Colony-morphology screening uncovers a role for the *Pseudomonas aeruginosa* nitrogen-related phosphotransferase system in biofilm formation

Matthew T. Cabeen, Sara A. Leiman and Richard Losick

Supplementary Figure Legends

Figure S1. The *adcA* gene is not required for colony wrinkling and shows induced expression in biofilm mutants. A. Colony morphologies (4 d) of cells with the *amrZ* deletion alone or in combination with an *adcA* deletion.
B. Expression of a P_{adcA}-lux reporter in the listed strains. Luciferase activity was calculated by normalizing luminescence to culture optical density. Results represent the average of 3 biological replicates (each having 4 technical replicates) using cultures grown on M6301-1% agar for 24 or 48 hours. Error bars show the standard deviation. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$ compared to the *amrZ* mutant. Luciferase activity was also assessed at day 3 and 4; the results were comparable to the results shown for day 2.

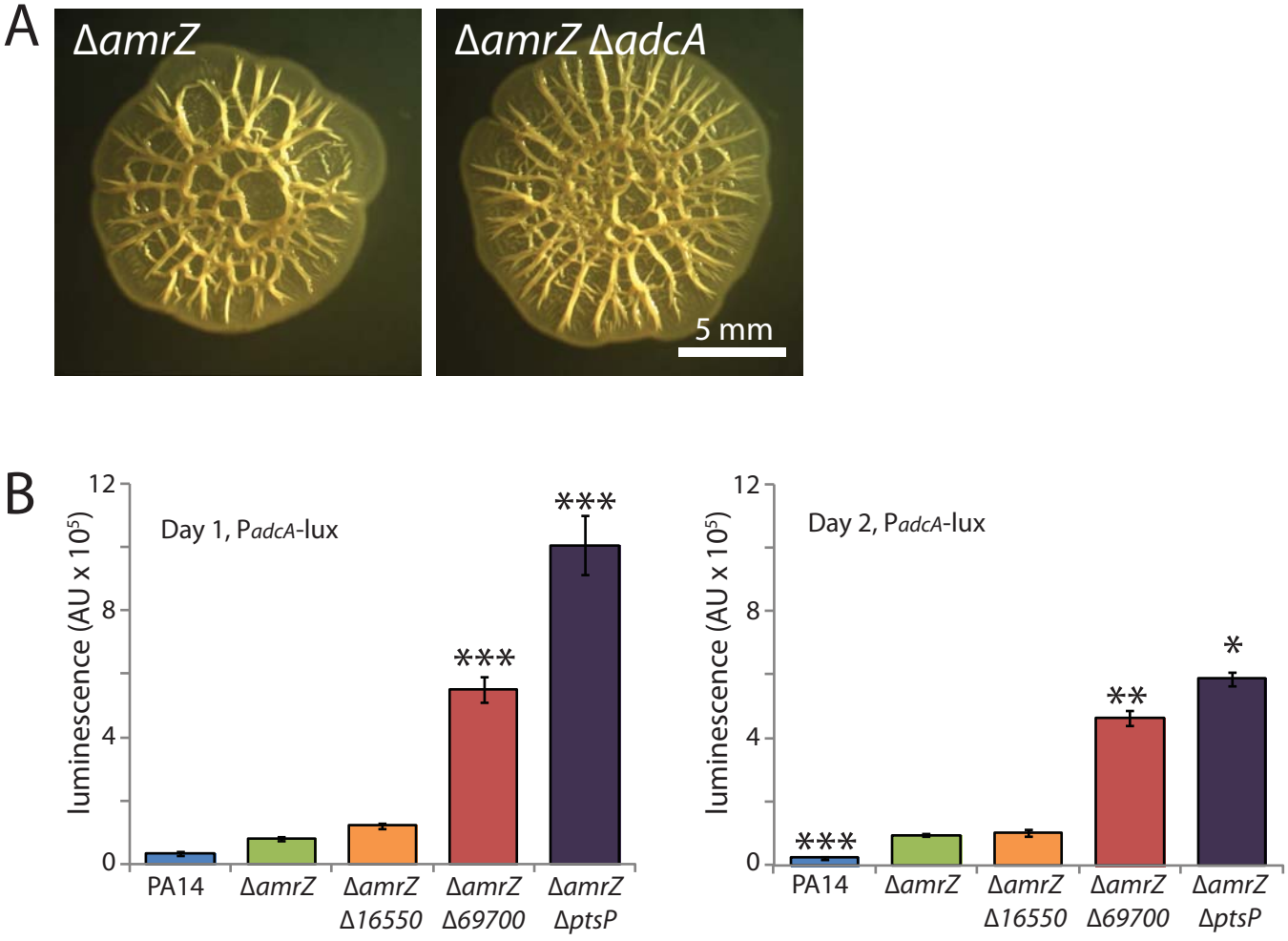
A
ΔamrZ
ΔamrZ ΔadcA
5 mm
B
Day 1, PadcA-lux
luminescence (AU x 10^5)
12
8
4
0
***
***
PA14
ΔamrZ
ΔamrZ Δ16550
ΔamrZ Δ69700
ΔamrZ ΔptsP
Day 2, PadcA-lux
luminescence (AU x 10^5)
12
8
4
0
***
**
*
PA14
ΔamrZ
ΔamrZ Δ16550
ΔamrZ Δ69700
ΔamrZ ΔptsP

Figure S1

Table S1. Summary of recovered transposon mutants

Gene	Colony Wrinkling Phenotype[a]	PA14 gene number	# of independent hits	Comments[b]
Mutants with reduced colony wrinkling compared to parental				
PA14_03370	reduced	03370	1	In operon downstream of a newly annotated open reading frame.
PA14_06640	reduced	06640	1	
PA14_07780	smooth	07780	1	First gene in a 3-gene operon; hit in sense orientation, may read into *PA14_07790* and *djlA*.
PA14_16550	smooth	16550	1	
PA14_20520	smooth	20520	1	Second gene in 2-gene operon with *PA14_20510*.
PA14_21850	reduced	21850	2	Second gene in 2-gene operon with *PA14_21860*.
PA14_24620	reduced	24620	1	
PA14_49880	reduced	49880	1	First gene in 2-gene operon with *PA14_49890*.
PA14_57170	smooth	57170	1	
acpD	reduced	22490	1	
aldG	reduced	33890	1	First gene in 2-gene operon with *PA14_33900*.
algK	smooth	18520	1	Fourth gene in an 8-gene alginate-related operon spanning from *PA14_18580* to *PA14_18470.*
aprA	reduced	48060	1	
argC	smooth	08480	1	First gene in 3-gene operon with *PA14_08490* and *PA14_08500*.
argG	smooth	18740	1	
clpS	reduced	30210	1	First gene in 2-gene operon with *PA14_30230*.
clpX	flatter/shiny	41230	2	
cupA1	smooth	37060	1	First gene in 6-gene *cupA* operon; last gene, *PA14_36990*, encodes an EAL-containing protein that degrades cyclic-di-GMP (Kulasakara *et al.*, 2006).

cupA4	smooth	37010	4	Fourth gene in 6-gene *cupA* operon.
cupA5	smooth	37000	2	Fifth gene in 6-gene *cupA* operon.
cyaB	reduced	22620	1	
cysD	translucent, small wrinkles	57720	1	First gene in 2-gene operon with *PA14_57710*.
cysH	reduced	41840	1	First gene in 2-gene operon with *PA14_41860*.
fadB	reduced	25080	1	First gene in 2-gene operon with *PA14_25090*.
fadD1	smooth	21370	1	Hit in sense orientation at 3' end of gene; may read into *PA14_21380*.
fimV	reduced	23830	1	
glnE	reduced	66270	1	
hisA/67900[c]	reduced	67890	1	Between third and fourth gene in 5-gene operon spanning from *PA14_67930* to *PA14_67880*.
hpd	smooth	53070	2	
kdpD	reduced	43350	1	
lemA (*gacS*)	smooth	52260	2	First gene in 2-gene operon with *PA14_52270*.
leuD	reduced	23760	1	Second gene in 2-gene operon with *PA14_23750*.
mocA/15000[c]	smooth	14990	1	Might overexpress *mocA* and/or knock out *PA14_15000*.
mtlR	flatter/shiny	34440	1	First gene in 7-gene operon spanning to *PA14_34350*.
mutS	reduced	17500	1	
nadB	reduced	54450	3	
nuoD	reduced	29990	1	Second gene in 4-gene operon between *PA14_30010* and *PA14_29970*.
pelA	smooth	24480	5	First gene in 7-gene *pel* operon.
pelB	smooth	24490	1	Second gene in 7-gene *pel* operon.
pelD	smooth	24510	2	Fourth gene in 7-gene *pel* operon.
pelF	smooth	24550	1	Sixth gene in 7-gene *pel*

				operon.
phhR	smooth	52980	2	
phoQ	smooth	49170	1	Hit in the last gene of the 2-gene operon.
phzA1/A2	reduced	09480/39970	1	Sequencing unable to discriminate between the homologous *phzA1* and *phzA2* genes.
pilF	reduced	14850	1	Third gene in 8-gene operon between *PA14_14820* and *PA14_14910*.
pilG/H[c]	reduced	05320/05330	1	Between first 2 genes in 3-gene operon with *PA14_05340*.
pilI	reduced	05340	1	Last gene in 3-gene operon.
pilS	reduced	60250	2	Second gene in 3-gene operon between *PA14_60240* and *PA14_60260*.
pilW	reduced	60290	3	Second gene in 4-gene operon between *PA14_60280* and *PA14_60310*.
pilX	smooth	60300	1	Third gene in 4-gene operon between *PA14_60280* and *PA14_60310*.
pilY1	reduced	60310	12	Fourth gene in 4-gene operon between *PA14_60280* and *PA14_60310*.
potF	smaller and tighter wrinkles	30570	2	Hit in sense orientation near 3' end of gene; may read into *PA14_30560*.
pqsB	reduced	51420	1	Second gene in 5-gene operon between *PA14_51430* and *PA14_51380*.
pqsL	reduced	09700	1	
pqsR	smooth	51340	1	
pstB	reduced /shiny	70810	1	
ptsP	reduced	04410	2	Second gene in 2-gene operon, after *PA14_04390*

				(*nudH*).
pvrR	smooth	59790	2	Second gene in 2-gene operon, after *PA14_59800*.
pvrS	reduced	59800	2	First gene in 2-gene operon, before *PA14_59790*.
pvdL	smooth	33280	1	Second gene in 2-gene operon, after *PA14_33270*.
rfbA	reduced	68200	1	First gene in 2-gene operon, before *PA14_68210*.
secB	reduced	67720	1	
serB	reduced	65560	1	
thrC	smooth/shiny	16090	1	Second gene in 2-gene operon, after *PA14_16070*.
zwf	reduced	23070	1	First gene in 3-gene operon extending to *PA14_23090*.
Mutants with enhanced colony wrinkling compared to parental				
bifA	hyper	56790	1	
bswR	hyper	28130	1	First gene in 2-gene operon, before *PA14_28120*.
cbrA	hyper	62530	1	Second gene in 2-gene operon, after *PA14_62520*.
cbrB	hyper/shiny	62540	1	
hslV	hyper	66770	1	
orfH	hyper	23380	1	
PA14_21290	slight hyper	21290	1	
PA14_28430	hyper	28430	1	Hit in sense orientation near 3' end of gene; may read into *PA14_28420*, which encodes a LysR-family transcriptional regulator.
PA14_28810	hyper	28810	2	Both hits in sense orientation near 3' end of gene; may read into *PA14_28820*.
PA14_41450	hyper	41450	1	May interrupt promoter of operon; *acnB* is downstream gene.
PA14_64320	hyper	64320	1	First gene in 2-gene operon, before *PA14_64335*.
PA14_66100	hyper	66100	1	Second gene in 2-gene operon, after *PA14_66090*.

PA14_69700	hyper	69700	2	Fourth gene in a 6-gene operon between *PA14_69660* and *PA14_69720*.
pcm	hyper	17460	1	Second gene in 2-gene operon, after *PA14_17450*.
pilK/chpA[c]	hyper-wrinkled, hyper spreader	05380/05390	1	Between second and third genes of a 7-gene operon between *PA14_05360* and *PA14_05430*.
pilR	hyper	60260	1	Last gene in 3-gene operon beginning with *PA14_60240*.
recG	hyper	70570	1	Second gene in 2-gene operon, after *PA14_70560*. Hit in sense orientation near 3' end of gene; may read into *PA14_70580*, which is also known as *sadB* and has a known role in biofilm formation (Caiazza & O'Toole, 2004).

Notes:

[a]Colony morphology phenotypes are not discrete but rather form a continuum from perfectly smooth and flat to highly wrinkled, while occasionally displaying other morphological features. Here, we use "smooth" to designate a morphology with no distinguishable wrinkles, "reduced" to designate a morphology with distinguishable wrinkles but with noticeably less wrinkling than the parental strain, and "hyper" to designate a morphology with noticeably more wrinkling than the parental strain. We use other descriptors (e.g., "shiny" and "translucent") to denote additional prominent morphological features.

[b]Transcriptional unit data was taken from (Wurtzel *et al.*, 2012). Genes with no comments are transcribed singly.

[c]The transposon inserted in the intergenic region between the 2 listed genes.

Table S2. *Escherichia coli* strains used in this study.

Strain	Relevant genotype or description	Source or reference
MTC27	SM10 (F- *endA1 hsdR17 supE44 thi-1* λ- *recA1 gyrA96 relA1*); *E. coli* mating strain for conjugation with *P. aeruginosa*	(Simon, 1983)
MTC33	SM10 pBT24 (mating strain for Tn mutagenesis), $Gent^R$	(Kulasekara *et al.*, 2005)
MTC570	SM10 pEXG2-Δ*amrZ*, $Gent^R$	This study
MTC1315	SM10 pEXG2-Δ*bifA*, $Gent^R$	This study
MTC1336	SM10 pEXG2-Δ*16550*, $Gent^R$	This study
MTC1346	SM10 pEXG2-Δ*69700*, $Gent^R$	This study
MTC1348	SM10 pEXG2-Δ*ptsP*, $Gent^R$	This study
MTC1408	SM10 CTX-1-*16550*, Tet^R	This study
MTC1529	SM10 CTX-1-P_{adcA}-lux, Tet^R	This study
MTC1551	SM10 CTX-1-*ptsP*, Tet^R	This study
MTC1552	SM10 pEXG2-Δ*adcA*, $Gent^R$	This study
MTC1595	SM10 pEXG2-$ptsP_{\Delta GAF}$, $Gent^R$	This study
MTC1597	SM10 pEXG2-Δ*ptsO*, $Gent^R$	This study
MTC1632	SM10 pEXG2-Δ*ptsN*, $Gent^R$	This study
MTC1699	SM10 pEXG2-Δ*wspF*, $Gent^R$	This study

Table S3. Plasmids used in this study.

Plasmid	Description	Source or reference
pCTX-1	mini-CTX-1, integrative tet^R plasmid for *P. aeruginosa*	(Hoang *et al.*, 2000)
pCTX-1-lux	CTX-1 reporter construct containing *luxA-E*	(Becher & Schweizer, 2000)
pCTX-1-*16550*	CTX-1 containing the *PA14_16550* gene	This study

pCTX-1-*ptsP*	CTX-1 containing the *ptsP* gene	This study
pCTX-1-P_{adcA}-lux	CTX-1 lux reporter driven from *adcA* promoter	This study
pEXG2	Integrating suicide plasmid for *P. aeruginosa*, $gent^R$, with sucrose counterselection	(Rietsch *et al.*, 2005)
pEXG2-Δ*amrZ*	EXG2 containing flanking sequences of *amrZ*	This study
pEXG2-Δ*bifA*	EXG2 containing flanking sequences of *bifA*	This study
pEXG2-Δ*16550*	EXG2 containing flanking sequences of *PA14_16550*	This study
pEXG2-Δ*69700*	EXG2 containing flanking sequences of *PA14_69700*	This study
pEXG2-Δ*ptsP*	EXG2 containing flanking sequences of *ptsP*	This study
pEXG2-Δ*adcA*	EXG2 containing flanking sequences of *adcA*	This study
pEXG2-$ptsP_{\Delta GAF}$	EXG2 containing flanking sequences of the GAF-encoding domain of *ptsP*	This study
pEXG2-Δ*ptsO*	EXG2 containing flanking sequences of *ptsO*	This study
pEXG2-Δ*ptsN*	EXG2 containing flanking sequences of *ptsN*	This study
pEXG2-Δ*wspF*	EXG2 containing flanking sequences of *wspF*	This study

Table S4. Primers used in this study.

Primer Name or No.	**Sequence (5'-3')**
Rnd1-ARB1	GGCCACGCGTCGACTAGTACNNNNNNNNNNAGAG
Rnd1-ARB2	GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC
Rnd1-ARB3	GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT
Rnd1-TnM20	TATAATGTGTGGAATTGTGAGCGG
Rnd2-ARB	GGCCACGCGTCGACTAGTAC
Rnd2-TnM20	ACAGGAAACAGGACTCTAGAGG
BT20TnMseq	CACCCAGCTTTCTTGTACAC
259	**GGGCTTCGGC** GGTGCCGAGGAAGCGTTCATTG
260	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** CGCTGAGGCGGTTGACCAG
261	**CCTCGGCACC** GCCGAAGCCCACGCGTAG
262	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** GGCATCCGTGGCTCAGCG

547	**TGCGCACCCGTGGAAATTAATTAAGGTACCGAATTC** CGTGGTGAACCTGAACAACCTG
548	**GGGGCCTTCCTTGTTCGTC** GTCGCGCCTGCTTGAGG
549	**GACGAACAAGGAAGGCCCC** CCTCAAGCAGGCGCGAC
550	**TTATACGAGCCGGAAGCATAAATGTAAAGCAAGCTT** GATGCCTGCCTGGTCGAC
573	**GACGCTCAGG** GGTGTTTCCTCCGCTCGC
574	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** CTGGAGCAGCCGCACTTC
575	**AGGAAACACC** CCTGAGCGTCAACCCCG
576	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** GGCCTCGACGAACACGTC
593	**CGAGGAGAAG** AAGCGTCTCGGTTCTCGCG
594	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** CTTCGTTCAGGACAAGCGCC
595	**CGAGACGCTT** CTTCTCCTCGCCGCTGC
596	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** CTTGTTGCCCTTGCCGCG
597	**CCGGGTTGAT** GCTCGGGGCCTTGTCTCC
598	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** TTGCCGGCCCTGTATCCTC
599	**GGCCCCGAGC** ATCAACCCGGCGGCTACC
600	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** GCGCTGCTGCAACTCCAG
607	**TCTAGAACTAGTGGATCCCCCGGGCTGCAG GAATTC** CGCCGCAACAGTCCATGG
608	**CCCCCTCGAGGTCGACGGTATCGAT AAGCTT** CGGGGTTGACGCTCAGG

609	**ATGTCCCAGC** GGAGACAAGGCCCCGAGC
610	**CCCCCTCGAGGTCGACGGTATCGAT AAGCTT** AAGCGCCGCACGACGAAG
611	**CCTTGTCTCC** GCTGGGACATCCTCGAAATCGC
612	**TCTAGAACTAGTGGATCCCCCGGGCTGCAG GAATTC** GGGGTGACTCCATCGGTTGG
641	**GACGGTATCGATAAGCTTGATATC GAATTC** CTGGGCGTCCTGCTGCAC
642	**TTTACCGGCAGATTTCTAAAGAAGAATTGG GGATCC** GACGCGCTTCTTTCGTGGTC
645	**CAAAGAGCCA** GACGCGCTTCTTTCGTGGTC
646	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** CCACCGTGGTGTAGCCGG
647	**GAAGCGCGTC** TGGCTCTTTGTCATCACCCGG
648	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** TGAGGCGGGTCAGGTGCAG
676	**TGCCCAGGCC** CTTGGCGGAGTTCACTTCCTG
677	**CTCCGCCAAG** GGCCTGGGCAAGCTCGG
678	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** ATCACCGTGGGGATGCCC
679	**CAGGCGCATG** AGGGGAGGGGAGATCAGCGG
680	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** AGGCGGGGAATGCTCGTCC
681	**CCCCTCCCCT** CATGCGCCTGATCATCGTCAGC
682	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** CCAGTAGGGGTTCGGCAGGC
683	**GAAACGGTTG** GGGCATCTTGGGGAGTCTGTC

684	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** GACCACCGACACCCGTTCG
685	**CAAGATGCCC** CAACCGTTTCGACGAAGGCGAG
686	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** GCGCATCCTGCTGTTCGTC
723	**ATCGAATACC** GGCATGTCATTGACGATTCCG
724	**TGCGCACCCGTGGAAATTAATTAAGGTACC GAATTC** CTGGTGGTGATGCCTCTCG
725	**ATGACATGCC** GGTATTCGATTAGCCGGGCG
726	**TTATACGAGCCGGAAGCATAAATGTAAAGC AAGCTT** GATAGGCCTCGTCGCGTTG

NB: The listed primer sequences may include 5' overlaps for isothermal assembly and/or stitch PCR (in boldface type). The 3' end is complementary to the target genomic sequence

Modes of strain construction

***P. aeruginosa* strains**

MTC590

MTC1 was mated with MTC570: 50 µL of an overnight LB culture of MTC570 was spot-dried on an LB plate, and 100 µL of an overnight LB culture of MTC1 was subsequently dried on top of the first spot. The plate was incubated at 37°C overnight, and the resulting colony was scraped up with a sterile loop and resuspended in 500 µL sterile phosphate-buffered saline (PBS). An aliquot (typically 100 µL) of the suspension was spread on LB agar containing 75 µg/ml gentamycin and 25 µg/ml irgasan to select for *P. aeruginosa* transformants with integrated pEXG2-Δ*amrZ* plasmid. Several of the resulting colonies were then inoculated into plain LB and grown at 37°C for 4-6 hours to accumulate second crossovers. Aliquots (typically 25 µL and 100 µL) of the LB culture were then spread on LB plates containing 6% sucrose to select against the plasmid. A number of the sucrose-resistant colonies arising were then patched on LB and LB with 20 µg/mL gentamycin. At least 2 sucrose-resistant, gent-sensitive clones were then streaked for single colonies, checked by PCR for presence of the desired deletion, and frozen at -80°C in 25% glycerol.

MTC1240, 1241, 1281, 1284

These strains were isolated from the transposon mutagenesis experiment, and the locations of the transposons were determined by sequencing out from the transposon arm, as described in the Materials and Methods.

MTC1381

Constructed like MTC590, but MTC590 was mated with MTC1336.

MTC1387

Constructed like MTC590, but MTC590 was mated with MTC1348.

MTC1398

Constructed like MTC590, but MTC590 was mated with MTC1346.

MTC1448

Constructed like MTC590, but MTC1 was mated with MTC1336.

MTC1450

Constructed like MTC590, but MTC1 was mated with MTC1348.

MTC1512

Constructed like MTC590, but MTC1 was mated with MTC1346.

MTC1521

Constructed like MTC590, but MTC1 was mated with MTC1315.

MTC1522

Constructed like MTC590, but MTC1381 was mated with MTC1315.

MTC1523

Constructed like MTC590, but MTC1512 was mated with MTC1315.

MTC1525

Constructed like MTC590, but MTC1450 was mated with MTC1315.

MTC1533

MTC1 was mated with MTC1529 as described above for MTC590, and a 10-µL aliquot of the PBS-resuspended mating mix was spread on LB plates with 75 µg/mL tetracycline and 25 µg/mL irgasan to select for *P. aeruginosa* transformants. At least 2 colonies were then re-

streaked for single colonies on LB-tet (25 µg/mL), grown in LB overnight at 37°C, and stored in 25% glycerol at -80°C.

MTC1535

Constructed like MTC1533, but MTC590 was mated with MTC1529.

MTC1537

Constructed like MTC1533, but MTC1381 was mated with MTC1529.

MTC1539

Constructed like MTC1533, but MTC1398 was mated with MTC1529.

MTC1541

Constructed like MTC1533, but MTC1387 was mated with MTC1529.

MTC1543

Constructed like MTC1533, but MTC1387 was mated with MTC1551.

MTC1549

Constructed like MTC1533, but MTC1381 was mated with MTC1408.

MTC1562

Constructed like MTC590, but MTC590 was mated with MTC1552.

MTC1603

Constructed like MTC590, but MTC590 was mated with MTC1597.

MTC1605

Constructed like MTC590, but MTC590 was mated with MTC1595.

MTC1649

Constructed like MTC590, but MTC1 was mated with MTC1632.

MTC1651

Constructed like MTC590, but MTC590 was mated with MTC1632.

MTC1666

Constructed like MTC590, but MTC1651 was mated with MTC1348.

MTC1709

Constructed like MTC590, but MTC1 was mated with MTC1699.

MTC1711

Constructed like MTC590, but MTC1450 was mated with MTC1699.

MTC1713

Constructed like MTC590, but MTC1 was mated with MTC1597.

MTC1714

Constructed like MTC590, but MTC1448 was mated with MTC1699.

MTC1716

Constructed like MTC590, but MTC1512 was mated with MTC1699.

***E. coli* strains**

MTC570, 1315, 1336, 1346, 1348, 1552, 1595, 1597, 1632, 1699

The appropriate pEXG2-derived knockout plasmids (listed in Table S2) were electroporated into SM10 (MTC27), and transformants were selected on LB plates containing 20 µg/mL gentamycin.

MTC1408, 1529, 1551

The appropriate mini-CTX-1 or mini-CTX-1-lux derivatives were electroporated into SM10 (MTC27), and transformants were selected on LB plates containing 25 µg/mL tetracycline.

Modes of plasmid construction

All plasmids constructed in this study were assembled from purified PCR products (using the primers listed in Table S3) and restriction enzyme-cleaved plasmid backbones by using isothermal assembly (Gibson *et al.*, 2009).

pCTX-1-*16550*

The *PA14_16550* gene was PCR-amplified from PA14 chromosomal DNA using primers 607 and 608. The resulting fragment was assembled into EcoRI/HindIII-cleaved mini-CTX-1.

pCTX-1-*ptsP*

The *ptsP* gene was PCR-amplified from PA14 chromosomal DNA using primers 609 and 610, and the upstream promoter was amplified using primers 611 and 612. The promoter was PCR-stitched to the CDS using primers 610 and 612. The resulting fragment was assembled into EcoRI/HindIII-cleaved mini-CTX-1.

pCTX-1-P_{adcA}-lux

The *adcA* promoter was PCR-amplified from PA14 genomic DNA using primers 641 and 642 and assembled into EcoRI/BamHI-cleaved mini-CTX-1.

pEXG2-Δ*amrZ*

The upstream and downstream flanking sequences of the *amrZ* gene were amplified from PA14 chromosomal DNA using primer pairs 259/260 and 261/262, respectively. A fragment containing the *amrZ* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 260 and 262. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*bifA*

The upstream and downstream flanking sequences of most of the *bifA* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 547/548 and 549/550, respectively. A fragment containing the *bifA* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 547 and 550. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*16550*

The upstream and downstream flanking sequences of the *PA14_16550* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 573/574 and 575/576, respectively. A fragment containing the *PA14_16550* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 574 and 576. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*69700*

The upstream and downstream flanking sequences of the *PA14_69700* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 593/594 and 595/596, respectively. A fragment containing the *PA14_69700* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 594 and 596. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*ptsP*

The upstream and downstream flanking sequences of the *ptsP* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 597/598 and 599/600, respectively. A fragment containing the *ptsP* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 598 and 600. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*adcA*

The upstream and downstream flanking sequences of the *adcA* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 645/646 and 647/648, respectively. A fragment containing the *adcA* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 646 and 648. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-*ptsP*$_{\Delta GAF}$

The upstream and downstream flanking sequences of the sequence encoding the GAF domain of PtsP were amplified from PA14 chromosomal DNA using primer pairs 598/676 and 677/678, respectively. A fragment containing the GAF-domain-encoding deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 598 and 678. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*ptsO*

The upstream and downstream flanking sequences of the *ptsO* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 683/684 and 685/686, respectively. A fragment containing the *ptsO* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 684 and 686. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*ptsN*

The upstream and downstream flanking sequences of the *ptsN* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 679/680 and 681/682, respectively. A fragment containing the *ptsN* gene deletion was generated by stitch PCR using the initial fragments as self-priming templates with primers 680 and 682. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

pEXG2-Δ*wspF*

The upstream and downstream flanking sequences of the *wspF* coding sequence were amplified from PA14 chromosomal DNA using primer pairs 723/724 and 725/726, respectively. A fragment containing the *ptsN* gene deletion was generated by stitch PCR using the initial

fragments as self-priming templates with primers 724 and 726. The resulting deletion fragment was assembled into EcoRI/HindIII-cleaved pEXG2.

References

Becher, A. & H. P. Schweizer, (2000) Integration-proficient *Pseudomonas aeruginosa* vectors for isolation of single-copy chromosomal *lacZ* and *lux* gene fusions. *Biotechniques* **29**: 948-950, 952.

Caiazza, N. C. & G. A. O'Toole, (2004) SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14. *J Bacteriol* **186**: 4476-4485.

Gibson, D. G., L. Young, R. Y. Chuang, J. C. Venter, C. A. Hutchison, 3rd & H. O. Smith, (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* **6**: 343-345.

Hoang, T. T., A. J. Kutchma, A. Becher & H. P. Schweizer, (2000) Integration-proficient plasmids for *Pseudomonas aeruginosa*: site-specific integration and use for engineering of reporter and expression strains. *Plasmid* **43**: 59-72.

Kulasakara, H., V. Lee, A. Brencic, N. Liberati, J. Urbach, S. Miyata, D. G. Lee, A. N. Neely, M. Hyodo, Y. Hayakawa, F. M. Ausubel & S. Lory, (2006) Analysis of *Pseudomonas aeruginosa* diguanylate cyclases and phosphodiesterases reveals a role for bis-(3'-5')-cyclic-GMP in virulence. *Proc Natl Acad Sci U S A* **103**: 2839-2844.

Kulasekara, H. D., I. Ventre, B. R. Kulasekara, A. Lazdunski, A. Filloux & S. Lory, (2005) A novel two-component system controls the expression of *Pseudomonas aeruginosa* fimbrial cup genes. *Mol Microbiol* **55**: 368-380.

Rietsch, A., I. Vallet-Gely, S. L. Dove & J. J. Mekalanos, (2005) ExsE, a secreted regulator of type III secretion genes in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* **102**: 8006-8011.

Simon, R., Prieffer, U., and Puhler, A., (1983) A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram-negative bacteria. *Biotechnology (N.Y.)* **1**: 784-790.

Wurtzel, O., D. R. Yoder-Himes, K. Han, A. A. Dandekar, S. Edelheit, E. P. Greenberg, R. Sorek & S. Lory, (2012) The single-nucleotide resolution transcriptome of *Pseudomonas aeruginosa* grown in body temperature. *PLoS Pathog* **8**: e1002945.