**Supplemental materials**

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

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| **Strain or Plasmid** | **Relevant Characteristics** | **Reference/Source** |
| PAO1 | Wildtype *P. aeruginosa* strain | Held et al., 2012 |
| PAO1-AzEvB8 | PAO1 strain with *mexR* E118\* and 20kb deletion (*mexF - antC*) mutations | Jorth *et al.,* 2017 |
| PAO1-AzEvB8 Δ*mexAB* | PAO1-AzEvB8 deletion of *mexAB* genes | This study |
| PAO1 Δ*mexR* | PAO1 strain with a complete deletion of the *mexR* gene | This study |
| PAO1 Δ*mexAB* | PAO1 strain with a complete deletion of the *mexA* and *mexB* genes | This study |
| PAO1 Δ*mexEF* (RP05) | PAO1 strain with a complete deletion of the *mexE* and *mexF* genes | This study |
| PAO1-AzEvB8 *rhlA*::GFP | PAO1-AzEvB8 with *rhlA* GFP transcriptional reporter at *attB* site | This study |
| PAO1 Δ*mexR* *attB*::*rhlA-gfp* | PAO1 Δ*mexR* with *rhlA* GFP transcriptional reporter at *attB* site | This study |
| PAO1 Δ*mexAB* *attB::rhlA-gfp* | PAO1 Δ*mexAB* with *rhlA* GFP transcriptional reporter at *attB* site | This study |
| PAO1 Δ*mexEF* *attB::rhlA-gfp* | PAO1 Δ*mexEF* with *rhlA* GFP transcriptional reporter at *attB* site | This study |
| *E. coli* DH5ɑ | *E. coli* strain used for cloning | Taylor et al., 1993 |
| *E. coli* DH5ɑpEX18Gm | E. coli cloning strain used to propagate pEX18Gm | Gift from Pradeep K. Singh Lab |
| *E. coli* DH5ɑpEX18Gm*::ΔmexAB* | Suicide plasmid carrying Δ*mexAB* deletion construct | This study |
| *E. coli* DH5ɑpEX18Gm*::ΔmexEF* | Suicide plasmid carrying Δ*mexEF* deletion construct | This study |
| *E. coli* DH5ɑpEX18Gm*::ΔmexR* | Suicide plasmid carrying Δ*mexR* deletion construct | This study |
| *E. coli* SM10(λpir) | *E. coli* mating strain | Chung et al., 1989 |
| *E. coli* SM10(λpir)pEX18Gm*::ΔmexR* | *E. coli* mating strain used to transform *pEX18Gm::ΔmexR* into *P. aeruginosa* | This study |
| *E. coli* SM10(λpir) pYL122 | E. coli mating strain carrying pYL122 with *rhlA-gfp* promoter fusion | Lequette and Greenberg, 2005 |
| pYL122 | Plasmid containing a *rhlA*-*gfp* promoter fusion in a mini-CTX-*lacZ* backbone | Lequette and Greenberg, 2005 |
| *E. coli* DH5ɑpEX18Gm*::ΔmexEF* (RP29) | pUC18-miniTn7T2.1-Gm-GW bearing Δ*mexEF*, Gmr  | This study |
| *E. coli* S.17.1 (λpir) pEX18Gm*::ΔmexEF* (RP12) | pUC18-miniTn7T2.1-Gm-GW bearing Δ*mexEF*, Gmr | This study |
| pEX18Gm | Suicide plasmid for generating *P. aeruginosa* mutants | Hoang et al., 1998 |
| pEX18Gm::Δ*mexAB* | Suicide plasmid carrying Δ*mexAB* deletion construct | This study |
| pDONRPEX18Gm::Δ*mexEF* (pRP12) | Suicide plasmid carrying Δ*mexEF* deletion construct | This study |
| pEX18Gm::Δ*mexR* | Suicide plasmid carrying Δ*mexR* deletion construct | This study |

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| **Primers** | **Sequence 5'-3'** |
| mexAB-KO-UP-F | ACAAGCACCTGCGCAGCG |
| mexAB-KO-UP-R | CATAGCGTTGTCCTCATGAGCG |
| mexAB-KO-DN-F | GAAAAGGGGCAATGATATGAAAC |
| mexAB-KO-DN-R | GTCGAACAGGCCGGACAG |
| mexAB-KO-Chk-F | GTGTACTGGTTCCGGCCCT |
| mexAB-KO-Chk-R | CAGCCGGACAGAACGACAG |
| pEX18Gm-F | CTGGCCGTCGTTTTACAAC |
| pEX18Gm-R | TCATGGTCATAGCTGTTTC |
| mexR-KO-UP-F | gtaaaacgacggccagTCTGCGCTTCCAGGGTCAC |
| mexR-KO-UP-R | aatatcctcGGGGTAGTTCATTGGTTTGGC |
| mexR-KO-DN-F | actaccccGAGGATATTTAAGAACATTCTTTTCGAAGC |
| 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 G***GG 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 | TCAGCGGGGTTCAGTACG |
| pYL122-chk-R | CGATAGAGTTTGACAGTGTGTTGC |

Note *attB*1 and *attB*2 recombination sites are highlighted in grey