Djilali Bounaama University of Khemis Miliana. **First semester/2025-2026**
FNLSES

Department of Biology

Module Manager: Prof. Guetarni H.

Cycle: Microbiology degree

PW3 : Microscopic Characterization of Fungi

Aims

The goal of this practical session is to familiarize students with the basic techniques for the observation and microscopic characterization of fungi (mycetes) from various sources (cultures, clinical samples).

Principle

Microscopic characterization of fungi focuses on directly observing and analyzing the morphology of fungal components, including hyphae, spores, and yeast cells, to identify them. Identification relies on evaluating specific attributes such as size, shape, arrangement, and septation of the fungal structures, which serve as consistent and distinctive diagnostic markers for various genera and species.

Procedure

Observation of Molds

Slide Preparation with Cotton Blue (Puncture or Fragment Method)

- Place a small drop of Lactophenol Cotton Blue (LPCB) in the center of a slide.
- Using a sterile inoculating loop or mounting needle (previously sterilized by flaming), gently pick up a small fragment of the mold colony (isolated from different medium : Environmental air and contaminated surfaces, soil, organic matter, food, crops, clinical and pathogenic sources), taking care not to disperse the spores.
- Deposit and carefully spread the fragment in the LPCB drop.

- Cover with a coverslip, avoiding air bubbles. Wipe off excess stain with absorbent paper.

Microscopic Observation

- Start observation at low magnification (X10 or X40) to locate the general structure.
- Switch to 40 magnification to observe specific structures:
    - **Hyphal morphology** (partitioned or coenocytic).
    - **Sporulation structure** (conidiophores, phialides, aspergillar heads, sporangiophores, and the shape of the spores (conidia or sporangiospores).

Observation of Yeasts

Wet Mount Preparation

- Place a small drop of sterile distilled water or saline solution on a slide.
- Using a sterile inoculating loop, pick up a small sample of the yeast culture (or an isolated colony).
- Gently mix in the drop to obtain a thin, homogeneous suspension.
- Cover with a coverslip.

India Ink Staining (for the Capsule)

- On another slide, mix the yeast suspension with a drop of India Ink.
- Create a thin smear and allow to air dry (no heat fixation).
- Observe at 100 oil immersion. The yeast appears white, the background black, and the capsule (if present) forms a clear halo around the cell.

Microscopic Observation

- Observe at 40 then 100 (immersion).
- Characterize:
    - Shape and size of the cells (round, oval).
    - Mode of reproduction (budding or fission).
    - Presence of pseudohyphae (for *Candida*).

Required work

Write a report, including the results obtained during this work.