Understanding How the Pel Polysaccharide Is Modified for Use in The *Pseudomonas Aeruginosa* Biofilm

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Biofilms are communities of microbial cells surrounded by an extracellular matrix that enables the embedded microbes to resist the immune system and antibiotic therapies. The Gram-negative bacterium *Pseudomonas aeruginosa* predominantly exists as a biofilm and is commonly found in chronic infections, including the lungs of individuals with Cystic Fibrosis (CF). The Pel polysaccharide is a component of biofilms formed by some clinical CF strains and can sequester the aminoglycoside antibiotics used to treat *Pseudomonas* infections.

After synthesis and transport of the α-1,4-linked N-acetyl-galactosamine polysaccharide across the inner membrane, PelA partially deacetylates and hydrolyzes the polymer into a range of a low- and high-molecular weight polysaccharides prior to its export out of the cell. The hydrolase activity of PelA is required for generating the low-molecular weight secreted form of Pel, which is important for the biomechanical properties of the biofilm and *P. aeruginosa* virulence. Determining the mechanism(s) of how PelA modifies the Pel polysaccharide is essential for understanding how the biofilm of *P. aeruginosa* is regulated during infection. To first gain insight into how the deacetylase and hydrolase activities are coordinated, we determined the structure of PelA from the thermotolerant ortholog *Pseudomonas thermotolerans* (PtPelA) using single wavelength anomalous dispersion to 2.1 Å resolution. Comparing the structure of PtPelA to known structures using the DALI server suggests that the domain organization and the full-length structure of PtPelA is unique. The structure revealed an electronegative groove that extends from the deacetylase to the hydrolase domain of PtPelA, highlighting a potential path for Pel to move through the enzyme as it is modified. Mutation of residues critical for deacetylase activity results in decreased hydrolase activity in a biofilm disruption assay, suggesting that the deacetylation of Pel can precede hydrolysis. Experiments to determine how full-length PelA binds Pel and to further understand the mechanisms of deacetylation and hydrolysis of the polymer are currently underway.