# Using Enzymes to Improve Antibiotic Effectiveness on *Staphylococcus epidermidis* Biofilm Removal

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## ABSTRACT

The effectiveness of five different enzymes as treatments against Staphylococcus biofilm growth was measured in the presence of antibiotics and alone. Protease was the least effective enzyme in biofilm removal with all antibiotics, and pectinase was the most effective with dicloxacillin and clindamycin. Also, dicloxacillin was the most effective antibiotic. S. epidermidis was resistant to ciprofloxacin except in the presence of amylase and pectinase. Overall, there was a statistically significant difference among the treatments on biofilm absorbance (ANOVA; df=23; F=2.06; p=0.009). Because of limited growth in the biofilm assay, no significant difference was found between the treatment groups and the control. However, a zone of inhibition study found that planktonic growth was unaffected by enzymes but inhibited by all antibiotics tested. Results support that enzymes may aid in antibiotic effectiveness against biofilm growth, and more extensive testing is warranted to explore why the impact differs in the biofilm structure.


## INTRODUCTION

Biofilms are communities of bacteria that infect indwelling medical devices; some biofilms are pathogenic, causing diseases (Donlan, 2001). Biofilms have developed antibiotic resistance because they are covered in extracellular polymeric substances (EPS) that form a matrix made up of polysaccharides and proteins (Cloete et al, 2010). This matrix helps protect the cells inside the biofilm and facilitates communication between the cells through physical and chemical signals (What is Biofilm, 2005). The EPS matrix is strong, making it difficult to break down. Therefore, new effective methods of biofilm removal are desperately needed. Because the EPS matrix is made up of polysaccharides and proteins, a possible method is to use enzymes such as polysaccharases and proteases to disrupt and break down the matrix structure. This was the focus of this study.

The purpose of this experiment was to use certain enzymes to break down the matrix of the biofilm Staphylococcus epidermidis in order to make antibiotics more effective. The antibiotics were then applied to the biofilm, and it was observed if they became more effective in the removal of the biofilm. The research hypothesis tested was that if different kinds of enzymes were applied to the biofilm Staphylococcus epidermidis, then antibiotics would be able to remove the biofilm more effectively.

## METHODOLOGY

This experiment measured the amount of Staphylococcus epidermidis that can be removed through the use of enzymes and antibiotics. The treatment group consisted of biofilms that were treated with enzymes alone, those that were treated with antibiotics alone, and those that were treated with a combination of both. These enzymes included amylase, cellulase, emporase, pectinase, and pro-

tease (Orgaz et al, 2006). The control group consisted of biofilms that received no treatment. Antibiotics used in this experiment included clindamycin, ciprofloxacin, and dicloxacillin. The dependent variable of this experiment was the amount of biofilm that was removed through treatment, which was determined by measuring the absorbance of the biofilm solutions (Merritt et al, 2005).

The first step of this project was to grow the biofilms for S. epidermidis by modifying the microtiter plate biofilm assay (Merritt et al, 2005). In this experiment, bacteria were grown in 24-well plates and allowed to incubate at 37 degrees Celsius for five days. After the incubation period, the treatments were added. After allowing the biofilms to incubate for another 24 hours, the wells were washed to get rid of planktonic bacteria. The remaining bacteria (biofilms) were then stained with crystal violet solution and shaken dry. After 24 hours, 70% ethanol was added to each well to detach the stained biofilm. The solutions from each well were then transferred to a cuvette, and the absorbance was measured using a SpectroVis and Vernier probe.

A separate part of this experiment included a zone of inhibition study. This was done to observe planktonic bacteria growth and verify antibiotic effectiveness. The bacteria were grown on nutrient agar plates, and each treatment of enzymes, antibiotics, and all combinations of both were streaked onto the plates. After incubation, the zones of inhibition were measured.

Aseptic technique was used throughout the experimentation. All enzymatic solutions were filter-sterilized, and filter-sterilized distilled water was used in making the antibiotic solutions. After the experiment was finished, anything that came in contact with the S. epidermidis bacteria was soaked in a 10 % bleach solution.

## RESULTS AND DATA ANALYSIS

### Part 1. Zone of Inhibition Study

For this project, a zone of inhibition study was done to compare the effects of the treatments on planktonic bacteria growth to those on the biofilms. This allowed for the antibiotic effectiveness to be verified. Most treatments showed a large zone of inhibition, meaning the concentrations of the treatments inhibited growth. However, the enzymes had no zones of inhibition, so they had no effect on the growth of the bacteria on their own. The most effective treatment was the dicloxacillin only, which almost completely inhibited the growth of the bacteria. Each enzyme was also tested with each antibiotic. An interesting observation was that the addition of enzymes did not alter the growth of planktonic bacteria. Each antibiotic performed similarly with enzymes when compared to the control of the antibiotic alone.

### Part 2. Biofilm Growth Study

The mean biofilm absorbance and standard deviation for each treatment group was calculated. The groups treated with protease and a combination of dicloxacillin and protease showed the highest average absorbance, meaning the most biofilm was present in these groups. The groups treated with dicloxacillin only had the lowest average absorbance as well as the lowest standard deviation, meaning there was the least amount of variance in this group. The

***Student Researcher Q &A***

*Carmen Candal has selected to attend the University of Georgia starting Fall 2012. She plans to study Animal Sciences as a step towards her goal of becoming a veterinarian. When asked about her experiences, Carmen shared the following tidbits to help future students.*

*Q: What advice would you give to students developing their first research projects?*
*A: I would suggest that students start by reading broadly on an area that interests them and then get more specific. I also found success by expanding on work that I had done before.*

*Q: What was the most rewarding part of the research process?*
*A: I feel better prepared for college and can see how research is applicable to the real-world. My mom said that she sees posters just like the ones I've presented when she is at the CDC. Presenting my work has helped me become more confident and improved my communication skills.*

group treated with protease only showed the most variance.

The average absorbance of biofilms that were treated with enzymes only compared to the control were calculated. The absorbance of the biofilms treated with protease (0.842) was much higher than any other group, and biofilms treated with cellulase showed the least absorbance (0.0745). For antibiotics alone, dicloxacillin was the most effective, and these biofilms had the lowest absorbance. In contrast, ciprofloxacin was the least effective antibiotic, and these biofilms had the highest absorbance, as shown in the following figures.

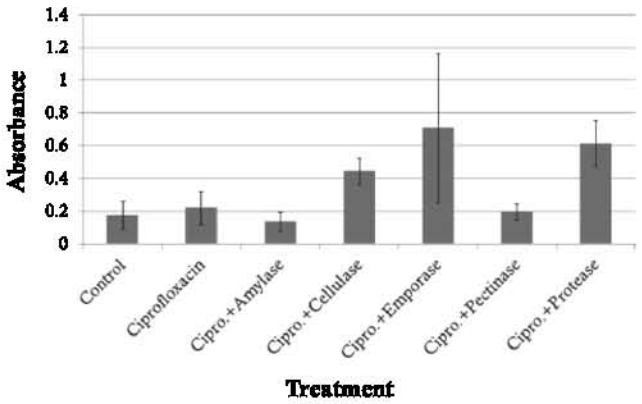


**Figure 1. Average absorbance of biofilms treated with Clindamycin and enzymes**

Figure 1 shows the average absorbance of biofilms treated with a combination of clindamycin and enzymes compared to biofilms treated with clindamycin only and the control. The graph shows that the biofilms treated with a combination of clindamycin and protease had the highest absorbance, and the biofilms treated with clindamycin only had the lowest.

Figure 2 shows the average absorbance of biofilms treated with a combination of ciprofloxacin and enzymes compared to biofilms treated with ciprofloxacin only, as well

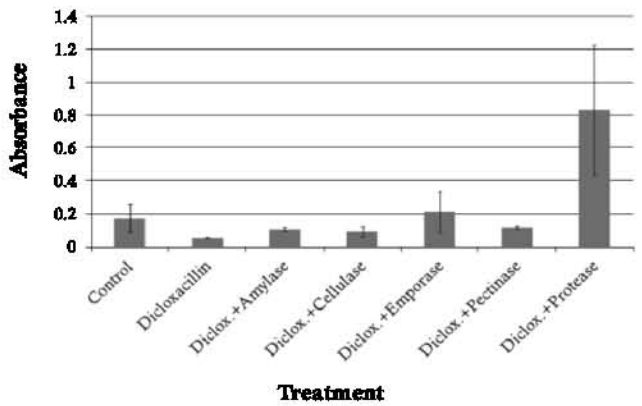


**Figure 2. Average absorbance of biofilms treated with Dicloxacillin and enzymes**

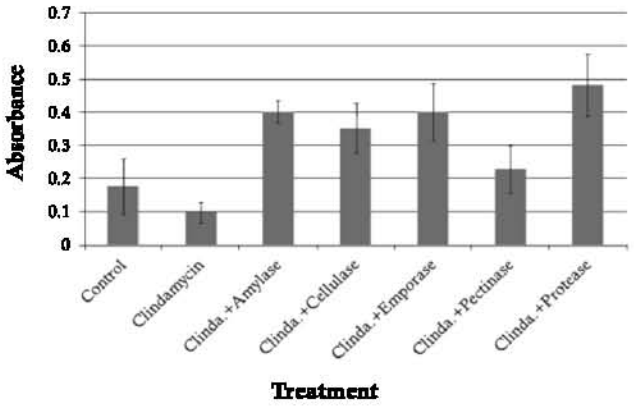


**Figure 3. Average absorbance of biofilms treated with Clindamycin and enzymes**

as the control. The biofilms treated with ciprofloxacin and emporase had the highest absorbance, and those treated with ciprofloxacin and amylase had the lowest.

Figure 3 shows the average absorbance of biofilms treated with a combination of dicloxacillin and enzymes compared to biofilms treated with dicloxacillin only, as well as the control. There was not much variance between these groups, except in the biofilms treated with dicloxacillin and protease, in which the average absorbance appears to be much higher than in the rest of the groups. Those biofilms treated with dicloxacillin only had the lowest absorbance.

Several trends were observed in the results. For instance, biofilms treated with protease, whether alone or in combination with another antibiotic, had the highest absorbance (Figures 1, 2, and 3). This most likely means that protease may actually aid S. epidermidis biofilm formation rather than inhibit it. Biofilms treated with dicloxacillin, whether alone or in combination with some enzyme, had the lowest absorbance (Figures 2 and 5). This most likely means that dicloxacillin is the most effective antibiotic of those tested in biofilm removal. Ciprofloxacin was the least effective antibiotic in reducing the absorbance of the biofilms (Figure 2).

For this experiment, an ANOVA test was used for inferential statistics to test for significance. A p-value of 0.05 was used, and the degrees of freedom were 23. The value calculated from the ANOVA test was 0.009. Because this is less than 0.05, the null hypothesis is rejected. Therefore, there was a statistically significant difference in biofilm growth by treatments of antibiotics and enzymes. Further post-hoc tukey tests show that each enzyme actually increased biofilm growth as increased absorbance was observed when they were added to the antibiotic treatment compared to the antibiotic alone.

## DISCUSSION AND CONCLUSIONS

The purpose of this experiment was to determine if there was a significant difference in the treatments on the growth of biofilms. The results from the ANOVA test showed that there was a significant difference in the absorbance of biofilms receiving varying treatments of antibiotics and enzymes ($df=23$; $F=2.06$; $p=0.009$). However, there was not a significant difference when compared to the control group. In other words, the biofilms treated with enzymes and/or antibiotics did not have a significantly lower absorbance than the biofilms that received no treatment. Therefore, the methods of biofilm removal used in this experiment were not effective.

The biggest problem in the experiment was getting the biofilms to grow and adhere to the well plates. There would appear to be a biofilm forming, but when the wells were stained, the biofilms would wash out. To try and fix this problem, the biofilms were allowed five days to incubate instead of the normal 48 hours. This was done to give them more time to grow and adhere to the surface of the well plates so quantifiable data could be collected. This method worked, and some quantitative results were collected, making it possible to use descriptive and inferential statistics to analyze the data.

To further the research, a wider variety of enzymes and antibiotics could be used. To apply this to more relevant medical applications, these methods could be used to try and overcome antibiotic resistance in pathogenic strains of bacteria such as Staphylococcus aureus rather than the non-pathogenic S. epidermidis.

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