The role of extracellular polymeric substances in *Pseudomonas aeruginosa* biofilm architecture

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen. It causes chronic lung infections in the cystic fibrosis patients. These infections become highly resistant to antibacterial treatments. Bacteria develop this resistance because they become protected inside biofilms. Biofilms are microbial communities enmeshed in a partially self-produced and partially recruited, impregnable extracellular matrix. The matrix is composed of extracellular DNA, proteins, lipids and exopolysaccharides. The exopolysaccharides play an imperative role in architecture of the biofilm matrix. *P. aeruginosa* produces three distinct exopolysaccharides; Psl, Pel and alginate. In this study, non-mucoid strain PAO1 and mucoid (producing excessive alginate) strain PDO300 of *P. aeruginosa* were used to generate mutants deficient in one or more exopolysaccharides. Role of these three exopolysaccharides in biofilm formation was investigated. Results showed that the absence of alginate altered the architecture of biofilms in PDO300 as well as in PAO1, when compared to biofilms formed by the respective parent strains. Psl was found indispensable for mushroom-like shape of the biofilms in both strains. Pel was required for the compactness of the biofilms, but PAO1 formed mushroom-like structures even in the absence of Pel. However, Pel-deficient PDO300 did not form mature biofilm, suggesting differential role of Pel in the two strains. Psl-only as well as Pel-only, producing mutants were able to formed multilayer biofilm. Production of one type of exopolysaccharide appeared to influence production of the other types of exopolysaccharide. Psl-deficient mutants increased the production of Pel, while Pel-deficient mutants showed a ten-fold increase in the production of alginate. Furthermore, absence of negatively charged alginate in the biofilm was compensated by eDNA. Regulation of exopolysaccharide biosynthesis operons showed a high expression of *psl* operon in PAO1, whereas its expression in PDO300 was surprisingly low and confined to a few cells near the base. A high and uniform expression of the *algD* operon in PDO300 was observed at all times during biofilm development. A low expression of *algD* operon was also detected in PAO1. Expression of the *pel* operon was confined to the stalk of PDO300 and PAO1. The role of PelF, the only glycosyltransferase encoded by *pel* operon, in Pel biosynthesis was investigated and found to be a soluble glycosyltransferase which uses UDP-glucose towards Pel biosynthesis. Site directed mutagenesis revealed that conserved R-325 and K-330 were essential for the PelF activity.

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