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

FNLSES

Department of Biology

Module Manager: Prof. Guetarni H.

Cycle: Microbiology degree

DW1: Mastery of Mold Identification Techniques

1. Core Identification Methods

Mold identification relies on combining morphological and molecular (DNA-based) techniques.

1.1. Macroscopic and cultural analysis

- **Media**: Standardized media like MEA (Malt Extract Agar) or CYA (Czapek Yeast Agar) at 25 °C are used.
- **Characteristic:**

  -*Growth Rate* : Helps distinguish fast-growing (*Rhizopus*) from slower-growing (*Penicillium*) genera.

  - *Colour* :The colour of the sporulating surface and the pigmentation of the colony reverse are crucial.

  - *Texture* **:** Described as powdery**,** velvety, or cottony.

1.2. Microscopic Analysis

- **Preparation**: The Tape-Lift Method is preferred as it uses adhesive tape to sample the colony gently, preserving the conidiophores (spore-bearing stalks).
- **Staining**: Lactophenol Cotton Blue is used. The Lactophenol acts as a fixative, while the Cotton Blue stains the fungal cell walls.
- **Key structures**: Observe if the mycelium is septate (has cross-walls) or non-septate, and identify the specific asexual fruiting structures.

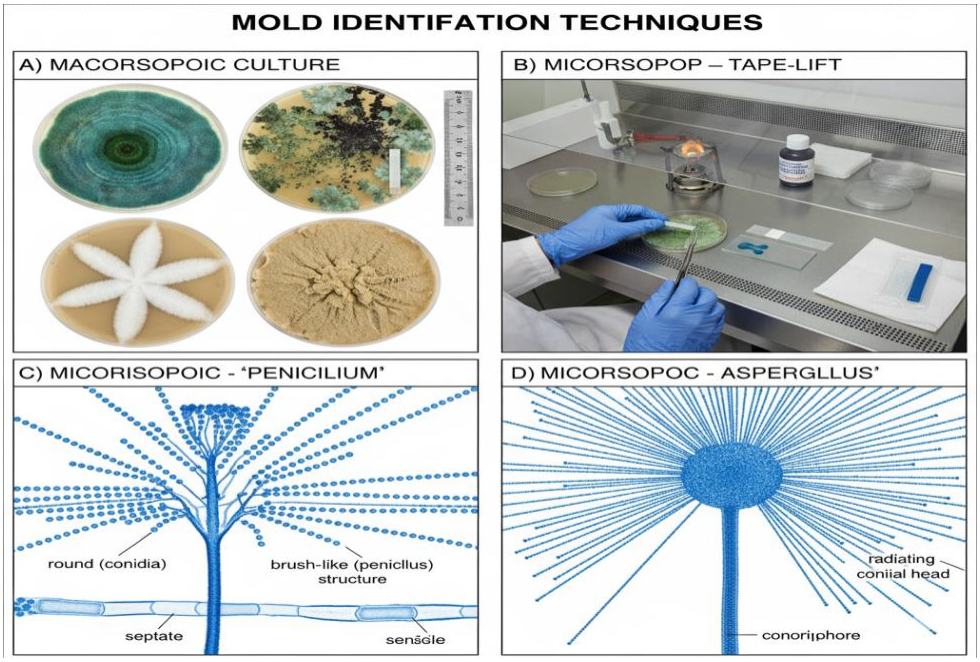
1.3. Molecular Confirmation

- **Target**: The ITS (*Internal Transcribed Spacer*) region of the ribosomal DNA is the standard molecular target.
- **Process**: DNA extraction is followed by PCR (Polymerase Chain Reaction) to amplify the ITS region.
- **Final step**: Sequencing and bioinformatic comparison provide definitive species identification (requiring 99 % similarity).

2. Illustrative case studies (Keys to genus ID)

Penicillium

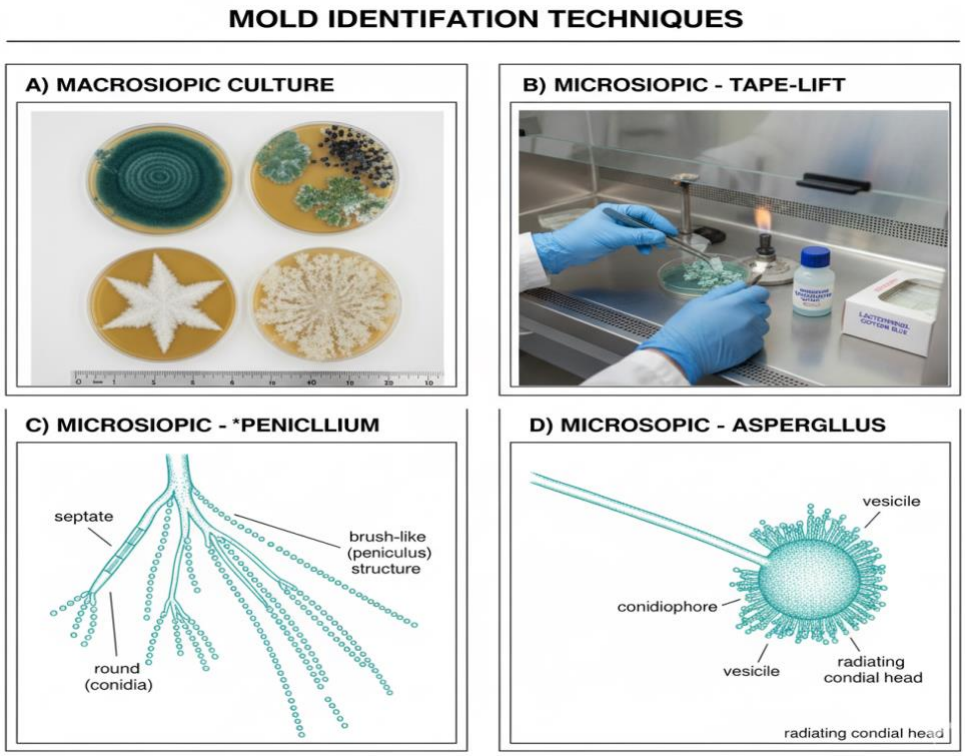
- **Macroscopic keys**: Medium growth; velvety/powdery texture; blue-green colour; pale reverse.



- **Microscopic keys**: Penicillus (brush-like structure); septate mycelium; conidia in long chains.

Aspergillus

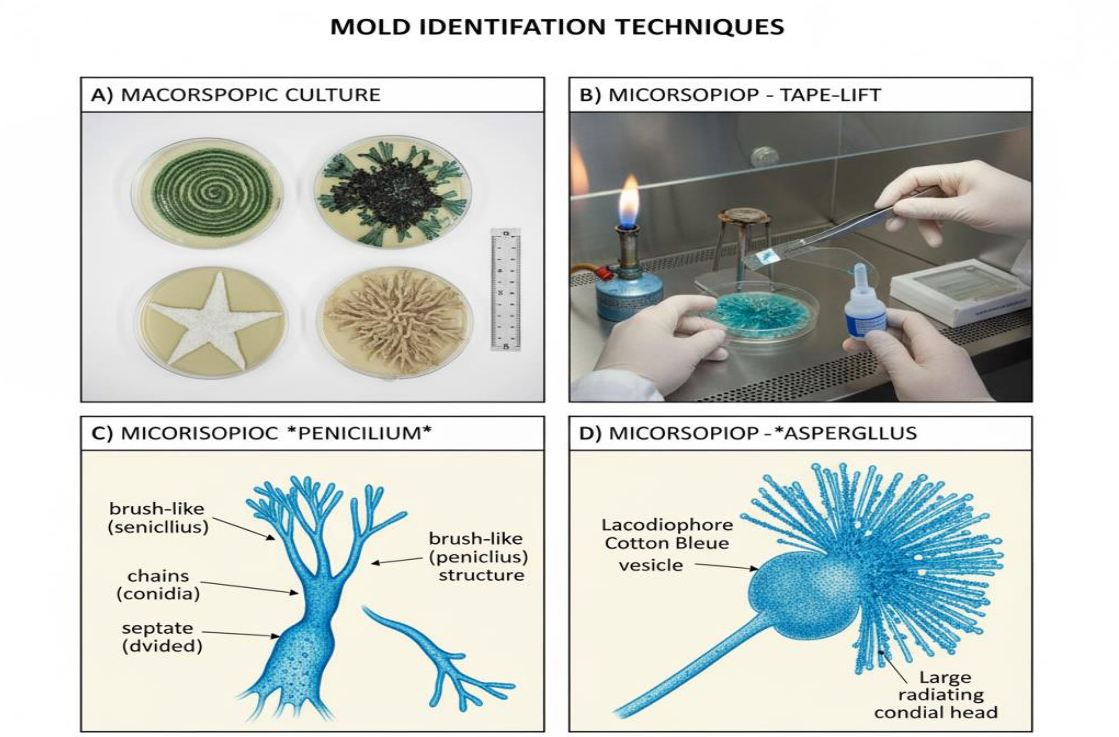
- **Macroscopic keys**: Fast growth; powdery texture; colours highly variable (Black, Yellow, Blue-Green); reverse often pigmented.



- **Microscopic keys**: Conidiophore with a terminal vesicle (swollen head); radiating Conidial Head; septate mycelium.

Rhizopus

- **Macroscopic keys**: Fast growth; cottony texture; dark gray; distinctive Rhizoids visible.
- **Microscopic keys**: Sporangia (spore sacs) on sporangiophores; non-septate mycelium.



Last Name :……

Family name : ……

Groupe :…….

3. **Practice Questions**

Question set 1: Genus Identification Keys

1. **Isolate A**: Macroscopic: Fast, cottony, dark gray, rhizoids. Microscopic: Non-septate mycelium, sporangia. **Probable Genu :**…….

2. **Isolate B**: Macroscopic: Medium, velvety, blue-green, pale reverse. Microscopic: Septate mycelium, Penicillus structure. **Probable Genus**:……..

3. **Isolate C**: Macroscopic: Fast, powdery, charcoal black. Microscopic: Septate mycelium, spherical conidial head on a vesicle. **Probable Genus**: ……..

Question set 2: Technical and Methodological Understanding

1. **Staining**: What is the dual function of the Lactophenol component in the Lactophenol Cotton Blue stain?
   - **Answer**:…….. ……..

2. **Technique Rationale**: Why is the Tape-Lift method superior to preparing a simple smear?
   - **Answer**: ……

3. **Molecular Target**: Which specific rDNA region is the standard target, and why is it preferred?
   - **Answer**: ……

4. **Conclusion confidence**: If your sequencing shows 99.8 % similarity to *A. fumigatus* and 95.5 % to *A. lentulus*, what is your conclusion?
   - **Answer**: ……...