Exercise 1: Bacteria

Part 1. Bacterial Diversity

Goals

- Recognize prokaryotes by their lack of intracellular structure.
- Identify different bacteria by their forms.
- Differentiate cyanobacteria from other bacteria.
- Describe the ecological and evolutionary importance of cyanobacteria.
- Describe the medical importance of bacteria.

The one time "Moneran" kingdom has recently been divided into two domains, the Archaea and Bacteria. Both domains are prokaryotic (no nuclear membrane). This lab focuses on Bacteria.

Domain Bacteria ("true bacteria")

Characteristics:

- Prokaryotic unicellular organisms
- Cell walls contain peptidoglycan
- Membrane lipids contain straight-chain fatty acids

Examples:

- Aerobic, endospore forming – Bacillus
- Aerobic - Streptococcus, Staphylococcus
- Anaerobic, endospore forming - Clostridium
- Anaerobic, phototrophic - Rhodospirillum rubrum
- Facultatively aerobic, heterotrophic - Escherichia coli,
- Phototrophic blue-green algae (cyanobacteria) - Oscillatoria, Anabaena

Human Bacteria

The average human has over 1 kg (over 2 lbs) of bacteria in and on their body. In fact, without bacteria we would not be able to survive. We are not able to independently break down all the foods that we ingest or synthesize all of the vitamins we need to survive. Bacteria help us to do this by breaking foodstuffs down into compounds that we can absorb. This symbiotic relationship is very important to our survival, and often becomes disturbed when we take broad-spectrum antibiotics.

There are several types of bacteria that live in our mouths. Some are good and necessary, others are not good and often result in infection. One example, "strep throat", is caused by a Streptococcus strain, a long chain (strep) of round (coccus) bacteria. Bad breath may be a result of active bacteria breaking down foodstuffs in our mouths.

A different Streptococcus, S. mutans, causes tooth decay. S. mutans is facultatively anaerobic, and forms a sticky capsule from sucrose (the sugar most often found in foods), which it uses to stick to teeth. Brushing your teeth mechanically removes this sticky material, called plaque. If the plaque is not removed, the bacterial waste product, lactic acid, destroys tooth enamel. Commercial mouthwashes and toothpastes are often advertised as having antibacterial activity. They kill the bacteria in the mouth and thus prevent plaque and tooth decay from occurring.
Bacterial Forms
Bacteria are typically not as structurally complex as eukaryotes. They usually conform to three basic cell shapes, which we can use to identify them. Many bacteria also have associated flagella, cilia, and reproductive structures (conjugation tubes). Others form colonies consisting of chains or clusters.

Figure 1. Bacterial forms.

View the prepared slides of medically important bacteria. For each, draw the bacteria, illustrating shape and association with other bacteria. Identify the proper bacterial form.

1. *Diplococcus pneumoniae*

What diseases do you think this bacterium causes? _________________________________

Based on its name, what do you predict the shape and arrangement of these cells will be?

Form: _________________________  Make a sketch:

2. *Borelia burgdorferi*

This bacterium causes Lyme disease. Lyme disease was named in 1977 when arthritis was observed in children near Lyme, CT. *Borelia burgdorferi* is transmitted to humans by the bite of infected deer ticks. More than 23,000 infections were reported in the United States in 2002 and continue to rise with the deer population.

Form: _________________________  Make a sketch:
3. *Clostridium botulinum*

What disease do you think this organism causes? _______________________________

Form: _____________________________ Make a sketch:

The toxin produced by *Clostridium botulinum* can cause serious and often fatal paralysis from ingesting contaminated foods. For many years, this toxin has been used to paralyze muscles to alleviate unwanted muscle spasms. More recently, dermatologists and plastic surgeons have been using this toxin for the treatment of wrinkles.

**Cyanobacteria**

Cyanobacteria (formerly called blue-green algae) are photosynthetic aquatic bacteria. They are not true “algae,” because they do not have chloroplasts. They are typically unicellular but often grow in colonies large enough to see. Cyanobacteria are one of the largest and most important groups of bacteria on earth. Oxygenic photosynthesis first evolved in cyanobacteria at least 2.8 billion years ago! This innovation was responsible for changing atmospheric concentrations of oxygen and paving the way for life as we know it today. All eukaryotic chloroplasts (including those of plants) are descendents of an engulfed (but not digested) cyanobacterium. Today cyanobacteria are a natural source of nitrogen fertilizer (via atmospheric *nitrogen fixation*) in the cultivation of rice and beans. **WATCH OUT!** Some cyanobacterial blooms in lakes actually produce toxins that can kill organisms (such as dogs) that ingest them!

If cyanobacteria have no chloroplasts, how do they photosynthesize? ________________________________

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Figure 2. Cyanobacteria.
Why do we consider the bacteria in Figure 2 colonial and not multicellular? Explain.

4. *Anabaena*

Make a wet mount of *Anabaena* from the culture provided. Note the enlarged **heterocysts**. Heterocysts are specialized cells involved in nitrogen fixation (capture of atmospheric nitrogen). Enlarged ** akinetes** (elongate, tube-shaped reproductive cells) may also be present. Make a sketch and label the heterocysts:

The process of N-fixation can only occur in an anaerobic environment. How is *Anabaena*, which produces food by oxygenic photosynthesis, able to fix nitrogen?

5. *Oscillatoria*

Make a wet mount of *Oscillatoria* and make a sketch:

Do you notice anything unusual about the *Oscillatoria*? Oscillatoria colonies can actually move in a gliding fashion without flagella. They use an unknown mechanism that may involve motor proteins.

Although we just observed a few examples, cyanobacteria come in a large variety of shapes and sizes.
Part 2. Effectiveness of Antibacterial Substances

Ever wondered whether that “antibacterial” soap that you use each day is really killing bacteria on your hands? During this part of the lab, we will test the effectiveness of different types of antibacterial substances. You will generate hypotheses about which substances are most effective, and assemble and analyze data to reach conclusions about your hypotheses. The final product for this part of the lab will be a short lab report.

Goals
- Learn how to inoculate plates and test antimicrobial agents.
- Refresh your understanding of hypothesis generation and experimental testing.
- Analyze data and create scientific graphs in MSExcel.
- Interpret graphs and use them to support conclusions.
- Write figure captions and conclusions using scientific language.

Each group will conduct tests on 3 types of products:
- 1. 10% soap solutions: antibacterial vs. non-antibacterial
- 2. Household cleaners: bleach vs. ‘Febreze’
- 3. Medical disinfectants: hydrogen peroxide vs. ‘Bactine’

Hypotheses
Not every scientific investigation begins with a hypothesis, but we have designed this lab so that you can easily create some. In consultation with your group member(s), write 3 hypotheses (and rationale) for each different type of product comparison listed above. You will use these in your final lab report.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Hypothesis</th>
<th>Rationale</th>
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<tbody>
<tr>
<td>1. 10% soap solutions</td>
<td></td>
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</tr>
<tr>
<td>2. Household cleaners</td>
<td></td>
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<tr>
<td>3. Disinfectants</td>
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Of the three types of products, which do you think will be most effective?
Methods
The effectiveness of antibacterial substances (including medicinal antibiotics) is typically assessed using the disc method. In this technique, a filter paper disc is saturated in a putative antibacterial test compound. The disc is then placed on an agar plate that is completely covered with bacteria (called a bacterial "lawn"). The test compound will diffuse into the surrounding agar, poisoning the environment for bacteria (if it is truly antibacterial). After a specified period of time, the bacterial colonies will grow up in a continuous surface except where growth has been inhibited by the test compound. The circular area lacking bacterial growth around the test disc is called the "zone of inhibition." The larger the diameter of the zone of inhibition, the more effective the test compound is inferred to be.

Your group will be supplied with 3 Petri plates (1 for each type of product). You will analyze and write-up the data from your entire lab section, with each group acting as one replicate sample.

What factors will you need to control for? What other factors in the experimental setup might affect the bacterial growth other than the test substance? Brainstorm with member(s) of your group, and share with the class:

How will you control for these? List your control(s):

1.

2.

Procedure
1. Obtain a Petri dish filled with agar (bacteria food)
2. Divide your dish into 4 quadrants by drawing on the bottom (outside) with a black marker. Label each quadrant with an abbreviation for the test substance (e.g., control = C). Also label the bottom with your name in small letters along the outer edge.
3. Make a bacterial lawn on the agar by adding 250 uL of E. coli culture to your plate. Spread it evenly across the surface.
   - Note: Escherichia coli is a common intestinal bacterium that on average comprises 0.1% of the total bacteria in an adult human. The O157:H7 strain of E. coli is dangerous, but other E. coli strains keep us healthy by producing vitamins and defending us against foreign invaders.
4. Saturate each disc in the test substance, but be sure to get excess substance off the disc so that it doesn’t run all over the dish.
5. Place each disc in the center of its quadrant on the agar.
6. Your Petri dishes will be incubated in proper growth conditions until next week.
List of Terms for Exercise 1

- Prokaryote
- Domain Bacteria
- Coccus
- Bacillus
- Spirilla
- Nitrogen fixation
- Cyanobacteria
- Heterocyst
- Bacterial lawn
- Zone of inhibition
- *Escherichia coli*

Reading Scientific Articles Homework

In order to help you learn how to read scientific journal articles, we will discuss the following article in lab next week. The article is available on the course website.


Answer the following questions on a separate sheet of paper in preparation for next week’s lab. Failure to do so will result in a point penalty off of your final report for this exercise.

1. Read the abstract. State the main take-home message in one sentence.

2. What is the objective of this study?

3. List 3 types of bacteria and 3 antibiotics that the authors of the study tested.

4. What statistical test(s) did the authors use?

5. Do the data in Figure 3 support the statement that “there is no difference between manual and computerized (Aura) measurements?” Why or why not?

6. A correlation of $r = 0$ means that there is no relationship between two variables. A correlation of $r = 1.0$ means that there is a very tight relationship. What do the author’s correlation values indicate about the relationship between computerized and manual measurements?

7. How could you use the zone of inhibition assay to monitor antibiotic resistance in your hospital?

8. What did the authors conclude?