Major Project Marcus Stevens December 25, 2015

*The Effects of Phosphate on the Bacterial Growth of E. Coli & Staph*

1. Introduction

Escherichia coli (*E. coli*) bacteria is the most well-understood bacterium in the world, and is one of the most vital models of an organism in many fields of research, most commonly molecular biology, genetics, and biochemistry. The bacterium was originally discovered in 1885 by the German bacteriologist Dr. Theodor Escherich. Initially, the bacterium was termed *Bacterium coli,* however, it was later named to honor Dr. Escherich. It is extremely easy to grow under optimal conditions, and research strains are very safe to work with. As with many species of bacteria, *E. coli* grows quickly, which allows for many generations to be studied in a short period of time. In fact, under ideal conditions, *E. coli* cells have the potential to double in number after only 20 minutes. (medicine.jrank.org)

Furthermore, large quantities of *E. coli* bacteria can be grown in a relatively small space. These are important characteristics when considering the conditions in a genetic experiment, which often involve selecting a single bacterial cell from among millions of candidates, then allowing it to reproduce into high numbers again to perform additional controlled experiments. Moreover, the organism can be used to study [bacterial growth](http://science.jrank.org/pages/2571/Escherichia-coli.html) in the presence of [oxygen](http://science.jrank.org/pages/4970/Oxygen.html) ([aerobic](http://science.jrank.org/pages/93/Aerobic.html) growth) and in the absence of oxygen ([anaerobic](http://science.jrank.org/pages/323/Anaerobic.html) growth). The ability of *E. coli* to grow aerobically and anaerobically classifies the bacterium as a facultative anaerobe. (medicine.jrank.org)

*E. coli* was involved in many vital techniques that were initially developed in bacterium, such as molecular cloning and overexpression of cloned genes. Revolutionary experiments that brought the details of fundamental biological processes to light such as DNA replication, [transcription](http://medicine.jrank.org/pages/2878/Transcription.html), and [translation](http://medicine.jrank.org/pages/2906/Translation.html), were performed to their full potential with *E. coli*. The huge amount of structural, biochemical, genetic, and behavioral information extracted from *E. coli* has made it one of the greatest the greatest bacterial model systems for scientific studies. The bacterium is still as prevalent as it has ever been in almost all modern day laboratories. Hospital [laboratory scientists](http://science.jrank.org/pages/2571/Escherichia-coli.html) are concerned with *E. coli,* since the bacterium is the primary cause of human urinary tract infections, as well as [pneumonia](http://science.jrank.org/pages/5361/Pneumonia.html), and traveler's diarrhea. Even research efforts that focus on other organisms, including humans or crop plants, use *E. coli* extensively as a tool to facilitate cloning and DNA sequencing. (medicine.jrank.org)

*E. coli* normally live in the intestines of people and animals. It constitutes approximately 0.1% of the total bacteria in the adult intestinal tract. The majority of E. coli is harmless, and is essential to many organisms because of the multitude of functions, such as the digestion of food, and the production of [vitamin](http://science.jrank.org/pages/7244/Vitamin.html) K and B—complex vitamins, that it provides in the human intestinal tract.

As well as providing for efficient intestinal function, E. coli is also extremely pathogenic, meaning they can potentially cause illness, such as diarrhea or sickness outside of the intestinal tract. These types of E. coli are transmitted through contaminated food, water, or through contact with various organisms. E. coli consists of a diverse group of bacteria.

Staphylococcus epidermidis is a gram-positive, coagulase-negative cocci that is a part of the human body’s normal flora. Consequently, it is a true “opportunistic” pathogen, as it requires a susceptible breach in the host’s defenses in its immune system. It is one of the leading pathogens of nosocomial infections, particularly associated with foreign body infections. Those most susceptible to infection are intravenous drug users, newborns, elderly, and those using catheters or other artificial appliances. (uconn.edu)

The organism produces a glycocalyx "slime" that acts as a glue adhering it to plastic and cells, and also causes resistance to phagocytosis and some antibiotics. The S. epidermidis family contributes to approximately 65-90% of all staphylococci recovered from human aerobic flora according to uconn.edu. It is approximately 0.5 to 1.5 micrometers in diameter. While S. epidermidis is a facultative anaerobe (an [organism](https://en.wikipedia.org/wiki/Organism) that makes [ATP](https://en.wikipedia.org/wiki/Adenosine_triphosphate) by [aerobic respiration](https://en.wikipedia.org/wiki/Aerobic_respiration) if [oxygen](https://en.wikipedia.org/wiki/Oxygen) is present, but is capable of switching to [fermentation](https://en.wikipedia.org/wiki/Fermentation_%28biochemistry%29) or [anaerobic respiration](https://en.wikipedia.org/wiki/Anaerobic_respiration) if oxygen is absent), it grows best in aerobic conditions. The hosts for the organism are humans and other warm-blooded animals as well.

The organism produces glycocalyx slime layers, which forms a hydrophobic biofilm. This film is adhesive to hydrophobic biopolymers of prosthetics, creating diseases such as endocarditis. The biofilm of S. epidermidis consists of clusters of cells that are embedded in extracellular slime substance that is up to 160 micrometers thick, exceeding 50 cells. (uconn.edu)

While there is much research regarding S. epidermidis’ virulence factor, little has been done to understand its mode of action. Infections are associated with intravascular devices such as prosthetic heart valves, shunts, etc., but also commonly occur in prosthetic joints, catheters, and large wounds. There has been attempts to treat S. epidermidis, however, it has developed resistance to many common antibiotics such as methicillin, novobiocin, clindamycin, and benzyl penicillin every time. Subsequently, antibiotics like vancomycin & rifampin are now used to treat these infections.

A significant study of neonatal infections was conducted in Naples between January 1996 and December 1998. Results indicated that out of 184 infections, 56 were directly attributed to S. epidermidis (approximately 30.4%). Of these, S. epidermidis was the primary causative pathogen leading to bloodstream infections (approximately 39.8%), surface infections (29.8%), and meningitis (58.3%). Percentages indicate that there was a significant number of infections caused by S. epidermidis out of total infections of that type. (Villari, et al. 2000)

The Gram stain test, developed in the 1800s by Hans Christian Gram, is a method for classifying different types of bacteria using a chemical stain and viewing through a microscope the results on the bacteria’s protective cell wall. Most bacteria are classified into two groups—Gram-positive or Gram-negative—depending on whether they retain a specific stain color.  Gram-positive bacteria retain a purple-colored stain, while Gram-negative bacteria appear pinkish or red. (nih.gov) In Gram Positive bacteria, there is a thick, multilayered *Peptidoglycan layer* and a periplasmic space. This thick wall ensures that the stain does not leak from the cell, causing the cell to appear purple. However, Gram Positive bacteria cells are characterized by an extremely thin *Peptidoglycan layer* anda second permeable phospholipid bilayer. Therefore, the purple stain that the Gram Positive bacterium retained is not present because of the thin peptidoglycan wall.

Gram-negative bacteria can cause many types of infections and are spread to humans in a variety of ways. Several species, including Escherichia coli, are common causes of food–borne disease. (nih.goav)

*Staphylococcus aureus* is a gram-positive bacteria, which means that the cell wall of this bacteria consists of a very thick peptidoglycan layer. They form spherical colonies in clusters in 2 planes and have no flagella. (kenyon.edu)

Currently, most research on this bacterium involves the proteomics of Staph. Staph’s resistance to antibiotics has become an increasing problem for today’s society and more research is needed to find our next “super drug”. There is extensive research focusing on the stress and starvation proteins to predict the physiological state of a cell population so that we may better understand and find another tactic to combat this bacterium. (nih.org)

Phosphates, like nitrates, are extremely vital nutrients for algae growth. Perhaps one of the most important energy components in a cell, Andesine Triphosphate (ATP), is partially made up of phosphate. ATP is utilized by all organisms because of its ability to generate energy, including bacteria. Therefore, phosphate is essential to all organisms. This especially is true when talking about bacterial growth.

Phosphates occur in minute quantities in all aquatic environments, and for the most part, are necessary for life to be sustained and to thrive. However, phosphates are detrimental to aquatic life when it occurs in abundance. Phosphates, in the form of fertilizers, are essential to agriculture. But these harmful fertilizers seep through land water and flow into streams, creeks, ponds, lakes and other larger bodies of water. When the fertilizers are exposed to these bodies of water, the phosphates and other dissolved minerals are enriched in the ecosystem. This is the process of eutrophication.

With an excess amount of phosphates in a system, an algal bloom could occur. Algal blooms are rapid increases in algae populations, in most cases causing the detrimental effects Harmful algal blooms have the potential to affect humans as well, making some seafood, like shellfish, unsafe by releasing toxins that cause fatal illnesses, even contaminating our drinking water. Since phosphates are nutrients, they may have the same effect on bacteria, causing them to grow exponentially.

In this experiment, the effects of different amounts of phosphates on two types of bacterial populations will be tested. The species of bacteria that will be tested are Escherichia coli (*E. coli*) and Staphylococcus epidermis (*Staph*). *E. coli* bacteria is the most well understood bacterium in the world, and is one of the most vital models of an organism in many fields of research. *Staph* is a [gram-positive](https://en.wikipedia.org/wiki/Gram-positive) [coccal](https://en.wikipedia.org/wiki/Coccus) [bacterium](https://en.wikipedia.org/wiki/Bacterium) is a true “opportunistic” pathogen, as it requires a susceptible breach in the host’s defenses in its immune system. It is one of the leading pathogens of nosocomial infections, particularly associated with foreign body infections. The mentioned bacteria were exposed to various concentrations (0%, .01%, .1%, & 1%) of a ammonium phosphate solution (NH4)3 PO4 at 10% concentration. The bacteria solutions contained either *E. coli* or *Staph* (microbe)*,* water (sterile fluid), and 10% concentration ammonium phosphate (variable). The solutions were contained in test tubes and later transferred onto agar plates for growth.

The results were studied after the plates were incubated in a controlled environment for 24 hrs. The rate of bacterial growth precipitously increased over the course of that 24 hours. Observations of the number of colonies in each of the agar plates were made and recorded.

**Purpose:** The purpose of this experiment is to study how *E. coli* and *Staph* bacteria grows with varying levels of phosphate exposure.

**Purpose:** The second purpose of this experiment is to better understand which bacteria, Gram Positive *Staph* or Gram Negative *E. coli*, are able to grow better when exposed to multiple ammonium phosphate concentrations.

**Null Hypothesis:** When varying levels of phosphate is applied to *E. coli* and *Staph*, the phosphate will not significantly affect the bacteria’s growth; nor will the phosphate affect either bacteria (Gram Positive & Gram Negative) differently.

**Alternative Hypothesis:** When four different concentrations of ammonium phosphate are applied to *E. coli* and *Staph*, there will be significant growth with both bacteria. Furthermore, the Gram Positive *Staph* bacteria will grow considerably more than the Gram Negative *E. coli* bacteria.

1. Materials & Methods (Procedure)

**Materials:** 10% concentration ammonium phosphate, *E. coli*, *Staph*, sterile water, 10 test tubes, a P200 (20-200 μl) micropipet, P1000 (200-1000 μl) micropipet, one 10ml pipet, sterile disposable plastic pipet tips, one Bunsenburner, many sterile spreader bars, 56 agar plates, one incubator, Klett \_\_\_\_\_\_\_, one sharpie marker, one vortex machine, test tube rack, alcohol (for sterilizing spreader bars).

**Procedure:**

1. 56 agar plates were obtained to grow the bacteria/phosphate solutions in.
2. Each agar plate was labeled with a sharpie marker according to its concentration and species.
3. *E. coli* and *Staph* bacteria, specially prepared to react correctly in the phosphate solution, was obtained.
4. 56 spreader bars were acquired and sterilized. This was doneby dipping the ends of them into a container of alcohol and putting them into fire for a few seconds using the Bunsen burner. This sterilizes the spreader bars, assuring the controlled spreading of bacteria/phosphate solution.
5. Each bacterium was put into its own, separate test tube, containing only that species of bacterium.
6. 8 test tubes were separated and labeled according to its bacteria and concentration of phosphate. One test tube was used to contain the sterile fluid (water). All test tubes were assorted on a rack.
7. One 10 ml pipet was used to transfer 8.9mls of sterile fluid into each of the eight test tubes used in the experiment.
8. Once the sterile water was transferred into each test tube, one p200 micro-pipet was used to transfer .1mls of *E. coli* and .1mls of *Staph* into their respective test tubes.
9. Once each test tube was filled with 9mls of bacteria/water solution, the ammonium phosphate was then added.
10. First, 0mls of phosphate were added, and 1ml of water was added to each test tube labeled 0% [ ] using the p1000 micro-pipet. Let the tubes sit for approximately 30 seconds, then vortex them.
11. Second, .01mls of phosphate (using p200 micro-pipet) and .99mls of water (using p1000 micro-pipet) were added to each test tube labeled .01% [ ]. Let the tubes sit for approximately 30 seconds, then vortex them.
12. Third, .1mls of phosphate (using p200 micro-pipet) and .9mls of water (using p1000 micro-pipet) were added to each test tube labeled .1% [ ]. Let the tubes sit for approximately 30 seconds, then vortex them.
13. Fourth, 1ml of phosphate and no water was added to each test tube labeled 1% [ ] using the p1000 micro-pipet. Let the tubes sit for approximately 30 seconds, then vortex them.
14. In total, 10mls of bacteria/ammonium phosphate solution was added to each test tube.
15. Once all of the tubes were filled, 100μl (.1mls) of each solution was added to their respective agar plates with a **p200** micro-pipet.
16. First, .01mls of the 0% [ ] solution was transferred into each of the seven 0% [ ] agar plates. As soon as the solution is transferred onto each of the plates, spread the solution using the sterile spreader bars on each of the seven plates (repeat this for each of the following solutions being transferred onto agar plates).
17. Second, .01mls of the .01% [ ] solution was transferred into each of the seven .01% [ ] agar plates.
18. Third, .01mls of the .1% [ ] solution was transferred into each of the seven .1% [ ] agar plates.
19. Fourth, .01mls of the 1% [ ] solution was transferred into each of the seven 1% [ ] agar plates.
20. When all of the solutions were equally spread, the agar plates were ready to be put into the incubator for 24 hours.
21. Once the plates were done, the colonies of the bacteria were counted and recorded.
22. The data was put through the ANOVA program to calculate if the phosphate made a significant impact on the bacterial growth of *E. coli* and *Staph.*
23. Once the ANOVA data was analyzed the, the data will be put through the Dunnet’s Test to obtain a more accurate conclusion form the data.
24. Results

**Graphs & Tables:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **E. Coli** | **Plate 1** | **Plate 2** | **Plate 3** | **Plate 4** | **Plate 5** | **Plate 6** | **Plate 7** |
| **0% [ ]** | **56** | **79** | **87** | **81** | **85** | **71** | **66** |
| **.01% [ ]** | **81** | **67** | **60** | **94** | **101** | **88** | **93** |
| **.1% [ ]** | **109** | **95** | **127** | **129** | **87** | **99** | **78** |
| **1% [ ]** | **147** | **156** | **144** | **142** | **105** | **129** | **103** |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Staph** | **Plate 1** | **Plate 2** | **Plate 3** | **Plate 4** | **Plate 5** | **Plate 6** | **Plate 7** |
| **0% [ ]** | **114** | **95** | **102** | **99** | **127** | **98** | **112** |
| **.01 [ ]** | **210** | **177** | **165** | **173** | **167** | **199** | **204** |
| **.1% [ ]** | **198** | **233** | **182** | **225** | **196** | **209** | **226** |
| **1% [ ]** | **319** | **340** | **329** | **303** | **296** | **280** | **302** |

**The ANOVA Calculations:**

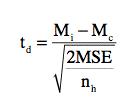
To generate a proper analysis and deeper understanding of the null hypothesis, the ANOVA program was used. The program analyzes the data recorded to show the statistical probability on whether the null hypothesis is most likely true or false. The ANOVA program produces a P-value. The P-value is a measure of the strength of the evidence that goes against the null hypothesis. It also indicates whether there is enough evidence to express the null hypothesis as either a true or false statement. The P-value will be .05 or above if there is a somewhat reasonable chance that the null hypothesis can be classified as correct. If the P-value is under .05, the null hypothesis is can be disregarded.

The data in this experiment was put through the ANOVA program to calculate the P-value. The program expressed the **P-value** for the E. coli bacteria as **8 to the negative sixth power (\*e-060**, **not** above **0.05**. This is well under the cutoff point, therefore, the null hypothesis is extremely likely to be false in this experiment. The ANOVA program expressed a **P-value** of **0**, therefore, the null hypothesis is once again extremely likely to be false.

To further test the results, a procedure for comparing each experimental mean with the control mean was use. This is called Dunnett’s, after the statistician who developed it. This test is much more powerful and accurate than any other test designed to test the mean with any other mean. It was conducted by computing a t-test between each experimental group and the control group.

**T-Test Formula:**

|  |  |  |
| --- | --- | --- |
|  | **E. coli** | **Staph** |
| **Control Mean** | **75** | **106.714** |
| **.01 % [ ]** | **83.4286** | **353.333** |
| **.1% [ ]** | **103.429** | **355.81** |
| **1% [ ]** | **132.286** | **425.143** |
| **MS** | **290.952** | **316.131** |
| **Critical Value** | **2.51** | **2.51** |



Mi is the mean of the experimental group, Mc is the mean of the control group, [MSE](http://davidmlane.com/hyperstat/A67017.html) is the mean square error as computed from the [analysis of variance,](http://davidmlane.com/hyperstat/B84377.html) and nh is the [harmonic mean](http://davidmlane.com/hyperstat/A33018.html) of the [sample sizes](http://davidmlane.com/hyperstat/A104571.html) of the experimental group and the control group. The [degrees of freedom](http://davidmlane.com/hyperstat/A42408.html) (df) for the test are equal to N-a where N is the total number of subjects in all groups and "a" is the number of groups (including the control).

**Staph Sample Problem .01% [ ]:**

2.73041 = \_\_\_\_\_\_\_\_\_353.333 – 106.714\_\_\_\_\_\_\_\_\_

­

7

**E. coli Sample Problem .01% [ ]:**

.10139 = \_\_\_\_\_\_\_\_\_83.4286 – 75\_\_\_\_\_\_\_\_\_

­

7

The critical value for the .05 alpha was **2.51** (this number varies depending on the number of groups and samples per group in the experiment). There were only four groups and seven samples per group for each bacteria in this experiment.

|  |  |  |
| --- | --- | --- |
| **Dunnet’s Test Results** | **E. coli** | **Staph** |
| **.01% [ ]** | **.10139** | **2.73041** |
| **.1% [ ]** | **.34199** | **2.75783** |
| **1% [ ]** | **.68912** | **3.52544** |

1. Conclusion

It was hypothesized that when varying levels of phosphate is applied to *E. coli* and *Staph*, the phosphate will not significantly affect the bacteria’s growth; nor will the phosphate affect either bacteria (Gram Positive & Gram Negative) differently. This statement, however, can be easily disregarded when applying to the Staph, but can be accepted when considering the E. coli. This experiment’s results were run through numerous trials and data evaluation programs/tests to assure that the most accurate conclusion possible can be drawn from it. If the null hypothesis were true with the Staph, this would not be the case with the acquired data, and all colonies treated and untreated with the ammonium phosphate solution would have more consistent numbers of colonies throughout each trial. The ANOVA program provided a statistical analysis that could is much more accurate than a human observation. It provided a P-value of only 8-6, which indicates that the probability of the null hypothesis being true is next to none for the *E. coli* bacteria. Furthermore, when the *Staph* bacteria’s data was run the ANOVA, it had a P-value of 0 which also indicates that the phosphate solution had a significant effect on the bacterial growth*.*

Since the ANOVA program does not provide as accurate of a conclusion that other tests do, the Dunnet’s Test was used to further back up the results. It’s conclusions about both bacteria corresponded with the ANOVA’s conclusions. The data showed that the T-Value for both tests was well above the critical value used analyze whether the variable was significant or not. Therefore, the phosphate solution did have a significant impact on the bacterial growth of both species according to this data.

The T-critical value for the .05 alpha was **2.51**. Since the **T-Value** wasmore than the critical value for every concentration of phosphate on staph, the results appear to be significant for this bacteria*.* This supports the data acquired in the ANOVA and acts as more convincing data that was recorded for this experiment. However, the results for the *E. coli* bacteria appear not significant, as the t-value for each concentration was under the critical value. This contradicts the previous analysis that the ANOVA produced and was very surprising. As a result of the Dunnet’s Test being much more powerful and accurate than any other test designed to test the mean with any other mean, the final conclusion of this experiment is based upon this test as opposed the ANOVA.

However, it was also hypothesized that when four different concentrations of ammonium phosphate are applied to *E. coli* and *Staph*, there will be significant growth with both bacteria. Furthermore, the Gram Positive *Staph* bacteria will grow considerably more than the Gram Negative *E. coli* bacteria. This alternative hypothesis is supported by the Staph’s recorded data, but not the E. coli’s. It is unclear why the Gram-Positive bacteria grew so much more than the Gram-Negative.

The phosphate solution’s affect was more pronounced for Gram-positive bacteria, probably due to the lack of additional permeability barriers, particularly the outer membrane of Gram-negative bacteria. As a result, the phosphate will stay effective for a longer period of time. Nevertheless, the outer membrane itself does not provide resistance to antimicrobial agents as it only decreases permeability.

With regard to the materials and procedure, the amount of time that the bacteria were incubated might be able to be changed. There could have been a time of exponential growth with the *Staph* bacteria that may have happened with the *E. coli* if it was incubated at a different amount of time. Other types of bacteria could be grown in the future that are both Gram-Positive and Gram-Negative to see if the phosphate solution would have the same effect on the two types of bacteria. Other than these changes, no other limitations in the procedure, materials, and the rest of the experiment can be mentioned.

**Bibliography**

“E. coli (*Escherichia Coli*).” *cdc.gov.* The Center for Disease Control & Prevention. 6 Nov. 2015. Web. 20 May. 2015.

“Escherichia coli - Importance In Laboratory.” *jrank.org*. Jrank. n.d. Web. 23 May. 2016.

“Escherichia coli.” *jrank.org.* Jrank. n.d. Web. 21 May. 2016.

Villari, Sarnataro, Iacuzio. “Molecular Epidemiology of Staphylococcus epidermidis in a Neonatal Intensive Care Unit over a Three-Year Period.” Journal of Clinical Microbiology. Vol. 38, No. 5 May, 2000. 30 May, 2016.

# Bukhari, Mohammad. “Staphylococcus epidermis.” *uconn.edu.* 27 sept, 2004. Web. 30 May, 2016.

# “Experimental Staph Vaccine Broadly Protective in Animal Studies.” *archive.org.* National Institute of Allergy and Infectious Diseases.n.d. Web. 24 May. 2016.

# “Gram-negative Bacteria.” *nih.gov.* National Institute of Allergy and Infectious Diseases. n.d. Web. 23 May. 2016.

Mallette, [M. F.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mallette%20MF%5Bauth%5D),  [Cowan](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cowan%20CI%5Bauth%5D), Cynthia I., and Campbell, [J. J. R.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Campbell%20JJ%5Bauth%5D)  “Growth and Survival of Escherichia Coli In Medium Limited In Phosphate.” *nih.gov.* PMC. n.d. Web. 23 May. 2016.

# 

# 