Applied and Environmental Microbiology, May 2007, p. 3428–3430 Vol. 73, No. 10
0099-2240/07/$08.00+0 doi:10.1128/AEM.02586-06

Effect of Exogenous Reductant on Growth and Iron Mobilization from Ferrihydrite by the *Pseudomonas mendocina ymp* Strain[▽]

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Received 7 November 2006/Accepted 13 March 2007

Growth of the *Pseudomonas mendocina ymp* strain on insoluble ferrihydrite is enhanced by exogenous reductants with concurrent increase in soluble iron concentrations. This shows that exogenous reductants play a substantial role in the overall microbial iron bioavailability. The exogenous reductants may work together with the siderophores, Fe-scavenging agents, to facilitate ferrihydrite dissolution.

Iron (Fe) is essential for microbial growth. Under oxic conditions at circumneutral pH (where most life exists), Fe occurs as extremely insoluble Fe(III) (hydr)oxides (e.g., ferrihydrite, hematite, and goethite) (14). Microorganisms sense environmental Fe deficiency and produce low-molecular-weight Fe-chelating agents, siderophores, to solubilize Fe(III) (hydr)oxides and transport Fe into cells (2, 5, 15). Siderophores bind Fe^{3+} with extremely high affinity, and this high formation constant between siderophore and Fe^{3+} is believed to be the driving force that facilitates the dissolution of Fe(III) (hydr)oxides (3, 5, 8). Thermodynamically, siderophores are very capable of dissolving Fe(III) (hydr)oxides; however, the rate of in vitro dissolution is much slower than the in vivo microbial Fe uptake rate (5–7). In the presence of a reducing agent, siderophores have been shown to dissolve Fe(III) (hydr)oxides at a faster rate (1, 9, 12). This increased dissolution resulting from a combined effect of siderophore and reductant could potentially be the mechanism that suffices for the microbial demand for Fe (7).

Our recent studies suggest that extracellular reductants are important metabolites produced by bacteria under Fe-deficient conditions. We have determined that the *Pseudomonas mendocina ymp* strain, a common soil bacterium that is a strict aerobe, produces a reductant (in addition to siderophore) when grown on Fe(III) (hydr)oxide (4–7). The structure of the *P. mendocina* reductant is unknown, but it may be similar to that produced by *Pseudomonas putida*, pyridine-2,6-bis(monothiocarboxylate) (PDTC) (10, 11). PDTC is a bifunctional molecule capable of reducing Fe(III) (hydr)oxide and subsequently binding Fe (S. Dhungana, C. R. Anthony III, and L. E. Hersman, submitted for publication). The mineral surface-normalized rate of ferrihydrite dissolution by PDTC via reductive mechanism has recently been characterized, and it has been shown to be 2 orders of magnitude faster than that seen for siderophore-only facilitated dissolution. With the observed importance of reductant in Fe(III) (hydr)oxide dissolution and Fe bioavailability, we have investigated the influence of externally supplemented reductant on the growth of the *Pseudomonas mendocina ymp* strain. The total soluble Fe in bacterial growth media along the growth curve time points was quantified to further explore the relationship between reductant, Fe availability, and bacterial growth. The hypothesis that the growth of *P. mendocina* on ferrihydrite will be enhanced in the presence of the exogenous reductant due to increased solubility and availability of iron was examined by looking at the *P. mendocina* growth and total dissolved Fe along the growth curve.

The bacterium used for this study, the *Pseudomonas mendocina ymp* strain, was isolated as part of the Yucca Mountain Project from sediment in a surface holding pond of a drilling operation at the Nevada Test Site (6). Ferrihydrite used in these experiments was synthesized by precipitation with alkali according to methods described by Cornell and Schwertmann (1a). Complete characterization of synthesized ferrihydrite is described elsewhere (6). All experiments were carried out in Fe-deficient minimal growth medium (FeDM), as previously described (6). Briefly, the FeDM consisted of the following (g liter^{-1}): K_2HPO_4, 0.5; NH_4Cl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.05; succinic acid disodium salt anhydrous ($C_4H_4Na_2O_4$), 5; trace elements, 0.125 ml (0.005 g $MnSO_4 \cdot H_2O$, 0.0065 g $CoSO_4 \cdot 7H_2O$, 0.0023 g $CuSO_4$, 0.0033 g $ZnSO_4$, and 0.0024 g MoO_3 per 100 ml of distilled, deionized water); H_2O, 1.0 liter (pH 7.4 [not buffered], because pH generally increases to 8 in experiments of this type). All Teflon flasks, tubing, and plastic sampling cups used to load samples in the graphite furnace atomic absorption (GFAA) spectrophotometer were acid washed with a trace metal-grade concentrated HNO_3 and rinsed with ultrapure water. Teflon Erlenmeyer flasks (250 ml) containing 30 ml of FeDM were used for all experiments, and each experiment was carried out in triplicate. To these flasks were added various Fe sources: FeEDTA, ferrihydrite, or a no-added-Fe control. For the FeEDTA growth studies, FeEDTA was added to yield 67 μM concentrations of Fe. For the Fe acquisition experiments, ferrihydrite was added so that the effective surface area was 29 m^2 liter^{-1}. The reductants, ascorbate and cysteine, were added so that the final concentration of the reductant was 67 μM. At every time point, 1 ml of sample was removed for reading of the optical density at 600 nm (OD_{600}). Ferrhydrite particles used in these experiments settle at the bottom of the

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▽ Published ahead of print on 23 March 2007.

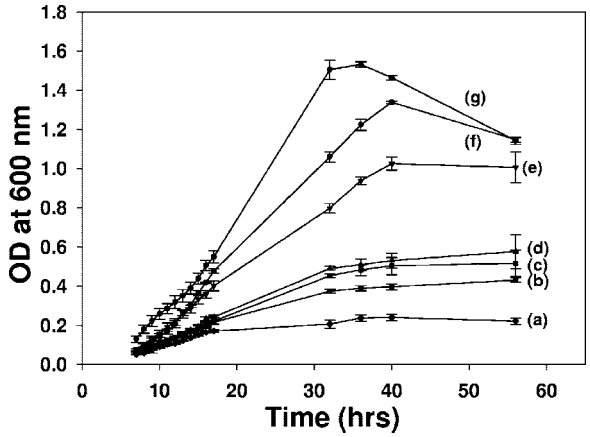

FIG. 1. *P. mendocina* growth under various conditions. (a) No iron. (b) Ascorbate (67 μM) with no iron. (c) Ferrihydrite (29 m^2 $liter^{-1}$). (d) Cysteine (67 μM) with no iron. (e) Ferrihydrite (29 m^2 $liter^{-1}$) plus 67 μM ascorbate. (f) Ferrihydrite (29 m^2 $liter^{-1}$) plus 67 μM cysteine. (g) FeEDTA (67 μM). The error bar represents ±1 standard deviation.

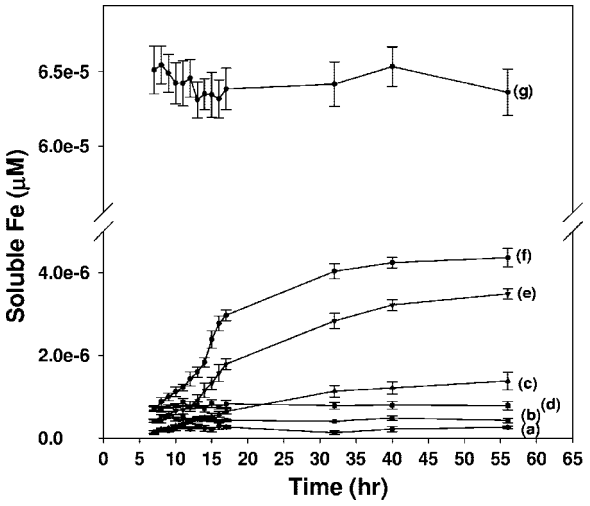

FIG. 2. Soluble Fe present in the growth media during *P. mendocina* growth under various conditions. (a) No iron. (b) Ascorbate (67 μM) with no iron. (c) Ferrihydrite (29 m^2 $liter^{-1}$). (d) Cysteine (67 μM) with no iron. (e) Ferrihydrite (29 m^2 $liter^{-1}$) plus 67 μM ascorbate. (f) Ferrihydrite (29 m^2 $liter^{-1}$) plus 67 μM cysteine. (g) FeEDTA (67 μM). In the absence of *P. mendocina*, 67 μM ascorbate and 67 μM cysteine dissolve very little iron, which cannot be distinguished from the background iron levels present in the media (statistically insignificant). The error bars represent ±1 standard deviation.

culture flask and do not interfere with sampling or OD readings. Following OD reading, the sample was sterile filtered and used for dissolved-Fe analysis. The amount of dissolved Fe in all samples was analyzed using a Perkin-Elmer Analyst 600 GFAA spectrophotometer, equipped with a built-in AS-800 Autosampler. Each sample analysis included three replicate runs, and the samples having a >3.0% relative standard deviation were reanalyzed.

P. mendocina growth under various conditions is summarized in Fig. 1. When the ferrihydrite was the source of Fe, microbial growth was observed that slightly exceeded that of the no-added-Fe control (a very limited amount of growth did occur in the control medium). Total-dissolved-Fe analysis indicated the presence of some dissolved Fe in the media when *P. mendocina* was grown in ferrihydrite compared to the no-Fe control (Fig. 2, curves a and d). This observation clearly suggested some Fe being mobilized from ferrihydrite by the *P. mendocina*.

When *P. mendocina* was grown on ferrihydrite supplemented with external reductant, a significant increase in growth was observed (Fig. 1, lines e and f). Both cysteine- and ascorbate-supplemented media displayed much increased growth compared to media that solely contained ferrihydrite or cysteine and the ascorbate controls (no ferrihydrite). The levels of growth in these reductant-supplemented media were only slightly lower than that seen when *P. mendocina* was grown in the Fe control (67 μM FeEDTA). Growth curves shown in Fig. 1, curves b and d, serve as a control for Fe contamination that could have been present in the added reductant (ascorbate or cysteine). A slightly higher growth rate compared to that of the no-Fe control is indicative of only slight Fe contamination. Total-dissolved-Fe analysis verified that there was a trace amount of Fe contamination in the added stock solution of reductants, but this was not significant enough to influence the bacterial growth. The levels of total soluble Fe in the reductant-supplemented growth media were much elevated, which in turn significantly facilitates bacterial growth (Fig. 2, curves e and f). The observed increase in the bacterial growth results solely from the ability of ascorbate and cysteine to reduce and solubilize Fe(III) from ferrihydrite. Our data indicate that the bacteria metabolized neither ascorbate nor cysteine, and even if they were metabolized, the succinate concentration (31 mM) was approximately 500 times the ascorbate or cysteine concentration (67 μM), which would make ascorbate or cysteine an insignificant carbon source for *P. mendocina* growth.

The presence of a reductant, whether externally present in the medium or specifically produced by bacteria, significantly improves the solubility of ferrihydrite and highly enhances the microbial growth. Microbial bioavailability of iron is believed to be fully dependent on siderophores; however, our findings clearly show that a reductant alone can play a considerable role in iron dissolution and resulting microbial growth promotion. A chelating agent of some nature is often required to keep iron mobilized from the mineral surface in the solution, and it is very possible that the exogenous reductants are working together with the siderophores produced by *P. mendocina* to facilitate ferrihydrite dissolution. *P. mendocina* is known to produce five siderophores when grown on an iron mineral (6), and thus the interaction between the reductant and siderophore is very likely; however, that conclusion is beyond the scope of this study. In abiotic studies, reductant not only appears to increase the concentration of Fe solubilized by siderophore but also allows the dissolution to be accomplished at a faster rate, which is critical to meeting the demands of a rapidly growing bacterial culture (1, 6, 8, 13). The involvement of a reductant in environmental Fe(III) (hydr)oxide dissolution is of significant interest as the reductant can change the redox states of other redox-active metal ions (e.g., toxic metals) and substantially influence their speciation and environmental mobility.

We thank Patricia A. Maurice at the University of Notre Dame for supplying us with well-characterized ferrihydrite samples.

We also thank the DOE-BES, DOE-NABIR, and LANL-LDRD programs for financial support.

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