

Experiment 8: Observation and Enumeration of Molds from Spoiled Bread

Abstract

This experiment introduces the basic knowledge of molds commonly found in spoiled food. Students will practice staining molds with lactophenol cotton blue to observe their morphology under the microscope. They will also enumerate the fungal population from spoiled bread using 3M[®] Yeast/Mold Petrifilm and apply the dilution technique.

Objectives

Practice lactophenol cotton blue staining to observe molds from spoiled bread.

Enumerate the population of molds using yeast/mold Petrifilm.

Apply dilution techniques for microbial enumeration.

Major Experimental Materials

Spoiled bread

Lactophenol cotton blue stain

Plastic tape

Buffered peptone water (100% and 0.1%)

3M[®] Yeast/Mold Petrifilm

Chopsticks, sterile pipettes, stomacher

Introduction of Fungi

- Fungi are dimorphic organisms:
- Yeast phase grows best at 35–37 °C.
- Mold phase grows best at 25 °C.
- Common molds in spoiled bread:
- *Aspergillus* (100X): found in breads, grains, vegetables, dairy.
- *Rhizopus* (100X): found in breads, vegetables.
- *Penicillium* (400X): found in breads, fruits, vegetables, dairy.
- Molds are composed of hyphae forming a fuzzy mat called mycelium. Specialized aerial hyphae bear conidia (spores).

Procedure

Part A: Lactophenol Cotton Blue Staining

Obtain mold-contaminated bread.

Food Microbiology Laboratory DR Nisreen Kadim (8) 2025

- Apply tape to your index finger (sticky side out).
- Gently press tape onto mold surface.
- Place one drop of lactophenol cotton blue stain on a clean slide.
- Press sticky side of tape onto the stain.
- Observe mold morphology under 10X and 40X objectives.

Part B: Enumeration of Molds in Bread
Weigh 15 g of spoiled bread.

- Place into 50 ml buffered peptone water.
- Stomach (blend) for 2 minutes.
- Prepare dilutions (10^{-3} and 10^{-5}).
- Spread plate onto yeast/mold Petrifilm.
- Incubate and count colonies.

Results (Expected)

Microscopy:

Aspergillus: septate hyphae, conidial heads. Rhizopus: non-septate hyphae, sporangia.

Penicillium: brush-like conidiophores. Enumeration: Colonies appear as colored/fuzzy spots on Petrifilm. Record CFU/g of bread sample.

★ Review Questions

1. Name and draw three possible molds in spoiled bread. 2. Briefly describe their structures (hyphae, spores, conidia). 3. Why is dilution necessary in microbial enumeration? 4. Compare yeast

