**Overexpression of *fetA* (*ybbL*) and *fetB* (*ybbM*), encoding an iron exporter, enhances resistance to oxidative stress in *Escherichia coli***

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## Supplemental Information

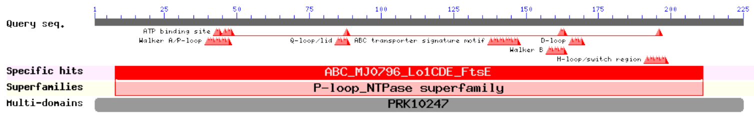
**Table S1**. Plasmids isolated for the $H_2O_2$ tolerant clones.

| Strain | Plasmid Library | Genomic Region/ Size * | Genes in plasmid ** |
|---|---|---|---|
| 1 | 2MgL | 1552,357 - 1,555,984 (3,627) | (*maeA*), *sra*, *bdm*, *osmC*, (*ddpF*) |
| | 4MgL | 4,032,316 - 4,029,152 (-3,164) | *pepQ*, *yigZ*, (*trkH*) |
| 2 | 2MgL | 4,412,412 - 4,414,354 (1,942) | (*aidB*), *yjfN* |
| | 4MgL | 127,628 - 124,611 (-3,017) | (*aceE*), *aceF* |
| 3 | 2MgL | 513,453 - 517,242 (3,789) | (*cueR*), *ybbJ*, *qmcA*, *ybbL*, *ybbM*, (*ybbN*) |
| | 4MgL | 769,180 - 765,615 (-3,565) | (*mngA*), (*mngB*) |
| 4 | 2MgL | 517,376 - 513,076 (-4,300) | *cueR*, *ybbJ*, *qmcA*, *ybbL*, *ybbM*, (*ybbN*) |
| | 4MgL | 366,152 - 369,903 (3,751) | (*lacI*), *mhpR*, *mhpA*, (*mhpB*) |
| 5 | 2MgL | 517,376 - 513,076 (-4,300) | *cueR*, *ybbJ*, *qmcA*, *ybbL*, *ybbM*, (*ybbN*) |
| | 4MgL | 362,352 - 367,016 (4,664) | *lacZ*, *lacI*, (*mhpR*) |
| 6 | 2MgL | 517,376 - 513,076 (-4,300) | *cueR*, *ybbJ*, *qmcA*, *ybbL*, *ybbM*, (*ybbN*) |
| | 4MgL | 2,820,268 - 2,823,783 (3,515) | *recA*, *ygaD*, *mltB* |

* A negative sign indicates that the fragment is included in the opposite orientation

** Genes included are in parenthesis are incomplete genes on the isolated plasmids

**A**




| Motif | Sequence in FetA | Residue in FetA |
|---|---|---|
| Walker A | GPSGCGKS | 40 – 47 |
| Q-loop | YCAQ | 85 – 88 |
| ABC motif | LSGGEKQRIS | 138 – 147 |
| Walker B | VLLLDE | 158 – 163 |
| D-loop | SALD | 166 – 169 |
| H-loop | LWVTHDK | 192 – 198 |

**B**

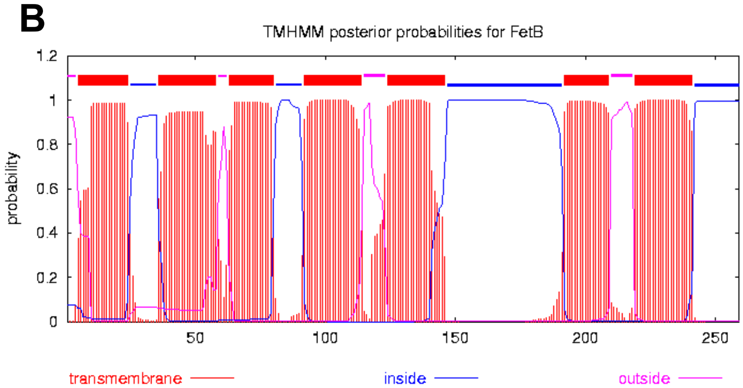



**Fig. S1. Bioinformatic analysis of FetA and FetB.** *(A)* pBLAST of FetA reveals specific hits for ABC transporters and the P-loop-NTPase superfamily (1). The conserved domains in FetA were predicted using the NCBI Conserved Domain Database (6). *(B)* The transmembrane domains for FetB and their internal localization were predicted using the TMHMM v. 2.0 server (4).

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ALS3          MDLKWDDFFN------------DYEWLIVFLKGMVKPAAALVVVLLAVILSYSQNLSLEG 48
Star2         MMASMAALLQRLLVVVNQVDPGAPGFWREFLVGMLKPVAATAVVAMAVALSFTQRLGLEG 60
FetB          --------------------------MNSHNITNESLALALMLVVVAILISHKEKLALEK 34
                                           .     . . *  :* :*: :*..:.*.**

ALS3          EMIYSVSRSFLQLSVIGFVLQFIFNQENSGWIILAYLFMVSVAGYTAGQRARHVPRGKYV 108
Star2         EMLYAMARAFLQLSVIGFVLQFIFTQKSAAWILLAYLFMVTVAGYTAGQRARHVPRGKHI 120
FetB          DILWSVGRAIIQLIIVGYVLKYIFSVDDASLTLLMVLFICFNAAWNAQKRSKYIAKAFIS 94
              ::::::.*:::** ::*:**::**. ..:.  :*  **:   *.:.* :*::::.:.

ALS3          AGLSILAGTSITMFLLVLLNVFPFTPRYMIPIAGMLVGNAMTVTGVTMKQLRDDIKMQLN 168
Star2         AAVSILAGTSVTMALLVALRVFPFTPRYIIPVAGMMVGNAMTVTGVTMKKLREDVGMQRG 180
FetB          SFIAITVGAGITLAVLILSGSIEFIPMQVIPIAGMIAGNAMVAVGLCYNNLGQRVISEQQ 154
              : ::* .*:.:*: :*:    : * *  :**:***:.****...*:  ::* : :  :

ALS3          LVETALALGATPRQATLQQVKRALVISLSPVLDSCKTVGLISLPGAMTGMIMGGASPLEA 228
Star2         VVETALALGATPRQATARQVRRSLVIALSPVIDNAKTVGLIALPGAMTGLIMGGASPLEA 240
FetB          QIQEKLSLGATPKQASAILIRDSIRAALIPTVDSAKTVGLVSLPGMMSGLIFAGIDPVKA 214
               ::  *:*****:**:   :: ::  :* *.:*..*****::*** *:*:*:.* .*::*

ALS3          IQLQIVVMNMMVGAATVSSITSTYLCWPSFFTKAYQLQTHVFSSD 273
Star2         IQLQIVVMNMLMGASTVSSILSTYLCWPAFFTGAFQLNDAVFAAD 285
FetB          IKYQIMVTFMLLSTASLSTIIACYLTYRKFYNSRHQLVVTQLKKK 259
              *: **:*  *::.::::*:* : ** :  *:.  .**    :  .
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**Fig. S2. Protein homology between FetB, ALS3 and Star2.** The sequence alignment of FetB, ALS3 and Star2. Sequence alignment was performed using ClustalW (2, 5). The consensus symbols are represented as generated by ClustalW (* indicates a fully conserved residue, : indicates residues with strongly similar properties, and . indicates conservation of residues with weakly similar properties).

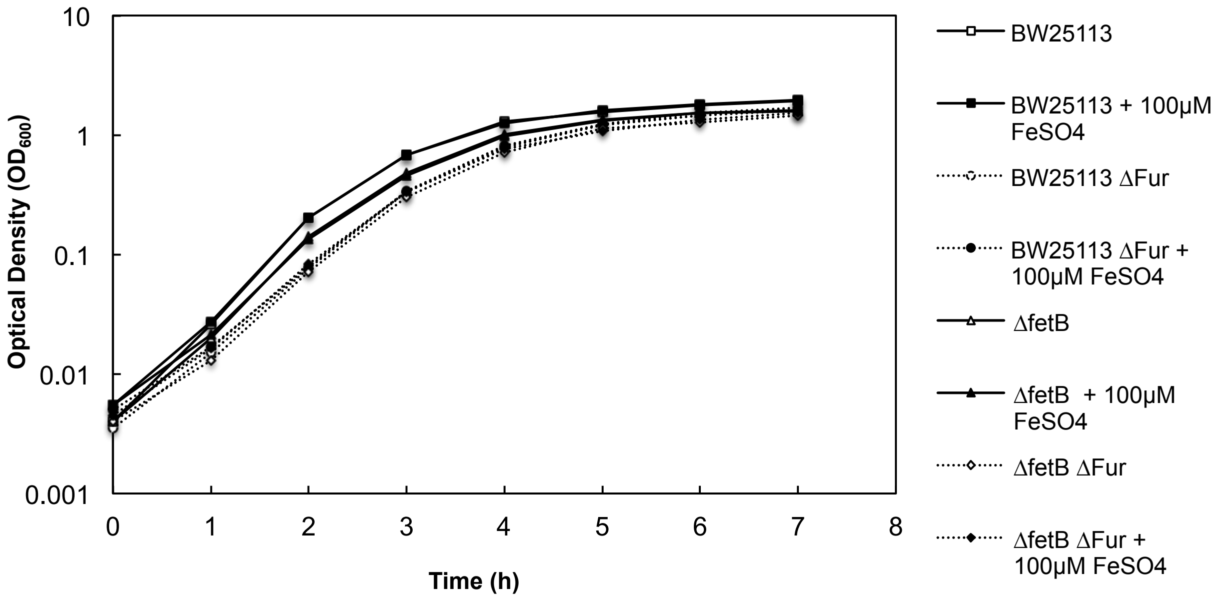


**Fig. S3. Growth of *E. coli* BW25113 strains in LB media with 100 µM $FeSO_4$.** Overnight cultures grown in LB were diluted 100-fold in fresh LB medium and grown for 2 hours to early exponential phase. The cultures were then diluted 100-fold into 20 ml fresh LB containing 100 µM $FeSO_4$ (filled markers) or no additives (empty markers) and growth was monitored spectrophotometrically. Dashed lines represent the Δ*fur* strains. The strains are marked as follows: WT BW25113 squares, BW25113 Δ*fur* circles, BW25113 Δ*fetB* triangles, BW25113 Δ*fetB* Δ*fur* diamonds. Experiments were performed in duplicate and the average is shown.

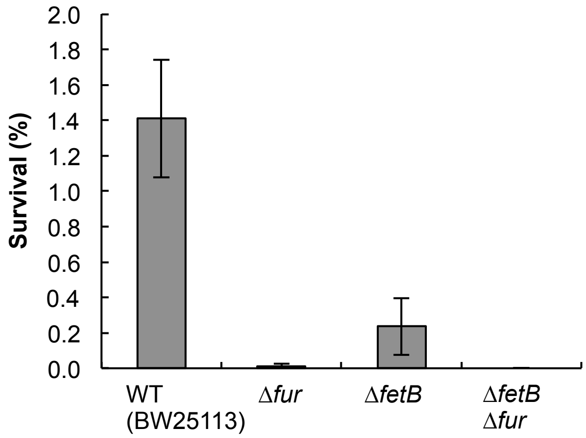


**Fig. S4. Survival of the WT BW25113, BW25113 Δ*fur*, BW25113 Δ*fetB*, and the BW25113 Δ*fetB* Δ*fur* strains after 30 min 4 mM $H_2O_2$ stress in LB supplemented with 30 µM $FeSO_4$.** The Δ*fur* knockouts exhibit increased sensitivity to $H_2O_2$ stress when supplemented with iron. The survival rate of the BW25113 Δ*fur* Δ*fetB* strains could not be determined as it was below the resolution of the plating assay. Data are means ± SEM ($n$ = 3).

***Investigating the protective role of manganese against oxidative stress by $H_2O_2$***

The role of manganese in $H_2O_2$ stress was investigated by culturing the BW25113, Δ*fetA* and Δ*fetB* with the pCntl and pF3 (*fetA*-*fetB*) overexpression plasmids in minimal media supplemented with 30 μM $MnCl_2$. Manganese provided a protective effect to all strains, as evidenced in Fig. S5 A and B (compared to Fig. 3A & B). The Δ*fetA* and Δ*fetB* strains have higher tolerance to $H_2O_2$ stress when supplemented with manganese, indicating that the metal is providing this protective effect. Furthermore, this suggests that FetAB does not facilitate manganese transport as tolerance was increased in the knockouts when manganese was present.

The data presented in this study shows that FetAB transports iron. However, iron importers have been shown to also facilitate manganese import, such as a SitABCD homologue in *E. coli* (7) and the *Streptococcus pyogenes* MtsABC transporter (3). We investigated the effects of manganese (Fig. S5A & B) and iron (Fig. 4 A & B) individually, and show that iron is the substrate of FetAB. We also investigated the effects of both metals (Fig. S5 C & D), to determine if the beneficial effect of manganese can counteract the detrimental effect of iron when coupled with $H_2O_2$ stress. As shown in Fig. S5C, iron is more detrimental to the Δ*fetA* and Δ*fetB* strains compared to the WT strain, even when manganese is present. This is in agreement with our earlier data, where iron (with no manganese) was shown to be more detrimental to the knockout strains. Manganese cannot abolish the extra ROS species created from the iron, but does provide some protective effect against $H_2O_2$ stress. Thus, the FetAB transporter does not appear to have a role in manganese transport, but clearly has a role in iron export.

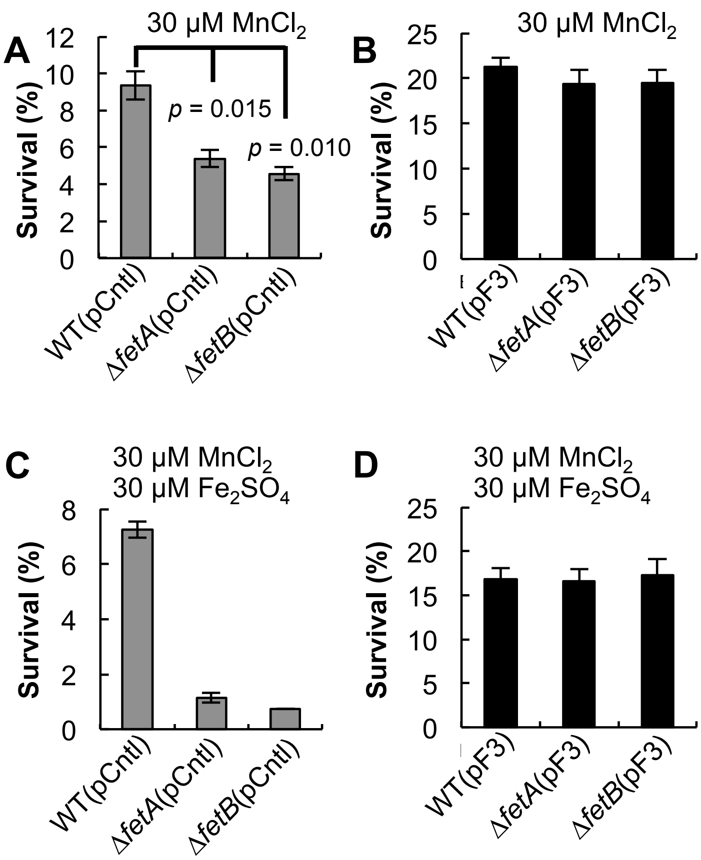


**Fig. S5**. Survival under $H_2O_2$ stress in M9 minimal media with 30 μM $MnCl_2$ or 30 μM $MnCl_2$ and 30 μM $FeSO_4$. *(A)* Survival rates of *E. coli* BW25113 (WT), Δ*fetA* and Δ*fetB* strains with a control plasmid (pCntl) after a 30 minute exposure to 4 mM $H_2O_2$ at 37 °C in M9 minimal media with 30 μM $MnCl_2$. *p*-values indicate the statistical significance of a Student's t-test performed between the WT and the KO strains. *(B)* Survival rates of *E. coli* BW25113 (WT), Δ*fetA* and Δ*fetB* strains with plasmid pF3 (*fetA* and *fetB* overexpression) after a 30 minute exposure to 4 mM $H_2O_2$ at 37 °C in M9 minimal media with 30 μM $MnCl_2$. *(C)* Survival rates of *E. coli* BW25113 (WT), Δ*fetA* and Δ*fetB* strains with a control plasmid after a 30 minute exposure to 4 mM $H_2O_2$ at 37 °C in M9 minimal media with 30 μM $MnCl_2$ and 30 μM $FeSO_4$. The *p*-values between the WT and KO strains are less than 0.001. (D) Survival rates of *E. coli* BW25113 (WT), Δ*fetA* and Δ*fetB* strains with plasmid pF3 (*fetA* and *fetB* overexpression) after a 30 minute exposure to 4 mM $H_2O_2$ at 37 °C in M9 minimal media with 30 μM $MnCl_2$ and 30 μM $FeSO_4$. For (A) – (D), the means of three biological experiments, each with three technical replicates are shown, with error bars indicating the SEM.

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