Protocol 5: Bacterial Culture

Goal: Grow up a large quantity of bacteria from a single isolated bacterial colony for use in future experiments (sequencing, freezing, antibiotic resistance, spatial evolution, etc.).

During this lab you should learn to:

- Learn to characterize bacterial colonies based on their physical characteristics
- Learn to pick colonies for growth in culture.
- Safely operate a Bunsen burner

Introduction:

We would like each individual to identify a colony for growth. In order to maximize the chances of these colonies being interesting, we can look at the characteristics of the colonies to try to identify unique types of colonies.

Bacterial colonies take on many shapes and sizes depending on their species and growth conditions. The FORM is the overall shape of the bacterial colony. They can be circular, irregular, filamentous (filaments intersect substantially), or rhizoid (filamentous but not intersecting). You can also see forms that are combinations of these basic forms. The ELEVATION describes the vertical structure of the colony. This may be difficult to ascertain in our samples so we will not concern ourselves with this characteristic. The MARGIN describes the appearance of the outer edge of the colony.

If the colony is sufficiently large, the color can be determined as well. Size, opacity, and texture can also be useful for characterizing a colony.

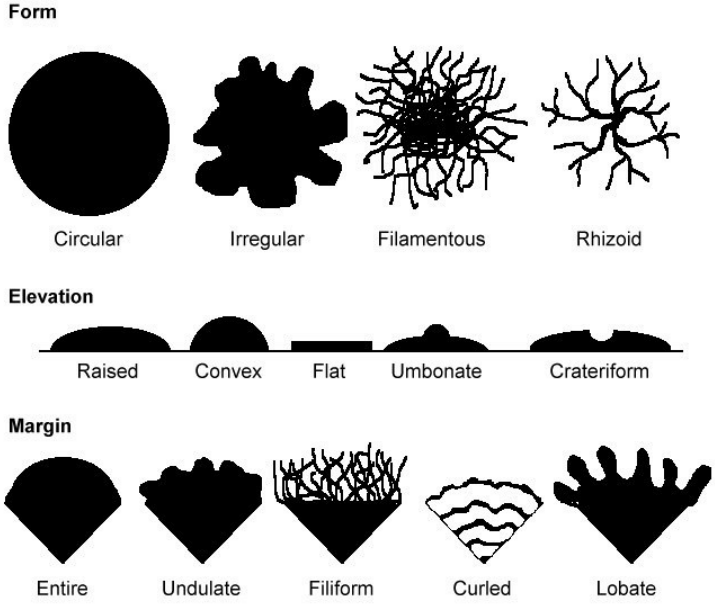

Figure 1: Examples of three characteristics of bacterial colonies are shown. (https://microbeonline.com/colony-morphology-bacteria-describe-bacterial-colonies/).

Important Guidelines for using inoculation loop:

1. Always sterilize in Bunsen burner flame before using to move bacteria
2. Allow loop to cool briefly before use.

Protocol:

Materials needed:

100mm plastic dish with LB Agar with bacteria cultured from water sample
Inoculation loop or pipette tip
5-10 mL LB broth in 15 mL conical tube
Bunsen burner

Step-by-step Instructions:

Prior to doing any lab work, please clean your station by wiping everything in your area down with Lysol wipes and ethanol.

1. Get together into large groups of 7-8 people.
2. Look around at your group members' plates to get a sense of the diversity and distribution of characteristics of the colonies generated.
3. Look at all bacteria colonies on your plate and make note of their characteristics in the table below. If you have no colonies, you may assess a lab mate's plate.
4. As a group, come up with an algorithm to choose a diverse set of colonies to pick. Each person should pick a single colony and their colony need not be from their own plate.

DO NOT TOUCH COLONIES UNTIL YOU SHOW YOUR SELECTIONS TO INSTRUCTOR OR TA FOR APPROVAL!

5. Sterilize an inoculating loop using a Bunsen burner. Once it has cooled for at least 30 seconds, scoop up your chosen colony and put it directly into the 15 mL conical tube containing LB.
6. Replace the lid and vortex the tube breaking up the colony.
7. Label the tube with a marker (not a sticker) and put it into the shaking incubator overnight.
8. Sterilize and cool the loop before putting it away for the day.

Sample Source Location:

Form	**Margin**	**Color**	**Size (*relatively* small, medium, or large)**	**Surface (wet/dry)**	**Who picked this colony?**

Before you leave, add your colonies to the class data spreadsheet.