**Supplemental Materials**

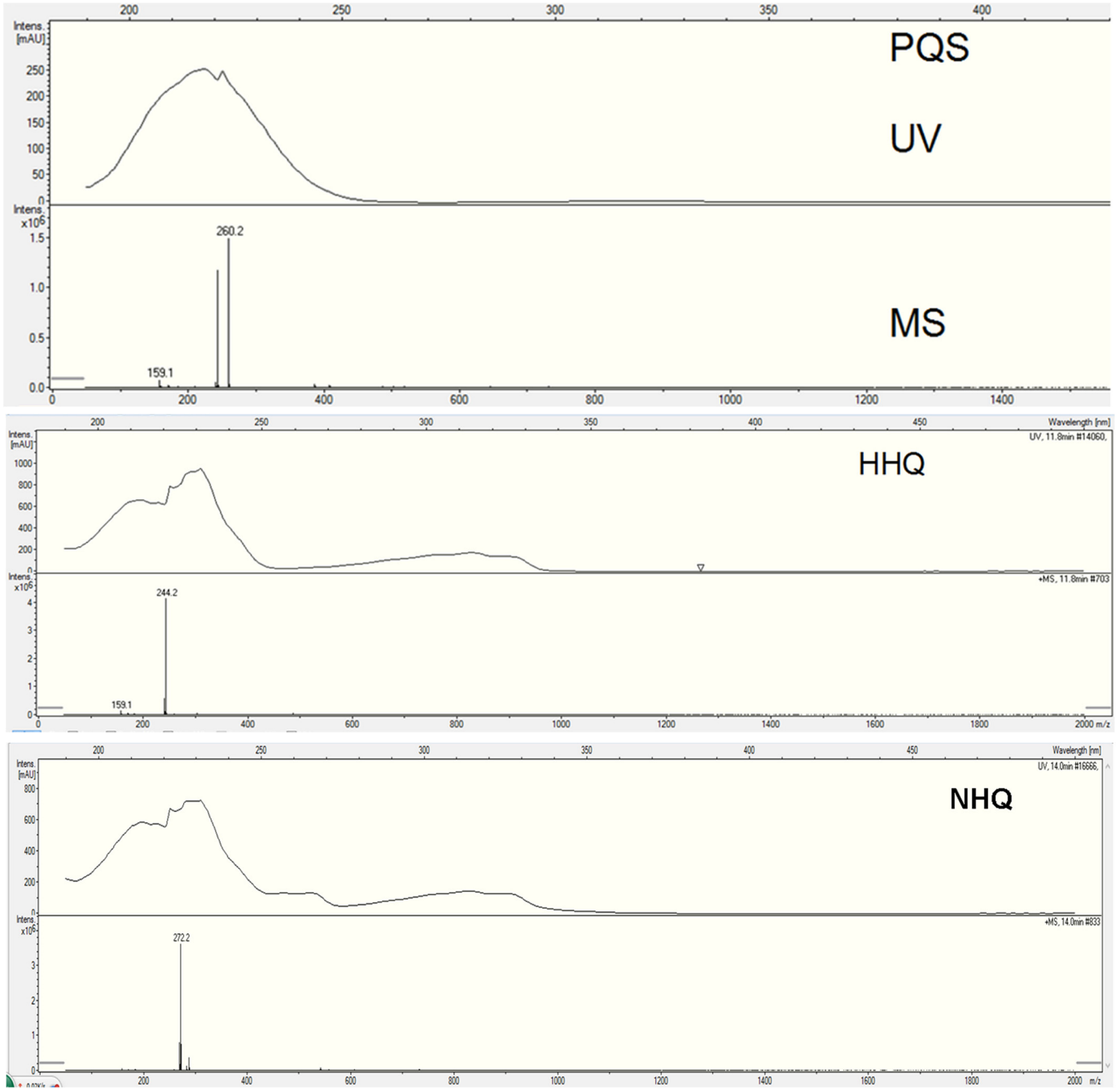



**Figure S1.** Mass spectra of PQS, HHQ, and NHQ. The bacteria were cultured in TSB medium until the $OD_{600}$ reached 3.5. The secondary metabolites were extracted using ethyl acetate, which has a moderate polarity and low boiling point. Then, 5 mL of the bacterial culture solution was extracted three times using the same volume of acidified ethyl acetate (containing 0.01% glacial acetic acid), and the extract was dried using a centrifugal concentrator. The extract was analyzed via ultra-performance liquid chromatography (UPLC) (Waters ACQUITY, USA) using a coupled MicroTOF-MS system (Bruker Daltonics GmbH, Bremen, Germany). A 2.1 mm × 150 mm reversed phase chromatographic column (Waters, BEH C18,1.7 μm, USA) with a flow rate of 250 μL $min^{-1}$ was used.

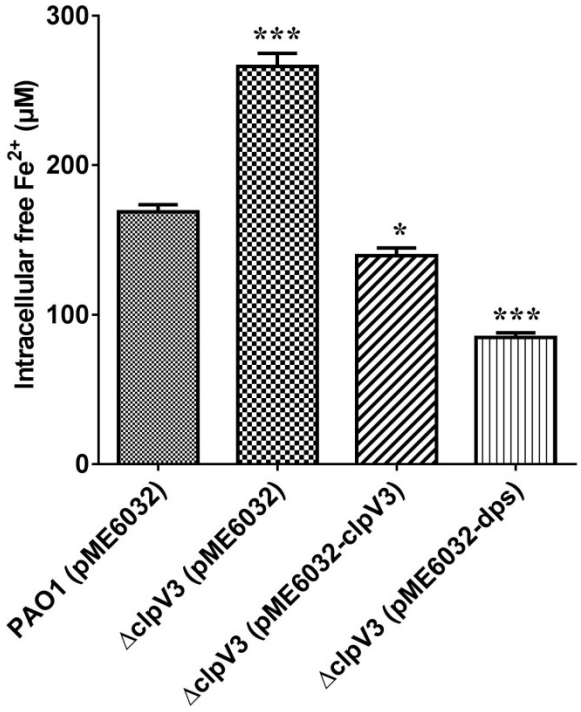



**Figure S2.** Intracellular free $Fe^{2+}$ concentration of *P. aeruginosa* strains. The strains were cultured in TSB medium, and the intracellular free $Fe^{2+}$ was determined via whole-cell EPR analysis. The data shown are the average of three independent experiments; and the error bars indicate the SD of the three independent experiments. * denotes $P<0.05$, and *** denotes $P<0.001$.

**Table S1.** Bacterial strains and plasmids used in this study.

| Strains and Plasmids | Relevant Characteristics * | Source |
|---|---|---|
| Strains | | |
| *P. aeruginosa* | | |
| PAO1 | Wild-type | Laboratory collection |
| Δ*clpV3* | *clpV3* deletion mutant in PAO1 | This study |
| Δ*hsiB3C3* | *hsiB3*/*hsiC3* double deletion mutant in PAO1 | This study |
| Δ*hcp3* | *hcp3* deletion mutant in PAO1 | This study |
| Δ*icmF3* | *icmF3* deletion mutant in PAO1 | This study |
| Δ*tseF* | *tseF* deletion mutant in PAO1 | This study |
| Δ*icmF1* | *icmF1* deletion mutant in PAO1 | This study |
| Δ*clpV1* | *clpV1* deletion mutant in PAO1 | This study |
| Δ*icmF2* | *icmF2* deletion mutant in PAO1 | This study |
| Δ*clpV2* | *clpV2* deletion mutant in PAO1 | This study |
| Δ*pqsA* | *pqsA* deletion mutant in PAO1 | This study |
| Δ*pqsH* | *pqsH* deletion mutant in PAO1 | This study |
| Δ*oxyR* | *oxyR* deletion mutant in PAO1 | This study |
| Δ*rpoS* | *rpoS* deletion mutant in PAO1 | This study |
| Δ*clpV3*Δ*pqsH* | *clpV3*/*pqsH* double deletion mutant in PAO1 | This study |
| Δ*hsiB3C3*Δ*pqsH* | *hsiB3*/*hsiC3*/*pqsH* triple deletion mutant in PAO1 | This study |
| Δ*icmF3*Δ*pqsH* | *icmF3*/*pqsH* double deletion mutant in PAO1 | This study |
| Δ*tseF*Δ*pqsH* | *tseF*/*pqsH* double deletion mutant in PAO1 | This study |
| PAO1-V | PAO1 with pME6032 | This study |
| PAO1-*ahpCF* | PAO1 with pME6032-*ahpCF* | This study |
| PAO1-*dps* | PAO1 with pME6032-*dps* | This study |
| Δ*tseF*-V | Δ*tseF* with pME6032 | This study |
| Δ*tseF*-*tseF* | Δ*tseF* with pME6032-*tseF* | This study |
| Δ*clpV3*-V | Δ*clpV3* with pME6032 | This study |
| Δ*clpV3*-*clpV3* | Δ*clpV3* with pME6032-*clpV3* | This study |
| Δ*icmF3*-V | Δ*icmF3* with pME6032 | This study |
| Δ*icmF3*-*icmF3* | Δ*icmF3* with pME6032-*icmF3* | This study |
| Δ*hsiB3C3*-V | Δ*hsiB3C3* with pME6032 | This study |

| | | |
|---|---|---|
| Δ*hsiB3C3*-*hsiB3C3* | Δ*hsiB3C3* with pME6032-*hsiB3C3* | This study |
| Δ*rpoS* (pME6032) | Δ*rpoS* with pME6032 | This study |
| Δ*rpoS* (pME6032-*rpoS*) | Δ*rpoS* with pME6032-*rpoS* | This study |
| Δ*clpV3*(pME6032-*ahpCF*) | Δ*clpV3* with pME6032-*ahpCF* | This study |
| Δ*clpV3* (pME6032-*dps*) | Δ*clpV3* with pME6032-*dps* | This study |
| *E. coli* | | |
| DH5α | FΦ80Δ*lacZ*ΔM15/Δ(*lacZYA-argF*)*U169recA1 endA1 hsdR17* | Laboratory collection |
| S17-1 | F$^-$*thi pro hsdR* [RP4-2 Tc::Mu Km::Tn7 (Tp Sm)] | Laboratory collection |
| Plasmids | | |
| p34s-Gm | Amp$^r$; Gm resistant cassette carrying vector | [70] |
| pK18*mobsacB* | Km$^r$; *sacB*-based gene replacement vector | [71] |
| pK-*clpV3* | Km$^r$, Gm$^r$; Δ*clpV3*::Gm in pK18*mobsacB* | This study |
| pK-*hsiB3C3* | Km$^r$, Gm$^r$; Δ*hsiB3C3*::Gm in pK18*mobsacB* | This study |
| pK-*hcp3* | Km$^r$, Gm$^r$; Δ*hcp3*::Gm in pK18*mobsacB* | This study |
| pK-*icmF3* | Km$^r$, Gm$^r$; Δ*icmF3*::Gm in pK18*mobsacB* | This study |
| pK-*tseF* | Km$^r$, Gm$^r$; Δ*tseF*::Gm in pK18*mobsacB* | This study |
| pK-*pqsA* | Km$^r$, Gm$^r$; Δ*pqsA*::Gm in pK18*mobsacB* | This study |
| pK-*pqsH* | Km$^r$, Gm$^r$; Δ*pqsH*::Gm in pK18*mobsacB* | This study |
| pK-*icmF1* | Km$^r$, Gm$^r$; Δ*icmF1*::Gm in pK18*mobsacB* | This study |
| pK-*clpV1* | Km$^r$, Gm$^r$; Δ*clpV1*::Gm in pK18*mobsacB* | This study |
| pK-*icmF2* | Km$^r$, Gm$^r$; Δ*icmF2*::Gm in pK18*mobsacB* | This study |
| pK-*clpV2* | Km$^r$, Gm$^r$; Δ*clpV2*::Gm in pK18*mobsacB* | This study |
| pK-*oxyR* | Km$^r$, Gm$^r$; Δ*oxyR*::Gm in pK18*mobsacB* | This study |
| pK-*rpoS* | Km$^r$, Gm$^r$; Δ*rpoS*::Gm in pK18*mobsacB* | This study |
| pME6032 | Shuttle vector between *Pseudomonas* and *E. coli* containing *lacI*$^q$-*Ptac* fragment for gene expression; source of *tetA* gene cassette, Tc$^r$ | [72] |
| pME6032-*tseF* | *tseF* cloned into pME6032 for complementation | This study |
| pME6032-*clpV3* | *clpV3* cloned into pME6032 for complementation | This study |
| pME6032-*icmF3* | *icmF3* cloned into pME6032 for complementation | This study |

| | | |
|---|---|---|
| pME6032-*hsiB3C3* | *hsiB3C3* cloned into pME6032 for complementation | This study |
| pME6032-*rpoS* | *rpoS* cloned into pME6032 for complementation | This study |
| pMini-CTX::*lacZ* | Ω-*FRT*-*attP*-MCS, *ori*, *int*, *oriT*, $Tc^r$ | [63,64] |
| pFLP2 | $Amp^r$; source of Flp recombinase | [73] |
| pMP-64 | 508 bp upstream region of *PA2364* (*H3-T6SS left*) in pMini-CTX::*lacZ* | This study |
| pMP-65 | 508 bp upstream region of *PA2365* (*H3-T6SS right*) in pMini-CTX::*lacZ* | This study |
| pMP-G3 | 1307 bp upstream region of *vgrG3* in pMini-CTX::*lacZ* | This study |
| pMP-*pqsA* | 1105 bp upstream region of *pqsA* in pMini-CTX::*lacZ* | This study |
| pMP- *pqsH* | 1311 bp upstream region of *pqsH* in pMini-CTX::*lacZ* | This study |

*$Tc^r$, $Gm^r$, $Km^r$ and $Amp^r$ represent resistance to tetracycline, gentamicin, kanamycin, and ampicillin, respectively.

**Table S2.** Primers used in this study.

| Primiers | 5′-3′ Sequence * | |
|---|---|---|
| clpV3 up F | CGTGTCTCTAGAACCTGTTCCAGCACCGCCTGCTCAG | |
| clpV3 up R | GCTTCCTCCAGGTCGATGTCCAGCAGCTCCAGCAG | To generate |
| clpV3 low F | CTGGACATCGACCTGGAGGAAGCCACCCTGGAAGG | pK-V3 |
| clpV3 low R | CACGACAAGCTTGCCGTCGGCAAACTCTTTCGGTAC | |
| hsiB3C3 up F | TGTCTCTAGATCATACGACTCCCCTTCGACG | |
| hsiB3C3 up R | CTCGAGCTGGAAGTGCAGCTCCTTCTTCTCGATGGC | To generate |
| hsiB3C3 low F | GAGCTGCACTTCCAGCTCGAGGAACTCAG | pK-B3C3 |
| hsiB3C3 low R | TGACAAGCTTGAGCCCGTAGGCGAGGATG | |
| hcp3 up F | CGTGTCTCTAGACACTCCAAGTACCTCTGGGGCAACG | |
| hcp3 up R | GCGGTCAGCTCCAGGCTGTCGCCTTTGATGTCGCTGC | To generate |
| hcp3 low F | GGCGACAGCCTGGAGCTGACCGCGCAGAAGGACGAC | pK-P3 |
| hcp3 low R | CACGACAAGCTTCTCGAACTGGACGATCGCGCAGGAC | |
| icmF3 up F | CGTGTCTCTAGAGATGGTCGAGCGCGCCCTCGAACTG | |
| icmF3 up R | CAGGACCAGCGGCAGCAGACGCCAGGTGGAGCACAAC | To generate |
| icmF3 low F | GGCGTCTGCTGCCGCTGGTCCTGCAACTGCCGGTG | pK-F3 |
| icmF3 low R | CACGACAAGCTTCGCGCGCAACGCTTCGTCTAGCTTC | |
| tseF up F | CTCGTCTAGACGACAACGCCCTGCCCTAC | |
| tseF up R | CAGCGCCAGCGGTCGCCACGAACCAGGTCGAG | To generate |
| tseF low F | CGTGGCGACCGCTGGCGCTGGTGGTGGAC | pK-*tseF* |
| tseF low R | CTCGAAGCTTTGCGGGGAGGCTTCTTGTG | |
| pqsA up F | CTCGAGATCTCTCGCCCAGTGTACTACG | |
| pqsA up R | GATAAAGGGTGTCCGAAGGCGAGTCGTTCAAC | To generate |
| pqsA low F | CTCGCCTTCGGACACCCTTTATCACGACAAC | pK-*pqsA* |
| pqsA low R | CTCGAAGCTTGCAGCAGTTCATCCAGAC | |
| pqsH up F | CTCGAGATCTACGGCGAGGTAGTTGTTG | |
| pqsH up R | CCTCAGCTCGACCAGCAGCCAGTCGATG | To generate |
| pqsH low F | GCTGCTGGTCGAGCTGAGGAATACCCTCGTTC | pK-*pqsH* |
| pqsH low R | CTCGAAGCTTGCGAAGACCTGGCGAATC | |
| icmF1 up F | CGTGTCTCTAGACGCTTCCTCGCCGACGACATCAAGG | |
| icmF1 up R | TGGTCGAGCAGGATGATCACGTACCAGGGCAACTCGTAC | To generate |
| icmF1 low F | GTACGTGATCATCCTGCTCGACCAGTCGGACCTGGAAC | pK-F1 |
| icmF1 low R | CACGACAAGCTTGCCGGGAAAGAGCGGGATCACCTC | |

| | | |
|---|---|---|
| clpV1 up F | GTGCGAATTCACCGCACGCTACTACTACACCG | |
| clpV1 up R | CAGCGCAACGCGACTGAT | To generate |
| clpV1 low F | ATCAGTCGCGTTGCGCTGGACGCGATCCTCACCAACA | pK-V1 |
| clpV1 low R | GTGCAAGCTTGGCGGAACCAGTAGTAGATGC | |
| icmF2 up F | CGTGTCTCTAGACGAACAGCTCGAGGAGCGGATGATC | |
| icmF2 up R | GAGCACCATCACCAGGAGTACCAGCAACAGCGTCCAG | To generate |
| icmF2 low F | CTGGTACTCCTGGTGATGGTGCTCAAGGCCGACCTG | pK-F2 |
| icmF2 low R | CACGACAAGCTTCGGTTCGTTCATTGGCGGATCACCAC | |
| clpV2 up F | CGTGTCTCTAGAACCTGATCGGCTTGTTCGGTGCCAG | |
| clpV2 up R | CAGCAAACGGTCGGTGTCGGCATCCAGCGCCTGAATC | To generate |
| clpV2 low F | GATGCCGACACCGACCGTTTGCTGGAAGCGATGGC | pK-V2 |
| clpV2 low R | CACGACAAGCTTGCAGTTCTGCACCACGAACGCCTG | |
| oxyR up F | CGTGTCTCTAGATGCTCGGCGTCGAGCATGATCCGTC | |
| oxyR up R | CGGAACGGCACGACCGAGAGGGTCGGTTGGCTGAC | To generate |
| oxyR low F | GACCCTCTCGGTCGTGCCGTTCCGTACCGTTGCCATCG | pK-*oxyR* |
| oxyR low R | CACGACAAGCTTTGGCCAGGGCCTGTTGGCTCAACTG | |
| rpoS up F | CGAGCAGGATCCTATCCAATTTGGTGGGCGGGCATC | To generate |
| rpoS up R | GACCTCTTCCAGCTGCTCGTCGGCAGACGACTCG | pK-*rpoS* |
| rpoS low F | GCCGACGAGCAGCTGGAAGAGGTCGGCCAGGAAATC | |
| rpoS low R | GCTCGAGCATGCATCCATCCGGACGAGTGCATCGAC | |
| tseF F | CTCGGAATTCATGGCGGCATCCGGCAAG | To generate |
| tseF R | CTCGCTCGAGGATGCGCGCCTAGGGCTC | pME6032-*tseF* |
| clpV3 F | CTCGGAGCTCCTGCGGCAGCCGGAGGTAG | To generate |
| clpV3 R | CTCGAGATCTGTCCTACTCCAACACCCACTCC | pME6032-*clpV3* |
| icmF3 F | CTCGGAATTCATGAGCGGCGCGACGCTG | To generate |
| icmF3 R | CTCGAGATCTCATGGGGTACCTCCGGTGCTG | pME6032-*icmF3* |
| hsiB3C3 F | CTCGGAGCTCTCCCCCACCATTGCATCG | To generate |
| hsiB3C3 R | CTCGGGTACCGAGGTGCGCTCCGAGTTG | pME6032-*hsiB3C3* |
| rpoS F | CTCGGAATTCTGAGTCGAACTCATGCAAGGG | To generate |
| rpoS R | CTCGAGATCTTTCCGTCACTGGAACAGCG | pME6032-*rpoS* |
| aphCF F | CTCGGAGCTCCCCATTCAACTGAGAGAGGAAC | To generate |
| aphCF R | CTCGGGATCCGCTGGCCGGGTCACTCCG | pME6032-*aphCF* |
| dps F | CTCGGAATTCATGGAAATCAATATCGGAATCGG | To generate |
| dps R | CTCGAGATCTCGATCGACGATCAGCTGG | pME6032-*dps* |

| | | |
|---|---|---|
| PA2364 F | TGTCGGTACCGAAGCGCAGCTCGACGTTC | To generate |
| PA2364 R | TGTCGAATTCCGGGACCAGCTCCAGGCTC | pMP-64 |
| PA2365 F | TGTCGGTACCCGGGACCAGCTCCAGGCTC | To generate |
| PA2365 R | TGTCGAATTCGAAGCGCAGCTCGACGTTC | pMP-65 |
| vgrG3 F | CCTGCTCGAGATCCTCGCCACCAGCAAC | To generate |
| vgrG3 R | TGTCCTGCAGGGTGGGACGGGGCATTAGTG | pMP-G3 |
| pqsA F | CTCGGTCGACAACCCACCGGCGAAACCG | To generate |
| pqsA R | CTCGCTGCAGGCATTGCAGCCGGCTGAG | pMP-*pqsA* |
| pqsH F | CTCGCTCGAGGGAGGGTGAAGGTGATGCC | To generate |
| pqsH R | CTCGAAGCTTGGTACCGATGGGCCTGATC | pMP- *pqsH* |

*Underlined sites indicate restriction enzyme cutting sites added for cloning.

**Supplementary References**


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