Exercise 3.2 – Simple Stain: Basic Dye

LEARNING OUTCOMES

1. List several basic dyes used in simple staining.
2. Describe the steps for preparing a simple stain.

One of the easiest techniques to use when visualizing cells under the microscope is to prepare a *simple stain.* Simple stains utilize one type of dye and result in cells that are all the same color, regardless of bacterial type. The most common simple stains use basic, or alkaline, dyes. These are dyes that contain a positively charged chromophore that are attracted by the negative charge of bacterial cells. When viewed microscopically, pigmented cells are visible against a white background or field. Common dyes used in simple staining are crystal violet, methylene blue, and the pink dye safranin.

Although simple staining is quick to do, the information that it provides about cells is limited. Since all bacteria are the same color upon completing the procedure, we only learn the size, shape, and arrangement of cells. Because of this, most microbiology laboratories do not perform simple staining alone and instead use a combination of dyes in multi-step differential staining procedures. These procedures tell us the same information that simple stains provide while also distinguishing between bacteria based on the type of composition of the cell wall, the presence or absence of endospores, etc. Two of the most common differential staining methods, the Gram stain and acid-fast stain, include the same dyes that we are using in this simple staining exercise.

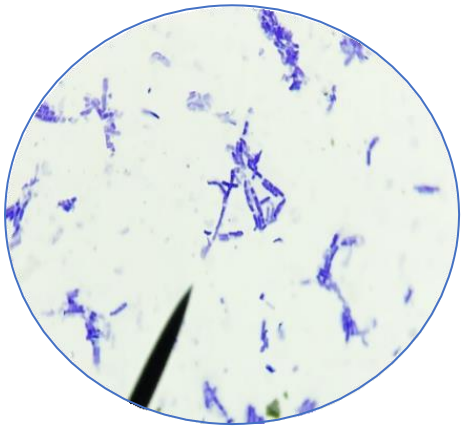

Figure 3.4: Simple stain of Bacillus megaterium using methylene blue dye (1000x)

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OBJECTIVE

Stain cells with a basic dye to determine cell size, shape, and arrangement.

MATERIALS

- SLIDES: *Bacillus cereus* and *Staphylococcus aureus* heat-fixed smears
- SOLUTIONS: Methylene blue; safranin (from Gram stain kit)
- EQUIPMENT: Stain pan, rack, wash bottle, bibulous paper, clothespin/slide holder

PROCEDURE – STUDENTS WORK IN PAIRS

IMPORTANT: If your staining pan becomes full, empty it into the sink at the center of your bench. ***Never carry a full pan across the room****!*

1. Place the heat-fixed smear of *B. cereus* on the rack over the staining pan, smear side up.
2. Cover the smear entirely with methylene blue and let stand for five minutes.
3. Using the clothespin or slide holder, rinse both sides of the slide with water.
4. Blot the slide gently in the bibulous paper booklet and put the slide aside.
5. Dispose of the pan water in the bench sink.
6. Place the heat-fixed smear of *S. aureus* on the rack over the staining pan, smear side up.
7. Cover the smear entirely with safranin and let stand for one minute.
8. Using the clothespin or slide holder, rinse both sides of the slide with water.
9. Blot the slide gently in the bibulous paper booklet and put the slide aside.
10. Dispose of the pan water in the bench sink.
11. View the stained slides microscopically under oil immersion and complete the lab report.