Prof : MOUSSI ABDELHAMID

Mohamed Khider University of Biskra
Faculty of Exact Sciences and Natural and Life Sciences
Department of Natural and Life Sciences

General Microbiology

PRACTICAL WORK N° 2

METHOD FOR ISOLATION AND ENUMERATION OF BACTERIA

I. INTRODUCTION

Bacteria are omnipresent in the environment (air, water, soil) in the form of complex mixtures of microorganisms belonging to different species. To study their morphological, biochemical, and physiological characteristics, it is essential to obtain pure cultures derived from a single parental cell.

Isolation and enumeration of bacteria allow the assessment of microbial load in a sample and the selection of colonies for further analysis.

II. MATERIALS AND REAGENTS

- **Equipment:**
  - Sterile Petri dishes
  - Sterile graduated pipettes
  - Bunsen burner
  - Water bath
  - Erlenmeyer flasks
  - Sterile distilled water
  - Sterile physiological saline solution
- **Culture media:**
  - Nutrient agar (NA) or Plate Count Agar (PCA) (optional, for enumeration)

III. WORKING METHOD

The isolation can be performed directly from the water or from its dilutions. The dilutions are carried out using the standard method with sterile water or sterile physiological water. Dilutions are not necessary in the case of low-polluted water.

III.1. Isolation of Airborne Bacteria

1. Melt the NA medium in a water bath, then allow it to cool to 45°C.
2. Pour the NA medium into a sterile Petri dish under aseptic conditions.
3. Leave the dish open in the laboratory air for 5 minutes, away from the Bunsen burner.
4. Close the dish and incubate at 35°C for 24 to 48 hours.

III.2. Isolation of Waterborne Bacteria

1. Take 1 mL of the sample (or a dilution) and place it in a sterile Petri dish.
2. Add the molten NA medium (cooled to 45°C) over the sample under aseptic conditions.
3. Gently homogenize by circular movements.
4. Incubate at 35°C for 24 to 48 hours.

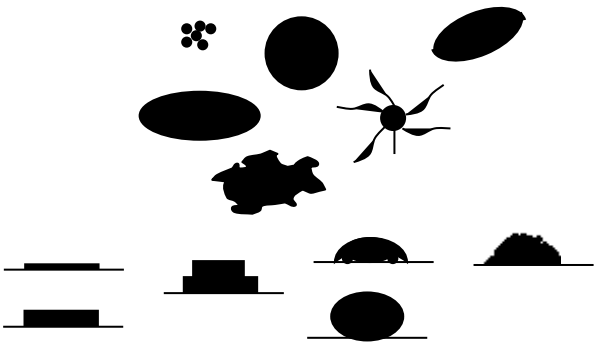
III.3. Isolation of Soil Bacteria

1. Weigh 1 g of soil and carefully grind it in 100 mL of sterile water in an Erlenmeyer flask.
2. Homogenize and then transfer 1 mL of the suspension (or a dilution) into a sterile Petri dish.
3. Add the molten NA medium (cooled to 45°C) over the sample under aseptic conditions.
4. Homogenize and incubate at 35°C for 24 to 48 hours.

IV. RESULTS ANALYSIS

IV.1. Macroscopic Observation

- **Growth density:**
  - Dense growth: > 100 colonies
  - Moderate growth: 10 to 100 colonies
  - Weak growth: < 10 colonies
- **Colony characteristics:**
  - Shape: circular, filamentous, irregular...
  - Elevation: flat, convex, raised...
  - Margin: entire, wavy, dentate...
  - Color and texture (smooth or rough)
  - Presence or absence of odor



IV.2. Enumeration of Bacteria

- Count the colonies using a colony counter.
- Calculate the concentration in CFU/mL or CFU/g:

$$CFU/mL = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}$$

V. REPORT

- **Observation of Colony Characteristics:**

Note the shape, elevation, margin, color, texture, and odor of the colonies.

- **Calculation of Bacterial Concentration:**

Use the CFU/mL or CFU/g formula to determine the bacterial load in the sample.