Roles of Siderophores, Oxalate, and Ascorbate in Mobilization of Iron from Hematite by the Aerobic Bacterium *Pseudomonas mendocina*†‡

Carolyn A. Dehner,¹ Jonathan D. Awaya,¹ ‡ Patricia A. Maurice,² and Jennifer L. DuBois¹*

Department of Chemistry and Biochemistry, University of Notre Dame,¹ and Department of Civil Engineering & Geological Sciences, University of Notre Dame,² Notre Dame, Indiana

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In aerobic, circumneutral environments, the essential element Fe occurs primarily in scarcely soluble mineral forms. We examined the independent and combined effects of a siderophore, a reductant (ascorbate), and a low-molecular-weight carboxylic acid (oxalate) on acquisition of Fe from the mineral hematite (α-Fe₂O₃) by the obligate aerobe *Pseudomonas mendocina* ymp. A site-directed ΔpmhA mutant that was not capable of producing functional siderophores (i.e., siderophore− phenotype) did not grow on hematite as the only Fe source. The concentration of an added exogenous siderophore (1 μM desferrioxamine B [DFO-B]) needed to restore wild-type (WT)-like growth kinetics to the siderophore− strain was ~50-fold less than the concentration of the siderophore secreted by the WT organism grown under the same conditions. The roles of a reductant (ascorbate) and a simple carboxylic acid (oxalate) in the Fe acquisition process were examined in the presence and absence of the siderophore. Addition of ascorbate (50 μM) alone restored the growth of the siderophore− culture to the WT levels. A higher concentration of oxalate (100 μM) had little effect on the growth of a siderophore− culture; however, addition of 0.1 μM DFO-B and 100 μM oxalate restored the growth of the mutant to WT levels when the oxalate was preexcreted with the hematite, demonstrating that a metabolizing culture benefits from a synergistic effect of DFO-B and oxalate.

Iron (Fe) is essential for almost all life. However, in aerobic, circumneutral environments, Fe is bound primarily in scarcely soluble minerals and amorphous solids [e.g., the solubility product (K_{SP}) for amorphous Fe(OH)₃ is 10^{-38}] (53) and is therefore poorly bioavailable. Aerobic microorganisms directly transform mineral-bound Fe(III) into soluble, bioavailable forms (1), overcoming significant kinetic and thermodynamic barriers to mineral dissolution and serving as primary transporters of Fe from the geosphere into global biogeochemical cycles.

A primary means by which aerobic microorganisms enhance Fe mobility and bioavailability is by secreting siderophores, which are structurally diverse, low-molecular-weight chelating agents with extremely high affinities for Fe(III) (12, 27, 37, 40). Fe(III)-siderophore stability constants can be as high as 10^{52} (1, 40), which is many orders of magnitude higher than the stability constants for low-molecular-weight organic acids, such as oxalic acid [for Fe(III) + 3 oxalate ⇌ Fe(oxalate)₃, K = 10^{18.6}] (45). While their high affinity for Fe(III) is clearly important for helping siderophores mobilize Fe from Fe(III) (hydr)oxides in the aqueous phase, the mechanisms of Fe mobilization appear to be complex and are the subject of much recent study (14, 17, 18, 26, 28, 49). In particular, the role of siderophores in ligand-promoted dissolution mechanisms has undergone careful evaluation *in vitro*. The model is described simply here as follows for amorphous Fe(OH)₃ and has been described in detail by Kraemer (26): Fe(OH)₃ + 3H⁺ ⇌ Fe(III) + 3H₂O (K_{SP}) (equation 1); Fe(III) + H₂L ⇌ FeL + 3H⁺ (K_{FeL}) (equation 2); and Fe(OH)₃ + H₄L ⇌ FeL + 3H₂O (K_{eq} = K_{SP}K_{FeL} = [FeL][H₂L]) (equation 3). The concentration of the solubilized FeL complex, according to equation 3, is determined as follows: [FeL] = [H₂L]K_{SP}K_{FeL}. The estimated concentration of siderophores in carbonic soil (~10^{-8} to 10^{-7} M), combined with their strong affinities for Fe(III) (39), suggests that [FeL] could in principle easily be micromolar or higher and could support vigorous bacterial growth. However, the trishydroxamate siderophores that have been studied most to date absorb only weakly to Fe(III) (hydr)oxide minerals, likely due to steric constraints, although charge repulsion may also play a role for positively charged siderophores, such as desferrioxamine B (DFO-B) (6, 26, 41, 42). Therefore, it has been proposed that siderophores act primarily in conjunction with other molecules, such as simple plant-derived carboxylic acids or reductants, which interact more strongly with mineral surfaces and release Fe directly through ligand-promoted and/or reductive mechanisms (52). This proposed “synergistic effect,” in which the combined effect of various elements is greater than the sum of the individual effects, suggests that an interaction of biogenic molecules may overcome kinetic and thermodynamic barriers to the release of Fe from minerals in the presence of siderophores. The role of the siderophore in such a synergistic system is not a direct role in surface processes; rather, the siderophore maintains a low concentration of aqueous Fe in equilibrium with the mineral (an Fe sink), thus driving the reaction toward more dissolution (26, 41). Only a low concentration of a siderophore relative to the concentra-
tions of surface-reacting organic species is required to promote efficient dissolution (26).

The synergistic effect has been observed directly in in vitro, abiotic experiments using combinations of microbe-derived siderophores and simple organic acids. A combination of environmentally relevant concentrations of oxalate (1 to 80 μM) and DFO-B (40 μM), for example, doubled the rate of Fe(III) hydroxide mineral (goethite) dissolution compared with the rate when only oxalate or DFO-B was present in a recent in vitro study (6). Actively metabolizing aerobic bacteria, which can move Fe from solution into cells and recycle or release new siderophores back into the medium, might be expected to promote the synergistic siderophore-carboxylic acid interaction even further in a batch system. Likewise, it has been suggested that organic reductants may work synergistically with siderophores. In particular, a recent study showed that exogenously added reductants significantly enhance the bioavailability of Fe to an aerobic siderophore-producing bacterium, *Pseudomonas mendocina* ymp (15), isolated from the Nevada Test Site and used in the work described here.

As an obligate aerobe, *P. mendocina* ymp does not have dissimilatory reduction pathways, so that its use of iron (hydr)oxide minerals is only for acquisition of nutritional Fe and not for cellular respiration. In contrast to dissimilatory Fe-reducing bacteria, which require millimolar concentrations of Fe (2, 29–31, 36, 43, 50), *P. mendocina* ymp requires micromolar concentrations (19, 20, 24, 32–34). Previously, this strain’s ability to dissolve and use various mineral forms of Fe was quantified in a series of microbial growth studies (23, 24, 32–34). Previously, this strain’s ability to dissolve and use various mineral forms of Fe was quantified in a series of microbial growth studies (23, 24, 32–34). Previously, this strain’s ability to dissolve and use various mineral forms of Fe was quantified in a series of microbial growth studies (23, 24, 32–34). Previously, this strain’s ability to dissolve and use various mineral forms of Fe was quantified in a series of microbial growth studies (23, 24, 32–34).

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In this work we used the wild-type (WT) strain *P. mendocina* ymp along with a mutant with a site-directed markerless mutation that was not capable of producing siderophores (ΔpmhA mutant with the siderophore− phenotype) (3) in a series of experiments examining siderophore use and potential synergistic effects with either a simple carboxylic acid (oxalate) or an exogenous reductant (ascorbate). Both ascorbate and oxalate are plant products that are frequently found in the shallow subsurface; their effects on in vitro Fe (hydr)oxide dissolution have been well described (6).

### MATERIALS AND METHODS

**Materials and strains.** The mineral hematite (α-Fe2O3) was prepared in P. Maurice’s geochemistry laboratory at the University of Notre Dame using the method of Cornell and Schwertmann (10). The specific surface area was determined by gas adsorption (Brunauer, Emmett, and Teller method) to be 36,730 m²/g (5).

*P. mendocina* ymp was obtained from Larry Hersman (Los Alamos National Laboratory, Biology Division) and maintained as a frozen stock in 30% glycerol (−80°C). A siderophore− mutation was created in *P. mendocina* ymp locus Pmen2871 (pmhA) as previously described (3). The pmhA gene is predicted to encode an ornithine monoxygenase involved in generating the Fe-chelating hydroxamate portions of *P. mendocina*’s siderophore. The ΔpmhA mutant was previously shown to have a siderophore− phenotype (3). Analytical-grade reagents were obtained from Sigma unless otherwise noted; 250-ml acid-washed glass flasks and ultrapure distilled 18-M Q Millipore) were used in growth experiments to decrease potential Fe contamination. UV/visible spectra were measured with a Cary 50 spectrophotometer.

**Construction of *P. mendocina* ymp biosensor.** A lacZ transcriptional fusion was constructed using the vector pUC18-mini-Tn7T-Gm-lacZ (7), which contains a transposon that integrates in the shallow subsurface; their effects on in vitro Fe (hydr)oxide dissolution have been well described (6).

### TABLE 1. Plasmids, primers, and strains used in this study

<table>
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<th>Primers, strain, or plasmid</th>
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<td>Plasmid containing site-specific transposable element; used for determination of integration site</td>
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<td>pTNS2</td>
<td>Helper plasmid for pUC18</td>
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the *P. mendocina* ymp chromosome 25 bp downstream of the glnS gene was verified by commercial sequencing by SeqWright (data not shown).

**β-Galactosidase assay.** β-Galactosidase expressed under *pmhA* control was quantified by the assay previously described by Miller and Herskberger (35). Briefly, 100-μl aliquots of cells were added to 1 ml of Z buffer (60 mM Na2HPO4·7H2O, 40 mM NaH2PO4·H2O, 10 mM KCl, 1 mM MgSO4·7H2O, 50 mM β-mercaptoethanol), 20 μl of 0.1% SDS, and 40 μl chloroform. After the chloroform settled, 100-μl samples of permeabilized cells were transferred into 20 μl of ortho-nitrophenyl-β-D-galactopyranoside (4 mg/ml) and incubated at room temperature for 20 min before the reaction was quenched with 50 μl of 1 M Na2CO3. The absorbance at 420 nm (A420) was determined with a Varian Cary 50 spectrophotometer, and the activity, expressed in Miller units, was determined as follows: 1 Miller unit = 1000 × (A420/T × V × A0.5), where T is the reaction time (in minutes) and V is the volume of culture used in the assay (in milliliters).

**Growth conditions.** WT and genetically modified *P. mendocina* ymp strains were grown in LB broth until the optical density at 600 nm (A600) was 0.8 (mid-logarithmic growth) and then inoculated (1:100) into acid-washed 250-ml shake flasks containing 50 ml of sterile, iron-deficient growth medium. The medium was prepared by adding the following components to 1.0 liter deionized (Millipore) H2O: 0.5 g of KH2PO4, 1.0 g of NH4Cl, 0.2 g of MgSO4·7H2O, 0.055 g of CaCl2·5H2O, 5.0 g of succinic acid (disodium salt, anhydrous), and 0.125 g of K2HPO4·3H2O·H2O (containing 19.7 mg of divalent, denitized water) 0.005 g of MnSO4·H2O, 0.0065 g of CoSO4·7H2O, 0.0023 g of CuSO4·5H2O, 0.0033 g of ZnSO4, and 0.0024 g of MoO3. The pH was adjusted to 7.2 as previously described (15). For Fe-depleted ([Fe(−)]) conditions, the medium was used as prepared, and the trace amount of Fe present supported minimal culture growth (15). Hematite cultures were amended with 0.125 g (91.82 m² liter⁻¹) hematite; Fe-replete [Fe(+)]) cultures were amended with Fe(EDTA) (EDTA) at a final concentration of 50 μM.

For simple carboxylic acid experiments, sodium oxalate was added from a concentrated stock to flasks and equilibrated for 24 h with medium and hematite before inoculation to allow ligand-promoted dissolution to occur. For reduced experiments, ascorbate was added at the time of inoculation because reductive dissolution tends to occur quickly and does not require extensive incubation (24 h of incubation of hematite with ascorbate actually decreases the bioavailability of the constituent Fe). All medium additives except hematite were sterile filtered and added from 100× stock solutions. Cultures were incubated at 30°C at 100 rpm for 42 h, and 600-μl samples were removed every 6 h to measure the optical density, the siderophore concentration (Chromato Azurol S [CAS] assay) (see below), and the reduction activity (ferrozine assay) (see the supplemental material). All cultures were grown in triplicate in the dark. The pH at the end of the experiments was 9.

Under Fe-limited conditions, the rate and extent of *P. mendocina* culture growth are dependent on Fe bioavailability. Thus, culture growth has been used in several previous carefully controlled studies as an indirect indication of Fe bioavailability (15, 20, 23, 24). Growth curves were used to provide similar sets of growth depend on Fe bioavailability. Thus, culture growth has been used in several previous carefully controlled studies as an indirect indication of Fe bioavailability (15, 20, 23, 24). Growth curves were used to provide similar growth conditions.

**RESULTS**

**Gene expression in WT and siderophore− *P. mendocina* strains in response to iron.** Using the *PmnhA-lacZ* reporter strain, it was found that Fe(−) growth conditions stimulate vigorous upregulation of *pmnhA* in the WT and siderophore− strains (Fig. 1A). The *pmnhA* promoter contains a ferric uptake regulator (Fur) box, and the *pmnhA* gene encodes ornithine monooxygenase, an enzyme which typically catalyzes the first committed step of hydroxamate siderophore biosynthesis (3). The Fur protein is an Fe-binding, negative transcriptional regulator that binds to and suppresses downstream transcription from Fur box-containing promoters in the presence of adequate cellular Fe. Consequently, expression of the *PmnhA-lacZ* reporter is expected to occur under the same conditions of Fe deprivation that stimulate siderophore biosynthesis. Figure 1A shows that the regulatory pathways controlling siderophore production are active in both the WT and siderophore− mutant strains under Fe(−) conditions, although the latter strain produces no functional siderophore (3).

The siderophore− phenotype does indeed seem to be the result of a localized mutation in the *pmnhA* gene. Further, under Fe(+) conditions, both the WT and mutant strains have minimal *PmnhA-lacZ* activity. Fe(EDTA) is therefore an efficiently metabolized Fe source that does not require siderophore production in order to be utilized.

**Culture growth and siderophore production.** WT and siderophore− cultures of *P. mendocina* were grown in Fe(−) medium amended with hematite. Hematite is a well-characterized, environmentally ubiquitous mineral form of Fe that has been shown to be used as an Fe source by *P. mendocina* (22). The growth kinetics and final culture density of WT *P. mendocina* cultures grown with hematite are only slightly slower and lower, respectively, than those of cultures grown in media containing Fe(EDTA) (Fig. 1B). In contrast, siderophore− *P. mendocina* cultures appeared to be unable to acquire as much Fe from hematite. After 42 h, the final optical density of these cultures was only ~50% of the final optical density of WT *P. mendocina* ymp cultures grown with hematite. Figure 1 therefore indicates that secreted siderophores are necessary for acquiring Fe to support substantially more than basal [Fe(−)] levels of growth on hematite. Hematite had a small growth-enhancing effect even in the absence of siderophores, and even the trace (or baseline) amount of Fe in the Fe(−) growth medium supported a minimal level of growth of the WT and siderophore− strains. Subsequent control experiments showed that this was due in large part to the small amount of LB broth in the inocula.

The amount of an exogenous siderophore (DFO-B) needed to restore the growth of the siderophore− mutant to WT levels on hematite was also determined. As shown in Fig. 2, when
grown to saturation in shake flasks with hematite. WT *P. mendocina* cultures produced a cumulative or endpoint siderophore concentration of \( \frac{1}{50} \) \( \frac{1}{9262} \) M (\( A_{600} = 2.0; \frac{1}{11011} = 2.8 \) \( \frac{1}{11003} \) CFU/ml). In contrast, when grown under the same conditions, the siderophore \( \frac{1}{11002} \) mutant required only 1 \( \frac{1}{9262} \) M DFO-B added along with the initial bacterial inoculum to exhibit WT levels of growth (Fig. 1B). If the two types of siderophores act equivalently, then *P. mendocina*’s siderophores appear to accumulate at concentrations that far exceed the concentration needed for optimal growth. Addition of DFO-B at concentrations more typical of hydroxamate siderophores in carbonic soil (0.01 to 0.1 \( \frac{1}{9262} \) M) only partially restored growth to WT levels (data not shown). Siderophore production exhibited an apparently logarithmic time course, and the highest cumulative concentration was detected during the stationary growth phase. Siderophores were produced most rapidly and accumulated to the greatest extent in Fe(\(+\)) cultures.

Growth in the presence of added siderophore plus exogenous reductant. Siderophore \( \frac{1}{11002} \) cultures were supplemented with ascorbate to examine the effects of a reductant in the absence of either endogenously produced or added siderophore. Addition of 50 \( \mu M \) ascorbate to cultures grown with hematite at the time of inoculation of the flasks fully restored WT hematite-supported levels of growth (Fig. 3). With an environmentally relevant reductant concentration, *P. mendocina* clearly did not require siderophores to grow efficiently; rather, the ascorbate appeared to promote sufficient hematite dissolution without siderophores.

To examine further the relationship of reductant and siderophore, siderophore \( \frac{1}{11002} \) cultures were grown in the presence of hematite and concentrations of ascorbate or DFO-B that were both environmentally relevant and significantly lower than the concentrations found to support WT levels of growth (10 \( \mu M \) ascorbate and 0.1 \( \mu M \) DFO-B; 20% and 10% of the amounts needed to restore growth to WT levels). The growth kinetics of the mutant with ascorbate plus DFO-B indicated that the combination of these compounds provided no advantage over the summed effects of the compounds added individually (Fig. 3). Under these conditions, the siderophore and reductant interact in a way that appears to be simply additive rather than synergistic.

Growth in the presence of siderophore and oxalate. A similar set of experiments was carried out using the siderophore \( \frac{1}{11002} \) mutant, oxalic acid, and DFO-B. Sterile-filtered oxalate was added to the hematite-containing growth medium 24 h prior to culture inoculation in order to allow the oxalate to begin to react with the mineral surface. After 24 h of incubation, the medium alone contained 5.2 ± 2.1 µg/liter Fe, the medium
...of meteoric water with hematite contained 4.6 ± 1.9 μg/liter Fe, and the medium with hematite and oxalate contained 7.2 ± 2.3 μg/liter Fe (all values are near the detection limit). Unlike ascorbate, oxalic acid alone (100 μM) had very little effect on the siderophore− culture (Fig. 4). This reflects the fact that the concentration of Fe in a medium with hematite and oxalate (no bacteria) was the same as the concentration in the Fe(−) medium. The suboptimal concentration of DFO-B used previously (0.1 μM) was then added to cultures of the siderophore− mutant grown in the presence of oxalate (100 μM). Although the oxalate alone did not have an appreciable effect on the growth kinetics and final culture density, a clear advantage over DFO-B alone was observed when a small amount of DFO-B was also present. This effect was not seen when oxalate was added simultaneously with DFO-B and the bacteria (data not shown), indicating that pretreatment is important to allow time for the initial labilization of surface iron from hematite by the adsorbed oxalate, as previously shown by Reichard et al. in abiotic studies (41).

**DISCUSSION**

Although siderophores are essential for Fe uptake by many aerobic bacteria, they are inefficient at releasing Fe from the surfaces of the mineral species that are the most common terrestrial sources of Fe (6, 9, 22, 25, 27). They also inevitably diffuse away from the source of production and into the greater geologic matrix. These factors seem to limit the utility of siderophores to their microbial producers and to influence their potential impacts on the Fe cycle in the environment, leading to questions about how these molecules behave in terrestrial contexts. Using WT strain *P. mendocina* ymp, a terrestrial obligate aerobe isolated from a carbon-poor site (21), and a siderophore− mutant, we investigated the role of siderophores in conjunction with environmentally available small molecules in supplying growing cultures with Fe from a common mineral, hematite.

Siderophores are clearly important for efficient acquisition of Fe from hematite by highly metabolically active cultures of *P. mendocina*. Strategies that include endogenous production of a reductant, as postulated by Hersman et al. (24), or attachment of the bacteria to the mineral surface do not by themselves compensate for siderophore loss. Indeed, preliminary biochemical studies performed in our lab showed that there was a negligible amount of Fe-reducing activity in the culture supernatant compared to the cell-associated activity (see the supplemental material). This activity was also NAD(P)H dependent and sensitive to boiling and guanidinium chloride treatment, suggesting that it could have been the activity of a cell-associated enzyme that “leaked” from lysed cells.

Moreover, our experiments performed with active *P. mendocina* cultures confirmed that previous in vitro observations of the effects of siderophores and carboxylic acids, alone and in combination, provided relevant insight into aerobic microbial Fe acquisition. For the siderophore− strain, significantly less DFO-B was needed to support optimal growth on hematite in the presence of an added carboxylic acid. Alone, a concentration of oxalate representative of the concentration in caronic soil (100 μM) (47) had a negligible effect on the growth of a siderophore− culture on hematite. However, addition of 0.1 μM DFO-B to the oxalate-containing culture (10-fold less than the optimal concentration and an environmentally relevant concentration) had a pronounced effect on the growth kinetics of the siderophore− strain. This suggests that there is a synergistic relationship in vivo between the siderophore and oxalate.
for mobilizing Fe from hematite. Similar concentrations of a siderophore and oxalate were shown to significantly accelerate mineral (goethite) dissolution in vitro, although at a lower pH (6). Hence, the in vivo results support the conclusion that environmentally relevant concentrations of carboxylic acids, in combination with low concentrations of a siderophore, provide a readily bioavailable Fe supply.

Notably, this effect was observed only when oxalate was prereacted with the hematite for several hours prior to addition of DFO-B and inocula, although this incubation did not appear to result in a greater concentration of dissolved Fe. It is possible that some dissolution did occur, but as the value obtained was near the detection limit of atomic absorption spectroscopy, the amount dissolved was not detectable. Alternatively, oxalate might have associated with and labilized the surface iron without effecting its release. In either case, the need to equilibrate the mineral with oxalate in order to see the synergistic effect suggests that the oxalate-siderophore process could be a sequential reaction, in which the slowest and hence rate-limiting step appears to be labilization of surface Fe species following adsorption of the ligand to the mineral surface. Prereaction with oxalate, which dissolves Fe through a ligand-promoted surface-controlled mechanism, overcomes this slow initial step that can otherwise hinder acquisition of Fe from hematite by siderophores. In contrast, prereaction with ascorbate had no enhancing effect (data not shown), possibly because Fe could be quickly reduced, reoxidized, and redeposited.

Synergistic effects between a surface-acting reductant and a siderophore have also been proposed and studied in vitro (4, 13, 22, 48). Using conditions similar to those described here, it was previously shown that a common plant-derived reducing agent (ascorbic acid) could significantly enhance the bioavailability of Fe from ferrihydrite to P. mendocina (15). Using either Fe(EDTA) or an equimolar amount of ascorbate in the presence of ferrihydrite, two cultures grew rapidly and to a final density that was roughly three times that observed for cultures grown with ferrihydrite (K_{sp} = 10^{-39}) alone. It was not clear whether ascorbate alone or ascorbate acting in conjunction with siderophores was responsible for the observed growth enhancement in this previous study. Dhungana et al. (15) also showed that ascorbate added with Fe(EDTA) had no effect on growth and was not likely used as a carbon source; this result was reproduced here (data not shown).

We examined more closely the individual and coupled roles of ascorbate and the siderophore in promoting growth of a culture on hematite. If the reductant and siderophore are capable of working synergistically to increase the bioavailability of Fe from hematite, then the siderophore−mutant should grow significantly better when both compounds are present. However, when both DFO-B and ascorbate are present, the effect is no greater than additive. Further, in contrast to oxalate, ascorbate alone (50 μM) can restore growth of a siderophore−culture to WT kinetics on hematite. There are at least two possible explanations for the finding that ascorbate alone is sufficient. First, the reductively mobilized Fe(II) could be directly taken up by the cells, either via a membrane-bound ferrous iron transporter or via a simple porin that allows passage of Fe(II) in its aquated form. In an aerobic environment, the Fe(II) would have to be generated very close to the cell surface in order to avoid reoxidation of the iron. Spiro and Saltman have estimated that the half-life of free Fe(III) is only a few seconds in an aqueous environment in which the pH is greater than 3 or 4 (46). Moreover, known mechanisms for transporting Fe(III) across the bacterial outer membrane require that it be bound in an organic ligand. A second role for ascorbate could therefore be to bind and thereby stabilize the released Fe(II) prior to uptake or to chelate reoxidized Fe(III) and assist in its passage through outer membrane transporters. Analogously, ascorbate has long been known to enhance Fe absorption by the intestine by forming chelates with Fe(II) and Fe(III) that stabilize Fe in soluble, bioavailable forms.

The results presented here can be understood in light of at least three well-defined roles that siderophores could serve in a bacterium-mineral system (Fig. 5). First, distinct from simple chelators, true siderophores transport Fe(III) through the bacterial outer membrane in receptor-mediated processes. The receptors have been described as receptors that are highly specific for their cognate ferrisiderophores, binding in a one-siderophore—one-receptor fashion, although specificity is likely not rigorous or universal (19, 21). Simple Fe(III)-ligand complexes that structurally resemble a receptor's ferrisiderophore complex, for example, have been shown to use the receptor for entry into the cell (38). Ferric complexes of synthetic EDTA and nitrilotriacetic acid (NTA) are also efficiently taken up by P. mendocina. Although the mechanisms may not be entirely understood, it is expected that a ligand is necessary for transporting Fe(III) through the outer membrane. Second, siderophores could adsorb to hematite and directly promote dissolution. Hersman et al. (22) showed that the siderophore produced by P. mendocina is able to enhance directly hematite dissolution at pH 3. However, proton-promoted dissolution of hematite, which would release Fe(III) into solution for uptake.
by the siderophore, is likely far faster at pH 3 than at the circumneutral pH used in the biotic experiments described here. Moreover, direct DFO-B adsorption to Fe oxides has been observed to be weak, at least at pH values less than 5 (27), although the hematite-sorpitive abilities of the siderophore produced by *P. mendocina* are unknown. Finally, siderophores might promote the solubility of the mineral (κ_{sp}) in batch systems by forming thermodynamically strong complexes with the released Fe in solution, which would be further enhanced by synergistic contributions from other ligands that interact directly with the mineral. Metabolizing bacteria are expected to augment this dissolution by importing, removing the Fe from and resecreting or producing more of the siderophore, steadily replenishing the soluble siderophore pool, and sustaining far-from-equilibrium conditions with respect to mineral dissolution. The relatively low concentration of DFO-B required to stimulate WT-like growth in the siderophore− mutant and the clear synergistic effect with oxalate both support the latter role. At the same time, the same exogenous siderophore pool must also support the trafficking of Fe(III) through the bacterial outer membrane. The fact that such a small amount is needed, even given that DFO-B is not the strain’s native siderophore, further suggests that *P. mendocina* may recycle its deferrated DFO-B back into the medium.

In summary, siderophore production and release are important for acquisition of Fe from hematite. Our findings suggest that siderophore production is more or less important depend-ant and the clear synergistic effect with oxalate both support the latter role. At the same time, the same exogenous siderophore pool must also support the trafficking of Fe(III) through the bacterial outer membrane. The fact that such a small amount is needed, even given that DFO-B is not the strain’s native siderophore, further suggests that *P. mendocina* may recycle its deferrated DFO-B back into the medium.

References