

Title: "Multiple Roles of Extracellular Polymeric Substances on Susceptibility of Biofilm and Detached Clusters"

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### **Supporting Information**

Figure S1 – *P. aeruginosa* strains on agar plates and under SEM. (a) EPS-; (b) WT; (c) EPS+.

Figure S2 – Flow cell system set up

Figure S3 – Relationship between biofilm structural parameters and viability ratio

Figure S4 – Spatial distribution of biomass through horizontal cross-sections. Biomass content in each cross-section determined using COMSTAT. Biofilm thickness is normalized for strain comparison. Error bars represent one standard error.

Construction of isogenic *P. aeruginosa* mutant strains via allelic exchange

SEM imaging of the bacterial cells

CLSM imaging

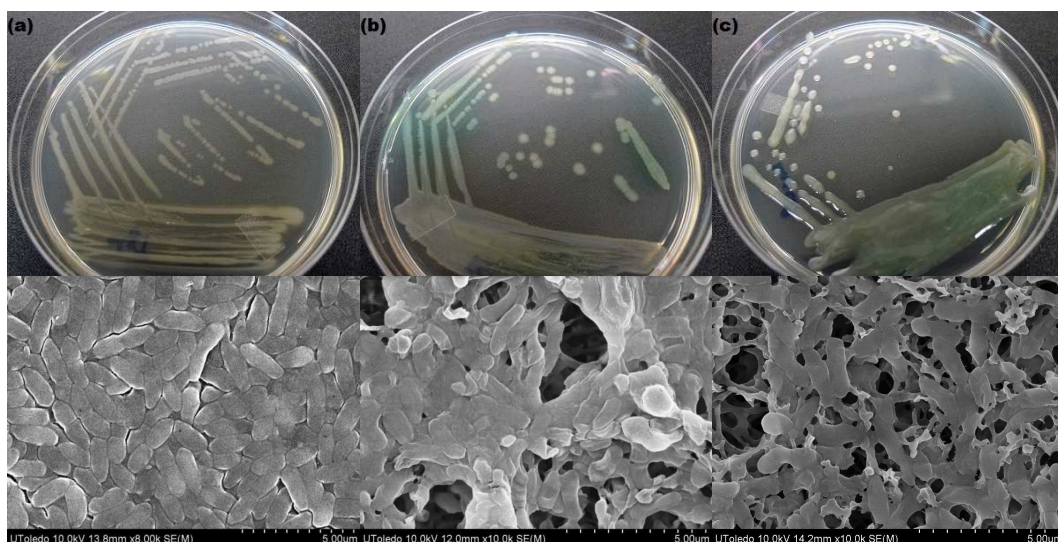


Figure S1 – *P. aeruginosa* strains on agar plates and under SEM. (a) EPS-; (b) WT; (c) EPS+.

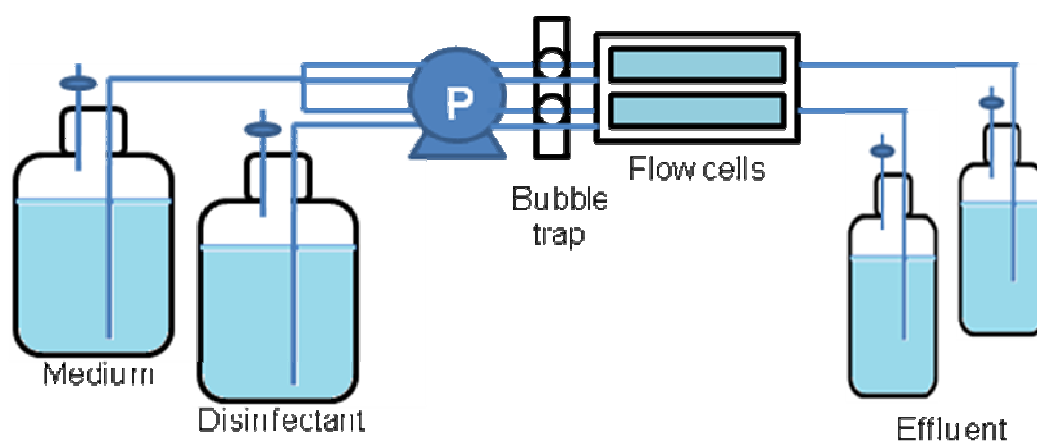


Figure S2 – Flow cell system set up

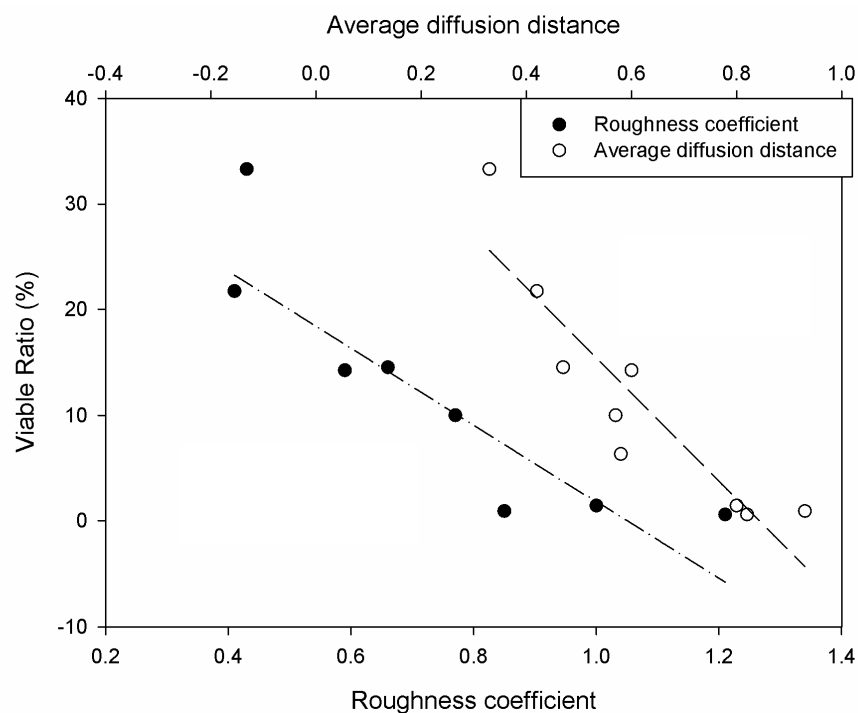


Figure S3 – Relationship between biofilm structural parameters and viability ratio

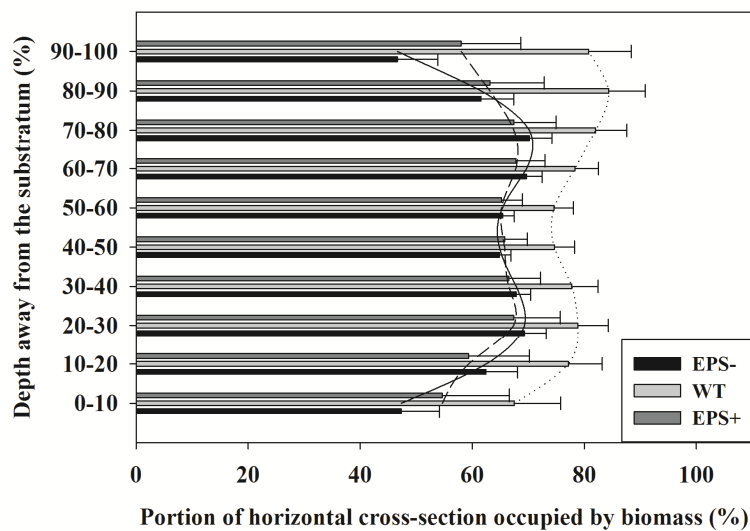


Figure S4 – Spatial distribution of biomass through horizontal cross-sections. Biomass content in each cross-section determined using COMSTAT. Biofilm thickness is normalized for strain

comparison. Error bars represent one standard error ( $N \geq 15$ ).

#### Construction of isogenic *P. aeruginosa* mutant strains via allelic exchange

All isogenic mutant strains were constructed in wild-type *P. aeruginosa* strain PAO1. Two mutants were constructed, the first using a *mucA22* allele (the most common for overproduction of alginate EPS in patients afflicted with cystic fibrosis) (Devries and Ohman 1994) and PAO1 *algT(U)* (for little or no alginate EPS production). Both mutants were constructed by genetic disruption of the *mucA* and *algT(U)* coding sequence with an 850-bp gentamicin resistance ( $Gm^R$ ) cassette from pUCGM (Schweizer 1993) and with the gene replacement vector pEX100T (Schweizer and Hoang 1995).

#### SEM imaging of the bacterial cells

All tested bacterial strains with EPS were visualized using scanning electron microscopy (SEM). Strains were grown to the late exponential phase, and planktonic suspensions were prepared as above. Suspensions were aseptically filtered through 0.45  $\mu\text{m}$  nitrocellulose membranes to capture bacteria (0.45  $\mu\text{m}$  pore size, Millipore). Membranes were sputter coated and viewed using a Hitachi S-4800 High Resolution SEM at 30 kV.

#### CLSM imaging

Biofilms were imaged using a Leica TCS SP5 laser scanning confocal microscope (Leica Microsystems, Bannockburn, IL) at 20X and 63X magnification with a scan rate of 400Hz in

sequential scan mode to eliminate any spectral overlap in the individual fluorophores. Specifically, the SYTO 9 and PI were excited with a 488 laser line and subsequently collected at 510-535 nm and 605-650 nm respectively. The ConA-Alexa 633 was excited with a 633 laser line and collected in the 650-690 nm range. Z-stacks were acquired in the 512 X 512 format in 1  $\mu$ m steps.

### **Reference:**

- Devries, C.A. and Ohman, D.E. (1994) Mucoid-to-Nonmucoid Conversion in Alginate-Producing *Pseudomonas-Aeruginosa* Often Results from Spontaneous Mutations in *AlgT*, Encoding a Putative Alternate Sigma-Factor, and Shows Evidence for Autoregulation. *Journal of Bacteriology* 176(21), 6677-6687.
- Schweizer, H.P. (1993) 2 Plasmids, X1918 and Z1918, for Easy Recovery of the Xyle and LacZ Reporter Genes. *Gene* 134(1), 89-91.
- Schweizer, H.P. and Hoang, T.T. (1995) An Improved System for Gene Replacement and Xyle Fusion Analysis in *Pseudomonas-Aeruginosa*. *Gene* 158(1), 15-22.