Lab Exercise 7: Microbial Motility

Background

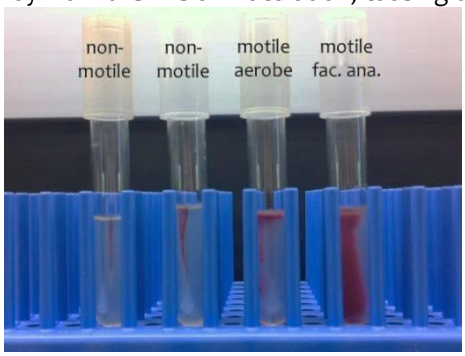
Many prokaryotes, single-celled eukaryotes and differentiated specialized cells of multicellular organisms (*e.g.*, sperm cells) are capable of independent movement due to a special organelle, the **flagellum** (plural: **flagella**). Bacterial flagella are long, thin (~20 nm) structures that are usually 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 even 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**. Conversely, 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 265 km/h (165 mph).

Microscopically, motility can be observed using a type of slide called a **wet mount**, wherein a drop of viable cells is placed on a glass slide with a coverslip- without being heat fixed. These wet mounts must be observed shortly after they are made, as the heat of the microscope tends to dry the preparations and make motility impossible. For the beginner especially, true motility under the microscope must be differentiated from **Brownian motion** of cells due to 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 called **motility media**, which contains 2,3,5-triphenyltetrazolium chloride (TTC) dye. It is important to note that the TTC dye will detect metabolic byproducts of the living cells and turn the medium red in any location where the cells are present- it does not turn red in response to motility. 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 below).



Bacterial inoculations into semi-solid motility medium. 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. Motile aerobes have growth only along the line of inoculation within the media (because they cannot grow without oxygen) but exhibit motile growth on the surface of the agar, where oxygen is present.

Introduction

In this lab each team will be looking at four organisms, *Staphylococcus epidermidis* and a *Proteus* species, which are both capable of growing without oxygen; and *Pseudomonas aeruginosa* and *Micrococcus luteus*, which cannot grow without oxygen. The wet mount and motility media methods will be used to determine true motility for each of these organisms. The inoculation of *Pseudomonas aeruginosa* and *Micrococcus luteus* into motility media will allow you to see motility of organisms that are incapable of growing within the motility media because of their oxygen requirements. Additionally, all organisms will be inoculated onto the surface of nutrient agar plates in order to look for characteristic **swarming motility**.

Objectives

1. Make a wet mount preparation for viewing live microorganisms.

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2. Understand principle behind motility media and determine motility based on inoculation results.
3. Observe the oxygen-dependent motility of certain organisms in motility medium.
4. Observe swarming motility on solid media.

Throughout this lab, students will work in teams of four. As a team, motility inoculations will be completed for all organisms.

Wet Mount Protocol

Team Supplies	Individual Supplies
Staphylococcus epidermidis	Microscope slides
Proteus sp.	Glass coverslips
Pseudomonas aeruginosa	Inoculating loop
Micrococcus luteus	

1. Use aseptic technique to transfer 4–5 loopfuls of the organism to the center of the 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 only one coverslip is used.
3. Examine the slide using the 10x objective lens. Because this is an unstained preparation, it may be difficult to find 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. Increase the magnification to observe motility.
4. Determine the motility of the organism. Look for Brownian and current movement too.

Motility Media Protocol

Team Supplies	Individual Supplies
S. epidermidis	tube of semi-solid motility media with 2,3,5-triphenyltetrazolium chloride (TTC) dye
Proteus sp.	Inoculating needle
Pseudomonas aeruginosa	
Micrococcus luteus	

1. Using the INOCULATING NEEDLE, transfer the organism 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 the needle along the inoculation point.
2. Incubate your inoculation at 37°C for 48 hours.

Swarming Motility Protocol

Team Supplies	Individual Supplies
S. epidermidis	nutrient agar plate
Proteus sp.	Inoculating loop
Pseudomonas aeruginosa	
Micrococcus luteus	

1. Using the inoculating loop, transfer one loopful of organism to the center of the agar.
2. Place a single loopful of the organism onto the middle of the agar plate.
3. Incubate the inoculation at 37°C for 48 hours.

Data Collection and Analysis

1. Record the wet mount observation for the organism used in this lab.
2. After incubation of the Motility Media, compare the tubes- look for the location of red coloration as an indicator of motility.
3. Observe and evaluate the motility of the organism on the nutrient agar. Identify swarming motility.
4. After incubation, determine the motility of all four of the organisms used in this lab.

Discussion

1. Did the medium inoculations concur with the wet mount slide results?
2. How did the motility of the two motile organisms differ? To what is this attributable?
3. Differentiate between the following types of movement observed in a wet mount: true motility, Brownian motion, water current movement.
4. 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?
