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Supplementary Materials for

Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms

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The PDF file includes:

table S1. Strains and plasmids used in this study.

Strain	Description	Reference or Source
E. coli BL21-CodonPlus® (DE3)-RP	F$^-$ *omp*T *hsd*S(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DE3) *end*A [*arg*U *pro*L Cam^r]	Stratagene
P. aeruginosa PAO1	*P. aeruginosa* wild-type strain; serotype O5	(*76*)
PAO1 P_{BAD}*psl*	*psl-ara*C-P_{BAD} promoter replacement. Expression of *psl* operon upon induction with L-arabinose	(*49*)
PAO1 P_{BAD}*psl* Δ*pelF*	In-frame deletion of *pelF* in P_{BAD}*psl* background	(*60*)
PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel*	*pel-ara*C-P_{BAD} promoter replacement. Expression of *pel* operon upon induction with L-arabinose	(*47*)
PA14	*P. aeruginosa* wild-type strain; serotype O19	(*77*)
Pa 62	*P. aeruginosa* isolate (environmental, soil)	(*78*)
X13273	*P. aeruginosa* isolate (clinical, blood)	(*78*)
MSH3	*P. aeruginosa* isolate (environmental, water)	(*78*)
MSH10	*P. aeruginosa* isolate (environmental, water)	(*78*)
19660	*P. aeruginosa* isolate (clinical, blood)	(*78*)
CF127	*P. aeruginosa* isolate (clinical, from cystic fibrosis patient)	(*78*)
IMR-90	normal human female lung fibroblasts; diploid; stable	ATCC® CCL-186™

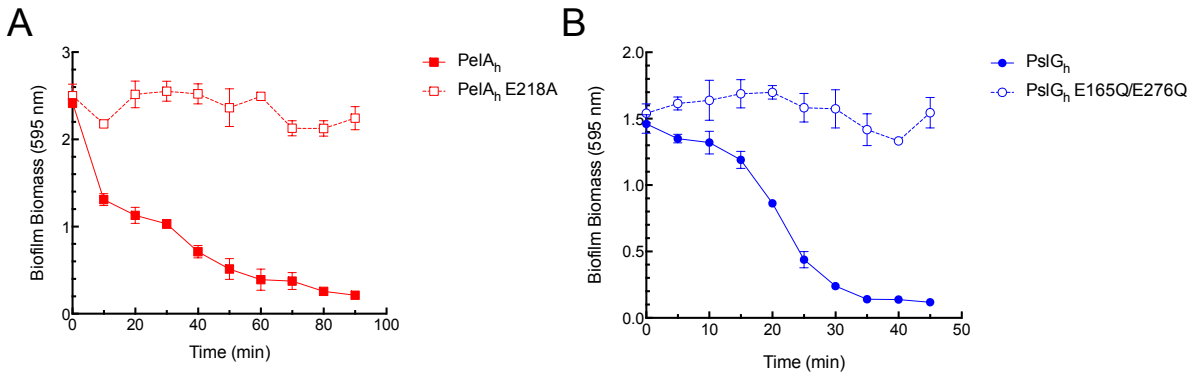

fig S1. Time course disruption of *P. aeruginosa* biofilms. Crystal violet staining of biofilms following the exogenous addition of glycoside hydrolases (**A**) $PelA_h$ and (**B**) $PslG_h$ on their respective exopolysaccharide. Each data point represents the mean from $n = 3$ crystal violet microtiter plate wells. Error bars indicate SEM.

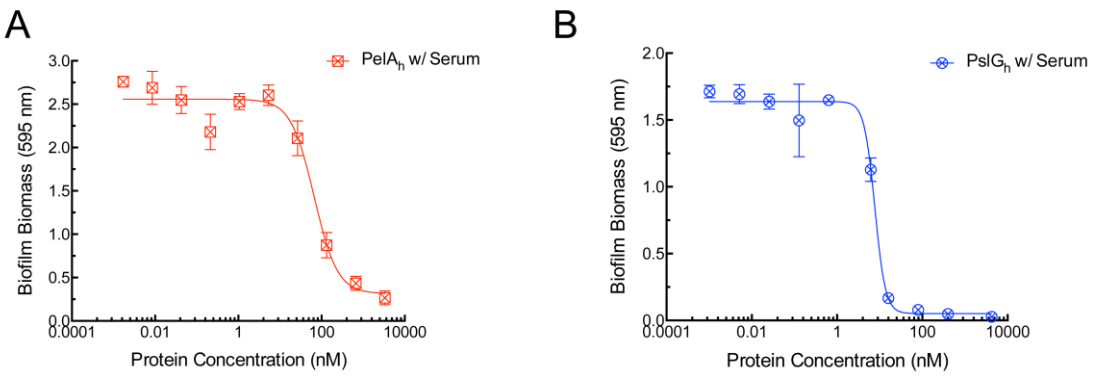

fig. S2. *P. aeruginosa* biofilm disruption by glycoside hydrolases in the presence of serum. Dose-response curves to examine the dispersal of biofilm biomass by the exogenous treatment of each glycoside hydrolase and variant. Each data point represents the mean from three independent experiments of $n = 3$ crystal violet microtiter plate wells. EC_{50} values were calculated using non-linear least-squares fitting to a dose-response model. Error bars indicate SEM.

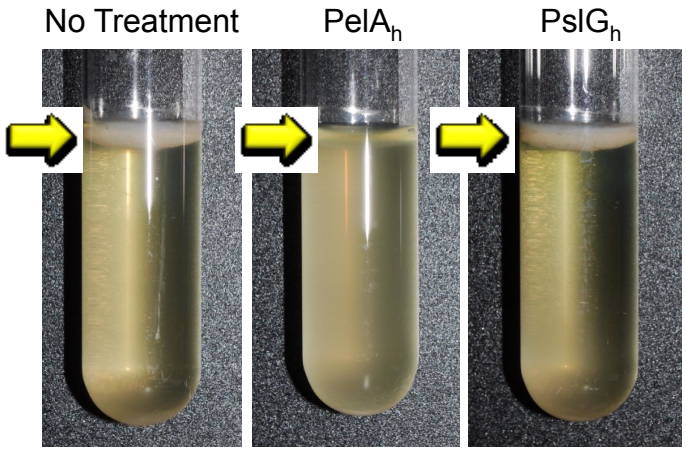

fig. S3. **Biofilm prevention in standing culture pellicle assay**. Biofilm formation at the air-liquid interface was examined in Pel-dependent culture following incubation with $PelA_h$ and $PslG_h$. Arrows indicate the location of the air-liquid interface where biofilm formation occurs.

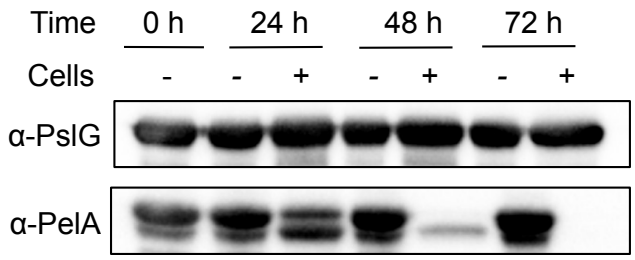

fig. S4. **Protein stability of $PelA_h$ and $PslG_h$ in *P. aeruginosa* culture.** Western blotting using α-PelA and α-PslG to detect the presence of exogenously applied $PelA_h$ and $PslG_h$ at various time points during incubating with P. aeruginosa Pel and Psl biofilm formation, respectively. Incubation of each glycoside hydrolase in the absence of *P. aeruginosa* culture (Cell -) was utilized for comparison.

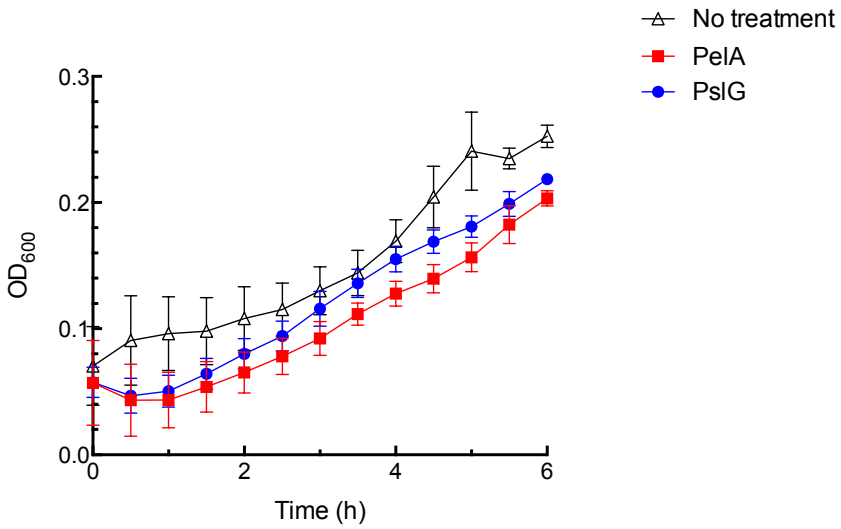

fig. S5. The growth of *P. aeruginosa* in the presence of glycoside hydrolases.. Growth curve with *P. aeruginosa* PAO1 in the presence of $PslG_h$ and $PelA_h$ over 6 h at 37 °C.

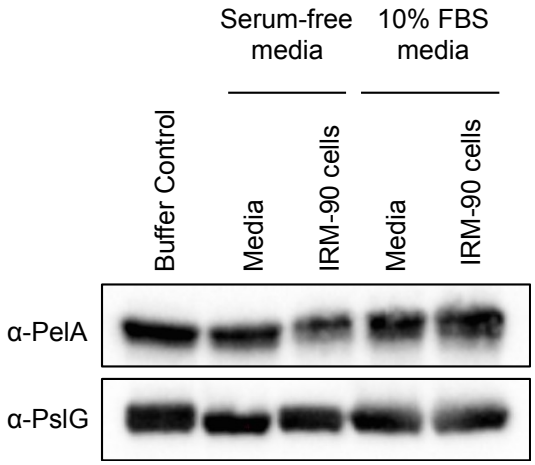

fig. S6. Protein stability of $PelA_h$ and $PslG_h$ in mammalian cell culture. Western blotting of exogenously added $PelA_h$ and $PslG_h$ after 48 h incubation in IMR-90 cell culture in the presence and absence of 10% FBS in the media.