Lab Exercise 7: Microbial Motility

Background

Many prokaryotes, single-celled eukaryotes and differentiated specialized cells of multicellular organisms (*e.g.*, sperm cells) are capable of independent movement due to a special structure, the **flagellum** (plural: **flagella**). Bacterial flagella are long, thin (~20 nm) structures that are usually not visible with the light microscope, except after staining with special flagellar stains which increase their diameter. The number and arrangement of flagella on a cell will vary among species and within a species when environmental conditions change. For instance, members of the genus *Rhodospirillum* will have a single **polar** or **monotrichous flagellum** in a liquid environment (*i.e.*, when grown in broth) but will increase the number of flagella on solid media to cover the entire body surface in a **peritrichous arrangement**. Additional flagellar arrangements include **amphitrichous,** where single flagella are located on both sides of the cell, and **lophotrichous**, where a tuft of flagella exists on one side of the cell. Although the physiological response to an environment that allows the bacterial cell to produce different flagellar arrangements is interesting, it will not be covered in this laboratory activity.

Flagella allow cells to move toward (positive) or away from (negative) a stimulus in the environment, through a process known as **taxis** (plural: **taxes**). If the stimulus is chemical, the process is referred to as **chemotaxis**. If the chemical is noxious, the bacterium will move away from it in a process called **negative chemotaxis**. Likewise if the chemical is beneficial the bacterium will move toward it in a process called **positive chemotaxis**. Similarly, bacteria are capable of exhibiting **positive phototaxis** and **negative phototaxis**: movement toward or away from light. These taxes are the fundamental way that bacteria respond to their environment and can allow bacterial cells to move quite quickly.

Flagellar rotation can move a cell through liquid media at up to 60 body lengths/second (*i.e.* about 0.00017 km/h). Although this may seem slow, in terms of the number of body lengths moved per second, it is extremely fast. A cheetah moves at a maximum rate of about 110 km/h, which represents only about 25 body lengths/second. Therefore, a bacterium can "run" approximately 2.4 times faster than a cheetah, or the equivalent of 165 mph.

Microscopically, one can observe motility using a type of slide called a **wet mount**, whereby a drop of <u>viable</u> cells is placed on a glass slide with a coverslip- without being heat fixed. These wet mounts should be observed shortly after their construction, as the heat of the microscope tends to dry the preparations. For the beginner especially, true motility under the microscope must be differentiated from **Brownian motion** of cells due to the molecular bombardment which causes cells to shake but not move in any vectorial way. Cells can also appear to move because of currents created under the cover slip. Neither of these is considered true motility

Another method for determining motility involves inoculation of a semisoft agar deep medium containing 2,3,5-triphenyltetrazolium chloride (TTC) dye. The agar concentration of this medium is 0.4%, which does not inhibit bacteria from moving through the medium. If the organism is motile, it will "swim" away from the line of inoculation, causing the medium to become turbid (Figure 1). In addition, the TTC dye will detect metabolic byproducts of the living cells and turn the medium red in any location where the cells are present.

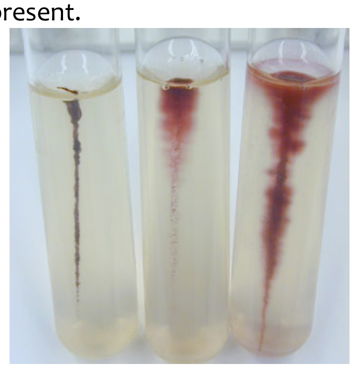

Figure 1: Bacterial inoculations into semi-solid motility medium. Non-motile (left) and motile (middle and right) are shown. Because of the addition of 2,3,5-triphenyltetrazolium chloride into the medium, all growth is red. Growth away from the line of inoculation is indicative of motility whereas growth only along the inoculation line indicates a non-motile organism.

Introduction

In today's lab you will be looking at two organisms: *Staphylococcus aureus* and *Proteus vulgaris*. You will use both the wet mount and motility media methods to determine true motility for each of these organisms. One of them is motile and it will be up to you to determine which.

Objectives

1. Make a wet mount preparation for viewing live microorganisms.
2. Understand principle behind motility media and be able to determine motility based on inoculation results.

Wet Mount Protocol

Team Supplies	Individual Supplies
Culture of *Staphylococcus aureus*	Microscope slides
Culture of *Proteus sp.*	Glass coverslips
	Inoculating loop

1. Use aseptic technique to transfer 4–5 loopfuls of one of your organisms to the center of your slide. Do not spread out the drop of liquid
2. Gently place a coverslip onto the glass slide. Coverslips tend to stick together, so make sure you have only one coverslip.
3. First examine the slide using your 10x objective lens. Because this is an unstained preparation, you might have some difficulty finding the focal plane. To help with focusing, try to focus near the edge of the drop of water, since most bacteria will be drawn to the edge by surface tension.
4. Use either your 40× or 100× objective- remember that only the 100× is an oil immersion lens. Look for true motility and determine whether your organism is motile or non-motile.
5. Repeat steps 1–4 using your second organism.

Motility Media Protocol

Team Supplies	Individual Supplies
Culture of *S. aureus*	2 tubes of semi-solid motility media with 2,3,5-triphenyltetrazolium chloride (TTC) dye
Culture of *P.* sp.	Inoculating needle

1. Using your INOCULATING NEEDLE, transfer one of your organisms into a tube of motility media. Be very careful to stab directly into the medium about 2/3 of the way down. Take special care to withdraw your needle along the inoculation point.
2. Repeat step 1 with your second organism, using an uninoculated tube.
3. Incubate both tubes at 25°C for 48 hours.
4. After incubation, compare the tubes- look for turbidity and red coloration as indicators of motility.

Data Collection and Analysis

1. Record your wet mount observations for the organisms used in this lab.
2. After incubation, draw the appearance of the motility media with TTC dye for all four of the organisms used in this lab.
3. Determine the motility of both organisms based on wet mount and motility media inoculations.

Discussion

1. Did the medium inoculations concur with the wet mount slide results?
2. If you compared two motile bacterial species and determined one was considerably more motile than the other, which arrangement of flagella would you expect to be associated with the highly motile species? How would you confirm this hypothesis?
3. Differentiate between the following types of movement observed in a wet mount: true motility, Brownian motion, water current movement.

What concentration of agar is used in a semisolid medium for motility determination? How does that compare to a typical solid medium? Why is that?