EXPERIMENT 4

Bacterial Physiology

INTRODUCTION

In order for suitable growth and division, a microorganism must be placed into a favorable environment. Bacterial growth refers to an increase in cell number rather than cell size. Bacteria replicate by means of **binary fission**, an asexual mechanism in which a cell first consumes nutrients, and then duplicates its genetic information into two daughter clone cells.

The growth of bacteria in broth culture is usually represented by a bacterial growth curve (Figure 1). The growth curve contains four different phases of growth, each with various events occurring.

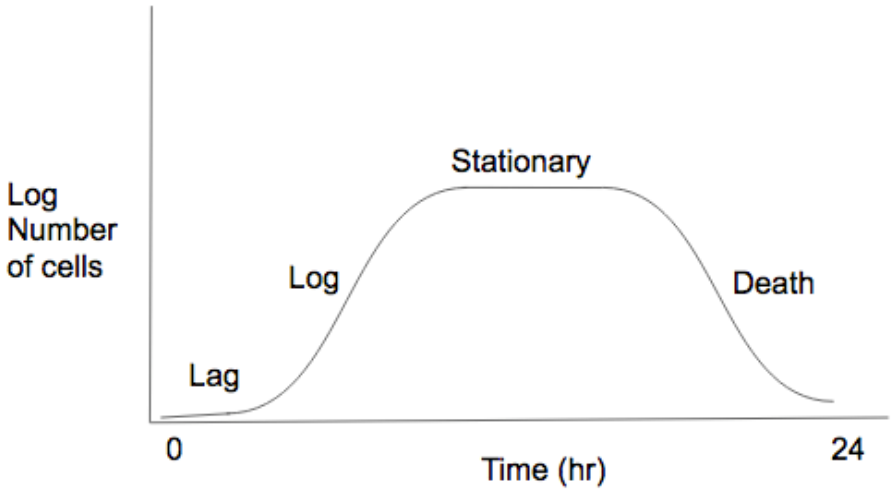

Figure 1. Bacterial growth curve.

Lag phase- in this phase, bacteria is getting acclimated to the environment it has been introduced to and begins to gather nutrients required for cell division. Bacteria increase in size and synthesize mostly enzymes and proteins at this stage.

Log phase- also known as the exponential phase, where the bacteria clone themselves at a constant rate and are metabolically active. This stage is ideal to perform experiments on bacteria.

Stationary phase- during this stage, the number of dying cells equal the number of growing cells, where there is no net increase in cell number. Here, the medium becomes nutrient limited and the bacterial metabolic products accumulate. Endospores begin to form in some genera of bacteria.

Death phase- this stage signifies a decline in cell number. The medium becomes depleted and saturated with toxic substances and results with the lysis of cells. Spore producing bacteria form mature endospores and release them into the medium.

Bacterial growth and replication depend on many physical and chemical factors from the environment. Physical factors include temperature, oxygen requirement, osmotic pressure, pH, radiation, and much more. Chemical factors include carbon and nitrogen sources, vitamins, autoinhibitory compounds, and many more.

Each bacterial species has its own preference for optimal conditions that will allow the maximum growth and usually reflects its environment. The survival and growth of microbes is a function of various activities including nutrient assimilation, catabolism, and synthesis of cellular structures performed by specific enzymes. The enzymes each function best in certain temperatures, osmotic pressure, and redox potential. In this experiment we will investigate three environmental factors: **temperature, salinity (osmotic pressure), and oxygen.**

Temperature

Depending on the optimum temperature of their growth, bacteria are divided into four groups including ***psychrophiles***, ***mesophiles***, ***thermophiles***, and extreme thermophiles or ***hyperthermophiles***. Psychrophiles grow best at temperatures below 20°C to subzero. Mesophiles grow best between 20°C and 45°C. Thermophiles grow optimally above 45°C. Hyperthermophiles grow above temperatures of 75°C. Organisms that fluctuate between 0°C and 35°C are considered psychoduric mesophiles, or psychrotrophs. Figure 2 depicts the differentiation between the bacterial "philism" towards optimal temperatures. In this lab, you will be testing various bacteria under increasing temperatures in order to determine their temperature optimum.

Figure 2. Table indicating optimum temperatures and corresponding bacterial classification.

Classification	Temperature Range (°C)
Psychrophiles	**-5 - 20**

Psychrotrophs	**-4 - 35**
Mesophiles	**15 - 40**
Thermophiles	**45 - 80**
Hyperthermophiles	**65 - 105**

Salinity

Salts in the environment, in soil, lake, ocean, and even the body can drastically affect the growth of bacteria. Salt concentration determines **osmotic pressure**, which helps regulate the water movement inside and outside the cells. Too high osmotic pressure will result in water loss and cell shrinkage. In low osmotic environments, cells will swell with incoming water and burst. A higher salt concentration results in a higher osmotic pressure against the cells of bacteria. Bacteria can be classified based on their tolerance to salt concentrations. Those that cannot withstand salt at all are considered **nonhalophiles.** Bacteria that tolerate 0.5-3% salt concentrations are considered **moderate halohiles**. Bacteria that are salt loving and can withstand high salt concentrations of 3-15% are considered **halophiles**. Organisms that do not prefer salty environments but can tolerate it are termed **haloduric**. In this lab, you will be testing various bacteria under increasing salt concentrations in order to determine their salt optimum.

Oxygen

Organisms can grow with or without oxygen (Figure 3). Bacteria can be classified based on oxygen requirements where **obligate aerobes** are those that require the presence of oxygen in order to grow. While **obligate anaerobes** are those that cannot grow in the presence of oxygen. **Facultative anaerobes** are able to grow in either the presence or absence of oxygen. Facultative anaerobe undergo respiration in the presence of oxygen and undergo fermentation in the absence of oxygen. There are also bacteria that are **aerotolerant** fermenters that do not use oxygen but tolerate its presence and undergo fermentation in both environments. Lastly, there are **microaerophiles** that are bacteria suited to only small or reduced amounts of oxygen, conditions not found in air. Microaerophiles lack the enzymes to protect them from toxic effects of oxygen. Oxygen tolerance is usually determined using agar deep tube (refer back to Appendix P.2, Figure 2c). In this lab, you will be testing various bacteria in an agar deep tube in order to determine their oxygen requirements, based on figure 3.

The oxygen requirements of a microbe can be tested by inoculating the bacteria in an agar deep tube. Anaerobic bacteria require reducing conditions to grow. If anaerobic bacteria are found within a medium with positive redox potential, they will fail to grow, as their enzymes will not function. Reducing conditions are one that contains agents such as **thioglycollate** that serves to remove the oxygen and decreases the redox potential of the agar medium. **Redox indicators** are chemical agents that when added to media can detect the presence of oxygen. Methylene blue is a redox indicator that is colorless when reduced, and blue when oxidized.

In addition to the agar deep tube, oxygen tolerance can be determined using the **Catalase test** and the **Oxidase test**. Since oxygen is a highly toxic gas cells must protect themselves from the generation of cellular toxins such as hydrogen peroxide and superoxide radicals which occurs when two electrons reduce oxygen during respiration. Catalase is an iron containing protein that binds first a molecule of hydrogen peroxide and then reacts with another to form oxygen and water, in order to remove the hydrogen peroxide. The catalase test involves taking a portion of a bacterial colony and using 3% H_2O_2 in order to observe generation of bubbles for a positive test (Figure 4).

The Oxidase test is for the presence of cytochrome C. Cytochrome C is an enzyme that is able to oxidize the oxidase test reagent. The test reagent in the reduced state is colorless, and in the oxidized state is deep blue (Figure 5).

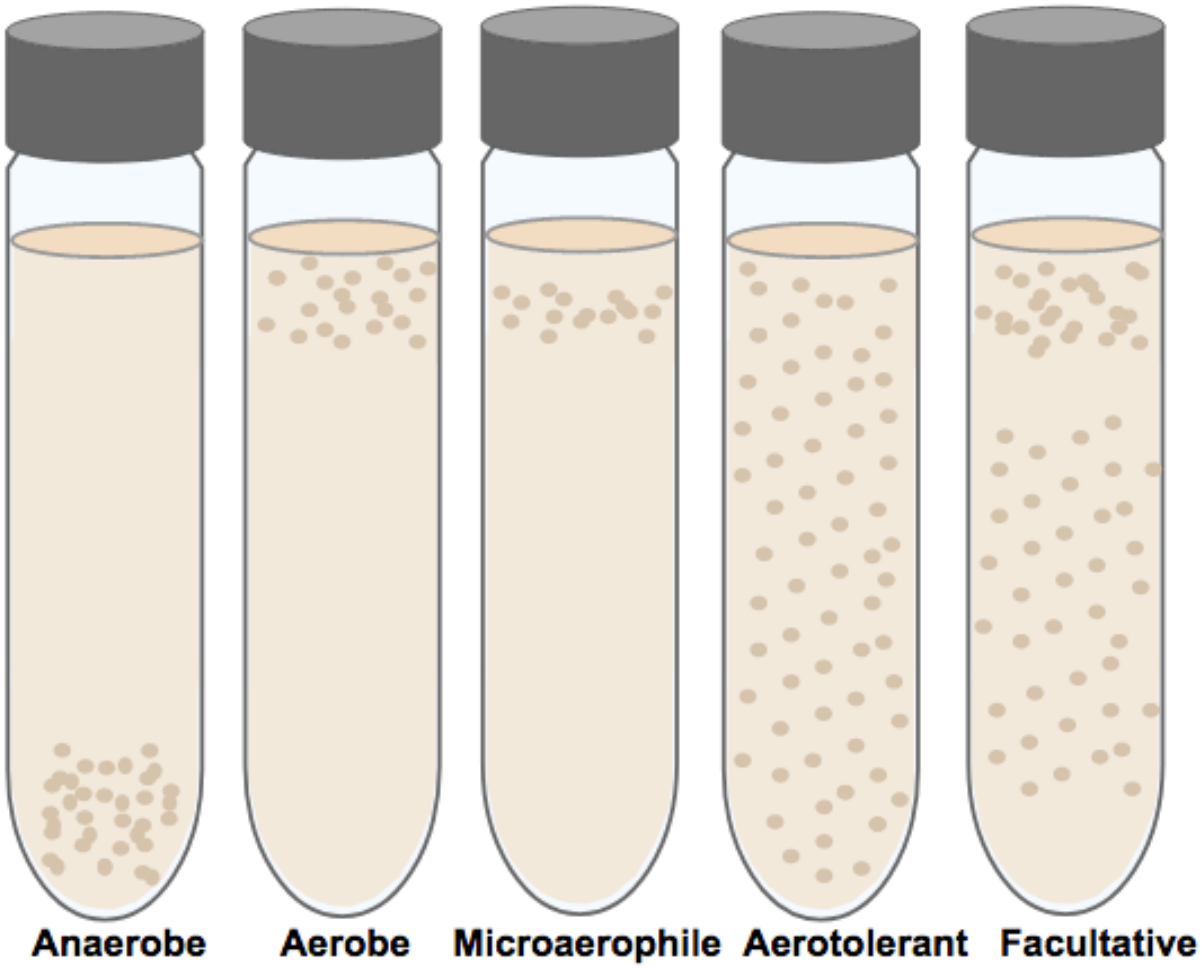

Figure 3. Oxygen requirements of the indicated microbes inoculated in agar deep.

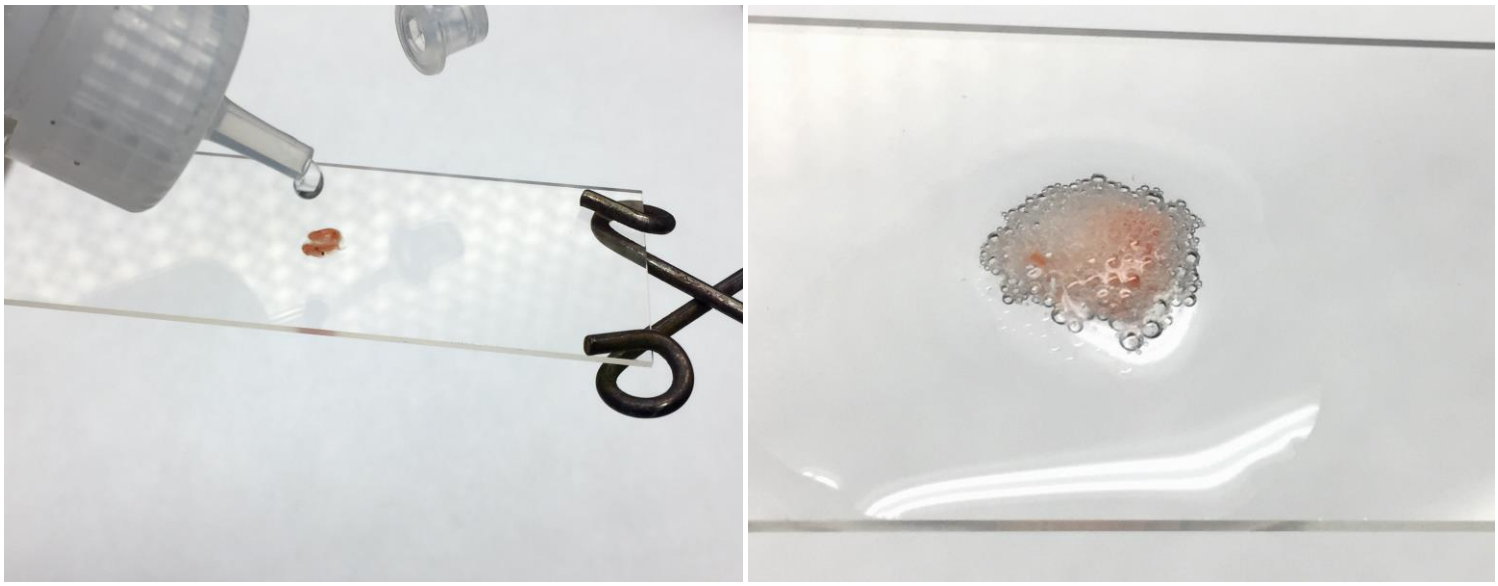

Figure 4. Performing the Catalase test by dropping H2O2 onto a smear and observing for generation of bubbles (oxygen).

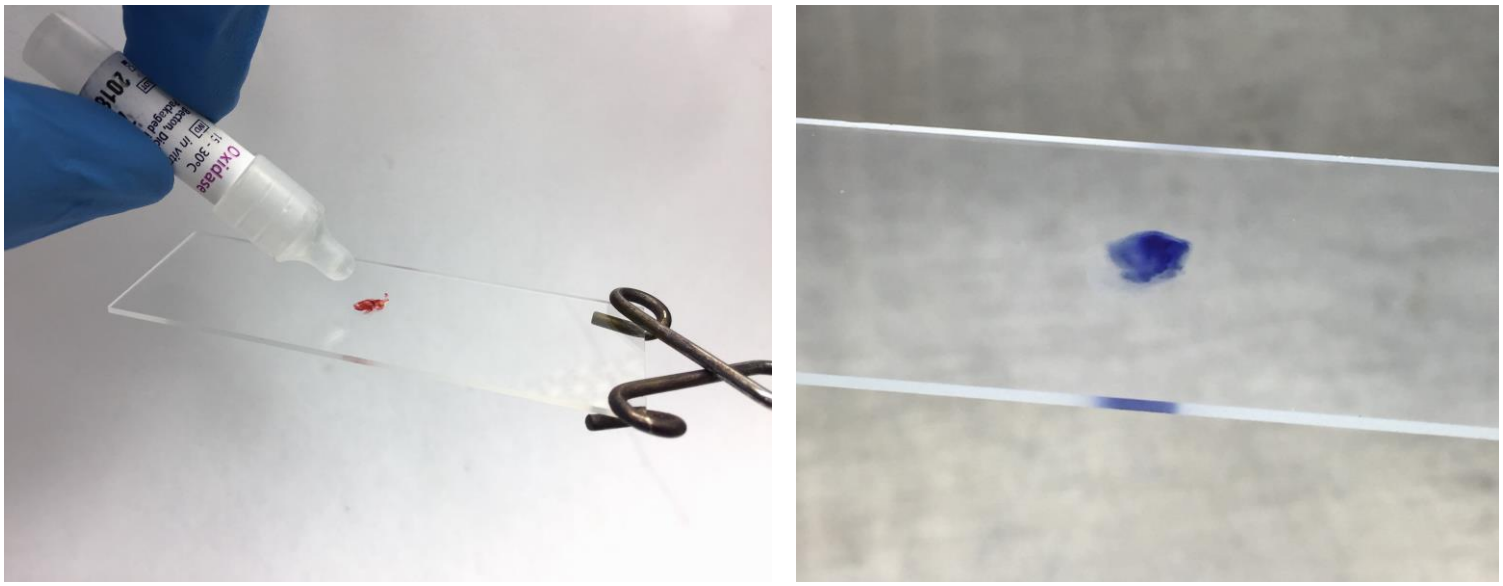

Figure 5. Performing the Oxidase test with the reagent by adding one drop and observing for generation of a blue/violet color within seconds.

Materials:

1. **5 BHI slants per groups**
2. **1 Thioglycollate agar deep tube molten at 45°C**
3. **1 Nutrient Agar deep tube with Methylene blue, molten at 45**
4. **5 BHI broth tubes with NaCl concentrations of 0, 3, 7, 15, 25%.**
5. **Catalase reagent (3% H_2O_2)**
6. **Oxidase Reagent**
7. **Culture broth tube of known bacteria**

Procedures Week 1:

1. Label your notebook with the number of your known bacteria.

2. **Label ALL** agar tubes and broth tubes to be inoculated with the contents of the tube, specimen number, and proper incubation temperatures.

3. Inoculate 5 BHI slants with your bacteria. Incubate each at 4°C, 30°C, 37°C, 45°C, and 55°C.

4. Inoculate 5 BHI broth tubes containing 0%, 3%, 7%, 15%, and 25% NaCl. Incubate these tubes at the temperature indicated by your TA.

5. The molten thioglycollate tube and Nutrient agar deep tubes are inoculated in stepwise addition of drops, from the bottom of the tube to the top of the agar surface. Allow solidifying and incubating at the temperature indicated by your TA.

Procedures Week 2:

1. Once enough growth has been established, observe and identify the BHI slants and broth tubes for growth.

2. Identify the amount of growth by placing a +++, ++, +, or – in your worksheet depending on the amount of growth that occurred in the tubes.

3. Refer to Appendix Figure 4 to identify the types of growth found in the BHI broth tubes.

4. Aseptically remove some bacterial growth from the tube with the best growth and place onto two slides.

5. Perform the oxidase test by placing a drop of the reagent onto the first slide. Observe for one minute or less for the cells to turn blue, no color change means a negative result for presence of Cytochrome C oxidase enzyme.

6. Perform the catalase by placing a drop or two of the catalase reagent (hydrogen peroxide) directly onto the second slide and observe for generation of bubbles.

Bacteria ID_____

Part I. Temperature Optimum (1 point)
Directions: Place a +++, ++, +, or -, depending on the amount of growth that occurred in the tubes.

Temperature	Growth
4°C	
30°C	
37°C	
45°C	
55°C	

Part II. Salinity Optimum (1 point)
Directions: Place a +++, ++, +, or -, depending on the amount of growth that occurred in the broth tubes. Indicate the type of growth pattern observed in each tube. Refer to practice section P.2, Figure 4 for growth patterns.

Salinity %	Growth (+/-)	Pattern
4°C		
30°C		
37°C		
45°C		
55°C		

Part III Oxygen Optimum (1 point)

Directions: Place a +++, ++, +, -, depending on the amount of growth/reaction that occurred in the agar deep tubes. Indicate any development of growth and color.

	Thioglycollate Agar	Nutrient Agar
Top		
Middle		
Bottom		

Part IV Catalase and Oxidase Tests (2 points)

Directions: Record your results for catalase and oxidase tests.

		Reaction (Positive or Negative)
Catalase Test	**Bubbles:**	
Oxidase Test	**Color:**	

Part V Bacteria Classification (2 points)

Directions: Classify the organism tested based on it's environmental optimums using the correct terms.

Environmental Factor	Optimum	Classification
Temperature °C		
Salinity (%NaCl)		
Oxygen		

Part VI Questions (3 points)

Directions: Answer the following question using full sentences. Refer back to your results.

1. Why would anaerobes be catalase negative?

2. Is it possible to observe an aerobe with a negative oxidase test?

3. Based on the results obtained from the oxygen requirements of your microbe, explain its relationship with oxygen (facultative, obligate, etc.) and the characteristics that led you to that conclusion (Catalase, Oxidase, Agar deep tubes).

4. What is the function of the Sodium Thioglycollate in the deep agar medium?

5. What is the role of the Methylene blue dye in the Nutrient agar deep tube?