**Supplemental materials**

Table S1 Strains and plasmids used in this study

| Strain or plasmid | Relevant characteristics | Reference |
|---|---|---|
| *P. aeruginosa* strains | | |
| PAO1 | Wild type | ATCC |
| PAO1-Sm | Spontaneous $Sm^r$ mutant of PAO1 | (1) |
| PAO1ΔT | *mvaT*::Gmr mutant of PAO1 | (2) |
| PAO1ΔU | *mvaU*::Tetr mutant of PAO1 | (2) |
| PAO1ΔTΔU | *mvaT*::Gmr *mvaU*::Tetr mutant of PAO1 | (2) |
| PAO1-pUCPT | Carrying complementation plasmid for *mvaT* gene | (2) |
| PAO1-pUCPU | Carrying complementation plasmid for *mvaU* gene | (2) |
| ΔT- pUCPT | Carrying complementation plasmid for *mvaT* gene | (2) |
| ΔT-pUCPU | Carrying complementation plasmid for *mvaU* gene | (2) |
| ΔU- pUCPT | Carrying complementation plasmid for *mvaT* gene | (2) |
| ΔU- pUCPU | Carrying complementation plasmid for *mvaU* gene | (2) |
| ΔTΔU-pUCPT | Carrying complementation plasmid for *mvaT* gene | (2) |
| ΔTΔU-- pUCPU | Carrying complementation plasmid for *mvaU* gene | (2) |
| Plasmids | | |
| pUCP 18 | *E. coli-P. aeruginosa* shuttle vector | (3) |
| pUCP-T | Complementation plasmid for *mvaT* gene, derived from pUCP18 | (2) |
| pUCP-U | Complementation plasmid for *mvaU* gene, derived from pUCP18 | (2) |

Table S2 Primers used in this study

| Gene name | Type | Oligonucleotide sequence (5'-3') | Source or reference |
|---|---|---|---|
| Primers for knockout of *mvaT* and *mvaU* genes | | | |
| up *mvaT* | Fw | CATAAGCTTCGCGGGCGATCGGGGCGA AAG | (2) |
| | Rev | ATCGAATTCTTGTCTGTGCTGAGTGGCGGT | |
| down *mvaT* | Fw | ATCGAATTCACGAAGAACGCCAGCCCAGTG | (2) |
| | Rev | CATGGATCCGATGTCCGCGCCACCATTGCC | |
| up *mvaU* | Fw | CCCAAGCTTCAAGGCGATCTTCAAGCCGATCTA | (2) |
| | Rev | CGGAATTCTTCGTTTCACTCCCGTTCTAAAAA | |

| | | | |
|---|---|---|---|
| down *mvaU* | Fw | CGGAATTCAGCCGGTTTTCCCGACGGCATCCT | (2) |
| | Rev | CGGGATCCCCGCCATTGTCGCATTCGCGCAGC | |
| Primers for detection of *mvaT* and *mvaU* genes | | | |
| *mvaT* | Fw | CATGGATCCAGCACAGACAAGGTACCTGAC | (2) |
| *mvaT* | Rev | ATCGGATCCTTAGCCGAGCAGGGTGGCCCA | |
| *mvaU* | Fw | GGATCCTTTAGAACGGGAGTGAAACGAATG | (2) |
| *mvaU* | Rev | GGATCCTTAGCGTTGCAGCCAGGATTCGAC | |
| Primers for construction of *lacZ* transcriptional fusion plasmids | | | |
| up *phzA1* | Fw | CATGCCATGGCCTGTTCCAGAGCCTTT | This study |
| | Rev | CGGGATCCAGAGGGCTCTCCAGGTAT | |
| up *phzA2* | Fw | CATGCCATGGCATCGGCCTGCTCAACTGAAT | This study |
| | Rev | CGGGATCCTGCGAATCTCCGCCAGTTCGAAT | |
| up *phzH* | Fw | CATGCCATGGTCGAACGTTGCCACGAAATC | This study |
| | Rev | CGGGATCCAGGGAAACTCCTATAATTG | |
| up *phzM* | Fw | CATGCCATGGTCATCCCGGGTTTCTTT | This study |
| | Rev | CGGGATCCCTTTTATTCTCTCTCGTTAC | |
| up *phzS* | Fw | CATGCCATGGTACCAGACCCACCCGATGT | This study |
| | Rev | CGGGATCCGGGTGCTTCCTTTTCTCGAGT | |
| up *rhlR* | Fw | CATGCCATGGTGCAGTAAGCCCTGATCGAT | This study |
| | Rev | CGGGATCCACGGTGCTGGCATAACAGATA | |
| up *lasR* | Fw | CATGCCATGGCCGAACTGGAAAAGTGGCTAT | This study |
| | Rev | CGGGATCCAGCGCTACGTTCTTCTTAAAC | |
| up *pqsR* | Fw | CATGCCATGGCCCTTATTCCTTTTATTGGG | This study |
| | Rev | CGGGATCCCAAGGCCGCGGATTCTAAC | |
| Primers for Real-Time PCR | | | |
| *oprL* | Fw | CCAACAGCGGTGCCGTTGA | |
| | Rev | GCCATATTGTACTCGCGGGT | |
| *phzA1* | Fw | AACGGTCAGCGGTACAGGGAAAC | This study |
| | Rev | ACGAACAGGCTGTGCCGCTGTAAC | |
| *phzA2* | Fw | CTGTAACCGTTCGGCCCCCTTCATG | This study |
| | Rev | ATGCGAGAGTACCAACGGTTGAAAG | |
| *phzH* | Fw | GCTCATCGACAATGCCGAACT | This study |
| | Rev | GCGGATCTCGCCGAACATCAG | |
| *phzM* | Fw | AGCAACCTGGCATTCCACGAG | This study |

| | | | |
|---|---|---|---|
| | Rev | TGCAGGATGGCCTTGGTCAATT | |
| *phzS* | Fw | CCGAAGGCAAGTCGCTGGTGA | This study |
| | Rev | GGTCCCAGTCGGCGAAGAACG | |
| *lasI* | Fw | ATGATCGTACAAATTGGTCGGCGCG | This study |
| | Rev | CGCTCCTTGAACACTTGAG | |
| *lasR* | Fw | CTGTGGATGCTCAAGGACTAC | (4) |
| | Rev | AACTGGTCTTGCCGATGG | |
| *rhlI* | Fw | CGGCATCAGGTCTTCATCG | (4) |
| | Rev | GTAGCGGGTTTGCGGATG | |
| *rhlR* | Fw | CGGTCTGCCTGAGCCATC | (4) |
| | Rev | GCCAGCGTCTTGTTCGG | |
| *pqsA* | Fw | GACCGGCTGTATTCGATTC | (4) |
| | Rev | GCTGAACCAGGGAAAGAAC | |
| *pqsR* | Fw | ATCGACGAGGAACTGAAGA | (4) |
| | Rev | CTGATCTGCCGGTAATTGG | |
| *pqsH* | Fw | GCGCGGATCGAGTTCATC | (5) |
| | Rev | CAGGGCGATTCCCACTGA | |
| *pqsE* | Fw | GGATGCCGAATTGGTTTG | This study |
| | Rev | GGTCGTAGTGCTTGTGGG | |
| *qsrO* | Fw | ATGCTTACGTTTTGGGCTAT | This study |
| | Rev | ATGGAAATGGATTCTTTTGAGTT | |

Fig S1

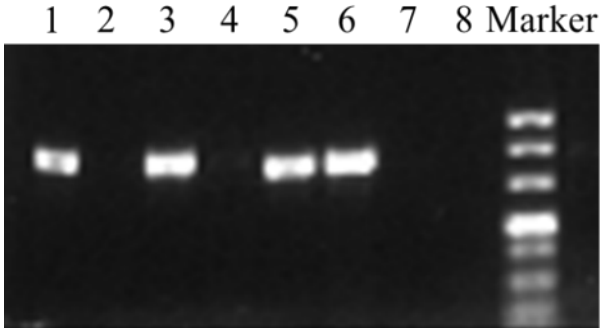


**Fig S1 Agarose gel electrophoresis of *mvaT* and *mvaU* PCR products in PAO1 and the relevant knockout mutants.** 1-4: Detection of *mvaT* gene, 5-8: Detection of *mvaU* gene. 1. PAO1, 2. PAO1ΔT, 3. PAO1ΔU, 4. PAO1ΔTΔU, 5. PAO1, 6. PAO1ΔT, 7.

PAO1ΔU, 8. PAO1ΔTΔU. Marker: DL500 (500 bp, 400 bp, 300 bp, 200 bp, 150 bp, 100 bp, 50 bp).

Fig S2

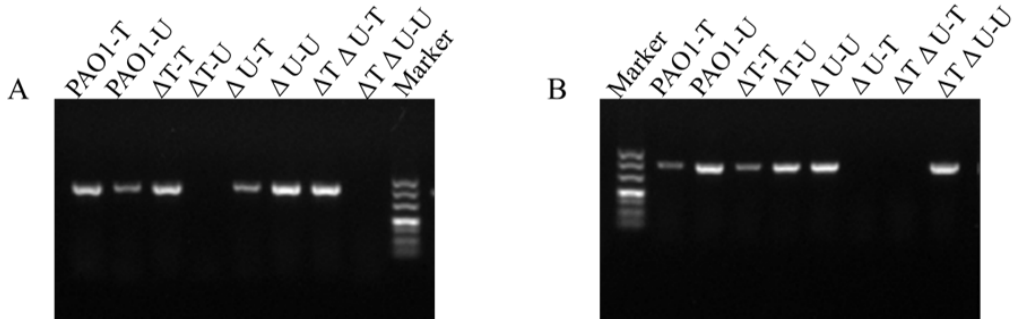


**Fig S2 Agarose gel electrophoresis of *mvaT* and *mvaU* PCR products in PAO1 and the knockout mutants after plasmid complementation.** A: Agarose gel electrophoresis images of *mvaT*. B: Agarose gel electrophoresis images of *mvaU*. Marker: 500 bp, 400 bp, 300 bp, 200 bp, 150 bp, 100 bp, 50 bp.

Fig S3

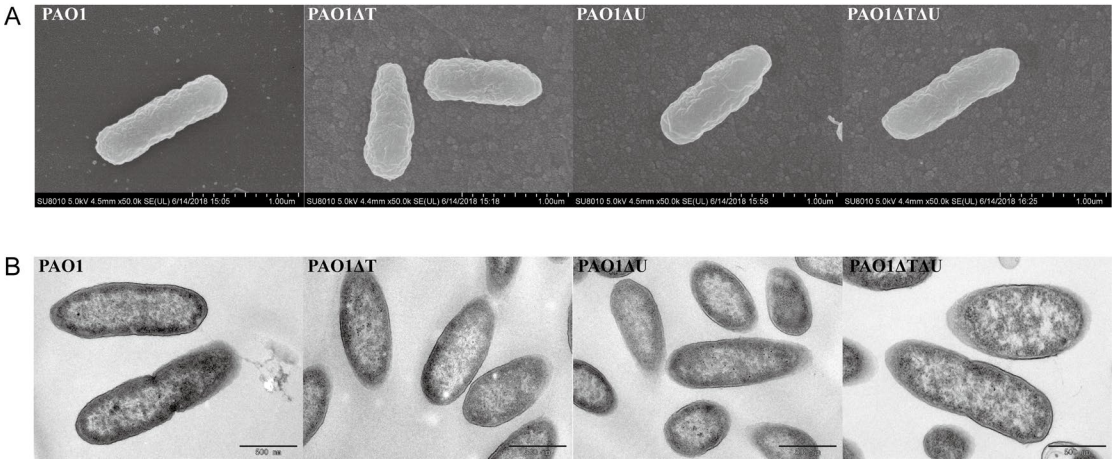


**Fig S3 Images from SEM (A) and TEM (B) for PAO1 wild type and the *mvaT mvaU* knockout mutants.** 1 mL of overnight cultures were centrifuged at 6000×g for 3 min and supernatants were discarded. Bacterial pellets were resuspended and washed in 1 mL 2.5% glutaraldehyde in phosphate-buffered saline (PBS). Tubes were fixed overnight at 4℃ and fixatives were removed by centrifuged again at 6000×g for 3 min.

SEM was conducted by using a scanning electron microscope (FEI Quanta 2000), and TEM was conducted by using a transmission electron microscope (JEOL JEM-1200EX).

Fig S4

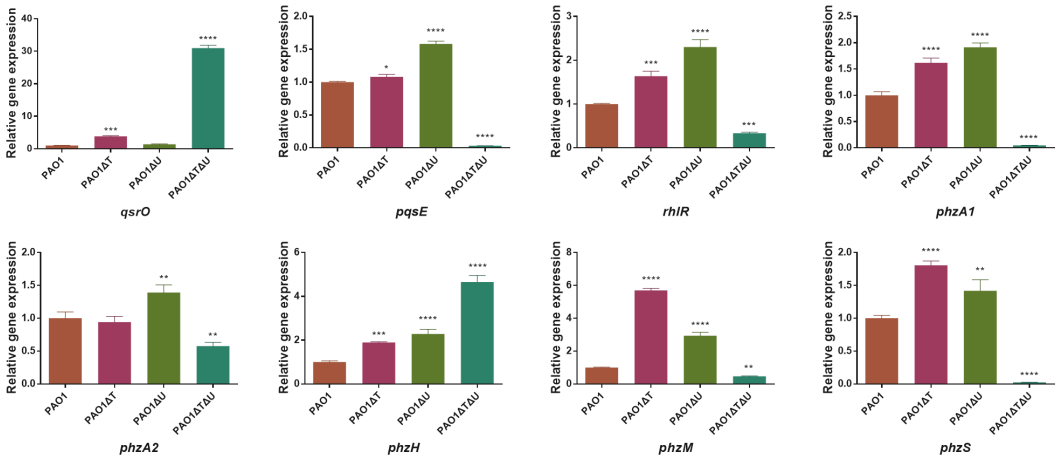


**Fig S4. Effect of *mvaT mvaU* knockout mutation on representative gene expression in early stationary phase detected by Real -Time PCR.** Data was calculated with one-way ANOVA and Bonferroni's multiple comparisons, in comparison with *P. aeruginosa* PAO1, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Reference


1. Itoh Y. 1997. Cloning and characterization of the aru genes encoding enzymes of the catabolic arginine succinyltransferase pathway in Pseudomonas aeruginosa. J Bacteriol 179:7280-90.
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pUC18/19. Gene 97:109-21.

4. El-Mowafy SA, Abd El Galil KH, El-Messery SM, Shaaban MI. 2014. Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in Pseudomonas aeruginosa. Microb Pathog 74:25-32.

5. Viducic D, Murakami K, Amoh T, Ono T, Miyake Y. 2016. RpoN Modulates Carbapenem Tolerance in Pseudomonas aeruginosa through Pseudomonas Quinolone Signal and PqsE. Antimicrob Agents Chemother 60:5752-64.