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

Department of Biology

Module Manager: Prof. Guetarni H.

Cycle: Microbiology degree

DW3: Mastering Microcultures

1. Objectives

The purpose of this directed work is to provide a comprehensive understanding of the microculture technique, also known as the slide culture method, for examining fungal structures.

2. Theoretical analysis

A. Problem of Identification

Mold identification in the laboratory typically involves examining their asexual reproductive structures, such as conidiophores, phialides, and conidia, as well as their vegetative structures like mycelium.

- Traditional culture (on an agar plate): The prolific growth of aerial mycelium combined with heavy sporulation often complicates the sampling process for microscopic analysis. Techniques like crushing or removing fragments of the colony (e.g., the tape mount technique) can damage delicate structures, potentially leading to inaccurate observations.

B. Principle of microculture

The principle of microculture entails the cultivation of a minimal fragment of fungal material on a limited nutrient medium, such as Potato Carrot Bile (PCB) agar or a low-glucose Sabouraud medium, which is directly placed on a glass slide and covered with a coverslip within a humid chamber. This controlled environment supports the growth of the fungus on a confined surface, encouraging planar development and facilitating the formation of intact, observable fruiting structures that can be studied *in situ.*

3. Manipulation and protocol

A. *Required Materials*

- Fungal specimens for study (e.g., *Aspergillus*, *Penicillium*, *Fusarium*).

- Sterile glass slides and coverslips.

- Sterile agar blocks (e.g., Sabouraud or PCB), cut into squares approximately 5 mm by 5 mm.

- Humid chamber consisting of a sterile Petri dish equipped with a piece of wet filter paper or cotton moistened with sterile water at the base.

- Sterile platinum loop or straight wire.

- Lactophenol Cotton Blue (utilized as a mounting and staining medium).

- Pasteur pipette or dropper.

- Bunsen burner or micro-incinerator for sterilization purposes.

- Optical microscope for observation.

B. Stepwise procedure

* Preparation of the Humid Chamber

- In a sterile Petri dish, place a filter paper or square piece of cotton, ensuring it is adequately moistened with sterile water to create a humid environment conducive to fungal growth.

* Assembly of the microculture

- Position two thin glass rods or a single thick glass slide in the center of the humid chamber to serve as a support platform.

- Place a sterile glass slide horizontally on the support structure.

- Carefully position a sterile agar block in the center of the slide.

* Inoculation process:

- Using a sterilized straight wire, collect a minimal amount of inoculum from the youngest growth zone at the periphery of the fungal colony.

- Inoculate the agar block at four evenly spaced points along its side faces, avoiding contact with the uppermost surface of the agar square to maintain even distribution of inoculum.

* Application of coverslip

- Gently position a sterile coverslip over the agar block, ensuring no air bubbles are trapped between the surfaces. It is critical to achieve perfect contact between the coverslip, agar block, and inoculum for optimal results.

* Incubation

- Securely close the Petri dish containing the humid chamber.

- Incubate under appropriate conditions specific to the fungal strain, typically at 25 °C to 30 °C, for a duration of three to seven days. The moistened filter paper ensures a saturated humid environment necessary for successful fungal cultivation.

4. Microscopic examination

A. Preparation of the permanent slide

Following the incubation period, the fungal culture will have developed along the agar surface and beneath the coverslip, forming reproductive structures primarily along the periphery of the agar block.

* Removal of the agar block

- Carefully lift the coverslip and place it, with the culture side facing upward, onto a separate glass slide.

- Dispose of the agar block in an appropriate container for contaminated waste.

* Staining and mounting

- Deposit a drop of Lactophenol Cotton Blue (LPCB) staining solution onto the slide previously holding the agar block.

- Using a sterile wire loop, gently extract a small fragment of mycelium that has grown on the slide (in the region where the agar block was situated) and immerse it in the LPCB drop. Cover this preparation with a clean coverslip to create the first mounting slide (identified as Slide No. 1).

- Apply a drop of LPCB directly onto the underside of the coverslip removed during Step 1 (the side where fungal growth occurred), then cover this with a clean glass slide using an inversion mounting technique. This forms the second mounting slide (identified as Slide No. 2).

* Microscopic observation

- Examine both prepared slides under an optical microscope, beginning with 10x magnification, and proceed to higher magnification (e.g., 40x) for detailed observation..

* Observation criteria

Microscopic examination should facilitate the identification and characterization of the following elements :

Criterion	Expected Description	Examples
Mycelium	Is it septate (divided by walls) or siphonaceous (non-septate)?	Hyaline fungi (septate) or Zygomycetes (siphonaceous).
Conidiophore	Shape, size, and branching pattern.	Simple (*Scopulariopsis*), brush-like (*Penicillium*), club-shaped (*Aspergillus*).
Conidia	Type of sporulation (blastic, thallic), shape, size, color, and arrangement.	Microconidia, macroconidia, arthrospores, chlamydospores.
Sexual Organs	Presence or absence of asci, basidia, etc. (rarely observed in routine analysis).	

Last name :……..

First name :…….

Group : ……..

5. Synthesis questions

1. What is the primary benefit of using the microculture method over a standard colony pick-up on classic agar for identifying mold species?

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2. Why must the microculture be incubated in a humid chamber?

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3. What function does Lactophenol Cotton Blue (LPCB) serve during microscopic analysis?

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4. When you observe septate hyphae and conidiophores that culminate in an "*Aspergillus* head" structure (a vesicle bearing phialides and conidia), which mold genus would you suspect first?

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