

Supplementary Information - Methods

Cloning methods. The genes of interest were cloned into vectors using the Gibson assembly technique. Briefly, genes were amplified with PCR primers with 20 bp overlap with the vector insertion site sequence with KAPA-HiFi HotStart (Kapa-biosystems) and stitched together with the Gibson assembly kit (New England Biolabs). The resulting plasmids were transformed into *E. coli* TOP10. For *E. coli* BqsR expression, the pET15b-BqsR plasmid was transferred to *E. coli* BL21 expression strain. The pMQ72 was electroporated into electrocompetent *P. aeruginosa* PA14 (1) and into *P. aeruginosa* PA14 $\Delta bqsR$. The point mutants were generated through site-directed mutagenesis. For the point mutants expressed from the *glmS* locus in the *P. aeruginosa* $\Delta bqsR$ strain, tetraparental conjugation was used to integrate the pUC18T-mini-Tn7T-BqsR plasmid and the point mutation plasmids (separately) into *P. aeruginosa* PA14 $\Delta bqsR$. Complementation of the mutants $\Delta bqsR$, $\Delta bqsS$, and $\Delta bqsRS$ were performed using the pUC18T-mini-Tn7 plasmid that included the 1 Kb upstream sequence of the *bqs* operon, and *bqsR*, *bqsS*, or *bqsRS*. These constructs were electroporated into *E. coli* TOP10 and tetraparental conjugation used to conjugate *P. aeruginosa* $\Delta bqsR$, $\Delta bqsS$, and $\Delta bqsRS$ mutants.

BqsR purification. BqsR was expressed heterologously in *E. coli* with an N-terminal His-tag, by inoculating LB/carbenicillin 50 ($\mu\text{g ml}^{-1}$) with 1% inoculum from an overnight culture. Cells were grown to $A_{600} = 0.4 - 0.5$ and BqsR expression induced with 1 mM IPTG and shifted to 16 °C overnight. Cells were pelleted at 5000 x g for 30 min and resuspended twice with wash buffer (500 mM KCl, 20 mM imidazole, 10% glycerol, and 20 mM Tris pH 8). DNase ($2.5 \text{ units ml}^{-1}$) lysate, 5 mM MgSO_4 , and 130 μM CaCl_2 were added to the suspension. Cell lysate was generated with 14,000 psi on the Avestin Emulsiflex C3 and protease inhibitors added. BqsR was purified on Akta FPLC using 5 ml HisTrap HP (GE Healthcare). Column-bound BqsR was washed with 100 mM imidazole to remove contaminating proteins and eluted with 200 mM imidazole. After loading 1 mg protein (determined by the Bradford assay) per lane, the 200 mM imidazole fraction contained one band, confirmed by MALDI-TOF to be BqsR. BqsR was dialyzed overnight with 3 changes of dialysis buffer (50% glycerol, 300 mM KCl, 50 mM KPO_4 pH 7.4), concentrated, and flash-frozen BqsR aliquots stored at -80 °C.

Gel shift assays. Double stranded DNA was generated by boiling equimolar concentrations of single stranded Cy5 labeled reverse complementary DNA sequences (approximately 60 bp in length) for 10 min and allowing to slowly come to room temperature over an hour. Various concentrations of BqsR were incubated with 5 nM double stranded DNA (both strands 5'-labeled with Cy5) in reaction buffer (15 mM KCl, 10% glycerol, 1 mM MgSO_4 , 50 mM KPO_4 pH 7.4) for 20 minutes at room temperature. 5 μl of reaction was loaded into 15-well 8% acrylamide gel (0.5x TBE, 29 acrylamide: 1 bis-acrylamide [Bio-Rad]) and run at 75 V at room temperature. DNA was visualized with Storm 860 in fluorescence mode using the 635 excitation laser. Through the duration of the experiment, the reaction and gel were shielded from light with aluminum foil.

Transcription start site determination. cDNA was generated using gene specific primer 1 and Superscript II (Invitrogen) including the RNase inhibitors, RNasin (Promega) in the reaction. The mRNA template was degraded with RNase H. The cDNA ligated to adaptor DNA (WNp210) with an inverted 3' T. A nested PCR reaction was performed with gene specific primer 2 and a primer complementary to the adaptor sequence (WNp213). The PCR reaction was TOPO cloned and sent for sequencing. The transcription start site is the junction between the adaptor sequence and the gene specific sequence.

Fe(II) shock conditions. Aerobic cultures of *P. aeruginosa* wt-pMQ72 and $\Delta bqsR$ -pMQ72 were grown in 3 ml MOMM supplemented with 100 ($\mu\text{g ml}^{-1}$) gentamycin at 37 °C for 36 hours. These cultures were used to inoculate 20 ml MOMM supplemented with 100 ($\mu\text{g ml}^{-1}$) gentamycin and 1% arabinose with 1% inoculum. The cells were grown anaerobically to early exponential phase (Beckman spectrophotometer 20 A500 = 0.2). When the cells reached early exponential phase, a total of 9 ml was removed and the culture returned to 37 °C. 4.5 ml culture was added to 9 ml of RNAprotect for a before Fe(II) shock time point. 200 μM ferrous ammonium sulfate added anaerobically to the remaining 4.5 ml and incubated for 30 minutes at room temperature then combined with 9 ml RNAprotect. The cells were incubated with RNAprotect for 5 minutes and centrifuged for 10 minutes at 5000 x g. The supernatant was discarded and the pellets stored at -80 °C for future RNA extraction.

Consensus sequence generation. For the final version of the PWM shown in Figure 3B the seeding sequences were from *Pseudomonas aeruginosa* PA14 and were validated by Fe(II) shock qRT-PCR experiments that showed upregulation in WT but not in a $\Delta bqsR$ mutant. The 500 bp upstream from the following gene translation start sites used for the final PWM were *bqsP*, PA14_04180, PA14_04270, PA14_01240, PA14_07070, PA14_01250, *dsbB*, *arnB*, PA14_32270, PA14_063110, PA14_32590, and *oprH*.

mRNA isolation and qRT-PCR data analysis. The threshold cycle values of *recA* and *clpX* were used as endogenous controls (2). Fold changes were calculated using the $\Delta\Delta C_t$ method. Briefly, all samples were normalized to each other by subtracting the C_t value for the control gene *recA* in the shock condition from the control condition ($\Delta C_t \text{ recA}$). This value was then converted from the \log_2 using the following formula: $a = 2^{C_t \text{ recA}}$. The change in the genes of interest was calculated by subtracting the C_t value of the gene from the shock condition from the C_t value for the gene in the control condition ($\Delta C_t \text{ gene}$). This value was then converted from the \log_2 using the following formula: $b = 2^{\Delta C_t \text{ gene}}$. The final relative fold change is calculated by: fold change = b/a . To ensure *recA* was constant in all conditions tested, the relative fold change for the internal control *clpX*, whose expression was also expected to remain constant across all our treatments, was calculated as described above. Only those samples with a *clpX* fold change between 0.5 – 2 were used. \log_2 of the final fold change was reported. Results were compared with an unpaired 2-tailed t-test assuming unequal variances.

Growth Curves. Biological replicates of PA14, $\Delta bqsR$, $\Delta bqsS$, and $\Delta bqsRS$ were prepared in MOMM+ 5 μM Fe(II) or 100 μM Fe(II) and moved into the anaerobic chamber. Cultures incubated at 5 μM Fe were diluted to an $\text{OD}_{500}=0.02$ in wells containing MOMM+ 5 μM Fe(II). Cultures incubated at 100 μM Fe(II) were diluted to an $\text{OD}=0.02$ in wells containing MOMM+100 μM Fe(II), MOMM+100 μM Fe(II) with 190 μM ferrozine, MOMM + 100 μM Fe(II) with 5 mM spermidine, or MOMM + 100 μM Fe(II) with 10 mM spermidine. The well plate was incubated at 37°C in the anaerobic well plate reader and absorbance was read at 500 nm every 2 hours over the course of 36 hours.

Spermidine quantification by HPLC analysis.

In order to ensure sufficient spermidine production for visual quantification, 5 mM arginine and 5 mM methionine were supplemented to the MOMM media to provide the precursors for spermidine synthesis; this medium will be subsequently referred to as MOMM+AA. Biological triplicates of PA14, $\Delta bqsR$, $\Delta bqsS$, and $\Delta bqsRS$ were in 5 mL MOMM+AA with either 5 μM Fe or 75 μM Fe and diluted to an $\text{OD}_{500}=0.01$ in balch tubes containing 20 mL MOMM+AA with either 5 μM or 75 μM Fe(II). Cultures were stoppered and grown shaking at 37°C until $\text{OD}\approx 0.7$. Cells were pelleted in two centrifugations in 15 mL falcon tubes at 6800 rpm for 15 minutes at 4°C, and supernatant was removed. Pellets were then suspended in 1 mL 1M NaCl and 0.1M HEPES pH=7.2 and incubated for 10 minutes at 37°C shaking. Suspension was transferred to a 2 mL microfuge tube and centrifuged at 8000rpm for 10 minutes. Supernatant was removed, and pellet was suspended in 1 mL 10% trichloroacetic acid and incubated at 4°C for 3 hours. Suspension was again centrifuged at 8000 rpm for 10 minutes and supernatant was siphoned off and stored in a glass vial at 4°C. 1,3-diaminopropane was added to the supernatants at a concentration of 30 $\mu\text{g}/\text{ml}$ as an internal standard for subsequent dabsylation and HPLC analysis of polyamines. 4 μL of each sample was dabsylated in triplicate and analyzed by HPLC. Results were normalized by OD_{500} .

Tobramycin MIC rescue experiments. Ferrozine and spermidine were tested for their ability to rescue tobramycin sensitivity. Cultures were prepared and analyzed as usual, but with the addition of 140 μM ferrozine or 10 mM spermidine to MOMM + AA + 75 μM Fe(II), with tobramycin concentrations ranging from 0-8 $\mu\text{g}/\text{ml}$.

1. Choi K, Kumar A, & Schweizer H (2006) A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: Application for DNA fragment transfer between chromosomes and plasmid transformation. *Journal of Microbiological Methods* 64(3):391-397.
2. Dietrich LEP, Price-Whelan A, Petersen A, Whiteley M, & Newman DK (2006) The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Molecular Microbiology* 61(5):1308-1321.