Diversity and Abundance of Bacteria in Natural Settings

Lab Goals: The purpose of this lab is to introduce students to the diversity and abundance of bacteria in natural settings. This will involve students working in the lab as well as outdoors. Students will be required to go outside to collect both soil and water samples. Students will work in groups of four and each group will be required to obtain one water sample and one soil sample. Samples will then be brought back to lab where students will dilute both soil and water in sterile phosphate buffered saline in order to plate on nutrient agar. Plates will be grown at 20°C for 24 hrs. and then be used to quantify diversity and abundance. Students will be asked to answer questions based on the diversity and abundance observed and compare this with what they have previously been told about bacteria and terrestrial and aquatic habitats.

Lab Intro: Bacteria are some of the most diverse organism on the planet. Ecologically, bacteria plan an important role in both terrestrial and aquatic habitats. The majority of these bacteria are beneficial and are involved in degradation of biomass, recycling of nutrients, as well as catalyzing other biogeochemical cycles. As such, their abundance and diversity can be important for determining how well ecosystem function. During the course of the semester you have learned about what we know about the diversity of bacteria in natural settings. You were introduced to global estimates of bacterial abundance (Whitman et al., 1998) as well as the great plate anomaly (Schmidt, 2006). In addition, you have been introduced to numerous studies that have estimated bacterial diversity in numerous settings (Fierer et al., 2007; Newton et al., 2011). However, today we are going to do our own survey of both terrestrial and aquatic bacteria. During our survey we will use culture dependent methods to determine both the diversity and abundance of bacteria in a nearby forest and stream. At the end of this lab you will use your finding to compare with other studies and discuss the possible variations.

Part 1: Field Collections

Our first step today will be to obtain environmental samples. We will continue to work in groups of four and each group will be required to procure one soil and one water samples from our study location. Each group should have a 5 gal bucket that includes the following supplies:

1. 1 x 1 L Nalgene Bottle
2. 1 x Metal Soil Core
3. 2 x Small WhirlPak Bags
4. Assorted Gloves
5. 1 x Spray Bottle of 70% EtOH
6. 2 x Lab Spatula
7. 1 x Box of Kimwipes
8. 1 x Black Sharpie

Once you have checked your bucket for the above supplies, your instructor will lead all groups to the field sites (Dunn Woods & Jordan River). After arriving to the field site the instructor r will demonstrate how to do the needed field sampling.

Sample	Location	Description

Part 2: Sample Processing

Once you have obtained your sample and returned to the lab, it is be time to process your samples for our analyses. In order to grow the soil and water bacteria on agar media we will first need to use a series of serial dilutions in order to obtain plates with observable colonies of bacteria.

Soil Bacteria

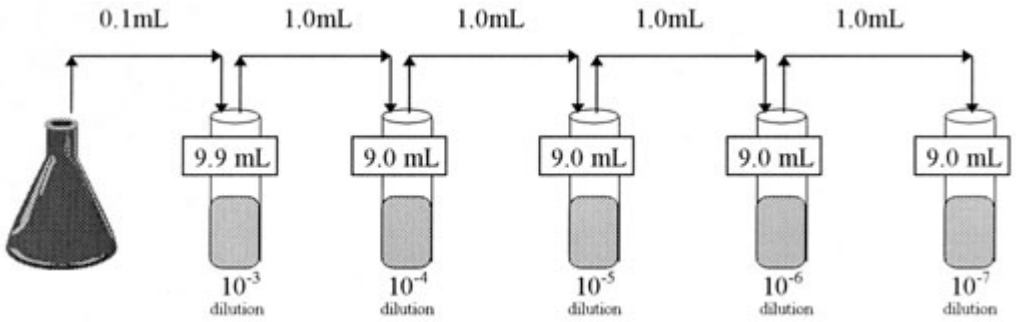
Materials Needed:

Soil Sieve
500 mL Sterile Flask with 100 mL sterile Phosphate Buffered Saline (PBS)
5 Milk Bottles each with 9.0 mL sterile PBS
1 Milk Bottle with 9.9 mL sterile PBS
Assorted Serological Pipettes
3 Nutrient Agar Plates
Glass Spreader with EtOH sterilizing container

Procedure:

1. Because soil has texture (clumps, etc.) you first need to sieve it through a 2 mm soil sieve, your instructor will demonstrate how to use the sieve.
2. Sieve your soil sample and collected sieve soil on a sterile sheet of aluminum foil
3. Using the soil scale in the front of the lab, measure 1 gram of sieved soil and add to a flask with 100 mL of sterile PBS
4. Shake the flask for 5 min in order to homogenize the soil/PBS slurry
5. Using a sterile serological pipette, transfer 0.1 mL of the slurry into a milk bottke with 9.9 mL sterile PBS
6. Tightly seal the bottle and shake for 1 min

7. Following the dilution diagram below, continue diluting your slurry sample until you achieve an 10^{-7} dilution. Make sure to use a new, sterile pipet for each transfer



8. Using a sterile 1.0 mL pipet, transfer 0.1 mL of each of the following dilutions to a nutrient agar plate: 10^{-5}, 10^{-6}, 10^{-7}. Make sure to use a fresh pipette and plate for each dilution.
9. Spread each dilution and allow the sample to soak into the plate before inverting
10. Once the plates are ready to be inverted, label, and store in the 25°C incubator for 24 hrs.
11. After 24 hrs. plates should be transferred to the 4°C fridge until analysis next week.

Predictions:

1. What density of bacteria do you expect to discover in this soil sample?

2. On a gauge of 1-10, what prediction do you have for the diversity of bacteria you will find in this sample? Why?

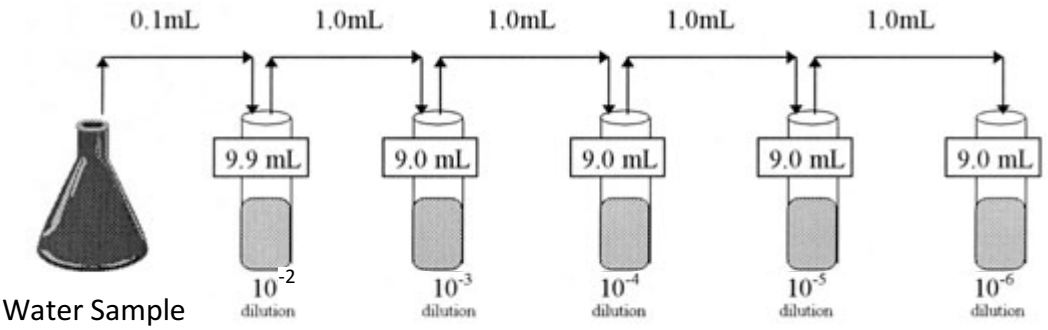
Water Bacteria

Materials Needed:

5 Milk Bottles each with 9.0 mL sterile PBS
1 Milk Bottle with 9.9 mL sterile PBS
Assorted Serological Pipettes
3 Nutrient Agar Plates
Glass Spreader with EtOH sterilizing container

Procedure:

1. Because water samples are already homogenized there is need to process the sample prior to dilution.
2. Similar to above, dilute the water samples using the following dilution scheme:



3. Using a sterile 1.0 mL pipet, transfer 0.1 mL of each of the following dilutions to a nutrient agar plate: 10^{-4}, 10^{-5}, 10^{-6}. Make sure to use a fresh pipette and plate for each dilution.
4. Spread each dilution and allow the sample to soak into the plate before inverting
5. Once the plates are ready to be inverted, label, and store in the 25°C incubator for 24 hrs.
6. After 24 hrs. plates should be transferred to the 4°C fridge until analysis next week.

Predictions:

1. What density of bacteria do you expect to discover in this water sample?

2. On a gauge of 1-10, what prediction do you have for the diversity of bacteria you will find in this sample? Why?

3. Which sample do you expect to be more diverse? Which do you think will contain the most bacteria? Elaborate

Part 3: Obserations

Now that our samples have had time to grow, we are able to make observations and measure diversity and density. Please retrieve your plates from the fridge. Remember, we want to count colonies on plates that have 30 – 300 colony forming units (CFUs). Please record calculations in the space give.

Abundance – Group Data:

Sample	Colonies	Plate Dilution	CFUs per g/ml in Sample

Calculations:

Abudance – Class Data (Averages):

Sample	CFUs per g/ml in Sample

Calculations:

Diversity is a bit harder to measure via growth-dependent methods. Here, we will just use a morphotype designation to measure diversity. Look at the bacteria on each of your plates.

What features distinguish these organisms?

Using these features, count the number of different 'morphotypes' on your plates. Describe each morphotype in your group the morphotype table below

Diversity – Group Data:

Sample	# of Different 'Morphotypes'

Diveristy – Class Data (Averages):

Sample	# of Different 'Morphotypes'

Morphotype Table

Morphotype 'Name'	Color & Shape	Other Features

Part 4: Discussion

1. For the soil samples, how did the observed data (class averages) compare with your predictions? Was the abundance higher or lower? What about diversity?

2. For the water samples, how did the observed data (class averages) compare with your predictions? Was the abundance higher or lower? What about diversity?

3. Why do you think your predictions differed from your observed data?

4. How might you obtain a better estimate of bacterial abundance in natural environments?

5. What about a better estimate of bacterial diversity?

6. If you were to guess, which habitat would contain a more diverse community of bacteria: soil or water? Why? (Use concepts and terms from your community lecture)