Unravelling the Genome-Wide Contributions of Specific 2-Alkyl-4-Quinolones and PqsE to Quorum Sensing in *Pseudomonas aeruginosa*

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Supplementary Material

Table S1. Genes whose transcription is controlled by HHQ, PQS and/or PqsE

PA number [a]	Gene name [a]	HHQ [b]	PQS [c]	PqsE [d]	Product name [a]
PA0051	*phzH*			3.2	Potential phenazine-modifying enzyme
PA0083	*tssB1*			-5.1	TssB1
PA0084	*tssC1*			-3.5	TssC1
PA0129	*bauD*		-5.5		Amino acid permease
PA0130	*bauC*		-5.5		3-oxopropanoate dehydrogenase
PA0131	*bauB*		-7.5		BauB
PA0132	*bauA*		-6.9		Beta-alanine:pyruvate transaminase
PA0187[J‡]				6.0	Hypothetical protein
PA0243[‡]				3.2	Probable transcriptional regulator
PA0263	*hcpC*		-4.3		Secreted protein Hcp
PA0269[J‡]				3.3	Conserved hypothetical protein
PA0270[‡]				2.8	Hypothetical protein
PA0271[J‡]				3.4	Hypothetical protein
PA0298	*spuB*			-2.8	Glutamylpolyamine synthetase
PA0299	*spuC*			-2.6	Polyamine:pyruvate transaminase
PA0303	*spuG*			-2.8	Polyamine transport protein PotH
PA0328	*aaaA*			-3.7	arginine-specific autotransporter, AaaA
PA0423	*pasP*		2.5		PasP
PA0431				2.7	Hypothetical protein
PA0432	*sahH*			2.9	S-adenosyl-L-homocysteine hydrolase
PA0471	***fiuR***		2.7		FiuR
PA0472	***fiuI***		2.5		FiuI
PA0480[J‡]				2.7	Probable hydrolase
PA0509	*nirN*		-16.0	-16.0	NirN
PA0510	*nirE*		-18.8	-13.1	NirE
PA0511	*nirJ*		-50.3	-22.1	Heme *d1* biosynthesis protein NirJ
PA0512	*nirH*		-22.4	-14.4	NirH
PA0513	*nirG*		-17.2	-13.5	NirG
PA0514	*nirL*		-27.2	-16.1	Heme *d1* biosynthesis protein NirL
PA0515	*nirD*		-75.3	-26.8	Probable transcriptional regulator
PA0516	*nirF*		-27.3	-12.8	Heme *d1* biosynthesis protein NirF
PA0517	*nirC*		-87.7	-21.5	Probable *c*-type cytochrome precursor
PA0518	*nirM*		-109.9	-28.6	Cytochrome *c551* precursor
PA0519	*nirS*		-101.2	-41.9	Nitrite reductase precursor
PA0520	*nirQ*		-4.7	-3.0	Regulatory protein NirQ
PA0521	*nirO*		-3.6	-3.2	Probable cytochrome *c* oxidase subunit
PA0523	*norC*		-32.4	-25.6	Nitric-oxide reductase subunit C
PA0524	*norB*		-41.0	-34.3	Nitric-oxide reductase subunit B
PA0525	*norD*		-7.2	-7.4	Probable denitrification protein NorD
PA0526			-20.1	-12.4	Hypothetical protein
PA0546	*metK*			3.0	Methionine adenosyltransferase
PA0746				-2.6	Probable acyl-CoA dehydrogenase

PA number [a]	Gene name [a]	HHQ [b]	PQS [c]	PqsE [d]	Product name [a]
PA0792	*prpD*		2.9		Propionate catabolic protein PrpD
PA0794			-3.7	-3.2	Probable aconitate hydratase
PA0795	*prpC*			-3.5	Citrate synthase 2
PA0796	*prpB*			-4.6	Carboxyphosphonoenolpyruvate phosphonomutase
PA0848[§]	*aphB*			2.5	alkyl hydroperoxide reductase, AhpB
PA0852	*cbpD*			4.1	Chitin-binding protein CbpD precursor
PA0918			-14.3		Cytochrome *b561*
PA0931	***pirA***		2.6		Ferric enterobactin receptor PirA
PA0997*[⌡‡◊]	*pqsB*	6.7	17.5		PqsB
PA0998*[⌡‡◊]	*pqsC*	5.5	16.1		PqsC
PA0999*[⌡‡◊]	*pqsD*	5.8	15.7		3-oxoacyl-[acyl-carrier-protein] synthase III
PA1000*[⌡◊]	*pqsE*			22.8	Quinolone signal response protein
PA1001*[⌡◊]	*phnA*			26.2	Anthranilate synthase component I
PA1002*[⌡◊]	*phnB*			22.4	Anthranilate synthase component II
PA1070	*braG*			-3.5	Branched-chain amino acid transport protein BraG
PA1071	*braF*			-3.1	Branched-chain amino acid transport protein BraF
PA1072	*braE*			-4.0	Branched-chain amino acid transport protein BraE
PA1073	*braD*			-5.3	Branched-chain amino acid transport protein BraD
PA1134			3.3		Hypothetical protein
PA1172	*napC*		-5.7		Cytochrome *c*-type protein NapC
PA1173	*napB*		-8.9		Cytochrome *c*-type protein NapB precursor
PA1174[§]	*napA*		-6.5		Periplasmic nitrate reductase protein NapA
PA1195			-3.4		Hypothetical protein
PA1212[‡]				2.8	Probable MFS transporter
PA1213[⌡]				3.7	Hypothetical protein
PA1214				3.6	Hypothetical protein
PA1215				4.1	Hypothetical protein
PA1216*				4.9	Hypothetical protein
PA1217				5.8	Probable 2-isopropylmalate synthase
PA1218[⌡]				5.5	Hypothetical protein
PA1245[§⌡]	***aprX***		3.9		AprX
PA1300			11.1		Probable sigma-70 factor, ECF subfamily
PA1301[‡]			6.1		Probable transmembrane sensor
PA1318[⌡]	***cyoB***		2.9		Cytochrome *o*-ubiquinol oxidase subunit I
PA1551	*fixG*		-3.7		Probable ferredoxin
PA1562	*acnA*		-2.6		Aconitate hydratase 1
PA1565	*pauB2*		-9.7	-3.8	FAD-dependent oxidoreductase
PA1583	*sdhA*		-2.6		Succinate dehydrogenase (flavoprotein subunit)
PA1602			-2.7		Probable oxidoreductase
PA1659	*hsiF2*		-2.5		HsiF2
PA1706	*pcrV*		2.7		Type III secretion protein PcrV
PA1707	*pcrH*		3.1		Regulatory protein PcrH
PA1708	*popB*		5.6		Translocator protein PopB
PA1709	*popD*		3.0		Translocator outer membrane protein PopD precursor

PA number [a]	Gene name [a]	HHQ [b]	PQS [c]	PqsE [d]	Product name [a]
PA1710	*exsC*		3.5		ExsC exoenzyme S synthesis protein C precursor
PA1711	*exsE*		3.1		ExsE
PA1712	*exsB*		2.6		Exoenzyme S synthesis protein B
PA1718	*pscE*		4.3		Type III export protein PscE
PA1869				2.8	Probable acyl carrier protein
PA1888				2.8	Hypothetical protein
PA1897			-3.1		Hypothetical protein
PA1901[♩]	*phzC2*			5.5	Phenazine biosynthesis protein PhzC
PA1902[§]	*phzD2*			7.5	Phenazine biosynthesis protein PhzD
PA1903[♩]	*phzE2*			8.8	Phenazine biosynthesis protein PhzE
PA1904	*phzF2*			10.3	Probable phenazine biosynthesis protein
PA1905	*phzG2*			9.7	Probable pyridoxamine 5'-phosphate oxidase
PA1912	*femI*		3.6		ECF sigma factor, FemI
PA1946	*rbsB*			-2.6	Binding protein component precursor of ABC ribose transporter
PA2014	*liuB*			-2.6	Methylcrotonyl-CoA carboxylase, beta-subunit
PA2030				3.7	Hypothetical protein
PA2031*				3.7	Hypothetical protein
PA2033[♩]			17.6		Hypothetical protein
PA2034			5.5		Hypothetical protein
PA2041				-4.7	Amino acid permease
PA2066[♩‡]				3.0	Hypothetical protein
PA2068[♩]				2.7	Probable MFS transporter
PA2069*[♩]				3.9	Probable carbamoyl transferase
PA2193*	*hcnA*			3.6	Hydrogen cyanide synthase HcnA
PA2194*	*hcnB*			3.1	Hydrogen cyanide synthase HcnB
PA2195*	*hcnC*			3.0	Hydrogen cyanide synthase HcnC
PA2264			-3.4		Conserved hypothetical protein
PA2265			-3.7		Gluconate dehydrogenase
PA2274*[♩]				5.4	Hypothetical protein
PA2300*[♩◊]	*chiC*			18.7	Chitinase
PA2383			4.5		Probable transcriptional regulator
PA2384[§]			42.2		Hypothetical protein
PA2385	***pvdQ***		29.1		3-oxo-C_{12}-homoserine lactone acylase PvdQ
PA2386	***pvdA***		113.8		L-ornithine N5-oxygenase
PA2389	***pvdR***		7.3		PvdR
PA2390	***pvdT***		4.1		PvdT
PA2391	***opmQ***		3.3		Probable outer membrane protein precursor
PA2392	***pvdP***		7.9		PvdP
PA2393			55.0		Probable dipeptidase precursor
PA2394	***pvdN***		45.6		PvdN
PA2395	***pvdO***		27.3		PvdO
PA2396	***pvdF***		49.0		Pyoverdine synthetase F
PA2397	***pvdE***		48.2		Pyoverdine biosynthesis protein PvdE
PA2398	***fpvA***		16.4		Ferripyoverdine receptor

PA number [a]	Gene name [a]	HHQ [b]	PQS [c]	PqsE [d]	Product name [a]
PA2399	***pvdD***		18.2		Pyoverdine synthetase D
PA2400	***pvdJ***		21.9		PvdJ
PA2402			23.1		Probable non-ribosomal peptide synthetase
PA2403			4.7		Hypothetical protein
PA2404			3.6		Hypothetical protein
PA2405[§]			3.9		Hypothetical protein
PA2406			3.4		Hypothetical protein
PA2407			4.6		Probable adhesion protein
PA2408			3.7		Probable ATP-binding component of ABC transporter
PA2409			3.5		Probable permease of ABC transporter
PA2410			3.0		Hypothetical protein
PA2411			30.2		Probable thioesterase
PA2412[§J]			70.7		Conserved hypothetical protein
PA2413[J]	***pvdH***		43.1		L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase
PA2424	***pvdL***		26.2		PvdL
PA2425	***pvdG***		11.6		PvdG
PA2426	***pvdS***		43.9		Sigma factor PvdS
PA2427			21.3		Hypothetical protein
PA2444	*glyA2*			-3.9	Serine hydroxymethyltransferase
PA2448[‡]				3.7	Hypothetical protein
PA2451			5.1		Hypothetical protein
PA2452			48.2		Hypothetical protein
PA2481			-2.9		Hypothetical protein
PA2482			-2.7		Probable cytochrome *c*
PA2509[J‡]	*catB*			4.3	Muconate cycloisomerase I
PA2511[‡]	*antR*			3.2	AntR
PA2531			3.2		Probable aminotransferase
PA2554				-2.7	Probable short-chain dehydrogenase
PA2555				-2.8	Probable AMP-binding enzyme
PA2557				-3.3	Probable AMP-binding enzyme
PA2564	*tam*			4.6	Hypothetical protein
PA2565				3.5	Hypothetical protein
PA2570*[J◊]	*lecA*			26.3	LecA lectin
PA2588				2.7	Probable transcriptional regulator
PA2682				3.0	Conserved hypothetical protein
PA2765			-2.9		Hypothetical protein
PA2862	*lipA*			-8.7	Lactonizing lipase precursor
PA2953			-2.9		Electron transfer flavoprotein-ubiquinone oxidoreductase
PA3032[§]	*snr1*			5.4	Cytochrome *c* Snr1
PA3120	*leuD*		-2.5		3-isopropylmalate dehydratase small subunit
PA3121	*leuC*		-3.6		3-isopropylmalate dehydratase large subunit
PA3190	*gltB*			-3.3	Probable binding protein component of ABC sugar transporter
PA3192	*gltR*			-2.6	Two-component response regulator GltR
PA3195*	*gapA*			-4.6	Glyceraldehyde 3-phosphate dehydrogenase

PA number [a]	Gene name [a]	HHQ [b]	PQS [c]	PqsE [d]	Product name [a]
PA3300	*fadD2*			-2.5	Long-chain-fatty-acid-CoA ligase
PA3361*[◊]	*lecB*			8.5	Fucose-binding lectin LecB
PA3369*				3.1	Hypothetical protein
PA3391	*nosR*		-4.6	-4.1	Regulatory protein NosR
PA3392*	*nosZ*		-72.7	-58.8	Nitrous-oxide reductase precursor
PA3393	*nosD*		-8.8	-9.0	NosD protein
PA3394	*nosF*		-6.3	-5.8	NosF protein
PA3395	*nosY*		-3.9	-3.2	NosY protein
PA3396	*nosL*		-3.4	-2.7	NosL protein
PA3407	***hasAp***		76.6		Heme acquisition protein HasAp
PA3408	***hasR***		6.4		Heme uptake outer membrane receptor HasR precursor
PA3410	***hasI***		7.6		HasI
PA3441	*ssuF*		10.2		Probable molybdopterin-binding protein
PA3444	*ssuD*		4.2		Conserved hypothetical protein
PA3452	*mqoA*		2.6		Malate:quinone oxidoreductase
PA3478*	*rhlB*			3.6	Rhamnosyltransferase chain B
PA3479	*rhlA*			3.6	Rhamnosyltransferase chain A
PA3484	*tse3*			-2.6	Tse3
PA3520*[§]				4.0	Hypothetical protein
PA3524	*gloA1*		-2.7		Lactoylglutathione lyase
PA3530	***bfd***		18.6		Bacterioferritin-associated ferredoxin Bfd
PA3568	*ymmS*			-8.9	Probable acetyl-CoA synthetase
PA3569	*mmsB*			-5.3	3-hydroxyisobutyrate dehydrogenase
PA3570	*mmsA*			-3.9	Methylmalonate-semialdehyde dehydrogenase
PA3600	*rpl36*		3.0		Conserved hypothetical protein
PA3601	*ykgM*		2.7		Conserved hypothetical protein
PA3602	*yerD*		-6.4		Conserved hypothetical protein
PA3709				-6.4	Probable MFS transporter
PA3710				-5.0	Probable GMC-type oxidoreductase
PA3734				4.8	Hypothetical protein
PA3784			-6.0		Hypothetical protein
PA3785			-7.0		Conserved hypothetical protein
PA3790	*oprC*		-3.7		Putative copper transport outer membrane porin OprC
PA3841	*exoS*		2.6		Exoenzyme S
PA3842	*spcS*		3.9		Specific *Pseudomonas* chaperone for ExoS, SpcS
PA3866			2.6		Pyocin protein
PA3870	*moaA1*		-2.7		Molybdopterin biosynthetic protein A1
PA3872	*narI*		-7.0	-5.0	Respiratory nitrate reductase gamma chain
PA3873	*narJ*		-3.6	-3.1	Respiratory nitrate reductase delta chain
PA3874	*narH*		-6.5	-3.8	Respiratory nitrate reductase beta chain
PA3899	*fecI*		3.4		FecI
PA3938	*tauA*		2.7		Probable periplasmic taurine-binding protein precursor
PA4023	*eutP*			-4.5	Probable transport protein
PA4024	*eutB*			-2.8	Ethanolamine ammonia-lyase large subunit

PA number [a]	Gene name [a]	HHQ [b]	PQS [c]	PqsE [d]	Product name [a]
PA4078*				12.1	Probable nonribosomal peptide synthetase
PA4131[§]			-5.8		Probable iron-sulfur protein
PA4132			-3.1		Conserved hypothetical protein
PA4133	*ccoN*		-5.2		Cytochrome c oxidase subunit (cbb3-type)
PA4134[‡]			-6.1		Hypothetical protein
PA4140[‡]			5.3		Hypothetical protein
PA4141*[§‡]			4.4	5.2	Hypothetical protein
PA4142[‡]			4.3	4.8	Probable secretion protein
PA4168	***fpvB***		4.0		Second ferric pyoverdine receptor FpvB
PA4175	***prpL***		3.7		PrpL, protease IV
PA4205*[J◊]	*mexG*			25.0	Hypothetical protein
PA4206*[J◊]	*mexH*			16.4	Probable RND efflux membrane fusion protein precursor
PA4207*[J◊]	*mexI*			18.5	Probable RND efflux transporter
PA4208*[J◊]	*opmD*			11.6	Probable outer membrane protein precursor
PA4209*[J◊]	*phzM*			4.1	Probable phenazine-specific methyltransferase
PA4210[◊]	*phzA*			10.2	Probable phenazine biosynthesis protein
PA4211*[◊]	*phzB*			5.6	Probable phenazine biosynthesis protein
PA4217*[§◊]	*phzS*			9.0	Flavin-containing monooxygenase
PA4218[§J]	***ampP***		22.6		AmpP
PA4219	***yfpB***		10.7		AmpO
PA4220[§]	***fptB***		30.3		Hypothetical protein
PA4221[§J‡]	***fptA***		23.6		Fe(III)-pyochelin outer membrane receptor precursor
PA4222[§J‡]	***pchI***		13.8		Probable ATP-binding component of ABC transporter
PA4223[§]	***pchH***		13.7		Probable ATP-binding component of ABC transporter
PA4224[§]	***pchG***		40.1		Pyochelin biosynthetic protein PchG
PA4225[§J‡]	***pchF***		37.0		Pyochelin synthetase
PA4226[§J‡]	***pchE***		28.0		Dihydroaeruginoic acid synthetase
PA4227[J‡]	***pchR***		11.2		Transcriptional regulator PchR
PA4228[§J‡]	***pchD***		34.1		Pyochelin biosynthesis protein PchD
PA4229[§J]	***pchC***		31.5		Pyochelin biosynthetic protein PchC
PA4230[§J]	***pchB***		47.3		Salicylate biosynthesis protein PchB
PA4231[§J‡]	***pchA***		44.0		Salicylate biosynthesis isochorismate synthase
PA4236[§]	*katA*		-5.5		Catalase
PA4333	*fumA*		-3.4		Probable fumarase
PA4366	*sodB*		-3.8		Superoxide dismutase
PA4370	***icmP***		2.6		Insulin-cleaving metalloproteinase outer membrane protein
PA4384				3.0	Hypothetical protein
PA4429			-2.9		Probable cytochrome *c1* precursor
PA4430			-3.1		Probable cytochrome *b*
PA4431			-2.8		Probable iron-sulfur protein
PA4467			12.9		Hypothetical protein
PA4468[§]	***sodA***		89.4		Superoxide dismutase
PA4469[§J]	***orfX***		136.5		OrfX
PA4470[§J]	***fumC1***		114.7		Fumarate hydratase

PA number [a]	**Gene name [a]**	**HHQ [b]**	**PQS [c]**	**PqsE [d]**	**Product name [a]**
PA4471§∫	***fagA***		58.6		FagA
PA4500				-2.6	Probable binding protein component of ABC transporter
PA4501	*opdD*			-5.4	Glycine-glutamate dipeptide porin OpdP
PA4502				-2.8	Probable binding protein component of ABC transporter
PA4519	*speC*			2.7	Ornithine decarboxylase
PA4570			57.4		Hypothetical protein
PA4587*§	*ccpR*		-12.3		Cytochrome *c551* peroxidase precursor
PA4613§‡	*katB*			2.7	Catalase
PA4623				2.5	Hypothetical protein
PA4648	*cupE1*			3.0	Pilin subunit CupE1
PA4708	***phuT***		4.2		Heme-transport protein, PhuT
PA4709	***phuS***		4.5		PhuS
PA4710	***phuR***		9.2		Heme/Haemoglobin uptake outer membrane receptor PhuR
PA4774				-2.5	Hypothetical protein
PA4810	*fdnI*		-3.0		Nitrate-inducible formate dehydrogenase, gamma subunit
PA4811§	*fdnH*		-4.5		Nitrate-inducible formate dehydrogenase, beta subunit
PA4812§	*fdnG*		-5.3		Formate dehydrogenase-O, major subunit
PA4880§			-10.4		Probable bacterioferritin
PA4896			3.5		Probable sigma-70 factor, ECF subfamily
PA5058	*phaC2*			2.5	Poly(3-hydroxyalkanoic acid) synthase 2
PA5082	*dguC*			-13.2	DguC
PA5098	*hutH*			-3.5	Histidine ammonia-lyase
PA5100	*hutU*			-3.2	Urocanase
PA5153				-2.6	Amino acid ABC transporter periplasmic binding protein
PA5154				-4.5	Probable permease of ABC transporter
PA5167	*dctP*			-3.4	DctP
PA5168	*dctQ*			-5.6	DctQ
PA5169	*dctM*			-7.0	DctM
PA5300	*cycB*		-3.4		Cytochrome *c5*
PA5355	*glcD*		-3.5		Glycolate oxidase subunit GlcD
PA5380	*gbdR*		-2.6		GbdR
PA5396			-4.3		Hypothetical protein
PA5397			-4.6		Hypothetical protein
PA5410	*gbcA*		-5.7		GbcA
PA5460§				3.7	Hypothetical protein

[a] PA number, gene name and product name are from the *Pseudomonas* Genome Database [13]. Genes previously reported controlled by iron-starvation are in bold characters [40,41]; genes controlled by PQS *via* a PqsR-independent and iron starvation-independent signalling pathway(s) are underlined. *, genes whose transcription was altered in the Δ*pqsR* mutant with respect to the wild type strain [10]; §, genes whose transcription was altered upon exogenous PQS provision [28]; ∫, genes whose transcription was altered in the Δ*pqsA* mutant with respect to the wild type strain [11]; ‡, genes whose transcription was altered upon PqsE overexpression [11]; ◊, genes whose transcription was altered in the Δ*pqsH* mutant with respect to the wild type strain [23]. RND, Resistance-Nodulation-Cell division; MFS, major facilitator superfamily.

[b] Fold change in gene expression in *P. aeruginosa* PAO1 Δ4AQ grown in LB supplemented with 40 µM HHQ with respect to the same strain grown in LB.

[c] Fold change in gene expression in *P. aeruginosa* PAO1 Δ4AQ grown in LB supplemented with 40 µM PQS with respect to the same strain grown in LB.

[d] Fold change in gene expression in *P. aeruginosa* PAO1 Δ4AQ grown in LB supplemented with 1 mM IPTG (to induce PqsE expression) with respect to the same strain grown in LB.

Table S2. Bacterial strains and plasmids

Strains/Plasmids	Relevant characteristics and plasmids construction	Reference/Source
E. coli		
DH5α	Cloning strain.	[55]
S17.1λ*pir*	Conjugative strain for suicide plasmids.	[56]
H1717	Mutant strain carrying the P*fhuF*::*lacZ* transcriptional fusion that does not produce the siderophore enterochelin. Used for the Fur titration assay (FurTA).	[51]
P. aeruginosa		
PAO1	Nottingham collection wild type strain.	
Δ*pqsA* Δ*pqsH*	*pqsA* and *pqsH* double mutant of strain PAO1.	[27]
Δ*pqsA* Δ*pqsE*	*pqsA* and *pqsE* double mutant of strain PAO1.	[11]
Δ*pqsR*	*pqsR* mutant of strain PAO1.	This study
Δ4AQ	Quadruple mutant of PAO1 carrying in frame clear deletions of the *pqsA*, *pqsH*, and *pqsL* genes, in which *pqsE* is under the control of an IPTG-inducible promoter.	This study
Δ5AQ	Quintuple mutant of PAO1 carrying in frame clear deletions of the *pqsA*, *pqsH*, *pqsL*, and *pqsR* genes, in which *pqsE* is under the control of an IPTG-inducible promoter.	This study
Δ*pvdS*	*pvdS* mutant of strain PAO1.	This study
Δ4AQΔ*pvdS*	Δ4AQ strain carrying a deletion of the *pvdS* gene.	This study
Δ5AQΔ*pvdS*	Δ5AQ strain carrying a deletion of the *pvdS* gene.	This study
Δ*pqsAHLE*	Quadruple mutant of PAO1 carrying in frame clear deletions of the *pqsA*, *pqsH*, *pqsL* and *pqsE* genes.	This study
Plasmids		
pBluescript II KS(+)	Cloning vector; ColE1 replicon; Ap^R.	Stratagene
pDM4	Suicide vector; *sacBR*; *oriR6K*; Cm^R.	[57]
pME6032	pVS1-p15A shuttle expression (IPTG-inducible) vector, Tc^R.	[58]
miniCTX-*lux*	Promoter-probe vector containing the *luxCDABE* operon; Tc^R.	[59]
pEXΔ*pvdS*	pEX18 derivative used to introduce the *pvdS* mutation in PAO1 wild type, Δ4AQ and Δ5AQ.	[53]
pDM4*pqsE*ind	pDM4 derivative for the generation of the *pqsE*-inducible strain Δ4AQ.	[11]

Strains/Plasmids	**Relevant characteristics and plasmids construction**	**Reference/Source**
pDM4Δ*pqsE*	pDM4 derivative for the generation of the PAO1 Δ*pqsAHLE* mutant strain.	[11]
pDM4Δ*pqsR*	pDM4 derivative used to introduce the *pqsR* mutation in PAO1 wild type and in the Δ5AQ strain.	[24]
miniCTX-P*pqsA*::*lux*	miniCTX-*lux* derivative used to insert the P*pqsA*::*lux* fusion in the chromosome of different *P. aeruginosa* strains.	[27]
pUCP18	pUC18 derivative containing a stabilising fragment for maintenance in *Pseudomonas*; Ap^R, *E. coli* / Cb^R, *P. aeruginosa*.	[60]
pUCP*pqsE*	pUCP18 derivative for *pqsE* complementation; Ap^R.	[11]
pBS*pqsL*UP	The DNA fragnment encompassing the upstream region of the *pqsL* gene originated with primers FW*pqsL*UP and RV*pqsL*UP (Table S3) was cloned in pBluescript II KS(+) by XhoI-EcoRI restriction.	This study
pBS*pqsL*DOWN	The DNA fragment encompassing the downstream region of the *pqsL* gene originated with primers FW*pqsL*DOWN and RV*pqsL*DOWN (Table S3) was cloned in pBluescript II KS(+) by EcoRI-XbaI restriction.	This study
pDM4Δ*pqsL*	pDM4-derived plasmid used to introduce the *pqsL* mutation in *P. aeruginosa* Δ*pqsA* Δ*pqsH*. It contains the DNA fragments encompassing the upstream region *pqsL* (extracted by XhoI-EcoRI restriction from pBS*pqsL*UP) and the downstream region of *pqsL* (extracted by EcoRI-XbaI restriction from pBS*pqsL*DOWN), and cloned in pDM4 by XhoI-XbaI.	This study
miniCTX-P*pqsH*::*lux*	miniCTX-*lux* derivative used to insert the P*pqsH*::*lux* fusion in the chromosome of different *P. aeruginosa* strains. Obtained by cloning in miniCTX-*lux* with restriction enzymes XhoI-PstI a DNA region encompassing the P*pqsH* promoter amplified with primers FWP*pqsH* and RVP*pqsH* (Table S3).	This study
miniCTX-P*pqsL*::*lux*	miniCTX-*lux* derivative used to insert the P*pqsL*::*lux* fusion in the chromosome of different *P. aeruginosa* strains. Obtained by cloning in miniCTX-*lux* with restriction enzymes XhoI-PstI a DNA region encompassing the P*pqsL* promoter amplified with primers FWP*pqsL* and RVP*pqsL* (Table S3).	This study

Strains/Plasmids	**Relevant characteristics and plasmids construction**	**Reference/Source**
miniCTX-P*pqsR*::*lux*	miniCTX-*lux* derivative used to insert the P*pqsR*::*lux* fusion in the chromosome of different *P. aeruginosa* strains. Obtained by cloning in miniCTX-*lux* with restriction enzymes HindIII-EcoRI a DNA region encompassing the P*pqsR* promoter amplified with primers FWP*pqsR* and RVP*pqsR* (Table S3).	This study
miniCTX-P*pchR*::*lux*	miniCTX-*lux* derivative used to test the ability of Fur to bind the P*pchR* promoter (FurTA; posistive control). Obtained by cloning in miniCTX-*lux* with restriction enzymes XhoI-HindIII a DNA region encompassing the P*pchR* promoter amplified with primers FWP*pchR* and RVP*pchR* (Table S3).	This study
miniCTX-P*phzA1*::*lux*	miniCTX-*lux* derivative used to insert the P*phzA1*::*lux* fusion into the chromosome of *P. aeruginosa* Δ*pqsAHLE*. Obtained by cloning in miniCTX-*lux* with restriction enzymes HindIII-BamHI a DNA region encompassing the P*phzA1* promoter amplified with primers FWP*phzA1* and RVP*phzA1* (Table S3).	This study
miniCTX-P*phzA2*::*lux*	miniCTX-*lux* derivative used to insert the P*phzA2*::*lux* fusion into the chromosome of *P. aeruginosa* Δ*pqsAHLE*. Obtained by cloning in miniCTX-*lux* with restriction enzymes EcoRI-PstI a DNA region encompassing the P*phzA2* promoter amplified with primers FWP*phzA2* and RVP*phzA2* (Table S3).	This study
pME-*pqsE*	pME6032 derivative for the IPTG-dependent expression on *pqsE*. Obtained by cloning in pME6032 with restriction enzymes EcoRI-SacI a DNA region encompassing the *pqsE* gene amplified with primers FW*pqsE* and RV*pqsE* (Table S3).	This study
pME-*pqsE*Δ1-6	pME6032 derivative for the IPTG-dependent expression of a mutated variant of *pqsE* lacking the first two codons (ATGTTG). Obtained by cloning in pME6032 with restriction enzymes EcoRI-SacI a DNA region encompassing the *pqsE* gene amplified with primers FW*pqsE*Δ1-6 and RV*pqsE*Δ1-6 (Table S3).	This study

Strains/Plasmids	Relevant characteristics and plasmids construction	Reference/Source
pME-*pqsE*NoFrame	pME6032 derivative for the IPTG-dependent expression of a mutated variant of *pqsE* out of frame. Obtained by cloning in pME6032 with restriction enzymes EcoRI-SacI a DNA region encompassing the *pqsE* gene amplified with primers FW*pqsE*NoFrame and RV*pqsE* (Table S3).	This study

Additional references for Table S2:

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58. Heeb S, Blumer C, Haas D. Regulatory RNA as mediator in GacA/RsmA-dependent global control of exoproduct formation in *Pseudomonas fluorescens* CHA0. J Bacteriol. 2002;184:1046-56.

59. Becher A, Schweizer HP. Integration-proficient *Pseudomonas aeruginosa* vectors for isolation of single-copy chromosomal *lacZ* and *lux* gene fusions. Biotechniques. 2000;29:948-50.

60. Schweizer HP. *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19. Gene. 1991;97:109-21.

Table S3. Oligonucleotides

Name	Sequence (5'-3') [a]	Restriction site [b]
FWP*pqsH*	CCGCTCGAGGGTCGTGCATGCTTGCCG	XhoI
RVP*pqsH*	TAACTGCAGTTGCTCCTTAGCAGCGGC	PstI
FWP*pqsL*	TCCGCTCGAGGATCGTCACCGTCAACTG	XhoI
RVP*pqsL*	TAACTGCAGCGTCATGGATGAGTCTCCG	PstI
FWP*pqsR*	TATAAGCTTAGGCCCTTGGTATTAACG	HindIII
RVP*pqsR*	TATGAATTCCCCTTATTCCTTTTATTG	EcoRI
FWP*pchR*	TATCTCGAGCGATCTCCGTGGATGCG	XhoI
RVP*pchR*	TATAAGCTTGGTCATCAGGTTTTCCTGTA	HindIII
FWP*phzA1*	GGCGGCAAGCTTCTCGTCGCTGTCGAT	HindIII
RVP*phzA1*	TTTCCCGGATCCGCTGACCGTTCATGC	BamHI
FWP*phzA2*	AGCGCAGAATTCCCACCGGCAGCGTTT	EcoRI
RVP*phzA2*	ACGCAACTGCAGGTTGTCGGTAAACCC	PstI
FW*pqsL*UP	CCGCTCGAGACCGTGGTCGATGGCGT	XhoI
RV*pqsL*UP	CCGGAATTCCGTCATGGATGAGTCTCC	EcoRI
FW*pqsL*DOWN	CCGGAATTCCTCGGCTGAACCCGCC	EcoRI
RV*pqsL*DOWN	TGCTCTAGACCTGTTCAATTACCCGAGCC	XbaI
FW*pqsB*	CCGCTCGAGCGACCAGGGCTATCGCA	XhoI
FW*pqsE*	TATGAATTCATGTTGAGGCTTTCGGCTCC	EcoRI
RV*pqsE*	TATGAGCTCTCAGTCCAGAGGCAGCGC	SacI
FW*pqsE*Δ1-6	TATGAATTCAGGCTTTCGGCTCC	EcoRI
RV*pqsE*Δ1-6	TATGAGCTCTGTCCCGTCTCAGTCCAG	SacI
FW*pqsE*NoFrame	TATGAATTCATGGTTGAGGCTTTCGGCTC	EcoRI
RV*pqsB*	CCGGAATTCCTTATGCATGAGCTTCTCC	EcoRI
FW16SRT	AGTACGGCCGCAAGGTTAAA	-
RV16SRT	CCCAACATCTCACGACACGA	-
FW*pchR*RT	CTCAGCGCACAGTTCCTTTC	-
RV*pchR*RT	CGAACACCTTGCGAAAGCC	-
FW*pvdS*RT	GGAACAACTGTCTACCCGCA	-
RV*pvdS*RT	GTAGCTGAGCTGTGCCTTGA	-
FW*lecA*RT	CAGGGCAGGTAACGTCGATT	-
RV*lecA*RT	CAACCCGGTATTGACCGGAA	-
FW*rhlA*RT	CATCTGCTCAACGACGACCGT	-
RV*rhlA*RT	TGCCGTTGATGAAATGCACG	-
FW*mexG*RT	CTGGCGAAGCTGTTCGACTA	-
RV*mexG*RT	TTGCTCCAGAAGGTGTGGAC	-
FW*nosR*RT	AGCTATCGGTGGTCAACGTG	-
RV*nosR*RT	GCGAGTTCGTTGAGCAGTTC	-

Name	**Sequence (5’-3’)** [a]	**Restriction site** [b]
FW*hcpC*RT	TATCAGGAAGGCCACGAGGA	-
RV*hcpC*RT	CCACTGGATCTCGACCTTGG	-
FW*aprX*RT	CTGCCGATCAACGTCTCCTT	-
RV*aprX*RT	TACCGTAGAACTTGGCGCTG	-
FW*sodA*RT	GCTGACCATGGAGATCCACC	-
RV*sodA*RT	CGACATCACGGTCCAGAACA	-
FW*pqsE*RT	CGGTGTTCCTGCTGCGTC	-
RV*pqsE*RT	GACGCCAGGACCTGTACG	-
FW*phnA*RT	GCGACATGCTCAAGGTCGAT	-
RV*phnA*RT	CTGCCGCGATAGCCATCTT	-
FW*pqsE-phnA*	GTGGGCAGAGCGTCGACT	-
RV*pqsE-phnA*	GTGCGGTACTCCAGACTTTC	-

[a] Engineered restriction sites are underlined.

[b] -, no restriction site introduced.

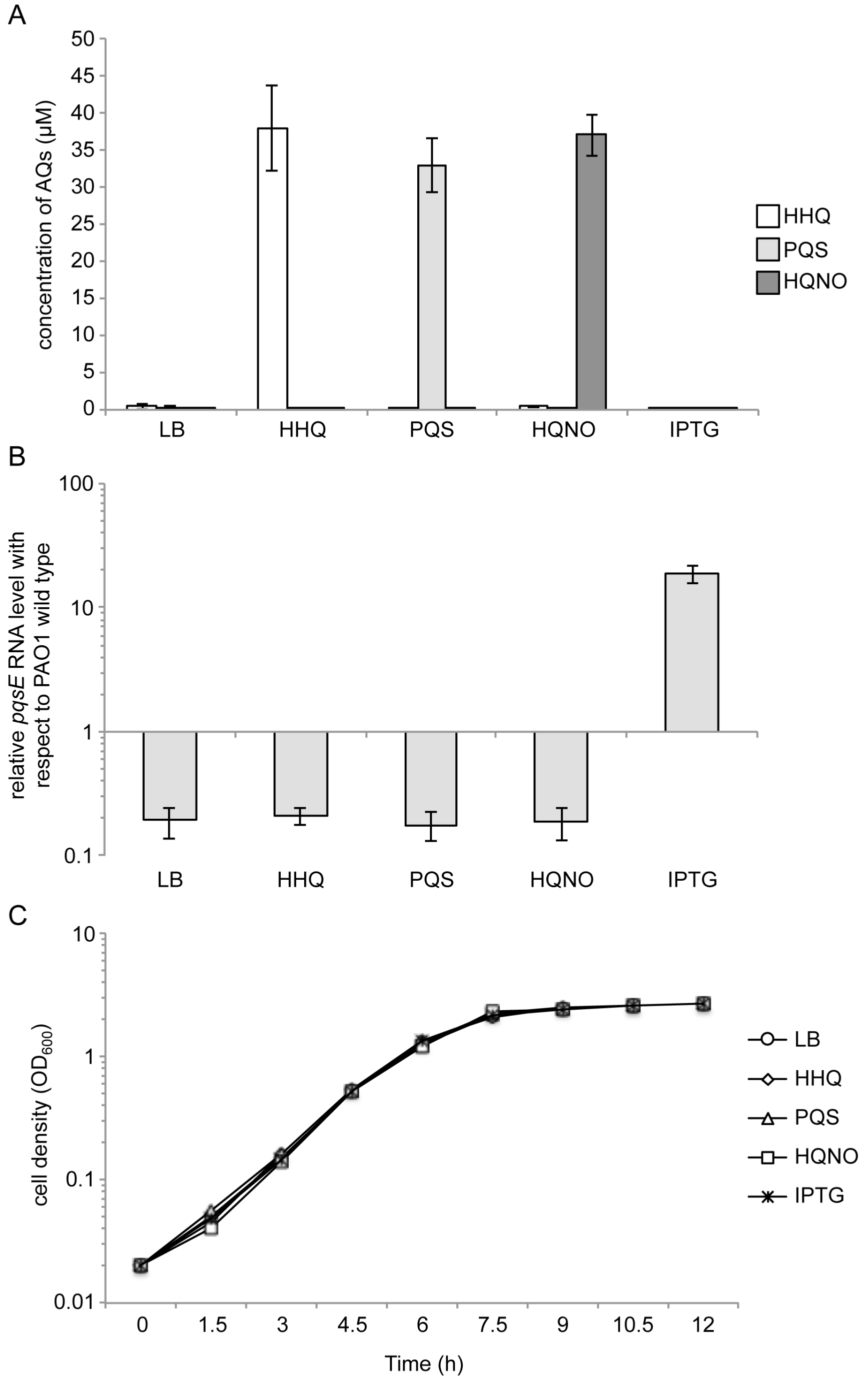

Fig S1. Validation of the *P. aeruginosa* Δ4AQ strain

(**A**) Levels of HHQ (white bars), PQS (light-grey bars) and HQNO (dark-grey bars) quantified by LC-MS/MS analysis in culture supernatants of *P. aeruginosa* Δ4AQ grown in LB or in LB supplemented with 40 µM HHQ, PQS, or HQNO, or with 1 mM IPTG, as indicated. AQ levels were quantified in supernatants from three independent cultures grown to OD_{600} 1.5. (**B**) Fold change in *pqsE* transcript levels measured by Real Time PCR in RNA extracted from Δ4AQ strains grown as in (**A**). Data were normalized to the *pqsE* RNA level in the wild type. (**C**) Growth curves of *P. aeruginosa* Δ4AQ grown in LB or in LB supplemented with 40 µM HHQ, PQS, or HQNO, or with 1 mM IPTG, as indicated.

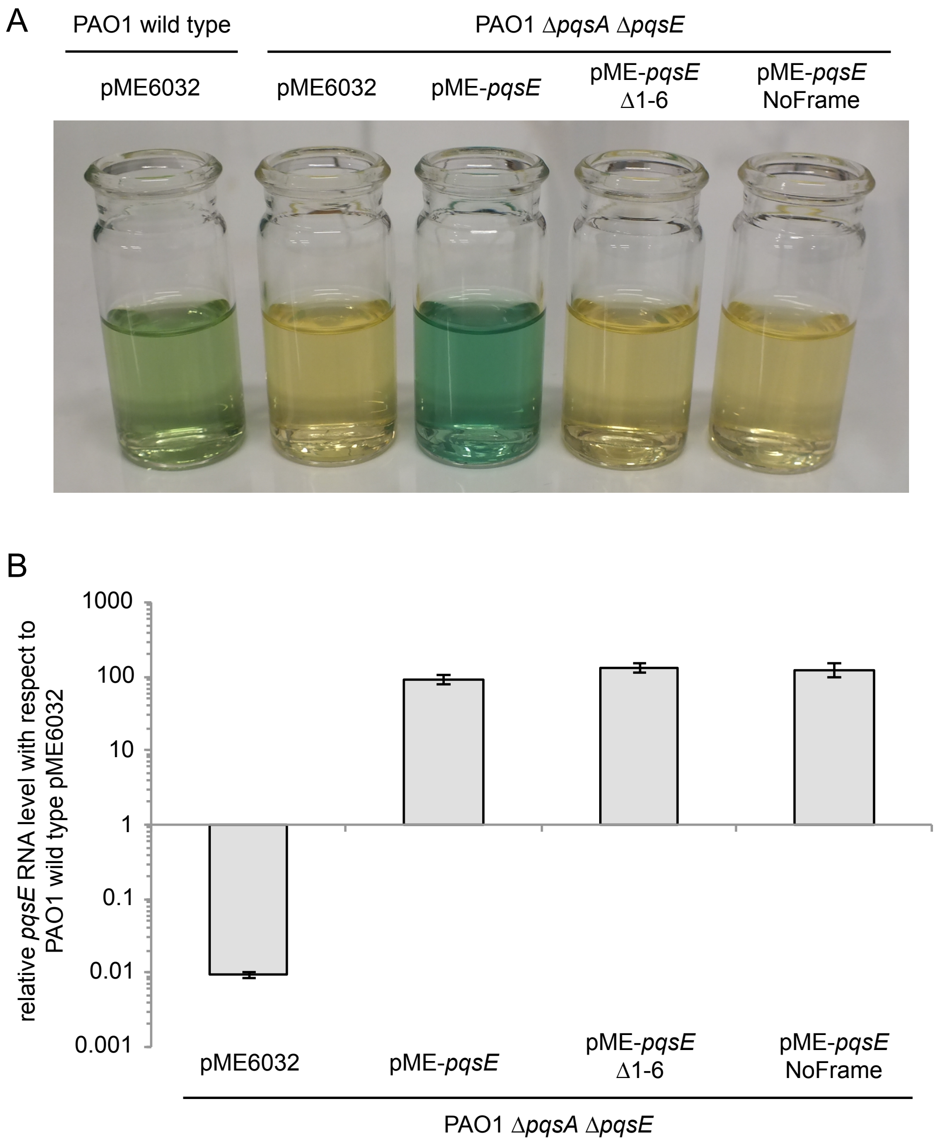

Fig S2. The *pqsE* RNA transcript does not promote pyocyanin production

Pyocyanin production (**A**) and *pqsE* RNA levels measured by Real Time PCR (**B**) in *P. aeruginosa* Δ*pqsA* Δ*pqsE* double mutant strains carrying the pME6032 empty vector or pME6032-derivative plasmids for IPTG-inducible expression of wild type *pqsE* (pME-*pqsE*), or *pqsE* mutated variants lacking the first two codons (pME-*pqsE*Δ1-6) or with a nucleotide insertion after the ATG to alter the protein frame (pME-*pqsE*NoFrame). Culture supernatants and total RNAs are from the indicated strains grown to an OD_{600} of 1.5 in LB supplemented with 1 mM IPTG. For the Real time PCR analysis, data are normalized to the *pqsE* RNA level measured in parallel in the *P. aeruginosa* PAO1 wild type.

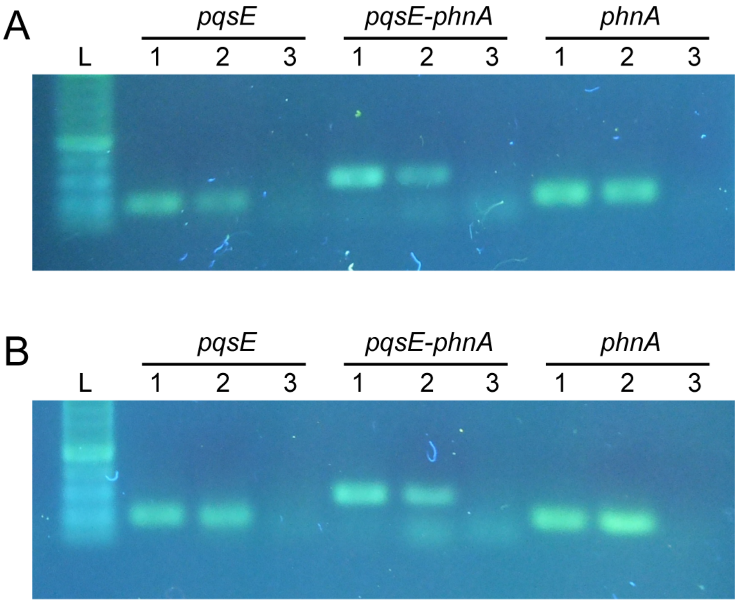

Fig S3. RT-PCR analysis showing co-transcription of *pqsE* and *phnA*

Amplification of cDNAs retro-transcribed from RNA extracted from (**A**) *P. aeruginosa* PAO1 wild type grown in LB and (**B**) *P. aeruginosa* Δ4AQ grown in LB supplemented with 1 mM IPTG, to an OD_{600} of 1.5. A 200 bp DNA region within the *pqsE* gene (*pqsE*), a 280 bp DNA region spanning from 97 bp upstream of the *pqsE* stop codon to 68 bp downstream of the *phnA* start codon (*pqsE-phnA*), and a 200 bp DNA region inside the *phnA* gene (*phnA*) were amplified from: 1, PAO1 genomic DNA (positive control); 2, cDNA; 3, the corresponding RNA (negative control). L, GeneRuler 100 bp DNA Ladder Plus (MBI Fermentas).

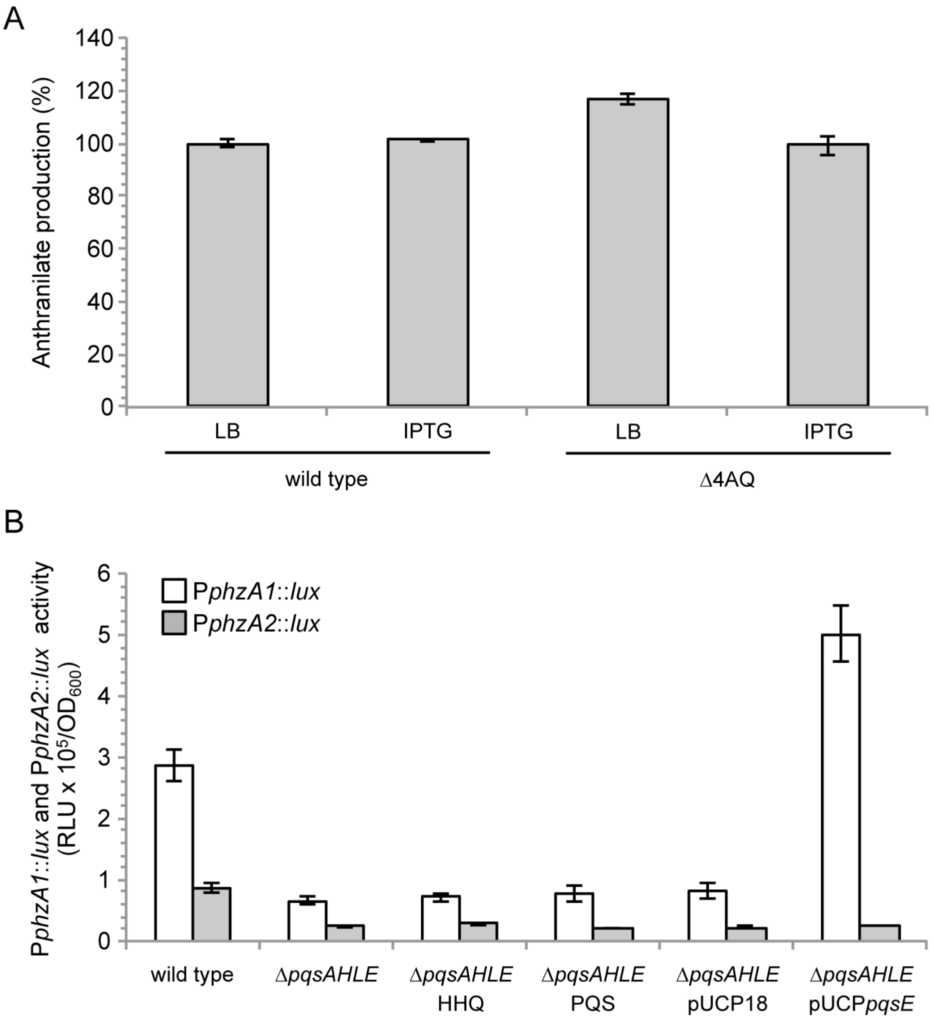

Fig S4. PqsE has negligible effect on anthranilate production and P*phzA2* activity, while it positively controls P*phzA1* activity

(A) Anthranilate was quantified by LC-MS/MS analysis in *P. aeruginosa* wild type and Δ4AQ grown to an OD_{600} of 1.5 in LB or in LB supplemented with 1 mM IPTG. Standard deviations are based on the mean values of three parallel cultures. (B) Maximal P*phzA1* and P*phzA2* promoter activity measured in *P. aeruginosa* PAO1 wild type and in the Δ*pqsAHLE* strain carrying the pUCP18 empty vector or the pUCP*pqsE* plasmid for constitutive expression of *pqsE*. Strains were grown in LB or in LB supplemented with 40 µM HHQ or PQS, as indicated. Promoter activity is reported as Relative Light Units (RLU)/OD_{600}.

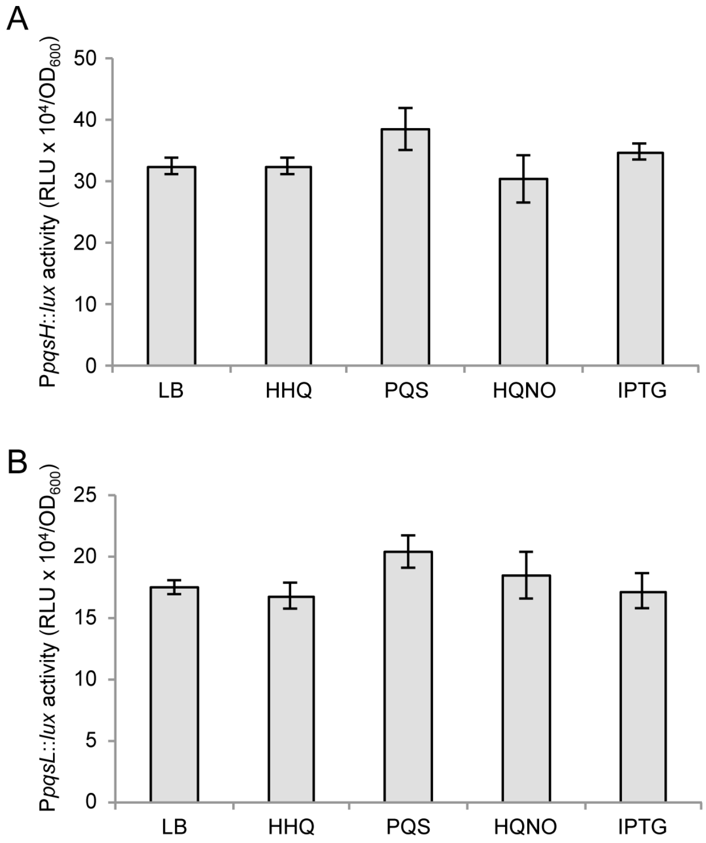

Fig S5. AQs and PqsE do not affect P*pqsH* and P*pqsL* activity

Maximal promoter activity measured in *P. aeruginosa* Δ4AQ strains carrying the transcriptional fusions (**A**) P*pqsH*::*lux* or (**B**) P*pqsL*::*lux*. Strains were grown in LB or in LB supplemented with 40 μM AQs or 1 mM IPTG, as indicated below the graphs. Promoter activity is reported as Relative Light Units (RLU)/OD_{600}.

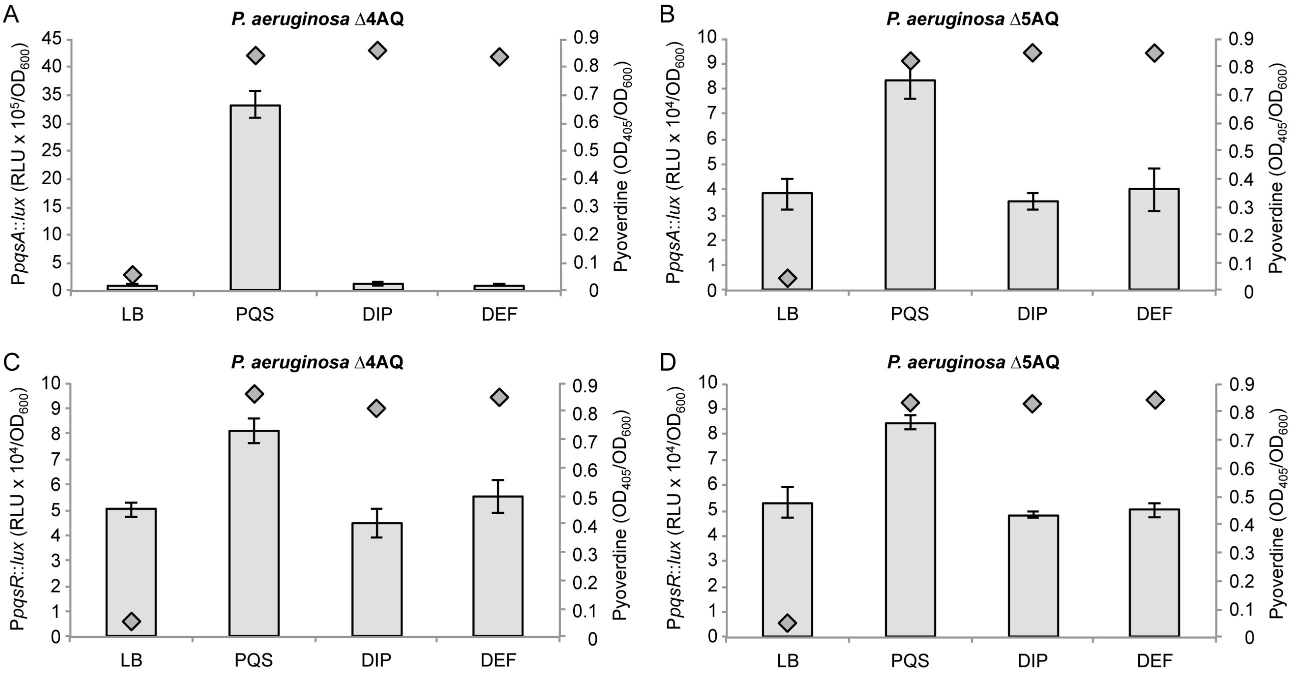

Fig S6. The iron-chelators 2,2'-dipyridyl and deferiprone do not increase P*pqsA* activity

Maximal promoter activity in strains carrying the transcriptional fusions P*pqsA*::*lux* (**A** and **B**) or P*pqsR*::*lux* (**C** and **D**). Strains were grown in LB or in LB supplemented with 40 µM PQS, 500 µM 2,2'-dipyridyl (DIP), or 160 µM deferiprone (DEF), as indicated. Diamonds indicate the pyoverdine levels measured in parallel in culture supernatants. Promoter activity is reported as Relative Light Units (RLU)/OD_{600}; pyoverdine levels are reported as OD_{405} normalized to cell density (OD_{600}).

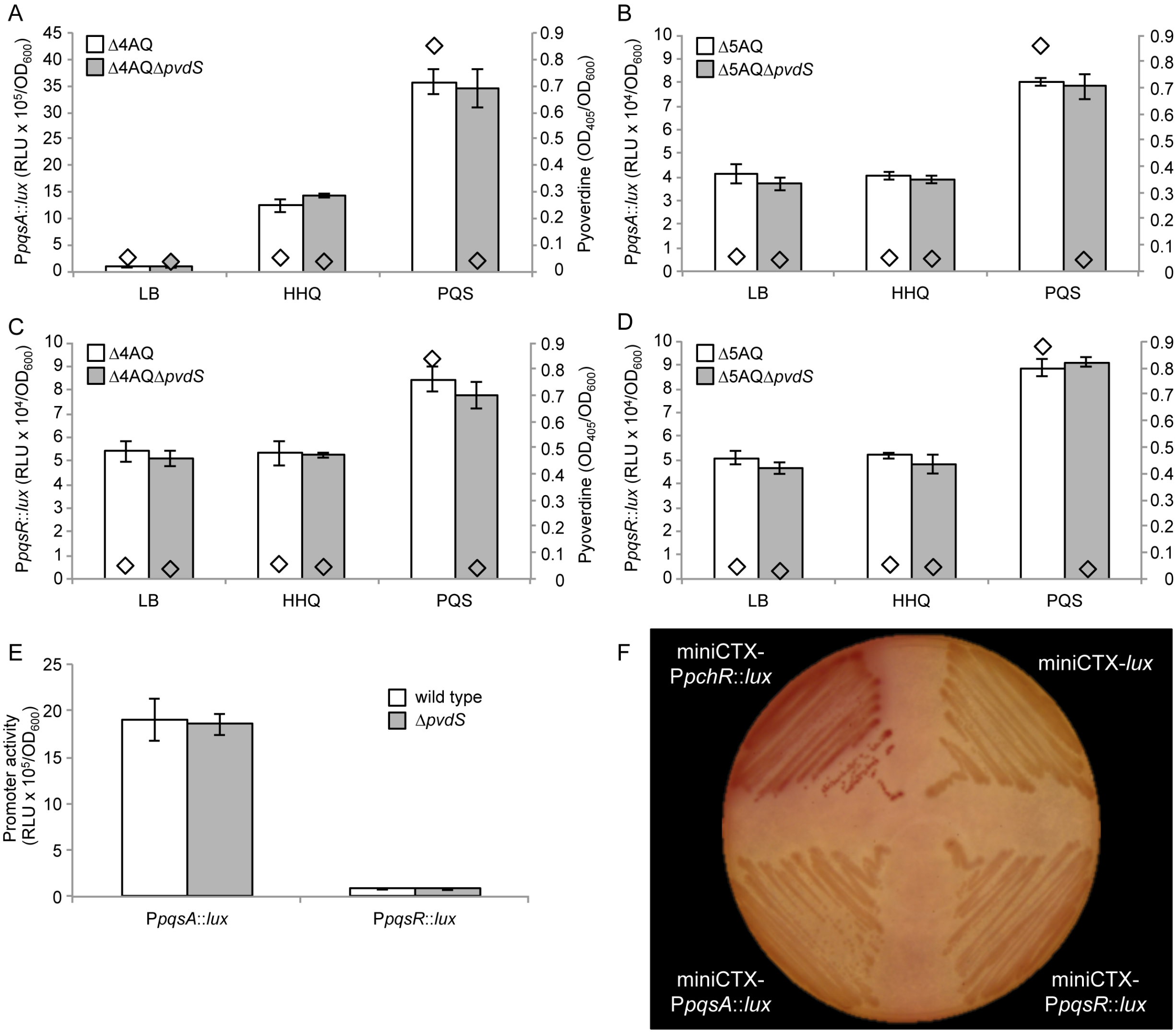

Fig S7. Impact of Fur and PvdS on the P*pqsA* and P*pqsR* promoter regions

(**A**-**D**) Maximal promoter activity in the strains carrying the transcriptional fusions P*pqsA*::*lux* (**A** and **B**) or P*pqsR*::*lux* (**C** and **D**). White bars indicate the *pvdS*-proficient genetic backgrounds (Δ4AQ and Δ5AQ); grey bars indicate the *pvdS*-mutant genetic backgrounds (Δ4AQΔ*pvdS* and Δ5AQΔ*pvdS*). Strains were grown in LB or in LB supplemented with 40 μM HHQ or PQS, as indicated. Diamonds indicate the pyoverdine levels measured in culture supernatants in the *pvdS*-proficient (white diamonds) or *pvdS*-mutant (grey diamonds) genetic backgrounds. Promoter activity is reported as Relative Light Units (RLU)/OD_{600}; pyoverdine levels are reported as OD_{405} normalized to cell density (OD_{600}). (**E**) Maximal P*pqsA*::*lux* and P*pqsR*::*lux* promoter activity in the wild type (white bars) and Δ*pvdS* (grey bars) strains grown in LB. Promoter activity is reported as Relative Light Units (RLU)/OD_{600}. (**F**) *E. coli* H1717 cells containing the plasmids indicated and grown for 24 h at 37ºC on McConkey agar supplemented with 10 μg ml^{-1} Tc and 20 μM $FeSO_4$. Red-staining indicates the ability to ferment lactose and hence the binding of Fur to the target promoter. miniCTX-P*pchR*::*lux*, positive control (red colonies); miniCTX-*lux*, negative control (white colonies).