

LAB 3: Morphological Characteristics of Bacteria

Protocols for Endospore Stain, Capsule Stain, Motility Stab and Wet Mount.

INTRODUCTION

Bacteria are characterized by the presence or absence of a number of different structures. Endospores, capsules and flagella are three such examples. Each of these structures is visible with light microscopy if the correct staining procedure is employed.

ENDOSPORES are survival structures. In poor growth conditions some genera may sporulate. Rather than dying, endospores survive in a dormant state. Endospores are unique to Bacteria and are formed by a limited number of bacterial genera. The soil bacteria within the genera *Bacillus* and *Clostridium* are the most familiar. The stepwise process of **sporulation** is triggered by poor growth conditions (see the discussion of the process of sporulation in your text). The transition from vegetative cell to endospore requires an environmental signal and then a series of steps. The endospore forms within the vegetative cell. A wall forms around a copy of the bacterial chromosome, capturing some ribosomes, proteins and DNA. The endospore forming *within* the cell can be visualized using the light microscope. As the sporulation process continues, layers form within the spore making it very dense. Exterior to the spore, the vegetative cell dies. At the completion of sporulation, oval spores are visible using light microscopy. Endospores cannot replicate. However they allow survival in lean times. In fact they are resistant to extreme environmental conditions such as high temperatures, dryness, toxic chemicals, and UV radiation. The dormant structure allows cell survival until conditions favorable to cell growth returns. Favorable growth conditions signal the process of endospore **germination**. Germination of a spore results in a break in the spore wall and the outgrowing of a new vegetative cell. The newly formed vegetative cell is capable of growth and reproduction.

The dense endospore is impenetrable by basic dyes. In the Gram stain the spore is not stained. In early stages of sporulation, it can be seen as a clear oval in the stained vegetative cell. A special differential stain can be used to color the endospore and differentiate it from the vegetative cell. In the Endospore Staining Procedure, heat is needed to drive the stain (Malachite Green) into the spore. Once stained, spores retain the green dye, whereas washing with water removes the malachite green from the vegetative cell. The counterstain, safranin, is used to color the vegetative cell. The size of the endospore and its position within the vegetative cell is characteristic for a given species.

CAPSULES are slippery structures found exterior to the cell wall of many bacteria. Capsules may be involved in bacterial virulence (cells with capsules are less likely to be phagocytized), or in bacterial adherence (such as the adherence of *Streptococcus mutans* to teeth). Capsules are normally composed of polymers of sugars and/or proteins. They have no net charge and will not bind charged dye particles.

Negative staining, where the background is stained revealing an unstained structure of interest, is useful for demonstrating capsules. In **Maneval's method**, cells are mixed on a slide with a drop of the pH indicator, congo red (pH 3 or below, the color is blue and at pH 5 and above, the color is red). Congo red does not penetrate the capsule and provides a colored background. The sample is then allowed to *air dry*. Note that heat fixing is not used for the capsule stain. Protein capsules are destroyed by heat. Heating samples will destroy protein

capsules. Heating can also dehydrate cells. As the cells dehydrate they will shrink and false capsule-like areas will form around the cell. After air drying the slide is flooded with Maneval's solution, a combination of acetic acid and acid fuchsin. The acetic acid lowers the pH in the sample and causes the Congo red to change from red to blue. The acid fuchsin penetrates through the capsule and stains the cell a bright red. The unstained capsule is clearly seen using the light microscope as white, in this red, white, and blue preparation.

FLAGELLA are structures that mediate bacterial **motility**. Motile bacteria may move toward a source of nutrition or away from a toxin by the process of **chemotaxis**. The number and mode of attachment of flagella, as well as their location on the cell surface are characteristic of a species. Flagella arrangements include **monotrichous** - single flagellum located at one end of a cell, **amphitrichous** - single flagellum located at both ends of a cell, **lophotrichous**- a group of flagella at one cell end, and **peritrichous** -where flagella are found around the cellular perimeter. Flagella are too thin to be seen in the light microscope without the use of special staining procedures. These technically difficult procedures are used to load molecules onto the flagella to increase their width.

~ Figures 3.33, 3.34, 3.35, 3.36

The presence of flagella indicate that the bacterium is capable of motility. But motility can also be detected by direct visualization of bacteria in a **wet mount**. Motility is characterized by directional movement of organisms. It must be distinguished from Brownian motion or streaming. In Brownian motion, a consequence of molecular vibration, cells wiggle in place. In streaming, a current carries the bacteria, en masse, from one area of the slide to another.

An indirect method of testing for motility is through the use of a **motility stab**. A motility stab contains growth media and low percentage of agar in a test tube. To inoculate the test organism, the tube of semi-solid media is stabbed with a needle inoculated with bacteria. The tube is incubated. As the bacteria grow, nonmotile bacteria will grow only along the stab line. Motile bacteria will have the capacity to move around in the medium. A motility stab that is positive for motility will show a fuzzy stab line or no stab line (bacteria have moved throughout the tube). A motility stab that is negative for motility will show growth only along the stab line (bacteria cannot move away from the site of inoculation). ~ Figure 5.25.

Additional mechanisms of bacterial locomotion include gliding and motion by axial filament contraction. Gliding is movement of bacteria along solid surfaces by an unknown mechanism. Axial filament contraction occurs in spirochetes. These unusual spiral-shaped bacteria have a filament that stretches from end to end of the bacterial cell. When the filament contracts the organism moves.

OBJECTIVES:

- To learn the role of the bacterial structures: endospore, capsule, and flagella.
- To learn to recognize endospores and complete the Endospore Stain effectively.
- To learn to recognize bacteria with capsules and complete the Capsule Stain effectively.
- To learn to recognize motile bacteria in a wet mount and in a motility stab. To learn the motility stab protocol.
- To learn how recognition of bacterial structures are used in bacterial classification.

/ Assignment 1 Check Point:

For today's lab, bacteria were cultured on skim milk plates. This media was chosen because the nutrients encourage capsule formation in some bacteria. Observe your plates before you begin the staining lab. Do the colonies appear differently than when grown on nutrient agar? Can you see zones of clearing around the colonies? What does this indicate? ~ Page 86.

Protocol: Endospore Stain

MATERIALS/Group

- 3 slides
- malachite green
- safranin
- blotting paper strips - cut to the size of the glass slide
- Coffee cans with boiling water - 6/lab
- Demonstration slides showing cells with and without endospores.

PROCEDURE

1. Prepare a bacterial smear on a slide as described previously. Air dry and heat fix.
1. Place slide in staining tray. Cover slide with blotting paper and saturate the paper with malachite green.
1. Using slide holder, transfer slide to a rack over a boiling water bath. Heat the slide gently over the water bath for 3 - 5 minutes. The sample should steam. Do not allow blotting paper to dry while heating. Add drops of stain to keep paper moist.
3. Transfer slide to rack over staining tray. Use water to rinse sample. Remove blotting paper and discard.
4. Counterstain sample with Safranin for 30 seconds.
5. Wash sample with water, drain, using blotting paper to blot dry, and examine under oil-immersion objective. Endospores will stain green and vegetative cells will stain red. Note the position of endospores within the vegetative cells. Record Observations.

Protocols for detection of motility

MATERIALS/group

3 slides
3 cover slips
3 tubes motility agar
Demonstration slides showing cells stained with flagella stain.
Demonstration slides showing motile and nonmotile organisms

Protocol: Observation of motility in a Wet Mount

1. Prepare wet mounts using wet mount protocol: place one drop of water on slide; add a small loopful of culture. Mix, cover with cover slip.
1. Observe sample under 40X and 100X magnification. Look for directional motion of cells.
1. Record observations.

Protocol: Motility Agar Test

1. Use a wire needle rather than an inoculating loop to transfer culture in this protocol.

After flaming the wire needle, transfer a small inoculum to the motility stab agar. Inoculate the agar piercing vertically through the agar in a non wavering motion. The needle should penetrate to within one centemeter of the bottom of the motility test agar tube. Remove the needle along the *same stab line*.
2. Incubate tubes appropriately for your sample.
(For most organisms in BSCI 223: incubation at 37°C for 48 hours will allow cell growth.)
3. Observe cultures. Motile cultures will move away from the stab line and grow throughout the culture media. Non-motile cultures will grow only along the stab line.
4. Record observations. Make conclusions regarding the motility of your organism.

Protocol: Capsule Stain - Maneval's Method

MATERIALS/group

3 slides

Congo Red

Maneval's Solution

Demonstration slides showing organisms with and without capsules.

PROCEDURE

1. Place a few drops of Congo Red on a clean slide.
 └ NO WATER DROP IN THIS SAMPLE PREPARATION
2. Mix in a small amount of culture.
3. AIR DRY. └ DO NOT HEAT FIX.
4. Place slide on rack of staining tray. **Gently** flood smear with Maneval's Solution - wait 5 minutes.
5. Lift slide and gently pour off excess stain.
Rinsing with water is the next step. Rinsing must be done with great care. As the sample was not heat fixed to the slide, the sample preparation is fragile. Suggestion for careful rinsing:
 - Place slide on bottom of staining tray.
 - Add water to tray in the opposite corner from the placement of the slide.
Add the water SLOWLY until a large puddle forms.
 - Tilt staining tray and allow water to rinse the slide. Tilt the tray to allow rinse to collect in tray opposite from the slide. Remove slide from tray.
6. Place slide on absorbent paper. Allow sample to AIR DRY. └ Do not blot.
7. Examine with oil immersion lens. Compare to prepared demonstration slides
8. Record observations.

/ *Assignment 1 Check Point*

Using these protocols and control cultures allows you to see positive and negative results for the presence of endospores, capsules and flagella.

Using these protocols what can you learn about your environmental isolates?

At this point do you know enough about your organisms to differentiate them?

Exercise 3 _____ Laboratory Report Questions

Morphological Characteristics of Bacteria

Name _____

Results:

Draw observations for control cultures and environmental isolates.

Endospore Stain

Wet Mount

Motility Stab

Capsule Stain

Questions:

1. In the Gram stain and the endospore stain, heat fixing is used. In the capsule stain the cells are not heat fixed. Why?

1. In a Gram stained preparation of *Bacillus cereus*, a clear oval is seen within a purple vegetative cell. Explain.

1. In inoculating a motility stab, your partner stabs the inoculating loop into the agar three times coming in and out at different sites. How might this sloppy technique effect the determination of motility?

1. Define:
Negative Staining

Peritrichous