Bacterial Isolation

Introduction:
There are approximately 10,000 named species of microbes. It is estimated that there are between 10,000 and 100,000 more unidentified species for every identified one. Not only are there many types of bacteria, there are a lot of individual bacteria. A single spoonful of soil can have 100 million individual bacteria. A scraping of your gums can yield 1 million bacteria per $cm.^2$ (A cm^2 is about the size of your little fingernail). The bacteria in and on our bodies makes up about 10% of our dry body weight.

Most of the currently known species of bacteria have been identified using traditional microbiological techniques such as the gram stain reaction, morphology, and metabolic reactions. Bacteria rarely live alone but in communities with other bacteria. This is true both in the environment and in and on our bodies. This class focuses on the role of bacteria in disease. Isolating a single bacterium species is the first step in identifying the bacteria possibly responsible for a disease process.

The first requirement for physically isolating a bacterium is that it can be cultured in the laboratory. This requires knowledge of optimal temperature for growth, optimal oxygen requirements, and optimal nutritional needs. We work with a very limited number of bacteria in this course. The bacteria we work with are also very easy to culture in the lab. Most bacteria are not this agreeable!

There are two main ways to isolate organisms.
1. Streaking for isolation on an agar plate
2. The pour plate method

Streaking for isolation on an agar plate involves the successive dilution of organisms until you have the cells at a low enough density that single cells are physically isolated spatially to give rise to recognizable individual colonies. In the pour plate method, you dilute your sample sufficiently before you add it to molten cooled agar and then pour this mixture in a dish. The isolated cells give rise to individual colonies growing in the agar itself. This technique can be a little tricky. If the melted agar is too hot you kill all the bacteria. If the melted agar is too cool you end up with a big lump in your Petri dish. The streaking method yields individual colonies on the surface of the agar. This technique is much faster and easier to master.

Overview:
You will be given a broth sample containing three organisms, *Staphylococcus xylosus, Serratia marsescens,* and *Escherichia. coli*. All three organisms readily grow aerobically on tryptic soy agar (TSA). However, *S. marsescens* only forms a red pigment at 35˚C or less and optimally at room temperature (25˚C). It grows very rapidly at all temperatures into medium sized colonies. *E. coli* has a tan appearance at all temperatures and is also a rapid grower that forms large colonies. *S. xylosus* has a yellow-orange appearance at all temperatures, grows rapidly, and forms medium to large colonies. Your ability to isolate and see the three organisms on your plate is dependent on appropriate incubation conditions. Your plates will be incubated at room temperature for 48 hrs. You should be able to identify the three organisms based on colony size and pigment.

Materials:
- 1 mixed-culture in tryptic soy broth (TSB) tube containing *Staphylococcus xylosus, Serratia marsescens,* and *Escherichia. Coli* (all BSL 2)
- 3 TSA plates

Streaking for Isolation Procedure:

There are several methods of streaking for isolation. The vast majority of our students have been most successful with the quadrant method of streaking which is described below.

1. Label your plate with your name, date, section, and organism.
2. Use BSL 2 procedures to obtain a loop-full of organisms from your tryptic soy broth (TSB) tube. Refer to the aseptic technique protocol.
   - Be sure that you have adequately mixed your broth tube so the organisms are uniformly suspended in the broth.
3. Recap your TSB tube.
4. You can do the next part with your plate on the lab bench or holding it in your hand. You decide which works best. Lightly drag your loop back and forth across the surface of the agar. Refer to Figure 1.
   - The more you drag the more bacteria you deposit.
   - The general idea is to decrease the bacterial concentration with each swipe.
   - Four to five zigzags seems to work well.
   - Experiment with your different plates. Be sure to keep track of what you did on each plate.
5. If you are using an incinerator, sterilize your loop. If you are using plastic loops, discard your used loop in the cavicide container and obtain a new sterile plastic loop.
6. Do **NOT** go back into the original broth tube.
7. Touch your loop to the agar surface against the far end of your first streak. Repeat by dragging back and forth.
   - Do not drag into the center of your plate.
   - You should be able to see the faint indentations of your streaking line on the agar surface.
8. Using a sterile loop, repeat the procedure on your second streak.
9. Using a sterile loop, repeat the procedure on your third streak. Zigzag the last part into the center of the plate.
   - You should end up with isolated colonies somewhere in your last streak.
10. If you are using an incinerator, sterilize your loop. If you are using plastic loops, discard your used loop in the cavicide container.
11. Replace the lid on your plate.
12. Place your completed plates agar side up on the incubation rack on the front desk in the INCUBATE section.

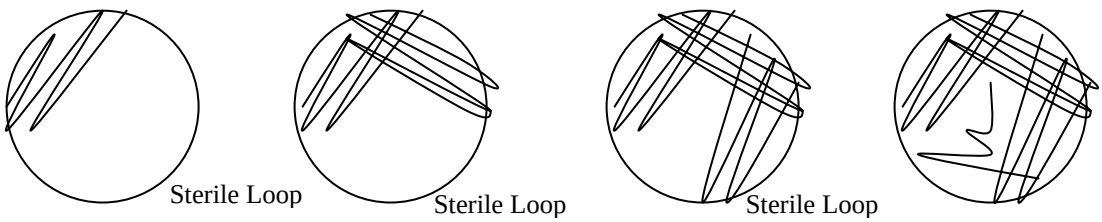

Figure 1: Quadrant Method of Streaking for Isolation

Notes:

- It is absolutely essential that you sterilize your loop between each streaking, either by using the incinerator or by obtaining a new sterile plastic loop. This is the most common mistake students make.
- Don't leave your plate open too long or extra bacteria from the environment will fall into your plate.
- Do not be disappointed if you do not get isolated colonies on your first try. This is a difficult procedure