## **Biofilm Development: Many characters, different plots**

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Biofilms are ubiquitous. They can be found on your driveway and in your mouth. Biofilms composed of *Escherichia coli* and *Staphylococcus aureus* are leading causes for nosocomial infections. Biofilms provide protection to bacteria from harsh environments and antibiotics. Understanding environmental factors that influence the development of biofilms may lead to better control methods. The purpose of this work is to study biofilm development under varying environmental conditions. The hypothesis is that surface chemistry, temperature, nutrients, and time influence the arrangement of cells within a biofilm, cell densities, and biofilm matrix production. Single-species biofilms of E. coli and S. aureus were developed on glass and polystyrene surfaces at 28°C and 37°C from log phase batch cultures. Cell arrangements and densities within the films were examined for early phase (1hr) and older (24hr) biofilms. The cells within the biofilms were fixed, stained with crystal violet, and then visualized by light microscopy. The films were examined at 30 random locations on each surface. Cell arrangement for each cluster of cells was categorized as individual, 2, 3, 4, or 5+ cells, and direct counts were performed. All experiments were performed in triplicate. Previous research by this group demonstrated that temperature plays a significant role in the arrangement of cells within the biofilm. Cells within biofilms developed at 28°C were predominantly clustered, whereas bacteria were mostly individual at 37°C. Work performed this summer indicated S. aureus biofilms had greater cell densities in general than their E. coli counterparts, polystyrene biofilms typically had more attached cells than glass biofilms, and biofilms developed at 28°C have greater cell densities then those developed at 37°C. The cell densities of early phase E. coli biofilms had lower cell densities than their older film counterparts (ANOVA p<0.05), but the opposite trend was observed for S. aureus biofilms, early phase had greater densities than older films (ANOVA p<0.05). Based on the data, it appears that S. aureus biofilms develop over time using different mechanisms than E. coli. Further research will examine the role of nutrients in these complex microbial-surface interactions and the role environmental factors play in the production of biofilm matrix polymers.

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