Determination of Bacterial Motility

Objectives:

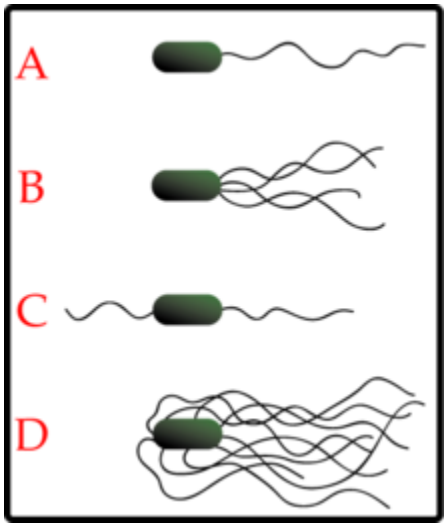
1. To recognize the arrangement of flagella on prepared slides of bacteria.
2. To determine bacterial motility using motility agar deeps.
3. To determine bacterial motility using a modified hanging drop wet mount slide.
4. To differentiate motile bacteria from non-motile bacteria.
5. To distinguish true bacterial motility from random Brownian movement.

Key Terms

motility, Brownian motion/movement, amphitrichous, peritrichous, monotrichous, lophotrichous, tetrazolium chloride (TTC), inoculating needle, flagellin

Introduction

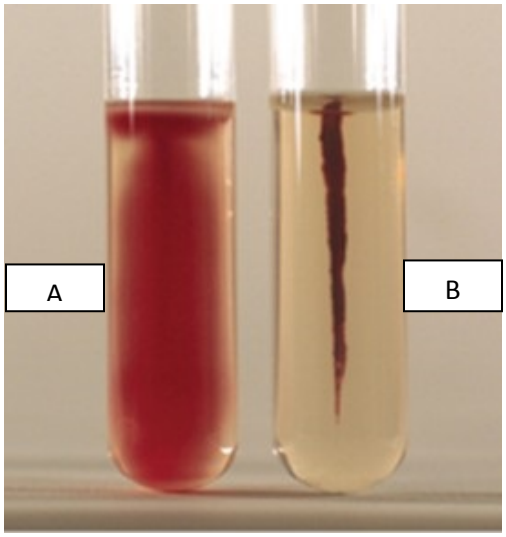
Motility is the ability of an organism to move by itself. Bacteria use propeller-like structures called flagella (singular flagellum). Bacteria can move towards or away from a stimulus (taxis). Moving towards chemicals (i.e. food) is chemotaxis. Moving towards light is phototaxis. Bacterial flagella are made of the protein flagellin. Flagellin is an antigen that can elicit an immune response. Flagellin can also be used to type different strains of bacteria (ex. *E. coli* O157 H7). "H" refers to the type of flagellar antigen. If bacteria possess flagella, the flagella can occur in four characteristic arrangements. A cell with a single flagellum has a monotrichous arrangement (A). Clusters of flagella are lophotrichous (B). One flagellum on each end of the cell is amphitrichous (C), and cells with flagella all around the cell have a peritrichous arrangement (D). Bacterial flagella are too small to be observed under the microscope. Heat-fixed bacteria can be stained using a special stain that makes the flagella appear thicker. The flagella of living bacteria cannot be directly observed under the microscope. Bacterial motility can be inferred by observation of the movement of living bacteria under the microscope or demonstrated by using motility agar.



Observing living bacteria under the microscope to determine motility can be challenging. All particles suspended in water move. This movement is called Brownian motion/movement. Brownian motion is the random, vibratory movement of particles caused by water molecules hitting those particles. In addition to Brownian motion,

some bacteria will exhibit true motility. True motility includes swimming directionally, tumbling, twisting, and spinning.

Observing living bacteria in motility agar is much easier. A bacterial sample will be stabbed into a motility agar deep using an inoculating needle. If the bacteria are motile, they will move away from the stab. If they are not motile, they will stay in the stab. Motility agar is softer than regular agar. Because the bacteria are essentially colorless and the motility agar is opaque, tetrazolium chloride (TTC) has been added to the agar. TTC is colorless in an oxidized state. When reduced, TTC turns red. Reduction occurs as a result of bacterial metabolism. The presence of a red color in the motility agar deep indicates bacterial growth. Note that some motile bacteria may not show motility because they cannot move through the agar, are inhibited by TTC, or are simply not motile at 37°C. Therefore, a false-negative result may occur.



Tube A is positive for motility.

Tube B is negative.

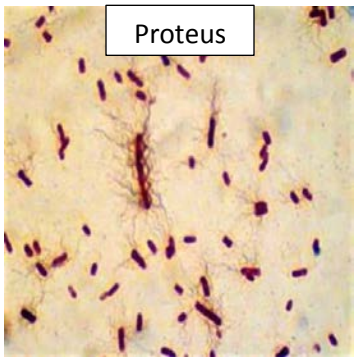
Materials

Flagella Stain

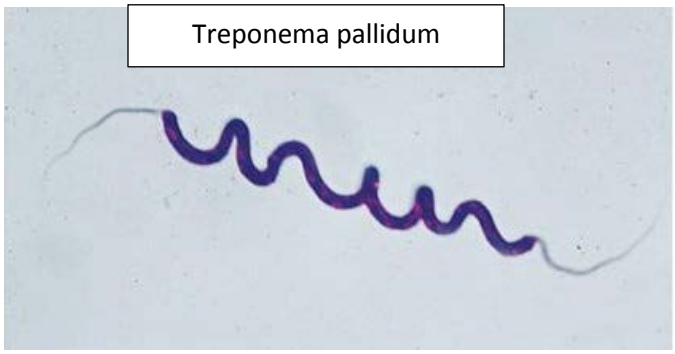
Live Organisms: None

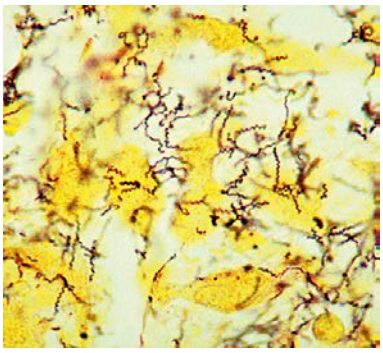
Procedure

1. This exercise is observation only. Observe the prepared slides of flagella stains:
Proteus
Spirillum
Borrelia
Aquaspirillum
Treponema pallidum (instructor demo only)



(Note: Your instructor may demonstrate these slides for you.)





Motility Agar

Live Organisms: *Escherichia coli; Micrococcus luteus; Aquaspirillum itersonii* (plate); *Corynebacterium xerosis; Staphylococcus aureus; Proteus mirabilis*

Per student: inoculating needle, one tube of motility agar

Procedure

1. Obtain a sample from one bacterial culture using the inoculating needle **(Note: do not use *Aquaspirillum*).**

2. Carefully stab a motility agar deep. Your instructor will demonstrate. It is important that your stab be "neat". Pull the needle out of the agar at the same angle at which the needle went into the agar deep. Place your deeps in the specified rack for incubation. The tubes will be incubated at least 48 hours.

Wet Mount Motility Slide

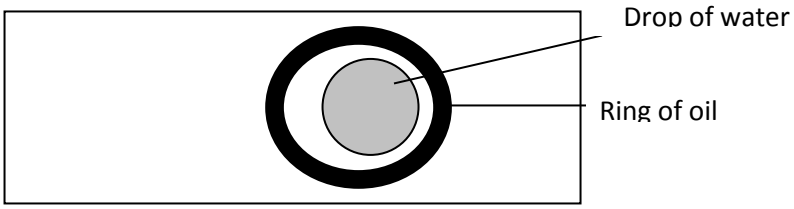
Live Organisms: *Escherichia coli; Micrococcus luteus; Aquaspirillum itersonii* (plate); *Corynebacterium xerosis; Staphylococcus aureus; Proteus mirabilis*

Per student: inoculating loop, two glass slides, two coverslips, immersion oil, water in bottles for suspending bacteria

Procedure

You will make at least two slides. Use the same organism that you stabbed into the motility agar deep and *Aquaspirillum*. Make one slide first and observe it, then make the second slide and observe that one. Do the bacteria appear to be moving by themselves or only Brownian motion?

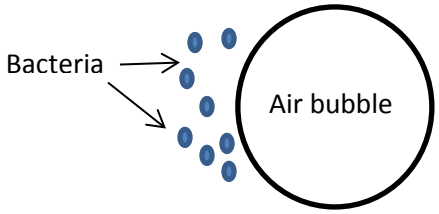
1. Instead of a traditional hanging drop slide, we will suspend the bacteria within a circle of immersion oil.



2. Carefully suspend the bacteria in the drop of water by touching the loop to the drop. Try to avoid mixing otherwise you will mix the oil and water together. Your suspension should be **slightly cloudy!** (Note: If using a broth culture, don't put a drop of water on the slide. Use several loopfuls of the broth culture instead.)

3. Apply a coverslip by "plopping" it on top. You want to trap air bubbles!

4. Focus on the edge of an air bubble. Close the iris. Go up to only 400x. Using oil is not necessary. On one side of the bubble will be air; on the other side will be the bacteria. Make sure that the 400x (high dry) lens is **clean!** Close the iris. Too much light makes the bacteria difficult to see.



Safety and Disposal

1. Today's lab requires goggles and aprons. Hair must be tied back and shoes must cover the entire foot.

2. Do not put inoculating needles in drawers. Return needles to the container they came from.

3. Put wet mount slides in the contaminated slide dish with disinfectant.

Observations and Results

	Motility Agar Deep	**Wet Mount Slide**
Organism	**Motile (+, -)**	**Motile (+, -)**
Aquaspirillum itersonii	~~	
Corynebacterium xerosis		
Escherichia coli		
Micrococcus luteus		
Proteus mirabilis		
Staphylococcus aureus		

Did the organism that you stabbed into the motility agar deep match the wet mount slide observation?

Concept Check

1. In a microscope, how can you distinguish true bacterial motility from random Brownian movement?

2. In a motility agar deep, how can you distinguish motile bacteria from non-motile bacteria?

3. What is the purpose of tetrazolium chloride (TTC)? Explain why TTC turns red?

4. Describe and identify the four arrangements of flagella found on bacteria.