

# Mycology

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# Fungi are eukaryotic cells

- each fungal cell has:
  - nucleus and nuclear membrane
  - endoplasmic reticulum
  - mitochondria
- most fungi are obligate or facultative aerobes



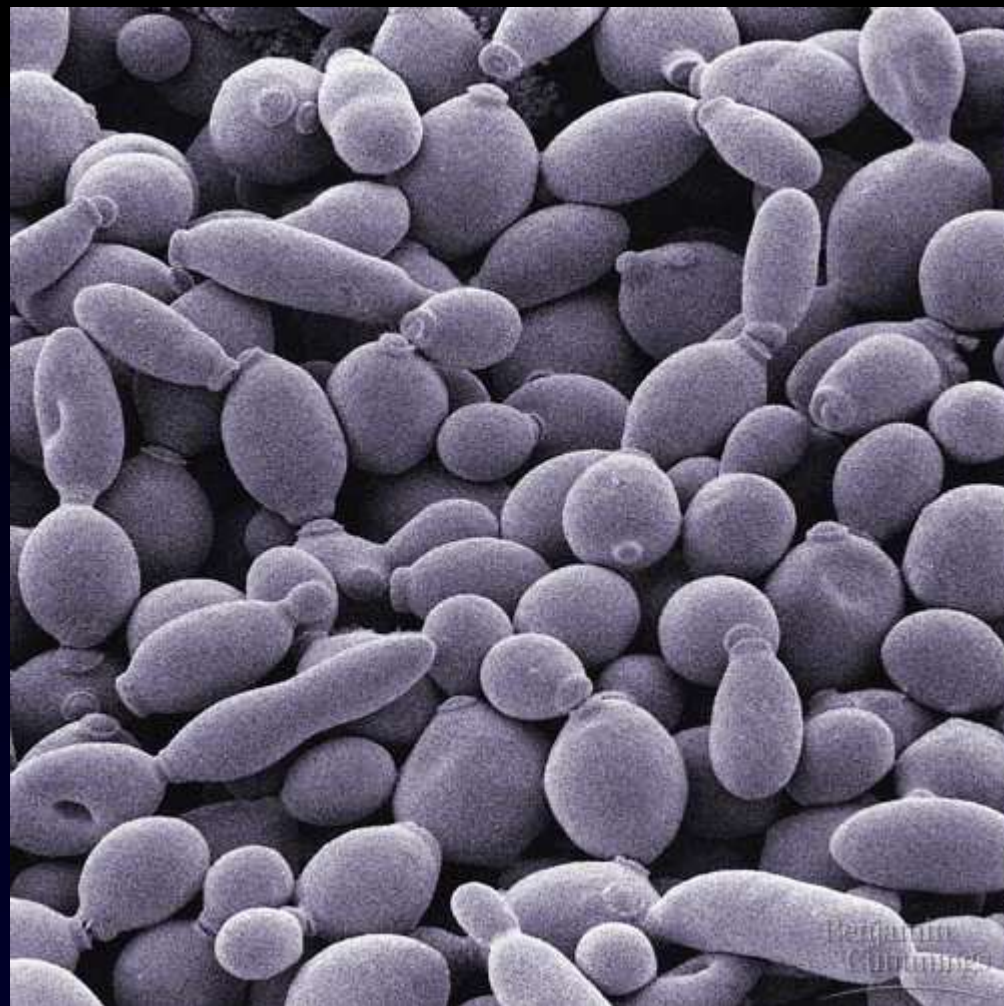
- most pathogenic fungi are exogenous
  - their natural habitats being water, soil, and organic debris
- the mycoses with the highest incidence (candidiasis and dermatophytosis) are caused by fungi that are part of the normal microbial flora or highly adapted to survival on the human host



# Fungi grows in 2 basic forms:

- Yeast:
  - unicellular growth pattern
  - can be spherical or ellipsoidal
  - they reproduce by budding
  - when the buds are not able to separate → pseudohyphae
- Mould (Mycelia):
  - produces multicellular filamentous colonies which contains the following:
    - Hyphae - cylindrical tubules composed of fungal cells attached end to end
    - Mycelium - the collective term for a mass of hyphae
    - Vegetative hyphae – penetrates the supporting medium and absorb nutrients
    - Aerial hyphae – project above the surface of the mycelium and bear the reproductive structures of the mould





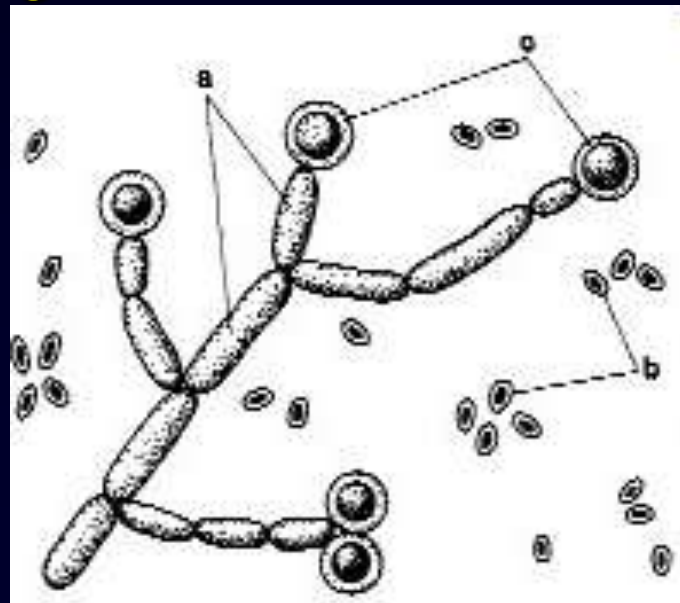


- Dimorphic:

- can grow either as yeast or a mould

- depending on the environmental conditions and temperature

- at body temperature they grow as yeast, at 25°C they grow as moulds



# Summary of important cell features of fungal cells

## Cell wall

- multilayered
- composed of carbohydrates, polysaccharides and some proteins (chitin, glucans, mannans, glucomannans, galactomannans)
- some are capsulated (*Cryptococcus neoformans* – stained by indian ink)

## Cell membrane

- bilayer
- composed of glycoproteins, lipids, and ergosterol (sterol)
- differs from mammalian cell membranes, which contain cholesterol





## Nucleus

- Membrane-bound (eukaryotic)
- Multichromosomal and can be haploid or diploid

## Cytosol complex

- contains several organelles
- nucleus, mitochondria, Golgi apparatus, ribosomes, a well-defined endoplasmic reticulum and other inclusions

## Shape and size

Yeasts - are oval to round; 2-10  $\mu\text{m}$  in diameter;

Moulds - are filamentous; 2-10  $\mu\text{m}$  in diameter, several hundred  $\mu\text{ms}$  in length



# Metabolism

- exclusively heterotrophic
- produces various metabolites - primary (e.g. citric acid, ethanol) and secondary (e.g. alpha amanitin, aflatoxin)
- doubling time is long (hours) compared with most bacteria (minutes)

# Staining properties

- false Gram-positive
- can be stained by
  - methenamine-silver
  - periodic acid Schiff-stain (PAS)



## Reproduction of the human pathogenic fungi

- Fungi can produce spores to enhance their survival
- Spores can be dispersed, they are more resistant and can germinate
- The spores can be used for both asexual and sexual reproduction

### Asexual reproduction

Asexual spores are mitosis progeny and genetically identical

Asexual spores are:

#### Conidia

- non-sexual bud produces from transformation of vegetative yeast or hyphal cell

#### Blastospore

- conidial formation through a budding process (yeast)

#### Arthrospore

- type of segmented spore. Very light → carried easily by air

#### Chlamydospore

- large thick wall produced from terminal or intracalary hyphal cells

#### Sporangiospore

- spore formed within a sac



# Sexual reproduction

Sexual spores are products of meiosis

Sexual spores:

Ascospore

- a spore formed within an ascus (a sac like)

Basidiospore

- a spore formed on basidia

Zygospore



# Diagnostic Laboratory Tests

## Specimens:

- scrapings from the skin and the nails
- hairs plucked from involved areas
- *Microsporum-infected* hairs fluoresce under Wood's light (365 nm) in a darkened room



## Culture:

- the identification of dermatophyte species requires cultures
- specimens are inoculated onto inhibitory mould agar or Sabouraud's agar plates containing cycloheximide and chloramphenicol
- species are identified on the basis:
  - colonial morphology (growth rate, surface texture, and any pigmentation)
  - microscopic morphology (macroconidia, microconidia)
  - nutritional requirements



## Sabouraud's agar

- glyucose, pepton, acidic pH
- morphologic characteristic of fungi are used for identification
- antibiotic (gentamycin, chloramphenicol) → inhibition of the bacteria
- long time incubation (for negative result) about 14 days

## Microscopy

- wet mount
- simple staining (eg. methylene blue)
- before native examination 30% KOH → keratolysis



# Biochemical reactions

- sugar fermentation
- sugar assimilation test
- nitrate assimilation test

# Serology

# Molecular biology





# Therapy of fungal infection

## Polyenes

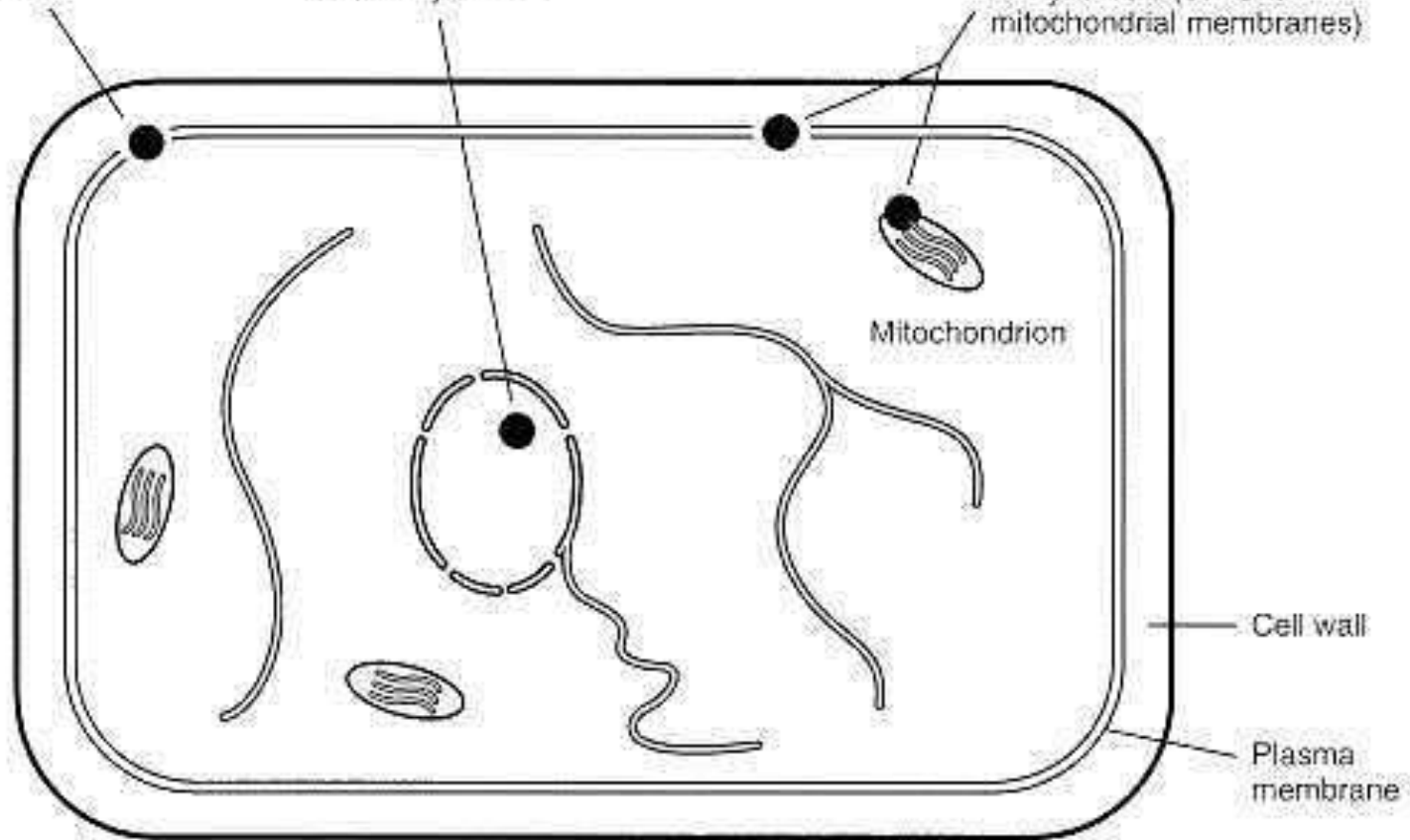
Integration into cell membrane

## 5-Fluorocytosine

Interruption of DNA & RNA synthesis

## Azoles

Interruption of sterol biosynthesis (cell and mitochondrial membranes)



## **Azoles** (e.g., fluconazole)

- act by inhibiting **ergosterol** biosynthesis
- the more recent triazole derivatives, such as fluconazole, itraconazole, and voriconazol, owe their antifungal activity to inhibition of cytochrome P450-dependent  $14\alpha$ -sterol demethylase
- the earlier imidazole derivatives, such as miconazole, econazole and ketokonazole, have a complex mode of action, inhibiting several membrane-bound enzymes as well as membrane lipid biosynthesis

## **Allylamines/Thiocarbamates** (e.g., naftifine, terbinafine/tolnaftate)

- act by inhibiting early steps of ergosterol biosynthesis
- target: squalene epoxidase

**Morpholines** (e.g., amorolfine) - Inhibit ergosterol biosynthesis (inhibition of  $\Delta 14$ -reductase and  $\Delta 7$ - $\Delta 8$ -isomerase)



## **Polyenes** (e.g., amphotericin B, nystatine)

- the polyene antifungal compounds bind to ergosterol
- resulting in the production of aqueous pores that lead to altered permeability leakage of vital cytoplasmic components, and death of the organism

Ambisome® = liposomal amphotericin B

## **Nucleotide** (e.g., 5-fluorocytosin) - Inhibits DNA and RNA synthesis

## **Grisans** (e.g., griseofulvin) –

- interacts specifically with tubulin and acts as a mitotic poison
- inhibits microtubular function

## **Chitin synthase inhibitors**

- Inhibit cell wall synthesis

## **Glucan synthesis inhibitors (Caspofungin)**

- Inhibit cell wall synthesis



# Classification of fungal disease

## Fungal allergies

- “sick building syndrome”, generally occur in individuals with other allergies

## Mycotoxicosis

- may result from ingestion of fungal-contaminated foods (generally an animal problem) or mushrooms



## Superficial mycoses

- infections limited to the outermost layers of the skin and hair

## Cutaneous mycoses

- infections that extend deeper into the epidermis, as well as invasive hair and nail diseases



## Subcutaneous mycoses

- infections involving the dermis, subcutaneous tissues, muscle and fascia

## Systemic mycoses

- infections that originate primarily in the lung but may spread to many organ

## Opportunistic mycoses

- infections in immunocompromised patients



# Clinical manifestations suggestive of fungal infection

- flu-type infection: that has lasted longer than a viral flu
- chronic respiratory problem: with weight loss and night sweats
- fever of unknown origin: that does not respond to antibacterial agents



- any infection with negative bacterial cultures: that does not respond to antibiotics and that does not appear viral
- signs of meningitis
- exposure to dust with bird or bat guano





# Predisposal factors of fungal infections

## Diseases

- diseases causing immunosuppression: tumours, AIDS, steroid therapy, diabetes mellitus
- trauma: burnt wounds - moulds, stabbed injuries - subcutaneous mycosis
- iatrogenic mycosis: during the immunosuppression therapy: irradiation, corticosteroid therapy, administration of broad spectrum antibiotics, cytostatic therapy
- nosocomial infections: fungi associated to plastic surfaces (catheters)
- „voluntarily risks”: alcoholism, drug abusers, microbiologists, mycologists



## Superficial mycoses

- affect the outermost layer of skin and hair
- generally do not induce a cellular response to the infection

### **Malassezia furfur - Pityriasis versicolor**

- yeast, required lipid in the medium of growth
- rarely may cause an opportunistic fungemia, folliculitis
- is a chronic mild superficial disease of the stratum corneum epidermidis
- hyper- or hypopigmented maculae occur on the skin
- Diagnosis: direct microscopic examination of scrapings of infected skin treated with 10-20% KOH
- Treatment: selenium sulfide, azoles



## Subcutaneous mycosis

- the fungi that cause subcutaneous mycoses normally reside in soil or on vegetation
- they enter the skin or subcutaneous tissue by traumatic inoculation with contaminated material
- extension via the lymphatics draining the lesion is slow except in sporotrichosis.
- in rare cases they become systemic and produce life-threatening disease

## Sporothrix schenckii

- is a thermally dimorphic fungus that lives on vegetation: grasses, trees, sphagnum moss, rose bushes, and other horticultural plants
- initial lesions is usually on the extremities
- initial lesion develops as a granulomatous nodule that may progress to form necrotic lesion
- treatment: itraconazole, azoles, amphotericin B



# Cutaneous mycoses

- infect only the superficial keratinized tissue (skin, hair, nails)
- most are unable to grow at 37°C or in the presence of serum
- 25°C on Sabourod's dextrose agar for 2 weeks
- are classified as: geophilic, zoophilic, anthropophilic depending on their usual habitat



- infections begin in the skin after trauma and contact
- **risk factors:** moisture, warmth, specific skin chemistry, composition of sebum, perspiration, youth heavy exposure, genetic predisposition
- trichophytin: is a crude antigen preparation that can be used to detect immediate or delayed type hypersensitivity to dermatophytic antigens



## **Tinea Pedis (Athlete's Foot): *Trichophyton rubrum*, *Epidermophyton floccosum***

- tinea pedis is the most prevalent of all dermatophytoses
- a chronic infection of the toe webs
- other varieties are: the vesicular, ulcerative, and moccasin types, with hyperkeratosis of the sole.
- there is itching between the toes and the development of small vesicles that rupture and discharge a thin fluid
- the skin of the toe webs becomes macerated and peels
- chronic form: peeling and cracking of the skin are the principal manifestations, accompanied by pain and pruritus



# Tinea unguium (Onychomycosis): Trichophyton rubrum, Epidermophyton floccosum

- nail infection may follow prolonged tinea pedis
- the nails become yellow, brittle, thickened, and crumbly
- treatment: nail infections are the most difficult to treat
- requiring months of oral itraconazole or terbinafine as well as surgical removal of the nail
- relapses are common



Tinea corporis (*Microsporum canis*) – ringworm  
Tinea cruris and Tinea manus (*Trichophyton rubrum*,  
*Epidermophyton floccosum*)

- dermatophytosis of the glabrous skin commonly gives rise to the annular lesions with a clearing, scaly centre surrounded by a red advancing border that may be dry or vesicular
- the dermatophyte grows only within dead, keratinized tissue
- fungal metabolites, enzymes, and antigens diffuse through the viable layers of the epidermis to cause erythema, vesicle formation, and pruritus
- the lesions expand centrifugally, and active hyphal growth is at the periphery, which is the most likely region from which to obtain material for diagnosis





- treatment: itraconazole and terbinafine
- topical preparations may be used: miconazole nitrate, tolnaftate, and clotrimazole
- applied for at least 2-4 weeks, the cures rates are usually 70-100%
- treatment should be continued for 1-2 weeks after clearing of the lesions



# Tinea capitis (Microsporum sp.) and Tinea barbae (Trichophyton rubrum, T. mentagrophytes)

- tinea capitis is dermatophytosis of the scalp and hair
- infection of the hair takes place just above the hair root
- the infection produces dull gray, circular patches of alopecia, scaling, and itching
- in prepubescent children, epidemic tinea capitis is usually self limiting



- **favus:** an acute inflammatory infection of the hair follicle caused by *T. schoenleinii*
- **tinea barbae:** involves the bearded region - cattle
- **treatment:** scalp infections are treated with griseofulvin for 4 - 6 weeks
- shampoos and miconazole cream or other topical antifungal agents may be effective if used for weeks
- ketoconazole, itraconazole and terbinafine



# Candida infections



# Opportunistic mycoses

## **Candida albicans (yeast)**

- member of the normal flora of the: oral cavity, gastrointestinal, vagina
- non albicans: *C. krusei*, *C. lusitanae*

## **Candida infection:**

### **- in patients with normal immunity:**

- oral - soor
- vaginal
- interdigital

### **- in patients with immunodeficiency:**

- disseminated oral candidiasis
- oesophagitis
- pneumonia
- nephritis
- endocarditis and sepsis



# Cryptococcosis (Cryptococcus neoformans)

- is a yeast
- may be isolated from fruit, milk, vegetation and soil
- meningitis - found in very large numbers in dry pigeon faeces



# Aspergilloses (Aspergillus fumigatus)

- is a mould
- found in decaying vegetation
  - allergic bronchopulmonary aspergillosis
  - fungus ball → the fungus grows in a preexisting cavity (tuberculoid cavity) → surgical resection
- invasive form → spreading into the lung → necrotising pneumonia → dissemination to other organs (meningitis)



# Mucormycosis (Mucor mucedo)

- is a mould
- localisation in the wall of the vessels → prone to thrombosis → possible ischemic necrosis throughout the body
- forms: rhinocerebral, pulmonary, cutan, gastro-intestinal, central nervous system





# Systemic mycosis

- affect internal organs and may disseminate to multiple sites of the body
- are named as: deep mycoses
- are caused by pathogenic fungi, which can invade and cause disease in healthy or compromised hosts

## Histoplasmosis (*Histoplasma capsulatum*)

- is a dimorphic fungus
- does not have a capsule
- **source:** guano
- **can be classified as:** local, disseminated, acute, chronic, fulminant
- inhalation of the spores → pneumonia → chronic. inflammation → heals with calcification



# Coccidiosis (*Coccidioides immitis*)

- is a dimorphic fungus
- inhalation of the spores → 2/3 asymptomatic
  - influenza like disease
  - erythema nodosum
  - arthritis
  - dissemination: meningitis, osteomyelitis



# Blastomycosis (*Blastomyces dermatitidis*)

- dimorphic fungus
- is a pulmonary, disseminated and cutaneous fungal disease
- inhalation of the spores → dissemination → pulmonary manifestation → skin lesions (rarely asymptomatic infection)



# Paracoccidioides brasiliensis

- dimorphic fungus
- mother cells may produce 10 to 12 uniform or variably sized buds
- cause systemic granulomatous disease
- involves mainly the lungs, phagocytic mononuclear system, mucous membranes, skin, and adrenals
- is the most common endemic human mycosis in Latin America



- has been isolated from:
  - soil, animals such as armadillos and bats, dog food, and penguin faeces
  - has also been isolated from the intestinal contents of bats
- the respiratory route is the main portal of entry and the lung is the primary site of infection
- the fungus spreads to other parts of the lung through peribronchial lymphatic vessels and drains into regional lymph nodes



## ***Pneumocystis jiroveci*** previously known as ***Pneumocystis carinii***

Geographic Distribution: Worldwide, in humans and animals

- *Pneumocystis* pneumonia occurs in immunosuppressed individuals and in premature, malnourished infants.

Clinical presentation:

- These fungi are found in the lungs of mammals where they reside without causing overt infection until the host's immune system becomes debilitated. Then, an often lethal pneumonia (PCP=*Pneumocystis pneumonia*) can result

**Infantile pneumonia** - epidemic, in premature and malnourished infants

**Pneumonia of immunocompromised host** - sporadic, may affect any host with congenital or acquired immunodeficiency

**Extrapulmonary infection** - result of dissemination to other organs, including lymph nodes, spleen, bone marrow, liver, kidney, heart, brain, pancreas, skin and other organs

Laboratory identification

- Gomori methenamine silver stain method

The walls of the cysts are stained black and often appear crescent shaped or like crushed ping-pong balls

- treatment: Trimethoprim-sulfamethoxazole, pentamidine, atovaquone, echinocandins (micafungin), sordarins, azasordarins, terbinafine

