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General Microbiology

Practical Work N° 2: Microscopic Observations

Topic: Fresh State and Mobility, Simple Staining, and Gram Staining

I. Materials and Reagents

Materials

- Optical microscope
- Slides and cover slips
- Pasteur pipettes
- Microbiological loop
- Bunsen burner
- Absorbent paper
- Distilled water

Reagents and Stains

- Ethanol (95%)
- Methylene blue
- Crystal violet
- Lugol's iodine
- Diluted fuchsin (1/20) or safranin

**** immersion oil***

Samples

- Bacterial culture on agar or in liquid medium
- Plain (unsweetened) yogurt

II. Experimental Protocol

A. Fresh State Observation (Bacterial Mobility)

1. Place a small drop of sterile distilled water on a clean slide.
2. Collect a small amount of bacterial colonies from the agar (preferably from the edges) or a drop of broth culture.
3. Mix gently in the drop of water to obtain a homogeneous suspension.
4. Cover with a cover slip without trapping air bubbles.
5. Quickly observe under a microscope at 40x objective, reducing the diaphragm opening.
6. Note the presence of mobility (active movement) or Brownian motion (random molecular agitation).

B. Smear Preparation and Fixation

1. Clean the slide with alcohol to remove grease.
2. Place a drop of bacterial suspension on the clean slide.
3. Spread it evenly in a circular motion ("snail shell" pattern).
4. Allow to air dry.
5. Fixation:
    - **By heat**: Pass the slide through the Bunsen burner flame 3 to 4 times (bacteria-free side facing the flame).
    - **By alcohol**: Cover the slide with alcohol for 3 minutes, remove the excess, and allow to dry.

C. Simple Staining (Methylene Blue)

1. Cover the fixed smear with methylene blue for 1 minute.
2. Rinse gently with distilled water.
3. Dry between two sheets of absorbent paper.
4. Observe under a microscope at 100x objective with immersion oil.

D. Gram Staining

1. Cover the fixed smear with crystal violet for 1 minute.
2. Rinse with distilled water.
3. Apply Lugol's iodine for 1 minute to fix the stain.
4. Rinse with water.
5. Decolorize by tilting the slide and allowing alcohol to flow for 10 seconds. Rinse immediately.
6. Counterstain with diluted fuchsin (or safranin) for 1 minute.
7. Rinse, dry, and observe at 100x objective with immersion oil.
8. Interpret the results:
    - Gram positive bacteria: *Purple*
    - Gram negative bacteria: *Pink*

III. Results and Interpretation

- Compare bacterial mobility between yogurt and bacterial culture samples.
- Determine bacterial shape (cocci, bacilli) and arrangement.
- Differentiate between Gram-positive and Gram-negative bacteria based on staining reactions.