

MYCOLOGY-1

General properties of fungi &
Candidiasis

GENERAL MYCOLOGY

- Medical mycology - branch of medical science that deals with the study of medically important fungi
- 'fungus' is derived from Greek '*mykes*' meaning *mushroom* (a type of edible fungus)

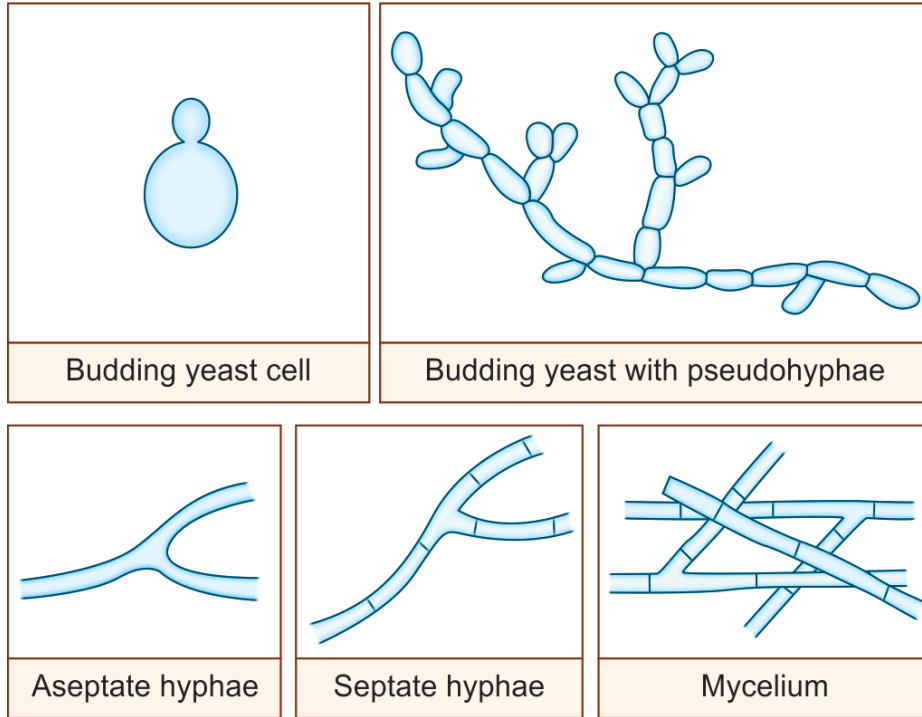
Fungi differ from bacteria & other eukaryotes

- Possess a rigid cell wall, composed of chitin, β -glucans and other polysaccharides
- Cell membrane contains ergosterol instead of cholesterol
- May be unicellular or multicellular
- Lack chlorophyll and divide by asexual and/or sexual means by producing spores

Morphological Classification of Fungi

- 1. Yeast:** Round to oval cells that reproduce by budding
 - *Cryptococcus neoformans* (pathogenic)
 - *Saccharomyces cerevisiae* (non-pathogenic)
- 2. Yeast-like:** Yeasts forming pseudohyphae (e.g. *Candida*)
 - Differentiated from true hyphae as they have constrictions at septa
- 3. Molds:** long branching filaments called hyphae
 - Hyphae - septate or nonseptate

Morphological forms of fungi



Classification of Fungi

- Based on the growth pattern in culture medium
- **Aerial mycelium:** It is the part of the mycelium which projects above the surface of culture medium
- **Vegetative mycelium:** It is the part of the mycelium that grows on the surface of the culture medium

Morphological Classification of Fungi

- 4. Dimorphic fungi:** exist as molds (hyphal form) in the environment at ambient temperature (25°C) and as yeasts in human tissues at body temperature (37°C)
- *Histoplasma capsulatum*
 - *Blastomyces dermatitidis*
 - *Coccidioides immitis*
 - *Paracoccidioides brasiliensis*
 - *Penicillium marneffeii*
 - *Sporothrix schenckii*.

Taxonomical Classification

- Based on the production of sexual spores
1. **Phylum zygomycota**: sexual spores – **zygospores**, and possess **aseptate** hyphae, e.g. *Rhizopus* and *Mucor*.
 2. **Phylum ascomycota**: Sexual spores - **ascospores** and possess **septate** hyphae, e.g. *Aspergillus*.
 3. **Phylum basidiomycota**: Sexual spores - **basidiospore** e.g. *Cryptococcus*
 4. **Phylum deuteromycota (Fungi imperfecti)**: sexual state is either absent or unidentified yet

Types of fungal spores

Sexual Spore	Observed in-
Zygosporeres	Zygomycetes
Ascospores	<i>Aspergillus</i>
Basidiospores	<i>Cryptococcus</i>
Asexual Spore	Observed in
Vegetative asexual spore	
Arthrospore	<i>Coccidioides Trichosporon</i>
Blastospore	<i>Candida</i>
Chlamyospore	<i>Candida albicans</i>

Types of fungal spores

Aerial asexual spore	
Conidiospore or conidia	<i>Aspergillus</i>
Sporangiospore	Zygomycetes
Microconidia	Dermatophytes
Macroconidia	Dermatophytes

CLASSIFICATION OF FUNGAL DISEASES

Superficial mycoses	Agents
Tinea versicolor	<i>Malassezia furfur</i>
Tinea nigra	<i>Hortaea werneckii</i>
Piedra	<i>Trichosporon beigeli</i> , <i>Piedraia hortae</i>
Dermatophytosis	<i>Trichophyton</i> , <i>Microsporum</i> , <i>Epidermophyton</i>
Subcutaneous mycoses	
Mycetoma	<i>Madurella mycetomatis</i> , <i>Pseudallescheria boydii</i> , etc
Sporotrichosis	<i>Sporothrix schenckii</i>
Chromoblastomycosis	<i>Phialophora verrucosa</i> <i>Fonsecaea pedrosoi</i>
Rhinosporidiosis	<i>Rhinosporidium seeberi</i>

CLASSIFICATION OF FUNGAL DISEASES

Systemic mycoses	
Histoplasmosis	<i>Histoplasma capsulatum</i>
Blastomycosis	<i>Blastomyces dermatitidis</i>
Coccidioidomycosis	<i>Coccidioides immitis</i>
Paracoccidioidomycosis	<i>Paracoccidioides brasiliensis</i>

CLASSIFICATION OF FUNGAL DISEASES

Opportunistic mycoses

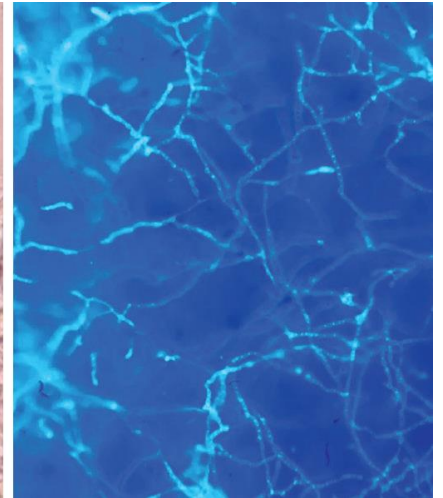
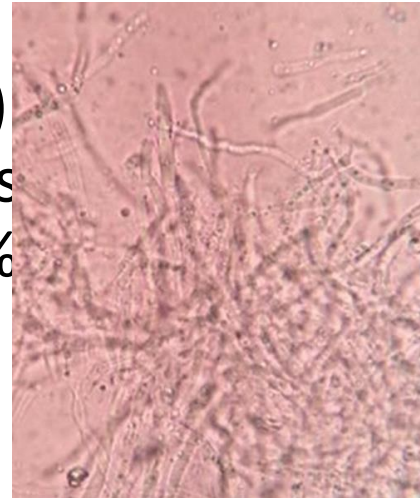
Candidiasis	<i>Candida albicans</i> , Other <i>Candida</i> species
Cryptococcosis	<i>Cryptococcus neoformans</i>
Zygomycosis	<i>Rhizopus</i> , <i>Mucor</i> , <i>Absidia</i>
Aspergillosis	<i>Aspergillus flavus</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus niger</i>
Penicilliosis	<i>Penicillium marneffeii</i> , Other <i>Penicillium</i> species
pneumocystosis	<i>Pneumocystis jirovecii</i>
Mycotoxicoeses	

LABORATORY DIAGNOSIS OF FUNGAL DISEASES

- **Specimen Collection**

- Depends on site of infection - skin scraping, hair, nail, sputum, etc.
- Systemic mycoses - blood sample, Cerebrospinal fluid (CSF), etc

- Demonstration of Fungal elements in the specimen
- **Potassium hydroxide (KOH) preparation:** Keratinized tissue specimens treated with 10% → digests keratin → fungal elements clearly seen
- 20–40% KOH - nail & hair



Microscopy

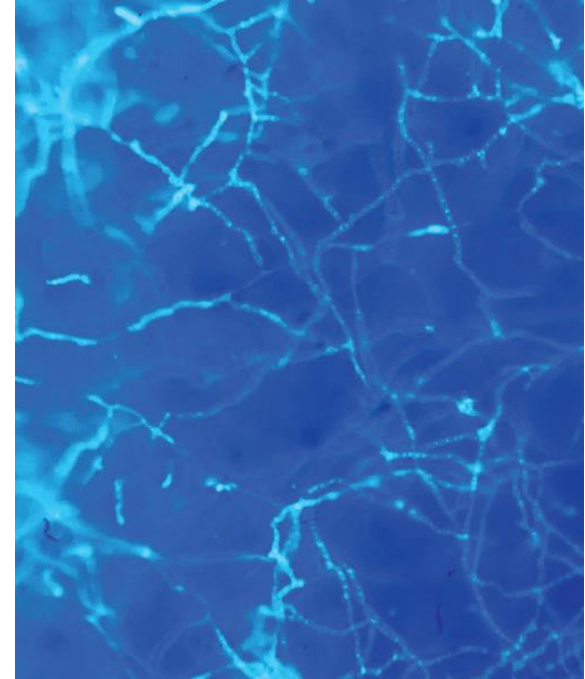
❖ KOH preparation

- **Biopsy specimens** - dissolved in 10% KOH in a test tube and examined after overnight incubation
- **Glycerol** (10%) can be added to prevent drying
- **DMSO** (dimethyl sulfoxide) - help in tissue digestion

❖ **Lactophenol cotton blue (LPCB):**

- Phenol acts as disinfectant
- Lactic acid preserves the morphology of fungi
- Glycerol prevents drying
- Cotton blue stains the fungal elements blue

- **Gram stain:** *yeasts & yeast like fungi appear* as gram-positive budding yeast
- **India ink and nigrosin stains:** negative stains for demonstration of capsule of *Cryptococcus neoformans*
- **Calcofluor white stain:** more sensitive
 - Binds to cellulose and chitin of fungal cell wall and fluoresce under UV light



Histopathological stains

- **P**or demonstrating fungal elements from biopsy tissues
- **P**eriodic acid schiff (PAS) stain:
 - PAS positive fungi appear magenta/deep pink, whereas the nuclei stain blue
 - PAS which stains only the live fungi
- **M**asson fontana stain: for pigmented (or pheoid) fungi
- **H**ematoxylin and Eosin stain
- **M**ucicarmine stain: for staining the carminophilic cell wall of *Cryptococcus* and *Rhinosporidium*

Histopathological stains

- **Gomori methenamine silver (GMS) stain:**
 - Alternative to PAS
 - Stains both live and dead fungi
 - Stains the polysaccharide component of the cell wall
 - Fungi appear black & background pale green color?

Culture Media

- **Sabouraud's dextrose agar (SDA):**
 - Most commonly used medium
 - Contains peptone (1%), dextrose (4%) and pH of 5.6
 - May not support some pathogenic fungi
- **Neutral SDA (Emmons' modification):**
 - Differs from original SDA in having neopeptone (1%) and dextrose (2%) and pH of 7.2

Culture Media

- **Corn meal agar and rice starch agar:** Nutritionally deficient media used for stimulation of chlamydospore production
- **Brain heart infusion (BHI) agar and blood agar:** Enriched media, used for growing fastidious fungi like *Cryptococcus* and *Histoplasma*
- **Niger seed agar and bird seed agar:** selective growth of *Cryptococcus*
- **CHROMagar *Candida* medium:** selective as well as 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
- **BOD incubators (biological oxygen demand):** capable of maintaining low temperature
- **Incubation time:** 2–3 weeks
- **Antibiotics** - cycloheximide (actidione), chloramphenicol and gentamicin - added to the culture media to inhibit bacterial growth

Culture Identification

- Based on macroscopic appearance of the colonies grown on culture and microscopic appearance
- **Macroscopic Appearance of the Colony**
- **Rate of growth:**
 - Rapid growth (<5 days) - saprophytes, yeasts and agents of opportunistic mycoses
 - Slow growth (1–4 weeks): dermatophytes, agents of subcutaneous and systemic mycoses

Culture Identification

- **Pigmentation:** reverse side of the culture media
- **Texture:** Refers to how the colony would have felt if allowed to touch - glabrous (waxy/leathery), velvety, yeast like, cottony or granular/powdery
- **Colony topography:** rugose (radial grooves), folded, verrucose or cerebriform (brain-like)

- **Teased mount:**
 - LPCB tease mount
 - **Identification is based on :**
 - **N**ature of hyphae (septate or aseptate, hyaline or phaeoid, narrow or wide) and **T**ype of sporulation (conidia or sporangia)
- **Slide culture:** in situ microscopic appearance of the fungal colony



Microscopic Appearance of Fungi

- **Cellophane tape mount:**
 - Impressions taken by placing the cellophane tape on the colonies → LPCB mount is made
 - Easy to perform than slide culture and in-situ fungal morphology maintained

Other Methods of Identification

- **For *Candida*:** Germ tube test, Dalmau plate culture, carbohydrate fermentation and carbohydrate assimilation tests are done
- **For dermatophytes:** Hair perforation test, dermatophyte test medium and dermatophyte identification medium are used
- **Urease test** can be done for the fungi that produce urease enzyme, e.g. *Cryptococcus*

Other Methods of Identification

- **Immunological Methods** - to detect antibody or antigen from serum and/or other body fluids
- **Antibody detection** - ELISA, immunodiffusion test, agglutination test, and complement fixation test (CFT)
- **Antigen detection** - latex agglutination test for cryptococcal antigen from CSF
- **Immunohistochemistry**: detecting antigens (e.g. proteins) on cells of a tissue section

Other Methods of Identification

- **Tests for Metabolites** - in body fluids by gas liquid chromatography
- **Tests to Demonstrate Delayed Hypersensitivity**
 - Skin tests - *Histoplasma, Blastomyces, Coccidioides, Paracoccidioides, Dermatophyte, Sporothrix and Candida.*
- **Molecular Methods**
 - Polymerase chain reaction (PCR) and its modifications
 - DNA sequencing methods

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CANDIDIASIS

CANDIDIASIS

- Yeast like fungus that produces pseudohyphae
- **Species of *Candida*:**
 - *Candida albicans*: most common and most pathogenic
 - Other *Candida* species which can occasionally cause infection such as—*C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. kefyr*, *C. guilliermondii* and *C. viswanathii*

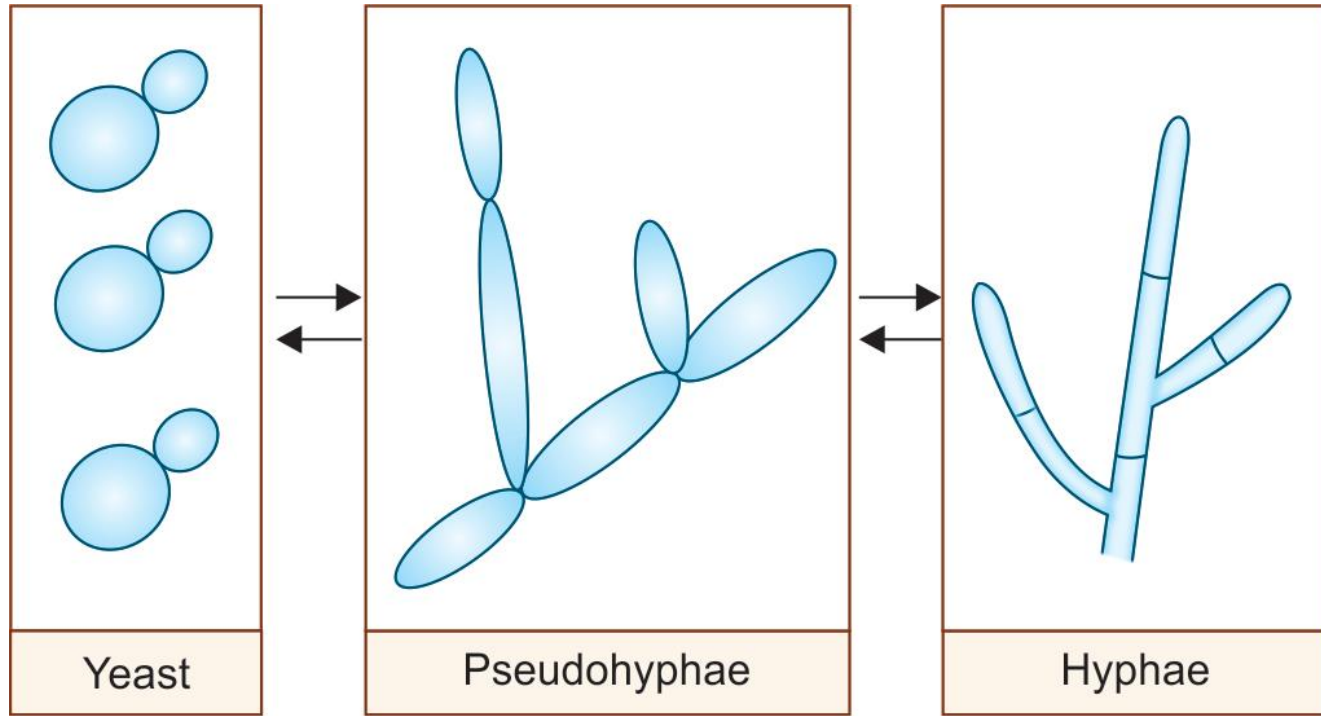
Pathogenesis

- Worldwide in distribution. Most common fungal infection
- **Predisposing Factors**
 - **Physiological state:** Extremes of age (infancy, old age), pregnancy
 - **Low immunity:** steroid or immunosuppressive drugs, post-transplantation, malignancy, HIV-infected people
 - **Patients on broad spectrum antibiotics**
 - **Others:** Diabetes mellitus, febrile neutropenia and zinc or iron deficiency

Virulence Factors

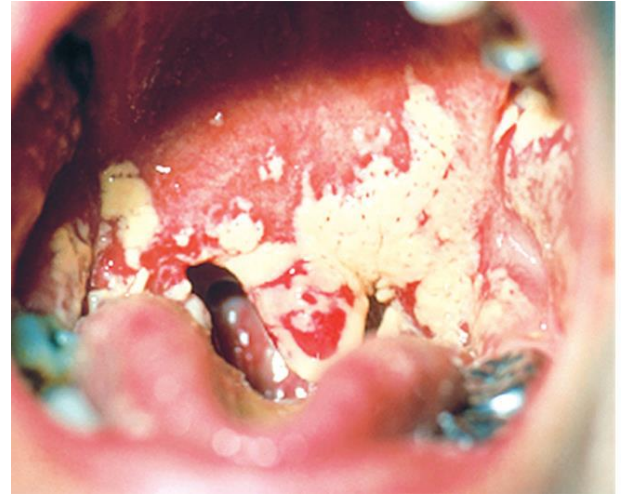
- **Adhesins**
- **Enzymes** - aspartyl proteinases and serine proteinases - tissue invasion
- **Toxins:** Glycoprotein extracts of Candida cell wall are pyrogenic
- **Pseudohyphae:** Presence of pseudohyphae indicates active infection
- **phenotypic switching** - Ability to transform between three phenotypic forms in the tissue - yeast (blastospores), pseudohyphae, and true hyphae

Phenotypic switching of *Candida*



- **Mucosal candidiasis:**

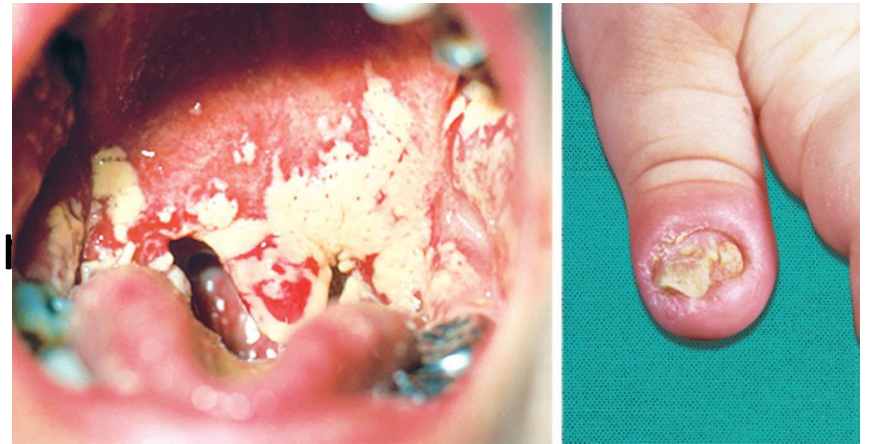
- Oropharyngeal candidiasis : white, adherent, painless patch
- Vulvovaginitis: pruritus, pain, and vaginal discharge (whitish curd like in severe cases)
- Balanitis and balanoposthitis
- Esophageal candidiasis



Clinical Manifestations

- **Chronic mucocutaneous candidiasis**
 - Infants and children with deficient CMI
 - Lesions on hair, nail, skin, and mucous membrane
 - Usually resistant to treatment
- **Cutaneous candidiasis:**
 - Intertrigo: erythema and pustules in the skin folds; associated with tight fitting undergarments and sweating

- Paronychia and onychomycosis
- Diaper candidiasis
- Perianal candidiasis
- Erosio interdigitalis blastomycetorum
- Generalized disseminated cutaneous candidiasis



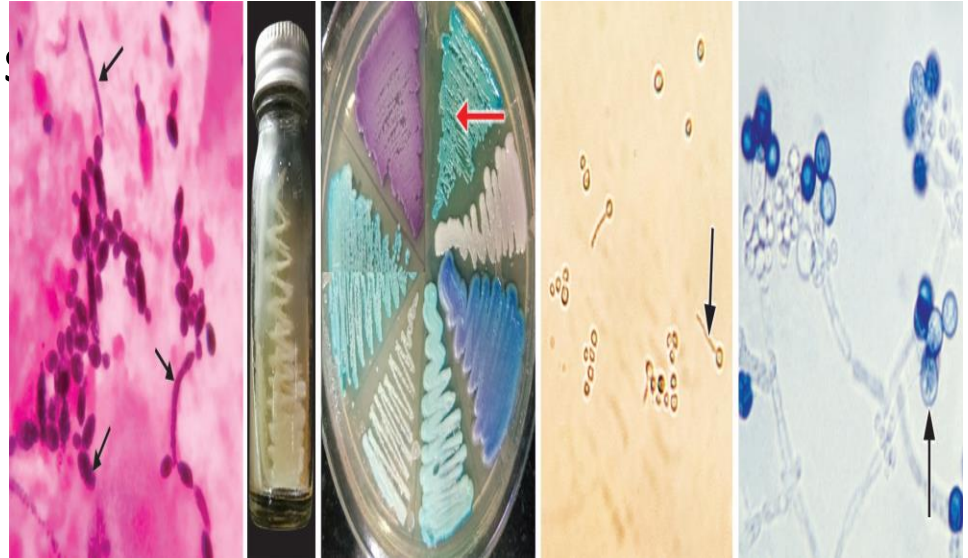
Clinical Manifestations

- **Invasive candidiasis:** hematogenous or local spread
 - Urinary tract infection
 - Pulmonary candidiasis, Septicemia
 - Arthritis and osteomyelitis, Meningitis
 - Ocular—keratoconjunctivitis and endophthalmitis
 - Hepatosplenic candidiasis, Disseminated candidiasis
 - Nosocomial candidiasis (mainly by *C. glabrata*).

Clinical Manifestations

- **Allergic candidiasis includes:**
 - **Candidid:** allergic reaction to metabolites of *Candida* - vesicular lesions in the web space of hands and other areas
 - Similar dermatophytid reaction
- **Other allergic reactions include:** Gastritis, irritable bowel syndrome and eczema

- **Specimen Collection**
- whitish mucosal patches on skin and nail scrapings, sputum, urine or blood
- **Direct Microscopy**
- Gram staining - gram-positive oval budding yeast cells with pseudohyphae



Culture

- SDA with antibiotic supplements at 37°C
- Grow in bacteriological culture media -blood agar
- Blood culture bottles (conventional and automated blood)
- Colonies - creamy white, smooth, and pasty with typical yeasty odor

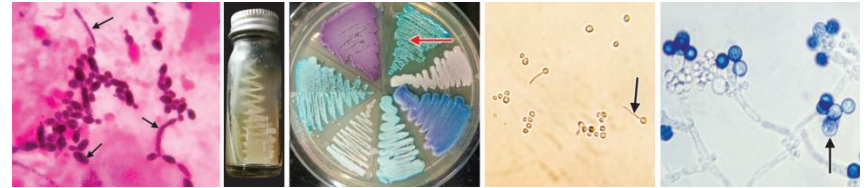
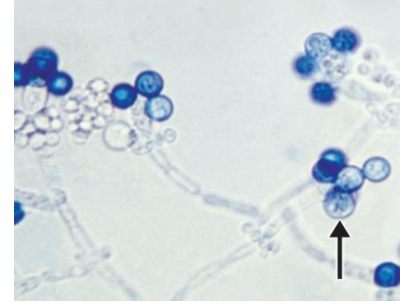


Tests for Species Identification

- **Germ tube test:** specific test for *C. albicans*
- Also called Reynolds Braude phenomenon
- **Germ tubes** - long tube like projections extending from the yeast cells
- Differentiated from pseudohyphae as there is no constriction at the origin



- **Dalmau plate culture:** Culture on cornmeal agar → *C. albicans* produces thick walled chlamydospores



- **CHROMagar:** Different *Candida* species produce different colored colonies

Tests for Species Identification

- **G**rowth at 45°C:
 - It differentiates *C. albicans* (grows) from *C. ubliniensis* (does not grow at 45°C)
- **C**arbohydrate fermentation test
- **C**arbohydrate assimilation
- **M**olecular methods

Immunodiagnosis

- **Antibody detection:** ELISA, latex agglutination tests - antibodies against cell wall mannan antigen
- **Antigen detection:** cell wall mannan and cytoplasmic antigens - ELISA
- **Enzyme detection:** *enolase*, *aspartate* proteinase
- **Test for metabolites:** mannitol, arabinitol
- G test is done for detection of β -1-3 -D-glucan

Treatment

- Cutaneous candidiasis or oral thrush: topical azole
- Esophageal and vulvovaginal candidiasis: oral fluconazole or caspofungin
- Disseminated candidiasis: liposomal amphotericin B or caspofungin
- *C. glabrata* and *C. krusei* exhibit intrinsic resistance to azoles

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