

# Ashley Bush

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Major: Biology

University: Southwest Missouri State University

Faculty Mentor: Dr. Lesa Beamer

Mentor Department: Biochemistry

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## **Expression and purification of AlgX in *Pseudomonas aeruginosa***

Ashley R. Bush, Catherine A. Regni, Lesa J. Beamer, Aniruddha Raychaudhuri and Peter A. Tipton

*Pseudomonas aeruginosa* is a Gram-negative bacterium that is the leading cause of hospital acquired infections. While this bacteria is present in water and soil, this bacteria only severely affects severely ill patients, such as those with cystic fibrosis. In cystic fibrosis patients, the bacteria will lodge in the lungs and form a coating, called a biofilm, around itself to protect it from the natural defenses of the body and antibiotics. This biofilm is formed by exopolysacchride sugar, known as alginate. The goal of this project is to, by isolating some of the proteins that help produce the biofilm, find the three-dimensional structure of those proteins. This will make it easier for various pharmaceutical research companies to create a drug to inhibit the specific active site in the proteins, preventing biofilm formation and allowing the infection to be treated with traditional antibiotics. The proteins encoded by two genes were picked for this study, algK and algX. Both are believed good targets because they have been shown in previously written papers to be critical to formation of the biofilm in *P. aeruginosa*. (1, 2) The cloned genes (in the form of a plasmid) previously obtained were used in the transformation step into *E. coli*. The lab protocol for general transformations was followed. To show that the bacteria were actually producing the protein we needed, the growth tubes were harvested; the cells lysed, and run on a 10% acrylamide gel to check for protein expression in the experimental versus control sample. The algK gel was inconclusive, however algX clearly overexpresses in large quantities. While most of the protein is insoluble, enough is soluble to warrant complete purification of the protein to set up crystallization trays. The current problem is how to optimize purification, because it co-elutes from a nickel column with contaminating proteins. A slow concentration bump of the buffers used to purify is being tested to see if it helps with this problem.

References 1. Antonette Robles-Price, Thiang Yian Wong, Havard Sletta, Svein Valla, Neal L. Schiller. 2004. AlgX Is a Periplasmic Protein Required for Alginate Biosynthesis in *Pseudomonas aeruginosa*. *J. of Bacteriology*. 186:7369-7377. 2. Jain, Sumita, Franklin, Michael J., Ertesvag, Helga, Valla, Svein, Ohman, Dennis E. 2003. The dual roles of AlgG in C-5-epimerization and secretion of alginate polymers in *Pseudomonas aeruginosa*. *Molecular Microbiology*. 47. 1123-1133