mount:** A bit of fungal colony is teased out from the culture tube and the LPCB mount is prepared.
  - **Slide culture:** It is done to demonstrate the most accurate in situ microscopic appearance of the fungal colony.

## Immunological Methods

These tests are available to detect the antibody or antigen from serum and/or other body fluids.

- **Antibody detection** can be done by ELISA and agglutination test
- **Antigen detection:** Various fungal antigens can be detected in clinical specimens such as blood, CSF, urine, etc.
  - **Cryptococcal capsular antigen** from CSF by latex agglutination test
  - Detection of *Aspergillus* specific *galactomannan antigen* in patient's sera or urine (by ELISA)
  - **#-d-Glucan assay** by ELISA: It is a marker of all invasive fungal infections.

## Automation

Automated identification systems such as MALDI-TOF and VITEK are revolutionary in the accurate identification of yeasts and to some extent molds.

## Molecular Methods

Molecular methods useful in the diagnosis of fungal infections include—polymerase chain reaction (PCR), real-time PCR, and DNA sequencing methods.

# TREATMENT OF FUNGAL INFECTIONS

Some of the commonly used antifungal agents include—amphotericin B, caspofungin, griseofulvin, fluconazole and voriconazole.

Treatment of the important human fungal infections has been described in Chapter no 35.

### EXPECTED QUESTIONS

**1. I. Write short notes on:**

1. Laboratory diagnosis of fungal infections.
2. Dimorphic fungi.

**2. II. Multiple Choice Questions (MCQs):**

1. **All are yeast or yeast-like fungi, except:**
  - a. *Candida*
  - b. *Trichosporon*
  - c. *Cryptococcus*
  - d. *Trichophyton*
2. **All of the following are microscopic techniques for the diagnosis of fungal diseases, except:**
  - a. KOH mount
  - b. LPCB mount
  - c. India ink staining
  - d. CHROMagar identification
3. **All are systemic mycoses, except:**
  - a. Histoplasmosis
  - b. Blastomycosis
  - c. Dermatophytosis
  - d. Coccidioidomycosis

4. All are examples of molds, *except*:

- a. *Aspergillus*
- b. *Penicillium*
- c. *Candida*
- d. *Rhizopus*

**Answers**

1. d	2. d	3. c	4. c
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