Damron *et al.* Vanadate and triclosan synergistically induce alginate production by *Pseudomonas aeruginosa* strain PAO1

Supplemental Table 1. Bacterial strains, plasmids, oligonucleotides used in this study

Bacterial strains	Phenotype, genotype, description, or sequence [a]	Source and or reference [b]
P. aeruginosa		
PAO1	Alg$^-$ Prototroph	P. Phibbs, East Carolina University, Greenville, NC
PAO1Δ*algB*	Alg$^-$, PAO1 in-frame deletion of *algB* (PA5483)	(Damron *et al.*, 2009)
PAO1*algD*	Alg$^-$, PAO1 Tc^r insertion into *algD* (PA3540)	(Jacobs *et al.*, 2003)
PAO1*algE*	Alg$^-$, PAO1 Tc^r insertion into *algE* (PA3544)	(Jacobs et al., 2003)
PAO1Δ*algR* (PSL317)	Alg$^-$, PAO1 in-frame deletion of *algR* (PA5261)	(Lizewski *et al.*, 2004)
PAO1Δ*algW*	Alg$^-$, PAO1 in-frame deletion of *algW* (PA4446)	(Damron & Yu)
PAO1Δ*algU*	Alg$^-$, PAO1 in-frame deletion of *algU* (PA0762)	(Damron et al., 2009)
PAO1*algZ* (PAO6886)	Alg$^-$, PAO1 Tc^r insertion into *algZ* (PA5262)	(Yu *et al.*, 1997)
PAO1*kinB*	Alg$^+$, PAO1 Gm^r insertion into *kinB* (PA5484)	(Damron et al., 2009)
PAO1*mucB*	Alg$^+$, PAO1 Gm^r insertion into *mucB* (PA0764)	H. Yu, Marshall University

		School of Medicine, Huntington, WV
PAO1*mucC*	Alg$^-$, PAO1 Gm^r insertion into *mucC* (PA0765)	(Boucher *et al.*, 1997)
PAO1*mucD*	Alg$^+$, PAO1 Gm^r insertion into *mucD* (PA0766)	(Qiu *et al.*, 2007)
PAO1*mucE*	Alg$^-$, PAO1 Tc^r insertion into *mucE* (PA4033)	(Jacobs et al., 2003)
PAO1*mucP*	Alg$^-$, PAO1 Tc^r insertion into *mucP* (PA3649)	(Qiu et al., 2007)
PAO1*oprH*	Alg$^-$, PAO1 Tc^r insertion into *oprH* (PA1178)	(Jacobs et al., 2003)
PAO1Δ*phoP*	Alg$^-$, PAO1 in-frame deletion of *phoP* (PA1179)	R. Ernst, University of Maryland-Baltimore, Baltimore, MA
PAO1*pmrA*	Alg$^-$, PAO1 Tc^r insertion into *pmrA* (PA4776)	(Jacobs et al., 2003)
PAO1*pmrB*	Alg$^-$, PAO1 Tc^r insertion into *pmrB* (PA4777)	(Jacobs et al., 2003)
PAO1*prc* (PAO1-184)	Alg$^-$, PAO1 Tc^r insertion into *prc* (PA3649)	(Reiling *et al.*, 2005)
PAO1*rpoH*	Alg$^-$, PAO1 Tc^r insertion into *rpoH* (PA0376)	(Jacobs et al., 2003)
PAO1*rpoS*	Alg$^-$, PAO1 Tc^r insertion into *rpoS* (PA3622)	(Jacobs et al., 2003)

PAO1Δ*rpoN*	Alg$^-$, PAO1 in-frame deletion of *rpoN* (PA4462)	(Damron et al., 2009)
PA14	Alg$^-$ *algU*$^+$ *mucA*$^+$	F. Ausubel, Harvard Medical School, Boston, MA
PAK	Alg$^-$ *algU*$^+$ *mucA*$^+$	J. Goldberg, University of Virginia, Charlottesville, VA
PA103	Alg$^-$	J. Goldberg, University of Virginia, Charlottesville, VA
C0132	Alg$^-$, CF isolate, 1st colonizer, *algU*$^+$ *mucA*$^+$	(Qiu et al., 2007)
C3715	Alg$^-$, CF isolate, 1st colonizer, *algU*$^+$ *mucA*$^+$	(Qiu et al., 2007)
C7406	Alg$^-$, CF isolate, 1st colonizer, *algU*$^+$ *mucA*$^+$	(Qiu et al., 2007)
FRD1	Alg$^+$, CF isolate, *algT*$^+$*mucA22*	(Ohman & Chakrabarty, 1981)
FRD2	Alg$^-$, CF isolate, *algT18 mucA22*	(DeVries & Ohman, 1994, Ohman & Chakrabarty, 1981)
CF149	Alg$^-$, CF isolate, $algU_{C182T}$ $mucA_{374\square G}$	(Damron et al., 2009)

PAO581	Alg$^{+,}$ PAO1 *algU*$^{+}$ *mucA25*	(Qiu *et al.*, 2008)
E. coli		
DH5α	F^{-}, φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk^{-}, mk^{+}), *phoA*, *supE*44, λ^{-}, *thi*-1, *gyrA*96, *relA*1	Lab strain
TOP10	DH5α derivative	Invitrogen
Plasmids		
pRK2013	Km^{r} *Tra Mob ColE1*	(Figurski & Helinski, 1979)
miniCTX1	Gene delivery vector for inserting genes at the CTX phage *att* site on the *P. aeruginosa* chromosome; Tc^{r}	(Hoang *et al.*, 2000)
miniCTX-*lacZ*	MiniCTX1 carrying the complete *lacZ*; Tc^{r}	(Hoang *et al.*, 2000)
miniCTX-P*algU-lacZ*	Complete P*algU* promoter (541 bp upstream of ATG *Eco*RI/*Hind*III fused with *lacZ* for integration at the CTX phage *att* site in *P. aeruginosa*	(Damron et al., 2009)
miniCTX-P*algD-lacZ*	Complete P*algD* promoter (1,525 bp upstream of ATG) *Hind*III/*Bam*H1 fused with *lacZ* for integration at the CTX phage *att* site in *P. aeruginosa*	(Damron et al., 2009)
miniCTX-P*algU-algU*-HA-*mucA*	P*algU-algU*-HA-*mucA* generated by SOE-PCR *Eco*RI/*Hind*III in miniCTX1 for integration at the CTX phage *att* site in *P. aeruginosa*	This study

Oligonucleotides		
EcoR1-PalgU-F	GTGAATTCGGGCACCCCCTGTTCGATCA	This study
co-HAmucA-F	5'GCCAAGAGAGGTATCGCTATGTACCCATACGATGTTCCAGA TTACGCTAGTCGTGAAGCCCTGCA3'	This study
co-HAmucA-R	5'TGCAGGGCTTCACGACTAGCGTAATCTGGAACATCGTATGGGTA CATAGCGATACCTCTCTTGGC3'	This study
HindIII-mucA-R	5'TTAAGCTTTCAGCGGTTTTCCAGGCTG3'	This study

[a] Alg^-, non-mucoid phenotype and Alg^+, mucoid phenotype

[b] See references listed below.

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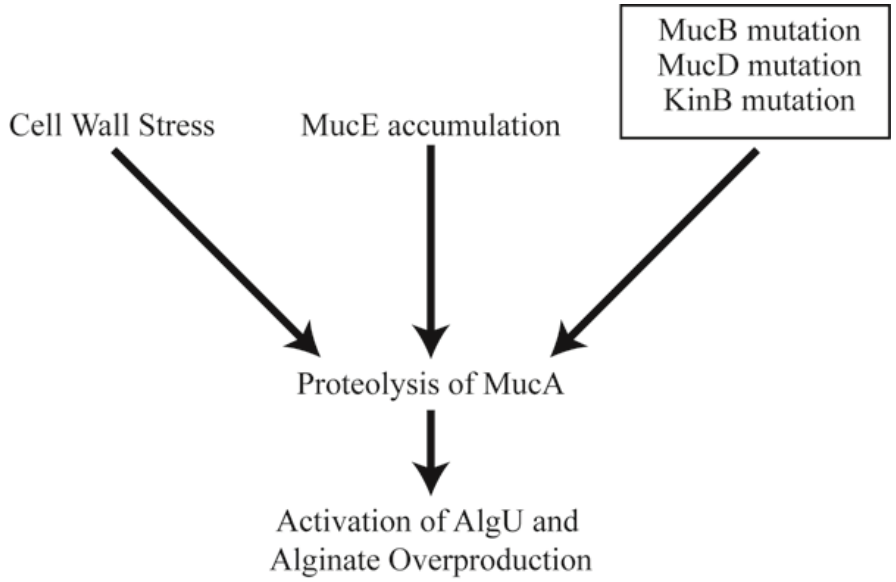

Figure S1. Signal transduction activates proteolytic degradation of MucA and alginate overproduction in *P. aeruginosa*. The degradation of wild type MucA is controlled by several proteases including envelope proteases AlgW and MucP. AlgW can be activated by cell wall stress (Wood *et al.*, 2006, Wood & Ohman, 2009), or when envelope protein MucE is overexpressed (Qiu et al., 2007). *mucB, mucD,* and *kinB* mutant strains also are mucoid due to the proteolytic degradation of MucA (see *Introduction*).

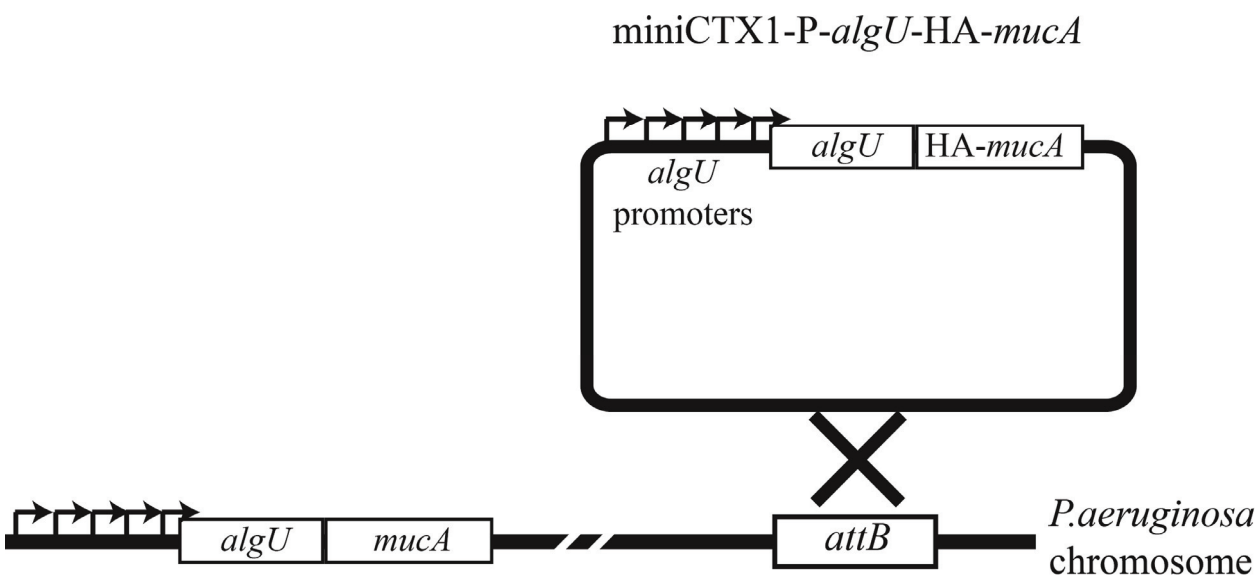

Figure S2. A schematic of miniCTX1-P-*algU*-HA-*mucA*. The single copy HA-*mucA* expressing construct was assembled with N-terminal HA-epitope tagged MucA that is expressed from the P*algU* promoters. The construct is integrated into the *attB* site of the *P. aeruginosa* chromosome. Specific cloning techniques are discussed in the text.

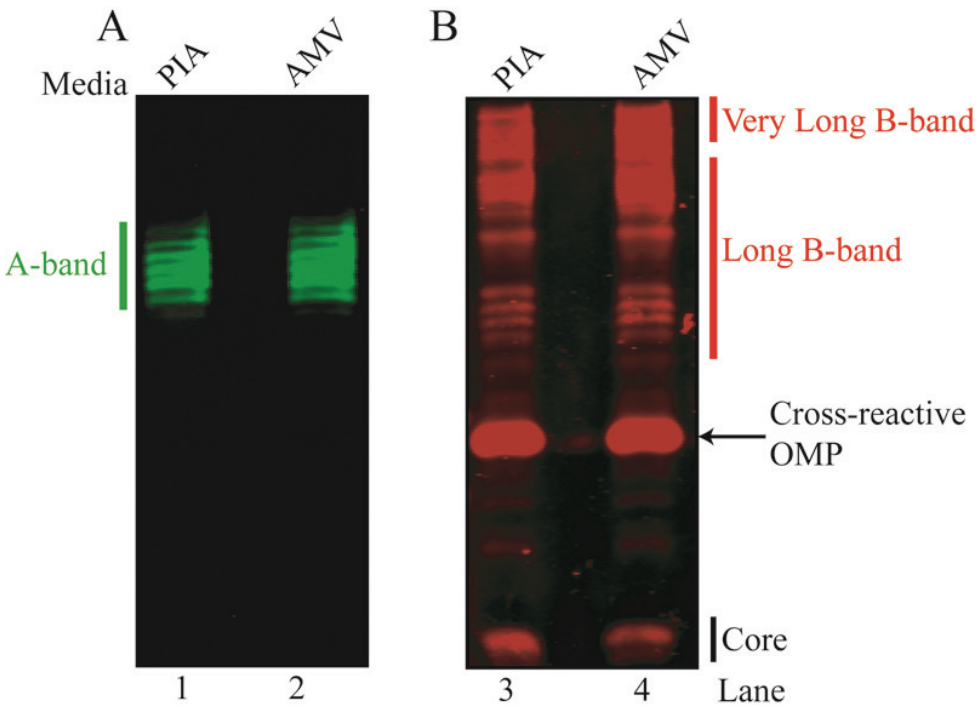

Figure S3. Amount of A- or B-band O-antigen of LPS is not altered in PAO1 on PIA-AMV. *P. aeruginosa* strain PAO1 was grown overnight at 37°C on either PIA or PIA-AMV. Bacteria from both of these plates were then transferred onto PIA or PIA-AMV and were grown overnight at 37°C. Cells were swabbed from these plates and suspended in 1 mL of Luria Broth to an OD_{600} of 0.5. CFU counts were performed (data not shown), and equal amounts of colony forming units were used for preparation of LPS. LPS was prepared as described (Hitchcock & Brown, 1983). Briefly, the cells were then pelleted at 20,800 x *g* and supernatant was removed. The pellets were suspended in 200 uL of 1X sodium dodecyl sulfate (SDS) (0.1 M Tris-HCl pH 6.8, 2% β-mercaptoethanol, 2% SDS, 10% glycerol) and boiled for 15 minutes. After samples cooled, 200 uL of 2X SDS buffer were added to each sample, and 10 µl was electrophoresed on a 12% SDS-polyacrylamide gel and transferred to 0.45 µm nitrocellulose. The blots were then analyzed using

monoclonal A-band specific antibody N1F10 (Lam *et al.*, 1989) and polyclonal serogroup O5 antiserum (Accurate Chemical & Scientific) for B-band LPS. The secondary antibody of goat anti-rabbit immunoglobulin G coupled to IRDye 680 (Li-Cor Biosciences) or goat anti-mouse immunoglobulin M coupled to IRDye 800 using the IRDye 800CW Protein Labeling Kit – High MW, per manufacturer's instructions (Li-Cor Biosciences) was used for detection. Blots were then scanned using the Li-Cor Odyssey infared imaging system and analyzed using Odyssey application software version 3.0.16 (Li-Cor Biosciences).Panel A indicates a representative of the A-band O-antigen LPS blots and Panel B indicates the B-band O-antigen LPS blots. Shown is a representative of three independent experiments.

Profile of A- or B-band O-antigen of LPS is unaltered in induced mucoidy of PAO1. During chronic infection of the CF airway, *P. aeruginosa* strains have altered LPS production. LPS is composed of lipid A, core polysaccharide and O-antigen polysaccharide. O-antigen is composed of the common antigen polysaccharide that is referred to as A-band and the serotype specific polysaccharide component known as B-band. Initial infecting strains are LPS-smooth and are able to synthesize complete LPS molecules with core, A- and B-band O-antigen. Isolates from the CF lung are commonly LPS-rough and do not make complete O-antigen molecules. These strains are serum sensitive and less virulent than LPS-smooth strains (Cryz *et al.*, 1984). Our data suggest that the PIA-AMV medium has little to no effect on O-antigen polysaccharide expression.

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