The Role of the FRE Family of Plasma Membrane Reductases in the Uptake of Siderophore-Iron in Saccharomyces cerevisiae*

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Saccharomyces cerevisiae takes up siderophore-bound iron through two distinct systems, one that requires siderophore transporters of the ARN family and one that requires the high affinity ferrous iron transporter on the plasma membrane. Uptake through the plasma membrane ferrous iron transporter requires that the iron first must dissociate from the siderophore and undergo reduction to the ferrous form. FRE1 and FRE2 encode cell surface metalloreductases that are required for reduction and uptake of free ferrous iron. The yeast genome contains five additional FRE1 and FRE2 homologues, four of which are regulated by iron and the major iron-dependent transcription factor, Aft1p, but whose function remains unknown. Fre3p was required for the reduction and uptake of ferrioxamine B-iron and for growth on ferrioxamine B, ferrichrome, triacetylfusarinine C, and rhodotorulic acid in the absence of Fre1p and Fre2p. By indirect immunofluorescence, Fre3p was expressed on the plasma membrane in a pattern similar to that of Fet3p, a component of the high affinity ferrous transporter. Enterobactin, a catecholate siderophore, was not a substrate for Fre3p, and reductive uptake required either Fre1p or Fre2p. Fre4p could facilitate utilization of rhodotorulic acid-iron when the siderophore was present in higher concentrations. We propose that Fre3p and Fre4p are siderophore-iron reductases and that the apparent redundancy of the FRE genes confers the capacity to utilize iron from a variety of siderophore sources.

Virtually every organism on earth requires iron as an essential nutrient. Although iron is the second most abundant metal in the crust of the earth, the bioavailability of iron can be extremely low. This poor bioavailability occurs because iron is rapidly oxidized in an aerobic environment to the ferric form (Fe(III)),¹ which is poorly soluble in water and forms precipitates of oxihydroxides. Microorganisms have the capacity to scavenge iron from insoluble precipitates by secreting and taking up siderophores, low molecular weight compounds that bind to Fe(III) with very high affinity and specificity. Siderophores are synthesized and secreted in the iron-free form, which then binds and solubilizes Fe(III) in the extracellular environment. The Fe(III)-siderophore complex is then recognized and selectively taken up by specific transport mechanisms. Many microorganisms synthesize one or a few types of siderophores, yet have the capacity to take up iron from a variety of siderophores secreted by other species of bacteria and fungi (1). Budding and fission yeast appear to be an exception; they neither synthesize nor secrete these compounds (2, 3). Saccharomyces cerevisiae can, however, recognize and take up iron from a variety of structurally distinct siderophores (4–10).

S. cerevisiae has two genetically separable systems for the uptake of siderophore-bound iron. One system depends on a family of homologous transporters of the major facilitator superfamily that is expressed as part of the AFT1 regulon and are termed ARN1, ARN2 (also TAF1), ARN3 (also SIT1), and ARN4 (also ENB1) (6–11). These transporters are expressed in intracellular vesicles. The individual ARN transporters exhibit specificity for different siderophores of the hydroxamate and catecholate classes; however, some siderophores, such as rhodotorulic acid, are not substrates of the ARN transporters (9). A second system of uptake for siderophore-bound iron depends on the high affinity ferrous iron (Fe(II)) transport complex, which is encoded by FET3 and FTR1 and is located on the plasma membrane (12–15). A low affinity Fe(II) transporter encoded by FET4 is also expressed on the plasma membrane (16). For the siderophore-bound Fe(III) to become a substrate for the Fe(II) transporter, the iron must be both reduced and dissociated from the siderophore. This is accomplished in a single step by the activity of plasma membrane reductase systems, which contain flavocytochromes and have the capacity to reduce siderophore-bound iron (4, 5, 17). FRE1 and FRE2 encode plasma membrane metalloreductases that can reduce oxidized forms of both iron and copper (18–23). Strains deleted for FRE1 exhibit only 10% of the Fe(III)-citrate reductase activity that is inducible in wild-type strains. Deletion of both FRE1 and FET4 results in cells that are completely lacking Fe(III)-citrate reductase activity and fail to grow on iron-poor media. The completed sequence of the S. cerevisiae genome revealed the presence of five additional genes with striking similarity to FRE1 and especially to FRE2. Four of these (FRE3, FRE4, FRE5, and FRE6) are greater than 35% identical to FRE2 and are regulated at the transcriptional level by Aft1p (24). The fifth homologue (FRE7) is regulated by exogenous copper ions through the Mac1p transcription factor. The functions of these new FRE family members have not been identified.

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¹ The abbreviations used are: Fe(III), ferric iron; Fe(II), ferrous iron; FOB, ferrioxamine B; FC, ferrichrome; TAF, triacetylfusarinine C; ENT, enterobactin; RA, rhodotorulic acid; BPS, bathophenanthroline disulfonate; HA, hemagglutinin; PCR, polymerase chain reaction.

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We have investigated the role of the FRE family of genes in the uptake of siderophore-bound iron. Although the FRE genes appear to have no role in the Arn-dependent uptake of siderophores, they were required for the uptake of siderophore-bound iron through the high affinity Fe(II) transport system. Although FRE1 and FRE2 encoded the majority of siderophore reductase activity, Fre3p could specifically facilitate reduction and uptake of iron bound to the trihydroxamid siderophores ferroxamine B (FOB), ferrichrome (FC), and triacetylferrixyanine C (TAFC) and to the dihydroxamid rhodotorulic acid (RA). Fre3p was expressed on the plasma membrane in a pattern consistent with its role in iron uptake through the plasma membrane Fe(II) transport system. Uptake of iron bound to the catecholate siderophore enterobactin (ENT) also occurred through the Fe(II) transport system and required either Fre1p or Fre2p. Expression of Fre4p was sufficient to facilitate the utilization of RA-bound iron when the siderophore was present in higher concentration.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Plasmids, and Media—** All strains were constructed in YPH499. Mating was performed by YHR245 MATa x YPH499 MATα (32°C for 5-6 days). Clones were screened by colony hybridization using a PCR-amplified FRE3 open reading frame as a probe. Yeast transformations were performed using the YEp351 vector (17). Cells were grown in 60-ml liquid YPD (1% yeast extract, 2% Bacto-peptone, and 2% dextrose) at 30°C with vigorous aeration until mid-log phase. Plates for low concentrations of Fe were prepared in triplicate, and the experiment was repeated three times. Error bars indicate the S.D.

**RESULTS**

**Reductive Uptake of Siderophore-Iron in Budding Yeast**

![Image](http://www.jbc.org/)

**Fig. 1.** Capacity of the FRE gene products to reduce Fe(III)-siderophores in FRE+ and fre− deletion strains. After culture in rich media or defined iron media containing 10 μM ferrous iron (inset), centraleldigenerated iron-Fe(II) in the exponential phase of growth were collected, washed, and assayed for Fe(III)-siderophore reductase activity in 1 mM Fe(III)-ferrichrome, -ferrioxamine B, -fusarinine, and rhodotorulic acid with ferrozine as the Fe(II) acceptor. Absorbance at 562 nm was measured. The experiments were repeated twice, and data from a representative experiment are shown. Inset, the fre1Δ2Δ and the fre1Δ2Δ strain were transformed with either a high copy number plasmid bearing the FRE3 gene (pFRE3) or the empty parent plasmid (pRS424) and the Fe(III)-ferrioxamine B reductase activity measured as described. Samples were prepared in triplicate, and the experiment was repeated three times. Error bars indicate the S.D.

sulfate, and the assay was performed as described (18) with the following modifications. Ferrozine was substituted for bathophenanthroline disulfonate (BPS) as the Fe(II) acceptor, and 1 mM Fe(III)-FC, -FOB, -TAFC, and -RA were the reductase substrates. Absorbance of the cell-free assay mixture was measured at 562 nm.

**Plate Assay and Uptake Assay—** For the plate assay, modified synthetic complete media were used in which copper and iron were omitted, and 1 μM copper sulfate and 100 μM BPS were added. Trace amounts of iron are supplied by agar (Difco). Plate assays for low concentrations of FC, FOB, TAFC, and ENT, and RA were performed as described (10) using 10 μM desferri-FC, -FOB, and -TAFC, and 5 and 10 μM ferric-ENT, and 15 μM ferri-RA (concentration with iron-chelating capacity equivalent to 10 μM Fe(III), FOB, TAFC, and ENT). Plate assays for high concentrations of FOB and RA were performed using 100 μM of the desferri-siderophore. Because of ligand exchange between the BPS and the siderophores, the exact concentration of the ferric-siderophore complex in the plate assay is not known. Uptake assays were performed as described (30) in Multiscreen Filtration plates (Millipore) using 1 μM each of 56Fe(III) and desferri-FOB and -ENT.

**Immunofluorescence—** Strains YPH499 pFRE3-ΔHA and YPH499 FET3-ΔHA PRT1-Myc were grown to mid-log phase in defined iron media containing 5 μM ferrous ammonium sulfate and 1 mM ferrozine to induce the expression of Fre3p. Cells were washed and prepared for immunofluorescence microscopy as described (15). Primary antibody was affinity-purified HA.11 (Babco) at 1:300, and secondary antibody was Cy3-conjugated polyclonal anti-mouse IgG from donkey (Jackson Immunolab). Images were acquired with a cooled CCD camera (Cooke) using IP Labs software (Scanalytics).
the Fe(II) transport system and the Arn1p and Arn3p transporters, displayed the lowest rate of reduction. FRE1 and FRE2 encoded 90–98% of the siderophore reductase activity, but there were measurable differences in the siderophore reductase activities of a fre1Δ fre2Δ strain and a fre1Δ fre2Δ strain that also carried a deletion of FRE3 (Fig. 1, inset). Deletion of FRE3 in the fre1Δ fre2Δ strain resulted in a 73% decrease in the reduction of FOB. This loss of FOB reductase activity was completely restored when the fre1Δ fre2Δ strain was transformed with either a high copy number plasmid containing the FRE3 gene (pFRE3) or the parent vector (pRS414) and grown to exponential phase in defined iron media containing 10 μM ferrous ammonium sulfate to induce expression of Fre3p. Uptake of 55Fe in the presence of either 1 μM FOB (gray bars) or 10 μM FOB (black bars) was measured. C, failure of FOB to stimulate growth in yeast deleted for ARN3, FRE1, FRE2, and FRE3. Congenic strains of the indicated genotype were plated in serial dilutions on synthetic iron-poor media containing 100 μM BPS (–) or 100 μM BPS and 10 μM desferri-FOB (+). Plates were incubated at 30 °C for 3 days.

The Role of FRE3 in Ferrioxamine B-mediated Iron Uptake and Growth—FOB-bound iron can be transported into the cell by both an Arn3p-dependent system and a Fre3p-dependent system that also involves an Fe(III) to Fe(II) reduction step. We tested the role of FRE1, FRE2, and FRE3 in the uptake of FOB-iron in strains both expressing Arn3p and deleted for Arn3p (Fig. 2A). When cells were grown in rich media, deletion of FRE3 alone resulted in an increased uptake of FOB-iron when compared with the congenic parent strain. This effect was consistently reproducible and suggested that deletion of FRE3 resulted in changes in the intracellular iron pool. Deletion of FRE1 and FRE2 resulted in dramatic decreases in FOB-iron uptake, both in an ARN+ and an arn3Δ background, indicating that Fre1p and Fre2p reductase activities contribute to FOB-iron uptake. Deletion of FRE3 in addition to FRE1 and FRE2 resulted in a small, additional decrease in FOB-iron uptake in both the ARN+ and the arn3Δ backgrounds, so that there was no detectable FOB-iron uptake in the arn3Δ fre1Δ fre2Δ fre3Δ strain. To determine whether Fre3p contributed significantly to FOB-iron uptake, Fre3p was overexpressed from a high copy-number vector in the arn3Δ fre1Δ fre2Δ fre3Δ strain (Fig. 2B). Fre3p overexpression resulted in a slight increase in uptake when FOB-iron was present at 1 μM and a larger increase in uptake when FOB-iron was present at 10 μM. These data indicated that Fre3p could facilitate the reductive uptake of FOB-iron.

To clarify further the role of FRE1, FRE2, and FRE3 in the utilization of FOB-iron, we examined the growth of FRE-deleted strains on iron-limited media containing FOB as an iron source (Fig. 2C). We used strains in which ARN-dependent FOB uptake was inactivated by deletion of ARN3; therefore, iron uptake was dependent on Fre3p and the high affinity Fe(II) uptake system. An arn3Δ fre1Δ fre2Δ strain did not grow in the absence of FOB, but grew as well as the FRE+ parent strain in the presence of FOB, indicating that the reductase activities encoded by FRE1 and FRE2 are not absolutely required for utilization of FOB-iron. An arn3Δ fre1Δ fre2Δ fre3Δ strain, however, failed to grow in the presence of FOB, confirming that Fre3p can facilitate the uptake and utilization of FOB-iron. This result also indicated that endogenous levels of expression of Fre4p, Fre5p, Fre6p, and Fre7p could not support the reductive uptake of FOB-iron. Deletion of FRE3 alone had no effect on growth on FOB, and transformation of the arn3Δ fre1Δ fre2Δ fre3Δ strain with a low copy number plasmid carrying either the FRE1 or FRE2 gene completely restored growth on FOB (data not shown). Fre1p, Fre2p, and Fre3p are not required for uptake of FOB through the ARN-dependent system, as an ARN+ fre1Δ fre2Δ fre3Δ strain grew well on FOB (Fig. 2C).

The Role of Fre3p in the Uptake of Ferrichrome, Triacetyl- fusarinarine C, and Rhodotorulic Acid—Because Fre3p could facilitate the reductive uptake of FOB-iron, we questioned whether structurally related siderophores were also substrates for Fre3p. The iron-binding sites of FC and TAFC are similar in structure to that of FOB in that they each consist of three bidentate hydroxamate ligands that bind Fe(III) in a six-coordinate complex. We examined the capacity of the FRE homologues to facilitate growth when FC and TAFC in low concentration (10 μM) were provided as iron sources (Fig. 3, A and B). Again, these experiments were performed using strains in which the ARN-dependent siderophore uptake systems were inactivated by deletion of ARN1 and ARN3 to test FC and by deletion of ARN2 to test TAFC. Deletion of either FRE3 alone or FRE1 and FRE2 together had no effect on FC-mediated growth (Fig. 3A). Deletion of FRE1, FRE2, and FRE3 in combination, however, resulted in a failure to grow on FC, indicating that Fre3p can facilitate reductive iron uptake from both FC and FOB. As was the case for FOB, deletion of FRE1, -2, and -3 in an ARN+ background did not impede FC-mediated growth, further supporting the role of the FRE genes in siderophore-iron uptake through the Fe(II) transport system but not through the ARN system. Similar results were obtained when a strain deleted for FRE1, -2, and -3 was tested for TAFC-mediated growth (Fig. 3B), indicating that Fre3p can
facilitate reductive iron uptake from each of the three trihydroxamate siderophore tested.

RA-mediated iron uptake in *S. cerevisiae* significantly differs from FOB-, FC-, and TACF-mediated uptake in that RA is not a substrate for the ARN transporters and RA-iron uptake occurs exclusively through the Fe(II) transport system (9). Also, RA is a dihydroxamate siderophore that coordinates Fe(III) in a RA₃Fe₂ complex (31). Despite these differences, Fre3p also facilitated RA-iron-mediated growth (Fig. 3C), as the fre1Δ fre2ΔFRE3Δ strain grew as well as the congenic parent strain on ferric-RA, whereas the fre1Δ fre2Δ fre3Δ strain did not grow.

**Localization of Fre3p to the Plasma Membrane**—Both components of the high affinity Fe(II) transport system, Fet3p and Fre4p, are expressed on the plasma membrane (10, 13–15). In contrast, the Arn1p, Arn3p, and Arn4p transporters are largely confined to intracellular vesicles that comigrate on density gradients with Pep12p, a protein of the late endosome (9, 10) (data not shown). To determine the cellular localization of Fre3p, we constructed a strain in which the chromosomal copy of *FRE3* carries a triple copy of the HA epitope at the carboxyl terminus. We confirmed that the epitope-tagged Fre3p was functional by expressing the HA-tagged Fet3p from a plasmid in a *FRE3*-deleted strain and observing restoration of FOB-iron uptake to wild-type levels. We grew the Fre3p-HAp strain and a strain expressing an HA-tagged version of Fet3p in media containing limiting amounts of iron (to induce Fre3p-HAp expression) and performed indirect immunofluorescence (Fig. 4). Fre3p was detected at the periphery of the cell in a pattern that was similar to that of Fet3p, indicating that both Fre3p and Fet3p were expressed on the plasma membrane. Fre3p was not detected in intracellular vesicles. This localization to the plasma membrane was consistent with a role for Fre3p in reductive iron uptake at the plasma membrane through the high affinity Fe(II) system. The absence of detectable Fre3p in intracellular vesicles is also consistent with genetic data indicating that Fre3p is not required for ARN-dependent siderophore uptake.

**Reductive Uptake of Fe(III)-Enterobactin**—ENT is a siderophore of the catecholate class that is synthesized and secreted by species of Gram-negative bacteria, such as *E. coli*. *S. cerevisiae* was recently reported to take up ENT through the ARN4 transporter (7), but it is not known whether ENT-bound iron can be taken up via a reductive mechanism that requires the high affinity Fe(II) transport system. We examined the uptake of ENT-bound iron in strains bearing a deletion of *ARN4* alone and deletions of all four *ARN* genes, both in strains with intact high affinity Fe(II) transport (*FET3*) and in strains lacking high affinity Fe(II) transport (*fet3Δ*, Fig. 5A). Deletion of *ARN4* in both *FET3*+ and *fet3Δ* backgrounds resulted in decreases in ENT-iron uptake to undetectable levels, confirming that uptake of ENT-iron is facilitated by Arn4p. Uptake of ENT-iron was increased over 10-fold in a *fet3Δ* strain, and this was associated with a large increase in the level of expression of the Arn proteins, especially Arn4p, in the *fet3Δ* strain (data not shown). This increase likely reflects the relative iron deprivation and subsequent Aft1p activation that results from the loss of high affinity Fe(II) uptake in *fet3Δ* strains. These results suggested that the majority of ENT-iron uptake occurs through Arn4p, but whether ENT-iron could also be taken up through a reductive mechanism remained unclear.

To evaluate ENT-iron uptake via the high affinity Fe(II) system, we tested the capacity of ENT to stimulate growth of strains on iron-limited media (Fig. 5B). Strains individually deleted for *ARN4* or *FET3* grew as well as the wild-type parent strain in the presence of ENT. Deletion of both *ARN4* and *FET3* resulted in a strain that did not grow in the presence of ENT, indicating that ENT is a substrate for both the ARN-dependent and FET3-dependent systems of iron uptake. To determine the role of the *FRE* genes in FET3-dependent ENT-iron uptake, we tested the capacity of ENT to stimulate growth of FRE-deleted strains that were also deleted for *ARN4* (Fig. 5C). In this experiment, the *arn4Δ FRE+* and the *arn4Δ fre1Δ* strains grew well on iron-limited media in the presence of ENT and very slowly in the absence of ENT. In contrast, the *arn4Δ fre1Δ fre2Δ* and the *arn4Δ fre1Δ fre3Δ* strains exhibited virtually no growth in the presence or absence of ENT. Expression of *FRE1* from a low copy number plasmid restored ENT-mediated growth to the *arn4Δ fre1Δ fre2Δ* strain (data not shown), indicating that either Fre1p or Fre2p is also required for the FET3-dependent utilization of ENT-iron.

**The Role of Fre4p in Rhodotorulic Acid Iron Utilization**—Fre4p, Fre5p, and Fre6p did not appear to have a role in the reductive uptake of low concentrations of siderophore-bound...
iron, because endogenous levels of expression of these proteins in fre1Δ fre2Δ fre3Δ strains did not provide sufficient activity to result in growth on low concentrations of siderophore. This apparent lack of function could occur because of the following: 1) FRE4, FRE5, and FRE6 are not expressed as functional enzymes on the plasma membrane; 2) the siderophores we tested were not substrates for Fre4p, Fre5p, and Fre6p; or 3) the tested siderophores were substrates but exhibited lower affinity for Fre4p, Fre5p, and Fre6p. We tested the last hypothesis by examining the growth of FRE-deleted strains on a higher concentration of RA and FOB. In Fig. 6, each of the six iron-regulated FRE genes was serially deleted, and the resulting strains were tested for the capacity to grow in the presence of 100 μM RA and FOB. Deletion of FRE1, FRE2, and FRE3 had no effect on the capacity of a high concentration of RA to stimulate growth on iron-limited plates (Fig. 6A). Deletion of FRE1, FRE2, FRE3, and FRE4, however, resulted in a strain that grew very slowly on iron-limited media in the presence or absence of 100 μM RA. In contrast, a high concentration of FOB did not stimulate growth of the arn3Δ fre1Δ fre2Δ fre3Δ strain.

These data indicate that Fre4p could specifically facilitate the reductive uptake of RA-iron, but not FOB-iron, through the Fe(II) transport system.

**DISCUSSION**

Uptake of siderophore-bound iron through the high affinity Fe(II) transport system requires the activity of plasma membrane reductases that are encoded by the FRE family of genes. Here we have shown that Fre1p and Fre2p can facilitate the reduction of ferric-siderophores of the trihydroxamate and dihydroxamate classes. This finding was consistent with previous observations that the plasma membrane reductase system of budding yeast exhibits an extremely broad range of substrate specificity that includes both ferric and nonferric electron acceptors (32). We also demonstrated that Fre3p could facilitate the reduction of FOB and that Fre3p and Fre4p could facilitate the reductive uptake and utilization of siderophore-iron, presumably by acting as ferric-siderophore reductases. Although Fre4p could facilitate utilization of RA-iron, we were not able to detect significant Fre4p-dependent RA-iron reductase or uptake activities. The most likely explanation for these observations is that S. cerevisiae can grow in the face of extremely low rates of iron uptake, and it is less likely that Fre4p facilitates iron uptake through an activity other than that of a reductase. Fre3p and Fre4p exhibit the most similarity to Fre2p with 72 and 56% identity, respectively, at the amino acid level (24). Fre5p and Fre6p exhibit lesser degrees of similarity to Fre2p (38 and 35%, respectively), and the function of these gene products remains unknown. Although the siderophore reductase activity exhibited by Fre3p is 40-fold lower than that of Fre1p and Fre2p, FRE3 and FRE4 mRNA transcripts are present in quantities roughly comparable to those of FRE1 and FRE2 (24). This observation suggests that the differences in activity are due less to differences in the rate of transcription and more to differences in the intrinsic properties of the proteins. We observed that overexpression of Fre3p did not result in increased Fre3p-specific reductase activity when compared with the activity associated with endogenous levels of expression. This observation suggests the possibility that the plasma membrane reductase system has additional components that may be present in limiting quantities.

Although Fre1p, Fre2p, Fre3p, and Fre4p could facilitate the reductive uptake of siderophore-iron, they were not functionally interchangeable. Fre3p and Fre4p did not facilitate growth on low concentrations of ferric-ENT, but Fre3p could facilitate growth on low concentrations of di- and trihydroxamates. Fre4p did not facilitate growth in the presence of low concentrations of catecholate siderophore or dihydroxamate siderophores nor on high concentrations of the trihydroxamate FOB. Yet Fre4p did facilitate growth on high concentrations of the dihydroxamate RA. These results suggest that the FRE reductases differ both in their specificities and affinities for different siderophores. Further evidence of differing specificities is seen in the case of the FRE3 deletion strain. Deletion of FRE3 in cells grown in rich media leads to an increased rate of
The reductase and transport complexes may impede the expression of the Fet3p-Ftr1p complex on the indicated electrode. Variations in activity coefficients, due to differences in buffer type or ionic strength, are generally assumed to be small. Reduction potentials (31) are listed in mV. Each reduction potential was measured at pH 7 in dilute aqueous solution versus a standard hydrogen electrode. Variations in activity coefficients, due to differences in buffer type or ionic strength, are generally assumed to be small.

The differences in specificity exhibited by the FRE reductases may be based on differences in the structure of the iron coordination site in the siderophores. Alternatively, differences in specificity may be accounted for by the reduction potentials of the siderophores. In Table I, the capacities of siderophores to stimulate growth of FRE-deleted strains and the reduction potentials (31) of these siderophores are presented. ENT had the most negative reduction potential and the most stringent reductase requirements, stimulating Arn4p-independent growth only when Fre1p or Fre2p was expressed. Although the data presented here suggest that Fre1p or Fre2p can reduce ENT-iron, ENT, with a reduction potential of −750 mV, is thought to be beyond the range of physiological reductants (31).

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| Strain | ENT | FOB | FC | TAPC | RA |
|--------|-----|-----|----|------|----|
| fre1Δ  | +   | +   | +  | +    | +  |
| fre1Δ fre2Δ fre3Δ | +   | +   | +  | +    | +  |
| fre1Δ fre2Δ fre3Δ | −   | +   | +  | +    | +  |

growth stimulation

| Strain | ENT | FOB | FC | TAPC | RA |
|--------|-----|-----|----|------|----|
| −750   | −468| −400| −468| −350|

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