

Supplemental material

Anr, and its activation by PlcH activity, in *Pseudomonas aeruginosa* host colonization and virulence

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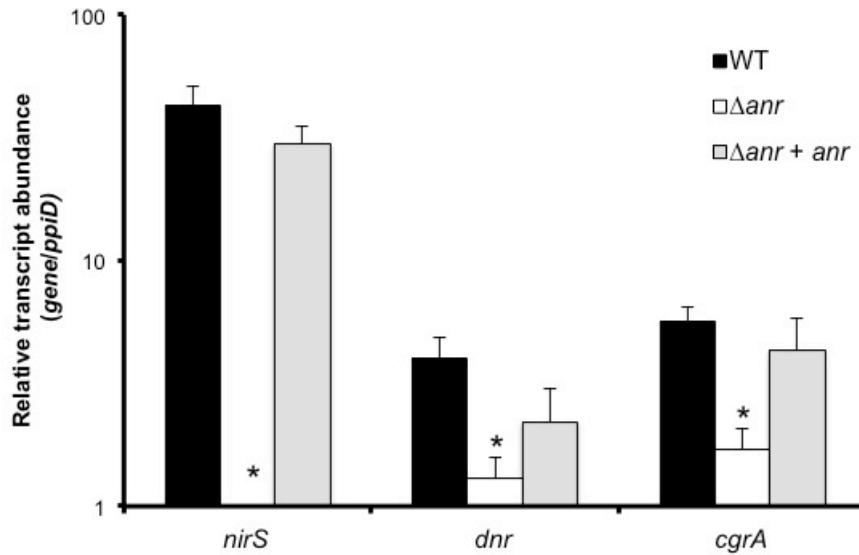


Fig. S1. Anr regulates the expression of *nirS*, *dnr*, and *cgrA* transcripts. Quantitative RT-PCR analysis of Anr-regulated transcripts (*nirS*, *dnr*, *cgrA*) normalized to the housekeeping gene *ppiD* in MOPS-glucose-surfactant medium, with additional choline (5 mM) and nitrate (5 mM). Data represent the means from three replicate cultures. * $P < 0.01$ difference from WT.

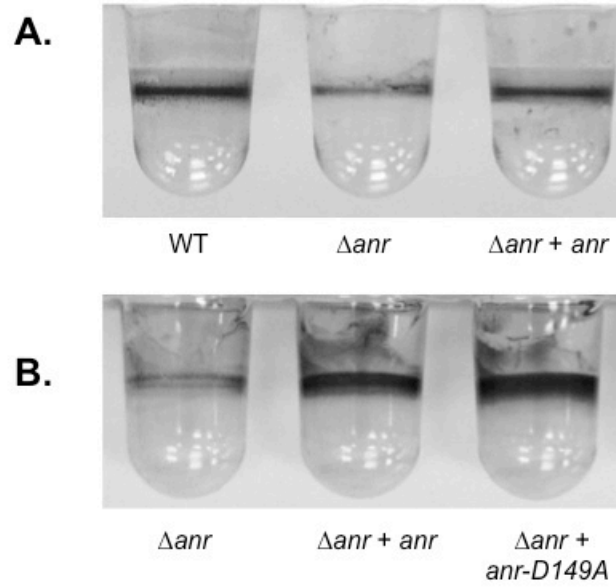


Fig. S2. Anr promotes *P. aeruginosa* PAO1 biofilm formation in different laboratory strains and in minimal glucose medium. A. PAO1 WT (DH2067), Δanr , and $\Delta anr + anr$ biofilms in M63-glucose after 24 h. **B.** Biofilms formed by PAO1 Δanr (DH1857), $\Delta anr + anr$, and $\Delta anr + anr-D149A$ strains after 24 h in MOPS-glucose.

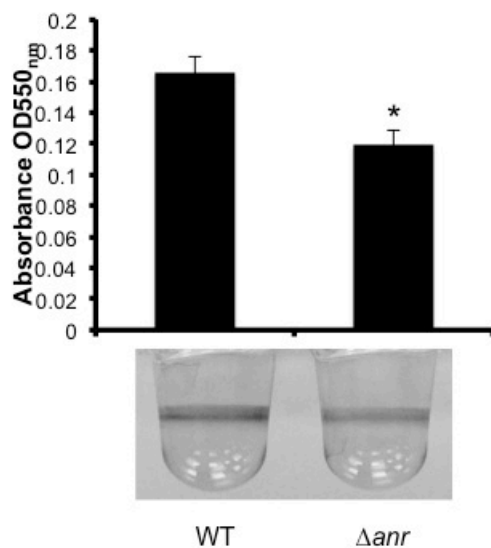


Fig. S3. Anr promotes *P. aeruginosa* PA14 biofilm formation. PA14 WT (DH2067) and Δanr biofilms in M63-glucose medium after 24 h. The difference is significant at $P < 0.05$.

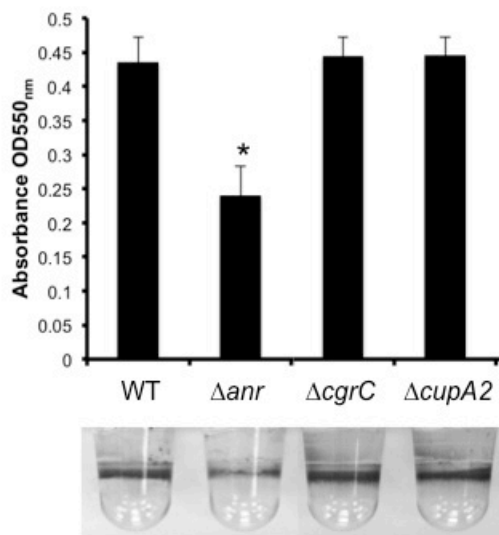


Fig. S4. Anr control of cup fimbriae does not affect biofilm formation. PAO1 biofilms in WT, Δanr , $\Delta cgrC$, and $\Delta cupA2$ strains after 24 h in MOPS-glucose medium.

Table S1. Reduction in expression of genes in *Pseudomonas aeruginosa* PAO1 Δ *plcHR* compared to wild type based on microarray data.

PA no.	Gene	Description	Fold change	Reference(s) indicating regulation by Anr
PA5475			-36.0	(1)
PA1673			-27.8	(1)
PA5027			-26.5	(1)
PA3337	<i>rfaD</i>	ADP-L-glycero-D-mannoheptose 6-epimerase	-24.9	(1, 2)
PA2753			-23.7	
PA4348			-23.0	(1)
PA0527	<i>dnr</i>	Global nitrogen utilization regulator	-22.9	(1, 3)
PA3880			-22.1	
PA1196			-21.8	
PA2119		Zn-dependent alcohol dehydrogenase	-19.5	(1)
PA0200			-18.5	(1)
PA4687	<i>ccpR</i>	Cytochrome c551 peroxidase precursor	-17.9	(1)
PA0519	<i>nirS</i>	Nitrite reductase precursor	-14.7	(1, 4)
PA1546	<i>hemN</i>	O ₂ -independent coproporphyrinogen oxidase	-13.8	(1, 5)
PA3006	<i>psrA</i>		-12.1	
PA4610			-11.6	
PA5232			-11.1	(1)
PA4577			-9.9	(1)
PA0141			-9.6	(1)
PA1556	<i>ccoO2</i>	Cbb-3 cytochrome c oxidase	-9.4	
PA5446			-9.3	
PA1746			-8.9	(1)
PA1557	<i>ccoN2</i>	Cbb-3 cytochrome c oxidase	-8.8	(1, 6)
PA4067	<i>oprG</i>	Outer membrane protein G	-8.2	(1) (7)
PA5427	<i>adhA</i>	Alcohol dehydrogenase	-7.9	(1)
PA1429			-7.3	
PA4571			-7.2	
PA4762	<i>grpE</i>	Heat shock protein	-7.2	
PA4352			-6.8	(1)
PA5053	<i>hslV</i>	Heat shock protein	-6.5	
PA2663	<i>ppyR</i>	Psl and pyoverdinin regulator	-6.2	
PA4542	<i>clpB</i>	Protease chaperone B	-6.1	
PA0201			-6.1	
PA1596	<i>htpG</i>	Heat shock protein G	-6.0	
PA3523			-5.6	
PA3678		Probable transcriptional regulator	-5.5	
PA2127	<i>cgrA</i>		-5.2	(1) (8)
PA5440	<i>yegQ</i>		-5.1	
PA3677			-5.1	

Table S2. Transcripts more highly expressed in *Pseudomonas aeruginosa* PAO1 Δ *plcH* compared to wild type

PA no.	Gene	Description	Fold change	Reference(s) indicating regulation by Anr
PA0059	<i>osmC</i>		9.2	
PA0355	<i>pfpL</i>	Intracellular protease	7.1	
PA0567			17.7	
PA1323			8.7	
PA1324			7.2	
PA1404			8.0	
PA1874			5.1	
PA1887			5.2	
PA1888			5.2	
PA2021			5.5	
PA2134			5.2	
PA2143			10.0	
PA2149			5.2	
PA2151			5.8	
PA2165		Amino acid peptidase/glucanase	5.2	
PA2166			5.7	
PA2167			5.4	
PA2168			5.4	
PA2170			5.6	
PA2171		Hypothetical	10.7	
PA2172			5.9	
PA2173			6.0	
PA2176		FixL-like	6.5	
PA2433			8.6	
PA2747			9.5	
PA3032	<i>snr1</i>	Cytochrome c	6.8	
PA3274			8.4	
PA3369			8.6	
PA3370			6.7	
PA3371			6.4	
PA3691			6.1	
PA3692	<i>lptF</i>	Lipotoxin	8.4	
PA3930	<i>cioA</i>	Cyanide-insensitive oxidase	6.8	(1, 9)
PA4648	<i>cupE1</i>	Pilin subunit <i>cupE1</i>	8.9	
PA4649			5.1	
PA4738			7.2	
PA4739			5.7	
PA4877			6.8	
PA4880		Probable bacteroferritin	16.6	
PA5212			5.3	
PA5481			8.5	
PA5482			15.3	

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