Lab Exercise 4: Pure Culture Techniques

Background

As you learned while investigating the ubiquity of bacteria, microbes exist everywhere and very rarely do they occur as a single species. Robert Koch was one of the first to recognize that isolating a microbe (in his case, a bacterium) away from other microbes was crucial for his own argument that microbes cause disease, as well as for understanding the characteristics of the microbe itself. His studies on *Bacillus anthracis* contributed to many of the laboratory techniques we still use today, including the method for isolating **pure cultures** of bacteria.

The most commonly used method in the laboratory for isolating microbes is the **streak plate** (Figure 1), which relies on the dilution of bacterial cells in a sample to the point at which a single cell can divide separately from the rest of the population, giving rise to a single **pure colony**. Additionally, because the colony is the result of the reproduction, or **binary fission**, of a single bacterial cell, the colony is actually millions of **clones** of that original cell and these identical copies of the original cell can be used for further study. Because these colonies are the result of the replication of a single viable cell, the cells that give rise to these colonies are often referred to as **colony forming units** (**CFUs**) rather than cells.

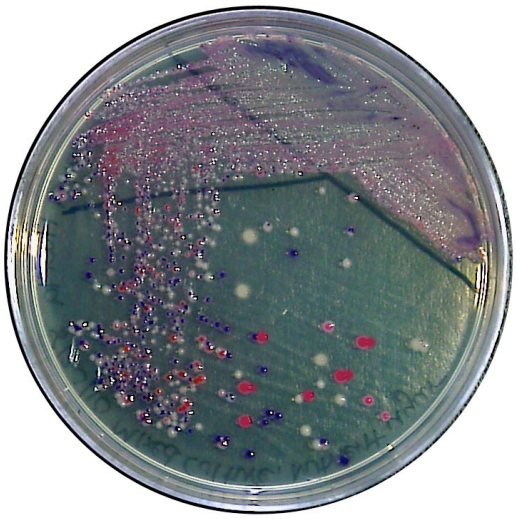

Figure 1: A representative streak plate of the three organisms for isolation in this lab: *Escherichia coli* (beige), *Serratia marcescens* (red) and *Chromobacterium violaceum* (purple). Dilution is achieved directly on one single plate as you drag colonies along the surface of the agar with your inoculating loop.

Introduction

In today's lab you will employ the streak plate method of isolating single colonies. Your inoculum will consist of a mixed culture of *Escherichia coli*, *Serratia marcescens* and *Chromobacterium violaceum*. These three bacterial species have been chosen for their ability to grow at similar temperatures, thrive on similar media and most importantly because the pigments produced by these bacteria make them easily distinguishable from one another. *E. coli* is considered to be a non-pigmented species because resultant colonies are beige in color. *S. marcescens* and *C. violaceum* are both pigmented, the former is red and the latter is purple.

Objectives

1. Perform a streak-plate to isolate bacterial cells of a mixed culture as discrete colonies/CFUs.

Protocol

Team Supplies	Individual Supplies
1 mixed culture of *Serratia marcescens*, *Escherichia coli* & *Chromobacterium violaceum*	1 nutrient agar plate
	Inoculating loop

1. Prepare your lab bench by removing extraneous items and cleaning the surface with table disinfectant.
2. Collect the necessary supplies: Petri dish, inoculating loop, lit Bunsen burner and mixed culture (in your test tube rack).
3. Label the bottom surface (containing the agar) of your sterile agar plate. Make sure to include your name, the date, the media type and the organism (mixed culture). Leave it agar side up in its lid on the counter in front of you.
4. Using the *Quadrant Method* illustrated in Figure 2 below, streak your plate.
    i. Finger vortex the mixed culture to resuspend the organisms.
    ii. Flame your loop until it is red-hot and let it cool briefly.
    iii. Remove the cap of mixed culture with the pinky of your dominant hand. Flame the mouth of the tube and continue to hold the cap in your pinky, do NOT place the cap down on the table.

iv. Insert the loop into the tube and remove a loopful of organisms.
v. Flame the mouth of the culture tube again. Return the cap to the tube and place the tube in the test tube rack.
vi. While holding the loop in your dominant hand, pick up the agar side of the Petri dish and streak the primary quadrant of the plate. Take care not to let your loop gouge the medium. As soon as you have performed this first streak, place the agar side back on top of the inverted lid.
vii. Flame sterilize your loop. **Do not retrieve a loopful of organism.**
viii. Repeat steps 5–7 to streak the secondary (2^{nd}) quadrant, putting your sterile loop into the 1^{st} quadrant (not the mixed broth culture).
ix. Repeat steps 5–7 to streak the tertiary (3^{rd}) quadrant, putting your sterile loop into the 2^{nd} quadrant.
x. If possible, repeat steps 5–7 to streak the quaternary (4^{th}) quadrant, putting your sterile loop into the 3^{rd} quadrant.

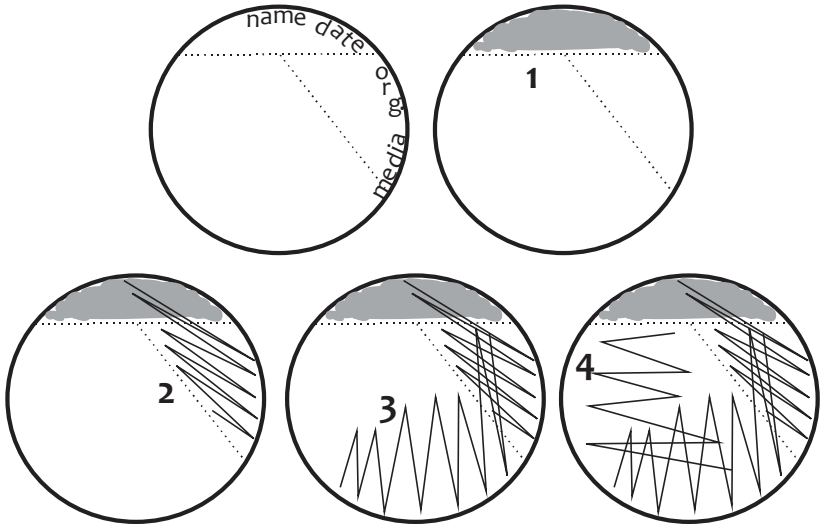

Figure 2: The Quadrant Method of streak plating for isolation. Plate guidelines (dotted lines) and label are drawn on the agar side of the Petri dish. These guidelines serve to keep subsequent quadrants from being contaminated by earlier streaks. The dark grey and black lines indicate proper streak technique for the 4 quadrants. Note that in quadrant 1 there is quite a bit of overlap of the streaks- as you are trying to get as much organism as possible into that area. In quadrants 2–4 however, there is no overlapping of streak lines, as the goal there is to dilute the organisms so that a single isolate can be found.

5. Incubate your plates inverted at **30°C** for 2 days.

Data Collection and Analysis

Make sure that your instructor sees your plate before you discard it.

Indicate the growth of the colonies on your plate by drawing them in your lab notebook. Were you able to get clear isolation of purple *C. violaceum*, red *S. marcescens* and non-pigmented *E. coli*?

Discussion

1. In regard to bacterial growth on solid media, define the terms "colony" and CFU.
2. Explain why dilution is a common approach employed in pure culture techniques.
3. Discuss the benefits and drawbacks to the streak plate method.
4. Provide two reasons why plates should be inverted during incubation.