ONLINE DATA SUPPLEMENT

A nanoscale characterisation of the interaction of OligoG with the cell surface of *Pseudomonas aeruginosa* and its effects on cell motility.

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MATERIALS AND METHODS

Bacterial Motility

Two methods (a plate and stab assay) were utilised to assess the effect of OligoG on bacterial motility. Overnight (O/N) cultures of *P. aeruginosa*, *Burkholderia* spp. and *S. aureus* (negative control) were grown in tryptone soya broth (Oxoid) at 37°C. Cultures were diluted 1:100 into MH broth supplemented with 0, 0.2, 0.5, 2, 6 and 10% OligoG and re-incubated for 18 h at 37°C. For the plate assay; Basal Medium 2 (BM2; E1) supplemented with 0, 0.2, 0.5, 2 and 6% OligoG was prepared and inoculated with 10 µl of PAO1 MH culture. Plates were incubated for 23 h at 37°C and the swarming distance recorded at 2, 5, 7, 13 and 23 h.

For the stab-assay, broth cultures of *Burkholderia* spp. and *S. aureus* were prepared as above. Five ml agar aliquots (for stab-cultures) were prepared using motility test agar (MTA; Mast Group Ltd., Bootle, United Kingdom) supplemented with 0, 0.2, 0.5, 2, and 6% OligoG. (Addition of 10% OligoG completely inhibited setting of the MTA and so was not used). Stab-cultures were inoculated from MH cultures and incubated for 24 h at 37°C. Motility was observed as a lateral diffuse spread of red colour subjectively scored from 0 to 4 (E2). Absence of growth beyond the inoculation tract was scored as 0 (non-motile), and growth throughout the agar was scored as 4 (highly motile). The agar concentrations in BM2 and MTA were 0.5% and 0.4% respectively.

AFM Surface Roughness measurements

AFM images were analyzed using Nanoscope data-processing software to obtain the Root Mean Square (RMS) surface roughness of a 250 nm^2 area selected from the middle of each PAO1 cell and each PAO1 cell treated with OligoG appearing in the AFM images.

RESULTS

AFM Surface Roughness measurements

Surface roughness measurements (derived from AFM images) demonstrated that OligoG treatment significantly reduced the observed surface roughness (3.67±1.02nm to 2.96±0.99nm; $P<0.05$); this change reflecting the OligoG binding and 'coating' of the cell surface observed in the AFM images.

References

E1. Overhage J, Lewenza S, Marr AK, Hancock REW. Identification of genes involved in swarming motility using a *Pseudomonas aeruginosa* PAO1 Mini-Tn*5*-lux mutant library. *J Bacteriol* 2007;189:2164-2169.

E2. Khan S, Tøndervik A, Sletta H, Klinkenberg G, Emanuel C, Onsøyen E, Myrvold R, Howe RA, Walsh TR, Hill KE, Thomas DW. Overcoming drug resistance with alginate oligosaccharides able to potentiate the action of selected antibiotic*s. Antimicrob Ag Chemother* 2012;56:5134-5141.

Figure Legends

Figure E1. *(A)* Mean zeta potential values for 10% OligoG, untreated PAO1 and PAO1 treated with 10% OligoG (post-wash) at various pH values in 0.1 M NaCl. *(B)* Cell size analysis of PAO1, PAO1 treated with 10% OligoG (pre-wash) and PAO1 treated with 10% OligoG (post-wash) in 0.1 M NaCl.

Figure E2. Typical size distribution by volume at pH 5, 0.01 M NaCl of PAO1 (black solid line); PAO1 treated with OligoG (pre-wash) (grey solid line); PAO1 treated with OligoG

(post-wash) (grey dashed line). (*A*) 0.2% OligoG. (*B*) 2% OligoG. (*C*) Their corresponding mean sizing values (nm).

Supplementary Data

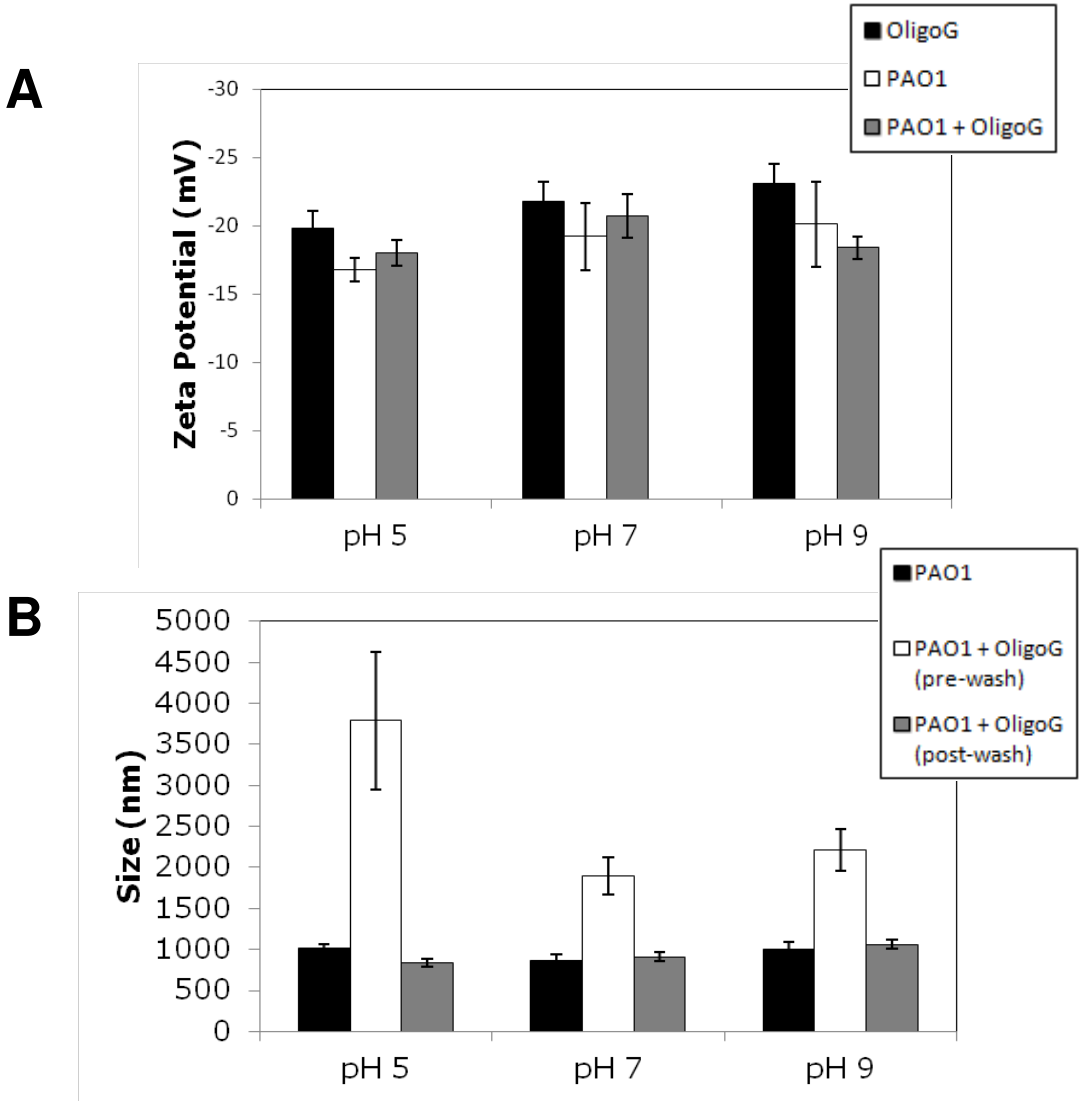
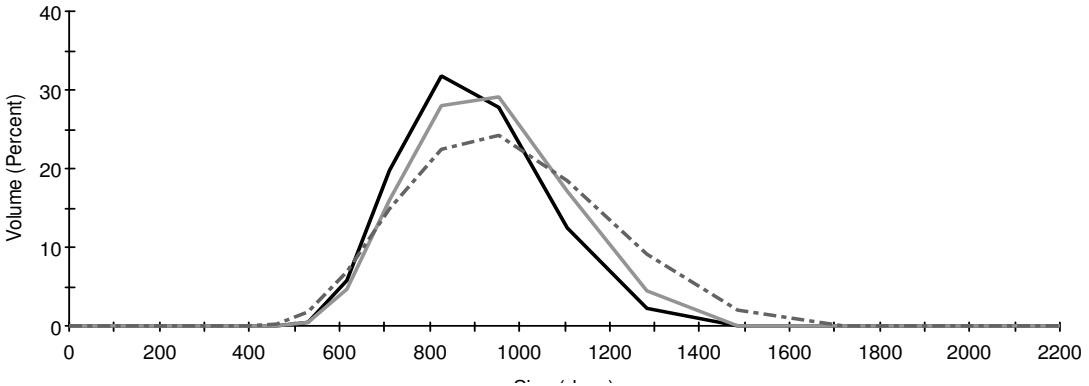
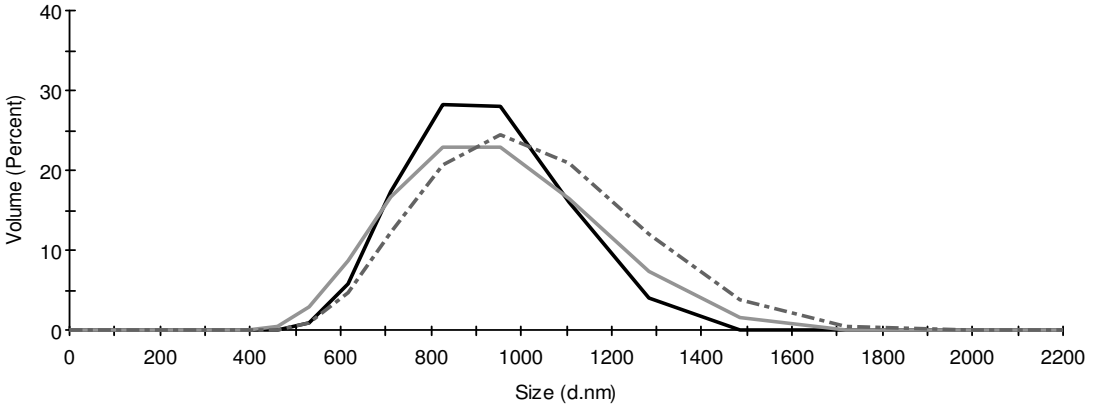


Figure E1. *(A)* Mean zeta potential values for 10% OligoG, untreated PAO1 and PAO1 treated with 10% OligoG (post-wash) at various pH values in 0.1 M NaCl. *(B)* Cell size analysis of PAO1, PAO1 treated with 10% OligoG (pre-wash) and PAO1 treated with 10% OligoG (post-wash) in 0.1 M NaCl.

A



B



C

	Mean Sizing (nm)		
	PAO1	PAO1 + OligoG (pre-wash)	PAO1 + OligoG (post-wash)
0.2% OligoG	837.0 ± 74.8	872.9 ± 61.3	1131.9 ± 137.5
2% OligoG	900.1 ± 49.4	970.9 ± 96.3	905.9 ± 71.6

Figure E2. Typical size distribution by volume at pH 5, 0.01 M NaCl of PAO1 (black solid line); PAO1 treated with OligoG (pre-wash) (grey solid line); PAO1 treated with OligoG (post-wash) (grey dashed line). (*A*) 0.2% OligoG. (*B*) 2% OligoG. (*C*) Their corresponding mean sizing values (nm).