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Biofilm Associated Staphylococcus Aureus Viability is Altered By Burkholderia Cenocepacia

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BIOFILM-ASSOCIATED *STAPHYLOCOCCUS AUREUS* VIABILITY IS ALTERED BY *BURKHOLDERIA CENOCEPACIA*

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BACKGROUND

Respiratory failure caused by chronic and recurrent microbial infections is the most common cause of death for people with cystic fibrosis (CF)[1], a disease causing the formation of thick mucus in the lungs[2]. Most bacteria can form biofilms, collections of sessile cells adhered to a surface by a secreted substance. Biofilm-associated cells develop antibiotic resistance at higher rates[3]. The thicker mucus in CF lungs is extremely difficult to clear via action of the mucociliary escalator and its presence fosters the formation of bacterial biofilms. *Staphylococcus aureus* and *Burkholderia cenocepacia* are two pathogens commonly found in the CF lung. Previous work in the Yoder-Himes laboratory established an antagonistic relationship between members of the *B. cepacia* complex and *S. aureus* biofilms[4]. To understand this antagonism, it is crucial to identify the biofilm changes occurring when *S. aureus* and *B. cenocepacia* interact. This work provides insight into the changes that may be responsible for the reduced viability of *S. aureus* in biofilms. Using crystal violet to measure biofilm biomass, confocal laser scanning microscopy, and assessing differences in antibiotic susceptibility, *S. aureus* and *B. cenocepacia* were examined in both monoculture and co-culture conditions. The results of this experiment indicate *S. aureus* and *B. cenocepacia* biofilm formation increases over time and is greater in nutrient-rich media. Additionally, *B. cenocepacia* inhibits biofilm formation of *S. aureus*. These findings provide information that can be used for understanding the interactions between pathogenic bacteria in the lungs of CF patients, leading to the development of more effective therapeutics.

METHODS

I. Crystal Violet assay to measure biofilm biomass
   i. Mid-logarithmic phase cultures were diluted into LB, TSB, 1% TSB, SCFSM, and M9 minimal medium supplemented with glucose and casamino acids. Dilutions of 10^6 CFU/mL were inoculated into a 96-well plate in 5 replicate wells per condition.
   ii. After incubation at 37°C for 3, 5, or 7 days, the plates were washed and stained with 0.1% crystal violet.
   iii. The stain was homogenized with 30% acetic acid and absorbances corresponding to total biomass were read using a spectrophotometer.

II. Confocal Laser Scanning Microscopy
   i. Mid-logarithmic phase cultures were diluted into TSB + glucose or SCFSM at 10^6 CFU/mL and mono- and co-culture conditions were inoculated in triplicate into 8-well chamber slides.
   ii. Chamber slides were incubated at 37°C for 3 or 7 days.
   iii. Biofilms were fixed with 1% formaldehyde and imaged using Nikon NIS-Element software and CLSM. Fluorescence was detected using GFP (487 nm) and TxRed (561 nm) lasers.

III. Antibiotic susceptibility assay
   i. Mid-logarithmic phase cultures were diluted into LB + glucose to 10^6 CFU/mL and inoculated into triplicate wells of a 96-well plate.
   ii. After incubation at 37°C for 1, 3, and 7 days, biofilms were treated with Vancomycin at 0, 0.5, 1.5, and 5.0 g/mL.
   iii. Biofilms were then sonicated to dislodge from 96-well plate surface, serially diluted, and plated. After 24 hours incubation, CFU/mL were manually counted.

RESULTS

HYPOTHESIS I: THE BIOMASSES OF *S. AUREUS* AND *B. CENOCEPACIA* WILL BE LARGER IN NUTRIENT RICH MEDIA AND INCREASE OVER TIME.

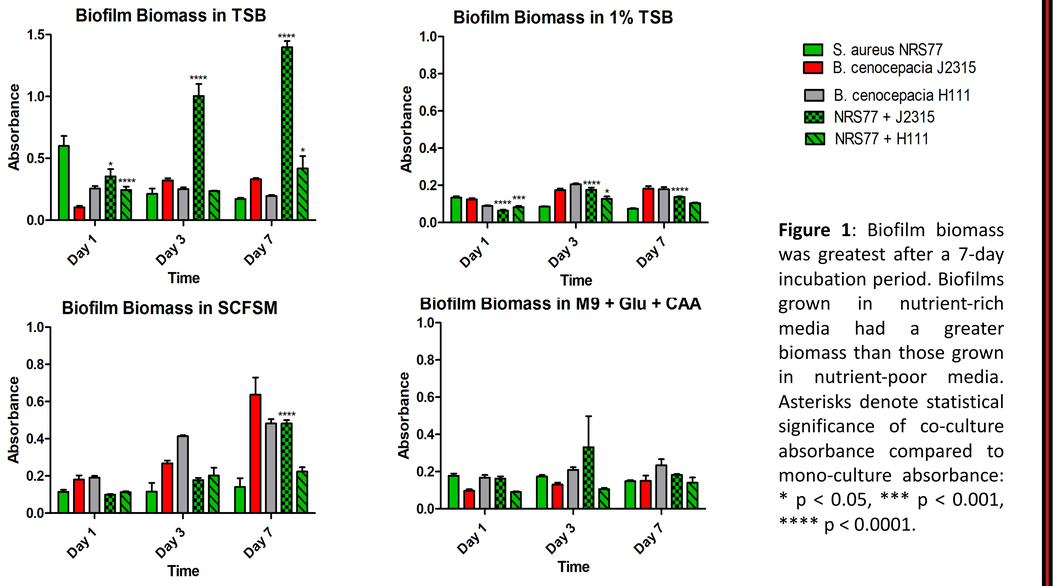

Figure 1: Biofilm biomass was greatest after a 7-day incubation period. Biofilms grown in nutrient-rich media had a greater biomass than those grown in nutrient-poor media. Asterisks denote statistical significance of co-culture absorbance compared to mono-culture absorbance: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

HYPOTHESIS IIN *S. AUREUS* BIOFILMS WILL BE STRUCTURALLY DIFFERENT IN MONO-CULTURE VERSUS IN CO-CULTURE WITH *B. CENOCEPACIA*

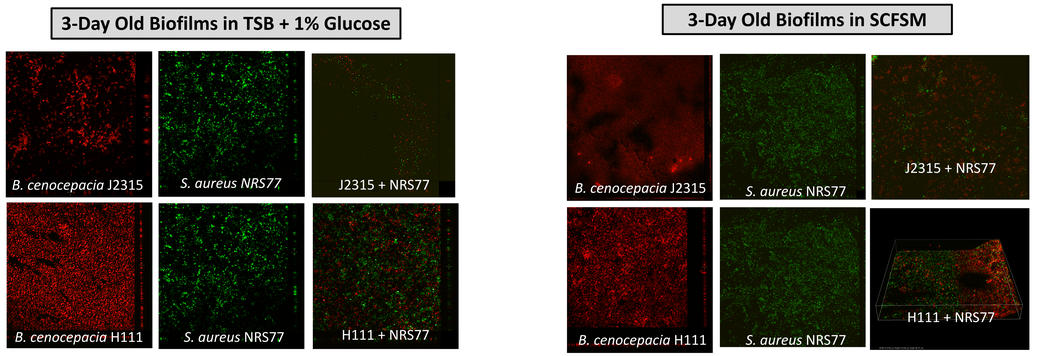

Figure 2: Representative images from confocal laser scanning microscopy of 3-day old biofilms indicate differences in biofilm formation of mono-culture versus co-culture biofilms. Biofilm structure is different depending on growth medium. The presence of *B. cenocepacia* (red) reduced the presence of *S. aureus* NRS77 (green) biofilms 3 days post co-inoculation.

HYPOTHESIS III: ANTIMICROBIAL RESISTANCE OF BIOFILM-ASSOCIATED *S. AUREUS* WILL INCREASE AFTER INCUBATION WITH *B. CENOCEPACIA*

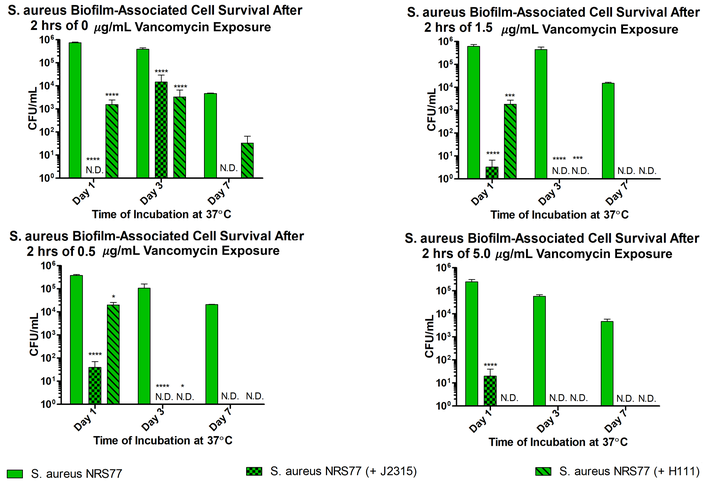

Figure 3. This pilot study did not demonstrate a clear trend in antibiotic resistance of *S. aureus.* However, this data supports the multi-strain *B. cenocepacia*-mediated reductive effect on *S. aureus* biofilm formation. Statistical analysis was performed using a 1-Way ANOVA. Asterisks denote statistical significance of co-culture CFU/mL compared to monoculture CFU/mL: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

CONCLUSIONS AND FUTURE DIRECTIONS

I. Biofilm biomass is dependent upon medium and co-occurring partner. Biomass does not indicate if one species makes up a greater proportion of the biofilm. Future work will aim to identify species-specific contributions to biofilm biomass.

II. Biofilm structure appears different across media types and in mono- versus co-culture conditions. Artifacts due to fixation method did not allow for enough biofilm surface to complete statistical analysis. A different method of fixation will be used in the future.

III. *S. aureus* antibiotic resistance did not follow a clear trend. This experiment serves as a pilot study to standardize growth conditions and effective antibiotic concentrations.

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