Medical Mycology

Dr.Bhavin Prajapati
Assistant Professor
Department of Microbiology

Learning Objectives

- Differentiate between fungi & bacteria
- Classify fungi
- Describe laboratory diagnosis of fungal infection
- Diseases caused by fungi

Definitions

- Mykos = mycete = fungus
- **Mycology**--scientific discipline dealing with fungi
- **Mycoses**--diseases caused by fungi in human or animals

General knowledge of the fungi

- Eukaryotic microorganisms
- Rigid cell walls: chitin, mannans and polysaccharide (glutan)
- Plasma membranes: ergosterol
- Chemotrophic
- Used in fermentation, production of antibiotics

Differenet from bacteria

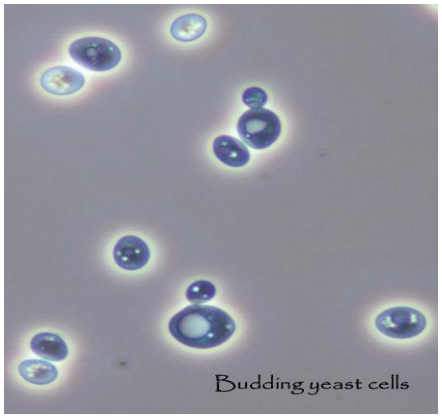
- Morphology –unicellular or multicellular
- Rigid cell wall
- Presence of sterol in cytoplasmic membrane
- Presence of true nuclei with nuclear membrane & paired chromosomes
- Reproduction: Sexual or asexual or both

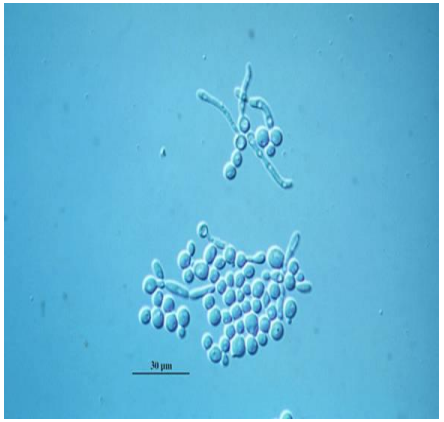
Classification of fungi

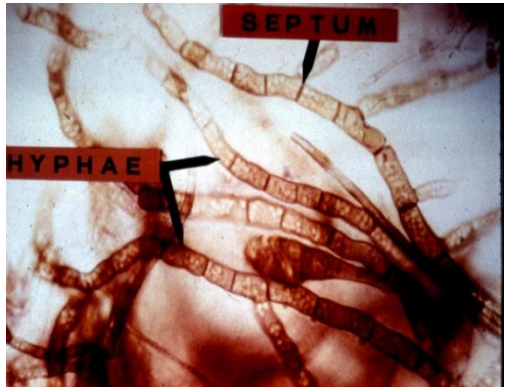
- Morphological
- Based on site of infection
- Taxonomic

Morphological classification

- Yeast
- Yeast like
- Mould
- Dimorphic







Clinical Classification

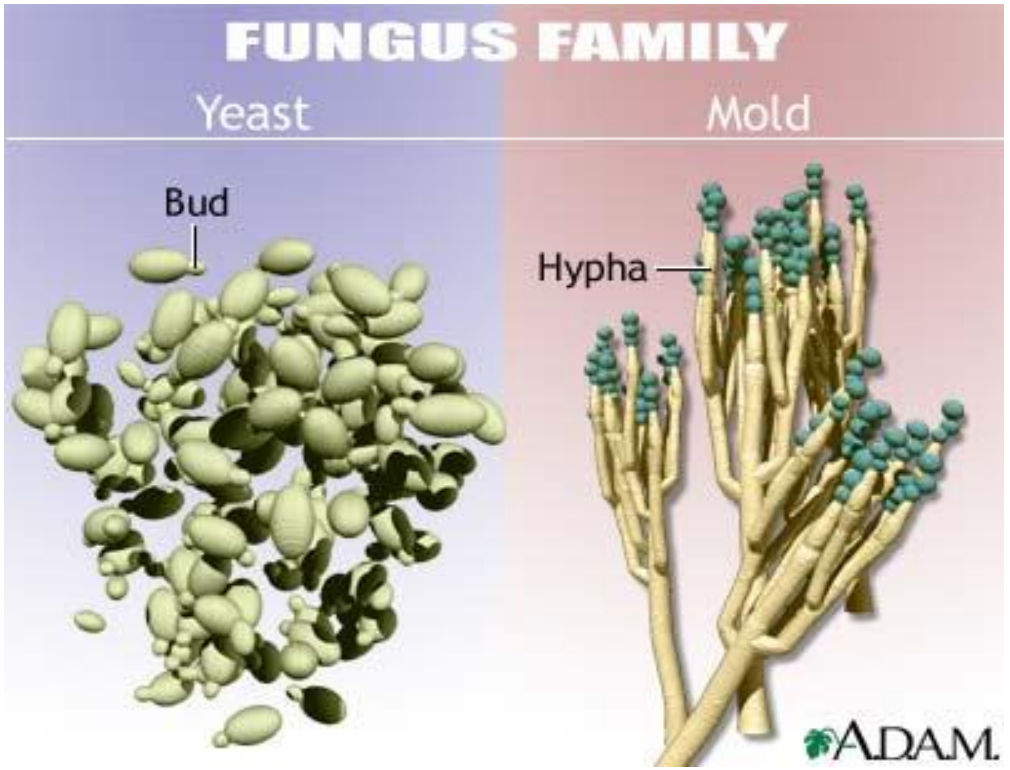
- Superficial
- Subcutaneous/cutaneous
- Systemic
- Opportunistic

Fungi-Taxonomic classification

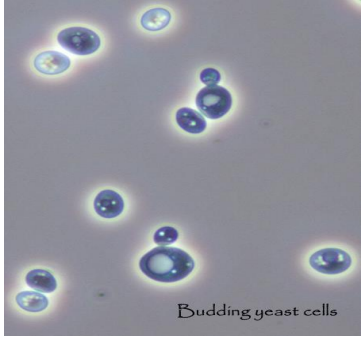
SEXUAL SPORE	CLASS
Zygospore	Zygomycetes
Basidiospore	Basidiomycetes
Ascospore	Ascomycetes
None/Unknown	Deuteromycetes ("Fungi Imperfecti")

Morphological classification

- Yeast
- Yeast like fungi
- Mould
- Dimorphic



1. Yeasts

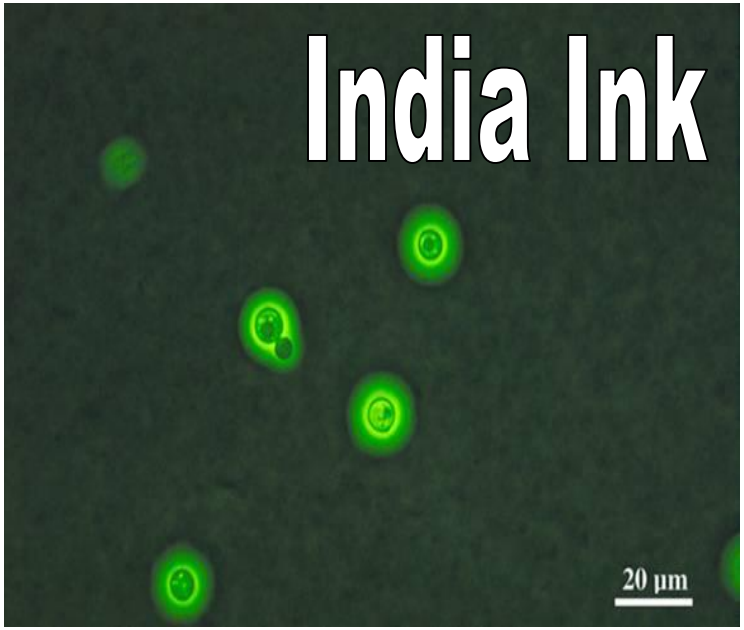


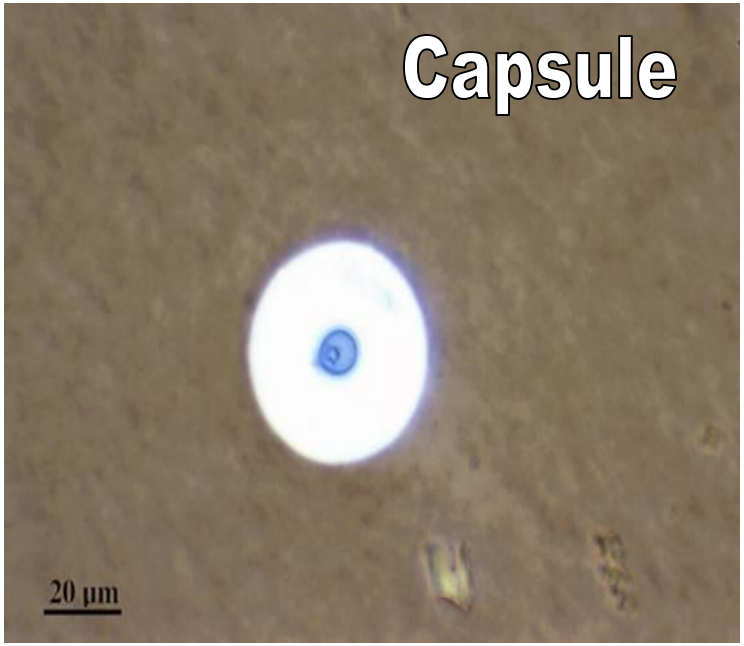
1. These occur in the form of round or oval bodies which reproduce by budding

2. Yeasts colonies resemble bacterial colonies in appearance and in consistency.

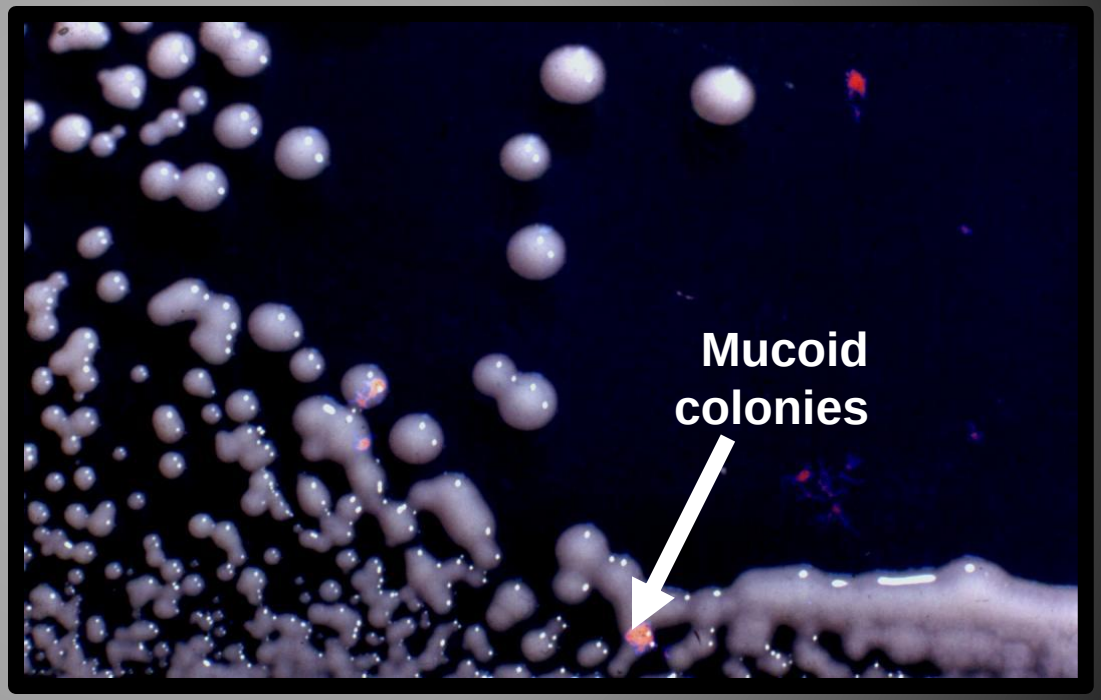
3. The only pathogenic yeast of medical importance is *Cryptococcus neoformans*.

Cryptococcus neoformans





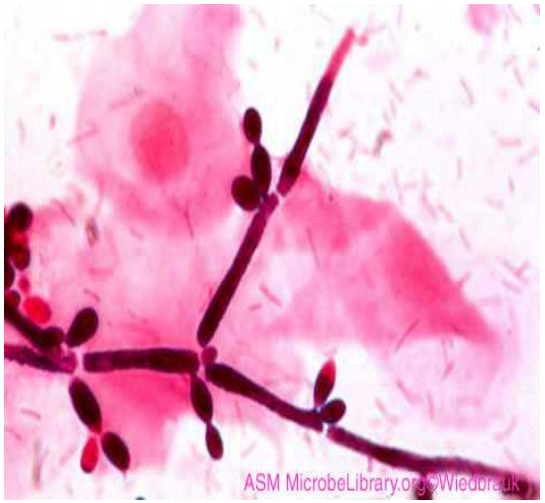
Yeast colonies



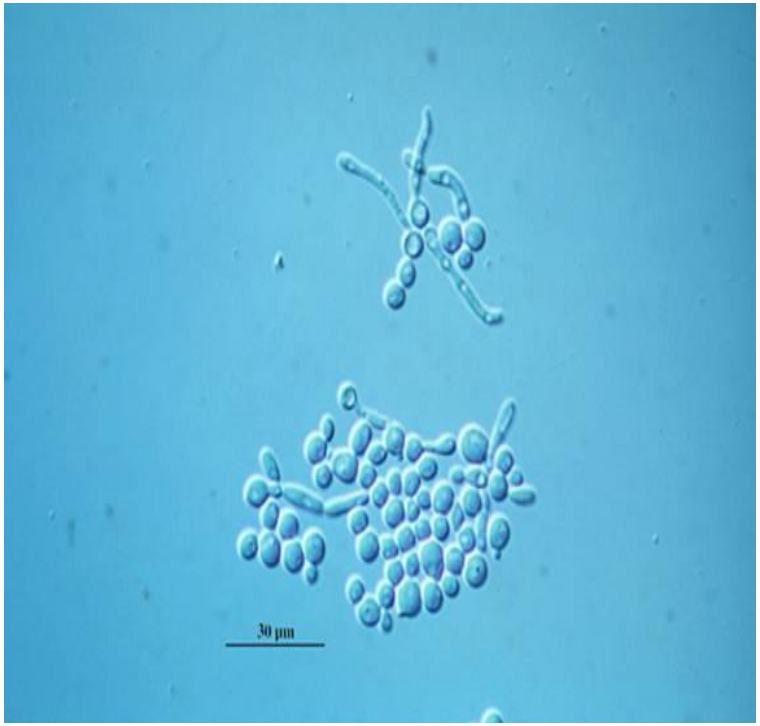
2. Yeast-Like

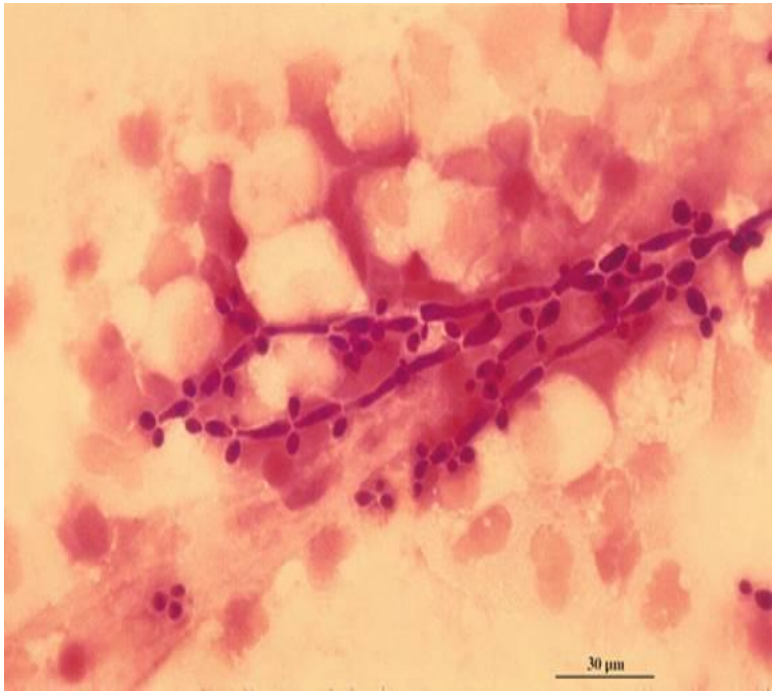
1. These are fungi which occur in the form of budding yeast-like cells and as chains of elongated filamentous cells which appear as broad septate hyphae. These are called as pseudomycelium.

2. Example is *Candida*.

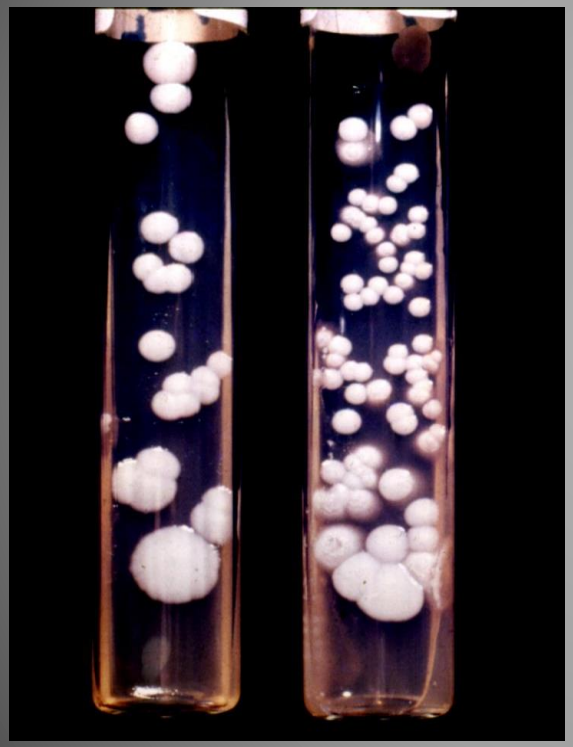


Candida albicans





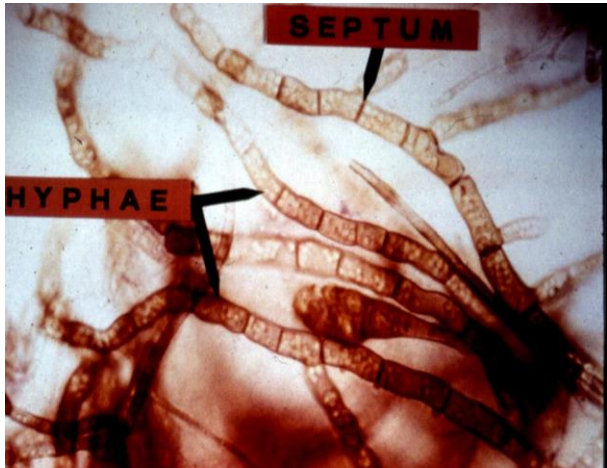
Candida Colonies



3. Moulds = filamentous

1. The basic morphological elements of filamentous fungi are long branching filaments or hyphae, which intertwine to produce a mass of filaments or mycelium .

2. Colonies are strongly adherent to the medium and unlike most bacterial colonies cannot be emulsified in water.



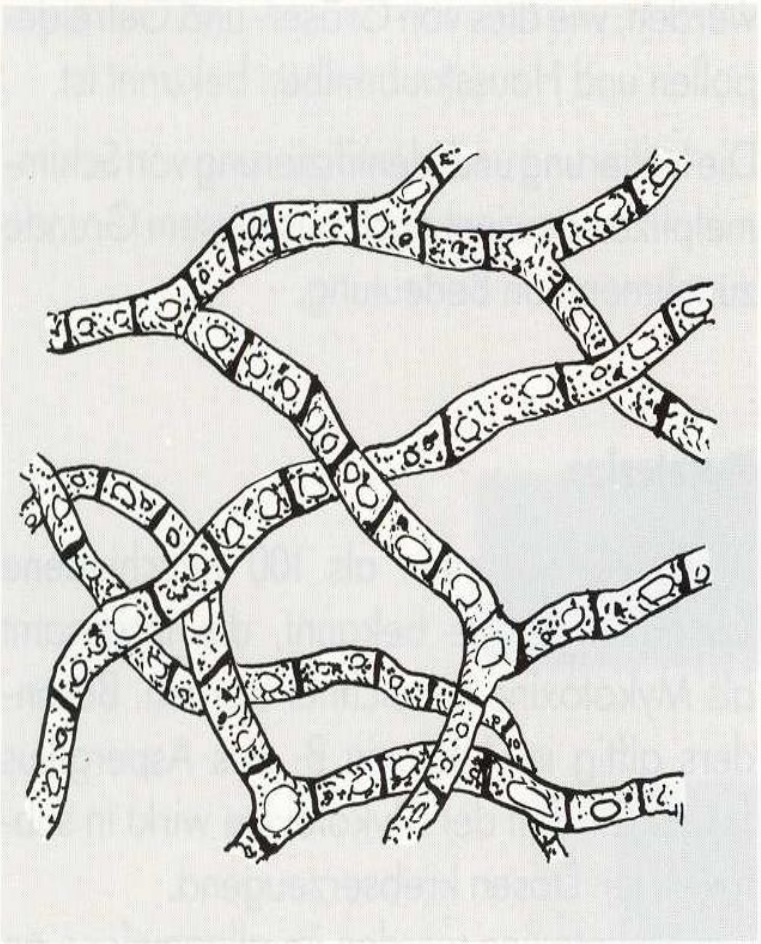


Abb. 47: Septiertes Myzel

mycelium: septate

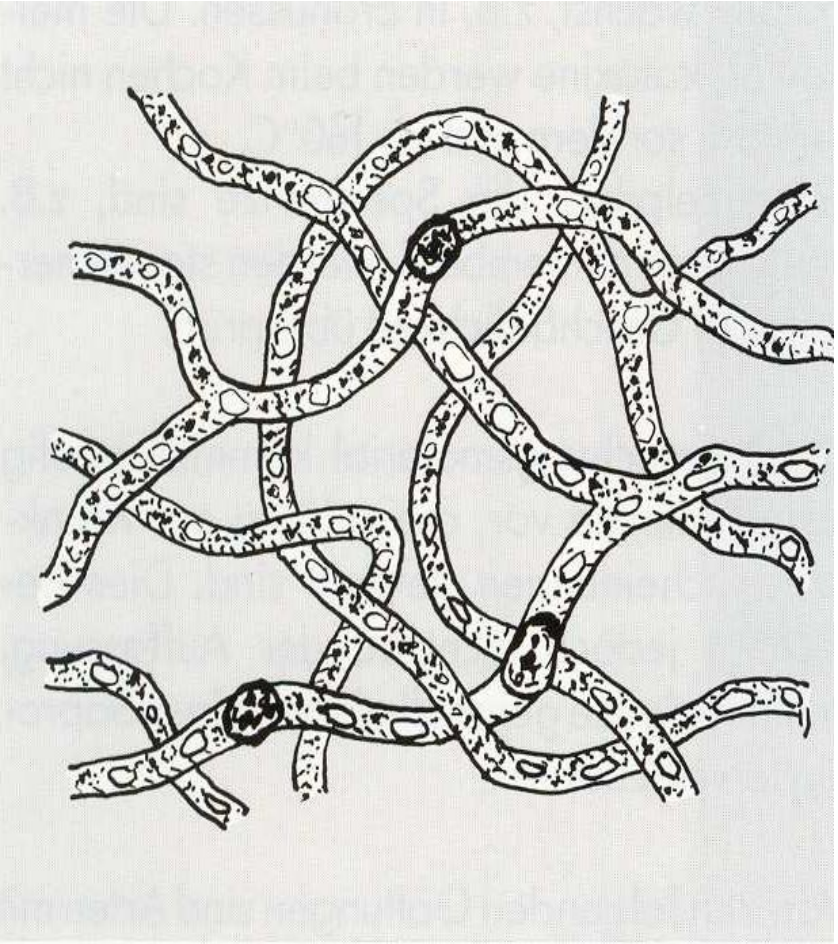


Abb. 48: Unseptiertes Myzel

mycelium: non septate

3. The surface of these colonies may be velvety, granular,powdery, or may show a cottony aerial mycelium.

4. Many fungi produce Pigmentation –

colony itself – obverse

of the underlying medium – reverse

Examples :

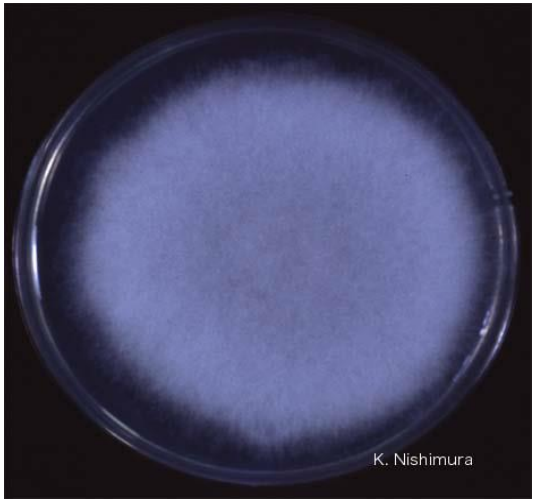
Aspergillus

Penicillium

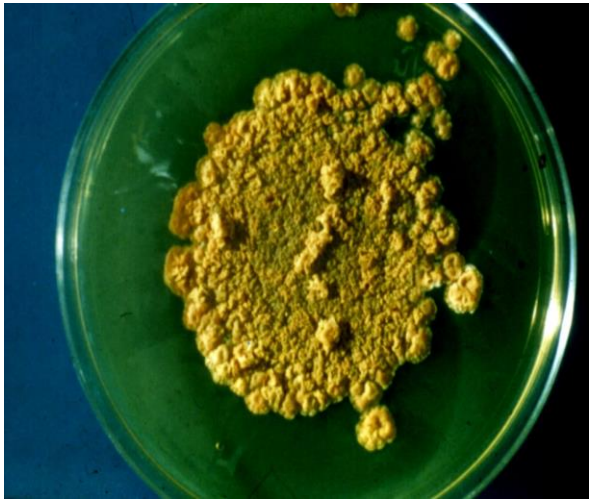
Fusarium

Rhizopus

cottony



powdery

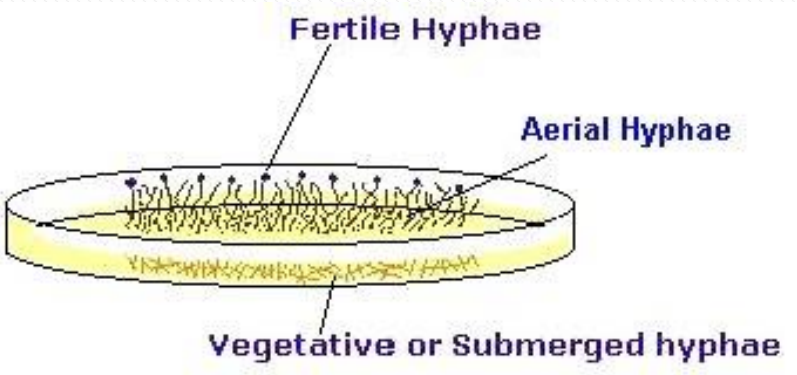


velvety

granular

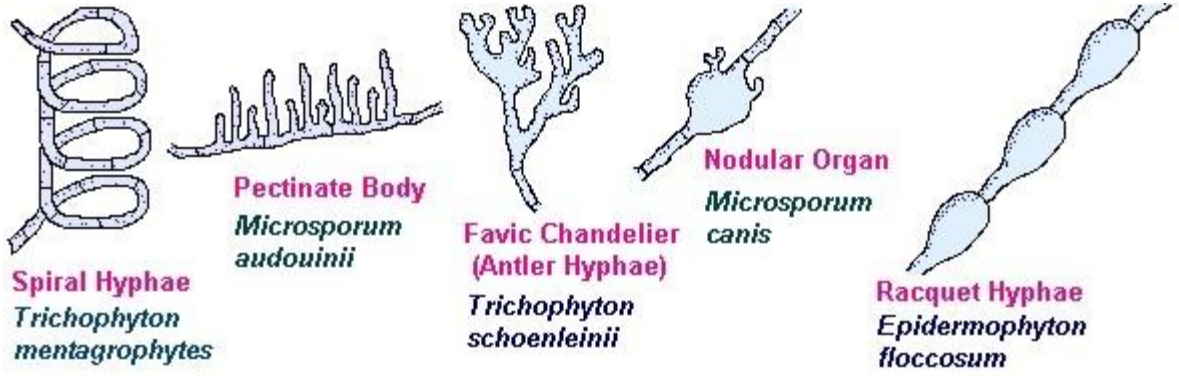
Types of hyphae :

- Hyphae –could be
  - Septate
  - Non-septate
- Based on pigmentation
  - Hyaline
  - Dematiceous
- Mycelium
  - Vegetative
  - Aerial / fertile



Special Hyphal structures

- Spiral
- Racquet
- Nodular
- Favic Chandelier
- Pectinate body



4. Dimorphic Fungi

Has two morphology –

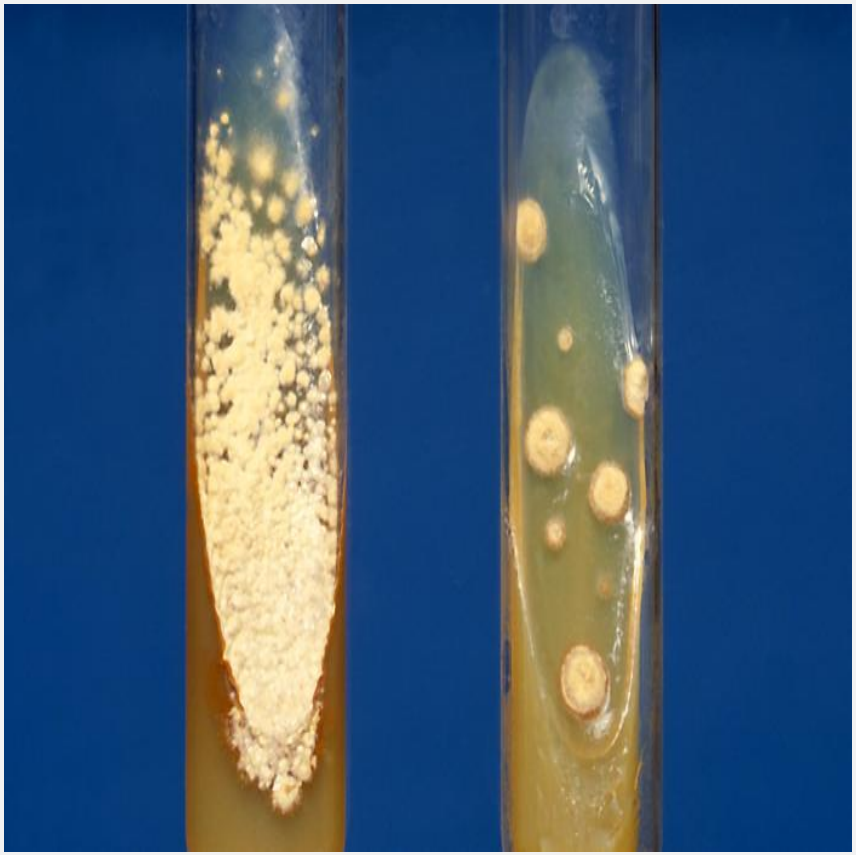
At 22-25^0 c –outside body – Mould

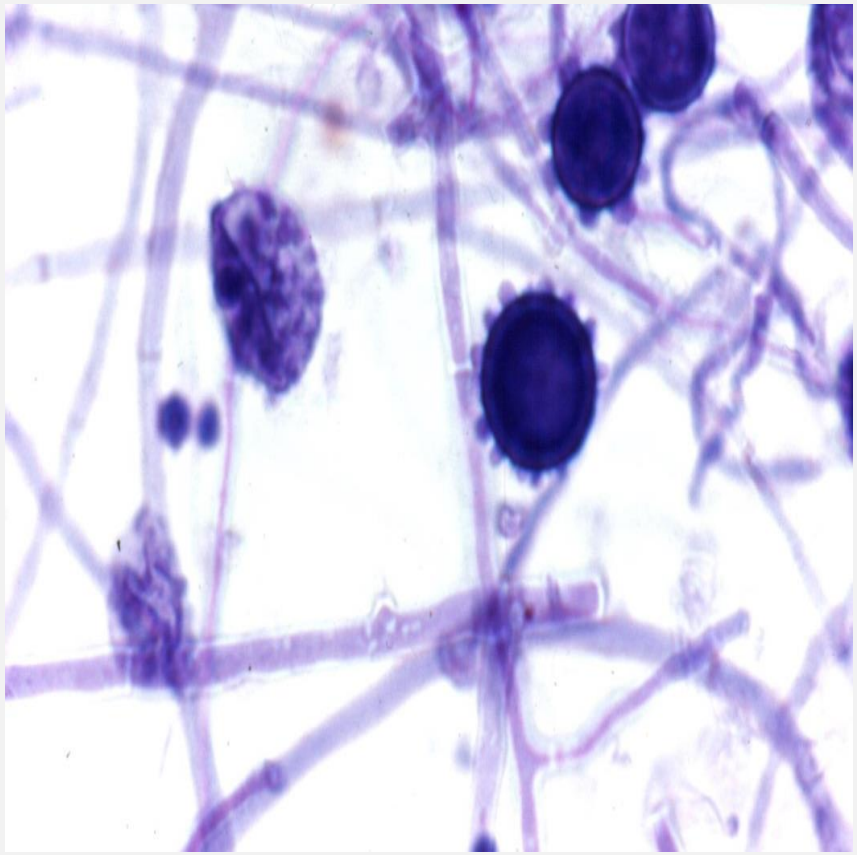
At 37^0 c – inside body – Yeast

Examples :

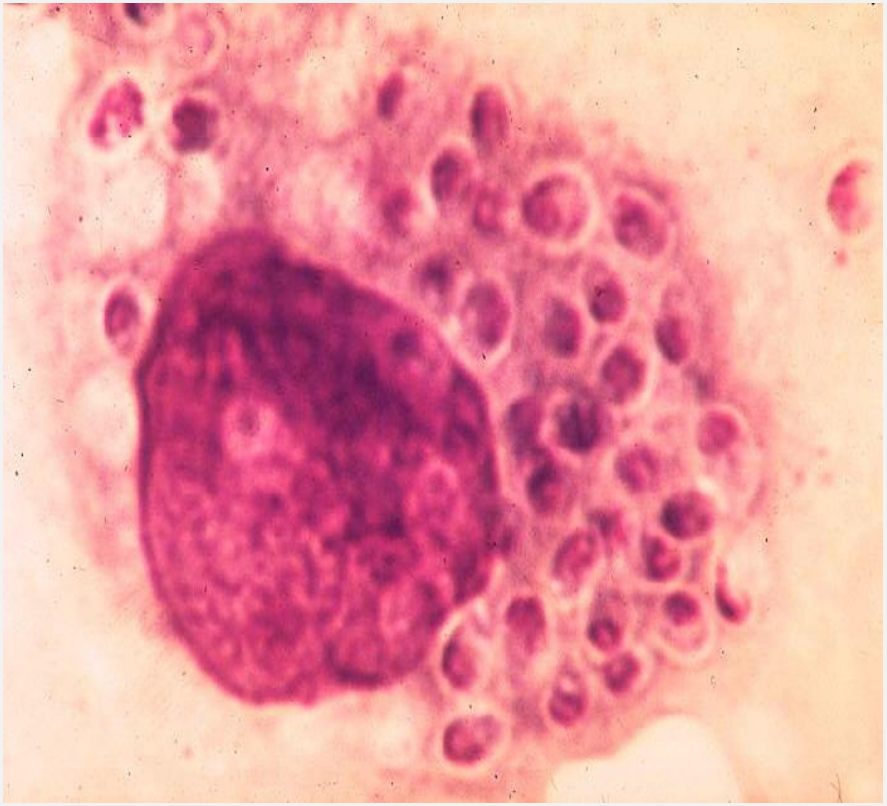
- Blastomyces dermatitidis
- Coccidioides immitis
- Histoplasma capsulatum
- Paracoccidioides brasiliensis
- Penicillium marneffei

Histoplasma capsulatum –22° –25°C





Histoplasma capsulatum – 37°c



Clinical Classification of Mycoses

- Superficial
- Subcutaneous
- Systemic
- Opportunistic

Superficial Mycoses

Surface infection & Cutaneous infection

Skin, hair and nails

Rarely invade deeper tissue

e.g. Dermatophytes,T.versicolor

Superficial mycoses

Surface infection	Cutaneous infection
Affecting dead layers of skin & its appendages	Affecting cornified layer of skin & its appendages
No inflammatory response	Inflammatory & allergic response
e.g. Tinea versicolor, Tinea nigra, Piedra	e.g. Dermatophytes

Subcutaneous Mycoses

- Confined to subcutaneous tissue and rarely spread systemically. Fungi present in soil introduced in body by trauma

- Examples :
  - Mycetoma
  - Chromoblastomycosis
  - Sporotrichosis
  - Rhinosporidiosis

Systemic Mycoses

- Involve lung and other deep viscera
- May become widely disseminated
- Mostly caused by dimorphic fungi
- Enter through inhalation, produces pneumonia and then enter into blood and spread to other organs

OPPORTUNISTIC FUNGI

Fungi are of low virulence
Saprophytes

Non pathogenic in healthy but can become pathogenic if person is immuno-compromised

- Examples
  - Candida
  - Aspergillus
  - Mucor

Predisposing condition

- Systemic diseases
  - Diabetes, cancer, lymphoma, leukaemia

- Patient on
  - Immunosuppressive drugs (renal transplant)
  - Corticosteroid therapy
  - X-ray irradiation (bone marrow suppression)
  - Broad-spectrum antibiotics

Taxonomic classification

- Based on type of sexual spore
  - Zygospore
  - Oospore
  - Ascospore
  - Basidiospore
- Classified in to
  - Zygomycetes
  - Ascomycetes
  - Basidiomycetes
  - Fungi imperfecti

Reproduction of Fungi

1. Sexual reproduction --Sexual spores

   Formed through a process involving the fusion of two parental nuclei followed by meiosis

2. Asexual reproduction--Asexual spores

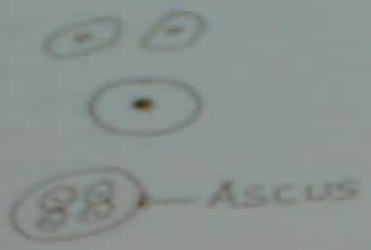
   The product of mitotic division of a single parent cell.

   Vegetative spores – thallospore

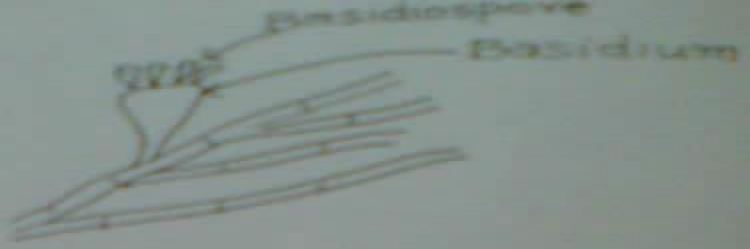
   Aerial spores

Sexual spore

- Formed after sexual fusion – Zygote formation
- Ascospore
  - Formed by a process called as free cell formation within a cell like structure called as ascus
- Basidiospore
  - Formed externally at the tip of basidium
- Zygospore
  - By fusion of two cells of equal size
- Oospore
  - By fusion of big female cell & small male cell



A. Ascospore



B. Basidiospore

C. Zygospore

D. Oospore

FIG. 7. SEXUAL SPORES

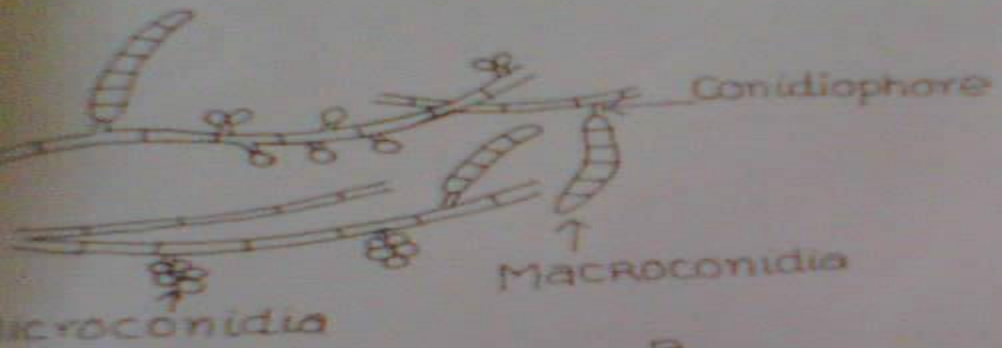
Asexual spores – Thallospore

- Develop directly from thallus- Hyphae

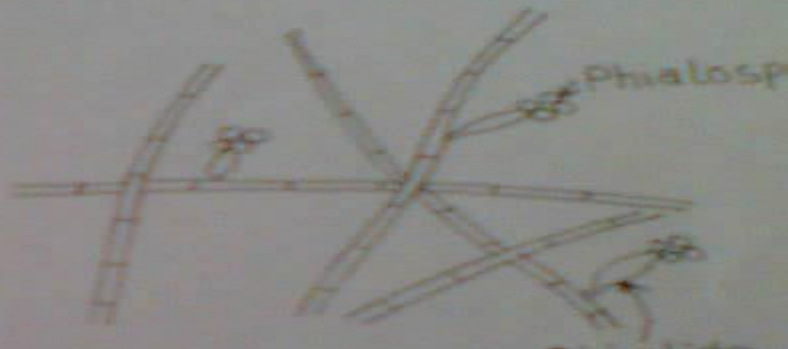
- *Arthrospores* – formed by disarticulation of vegetative cells of hyphae
- *Blastospore* – buds of yeast
- *Chlamydospores* – develop from hyphal cell in old cultures or under unfavorable condition

A. Blastospore B. Arthrospore C. Chlamydospore

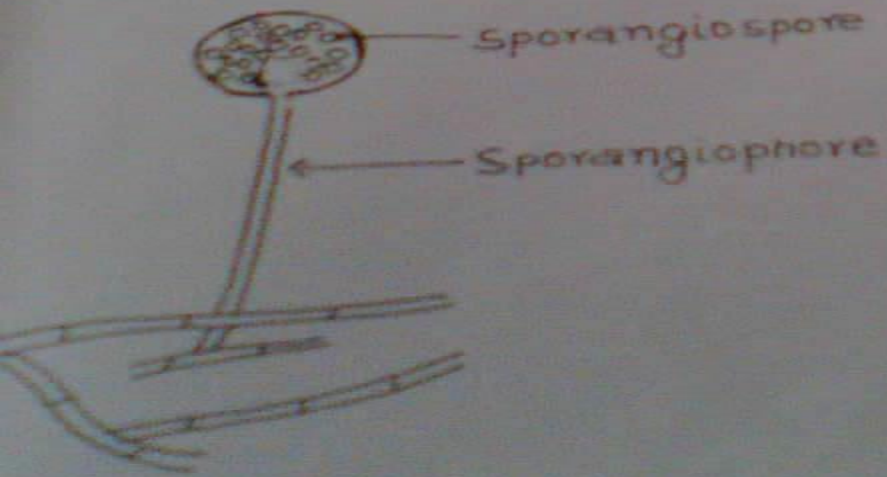
FIG. 5. THALLOSPORES



A. B.



C.

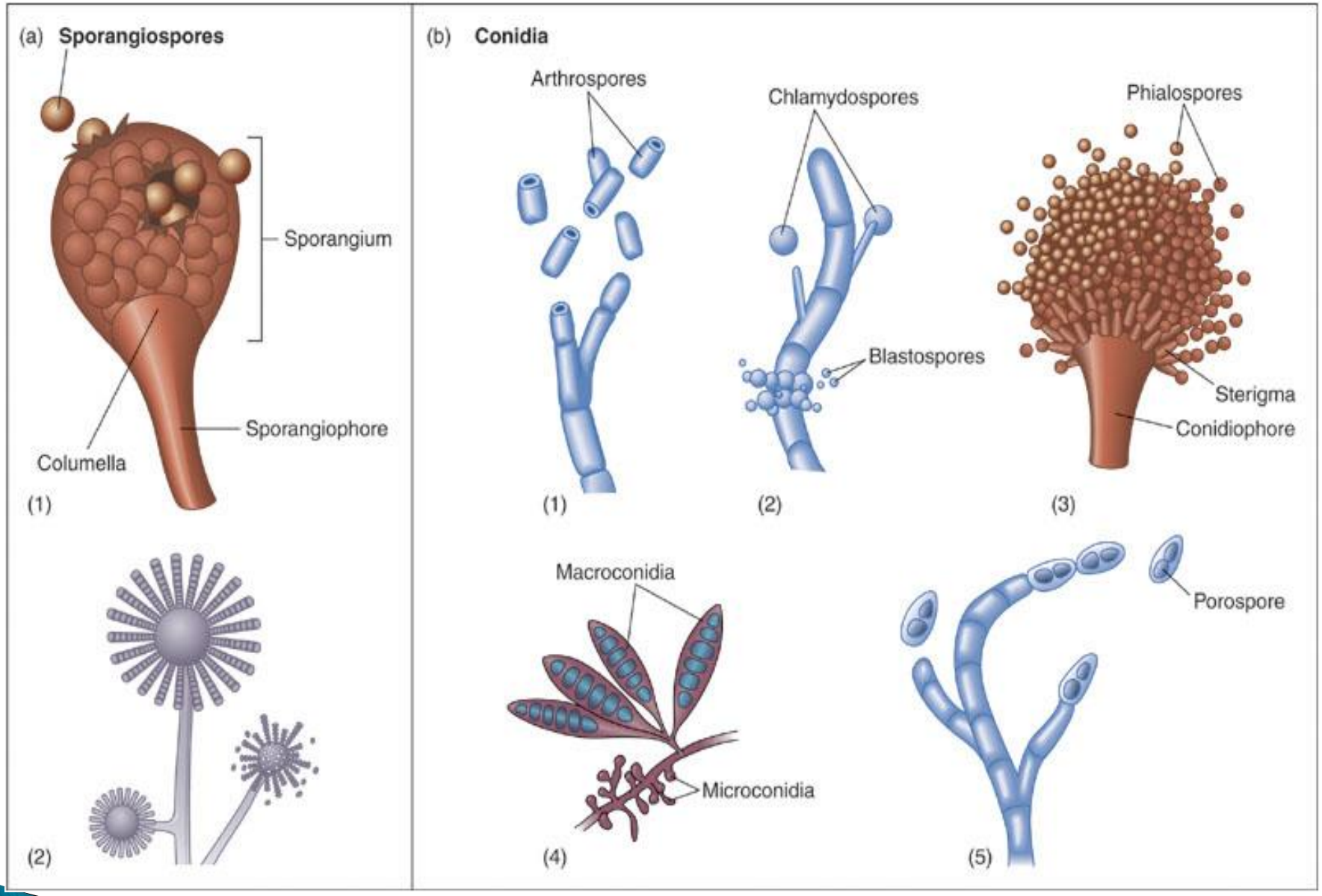


D. Sporangiospores

E. Aleurospore

Asexual spores – from special hyphal structure – aerial spores

- Sporangiospores
  - Spores formed in sporangia borne on sporangiophore (Intracellular)
- Conidia
  - Formed externally on phialides or specialized hyphal branches– conadiophore
  - Conidia – 2 types
    - Microconidia – unicellular
    - Macroconidia – multicellular



Laboratory to diagnosis of fungal infection

- Specimen collection and transport
- Specimen processing
  - Direct examination
  - Culture
  - Identification by growth
- Antigen/antibody detection

Specimen collection and transport

- must be material from the actual site of infection
- carefully collected without contamination
- in sufficient quantity
- collected before antifungal therapy

Specimen processing

- Specimen should be examined as soon as possible

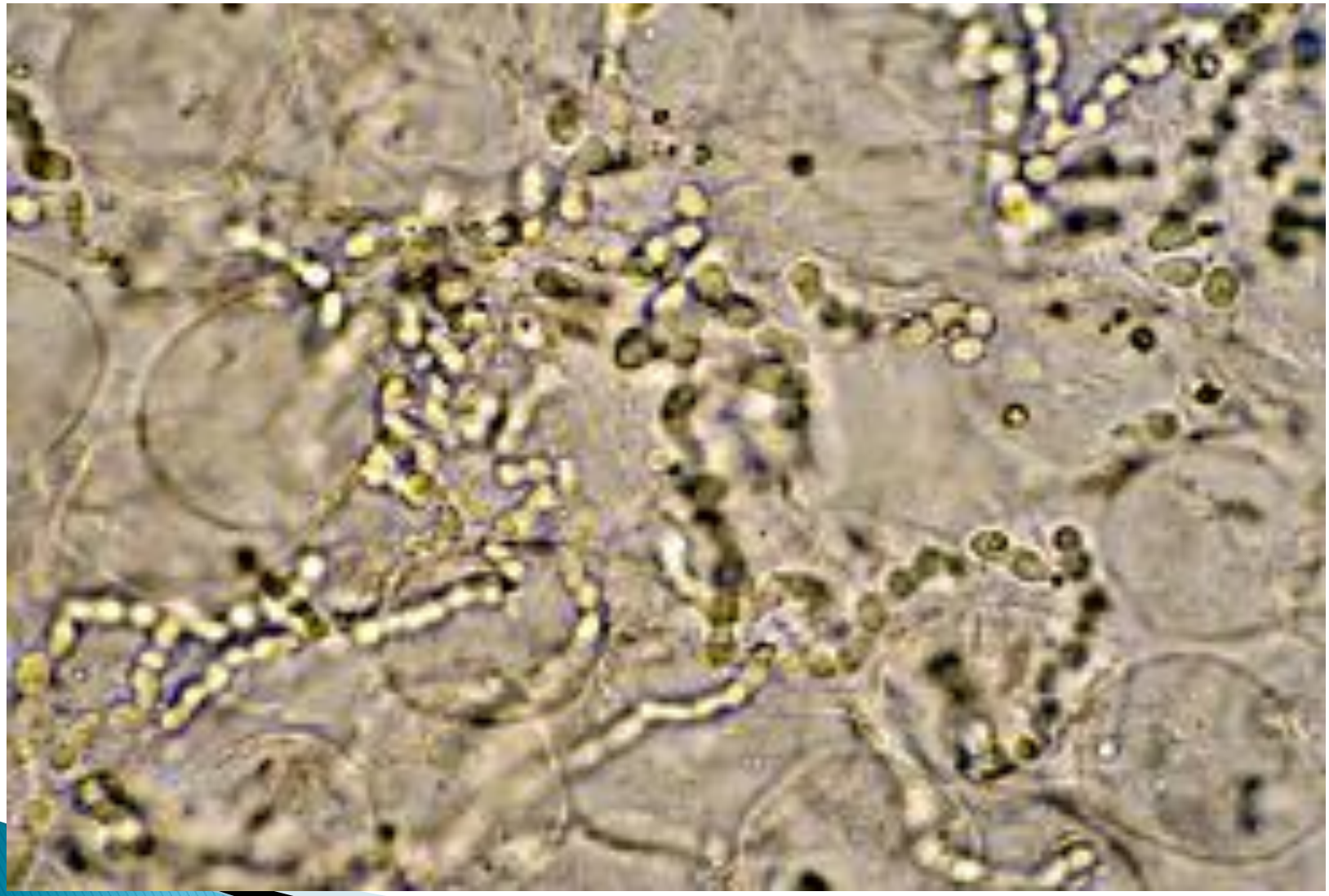
- Direct examination :
  - KOH mount
  - Calcofluor white
  - India ink
  - Gram stain

- Culture media
  - SDA
  - Brain-Heart infusion agar (BHIA)

KOH preparation

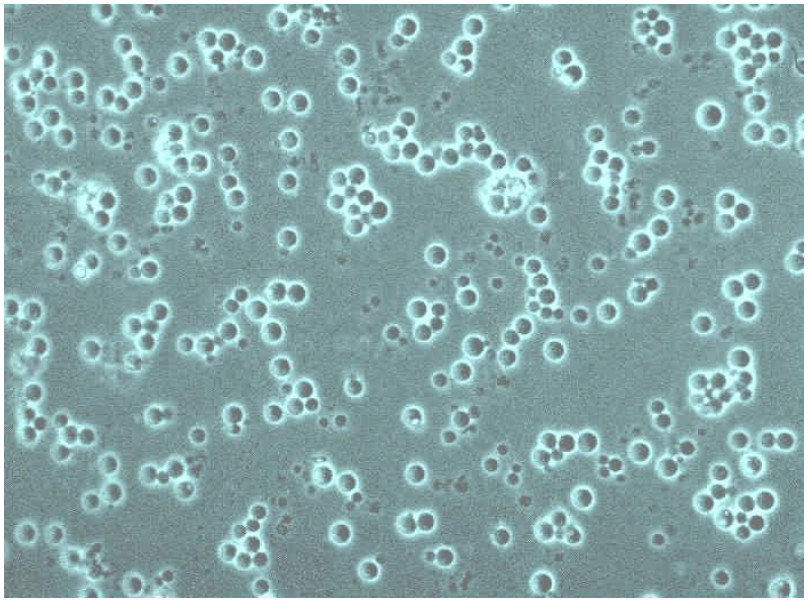
- Made for hair, nail or skin scraping or all thick material which requires digestion
- Consist of
  - KOH – 10 % – 70 ml
  - Glycerol – 20 ml

Direct wet mount (KOH)



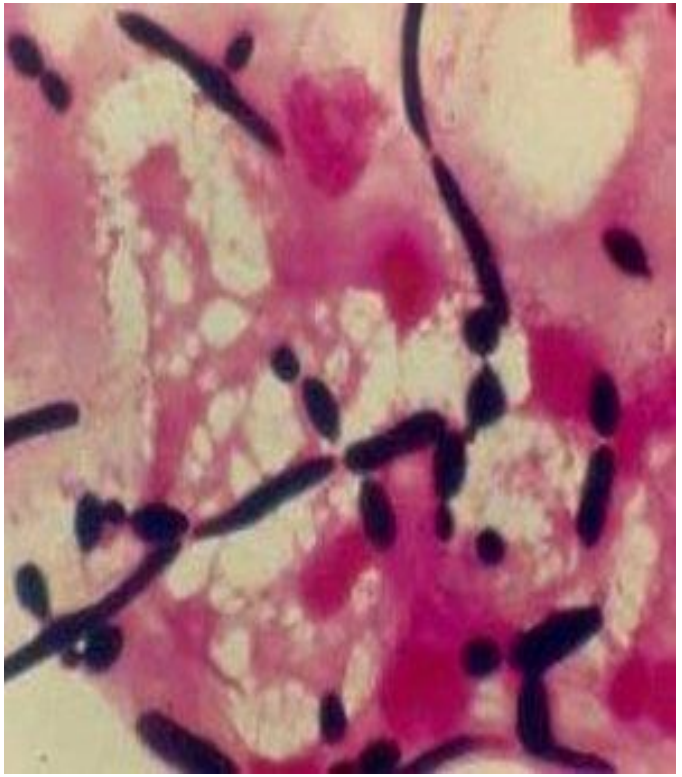
India ink preparation

- Negative stain to detect presence of capsule of Cryptococcus neoformans which appear as halo surrounding yeast cells

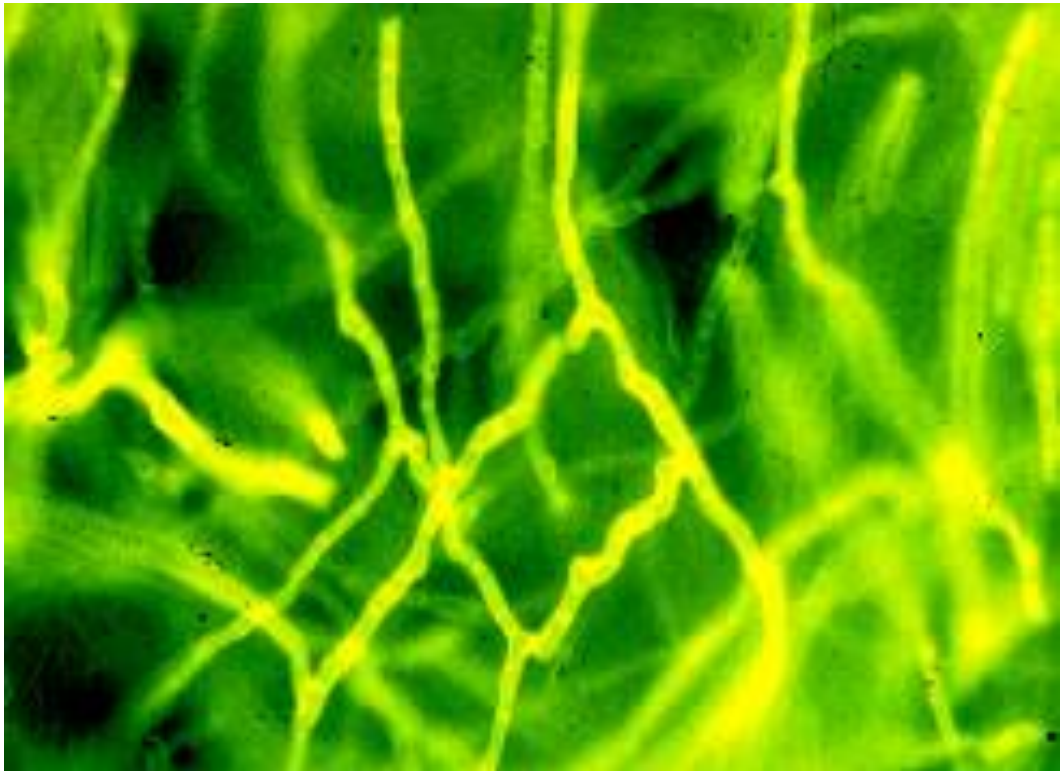


Gram stain

- Same procedure as for bacteriology
- Fungi are **gram positive**
- Budding yeast cells with or without psuedohyphae or hyphae are seen.



Nail: Calcofluor white stain



Culture of fungi

- Fungi are slowly growing taking days to weeks to grow
- Most of specimen may contain bacteria along with fungi
- Media should be prepared in such a way to avoid contamination
  - Antibiotics are added
  - Kept in screw-cap bottles
  - pH is kept acidic

Culture media

- Sabouraud's dextrose agar with antibiotics
  - Dextrose 40 gm 4 %
  - Poly peptone 10 gm 1 %
  - Agar 25 gm 2.5 %
  - DW 1000 ml
  - Antibiotics Chloramphenicol
    Gentamycin
    Cycloheximide
- Brain hear infusion agar
- Corn meal agar

Growth of fungus on SDA

Brain – heart infusion agar

- Enriched medium
- Mainly used for systemic fungi like Histoplasma
- Consist of
  - Calf brain infusion
  - Beef heart infusion
  - Peptone
  - Glucose
  - NaCl, Na_2HPO_4 & DW

Identify by colony characteristics

- Colony characters: Glabrous, velvety, yeast like, cottony, granular, pigmented
- Rate of growth
  - Rapid
    - 1-2 days - Mucor
    - 2-3 days - Yeast & yeast like fungi
  - Intermediate
    - 1-2 weeks - Dermatophyte
  - Slow
    - 2-4 weeks - Systemic

Growth of fungus on SDA

Microscopic examination after culture

- Tease mount
- Cellophane tape preparation
- Slide culture

Tease mount in LCB –Lactophenol Cotton Blue stain

Role of

- Lactic acid : Enhances penetration of sol. in hyphae
- Phenol : Inactivates the living cells
- Cotton blue : Does the staining
- Glycerol : Creates semipermanent preparation & reduces precipitation of stain

Antigen detection

- Used for Cryptococcus, Aspergillus, Histoplasma etc
- Detected by
  - CIEP
  - Latex agglutination
  - ELISA
  - PCR

- Thank you