Medical Mycology

Lab (1)

1-Introduction

Mycology - the study of fungi

Fungi - molds and yeasts

Molds - exhibit filamentous type of growth

Yeasts - pasty or mucoid form of fungal growth
50,000 + valid species; some have more than one name due to minor variations in size, color, host relationship, or geographic distribution

2. General considerations:

✱Fungi stain gram positive, and require oxygen to survive

✱ Fungi are eukaryotic, containing a nucleus bound by a membrane, endoplasmic reticulum, and mitochondria. (

✱ Fungi are heterotrophic like animals and most bacteria; they require organic nutrients as a source of energy.

✱ Fungi are dependent upon enzymes systems to derive energy from organic substrates

- saprophytes - live on dead organic matter
- parasites - live on living organisms

✱ Fungi are essential in recycling of elements, especially carbon.

Culturing

Media used for culturing fungi

- A wide range of media are used for growing fungi.
- Media will affect colony morphology and color
- Media generally contain a source of carbon, nitrogen and vitamins.
- Glucose (dextrose) is the most widely utilizable carbon source followed by Fructose and mannose .
- Nitrogen sources include peptone, yeast extract, malt extract, amino acids, ammonium and nitrate compounds.
- Two general types of culture media are essential to ensure the primary recovery of all clinically significant fungi from clinical specimens.
- One medium should be nonselective (such as potato dextrose agar ,Brain Heart Infusion Agar) that is, one that will permit the growth of virtually all clinically relevant fungi and other medium should be selective, specially tailored to isolate specific pathogenic fungi.

Culturing

- For optimal recovery of fungal pathogen, a battery of media should be used, and the followings are recommended:
- Media with or without cyclohexamide (Cycloheximide is added to inhibit the growth of rapidly growing contaminating molds.)
- Media with or without an antibacterial agent (Chloramphenicol, Gentamicin and Ciprofloxacin are commonly used antibacterial for this purpose).
- Antibacterial agents are used to kill the contaminating bacterial species. If the sample is taken from sterile site, it is not necessary to use media containing antibacterial agents.

Media

Potato-dextrose agar (PDA) and Corn-meal agar - are used in slide cultures; as they induce spore formation, which greatly aids in identification.

PDA is richer than cornmeal agar and is used for growing a wide range of fungi.

Czapek's agar: It is used for the subculture of *Aspergillus* species for their differential diagnosis.

Sabouraud's dextrose agar (SDA): Sabouraud's agar is sufficient for the recovery of dermatophytes from cutaneous samples and yeasts from vaginal cultures.

(SDA +chloramphenicol) where chloramphenicol inhibits bacterial growth.

Sabouraud's dextrose agar (2%) is most useful as a medium for the subculture of fungi recovered on enriched medium to enhance typical sporulation and provide the more characteristic colony morphology.

Mycosel/Mycobiotic agar: It is generally Sabouraud's dextrose agar with cycloheximide and chloramphenicol added.

- It is used for the primary recovery of dermatophytes.
- Chloramphenicol to inhibit bacterial growth, and cycloheximide to inhibit saprophytic fungi and some yeasts.

Brain heart infusion slant (BHI) - It is a nonselective fungal culture medium that permits the growth of virtually all clinically relevant fungi. It is used for the primary recovery of saprophytic and dimorphic fungi.

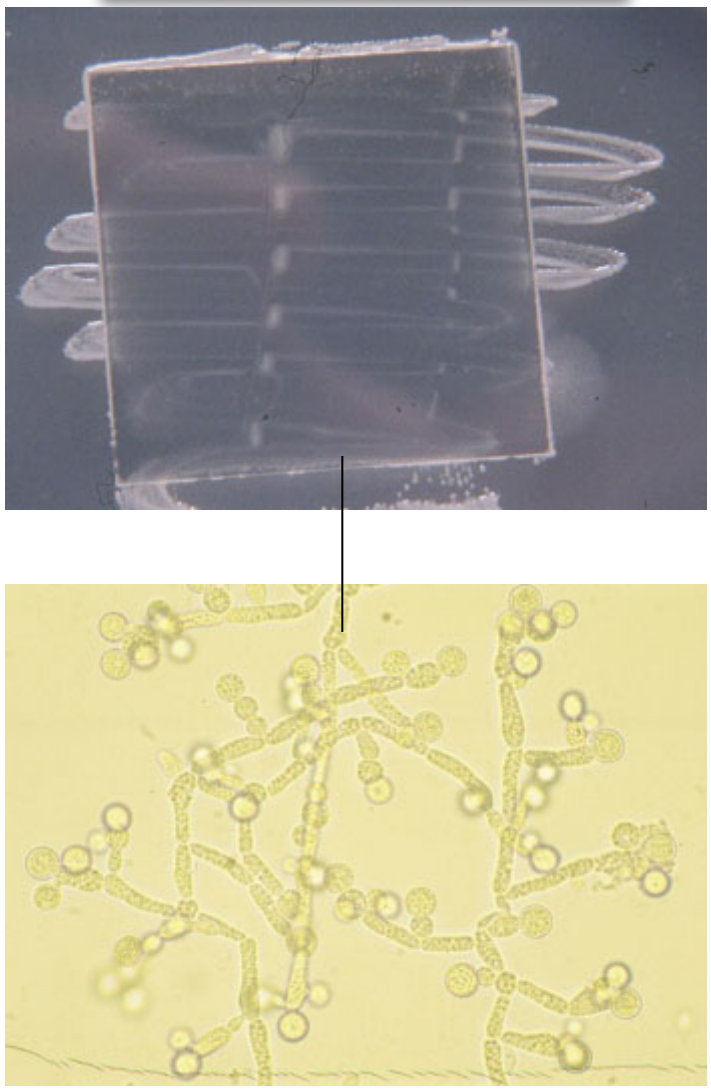
It is more enriched than Sab-Dex. Used in recovery of *H. capsulatum*.

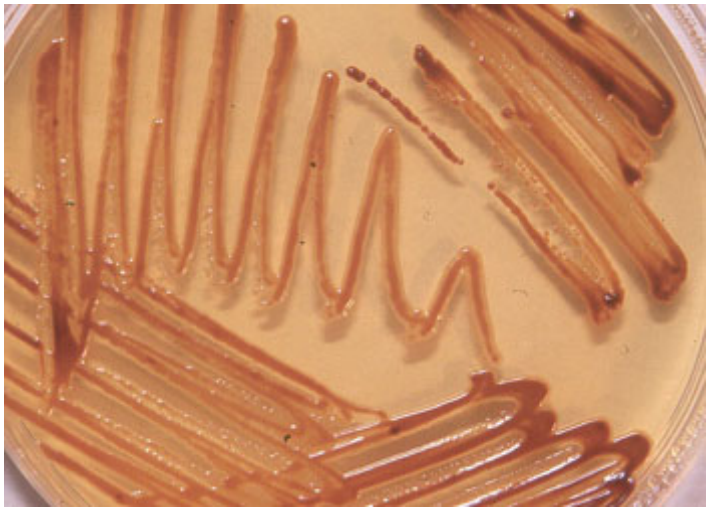
Birdseed Agar - used to isolate *Cryptococcus neoformans* from contaminated cultures.

SELECTIVE MEDIA

- Selective media
  - Corn meal agar (CMA) – sporulation, chlamydospore formation
  - Bird seed agar – cryptococcus, forms brown colonies
  - Brain Heart Infusion (BHI) agar – dimorphic & other fastidious fungi

Corn Meal Agar





Bird Seed Agar

Fungal Culture

- Specimens should be cultured on agar slants:
  - **Tubed media is used rather than plated media.**
  - Safe
  - Easy storage
  - Require less space
  - More resistant to drying during prolonged incubation
  - Less chance for spore release into the environment.
  - Blood cultures should be inoculated in to biphasic blood culture bottles

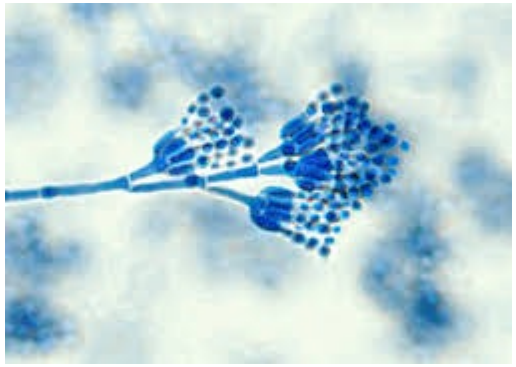
The agar in a tube is inoc ulated in a straight line.

Stains

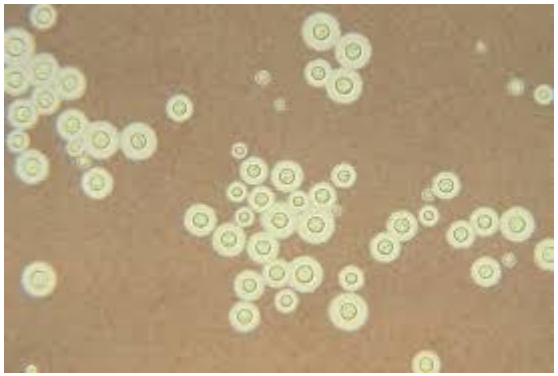
1. Lactophenol Cotton Blue (LPCB) - very popular for quick evaluation of fungal structures; will stain the chitin in cell walls of fungi.
2-Gram Stain - generally fungi are gram positive; Actinomyces and Nocardia are gram variable ..
3. India Ink - demonstrates the capsule of *Cryptococcus neoformans* in CSF specimens .

Stains

- **Periodic Acid - Schiff Stain (PAS) - stains polysaccharide in the cell wall of fungi. Fungi stain pink-red with blue nuclei.**
- **Gomori Methenamine Silver Stain - silver nitrate outlines fungi in black due to the silver precipitating on the fungi cell wall.**
- **Giemsa Stain - used for blood and bone marrow specimens**

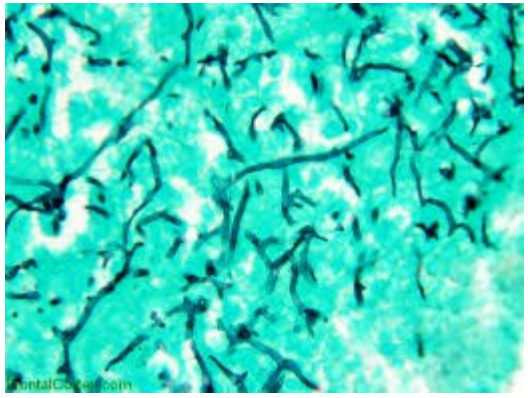


Lactophenol cotton blue

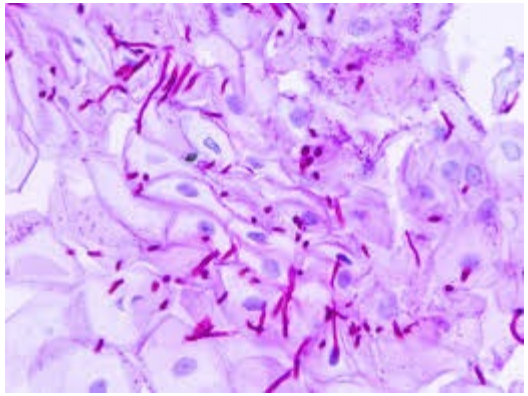


Indian ink

Gomori Methenamine Silver Stain



Periodic Acid - Schiff Stain (PAS)



Fungal Culture

- Temperature requirement
  - Majority of fungi – 37°C
  - Superficial mycosis – 30°C
  - Dimorphic fungi – 25°C & 37°C

- Incubation time
  - At least 4 weeks
  - Usually positive cultures are obtained in 7-10 days
  - Candida & Aspergillus - 24 to 72 hrs

Fungal growth requirements

a. Temperature - Room temperature (25-30 C) for most fungi.

•Notes: (1) *Nocardia sp*. and some dimorphic organisms grow best at 37 degrees C.
•(2) Any fungus capable of growing at 37 C, should be considered potentially pathogenic.

• b. Atmosphere - True fungi are aerobic; there are a few anaerobes among the bacteria-like fungi.

•c. Time - Some yeasts grow overnight. Saprophytes are fast growers (several days). Generally cultures are held at least 4 weeks.

•Dimorphic fungi are cultured both on 25 and 37 C

Excercise

- Understand the difference between the different media used in medical mycology.

- List the Stains used .