Requirements for Bacterial Growth:
Temperature, pH and Atmospheric Oxygen

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Introduction
Talking about bacterial growth, scientists refer to the increase in the number of cells, not the size of cells. Starting with an isolated cell, growing bacteria accumulate into colonies and populations of billions of cells in a very short time by the process of binary fission.

By understanding the conditions necessary for microbial growth, scientists can determine how to control the growth of microbes that cause disease and food spoilage; on the other hand, this information can be applied with respect to encouraging the growth of "helpful" microbes. The requirements for microbial growth can be divided into two distinct categories: physical and chemical. Physical requirements are: temperature, pH, salt, and osmotic pressure. Chemical requirements are: sources of carbon, hydrogen, nitrogen, oxygen and phosphorus (CHNOPS,) mineral elements (cofactors,) organic growth factors (vitamins as organic cofactors,) and energy.

The objective of this experiment is to determine how various changes in chemical and physical factors might affect the growth of five different bacteria, and based on the results classify bacteria with respect to temperature, pH and oxygen requirements.

Materials and Methods
The materials used in this experiment were: inoculating loops, tube racks, Bunsen burners, paper towels and soap, bleach bottles, waterbath at 55° Celsius, sterile Pasteur pipettes and bulbs, tryptic soy broth (pH3, 5, 7 and 9,) nutrient agar slant, nutrient agar plate, icebath, 500 ml beakers, heating block,and bacterial cultures including: *E. coli, Staphylcoccus aureus, Clostridium sporogenes, S. marcescens, B. stearothermophilus, Bacillus subtilis* and *Pseudomonas aeruginosa.*

For the temperature experiment, bacterial cultures (*S. marcescens, E. coli, B. stearothermophilus, B. subtilis, and S. aureus*) were transferred to nutrient agar slants using streaking technique, flaming the inoculating loopbefore and after every inoculation. Each inoculated slant was put into the incubator/ refrigerator at specific temperature (4°C, 25°C, 37°C and 60°C) for 3 days.

For the pH experiment, Tryptic soy broth with the assigned pH (pH 3, 5, 7 and 9) was inoculated with the same bacteria as in temperature experiment via liquid-to- liquid transfer (the loop was flamed before and after each inoculation). Inoculated broth tubes were placed into the incubator at 25°C for three days.

For the atmospheric oxygen experiment, each group was given a nutrient agar plate and 3 bacteria cultures: *S. aureus, Clostridium sporogenes, and Pseudomonas aeruginosa*. The nutrient agar plate is divided into 3 sections and marked whether the environment is oxygenated or not. Every section was inoculated with different bacteria via streaking technique (the loop was flamed before and after each inoculation.) The plates were placed into oxygenated and non-oxygenated environments for 3 days at 25°C. Non-oxygenated environment was achieved by placing the plates with Gaspack in an airtight container. After three days, the results of all three experiments were observed and recorded.

Results and Discussion

In the temperature experiment, four bacteria (*S. marcescens, E. coli, B. subtilis, Staph. aureus*) exhibited growth at 25°C and 37°C, and none of them grew at 4°C or 60°C. Based on these findings, we can conclude that these bacteria can be classified as moderate-temperature-loving mesophiles. Their enzymes responsible for catalysis of biochemical reactions work best in the 15°C - 45°C temperature range. The enzymes cease their functionality if the temperature of the environment is higher than 45°C. Moreover, high temperature destroys the lipids that make up biological membranes. If the temperature is lower than 15°C, the growth of cells is slowed by the reduction of the rate of chemical reactions and, also, by the reduction of fluidity of the cell membrane. In this case, the rate at which nutrients can cross into the cell is reduced as well. High temperatures can be microbicidal as they tend to cause irreversible and changes in the cell (lethal effect;) low temperatures on the other hand, are more often microbistatic, with growth rates returning to normal as temperature rises. For these bacteria, optimal growth temperature is 37°C, which does not always coincide with the optimal temperature for enzymatic activity. In the case of *S. marcescens,* optimal growth temperature is 37°C, but optimal temperature for enzymatic activity (not for all enzymes) is 25°C, because only at this temperature the orange-red prodigiosin pigment is produced. The name "prodigiosin" is derived from "prodigious" (*i.e.* something marvelous) (1, 2.) Prodigiosin is a secondary metabolite of *Serratia marcescens* (1). Because it is easy to detect, it has been used as a model system to study secondary metabolism (3). Prodigiosin production has long been known to be enhanced by phosphate limitation (2). In low phosphate conditions, pigmented strains have been shown to grow to a higher density than unpigmented strains. The pigment has no defined role in the physiology of producing strains, but has been reported to have antifungal, antibacterial, algicidal, antiprotozoal/antimalarial, immunosuppressive and anticancer activities (3). Although *B. stearothermophilus*

showed similar results as the other four bacteria with respect to positive growth at 25°C and 37°C, and absence of growth at 4°C, it also grew at 60°C (which was too high for the other four.) Based on this data *B. stearothermophilus* can be classified as a heat-loving thermophile with optimal enzymatic activity at 40°C-80°C temperature range.

In the pH experiment, none of the bacteria could be classified as acidophilic because at pH3 the growth was not observed. However, all four bacteria grew at pH5 and pH7 and only *S. marcescens, E. coli,* and *B. stearothermophilus* grew at pH9. Analyzing these results, we can conclude that *B. subtilis, Staph. aureus* are neutrophils, growing best at pH7 and not exhibiting any growth in the alkaline (pH9) or acidic (pH3) environments. *S. marcescens, E. coli,* and *B. stearothermophilus* all grew in a wide range of pH (pH5, 7, and 9) (4). However, this does not mean that all three bacteria can be classified as basophiles. Bacterial growth is considered normal in different pH due to the nature of the pH curve. Therefore, the determinant factor for classification is the optimal pH. *E. coli* can grow at a pH range of 4.4-10, however, the optimal pH is 6-7; therefore, it can be classified as neutrophil. *B. stearothermophilus* can grow at a pH range of 4-11, however, the optimal pH is 6.2-7.5; therefore, it can be classified as neutrophil. *S. marcescens* can grow at a pH range of 5-9, however, the optimal pH is 9; therefore, it can be classified a basophil.

In the atmospheric oxygen experiment, *Clostridium sporogenes* can be classified as an obligate anaerobe that requires complete absence of oxygen and uses molecules other than oxygen as the final electron acceptor. *Staph. aureus* is a facultative anaerobe because it can grow in presence or absence of oxygen. It is able to use nitrates or sulfates as final electron acceptors, or go through fermentation when oxygen is not present. *Pseudomonas aeruginosa* is an obligate aerobe, which requires the presence of oxygen for growth and uses oxygen as a final electron acceptor.

The growth of bacteria can be controlled in a number of ways including refrigerating, cooking, storing food into airtight containers, and pickling. All three experiments present limitations such as the risk on contamination, and not starting with the same number of cells in each experiment, agar media melting at higher temperatures, evaporation, and freshness of bacterial samples. However, the obtained data is very useful in controlling bacterial growth, providing alternative ways to identify bacteria, and contributing to establishment of bacterial charts for bacterial classification.

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