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Temporal-spatial regulation of exopolysaccharides in mucoid and non mucoid *Pseudomonas* aeruginosa biofilms

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Pseudomonas aeruginosa forms biofilm at biotic and abiotic surfaces. These biofilms are resistant to antibiotic and disinfectant treatments. Biofilm matrix plays an important role in architecture and function of the biofilm. Three exopolysaccharides, *Psl*, Pel and alginate are important components of the biofilm matrix. Biosynthesis of these three exopolysaccharides is controlled by three operons which are under tight control of their respective promoters. Here, we have shown the temporal and spatial regulation of the *psl*, the *pel* and the *algD* operons. Promoters of the respective operons were fused with *gfp* gene and expression of the GFP was studied by observing biofilms through confocal laser scanning microscopy. In wild type non mucoid PAO1, the results showed that *psl* operon was expressed throughout the PAO1biofilm structure at all timepoints and nearly 90% of the biofilm was populated by the cells showing the *psl* operon expression. The cells showing expression of the *algD* operon were present only in the base of PAO1 biofilms. The biofilms formed by the mucoid strain PDO300 (an isogenic *mucA* mutant of PAO1) were populated by more than 90% cells which were expressing the *algD* operon. However, the cells expressing the *psl* operon were very few and present near the base of the PDO300 biofilms. Expression of the *pel* operon showed gradual increase with the age of the biofilm and was mainly observed in the cells near the attachment surface especially in the base of the stalk of the biofilms formed by both PAO1 and PDO300.

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