SUPPLEMENTARY INFORMATION

The *Bradyrhizobium japonicum* ferrous iron transporter FeoAB is required for ferric iron utilization in free-living aerobic cells and for symbiosis

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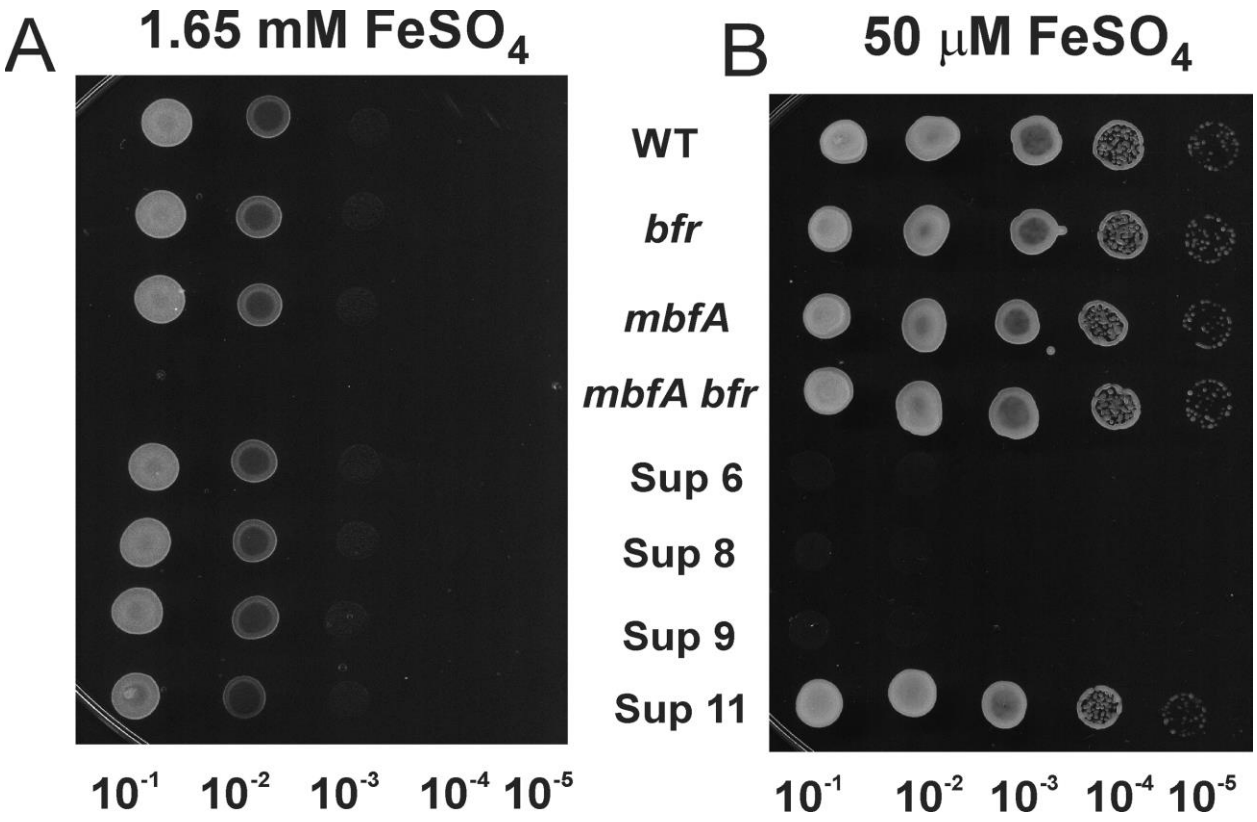

FIGURE S1. **Growth phenotypes of the *mbfA bfr* suppressor strains:** Cells of the wildtype, *bfr*, *mbfA* , *mbfA bfr* , Suppressor 6 (Sup6), Suppressor 8 (Sup8), Suppressor 9 (Sup9) and Suppressor 11 (Sup11) strains were grown in modified GSY medium supplemented with 100 nM heme. Cells were then serial diluted 10^{-1}- to 10^{-5}-fold and 10 μl spotted on plates containing either 1.65 mM $FeSO_4$ (A) or 50 μM $FeSO_4$ (B).

A

B

Suppressor	Genome location	Nucleotide change	Amino acid change	Gene
Sup 6	7180337-7180345	ΔGTACATCTT	Δ 571-WYIF-574 to Cys	*feoB*
Sup 8	7180313-7180314	Transposon insertion	Insertion between Ala563 and Thr564	*feoB*
Sup 9	7179986	C>A	Tyr454term	*feoB*
Sup 11	7178417	G>A	Glu40Lys	*feoA*

FIGURE S2. **Suppressor mutations occur within the *feoA* and *feoB* genes**. (A) Organization of *feoAB* operon of *B. japonicum* and positions of the suppressor mutations in the operon**.** Genetic orientation of the *feoA* and *feoB* genes in genome of *B. japonicum* USDA 110 is diagrammatically represented. The approximate positions of the suppressor mutations in the operon are indicated. Type of suppression and the respective suppressor number are also indicated. Inverted closed triangle represents a transposon insertion and open triangle indicates deletion. (B) Mutations in the *mbfA bfr* suppressor strains**.** Nucleotide sequence changes were identified by whole genome sequencing and confirmed by re-sequencing of PCR fragments that include the mutation.

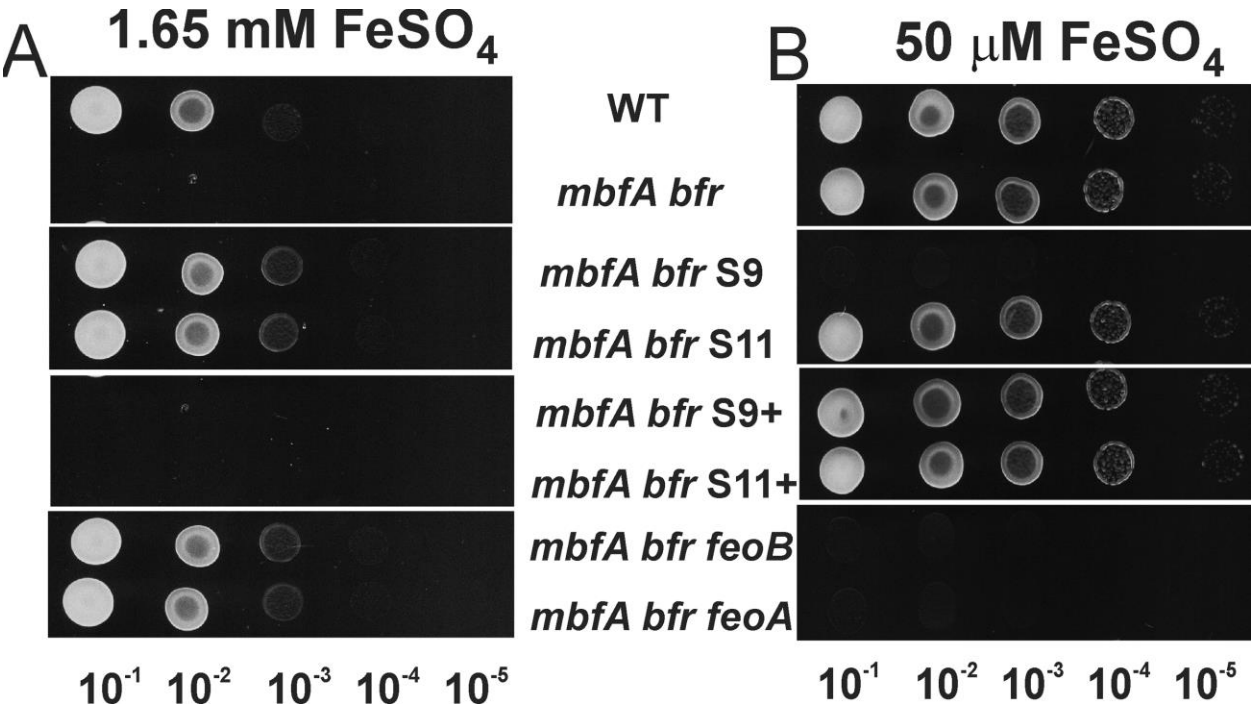

FIGURE S3: **Growth of mutants harboring *feoA* or *feoB* suppressor alleles or deletions in the *mbfA bfr* background.** Cells grown in liquid medium supplemented with 100 nM heme were serial diluted 10^{-1}- to 10^{-5}-fold and 10 μl was spotted on plates containing either 1.65 mM $FeSO_4$ (A) or 50 μM $FeSO_4$ (B). *mbfA bfr* S9 and *mbfA bfr* S11 denote the *mbfA bfr* mutant strain with suppressor alleles *feoB(Y454term)* and *feoA(E40K)* respectively. *mbfA bfr* S9+ denotes partial diploid which contain both the wildtype *feoB* allele and the *feoB(Y454term)* allele. Similarly, *mbfA bfr* S11+ strain contain both the wildtype *feoA* allele and the *feoA(E40K)* allele. Spotting at each iron condition was done on a single plate, and the image was separated for clarity of presentation.

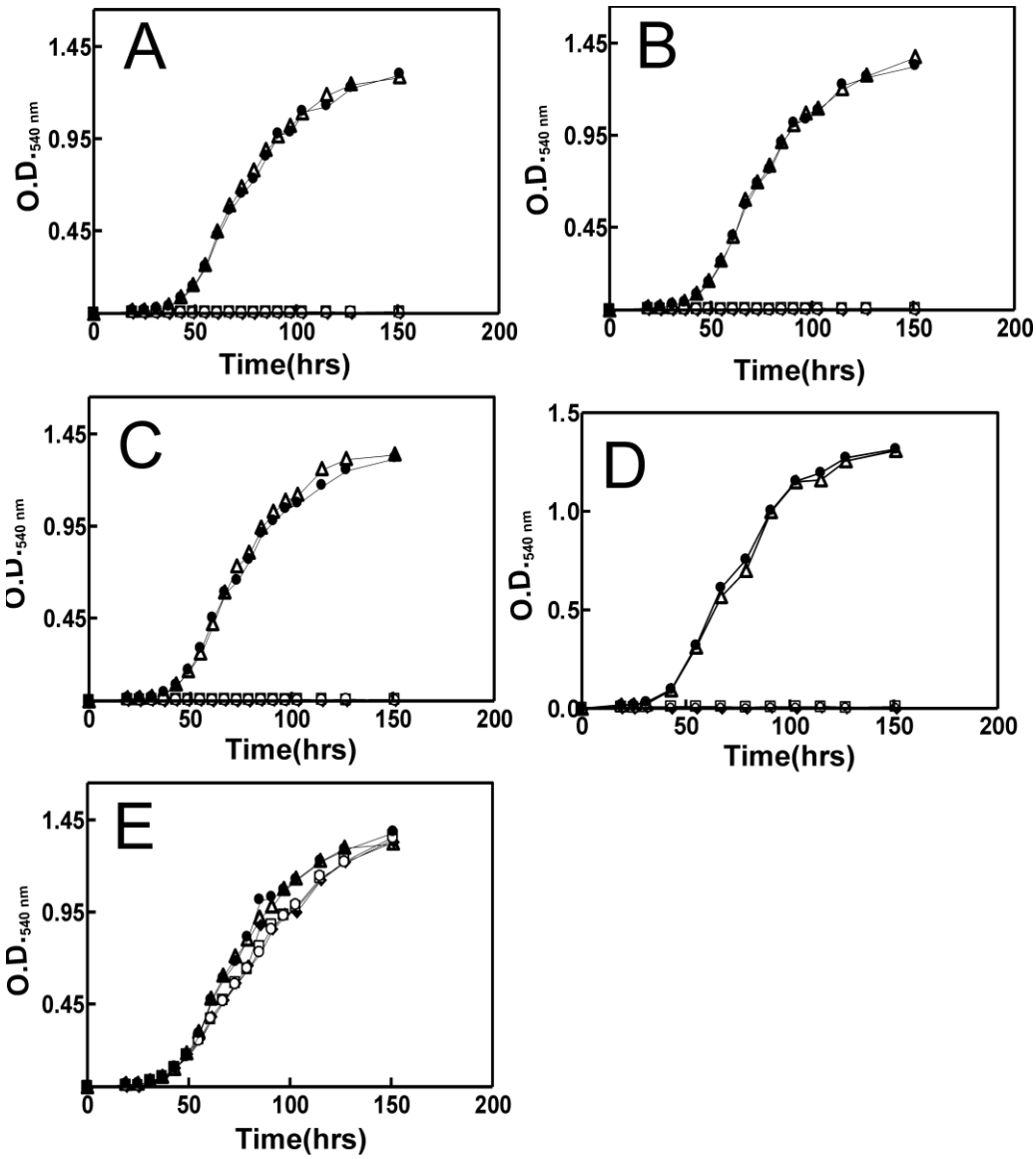

FIGURE S4. **Growth curves of wildtype and mutant strains in GSY medium with various ferric iron conditions.** Growth media were inoculated with 1×10^6 cells/ml of the wildtype (closed circles), *feoB* (open circles) *feoA* (closed diamonds), *feoB(Y454term)* (open squares) and *feoA(E40K)* (open triangles) strains. Strains were grown in modified GSY medium supplemented with either no added iron (A), 5 µM $FeCl_3$ (B), 50 µM $FeCl_3$ (C), 500 µM $FeCl_3$ (D) or 0.5 µM heme (E). Aliquots were taken at the indicated time points and the optical density was measured at 540 nm (OD_{540}).

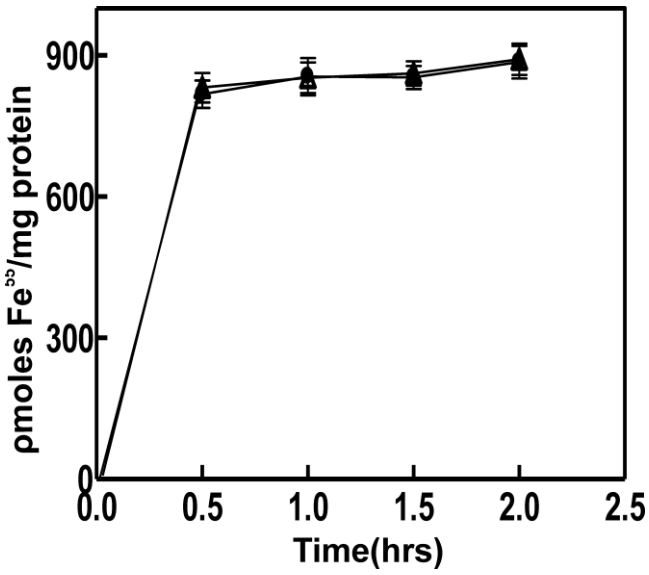

FIGURE S5. **Incorporation of radiolabel from radioactive iron in plant cytosol:** Roots with attached nodules incited by either the wildtype strain (closed circles) or *feoA(E40K)* strain (open triangles) were incubated with 5 µM $^{55}Fe^{2+}$ at time zero. At each time point, roots with attached nodules were removed, and bacteroid and plant cytosol fractions were prepared. Each time point is the average of three biological replicates ± S.D (*error bars*)