Role of AlgL in *Pseudomonas aeruginosa* alginate biosynthesis

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Pseudomonas aeruginosa is an opportunistic pathogen responsible for chronic lung infections in cystic fibrosis patients. These *Pseudomonas* infections are characterized by formation of surface-associated biofilms and overproduction of alginate exopolysaccharide. Alginate is synthesized, modified, and exported by a multi-protein complex that spans the inner and outer bacterial membranes. While most of the proteins within the biosynthetic complex have an established role in exopolysaccharide production, the role of the alginate lyase, AlgL, remains poorly understood. In this study, we determine the structure of AlgL and, through structural alignment with the homologous alginate lyase A1-III from *Sphingomonas* sp., identify active site residues important for alginate binding and catalysis. We demonstrate that both in a *ΔalgL* strain or when this strain is complemented with active site variants, induction of alginate expression is detrimental to the bacteria and results in a lethal phenotype. The active site point mutants Y256F, R249E, and R249A were found to negatively affect *P. aeruginosa* growth and viability *in vivo* by growth curve and colony forming unit analyses. Furthermore, transmission electron microscopy (TEM) images of whole cells demonstrated that the absence of AlgL and the catalytic point mutants Y256F, R249E, and R249A result in abnormal cell morphology, including membrane perturbations and build-up of substance within the periplasmic space. Approximately 5% of *ΔalgL P. aeruginosa* cells complemented with the K66A active site variant also demonstrated abnormal cell morphology. Structural determination of the K66A point mutant and alignment with *Sphingomonas* sp. A1-III show that K66 is part of a conformationally flexible lid loop region which probably directly interacts with alginate. The structural comparison suggests that this residue moves over 14 Å when the enzyme-substrate complex is formed. *In vitro* characterization of AlgL point mutant enzymatic activities is currently in progress. Combined, our results suggest that AlgL functions to degrade alginate that is not exported from the cell, thereby preventing its accumulation within the periplasmic space.