

Medical Mycology : BDS

Objectives

- Introduction
- Classification
- Definitions
- Reproduction
- Classification of fungal diseases
- Lab diagnosis
- Treatment

Introduction

- Eucaryotic protista
- Differs bacteria & other prokaryotes in many ways.
 - Rigid cell wall
 - Cell Wall :Chitin, mannan & other polysaccharides
 - Cytoplasmic membrane : Sterols
 - True nuclei within Nuclear membrane
 - Paired chromosomes
- Cell shows various degree of specialization

Thallophyta

(Division)

FUNGI
(No chlorophyll)

ALGAE
(Chlorophyll)

Pseudomycetes

Eumycetes (true fungi)

Schizomycetes
(bacteria)
e.g. *Nocardia*
Actinomyces

Myxomycetes
(slime molds)

(Phyla)

Ascomycetes

e.g. *Histoplasma*
• *Candida*
• *Blastomycosis*

Basidiomycetes

e.g. *Cryptococcus*
neoformans

Phycomycetes

e.g. *Mucor*
Rhizopus

Fungi Imperfecti

e.g.
• *Trichophyton*
• *Epidermophyton*

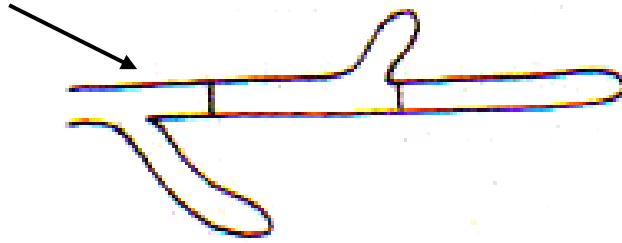
Definitions

- **Pseudohypha** – yeast produces a chain of elongated cells known as pseudohypha
- **Spores** – structures produced as a result of sexual or asexual reproduction of fungi
- **Asexual spores** – produced as a result of budding, fission & mitosis
- These are –
 - ✓ **Chlamydo spores** – are large with thick double refractile wall produced from hyphal cell
 - ✓ **Arthrospores** – produced as a result of fragmentation of hyphae. They are cuboidal, rectangular spores formed in chains
 - ✓ **Blastospores** – produced as a result of budding

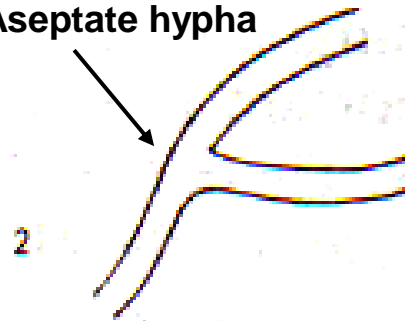
- ✓ **Sporangiospores** – present inside the sporangium
- ✓ **Conidia** – asexual reproductive structure present on hyphae called conidiophore
- **Sexual spores** – are produced as a result of meiosis
- These are –
 - ✓ **Ascospores** – these are inside a sac called ascus
 - ✓ **Zygosporangia** – sexual spores in zygomycetes
 - ✓ **Basidiospores** – formed on basidium

- Simplest type of fungus : unicellular budding yeast
- **Hypha**: Elongation of the cell produces tubular thread like structure.
- **Mycelium**: Tangled mass of hyphae : septate / nonseptate
- **Fungus form mycelia** : moulds / filamentous fungi
- Septa have holes : free flow of cytoplasmic material
- Mycelium divided into the vegetative mycelium : grows into medium & aerial mycelium which protrude from surface

Hyphal tip with lateral branching



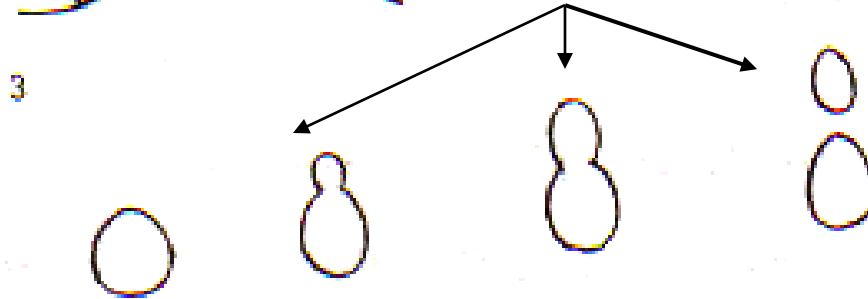
Aseptate hypha



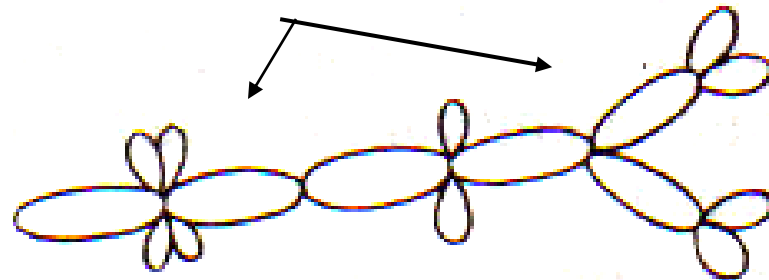
Septate Hypha



Yeast cell showing different stages of budding



Yeast pseudo mycelium



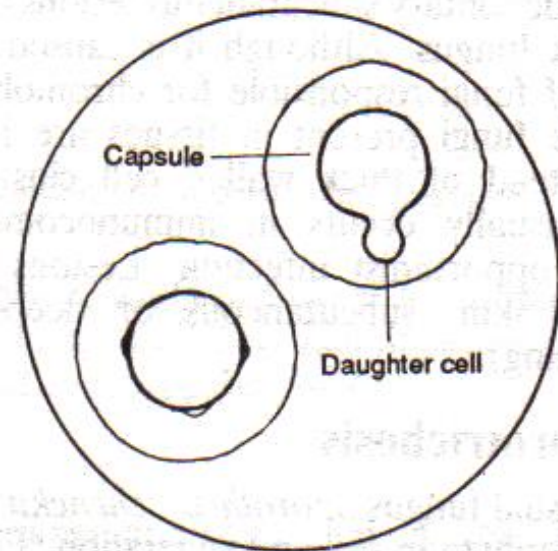
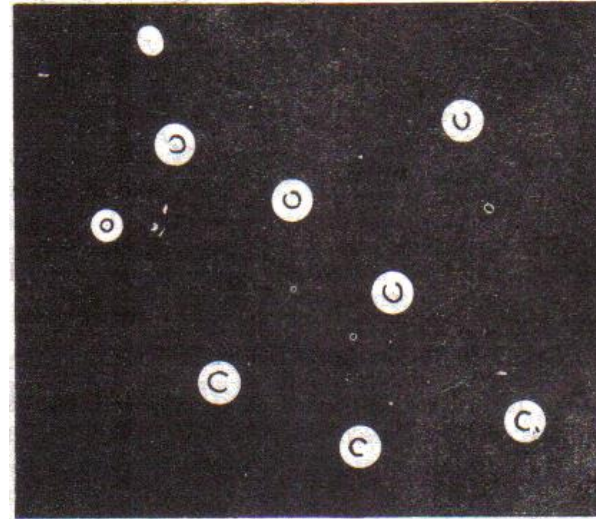
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Classification

- Depending on cell morphology : 4 Classes
 - 1) Yeast
 - 2) Yeast like
 - 3) Moulds
 - 4) Dimorphic

Classification

- **Yeast** : Unicellular fungi : spherical / ellipsoidal cells & reproduce by simple budding.
- On culture : form smooth, creamy colonies
- e.g. *Cryptococcus neoformans*



Cryptococcus neoformans

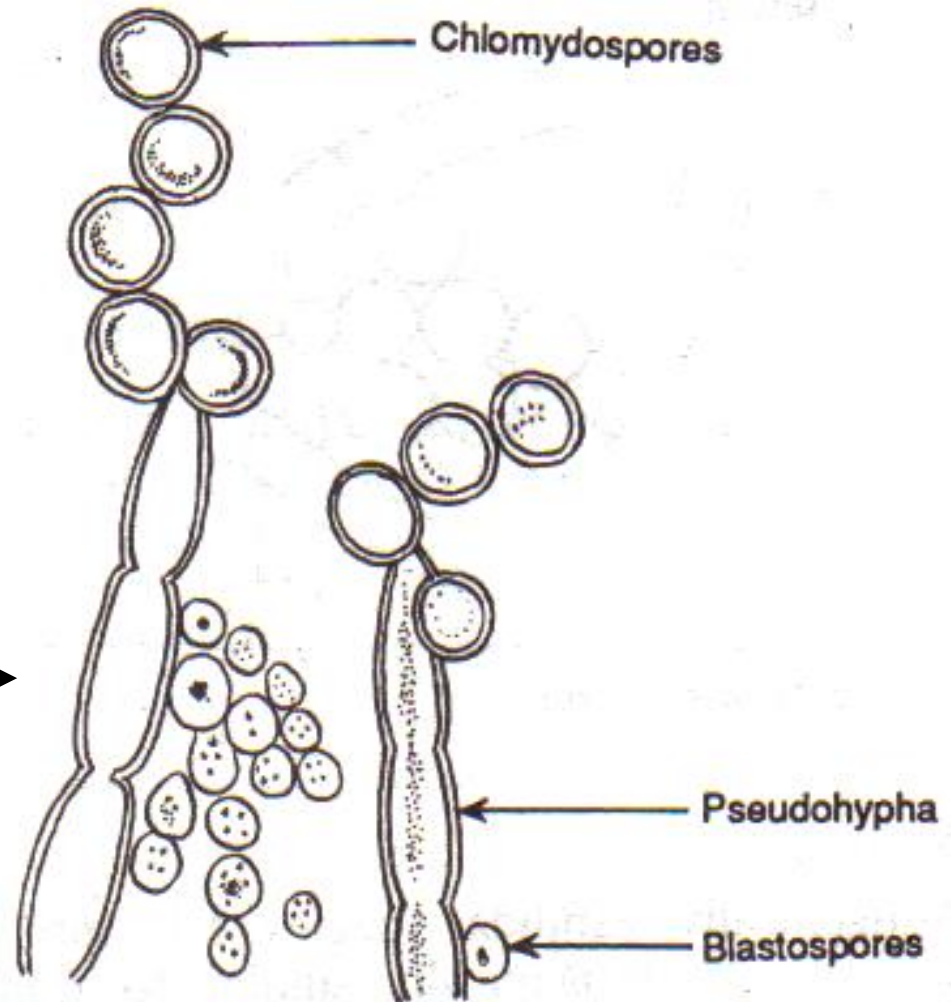


Courtesy of C. Halde

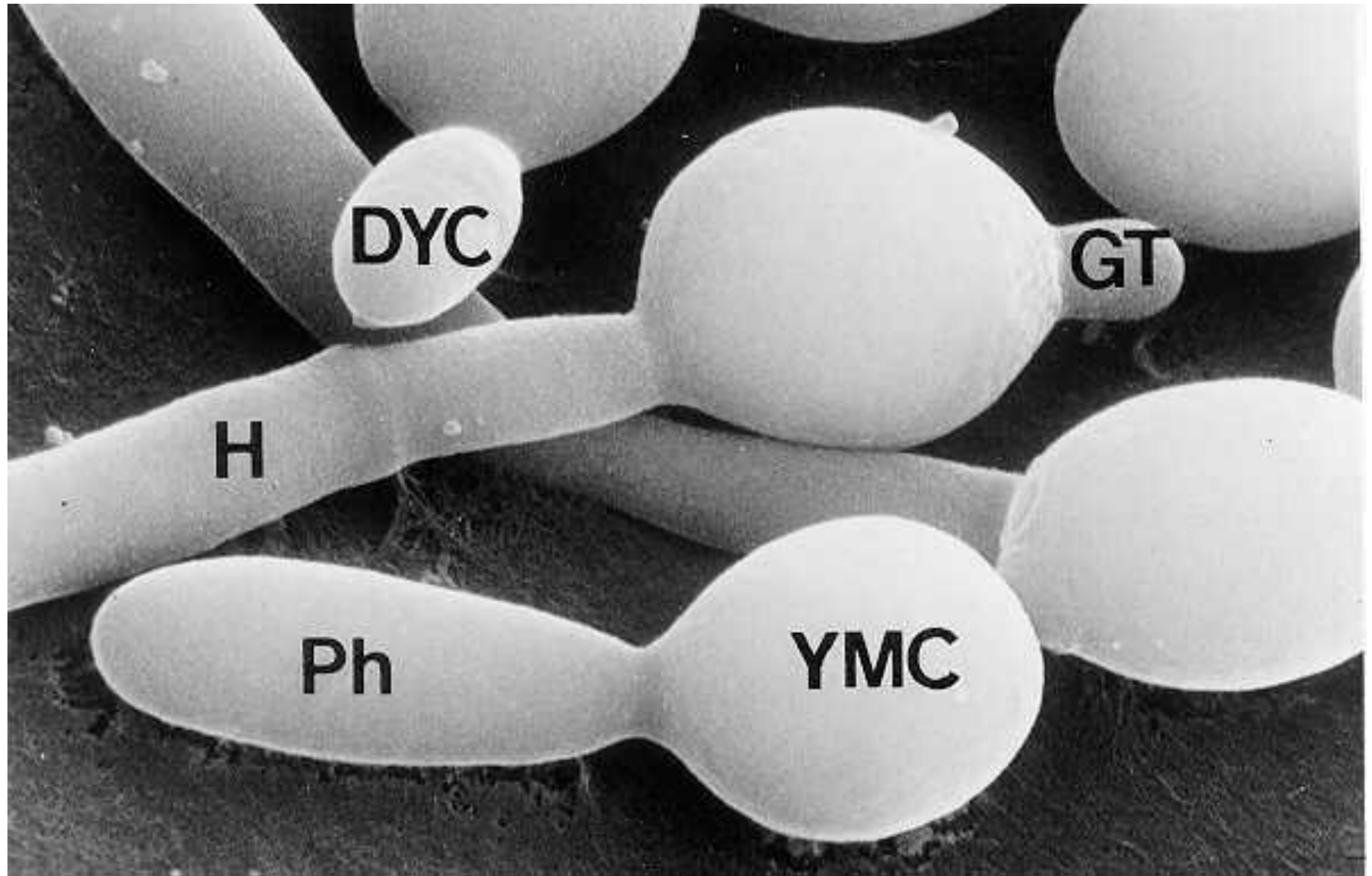
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Classification

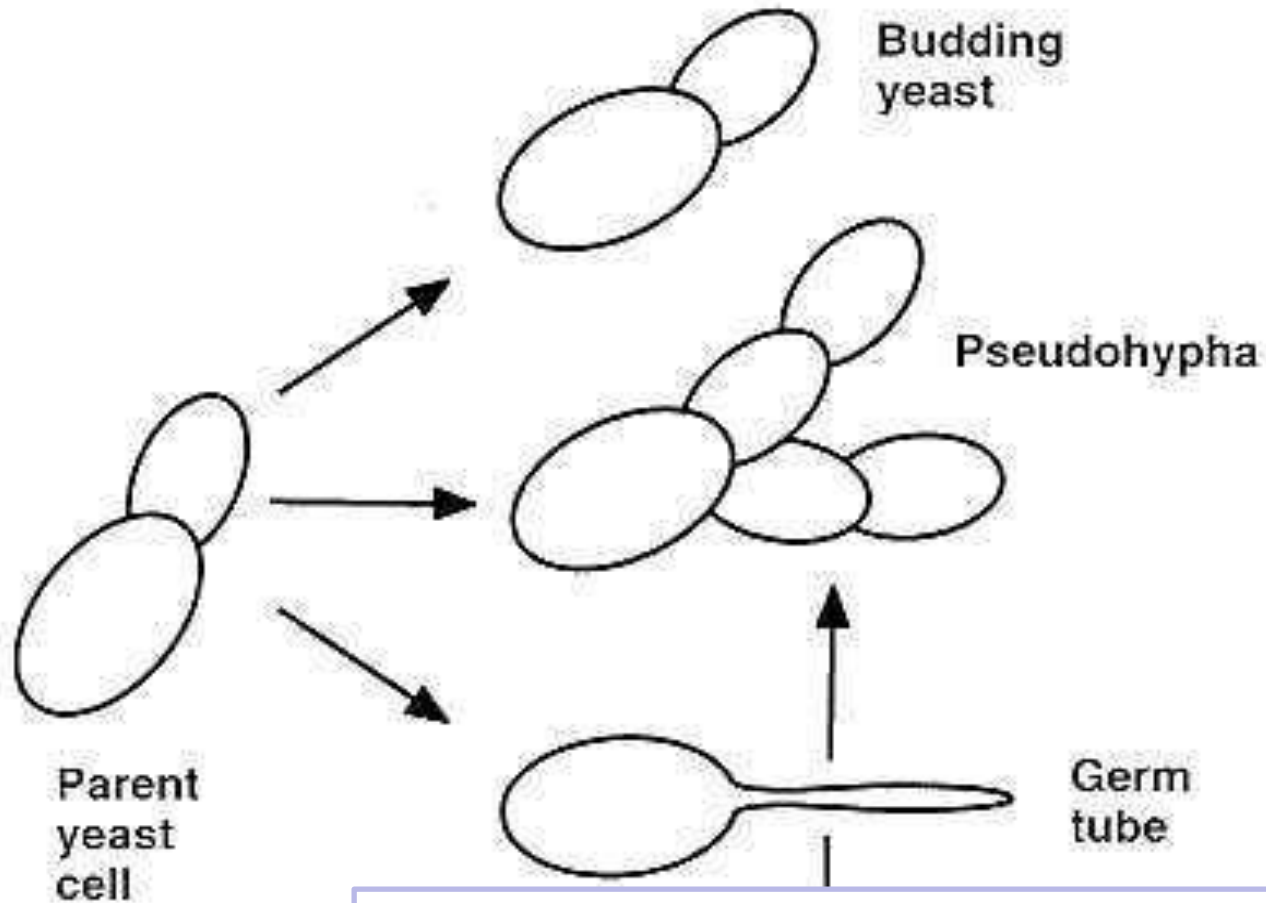
- **Yeast like fungi** :
Grow partly as yeast & partly as elongated cells resembling hyphae
- Hyphae form pseudomycelium
- **Pathogenic spp** :
Candida albicans



Features of *Candida albicans*



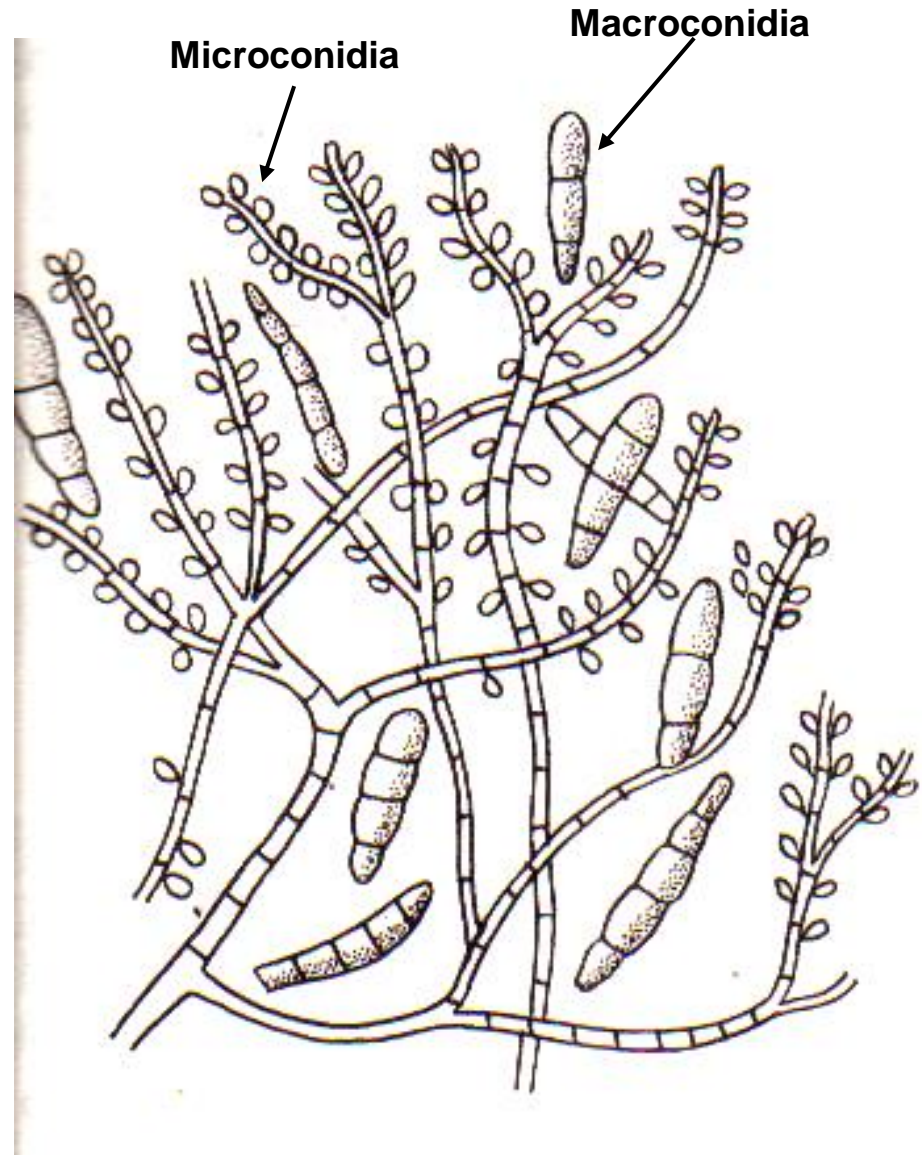
DYC: Daughter yeast cell, YMC: Yeast mother cell, Ph: Pseudohyphae, GT: Germ tube



Germination of *Candida albicans*

Classification

- **Moulds / filamentous fungi** : form true mycelia & reproduce by the formation of different types of spores
- e.g. Dermatophytes : **Pathogenic** moulds
Trichophyton spp,
Microsporum spp &
Epidermophyton



Classification

- **Dimorphic fungi** : Exist either as yeasts / as filaments, depending on the conditions of growth. Causing Systemic infections
- Yeast form (parasitic phase) : Host tissue & on culture at 37⁰C
- Filamentous form (Saprophytic phase) : Soil & in cultures At 22-25⁰C
- e.g. *Blastomyces spp*, *Coccidioides immitis*, *Paracoccidioides spp*, *Histoplasma capsulatum*

Reproduction & Sporulation

- Two types of spores : Sexual & Asexual
- Sexual spores: Oospores, Zygosporangium, Ascospore, Basidiospore
- Asexual spores: Aerial spores (conidiospores)
 - :Vegetative spores: -Blastospore
 - Arthrospore
 - Chlamydospore

Sexual Spores

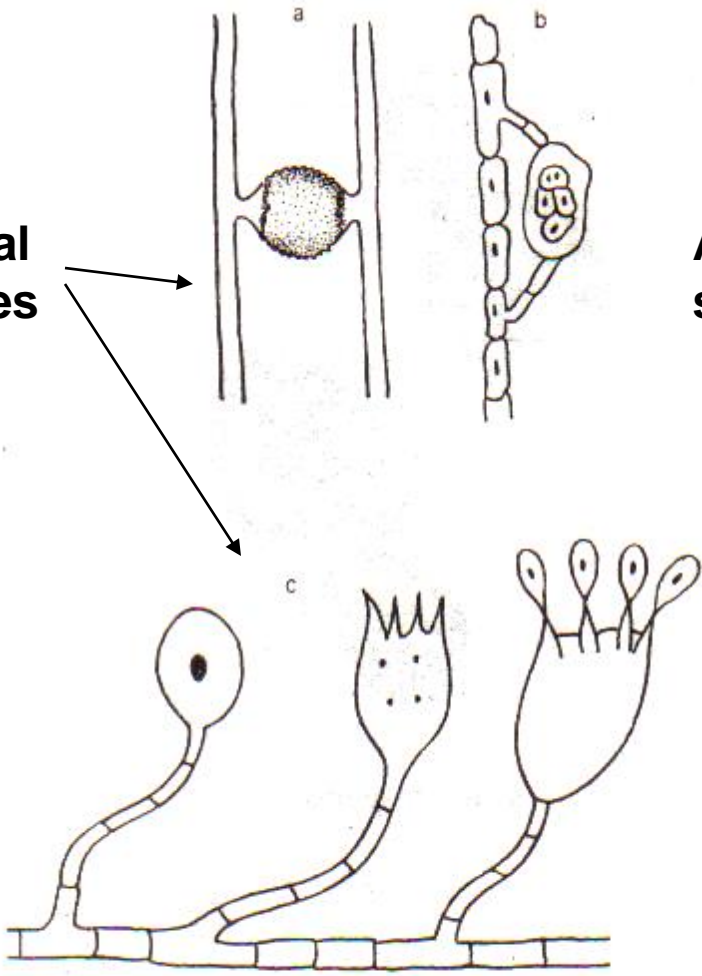


Fig. 64.4 Sexual spores : a. Zygosporangium, b. Ascospore, c. Basidiospore.

Asexual spores

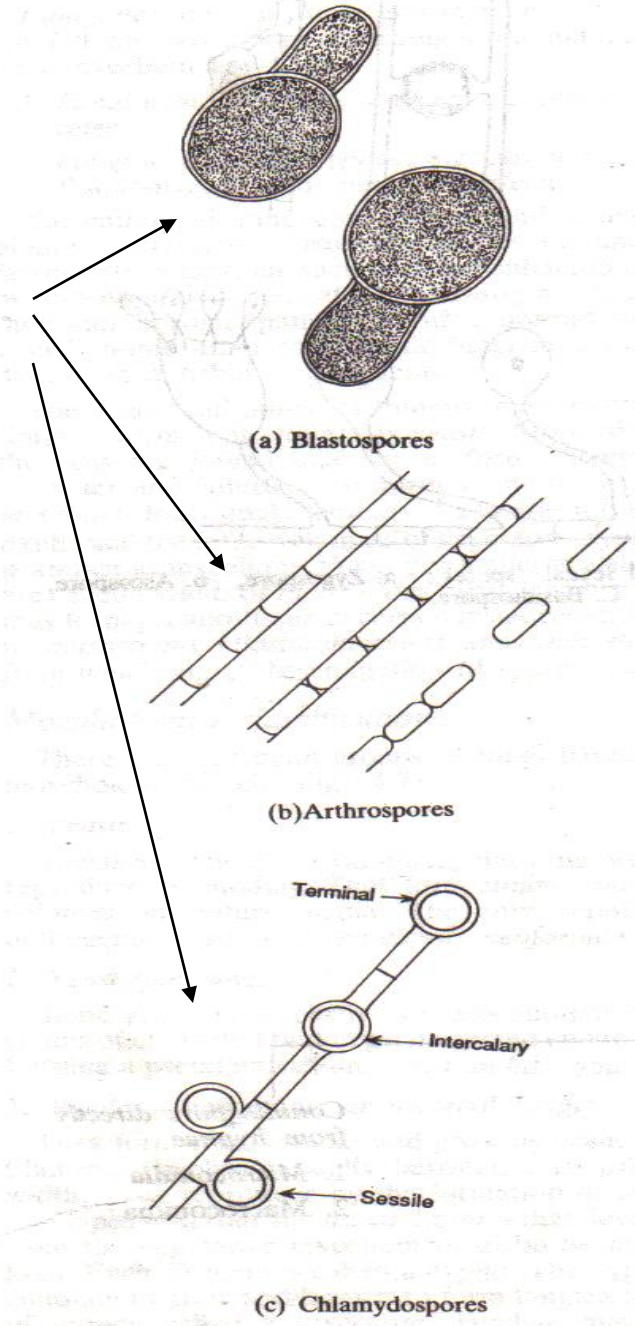


Fig. 64.5 Vegetative spores

Classification of fungal disease

- **Superficial mycoses** : Surface infections
 - : Mucosal infection by Yeast
 - : Keratin Infection Of skin
 - : Hair & Nails

Superficial infections are common & troublesome,
do not involve deeper tissue

Classification of fungal disease

- **Subcutaneous mycoses:** Traumatic implantation

Progressive local disease with tissue destruction & sinus formation.
Lesion spreads slowly via lymphatics

- 1) ***Mycotic Mycetoma***
- 2) ***Chromoblastomycosis***
- 3) ***Sporothrichosis***
- 4) ***Rhinosporidiosis***
- 5) ***Subcutaneous phycomycosis***

Classification of fungal disease

- Systemic Mycoses
- Soil fungi & acquired by inhalation & fungus may disseminate from the lung to internal organs.

- 1) Cryptococcosis
- 2) Blastomycosis
- 3) Paracoccidioidomycosis
- 4) Coccidioidomycosis
- 5) Histoplasmosis

Classification of fungal disease

- **Opportunistic Fungi**
- Saprophytic fungi from environment usually do not produce disease, may cause infection under special conditions :
Immunocompromised pts & in terminal stages of chronic disease
- Incidences are increased with wide use of antibiotics, steroids & Immunosuppressive drugs

Classification of fungal disease

- Opportunistic fungi

Yeast & Yeast like fungi : *Candida spp*

: *Torulopsis*

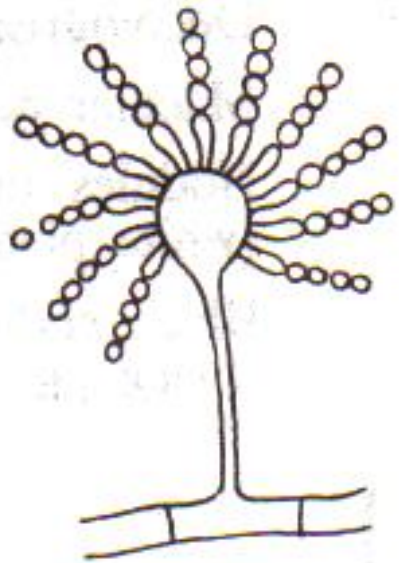
: *Cryptococcus*

Filamentous fungi :

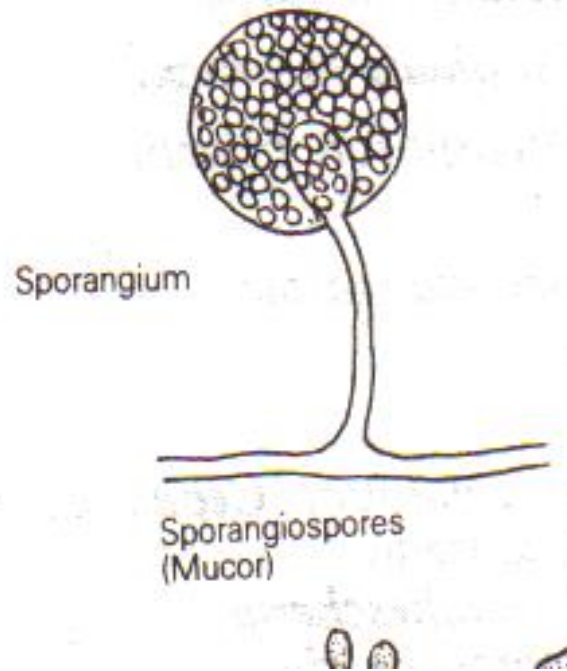
Aspergillus, Mucor , Rhizopus,

Absidia, Acrimonium, Fusarium,

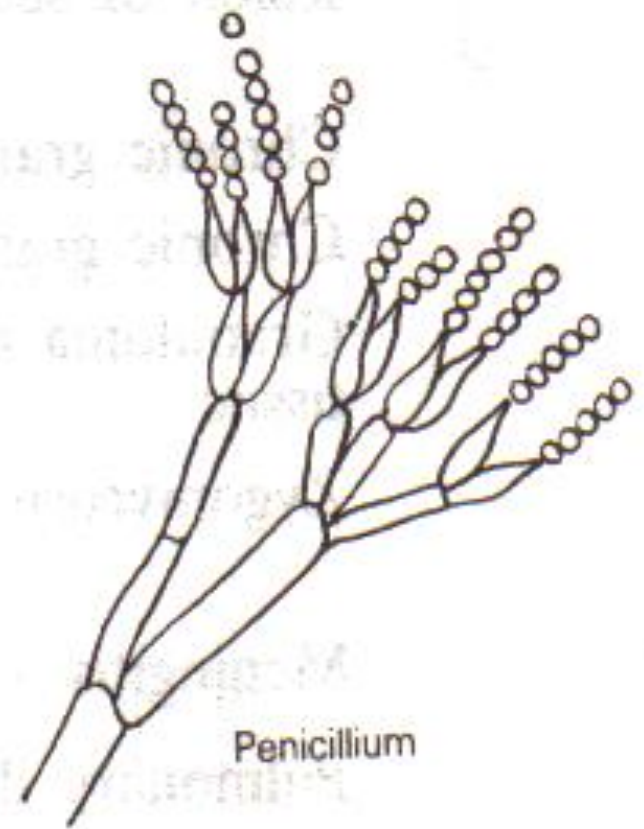
Penicillium, Geotrichum, Scopulariopsis



Conidiospores
(Aspergillus)



Sporangiospores
(Mucor)



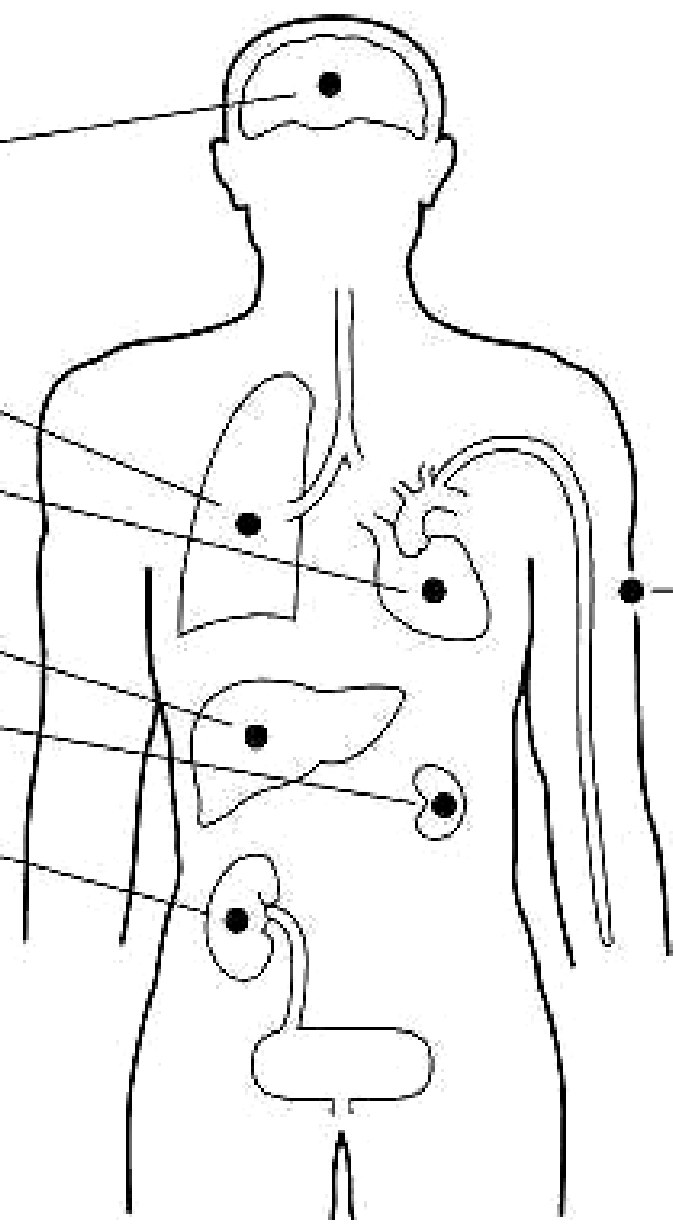
Penicillium

Opportunistic Miscellaneous fungi of Medical Importance

Deep mycoses

Superficial, cutaneous, subcutaneous mycoses

- Brain
- Lungs
- Heart
- Liver
- Spleen
- Kidney



- Superficial (hair, nail, skin)
- Cutaneous (hair, nail, skin)
- Subcutaneous

Portals of entry of pathogenic and opportunistic fungi causing deep mycoses.

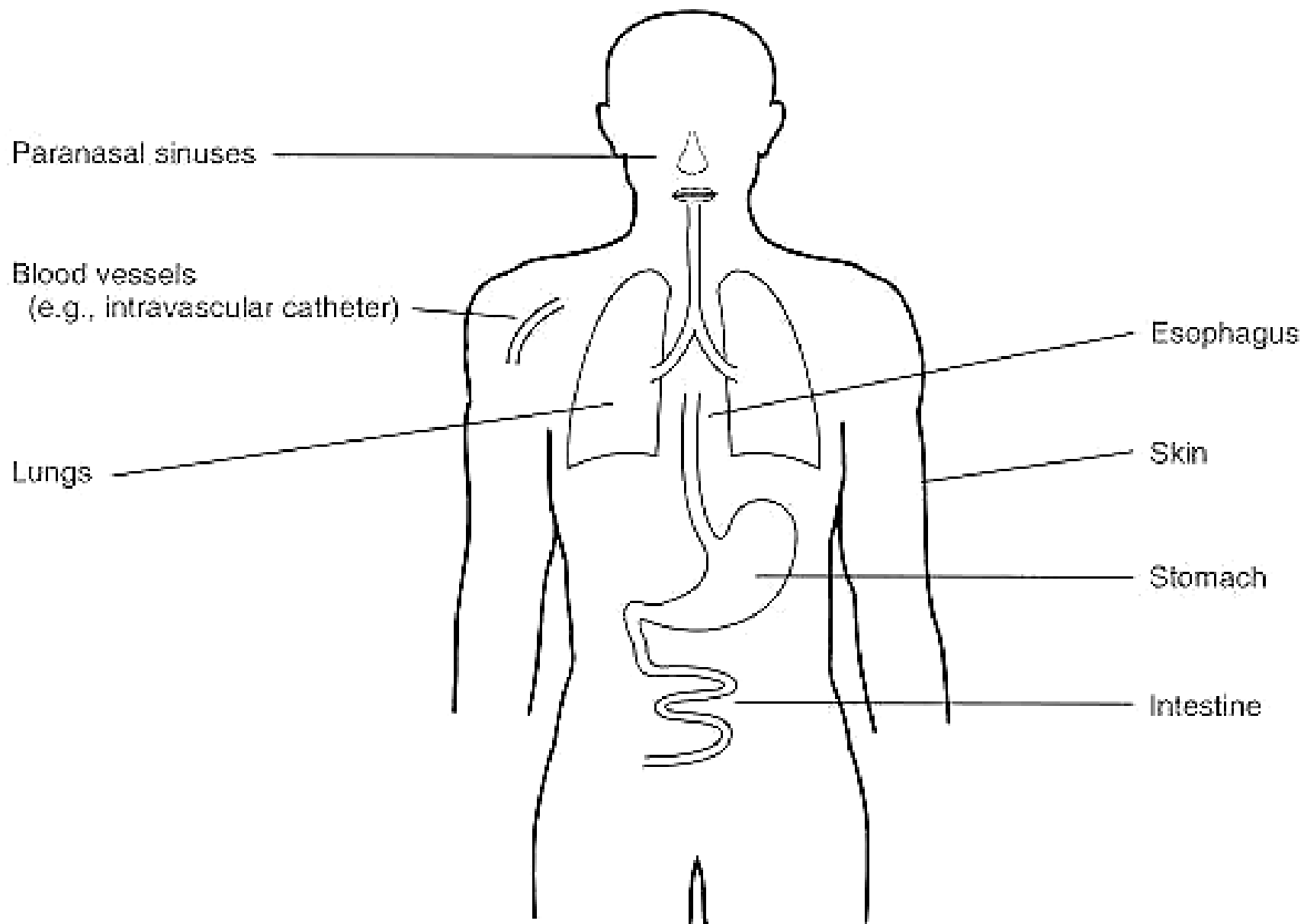


TABLE 74-1 Summary of Disease Mechanisms of Fungi

Source of Fungus	Clinical Classification	Mechanism of Entry
Endogenous	Opportunistic	Iatrogenic (indwelling lines, catheters, etc.)
Exogenous	Superficial	Trauma (personal hygiene?) Trauma Trauma
	Cutaneous	Inhalation
	Subcutaneous	Inhalation
	Systemic Opportunistic	(iatrogenic, trauma)

Laboratory Diagnosis of Fungal infections

- Microscopy
- Culture
- Serology
- Skin tests (for delayed hypersensitivity)

Specimens

- Specimen is collected from affected site
- In disseminated infections –blood needed
- Superficial infections – scrapings from lesions
- Nail clippings
- Hair from root / follicle
- Sputum, blood, pus, CSF etc.

Microscopy:

- Fungal structures detected by direct microscopic examination of material from lesion and study of morphology
- 1. Potassium Hydroxide (KOH) preparation:
- Skin scrapings + 10% KOH
- The alkali digests cells and other tissue materials enhancing fungal elements visualization
- 2. Gram Staining: yeast like *Candida* spp.

- 3. Calcoflour white (CFW) : gives non-specific fluorescence ; good visualization of morphology of fungus
- 4. India ink preparation: negative staining of capsulated yeast ; Cryptococcus
- 5. Methenamine Silver stain and periodic acid-Schiff (PAS) – for demonstrating fungi in tissues
- Lactophenol cotton Blue (LPCB): microscopy of fungal colonies teased on to a slide and mounted.

Culture :

- Most common medium: Sabouraud's Dextrose Agar (SDA); pH 5.4
- Addition of antibiotics prevents bacterial contamination
- Addition of cycloheximide (Actidione) – inhibits moulds contamination
- Cultures incubated in parallel at Room temp (22°C) for weeks and at 37°C for days.
- Other media – BHI agar / broth; Blood agar

- Detailed study of morphology of isolate is essential for identification.
- Growth characters important for identification are:
 - Rapidity of growth
 - Colour and morphology of the colony on the obverse
 - Pigmentation on the reverse
 - Morphology of hyphae (diameter, septa), conidia (spores) and other special structures

Slide culture:

- Difficult to perform practically but most ideal way to preserve the morphology “In Situ” for viewing under microscope

Serology :

- To demonstrate antigen / antibody in serum or body fluids:
- Antibody Detection by
- Agglutination / CFT / Immunodiffusion / CIEP / ELISA
- Antigen detection: by Latex agglutination for Cryptococcal capsular polysaccharide antigen, Candida , Aspergillus

Skin Tests:

- Used to detect Delayed type Hypersensitivity (DTH) for pathogens like Histoplasma / Coccidioides

- Newer rapid Diagnostic tests are:
 - Nucleic acid Hybridization
 - Polymerase Chain Reaction (PCR)

Treatment:

- Medications used topically for superficial skin / mucous memb infections e.g. Pimaricin for eye or skin infections
- Orally – Griseofulvin
- Amphotericin B, polymixin B – popular
- Itraconazole / Ketoconazole / Fluconazole