

EXERCISE 12

Streak Plate Technique and Colony Morphology

OBJECTIVES

At the conclusion of the exercise, you should...

1. be able to perform the T streak plate method for isolating bacteria.
2. learn the Quad streak plate method for isolating bacteria.
3. be able to streak bacteria for isolation from a mixture of bacteria.
4. list other methods for obtaining pure bacterial cultures.
5. recognize and describe different colony types of bacteria.
6. learn the proper way to label Petri plates.

INTRODUCTION

Microbes exist in mixed populations in nature. In order to study a given organism, it must be in a **pure culture** (containing one kind of microbe). This exercise will demonstrate how to isolate bacteria from a mixed culture, using the **T streak plate method**.

MATERIALS

Cultures:

TSB mixtures of one of the following pairs of bacteria:

Staphylococcus aureus & *Chromobacterium violaceum*

or

Staphylococcus aureus & *E. coli*

Media:

4 TSA plates

PROCEDURES

Day 1 (first isolation)

Technical Background

Isolation of bacteria by **dilution** techniques is necessary in order to obtain a pure culture for further studies. The streak plate is the most-used dilution technique. When a loopful of bacteria is streaked across the agar plate, the bacteria are distributed across the surface of the agar. The more streaks, the more the bacteria will be diluted until, in theory, only one cell is left to grow and give rise to a colony of the same bacteria. There are different types of streak patterns, the **T streak** method and the Quad streaking method. Other dilution methods include the **spread plate technique** and the **pour plate technique**. These will be introduced in later exercises.

Colony morphology will help determine whether a pure culture was obtained, since bacteria of the same species will produce nearly identical colonies. **Colony characteristics** should include: color, size, shape, margin and consistency (or texture). Use the following guidelines for describing colonies of bacteria. (Note: It is best to choose colonies that are isolated and not too crowded.) Some of these descriptions will have variations and combinations. For example, a colony could be described as very large, dark brown, slightly raised, slightly spreading, very mucoid.

Size: pinpoint, small, medium, large

Color: white, cream, tan, brown, black, purple, yellow, red, etc.

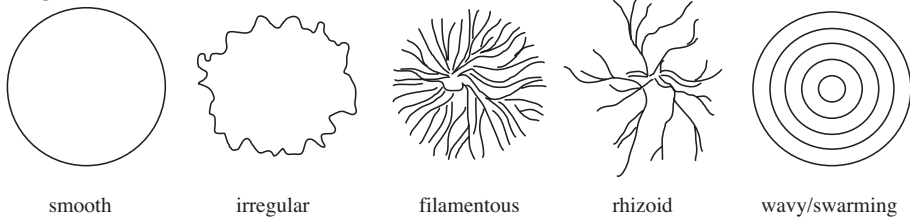
Shape (elevation): raised, flat, convex, dimpled (caved in), fried egg, growth into agar

Margin (edge): smooth, spreading (swarming), irregular, wavy, rhizoid, filamentous

Consistency (Texture): shiny, glistening, dry, powdery, wrinkled, rough, dull or matte, mucoid

COLONIAL MORPHOLOGY

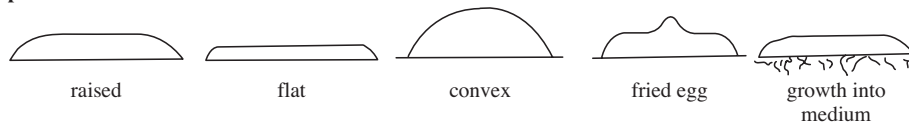
Margin



Margin continued



Shape



The mixture used for this exercise has two of the three different species of bacteria listed below. They each have characteristic colony morphology. See demonstrations provided.

S. aureus- medium-sized, yellow, slightly raised, smooth, shiny to dull colonies

C. violacium- medium-sized, dark purple, raised, gumdrop-like, smooth, shiny colonies

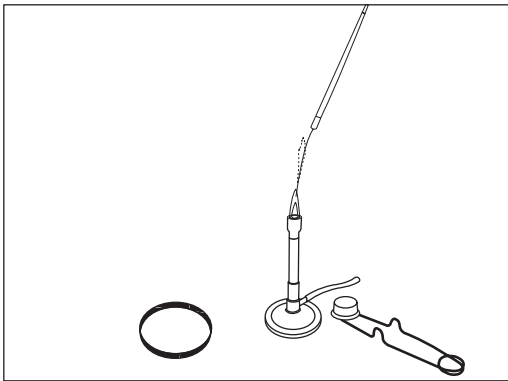
E. coli – medium-sized, grey, raised, smooth, shiny colonies

Performing a Streak Plate Technique

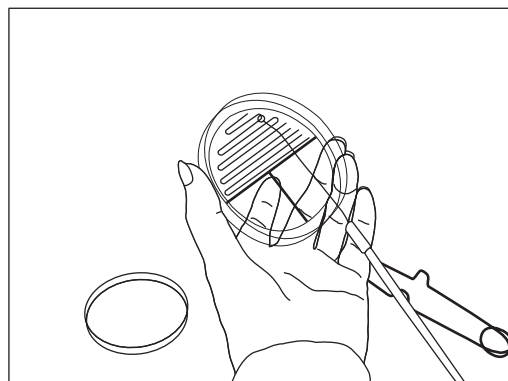
Observe the instructor's demonstration before you begin.

1. Label a TSA Petri plate on the bottom half around the edge with a Sharpie marking pen. The label should include any or all of the following: **Name**, **Date**, **Class**, **Section #**, **Exercise #**, and/or a short name of the exercise. One example for labeling is: J. Doe 6/8/98 Bio 210, #1, ex. 12, mix. This may vary with different schools or instructors.
2. Draw a "T" on the bottom of the plate to delineate the 3 areas for dilution of the organism (see T streak diagram below).
3. Follow aseptic technique for flaming and cooling the loop (as described in the aseptic technique exercise).
4. Mix the broth culture by gently swirling the test tube.
5. Obtain a loopful of the mixture (this will be the only time you will obtain the specimen).
6. Turn the Petri plate over, onto its top.
7. Pick up the bottom of the Petri plate with one hand, leaving the lid on the lab bench.
8. Starting at the edge of the agar, streak the **Primary inoculation area** (above the crossbar of the "T"). Hold the loop like you would hold a pencil and gently touch the surface of the agar. Be careful not to gouge it. Make as many streaks as possible, covering as much of the area without overlapping previous streaks.
9. Flame and cool the loop.
10. Turn the plate about a quarter turn.
11. Streak the **Secondary inoculation area** by going back into the primary area with your loop only once or twice. Streak this area in the same manner as the primary area, covering as much agar as possible without overlapping the previous streaks.
12. Flame and cool the loop.
13. Turn the plate another quarter turn.
14. Continue streaking as above, passing into the secondary area once or twice and then streaking out to the end of the third inoculation area. (See diagrams.)
15. Incubate the plate **upside down**, at 35°C until the next lab period.

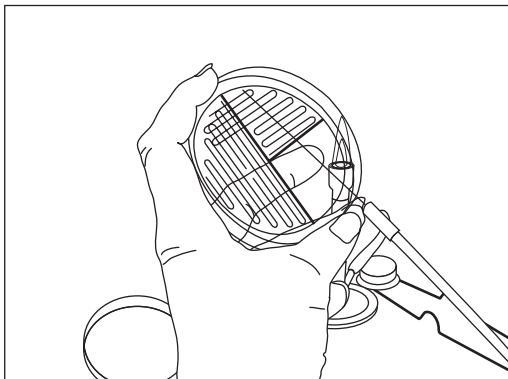
Streaking a plate with the Quad streak technique

STREAK PLATE PROCEDURE

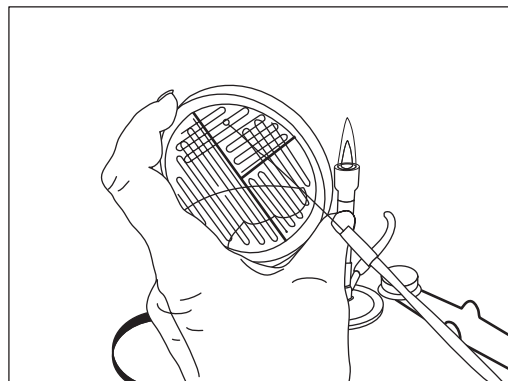
After flaming and cooling the loop, pick up a loopful of the culture or a portion of a colony from a fresh plate, then.....



Starting on one side, streak the primary area by streaking about 1/3 of the plate. Flame and cool the loop.....



Turn the Petri plate about 1/4 turn and streak the secondary area by going back into the primary area only once or twice and then streaking four to five times in the secondary area. Flame and cool the loop.....



Turn the plate another quarter turn and continue streaking by passing into the secondary area once or twice and then streaking out to the end of the plate (see diagram below).

PROCEDURES

Day 2 (second isolation)

Describing Colony Morphology

1. Examine the streak plate for the different colony types.
2. Circle and number the different types of colonies.
3. Describe the colony morphology using the guidelines listed in Day 1 procedures.
4. Perform a Gram stain on each of the isolated colonies.
(Hint: You can place as many as three colony types on the slide by drawing lines and using a small amount.)
5. If you did not obtain two distinct colony types in the third area of inoculation, restreak for isolation from mixed areas on the plate. Do this by taking a sample from the primary area and follow the T streak procedure using a new TSA plate.
6. If there are two distinct isolated colonies on the plate, restreak each colony type onto a new TSA plate. For each, take a small sample by touching the top of an isolated colony with a needle or a loop and transferring to the primary area of a new TSA plate. Streak for isolation as instructed previously.
7. Incubate the TSA plates at 35°C until the next lab.
8. Reincubate the original streak plates until the next lab period for more growth, if necessary.
9. Save all streak plates in the refrigerator until the exercise is completed.
10. Complete the morphology and Gram stain results table in the Data section.

PROCEDURES

Day 3 (final evaluation)

Evaluating Your Results

1. Examine all of the streak plates for isolated colonies.
2. Perform Gram stains on each of the colonies from the second isolation, if not done previously.
3. Compare the results with the original streak plate with the mixture of the bacteria.
4. If necessary, continue practicing streaking for isolated colonies of each of the colony types for up to a total of 2 plates after the initial mixture plate.

EVALUATION OF RESULTS

(EXERCISE 12: STREAK PLATE TECHNIQUE AND COLONY MORPHOLOGY)

Purpose

Data

Colony #	Color	Size	Shape (elevation)	Margin (edge)	Consistency	Gram Stain

CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. Why are Petri plate cultures incubated upside down?
2. Discuss reasons why only one colony type would appear after incubation, even though the original broth contained two different species of organisms.
3. What is the purpose of flaming the loop between the streak areas?
4. How could you tell if one of the colonies on the plate was a contaminate that fell in from the air?

