DspB mutants used to degrade *S. epidermidis* biofilms

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Site Information

Poulin Lab

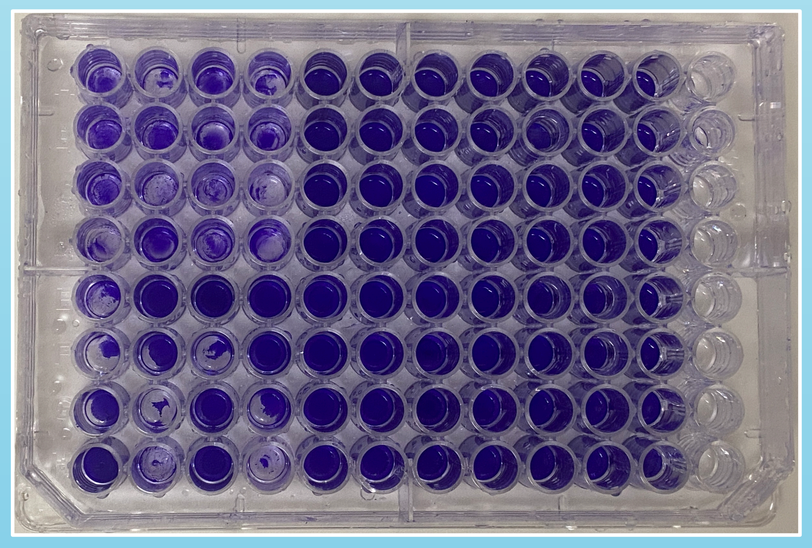
University of Maryland, College Park

Drs. Alex Peterson and Myles Poulin

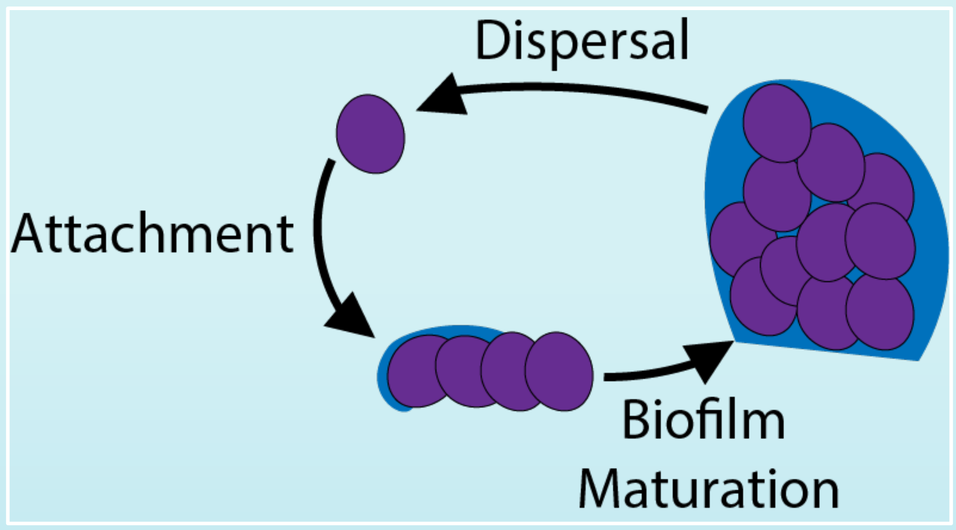
Investigate proteins involved in the synthesis and breakdown of bacterial biofilms

Introduction

- Biofilms are 3D communities of bacteria that form on surfaces which are impenetrable to most antibiotics and immune cells
- A major component of some biofilms is the polysaccharide poly-N-acetylglucosamine (PNAG)
- *Staphylococcus epidermidis* is a bacteria found on human skin, but can form pathogenic biofilms which are made of PNAG
- DspB is an enzyme that can cleave PNAG



Example of biofilm assay used to determine biofilm dispersal.
More purple → more cells remaining → less dispersal



Life cycle of a bacterial biofilm. The purple circles represent cells, and the blue outline represents the Extracellular Polymeric Substance (EPS)

Activities

My part of this project included inoculating, scaling, and inducing cell cultures to produce the DspB variants. Once we had enough of each variant, Dr. Peterson and I would use many dilutions of the enzymes to disperse mature *S. epidermidis* biofilms. Then through staining with crystal violet and absorbance spectroscopy, we quantified dispersal relative to enzyme concentration.

Discussion

- *S. epidermidis* biofilms were grown in 96 well plates
- Wild-type and mutant DspB were expressed, purified, and used to disperse the biofilms
- The mutant $DspB_{D242N}$ which can only degrade PNAG in certain conditions degraded biofilms less effectively than $DspB_{wt}$

Future Work

I'm going to continue my work in the lab, and I will be starting on a project to optimize the activity of DspB using site directed mutations and directed evolution. This will involve high throughput screening of 120 different mutants to look for increased activity with respect to the wild-type. I got to work on this particular project during the fall. I enjoyed working on this project, and being in a research lab has reinforced my interest in research and pursuing some kind of biological research as a career.

Acknowledgments

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