Study of Glycine and Alanine Coupled with Carboxylic Acids as Biofilm Inhibitors in Common Bacteria Strains

Sara Davila Severiano, Jacob Tondreau, Parker Kight, Dr. Linda Hensel, Dr. David Goode

Department of Biology, Mercer University 1501 Mercer University Dr., Macon, GA 31207

Introduction

- Biofilm starts at quorum sensing when a quorum is reached, and gene regulation is then induced. Gene regulation occurs through signal transduction, where a ligand reacts with a surface protein. which results in a response within the cell. In this case, the response is a virulent response through the creation of biofilm. Biofilm is observed when bacteria adhere to a surface and a film-like substance is secreted by the bacteria that offers protection to the colony.

Figure 1: ***Streptococcus mutans***

- Medical devices such as prosthetics, heart valves, and central venous catheters can contain infections and diseases linked to biofilm growth.[1]
- Some strains of bacteria have become resistant to traditional antibiotics. Drugs that competitively interfere with the quorum sensing molecule would target specific bacteria, leaving natural flora untouched, and preventing resistant strains from developing. These drugs could prevent biofilm development and infection on medical devices.
- The purpose of this study is to investigate the properties of a series of novel drug compounds (Ala-6, Ala-9, Ala-46, Gly-4, Gly-32, Gly-58), and investigate their effects on *Streptococcus mutans, Escherichia coli, and Bacillus subtilis* biofilm production.
- *Streptococcus mutans* has been identified as the leading cause of dental cavities.
- *S. mutans* has been identified as causing endocarditis and intracerebral hemorrhaging. [2]
- Multiple health-care industries including dentistry and medicine are impacted detrimentally by biofilm. Dental plaque is a common type of biofilm in dentistry which leads to numerous diseases, like periodontal disease and cavities.[3]

Method and Analysis

- Drugs synthesized through a dehydration synthesis between a carboxylic acid and an amino acid to form a secondary amide bond.[4]
- Glycine coupled with 1-Adamantanecarboxylic acid, octanoic acid, and 3-(4-Bromophenyl) propionic acid.
- Alanine coupled with benzoic acid, p-bromobenzoic acid, and p-nitrobenzoic acid.

Drugs Synthesized

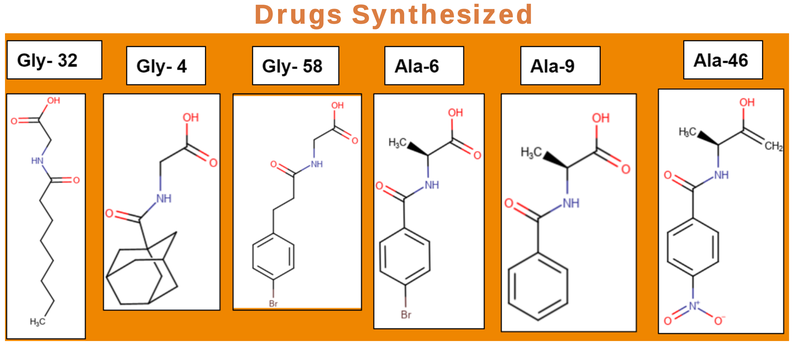

Figure 2: Novel drug compounds synthesized.

Abstract

Biofilm is a substance secreted by bacteria cells that offers protection to the bacteria colony, typically from host immune cells. Scientists have found biofilm to cause complications and infections in medical devices such as pacemakers and catheters. Biofilm production is stimulated when enough bacteria communicate through a process known as quorum sensing. This study aimed to identify possible amide coupled carboxylic acids and amino acids that resemble quorum-sensing signaling molecules as biofilm inhibitors in *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans, Escherichia coli, and Bacillus subtilis*. Crystal violet assays were used to test for biofilm inhibition; disk diffusion, congo red, and use-dilution assay; and planktonic assays were conducted to test for traditional antibiotic properties of bactericidal or bacteriostatic activity. Gly-4 and Gly-32 were found to be biofilm inhibitors in S. mutans, with 53% and 47% biofilm inhibition respectively; neither drug showed signs of traditional antibacterial properties. Future experiments are required to corroborate this study's findings, and to explore the efficacy of other drugs with similar functional groups to develop more sophisticated biofilm inhibitors.

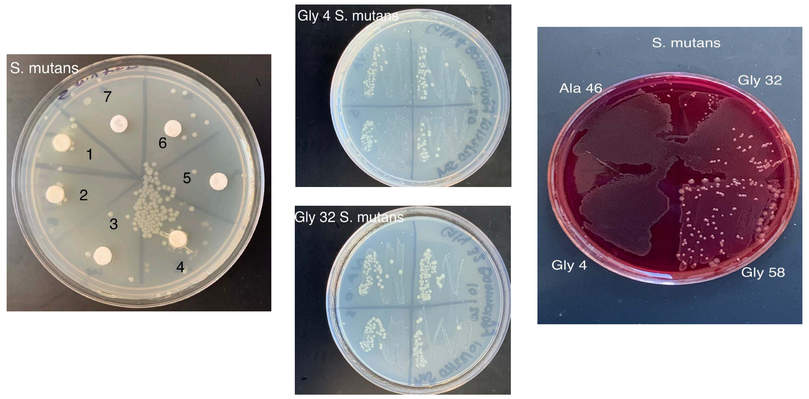

Figure 3: Gly-4 and Gly-32 on use-dilution, disk diffusion (1 and 2), and congo red assays, respectively, exhibiting lack of antimicrobial effects. [5]

Results

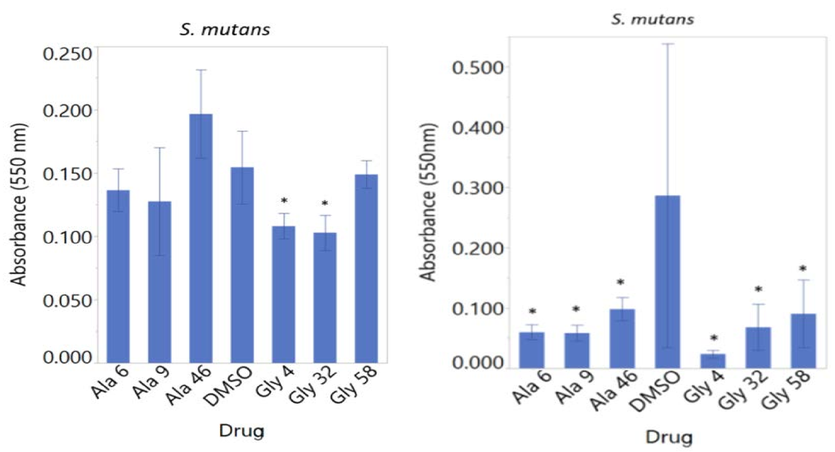

Figure 4: Crystal Violet Assay of *S.mutans.* Mean +/-SD, n=8-16. *Statistically different (p<0.05)

Species	Drug	Inhibition Run 1	Inhibition Run 2	Average % Inhibition
S. mutans	Gly-4	77.276	30.016	53.646
	Gly-32	62.335	33.414	47.875
	Gly-58	54.970	No difference	--
	Ala-9	65.600	No difference	--

Table 1: % inhibition. Cells labeled "No difference" are not statically difference in tested species from DMSO. (p<0.001, α= 0.05, n=16 for 1%DMSO; n=8 for all others).

Discussion

- A statistically significant difference across two trials was found in Gly-4 and Gly-32 in *S. mutans.*
  - Gly-4: 53.646% inhibition
  - Gly-32: 47.875% inhibition
- Both did not impede bacterial growth in the disk-diffusion assay
- In Use-dilution both drugs showed no sign of killing the bacteria

.

Limitations of Design

- Inconsistent CVA results. DMSO added incorrectly for initial *S. mutans, B.subtilis*
- Only one CVA can be considered valid, limiting the credibility of the rest of the study.

Future Plans

- More crystal violet assays, planktonic, and disk diffusion trials should be done with Gly-4 and Gly-32 in *S. mutans*
  - Determine what about Glycine is the cause for biofilm inhibition; Alanine was expected to inhibit biofilm as well due to its nonpolar nature.
- Determine the efficacy of Ala-46 as a biofilm accelerator in *S. mutans*
- New drugs of interest in *S. mutans* due to similar properties and functional groups
  - Alanine 4, 32, 22
  - Valine 4, 32, 22
  - Other nonpolar side chain amino acids

Acknowledgements:
We would like to thank Dr. Linda Hensel, Dr. David Goode, Dr. Denny, BOMM Lab TAs, and our peers.

References:

1. Donlan, R. M. (2001). Biofilms and Device-Associated Infections. *Emerging Infectious Diseases*, *7*(2), 277-281.
2. Mansour, T. R., Alam, Y., Dahbour, L., Alnemari, A., Jumaa, M., & Schroeder, J. L. (2017). Streptococcus Mutans: A Potential Risk Factor in Recurrent Hemorrhagic Stroke. Cureus, 9(5), e1264.https://doi.org/10.7759/cureus.1264
3. Seneviratne CJ, Zhang CF, Samaranayake LP. Dental plaque biofilm in oral health and disease. Chin J Dent Res. 2011;14(2):87-94. PMID: 22319749.
4. Goode, David. (2020). Department of Chemistry. List of Carboxylic Acids. Macon, GA: Mercer University.
5. Hensel, Linda. (2020B). Department of Biology. Testing Biofilm Inhibitors for Bacteriostatic, Bactericidal, and Biofilm Inhibition. Macon, GA: Mercer University..