Assessment of *Staphylococcus epidermidis* Biofilm Inhibition Using Essential Oils

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Introduction

Our skin is a host to many harmless microorganisms. Among these is *Staphylococcus epidermidis*. This bacterium, although harmless to our epithelia, is an opportunistic pathogen when entering the bloodstream. Due to *S. epidermidis'* prevalence, and the fact that it readily forms biofilms, its potential as an infectious agent is high among patients with pre-existing ailments. As we have previously found in UGRP-funded projects, essential oils are effective antibacterial agents. However, their effectiveness at preventing biofilm formation is an area that requires attention. In this project, S. epidermidis was cultured and further grown on plasma-treated polystyrene surfaces to form biofilms, whose growth was confirmed with crystal violet staining. To assess biofilm growth inhibition, the polystyrene surfaces will be treated with readily available Lemongrass, Clove Bud, and Cinnamon Leaf essential oils. Future experiments will involve genetic profiling of biofilm-forming vs. non-biofilm forming bacteria using droplet-digital Polymerase Chain Reaction (ddPCR). The targeted genes for the genetic profiling studies will be from the intracellular adhesion (ica) locus, which is responsible for biofilm formation. Results from this work have the potential to aid in prevention of biofilm formation. For this presentation, results related to biofilm growth optimization, as well as essential oil-mediated growth inhibition studies, will be presented.

Experimental

- *S. epidermidis* was cultured in Tryptic Soy Broth (TSB) medium and grown to an OD_{600} ~ 0.5.
- For the biofilm growth studies, 600 and 900 µL of the bacteria or TSB was transferred to the 48 well plate and the 24 well plate, respectively.
- Plates were filled with bacteria or TSB to account for positive and negative controls for biofilm growth assessment.
- The 48 and 24 well plates with bacteria and TSB were incubated between 30 minutes and 2 hours for biofilm growth studies.
- To test the inhibition of biofilm formation with lemongrass essential oil (LEO), varying dilutions of LEO (10x, 100x, and 1,000x) were added to wells containing both *S. Epidermidis* and TSB prior to incubation
- To assess biofilm growth inhibition by LEO, 24 well plates were incubated and shaken at room temperature for 1 hour.
- To measure the biofilm growth, plates were stained with crystal violet upon removing planktonic bacteria with Phosphate Buffered Saline (PBS), and further rinsed with deionized water
- The absorbance of each plate was measured at 590 nm using a Biotek microplate reader

Methods

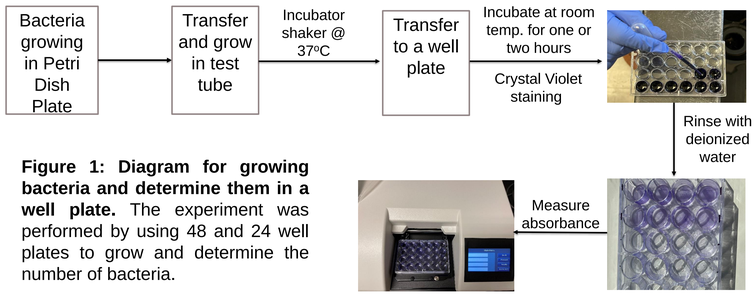

Figure 1: Diagram for growing bacteria and determine them in a well plate. The experiment was performed by using 48 and 24 well plates to grow and determine the number of bacteria.

Results

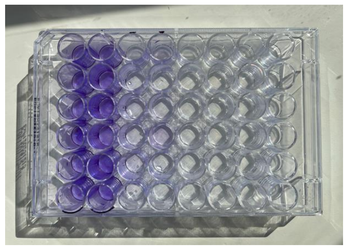

Figure 2: Biofilm Growth and Lemongrass Inhibition Study. Columns 1-2: *S. epidermidis* bacteria; Columns 3-4: Tryptic Soy Broth negative control. Wells were stained with Crystal Violet and rinsed with deionized water.

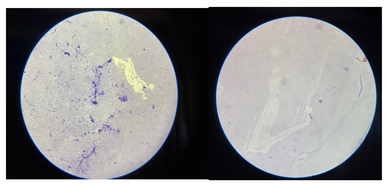

Figure 3: Light Microscope Images of Wells With Biofilm Growth. Both wells were incubated with *S. epidermidis,* stained with crystal violet, and rinsed with deionized water prior to image capture.

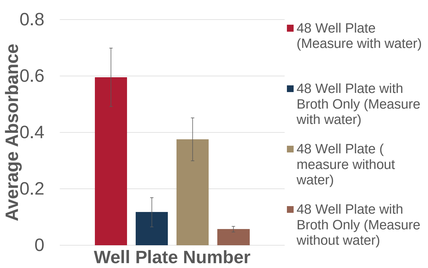

Figure 4: Effect of Solvent on Absorbance of Biofilms Stained With Crystal Violet. Absorbance of Positive and Negative control well plates were measured

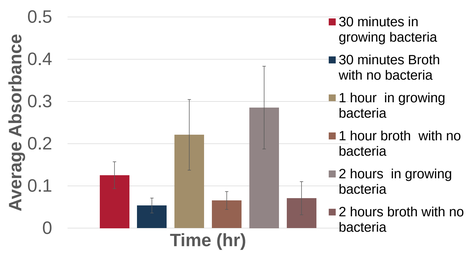

Figure 5: Biofilm Growth Time Study. Biofilm growth was assessed by measuring the absorbance upon staining with crystal violet after incubating bacteria in the 24 well plate. Significant differences in absorbance were found between 30 minutes and 1 hour, but not between 1 hour and 2 hours incubation.

Effect of Lemongrass on Biofilm Growth

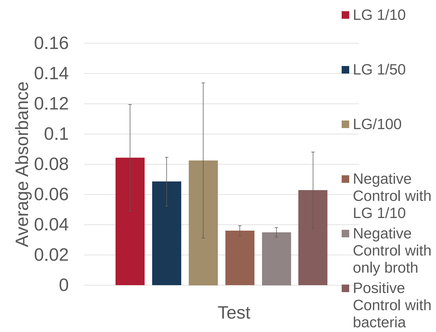

Figure. 6: Effect of Varying Lemongrass Concentrations on Biofilm Formation. 100 microliters Lemongrass diluted 1/10, 1/50, and 1/100 was added to 900 microliters of bacteria or broth.

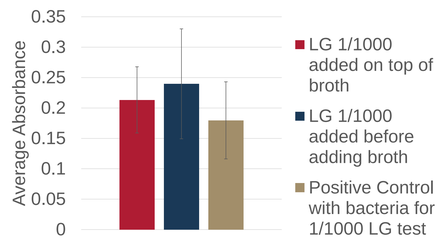

Figure. 7: Effect of Adding Lemongrass Prior to and After Bacteria on Biofilm Formation. 300 microliters Lemongrass diluted 1/1000 was added to 900 microliters of bacteria or broth, and a positive control of bacteria in absence of lemongrass was tested.

Conclusions

- The *S. epidermidis* Biofilm growth was significant on the polystyrene, plasma treated well plates, but there was no significant growth on the hydrophobic, non-plasma treated polystyrene well plates (Data Not Shown)
- The Biofilm growth significantly increased from 30 minutes of incubation to 1 hour of incubation on the plasma treated well plates at the 95% confidence level; however, there was no statistically significant difference in biofilm growth between the 1 hour well plate and the 2 hour well plate at the 95% confidence level
- The addition of the diluted lemongrass essential oil did not show significant biofilm growth inhibition in our initial studies. In the future, we will look for better options to enhance the lemongrass solubility
- Future studies will test Clove Bud and Cinnamon Leaf essential oils for biofilm growth inhibition
- In the future, Intracellular Adhesion (ica) genetic profiling of biofilm-forming *S. epidermidis* and planktonic bacteria will be performed using a droplet digital PCR.

References

- *Osong Public Health Res Perspect* **2018** 9(4): 160-166
- *Int J Antimicrob Agents* **2002** 19(6): 570-575
- *Clin Microbiol Infect* **2004** 10(12): 1081-1088
- *Microorganisms* **2019** 7(9): 345-358

Acknowledgments

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