

SUPPORTING INFORMATION

Elevated levels of the second messenger c-di-GMP contribute to antimicrobial resistance of *Pseudomonas aeruginosa*

Kajal Gupta, Julie Liao, Olga E. Petrova, K. E. Cherny, and Karin Sauer#

Pages 1 -2 Supplementary Table S1
Page 3 Supplementary Figure S1-S2

SUPPLEMENTARY TABLES

Table S1. Primers used.

Oligonucleotide	Sequence
PCR/qRT-PCR	
mreB-for	CTGTCGATCGACCTGGG
mreB-rev	CAGCCATCGGCTCTTCG
16S rDNA_f	G ACTCCTACGGGAGGCAGCAGT
16S rDNA_r	GTATTACCGCGGCTGCTGGCAC
brlR_RT-for	GCAACGACACCAGCACAC
brlR_RT-rev	GAAGCGTTCACAGAGCTG
pslA-RT-for	CACAACCGCATCGTCGACG
pslA-RT-rev	CTTGGAGTCGAGCCTGATC
mexA-for	CAGCAGCTCTACCAGATCGAC
mexA-rev	GTATTGGCTACCGTCTCCAG
mexE-for	GTCATCGAACAACCGCTG
mexE-rev	GTCGAAGTAGGCGTAGACC
Pser-up	CGAGTGTTTAAGGCAACGGTCTTGA
Pser-down	AGTTCGGCCTGGTGGAGCAACTCG
Mutagenesis	
PA4843delF1-Sac	GCGCGCGGAGCTCGCATAAGCATGCCGTAGTCC
PA4843delR1-Xba	GCGCGCGCTCTAGAGAGTTCGCTGATCGACTCG
PA4843delF2-Xba	GCGCGCGCTCTAGAGCGCTGTTCTCAAGCAAC
PA4843delR2-Hind	GCGCGCGCAAGCTTGCAAGGAGCAGGGTGTGAG
Cloning	
PbrlR-His6V5-pET-for	CACCCTCCCTTTGCGATGGGTTTCCAC
brlR-pET-rev	ATAGATGGGGATATACAGGTTCGAC
P120-brlR-CTX-XbaI-for	GCGCGCGCTCTAGACTCCCTTTGCGATGGGTTTCCAC
P120-brlR-CTX-XmaI-rev	GCGCGCGCCCCGGTCAATGGTGATGGTGATG
PA4929_pET-for	CACCATGAGCAATTCCGACG
PA4929_pET-rev	GGAGATACGCTCCGGTGCCAG
rbdA_pET-for	CACCATGAGGCAGAACCGG

rbdA_pET-rev	CCGGAGGTTCTGTCC
His/V5_EcoR1_rev	GCGCGCGAATTCTCAATGGTGATGGTGATG

Streptavidin bead assays

PmexAF*	GTAGTTCATTGGTTTGGCC
PmexAR	CATAGCGTTGTCCTCATG
PmexEF*	GGATCAGCATGTTTCATCG
PmexER	CTGTTCCATGCTTGACTC

*, primers were biotinylated; unbiotinylated primers were used for competition.

SUPPLEMENTARY FIGURES

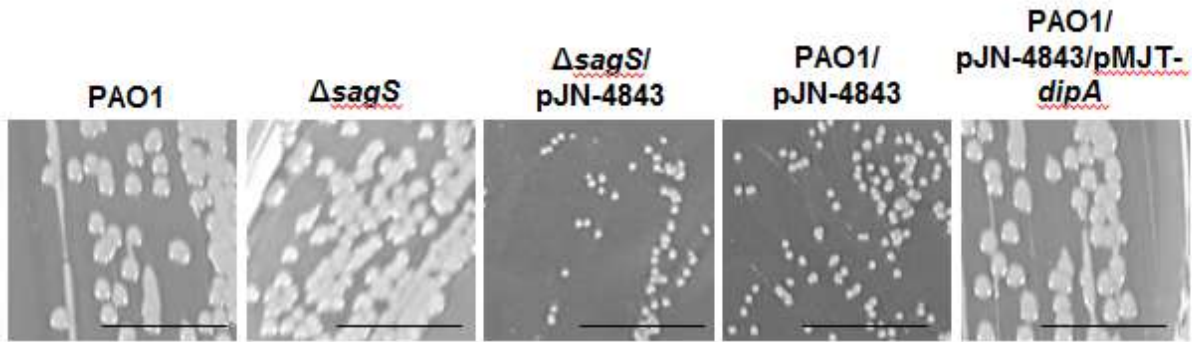


Figure S1. Appearance of colonies formed by *P. aeruginosa* PAO1, Δ *sagS*, strains overexpressing PA4843 or strains co-expressing PA4843 and *dipA*. Images were acquired following 24 hr of growth on LB agar. Black size bar = 1 cm.

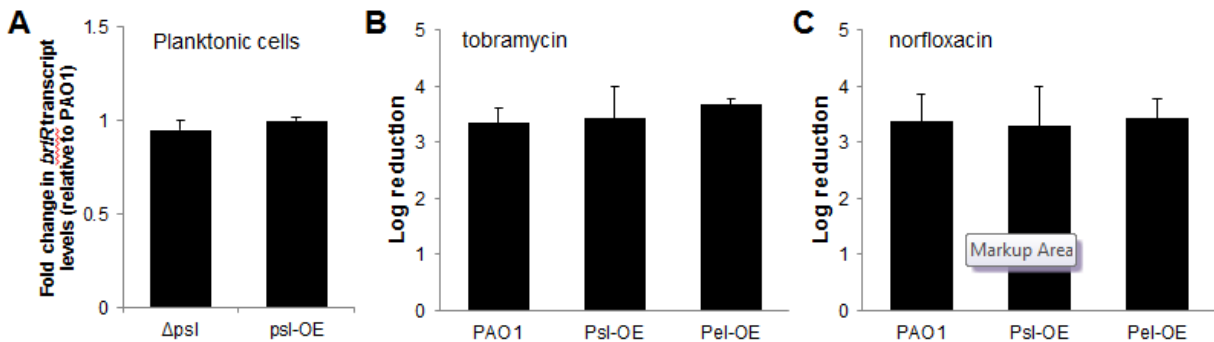


Figure S2. The polysaccharide Psl does not contribute to *brlR* gene expression nor do Psl and Pel contribute to resistance of exponential phase *P. aeruginosa* cells to tobramycin and norfloxacin. (A) Inactivation or overexpression of genes encoding the Psl biosynthetic operon does not affect *brlR* transcript levels in *P. aeruginosa* strains grown planktonically to exponential phase. (B, C) Susceptibility determination of exponential phase planktonic *P. aeruginosa* PAO1 cells and strains overproducing polysaccharides Psl and Pel (*Psl*-OE, *Pel*-OE) to (B) 50 μ g/ml tobramycin and (C) 150 μ g/ml norfloxacin. All strains were grown in LB in the presence of 1% arabinose. All planktonic cells were treated for 1 hr and experiments were done in triplicate. Error bars denote standard deviation.