

Microbiology Practical 03

Macroscopic and microscopic examination of Bacteria

Objectives:

- Observe macroscopic characteristics of bacterial colonies on Petri dishes.
- Perform a Gram stain to observe bacterial morphology and cell wall structure.

Part 1: Macroscopic examination of bacterial colonies

Materials:

- Petri dishes with bacterial cultures (nutrient agar)
- Marker pen

Method:

Observe the colonies with the naked eye and record the following features:

Characteristic	Examples
Shape	Circular, irregular, filamentous
Edge (margin)	Smooth, wavy, lobed
Elevation	Flat, raised, convex, crateriform
Surface texture	Smooth, rough, wrinkled, mucoid
Color	White, cream, yellow, red...
Transparency	Transparent, opaque, translucent
Shine	Dull, shiny, matte
Odor (with caution)	Characteristic, strong, none

Part 2: Gram Staining

Principle:

Gram staining differentiates bacteria into **Gram-positive** (thick wall, purple) and **Gram-negative** (thin wall, pink/red) based on cell wall structure.

Materials:

- Clean glass slides
- Inoculation loop
- Bunsen burner
- Stains: crystal violet, Lugol's iodine, alcohol (ethanol or acetone), safranin
- Distilled water

- Blotting paper
- Light microscope

Steps of the Gram Staining Procedure:

1. **Smear preparation:**
 - Place a drop of sterile water on a clean slide.
 - Pick a small amount of a colony with a sterile loop.
 - Spread the sample and let it air dry.
 - Heat-fix by quickly passing the slide 2–3 times over a flame.
2. **Staining:**
 - Stain with **crystal violet** (1 min), rinse gently.
 - Apply **Lugol's iodine** (1 min), rinse.
 - Decolorize with **alcohol** (10–15 sec), rinse immediately.
 - Counterstain with **safranin** (30 sec), rinse and dry.
3. **Microscopic observation (oil immersion $\times 100$):**
 - **Gram-positive:** purple
 - **Gram-negative:** pink/red
 - Note **shape** (cocci, bacilli) and **arrangement** (chains, clusters, single...).

Expected Results:

Strain	Gram Reaction	Shape	Arrangement	Macroscopic Observations
Example 1	+	Cocci	Clusters	Circular, opaque, cream-colored colonies
Example 2	–	Bacilli	Single	Flat, translucent colonies