MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE,
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DEPARTMENT OF VETERINARY PATHOLOGY

STUDY OF PATHOGENIC FUNGI

Key points related to Fungi

- ✓ **Eukaryotic, non-photo synthetic - microorganisms in the kingdom Fungi.**
- ✓ **Widely distributed in the environment.**
- ✓ **Cell wall contain chitin and other polysaccharides.**
- ✓ **Heterotrophs; produce exoenzymes and obtain nutrients by absorbtion.**
- ✓ **Branching hyphae and unicellular yeasts are the two major forms.**
- ✓ **Reproduce both sexually and asexually with the production of spores.**

- ✓ Grow aerobically at 25^0 C ; some molds are strict aerobes.
- ✓ Tolerate high osmotic pressure and low pH values.
- ✓ Grow on Sabouraud Dextrose agar (SDA) at pH 5.5.
- ✓ Resistant to antimicrobial drugs which are effective against bacteria.
- ✓ Majorities are saprophytes; some cause opportunistic infections.
- ✓ Dermatophytes are pathogens which cause ringworm in animals and humans.

Introduction

- There are more than 250,000 species in the Kingdom of fungi and less than 150 are known to pathogenic for animals and man.

- The three phyla in the kingdom
  - ✓ Ascomycota (ascomycetes)
  - ✓ Basidomycota (basidomycetes)
  - ✓ Zygomycota (zygomycetes)
  - ✓ Fungi imperfecti (Deuteromycetes)
    - Because sexual forms has not been found, constitute a heterogenous fourth group.
    - Although most fungi of Veterinary importance are dueteromycetes.

❖ *Two main morphological Fungal forms moulds and yeasts*

- ✓ **Moulds grow as branching filaments called hyphae.**
- ✓ **Unicellular yeasts have an oval or spherical appearance.**
- ✓ **However, dimorphic fungi occur in both moulds and yeasts forms.**

❖ *Pathogenic fungi are studied by following methods*

- ✓ **Direct microscopic examination**
- ✓ **Cultural methods for isolation and identification**
- ✓ **Biochemical reactions**
- ✓ **Histopathology**
- ✓ **Animal inoculation**
- ✓ **Cutaneous tests**
- ✓ **Serological reactions.**

❖ *Direct microscopic examination :*

- ✓ **Hair, skin scrappings, sputum, milk, cerebrospinal fluid, nasal discharge etc. can be examined directly under microscope for the presence of fungus.**
- ✓ **It is a rapid and reliable method for diagnosis of ringworm.**

❖ *Procedure :*

- ✓ **The suspected material is mixed with 2-3 drops of 10% KOH (to dissolve keratin) solution on the slide and allowed for 10-15 min.**
- ✓ **Thicker portion of skin, hair etc. should be gently heated.**
- ✓ **The coverslip is placed on the slide and examined under microscope.**

❖ *Observation :*

- ✓ **The fungal spores are found in cluster or rows either inside the hair shaft (endothrix) or around the hair shaft (ectothrix) in case of ring worm.**
- **Cerebrospinal fluid is examined for the presence of yeast, *Cryptococcus neoformans* in dog suffering from meningitis.**
- **Nasal discharge is examined for sporangium of *Rhinosporidium seeberi* in bullock having tumor in the nose.**

❖ *Cultural methods (Isolation and identification)*

✓ Isolation (Cultivation) :

- The suspected material is embedded superficially on the surface of Sabouraud's agar plate/slant and incubated at 25-28ºC in incubator or at room temperature for about seven days until the complete growth takes place.
- When yeast is to be cultivated from the suspected material, the inoculated medium is incubated at 37ºC.

✓ Identification :

- The identification is done by examination of growth by naked eye as well as by microscopical examination.

❖ *Examination of growth:*

✓The fungal culture is examined for following characters:

(a) Colour of colony:

- The top most surface of the culture appears black, green, yellow, blue etc. due to different colours of spores produced by different fungi.

(b) Rate of growth:

- Fungi like Aspergillus and Mucor grow within 3-6 days while ringworm fungi like Microsporum, Trichophyton take linger time for growth i.e. about 1-2 weeks.

(c) Surface structure :

- In fungi imperfecti, the aerial mycelium is compact, short, smooth, waxy and appears like velvet. In phycomycetes, the mycelium is coarse and loose.
- The aerial hypha is longer and looks like a cotton wool.

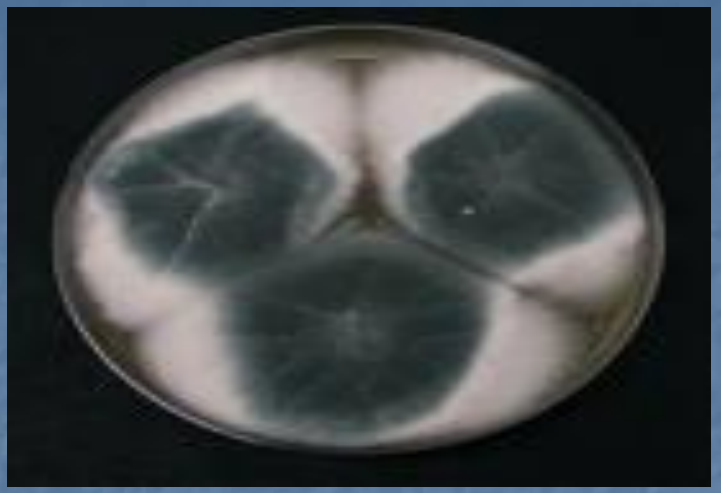
(d) Pigment production :

- **Certain fungi produce pigment and it is seen on the reverse side i.e., under the surface of the colony. Mainly the ringworm fungi produce the pigments as follow :**

- **Red pigment : *Trichophyton rubrum***
- **Violet pigment : *T. violaceum***
- **Sulfur yellow pigment : *T. sulfureum.***
- **Gypsum like pigment : *T. gypseum.***

Mucor

Aspergilus fumigatus

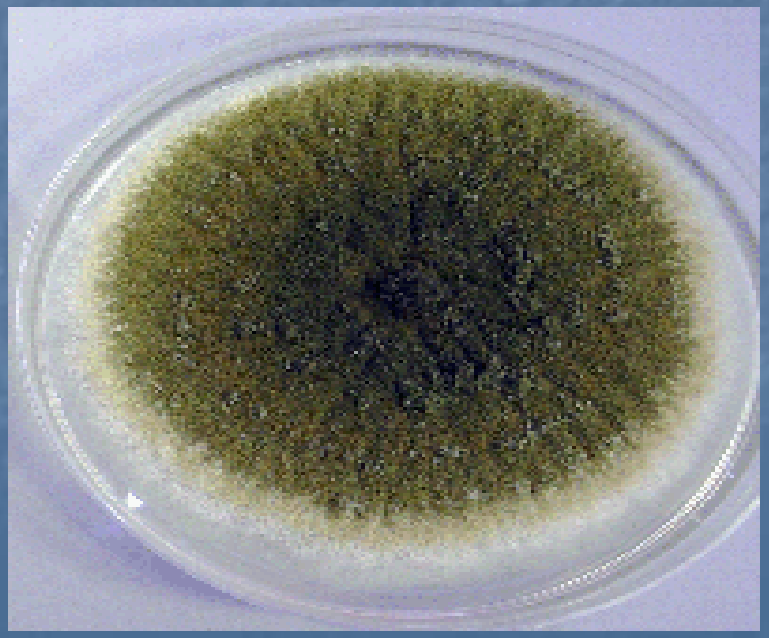


Aspergilus fumigatus

- Trichophyton

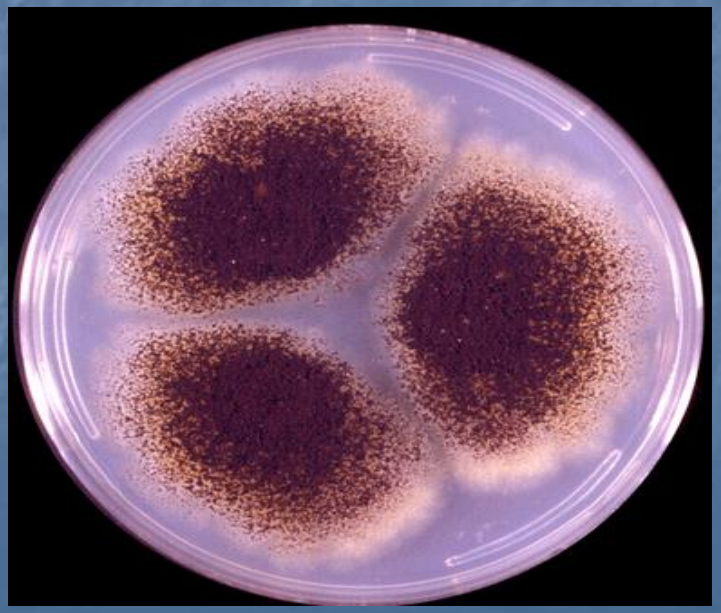
Microsporum gypsium

Aspergillus flavus



Aspergillus niger

Czapek dox agar-aspergillus niger



❖ *Microscopical examination :*

- **A piece of mycelium is removed with two teasing needles and transferred to a slide on which few drops of Lactophenol cotton blue are taken to stain the mycelium and spores.**

- **Mycelium is carefully teased and coverslip is placed over it.**

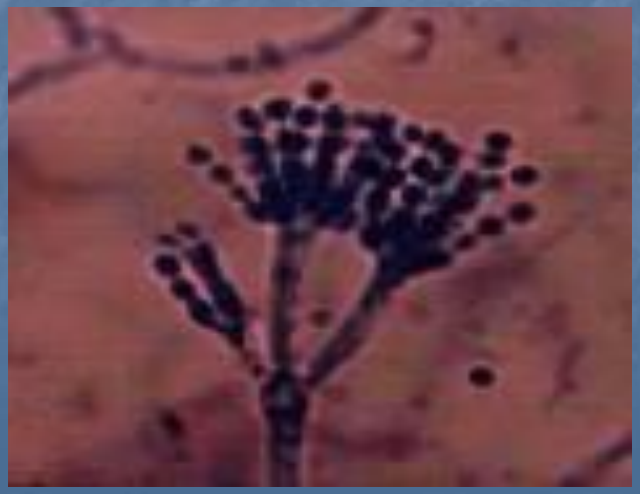
- **The slide is examined under low and high powers of microscope for following structures.**

- *Mucor*

- *Candida albicans*

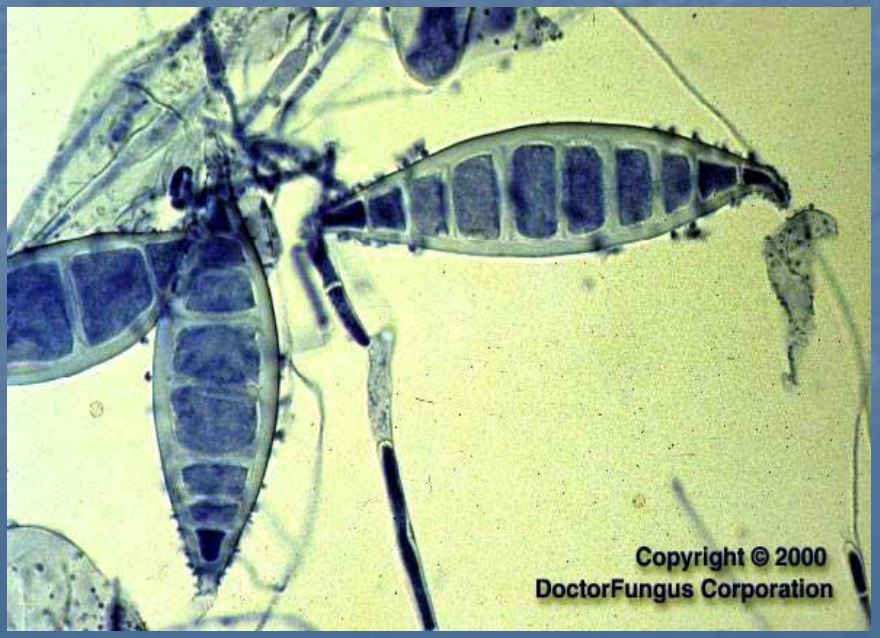
- *Trichophyton verucosum*

- *Penicillium*



Epidermophyton

Microsporum canis



❖ *Nature of mycelium:*

- ✓ **Septate mycelium is observed in**
  - **Ascomycetes - e.g. Penicillium, Aspergillus etc.**
  - **Basidiomycetes - e.g. Mushroom**
  - **Deuteromycetes - e.g. Cryptococcus, Candida etc.**

- ✓ **while non-septate mycelium is observed in**
  - **phycomycetes - e.g. Mucor, Rhizopus etc.**

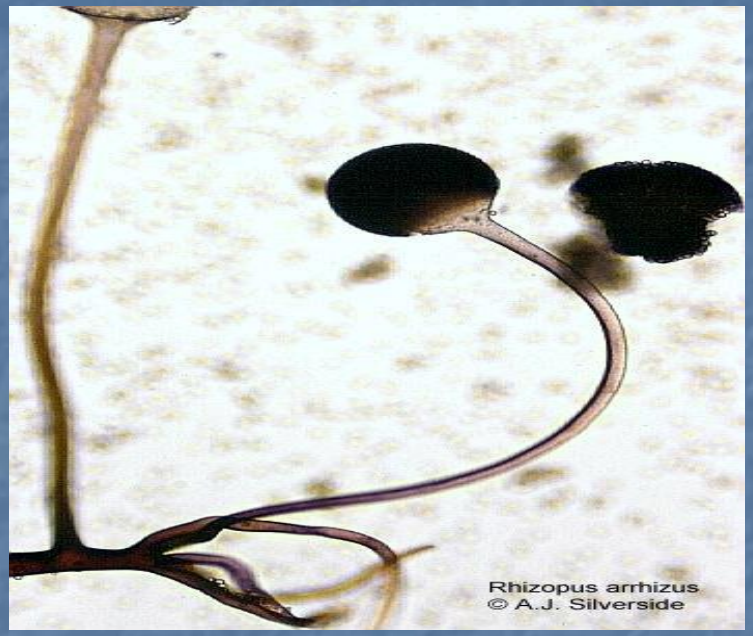
❖ *Types of spores:*

✓ **The different types of spores, viz.,**

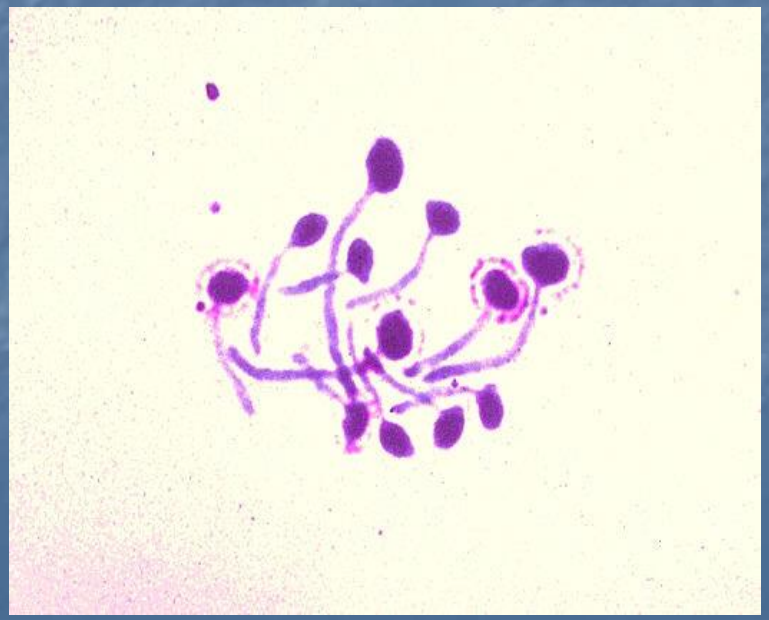
- **sporangiospore** – **Mucor**
- **conidia** -**Aspergillus and Penicillium**
- **arthrospore** - **Coccidioides**
- **chlamydospore and blastospore** - Candida

- ***For examination of yeast, the smear is prepared from yeast colony and stained by Gram's method.***
- ***Under microscope round or oval yeast cells are seen with double contoured walls.***
- ***Few cells show budding also.***

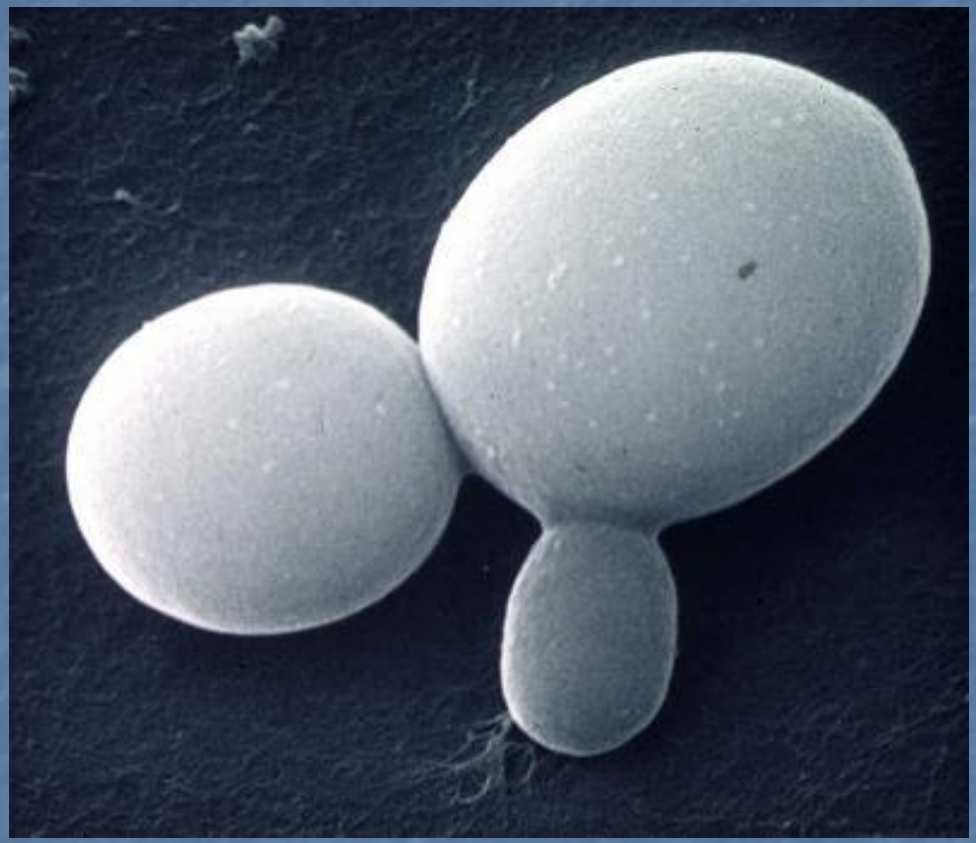
Rhizopus arrhizus



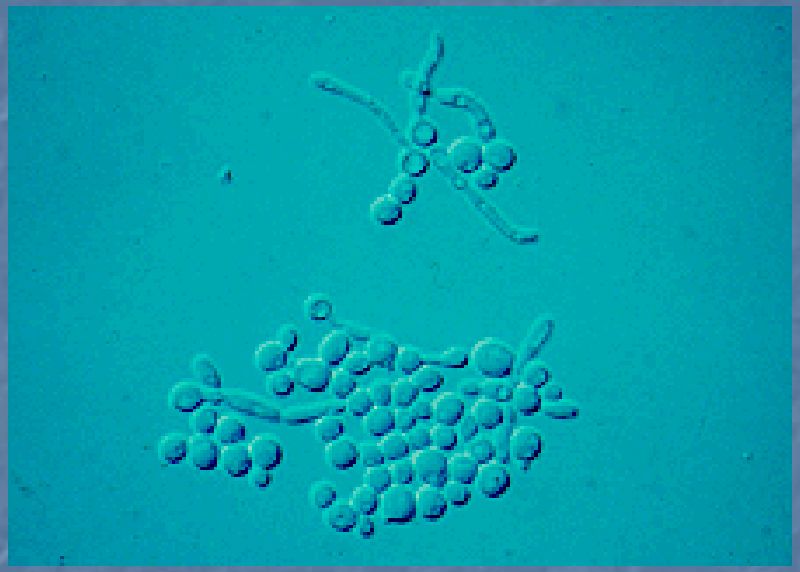
Germ tube production –Gram stain



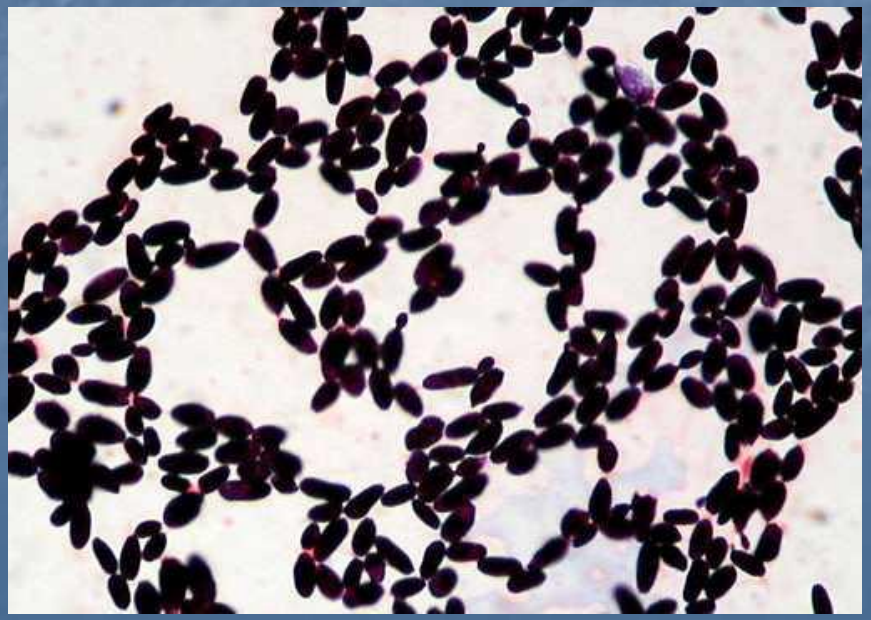
Scnning electron microscope of candida budding



Budding yeast cell- Idia ink preparation,no capsule



yeast stained with the **Gram stain**



THANK

YOU