

FUNGUS

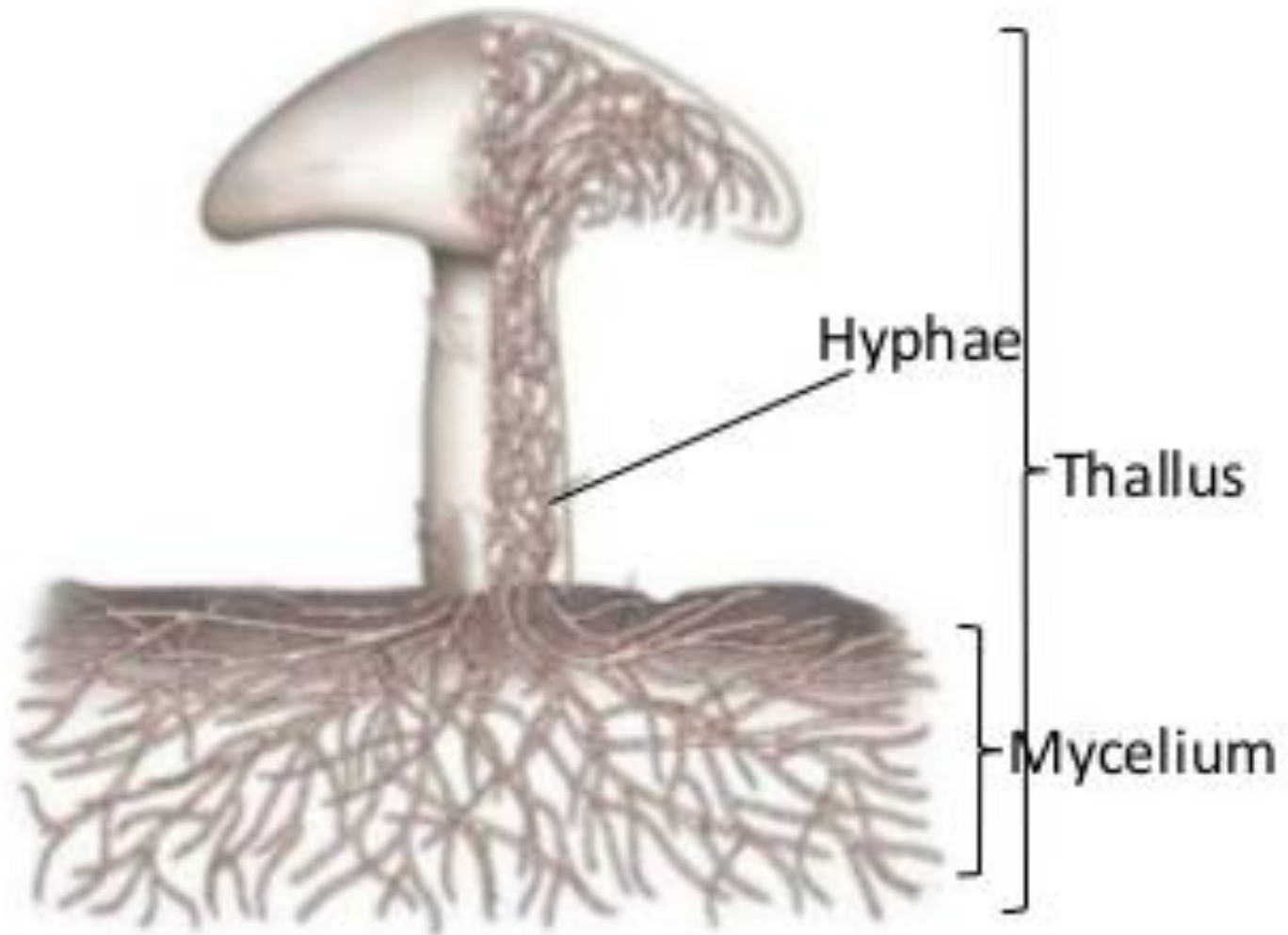


Dr. Poonam Shakya

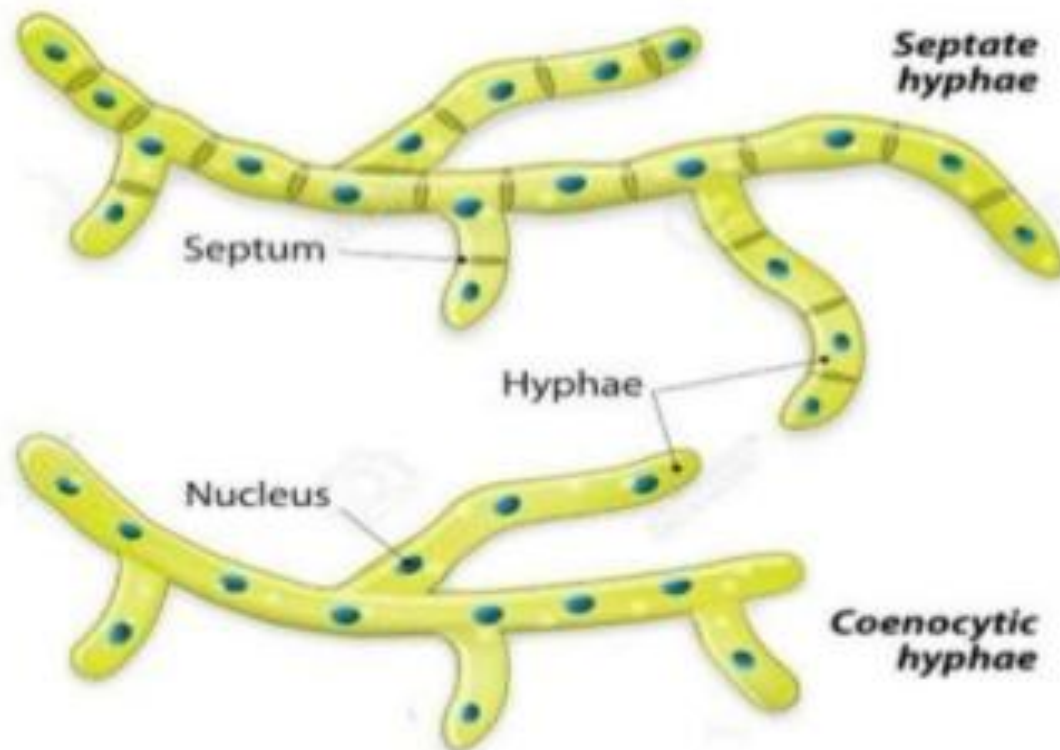
GENERAL CHARACTERISTICS OF THE FUNGI

- ▶ Eukaryotic and are therefore insensitive to most bacterial antibiotics
- ▶ Contain all the eukaryotic cell organelles
- ▶ Non-photosynthetic, Non-motile, Spore bearing
- ▶ Possess Cell wall of chitin
- ▶ Optimum pH is about 6, but they can tolerate more acidic conditions
- ▶ Strict aerobes
- ▶ Optimum temperature for growth of 20–30°C, but the pathogenic fungi causing systemic mycoses can tolerate 37°C.
- ▶ Two forms-Unicellular Yeasts & Multicellular Moulds

General Structure



- Hyphae are divided into cells by internal cross walls called septa (singular:-septum)
- Due to septa there are two types of hyphae :
 - 1)Septate hyphae
 - 2)Aseptate hyphae



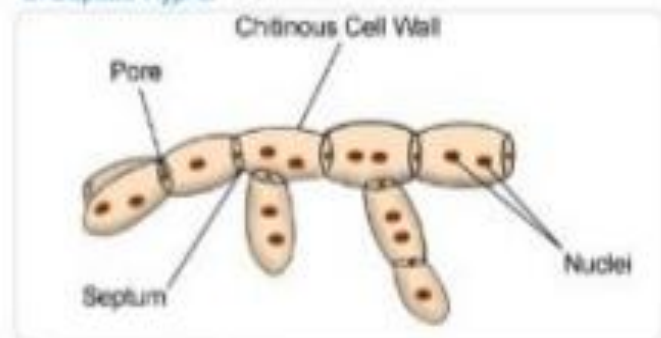
Classification

Morphological classification-

1) Mold-fungi which are structurally long filamentous & multicellular & form a network of hyphae/mycelium.

- Ex-
 - Dermatophytes | Septate
 - Aspergillus |
 - Mucor --Aseptate

a. Septate Hypha

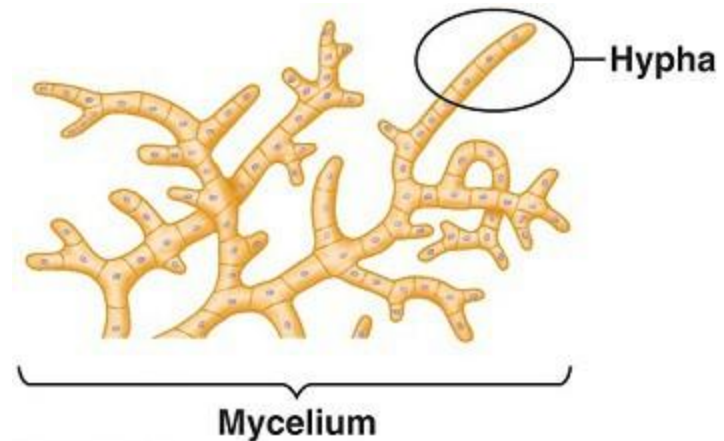


b. Coenocytic Hypha



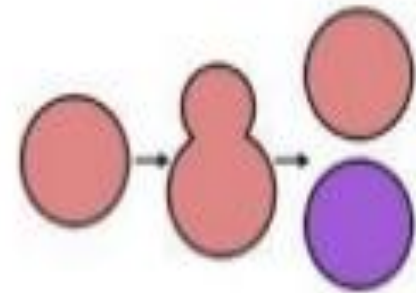
Molds

- ▶ Consists of the Hypha- a branching tubular structure 2-10 microns in diameter
- ▶ As growth begins hypha become intertwined to form Mycelium.
- ▶ Vegetative mycelium & Aerial mycelium
- ▶ Aerial mycelium produce spores or bears fruiting bodies



2) Yeast- unicellular
round/oval like fungi
reproduction by budding.

- Ex- Cryptococcus neoformans.



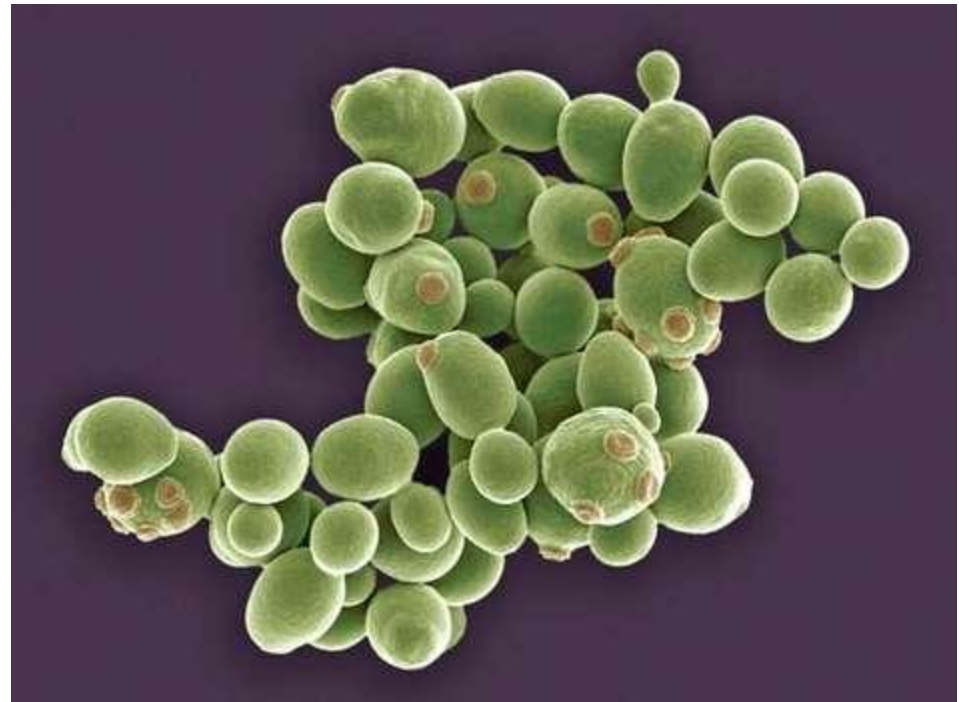
3) Yeast like fungus-
Fungus that reproduction
by budding, but bud fails
to separate from parent
cell & ultimately form a
structure that looks like a
chain of elongated cell.
They form
pseudohyphae.

- Ex- Candida



Yeasts

- ▶ Oval spherical cells
- ▶ 3-5 microns in diameter
- ▶ Grows well at 37°C



4) Dimorphic fungus

Fungi that can remain either mold/ yeast form depending upon the temperature of the environment.

- Ex- *Histoplasma capsulatum*
- 37C --> change to yeast form
- 25C --> mold form

CLASSIFICATION

- ▶ Kingdom *Fungi*
- ▶ Six phyla: different phyla are primarily characterized by different methods of sexual reproduction
 1. *Ascomycota*
 2. *Basidiomycota*
 3. *Chytridiomycota*
 4. *Zygomycota*
 5. *Microsporidiomycota* &
 6. *Glomeromycota*
- ▶ Members of *Chytridiomycota* and *Glomeromycota* are not known to be of veterinary or medical importance.
- ▶ Traditionally fungi with no known sexual stage have been formally grouped in the phylum *Deuteromycota*, also referred to as the Fungi Imperfecti.

Phyla	Class	Common name	Spore Vessels	Sexual spores	Representative genera
Ascomycota	Ascomycetes	Sac fungi	ascus	ascuspores	Penicillium
Basidiomycota	Basidiomycetes	Club fungi	none	basidiospores	Agaricus Amanita
Chytridiomycota	Chytridiomycetes	Chytrids	zoosporangium	zoospores	<i>Batrachochytrium</i>
Glomeromycota	Glomeromycetes	-	sporangium	glomerospores	gigaspora
zygomycota	Zygomycetes	Zygote fungi	sporangium	zygospores	Rhizopus

GENERAL FEATURES OF FUNGAL INFECTIONS

- ▶ Majority of the pathogenic fungi are found widespread in the environment as saprophytes or present as commensals associated with animals and humans
- ▶ Most are therefore opportunistic pathogens and predisposing factors often contribute to the establishment of fungal infections
- ▶ Upset in the normal flora of the host by prolonged administration of antibiotics,
- ▶ immunosuppression,
- ▶ concurrent infections,
- ▶ breaks in the skin or mucous membranes, perpetually moist areas of skin
- ▶ exposure to a large infective dose,
- ▶ mycotoxicoses occur due to animals ingesting preformed toxic metabolic products, termed mycotoxins, produced during fungal growth in animal feedstuffs

Laboratory Diagnosis

❧ Microscopy

❧ Culture

❧ Serology

❧ Skin test

❧ Antifungal susceptibility test

Direct Microscopic Examination

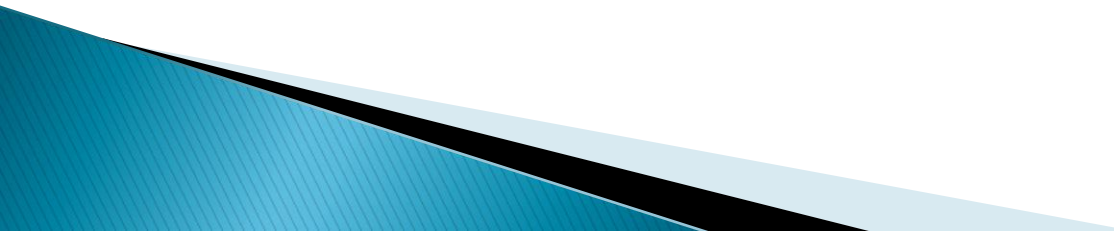
- ▶ Lacto phenol cotton blue staining
- ▶ 10–20% KOH wet preparations
- ▶ Calcofluor white (0.1%)- Fluorescence of fungal elements under fluorescence microscope
- ▶ India ink or nigrosin
- ▶ Gram or methylene blue stain
- ▶ Fluorescent antibody technique
- ▶ Periodic acid-Schiff
- ▶ Methenamine silver stain
- ▶ Wright or Giemsa stain

Lactophenol Cotton Blue



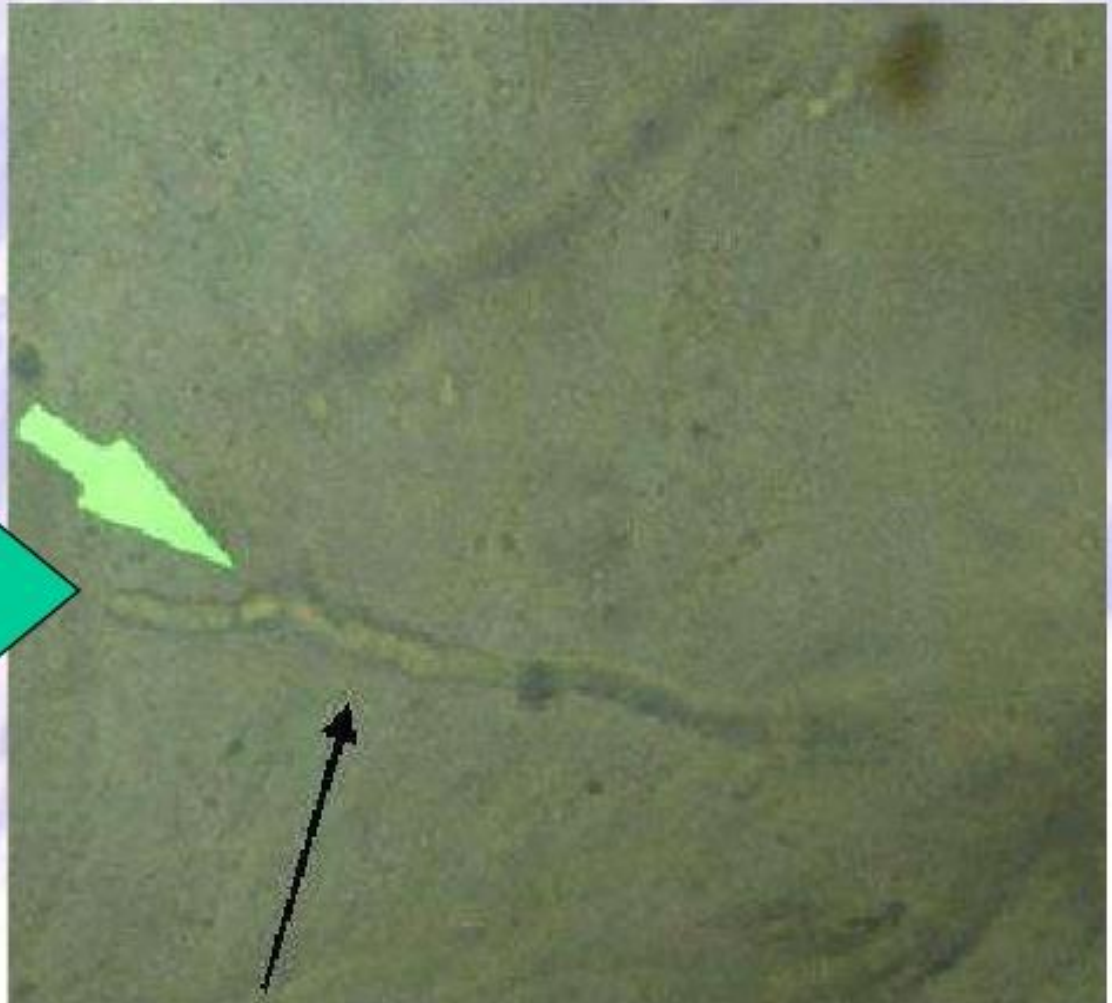
ASPERGILLUS ON LPCB MOUNT

KOH wet mount

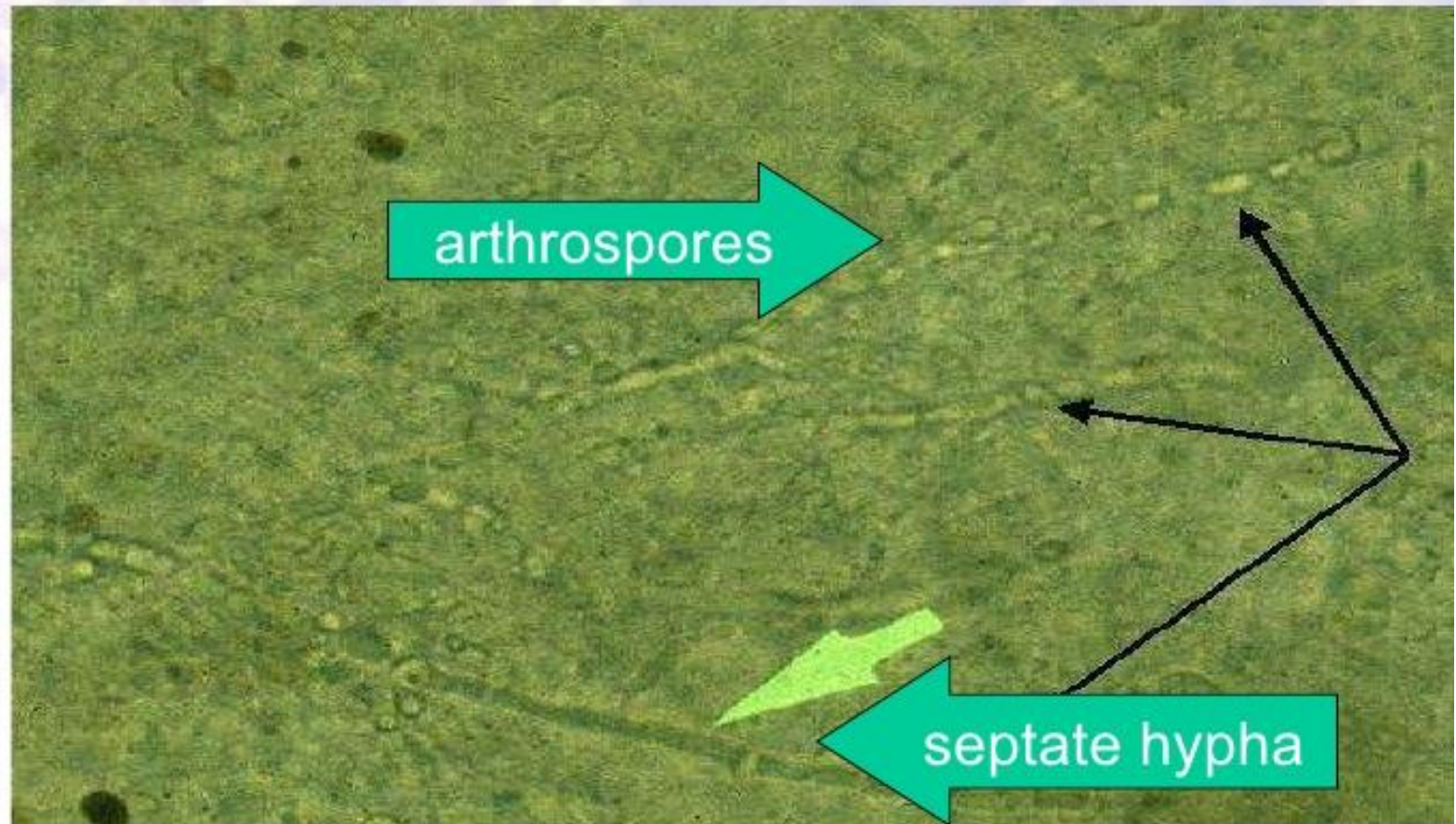
- ▶ Place 1-2 drops of 10–20% KOH on a microscope slide & add a small amount of the specimen to the drop of KOH and mix well
 - ▶ Place a cover slip on top of the preparation and press down gently
 - ▶ Allow to stand for 1-2 hours, or even overnight in a moist chamber; The KOH will partially digest the proteinaceous debris
 - ▶ Examine under phase contrast or under the low- and high-dry objectives of a light microscope
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KOH of skin scrapings

Septate hypha



KOH of skin scrapings



Cutaneous

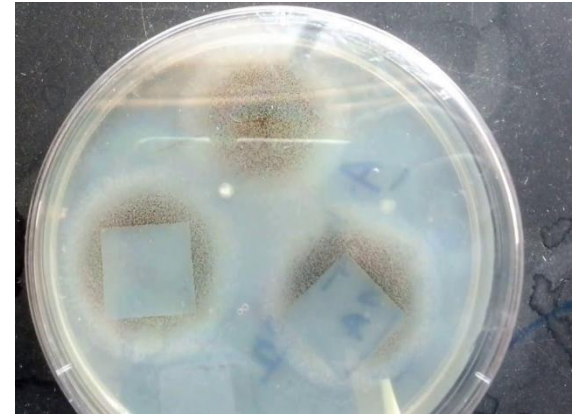
Selective Media

- ▶ Sabouraud dextrose agar- It has a pH of 5.6 and is therefore inhibitory to bacteria while supporting the growth of fungi that are acid-tolerant.



Other media

- ▶ Corn Meal Agar
- ▶ Bird Seed Agar
- ▶ BHI Agar



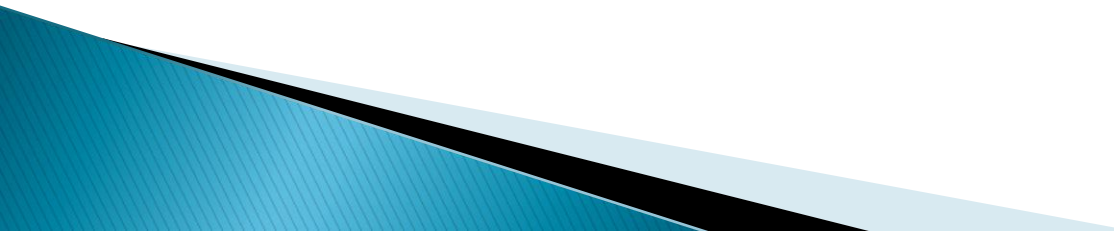
Corn Meal Agar

Bird Seed Agar

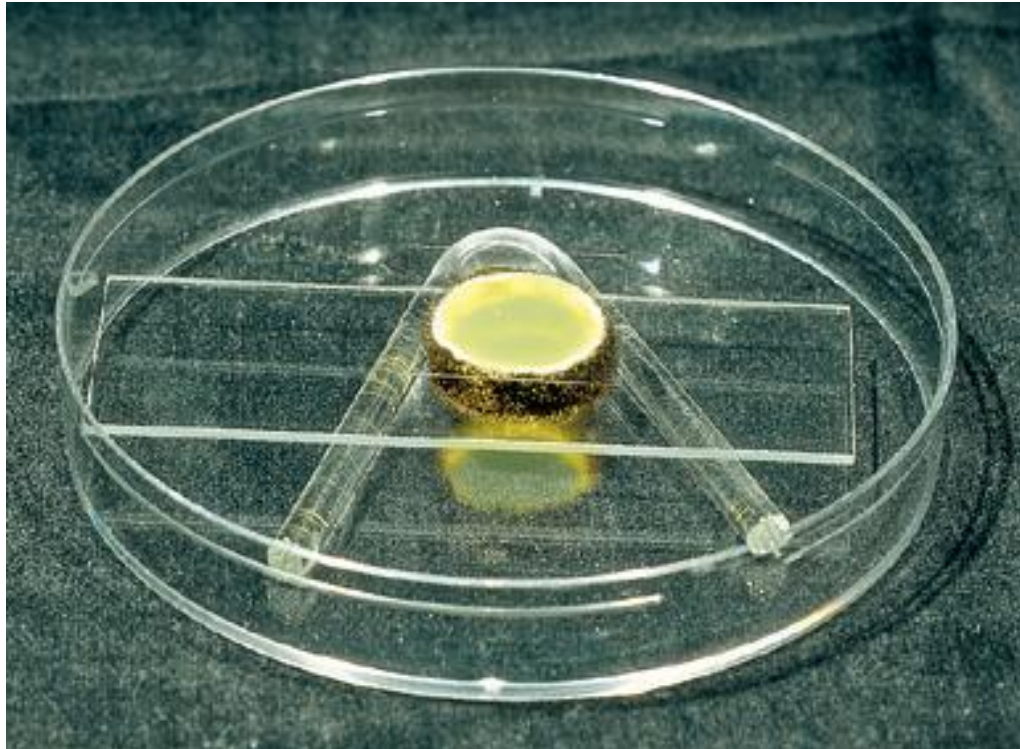
for the isolation of *Cryptococcus neoformans*



Sticky tape preparation

- ▶ **Clear tape** such as Sellotape
 - ▶ A 6 cm length of 2 cm wide tape
 - ▶ The adhesive side is then pressed firmly down, with the index finger, on the centre of the colony to be examined.
 - ▶ The fruiting heads and spores stick to the tape and can be gently pulled from the mat of mycelium.
 - ▶ The inoculated tape is placed over a drop of LPCB on a microscope slide.
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Slide culture technique



SEROLOGY

Precipitation test

**Latex
agglutination test**

**Complement
fixation test.**



2/10/2011

Dr.T.V.Rao MD

Skin Test

◆ Histoplasmosis	Histoplasmin
◆ Candidiasis	Candidin
◆ Blastomycosis	Blastomycin
◆ Sporotrichosis	Sporotrichin
◆ Dermatophytosis	Trichophytin

Other Methods

- ◆ PCR – Polymerase Chain Reaction
- ◆ RFLP - Restriction fragment length polymorphism
- ◆ Protein electrophoresis
- ◆ Nucleic acid probes
- ◆ Serotyping
- ◆ Karyotyping