Supplementary Material

Inhibiting the BfrB:Bfd Interactions in *Pseudomonas aeruginosa* Causes Irreversible Iron Accumulation in Bacterioferritin and Iron Deficiency in the Bacterial Cytosol

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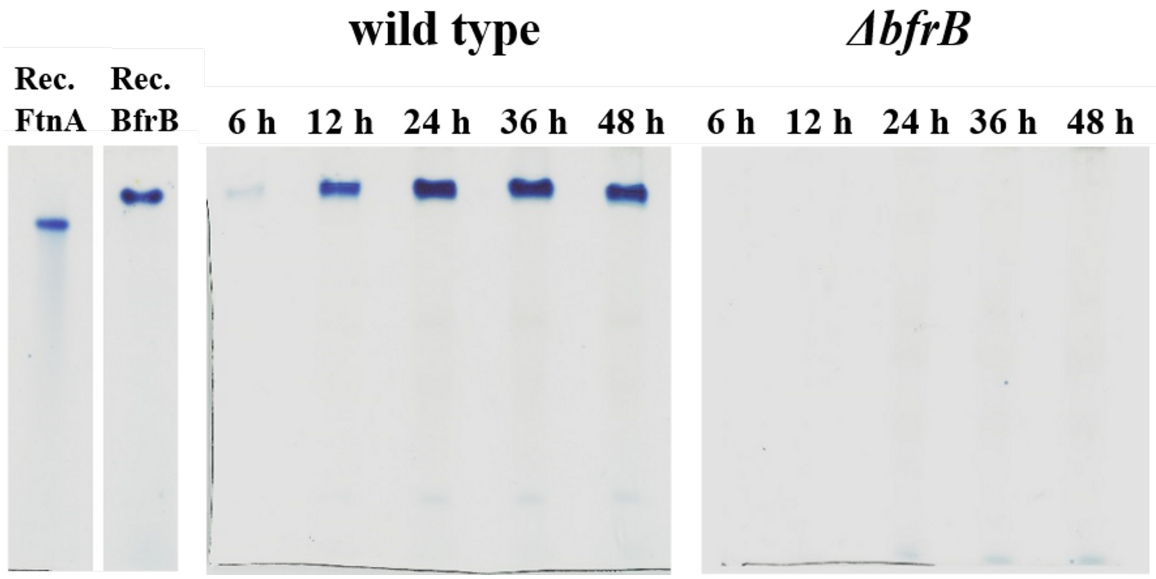

Figure S1. PAGE gels stained with Ferene-S. Left: Recombinant FtnA (Rec. FtnA) and recombinant BfrB (Rec. BfrB). Center: Lanes loaded with lysates of wild type *P. aeruginosa* cultured in PI media supplemented with 30 μM Fe show only iron-stained bands migrating with the electrophoretic mobility of BfrB. Right: Lanes loaded with lysates of Δ*bfrB* cultured in PI media supplemented with 30 μM Fe show that iron is not accumulated in FtnA, or in any other protein.

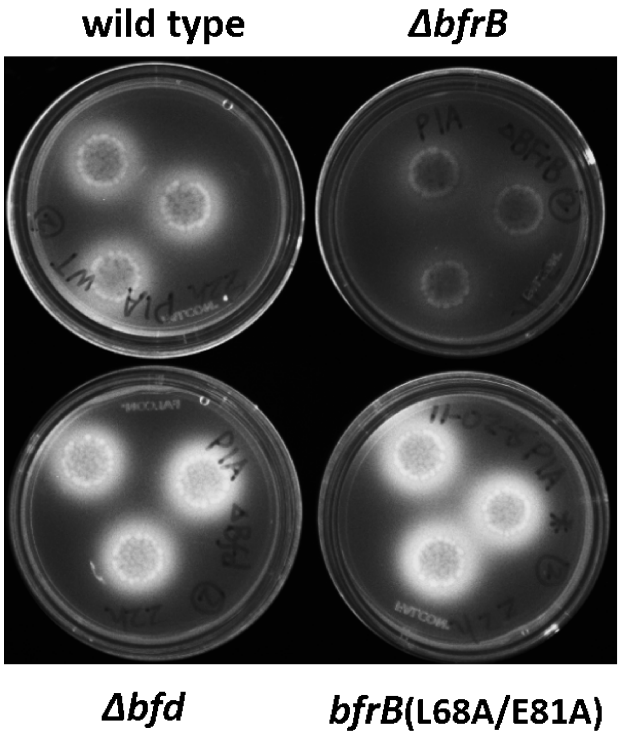

Figure S2. *P. aeruginosa* cells, wild type and mutants, were spotted on PIA plates and cultured for 22 h. Illuminating the plates with UV-light from the bottom reveals a fluorescent green-yellow color on the cells and their surrounding area caused by Pvd fluorescence. The comparison shows that Δ*bfd* and *bfrB*(L68A/E81A) cells secrete significantly more Pvd than the wild type and Δ*bfrB* cells.

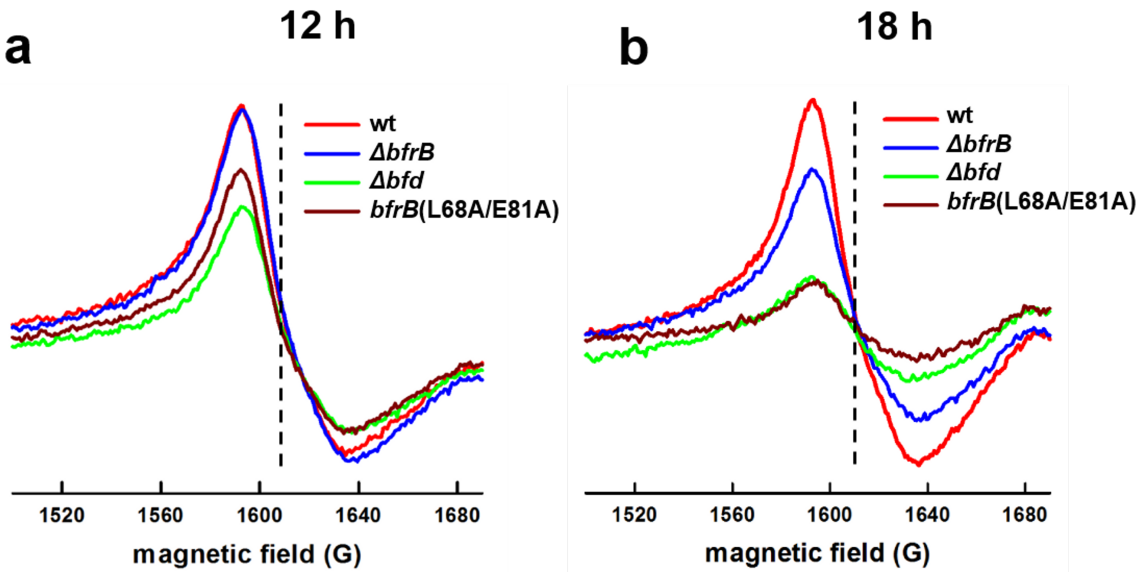

Figure S3. Whole-cell EPR spectra obtained from *P. aeruginosa* cells (wild type and mutants) harvested at (a) 12 h and (b) 18 h post inoculation. Cells were cultured in PI media supplemented with 10 μM Fe, and treated as described in Methods prior to EPR spectroscopic analysis. The vertical segmented line indicates $g = 4.3$.

Table S1. Plasmids

pEXG2	Allelic exchange vector with pBR origin; *sacB,* Gm^r	(1)
pEXG2 Δ*bfrB*	pEXG2 with Δ*bfrB* extending from +96 to +468 with respect to the translational start site; Gm^r	This study
pEXG2 Δ*bfd*	pEXG2 with Δ*bfd* extending from +22 to +222 with respect to the translational start site; Gm^r	This study
pEXG2 *bfrB*(L68A/E81A)	pEXG2 with the *bfrB* allele with the base substitutions C202G, T203C and A242C	This study
pUC18---mini---Tn7T---LAC	Suicide delivery vector for insertion at *att*Tn7 site, Gm^r	(2)
pUC18---mini---Tn7T---LAC *bfrB*	pUC18---mini---Tn7T---LAC with P_{lac}–driven *bfrB*	This study
pUC18---mini---Tn7T---LAC *bfd*	pUC18---mini---Tn7T---LAC with P_{lac}–driven *bfd*	This study
pTNS2	T7 transposase expression vector; R6K *ori*, *ori* T, Ap^r	(3)
pFLP2	Site---specific excision vector; *sacB, ori* T, Cb^r	(4)

Table S2. Primers

Primers used to make* pEXG2 Δ*bfrB:	
BfrB d1 XbaI	TAATAATCTAGAGCCTTGGCGATCTCGTGCTTGGTG
BfrB d2	GCTTCGTCGTGGTTCCCGATCAGTCGTCGCCTTTCATGCCGAATCCTGCC
BfrB d3	GGCAGGATTCGGCATGAAAGGCGACGACTGATCGGGAACCACGACGAAGC
BfrB d4b HindIII	TTAAATAAGCTTCCACATGCGCGAGTGGAGGAAGTACTG
Primers used to make* pEXG2 Δ*bfd:	
Bfd d1 XbaI	TAATAATCTAGACTCGCGAGCCCATGAAAAAGCCC
Bfd d2	GTTCTTCTGAAAGAGGGATAAAAAATATTTTTCAGGCTTGGCAGAGGCAGACGTACATGG
Bfd d3	CATGTACGTCTGCCTCTGCCAAGCCTGAAAAATATTTTTTATCCCTCTTTCAGAAGAACC
Bfd d4 HindIII	TAATAAAAGCTTCGCTGAGCATCGCCGGTTTC
Primers used to make* pUC18-mini-Tn7T-LAC *bfrB:	
BfrB 5' SpeI	TAA TAA ACT AGT ATG AAA GGC GAC AAG AAA GTC ATC CAG CAC CTC AAC
BfrB 3' HindIII	TAA TAA AAG CTT TCC CGA TCA GTC GTC TTC GTG CAT GTG
Primers used to make pUC18-mini-Tn7T-LAC bfd:	
Bfd 5' SpeI	TAA TAA ACT AGT ATG TAC GTC TGC CTC TGC CAA GGT GTT ACC
Bfd 3' HindIII	TAA TAA AAG CTT TCA GGC AGC GAC GAA CGC CGT AGT C

References

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3. Choi, K.-H., Gaynor, J. B., White, K. G., Lopez, C., Bosio, C. M., Karkhoff-Schweizer, R. R., and Schweizer, H. P. (2005) A Tn7-based broad-range bacterial cloning and expression system, *Nature methods 2*, 443-448.
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