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The Effects of Nutrient Availability on Pseudomonas aeruginosa Mono and Co-culture Biofilms

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The Effects of Nutrient Availability on *Pseudomonas aeruginosa* Mono and Co-culture Biofilms

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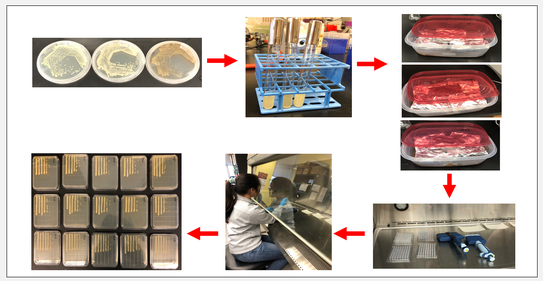
Introduction

Cystic Fibrosis (CF) is a genetic disorder characterized by faulty ion channels and result in thick mucus accumulation, especially in lungs. Mucus buildup provides ideal conditions for bacterial infections. *Pseudomonas aeruginosa* (PA) is the second most prevalent bacterium isolated from people with CF and has a high clinical importance[1]. Most CF pathogens form biofilms which make treatment of infections difficult. Biofilms are clusters of cells attached to a surface enclosed in a structured matrix. These structures are a means to provide shelter for bacteria from the environment, especially antibiotics and the immune system[2]. PA alone can form these biofilms, but communities of different bacterial species can also form biofilms together. Multispecies biofilms can form beneficial or antagonistic relationships with PA. In this study, we investigated the interaction between PA and two other important CF pathogens, *Staphylococcus aureus* (SA) and *Burkholderia cenocepacia* (BC). SA is the most prevalent CF pathogen and BC is arguably the most fatal. We tested the survival of these species in groups or alone in various nutrient conditions and in differing tobramycin concentrations. We chose tobramycin because it is an antibiotic commonly prescribed to treat PA infections. **Our results show that nutrient composition, antibiotic concentration, and time all had a significant effect on the interactions between PA mono-culture and co-cultured biofilms.** Understanding these interactions may set the stage for a better understanding of the clinical course of infection and how treatments can be altered for multi-species infections.

Methods



I. PA B84725, SA NRS77, and BC J2315 strains were streaked from the freezer stocks and placed in the incubator at 37°C overnight.

II. Overnights of each strain were obtained by isolating a colony of each into LB.

III. Mid-log cultures were diluted to 10^7 CFU/mL in the desired medium and transferred to multiple 96-well round bottom plates for the formation of mono and co-cultures biofilms.

IV. The 96-well round bottom plates were incubated at 37 ˚C for 1, 3, or 7 days.

V. Serial dilutions were performed for each day after treating each well with different antibiotic concentrations and sonicating the cells to obtain drip dilutions plates at different conditions. The drip dilutions plates were incubated at 37 ˚C for overnight.

VI. Each drip dilutions plate were counted based on CFU/mL.

VII. Steps I-VI were repeated for each media type: TSB, 1% TSB, M9-Glucose, and Artificial CF Sputum.

Results:

The Legend for the Graphs: PA B84725 Mono; PA B84725+SA NRS77; PA B84725+BC J2315

I. The Effects of Nutrients on PA B84725 Mono-Culture and Co-Culture Biofilms

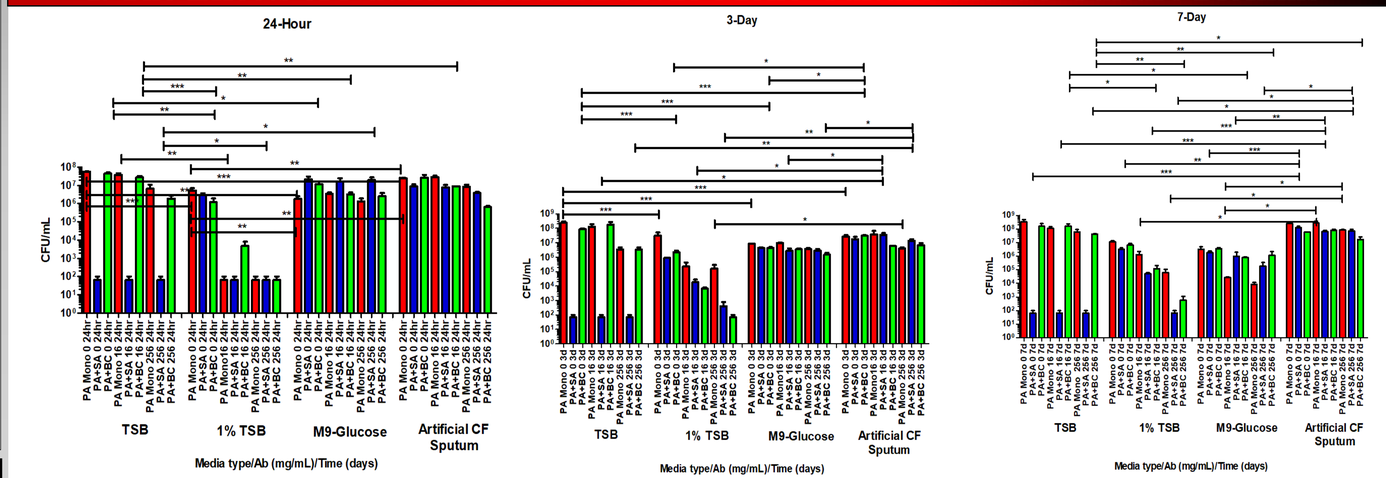


Figure 1: The effects of nutrient availability were tested by analyzing the data with a one-way ANOVA with Tukey's posttest comparing each media type at each antibiotic concentration and each condition. Asterisks indicated above show statistical significance of the relationships marked with a capped line.

II. Tobramycin Concentration Effect on PA B84725 in the Different Media

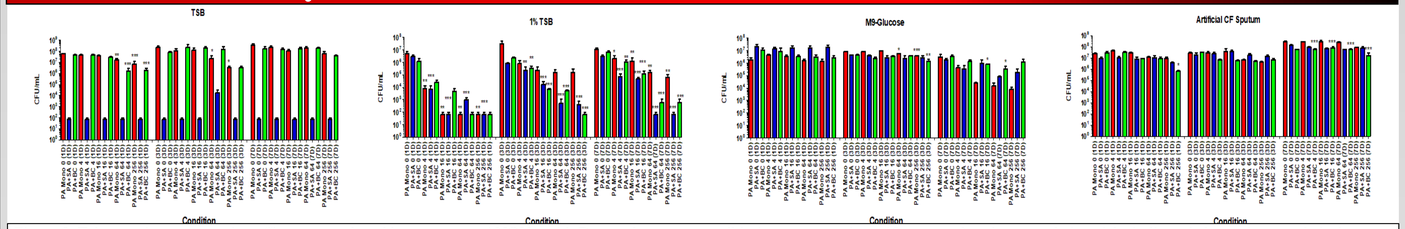


Figure 2: Tobramycin concentration effect was analyzed by a one-way ANOVA with Dunnett's posttest; antibiotics concentration of 0 was used as the control. Asterisks indicated above show statistical significance.

III. Co-Culture Effect on PA B84725 in the Different Media

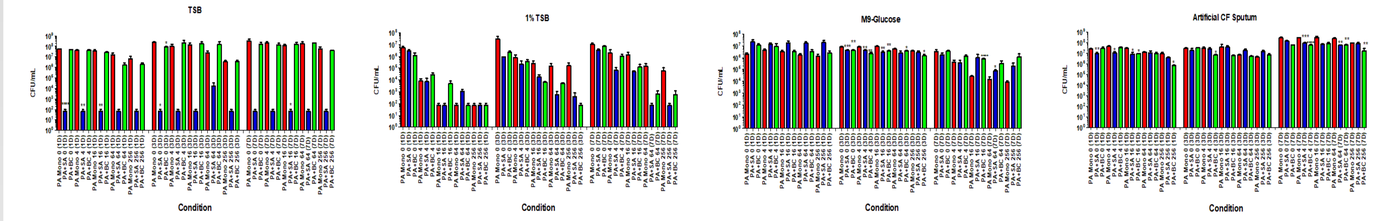


Figure 3: The co-culture effect was determined by analyzing the data with t-tests between mono and co-culture. Asterisks indicated above show statistical significance.

IV. The Effects of Time on PA B84725 in the Different Media

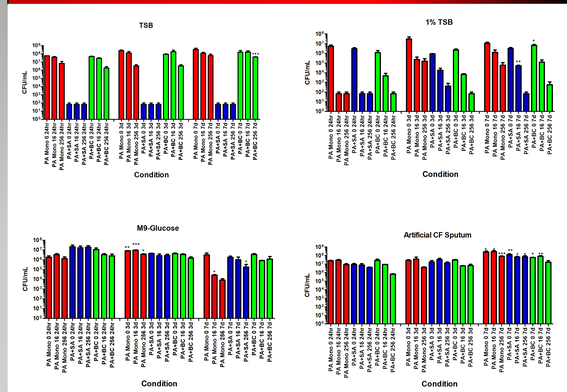


Figure 4: Time comparison between antibiotic concentrations were analyzed by one-way ANOVA with Dunnett's posttest using 24 hours as the control. Asterisks indicated above show statistical significance.

Conclusions/Future Studies

- The data shows that the variables studied, including time, nutrient availability, co-culture partner, and antibiotics, effect PA viability in biofilms.
- **Nutrient Availability:** Overall high nutrient availability increased PA numbers in monoculture. TSB had significantly less PA in co-culture with SA and BC, with SA eliminating PA altogether; however, the other media types, including artificial CF sputum, showed PA survival when grown in co-culture with SA and BC. Because artificial CF sputum medium has fewer nutrients than TSB, this suggests that lower nutrient availability shifts the co-culture effect from antagonistic to beneficial.
- **Antibiotic Concentrations:** Increasing antibiotic concentration significantly lowered PA viability in monoculture and in co-culture.
- **Co-Culture:** Co-culturing PA with SA or BC significantly lowered PA numbers across media types and Ab concentrations except in 1% TSB.
- **Time:** When the time is increased from 1 day to 3 days the increased antibiotic concentration had less of an effect on PA viability in monoculture and in co-culture. This suggests that biofilm maturation in monoculture and in co-culture can allow PA to withstand antibiotics.

Future Studies:

- To expand on the knowledge of how different variables affect each other by repeating the experiment with different strains of bacteria, antibiotics concentrations, time period, other media types (intermediates such as 50% M9-Glucose or Artificial CF Sputum).
- Develop a better understanding of the mechanism of PA, SA, and BC.

References & Acknowledgements

[1] Davies et al. (2007). Cystic fibrosis. *BMJ*, *335*(7632), 1255–1259.
[2] López et al. (2010). Biofilms. *Cold Spring Harbor perspectives in biology*, *2*(7), a000398.
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