BASIC CULTURE TECHNIQUE: STREAK PLATE

Principle and Purpose

The isolation of pure cultures of microorganisms is a technique essential to many types of experiments in microbiology as well as in the identification of potential pathogens. One very common way to isolate bacterial and other microbes is by employing the streak plate technique. In essence, the streak plate technique is a type of dilution scheme in which a single colony presumably arises from a single cell. In practice, a gradient of cells is established on the surface of an agar plate. The result is that confluent growth will occur in one part of the plate, but as the as the gradient lessens, single cells will be deposited well separated from other cells. These cells will give rise to individual colonies which can then be picked using an inoculating loop or needle and transferred to new media for maintenance of a pure culture.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the basic tenets of the streak plate technique; and
- Correctly use the streak plate technique to isolate discrete bacterial colonies.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms

- TSB mixture of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (Carolina Biological Supply), and *Chromobacterium violaceum* (Carolina Biological Supply)

Media

- TSA Petri dishes

Procedures

The exact streak plate method that one uses to generate isolated colonies is not too critical. What is critical is the generation of isolated colonies. However, to isolate colonies, some folks prefer the "three-phase" streak plate technique, whereas others prefer the "four-quadrant" (four-phase) technique. Other types of streak plates are also possible. They are all valid so long as single, well-separated colonies are produced.

The basic "three-phase" method is shown in Fig. 1. This will be the one described below and is very well explained in the video located at the following URL: https://youtu.be/pxqF-5QibQk. A typical result of this type of method is shown in Fig. 2. For comparison, Fig. 3 shows similar results obtained using both the "three-phase" and "four-quadrant" methods. In short, it does not matter how a student gets there so long as he/she does! However, here are some hints to help students generate that perfect streak plate:

- Use the entire surface of the agar plate;
- Use the tip of the loop – do not use it lying flat on the agar surface;
- Keep the streak lines "tight", i.e., close together;
- Do not cross over a prior streaked area more than once or twice; and

- Be sure to sterilize the loop between streaking a new area of the agar surface.

By following these hints as well as the general instructions detailed below, students will be able to master the art of the streak plate which will be a key skill for future work in this course

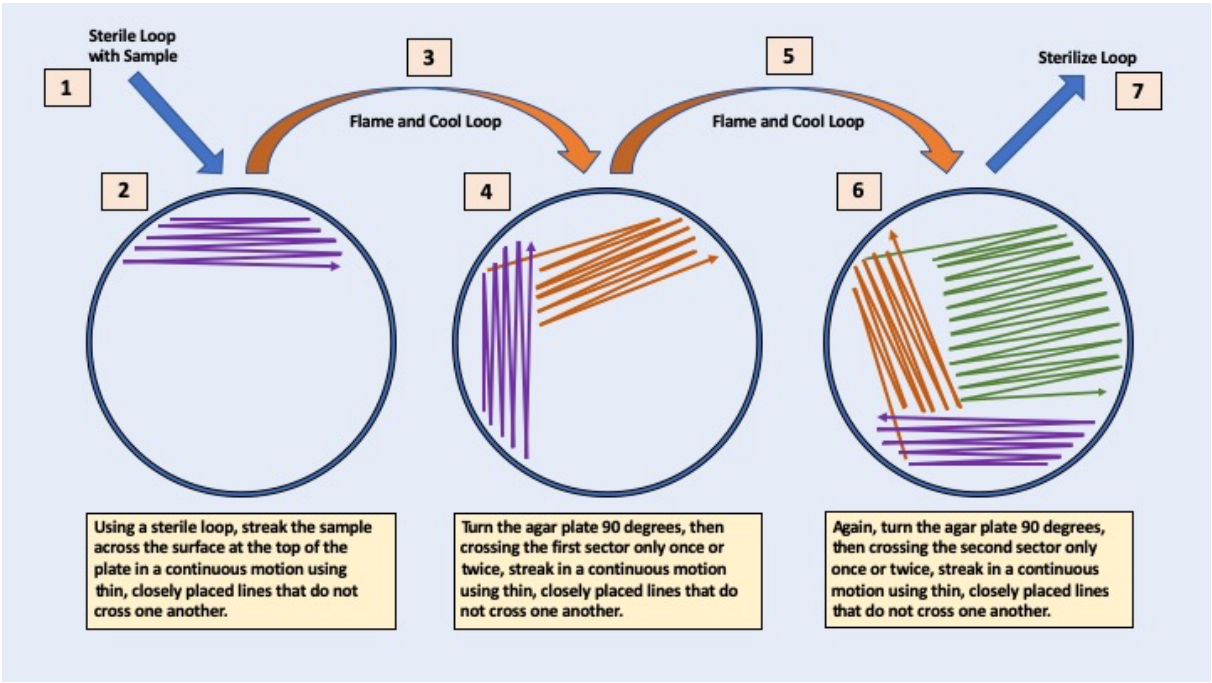


Figure 1. A diagrammatic depiction of the three-phase streak plate method. The individual steps noted above correspond with the directions given elsewhere in this exercise.

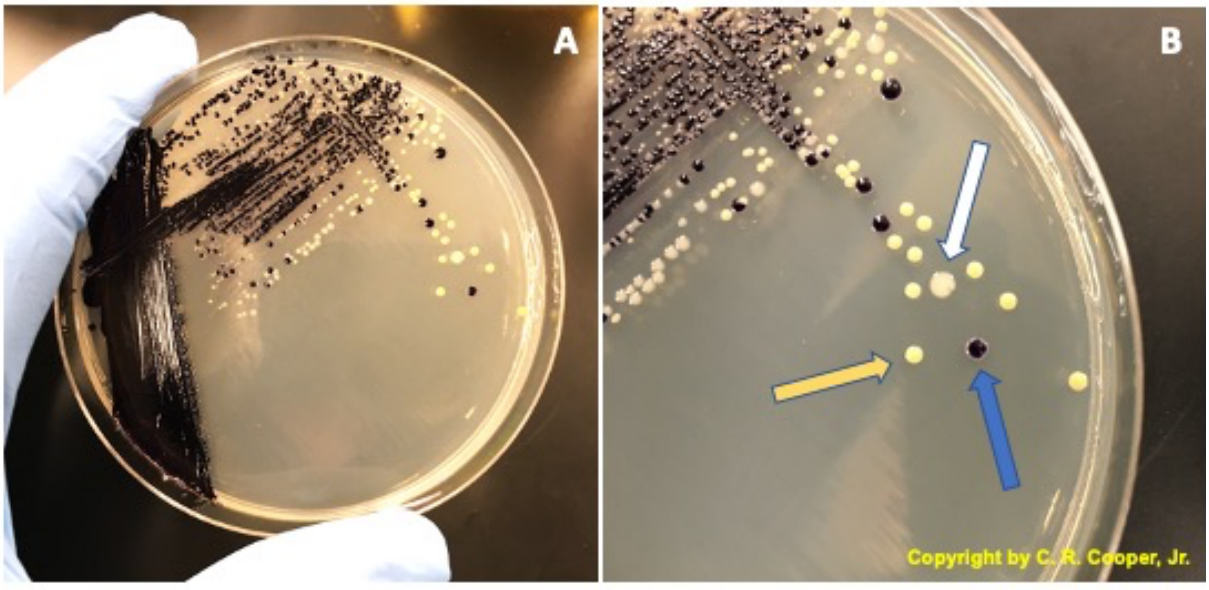


Figure 2. Typical results of the three-phase streak plate technique. A broth culture mixture of *Escherichia coli*, *Staphylococcus aureus*, and *Chromobacterium violaceum* was streaked onto a TSA plate and incubated for 24 hours at 37°C (A). In (B), a closer look at the plate shows isolated colonies of *E. coli* (white colony; white arrow), *S. aureus* (yellow colony; yellow arrow), and *C. violaceum* (purple colony; blue arrow).

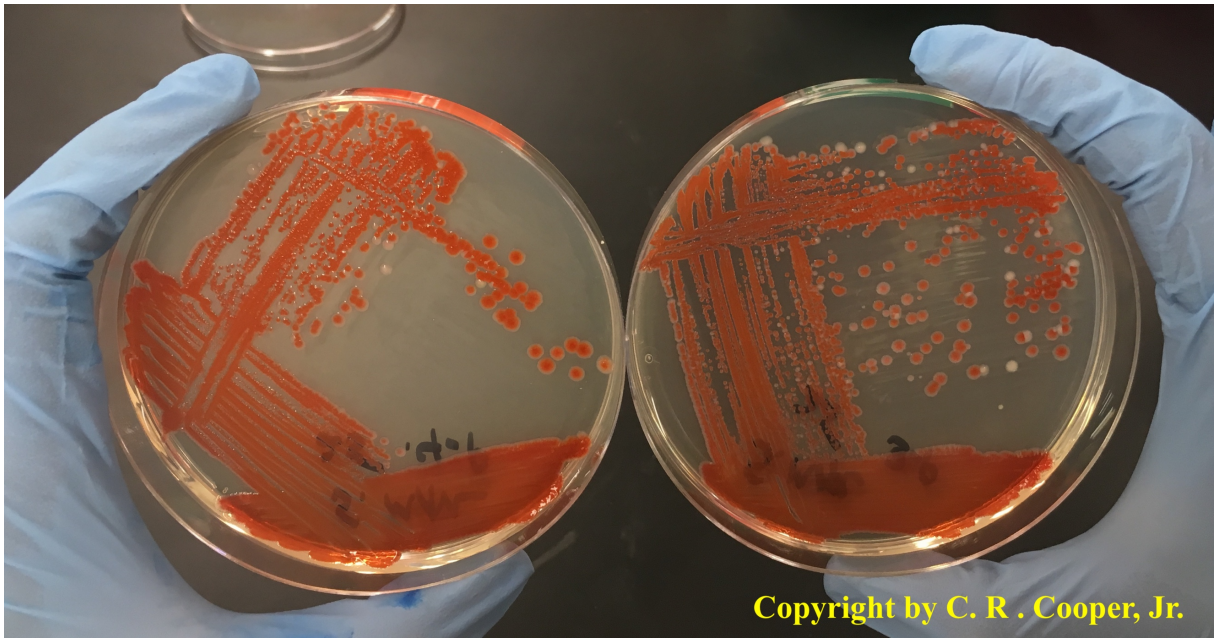


Figure 3. Isolated colonies from an *Escherichia coli/Serratia marcescens* mixture using the four-quadrant streak plate method (left) compared to the three-phase streak plate method (right).

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

Performing the Streak Plate ("Three-Phase" Method)

1) Obtain two TSA plates. On the bottom (i.e., the half containing the agar medium), label the plates with a name (or initials) and the date.

Note: If directed by the laboratory instructor, one of the TSA plates that can be used is that generated in the previous exercise (Part C in "*Basic Culture Technique: Aseptic Transfer*") should it not be contaminated. If it is contaminated or otherwise unusable, obtain a fresh plate.

2) A TSB culture of a mixture of *Escherichia coli, Staphylococcus aureus,* and *Chromobacterium violaceum* will be provided. To be sure that the bacterial cells are suspended, roll the tube in both palms ten times or more to suspend any sediment of cells that may have formed. Roll the tube quickly, but not so harshly that the broth splashes onto the tube cap or such that it rolls out of the hands causing leakage or breakage.
3) Using aseptic technique, sterilize a microbiological *loop* using either the gas burner or the Bacti-Cinerator (see the exercise entitled *Basic Microbiology Technique: Aseptic Transfer*).
4) While holding the loop between the thumb and forefinger, grasp the mixed bacterial culture in the other hand. With the hand holding the loop, curl the little finger around the tube cap and remove it. Do not set the cap down. Continue to hold in it in the curled finger.
5) Heat the opening of the culture tube by briefly passing it through the flame of the gas burner or, if using a Bacti-Cinerator, by holding the tube mouth next to the incinerator opening for 5-10 seconds.

6) Insert the cooled loop into the broth culture and withdraw it. The loop should contain a drop of liquid.

Note: To be sure the loop is cool, first touch it to the inside part of the glass tube above the medium. If the loop causes the medium to sizzle/hiss, it is too hot still. If this occurs, go back to step 3 and begin again.

7) Again, heat the end of the mixed culture tube, then replace the culture cap being held in the opposite hand. Place the culture tube in a rack.
8) Place a TSA plate on the bench top and raise the lid. DO NOT SET THE LID DOWN. Keep the lid positioned over the bottom part of the dish (thereby helping to prevent contamination from airborne microbes falling onto the plate). Insert the loop and place the drop of fluid it contains by lightly touching (not stabbing) the loop on the far surface of the plate. Using the *tip* of the loop (do not place the loop flat on the agar surface), spread/streak the liquid across the back third of the plate in a smooth back-and-forth motion being sure not to puncture the agar. Also, the back-and-forth strokes should be very close together. Use as much surface area as possible in this portion of the plate. Remove the loop and replace the lid of the Petri dish.
9) Sterilize the loop using a gas burner or Bacti-Cinerator.
10) After allowing the loop to cool, turn the plate about a third (60 degrees). Raise the petri dish lid (do not set it down on the bench surface). While keeping the lid positioned over the bottom part of the dish, insert the loop. Using the *tip* of the loop (do not place the loop flat on the agar surface) move it through the last few streaks of the first quadrant no more than twice. Streak across the second third of the plate in a smooth back-and-forth motion being sure not to puncture the agar. Again, the back-and-forth strokes should be very close together. Remember to use as much surface area as possible in this portion of the plate. Remove the loop and replace the lid of the Petri dish.
11) Sterilize the loop using a gas burner or Bacti-Cinerator.
12) After allowing the loop to cool, again turn the plate about a third (60 degrees). Raise the petri dish lid (do not set it down on the bench surface). While keeping the lid positioned over the bottom part of the dish, insert the loop. Using the *tip* of the loop (do not place the loop flat on the agar surface) move it through the last few streaks of the second quadrant no more than twice. Streak across the last third of the plate in a smooth back-and-forth motion being sure not to puncture the agar. Again, the back-and-forth strokes should be very close together. Remember to use as much surface area as possible in this portion of the plate Remove the loop and replace the lid of the Petri dish.
13) Sterilize the loop using a gas burner or Bacti-Cinerator.
14) Repeat this procedure (steps 1-13) using a second TSA plate.

Note: The purpose for performing this method on two separate TSA plates is simple. Students not having experience in this area are often timid and anxious about performing their first streak plate. They worry about making a mistake and failing in correctly carrying out the procedure. Well, making mistakes is how we learn and one only fails if one does not try to be successful. Hence, consider the first TSA streak plate a trial run to get the feel of the technique. Then use the second TSA to "go for the gusto"! Do your best! And if you feel you need to do so, ask your instructor if you can try streaking a third TSA plate.

15) Incubate all plates at 35-37°C for 36-48 hours.

Sneak a Peak! Students anxious to see their handywork may return to the laboratory and retrieve their plates after 18-24 hours and observe any growth that may have occurred. When grown at 35-37°C, colonies of *E. coli* appear white, whereas colonies of *S. aureus appear yellow,* and *C. violaceum* appears purple. However, further incubation will permit all colonies to be readily visible and identifiable. After taking a gander, return the plates to the incubator.

16) Retrieve the plates from the incubator.

Record any observations on the data report sheet attached to this document

Observing the Results: Again, when grown at 35-37°C, colonies of *E. coli* appear white, whereas colonies of *S. aureus appear yellow,* and *C. violaceum* appears purple. .

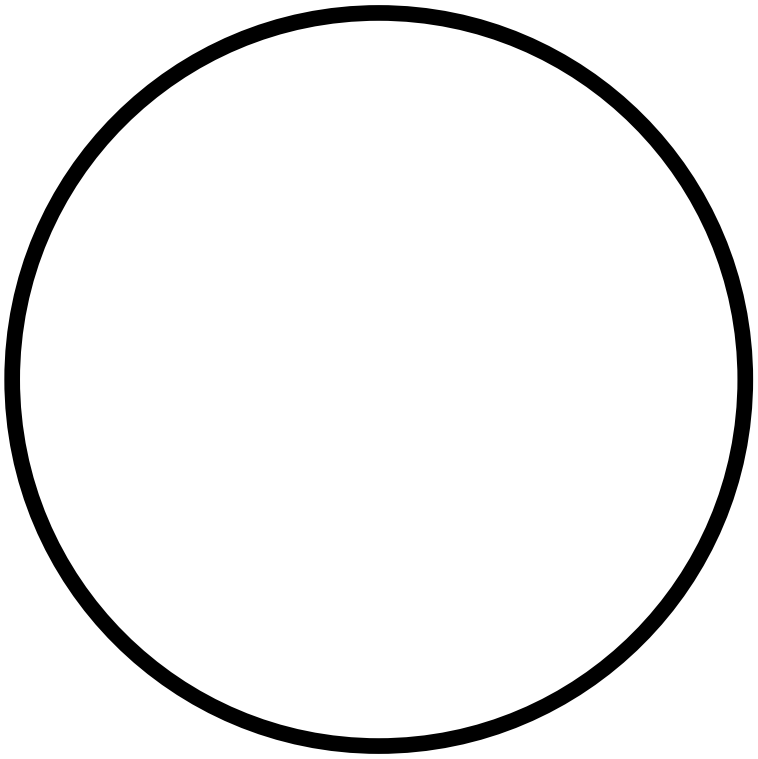
OPTIONAL: Comparing Streak Plate Methods

As previously noted, it does not matter how a student achieves the goal of obtaining isolated colonies, just so long as he/she is successful in doing so. Some persons are able to streak a plate in only one direction and obtain isolated colonies. Others are more comfortable using the "four-quadrant" method to generate single colonies. The follow part of this exercise is NOT required, but it will provide the means by which these two other methods can be compared to the "three-phase" method.

Students may repeat the streak plate procedure above with two other TSA plates. For the one plate, streak the mixed culture solely in one direction across the entire plate in one long motion. For the other TSA plate, using the above procedure as a guide, perform the streak plate using the "four-phase" method. The main difference shall be how far the plate is turned during each streak.

Incubate all plates at 35-37°C for 36-48 hours. Retrieve the plates from the incubator and compare the results of each method with each other. Which produces the most well-isolated colonies? If one of the optional methods works better, then use this approach in future studies.

Student Name: _____ **Date:** _____



Drawing of streak plate generated using the three-phase method.

1) What went right and what went wrong with your streak plate?

2) If you performed the optional procedure comparing the three-phase and four-quadrant streak plates, or any other technique, indicate so below and provide an appropriate drawing on the back of this page. How do the different methods compare?