Lab Exercise 3: Microbial Ubiquity

Background

The term **microbe** includes a wide range of organisms, from single cell **prokaryotes** (**Bacteria** and **Archaea**) to single and multi-celled eukaryotes (**fungi**, **algae**, **protozoa** and **helminths**). The term even includes non-living **viruses** and **prions**. As the name of this lab suggests, bacteria are **ubiquitous**, they are everywhere. They are in the air you breathe, the food and water you consume and they play an integral part in both causing sickness and maintaining good health. Bacteria are much more than the sum of the diseases they can cause, however. In fact, less than 1% of bacteria are classified as human pathogens. Many bacteria perform tasks in the environment that are absolutely essential to our survival and the continued existence of the earth as we know it. They play essential and sometimes exclusive roles in the **biogeochemical cycling of nutrients** like carbon, nitrogen, sulfur and many other inorganic substances into forms usable by higher organisms. Bacteria are also incredibly metabolically diverse- **bioremediating** not only waste materials, but in some cases substances that are dangerous. There are even bacteria out there that can metabolize 2,4,6-trinitrotoluene (TNT) and arsenic! Bacteria are some of the most widely distributed organisms in the biosphere. Based on their diverse metabolic needs and abilities, they are capable of thriving in a huge range of **ecological niches**: deep sea hydrothermal vents, soil and water, the surface of metal pipes and rocks, polar snow caps and the human body.

Many bacteria are part of your **normal flora**, those organisms that have been colonizing you since the moment of your birth and help you to remain both healthy and disease-free. Your skin houses many of these organisms, as well as potential pathogens, and it is important for you to be aware of the amount of microscopic life you are supporting. It is also important to be aware of the best way to make sure none of those organisms contaminate your experiments or hitch a ride home with you: wash your hands before and after beginning any lab work, and wash them well.

Introduction

In this laboratory exercise, sterile liquid and solid media will be exposed to a variety of habitats, looking to see differences in the variety and abundance of the resulting microbial growth. A variety of media will be used, including both liquid (**broth**) and solid (**agar**) **media**. Two types of **complex media** will be used that are used frequently in this course: **blood agar** and a complex **general nutrient agar**. These media are considered useful for culturing many species of bacteria and are complex because their exact chemical constituency is undefined (unknown).

Objectives

1. Properly expose sterile media to various microbial environments.
2. Properly label and incubate media.
3. Observe the diversity of bacteria and fungi in the environment.

Broth Inoculation Protocol

Individual Supplies
1 tube general nutrient broth
1 sterile cotton swab

1. Prepare the lab bench by removing any extraneous items and cleaning the surface with table disinfectant.
2. Label a tube of general nutrient broth with an *Inoculation Label* and rubber band. DO NOT TILT THE TUBE.
3. If your student laboratory number is even, rub a sterile cotton swab over some part of your body.
   If your student number is odd, rub a sterile cotton swab over some inanimate object.
4. Place the swab into the tube of nutrient broth and cap the tube.
5. Incubate the test tube in the appropriate rack in the 37°C incubator.

Plate Inoculation Protocol

Individual Supplies
1 general nutrient agar plate OR 1 blood agar (BA) plate

1. Prepare the lab bench by removing any extraneous items and cleaning the surface with table disinfectant.
2. Label the plate properly on the outer edge of the bottom surface. **Make sure to note the type of agar used.**
3. Expose the plate according to your student lab number and the assignment table below.

Exposure Method for Agar Plate	**Student #**	**Exposure Method for Agar Plate**	**Student #**
To the air in the laboratory for 30 min.	1, 9, 17, 25, 33	Moist lips pressed against medium	5, 13, 21, 29
Bathroom sample (doorknob, toilet seat, etc.)	2, 10, 18, 26,	Coins or cell phone pressed against medium	6, 14, 22, 30
Fingertips pressed lightly on medium	3, 11, 19, 27	Hair combed over medium (10 strokes)	7, 15, 23, 31
Blow dust onto exposed medium	4, 12, 20, 28	Vigorous cough onto blood agar	8, 16, 24, 32

4. Incubate the plate agar side p in the 37°C incubator.

Data Collection & Analysis

1. Examine the plate. Record the amount of growth as follows:
   - 0 no growth
   - \+ 1 to 10 colonies
   - ++ 11-50 colonies
   - +++ 51- 99 colonies
   - ++++ ≥ 100 colonies
2. Record any observations on the relative concentration of bacterial vs. fungal/mold growth.
3. Observe growth in the nutrient broth tube. If it is not immediately apparent, flick the bottom of the tube to disperse cells that might have settled. Save this tube for use in the Simple Staining Protocol.
4. Record all relevant data to the course website. Once these data have been collected, make sure to keep a copy of the results in your *Laboratory Notebook*.

Discussion

1. Using the number of colonies as an indicator, which habitat sampled by the class appears to have the most abundant microbial communities?
2. Were any habitats completely lacking in microbes?
3. In what ways do the macroscopic features of bacterial colonies differ from fungi/molds?
4. Why is the level of contamination measured as number of colonies rather than the size of colonies?
5. Should one be concerned to find bacteria on skin? How about fungi/molds molds?