Suppressing *E. coli* Biofilm Formation with Garlic Extract and Justifying Conclusions with the Philosophy of Science

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Abstract

Antibiotics have revolutionized human medicine by providing powerful tools to combat bacterial illnesses, but the rise of antibiotic resistance threatens to render them useless. One type of anibiotic resistance, called a biofilm, might be inhibited by quorum sensing inhibitors, which block bacterial communication. Because quorum sensing inhibitors do not rely on killing bacteria, it may take longer for bacteria to develop resistance against them. Garlic extract, which contains a quorum sensing inhibitor, is tested in this research for efficacy against the biofilm formation of *E. coli*, specifically the K-12 MG1655 strain, because *E. coli* is a major cause of food-borne illness. If garlic extract proves effective, it could help prevent *E. coli* biofilms from surviving cleaning procedures, protecting against ingestion. After testing a variety of concentrations, this research finds that garlic extract at a concentration of 16 μL / mL likely promotes biofilm formation but that it inhibits formation at a concentration of 64 μL / mL. However, the data are ultimately inconclusive. Thus, while additional research is required, garlic extract could prove valuable in preventing food-borne illness.

As this work is the combination of the science-based LOGOS program and the more philosophical Global Scholars Program, the logical backing for the scientific method is also considered through the philosophy of science. The value of the scientific method is shown through how it provides a way to maintain logical reliability despite its use of inductive reasoning and subjective perceptions. Based on that philosophy, a framework for logically sound scientific endeavor is used to interpret the empirical results of this research and come to the normative conclusion that everyone should participate in scientific endeavors.

Acknowledgements

This project would not have been possible without the support of all those I worked with and whose work I relied upon. Foremost among them are those at Park Tudor who spent countless hours helping me. Mr. Mark Dewart ensured that I had access to the equipment, supplies, and spaces I needed to conduct my experiments, and his advice on the initial form of this paper, which was for the LOGOS program, helped me convey my results clearly. Additionally, Dr. Kristin Chun, my mother, supervised nearly all of my lab work, which totaled over 150 hours, giving me the flexibility to conduct experiments more often that would be permitted by others' schedules. Finally, Mr. Bohrer's advice on the Global Scholars component of my paper pushed me to explore the fascinating philosophy of science and give my own voice to its normative conclusions.

Despite Park Tudor's central role in developing my project, it is not actually where my work began. That happened at Health & Science Innovations' Young Innovators Quest (YiQ) summer program. There, Mr. Luis Palacio's and Dr. Alfredo Lopez-Yunez's guidance nurtured the first seeds of my topic. Later, under the YiQ Scholars program, Ms. Dara Marquez identified and suggested agricultural biofilms as an interesting area of study, which led to my focus on *E. coli* biofilms, and Health & Science Innovations provided the UV Spectrophotometer I used to collect my data.

Dr. Gregory Anderson, my mentor, was another invaluable source of advice. I based my protocols on the ones he gave me, and he helped me consider how to setup my experiments.

Later, he helped me interpret my data and rule out sources of error. Dr. David Skalnik, my father, also helped by suggesting Dr. Anderson as a potential mentor.

In the early stages of my project, I was working with a group—consisting of Victor Xiao, Jay Sangani, Aysha Chaudhary, Haeli Juthani, and me— from the YiQ program. They assisted with the identification of the project topic, and Jay and Victor helped with the early stages of research.

Finally, my work stands upon the shoulders of those scientists who came before me. By releasing their findings publicly, and in many cases for free, they let me learn from their research and therefore make more informed choices regarding mine.

Outline

Research Question: Does garlic extract suppress biofilm formation by *E. coli* K12 MG1655, and how can the answer be relied upon when human perceptions are fallible?

I. Introduction

A. Dangers of Biofilms

1. Resilient

a. Antibiotic Resistance

b. Environmental Tolerance

2. Human Health Impacts

a. Immunodeficient Patients (*P. aeruginosa*)

i. Cystic Fibrosis

b. Produce (*E. coli*)

B. Possible Solution

1. Garlic Extract

a. How: Quorum Sensing Inhibition

b. Why: Weaker Selective Force

C. Foci of Past Research

1. Species

a. P. aeruginosa

2. Environments

a. Biomedical Implants

b. Cystic Fibrosis Patients' Lungs

3. Form

a. Synthesized Ajoene

b. Extracted Ajoene

D. Research Question for Empirical Research: Does garlic extract suppress biofilm formation by *E. coli* K12 MG1655?

E. Confounding Variables

1. Contamination
2. Impact of Surface on Growth
3. Growth Rate of Bacteria
4. Impact of Ethanol and Crystal Violet on Cuvettes
5. Differences in Media
6. Variations in Concentrations

a. Imprecise Volume Measurements

b. Non-Uniform Suspensions

F. LOGOS and Global Scholars

1. LOGOS: Empirical Research
2. Global Scholars: Philosophical Foundation of Science

G. Reliability of Results

1. Philosophy of Science

a. Human Condition

b. Types of Logical Reasoning

c. Goal of Reducing Subjectivity

i. Scientific Method

1) Methodological Controls

2) Peer Review

H. Value of Research

1. Food and Healthcare

a. Evidence for Efficacy or Inefficacy

2. Contribution to Another Perspective

II. Hypothesis: Garlic extract suppresses biofilm formation in *E. coli* K12 MG1655.

A. Ajoene Effective in Mammals

B. *E. coli* Uses Quorum Sensing

C. *E. coli* growth is Inhibited by Ajoene

III. Methods

A. Stock Materials and Methods

1. Bacteria

a. Procedure to Revive

b. Plating

2. Lysogeny Broth

a. Agar

b. Media

3. Sterilization

4. Colony Maintenance

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1. Day 1
    a. Culture and Control
    b. Sterilization
    c. Inoculation
    d. Incubation
2. Day 3
    a. Sample Creation
        i. Experimental
        ii. Vehicle Controls
        iii. Positive and Negative Controls
    b. Replicates
    c. Incubation
3. Day 4
    a. Rinsing
    b. Crystal Violet
    c. Rinsing
    d. Incubation
4. Day 5
    a. Ethanol Addition
    b. Incubation
    c. Quantitative Analysis

C. Control of Confounding Variables

1. Vehicle Controls
2. Contamination Controls
    a. Late Sterilization
    b. Flaming
    c. Negative Controls
3. Vortexing
4. Glass Cuvettes
5. Same Manufacturers

IV. Results

A. Trial 1

1. Graph
2. Explanation
3. Statistical Analysis
    a. Table
    b. Explanation

B. Trial 2

1. Graph
2. Explanation
3. Statistical Analysis
    a. Table
    b. Explanation

C. Trial 3

1. Graph
2. Explanation
3. Statistical Analysis
    a. Table
    b. Explanation

D. Trial 4

1. Graph
2. Explanation
3. Statistical Analysis
    a. Table
    b. Explanation

E. Trial 5

1. Graph
2. Explanation
3. Statistical Analysis
    a. Table
    b. Explanation

F. Overall Analysis

1. Graph
2. Explanation
3. Statistical Analysis

4. Table

V. Conclusion

A. Results Inconclusive

1. Fail to Support Hypothesis

a. Not Statistically Significant

2. Fail to Refute Hypothesis

a. Significant Differences Expected but not Guaranteed

B. Summary of Results

1. Suggest Suppression at 64 μL / mL

2. Suggest Promotion at 16 μL / mL

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1. Within Trials

a. Generally Significant

b. Not Independent

c. Lack of Consensus

2. Between Trials

a. Not Significant

b. Independent

3. Improved Methodology May Reduce Variation

D. Meaning

1. Suggestions

a. Link May Exist

b. Warn Against Improper Usage

2. Ultimately Inconclusive

VI. Discussion

A. Introduction

1. LOGOS and Global Scholars

a. LOGOS: Empirical Research

b. Global Scholars: Philosophical Foundation for the Scientific Method

2. Objection: Science Best Left to Experts

3. Objection: Science Unreliable

4. Everyone Should Participate in the Scientific Process Because It Is Reliable

B. Objection: Science Relies on Assumptions

1. Objective Truth or Reality Exists

a. Otherwise We Cannot Coexist

i. We Must Assume We Live in the Same World as Everyone Else

2. That Reality Is Intelligible

a. Otherwise We Cannot Predict the Future or Control Our Destinies

3. Perceptions Tainted by Human Condition

a. Some Parts Reflect Reality

b. Some Parts Reflect Bias

c. Best Explains Why We Only Agree Sometimes

C. Understanding Reality is Valuable

1. Need Understanding

a. Control of the Future is a Necessary Tool to Improve Human Lives

b. To Control Our Future, We Must Understand It

c. Assume Objective Reality Exists and It Is Intelligible

d. We Live In Reality, So It Prescribes Our Future

e. We Should Seek Understanding of Objective Reality

2. Health Implications of This Research

a. Treat Antibiotic-Resistant Infections

i. Cystic Fibrosis

ii. Medical Implants

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i. Medical Devices

ii. Food

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D. Science Overcomes Obstacle of Induction

1. Logical Reasoning

a. Inductive Reasoning

i. Definition and Examples

ii. No Logical Guarantees

b. Deductive Reasoning

i. Definition and Examples

ii. Logical Guarantees

iii. General Claim Relies on Induction

2. Science's Solution

a. Evidence Can Only Disprove

b. All Conclusions Tentative

E. Science Overcomes Obstacle of Subjectivity

1. Separate Subjective from Objective in Perceptions

a. Compare Perceptions

b. More Participants Weed Out More of the Subjective

c. Applied Generally: More Skeptical People Eliminate More Bias

2. Scientific Method

a. Theories Create Testable Predictions

b. Scientists Seek to Disprove Hypotheses and Theories

c. Peer Review

i. Reproducible Experiments

ii. Journal Peer Review

iii. Commentary on Published Work

F. Contribution of Perspectives Valuable

1. Elephant and Blind Men

2. Guidance for Future Research

G. Objection: Even Data Collection is Prone to Error

1. In Practice:
    a. A Single Counter-Instance Is Not Disproval
    b. Disproval is Tentative
2. Methodological Controls
    a. Controls
        i. Positive
        ii. Negative
    b. Variation
        i. Statistics
            1) Replicates
            2) Trials
3. Analysis of This Work's Results

H. Normative Conclusions

1. Federal, State, and Local Governments
    a. Promote Research
    b. Fund Research
2. Scientists
    a. Conduct Research
3. Non-Scientific Intellectuals (Humanists)
    a. Analyze and Critique Findings

VII. Bibliography

A. Works Cited

B. Works Consulted

VIII. Outcomes

A. LOGOS and Global Scholars Program

1. LOGOS Covered Scientific Research

2. Global Scholars Justified that Scientific Process

B. Topic Discovery and Selection

1. Natural Process

a. Started Early

i. Inspiration from Scientific American

1) Bacterial Food Collection

ii. Exploration at YiQ

1) Cancer Communication

2) Bacterial Communication

3) Quorum Sensing

iii. Refinement Through YiQ Scholars

1) Agricultural Bacteria

2) Biofilms

a) E. coli

2. Reflection

a. Benefits of Early Start

b. Benefits of Discussions with Others

C. Logistics and Practicalities of Laboratory Work

1. Lab Space and Equipment
    a. Park Tudor
        i. Space
        ii. Purchased Equipment
    b. YiQ
        i. UV Spectrophotometer
2. Supervision
    a. Mr. Mark Dewart
    b. Dr. Kristin Chun
3. Time Commitment
    a. Summer
    b. School Year
        i. Early
        ii. Break for College Applications
        iii. Resumption

D. Initial Experiments and Protocol Design

1. Response to Failure
    a. Contamination
        i. Late Sterilization
        ii. Flame
    b. Lack of Growth
        i. Aversion to Soap

c. Variation

i. Systematic Analysis

ii. Disposable Tubes

2. Advice

a. Mother

i. At Home

b. Dr. Gregory Anderson

i. Meetings

1) Proposed Protocols

ii. Email

1) Confusing Data

2) Potential Explanations

E. Data Analysis

1. Null Hypothesis

a. Doubting Results

b. Statistical Analysis

F. Writing

1. Organization

a. Zotero

i. Explanation

ii. Scientific Papers

b. Lab Notebook

i. Daily Chronology

ii. Data

iii. Documentation

iv. Protocols

2. Early Start from LOGOS

G. Personal Insights

1. Continued Work on Challenging Project

a. Challenges and Frustrations

i. Over a Year of Work

ii. Hours of Time After School

iii. Numerous Setbacks

1) Contamination

2) Variation

3) Statistical Significance

iv. Ultimately Inconclusive

b. I Continued and Would Do So Again

i. I Value Challenge

1) Learning Experience

a) Techniques

b) Limits

2) Knowledge of Capabilities

ii. I Wanted to Finish

1) Curiosity

iii. Work Felt Meaningful

2. Conclusions About Myself

a. Reluctant to Accept What I Think I Can Do

i. I Want Proof

b. Hesitant to Accept My Limits

i. I Want to Try to Push Past Them

c. Desire to Learn

i. About Myself

ii. About My Surroundings

d. Desire to Contribute to Something Beyond Myself

3. My View of Truth

a. Distrust Singular Sources

i. Myself

1) Abilities and Limits

ii. Anecdotal or Experiential Evidence

1) Quantitative Instead of Qualitative

iii. I Don't Trust Anyone; I Trust Everyone

iv. Why I Value Adding Myself

b. Accept Plurality of Sources

i. My Research's Assumptions

1) Spectroscopy

2) Volume Measurements

c. Tentative Conclusions

i. Willing to Second-Guess Assumptions

1) Variation from Test Tubes

H. Future Improvements

1. Professional Lab

a. Faster

b. Learned More Without

2. Shorter Experiments

a. Hard to Schedule

b. Tired After School

3. Wish Mentor More Involved

I. Overall Assessment

1. Wish for More Definitive Results

a. Better Equipment

b. Better Methodology

2. Satisfied with:

a. Time Management

b. Learning

IX. Appendix A: Raw Data[1]

A. Explanation

[1] The appendices are not included in this document but are available separately. They are not not needed for the arguments of this paper but are provided in the interest of transparency.

B. Trial 1 Table

C. Trial 2 Table

D. Trial 3 Table

E. Trial 4 Table

F. Trial 5 Table

G. Overall

1. Table

2. Explanation

X. Appendix B: Figures

A. Explanation

B. Trial 1

1. Cultures

2. Staining

C. Trial 2

1. Cultures

2. Staining

D. Trial 3

1. Explanation

2. Table

E. Trial 4

1. Explanation

2. Table

F. Trial 5

1. Explanation
2. Table

Introduction

While antibiotics, perhaps more than any invention of the twentieth century, have dramatically improved public health, those gains are now in jeopardy in the face of antibiotic-resistant strains of lethal bacterial diseases. From MRSA to salmonella to *E. coli*, resistant strains are expensive if not impossible to treat, so they pose a grave threat to public health.[2] The biofilm is one particularly dangerous form of antibiotic resistance. Biofilms are colonies of bacteria that anchor themselves to biotic or abiotic surfaces and then secrete a matrix of polysaccharides, proteins, and DNA around themselves.[3] This matrix protects the bacteria inside from a variety of environmental stressors, including host immune systems and antibiotics.[4] Additionally, once the biofilms of some strains, including salmonella, are established, they can withstand being soaked in disinfectants like sodium hydroxide.[5] This is particularly dangerous because salmonella is a common food-borne illness. Biofilms can also form on produce and survive cleaning procedures to infect whomever eats the contaminated produce.[6] Finally, biofilms can form inside the body. In particular, *P. aeruginosa* forms biofilms in the lungs of cystic fibrosis patients[7] despite high doses of antibiotics.[8]

Garlic extract has the potential to fight biofilms better than current antibiotics because it contains the compound ajoene, which inhibits quorum sensing. Quorum sensing is a type of bacterial communication that allows individual bacteria to measure the concentration of other

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2 United States. Congress. House. Committee on Energy and Commerce. Subcommittee on Health, 2012
3 Absalon, Van Dellen, & Watnick, 2011
4 Redelman, Chakravarty, & Anderson, 2014
5 Corcoran et al., 2014
6 Annous, Fratamico, & Smith, 2009
7 Jakobsen et al., 2012
8 Redelman, Chakravarty, & Anderson, 2014

bacteria around themselves. As part of this process, each bacterium releases signaling molecules, called autoinducers,[9] into its environment, so the concentration of signaling molecules is related to the concentration of bacteria.[10] This communication is critical for biofilm formation because high concentrations of autoinducers trigger behavioral shifts in bacteria, including the start of biofilm formation. Quorum sensing inhibitors, molecules that block quorum sensing, can prevent biofilm formation and reduce the virulence of the bacteria.[11] Additionally, because quorum sensing inhibition makes bacteria less dangerous instead of killing them, there is a weaker selective force favoring any resistant bacteria.[12] Therefore, it puts less evolutionary pressure on bacteria, so bacteria are less likely to develop resistance against it. Ajoene is an effective quorum sensing inhibitor and reduces biofilm formation by *P. aeruginosa* in mammalian systems,[13] so garlic extract is a promising candidate for future antibiotic treatments.

While previous research has examined quorum sensing inhibitors, including ajoene, as antibiotic treatment options, there are still gaps in science's understanding where additional research is needed. Past work has largely focussed on biofilms in the body, so *P. aeruginosa*, biofilms in cystic fibrosis patients' lungs, and biofilms on biomedical implants have been examined extensively. Additionally, much of the past research on ajoene has used extracted or synthesized ajoene. However, little or no research has been done on the effectiveness of raw garlic extract for inhibiting biofilms formed on produce. Research has been done on crude garlic extract as a quorum sensing inhibitor and as an inhibitor of *P. aeruginosa* biofilms,[14] but not as an inhibitor of *E. coli* biofilms. As a first step towards filling that gap, this research examines the

9 O'Loughlin et al., 2013
10 How bacteria "talk," 2009
11 O'Loughlin et al., 2013
12 Tay & Yew, 2013
13 Jakobsen et al., 2012
14 Rasmussen et al., 2005

impact of garlic extract on the biofilm formation of *E. coli*, a principal culprit in food-borne illness. Thus, this research seeks to answer the question, "Does garlic extract suppress biofilm formation by *E. coli* K-12 MG1655?"

In order to isolate the changes in biofilm formation due to the concentration of garlic extract, a number of confounding variables have to be controlled. Contamination has to be prevented as much as possible and detected if it does occur. The biofilm attachment surface must be kept identical across samples, and the growth in each sample needs to be approximately equal, excluding inhibitory effects by garlic extract. Changes to the cuvette by the compounds used also has to be prevented, the growth media must be constant, and errors in concentrations due to imprecise volume measurements or non-uniform solutions need to be minimized.

This work was performed as a merger of two programs: LOGOS, which covered the scientific research, and Global Scholars, which covered the philosophical underpinnings of the scientific process. The questions of how to illuminate truth when one's reason is chained by the human condition and how to overcome the unreliability of inductive reasoning is therefore examined through the philosophy of science. Ultimately, the scientific method's methodological controls and peer review processes are presented as solutions for reducing subjectivity in empirical research.

To more effectively combat antibiotic resistance, the healthcare and food industries need better antibiotic compounds. However, before those can be developed, let alone used, they have to be identified and tested. This research will contribute evidence of garlic extract's efficacy or inefficacy to that search. That evidence may help future researchers direct their resources more efficiently and expedite the creation of more effective antibiotics. Furthermore, this work will

contribute another perspective to a broader conversation, perhaps further reducing the subjectivity, and therefore increasing the reliability, of that conversation's conclusions.

Hypothesis

For this experiment, it is hypothesized that if the right concentration of garlic extract is added to a liquid culture of *E. coli* K-12 MG1655, biofilm formation will be reduced. This is based on research showing that ajoene reduces the amount of biofilm formed by *P. aeruginosa* in mammalian systems by inhibiting quorum sensing[15] and research showing that crude garlic extract reduces quorum sensing.[16] Since *E. coli* uses quorum sensing, it is believed that garlic extract will also reduce biofilm formation by *E. coli*.

[15] Jakobsen et al., 2012
[16] Rasmussen et al., 2005

Methods

For the stock colonies of bacteria used in this research, dehydrated *E. coli* K-12 MG1655 was ordered. The dehydrated bacteria were revived by adding liquid LB media to the filter paper and then spreading that inoculated liquid on a lysogeny broth (LB)[17] agar petri dish with a sterile, plastic inoculating loop. The LB agar plates were made by mixing powdered LB agar with deionized water in a ratio of 32.00 g per 1 L, per the manufacturer's instructions. That mixture was stirred until it dissolved as much as possible, and then it was sterilized. Sterilization was performed with a pressure cooker on for at least 10. mins. after its valve sealed. In the case of liquids, the cooker was slowly depressurized either by letting it cool or by depressing the valve no faster than once every 2 sec. The agar was then poured into petri dishes to make the plates for bacteria to grow on. Cultures were cycled every 2-4 wks. Each time, sterile wooden inoculating sticks were used to inoculate a new petri dish from the youngest existing colony.

Liquid cultures of bacteria were grown in liquid LB media. This was made by mixing the powdered form of the media with deionized water in a ratio of 20.00 g per 1 L, per the manufacturer's instructions, and then sterilizing the mixture.

On the first day of each experiment performed for this research, a culture and a negative control were made in liquid LB media. Two 125 mL Erlenmeyer flasks, each with 10.0 mL of media, were sterilized with the media inside. Then, one was inoculated with a sterile, wooden inoculating stick and the other was left as a negative control. Both were incubated at 37°C for 48 hrs.

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[17] Bertani, 2004

After 48 hrs. of incubation, on the 3rd day of each experiment, the culture was diluted by a factor of 100. Some of that diluted culture was split among 8 sterile glass test tubes. To 4 of those tubes, differing amounts of garlic extract were added to test different concentrations. To the other 4 tubes, 95% ethanol was added in the same amounts as the garlic extract. This functioned as the vehicle control as the garlic extract was dissolved in 95% ethanol. Once those mixtures were made up, each one was divided among five replicates. Each replicate contained 1 mL of solution in a plastic sterile test tube. There were also 5 replicates of the diluted culture and 5 of the negative control from the Erlenmeyer flask. All the replicates were incubated at 37°C for 24 hrs.

The next day, day 4, all of the replicate test tubes were emptied and rinsed 4 times with deionized water to eliminate any unattached bacteria. To rinse a tube, it was filled with water and then emptied. To each replicate tube, 2 mL of 0.100% crystal violet were added to stain the biofilm. After incubating for 30.0 mins. at room temperature, the replicates were emptied and rinsed 4 times to remove excess stain, revealing the stained biofilm as a purple ring where the interface between the culture and the air used to be. The tubes were left to dry upside-down overnight.

On the 5th and final day of the experiment, each replicate was banged on the table-top to shake out excess water, and each was filled with 3 mL of 95% ethanol to dissolve the stained biofilm. After a 15.0 min., or however long it took for the rings to dissolve, incubation at room temperature, a glass cuvette was filled with the solution and read with a UV Spectrophotometer at OD600 (600.0 nm). The cuvette was rinsed with 95% ethanol between each reading. Liquid was transferred using disposable plastic transfer pipets, with a new one used for each replicate.

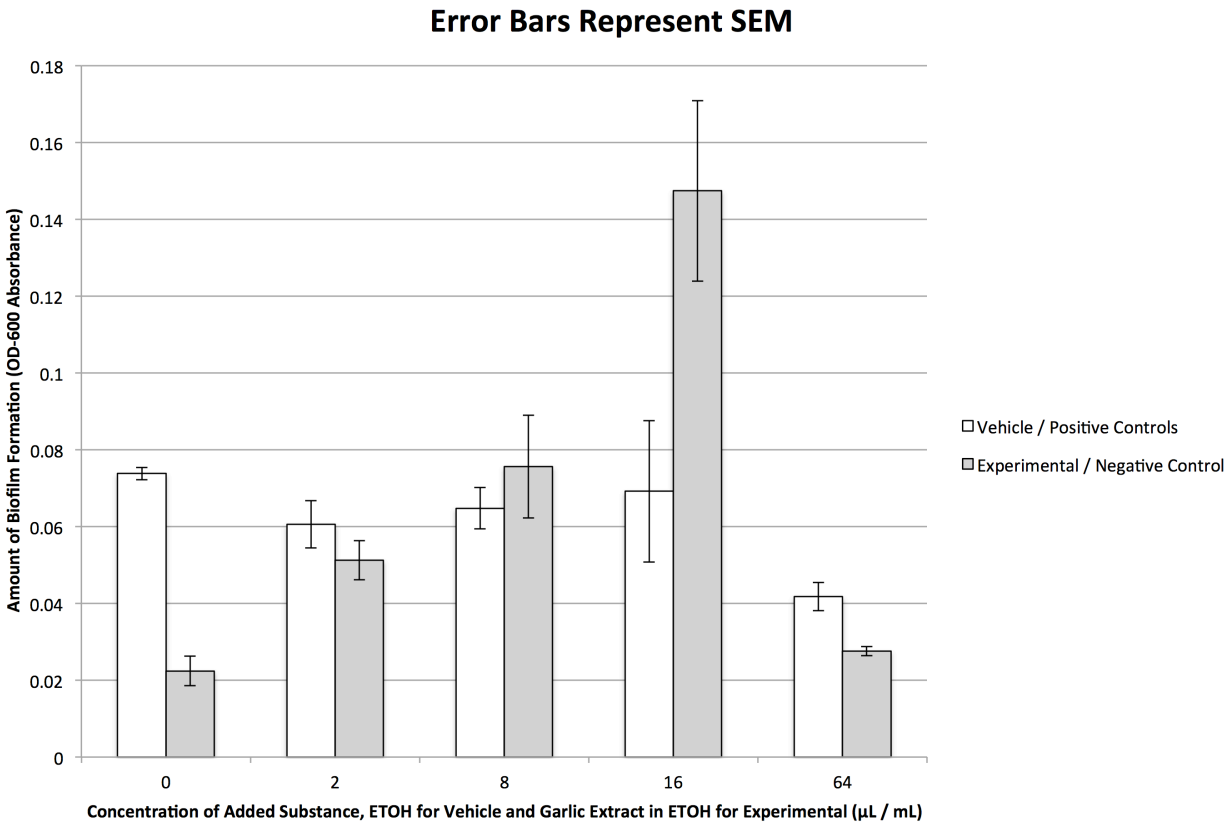
Overall, this protocol measured the amount of biofilm formed, the dependent variable, using crystal violet and UV spectroscopy when varying concentrations of garlic extract, the independent variable, were added.

To account for changes introduced by the ethanol the garlic extract was dissolved in, a set of vehicle controls, each one of which had the same concentration of ethanol as an experimental had garlic extract, were kept alongside the experimental samples. Without this measure, it would have been impossible to tell whether the differences measured were due to garlic extract or ethanol. In addition, to reduce contamination, media was sterilized after transfer when possible and the openings of tubes and bottles were flamed before and after adding or removing liquid. In case contaminant organisms did slip through, negative controls were treated just like the other samples and monitored for growth. This prevented any organisms from growing and changing measured growth through competition or biofilm formation without detection. Furthermore, to reduce variations in concentration, heterogeneous mixtures were vortexed or swirled before any liquid was removed and all replicates of a sample came from the same mixture. These measures helped prevent variations in concentrations obscuring actual results. Finally, a glass cuvette was used to avoid any changes to the cuvette surface by the ethanol or crystal violet, and all tubes were the same product from the same manufacturer to further reduce variation.

Results

Trial 1

Graph 1: Impact of Garlic Extract on Biofilm Formation (Trial 1)



In the graph above, experimental (gray) and vehicle control (white) samples are grouped together at each concentration tested (2.0, 8.0, 16, and 64 µL / mL). The bars at 0 µL / mL represent the negative (gray) and positive (white) controls.

In Graph 1, little difference is apparent at concentrations of 2.0 and 8.0 µL / mL. However, at 16 µL / mL, the experimental samples showed much greater biofilm formation than

the vehicle control. Conversely, at 64 μL / mL, there was less biofilm formation in the experimental than in the vehicle control. Error bars suggest that both of these differences are significant, though T-Tests will be used to know for sure.

Table 1: Trial 1 Statistical Analysis

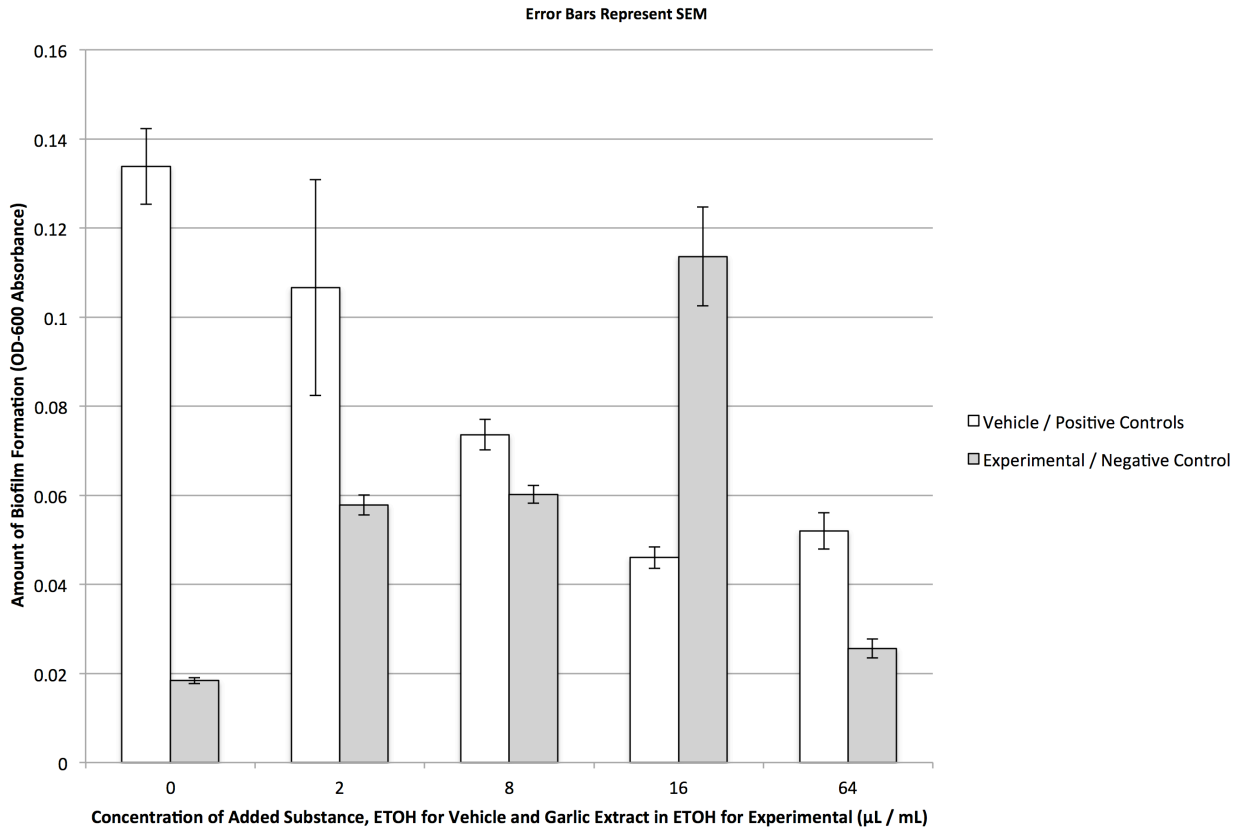
T-Test Table	NC	PC	E1	E2	E3	E4	V1	V2	V3	V4
NC	1.000									
PC	0.000	1.000								
E1	0.002	0.009	1.000							
E2	0.014	0.900	0.148	1.000						
E3	0.005	0.035	0.014	0.036	1.000					
E4	0.256	0.000	0.008	0.023	0.007	1.000				
V1	0.001	0.098	0.274	0.350	0.019	0.005	1.000			
V2	0.000	0.173	0.104	0.486	0.023	0.002	0.621	1.000		
V3	0.063	0.816	0.393	0.787	0.032	0.087	0.677	0.828	1.000	
V4	0.007	0.000	0.177	0.063	0.010	0.015	0.036	0.009	0.214	1.000

The table above can be used to find the results of a two-tailed T-Test assuming unequal variance between any two samples. Any given cell containing a number holds the p value from the T-Test between the samples indicated in the headers for its row and column. “E” indicates an experimental sample while “V” indicates a vehicle control one. The numbers following an “E” or a “V” indicate successively higher concentrations (i.e. “E3” indicates the experimental sample with a concentration of 16 µL / mL). Gray cells indicate values less than 0.05. Cells with borders are those that compare an experimental sample with the associated vehicle control. Half of the table is blank because it would be a mirror image of the completed half.

The p values shown in the boxed sections of Table 1 reveal that the differences apparent in Figure 1 (at 16 and 64 µL / mL) are indeed statistically significant. However, because the samples in this case were replicates, they were not independent samples. Thus, this does not demonstrate overall statistical significance

Trial 2[18]

Graph 2: Impact of Garlic Extract on Biofilm Formation (Trial 2)



The graph above has the same format and notation as that in Trial 1.

In Graph 2, little difference is apparent at a concentration 8.0 µL / mL. However, at 16 µL / mL, the experimental samples showed much greater biofilm formation than the vehicle control. Conversely, at 64 µL / mL and 2.0 µL / mL, there was less biofilm formation in the experimental samples than in the vehicle controls. Error bars suggest that both of these differences are significant, though T-Tests will be used to know for sure.

[18] Note that another trial was performed between trials 1 and 2, but because of human errors in carrying out the procedure, it was deemed flawed and the results were discarded.

Table 2: Trial 2 Statistical Analysis

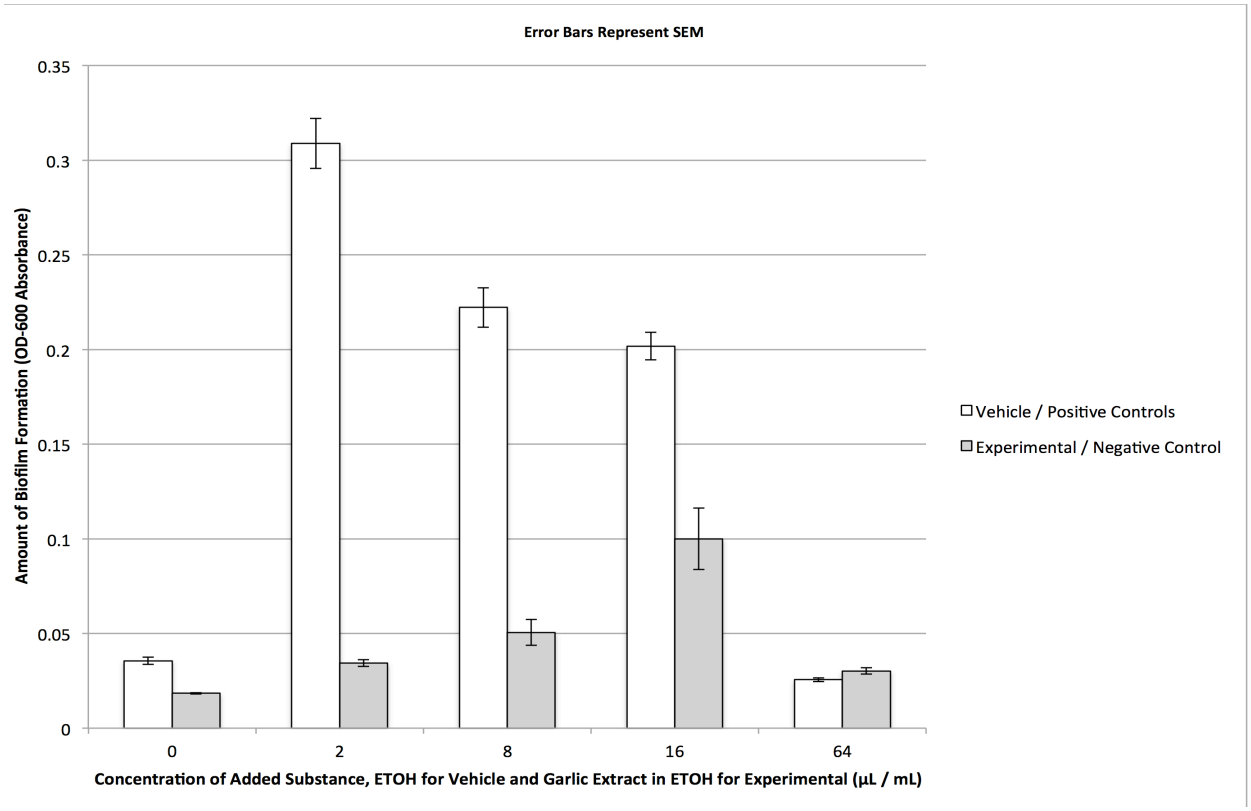
T-Test Table	NC	PC	E1	E2	E3	E4	V1	V2	V3	V4
NC	1.000									
PC	0.000	1.000								
E1	0.000	0.001	1.000							
E2	0.000	0.001	0.447	1.000						
E3	0.001	0.188	0.006	0.008	1.000					
E4	0.024	0.000	0.000	0.000	0.001	1.000				
V1	0.022	0.338	0.114	0.128	0.802	0.028	1.000			
V2	0.000	0.001	0.007	0.014	0.020	0.000	0.246	1.000		
V3	0.000	0.000	0.007	0.002	0.003	0.000	0.066	0.000	1.000	
V4	0.001	0.000	0.257	0.122	0.003	0.001	0.086	0.004	0.248	1.000

The table above has the same format and notation as that in Trial 1.

The p values shown in the boxed sections of Table 2 reveal that the differences apparent in Figure 2 (at 8. 0, 16 and 64 µL / mL) are statistically significant. However, because the samples in this case were replicates, they were not independent samples. Thus, this evidence does not demonstrate overall statistical significance.

Trial 3

Graph 3: Impact of Garlic Extract on Biofilm Formation (Trial 3)



The graph above has the same format and notation as that in Trial 1.

In Graph 3, little difference is apparent at a concentration of 64 µL / mL, though the experimental samples may have shown slightly more growth. However, at 2.0, 8.0, and 16 µL / mL, the experimental samples showed much less biofilm formation than the associated vehicle controls. Error bars suggest that these three differences are significant, though T-Tests will be used to know for sure. This sharply contrasts the results of Trials 1 and 2.

Table 3: Trial 3 Statistical Analysis

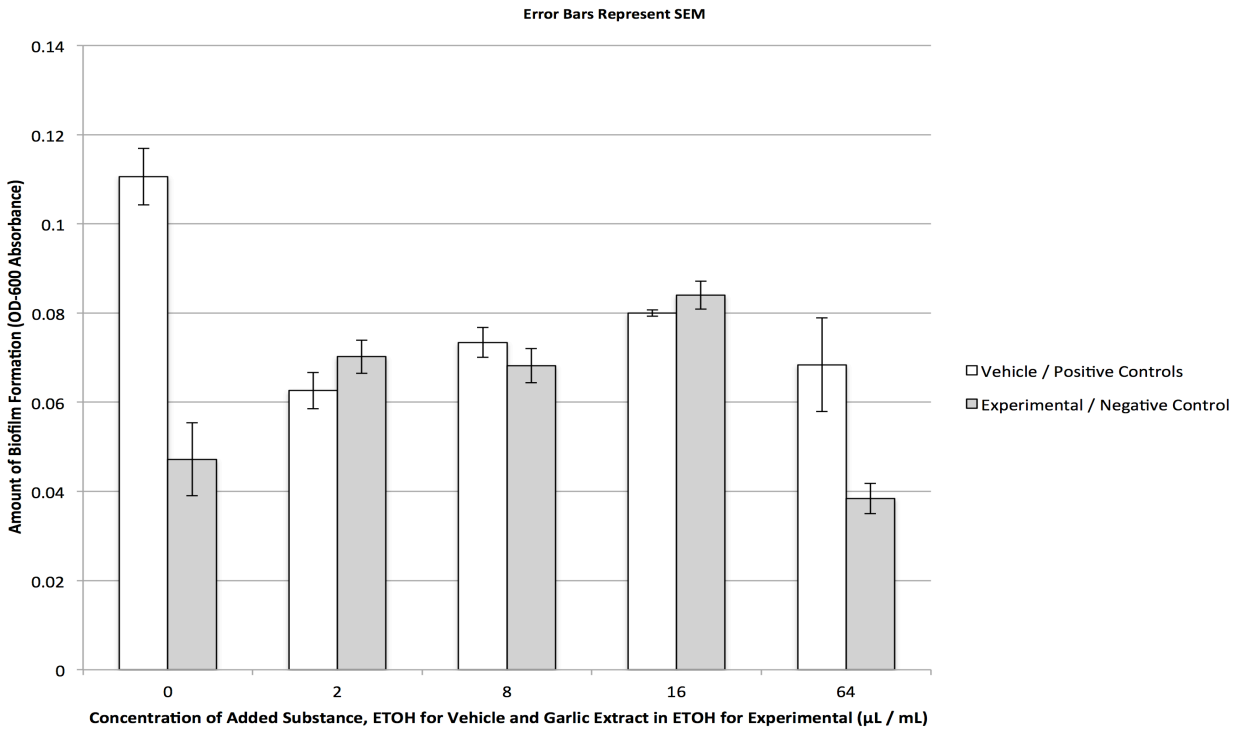
T-Test Table	NC	PC	E1	E2	E3	E4	V1	V2	V3	V4
NC	1.000									
PC	0.001	1.000								
E1	0.001	0.663	1.000							
E2	0.009	0.093	0.076	1.000						
E3	0.007	0.016	0.015	0.035	1.000					
E4	0.001	0.068	0.120	0.039	0.012	1.000				
V1	0.000	0.000	0.000	0.000	0.000	0.000	1.000			
V2	0.000	0.000	0.000	0.000	0.000	0.000	0.001	1.000		
V3	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.153	1.000	
V4	0.001	0.004	0.004	0.021	0.010	0.048	0.000	0.000	0.000	1.000

The table above has the same format and notation as that in Trial 1.

The p values shown in the boxed sections of Table 3 reveal that the differences apparent in Figure 3 (at all concentrations) are indeed statistically significant. However, because the samples in this case were replicates, they were not independent samples. Therefore, this does not demonstrate overall statistical significance.

Trial 4

Graph 4: Impact of Garlic Extract on Biofilm Formation (Trial 4)



The graph above has the same format and notation as that in Trial 1.

In Graph 4, little difference is apparent at any concentration, except for at 64 µL / mL. There, there was more biofilm formation in the vehicle control. Error bars suggest that this difference is significant, though T-Tests will be used to know for sure. At 2.0 and 16 µL / mL, it appears there was slightly more biofilm formation in the experimental samples, with the reverse true for 8.0 µL / mL. These results are far less clear than those of the first three trials, though the difference at 64 µL / mL matches that in Trials 1 and 2.

Table 4: Trial 4 Statistical Analysis

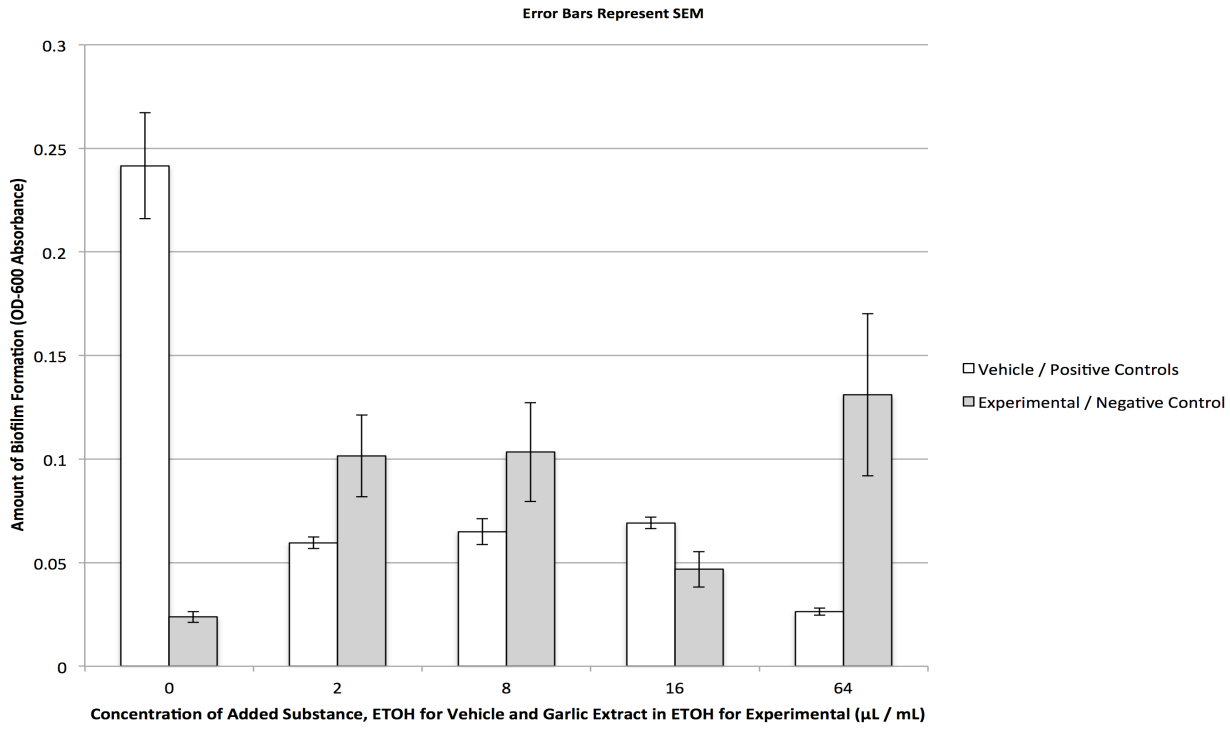
T-Test Table	NC	PC	E1	E2	E3	E4	V1	V2	V3	V4
NC	1.000									
PC	0.000	1.000								
E1	0.045	0.001	1.000							
E2	0.061	0.001	0.718	1.000						
E3	0.008	0.010	0.023	0.013	1.000					
E4	0.362	0.000	0.000	0.000	0.000	1.000				
V1	0.143	0.000	0.205	0.345	0.004	0.002	1.000			
V2	0.029	0.002	0.539	0.336	0.049	0.000	0.075	1.000		
V3	0.016	0.008	0.057	0.036	0.275	0.000	0.012	0.118	1.000	
V4	0.152	0.012	0.878	0.986	0.217	0.043	0.627	0.669	0.331	1.000

The table above has the same format and notation as that in Trial 1.

The p values shown in the boxed sections of Table 4 reveal that the difference apparent in Figure 4 at a concentration of 64 μL / mL is indeed statistically significant. However, because the samples in this case were replicates, they were not independent samples. Therefore, this does not demonstrate overall statistical significance.

Trial 5

Graph 5: Impact of Garlic Extract on Biofilm Formation (Trial 5)



The graph above has the same format and notation as that in Trial 1.

In Graph 5, there seems to have been more biofilm formation in experimental groups at concentrations of 2.0, 8.0, and 64 µL / mL. On the other hand, at 16 µL / mL, there appears to be less growth in the experimental samples. Error bars suggest that the three differences at 2.0 and 64 µL / mL may be significant, though T-Tests will be used to know for sure. The results at 2.0 µL / mL contrast those from Trials 2 and 3, and those at 64 µL / mL contradict those from Trials 1, 2, and 4. Some trials were excluded from those comparisons due to a lack of substantial difference between experimental and vehicle control samples.

Table 5: Trial 5 Statistical Analysis

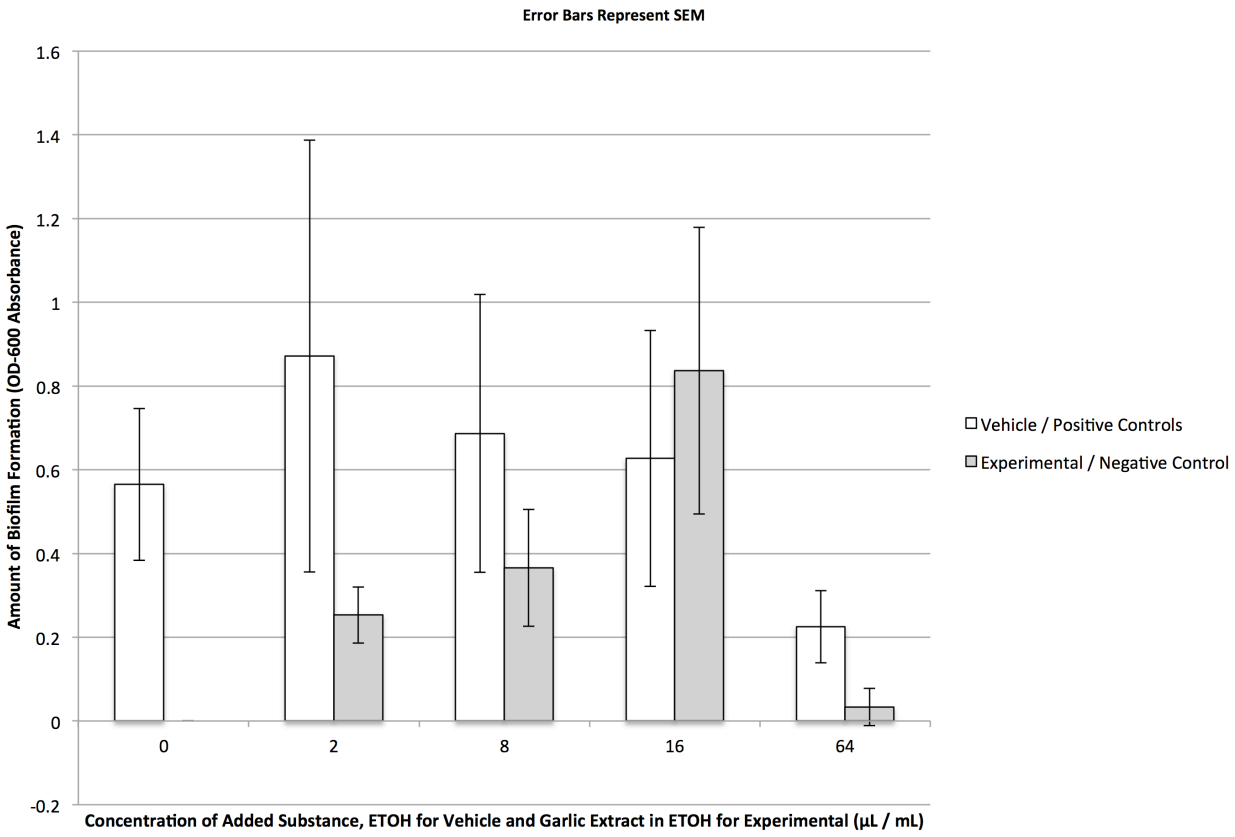
T-Test Table	NC	PC	E1	E2	E3	E4	V1	V2	V3	V4
NC	1.000									
PC	0.001	1.000								
E1	0.016	0.003	1.000							
E2	0.028	0.004	0.955	1.000						
E3	0.053	0.001	0.048	0.076	1.000					
E4	0.052	0.050	0.528	0.567	0.097	1.000				
V1	0.000	0.002	0.100	0.140	0.218	0.142	1.000			
V2	0.001	0.002	0.140	0.186	0.130	0.168	0.467	1.000		
V3	0.000	0.002	0.177	0.226	0.058	0.190	0.041	0.566	1.000	
V4	0.432	0.001	0.019	0.032	0.076	0.056	0.000	0.003	0.000	1.000

The table above has the same format and notation as that in Trial 1.

These p-values indicate that none of the differences observed in Figure 5 are statistically significant if replicates are taken as independent samples.

Overall

Graph 6: Impact of Garlic Extract on Biofilm Formation (All Trials)



The graph above has the same format and notation as that in Trial 1.

Graph 6, which combines data from all five trials, reveals that the differences between experimental and vehicle control samples are small compared to the variations represented by error bars. The data from the five trials were normalized before combined. Normalization was done by subtracting from the average readings in each trial the average value from the negative control and then dividing by the average value for the positive control.

Table 6: Statistical Analysis over Both Trials

T-Test Table	NC	PC	E1	E2	E3	E4	V1	V2	V3	V4
NC	1.000									
PC	0.010	1.000								
E1	0.005	0.056	1.000							
E2	0.019	0.147	0.416	1.000						
E3	0.065	0.861	0.199	0.306	1.000					
E4	0.257	0.017	0.176	0.095	0.105	1.000				
V1	0.125	0.822	0.288	0.389	0.936	0.180	1.000			
V2	0.076	0.868	0.269	0.430	0.782	0.132	0.758	1.000		
V3	0.073	0.733	0.293	0.487	0.683	0.135	0.681	0.897	1.000	
V4	0.064	0.028	0.356	0.176	0.139	0.572	0.222	0.181	0.190	1.000

The table above has the same format and notation as that in Trial 1.

The p values shown in the boxed sections of Table 3 reveal that the differences are not statistically significant.

Conclusion

Overall, these results do not conclusively answer the question of whether garlic extract suppresses biofilm formation by *E. coli* K-12 MG1655. The lack of statistically significant differences means that they fail to support the hypothesis, but that same uncertainty also means that they fail to refute it. The lack of significance could stem from methodological problems, so significant results were expected but not guaranteed. This means that the hypothesis could still be correct.

Despite this uncertainty, they do suggest that garlic extract at 64 μL / mL, might reduce biofilm formation. Surprisingly, they also indicate that at a concentration of 16 μL / mL, garlic extract may promote biofilm formation.

Across the trials that were conducted, which were conducted on different weeks and are therefore considered independent, the samples with 64 μL / mL garlic extract, which was dissolved in 95% ethanol, generally (in trials 1, 2, and 4) showed less biofilm formation than those with an equal amount of 95% ethanol. This can be seen in Graphs 1 -5 as the differences in white and gray bars at 64 μL / mL, and it suggests that the garlic extract may have been effective in inhibiting biofilm formation.

On the other hand, many trials (trials 1, 2, and 4) also showed an increase in biofilm formation at 16 μL / mL in the experimental samples compared to the vehicle controls. This is evident in Graphs 1-5 as the differences between white and gray bars at 16 μL / mL, and it suggests that garlic extract may have promoted biofilm growth at this concentration. However, for both of these suggestions, there was no consensus across all trials.

Additionally, T-Tests on the replicates from the trials (shown in the boxed sections of Tables 1-5) show that the differences at 16 and 64 μL / mL are generally statistically significant. However, because the samples used in a T-Test need to be independent, unlike the replicates used, the p values only indicate that the difference measured is likely not due to random variation within the trial. Variations affecting the entire group of samples could still be at play, which is why multiple trials were performed. Many trials found the same results, which suggests that the differences in the graphs reflect differences in the broader population, but T-Tests using the trials as independent samples (shown in the boxed sections of Table 6) indicate that the differences were not statistically significant. However, the similarities among trials suggest that a methodology with less variation might reveal significant differences.

While these results reveal no definite link between garlic extract concentration and biofilm formation, they do show that such a link may still exist. That suggestion marks garlic extract as a potential candidate for new antibiotics designed to thwart antibiotic resistance. Future research on garlic extract may produce powerful new antibiotic compounds to help treat an increasingly dangerous epidemic of antibiotic-resistant bacteria. Additionally, these results warn that if used improperly, such as at too low of a dosage, garlic extract or its derivative medicines could help bacteria form the very antibiotic-resistant biofilms the medication is designed to prevent. This warning may help dissuade patients from skipping their medications and avoid life-threatening resurgences of diseases. Ultimately, however, these results are inconclusive and fail to either support or refute the initial hypothesis.

Discussion (Findings Part C)

At this point, empirical research has been presented and conclusions have been drawn in accordance with the scientific method, but that methodology itself has not been justified. Those without a scientific background might argue that the practice of science is best left to experts and not everyday citizens, and some philosophers might argue that science's reliance on experience and subjective perceptions makes it unreliable. However, based on the philosophy of science, which forms "the logic with which scientific claims are grounded,"[19] everyone should participate in the scientific process because it offers the most reliable way to learn about the reality we live in.

Some logicians might object that the philosophy of science relies on assumptions that cannot be proven, but while they cannot be proven, there is justification for making them. Firstly, it assumes that an objective reality exists, or at least that the universe behaves as if one did. This is an assumption most everyone has to make, for human coexistence is impossible unless everyone lives in the same world, and a subjective reality by definition[20] differs for each person. Additionally, if no objective reality exists, then asking questions about the world is pointless since the answers would vary for everyone. Secondly, it assumes that reality is intelligible. If it were not, logical arguments about reality would be baseless, and prediction of the future—and therefore control of one's destiny—would be impossible. Additionally, this assumption fits with human intuition even if a proof is impossible. The universe does seem to be reasonable. Finally, it assumes that human perception is tainted by the subjectivity inherent in the human condition,

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[19] Stemwedel, 2014
[20] Stemwedel, 2011

specifically in humans' physical imperfections, and that some parts of our perception stem from an objective reality while others stem from human bias. This assumption explains why people only agree on some things. Areas of agreement are due to reality while areas of disagreement are caused by variations in subjectivity between individuals. It is also supported by the arguments of philosophers of science like Popper, who recognized that "no observation is free from the possibility of error."[21] Therefore, while the philosophy of science does rest on assumptions, those assumptions are reasonable and justified.

Research is inherently valuable because the perspective it contributes aids our societal quest for understanding our universe. In order to improve human life—a goal I assume to be morally right and worthy of attention—we need to be able to control, at least to some extent, the future. The past and the present are both immutable, so the future is the only realm in which we can effect changes that will help people. To control the future, we must understand how it is dictated, and assuming that an objective, intelligible reality exists, that reality will dictate our future since we live within it. Therefore, in order to control our future and improve human life, we must seek an understanding of the objective reality we live in. My research is one example. It highlights the impact of garlic extract on E. coli as an interesting area for future research, and if further research supports the hypothesis, garlic extract could end up being used to fight disease. A drug derived from garlic extract might be used to fight antibiotic-resistant infections in the lungs of cystic fibrosis patients or around medical implants, and it could also be used to sterilize surfaces in hospitals or fresh produce. Just like how my research into garlic extract could spawn products to protect human health, research is valuable because it helps improve people's lives.

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[21] Thornton, 2016

Anti-realists could argue that while objective reality seems to exist, that view can never be proven. Even if every possible test supports the idea that we live in an objective reality, our universe could still be an illusion whose physical laws are constant but artificial. There is no way to disprove this possibility because a perfect illusion by definition cannot be shown to be false; however, given that we seek to understand reality so we can affect the future, this possibility does not matter. If we lived in a perfect illusion, then models assuming an objective reality would predict the future, and therefore help us control it, just as well as if our universe were reality. This is a view supported by Stephen Hawking, called model-dependent realism, which circumvents the argument between "realist and anti-realist schools of thought"[22] over whether an objective reality exists. Instead, it claims that "it is pointless to ask whether a model is real, only whether it agrees with observations."[23] As long as it does, it will work to predict, and therefore control, the future. This means that while it is impossible to prove that an objective reality exists, assuming that one does is reasonable because, as a model, it works well to explain observations.

Science's reliance on experience, or inductive reasoning, is difficult to accept because induction is not logically guaranteed. However, there is no alternative. Generally, there are two ways to logically prove claims: inductively and deductively. Inductive reasoning draws conclusions from a variety of supporting examples. For example, if an observer sees only red apples, they might inductively conclude that all apples are red. This conclusion is not guaranteed, since there are of course green apples, because it is based on limited data. There could always be contradictory data that one has yet to find. On the other hand, deductive reasoning applies an assumed statement to an example of it. For instance, if one knows that all apples are red and is

[22] Hawking & Mlodinow, 2010
[23] Hawking & Mlodinow, 2010

asked what color a given apple is, they can confidently say it is red. The results of deductive reasoning are guaranteed to be true, given that its assumptions are true, since if all apples are red and there is a given apple, it must be red. Deduction, though, is crippled by its reliance on assumed statements,[24] which have to come from induction. Therefore, while deduction is more logically sound, we ultimately have to use induction to learn about our surroundings because we are given examples, not statements of fact.

Science is reliable because it represents a framework for logically sound reasoning despite the "shaky"[25] process of induction. Karl Popper achieved this by eliminating the idea of "proof" in science. Instead, he argued that science could only draw definitive conclusions about which hypotheses are wrong. Given evidence that contradicts a hypothesis, one can, "via deductive inference"[26] conclude it is false. The opposite, however, cannot be said. "It is logically impossible to conclusively verify a universal proposition by reference to experience."[27] Even the most widely accepted theory cannot be considered true; it has just "received a high measure of corroboration, and may be provisionally retained as the best available theory until it is finally falsified … or superseded."[28] Importantly, this does not mean induction is invalid in science. Rather, induction is used frequently. Its conclusions are just tentative.[29] This may not be a satisfying route to truth, but it does provide a reliable way for scientists to develop a clearer picture of reality without making potentially false assumptions like induction.

Philosophers of science have also devised ways to overcome the subjectivity in human perceptions, making scientific conclusions more reliable. Assuming that human perceptions are a

[24] Stemwedel, 2007
[25] Stemwedel, 2014
[26] Stemwedel, 2014
[27] Thornton, 2016
[28] Thornton, 2016
[29] Stemwedel, 2007

mixture of objective and subjective portions, those portions can be separated by comparing perceptions. Since everyone has their own subjectivities, areas of wide disagreement can be taken as subjective ones. For instance, since people may have many different reactions to a film, the quality of a film is likely subjective. Areas of wide agreement, on the other hand, are likely indicative of objective truth. For example, since most everyone will agree on how long a film is, its duration is likely an objective fact. One can "find out the difference between objective facts and subjective impressions … by actually sharing a world with other people whose subjective impressions … differ from our own,"[30] and the more agreement exists the more likely an idea is to be objective truth. Applied to science, this means that the more people there are participating in scientific inquiry, the more subjectivity is weeded out. Together, the solutions philosophers of science have devised for induction and subjectivity form the logical foundation of the practice of science.

That foundation is evident in the structure of the scientific method. "Scientific claims are falsifiable"[31] so they can be deductively disproven, and scientists seek contradictory evidence rather than inductive support. Additionally, the peer review process—including both the formal process for publication and the ensuing discussions in the scientific community—acts to reduce subjectivity by involving many experts. For example, scientific results must be reproducible so that the results are the same "no matter who conducts the experiment, and whether she conducts the experiment in this lab or some other lab."[32] A result's logic is also checked before publication by formal peer reviews—in which "manuscripts that have been submitted to journal editors are then sent to reviewers with relevant expertise for their evaluation"[33]—to reduce biased

[30] Stemwedel, 2011
[31] Stemwedel, 2006
[32] Stemwedel, 2011
[33] Stemwedel, 2011

arguments. After publication, the broader community of scientists also seeks to reproduce its findings to check for subjectivity in how experiments are conducted. These examples of scientific methodology all demonstrate how modern science is built upon the framework developed by the philosophy of science.

Because the scientific method relies on incorporating many perspectives, contributing to scientific discussions helps support them. Just like how the six blind men in an Indian folk tale must combine their perceptions to overcome the limitations of their senses,[34] we can create a more accurate understanding of reality by combining the results of research by many different people. My research, for example, is valuable because the data it contributes may help focus future research and add another perspective to the broader scientific discussion.

Some logicians may argue that science's reliability is destroyed by the fact that "no observation is free from the possibility of error."[35] Statistical tests like T-Tests based around the 95% confidence interval help, but there is always a chance that results are not in fact indicative of real differences. However, this is why "in practice a single … counter-instance is never sufficient methodologically to falsify a theory"[36] and even disproval is tentative. For example, had the results for this research shown with statistically significant differences that garlic extract promotes biofilm formation at all concentrations, the possibility would still exist that those were the results of chance.

This is why scientific methodologies strive to eliminate as much error as possible. In my research for instance, positive controls provided a benchmark against which all results were compared, eliminating many environmental variations that might have affected the growth of all

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[34] Blubaugh, 1965
[35] Thornton, 2016
[36] Thornton, 2016

samples. Similarly, the low optical density readings of negative controls show that there was negligible contaminating bacterial growth. Additionally, the statistical tests on the replicates in each trial indicate that most of the differences shown are unlikely to be caused by random variations between those replicates. Had I found significant differences, these safeguards would have enhanced the reliability of the resulting conclusions. As it is, the lack of significance despite safeguards raises the possibility that the hypothesis is false.

The tentative conclusions science draws can also be seen in the interpretation of my data. Their inconclusiveness means that they fail to support my hypothesis; however, that is not the same as refuting the hypothesis because it is entirely possible that the methodologies used were simply not precise enough to detect the difference that exists. Had I found significant differences showing that garlic extract promoted biofilm formation at all concentrations, those results would refute my hypothesis, though not conclusively since they would represent only a single counter-example. Generally, "absence of evidence is not evidence of absence."[37] This aphorism does not apply when one has "looked carefully for something that is predicted … and not observed anything,"[38] but that is not the case with my research for I have only searched in one way. Therefore, the lack of significant differences is not evidence of garlic extract's inefficacy and so does not constitute a refutation of my hypothesis.

Because of research's valuable contribution to the improvement of human life, everyone should support it. Local, state, and national governments should do so by funding scientific research. Universities and other research institutions should support their faculty, and research groups and individuals should carry out research. Finally, even those not directly involved with

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[37] Pickering, 2015
[38] Pickering, 2015

research should critically analyze the ideas others present, especially when they are presented as being scientific. Non-scientific intellectuals ("humanists"[39]), for instance, should "engage with it [science] critically."[40] They should challenge scientists' assumptions and search for flaws in research to further eliminate subjectivity and further enhance the reliability of science's findings. Everyday citizens, regardless of their trainings, should also apply careful reason to scientists' findings and the conclusions drawn from them by policy-makers, companies, and the media. The reliability of scientific theories rests upon them being critically tested, and their utility to everyday life rests upon their faithful application. Critical analysis of theories and their applications is essential for both of these processes, and since scientific inquiry is a powerful tool for the betterment of human lives, it should be supported by all.

[39] Horgan, 2013
[40] Horgan, 2013

Works Cited

Absalon, C., Van Dellen, K., & Watnick, P. I. (2011). A Communal Bacterial Adhesin Anchors Biofilm and Bystander Cells to Surfaces. *PLoS Pathogens*, *7*(8), e1002210. https://doi.org/10.1371/journal.ppat.1002210

Annous, B. A., Fratamico, P. M., & Smith, J. L. (2009). Scientific Status Summary. *Journal of Food Science*, *74*(1), R24–R37. https://doi.org/10.1111/j.1750-3841.2008.01022.x

Bertani, G. (2004). Lysogeny at Mid-Twentieth Century: P1, P2, and Other Experimental Systems. *Journal of Bacteriology*, *186*(3), 595–600. https://doi.org/10.1128/JB.186.3.595-600.2004

Blubaugh, D. (1965, 1957). Folk Tale: The Blind Men and the Elephant. Retrieved November 7, 2016, from https://www.peacecorps.gov/educators/resources/story-blind-men-and-elephant/

Hawking, S., & Mlodinow, L. (2010). *The grand design*. New York: Bantam Books.

Horgan, J. (2013, August 14). Should the Humanities Embrace Scientism? My Postmodern Response to Pinker’s Patronizing “Plea” Retrieved February 7, 2017, from https://blogs.scientificamerican.com/cross-check/should-the-humanities-embrace-scientism-my-postmodern-response-to-pinker-8217-s-patronizing-8220-plea-8221/

How bacteria "talk." (2009). TED. Retrieved from http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate?language=en#t-569373

Jakobsen, T. H., van Gennip, M., Phipps, R. K., Shanmugham, M. S., Christensen, L. D., Alhede, M., … Givskov, M. (2012). Ajoene, a Sulfur-Rich Molecule from Garlic, Inhibits Genes Controlled by Quorum Sensing. *Antimicrobial Agents and Chemotherapy*, *56*(5), 2314–2325. https://doi.org/10.1128/AAC.05919-11

O'Loughlin, C. T., Miller, L. C., Siryaporn, A., Drescher, K., Semmelhack, M. F., & Bassler, B. L. (2013). A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation. *Proceedings of the National Academy of Sciences*, *110*(44), 17981–17986. https://doi.org/10.1073/pnas.1316981110

Pickering, N. (2015). When Lack of Evidence Is Evidence of Lack. *Journal of Bioethical Inquiry*, *12*(4), 545–547. https://doi.org/10.1007/s11673-015-9675-z

Rasmussen, T. B., Bjarnsholt, T., Skindersoe, M. E., Hentzer, M., Kristoffersen, P., Kote, M., … Givskov, M. (2005). Screening for Quorum-Sensing Inhibitors (QSI) by Use of a Novel Genetic System, the QSI Selector. *Journal of Bacteriology*, *187*(5), 1799–1814. https://doi.org/10.1128/JB.187.5.1799-1814.2005

Redelman, C. V., Chakravarty, S., & Anderson, G. G. (2014). Antibiotic treatment of Pseudomonas aeruginosa biofilms stimulates expression of the magnesium transporter gene mgtE. *Microbiology*, *160*(Pt_1), 165–178. https://doi.org/10.1099/mic.0.070144-0

Stemwedel, J. D. (2006, December 2). Has the demarcation problem been solved? | Adventures in Ethics and Science. Retrieved February 4, 2017, from http://ethicsandscience.scientopia.org/2006/12/02/has-the-demarcation-problem-been-solved/

Stemwedel, J. D. (2007, February 26). What scientists believe and what they can prove (with a flowchart for Sir Karl Popper). | Adventures in Ethics and Science. Retrieved February 3, 2017, from http://ethicsandscience.scientopia.org/2007/02/26/what-scientists-believe-and-what-they-can-prove-with-a-flowchart-for-sir-karl-popper/

Stemwedel, J. D. (2011, July 20). The objectivity thing (or, why science is a team sport). Retrieved February 3, 2017, from https://blogs.scientificamerican.com/doing-good-science/httpblogsscientificamericancomdoing-good-science20110720the-objectivity-thing-or-why-science-is-a-team-sport/

Stemwedel, J. D. (2014a, April 4). What is philosophy of science (and should scientists care)? Retrieved from https://blogs.scientificamerican.com/doing-good-science/what-is-philosophy-of-science-and-should-scientists-care/

Stemwedel, J. D. (2014b, April 15). Pub-Style Science: philosophy, hypotheses, and the scientific method. Retrieved February 3, 2017, from https://blogs.scientificamerican.com/doing-good-science/pub-style-science-philosophy-hypotheses-and-the-scientific-method/

Tay, S., & Yew, W. (2013). Development of Quorum-Based Anti-Virulence Therapeutics Targeting Gram-Negative Bacterial Pathogens. *International Journal of Molecular Sciences*, *14*(8), 16570–16599. https://doi.org/10.3390/ijms140816570

Thornton, S. (2016). Karl Popper. In E. N. Zalta (Ed.), *The Stanford Encyclopedia of Philosophy* (Winter 2016). Metaphysics Research Lab, Stanford University. Retrieved from https://plato.stanford.edu/archives/win2016/entries/popper/

United States. Congress. House. Committee on Energy and Commerce. Subcommittee on Health. (2012). *Antibiotic resistance and the threat to public health : hearing before the Subcommittee on Health of the Committee on Energy and Commerce, House of Representatives, One Hundred Eleventh Congress, second session, April 28, 2010.* (Vols. 1–1 online resource (v, 1–127 pages) : illustrations). Washington : U.S. Government Printing Office,. Retrieved from http://purl.fdlp.gov/GPO/gpo34205

Works Consulted

10 Costly Food Recalls. (2009, March 12). Retrieved February 18, 2016, from http://money.howstuffworks.com/10-food-recalls.htm

Basnyat, B. (2014). Antibiotic resistance needs global solutions. *Lancet Infectious Diseases*, *14*(7), 549–550. Retrieved from https://ezproxy.butler.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&AuthType=ip&db=aph&AN=96785079&site=ehost-live&scope=site

Burnett, T. (2013, June 28). What is Scientism? Retrieved February 7, 2017, from https://www.aaas.org/page/what-scientism

Corcoran, M., Morris, D., De Lappe, N., O'Connor, J., Lalor, P., Dockery, P., & Cormican, M. (2014). Commonly Used Disinfectants Fail To Eradicate Salmonella enterica Biofilms from Food Contact Surface Materials. *Applied and Environmental Microbiology*, *80*(4), 1507–1514. https://doi.org/10.1128/AEM.03109-13

DeWaal, C., & Barlow, K. (2004, March). Outbreak Alert! Center for Science in the Public Interest.

Glossary Definition: Scientism. (n.d.). Retrieved February 7, 2017, from http://www.pbs.org/faithandreason/gengloss/sciism-body.html

Halverson, D. (2016, April 12). The Poverty of Scientism. Retrieved from https://www.partiallyexaminedlife.com/2016/04/12/the-poverty-of-scientism/

Harlander, S. (2002). Safety Assessments and Public Concern for Genetically Modified Food Products: The American View. *Toxicologic Pathology*, *30*(1), 132–134.

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the Phyllosphere. *Applied and Environmental Microbiology*, *69*(4), 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003

Manager, M. M. T. P. C. T. (n.d.). Statement From Foodborne Illness Victim Dana Dziadul. Retrieved February 11, 2016, from http://bit.ly/1x4ZsGk

Midelet, G., & Carpentier, B. (2004). Impact of cleaning and disinfection agents on biofilm structure and on microbial transfer to a solid model food*. *Journal of Applied Microbiology*, *97*(2), 262–270. https://doi.org/10.1111/j.1365-2672.2004.02296.x

Naganawa, R., Iwata, N., Ishikawa, K., Fukuda, H., Fujino, T., & Suzuki, A. (1996). Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. *Applied and Environmental Microbiology*, *62*(11), 4238–4242.

Number and Incidence of Infections by Year, 1996-2014 | FoodNet | CDC. (2015a, May 14). Retrieved February 10, 2016, from http://www.cdc.gov/foodnet/trends/2014/number-of-infections-by-year-1996-2014.html#table2b

Opal, S. M. (2007). Communal Living by Bacteria and the Pathogenesis of Urinary Tract Infections. *PLoS Medicine*, *4*(12), e349. https://doi.org/10.1371/journal.pmed.0040349

Parsek, M. R., & Greenberg, E. P. (2005). Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends in Microbiology*, *13*(1), 27–33. https://doi.org/10.1016/j.tim.2004.11.007

PM, D. D. O. 7/17/14 at 3:13. (2014, July 17). Silencing Slime. Retrieved February 19, 2016, from http://www.newsweek.com/2014/07/25/spray-keep-bacteria-laden-biofilms-growing-food-259323.html

Redelman, C. V., Hawkins, M. A. W., Drumwright, F. R., Ransdell, B., Marrs, K., & Anderson, G. G. (2012). Inquiry-based examination of chemical disruption of bacterial biofilms. *Biochemistry and Molecular Biology Education*, *40*(3), 191–197. https://doi.org/10.1002/bmb.20595

Salmonella Biofilms Extremely Resistant to Disinfectants. (2014, January 23). Retrieved February 11, 2016, from http://www.foodsafetynews.com/2014/01/salmonella-biofilms-extremely-resistant-to-disinfectants/

Salmonella Homepage. (2016, February 3). Retrieved February 10, 2016, from http://www.cdc.gov/salmonella/

Shetty, S., Thomas, B., Shetty, V., Bhandary, R., & Shetty, R. (2013). An in-vitro evaluation of the efficacy of garlic extract as an antimicrobial agent on periodontal pathogens: A microbiological study. *AYU (An International Quarterly Journal of Research in Ayurveda)*, *34*(4), 445. https://doi.org/10.4103/0974-8520.127732

Stockwell, V., Granatstein, D., & Grieshop, M. (2013, January). Antibiotic Residues on Plant Tissues. WSU Tree Fruit Research and Extension Center. Retrieved from http://www.tfrec.wsu.edu/pdfs/P2632.pdf

Technical Information | General Information | Salmonella | CDC. (2015b, March 9). Retrieved February 10, 2016, from http://www.cdc.gov/salmonella/general/technical.html

US Department of Commerce, B. E. A. (n.d.). Bureau of Economic Analysis. Retrieved February 11, 2016, from http://www.bea.gov/industry/gdpbyind_data.htm

What you need to know about OD600. (n.d.). Retrieved from http://bitesizebio.com/1005/what-you-need-to-know-about-od600/

WHO Estimates of the Global Burden of Foodborne Diseases. (2015). World Health Organization. Retrieved from http://apps.who.int/iris/bitstream/10665/200046/1/WHO_FOS_15.02_eng.pdf

Outcomes

In a break from the standard progression of the Global Scholars Program, I began working on what would become my project in the summer before my junior year. This was partly because of the dual nature of my work. My research was initially completed through the science-based LOGOS program and then later applied to Global Scholars, where I examined the philosophical justification for the scientific process. My initial inspiration came from an article in *Scientific American* about bacterial food collection, and during the Young Innovators Quest (YiQ) summer camp, that idea evolved into communication among cancer cells, bacterial communication, and finally quorum sensing. Through the ensuing YiQ Scholars program, I narrowed my topic to agricultural bacteria to arrive at my final topic of *E. coli* biofilms. This long period of topic development and refinement allowed me to start research with a strong foundation in my field. Additionally, discussing my ideas with my mentor and YiQ staff helped me identify a feasible project that I could also invest myself in. Without such a long period of preparation, the process as a whole would have been more difficult.

One of the major challenges I encountered when conducting research was a simple one: logistics. Unlike most Global Scholars projects, my research required a physical presence; instead of being able to research anywhere with an internet connection or at a library, I needed a lab space and equipment to run my experiments. Additionally, I needed to be supervised while researching, and my research was time-consuming. Thankfully, Park Tudor and YiQ were able to provide lab space and equipment, and Mr. Mark Dewart and Dr. Kristin Chun were able to supervise me. However, had they been unavailable or had I been unable to acquire the necessary

resources, my research would have been impossible. Still, even with their help, my research required a massive time commitment. I spent over 150 hours in the lab, and that excludes the time it took to conduct background research, write protocols, and analyze data. Even after spending a couple hours a day conducting experiments during sthe summer and the early part of the school year, I had more work to do when it came time to focus on college applications. I had to set my research aside and return to it after early application deadlines. That balancing of priorities and fluctuation of foci over time was characteristic of my Global Scholars experience as a whole.

One reason for the time-consuming nature of my research was the nature of my research itself; each trial took five days to run, with each day taking between one and three hours of lab work. Additional delays came from early failures. Contamination plagued my early work, which led to my sterilizing media at the last possible moment and flaming the openings of containers. I also saw an inexplicable lack of growth in some cases, which turned out to be due to soap residue, and when variation was masking whatever trends might exist, I systematically narrowed down the cause to reusing test tubes. In each of these cases, I learned to not be intimidated by failure but to take steps to diagnose or overcome it even when I am not sure what to do.

To help overcome and avoid those failures, I relied on advice from others. I reviewed my protocols with my mom at home to identify oversights, which helped experiments go more smoothly. I also met with Dr. Anderson to hear his thoughts on experimental techniques I was considering, and he responded via email to confusing data with potential explanations or troubleshooting ideas. This was one of my most important lessons: asking for help. Others' input helped me avoid ruts in my thinking, expediting my work. While I might have wanted to tackle

problems on my own, to not ask for help would have been to waste incredibly valuable resources.

Once experiments started completing successfully, I began analyzing the data they produced. Since I was aware of the biases implicit in the human condition—which argues that the animalistic portions of our nature, versus the rationality of our brains, distorts our perceptions—, I began with a null hypothesis and assumed there was no link between garlic extract and biofilm formation. I used quantitative optical density readings instead of qualitative impressions from sample color or ring thickness because their objectivity helped reduce bias. Furthermore, I evaluated the possibility that random chance was causing differences by running statistical analyses on my data. I focused on objective, empirical techniques to answer my question because I distrusted myself to eliminate the preconceived notions and desires that could taint my thought processes. That way, I, and hopefully others, could have confidence in the validity of my findings, whatever they would be.

Finally, after the data had been collected and analyzed, it came time to share my results by writing a paper to explain them (this document is one form of that paper). To prepare for that eventual end to the project, I began organizing relevant materials early. I stored scientific papers and other sources in Zotero, a free and open-source citation manager, to keep their citation information and my annotations ordered. That allowed me to easily switch between formatting styles and to quickly find the notes I had made on a source from months before. When I began conducting my own experiments, I kept a lab notebook with a minute-by-minute accounting of what I did and my results. That provided documentation of my work and let me review earlier procedures when conducting new experiments. My extended timeline demanded this kind of

thoroughness because when I actually began writing my paper, it had been almost a year since I had started researching. On the other hand, because of my participation in the LOGOS program, the body of my paper was due a month or so before the Global Scholars version. That pushed me to start writing earlier, avoiding a late panic to finish.

My work on this project has helped me better understand myself, and that understanding stems from my observations about how I reacted to the challenges of my work. I spent over a year on this project, including many long afternoons carrying out experiments after school and numerous setbacks from contamination to variation to an ultimate lack of statistical significance. In the end, my results were inconclusive, yet I continued my work and would do so again even knowing the end result. One reason for this perseverance was my view of challenges as learning experiences and ways to test my abilities. I learned a variety of technical skills—such as how to maintain sterility of samples—and about my limits—for example, my concentration began to lapse after three hours of lab work. In addition, I learned that I was capable of more than I had thought before. I doubted I would be able to maintain focus on a project for this long, but I proved to myself that I could. Another reason I continued working was my deep-seated curiosity. My research fascinated me, so I wanted to find the answer to my hypothesis. Finally, I continued because I felt my work was meaningful. Creating new knowledge is a pursuit worthy of time and devotion, even if it serves only to spur other, more profound discoveries.

From these observations, I have been able to draw a number of conclusions about myself. First, I am reluctant to accept what I think I can do. I want to actually try tackling a challenge before I allow myself to be confident I can overcome it. Conversely, I am also hesitant to accept my limits. Even after failure, I want to push past perceived limits and broaden my abilities. I also

enjoy learning and want to learn more, whether it be about myself and my limits or about my surroundings. Finally, I have a desire to contribute to something beyond myself. For my work to be meaningful, I want it to impact an entity that surpasses and will outlast me, such as the societal knowledge science creates.

From this project, I also learned more about my view of truth. I have discovered that I distrust singular sources of information, such as myself about my limits and abilities or anecdotal and experiential evidence—this is why I collected data quantitatively instead of with qualitative judgements. I trust everyone but no one because it is a consensus that reduces subjectivity, not any individual. Therefore, I value adding my own perspective, and as a result strengthening any consensus, to existing conversations. That acceptance of the convergence of a plurality of sources also explains assumptions I was willing to make when starting my experiments. I did not, for example, verify myself that spectroscopy worked the way the literature says it does, and I assumed that my pipets accurately measured volume. These assumptions were based upon the conclusions of many experts, so I accepted them. At the same time, though, I consider all my conclusions, irrespective of the evidence supporting them, to be tentative. When confronted with illogical results, I was willing to second-guess my assumption that washing and re-using tubes would work and discover that re-using test tubes had introduced variation.

Despite how well my project turned out overall, there are a number of changes I might make were I to do it over again. For one, working in a professional lab would have made my work so much easier and faster. I learned a lot by making my own lab and running experiments with improvised equipment, but a professional lab might have let me push further with my work and learn more that way. I am not sure which way would be better, but I would like to try both.

Additionally, I would try to make my experiments shorter. Once they started working, I was loath to change protocols, but my experiments were so long that they were hard to schedule. Because of how many days they took, I had to work after school when I was more tired and likely to err. It would have been much more pleasant and efficient to run shorter experiments when I could focus better: on the weekends. Finally, I wish I had involved my mentor more with my work. While he offered great insight into my work and results, and while his advice was invaluable, I think I could have learned so much more had I worked more closely with him.

Overall, while I did not expect to make any earth-shattering discoveries, I wish my results were more definitive. They are not clear enough to conclude anything one way or the other, and the fact that the question is still open frustrates me. That frustration reveals my deep-seated desire to understand my world. Perhaps with better equipment or a better methodology, I could have gotten more compelling data. Even so, I am pleased with what I learned and with my performance, especially my time management. For a few months, my research easily dwarfed any of the many other significant commitments I had, but I still found a way to work it in without undue stress. That is an achievement I am proud of, and one I hope to repeat elsewhere.