Interbacterial antagonism mediated by a released polysaccharide

Supplemental Information

Supplemental Methods

Surfactant activity

The drop collapsed assay was performed as previously described(1). Briefly, filter-sterilized spent media of PAO1, ΔP*psl* and *rhlA*::Tn were serially diluted (1:1) with water supplemented with 0.005% crystal violet. 20µl of each dilution was spotted onto a plastic 96-well plate lid and tilted at a 90° angle. The downward migration of the droplets, resulting from surfactant activity, was recorded. Reduced surfactant concentration by dilution eventually resulted in the beading of the droplets. Surfactant scores were calculated as the reciprocal of the greatest dilution at which there was still surfactant activity.

Protease assay

Skim milk plate assay was modified from an established protocol F. Casilag et al. (2). Briefly, 5µl of filter-sterilized PAO1, ΔP*psl* and *lasA*::Tn spent media, and 1mg/ml Proteinase K (Qiagen) were each spotted onto a milk agar plate (5% skim milk, 1.5% agar) and incubated overnight at 37˚C. Diameters of the clearing zone was measured and indicative of protease activity.

Staphylolytic assay

Modified from an established protocol (3), overnight cultures of *S. aureus* USA300 were resuspended in PBS, heat-killed at 100˚C for 10min and normalized to an OD_{600} of 1.0. They were incubated with PAO1, ΔP*psl* and *lasA*::Tn spent media at 37˚C for 7h. OD_{600} was measured using a spectrophotometer, as an indication of cell lysis.

Table S1 Primers

Name	Sequence	Description
pqsA-up_fwd	taaaacgacggccagtgccaGAAGCCTGCAAATGGCAG	for *pqsA* deletion
pqsA-up_rev	acagcctgaaGACAGAACGTTCCCTCTTC	
pqsA-down_fwd	acgttctgtcTTCAGGCTGTGGGGGTGAACC	
pqsA-down_rev	gctcggtacccggggatcctCGGATCACCGCCCAGCGC	
pvdA-up_fwd	taaaacgacggccagtgccaGTCAAGCGCAGATCGAGC	for *pvdA* deletion
pvdA-up_rev	gtggcgccgaTTCCAGTTCCTCTGGATTGG	
pvdA-down_fwd	ggaactggaaTCGGCGCCACGCCGCTAC	
pvdA-down_rev	gctcggtacccggggatcctCAACTGGCGTACCGCGGG	

Table S2 Screening for *P. aeruginosa* mutants with altered Psl distribution.

	Strains	Cell-associated (%)	Cell-free (%)
WT	PAO1	49.6	50.4
LipidA pathway	*pagP*::Tn	25.9	74.1
	phoP::Tn	15.9	84.1
	phoQ::Tn	17.4	82.6
	pmrB::Tn	18.6	81.4
	htrB1::Tn	16.4	83.6
	htrB2::Tn	17.3	82.3
	lpxO1::Tn	15.2	84.8
	lpxO2::Tn	15	85
	pagL::Tn	27.7	72.3
Acyl-transferase	*lptA*::Tn	40.7	59.3
	PW 1377	40.3	59.7
	lgt::Tn*	0	0
	plsB::Tn**	37.5	62.5
	PA4351::Tn	32	68
Acyl carrier protein	*acpD*::Tn**	45.9	54.1
	PA3334::Tn	36.7	63.3

* No Psl detected

** Showing the average of 3 mutants in the transposon library for the same gene.

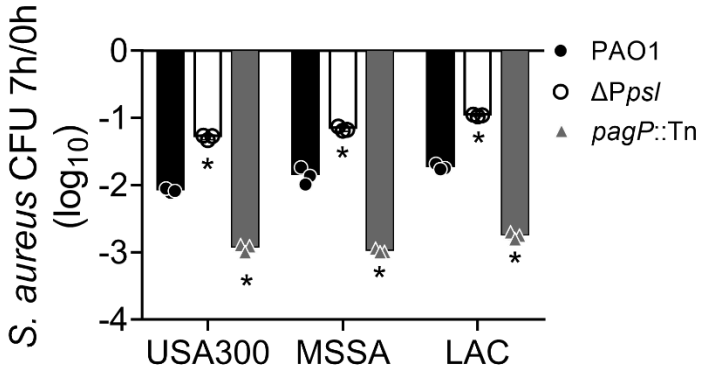

Figure S1. Psl production promotes killing of various *S. aureus* strains. Survival of *S. aureus* USA300, MSSA and LAC, was quantified when co-cultured for 7h with *P. aeruginosa* PAO1, Δ*Ppsl* or *pagP*::Tn. *S. aureus* survival was presented as CFUs normalized to the starting CFUs at 0h. Data presented as mean ± SD, individual points indicate the biological replicates (N = 3, n = 3). Significance was determined using a two-way ANOVA. *, $P<0.05$ compared to co-culture with PAO1.

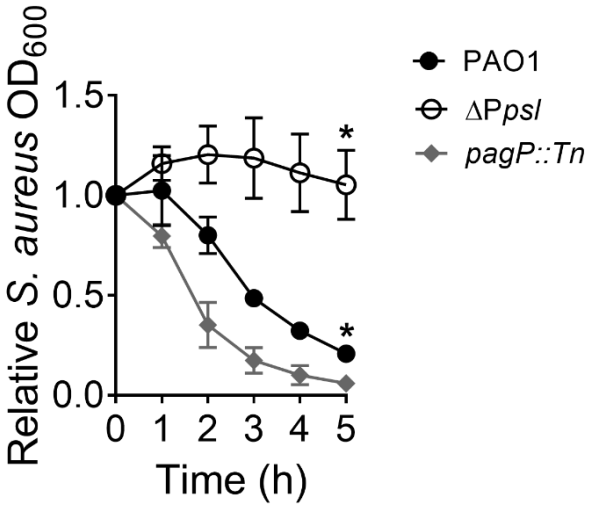

Figure S2. Cell-free Psl promotes *S. aureus* killing in *P. aeruginosa* spent media. Stationary phase *S. aureus* USA300 (normalized to OD_{600} = 1.5) was incubated with diluted spent media from PAO1, Δ*Ppsl* or *pagP*::Tn for 5h. *S. aureus* survival is presented as OD_{600} normalized to the starting OD_{600} at 0h. Data presented as mean ± SD, individual points indicate the biological

replicates (N = 4, n = 3). Significance was determined using a Student's t-test. *, *P*<0.05 compared to PAO1.

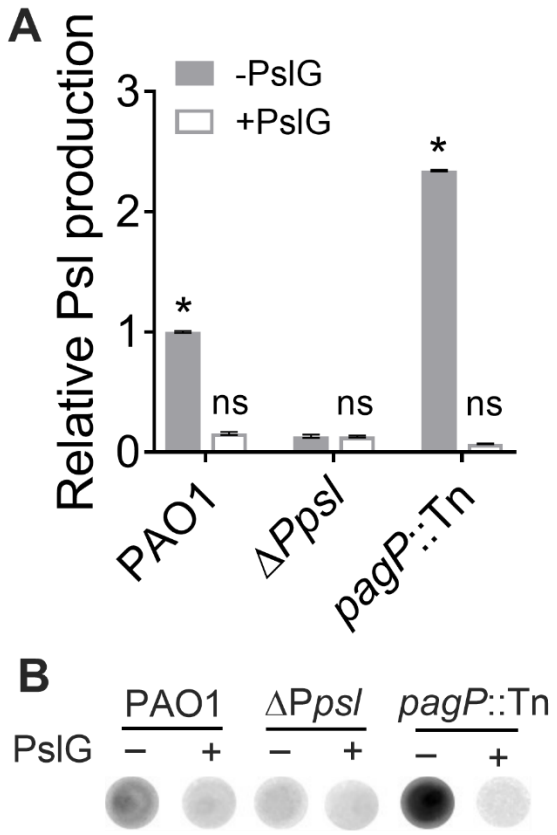

Figure S3: PslG degrades secreted cell-free Psl in *P. aeruginosa* spent media.

PAO1, ΔP*psl* and *pagP*::Tn spent media were treated with (+) or without (-) 100μM PslG for 1h at 37˚C to degrade Psl. Psl production was then measured by immunoblot assay and quantified by densitometry **(A)**, shown in a representative image **(B)**. Data presented as mean ± SD (N = 3, n = 3). Significance was determined using one-way ANOVA. ns, not significant; *, *P*<0.05 compared to ΔP*psl* - PslG.

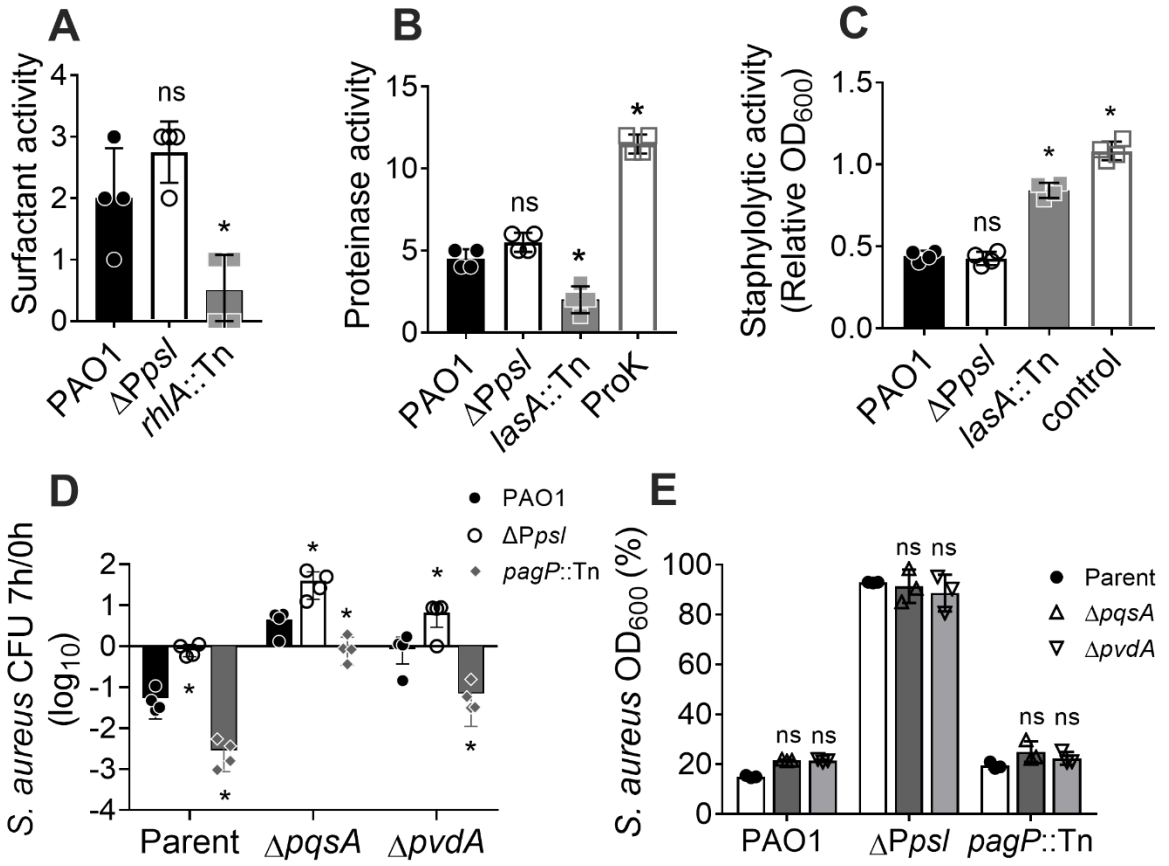

Figure S4. Psl-mediated killing is independent of known *P. aeruginosa* mechanisms of *S. aureus* killing. (A) Drop collapse assay was performed to quantify the surfactant activity of *P. aeruginosa* PAO1, ΔP*psl* and *rhlA*::Tn. Overnight *P. aeruginosa* spent media was serial diluted in half, and spotted on a plastic surface which was then tilted at a 90˚ angle. The surfactant activity score was calculated as the reciprocal of the greatest dilution at which there was still surfactant activity (collapse of the droplet). Significance was determined with one-way ANOVA. *, $P<0.05$ compared to PAO1 (N = 4, n = 3). **(B)** *P. aeruginosa* PAO1, ΔP*psl* and *lasA*::Tn spent media, as well as Proteinase K (ProK) were spotted on a skim milk agar plate and incubated at 37˚C overnight. The diameter (mm) of the clear zone was measured, as an indication of proteinase activity. Significance was determined with one-way ANOVA. *, $P<0.05$ compared to PAO1 (N = 4, n = 3). **(C)** The staphylolytic activity was measured by OD_{600} of heat-killed *S. aureus* USA300 incubated without (control) or with half-diluted PAO1, ΔP*psl* and *lasA*::Tn spent media for 7h.

Significance was determined with one-way ANOVA. *, *P*<0.05 compared to PAO1 (N = 4, n = 3). **(D)** *S. aureus* survivals, when cocultured with *pqsA* and *pvdA* mutants for 7hrs, were quantified by CFUs normalized to the starting CFUs at 0h**.** Significance was determined with Student's t-test. *, *P*<0.05 compared to PAO1 (N = 4, n = 3). **(E)** *S. aureus* survival, treated with 100µg/ml cell-free Psl purified from PAO1, Δ*psl*, *pagP*::Tn, Δ*pqsA*, Δ*psl*Δ*pqsA, pagP*::TnΔ*pqsA,* Δ*pvdA,* Δ*psl*Δ*pvdA and pagP*::TnΔ*pvdA* for 16h, was quantified by OD_{600} normalized to that of *S. aureus* grown alone. Significance was determined with two-way ANOVA. ns, not significant compared to parent (N = 3, n = 3). All data presented as mean ± SD, individual points indicate the biological replicates.

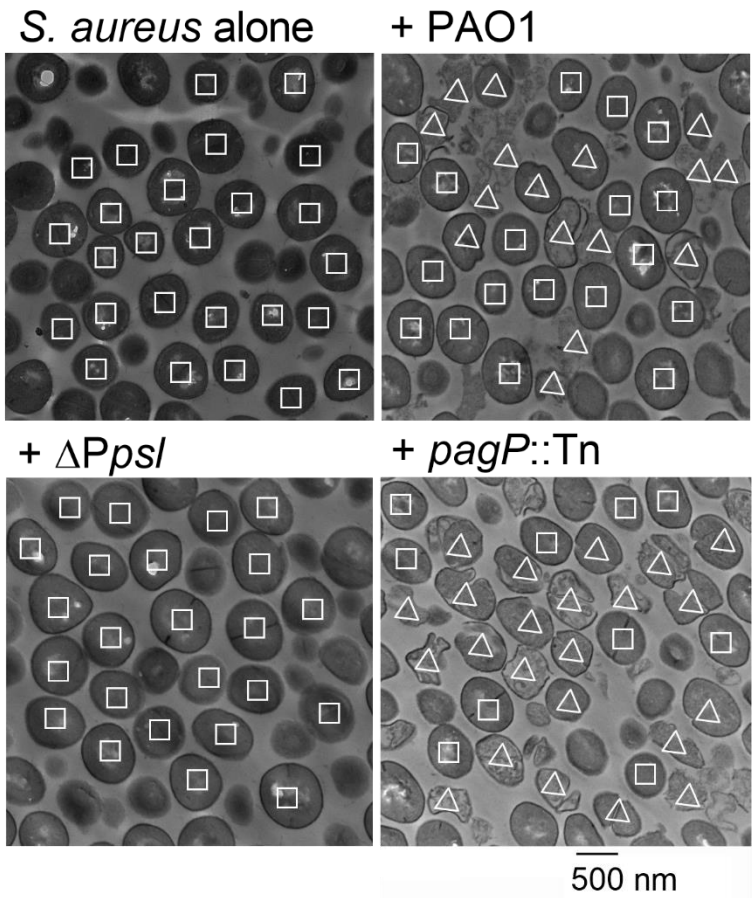

Figure S5 Labled *S. aureus* cells in TEM images. *S. aureus* USA300 was incubated with spent media from *P. aeruginosa* PAO1, Δ*psl* or *pagP*::Tn for 2h. Changes in cell morphology were

visualized by TEM. Square labels all intact cells, while triangle labels all cells with disrupted cell envelopes from Figure 3A.

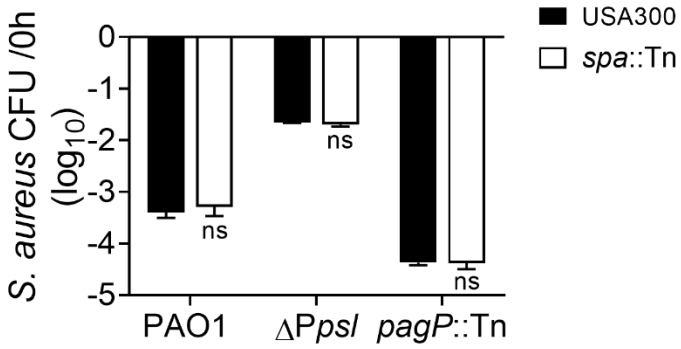

Figure S6. *S. aureus* SpA plays no role in Psl-mediated killing. The survival of *S. aureus* USA300 and *spa*::Tn, when co-cultured for 8h with *P. aeruginosa* PAO1, ΔP*psl* or *pagP*::Tn, was measured. *S. aureus* survival is presented as CFUs normalized to the starting CFU at 0h. Data presented as mean ± SD (N = 3, n = 3). Significance was determined with two-way ANOVA. ns, not significant compared to the parent.

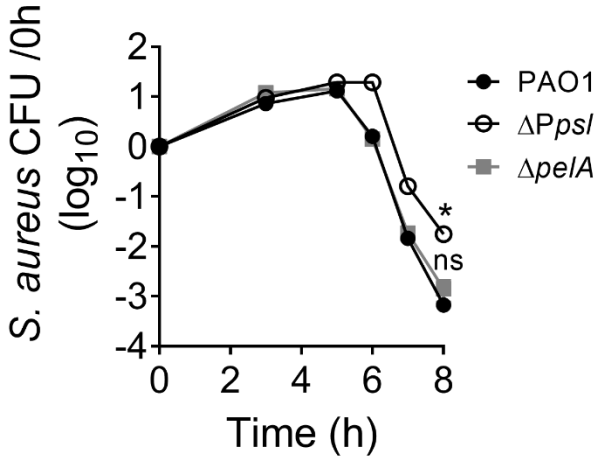

Figure S7. *P. aeruginosa* Pel production has no effect on *S. aureus* survival. *S. aureus* USA300 survival was measured when co-cultured for 8h with *P. aeruginosa* PAO1, ΔP*psl*, or a

pelA gene deletion mutant that does not produce Pel (Δ*pelA*). *S. aureus* survival is presented as CFUs normalized to the starting CFUs at 0h. Data presented as mean ± SD, individual points indicate the mean of biological replicates (N = 3, n = 3). Significance was determined with Student's t-test, compared to PAO1 *, $P<0.05$, ns, not significant.

References

1. Deziel E, Lepine F, Milot S, Villemur R. 2003. rhlA is required for the production of a novel biosurfactant promoting swarming motility in Pseudomonas aeruginosa: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology (Reading) 149:2005-13.
2. Casilag F, Lorenz A, Krueger J, Klawonn F, Weiss S, Haussler S. 2016. The LasB Elastase of Pseudomonas aeruginosa Acts in Concert with Alkaline Protease AprA To Prevent Flagellin-Mediated Immune Recognition. Infect Immun 84:162-71.
3. Kessler E, Safrin M, Olson JC, Ohman DE. 1993. Secreted LasA of Pseudomonas aeruginosa is a staphylolytic protease. J Biol Chem 268:7503-8.