Practical Work N° 4

Preparation of Culture Media (Nutrient Broth and Nutrient Agar)

I. Introduction

Culture media are essential tools in microbiology for isolating, purifying, growing, and studying microorganisms. An effective culture medium must:

- Provide all necessary nutrients (carbon sources, minerals, growth factors) in absorbable forms
- Maintain optimal physicochemical conditions (pH, osmolarity, redox potential)

Over hundreds of specialized media exist for different microbiological applications.

II. Classification

II.1. By composition

- Complex/Natural: Contains undefined biological components
- Semi-synthetic: Partially defined composition
- Synthetic: Precisely defined chemical composition

II.2. By function

- Basal media: Supports general bacterial growth.
- Enriched media: Provides additional nutrients (growth factors) for fastidious organisms.
- Selective media: Suppresses the growth of unwanted microbes and promotes the growth of target organisms.
- Differential media: Distinguishes microorganisms based on their biochemical characteristics.
- Transport media: Maintains and preserves specimens during transport without promoting growth.

II.3. By Consistency (Physical state)

- Liquid media (broths)
- Solid or agar medium (1.5-2% agar)
- Semi-liquid, semi-solid, or weakly gelled medium (0.4-0.8% agar)

III. Objective of the Practical Work

- Learn about the different types of culture media used in microbiology.
- Gain familiarity with the techniques for preparing culture media (nutrient broth and nutrient agar from basic constituents)

IV. Materials and Methods

IV.1. Materials

Glassware & Equipment :

- 2 Erlenmeyer flasks (500 mL)
- 100 mL graduated cylinders
- 180 mL sterile glass bottles with screw caps
- Petri dishes
- Screw-cap tubes and tube racks
- Precision balance

- Watch glass and spatula
- Magnetic stirrer with heating
- Magnetic stir bars
- Sterile graduated pipettes
- Bunsen burner
- Autoclave

Products :

For nutrient broth (NB):

- Peptone: 5 g/L
- Meat extract: 3 g/L (*Can be substituted with yeast extract for vegetarian alternative*)
- NaCl: 5 g/L
- Distilled water: 1 L

For Nutrient Agar (NA):

- Same as NB + Agar 15 to 20 g/L

IV.2. Methods

- Measure the appropriate amount of each dehydrated constituents for **200 mL of distilled water**. Add them to distilled water in **Erlenmeyer flasks**.
- Vigorously shake the mixture to promote dissolution.
- Place **magnetic stir bars** in the flasks and continue stirring using the **heated magnetic stirrer** until the powder is completely dissolved.
- Maintain a **gentle boil (for 1 minute)** while controlling the stirrer's temperature (**avoid overflow**).
- Using a sterile funnel, dispense each medium into 180 mL bottles, then, replace caps without tightening completely - a quarter turn is sufficient to allow air exchange during autoclaving.
- Media should be **autoclaved at 121°C for 20 minutes** for **sterilization** (This step is omitted in this session **due to time constraints** because autoclaving—including heating to 121°C, sterilization time, and pressure release—takes more than **2 hours**.)
- After autoclaving, proceed near a Bunsen burner as follows (for NA only):
  a. **Deep agar (Butt):** Prepare sterile test tubes. Using a sterile pipette, transfer 10 mL of still-liquid agar (about 50°C) into each tube. Maintain tubes perfectly vertical until solidification.
  b. **Slant agar:** In other sterile tubes, pour 5-7 mL of agar. Immediately after pouring, incline tubes at 45° using an appropriate rack. Allow to solidify in this position for 30 minutes.
  c. **Petri Plates:** Slightly open sterile Petri dishes near a Bunsen burner flame. Gently pour 15 mL agar in each plate. Let solidify on a flat surface."
- Before use, systematically verify that agar media present smooth surfaces without bubbles or cracks
- Label each container with the medium type (NB or NA), preparation date, and your name or group identifier, then store the prepared culture media under appropriate conditions (typically at low temperatures) to ensure stability and maximize shelf life.