

Environmental Isolates

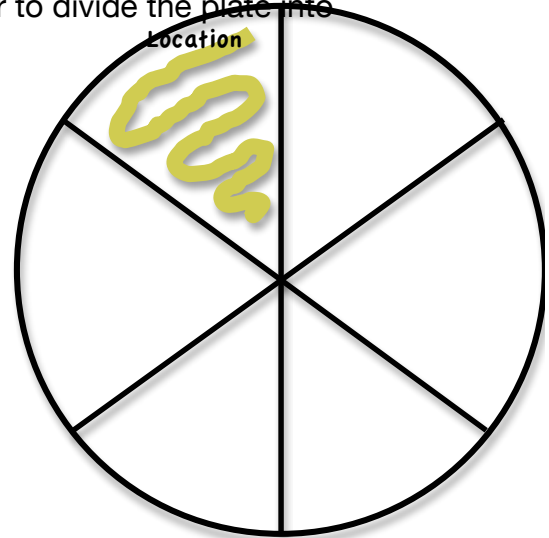
Almost every surface you come into contact with every day has bacteria on it. This exercise will give you an idea of the diversity of life we interact with every day without even knowing it.

Materials = Day 1

Sterile Cotton Swabs
LB Agar Plates
37°C Incubator
Sharpie

Protocol – Day 1

1. On the bottom of your LB agar plate, use a marker to divide the plate into 6 sections as shown.
2. Pick 6 locations you want to test (examples could include your hands, cell phone, door handle) and write the name of the location on the bottom of the plate. There will be one location per section of the agar plate.
3. Go to one of your 6 locations and using the cotton swab, swab the area to test, swabbing a similar size space for a similar time on each area.
4. Then gently swab the surface of the agar plate in a zig-zag pattern (it is easiest to start from the widest part of the section and move down).
5. Repeat with the remaining locations, using a fresh cotton swab each time
6. Incubate your plate face down (**agar side up**) at 37°C overnight. Remember to put your initials on your plate!



Question:

What location(s) do you think will harbor the most bacteria and why?

Part 2 Day 1:

As a class, come up with a question comparing the bacteria that will be found on different surfaces around campus. This could include comparing the same surface in different buildings or “clean” vs. “dirty” surfaces.

Take the second LB agar plate and swab the surfaces to answer the question the class has agreed on.

REMEMBER TO WASH YOUR HANDS BEFORE LEAVING THE LAB!

Materials – Day 2

Microscope
Glass microscope slides
Sterile sticks
Sterile water
LB agar plates
P200 pipettor and sterile tips

Protocol – Day 2

Look at your plates by eye:

- Count (if possible) and record the number of colonies that grew from each location
- Observe and record what the colonies from each location look like (i.e. size, color, texture)

Look at some of the bacteria on the microscope:

- Place a drop of water on a microscope slide
- Using a sterile stick, transfer a colony of bacteria from your plate to the drop of water and stir around gently to suspend/mix
- Look at the bacteria at 10X and 40X magnification. What do the bacteria look like?

Discussion and Questions:

1. Which location harbored the most bacteria?
2. Was your above prediction correct?
3. Did all locations have the same types of bacteria?
4. Did you get similar results as other groups?
5. Are these results surprising?

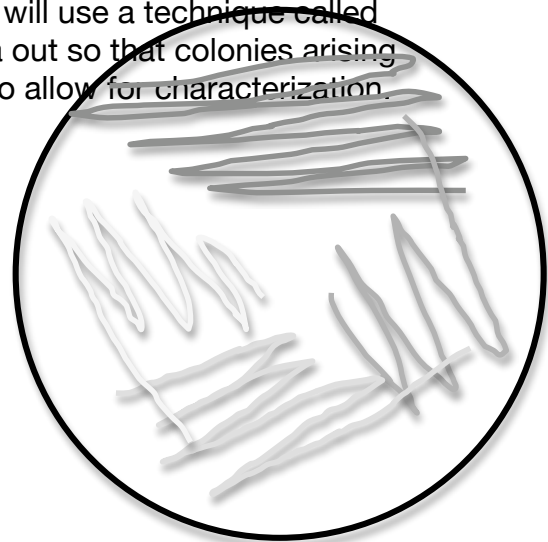
Day 2 part 2: Isolation of pure cultures

In order to look at pure colonies of bacteria, we will use a technique called “quadrant streaking.” This spreads the bacteria out so that colonies arising from a single bacterium are separated enough to allow for characterization.

1. Select one of your environmental isolates that grew well.
2. Label a new LB agar plate (on the agar side) with your initials, the date and where the isolate came from
3. Take a sterile wooden stick and gently scoop up a small quantity of the isolate from your plate.

Note: *Sticks should not be thrown into the biohazard waste, collect them in the waste stick receptacle and they will be sterilized.*

4. Gently streak the bacteria over the new plate in the pattern shown in the darkest grey.
5. Each successively lighter streak should be done with a fresh sterile stick. ONLY the first stick is to pick up bacteria from your other plate, the others are only to spread the bacteria you transferred with the first stick.
6. Place the plate in the 37° C incubator overnight, face down (**agar side up**).



Quadrant Streak