Medical mycology

Chapter 45

2013/14

Mycology

- myco = fungus
- -logy = study

Mycology

- Mycology: Study of fungi
- Fungal infections are termed **mycoses**
- Mycotoxicosis- intoxicaton by mycotoxins

- Mycetismus (mushrooms dose related poisoning)

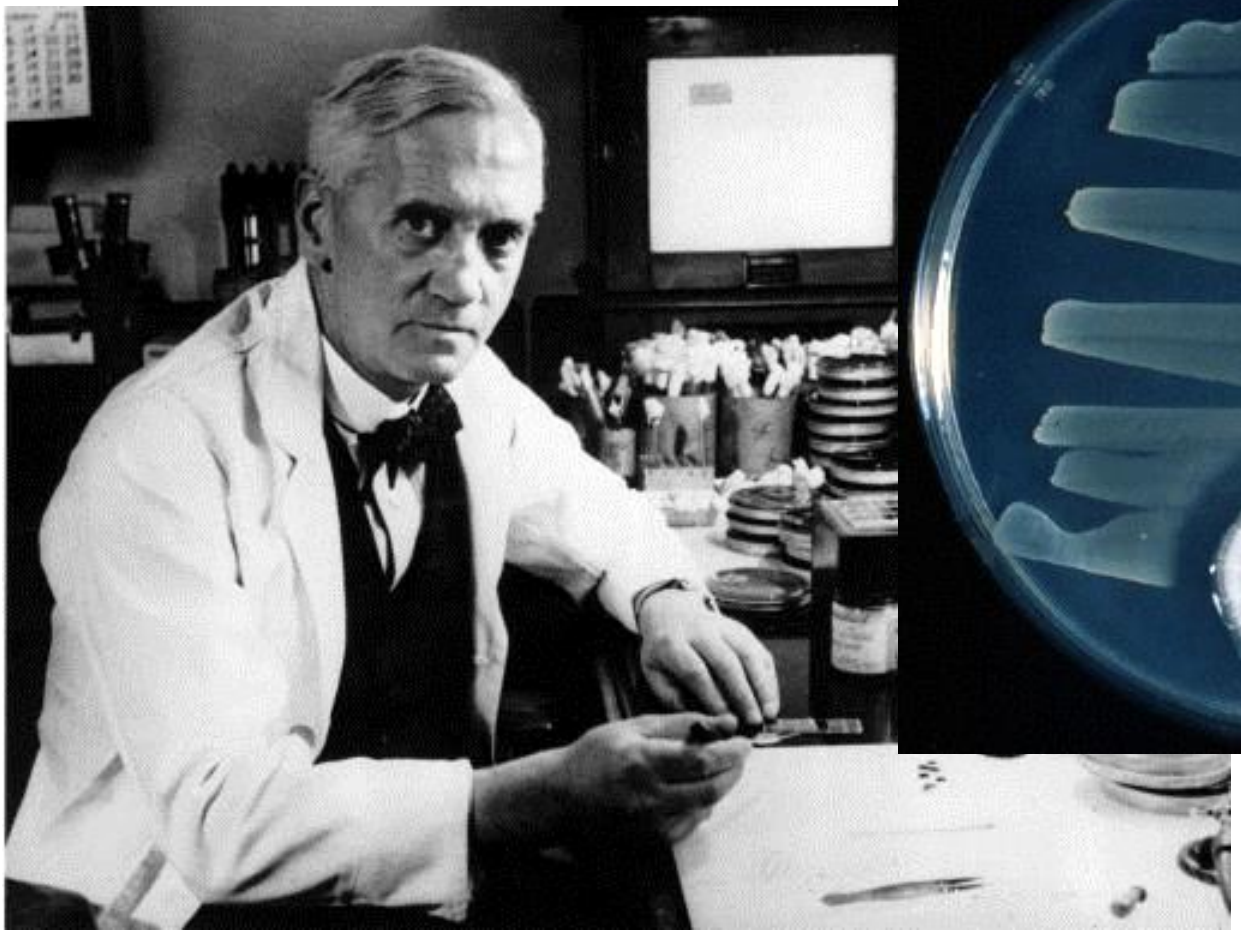
Fungi are eukaryotic, heterotrophic organisms that produce extracellular enzymes and absorb their nutrients.

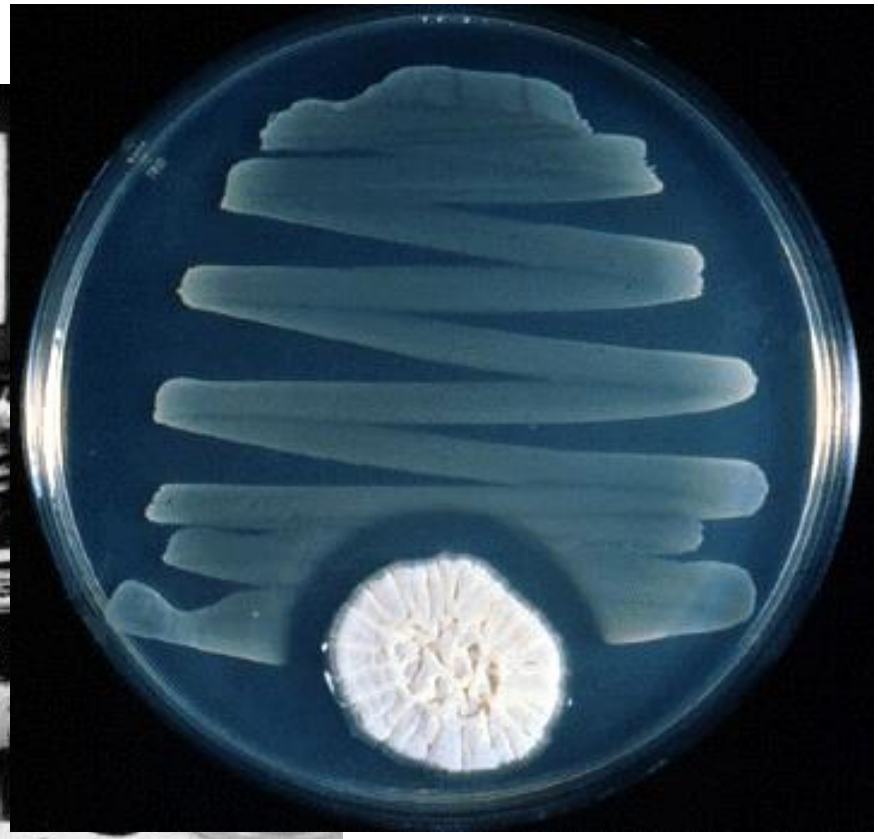
- Differ from bacteria and other prokaryotes and belong to Kingdom Mycota

  - Of the 80,000 fungal species less than 400 cause human disease and have medical importance
  - 50 species cause more than 90% fungal infections


BYLAND
BLUE

Fleming and Penicillin





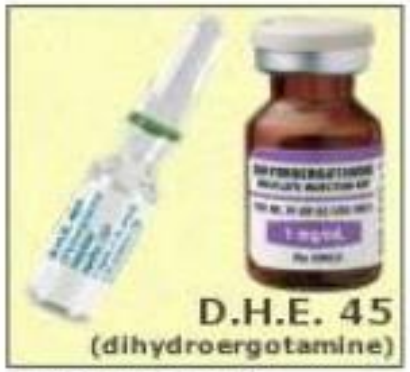
Useful Properties of Fungi

Source of food
e.g. mushrooms

Fermentation - Production of alcohol, bread, cheese
e.g. *Sacchromyces* spps

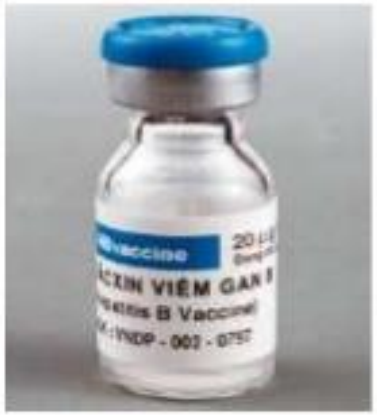
Antibiotic production
e.g. Penicillin from
Penicillium notatum

Useful Properties of Fungi



Photos: Teri Robert

Ergot from *Claviceps purpurea*, used to induce uterine contractions



Vaccines for Hepatitis B – *Sacchromyces cerevisiae*

Eukaryote cell

- At least one nucleus with nuclear membrane
- Endoplasmatic reticulum
- Mitochondria
- Secretory apparatus for secreting enzymes that degrade organic substrates into soluble nutrients

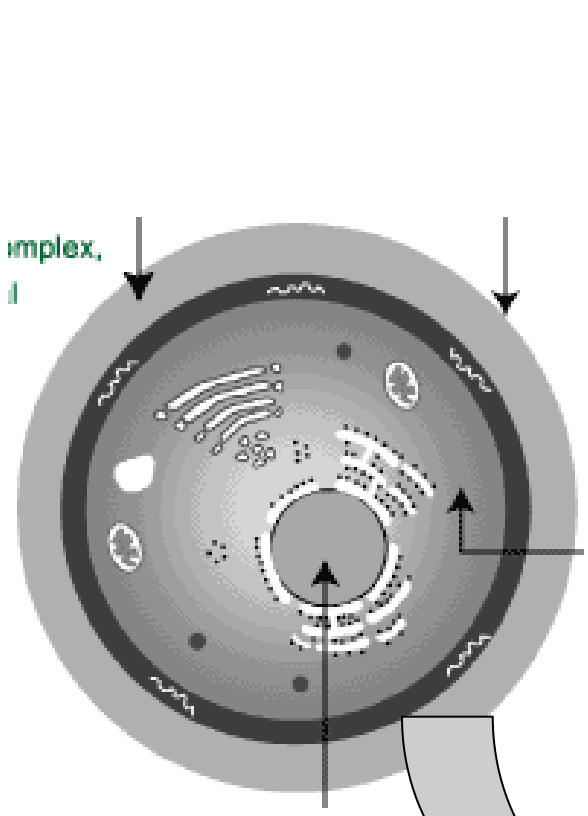
- Most fungi are obligate or facultative aerobes

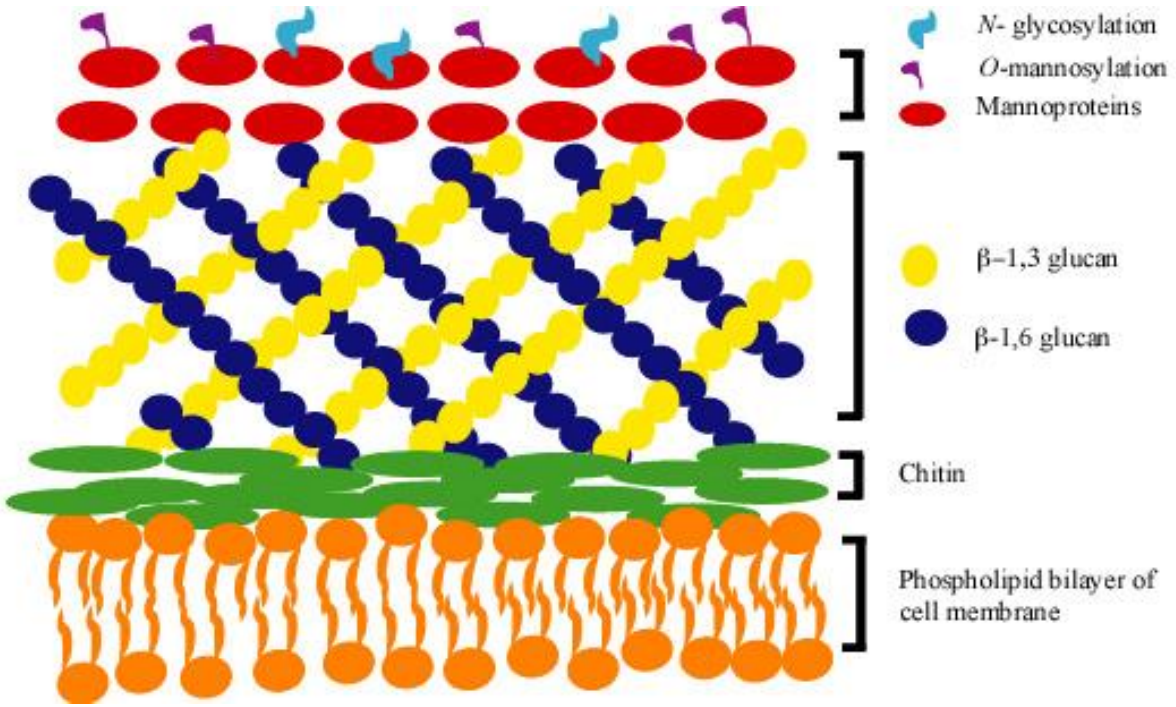
Fungi

- Cell wall containing chitin (rigidity & support) manan and other polysaccharides
- Cytoplasmic membrane contains ergosterols
- Posses true nuclei with nuclear membrane & paired chromosomes
- Unicellular or multicelular
- Divide asexually, sexually or by both

The Fungal Cell Wall

Rigid structure surrounding the cell





Cell walls are made up of <u>chitin</u> (polysaccharide)

Introduction to Medical Mycology

Most fungal pathogens are exogenous → live in water, soil and organic debris

- Fungi that are environmental saprobes can cause invasive disease in humans if they enter the human body
- Usually such organisms are carried in the air, inhaled into the pulmonary tree, and begin a localized invasive infection that may or may not disseminate further in the body

Introduction to Medical Mycology

- **Endogenous fungal pathogens**
- ***C. albicans*** is part of the normal human body flora, and it can become pathogenic if it moves from the body compartment where it performs its function as a normal commensal → predisposing factors
- Use of antibiotics, steroids, immunosupressive
- Causes can be cancer chemotherapy, radiation, trauma, concurrent viral ulcers

Introduction to Medical Mycology

Fungal pathogens can be divided into two general classes: **primary** pathogens

those caused by truly pathogenic fungi with the ability to cause disease in the normal human host when the inoculum is of sufficient size.

- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis*

Paracoccidioides brasiliensis

Opportunistic fungi, low virulence organisms, require the patient's defenses to be lowered before the infection is established.

- *Aspergillus sp.*
- *Candida albicans*

Cryptococcus neoformans

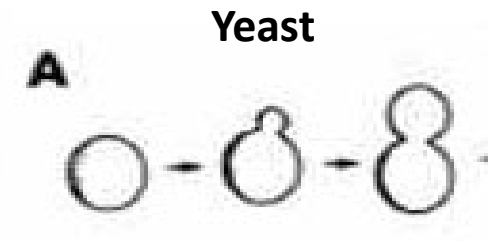
Classification

- Depending on cell morphology

- Yeasts
- Yeast like fungi
- Molds
- Dimorfic fungi

Classification of fungi

- Fungi grow in two basic forms: yeasts and molds
- Yeasts: unicellular fungi reproduce by budding or by fission
- Oval to round, spherical to elipsiod (diameter 3-15 µm), grow at 37°C
- The basic building block conidia (blastoconidia)

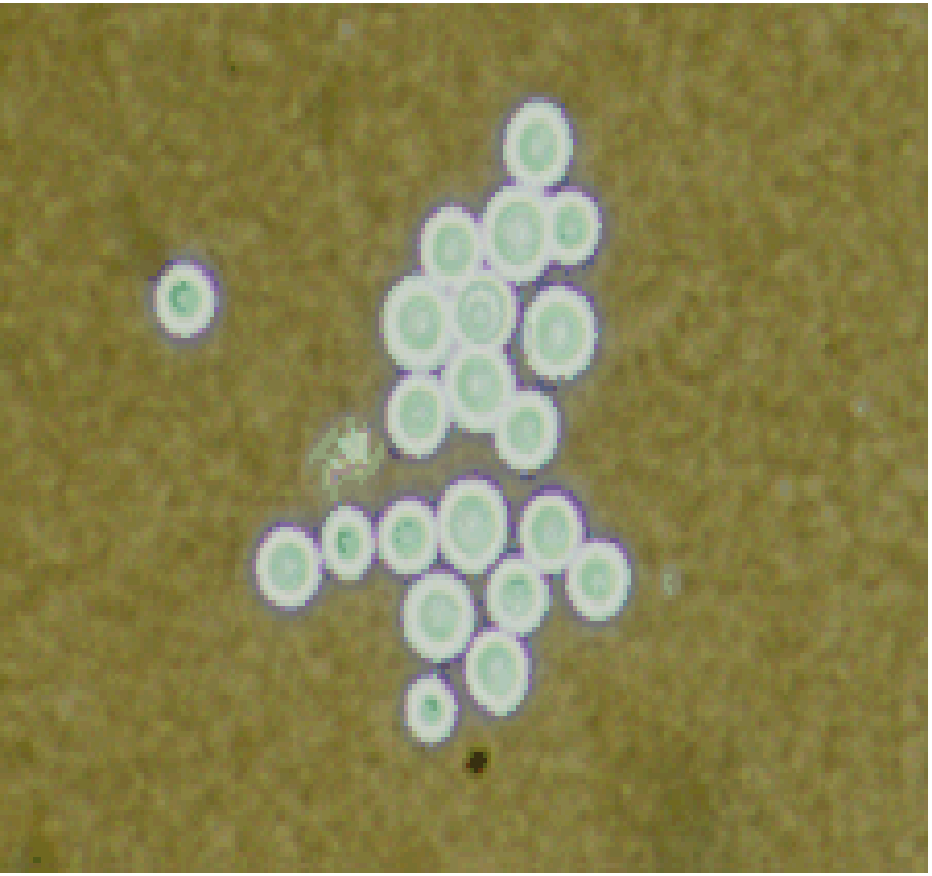


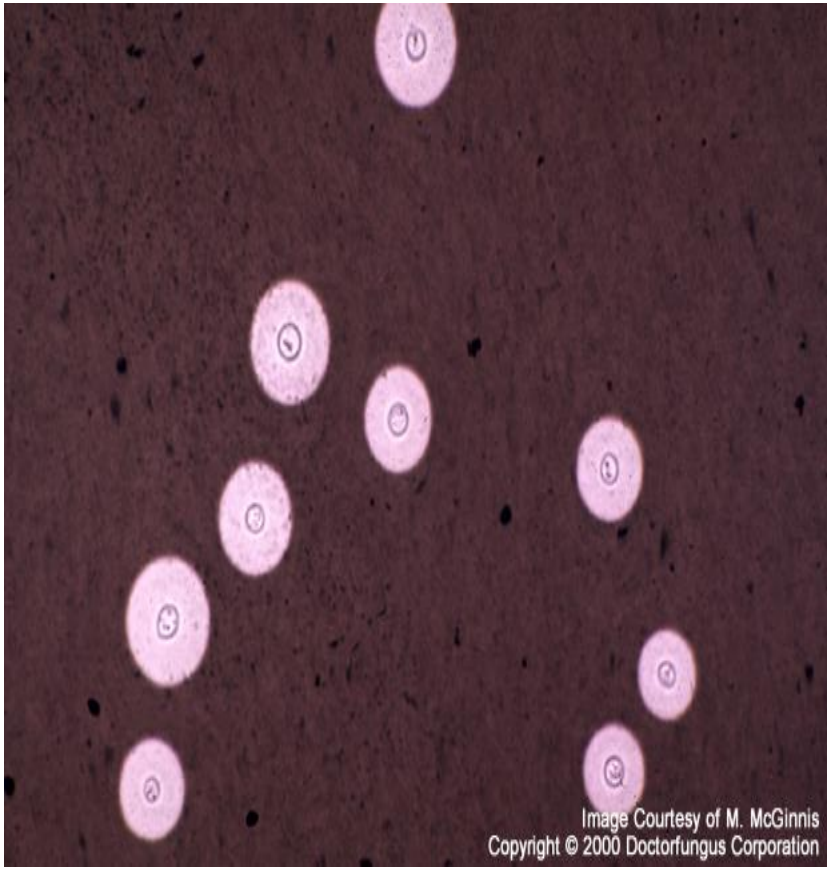
Yeasts

- Unicelular fungi which reproduce by budding

- On culture – pruduce smooth, creamy colonies e.g. *Cryptococcus neoformans* (capsulated yeast)

Capsulated Yeast / Cryptococcus neoformans (India ink test)





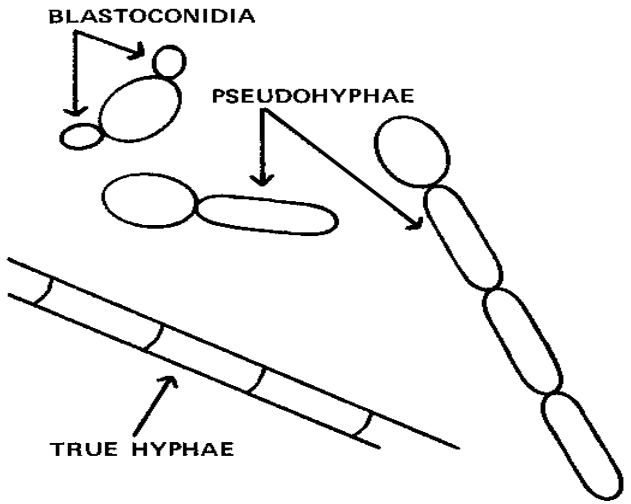
Yeasts

- Unicellular fungi with single nucleus
- Reproduce asexually, often by budding
- Have no flagella but possess all the eukaryotic organelles
- Some yeast are opportunistic pathogens, cause disseases in immuno-compromised individuals
- Yeast are used in preparation in the variety of foods

Yeast like fungi

- Grow partly as yeasts and partly as elongated cells resembling hyphae which are called pseudohyphae (chains of elongated buds of blastoconidia)

- *Candida albicans*

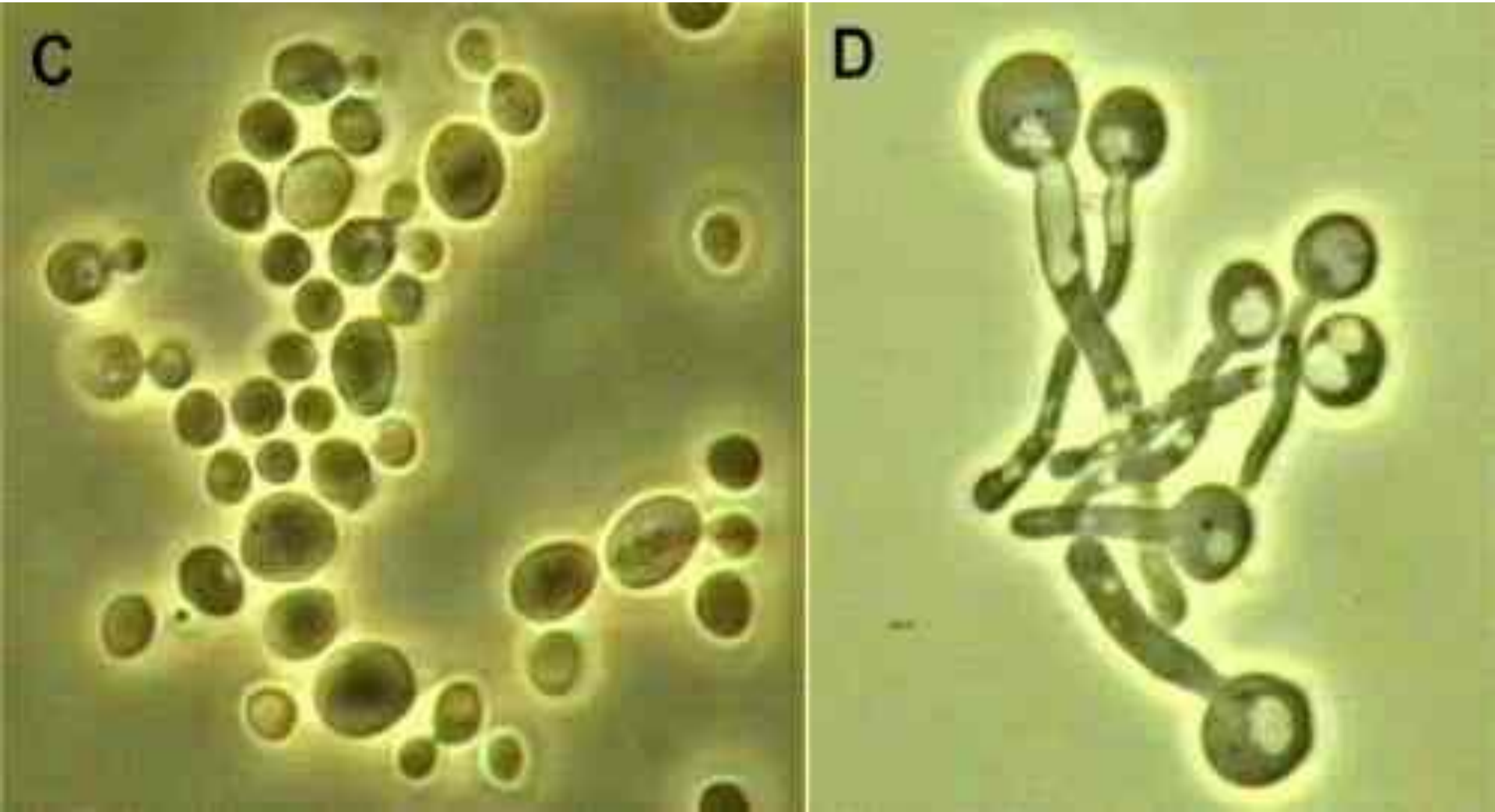


Candida Pseudohyphae

Yeast Reproduction

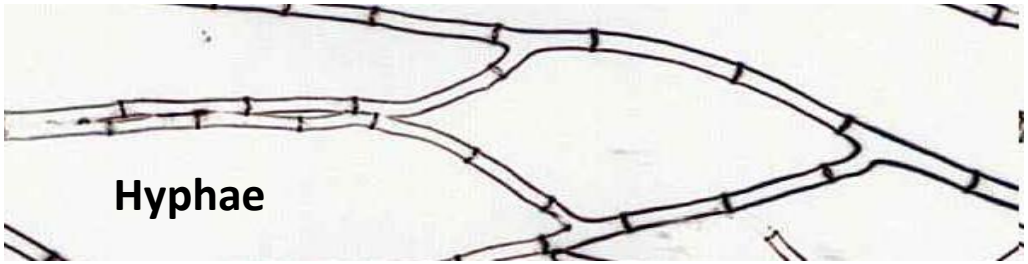
- FISSION
- “even” reproduction, nucleus divides forming two identical cells, like bacteria
- BUDDING
- “uneven” reproduction, parent cell’s nucleus divides and migrates to form a bud and then breaks away

Budding Yeast/Germ Tubes

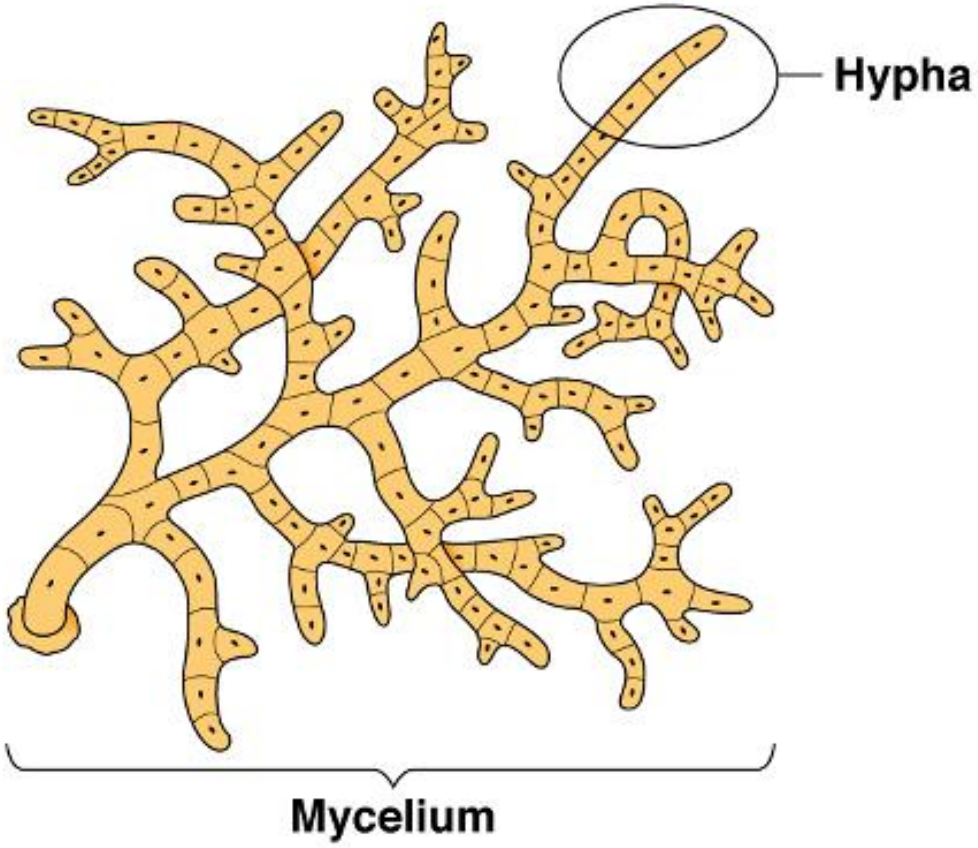


Classification of fungi

- Molds: multicellular fungi, filamentous, produce hyphae and mycelium
- Hyphae are branching cylindric tubules, varyng from 2-10 µm
- Mycelium is mass of hyphae

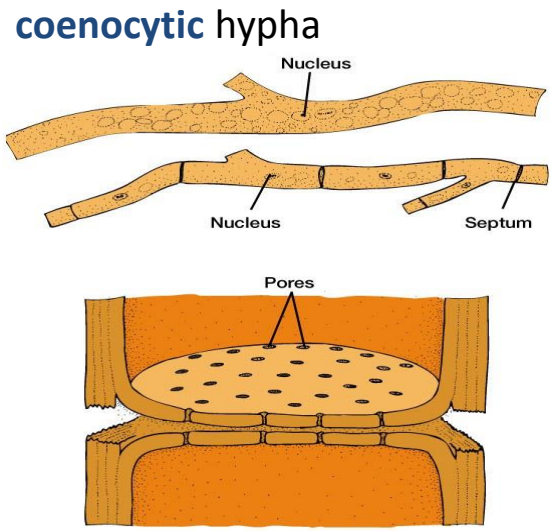


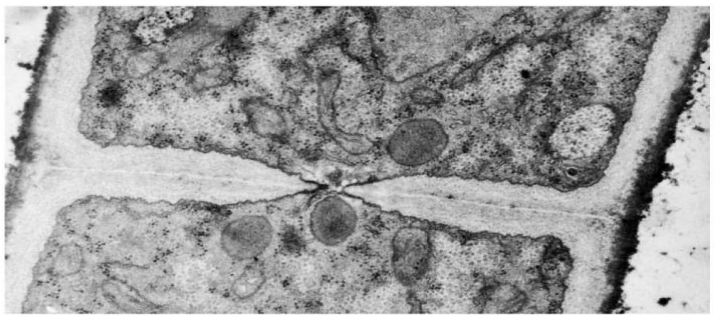
Hypha/Mycelium



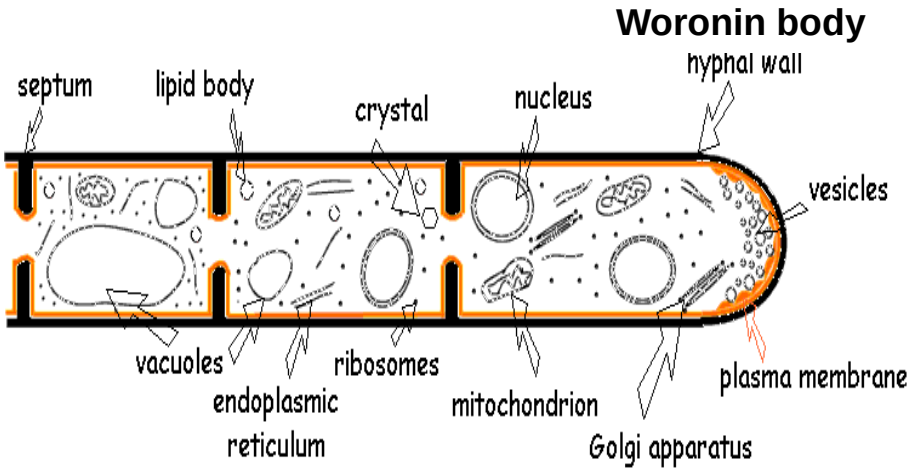
Molds

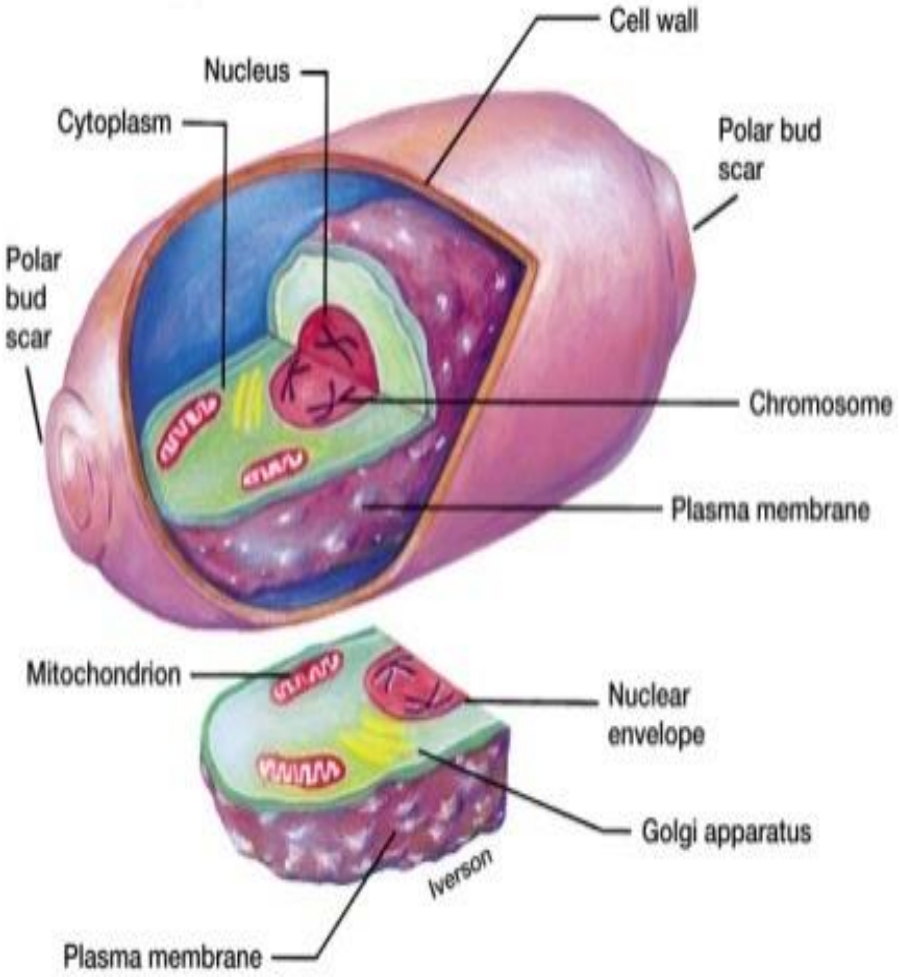
- Filamentous fungi where cells are called hyphae
- Hyphae often form a tangled mass or tissue-like aggregation called mycelia; may be **coenocytic** (no cross walls) or have septa (cross walls) → **septate**





Basic Structure

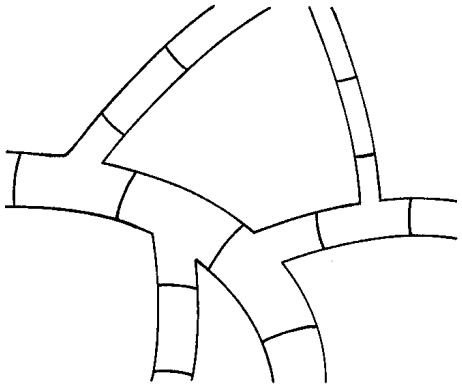




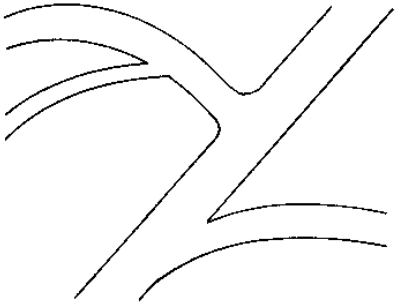
Hyphal Tip

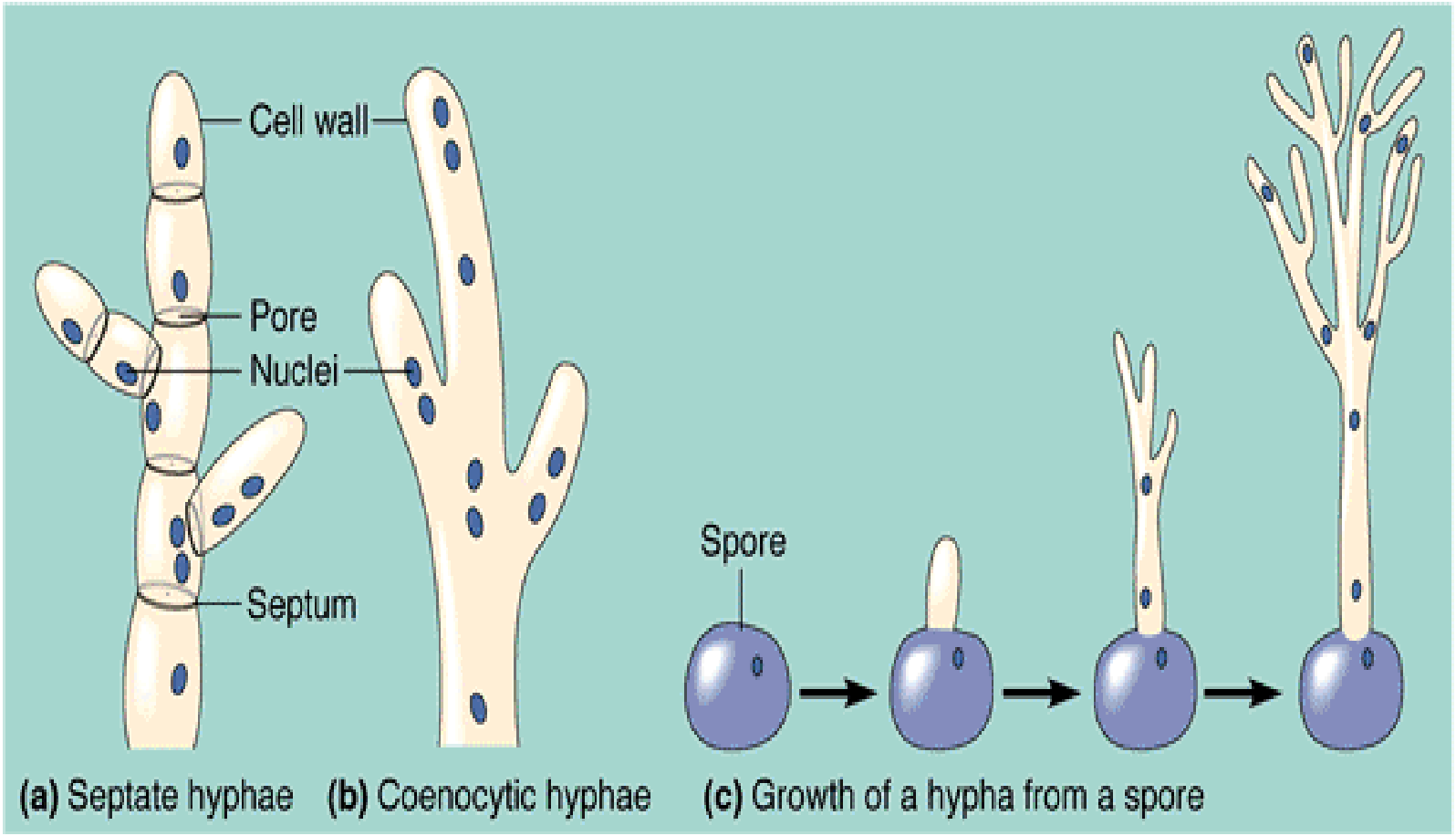
Hyphae

- Septate



- Aseptate

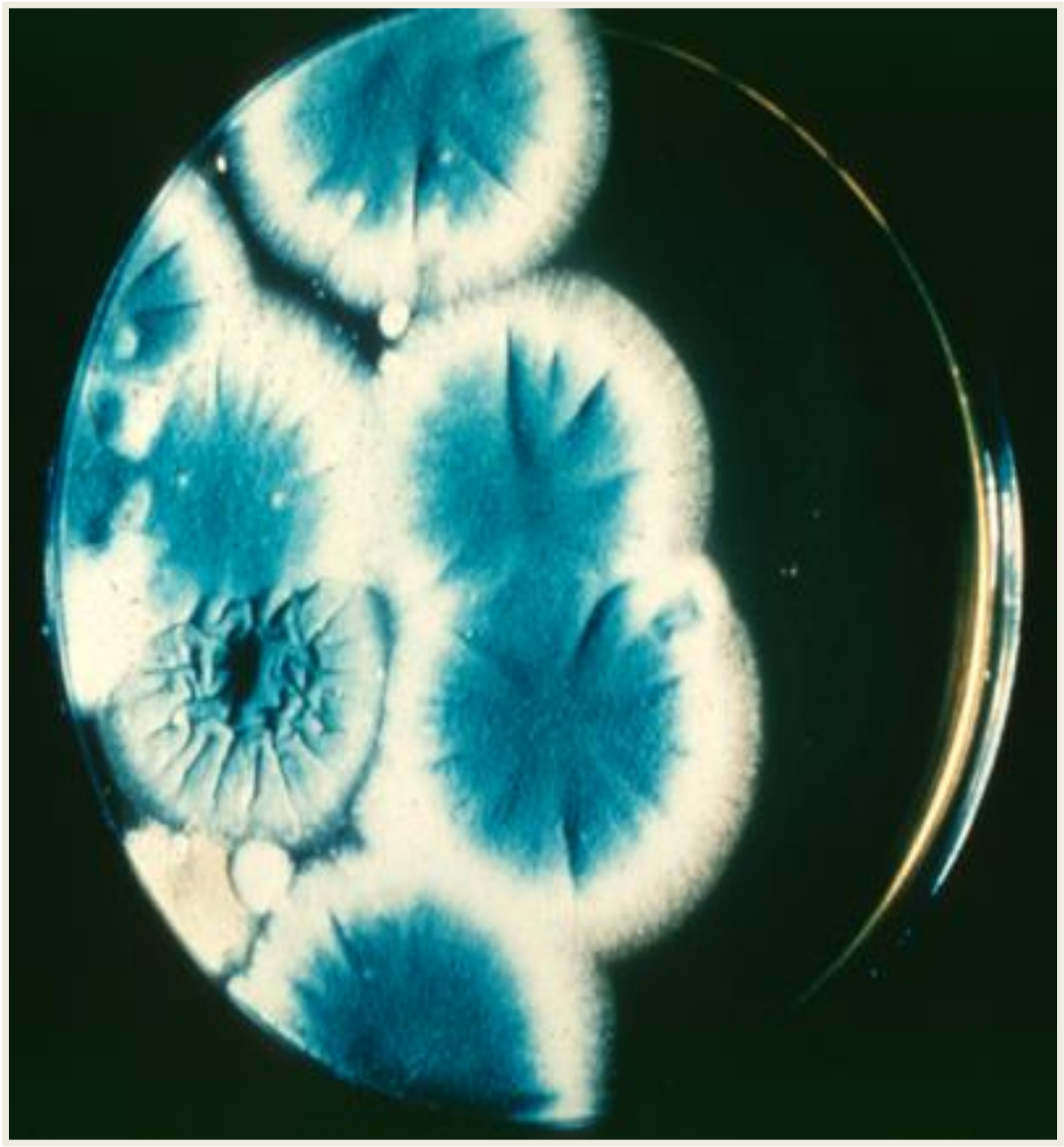




Molds

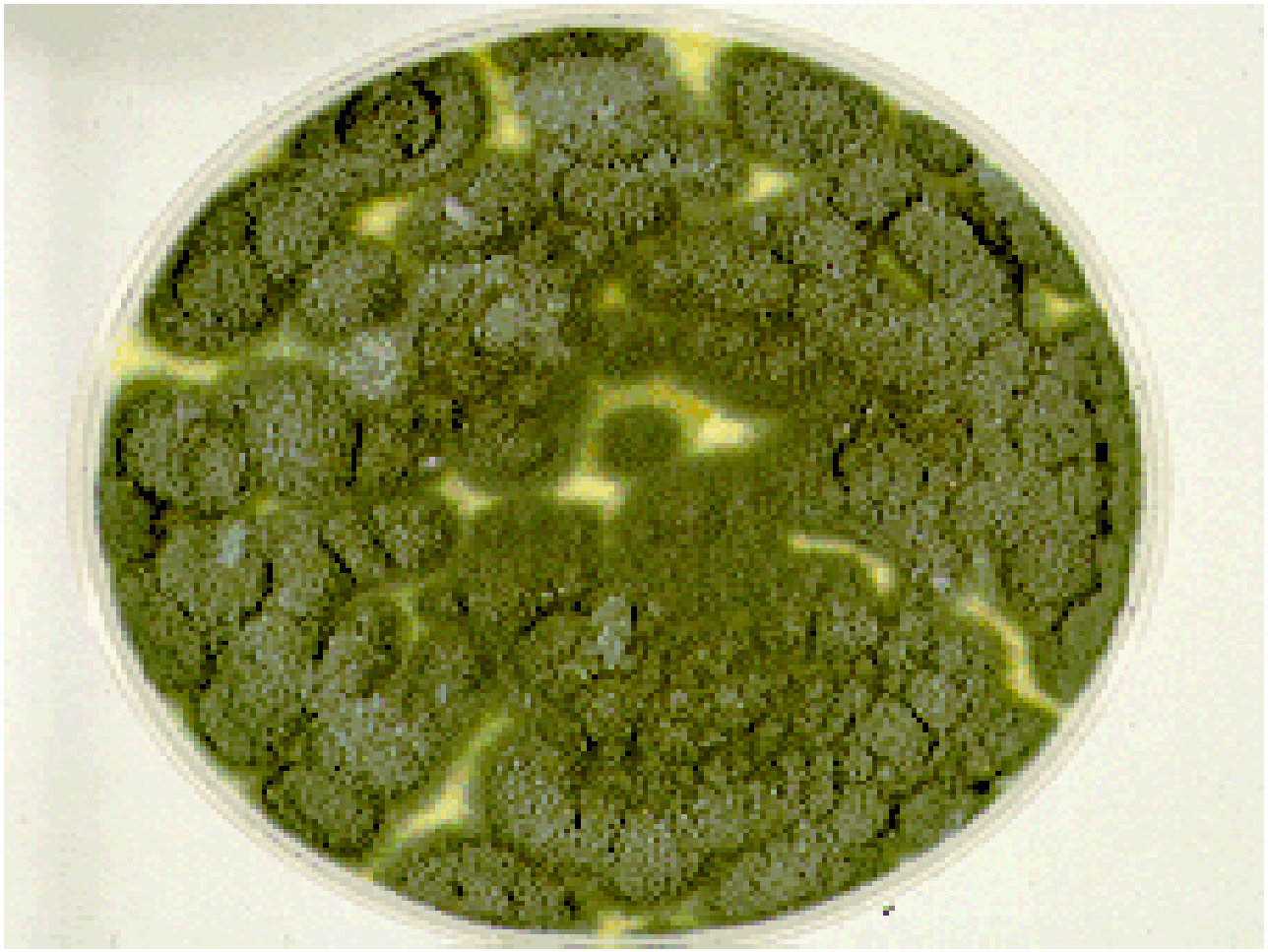
- Mycelium can be vegetative (basal)
- Composed of cells involved in catabolism and growth.

- Reproductive (aerial)
- Composed of cells involved in reproduction (produce spores)
- Reproduce by formation of different types of spores



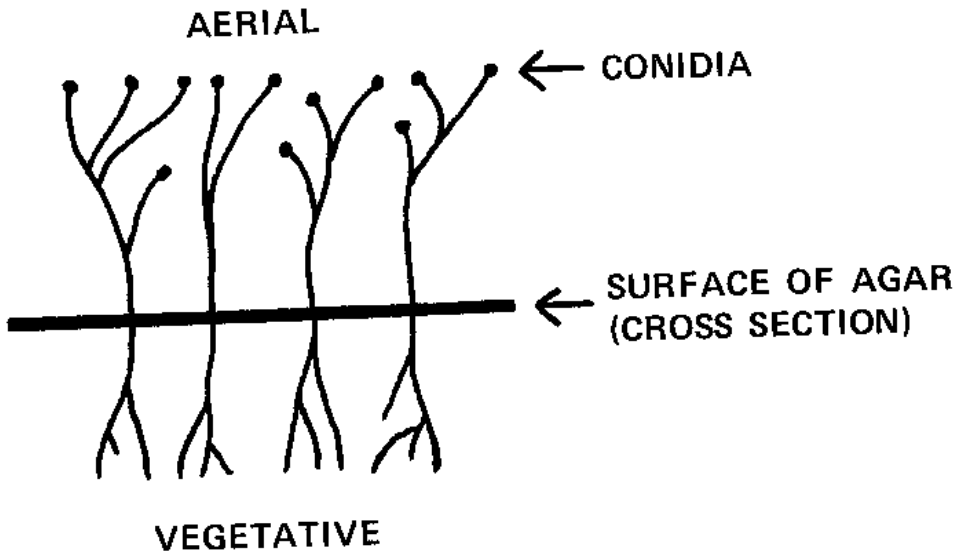
Aspergillus sp.

Penicillinum spp



Mycelium

- Mass of branching intertwined hyphae
  - Vegetative
  - Aerial
  - Fertile



Vegetative types

- Favic chandeliers
- Nodular organs
- Racquet hyphae
- Spiral hyphae

Dimorphic Fungi

- Dimorphic: grow in two forms, as moulds (environment) or yeasts (in human host)
- Dimorphic fungi (thermally dimorphic fungi) : mold phase & yeast phase
- Yeast Form
  - Parasitic form
  - Tissue form
  - Cultured at 37 C
- Mycelial Form
  - Saprophytic form
  - Cultured at 25 C
  - soil

Dimorphic Fungi

- Cause endemic mycoses - those fungus infections with a limited geographic distribution. They are all caused by dimorphic fungi, and caused systemic infections.
- Primary pathogen
- Low importance in Europe
- Coccidioidomycosis, Histoplasmosis, Blastomycosis, Paracoccidioidomycosis

Fungi That Cause Human Infection

- Yeast: *Candida albicans, Cryptococcus neoformans*
- Mould: *Aspergillus, Penicillium, Fusarium, Scedosporium*
- Dimorphic: *Histoplasma capsulatum*

Associated with Fungi

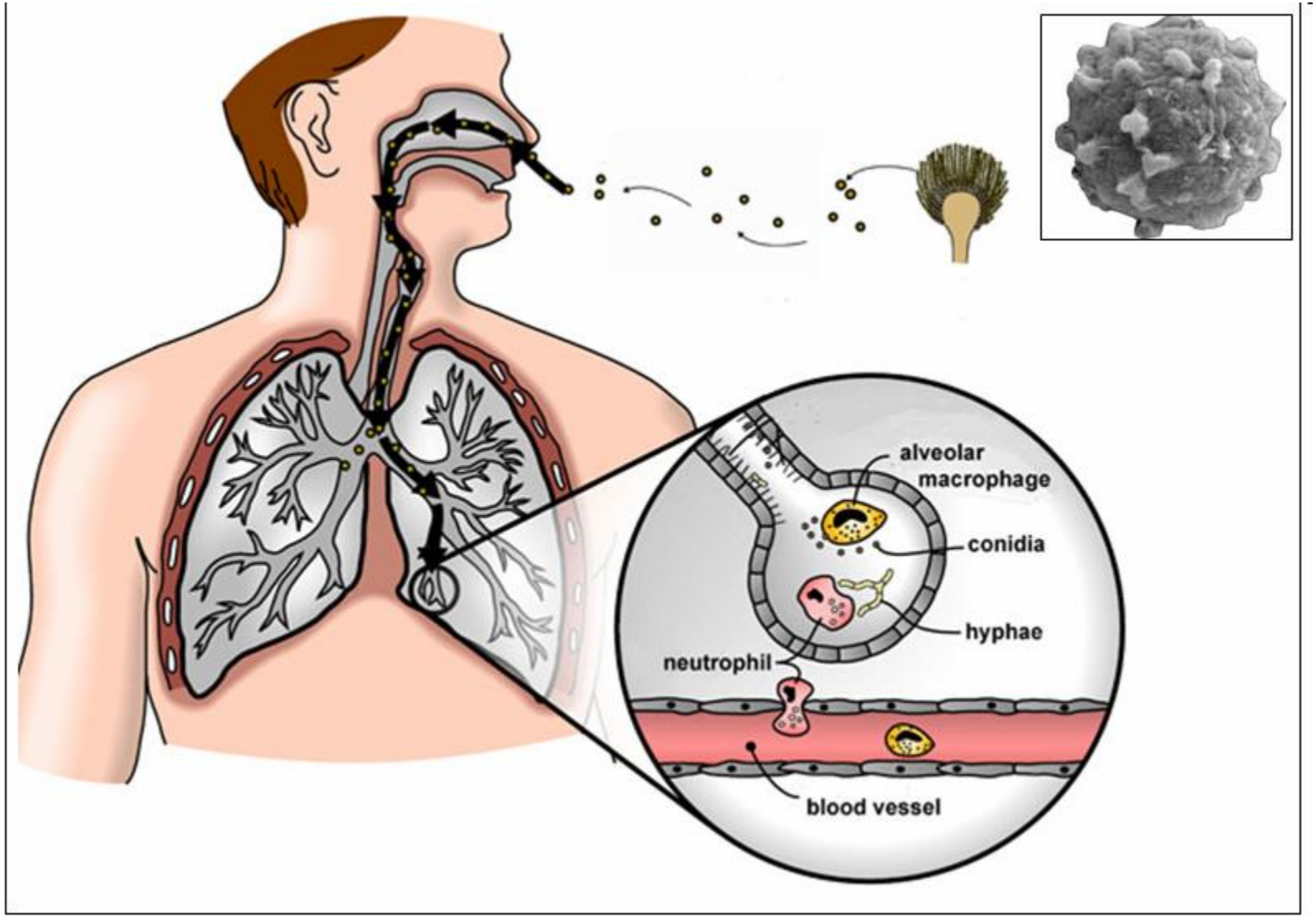
- **Sick building syndrome**
- **"Curse of the Pharaohs"**

"Cursed be those that disturb the rest of Pharaoh. They that shall break the seal of this tomb shall meet death by a disease which no doctor can diagnose." (Inscription reported to have been carved on an Egyptian royal tomb)

Pathogenesis – portal of entry

- SKIN
- HAIR
- NAILS
- RESPIRATORY TRACT
- GASTROINTESTINAL TRACT
- URINARY TRACT

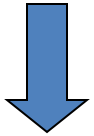
Primary Route of Infection



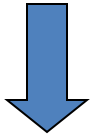
Virulence factors favor

fungal adhesion

colonization

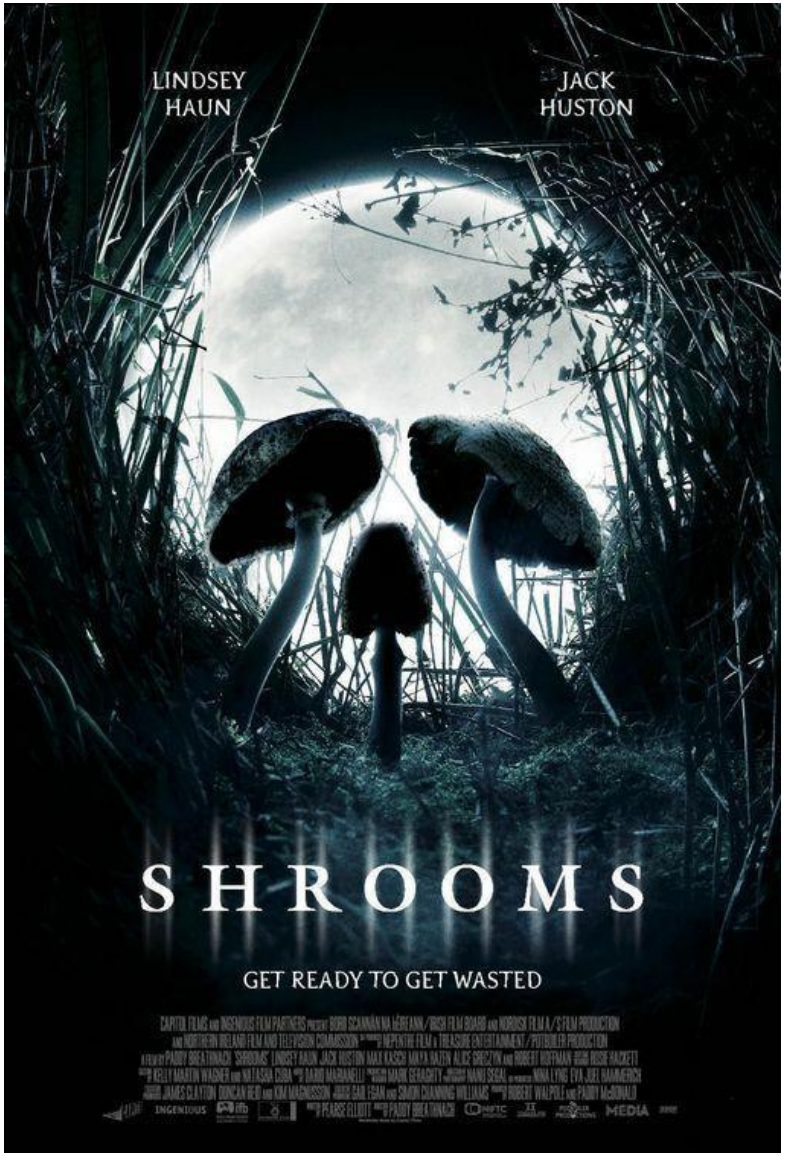


dissemination and the ability to survive in hostile environments



and elude the immune response mechanisms of the host

Mycotoxicosis



Mycotoxins

- Mycotoxins are low-molecular-weight secondary metabolites of fungi
- Often produced by food spoilage organisms or in basidiocarps (Mushrooms)
- mycotoxins are an important chronic dietary risk factor, cause acute or chronic intoxication and damage
- Aflatoxins - *Aspergillus* spp.; Citrinin – *Penicillium* spp.; Ergot Alkaloids – *Claviceps* spp. – Ergotism;

Mycotoxins

- Aflatoxins are a small group of mycotoxins produced by the fungi from the genus *Aspergillus,* produces a natural occurring human carcinogen, bis-furanocumarin that is found in aflatoxin.
- Aflatoxins are known contaminants on corn, peanuts, tree nuts, cottonseed and certain meats and milks.

Classification of Mycoses

- Clinical classification

- Superficial
- Cutaneous
- Subcutaneous
- Systemic (invading the internal organs)

Classification of human fungal infections

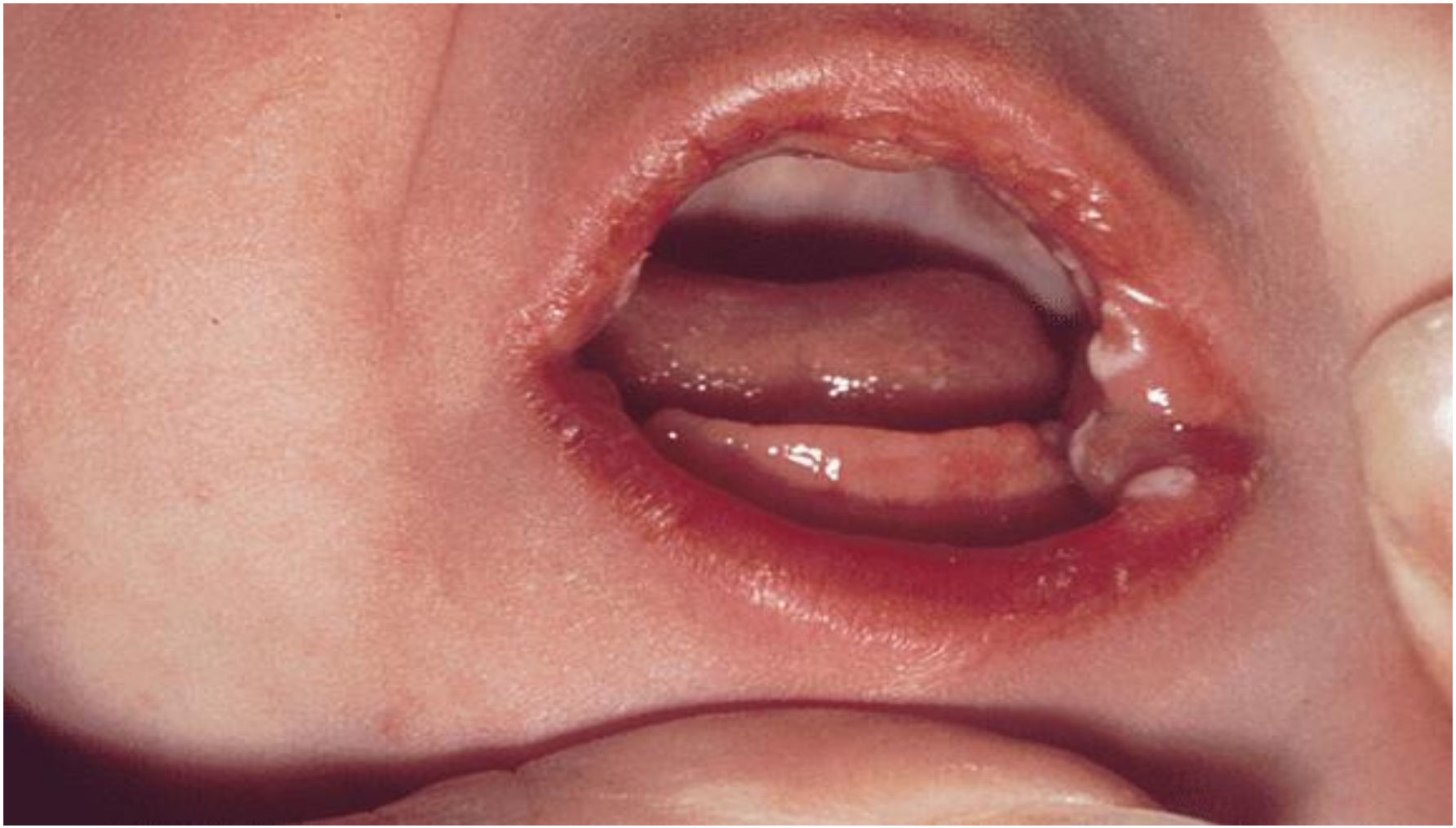
- Superficial: confined to the outermost layers of the skin and hair,

  ringworm (dermatophytes), thrush (*Candida* species),

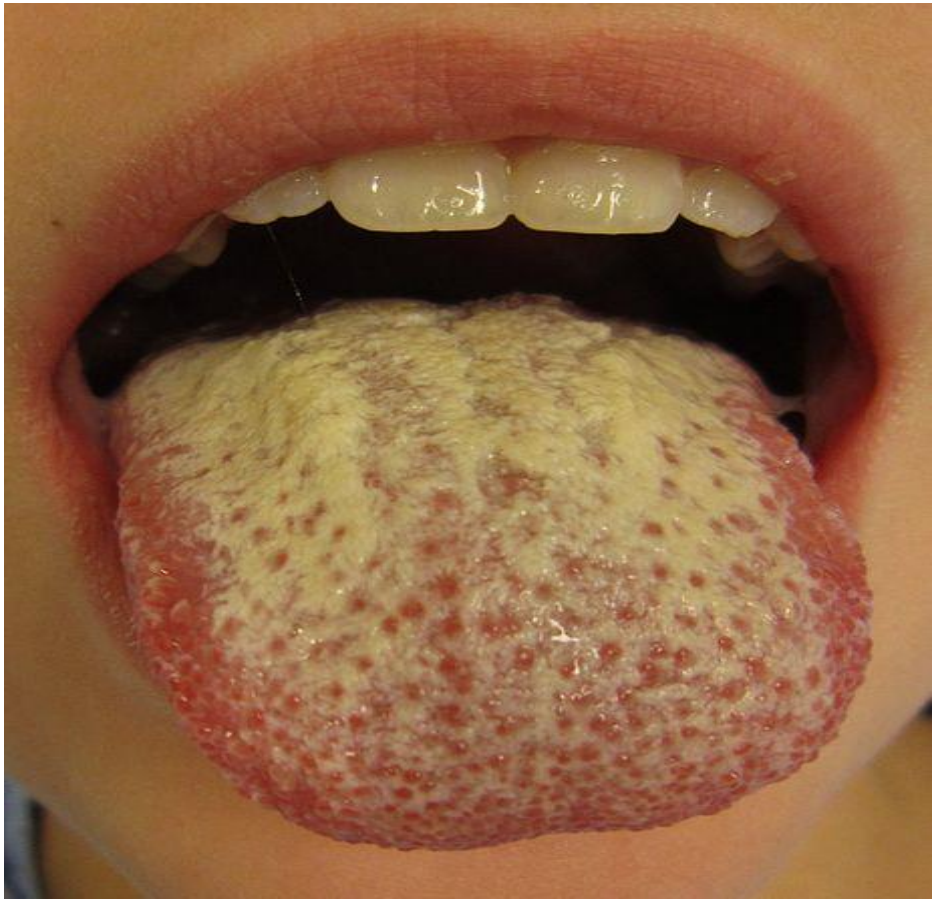
Tinea – describes an infection caused by a dermatophyte (ringworm fungi)

Dermatophyte include *Trichophyton*, *Microsporum* and *Epidermophyton*

Candida Trush



Oral candidiasis (trush)



Superficial and Cutaneous Mycoses

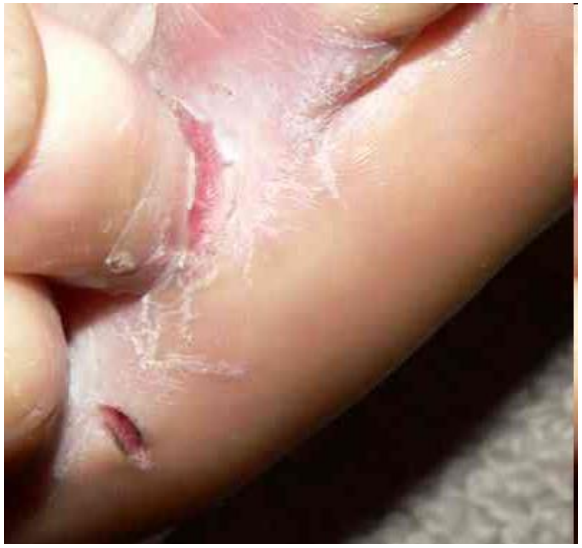
Skin, hair and nails

Rarely invade deeper tissue

Dermatophytes

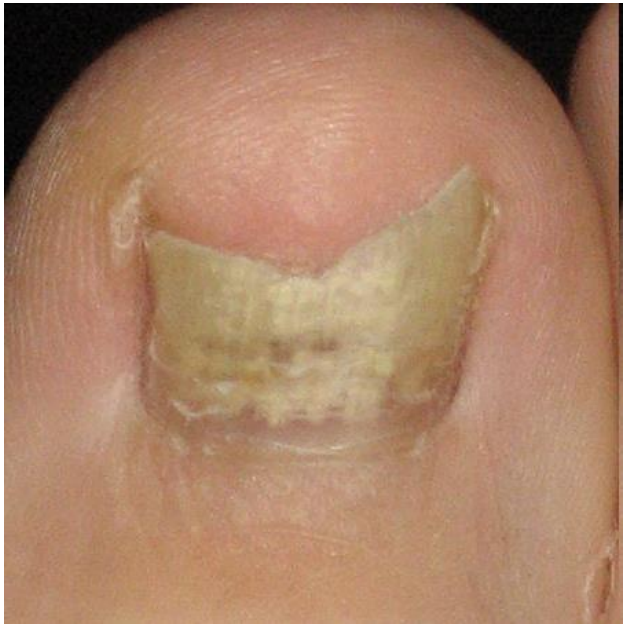
- Specialised pathogenic fungi
- Worldwide distribution
- Key feature is keratin degradation
- Tend to grow at 28 °C

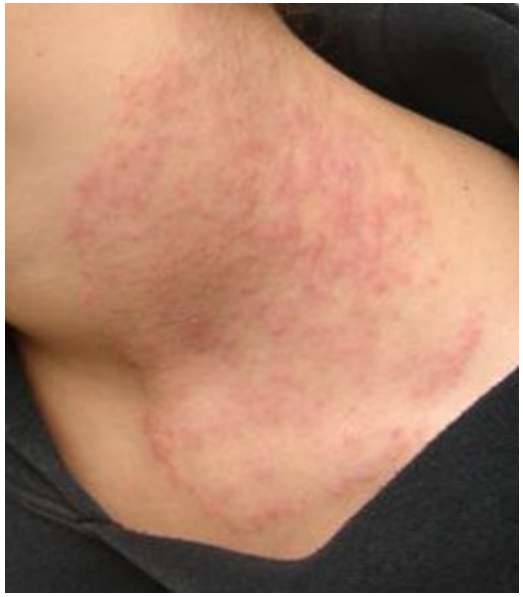
Dermatophytes



Tinea pedis

Onychomycosis

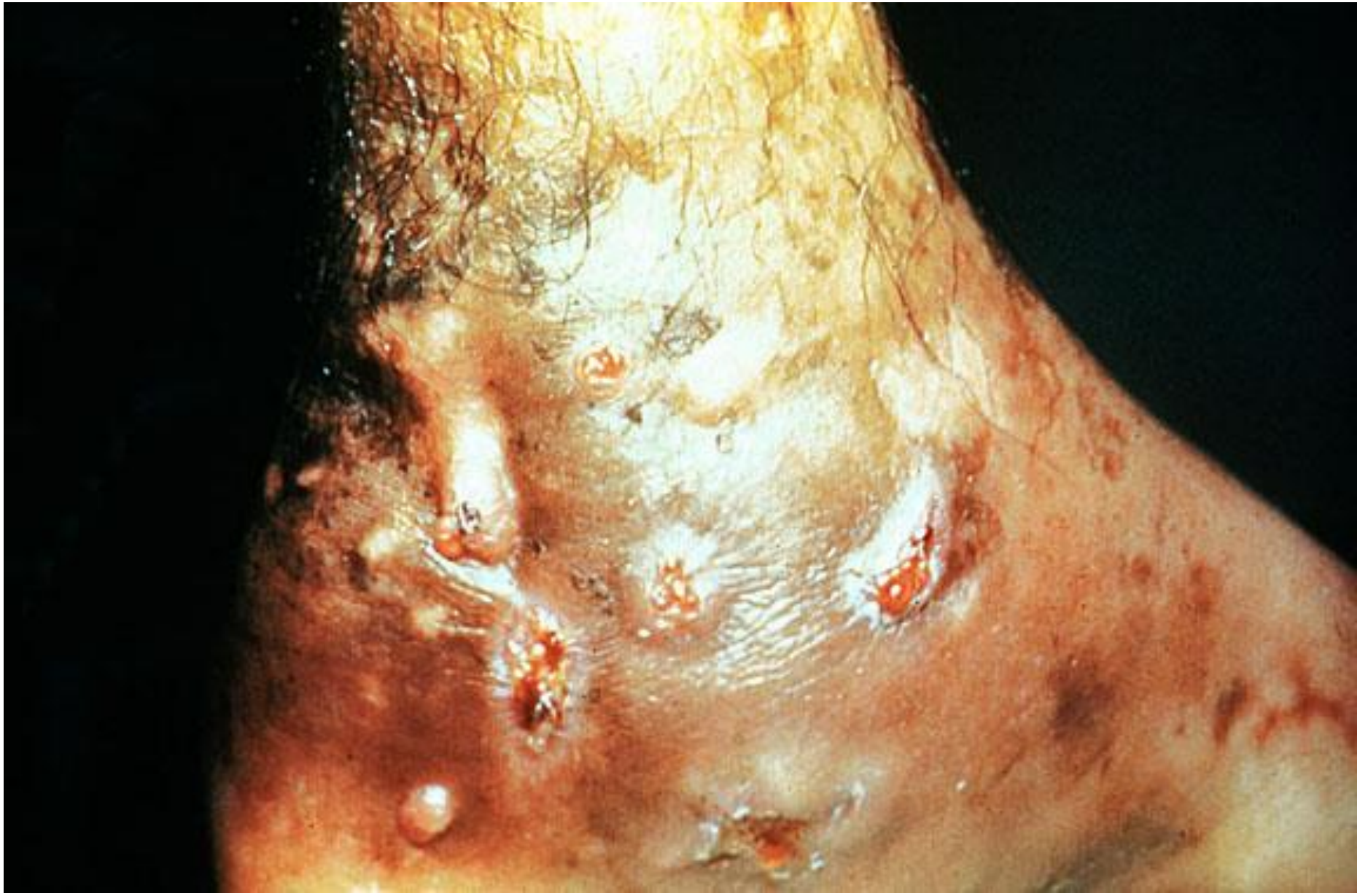




Tinea corporis

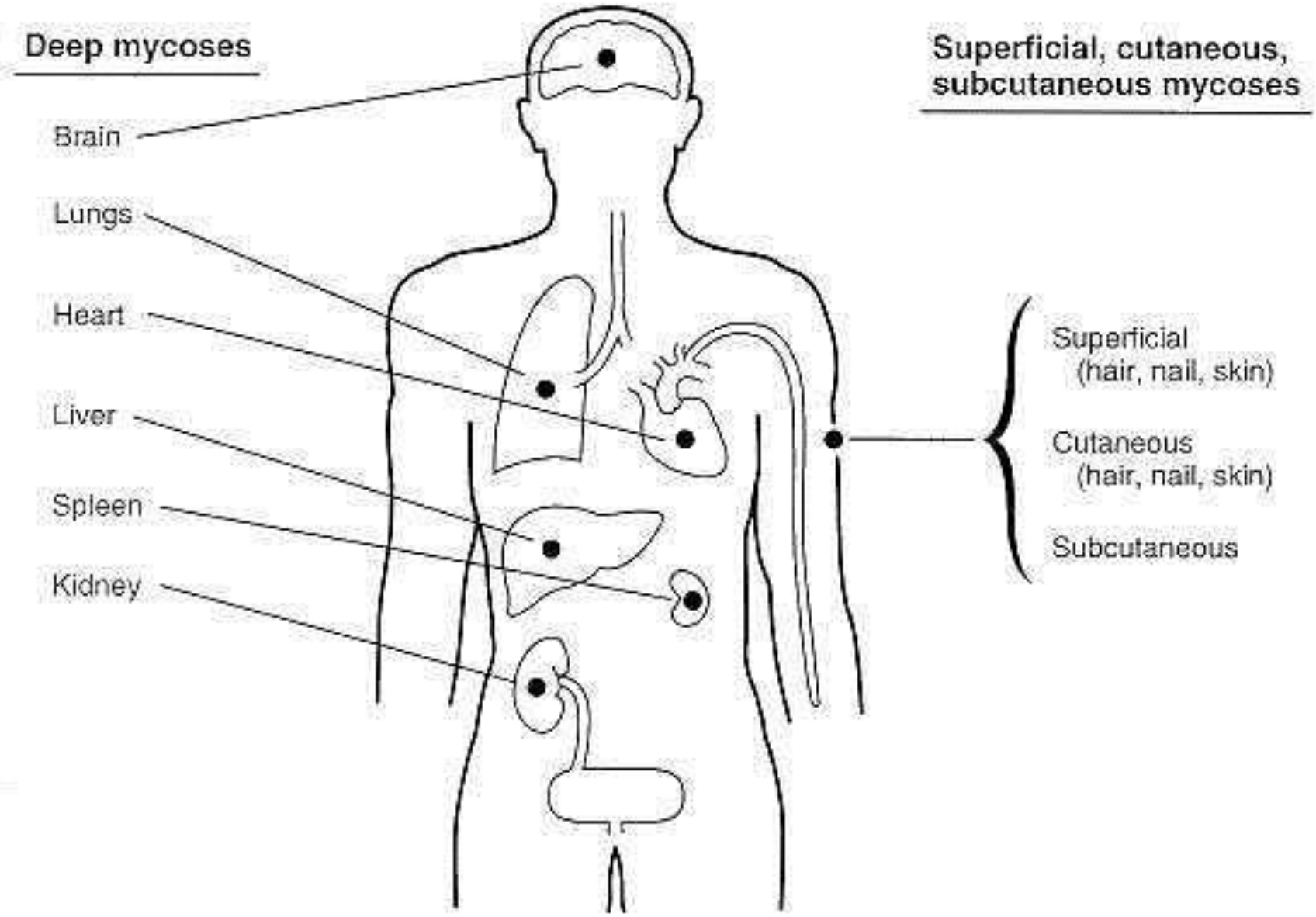
Subcutaneous Mycoses

- Confined to subcutaneous tissue and rarely spread systemically. The causative agents are soil organisms introduced into the extremities by trauma.
- Subcutaneous: involve the dermis of the skin, deep tissues or bone. Usually found in tropics/sub-tropics where caught walking barefoot eg, mycetoma.



Systemic Mycoses

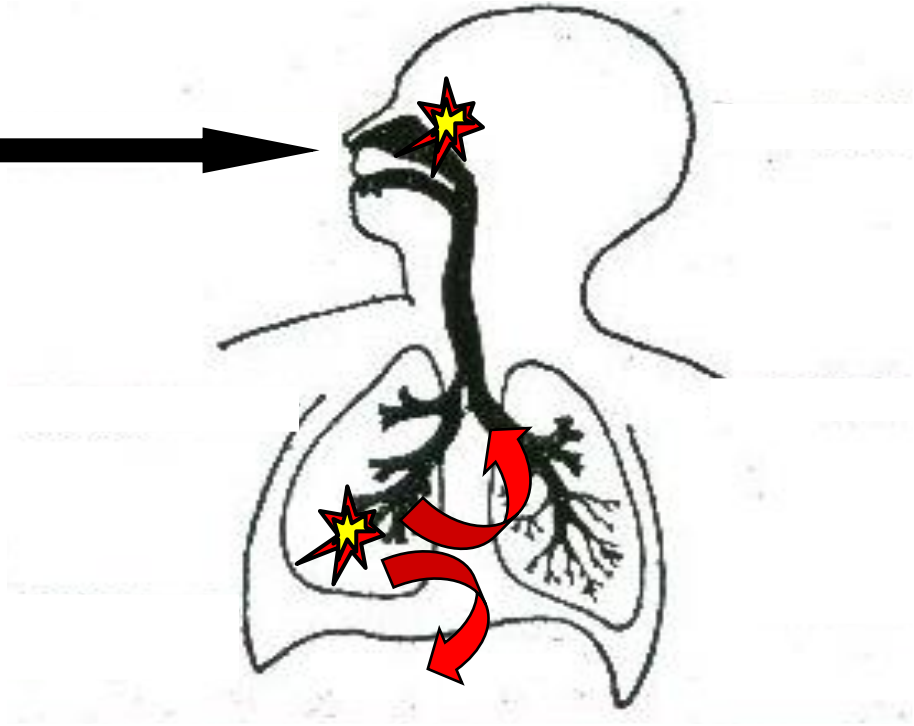
- Involve skin and deep viscera
- May become widely disseminated
- Predilection for specific organs
- Systemic: due to pathogenic (*Histoplasma*) or opportunistic (*Aspergillus*) fungi


Deep mycoses
Brain
Lungs
Heart
Liver
Spleen
Kidney
Superficial, cutaneous, subcutaneous mycoses
Superficial (hair, nail, skin)
Cutaneous (hair, nail, skin)
Subcutaneous

Inhalation of Fungal Spores

Spores
8 µm

2-4 µm



DISEASE SPECTRUM

ALLERGY

SINUSITIS

PULMONARY MYCOSES

DISEMINATED MYCOSES

LABORATORY DIAGNOSIS OF MYCOSES

- Direct microscopic examination
- Culture
- Fluorescent antibody
- DNA probes
- Biopsy and histopathology

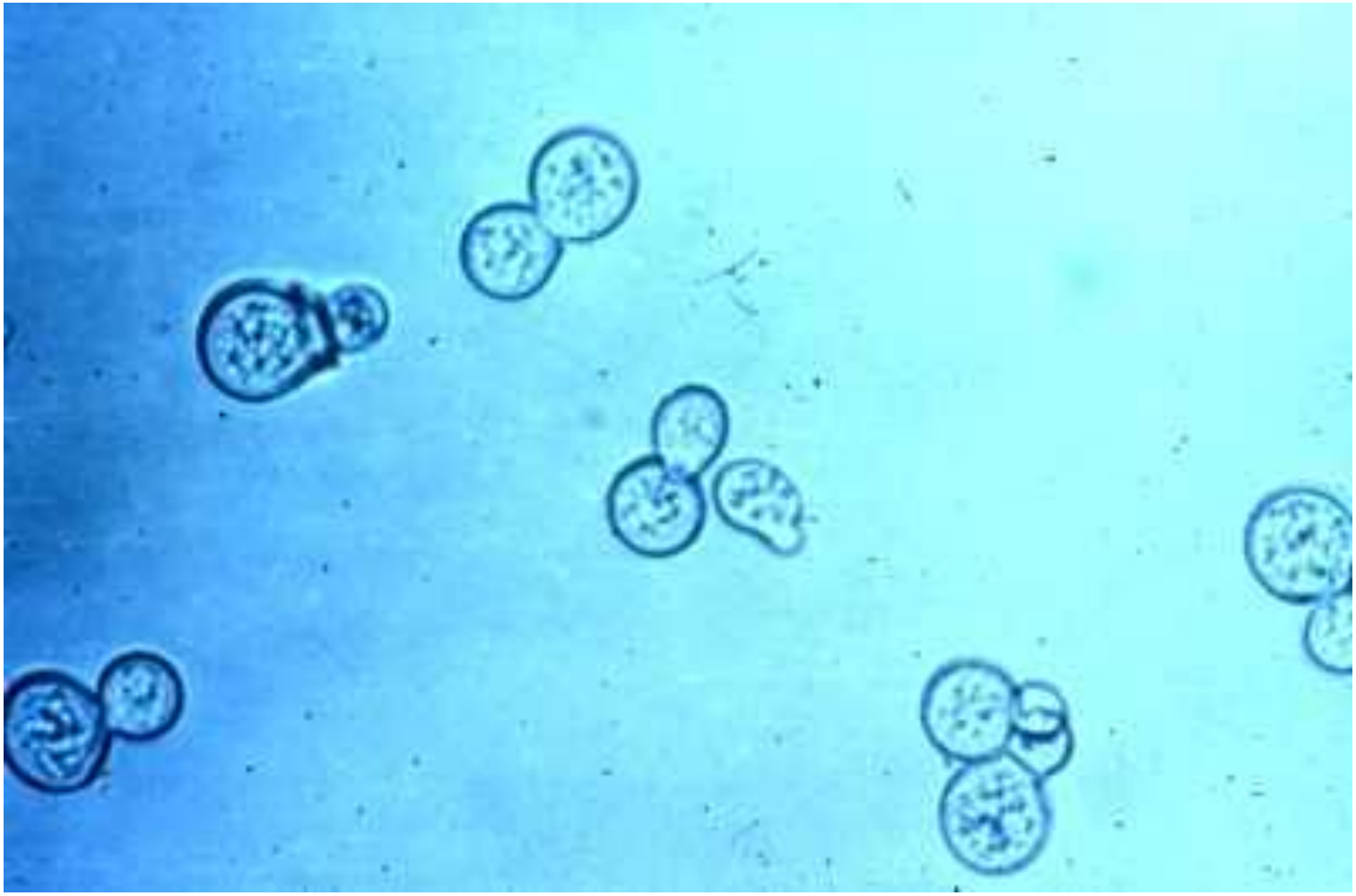
- Serology

Direct microscopic examination

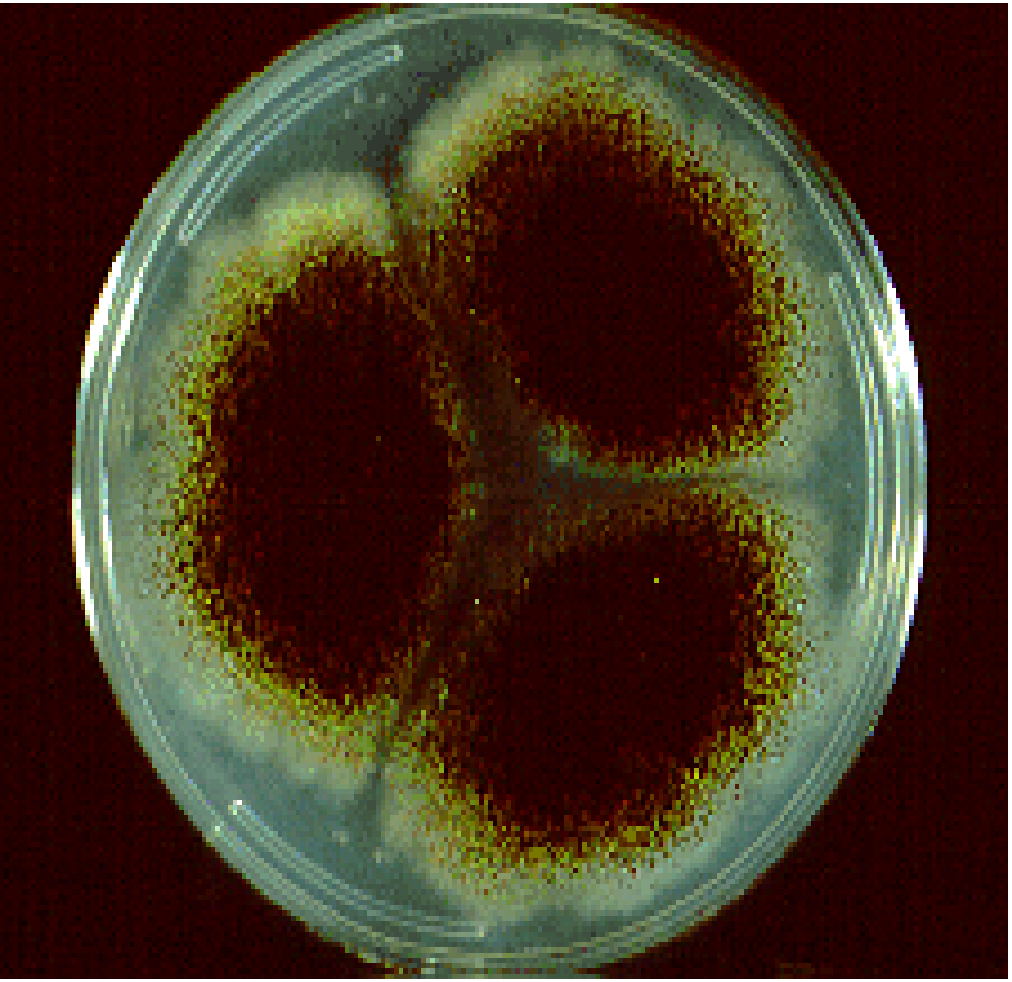
Direct examination:

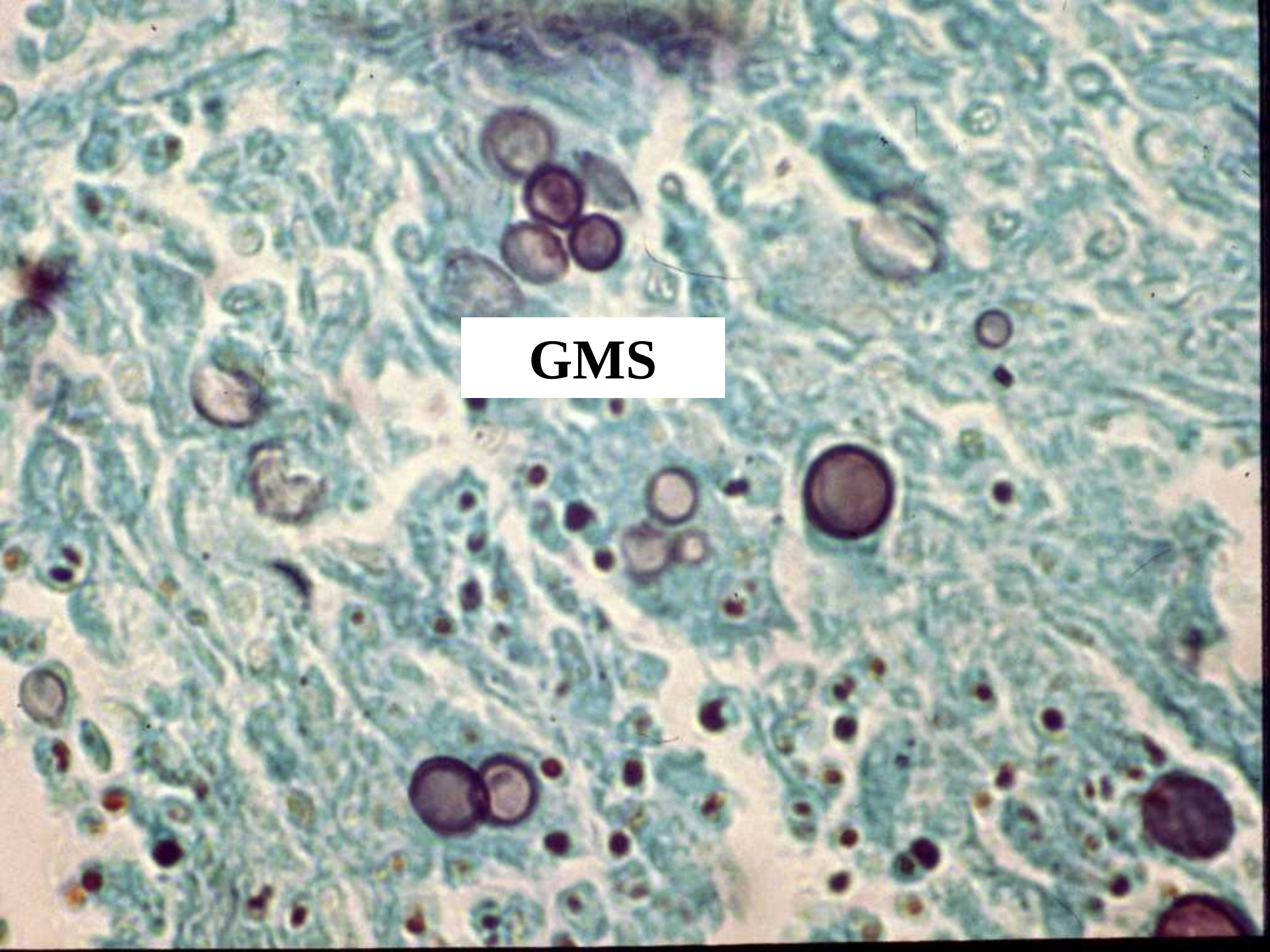
- wet mount
- 10% KOH preparation wet mount
- Lactophenol cotton blue wet mount
- Gram or Gomori stain
- India ink stain
- Fluoroscent antibody

KOH Wet Mount

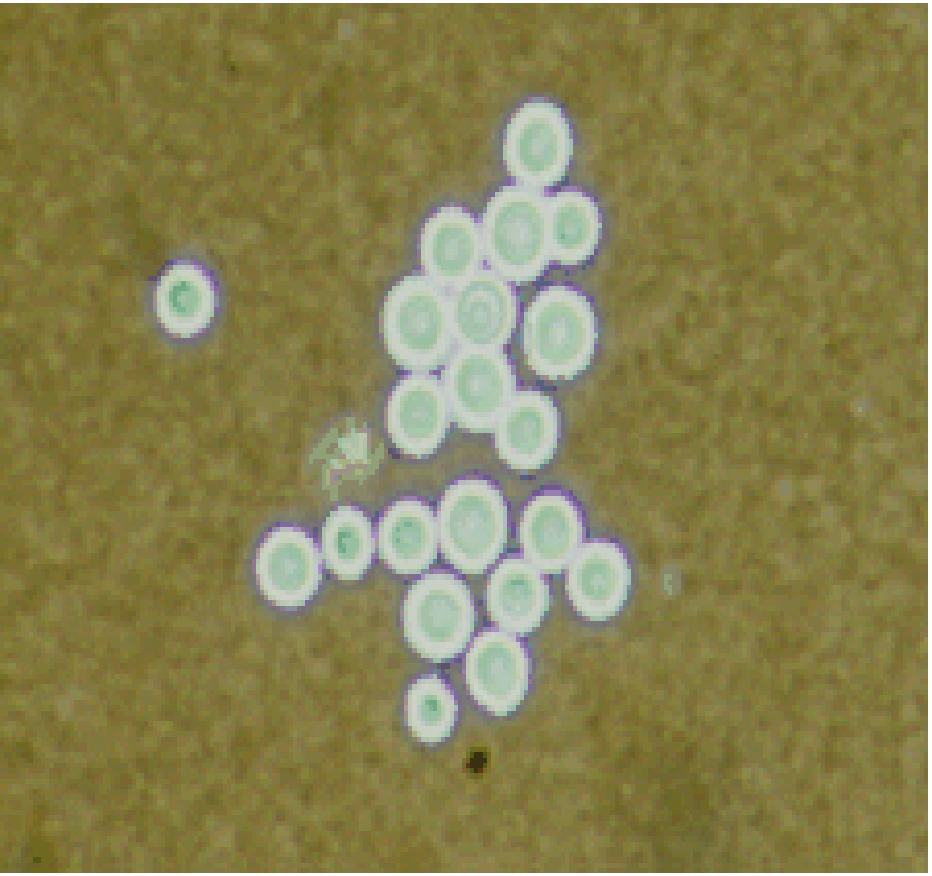


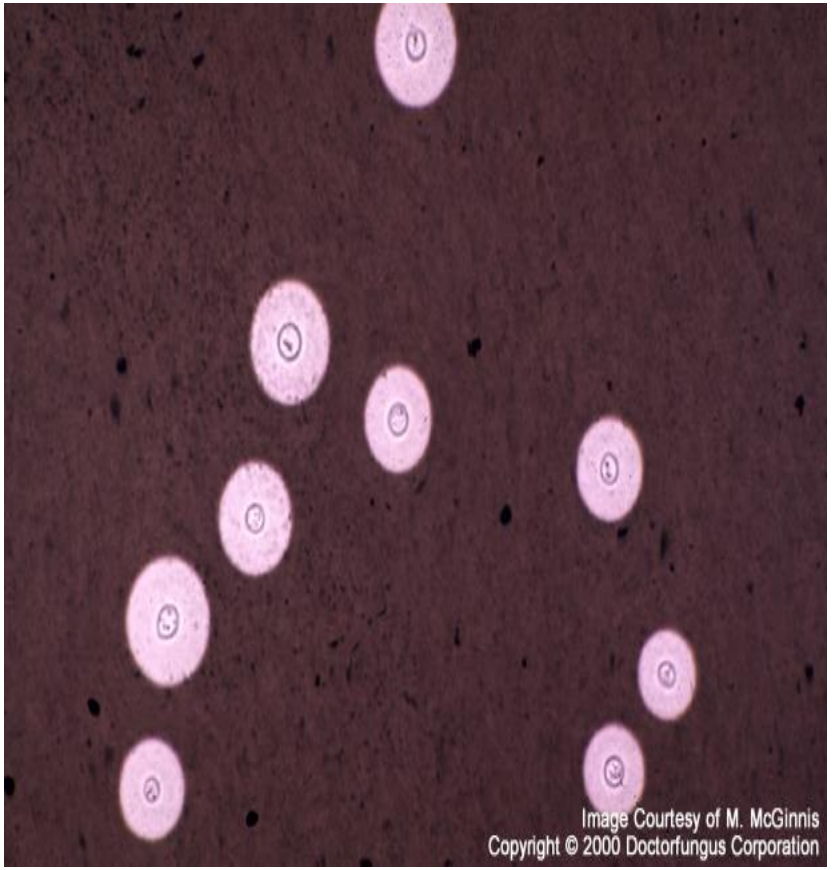
1-Aspergillus niger growth
2- Wet preparation, Aspergillus



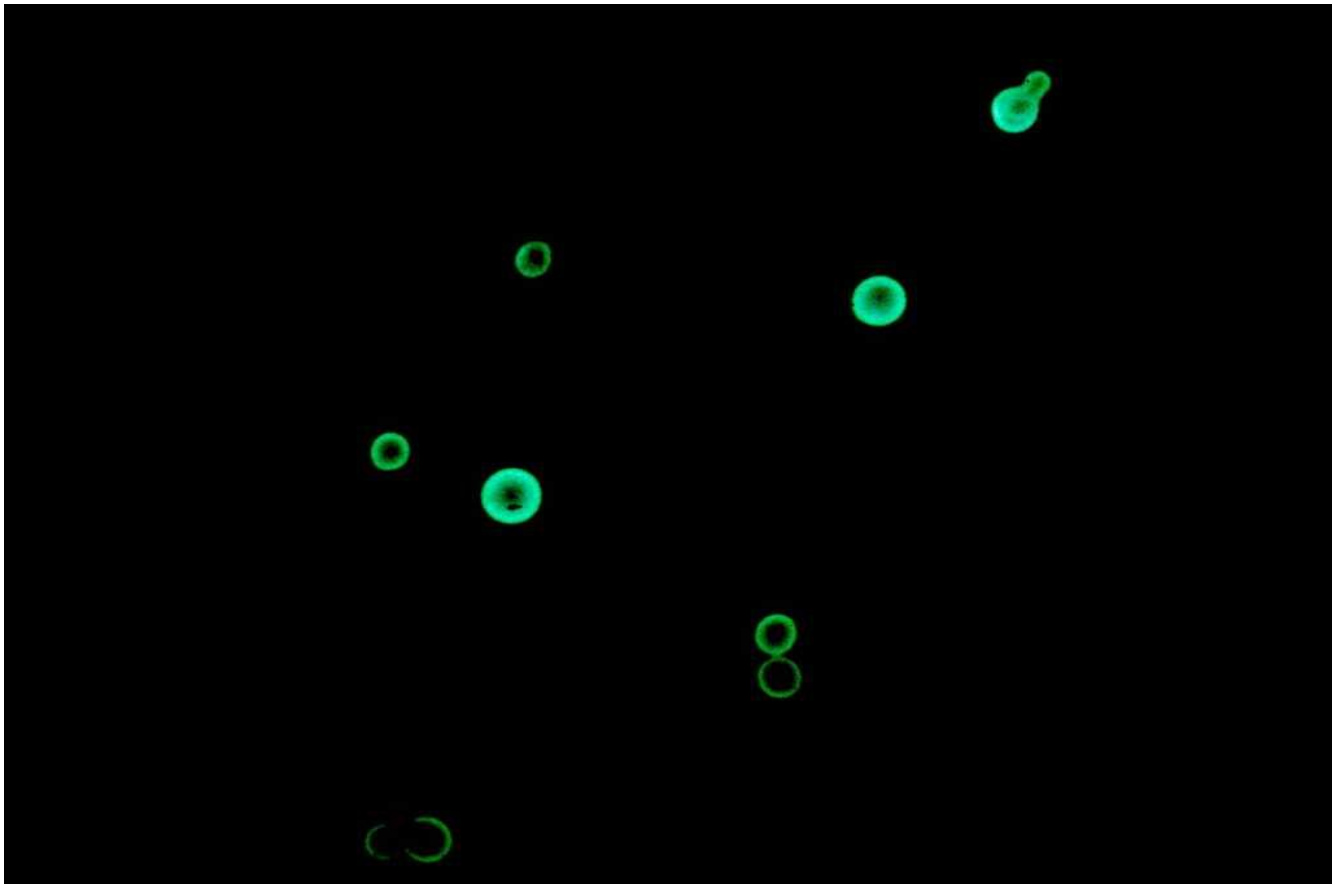

GMS

Capsulated Yeast / Cryptococcus neoformans (India ink test)





Fluorescent antibody



Growth and isolation of fungi

- Cultivation on traditional mycological medium
- Sabouraud's agar (pH 7.0) contains glucose and modified peptone – does not support bacteria

- With antibiotics (for non-sterile specimens)
- With cycloheximide (for dermatophytes)

Growth and isolation of fungi

- Fungal growth requirements

  a. Temperature - Room temperature (25-30 C) for most fungi.

    - Any fungus capable of growing at 37° C should be considered potentially pathogenic.

  b. Atmosphere - True fungi are aerobic

  c. Time - Some yeasts grow overnight. Saprophytes are fast growers (several days).

DNA probes (PCR)

- Rapid (1-2 Hours)
- Species specific
- Expensive

Serology

- Fungi are poor antigens
- Most serological tests for fungi measure antibody
- FUNGAL SEROLOGY ANTIBODIES

• Latex Agglutination	IgM
• Immunodiffusion	IgG
• EIA	IgG & IgM
• Complement Fixation	IgG

Identification of fungi

1. Appearance of the growth
2. Rate of growth
3. Colony pigmentation
4. Growth on media containing antifungal agents

Identification of fungi

1.Appearance of the growth

- surface and reverse surface of colony were observed
- delicate or hairlike hyphae

2. Rate of growth

- saprophytes : 3-5 days
- dimorphic fungi : 10 days or more
- dermatophytes : 14 days or more

Diagnosis of Fungal Infection

- **Microscopy** – direct staining of fungi in sections can distinguish between yeasts and molds

- **Culture** – can lead to diagnosis of the exact species. *Candida* can be grown in blood cultures but *Aspergillus* cannot

- **Serology** – direct detection of fungal antigens in serum samples. ELISA to detect galactomannan (invasive *Aspergillus* infection) or detection of ß-d-glucan (fungal infection)
- **Radiography** – direct observation of patients to spot characteristic signs of infection, e.g. halo signs, cavities
- **PCR** – assays target fungal ribosomal operon, nucleic acid extraction from blood or BAL.

ANTIFUNGAL THERAPY

Because they are eukaryotic, fungi are biochemically similar to the human host. Therefore it is difficult to develop chemotherapeutic agents that will destroy the invading fungus without harming the patient.

- Mammalian cells – cholesterol
- Fungal cells – ergosterol
- All eucariotic cells contain sterols

MECHANISMS OF ACTION

- Polyenes
- Azoles
- Griseofulvin
- 5 - flucytosine

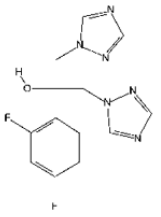
- Bind to ergosterol in cell membrane
- Interfere with ergosterol synthesis
- Forms a barrier to fungal growth (interferes with microtubule assembly)
- Inhibits RNA synthesis

PRIMARY ANTI-FUNGAL AGENTS

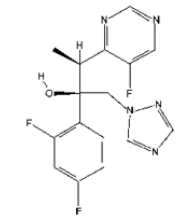
1. Polyene derivatives
   - Amphotericin B
   - Nystatin
2. Azoles
   - Ketoconazole
   - Fluconazole
   - Itraconazole
   - Voriconazole
   - Posaconazole

Antifungals

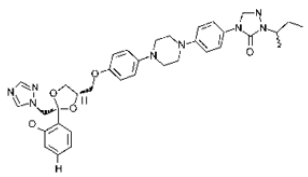
Azole Fungicides: Itraconazole Voriconzaole Posaconazole Fluconazole	Inhibit ergosterol biosynthesis – affect cell membranes
Echinocandins: Caspofungin Micafungin	•Inhibit 1,3-beta-glucan synthase, affects cell wall •Broad spectrum, low toxicity
Fluorinated Pyrimidines: Flucytosine (5FTC)	•Inhibit nucleic acid synthesis
Polyenes: Amphotericin B Nystatin	•Forms Pores in membranes by interacting with ergosterol •Toxic



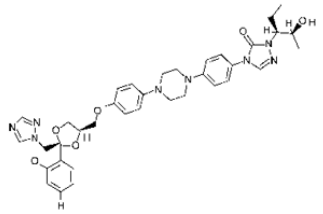
Fluconazole



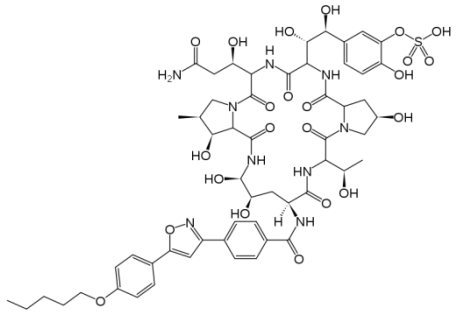
Voriconazole

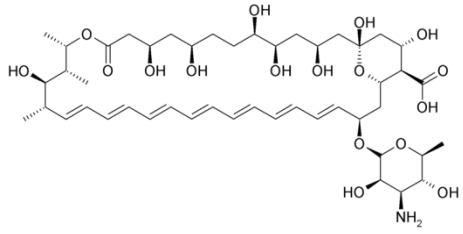


Intraconazole

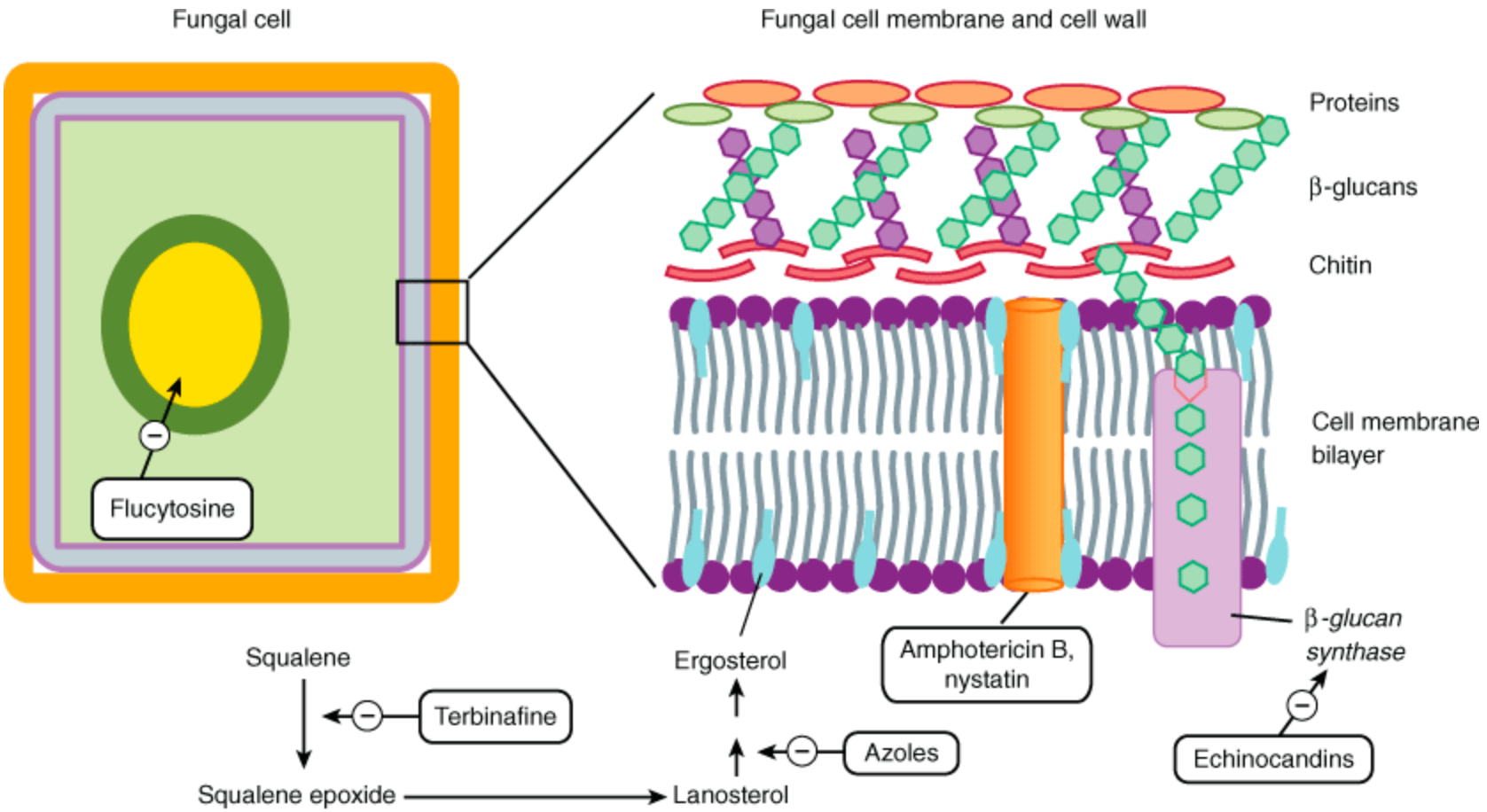


Posaconazole

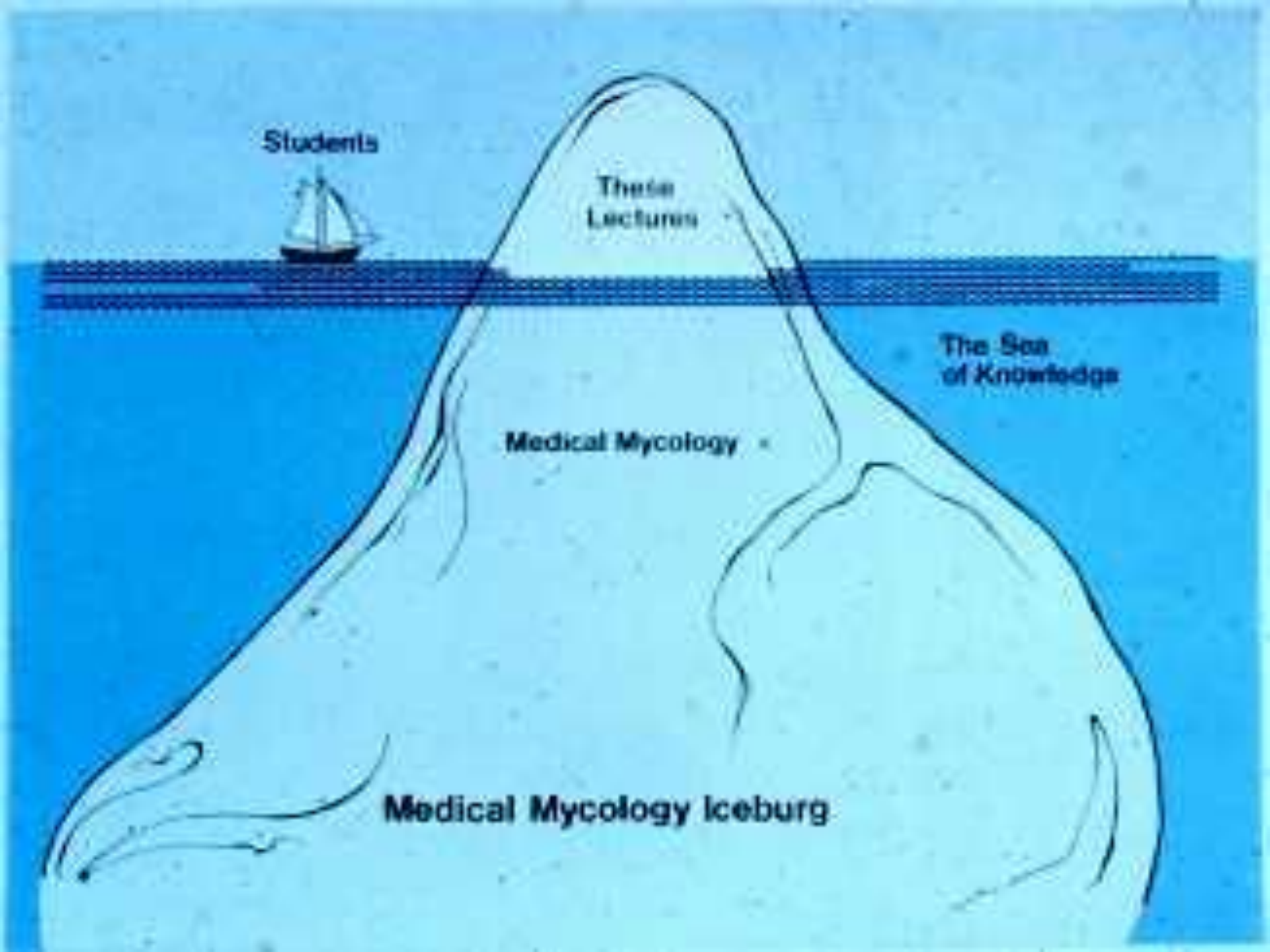




Antifungal Agents



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com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