Lab 2
Microbiology
January 17, 2010

Pure Cultures and Aseptic Technique

Today's lab involves the transfer of bacterial cultures from one type of **media** to another. Microbiologists often need to use bacteria grown in broth for experiments using agar plates. Recall that the development of solid media was the technology that allowed **Robert Koch** to study isolated bacterial species and to test a **Germ Theory** for disease. In addition, bacteria maintained on solid media need to be transferred to fresh solid or liquid media at regular intervals (once a week), to insure healthy growth.

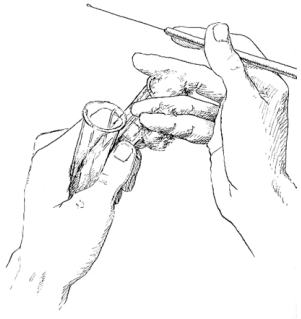
Successful transfer of bacteria requires the practice of **aseptic (sterile) technique**. Sterile technique insures that only the bacteria of interest and no **contaminants** grow on the plate. One common practice of lab microbiology is the separation of individual types of bacteria in a mixture (i.e. in a fluid sample from a wound). We can use techniques to spread a portion of the mixture so thinly that single organisms are separated from each other. Today, we will employ the **streak method** to transfer individual organisms present in the broth to the agar plates. We will practice with a culture containing only one type of organism and continue with a mixed culture. Our goal/purpose is to isolate pure cultures (isolated colonies) from a culture of billions of organisms. We will continue to use the streak method throughout the course.

Materials

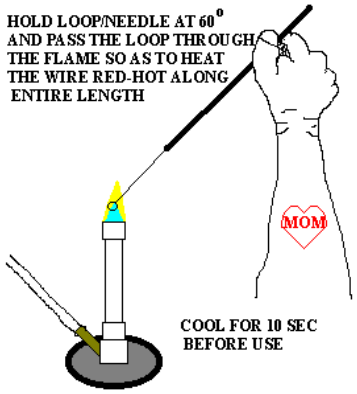
1 tube of *Micrococcus luteus* **and** one tube of *Serratia marcescens* broth culture (Each partner will use one pure culture)
1 tube mixed (2 types of bacteria present) culture
2 nutrient agar plates (each person should have 2 plates)
1 inoculating loop
Bunsen burner

PROCEDURE

1. Dr. Super will demonstrate how to hold the tubes of bacteria and loop using aseptic technique. The thumb and 2 adjacent fingers hold the loop much like a pencil is held for writing. The 2 remaining fingers, on the pinky side of the hand, remain free to hold the cap of the test tube for transfer.



2. Sterilize the loop in the flame of the Bunsen burner. Hold the loop at a near vertical position and allow the loop most of the stem to become red hot. (Always allow the loop to cool briefly before touching bacterial broth or plate.)



3. To make the transfer, hold the test tube as above in your free hand Remove the cap by clasping it with the 2 free fingers on the hand holding the inoculating loop. Rotate the tube and lift the cap, but DO NOT SET THE CAP DOWN.
4. Dip the sterilized loop into the broth, taking care to watch so the loop just dips under the surface. You should see a film of liquid in the space of the loop.
5. Set the tube back in a rack and replace the cap securely.
6. Using your free hand, place your NA plate on its lid in front of you.

7. Keeping your plate inverted, lift plate off lid and turn over. Working as quickly as possible, and streak some of the liquid culture along the top edge of the nutrient agar. Replace the plate on its lid ASAP.

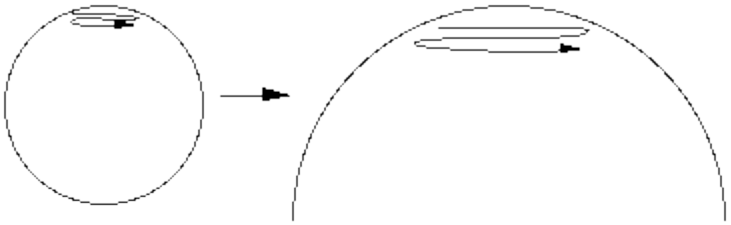
7. Flame the loop (red hot, 10 seconds) and allow to cool.

8. Turn the plate 90 degrees. Starting with the loop in the area in which you first streaked, streak again at a right angle to the first area streaked. (NOTE---You should **not** obtain more of the liquid culture for this or subsequent streaks.)

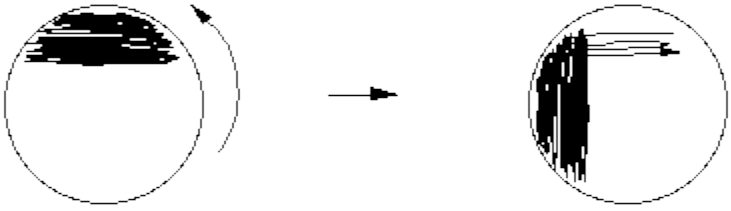
9. Repeat steps 8 and 9 two more times until you have 4 areas of streaking on your plate---each time taking care to start in the area of the last streak and to reduce the bacteria with each subsequent streak.

10. Repeat for each culture, *Micrococcus luteus* **, or** *Seratia marsescens,* **and** the mixed culture.

11. Incubate the plates inverted at room temperature. Observations will be made next week.



Start at one edge of the plate, moving the loop back and forth while pulling it back to streak about 1/4 of the plate. Sterilize the loop, and touch a clean part of the surface to cool it.



Rotate the dish 90˚ and streak one-half of the remaining surface, starting with two streaks that overlap the first area. Sterilize and cool the loop again.

Repeat for the third section. Remember NOT to overlap either previously inoculated section after the first two.