Table S1. Bacterial strains, plasmids and primers used in this study.

Strain	Description	Source
E. coli		
DH5α	Cloning strain; F^- Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *recA1 endA1 hsdR17* (r_K^-, m_K^+) *phoA supE44* λ^- *thi*-1 *gyrA96 relA1*	Invitrogen
SM10	Bi-parental mating strain; *thi thr leu tonA lacY supE recA*::RP4-2-Tc::Mu K_m λ*pir*, Kan^R, Tet^R	(1)
BTH101	BACTH strain; F^- *cya-99 araD139 galE15 galK16 rpsL1* (Str^R) *hsdR2 mcrA1 mcrB1*	Euromedex
P. aeruginosa		
PAO1	Wild-type strain	M. Parsek
PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel*	PAO1 Δ*wspF* (in-frame); Δ*pslBCD* (polar); *araC*-P_{BAD} inserted upstream of *pelABCDEFG*	(2)
GBW1	PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel* Δ*rmcA*	This study
GBW2	PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel* Δ*morA*	This study
GBW3	PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel* Δ*rmcA attTn7*::miniTn7T-Gm::*araC*-P_{BAD}::*rmcA*, Gen^R	This study
GBW4	PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel* Δ*morA attTn7*::miniTn7T-Gm::*araC*-P_{BAD}::*morA*, Gen^R	This study
GBW5	PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel* Δ*rmcA attTn7*::miniTn7T-Gm::*araC*-P_{BAD}::$rmcA^{C\text{-}VSV\text{-}G}$, Gen^R	This study
GBW6	PAO1 Δ*wspF* Δ*psl* P_{BAD}*pel* Δ*morA attTn7*::miniTn7T-Gm::*araC*-P_{BAD}::$morA^{C\text{-}VSV\text{-}G}$, Gen^R	This study
SMC232	UCBPP-PA14	(3)

SMC6279	PA14 Δ*rmcA* (in-frame)	(4)
SMC6289	PA14 Δ*morA* (in-frame)	(4)
SMC7006	PA14 *att*::DMS3$_{42}$	(5)
SMC2893	PelA KO	(6, 7)
SMC6607	Pel inducible, P_{pel} replaced with P_{bad}	(8)
SMC8808	PA14 Δ*rmcA att*::DMS3$_{42}$	This study
SMC8809	PA14 Δ*morA att*::DMS3$_{42}$	This study
SMC8810	PA14 pKNT25 GcbC R363E	This study
SMC8811	PA14 Δ*rmcA* pKNT25 GcbC R363E	This study
SMC8812	PA14 Δ*morA* pKNT25 GcbC R363E	This study
SMC8813	PA14 Δ*rmcA attTn7*::miniTn7T-Gm::*araC*-P_{BAD}::*rmcA*, Gen^R	This study
SMC8814	PA14 Δ*morA attTn7*::miniTn7T-Gm::*araC*-P_{BAD}::*morA*, Gen^R	This study
SMC8815	PA14 Δ*rmcA*:P_{tac}-*mKO*-κ	This study
SMC8816	PA14 Δ*morA*:P_{tac}-*mKO*-κ	This study
SMC8817	PA14 Δ*rmcA*:Psmc21-*gfp*	This study
SMC8818	PA14 Δ*morA*:Psmc21-*gfp*	This study
SMC8819	PA14 Δ*rmcA* Δ*pelA*	This study
SMC8820	PA14 Δ*morA* Δ*pelA*	This study
SMC8821	PA14 Δ*rmcA,* inducible Pel under the control of P_{BAD}	This study
SMC8822	PA14 Δ*morA* inducible Pel under the control of P_{BAD}	This study

Plasmid	Description	Source
Two-step allelic exchange		
pEX18Gm	Suicide vector for allelic exchange in *P. aeruginosa*, encodes SacB, Gen^R	(1)
pEX18Gm::Δ*rmcA*	pEX18Gm with *P. aeruginosa* PAO1 Δ*rmcA* allele cloned between the EcoRI and HindIII sites, Gen^R	This study
pEX18Gm::Δ*morA*	pEX18Gm with *P. aeruginosa* PAO1 Δ*morA* allele cloned between the SacI and HindIII sites, Gen^R	This study
pSMC21	pUCP-based plasmid containing gfpmut2, Ap^r , Cb^r , Km^r	(9)
pMQ30	Suicide vector; Gm^r, *sacB*, *URA3*, *CEN6/ARSH4*, *lacZα*	(10)
pKO PA14_07500	PA14_07500 knockout construct in pMQ30	(4)
pKO PA14_07500	PA14_60870 (*morA*) knockout construct in pMQ30	(4)
pKNT25 GcbC-R363E	GcbC protein with an I-site mutation	(11)
Complementation analysis		
pUC18T-miniTn7T-Gm	*aacC1* on miniTn7-based vector with transcriptional terminators at the right end of the Tn7 transposon; Amp^R, Gen^R	(12)
pUC18T-miniTn7T-Gm-pBAD	pUC18T-miniTn7T-Gm containing *araC*-P_{BAD} and a downstream MCS (SmaI-NotI-PstI-NcoI) cloned between the HindIII and SacI sites; Amp^R, Gen^R	This study

pTNS2	Helper plasmid encoding *tnsABCD*, Amp^R	(13)
p-miniTn7-*rmcA*	pUC18T-miniTn7T-Gm-pBAD with *P. aeruginosa* PAO1 *rmcA* fused to an upstream synthetic ribosome binding site, cloned between the SmaI and NotI sites; Amp^R, Gen^R	This study
p-miniTn7-*morA*	pUC18T-miniTn7T-Gm-pBAD with *P. aeruginosa* PAO1 *morA* fused to an upstream synthetic ribosome binding site, cloned between the NotI and Mph1103I sites; Amp^R, Gen^R	This study
p-miniTn7-*rmcA*$^{C\text{-}VSV\text{-}G}$	p-miniTn7-*rmcA* fused to a downstream VSV-G tag; Amp^R, Gen^R	This study
p-miniTn7-*morA*$^{C\text{-}VSV\text{-}G}$	p-miniTn7-*morA* fused to a downstream VSV-G tag; Amp^R, Gen^R	This study
BACTH assays		
pUT18C	Encodes T18 fragment of *B. pertussis* adenylate cyclase toxin on N-terminus, Amp^R	(14)
pKT25	Encodes T25 fragment of *B. pertussis* adenylate cyclase toxin on N-terminus, Kan^R	(14)
pUT18C::zip	BACTH positive control, pUT18C containing sequence coding for the leucine zipper region of yeast GCN4 protein, Amp^R	(14)
pKT25::zip	BACTH positive control, pKT25 containing sequence coding for the leucine zipper region of yeast GCN4 protein, Kan^R	(14)

pKT25::*pelD*	*P. aeruginosa* PAO1 *pelD* cloned into the XbaI and BamHI sites of pKT25, Kan^R	(15)
pUT18C::*rmcA*	*P. aeruginosa* PAO1 *rmcA* cloned into the XbaI and SmaI sites of pUT18C, Amp^R	This study
pUT18C::*morA*	*P. aeruginosa* PAO1 *morA* cloned into the XbaI and KpnI sites of pUT18C, Amp^R	This study
pBADGr	For expression of third protein in the BACTH assay, *ori araC*-P_{BAD} *dhfr*::Gm^R mob^+, Gen^R	(16)
pBADGr::*pelE*	*P. aeruginosa* PAO1 *pelE* cloned into the EcoRI and HindIII sites of pBADGr with synthetic RBS (GAGGAGGATATTC), Gen^R	(15)
pBADGr::*pelG*	*P. aeruginosa* PAO1 *pelG* cloned into the EcoRI and HindIII sites of pBADGr with synthetic RBS (GAGGAGGATATTC), Gen^R	(15)
pBADGr::*pelEG*	*P. aeruginosa* PAO1 *pelE* and *pelG* ORFs separated by a clean, unmarked deletion of *pelF* cloned into the EcoRI and HindIII sites of pBADGr with synthetic RBS (GAGGAGGATATTC), Gen^R	This study

Abbreviations: Amp, ampicillin; Kan, kanamycin; Gen, gentamicin; Str, streptomycin; Tet, tetracycline

Primers	Sequence*
Allelic exchange vectors	
*rmcA*PAO1upF	GGG **GAA TTC** GAA CCA CGA GAC CGA CGA GA
*rmcA*PAO1upR	*TAC CGG CGC TTC TCG GGG AGC* ACA CAG GAG AAG CAC GGC CTG
*rmcA*PAO1downF	GCT CCC CGA GAA GCG CCG GTA
*rmcA*PAO1downR	AGG **AAG CTT** GGA ATA ACC AAC GGT GCC AGG T
*morA*PAO1upF	AAT **GAG CTC** TCC TAC CTG GAA GCG CTG GA
*morA*PAO1upR	*TCA GCC CTC GTT GAA CAT GAA* CGA CGG GGT TAG CGA GGG GGT
*morA*PAO1downF	TTC ATG TTC AAC GAG GGC TGA
*morA*PAO1downR	GGG **AAG CTT** AGG TAG ACC GCG GCG TTG GC
miniTn7 complementation vectors	
miniTn7-pBAD-F	GGG **AAG CTT** TTA TGA CAA CTT GAC GGC TA
miniTn7-pBAD-R	GGG **GAG CTC** CCA TGG CTG CAG GCG GCC GCC CCG GGC AAA AAA ACG GGT ATG GAG AAA CAG TA
rmcA-miniTn7-F	GAG **CCC GGG *AGG AGG ATA TTC*** ATG CAG CGT CTG CAG GCC GT
rmcA-miniTn7-R	GGG **GCG GCC GC**T TAT ACC GGC GCT TCT CGG GG
morA-miniTn7-F	GGG **GCG GCC GC*A GGA GGA TAT TC***G TGT CGA CCC CCT CGC TAA C
morA-miniTn7-R	GGG **ATG CAT** TCA GCC CTC GTT GAA CAT GAA CA
rmcA-C-VSV-G-F	TGA ATA GAT TAG GAA AAT AAG CGG CCG CCT GCA GC
rmcA-C-VSV-G-R	TTT CAA TAT CTG TAT ATA CCG GCG CTT CTC GGG GA

morA-C-VSV-G-F	ATA GAT TAG GAA AAT GAA TGC ATG ATC GAA TTA GCT TCA A
morA-C-VSV-G-R	TCA TTT CAA TAT CTG TAT AGC CCT CGT TGA ACA TGA ACA G
BACTH vectors	
pelD-BACTH-F	GGG **TCT AGA** GAT GTC CGC GCA CAA GGA TTT CA
pelD-BACTH-R	GTT **GGA TCC** CCT AAA TAG CCA CTT GCT GAT CAT TC
rmcA-BACTH-F	GAT **TCT AGA** GAT GCA GCG TCT GCA GGC CG
rmcA-BACTH-R	GAG **CCC GGG** TTA TAC CGG CGC TTC TCG GG
morA-BACTH-F	GAG **TCT AGA** GGT GTC GAC CCC CTC GCT AA
morA-BACTH-R	GTG **GGT ACC** TCA GCC CTC GTT GAA CAT GAA C
pelE-BADGr-F	GGG **GAA TTC** GAG GAG GAT ATT CAT GAT CAG CAA GTG GCT ATT TAG C
pelE-BADGr-R	GAG **AAG CTT** TCA TGT CCA GTA TCT CGC CAG
pelG-BADGr-F	GGG **GAA TTC** GAG GAG GAT ATT CAT GGC CGG CAT CGG CTTC
pelG-BADGr-R	GAG **AAG CTT** TCA GCG ATT GAG CAT GAA GGT
pelF-del-BADGr-upR	*TCA TGC AAT CTC CGT GGC TT*C CGG AGC GGT GTG TTC GGT CAT
pelF-del-BADGr-downF	GAA GCC ACG GAG ATT GCA TGA
Sequencing primers	
M13F	GTA AAA CGA CGG CCA G
M13R	CAG GAA ACA GCT ATG AC
rmcA-SEQ-F	TGG ACA TGC CTG AGG ACA AG
rmcA-SEQ-R	CGC CTG TTT CAG CAC GTC AAA A

morA-SEQ-F	CGG ACT TCC TCG ACC CG
morA-SEQ-R	CGC CAT GGG CCA GGC T
miniTn7-SEQ-F	GCG GAT CCT ACC TGA CGC TT
miniTn7-SEQ-R	AAC TTC AGA GCG CTT TTG AA
pUT18C-SEQ-F	CTC GCC GGA TGT ACT GGA AAC
pUT18C-SEQ-R	CGG GGC TGG CTT AAC TAT GC
pKT25-SEQ-F	GGC GCG CAG TTC GGT GAC CAG CGG C
pKT25-SEQ-R	GGG ATG TGC TGC AAG GCG ATT AAG
pBADGr-SEQ-F	ATG CCA TAG CAT TTT TAT CC
pBADGr-SEQ-R	GAT TTA ATC TGT ATC AGG
rmcA-SEQ-int1	GGA GAT CGA GCG ACT CCA GG
rmcA-SEQ-int2	CGA GCA GCA GAT CGG CAT CG
rmcA-SEQ-int3	TCA ACC GCG CCT TCA GCG AG
rmcA-SEQ-int4	ATC GCC CTG TTC CCC AAG GA
morA-SEQ-int1	TGC TCG ACG ACA TGC AGA CC
morA-SEQ-int2	TGC GCC AGG CTC TTG TGG AG
morA-SEQ-int3	TGG CAC TGG TTC GAC ATC CG
morA-SEQ-int4	ACC GCC AGG AAG CCA ACC AG

*Restriction sites and Gateway *att* sequences are bolded; regions of complementary to the target amplicon are underlined; regions of reverse complementarity (to facilitate splicing) are italicized; synthetic ribosomal binding sites are in bold italics

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