Djilali Bounaama University of Khemis Miliana. **S1/2025-2026**

FNLSES

Department of Biology

Module Leader : Prof. Guetarni H.

Cycle : Microbiology Degree

PW 2: Isolation of Molds from Moldy Foodstuffs

Aims

This practical work focuses on the targeted isolation and detailed morphological analysis of filamentous fungi (molds) that contribute to the spoilage of various food products.

Principle

Contaminated food samples are utilized to isolate various strains of molds following their cultivation on microbiological media, such as Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA).

Procedure

A. Isolation and culturing phase

- Perform all inoculation steps within the protected zone near the Bunsen burner to maintain aseptic conditions.

- Sterilize the straight loop thoroughly and then collect a small sample of inoculum, consisting of mycelium and/or spores, from a visibly moldy section of the food sample (fruits, vegetables, cheeses, bread, grains, etc.).

- Inoculate the solid medium, such as PDA or MEA, using the streaking for isolation technique. This approach is designed to dilute the inoculum systematically, allowing for the development of distinct, isolated colonies.

- Incubate the inoculated plates in mesophilic conditions at a temperature range of 20-25 °C. Ensure the plates are partially sealed to allow proper gas exchange, and place them in darkness for an incubation period of 5 to 7 days.

B. Morphological characterization phase

- Macroscopic analysis: Observe the pure colonies daily. Note key macroscopic characteristics such as the color of both the surface and reverse sides, the texture (e.g., powdery, cottony, or velvety), and the radial growth rate.

- Microscopy preparation: Create a temporary wet mount by employing the tape-lift method combined with a drop of Lactophenol Cotton Blue. This preparation technique helps to preserve the structural integrity of fungal elements for microscopic examination.

- Microscopic analysis: Examine the prepared slide under an optical microscope at magnifications of x100 and x400. Focus specifically on the architecture of the fungal asexual sporulation structures for detailed observation and analysis.

-Generic identification of isolates : It should be performed based on distinct structural characteristics. This involves classifying *Penicillium* based on its brush-like conidiophore, *Aspergillus* on the presence of a vesicle structure at the tip of its conidiophores, and *Rhizopus* by identifying the characteristic rhizoids associated with its sporangiophores.

Required work

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