Biofilm CFU Reduction by LB Concentrations *in vitro*

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Introduction

- **What are Biofilms?** They are a community of bacterial microorganisms attached to a surface and encased in a 3D matrix of extracellular polymeric substances.
  - Leads to persistent mammalian infections → increased treatment cost, morbidity, and mortality in orthopedic patients.

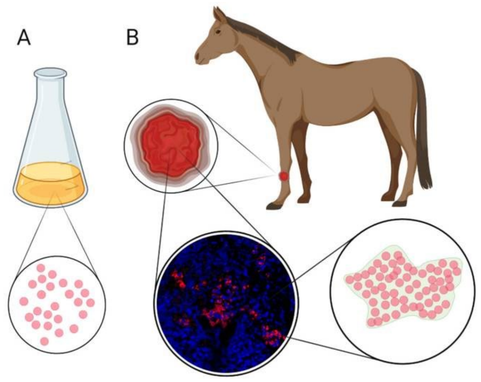

Figure 1

- Our lab uses *in vitro* techniques (A) to investigate how the animal's immune system would combat bacterial biofilms *in vivo* (B) (Figure 1).
- Biofilms treated with stem cells had discrepancies compared to untreated biofilms but had no outstanding differences quantitatively when measuring colony forming units (CFU) of bacteria.

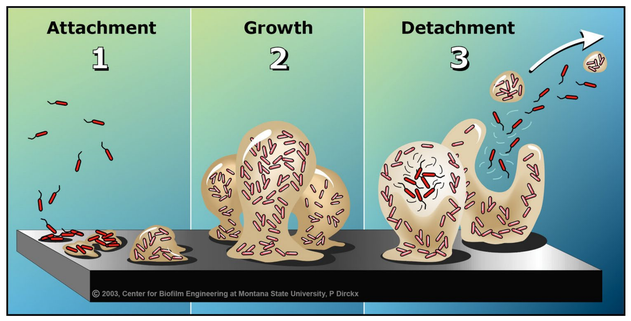

Figure 2: Growth cycle of a biofilm

- **My Question:** Were the *S. aureus* bacterial cells competing with the stem cells for nutrients in the *in vitro* environment?
  - This could be why there is a visible difference in biofilm size and structure but still allows it to maintain the bacteria's ability to disperse and form new colonies through CFU.
- Currently – there is no research on the competitive nature between biofilms and stem cells on nutrient consumption when *in vitro.*

Objective: To investigate the ability of variable lysogeny broth concentrations to establish *S. aureus* biofilm matrices *in vitro.*

Why use Lysogeny Broth (LB)? Commonly used nutritionally-rich media to promote bacterial growth.

→ Use Phosphate-buffered saline (PBS) to form varying concentrations of LB. PBS is a water-based salt solution and commonly used in substance dilution.

Hypotheses:

1. There will be no difference in CFU of *S. aureus* biofilms grown in lowered concentrations of LB broth.
2. There will be no difference in CFU between groups cultured in LB broth.

Materials

Lab Schedule:

Day -7	• Prepare tryptic soy agar (TSA) plates, sterile LB, and sterile 1 x PBS.
Day -3	• *S. aureus* culture setup.
Day 0	• Serially dilute *S. aureus* 10-fold to 10^{-7} and plate for biofilm setup.
Day 1	• Establish concentrations of LB:PBS and setup culture for *S. aureus* biofilms.
Day 2 & 3	• Digest 24 hour or 48 hour *S. aureus* biofilms, serial dilute each biofilm replicate, and plate CFU on TSA plates. • Photograph 24 hour plates for CFU quantification.
Day 4	• Photograph 48 hour plates for CFU quantification.

Statistical Analysis:

- Shapiro-Wilk normality test to see if the data had normal distribution.
- ANOVA with pairwise comparison using $\alpha = 0.05$ to see if data had significant results.

Results

Different LB concentrations had varying effects on the reduction of live bacteria of established *S. aureus* biofilms.

- When comparing between groups of LB concentrations at the 24-hour time point, there was no significant difference in CFU quantification.
- However, when comparing the 48-hour time point, the 50% LB had a higher CFU/biofilm count (mean = 1.27 x 10^{-9}) compared to the 0% LB CFU/biofilm count (mean = 6.00 x 10^{-8}) (P = 0.0062) (Figure 3).

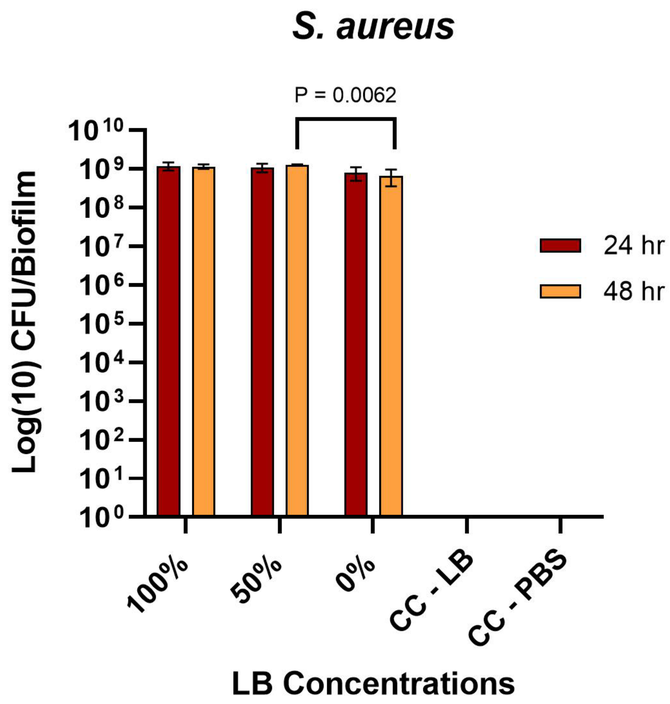

Figure 3: Live bacterial counts quantified by CFU analysis following 24 hours (maroon) or 48 hours (orange), of treatment by varying LB:PBS concentrations and contamination controls (CC) of LB and PBS. Bars represent means ± SD.

Results, cont.

Biofilms treated with a decreased amount of LB were slightly smaller and had a less defined extracellular matrix (Figure 4).

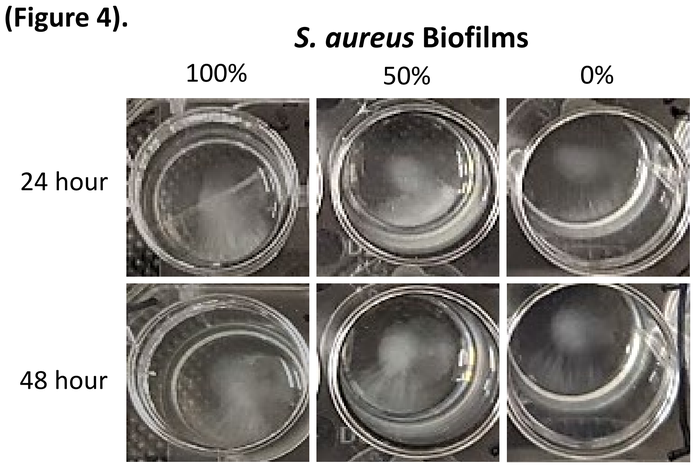

Figure 4: Standardized photographs of biofilms following 24 or 48 hours of culture treatment with varying LB concentrations.

Conclusion

- Variable LB:PBS concentrations did not effect live bacterial counts in biofilms for 24 hour cultures compared to the 100% LB standard treatment.
- Variable LB:PBS concentrations did, however, reduce live bacterial counts in biofilms grown in 0% LB compared to 50% LB for 48 hours.
- When performing photo analysis, the biofilms grown in low to zero amounts of LB appeared subjectively less organized and more transparent compared to 100% LB.

Discussion

- Discrepancy in lysogeny broth versus other mediums used in research studies, like stem cell media with varying amounts of fetal bovine serum (FBS) or equine serum included, will alter the rates of growth for biofilms.
- Comparing CFU in the adhered biomass versus the surrounding fluid following LB concentration treatments is needed to quantify biofilm dispersal.
- Larger experiments with more replicates might show significance when comparing between 100% LB and 0% LB during 48 hours. Currently P = 0.055.
- **Results show variable LB:PBS concentrations have some effect when the time point is higher than 24 hours.**
  - Preliminary data supports lowered concentrations of LB will not have an effect on biofilm growth & development when testing for 24 hours. However, lowered concentrations of LB may effect biofilm growth when testing for 48 hours or longer.

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Contact Information

Have questions? Email me at oliviab20@vt.edu for more info!

References

- Westgate, S. J., Percival, S. L., Knottenbelt, D. C., Clegg, P. D., & Cochrane, C. A. (2011). Microbiology of equine wounds and evidence of bacterial biofilms. *Veterinary Microbiology*, *150*(1-2), 152–159. https://doi.org/10.1016/j.vetmic.2011.01.003
- Jørgensen, Elin et al. "Biofilm and Equine Limb Wounds." *Animals : an open access journal from MDPI* vol. 11,10 2825. 27 Sep. 2021, doi:10.3390/ani11102825