

E X E R C I S E

8

Simple Staining

OBJECTIVES

At the conclusion of the exercise, you should...

1. know the advantages of staining bacteria.
2. explain the mechanism of staining.
3. perform a simple stain on the cultured bacteria provided.
4. perform a simple stain on the microorganisms from scrapings around the teeth and gums.
5. observe **pleomorphic** and **palisade** arrangements of bacteria.

Exercises Required

Exercise 1: The Brightfield Microscope

Exercise 5: Observing Bacteria

Exercise 6: Aseptic Technique

Exercise 7: Smear Preparation

INTRODUCTION

Bacteria are very small and transparent when observed with a wet mount preparation. In order to observe their cell characteristics, they need to be stained (dyed). This method consists of preparing a smear that is air dried and heat fixed (as in the smear preparation exercise) and adding a stain to the bacteria on the slide.

MATERIALS

Cultures:

Corynebacterium xerosis

Corynebacterium pseudodiphtheriticum

Supplies:

Microscope slides with frosted ends

Pencil

Gram Stain marking pen (or China grease marking pen)

Toothpicks (sterile)

Clothespins
Inoculating loop
Inoculating needle
Loeffler's methylene blue
Bibulous (blotting) paper

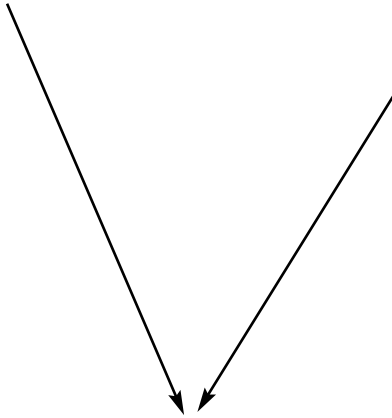
Technical Background

Bacteria are very small and transparent when observed with a wet mount preparation. In order to observe their cell characteristics, they need to be stained (dyed). This method consists of preparing a smear that is air dried and heat fixed, and adding a stain to the bacteria on the slide.

Simple staining is useful in determining the basic morphology of an organism. Simple staining involves only one reagent. Examples of simple stains are crystal violet, basic fuchsin, and methylene blue. **Pleomorphism** is a morphological characteristic that pertains to an organism's ability to demonstrate several different shapes. The *Corynebacteria* are rod-shaped, but when grown on certain media, they will appear club-shaped and needle-shaped. The *Corynebacteria* also exhibit **palisade arrangement**. This type of cell arrangement is described as a "picket fence" arrangement.

Bacteria are usually negatively charged anionic (-). The simple dyes used to stain bacteria have a positive charge cationic (+); therefore, they are attracted to each other. These dye molecules are also strongly colored so that our eyes can see the stained bacteria.

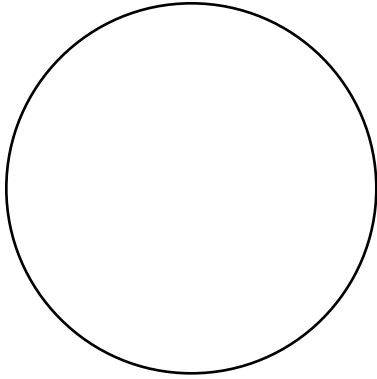
PROCEDURES

1. Prepare air-dried, heat-fixed smears of the *Corynebacterium* species
 2. Prepare a smear of material from your teeth as follows:
 - a. Put a loopful of water on a clean slide.
 - b. Use a sterile toothpick to scrape some material from between your teeth.
 - c. Smear this material in the water; air dry and heat fix.
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3. Place the slides on the staining rack.
 4. Flood the prepared smears with Loeffler's methylene blue for 1 minute.
 5. Pick up each slide with the clothespin and rinse the dye off with water.
 6. Blot using a piece of bibulous paper, then air dry.
 7. Examine under oil immersion.
 8. Draw your observations in the data section of Evaluation of Results.
 9. (Optional) Make a wet mount of your gum scraping without staining it, and compare to the stained slide

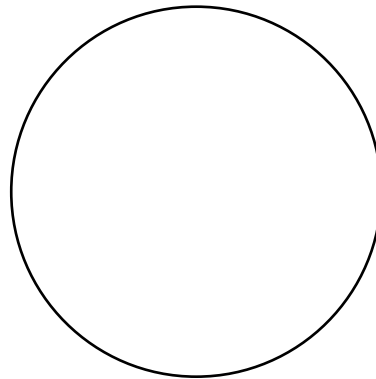
EVALUATION OF RESULTS (EXERCISE 8: SIMPLE STAINING)

Purpose

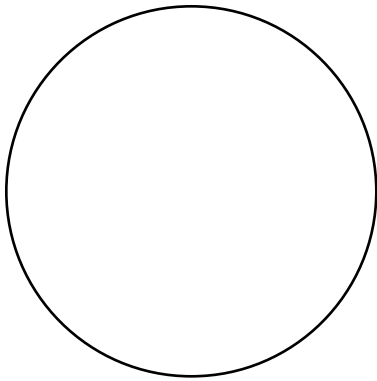
Data



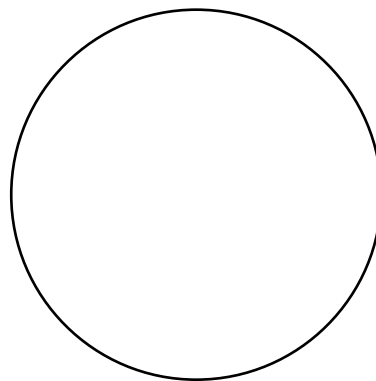
C. xerosis



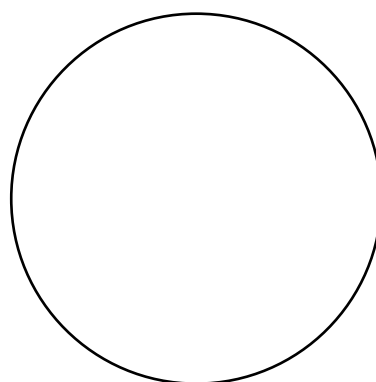
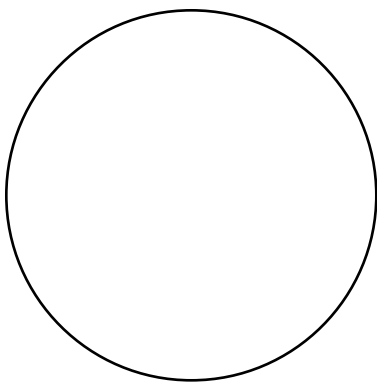
C. pseudodiphtheriticum



Your teeth scrapings



Your partner's teeth scrapings



CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. If you had made a wet mount of the scrapings from your teeth, would you have seen the same organisms as you did with the simple stain? Why or why not?
2. Why is the simple stain used for observing bacteria?
3. What shapes of bacteria were observed from the teeth and gum scrapings?
4. Use your textbook to find the names of 3 genera of bacteria that might be present in the scrapings from your teeth.

