Medical mycology

2014

Introduction

- There are over 100,000 species of fungi, but only about 100 of them cause diseases in humans
- Good fungus (mushrooms, Saccharomyces cereviseae is used to make the alcohol in beer and wine or make bread).
- Bad fungus (cause diseases)

Infections caused by fungi

- Mild (skin) or life-threatening
- Mortality rate in case of candidemia: 40%
- Mortality rate in case of invasive Aspergillus infection :70-80%

General properties of fungi

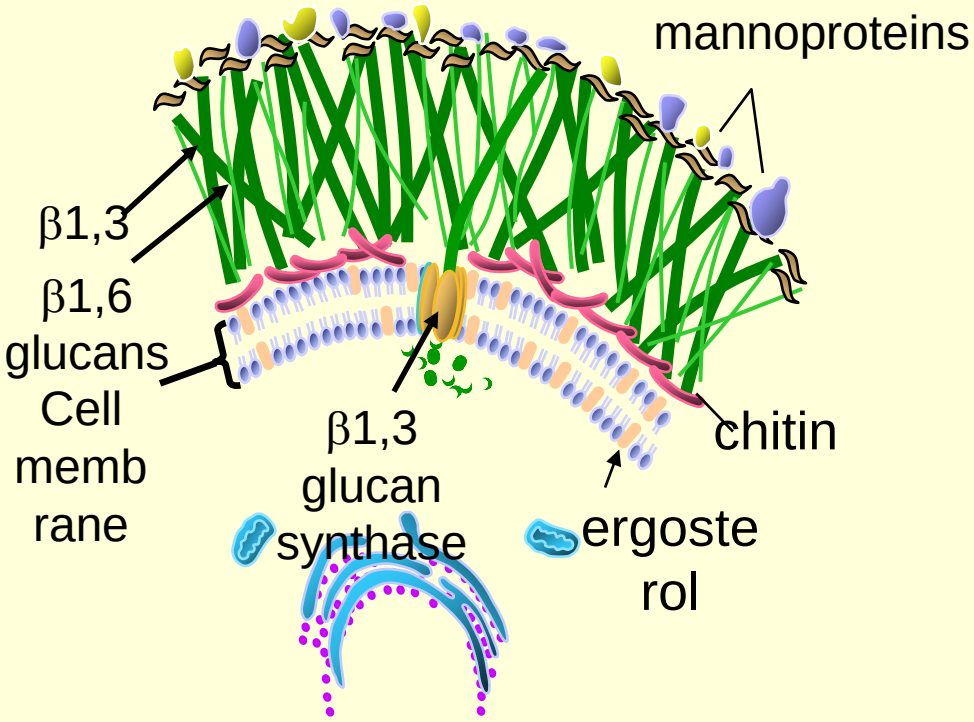
- eukaryotes
- ergosterols present in their plasma membrane
- two basic morphological forms, yeast and hyphae
- mass of hyphal elements is termed the **mycelium** (synonymous with **mould**).

The cell wall

Glucan. Glycans composed of glucose homopolymers. The cell wall glucan of *Candida* and *Saccharomyces* is a highly branched polymer consisting of ß-1,3 and ß-1,6 linkages

Chitin. Linear polymers of ß-1,4-D-GlcNAc, provide cross-linking and strength to the glucan scaffolding

mannan" and "mannoprotein (covalently linked to the glucan chains)



Asexual reproduction (Conidia production)

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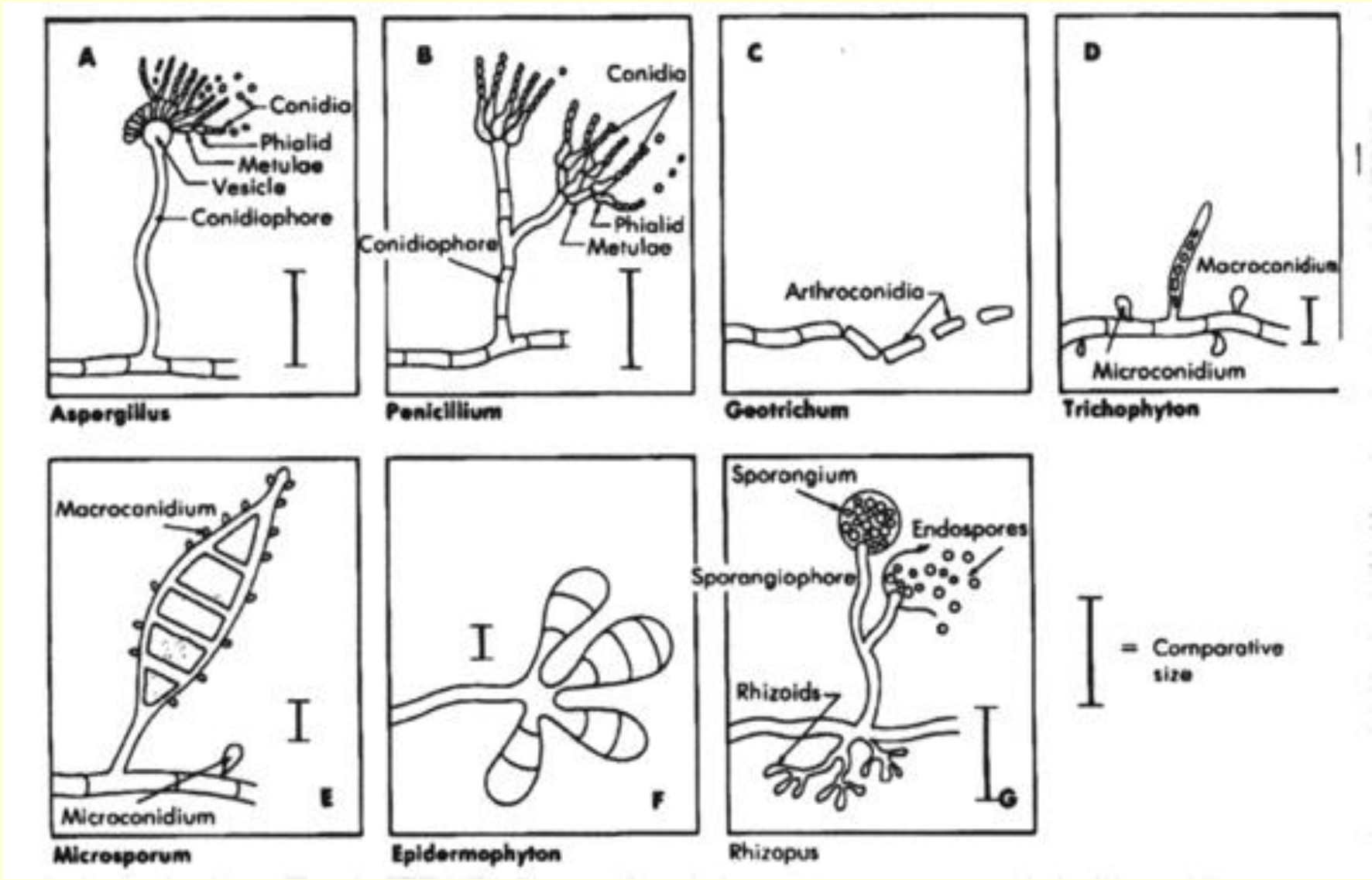
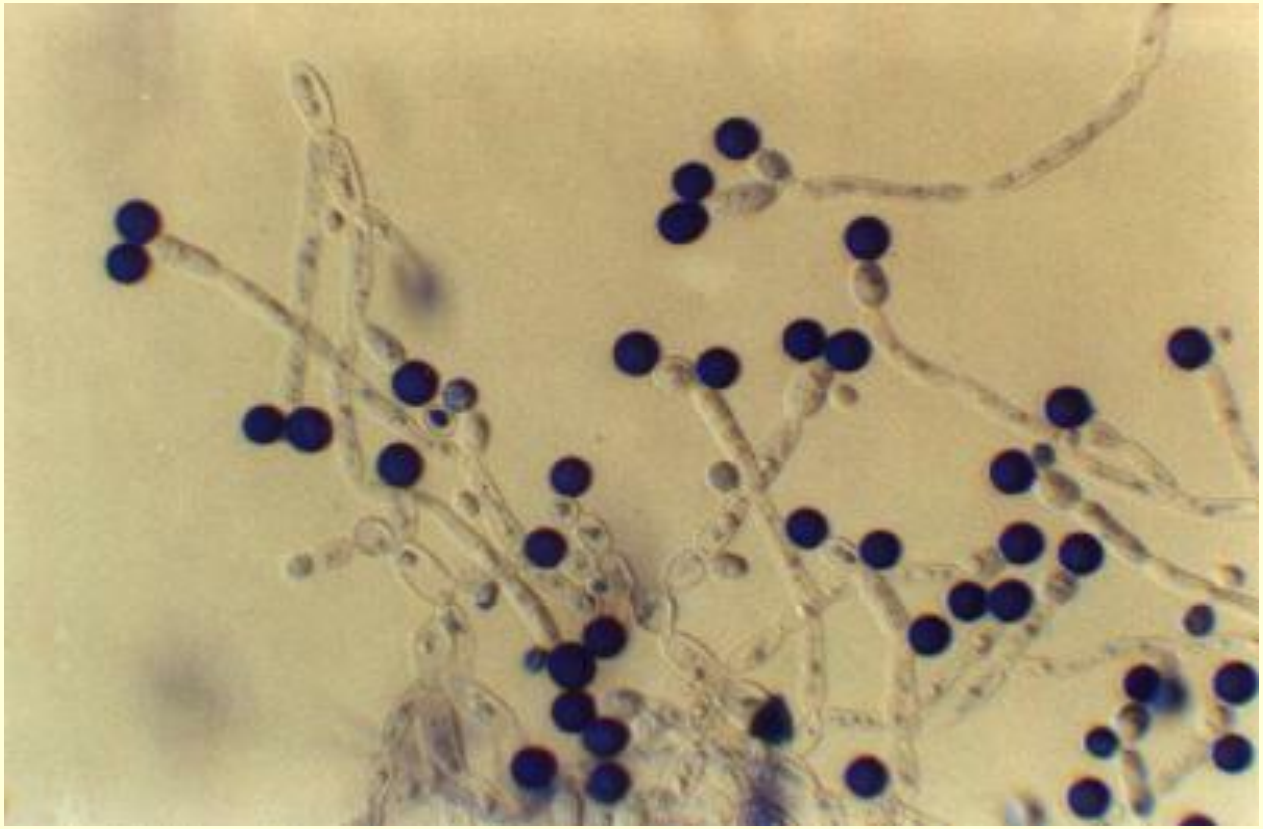


Arthrospores

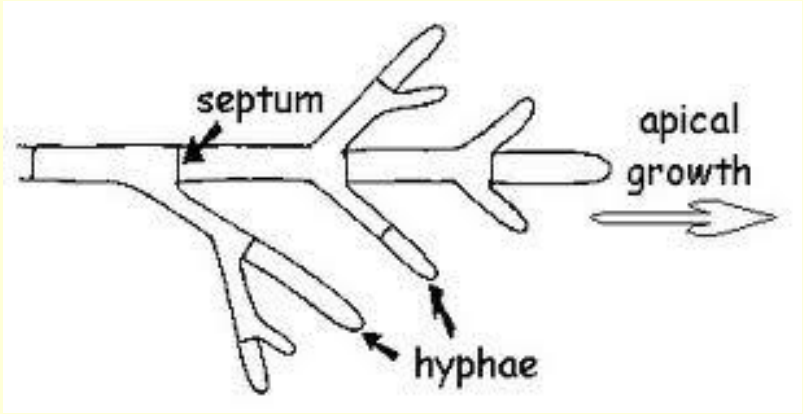
- Coccoides immitis
- Fragmentation of a hypha into individual cells
- Very easily enter into the lung

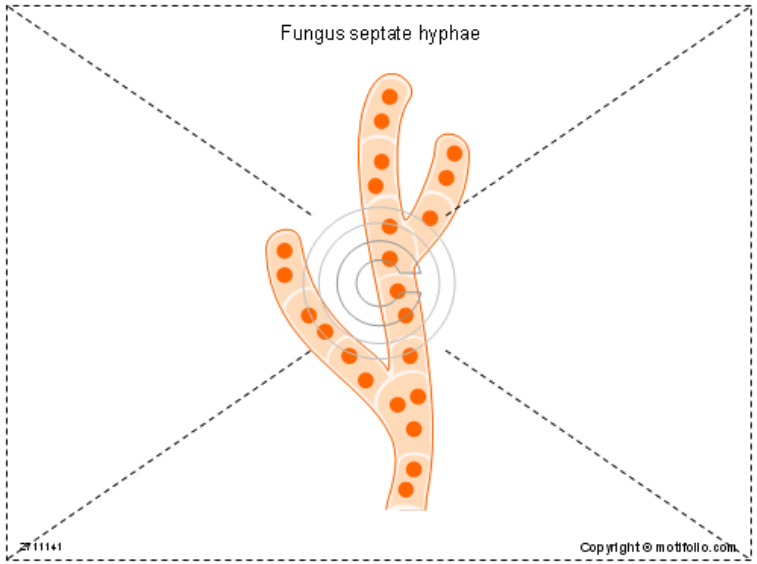
Chlamydospore

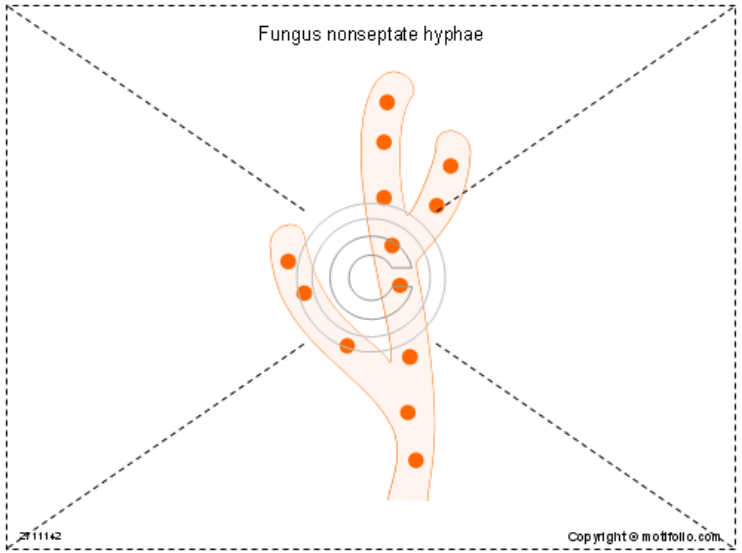


Morphology I. (Hyphae)

- **Hyphae** are branching, threadlike, tubular filaments that either lack cross walls (aseptate) or have cross walls (septate).

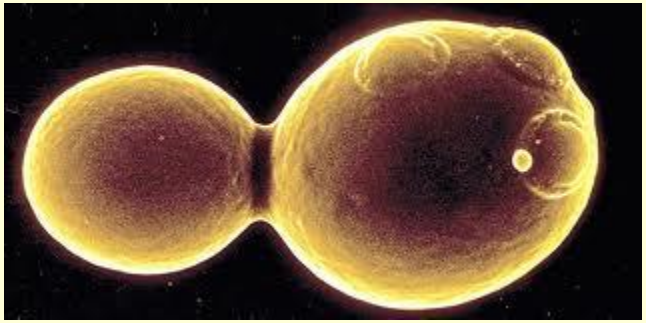


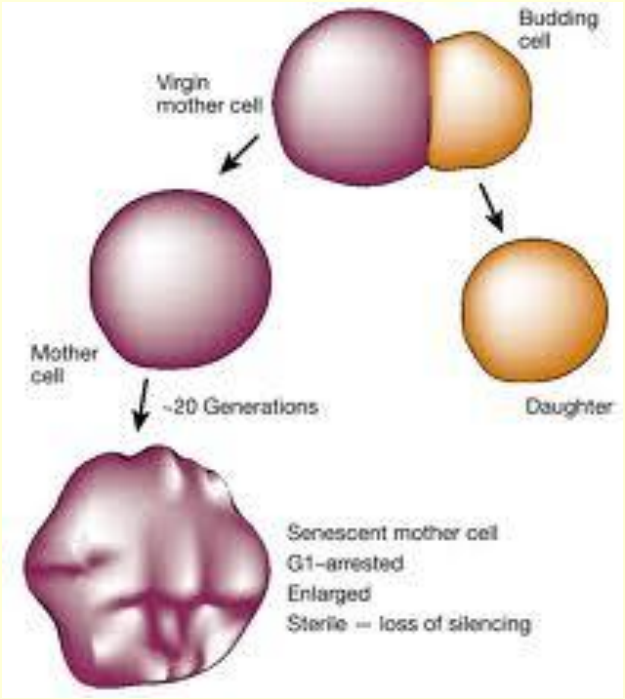




Morphology II. (yeast)

- Yeast (single cell)
- reproduce asexually by <u>blastoconidia</u> formation (budding***)***





Dimorphism

- Dimorphism is the condition whereby a fungus can exhibit either the yeast form or the hyphal form, depending on growth conditions
- For example: *C. albicans* at 37^{o}C growth as a yeast, but at 25^{o}C growth as a hypha, (but in tissues both forms may be present)
- Dimorphism is an important virulence factor

Virulence factors

Colonization

- ALS (agglutinin-like sequence) family
- hydrophobic-hydrophobic interaction
- Co-aggregation with aerobic and anaerobic bacteria (i.e. *Fusobacterium necrophorum*)
- Secreted phospholipases

Phenotypic switch

- During infection, the phenotypic switch between blastoconidia and hyphae is a virulence trait of ***C. albicans***

Secreted aspartic proteinases

- Differential expression of the *SAP* gene family during *C. albicans* infections has also been demonstrated using *in vitro* and *in vivo*.
- important role of *SAP4–6* in the pathogenesis of invasive candidasis.
- *SAP1–8* in **oral candidasis** patients (*C. albicans*)
- Inhibitors of Saps protect against *C. albicans* infection

Secreted phospholipases

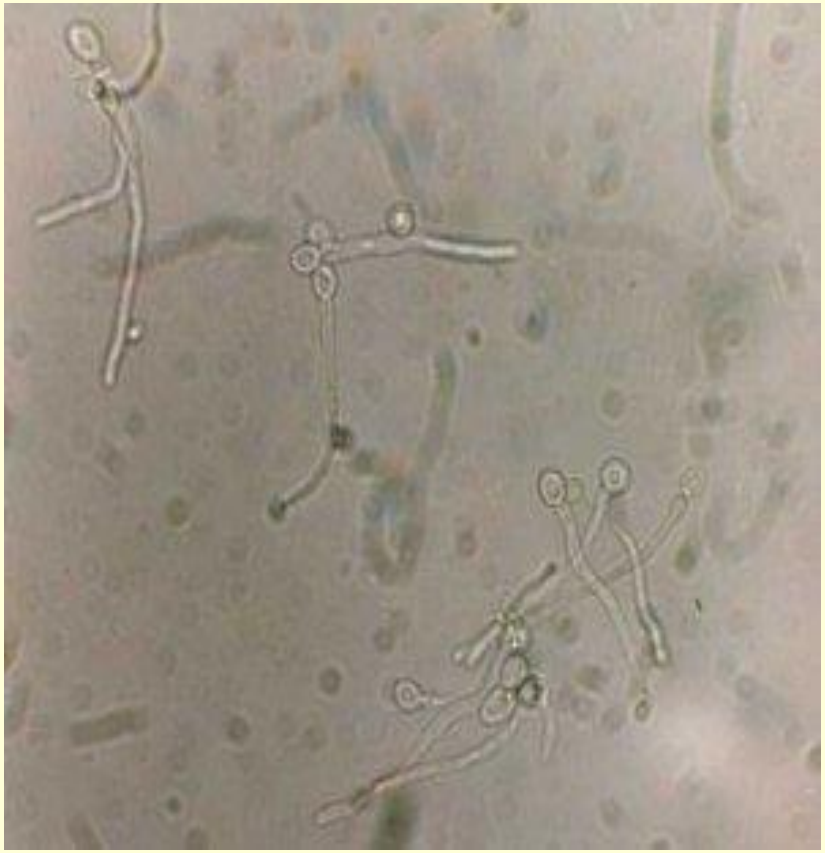
- They have a role in host cell **penetration**, adhesion to epithelial cells, **invasion** of human oral epithelium and perhaps interaction with host signal transduction pathways.
- In the dissemination of *C. albicans* by both the gastrointestinal and haematogenous routes
- ***C. albicans***,
- ***Aspergillus fumigatus***
- ***Cryptococcus neoformans***
- (*Clostridium* species, *Listeria monocytogenes*, *Pseudomonas* species, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Toxoplasma gondii* and *Entamoeba histolytica)*.

Esterases and lipases

- Hydrolysis of ester bonds of mono-, di- and triacylglycerols or even phospholipids
- *LIP1–10* were detected in case of *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*

germ tube

- *Candida albicans and Candida dubliniensis* can form 'germ tubes' when incubated for up to two hours in serum.
- Typical yeast cells, together with elongated structures



Immunity

- Cell-mediated response is the most important
- Neutrophils, macrophages, dendritic cells (DC)
- DC can discriminate between the yeast and hyphal forms and initiate T-helper cell immunity, which is required for long-term protection (*C. albicans*)
- ingestion of yeasts activates dendritic cells for interleukin 12 production (effective Th1 response), whereas ingestion of hyphae inhibited IL-12 and induced IL-4 production (ineffective Th2 response).
- **Neutropenia is the most important factor for invasive fungal infection**

- Laboratory diagnosis

Visualization of fungi in tissue preparations

- 1. Treatment with 10% potassium hydroxide (skin, hair)
- 2. Positive stain with
- a. Lactophenol cotton blue
- b. Grocott silver stain
- c. Hematoxylin
- d. Eosin

3. Negative stain with India ink

Culture of fungi on:

1. **Sabouraud's agar** (favors fungal growth because of low pH)
2. **Mycosel agar** (selective for pathogenic fungi because of chloramphenicol and cycloheximide in medium)

- (inoculation parallel with bacterial culture)

Visualization of cultured fungi (25°C and 37°C)

- 1. Colony morphology
- 2. Cellular morphology

- a. Hyphal morphology

- (1) Aseptate
- (2) Septate

Spore morphology

- **Conidiospore**
- **Sporangiospore**
- **Arthrospore**
- **Chlamydospore**

Yeast morphology

Size
Thickness of walls
Capsule
(presence/absence)

Identification of yeast by:

1. Biochemical tests (ID32C, API 20C)
2. CHROMagar Candida
3. Behavior in broth and serum (**germ tube** formation)
4. Dalmau-plate (behavior on cornmeal or rice agar) (**pseudohypha** formation)
5. MALDI-TOF
6. PCR-based methods (not available in many labs)

CHROMagar Candida

- With the inclusion of chromogenic substrates in the medium, the colonies of *C. albicans*, *C. tropicalis* and *C. krusei* produce different colors
- Only for presumptive identification
- This medium facilitates the detection of mixed yeast cultures in specimens

- After 24 or 48 h incubation

Serology

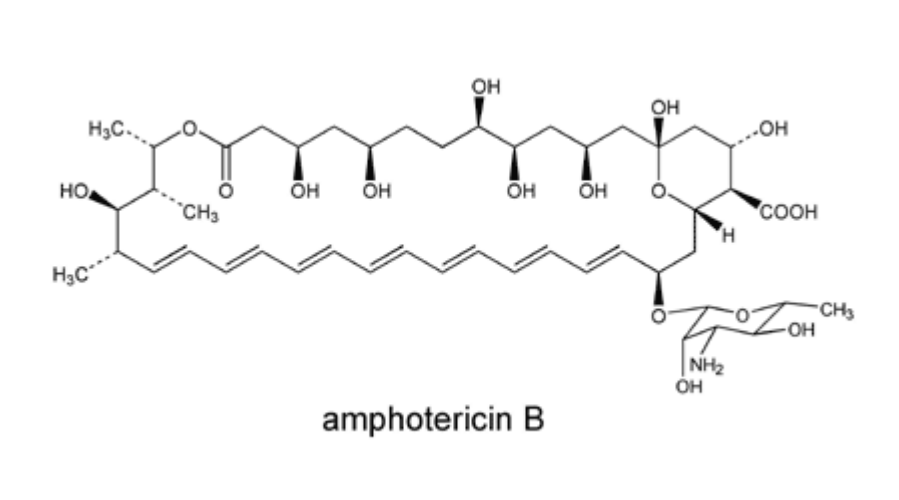
- Antigen detection is important in case of invasive infection (ELISA)
- Antibody: lesser important
- Mannan antigen detection for *Candida*
- Galactomannan antigen detection for *Aspergillus* species
- Repeated sampling is needed

Antifungal Agents

- Primary and secondary prophylaxis
- Empiric therapy in resistant febrile neutropenia
- Targeted therapy for proven fungal infections
- Fungicidal (killing) or fungistatic (inhibition of growth) effect
- Local or systemic treatment

Polyene antibiotics (macrolides) (fungicidal)

- Interaction with ergosterol, increased membrane permeability, cell death
- Amphotericin B (severe side effects)
- Nystatin (local)



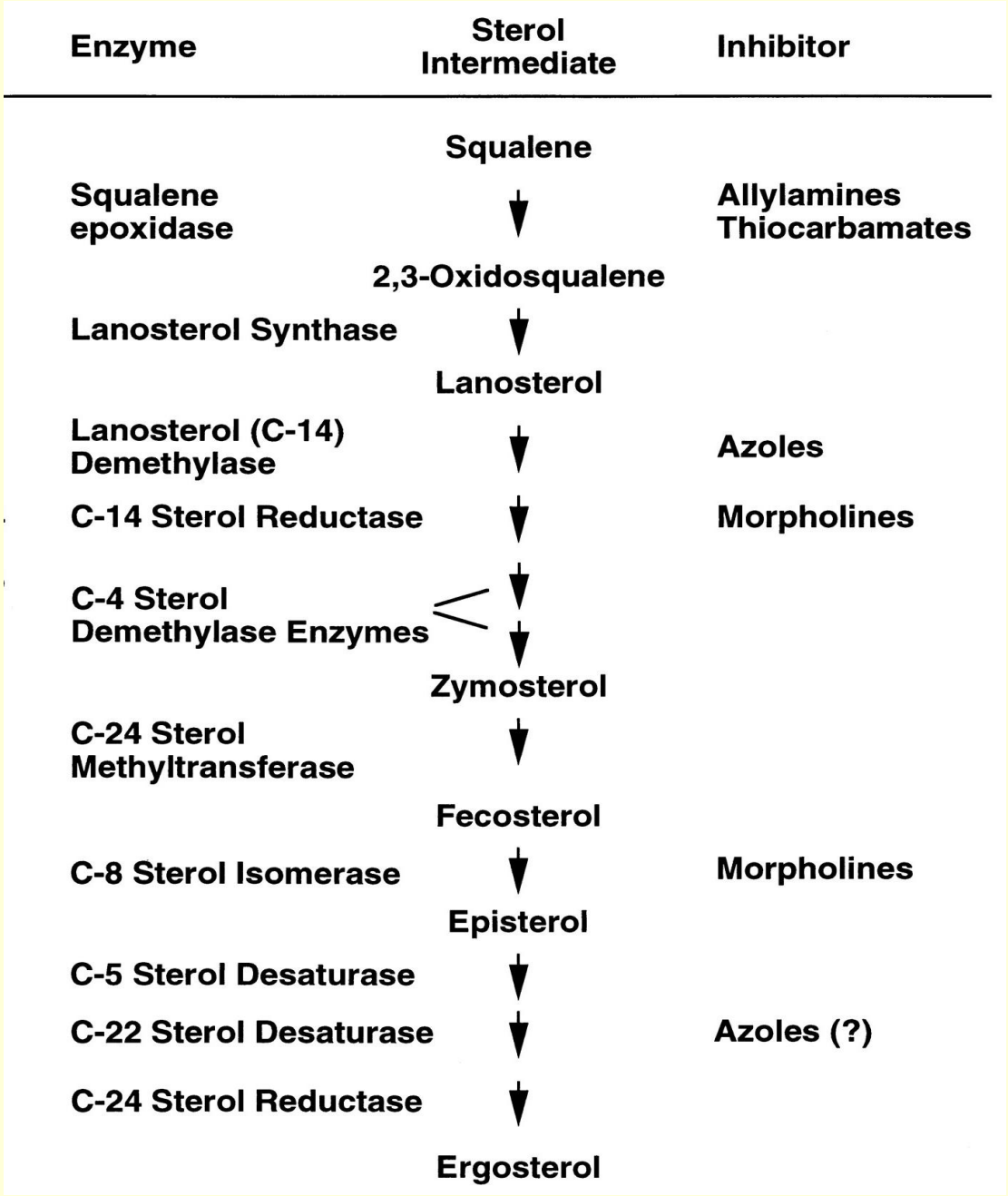
amphotericin B

- - very broad range of activity and is active against most pathogenic fungi
- - Lipid-associated amphotericin B and nystatin formulations (lesser side effects, but not significantly effective than conventional amphotericin B)

Azoles and triazoles

- target enzyme, the cytochrome P-450 lanosterol 14 -demethylase
- Fungistatic effect
- Fluconazole is the widely used, however, no activity against moulds
- Newer triazoles (voriconazole and posaconazole) posses better activity against moulds

Ergosterol biosynthetic pathway from squalene to ergosterol



Azoles and triazoles for local treatment

- Butoconazole (Suppository, Topical)
- Clotrimazole (Topical)
- Econazole (Topical)
- Fluconazole (Oral, Topical)
- Itraconazole (Oral)
- Ketoconazole (Oral, Topical)
- Miconazole (Topical)

Azoles and triazoles for systemic treatment

- Fluconazole PO, IV
- Itraconazole PO, IV
  Ketoconazole PO,Topical
- Posaconazole PO
  Voriconazole PO, IV

Fluoropyrimidines

- 5-fluorocytosine
- inhibition of DNA or RNA synthesis
- Mainly, for combination therapy

Glucan Synthesis Inhibitors (**echinocandins)**

- inhibiting the enzyme 1,3-beta glucan synthase
- Fungicidal effect against *Candida* species
- Fungistatic against *Aspergillus* species
  - Caspofungin IV **Cancidas**
  - Micafungin IV **Mycamine**
  - Anidulafungin IV Eraxis

Allylamines

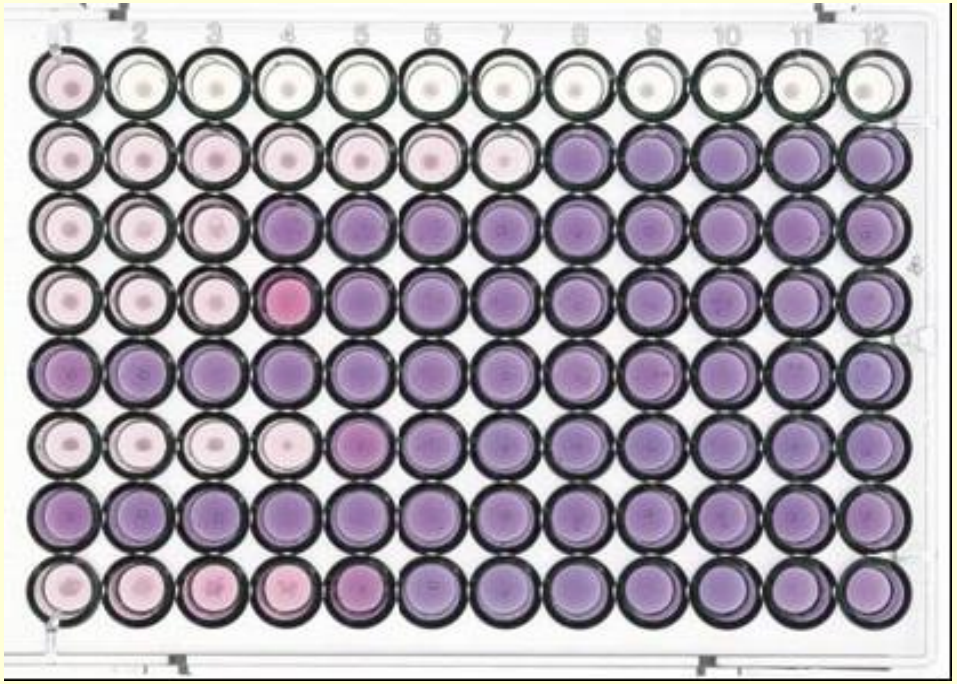
- Terbinafine - Binds to and inhibits squalene epoxidase blocking ergosterol synthesis.

Griseofulvin

- Causes disruption of the mitotic spindle by interacting with polymerized microtubules through binding to microtubule protein. Administered systemically for dermatophytic infections.

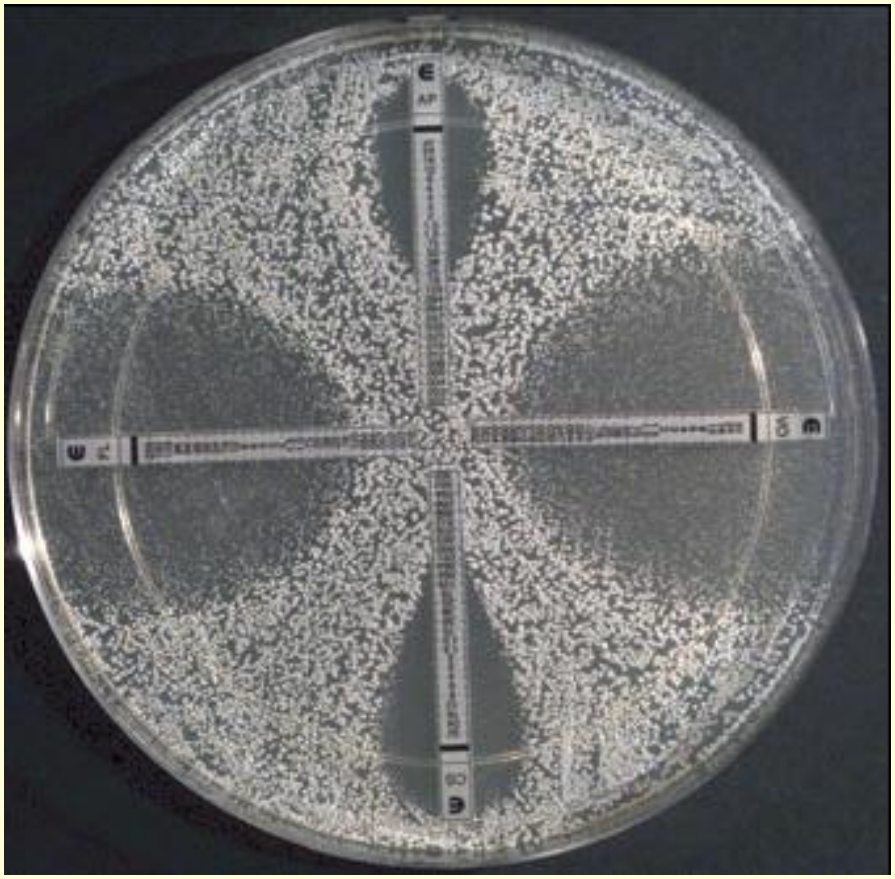
Susceptibility methods

Broth microdilution method



Etest method

- Well-defined MIC value
- Easy to handle



Disc diffusion method

- good correlation with the standard broth microdilution method