Exploring the Impact of Temperature and *Stevia Rebaudiana* Leaf Extract on Biofilm Formation and Bacterial Growth in *Pseudomonas* Strains: Insights Into Antibiotic-Resistant Infections

Amelia Morra and Dr. Stephanie Strand, Department of Biology, College of Wooster

Abstract

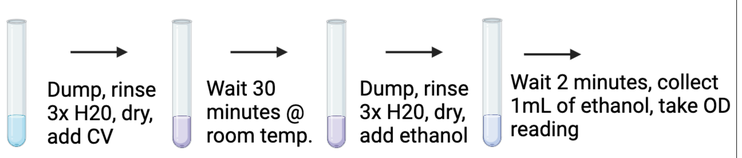
Biofilms coat the surfaces of bacterial cells, creating a matrix that contains an abundance of microorganisms. They play a major role in antibiotic-resistant infections. In this study we measured bacterial growth and biofilm formation in *Pseudomonas* strains. We examined these changes in response to different temperatures and exposure to Stevia leaf extract. The *Pseudomonas* strains tested showed different responses to varying temperature. Stevia extract inhibited biofilm formation in some of the strains and not others. Our findings suggest that closely-related *Pseudomonas* strains have similar regulation of biofilm formation in response to temperature and stevia extract.

Background

- Biofilms are sticky coatings secreted by some bacteria, they help bacteria adhere to surfaces such as a dogs bowl or your teeth. This protects bacteria from environmental stressors and harms such as antibiotics.
- Biofilms play a major role in bacterial antibiotic resistance. MRSA kills 9,000 people per year (CDC).
- Biofilm-forming genes have shown greater levels of expression in bacteria exposed to environmental stressors.
- Previous studies have shown that closely related bacteria show similar phenotypes in terms of biofilm formation and response to stress.
- Experts are trying to find ways of degrading these biofilms or preventing their initial formation. One compound being researched is Stevia leaf extract, from the plant *Stevia rebaudiana.*

Methods

Crystal Violet Assay: used to measure the amount of biofilm formation. Bacteria were grown in liquid cultures in test tubes for 36 hours at different temperatures. Then, the liquid culture was removed and the following procedure was conducted.



Results

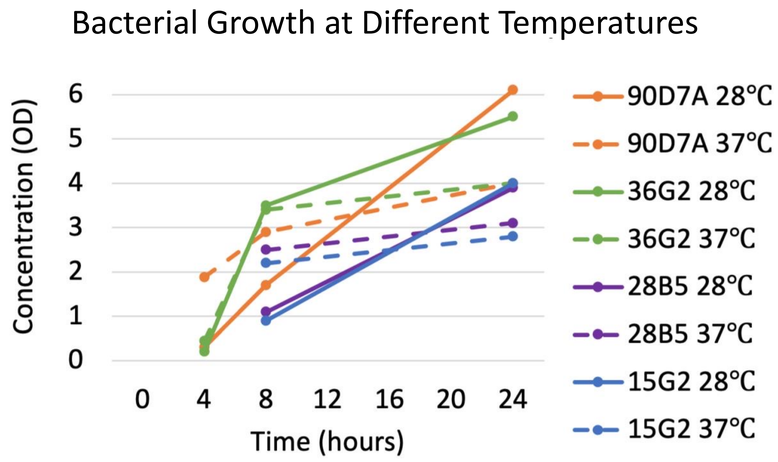


Figure 1. Bacterial growth over time when grown at different temperatures. *Pseudomonas* strains were incubated at different temperatures for 24 hours, while taking a measurement of the bacterial concentration every 4 hours.

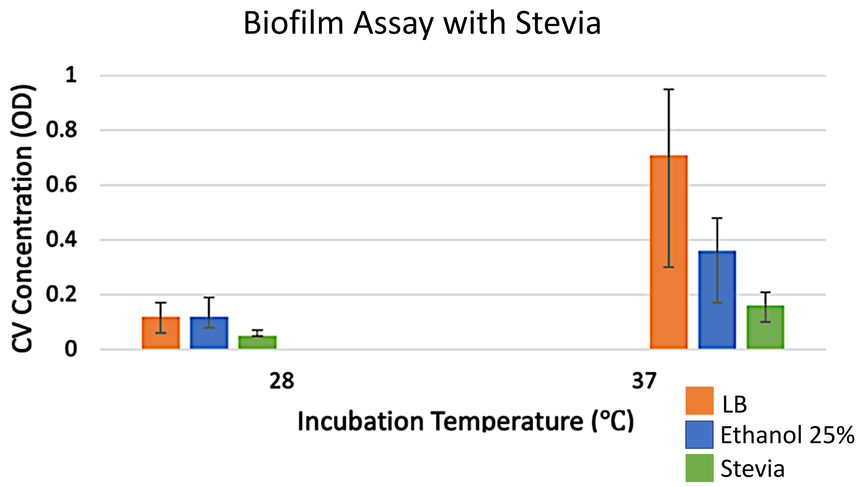


Figure 2. Biofilm formation when exposed to stevia extract. The *Pseudomonas* strain 15G2 was incubated in LB media with ethanol (blue), stevia extract (green), or no additive (orange). The more biofilm formation, the greater the Crystal Violet (CV) concentration of the assay.

Findings

- All four bacterial strains did not grow as well at 37°C, showing that this is not an ideal temperature for them and would be perceived as physiologically stressful (Figure 1).
- The strain 15G2 showed greater levels of biofilm formation at this stressful temperature as expected. Biofilms are often a stress response in bacteria (Figure 2).
- Biofilm formation in 15G2 was greatly inhibited by stevia, and partially inhibited by ethanol, a negative control for the stevia tincture (Figure 2).
- The biofilm assay was repeated for 36G2 and 28B5.

Discussion

- These findings were compared to a previously established phylogeny of *Pseudomonas* strains.
- While some bacteria within the same clade have the same response to increased temperature, all bacteria tested within the phylogeny showed inhibited biofilm formation when exposed to Stevia leaf extract.
- Our study suggests that phylogenetically related *Pseudomonas* strains show similar regulation of biofilm formation in response to environmental temperature and exposure to stevia extract.

Future Work

- Further studies can be done to examine biofilm formation in other *Pseudomonas* strains, expanding on this study.
- A study examining when biofilm formation begins during incubation and how long it takes to completely form is another area of future work.

References

- Centers for Disease Control and Prevention. 2022. Post-treatment Lyme disease syndrome. *Centers for Disease Control and Prevention*.
- Theophilus et.al. 2015. Effectiveness of *Stevia rebaudiana* whole leaf extract against the various morphological forms of *Borrelia burgdorferi in vitro. European Journal of Microbiology and Immunology.* 5(4).