Supplemental materials of

Complex iron uptake by the putrebactin-mediated and Feo systems in *Shewanella oneidensis*

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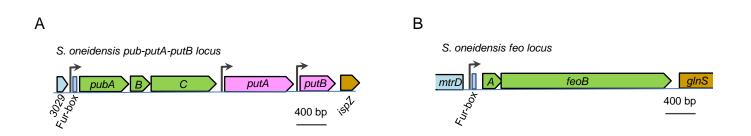


FIG S1 Gene organization of the *putA* and *feo* locus in *S. oneidensis*. (A) The *pub-putA-putB* locus. (B) the *feo* locus. Both loci are largely conserved in *Shewanella*. Promoters indicated by arrows are determined by the *lacZ* reporter system and Fur-boxes are given according to a published report (Fu H, Liu L, Dong Z, Guo S, Gao H. 2018. Appl Environ Microbiol 84:e00039-18, doi: 10.1128/AEM.00039-18). Drawn in scale.

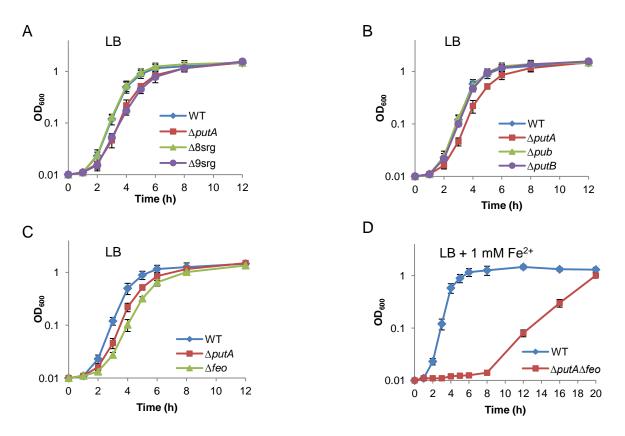


FIG S2 Growth of indicated strains in LB. Fresh medium indicated was inoculated with overnight cultures grown from a single colony by 1:100 dilution, and growth was determined by recording the optical density of cultures at 600 nm (OD_{600}). Δ 8srg and Δ 9srg, strains lacking all TBSRs but PutA and all TBSRs, respectively. Experiments were performed at least three times and the data were presented either as means \pm SEM.

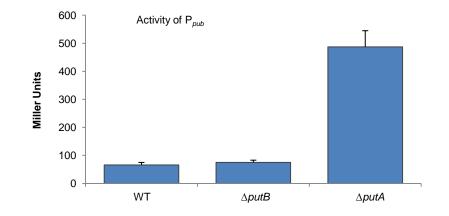
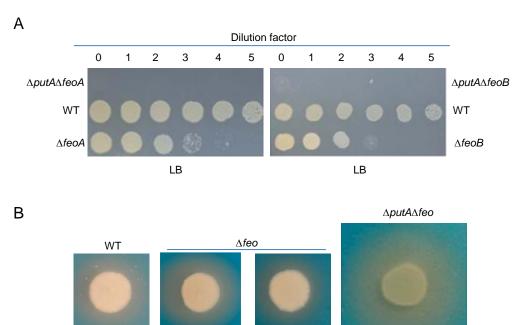


FIG S3 Impacts of PutA and PutB depletion on expression of the *pub* **operon.** Expression of the *pub* operon in the wild-type, $\Delta putA$, and $\Delta putB$ strains grown in LB to the mid-log phase was analyzed by an integrative *lacZ*-reporter.



LB + 2,2-dipyridy (50 μM) LB + hemin

FIG S4 Physiological impacts of *feoA* and *feoB* mutations. (A) Growth of indicated strains on LB plates. (B) Siderophore production of indicated strains grown in LB with 50 μ M 2,2-dipyridyl. All experiments were performed at least three times and the data were presented by a representative of similar results.

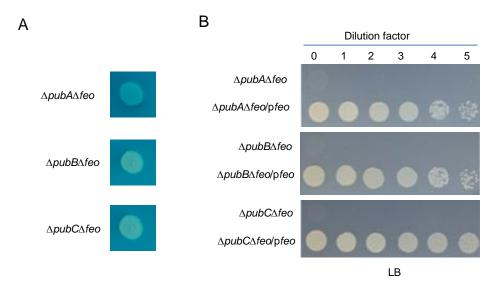


FIG S5 Physiological impacts of *pubA*, *pubB*, and *pubC* mutations. (A) Siderophore production of indicated strains grown in LB with 50 μ M 2,2-dipyridyl. (B) Growth of indicated strains on LB plates. All experiments were performed at least three times and the data were presented by a representative of similar results.

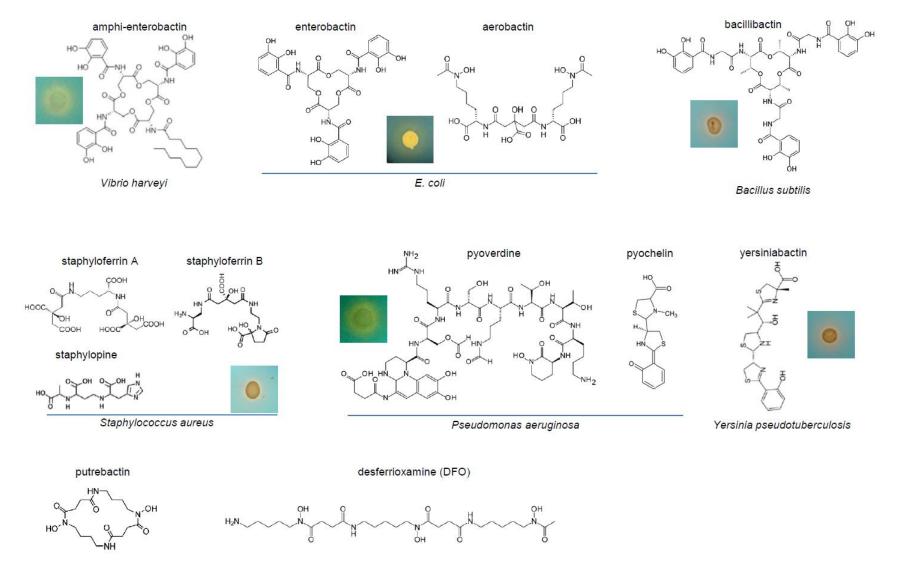


FIG S6 Siderophores in the supernatants of the indicated bacteria. All bacteria under test were grown in LB containing 50 μM 2,2dipyridyl to the stationary phase. The supernatants obtained by filtration were applied onto the CAS plates for siderophore detection. Siderophores produced by indicated bacteria are given according to published reports (Hider RC, Kong X. 2010. Nat Prod Rep 27:637-657, doi: 10.1039/B906679A; Wilson BR, Bogdan AR, Miyazawa M, Hashimoto K, Tsuji Y. 2016. Trends Mol Med 22:1077-1090, doi: 10.1016/j.molmed.2016.10.005).

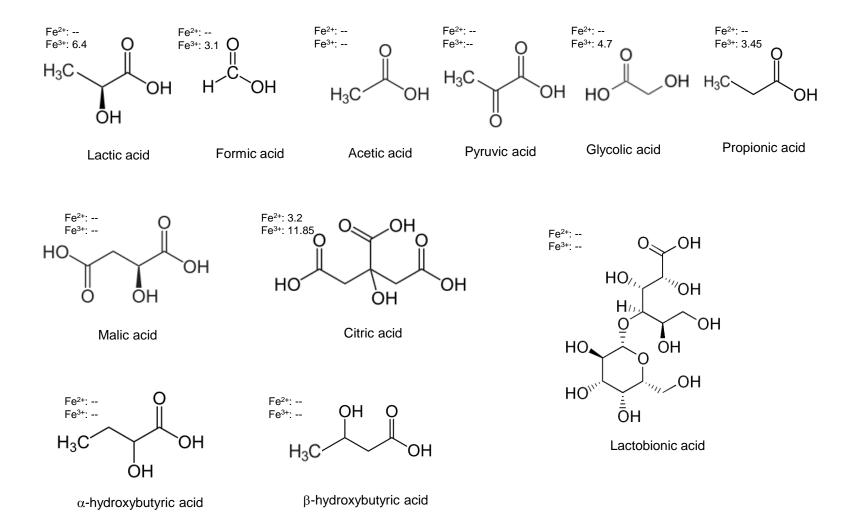


FIG S7 Chemicals examined for their role in promoting iron uptake. Stability constants of each chemical for Fe³⁺ and Fe²⁺ if available (Smith RM, Martell AE. 1989. Carboxylic Acids, p299-359, Critical Stability Constants: Second Supplement doi:10.1007/978-1-4615-6764-6_12. Springer US, Boston, MA).

Protein	Size (aa)	BLASTp E-value	Fur-regulation
PutA (SO_3033)	730	0	
SO_4743	706	5e-24	ATAAATAAGAATTGTTTTT
SO_3914	730	2e-20	GCAAATGATAATAATTATT
SO_1156	715	2e-20	
SO_0798	720	7e-12	GTAAATGAGAATTGTTATT
SO_1482	815	3e-11	GAGGATGGAAATCATTATC
IrgA (SO_4523)	663	1e-09	GGTATTGAAAATTATTATC
SO_4422	724	4e-09	GCAAATGGTAACGATTTTT
SO_4516	685	0.14	GTAAATGATATTGGTTATC

TABLE S1 TonB-dependent siderophore receptors in S. oneidensis