**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 ×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 |