Strains/Plasmids	Relevant characteristics and plasmids construction	Reference/Source
E. coli		
DH5a	Cloning strain.	[55]
S17.1λ <i>pir</i>	Conjugative strain for suicide plasmids.	[56]
H1717	Mutant strain carrying the <i>PfhuF</i> :: <i>lacZ</i> 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.	
ΔpqsA ΔpqsH	pqsA and pqsH double mutant of strain PAO1.	[27]
$\Delta pqsA \ \Delta pqsE$	pqsA and pqsE double mutant of strain PAO1.	[11]
$\Delta pqsR$	pqsR mutant of strain PAO1.	This study
Δ4AQ	Quadruple mutant of PAO1 carrying in frame clear deletions of the <i>pqsA</i> , <i>pqsH</i> , and <i>pqsL</i> genes, in which <i>pqsE</i> is under the control of an IPTG-inducible promoter.	This study
Δ5AQ	Quintuple mutant of PAO1 carrying in frame clear deletions of the <i>pqsA</i> , <i>pqsH</i> , <i>pqsL</i> , and <i>pqsR</i> genes, in which <i>pqsE</i> is under the control of an IPTG-inducible promoter.	This study
$\Delta pvdS$	pvdS mutant of strain PAO1.	This study
$\Delta 4$ AQ $\Delta pvdS$	$\Delta 4AQ$ strain carrying a deletion of the <i>pvdS</i> gene.	This study
$\Delta 5 AQ \Delta pv dS$	Δ 5AQ strain carrying a deletion of the <i>pvdS</i> gene.	This study
ΔpqsAHLE	Quadruple mutant of PAO1 carrying in frame clear deletions of the <i>pqsA</i> , <i>pqsH</i> , <i>pqsL</i> and <i>pqsE</i> genes.	This study
Plasmids		
pBluescript II KS(+)	Cloning vector; ColE1 replicon; Ap ^R .	Stratagene
pDM4	Suicide vector; <i>sacBR</i> ; <i>oriR6K</i> ; Cm ^R .	[57]
pME6032	pVS1-p15A shuttle expression (IPTG-inducible) vector, Tc ^R .	[58]
miniCTX- <i>lux</i>	Promoter-probe vector containing the $luxCDABE$ operon; Tc^{R} .	[59]
pEXA <i>pvdS</i>	pEX18 derivative used to introduce the <i>pvdS</i> mutation in PAO1 wild type, Δ 4AQ and Δ 5AQ.	[53]
pDM4 <i>pqsE</i> ind	pDM4 derivative for the generation of the <i>pqsE</i> -inducible strain Δ 4AQ.	[11]

Table S2. Bacterial strains and plasmids

Strains/Plasmids	Relevant characteristics and plasmids construction	Reference/Source
pDM4∆ <i>pqsE</i>	pDM4 derivative for the generation of the PAO1 $\Delta pqsAHLE$ mutant strain.	[11]
pDM4∆ <i>pqsR</i>	pDM4 derivative used to introduce the $pqsR$ mutation in PAO1 wild type and in the Δ 5AQ strain.	[24]
miniCTX-PpqsA::lux	miniCTX- <i>lux</i> derivative used to insert the PpqsA:: <i>lux</i> fusion in the chromosome of different <i>P. aeruginosa</i> strains.	[27]
pUCP18	pUC18 derivative containing a stabilising fragment for maintenance in <i>Pseudomonas</i> ; Ap^{R} , <i>E. coli</i> / Cb^{R} , <i>P. aeruginosa</i> .	[60]
pUCPpqsE	pUCP18 derivative for $pqsE$ complementation; Ap ^R .	[11]
pBS <i>pqsL</i> UP	The DNA fragmment encompassing the upstream region of the <i>pqsL</i> gene originated with primers $FWpqsLUP$ and RVpqsLUP (Table S3) was cloned in pBluescript II KS(+) by XhoI-EcoRI restriction.	This study
pBS <i>pqsL</i> DOWN	The DNA fragment encompassing the downstream region of the <i>pqsL</i> gene originated with primers FW <i>pqsL</i> DOWN and RV <i>pqsL</i> DOWN (Table S3) was cloned in pBluescript II KS(+) by EcoRI-XbaI restriction.	This study
pDM4∆ <i>pqsL</i>	pDM4-derived plasmid used to introduce the <i>pqsL</i> mutation in <i>P. aeruginosa</i> $\Delta pqsA$ $\Delta pqsH$. It contains the DNA fragments encompassing the upstream region <i>pqsL</i> (extracted by XhoI-EcoRI restriction from pBS <i>pqsL</i> UP) and the downstream region of <i>pqsL</i> (extracted by EcoRI-XbaI restriction from pBS <i>pqsL</i> DOWN), and cloned in pDM4 by XhoI-XbaI.	This study
miniCTX-P <i>pqsH</i> ::lux	miniCTX- <i>lux</i> derivative used to insert the P <i>pqsH</i> :: <i>lux</i> fusion in the chromosome of different <i>P. aeruginosa</i> strains. Obtained by cloning in miniCTX- <i>lux</i> with restriction enzymes XhoI-PstI a DNA region encompassing the P <i>pqsH</i> promoter amplified with primers FWP <i>pqsH</i> and RVP <i>pqsH</i> (Table S3).	This study
miniCTX-P <i>pqsL::lux</i>	miniCTX- <i>lux</i> derivative used to insert the P <i>pqsL::lux</i> fusion in the chromosome of different <i>P. aeruginosa</i> strains. Obtained by cloning in miniCTX- <i>lux</i> with restriction enzymes XhoI-PstI a DNA region encompassing the <i>PpqsL</i> promoter amplified with primers FWP <i>pqsL</i> and RVP <i>pqsL</i> (Table S3).	This study

Strains/Plasmids	Relevant characteristics and plasmids construction	Reference/Source
miniCTX-P <i>pqsR::lux</i>	miniCTX-lux derivative used to insert the PpqsR::lux fusion	This study
	in the chromosome of different P. aeruginosa strains.	
	Obtained by cloning in miniCTX-lux with restriction enzymes	
	HindIII-EcoRI a DNA region encompassing the PpqsR	
	promoter amplified with primers FWPpqsR and RVPpqsR	
	(Table S3).	
miniCTX-P <i>pchR::lux</i>	miniCTX-lux derivative used to test the ability of Fur to bind	This study
	the PpchR promoter (FurTA; posistive control). Obtained by	
	cloning in miniCTX-lux with restriction enzymes XhoI-	
	HindIII a DNA region encompassing the PpchR promoter	
	amplified with primers FWPpchR and RVPpchR (Table S3).	
miniCTX-P <i>phzA1::lux</i>	miniCTX-lux derivative used to insert the PphzA1::lux fusion	This study
	into the chromosome of <i>P. aeruginosa</i> $\Delta pqsAHLE$. Obtained	
	by cloning in miniCTX- <i>lux</i> with restriction enzymes HindIII-	
	BamHI a DNA region encompassing the <i>PphzA1</i> promoter	
	amplified with primers FWP <i>phzA1</i> and RVP <i>phzA1</i> (Table	
	S3).	
miniCTX-PphzA2::lux	miniCTX-lux derivative used to insert the PphzA2::lux fusion	This study
	into the chromosome of <i>P. aeruginosa</i> $\Delta pqsAHLE$. Obtained	
	by cloning in miniCTX- <i>lux</i> with restriction enzymes EcoRI-	
	PstI a DNA region encompassing the PphzA2 promoter	
	amplified with primers FWP <i>phzA2</i> and RVP <i>phzA2</i> (Table	
	S3).	
pME- <i>pqsE</i>	pME6032 derivative for the IPTG-dependent expression on	This study
pine pqse	pqsE. Obtained by cloning in pME6032 with restriction	This study
	enzymes EcoRI-SacI a DNA region encompassing the <i>pqsE</i>	
	gene amplified with primers $FWpqsE$ and $RVpqsE$ (Table	
	S3).	
pME- <i>pqsE</i> ∆1-6	pME6032 derivative for the IPTG-dependent expression of a	This study
pmL-pqsEA1-0	mutated variant of $pqsE$ lacking the first two codons	i ilis study
	(ATGTTG). Obtained by cloning in pME6032 with restriction	
	enzymes EcoRI-SacI a DNA region encompassing the $pqsE$	
	gene amplified with primers FW $pqsE\Delta 1$ -6 and RV $pqsE\Delta 1$ -6	
	(Table S3). (Table S3).	
	(14010-05).	

Strains/Plasmids	Relevant characteristics and plasmids construction	Reference/Source
pME-pqsENoFrame	pME6032 derivative for the IPTG-dependent expression of a	This study
	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	
	FWpqsENoFrame and RVpqsE (Table S3).	

Additional references for Table S2:

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