Exercise 3.3 – Negative Stain: Acidic Dye

LEARNING OUTCOMES

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

Some bacteria secrete a polysaccharide-rich structure external to the cell wall called a glycocalyx. If the glycocalyx is thin and loosely attached, it is called a slime layer; if it is thick and tightly bound to the cell, it is called a capsule. The glycocalyx can protect the cell from desiccation and can allow the cell to stick to surfaces like tissues in the body. They may also provide cells with protection against detection and phagocytosis by immune cells and contribute to the formation of a biofilm. In this way, the glycocalyx can serve as a virulence factor that contributes to the ability of an organism to cause disease.

Capsules can be detected using a *negative staining* procedure, in which an acidic dye stains the background rather than the encapsulated cells. Unlike simple staining with a basic dye, negative staining results in colorless cells that are easily seen against a colored background (Figure 3.5). Acidic dyes carry a negatively charged chromophore, thus they are repelled by the net negative charges on the bacterial cell. Examples of acidic dyes include the black dyes nigrosin and India ink, as well as the red dye eosin. Since capsules are destroyed by heat, this staining procedure is done without heat-fixing.

Although negative staining reveals the cellular morphology, size, and arrangement of cells, it does not differentiate between Gram-negative and Gram-positive bacteria. The term *negative* in negative staining technique refers to the negative charge of acidic dyes, while *Gram-negative* describes the type of bacterial cell wall.

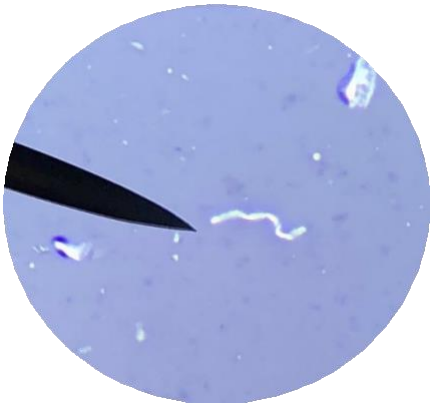

Figure 3.5: Negative stain of Rhodospirillum rubrum using nigrosin dye (1000x).

Exercise 3.3 – Negative Stain: Acidic Dye

OBJECTIVE

Stain cells with an acidic dye to determine cell size, shape, and arrangement.

MATERIALS

- EQUIPMENT: Glass slides, Kimwipes or Sta-Clear paper, inoculating loop, marker
- CULTURES: Broth culture of *Rhodospirillum rubrum*
- SOLUTIONS: Nigrosin

PROCEDURE

1. Close the cap and vortex the broth culture 5-10 seconds.
2. Wipe both sides with Kimwipes or Sta-Clear paper to reduce static charge.
3. Label the frosted edge of one slide with the organism number and NS for negative stain.
4. Place a small drop of nigrosin on slide near the labeled end.
5. Aseptically obtain a loopful of bacteria and close the culture tube.
6. Emulsify the bacteria on the loop in the nigrosin drop, but do not spread it over the slide.
7. Place the second slide in the dye at a 45^{o} angle in the nigrosin.
8. Pull the spreader slide across the bottom slide to spread the nigrosin toward the other end.
9. Dispose of the second slide in the disinfectant beaker.
10. Allow the nigrosin slide to air dry.
11. Repeat steps for the second culture.
12. View the stained slides microscopically under oil immersion and complete the lab report.