

2018

# Testing Bacterial Antibiotic Production under Carbohydrate and Protein Starvation

Briley Baird  
*Ouachita Baptist University*

Follow this and additional works at: [https://scholarlycommons.obu.edu/honors\\_theses](https://scholarlycommons.obu.edu/honors_theses)

 Part of the [Bacteria Commons](#), [Biochemistry Commons](#), and the [Microbiology Commons](#)

---

## Recommended Citation

Baird, Briley, "Testing Bacterial Antibiotic Production under Carbohydrate and Protein Starvation" (2018). *Honors Theses*. 650.  
[https://scholarlycommons.obu.edu/honors\\_theses/650](https://scholarlycommons.obu.edu/honors_theses/650)

This Thesis is brought to you for free and open access by the Carl Goodson Honors Program at Scholarly Commons @ Ouachita. It has been accepted for inclusion in Honors Theses by an authorized administrator of Scholarly Commons @ Ouachita. For more information, please contact [mortensona@obu.edu](mailto:mortensona@obu.edu).

# **SENIOR THESIS APPROVAL**

This Honors thesis entitled

**“Testing Bacterial Antibiotic Production under Carbohydrate  
and Protein Starvation”**

written by

**Briley Baird**

and submitted in partial fulfillment of  
the requirements for completion of  
the Carl Goodson Honors Program  
meets the criteria for acceptance  
and has been approved by the undersigned readers.

Dr. Ruth Plymale, thesis director

Dr. Angela Douglass, second reader

Dr. Barbara Pemberton, third reader

Dr. Barbara Pemberton, Honors Program director

April 24, 2018

Testing bacterial antibiotic production under carbohydrate and protein starvation

An honors thesis by Briley Baird

Ouachita Baptist University

Spring 2018

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

## Summary

Bacteria produce antibiotics when they are under stress, including starvation stress. Bacteria were tested under carbohydrate and protein starvation against *Bacillus subtilis* and *Escherichia coli* (due to the respective Gram positivity and negativity), in order to check for antibiotic production. The bacteria being tested were isolated by past Microbiology classes and stored in a -80°C freezer in the basement of Jones Science Center at Ouachita Baptist University. These test bacteria were grown on tryptic soy agar (TSA) to produce isolated bacterial colonies. Samples of isolated test colonies were then grown under conditions of carbohydrate starvation (M9 salts agar with 0.1% glucose and 1% peptone) or protein starvation (M9 salts agar with 1% glucose and no peptone) in the presence of *Bacillus subtilis* and *Escherichia coli*. After five days of incubation at 28°C, antibiotic production by test bacteria was determined by measuring growth inhibition of *B. subtilis* or *E. coli*. Of the 27 bacteria tested, 10 were found to consistently produce the zones of inhibition. Three produced zones of inhibition under all conditions, two did so only under carbohydrate starvation conditions, and none only under protein starvation. Also, two bacteria only responded when grown in the presence of *B. subtilis*, while no bacteria responded when grown solely in the presence of *E. coli*.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

## Introduction

Bacteria are evolving. Every day, these tiny prokaryotes fight for survival against the multitudes of antibiotics specifically designed to kill them. Many lose the battle and die off. A lucky few, however, possess a genetic mutation that makes them resistant to the antibiotic. As the regular bacteria are killed by the antibiotics, this mutation allows the resistant bacteria to survive and thrive. Over time, the bacterial population changes to become resistant to antibiotics (Baym et al., 2016). Unfortunately for us, this process has created bacteria that are no longer affected by what we have always used to combat them. In keeping ourselves healthy, we have created antibiotic-resistant bacteria.

In August of 2016, a woman checked into a Reno, Nevada, hospital shortly after returning from India (Dall, 2017). Doctors discovered she was infected with a bacterium best known for causing urinary tract infections, *Klebsiella pneumoniae* (Branswell, 2017). In September of 2016, the woman died (Dall, 2017). This was due to the fact that the specific strain of *Klebsiella pneumoniae* was resistant to every single antibiotic available (Dall, 2017). Around the same time as the woman's death, the UN expressed an unprecedented amount of interest in combatting the global antibiotic resistance problem (Napolitano, 2016). Although this woman's infection may seem unique in being resistant to every available antibiotic, antibiotic resistant infections—bacterial infections untreatable by one or more antibiotics—are an increasing public health threat worldwide (Napolitano, 2016). Unless new antibiotics are isolated that can combat this growing issue, different species of bacteria will continue to become more resistant to our existing medicines until incidences like the Nevadan woman's death become commonplace.

For my thesis, I have contributed to a solution for this rising problem. Bacteria secrete antibiotics to inhibit the growth of other species of bacteria when they are starved (Cordero et al.,

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

2012). I have used this competition mechanism to my advantage to test the previously isolated antibiotic-producing bacteria for the ability to kill off known bacteria, specifically *Bacillus subtilis* and *Escherichia coli*. These bacteria were selected because they are common examples of the Gram + and Gram - bacteria, respectively. They were also selected because they are safe to handle.

Over the course of these experiments, I grew the test bacteria under carbohydrate and protein starvation. Some bacteria produced antibiotics to starvation of carbohydrates, while others respond to the starvation of proteins (Ochi and Ohsawa, 1984), (Gallo and Katz, 1972). To ensure the production of their antibiotics, I starved them of both nutrients, separately, to make sure they are affected. These bacteria were tested against *B. subtilis* and *E. coli* because they are examples of the two most common types of bacteria. *B. subtilis* is Gram positive, whereas *E. coli* is Gram negative. Gram positive and Gram negative bacteria have different cell wall structures, and, resultantly, have different susceptibilities to antibiotics. If the antibiotics killed either, or both, of these bacteria, then the antibiotics could potentially work against antibiotic-resistant bacteria of the same type (Gram + vs Gram -).

In addition to this, I also used my thesis to discuss the effects of bacteria and biofilm formation on contact lenses. Biofilms are groups of bacteria surrounded by a mucus-like matrix . If biofilms form on contacts, that could easily lead to bacterial infections in the eyes (Bruinsma et al., 2001).

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

## Materials and Methods

Bacteria

Twenty-seven test bacteria were obtained from the -80 °C freezer located in the basement of Jones Science Center. These 27 bacteria had been isolated from soil samples by previous microbiology classes at Ouachita Baptist University, and had all been found to successfully produce antibiotics under certain conditions. However, they had not ever been tested under the set of conditions used in these experiments. These bacteria were: Hall, Feather, JLM, Red, Compton, Jackson, Hargis, Lewis, Small, Ach!, Run!, Bubbles, Janet, Smith, Darquez, Ewok, Lafawnduh, Blaneece, Games, LEO, Jamison, HegiBarfield, Archer, HolcombRubin, S2B1, S4B1, and S6B3.

Media

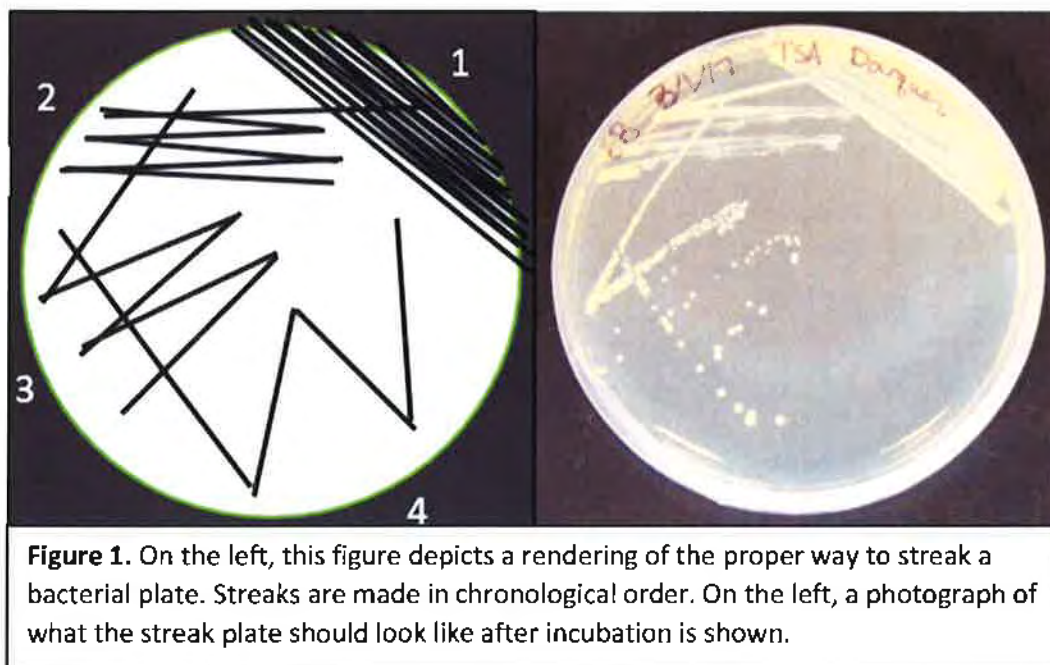
There were four different kinds of media used in these experiments. Tryptic Soy Agar (TSA) was used. Tryptic Soy Broth (TSB) was used. These were used for routine upkeep of the test bacteria. For the actual swab patches, M9 salts salts 1% Glucose – 0% Peptone Agar and M9 salts 0.1% Glucose – 1% Peptone Agar were used.

Growing Test Bacteria from Frozen Samples

Samples of all the needed bacteria were preserved in a -80 °C freezer since their original isolation. From these samples, the bacterial colonies needed for testing purposes were grown. Small amounts the frozen bacteria were streaked onto individual TSA plates. This was done in a sterile environment, created by the updraft of a flame from a Bunsen burner. They were streaked in the pattern depicted in Figure 1. The streak plates were incubated for five days at 25 °C.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

Afterwards, the freshly grown colonies were used to grow two other TSA streak plates labeled “primary” and “back-up.” All of the plates were then stored in a 4°C refrigerator.



### Swab patches

Following the growth of the streak plates, swab patches were made. In order to plate the background bacterium on the agar, *B. subtilis* and *E. coli* were grown in TSB for 24 hours at 37°C before making the swab patches. After the 24 hours, sterile cotton swabs were used to cover the entire surface of the agar with the desired bacterium. The agar plates were then allowed to sit for 15 minutes at room temperature to allow the bacteria to anchor themselves to the agar.

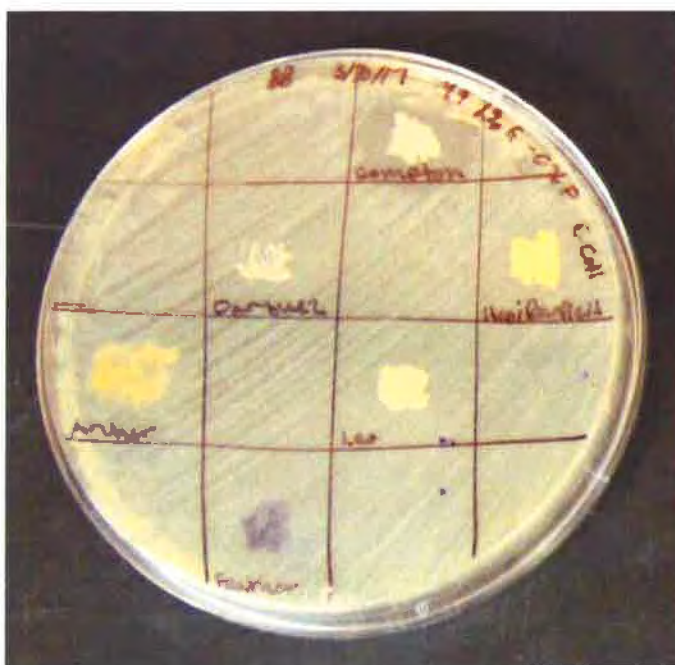
After the waiting period, the test bacteria were plated in a small square approximately 5mm in diameter with a sterilized loop on each of the four variations of medium-background bacteria combinations. Six bacteria were plated on each medium-background bacterium combination on each plate, creating the swab patches. They were plated in the pattern shown in Figure 2, with each test bacterial patch not being in direct contact with any other test bacterial



## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

patch. This entire process was also done beneath a Bunsen burner updraft to increase sterility. The swab-patch plates were then incubated for five days at 25°. After incubation, the swab-patch plates were examined for zones of inhibition around the different test bacteria. The diameters of each bacterial patch were measured as well as the diameters of each zone of inhibition plus bacterial patch, when applicable. All results were recorded, and each test bacterium was tested under all medium-background bacterium combinations three times for triplicate results.

The test bacteria were plated on two types of agar (M9 salts 1% Glucose – 0% Peptone and M9 salts 0.1% Glucose – 1% Peptone) in the presence of two types of background bacteria (*Bacillus subtilis* and *Escherichia coli*) for a total of four different kinds of swab patches: M9 salts 1% Glucose – 0% Peptone agar with *B. subtilis* background bacteria M9 salts 1% Glucose – 0% Peptone agar with *E. coli* background bacteria, M9 salts 0.1% Glucose – 1% Peptone with *B. subtilis* background bacteria, and M9 salts 0.1% Glucose – 1% Peptone with *E. coli* background bacteria.



**Figure 2.** This figure is a photograph of an incubated spread patch. As seen in the photo, three horizontal lines and three vertical lines are drawn on the casing to create twelve separate areas. Six different bacteria are plated with a sterile ring on each spread patch, making sure to place the diagonal from each other so that none of the bacterial squares are bordering each other. As shown in the photo, the bacteria are plated in a square shape. When the bacteria produce zones of inhibition, the fan out from the bacteria in circular shape, as seen with the bacterium labeled Compton.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

Each of the 27 test bacteria were assayed using swab-patch assays on each of the four medium-background bacterium combinations: M9 salts 1% Glucose – 0% Peptone agar with *B. subtilis* background, M9 salts 1% Glucose – 0% Peptone agar with *E. coli* background, M9 salts 0.1% Glucose – 1% Peptone with *B. subtilis* background, and M9 salts 0.1% Glucose – 1% Peptone with *E. coli* background. All of these tests were performed in triplicate.

Of the 27 bacteria, 17 did not produce antibiotics under any of the four medium-background bacterium combinations. The 17 that did not produce antibiotics are: Hall, Feather, JLM, Lewis, Ach!, Run!, Bubbles, Janet, Smith, Darquez, Ewok, Lafawduh, Games, LEO, HegiBarfield, HolcombRuhin, S2B1, and S6B3. The 10 bacteria that produced antibiotics under one or more sets of test conditions are: Red, Compton, Jackson, Hargis, Small, Blaneecce, Jamison, Archer, S2B1, and S6B3. The results from Trials 1, 2, and 3 are depicted in Tables 1, 2, and 3, respectively.

By averaging the results from the three trials, mean values for the antibiotic production zones created by the 10 bacteria were calculated. These values are depicted in Figure 3. Of the 10 bacteria that produced zones of inhibition, Compton, Jackson, Jamison, S2B1, and S6B3 produced antibiotics that inhibited both *B. subtilis* and *E. coli* under both peptide and carbohydrate starvation conditions. Red and Small both inhibited *B. subtilis* under carbohydrate and protein starvation, but only inhibited *E. coli* under peptide starvation. Hargis and Archer inhibited the growth of the *B. subtilis* under protein starvation and peptide starvation. Blaneecce responded to two of the test conditions, inhibiting growth of both *E. coli* and *B. subtilis* when starved of peptides but not when starved of carbohydrates.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

0.1% Glucose - 1% Peptone					1% Glucose – 0% Peptone				
Name	B. subtilis bckgrnd		E. coli bckgrnd		Name	B. subtilis bckgrnd		E. coli bckgrnd	
	Bacteria	Zone	Bacteria	Zone		Bacteria	Zone	Bacteria	Zone
Red	9mm	15mm	10mm	none	Red	7mm	14mm	10mm	28mm
Compton	4mm	none	6mm	none	Compton	6mm	14mm	7mm	14mm
Jackson	8mm	11mm	10mm	13mm	Jackson	6mm	9mm	9mm	20mm
Hargis	5mm	none	11mm	none	Hargis	8mm	9mm	7mm	none
Small	7mm	none	8mm	none	Small	5mm	none	9mm	13mm
Blaneece	5mm	none	6mm	none	Blaneece	8mm	10mm	10mm	none
Jamison	8mm	23mm	7mm	19mm	Jamison	4mm	11mm	7mm	10mm
Archer	8mm	16mm	5mm	none	Archer	5mm	10mm	12mm	none
S2B1	5mm	none	5mm	none	S2B1	4mm	6mm	5mm	25mm
S6B3	11mm	20mm	9mm	12mm	S6B3	5mm	9mm	10mm	25mm

**Table 1.** This table depicts the Trial 1 results of the 10 bacteria that produced antibiotics under any of the four sets of test conditions. The measurements listed refer to the diameter of the bacterial growth on the swab patches (denoted "Bacteria"), and, when applicable, the diameters of the bacterial growth on the swab patches plus the zone of inhibition (denoted "Zone"). "Glucose Starvation Conditions" refers the swab patches with M9 salts 0.1%G-1%P Agar. "Protein Starvation Conditions" refers to the swab patches with M9 salts 1%G-0%P Agar. Bacteria are arranged in the order that they were tested.

0.1% Glucose - 1% Peptone					Protein Starvation Conditions				
Name	B. subtilis bckgrnd		E. coli bckgrnd		Name	B. subtilis bckgrnd		E. coli bckgrnd	
	Bacteria	Zone	Bacteria	Zone		Bacteria	Zone	Bacteria	Zone
Red	8mm	10mm	10mm	none	Red	8mm	11mm	9mm	28mm
Compton	8mm	10mm	11mm	none	Compton	8mm	none	11mm	22mm
Jackson	8mm	16mm	15mm	17mm	Jackson	7mm	8mm	10mm	25mm
Hargis	8mm	12mm	10mm	none	Hargis	8mm	14mm	7mm	none
Small	13mm	19mm	10mm	none	Small	9mm	13mm	8mm	15mm
Blaneece	7mm	none	8mm	none	Blaneece	11mm	13mm	20mm	43mm
Jamison	8mm	20mm	7mm	16mm	Jamison	9mm	17mm	7mm	8mm
Archer	7mm	21mm	9mm	none	Archer	9mm	19mm	12mm	none
S2B1	7mm	17mm	11mm	25mm	S2B1	9mm	20mm	11mm	33mm
S6B3	12mm	28mm	11mm	18mm	S6B3	11mm	21mm	12mm	26mm

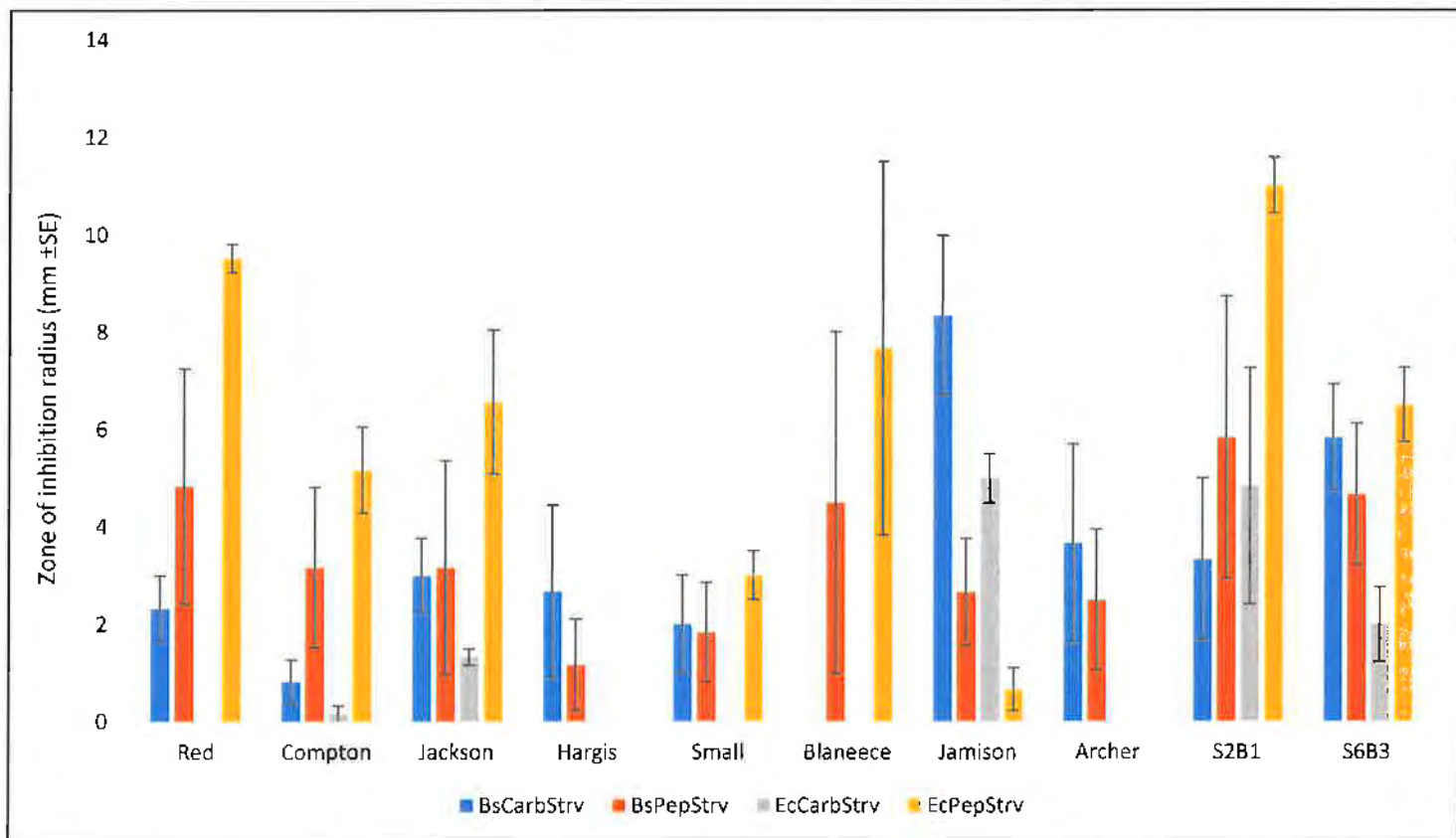
**Table 2.** This table depicts the Trial 2 results of the 10 bacteria that produced antibiotics under any of the four sets of test conditions. The measurements listed refer to the diameter of the bacterial growth on the swab patches (denoted "Bacteria"), and, when applicable, the diameters of the bacterial growth on the swab patches plus the zone of inhibition (denoted "Zone"). "Glucose Starvation Conditions" refers the swab patches with M9 salts 0.1%G-1%P Agar. "Protein Starvation Conditions" refers to the swab patches with M9 salts 1%G-0%P Agar. Bacteria are arranged in the order that they were tested.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

<u>0.1% Glucose - 1% Peptone</u>					<u>Protein Starvation Conditions</u>				
	<u>B. subtilis bckgrnd</u>		<u>E. coli bckgrnd</u>			<u>B. subtilis bckgrnd</u>		<u>E. coli bckgrnd</u>	
<u>Name</u>	<u>Bacteria</u>	<u>Zone</u>	<u>Bacteria</u>	<u>Zone</u>	<u>Name</u>	<u>Bacteria</u>	<u>Zone</u>	<u>Bacteria</u>	<u>Zone</u>
Red	12mm	18mm	9mm	none	Red	9mm	16mm	12mm	32mm
Compton	10mm	13mm	12mm	13mm	Compton	9mm	none	9mm	22mm
Jackson	12mm	19mm	13mm	16mm	Jackson	8mm	14mm	12mm	29mm
Hargis	9mm	21mm	10mm	none	Hargis	9mm	21mm	7mm	none
Small	10mm	16mm	10mm	none	Small	10mm	14mm	13mm	20mm
Blaneece	11mm	none	8mm	none	Blaneece	8mm	10mm	17mm	40mm
Jamison	10mm	33mm	9mm	18mm	Jamison	9mm	17mm	7mm	none
Archer	8mm	none	9mm	none	Archer	8mm	11mm	7mm	none
S2B1	10mm	20mm	10mm	25mm	S2B1	9mm	21mm	11mm	35mm
S6B3	14mm	24mm	13mm	15mm	S6B3	13mm	22mm	14mm	24mm

**Table 3.** This table depicts the Trial 3 results of the 10 bacteria that produced antibiotics under any of the four sets of test conditions. The measurements listed refer to the diameter of the bacterial growth on the swab patches (denoted "Bacteria"), and, when applicable, the diameters of the bacterial growth on the swab patches plus the zone of inhibition (denoted "Zone"). "Glucose Starvation Conditions" refers the swab patches with M9 salts 0.1%G-1%P Agar. "Protein Starvation Conditions" refers to the swab patches with M9 salts 1%G-0%P Agar. Bacteria are arranged in the order that they were tested.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION



**Figure 3.** The average diameters of the bacterial patches combined with the zones of inhibition over the three trials were calculated for all four sets of conditions (M9 salts 0.1% Glucose – 1% Peptone with *B. subtilis* background bacteria [BsCarbStrv], M9 salts 1% Glucose – 0% Peptone agar with *B. subtilis* background bacteria [BsPepStrv], M9 salts 0.1% Glucose – 1% Peptone with *E. coli* background bacteria [EcCarbStrv], and M9 salts 1% Glucose – 0% Peptone agar with *E. coli* background bacteria [EcPepStrv]), converted to radii, and graphed in this figure. Standard Error is accounted for and depicted in the graph as well.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

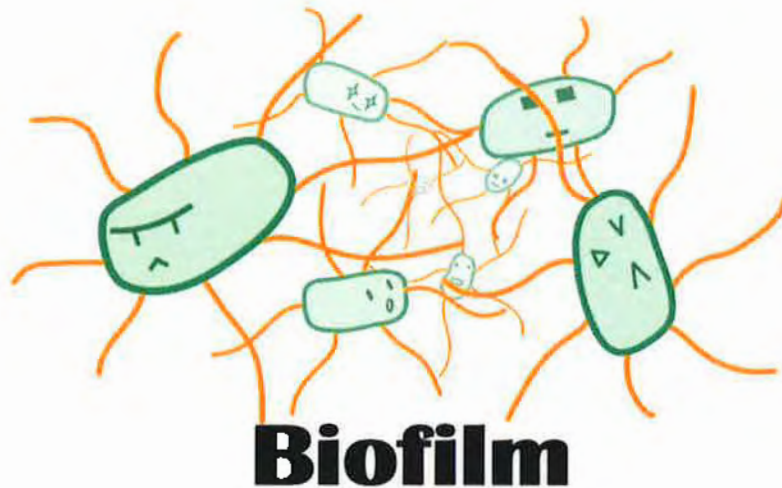
## Discussion

This research began with 27 test bacteria that had been deemed strong candidates to produce antibiotics under starvation conditions by previous microbiology classes. In the end, only 10 of these actually did so. By no means, however, does that make this research a failure. Quite the opposite is true. Because of this research, these 10 bacteria are now known to definitively produce antibiotics under at least one of the four sets of medium-background bacterium conditions tested. Of the two kinds of starvation tested, all 10 of the bacteria produced antibiotics when starved for proteins. Nine out of the 10 responded to carbohydrate starvation. Out of these nine, four (Red, Compton, Jackson, and S2B1) consistently produced larger zones of inhibition when starved of protein starvation than when starved of carbohydrates. Because of this, it can be concluded that protein starvation is a slightly more effective method to induce antibiotic production in most bacteria than carbohydrate starvation. This suggests that proteins may be more significant for bacterial survival than carbohydrates. Bacterial cells combat protein starvation using the stringent response. This data suggest that the stringent response might be more effective than the catabolite repression, bacterial cells' mechanism to combatting carbohydrate starvation.

Overall, the bacteria inhibited the growth of the *B. subtilis* more effectively than *E. coli*, with two of the bacteria not producing zones of inhibition against *E. coli* under either starvation condition and three bacteria (Small, Red, and Blancece) only producing zones of inhibition against *E. coli* when starved of proteins instead of carbohydrates. This was to be expected, as *E. coli* is a Gram negative bacterium. The presence of the extra outer membrane that causes it to be Gram negative increases the bacterium's resistance to antibiotics. On the other hand, *B. subtilis* is Gram positive, making it more susceptible to antibiotics than *E. coli*.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

All 10 of the bacteria that proved to be effective antibiotic producers under some or all sets of starvation/background combination conditions could, after further experimentation, potentially be used to fight the rising problem that is antibiotic-resistant bacteria. The antibiotics that the 10 test bacteria produced to inhibit the growth of *E. coli* and/or *B. subtilis* could be harvested and used against antibiotic-resistant bacteria. Before this could be done, however, more experimentation needs to be done. The next immediate step will be to test these newfound antibiotics for eukaryotic toxicity. This will be done by growing the test bacteria in the presence of eukaryotic cells in order to see if the eukaryotes remain viable. If the test bacteria prove toxic, testing with them will cease. If the bacteria prove safe for eukaryotic cells, then bacterial genome sequencing will follow. This will be done by RNA sequencing. Since these test bacteria were simply harvested from soil, it's possible that some or all of the bacteria have already been discovered. Sequencing is needed to determine if this is the case.



### Biofilms and Contact Lenses

Have you ever stepped on a rock in a rushing river and noted its slippery surface? Have you ever run your tongue over your teeth after going a little too long between brushes and felt an extra layer of slickness? Have you ever looked out over a small pool of water only to see a layer of green pond scum covering patches of the surface? If your answer to any of these is yes, then you're familiar with biofilms. Caused by the accumulation of certain kinds of bacteria, biofilms are found all throughout life (Watnick and Kolter, 2000). Biofilms, which act as anchoring mechanism with added bonus of increased antibiotic resistance, are a coalition of bacteria embedded in a secreted polymeric matrix (Watnick and Kolter, 2000). Because of this protection, bacteria that form into biofilms are often more of a threat to humans than bacteria that do not form biofilms, and one particular facet of life where biofilms can cause major damage is contact lenses.



## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

Biofilms are protective coverings that some kinds of bacteria produce in order to help them stay anchored in place, trap food, and surround themselves with other species of bacteria that they can benefit from being in close proximity with. While not the main focus of biofilms, an added bonus is their increased resistance to antibiotics. In order for biofilm formation to commence, large amounts of bacteria must become densely packed on a surface (Donlan, 2002).

Once enough bacteria grow or migrate to one spot, quorum signaling will initiate the biofilm formation (Miller and Bassler, 2001). Quorum signaling is a group behavior signaling mechanism that bacteria can use to communicate with each other (Miller and Bassler, 2001). Biofilms begin as a single species, but over time, other species are incorporated into the biofilm. Because many biofilms contain multiple kinds of bacteria, it's imperative for the biofilm synthesis stimuli to be capable of interspecies communication (Watnick and Kolter, 2000). While quorum signaling is mainly used between bacteria of the same species, intraspecies communication is still possible. Once stimulated, gene regulation is altered, and the bacteria begin to secrete a matrix composed of mainly polysaccharide materials (Donlan, 2002). The matrices secreted from each individual bacterium combine with each other to form a larger, all-encompassing matrix that draws in nearby fungi, protozoa, inorganic objects, and everything it touches (Donlan, 2002). Figure 1 is a close-up photo of a biofilm.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION



**Figure 1.** This figure shows a photograph taken of individual bacterial cells anchored to a surface and each other. The matrix visible around the individual cells is the secreted biofilm components that have merged from each bacterium to form a larger biofilm.

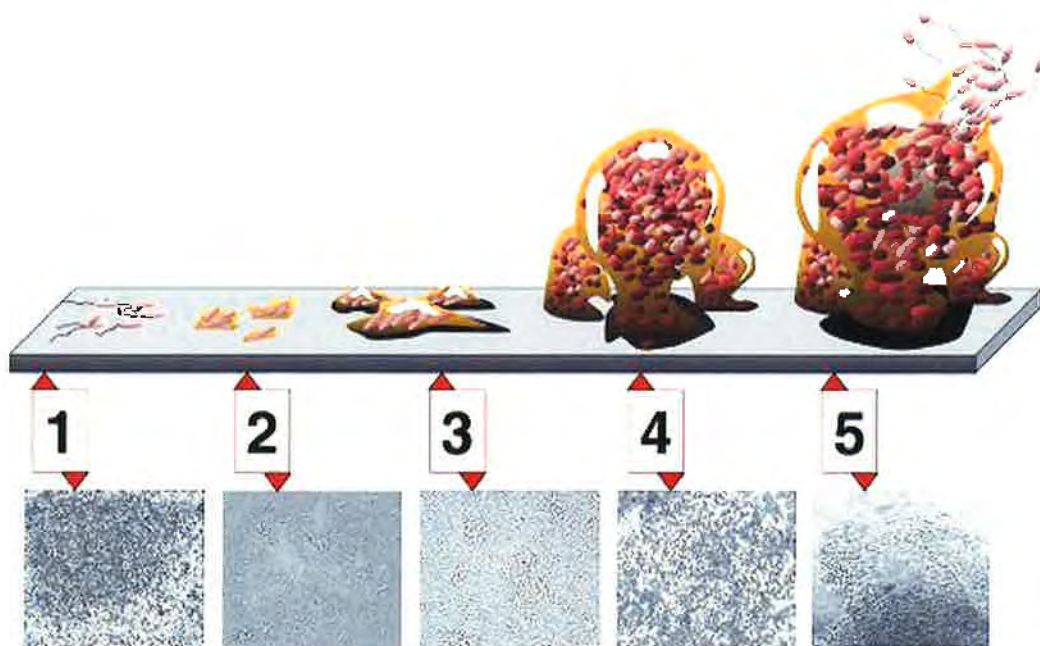
Many different conditions determine if biofilms can form. Perhaps the most necessary condition is the presence of an aqueous environment (Donlan, 2002). The solid-liquid interphase provides the ideal parameters for biofilm attachment and formation (Donlan, 2002). Other factors that affect the attachment of microbes, and, consequently, the formation of biofilms include temperature, levels of nutrients present, pH, and ionic strength (Donlan, 2002). If any of these factors vary outside of what certain species of bacteria can survive in, no microbes will anchor there in the first place.

The biofilms themselves create a largely self-sufficient environment for bacteria (Donlan, 2002). Because the biofilms are often composed of multiple species of bacteria, nutrients can be recycled between the species to maintain life (Donlan, 2002). That being said, it would not be safe to assume that the bacteria never face any internal threats. It's not uncommon that the bacteria will multiply too rapidly and deplete the nutrients from the area (Watnick and Kolter, 2000). When this happens, it becomes necessary for some of the bacteria to migrate to a new, nutrient-rich area. When this overcrowding occurs, quorum signaling will cause some of the bacteria to shift into a mobile form and leave the biofilm in search of a new home (Donlan, 2002). This is the most common ideal life cycle of bacteria inside a biofilm, which is depicted in

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

Figure 2. However, sometimes the bacteria are spread to new areas in different ways. Sometimes, outside forces will break off a piece of the biofilm. When this happens, the bacteria can reattach themselves and start over, growing a fresh biofilm at a new location (Donlan, 2002). One environment that biofilms particularly thrive in is the semi-aqueous surface of modern day soft contact lenses (Bruinsma et al., 2001).

The concept of contact lenses has been around for many years. Leonardo da Vinci is credited with coining the idea for contacts way back in 1508 (“A Brief History”, 2015). Despite the early schematics, the first contact lenses, which were made from glass and covered the entire eye, were not actually created until 1887 (“A Brief History,” 2015). In 1939, contacts shifted to be made from plastic, but it wasn’t until 1948 that the lens design changed to only cover the cornea instead of the entire sclera (“A Brief History,” 2015).



**Figure 2.** This figure depicts the life cycle of a biofilm. The upper images are representations of the different steps while the lower images are actual photographs of biofilms at the various stages. Step one is the bacteria anchoring themselves to the surface. Step two is the individual colonies secreting their biofilms. Step three is the biofilms growing and spreading. Step four is the once the biofilms have combined and grown into one massive biofilm. Finally, step five shows some of the bacteria being sent out to colonize new areas once the current biofilm has reached maximum capacity.

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

In 1987, disposable soft contacts finally became available, and contacts remained relatively unchanged until 2002, when the first silicone hydrogel contacts became commercially available (“A Brief History,” 2015). The silicone hydrogel contact lenses are the most common kind of contacts in today’s society. Unfortunately, they are also the type of contacts that biofilms most easily form on.

Contact lens cases are ideal environments for biofilm formation since they often hold contact solution, creating a solid-liquid interphase (McLaughlin-Borlace et al., 1997). From that point, it is as easy as the biofilm bacteria shifting into their mobile form to travel from the lens case to the actual contact lens itself (McLaughlin-Borlace et al., 1997). The common soft contact is made of a hydrogel material (Kacker, et al, 2017). According to the Merriam Webster dictionary, a hydrogel is “a gel composed of usually one or more polymers suspended in water” (2017). This watery solid is the ideal environment for biofilms, so once they are introduced to a contact lens, the biofilm bacteria thrive.

If immediate care isn’t taken, these biofilms can infect the eye when the contact lens user puts in their contacts. This can lead to a bacterial infection in the cornea of the eye (Bruinsma et al., 2001). Common ocular infections caused by biofilms are microbial keratitis, infiltrative keratitis, acute red-eye, and contact lens peripheral ulcers (Kacker et al., 2017). In the case of microbial keratitis, as many as 66% of cases can be linked to biofilm formation on contact lenses (Kacker et al., 2017). If not treated, bacterial infections of the eye can lead to corneal scarring, vision loss, or even permanent blindness (Kacker et al., 2017).

According to McLaughlin-Borlace et al., the best way to prevent contact lens contamination is to minimize handling of contacts (1997). Every time a user takes contacts in and out of their eyes and stores them in a lens case, the risk for some sort of contamination goes

## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

up. The safest route would be for contact lens users to wear single-use contacts. This allows for a fresh, sterile pair every time they put in their contacts. If the user prefers some other kind of contact lens, then the kind of lens solution can make a big difference (Kacker et al., 2017). It's a common misconception that water can be used in place of lens solution. This is completely false, as even purified water does not contain the chemicals needed to break down bacteria and other things that may be adhering to the contacts (Heiting, 2017). According to Kacker et al., lens solutions that contain hydroxyalkylphosphonate, boric acid, edetate disodium poloxamine, and sodium borate are fairly effective for preventing the growth of biofilms on the lenses. Other lens solutions, such as those containing a cocktail of purified water, HPMC, CMC, EDTA and borax in sodium chloride base, are not as useful against biofilm formation, but they are still a much better alternative to using pure water as lens solution (Kacker et al., 2017).

Despite all of this, the best way to treat contact lenses or lens cases that form biofilms is disposal. Once biofilms form, they can be tricky to remove due to their sticky matrix and difficulty to penetrate (Kacker et al., 2017). It's always the safest route to throw away that case and pair of contacts for a new pair. When it comes to your vision, it's always better to be safe than sorry.

### Acknowledgements

First and foremost, I'd like to thank Dr. Ruth Plymale from Ouachita Baptist University for allowing me to work on her research team. If it wasn't for her, I never would have been able to participate in this awesome and captivating research. I'd also like to thank the Arkansas INBRE foundation for funding this research, and for allowing me to present my findings at their conference. I'd also like to thank the entire biology department at Ouachita Baptist University for allowing me access to the equipment and tools needed to perform this research, and for transporting me to the Arkansas INBRE conference to present my research, as well as the other undergraduate members of Dr. Plymale's research team who helped me on occasion with learning procedures and protocols.

## References

- Baym, B., Lieberman, T.D., Kelsic, E.D., Chait, R., Gross, R., Yelin, I., Kishony, R. (2016, September 9). Spatiotemporal microbial evolution on antibiotic landscapes. *Science*, 353(6204), pp. 1147-1151. Retrieved from <http://science.sciencemag.org/content/353/6304/1147.full>
- Branswell, H. (2017, January 12). A Nevada woman dies of a superbug resistant to every available antibiotic in the US. Retrieved February 04, 2017, from <https://www.statnews.com/2017/01/12/nevada-woman-superbug-resistant/>
- Bruinsma, G.M., van der Mei, H.C., Busscher, H.J. (2001). Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. *Biomaterials*. (22), pp. 3217-3224. Retrieved from <https://pdfs.semanticscholar.org/d65b/bfeebebe63d766660af6307263dd872af816.pdf>
- Contact Lens Manufacturers Association. (2015, August). A Brief History of Contact Lenses. Retrieved January 10, 2018, from <http://www.contactlenses.org/timeline.htm>
- Cordero, O.X., Wildschutte, H., Kirkup, B., Proehl, S., Ngo, L., Hussain, F., Le Roux, F., Mincer, T., Polz, M.F. (September 7, 2012). Ecological Populations of Bacteria Act as Socially Cohesive Units of Antibiotic Production and Resistance. *Science*, 337(6099), pp. 1228-1231. Retrieved from <http://science.sciencemag.org/content/337/6099/1228>
- Dall, C., (Jan 13, 2017). Pan-resistant CRE reported in Nevada. *CIDRAP News*. Retrieved February 04, 2017, from <http://www.cidrap.umn.edu/news-perspective/2017/01/pan-resistant-cre-reported-nevada>



## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

Donlan, Rodney M. (2002). Biofilms: Microbial Life on Surfaces. *Emerg Infect Dis*, 8(9), pp.

881-890. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2732559/>

Gallo, M., Katz, E. (February 1972). Regulation of Secondary Metabolite Biosynthesis:

Catabolite Repression of Phenoxazinone Synthase and Actinomycin Formation by

Glucose. *Journal of Bacteriology*, 109(2), pp. 659-667. Retrieved from

<http://jb.asm.org/content/109/2/659.short>

Heiting, O. G. (2017, November 27). Can Contacts Be Stored in Water? Retrieved January 10,

2018, from <http://www.allaboutvision.com/contacts/faq/store-cls-water.htm>

Hyrdogel. (n.). In Merriam-Webster online. Retrieved from <https://www.merriam->

[webster.com/dictionary/hydrogel](https://www.merriam-webster.com/dictionary/hydrogel)

Kacker, S., Suman, E., Kotian, M.S. (2017). Bacterial and fungal biofilm formation on contact

lenses and their susceptibility to lens care solutions. *Indian Medical Journal*. 35 (1). pp.

80-84. Retrieved from

[http://web.a.cbscohost.com/ehost/detail/detail?vid=0&sid=f41a8ce2-2fa1-43b5-9f45-](http://web.a.cbscohost.com/ehost/detail/detail?vid=0&sid=f41a8ce2-2fa1-43b5-9f45-09b54a55d385%40sessionmgr4007&bdata=JnNpdGU9ZWwhvc3QtbGl2ZQ%3d%3d#AN)

[09b54a55d385%40sessionmgr4007&bdata=JnNpdGU9ZWwhvc3QtbGl2ZQ%3d%3d#AN](http://web.a.cbscohost.com/ehost/detail/detail?vid=0&sid=f41a8ce2-2fa1-43b5-9f45-09b54a55d385%40sessionmgr4007&bdata=JnNpdGU9ZWwhvc3QtbGl2ZQ%3d%3d#AN)

[=28303823&db=cmedm](http://web.a.cbscohost.com/ehost/detail/detail?vid=0&sid=f41a8ce2-2fa1-43b5-9f45-09b54a55d385%40sessionmgr4007&bdata=JnNpdGU9ZWwhvc3QtbGl2ZQ%3d%3d#AN)

McLaughlin-Borlace, L., Stapleton, F., Matheson, M., Dart, J.K.G. (1998). Bacterial biofilm on

contact lenses and lens storage cases in wearers with microbial keratitis. *Journal of*

*Applied Microbiology*. 84, pp. 827-838. Retrieved from

<http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.1998.00418.x/epdf>



## TESTING BACTERIAL ANTIBIOTIC PRODUCTION

Miller, M.B., Bassler, B.L. (2001). Quorum Sensing in Bacteria. *Annual Review of*

*Microbiology*, 55, pp. 165-199. Retrieved from

<https://pdfs.semanticscholar.org/fe53/fe0c9fb34956af6f3b0d4d02eab304399a9a.pdf>

Napolitano, G. (2016, September 21). At UN, global leaders commit to act on antimicrobial resistance. Retrieved February 04, 2017, from

[http://www.un.org/apps/news/story.asp?NewsID=55011#.WJD\\_zBsrLIU](http://www.un.org/apps/news/story.asp?NewsID=55011#.WJD_zBsrLIU)

Ochi, K., Ohsawa, S. (October 1984). Initiation of antibiotic production by the stringent response of *Bacillus subtilis* Marburg. *Journal of General Microbiology*, 130(10), pp. 2473-2482.

Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/6439818> \

Watnick, P., Kolter, R. (May 2000). Biofilm, City of Microbes. *Journal of Bacteriology*,

182(10), 2675-2679. Retrieved from <http://jb.asm.org/content/182/10/2675.short>