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The Effects of Farnesol, a Quorum Sensing Molecule from Candida albicans, on Alcaligenes faecalis

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by
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ABSTRACT

Quorum sensing molecules have become a recent focus of study to learn if and how they can be used, both on their own and in conjecture with current antimicrobial methods, as a means of bacterial control. One such quorum sensing molecule is the sesquiterpene alcohol, Farnesol, which is synthesized and released by the fungus, *Candida albicans*. In most in-vivo cases, our laboratory has shown that *Alcaligenes faecalis* overtakes *C. albicans*, preventing its growth. However, as a way to counteract this inhibitory effect, Farnesol may be one way that *Candida* has found to fight back. In this study, we focused on the inhibitory properties of Farnesol for growth and motility of *A. faecalis*, as well as, the molecule's ability to prevent *Alcaligenes* from creating biofilms and/or degrading them once they have already been established. Our experiments show evidence that Farnesol is able to inhibit both the growth and motility of *A. faecalis*, and determination of the specific concentrations of Farnesol needed to see the largest effects on *A. faecalis* biofilms. Our hope is that in future studies, we will be able to add varying concentrations of the Farnesol to known and widely used antibiotics in order to increase the effectiveness of antibiotics against bacterial strains, both in the *Alcaligenes* genus and in other genus, that have previously been considered "antibiotic resistant".

ACKNOWLEDGEMENTS

First and foremost, I want to thank Dr. Sean Fox with the Health Sciences (Microbiology) Department at East Tennessee State University for allowing me to learn and work in his research lab for the past two years. I feel that the work I have been allowed to take part in have greatly expanded my knowledge and confidence in the field of Microbiology and have been instrumental in cultivating my clinical laboratory abilities. The time that has been invested in me has been greatly appreciated. I also want to thank Dr. Ranjan Chakraborty for his guidance support throughout my research as well as my undergraduate studies. I would also like to thank Robin Grindstaff for always being willing to answer my questions and lead me in the right direction for lab resources, techniques, and procedures. I want to thank everyone who has been a part of my undergraduate studies as well as my research for continuously cultivating my abilities and ensuring my understanding Microbiological research.

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INTRODUCTION

Antibiotics and Antibiotic Resistance:

Antibiotics have been in use since the 1940s when Penicillin was first released on the market, almost 12 years after Alexander Fleming first recognized its bacteria-fighting capabilities [1]. Since it was first released as a treatment for infections, there have been several advancements in the field of antibiotic treatments with numerous new medications being marketed for the public. The popularity and the use of these medications has continued to increase and with that comes the potential misuse of those medications. Bacteria naturally possess the ability to develop resistance in their typical environment; however, the misuse and overuse of antibiotics have expedited the resistance rate by accelerating the fast-adapting abilities of bacteria [2]. These adaptations have led to the need for more and more antibiotics to be researched and developed in order to keep up with the ever-growing resistance problems. However, producing antibiotics is not as economically advantageous as producing medications for chronic illnesses such as hypertension. Therefore, less funding has gone toward the research and production of new antibiotics by large pharmaceutical companies because they stand to make limited profit from the products [3]. This is true for antibiotics used against a wide spectrum of infections, but especially against Gram (-) bacteria. Gram (-) bacteria tend to possess faster resistance rates with no way to pinpoint exactly when the resistance will manifest itself, rather than many of the Gram (+) bacteria that are currently causing issues globally such as Methicillin Resistant *Staphylococcus aureus*. Many of the current Gram (-) bacteria already have strains that are resistant to most of the antibiotics currently available to patients. Since large pharmaceutical companies have severely decreased the amount of resources and time that they put forth to create new antibiotics, some smaller companies have tried to take over research and

production of new treatment methods. However, many new antibiotics do not get past the first two phases of research because the cost of the clinical trials in phase 3 is so expensive that many small companies and investors cannot afford to follow through the process [3].

As published by the Centers for Disease Control in 2013, the world, as a whole, has entered into the "post-antibiotic era" [3]. The medical world is in desperate need of new methods for treating both simple and potentially deadly infections. Therefore, researchers have begun looking at how we can amplify the tools (medications) that are already available by also exploiting the ability of microorganisms to use secreted molecules to inhibit other microbes.

Alcaligenes faecalis:

Alcaligenes faecalis is an aerobic, motile, Gram (-) bacilli bacterium found ubiquitously in the environment, specifically in water sources, soil and the normal gastrointestinal flora of humans. The *A. faecalis* species is the most common species in the genus to be isolated and cultured in a lab, but it is rarely cultured from clinical specimens [4] [5]. Due to its regular presence in the normal flora of human hosts, this bacterium rarely causes issues; however, *A. faecalis* is an opportunistic pathogen that can cause bacterial infections in immunocompromised hosts such as patients in hospitals or patients with chronic infections such as Cystic Fibrosis [4]. This pathogen has been associated with several types of infections including but not limited to skin and soft tissue infections, urinary tract infections, and pneumonia [5] [6] [7]. This bacterium is also commonly seen in nosocomial infections particularly after surgery/invasive procedures and due to interactions with fluids via nebulizer treatments and respirators [5]. Though it is still quite rare for *A. faecalis* to cause life-threatening septicemia, the occurrences of cases such as this has been on the rise. In July of 2019, at Square Hospitals Limited in Bangladesh, a patient was diagnosed with a bloodstream infection of *A. faecalis* that was resistant to all currently

available antibiotics, including the "reserve" antibiotics such as carbapenems and tigecycline [8]. This bacterium is quickly morphing into yet another pan-resistant bacterium that cannot be treated by the antibiotics we currently have available. Medicine needs a method for strengthening the antibiotics that are currently commercially available and creating new methods to fight back against such resistant infections. This study will begin to look at how *Candida albicans* may be able to fight back against *Alcaligenes faecalis* infections.

Candida albicans:

Candida albicans is a fungus that is found commonly in healthy human microbial flora. Even so, this fungus is known as an opportunistic pathogen (much like *A. faecalis*) as it can cause illness in a human host, especially one that is immunocompromised due to illnesses, infections, or treatments for other diseases [9]. Fungi can have several similar effects on the human body as most bacteria. They can also interact with bacteria in the host body, resulting in either the amplification or hindrance of one or both microbes. Several interactions such as this have been studied regarding *Candida*'s interactions with *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus*, etc. [10]. The research discussed in this report will look at the interactions between *C. albicans* (particularly the quorum sensing molecule it produces, Farnesol) and *A. faecalis*.

Quorum Sensing Molecules (Farnesol):

Quorum sensing is one of the many ways that bacteria communicate with one another. It was once thought to only occur in eukaryotic cells; however, it has been well documented in several bacterial genera [11]. If one bacterium turns on all of its virulence factors, it cannot cause severe damage to the human host and that one singular bacterium would easily be killed or expelled by the host's immune system. However, if hundreds or thousands of bacteria are

present, they can release cell-population density signaling molecules that alert bacteria that enough individual cells are now present to cause damage. Once the number of bacterial cells inside the host reaches a certain threshold, the concentration of the cell density signaling molecules is also high enough to trigger expression of potentially harmful virulence factors. This process of quorum sensing communication is used by several different types of bacteria to carry out many biological functions including competence, motility, luminescence, etc. In the case of Farnesol produced by *C. albicans*, the molecule causes inhibitory functions against some competing bacteria [11].

Farnesol is a sesquiterpene alcohol that is produced by the fungus *C. albicans* via the biosynthetic sterol pathway using farnesyl pyrophosphate (Fpp) as the starting material [12]. Farnesol controls several of the virulence factors that *Candida* can use against other competing fungi and some bacteria. These include, but are not limited to, biofilm formation and *Candida*'s ability to alternate between its three morphological states [13].

Objectives:

The research in this report examines the potential inhibitory functions of Farnesol production by *C. albicans* on the growth, motility, biofilm formation, and biofilm degradation of *A. faecalis* in vitro. Normally, when the two bacteria are grown together, *A. faecalis* overtakes *C. albicans* and inhibits its growth. Therefore, we looked at a possible countermeasure that *Candida* could use to fight back, and potentially inhibit, the growth of the *Alcaligenes* by using a molecule naturally produced by the fungus.

MATERIALS AND METHODS

Microorganisms and Culture Conditions. *C. albicans* strain SC5314 was routinely cultured on Yeast Peptone Dextrose (YPD) agar and broth and grown at 37ºC. *A.faecalis* strain ATCC 8750 and *A. viscolactis* was routinely cultured on Luria Bertani (LB) agar and broth and grown at 37ºC or room temperature respectively. For co-cultures, Brain Heart Infusion (BHI) agar and broth were used.

Inhibition of Growth. For disc diffusion tests, a lawn of *A. faecalis* was grown on an agar plate using a liquid culture grown 24 hours prior. Two filter disks: 1 containing 20µl of 4 molar Farnesol and 1 containing 20µl of *C. albicans* (also from a liquid broth grown 24 hours before) were placed 3 cm apart on the agar plate on top of the *A. faecalis* lawn. The plate was incubated at 37ºC for 24 hours and monitored for zones of inhibition (ZOI). For line streak tests, on two separate BHI plates, a line of the liquid broth containing *A. faecalis* was drawn down the center using a sterile Q-tip for each plate. On the first plate, a line was also drawn immediately adjacent to the *Alcaligenes* line, but not touching, using Farnesol. On the second plate, the same technique was utilized with Phosphate Buffered Saline (PBS) acting as a control in place of the Farnesol. The two plates were incubated at 37ºC for 24 hours. For spot tests, a liquid culture of *A. faecalis* grown overnight was used to make a lawn by spreading 100µl on an agar plate, allowed to dry, then 10µl of 4 molar Farnesol was spotted onto the lawn. The plate was then incubated at 37ºC for 24 hours.

Inhibition of Motility. Five tubes of liquid motility agar deeps were prepared. Two of the deeps contained 200µl of *C. albicans* from a liquid overnight culture along with the cooled molten motility agar. Two other deeps contained 25µl of Farnesol along with the normal motility agar media. The last deep contained only the normal motility agar. The broths were mixed,

respectively, chilled, and allowed to solidify and set for 24 hours. After the deeps were set, an overnight culture of *A. faecalis* liquid broth was used to aseptically stab into the center of 1 *C. albicans* deep, 1 Farnesol deep, and the control deep. The process was repeated by aseptically stabbing the 2 remaining tubes (1 *C. albicans* and 1 Farnesol) with a liquid overnight broth culture of *A. viscolactis*. All five tubes were incubated at 37ºC (*A. faecalis*) or room temperature (*A. viscolactis*) for 24 hours.

Liquid Co-Culture. 25 µl of fresh overnight cultures of *A. faecalis*, was added to two broth tubes and 60 µl Farnesol was added to one tube forming a co-culture. Both tubes were placed in the 37ºC incubator with shaking (250rpm) for 24 hours. Each tube was serially diluted, plated onto agar plates containing either Kanamycin (50ug/ml) or Amphotericin B (25ug/ml), incubated for 24-hours at 37ºC, and colony forming units (CFUs) were enumerated. Three independent trials were performed.

Biofilm Growth. Prevention of Biofilm Attachment**.** 96 well plates were filled with broth only (blank control, Row A) or broth inoculated with an overnight culture of *A. faecalis* equilibrated to an OD600 of 0.01 (~1x106 cells/ml). Varying amounts of stock Farnesol (30µl of Farnesol and 1500µl broth) were added to Rows B-H of the 96 well plate (C – 15 microliters, D – 7.5 microliters, E – 3.8 microliters, F – 1.8 microliters, G – 0.9 microliters, H – 0.5 microliters). The 96 well plate was incubated at 37ºC for 24 hours and divided into 3 sections for processing. The first section, columns 1 – 4 was processed for OD600 readings. The second section, columns 5 – 8, was processed for MTT testing and OD 450 readings. Broth was removed from the wells, washed with 200µl of PBS, and PBS/MTT (200µl and 10µl respectively) was added to the wells. The well plate was then incubated at 37ºC for 15 minutes, 100µl of Dimethylsulfoxide (DMSO) was added, and OD450 reading was taken. The third section, columns 9 – 12, was processed for

Crystal Violet (CV) staining and read at OD 600. Broth was removed from the wells, washed with 200 µl of PBS, 200µl of diluted 0.01% CV was added to all wells and incubated at room temperature for 5 minutes. Excess CV was washed off with 200µl of PBS, allowed to dry in the 37^{o}C for 15 minutes, 200µl of 33% Glacial Acetic Acid was added to lyse the cells open and the OD600 reading was taken. This procedure was performed in 3 trials to ensure accuracy.

Inhibiting pre-formed biofilms. The process for this experimentation was performed similar to the process used to for biofilm attachment, however, the Farnesol was not added until after the A. faecalis biofilms had grown in the 37^{o}C incubator for 24 hours. After those 24 hours, the same varying amounts of Farnesol were added and the well plate was again placed in the 37^{o}C incubator for an additional 24 hours and processed for OD600, MTT, and CV staining.

RESULTS AND DISCUSSION

Inhibition of Growth:

Previously in our laboratory, we have shown that when *A. faecalis* is spotted onto *C. albicans* lawns, zones of inhibition (ZOI) are apparent after 24 hours. However, upon further incubation of those lawns, *C. albicans* can breach the ZOI of *A. faecalis*, thus indicating that *C. albicans* is initially inhibited, but may employ counter measures to fight *A. faecalis*. Though *A. faecalis* normally inhibits the growth of *C. albicans* and grows abundantly, even in the presence of the fungus, these experiments were completed to test *Candida*'s ability to fight back using Farnesol. In our disc diffusion test, the agar plate with the lawn of *A. faecalis* and the disks containing 4 molar Farnesol and *C. albicans,* respectfully, were analyzed for ZOI surrounding each disk. The disk soaked in *C. albicans* did not result in any inhibition of *A. faecalis* growth; however, there was a slight zone of inhibition surrounding the Farnesol disk (Figure 1).

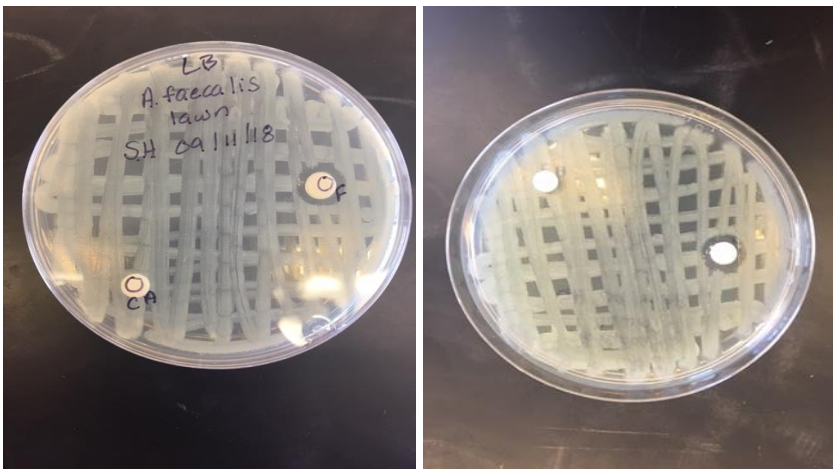


Figure 1: Agar plates inoculated with a lawn of *A. faecalis* and the disks placed over the lawn containing *C. albicans* (labeled CA) or containing Farnesol (labeled F). There is an obvious zone of inhibition around the F disk.

In the streak line test plates, the plate with the *A. faecalis* and PBS streaks resulted in normal overgrowth of the bacterial with no growth inhibition when challenged with PBS (Figure 2). However, the plate containing the *A. faecalis* streak line and Farnesol streak line did result in inhibition of the bacterial growth. The bacteria grew along its streak and in the opposite direction, but it did not cross the barrier created by the Farnesol (Figure 3).

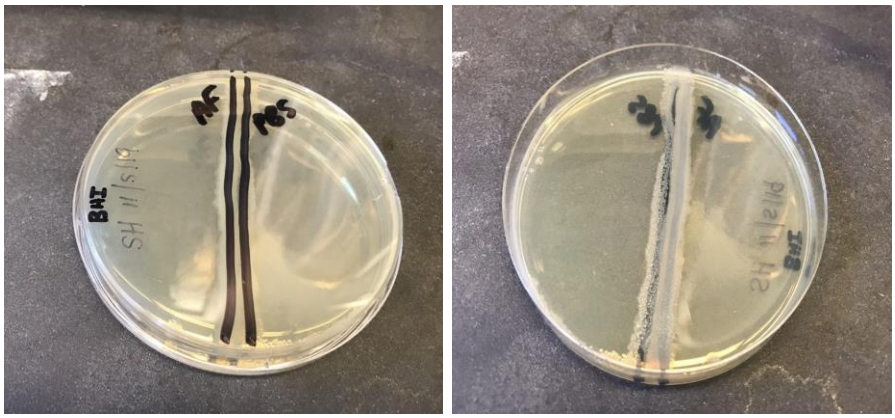


Figure 2: Growth lines of *A. faecalis* next to the PBS streak. This served as the control for the experiment.

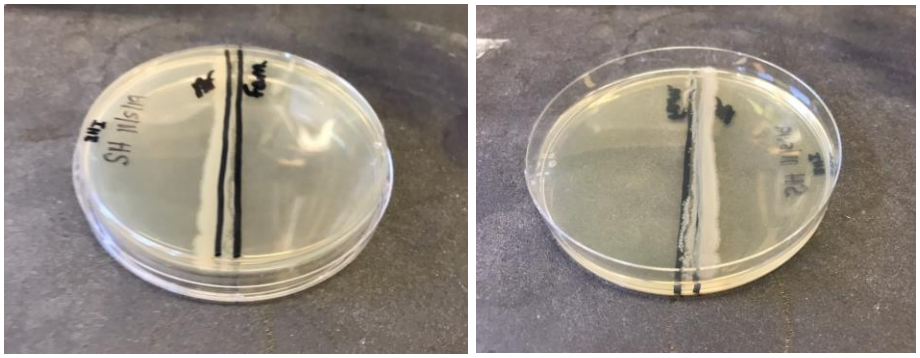


Figure 3: Line streak plates on agar of *A. faecalis* challenged with Farnesol.

Similar results lending to Farnesol's inhibitory properties was seen upon viewing the two agar plates that were seeded with the *A. faecalis* liquid culture and the *A. faecalis* + Farnesol liquid culture, respectfully. There was an obvious difference in the growth with the plate containing just the bacteria presenting with significantly more growth verses the growth of A. faecalis with diluted Farnesol added (Figure 4).

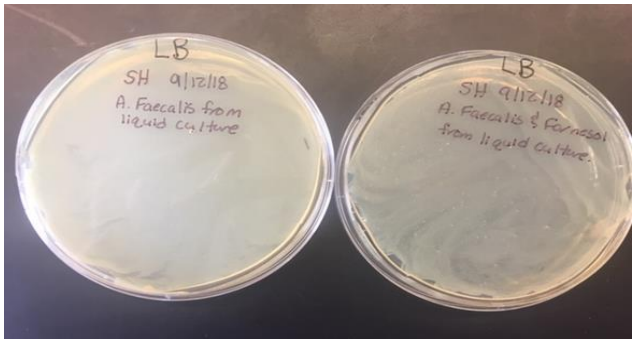


Figure 4: Agar plate growth inhibition by Farnesol. On the left contains the inoculum of *A. faecalis* in LB. The LB plate on the right contains the inoculum of *A. faecalis* and Farnesol and has less growth than the plate on the left.

These tests demonstrate that *A. faecalis* could be inhibited by *C. albicans'* quorum sensing molecule, Farnesol, and primarily examined growth. We then wanted to determine, visually, the ability of *Candida* to inhibit a more contact-dependent manner of motility.

Inhibition of Motility

A. faecalis is usually an extremely motile bacteria and it is easily able to spread across its environment. Motility agar deeps were used to determine how motile the bacteria were in the

presence of *C. albicans* or its molecule, Farnesol. In the motility deep containing Farnesol, there is a clear red stab line from the *A. faecalis*; however, it does not spread significantly away from that immediate stab line (photo 1, Figure 5). The motility deep that contained the *C. albicans* showed significant growth at the top of the agar as well as minor growth near the beginning of the stab line before diminishing towards the middle/bottom of the tube (photo 2, Figure 5). The agar was also more turbid than the others. There is an obvious difference between the two tubes previously described and the control tube which contained only the motility agar. In this tube, there is a definite red stab line from the inoculation as well as notable spreading/radiation outwards (photo 3, Figure 5). We were also interested to see if inhibition by *Candida* and Farnesol was limited to *A. faecalis* or was shared by other species in the genus, particularly *A. viscolactis*. As for the deeps inoculated with *A. viscolactis*, there was no growth at any point in the tubes. However, there was not much growth in the original LB broth of *A. viscolactis* that had been prepared beforehand either. *A viscolactis* has a very slow generation time and specific temperature dependent growth characteristics so these could play a role in the differences seen between *A. faecalis* and *A. viscolactis*.

Figure 5: Motility agar deeps. The Farnesol tube (far left) showed no growth emanating from the stab line. The *C. albicans* tube (middle) showed growth at the top of the motility agar deep. The control tube (far right) does show growth radiating from the stab line.

From these results, it can be inferred that the Farnesol did inhibit the motility of the *A. faecalis.* Though the experimentation with the *A. viscolactis* did not present any results, working with other species in the *Alcaligenes* genus is an option for further research in the future.

Liquid Co-Culture

We then wanted to turn our attention to finding out if Farnesol could inhibit the liquid growth of A. faecalis by enumerating the CFUs that *A. faecalis* can produce after exposure to Farnesol. The ability to do this would demonstrate that Farnesol does have the ability to restrict planktonic growth of the bacteria. For all three trials, the control, *A. faecalis* alone, and the experimental, *A. faecalis* with Farnesol, were grown in planktonic liquid culture for 24 hours at 37ºC, serially diluted, and plated. The plates from Trial 2 are pictured below in Figure 6.

Figure 6: These pictures show the liquid cultures for trial 2 of this experiment. The picture on the left shows the control plates from the liquid cultures containing only A. faecalis. The order of the plates is top left (#1), bottom left (#3), bottom right (#5), top right (#7), and right center (#9). The picture on the right shows the plates containing growth from the liquid culture containing *A. faecalis* and Farnesol. The plate order is as follows: bottom left (#1), top left (#3), top right (#5), bottom right (#7), right middle (#9).

These results show that adding the Farnesol to the broth along with the *A. faecalis* before dilutions and plating, does indicate inhibition of *A. faecalis* growth, as opposed to the *A. faecalis* by itself (Figure 7). There is an average 11% decrease between the colony growth of the *A. faecalis* alone verses the *A. faecalis* with Farnesol growth. Further research could be done at a

later date to look at differing concentrations of Farnesol to see if increased amounts affect the CFU growth of *A. faecalis* differently.

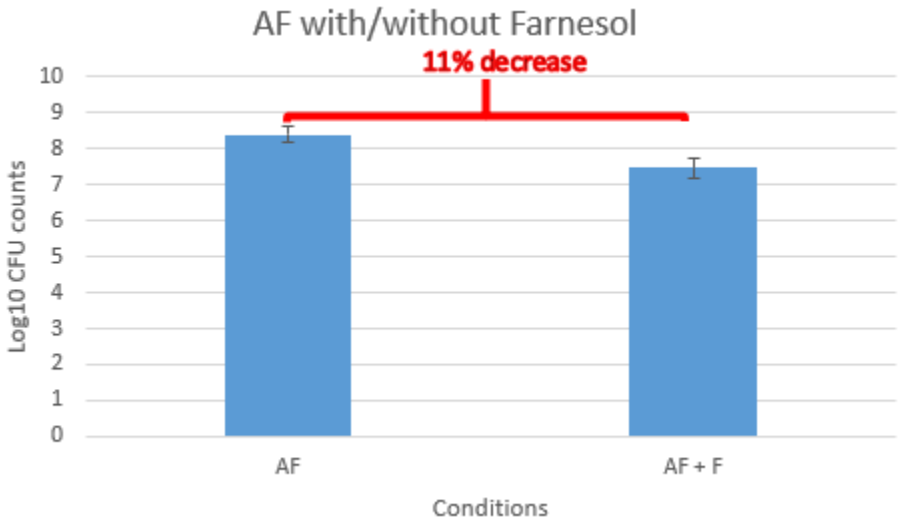


Figure 7: Log_{10} CFU counts for the liquid cultures performed using *A. faecalis* (AF) as a control and *A. faecalis* plus Farnesol (F). These are graphed above along with the standard deviations. There is an average 11% difference between the colony growth on the AF plates and the AF+F plates proving that the data obtained was statistically significant.

Prevention of Biofilm Attachment

Knowing that Farnesol inhibited planktonic growth of *A. faecalis*, we then wanted to examine any possible inhibitory effects on *A. faecalis* biofilm growth. Biofilms form in discreet, but fluid stages. The first stage of biofilm growth involves attachment of cells to a substrate. Thus, our first step was to test whether Farnesol could inhibit *A. faecalis* from initial attachment and subsequently inhibit biofilm growth by denying this primary step. In order to quantitate biofilm attachment, Farnesol was added to 96 well plates at the same time as inoculating the plate with *A. faecalis*. These biofilms were then processed in three different ways: OD595 readings for biofilm turbidity (columns 1-4), MTT readings at OD450 (columns 5-8) for cell viability, and Crystal Violet readings at OD595 (columns 9-12) for biofilm mass. For the first 4 columns, taking this normal OD595 reading shows the turbidity in the wells to see if the bacteria were growing and forming biofilms. However, this reading shows the cell density only, without

differentiating between living and dead cells. This also allowed us to determine which concentration of Farnesol would result in the least cell density. As seen in Figure 8, the concentration at which cell density was the lowest is 50µM, with cell density increasing as the graph spreads out in either direction. During the first analysis of these results, it was assumed that the higher concentration of Farnesol would result in a lower cell density; however, upon further research, 50µM is the concentration at which Farnesol would be seen at physiologically relevant levels in humans. It can be inferred that at the lower concentrations, there was not enough Farnesol molecules present to affect the *A. faecalis*. As for the higher concentrations, there is a possibility that the Farnesol and bacteria interacted in a different manner or the culture becomes over saturated, causing the cell density to be higher.

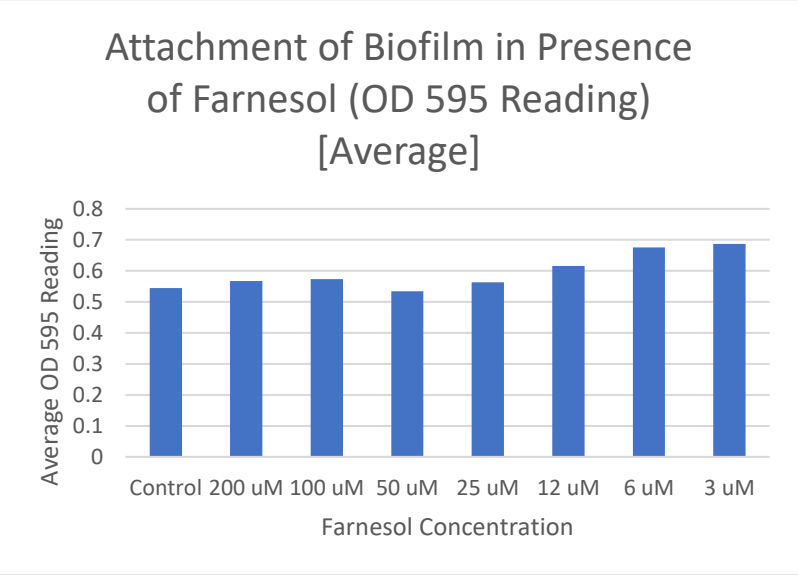
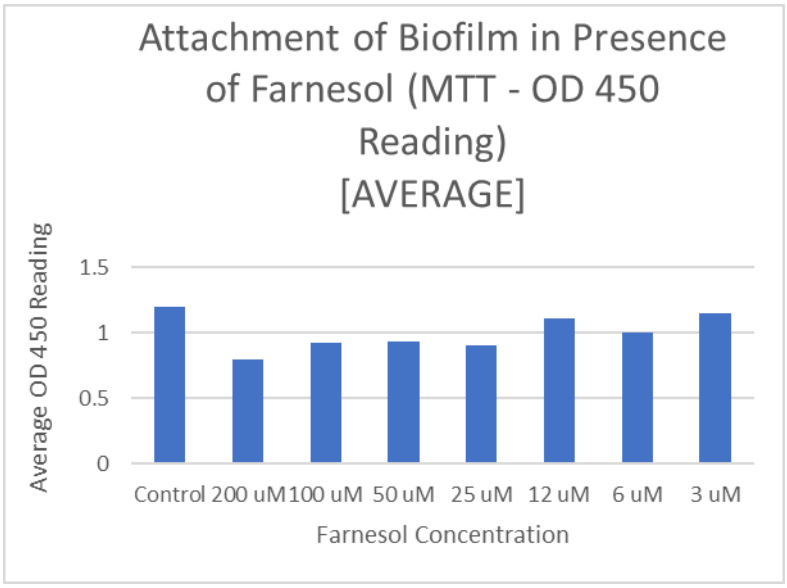


Figure 8: This graph shows the average OD 595 readings (columns 1-4 in well plate) for the three biofilm attachment trials.

The MTT test for columns 5-8, requires use of MTT. This is a yellow dye that is reduced by metabolically active cells to create a dark blue/black colored product called Formazan (15). This test differs from the original OD test because it allows us to look only at the living bacteria; due to the fact that the cells can only break down the MTT if they are alive and metabolically

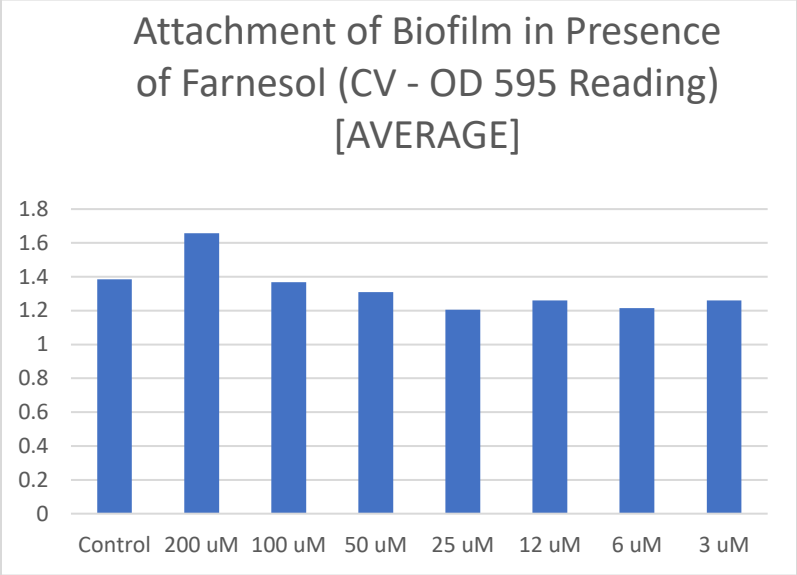
active. Only the cells that are attached in the biofilm are being tested because the other cells have been removed and washed away during the experimental process. Again, the ideal concentration of Farnesol was centered around the 50µM region with almost equal results stemming from the 100µM and 25µM region. For this particular experiment, it is possible that the cells that are metabolically active would have a different reaction to the presence of Farnesol than the cells that are present and counted in the original OD595 reading but are dead. These results can be seen graphically in Figure 9.



Graph 9: Average MTT - OD 450 readings (columns 5-8 in well plate) for the three biofilm attachment trials.

As for the final 4 columns (columns 9-12), the Crystal Violet (CV) testing allows a visual representation of the biofilms and allows the overall mass of the *A. faecalis* biofilms to be analyzed. The amount of CV that is absorbed and released once the cells are lysed, tells if the ability to create biofilm mass is still present in the cells, even in the presence of Farnesol. As expressed in Figure 10, the ideal Farnesol concentration is closer to 25µM rather than the 50µM seen in the other experiments. As for the stark increase in cell mass at the 200µM region as opposed to the other concentrations, there was an outlier for Trial 2 of this test. In future testing,

this test could be replicated to rectify the outlier; however, with this being preliminary data, the outlier could not be eliminated from the analysis.



Graph 10: Average Crystal Violet - OD 595 readings (columns 9-12 in well plate) for the three biofilm attachment trials.

Overall, the tests did show that the ideal concentration of Farnesol to work with and analyze in future studies, is around the 25µM – 100µM range with special attention to 50µM. These tests were used to determine what differences in concentrations of Farnesol would affect *A. faecalis* in these different biofilm quantitative methods.

Biofilm Degradation

After demonstrating that Farnesol inhibited the attachment phase of *A. faecalis* biofilms, we then wanted to examine any possible inhibitory effects on mature *A. faecalis* biofilm growth. Mature biofilms can be up to 1000X more resistant to antibiotics and are very difficult to eradicate. Thus, our next step was to test whether Farnesol could inhibit *A. faecalis* pre-formed biofilms. In order to quantitate biofilm attachment, Farnesol was added to 96 well plates only after *A. faecalis* was allowed to form biofilms for 24 hours. These biofilms were then processed in the same three ways as the attachment biofilms. These experiments look more at what effect

varying concentrations of Farnesol have on degrading *A. faecalis* biofilms that are already growing and have been doing so for 24 hours. We wanted to see if the same "ideal" concentrations remained constant between both experiments.

For the original OD 595 readings, looking at turbidity and cell density, it is seen that the ideal concentration of Farnesol was 200µM with 25µM coming close behind. The 25µM was to be expected considering that it was one of the "ideal" concentrations listed in the attachment trials. However, the 200µM region was not expected. However, this may be due to the fact that a higher concentration of Farnesol was needed to break up the thicker growth of biofilms. The results from this experiment are pictured in Figure 11. The OD595 reading for the control which contained no Farnesol, was also lower than expected; however, according to the data collected, all three trials resulted in the same pattern (much lower control OD595 readings than with the readings for different Farnesol concentrations). At this point in time, the lab was in the process of ordering new Farnesol stocks, and it is a possibility that the Farnesol that was used for this set of experiments was already deactivated, causing the much lower numbers in the control OD595 than the experimental values. Farnesol activity is very dependent on light and environmental conditions in which it is located. This anomaly could be studied more in future experiments, and the test itself could be run through more trials to ensure it was not researcher error.

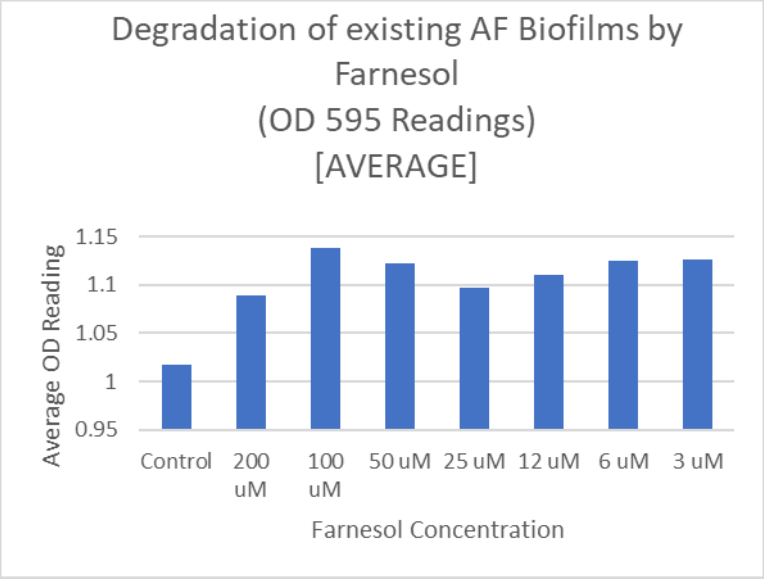


Figure 11: Effects of varying concentrations of Farnesol on degradation of *A. faecalis* biofilms through OD595 readings.

The results for the MTT test and OD450 readings for the degradation of existing biofilms looked much like the results seen for the normal OD595 readings. These results can be seen below in Figure 12. There is a significant drop in the number of metabolically active cells (in comparison to the other concentrations) at the 200µM Farnesol concentration. Again, this leads to the notion that a higher concentration works to degrade the cells because the biofilm has already been formed and needs more Farnesol to degrade it. However, the rest of the concentrations follow a similar pattern as the original OD595 readings as well, decreasing in the number of metabolically active cells until it reaches its lowest at 25µM and then slowing inching back up.

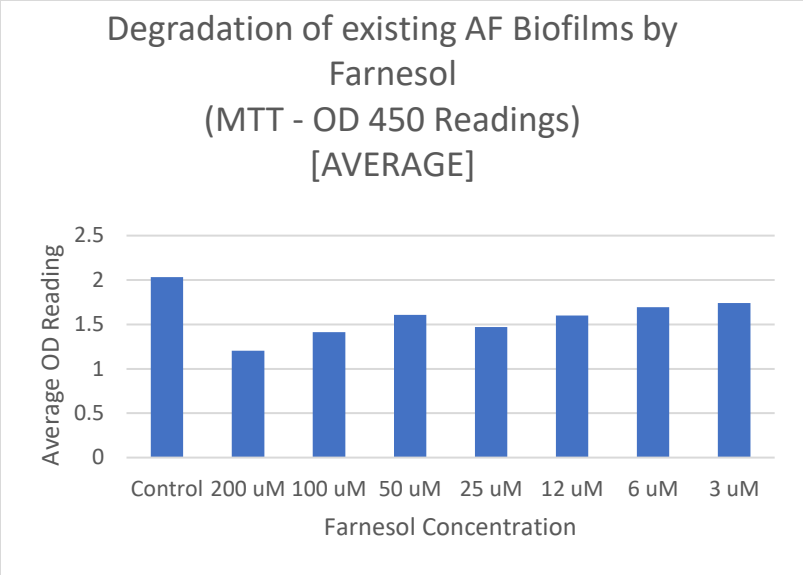


Figure 12: Effects of varying concentrations of Farnesol on degradation of *A. faecalis* biofilms through reduction of MTT and OD450 readings.

Lastly, we examined how varying concentrations of Farnesol would affect the overall cell mass of *A. faecalis* by staining with Crystal Violet and determining the OD595 readings. Though the 200µM concentration was not as low as expected after reviewing the averaged results of the previous two experiments, the results did follow a similar trend of having the 25µM concentration depleting the largest amount of cell mass. These results can be seen in Figure 13.

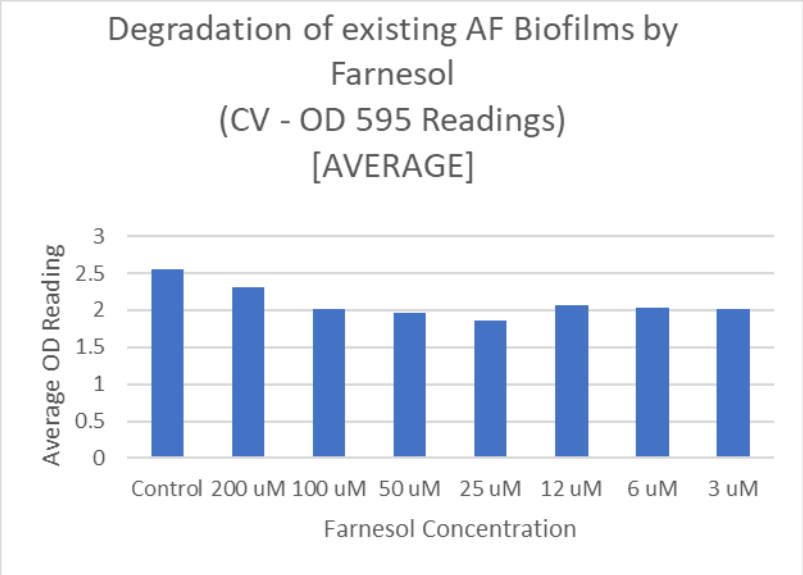


Figure 13: Effects of varying concentrations of Farnesol on degradation of *A. faecalis* biofilms through Crystal Violet staining and OD595 readings.

The overall analysis of these results shows that although the 50µM concentration range of Farnesol was the "ideal" concentration when preventing *A. faecalis* from creating a biofilm, the "ideal" concentration for invoking the degradation of already formed *A. faecalis* biofilms is closer to the 200µM or 25µM regions. Further research would be able to look at these trials, replicate them, and attempt to test more concentrations. We can also look further at the 200µM concentration to see how it causes a reduction of *A. faecalis* in all trials, even though the 50µM – 100µM concentrations had increased cell amounts.

CONCLUSION

As a society, we are quickly approaching a time when the antibiotics we have come to rely on so heavily, are no longer going to be able to treat even the simplest of infections. Quorum sensing molecules such as Farnesol are one of the potentials that researchers have found that may offer a solution to this widespread problem. Our results from this preliminary research can continually be expanded in the future. Now that we have determined that there is evidence supporting Farnesol's ability to inhibit growth, motility, and biofilm formation/degradation of *Alcaligenes faecalis*, there are several avenues that we can explore in future studies. These include, but are not limited to, adding the "ideal" Farnesol concentrations to currently used antibiotics to act as a synergistic form of treatment for infections while also testing Farnesol's effects on other bacteria (both in the *Alcaligenes* genus and others). The hope is that this research gives a starting point for more advances to be made in the field of alternatives to antibiotic treatments.

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