

## Supplemental Materials

**Table S1.** Strains, plasmids, and primers used in this study.

Strain or Plasmid	Relevant Characteristics	Reference/Source
PAO1	Wildtype <i>P. aeruginosa</i> strain	Held et al., 2012 [49]
PAO1-AzEvB8	PAO1 strain with <i>mexR</i> E118* and 20kb deletion ( <i>mexF-antC</i> ) mutations	Jorth et al., 2017 [20]
PAO1-AzEvB8 $\Delta$ <i>mexAB</i>	PAO1-AzEvB8 deletion of <i>mexAB</i> genes	This study
PAO1 $\Delta$ <i>mexR</i>	PAO1 strain with a complete deletion of the <i>mexR</i> gene	This study
PAO1 $\Delta$ <i>mexAB</i>	PAO1 strain with a complete deletion of the <i>mexA</i> and <i>mexB</i> genes	This study
PAO1 $\Delta$ <i>mexEF</i> (RP05)	PAO1 strain with a complete deletion of the <i>mexE</i> and <i>mexF</i> genes	This study
PAO1 <i>mexE</i> Tn mutant	PAO1 Two-Allele transposon library strain PW5180; <i>mexEH04::ISphoA/hah</i>	Held et al., 2012 [49]
PAO1 <i>mexF</i> Tn mutant	PAO1 Two-Allele transposon library strain PW5184; <i>mexFG01::ISphoA/hah</i>	Held et al., 2012 [49]
PAO1 <i>oprN</i> Tn mutant	PAO1 Two-Allele transposon library strain PW5186; <i>oprNC04::ISphoA/hah</i>	Held et al., 2012 [49]
PAO1 <i>mexR</i> Tn mutant	PAO1 Two-Allele transposon library strain PW9404 <i>mexRG01::ISphoA/hah</i>	Held et al., 2012 [49]
PAO1 PA3303 Tn mutant	PAO1 Two-Allele transposon library strain PW6550; PA3303H03: <i>ISphoA/hah</i>	Held et al., 2012 [49]
PAO1-AzEvB8 <i>rhlA::GFP</i>	PAO1-AzEvB8 with <i>rhlA</i> GFP transcriptional reporter at <i>attB</i> site	This study
PAO1 $\Delta$ <i>mexR</i> <i>attB::rhlA-gfp</i>	PAO1 $\Delta$ <i>mexR</i> with <i>rhlA</i> GFP transcriptional reporter at <i>attB</i> site	This study
PAO1 $\Delta$ <i>mexAB</i> <i>attB::rhlA-gfp</i>	PAO1 $\Delta$ <i>mexAB</i> with <i>rhlA</i> GFP transcriptional reporter at <i>attB</i> site	This study
PAO1 $\Delta$ <i>mexEF</i> <i>attB::rhlA-gfp</i>	PAO1 $\Delta$ <i>mexEF</i> with <i>rhlA</i> GFP transcriptional reporter at <i>attB</i> site	This study
<i>E. coli</i> DH5 $\alpha$	<i>E. coli</i> strain used for cloning	Taylor et al., 1993 [52]
<i>E. coli</i> DH5 $\alpha$ pEX18Gm	<i>E. coli</i> cloning strain used to propagate pEX18Gm	Gift from Pradeep K. Singh Lab
<i>E. coli</i> DH5 $\alpha$ pEX18Gm:: $\Delta$ <i>mexAB</i>	Suicide plasmid carrying $\Delta$ <i>mexAB</i> deletion construct	This study
<i>E. coli</i> DH5 $\alpha$ pEX18Gm:: $\Delta$ <i>mexEF</i>	Suicide plasmid carrying $\Delta$ <i>mexEF</i> deletion construct	This study
<i>E. coli</i> DH5 $\alpha$ pEX18Gm:: $\Delta$ <i>mexR</i>	Suicide plasmid carrying $\Delta$ <i>mexR</i> deletion construct	This study
<i>E. coli</i> SM10( $\lambda$ pir)	<i>E. coli</i> mating strain	Chung et al., 1989 [54]

<i>E. coli</i> SM10(λpir) pEX18Gm::ΔmexR	<i>E. coli</i> mating strain used to transform pEX18Gm::ΔmexR into <i>P. aeruginosa</i>	This study
<i>E. coli</i> SM10(λpir) pYL122	<i>E. coli</i> mating strain carrying pYL122 with rhlA-gfp promoter fusion	Lequette and Greenberg, 2005 [55]
pYL122	Plasmid containing a rhlA-gfp promoter fusion in a mini-CTX-lacZ backbone	Lequette and Greenberg, 2005 [55]
<i>E. coli</i> DH5α pEX18Gm::ΔmexEF (RP29)	pUC18-miniTn7T2.1-Gm-GW bearing ΔmexEF, Gm <sup>r</sup>	This study
<i>E. coli</i> S.17.1 (λ <sub>pir</sub> ) pEX18Gm::ΔmexEF (RP12)	pUC18-miniTn7T2.1-Gm-GW bearing ΔmexEF, Gm <sup>r</sup>	This study
pEX18Gm	Suicide plasmid for generating <i>P. aeruginosa</i> mutants	Hoang et al., 1998 [51]
pEX18Gm::ΔmexAB	Suicide plasmid carrying ΔmexAB deletion construct	This study
pDONRPEX18Gm::ΔmexEF (pRP12)	Suicide plasmid carrying ΔmexEF deletion construct	This study
pEX18Gm::ΔmexR	Suicide plasmid carrying ΔmexR deletion construct	This study

Primers	Sequence 5'-3'
mexAB-KO-UP-F	ACAAGCACCTGCGCAGCG
mexAB-KO-UP-R	CATAGCGTTGTCCCTCATGAGCG
mexAB-KO-DN-F	GAAAAGGGCAATGATATGAAAC
mexAB-KO-DN-R	GTCGAACAGGCCGGACAG
mexAB-KO-Chk-F	GTGTACTGGTCCGGCCCT
mexAB-KO-Chk-R	CAGCCGGACAGAACGACAG
pEX18Gm-F	CTGGCCGTCGTTTACAAC
pEX18Gm-R	TCATGGTCATAGCTGTTTC
mexR-KO-UP-F	gtaaaacgacggccagTCTGCCTTCCAGGGTCAC
mexR-KO-UP-R	aatatcctcGGGTAGTCATTGGTTGGC
mexR-KO-DN-F	actaccccGAGGATATTAAAGAACATTCTTCGAAGC
mexR-KO-DN-R	aacagctatgaccatgaCACCAACAGCGTGGACAC

oRP_21(PAO1_mexEFupF01)	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CGC AAG CGC AAG GTG GTC C
oRP_22(PAO1_mexEFupR01)	CTC TGG CAG GCC TTT GTC GTT GGC GGG TAG CGC CAG GAG AAG TG
oRP_23(PAO1_mexEFdownF01)	CGC CAA CGA CAA AGG CCT G
oRP_24(PAO1_mexEFdownR01)	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTC CAG CTG ACG GCG GAT G
oRP_27(PAO1_mexEFseqF01)	CAA GCG CAA GGT GGT CCT G
oRP_28(PAO1_mexEFseqR01)	CGA ACA GGT CAA GCT CCC AG
pYL122-chk-F	TCAGCGGGGTTCACTACG
pYL122-chk-R	CGATAGAGTTGACAGTGTGTTGC

Note *attB1* and *attB2* recombination sites are highlighted in grey