**Supplemental Table S1 Bacterial strains and plasmids used in the present study**

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| --- | --- | --- |
| **Bacterial strains or plasmids** | **Characteristics** | **Source or reference** |
| ***Escherichia coli* strains** |  |   |
| Pir1 | F*- ∆lac169 rpoS(Am) robA1 creC510 hsdR514* endA recA1 uidA(∆MluI)::pir-116  | Invitrogen |
| MG4 | pKDT17 | Addgene |
| ***Pseudomonas aeruginosa s*trains**  |   |  |
| PAO1 |  Wild-type | Manoil Lab UW |
| ∆pmtA | PAO1*∆*PA2140 | This study |
| pmtA:pmtA | PAO1, *∆*PA2140, Gmr | This study |
| **Plasmids** |   |   |
| pEX18GmAF047518.1 | Gene replacement vector; *sacB*; Gmr | Schweizer lab |
| pUC18R6K-mini-Tn7T-GmDQ153108.1 | Gene replacement vector; Gmr | Addgene |
| pGEX-6p-*pmtA*  | pGEX-6p-1, PA2140 | This study |

**Supplemental Table S2 Primers used in the present study**

|  |  |
| --- | --- |
| Primer | Sequence |
| Deletion of PA2140 |  |
| 1\_PA2140\_del\_F   | TGCAGGTCGACTCTAGAGGTGTCGGTACCGGTGTTC  |
| 1\_PA2140\_del\_R   | GTTCATGGGCAGTCTCCAG |
| 2\_PA2140\_del\_F   | TGGAGACTGCCCATGAACATCTCGCCCTGAGGAGCG |
| 2\_PA2140\_del\_R   | CAGCTATGACCATGATTACGTTGATGGAATTCGGGCAG |
| Screen for inserts |  |
| 2\_PA2140\_test\_F | CTGCTGATCCTGCTCCTG |
| 2\_PA2140\_test\_R  | CAGTTCGGCGCTGATCTG |
| Sequencing  |  |
| PA2140\_Tn7\_F | ATGAACAGCGAAACCTGTGC |
| PA2140\_Tn7\_R | GGCCTTCGCGAGGTACGCTCCTCAGGGCGAGATC |
| Cloning of PA2140 |  |
| PPA2140\_Tn7\_F | TGGATCCCCCGGGCTGCAAACCAATGGAACCTGCGC |
| PA2140\_Tn7\_R | GGCCTTCGCGAGGTACGCTCCTCAGGGCGAGATC |
| Sequencing  |  |
| pTn7GM\_F | TGCTGTTGACAAAGGGAATC |
| pTn7GM\_R | GTAGCGTCGTAAGCTAATACG |
| RT-PCR |  |
| qPCR\_PmtA\_F | ACCTGTGCCTGTCCCAAAT |
| qPCR\_PmtA\_R | CGGGAAAGGTCTCCTTCAAC |
| qPCR\_RNApol\_F | TGATTTCGGTCAGGGACTTC |
| qPCR\_RNApol\_R | GATGACCTGGAACTGACCGT |
| qPCR\_phzH\_F | TTATCCGACTTCTGCCAACC |
| qPCR\_phzH\_R | GAGAGCCCGTACAACCTGAG |
| qPCR\_phzM\_F | CAAGTTGTTACCGGGGAATG |
| qPCR\_phzM\_R | AGATCTCGAAGGCCACCAG |

**Figure S1 Schematic representation of *P. aeruginosa pmtA* knock-out strain construction.** Schematic indicates the generation of the *ΔpmtA* mutant.

Figure S2. Confirmation of Tn7 insertion into clean deletion mutant by colony PCRLanes 2, 3, and 4 used the pTn7GM\_F and pTnGM\_R primers, while lanes 5 and 6 used the PPA2140\_Tn7\_F and PA2140\_Tn7\_R primers with GoTaq Green Master Mix as previously described. Lane 1, 100 bp ladder; lane 2, pUC18R6K-mini-Tn7T-Gm-promoterPA2140 transformant (979 bp); lane 3, complemented *ΔpmtA* mutant (*ΔpmtA:pmtA*); lane 4, pUC18R6K-mini-Tn7T-Gm empty vector (261 bp); lane 5, wild-type PAO1 (781 bp); and lane 6, complemented *ΔpmtA* mutant *ΔpmtA:pmtA*,(781 bp)

**Figure S3.** *P. aeruginosa* strains PAO1, *ΔpmtA,* and *ΔpmtA:pmtA* grow at similar rates**.** Single colonies of the *P. aeruginosa* strains were each grown overnight in 5 mL of LB media at 37°C with vigorous shaking. These cultures were transferred to **A.** 50 mL LB media at a dilution of 1:100 and grown at 37°C with vigorous shaking. Growth was measured at 2, 4, 6, 24, and 30 hours by removing 250 μL of culture in triplicate and transferring to a NUNC 96 well plate. The plate was measured using a Spectramax microplate reader. **B.** 1 mL of 1:100 diluted cultures were transferred to a NUNC 96 well plate in triplicate. Growth was monitored over 15 hours at 37°C with 5s shaking every 5 min in a Spectramax microplate reader at OD600. C. Cultures were grown in M9 salts media in a 24 well plate and monitored over 15 hours at 37°C with 5s shaking every 1 hour in a Spectramax microplate reader at OD600. The data are presented as the average of three biological replicates (+/- standard error of the mean) and are representative of three separate experiments.

### Figure S4. PmtA is not required for survival in oxidative stress conditions.

Overnight cultures were diluted at 1:100 and grown for 2 hours. Cultures were adjusted to 0.08 OD at OD600. 600 μL of culture in triplicate were put in a 24-well plate containing either LB (A) or M9 salt media (B). Hydrogen peroxide to a final concentration of 10 mM or water was added to the wells. **A.** Growth was measured at OD600 every hour with shaking before and after measurement in a Spectramax microplate reader. The data are presented as the average of three biological replicates (+/- standard error of the mean) and are representative of three separate experiments.

Figure S5. PmtA is not required for survival in heavy metal stress conditions. Overnight cultures were diluted at 1:100 and grown for 2 hours in LB or M9 salt media. Cultures were adjusted to 0.08 OD at OD600. 600 μL of culture in triplicate were put in a 24-well plate. Zinc chloride (A&B) to a final concentration of 200 μM, or cadmium chloride (C&D) to a final concentration of 100 μM.Growth was measured at OD600 every hour with shaking before and after measurement in a Spectramax microplate reader. The data are presented as the average of three biological replicates (+/- standard error of the mean) and are representative of three separate experiments.

**Figure S6. PmtA does not play a role in iron uptake.** Wild-type PAO1, *ΔpmtA,* and *ΔpmtA:pmtA* cultures were grown overnight in M9 salt media, diluted at 1:100 and grown in M9 salt media until they reached an OD600 of 0.08. 600μL of culture in triplicate were put in a 24-well plate. M9 salt media (orange), EDDA to a final concentration of 150μM (Blue), EDDA + FeCl2 (Black) to a final concentration of 150μM and 50μg, or EDDA+ Transferrin (purple) to a final concentration of 150μM and 200μg. Growth was measured at OD600 every hour with shaking before and after measurement in a Spectramax microplate reader. The circles indicate PAO1, the squares indicate *ΔpmtA,* and the triangles indicate *ΔpmtA:pmtA.* The data are presented as the average of three biological replicates (+/- standard error of the mean) and are representative of three separate experiments