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Role of bacterial siderophores in dissolution of hornblende

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Abstract—Hornblende, a common mineral in granitic soils, may act as a source for a variety of metals needed by bacterial species for enzyme function (e.g., Fe, Zn, Mn, Cu, Co, Mo, V, Ni). A species of the bacterial genus *Streptomyces* was cultured from an Adirondack soil and isolated because of its ability to grow robustly in low Fe medium with hornblende present. Studies with unbuffered culture medium, to discover whether *Streptomyces* sp. cultures affected solution pH, showed a decrease of 2.0 pH units in 21 d, then an increase of 3.0 pH units at 56 d. Cells that adhered to the hornblende surface at 56 days were difficult to remove, presumably because of mycelial growth deep into pits and cracks. Decreases and increases in pH may have been due to production of organic acids and ammonia respectively. Increases in pH could also have been related to release of components during death of organisms. In a buffered medium, *Streptomyces* sp. increased the initial Fe release rate from hornblende approximately fivefold over that of an abiotic control. A catechol derivative, produced by the *Streptomyces* sp. and characterized by chromatography and mass spectrometry, is presumed to cause this Fe release enhancement. Hornblende dissolution was also analyzed in the presence of a commercially available hydroxamate siderophore, desferrioxamine mesylate (DFAM). DFAM is the methane sulfonate form of one of many siderophores known to be a product of streptomycetes. The rate of Fe release obtained when incubating the hornblende with 24 μm of DFAM was similar to the rate observed in the presence of the *Streptomyces* sp. isolate. Higher concentrations of DFAM increased the dissolution rate nonlinearly, described by the rate equation $R = (7.6 \times 10^{-13})C^{0.47}$, where R is the release rate of Fe ($\text{mol}/\text{m}^2\text{s}$), and C is the concentration (mol/l) of DFAM. The DFAM also increased release of Al and Si from hornblende into solution; however, these release rates were not increased by addition of the *Streptomyces* sp. alone. Preferential release of Al and Si in the presence of DFAM, but not in the presence of bacteria alone, may be related to the difference in selectivity of catechol vs. hydroxamate siderophores. Addition of *Streptomyces* sp. in the presence of DFAM at three concentrations consistently enhanced Fe release approximately two to threefold the rate with siderophore alone. Recycling of siderophore molecules or enhanced production of one siderophore by microorganisms in the presence of other siderophores makes it difficult to predict a priori release rates when both siderophore and bacteria are present, as would be the case in natural soils. Copyright © 1999 Elsevier Science Ltd

1. INTRODUCTION

Relatively few studies have quantified the effects of soil organisms on silicate weathering or identified the mechanisms of these effects, despite the fact that the role of microorganisms in mineral weathering has been the subject of investigation for many years (for a recent review see Barker et al., 1997). Proposed mechanisms for dissolution of minerals by lichens, fungi, and bacteria include the use of microbially produced organic or mineral acids (Duff et al., 1963; Keil and Schwartz, 1980; Barman et al., 1992; Bosecker, 1993; Barker et al., 1997), alkaline metabolites (Aristovskaya and Kutuzova, 1968; Kutuzova, 1973), polysaccharide slimes (Malinovskaya et al., 1990), chelates (Watteau and Berthelin, 1994; Holmen and Casey, 1996; Ullman et al., 1996), and oxidation or reduction of metals in the mineral (Ivarson et al., 1978, 1979, 1980; Rickard, 1973). Almost all of these proposed microbial effects (with the possible exception of alkaline metabolite excretion) predict enhancement of field weathering rates. Indeed, most studies emphasize short-chain organic acids and the effect of lower pH and/or enhanced chelation (e.g., Robert and Berthelin, 1986). For most minerals, both a decrease in pH and

chelation should increase the weathering rate (White and Brantley, 1995).

The effects of pH and chelation may be especially important for mobilization of micronutrients (e.g., Fe, Mn, Cu, Zn, V, Mo, Ni, Co) by soil bacteria. Where some of these elements are important in the structure and function of bacterial enzymes, coenzymes, and cofactors, a “garbage mineral” such as hornblende may represent a common source of these limiting trace metals. For example, Fe, K, Mn, Mg, and Ca often limit bacterial growth, and all of these elements have the potential to be mobilized from hornblende. Of these elements, Fe is particularly in short supply because Fe^{3+} is unavailable to cells in aerobic environments due to low solubility of Fe oxyhydroxides near neutral pH. In this paper, we report studies on the rate and mechanism of Fe mobilization from the hornblende surface by a streptomycete isolated from a hornblende-rich Adirondack soil. This bacterium, like many other species, releases a multidentate Fe(III)-specific chelate (a siderophore) that enhances the dissolution of hornblende.

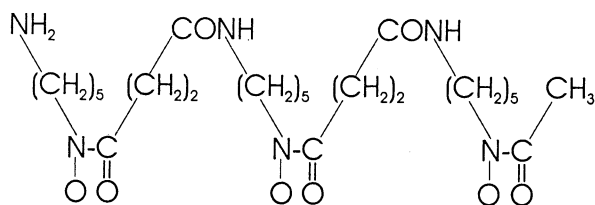
1.1. Siderophores

The term siderophore (Greek meaning “iron bearer”) denotes a virtually Fe(III)-specific ligand that is produced by aerobic

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bacteria and fungi growing under low iron conditions (Neilands, 1982). Their biosynthesis is tightly controlled by levels of iron; for example, their production is repressed when iron is abundant in the environment. Page and Huyer (1984) showed that siderophore production by the nitrogen-fixing soil microbe *Azotobacter vinelandii* could be unrepressed by the presence of Fe-containing minerals, with increasingly stronger siderophores produced in response to increasingly insoluble iron sources. So-called minimal media (e.g., Maniatis et al., 1982) generally have only micromolar quantities of iron, a concentration low enough to induce siderophore production (Neilands, 1982). When Fe concentrations are approximately an order of magnitude above micromolar, siderophore-independent, low-affinity Fe uptake systems (e.g., organic acids) are sufficient for obtaining iron from the environment (Neilands, 1982; Page, 1993).

Siderophores are relatively low molecular weight ($M_r < 1500$), and are divided into classes based on their chelating structures. Most are of either the hydroxamate (e.g., ferrioxamines) or catechol class; others include carboxylates and pyoverdins. The siderophore association constants for Fe(III) are in the range of 10^{12} to 10^{52} (Neilands, 1982; Page, 1993); those with hexadentate coordination of Fe(III) have higher affinity than those with tetradentate or bidentate coordination (Page, 1993). The commercially available siderophore derivative used in this study, desferrioxamine mesylate (DFAM), is the methane sulfonate form of the hydroxamate siderophore desferrioxamine B. Desferrioxamine B has the chemical formula $C_{25}H_{48}N_6O_8$, and is a linear trihydroxamate, hexadentate ligand with the following structure:



DFAM has an association constant of $10^{30.6}$ M (Dionis et al., 1991), chelating Fe^{3+} as a 1:1 chelate complex (Jalal and van der Helm, 1991; Matzanke, 1991).

1.2. Mechanism of Iron Uptake

In studies using radiolabelled ^{55}Fe -ferrioxamines and Cr(III)- or Ga(III)- substituted ferrioxamine complexes, all three ferrioxamines were taken up by *Streptomyces pilosus* at comparable rates, indicating that the entire siderophore-Fe(III) complex is taken up by the cell before Fe reduction takes place (Muller and Raymond, 1984). Upon reduction of the Fe(III) to Fe(II), the siderophore releases Fe. The fate of the siderophore may vary in different organisms; it may be recycled, or its hydrolysis may accompany reduction of Fe(III) (Neilands, 1982; Hughes and Poole, 1989). Imbert et al. (1995) and Muller and Raymond (1984) showed that uptake of the siderophore-Fe complex is energy dependent.

Considerable information is available concerning Fe-sid-

erophore interactions with pathogenic bacteria (Neilands, 1980; Braun et al., 1984; Hider, 1984; Winkelmann, 1991), and several recent publications have investigated microbial interactions with Fe(III)-(hydr)oxide coatings on mineral surfaces (Adams et al., 1992; Hersman et al. 1996; Maurice et al. 1996; Grantham et al. 1997). Watteau and Berthelin (1994) examined dissolution of Fe and Al from goethite, biotite, and pyrite by fungal siderophores and aliphatic acids. Hersman et al. (1995) investigated siderophore-promoted dissolution of hematite using a siderophore isolated from a *Pseudomonas* sp. Enhanced dissolution of hematite was observed at a siderophore concentration assumed to be present in bacterial microniches in natural soils, as calculated by these researchers ($240 \mu M$ of siderophore). Holmen and Casey (1996) investigated dissolution of goethite in the presence of acetohydroxamic acid, a hydroxamate siderophore analog. Overall, however, as pointed out by Stone (1997), little work has been completed on siderophore-promoted dissolution of minerals.

1.3. Actinomycetes

The actinomycetes are a numerous and widely distributed group of soil microorganisms, composing from 10 to 50% of the soil microflora community over a broad range of soil conditions, with *Streptomyces* the numerically dominant genus (Alexander, 1977). Much of the following review of the actinomycetes is summarized from Alexander (1977). Actinomycetes are less abundant in waterlogged and acidic soils, and may be present in higher than normal numbers in drier, alkaline soils. Although they are classified as bacteria, they are fungus-like in appearance and growth. Whereas most bacteria grow by asexual fission of individual cells, the vegetative cells of most actinomycetes produce slender, branched filaments, or hyphae, that are collectively termed mycelia. These structures are smaller and of a different morphology and composition than their fungal counterparts. Within these hyphae are formed asexual spores called conidia; the spores are formed when conditions are no longer optimal for vegetative growth, and germinate when conditions are again favorable.

Actinomycetes are heterotrophic, and are valuable decomposers of organic matter in soil communities. Members of the streptomycete family of actinomycetes, which includes streptomycetes, are responsible for production of the compound causing the musty odor prominent in freshly turned soils; many also produce antibiotics that inhibit the growth of bacteria and fungi (Alexander, 1977; Locci, 1989). A species of the genus *Streptomyces* was chosen for studies on the release of Fe and other elements from hornblende based on its ability to grow in metal-poor medium in the presence of powdered hornblende (see Section 2). Species of streptomycetes are known to produce siderophores, primarily of the hydroxamate class (Hughes and Poole, 1989; Winkelmann, 1991; Imbert et al., 1995).

2. MATERIALS AND METHODS

2.1. Isolation of *Streptomyces* Species

The streptomycetes species used in these experiments was isolated from hornblende-containing unsaturated soil from Gore Mountain, in the Adirondack Mountains of New York. Soils were collected in 10-gallon plastic containers in autumn 1996. Approximately 30 g of soil were placed into small mason jars and enriched with 30 mL of

1.5% (w/v) glucose–beef extract medium. Into each jar was placed a cut slab ($2 \times 5 \times 0.2$ cm) of autoclaved hornblende, also collected from Gore Mountain. The jars were covered with aluminum foil and allowed to incubate at 37°C for 8 weeks to allow formation of biofilms. The slabs were removed from the soils, rinsed with sterile medium to remove loose inorganic material, and scraped with sterile spatulas. The material adhering to the spatulas was used to inoculate 2 mL of glucose–beef extract medium. Dilutions of these cultures were made and streak-plated onto glucose–beef extract agar plates supplemented with 0.4% (w/v) powdered hornblende (>400 mesh) suspension.

Individual colonies (isolates) that developed were picked and inoculated into glucose–beef extract liquid medium. Eight different isolates obtained in this manner were then cultured in metal-poor medium composed of 0.5% (w/v) glucose, 1% (v/v) 100X Wolfe's Mineral elixir (Wolin et al., 1963) prepared without compounds containing elements found in hornblende ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl, CaCl_2 , $\text{AlK}(\text{SO}_4)_2$, and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$), and 0.2% (v/v) B-vitamin solution, with 0.4% (w/v) suspension of hornblende (>400 mesh) added. Control cultures consisted of medium with no added hornblende. Of the eight isolates, two grew robustly in the hornblende-enriched medium, one of them is described in this paper.

2.2 Preparation of Media Used in Growth Experiments

Some growth experiments were conducted using an unbuffered medium to ascertain whether the isolate could cause pH changes. The medium contained a 0.5% (w/v) glucose and 0.5% (w/v) casamino acids solution supplemented with 1% (v/v) Wolfe's Mineral Elixir prepared without FeSO_4 , and 0.2% (v/v) B-vitamin solution. The glucose–casamino acids solution was first treated with Chelex-100 (Bio-Rad Laboratories), a cation-exchange resin used to remove Fe and other trace metals. Control cultures were grown in the same medium composition, but without Chelex treatment and with FeSO_4 in the mineral elixir.

A buffered medium was chosen for growth studies at constant pH: this medium was a modified M9 medium, denoted MM9 (Maniatis et al., 1982; Schwyn and Neilands 1987), consisting of 6.0 g/l Na_2HPO_4 , 0.3 g/l KH_2PO_4 , 0.5 g/l NaCl, 1.0 g/l NH_4Cl , and 6.06 g/l (50 mM) Tris base, pH 7.4. This solution was autoclaved and allowed to cool to 25°C. It was then supplemented with 2% (v/v) Chelex-100 treated 10% (w/v) casamino acids (Difco Laboratories), 0.2% (v/v) 1 M MgSO_4 , 1% (v/v) filter-sterilized 20% (w/v) glucose, and 0.01% (v/v) 1 M CaCl_2 ; these solutions were prepared and sterilized separately.

All glassware used was soaked for > 2 h in a Nochromix (GODAX Laboratories, Inc.) acid bath and rinsed 6 times with distilled H_2O and 6 times with deionized H_2O ; plasticware was soaked sequentially in HCl and HNO_3 acid baths [1:10 (v/v) dilution with deionized H_2O], with a minimum of 12 rinsings, as above, between and after acid soaks.

2.3. Growth Conditions

2.3.1. Unbuffered experiments

In the unbuffered studies, 1 cm diameter hornblende discs polished to 0.25 μm were autoclaved in 5.0 mL of medium in each of eight 25 mL culture tubes. Half of the tubes were inoculated with *Streptomyces* sp., and half were not inoculated. The cultures were incubated for either 21 or 56 d. After incubation, the discs were removed, and the medium was filtered through a 0.2 μm filter and analyzed by inductively coupled plasma–atomic emission spectroscopy (ICP–AES) (Leeman Laboratories). The hornblende discs were rinsed in distilled water, then ultrasonically washed in ultrapure Optima acetone (Fisher Laboratories) for 20 min. The samples were then cleaned by ultraviolet ozone cleaning and imaged under a Leica differential interference contrast (DIC) microscope.

2.3.1. Buffered experiments

In experiments using buffered medium, sterile 500 mL flasks containing 60 mL of medium and either 2.0 g of hornblende powder (250 to 429 μm) or no hornblende were inoculated with 1.0 mL *Streptomyces* sp. from 1 to 3 week tube cultures in the same medium. Cultures were covered and incubated without agitation at room temperature.

Four sets of experiments (#1, #2, #3, #4) using buffered medium were conducted. The first set (#1; Tables 1 and 2) was a pilot experiment to determine whether the presence of the isolate affected dissolution. In this experiment three flasks contained hornblende and two flasks contained only medium. One flask of each type was inoculated with the isolate. At 7d intervals, 6.0 mL samples were withdrawn from each flask; the samples were centrifuged to pellet the cells, and the supernatants were filtered through a 0.2 μm filter. The pH of each sample was determined from a 1.0 mL aliquot, and 0.5 mL aliquots were analyzed for siderophore. The remainder of each sample was analyzed for cations by ICP–AES. A total of 3 samples were analyzed from each flask.

In the second set of experiments (#2; Tables 1–3), the hydroxamate siderophore desferrioxamine mesylate (DFAM, Sigma Chemical Co.) was added at concentrations of 24 μM or 240 μM to triplicate flasks with hornblende. This commercially available siderophore derivative was used because the siderophore produced by the isolate could not be collected in large enough quantities for experiments without bacteria. One flask of each concentration of siderophore was inoculated with the isolate; a control flask had medium and hornblende only. At approximately 24 h intervals over 5 d, 6.0 mL samples were withdrawn from each flask and treated as described above for Experiment #1. Aliquots were removed to measure pH and the remainder of each sample was analyzed by ICP–AES.

In the third set of experiments (#3; Tables 1–3), DFAM (24, 120, or 240 μM) was added to duplicate flasks with hornblende. One flask of each siderophore concentration was inoculated with the isolate, as was a flask containing hornblende with no added siderophore; control flasks contained medium only, medium + isolate, and medium + hornblende (no inoculum). Samples of 8.0 mL each were removed every 2 d for 10 d; the samples were filtered through a 0.2 μm filter, and 1.0 mL aliquots were removed for pH determination. The remainder of each sample was analyzed by ICP–AES. After 21 d, 1.0 mL aliquots were removed for pH determination, 0.5 mL aliquots were removed for siderophore assays, and 5.0 mL were submitted for ICP analysis; the remainder was reserved for high-performance liquid chromatography (HPLC) analysis of siderophores.

In the fourth set of experiments (#4; Tables 1–3), duplicate flasks of medium contained hornblende, hornblende + isolate, hornblende + 24 μM DFAM, hornblende + 24 μM DFAM + isolate, hornblende + 240 μM DFAM, or hornblende + 240 μM DFAM + isolate. Triplicate flasks of medium contained hornblende + 120 μM DFAM or hornblende + 120 μM DFAM + isolate. At approximately 24-h intervals over 5 d, 6.0 mL samples were removed and filtered through 0.2 μm filters for analysis by ICP–AES; 1.0 mL aliquots from Days 1, 3, and 5 were pH tested and analyzed for glucose content using a YSI 2700 Select Biochemical Analyzer. After 7 d, cell pellets were collected by filtration onto preweighed nitrocellulose filters (Whatman International, Ltd.) and dried at 100°C overnight. The masses of these pellets were then measured to determine the dry cell mass of each culture.

2.4. Siderophore Assays

A universal assay developed by Schwyn and Neilands (1987) was used to detect the presence of siderophores in culture fluids. The assay is based on the decrease in absorbance at 630 nm of a chrome azurol S (CAS) dye–Fe (III) complex when the iron is removed by a strong chelator, such as a siderophore, changing the color of the solution from blue to orange. The assay solutions were prepared as described by Schwyn and Neilands (1987) (chemicals from Sigma Chemical Co. or laboratory stocks). For the assay, 0.5 mL aliquots of CAS assay solution were added to 0.5 mL of culture supernatants, blanks, and DFAM standards. The samples appeared to reach equilibrium within 1 h, at which time absorbances were read at 630 nm in a Beckman DU640 UV/VIS spectrophotometer.

Upon confirmation of the presence of siderophore by the CAS assay, a hydroxamate specific assay (Arnold and Viswanatha, 1983) was used to test the culture supernatants for the presence of a hydroxamate siderophore. This assay is based on the competition for Fe(III) ions of a bis(mercaptoacetato-S,O)hydroxoirion(III) complex; removal of Fe(III) from the purple dye complex results in a decrease in absorbance at 532 nm. Assay solutions were prepared as described by Arnold and Viswanatha (1983) (chemicals from Sigma Chemical Co. or laboratory

Table 1. Hornblende dissolution experiments in abiotic buffered medium with and without DFAM.^a

Sample name ^b	Time (d)	pH				³ [Fe] mol/L ($\times 10^{-5}$)				[Si] mol/L ($\times 10^{-5}$)				[Al] mol/L ($\times 10^{-5}$)			
		#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4
Hornblende only	0	7.47	–	7.34	7.38	0.04	–	0.02	0.02	2.49	–	5.34	4.99	0.15	–	0.22	0.30
	1	–	7.22	–	7.41	–	0.13	–	0.08	–	4.27	–	5.34	–	0.19	–	0.22
	2	–	–	7.33	–	–	0.16	0.07	0.12	–	4.27	6.41	5.70	–	0.22	0.19	0.22
	3	–	–	–	7.33	–	0.18	–	0.14	–	4.63	–	5.88	–	0.26	–	0.24
	4	–	–	7.30	–	–	0.22	0.14	0.21	–	4.98	6.77	6.41	–	0.26	0.30	0.22
	5	–	7.20	–	7.32	–	0.31	–	0.18	–	5.34	–	6.41	–	0.37	–	0.35
	6	–	–	7.32	–	–	–	0.20	–	–	–	7.12	–	–	–	0.37	–
	7	7.30	–	–	–	0.16	–	–	–	–	4.63	–	–	–	0.26	–	–
	8	–	–	7.31	–	–	–	0.25	–	–	–	7.48	–	–	–	0.41	–
	10	–	–	7.32	–	–	–	0.29	–	–	–	8.55	–	–	–	0.30	–
	21	7.37	–	7.29	–	0.27	–	0.30	–	6.05	–	12.5	–	0.33	–	0.37	–
HB + 24 μ M DFAM	0	–	–	7.34	7.38	–	–	0.02	0.02	–	–	5.34	4.99	–	–	0.22	0.30
	1	–	7.22	–	7.45	–	0.34	–	0.36	–	4.63	–	6.23	–	0.48	–	0.56
	2	–	–	7.30	–	–	0.66	0.59	0.54	–	5.70	7.48	6.77	–	0.63	0.82	0.80
	3	–	–	–	7.35	–	0.84	–	0.77	–	6.77	–	7.48	–	0.82	–	0.95
	4	–	–	7.31	–	–	1.09	0.95	0.96	–	8.55	8.90	9.08	–	1.00	1.15	1.22
	5	–	7.22	–	7.33	–	1.27	–	1.01	–	8.55	–	8.55	–	1.19	–	1.09
	6	–	–	7.33	–	–	–	1.13	–	–	–	9.97	–	–	–	1.26	–
	8	–	–	7.32	–	–	–	1.29	–	–	–	10.3	–	–	–	1.33	–
	10	–	–	7.33	–	–	–	1.36	–	–	–	10.7	–	–	–	1.30	–
	21	–	–	7.29	–	–	–	1.68	–	–	–	13.9	–	–	–	1.30	–
	HB + 120 μ M DFAM	0	–	–	7.34	7.38	–	–	0.02	0.02	–	–	5.34	4.99	–	–	0.22
1		–	–	–	7.45	–	–	–	0.55	–	–	–	6.41	–	–	–	0.72
2		–	–	7.32	–	–	–	1.20	0.93	–	–	8.55	7.36	–	–	1.15	0.96
3		–	–	–	7.35	–	–	–	1.28	–	–	–	8.07	–	–	–	1.19
4		–	–	7.32	–	–	–	1.97	1.50	–	–	10.7	9.38	–	–	1.70	1.45
5		–	–	–	7.35	–	–	–	1.89	–	–	–	9.97	–	–	–	1.63
6		–	–	7.32	–	–	–	2.69	–	–	–	13.1	–	–	–	2.15	–
8		–	–	7.32	–	–	–	3.23	–	–	–	15.0	–	–	–	2.45	–
10		–	–	7.33	–	–	–	3.76	–	–	–	17.4	–	–	–	2.89	–
21		–	–	7.28	–	–	–	6.27	–	–	–	28.5	–	–	–	4.15	–
HB + 120 μ M DFAM		0	–	–	7.34	7.38	–	–	0.02	0.02	–	–	5.34	4.99	–	–	0.22
	1	–	7.22	–	7.42	–	0.84	–	0.69	–	5.34	–	6.41	–	0.63	–	0.76
	2	–	–	7.31	–	–	1.43	1.34	1.16	–	6.77	8.55	7.30	–	1.00	1.19	0.95
	3	–	–	–	7.35	–	1.97	–	1.94	–	8.55	–	8.19	–	1.37	–	1.30
	4	–	–	7.31	–	–	2.51	2.15	3.64	–	9.97	10.7	9.26	–	1.70	1.74	1.48
	5	–	7.23	–	7.35	–	3.40	–	2.38	–	12.8	–	10.2	–	2.30	–	1.72

^a Averages of 2 (exp. #1), 2 (exp. #2), 1 (exp. #3), or 2–3 (exp. #4) replicates from experiments as described in text. Dashes indicate conditions were not used in a particular experiment, or data was not obtained for the indicated time point.

^b Abbreviations: HB - hornblende; DFAM - desferrioxamine mesylate.

^c Error in concentrations estimated at ± 2 –5%.

stocks); 1 mL of assay reagent was added to 2 mL each of culture fluid, blank, or DFAM standard. The absorbance values were determined in a Beckman DU640 UV/VIS spectrophotometer.

Buffered culture supernatants were also tested for the presence of catechol siderophore, using a modified version of the Arnow reaction (Arnow, 1937; Neilands and Nakamura, 1991). In this assay, the test sample is treated with HCl, NaNO₂, and Na₂MoO₄·2H₂O, which results in a yellow color; subsequent addition of NaOH causes the color to change to red in the presence of catechol compounds. Reagents were prepared and reactions carried out as in Neilands and Nakamura (1991) using laboratory stocks and Catechin standard (Sigma Chemical Co.); absorbance values were determined in a Beckman DU640 UV/VIS spectrophotometer at a wavelength of 515 nm.

2.5. HPLC and Mass Spectrometry of Siderophores

Filtered supernatant solutions from experiments with *Streptomyces* sp. with and without hornblende were analyzed with reversed phase HPLC.

It was assumed that the siderophore was a hydroxamate, because streptomycetes are known to produce this class of siderophore (Neilands, 1981; Hughes and Poole, 1989; Winkelmann, 1991; Imbert et al., 1995). Therefore, a modified method by Cramer et al. (1984), who

analyzed the hydroxamate siderophore desferrioxamine B, was used. Reversed phase liquid chromatography with a mobile phase of 15% (v/v) acetonitrile and 85% (v/v) of 0.1% aqueous *o*-phosphoric acid (both of HPLC grade, Fisher Chemical Co.) and a 4 mm \times 250 mm Nucleosil C₁₈ column (Waters Inc.) were used on a Waters 2690 Separations Module using a dual wavelength absorbance detector set at 226 nm. At this wavelength the nonchelated standard siderophore desferrioxamine B is expected to have its maximum absorbance. The flow rate was set at 1 mL/min. The chelator EDTA and phosphate buffers were omitted in the mobile phase to minimize the number of components to avoid interference with the results of subsequent mass spectrometry analysis.

To reveal whether Fe-chelated siderophore was present, absorbance at 430 nm was also analyzed (e.g., Cramer et al., 1984). The samples were acidified to pH < 1 (500 μ L 6N *o*-phosphoric acid to 4 mL sample) to get narrow peaks and good separation. For the fraction collection the flow rate was set at 0.5 mL/min and the injection volume was 100 μ L. Under these conditions the siderophore had a retention time of 12.5 min.

Ten fractions were collected giving a total volume of 24 mL, which was evaporated using liquid nitrogen, freezing and then freeze drying to a volume of a few μ Ls. To eliminate the *o*-phosphoric acid, the sample was suspended in 1 mL acetonitrile and injected on a Supel-

Table 2. Hornblende dissolution experiments in buffered medium with DFAM and *Streptomyces*.^a

Sample name ^b	Time (d)	pH				³ [Fe] mol/L ($\times 10^{-5}$)				[Si] mol/L ($\times 10^{-5}$)				[Al] mol/L ($\times 10^{-5}$)				
		#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	
HB + <i>Streptomyces</i>	0	7.46	–	7.34	7.38	0.04	–	0.02	0.02	2.49	–	5.34	4.99	0.19	–	0.22	0.30	
	1	–	–	–	7.41	–	–	–	0.10	–	–	–	5.34	–	–	–	0.26	
	2	–	–	7.28	–	–	–	0.25	0.26	–	–	6.05	6.05	–	–	0.22	0.30	
	3	–	–	–	7.32	–	–	–	0.56	–	–	–	6.23	–	–	–	0.20	
	4	–	–	7.26	–	–	–	0.65	0.91	–	–	6.77	6.77	–	–	0.37	0.28	
	5	–	–	–	7.29	–	–	–	1.08	–	–	–	6.77	–	–	–	0.32	
	6	–	–	7.28	–	–	–	1.04	–	–	–	7.12	–	–	–	0.41	–	
	7	7.25	–	–	–	0.61	–	–	–	4.99	–	–	–	0.41	–	–	–	
	8	–	–	7.24	–	–	–	1.33	–	–	–	7.48	–	–	–	0.37	–	
	10	–	–	7.21	–	–	–	1.56	–	–	–	8.55	–	–	–	0.37	–	
	21	7.26	–	7.53	–	1.38	–	1.45	–	5.70	–	11.0	–	0.33	–	0.26	–	
HB + 24 μ M DFAM + <i>Streptomyces</i>	0	–	–	7.34	7.38	–	–	0.02	0.02	–	–	5.34	4.99	–	–	0.22	0.30	
	1	–	7.21	–	7.43	–	0.41	–	0.36	–	5.70	–	6.05	–	0.48	–	0.62	
	2	–	–	7.31	–	–	0.95	0.79	0.86	–	6.05	6.77	6.23	–	0.52	0.70	0.76	
	3	–	–	–	7.30	–	–	–	1.42	–	–	–	6.77	–	–	–	0.74	
	4	–	–	7.29	–	–	–	1.70	2.06	–	–	7.83	8.55	–	–	0.93	1.00	
	5	–	–	–	7.30	–	–	–	2.26	–	–	–	7.83	–	–	–	0.95	
	6	–	–	7.26	–	–	–	2.15	–	–	–	7.83	–	–	–	0.96	–	
	8	–	–	7.25	–	–	–	2.69	–	–	–	8.55	–	–	–	0.96	–	
	21	–	–	7.18	–	–	–	3.40	–	–	–	10.7	–	–	–	0.56	–	
	HB + 120 μ M DFAM + <i>Streptomyces</i>	0	–	–	7.34	7.38	–	–	0.02	0.02	–	–	5.34	4.99	–	–	0.22	0.30
		1	–	–	–	7.42	–	–	–	0.65	–	–	–	6.17	–	–	–	0.78
2		–	–	7.32	–	–	–	1.79	2.14	–	–	8.90	7.12	–	–	1.04	0.93	
3		–	–	–	7.31	–	–	–	3.57	–	–	–	8.19	–	–	–	1.24	
4		–	–	7.28	–	–	–	3.76	4.93	–	–	11.0	10.2	–	–	1.56	1.66	
5		–	–	–	7.29	–	–	–	5.67	–	–	–	9.85	–	–	–	1.66	
6		–	–	7.29	–	–	–	5.20	–	–	–	12.8	–	–	–	1.96	–	
8		–	–	7.26	–	–	–	6.09	–	–	–	14.6	–	–	–	2.15	–	
10		–	–	7.23	–	–	–	6.81	–	–	–	15.3	–	–	–	2.37	–	
21		–	–	7.47	–	–	–	8.24	–	–	–	17.8	–	–	–	2.30	–	
HB + 240 μ M DFAM + <i>Streptomyces</i>		0	–	–	7.34	7.38	–	–	0.02	0.02	–	–	5.34	4.99	–	–	0.22	0.30
	1	–	7.21	–	7.41	–	0.72	–	0.70	–	5.70	–	6.77	–	0.63	–	0.76	
	2	–	–	7.32	–	–	1.38	2.51	2.29	–	6.41	8.90	8.19	–	1.00	1.15	0.95	
	3	–	–	–	7.31	–	2.51	–	3.76	–	7.83	–	8.90	–	1.22	–	1.33	
	4	–	–	7.27	–	–	3.94	5.20	5.26	–	9.26	11.4	10.7	–	1.56	1.74	1.76	
	5	–	7.16	–	7.27	–	5.91	–	6.08	–	10.7	–	11.8	–	1.96	–	1.87	
	6	–	–	7.23	–	–	–	7.35	–	–	–	13.5	–	–	–	2.34	–	
	8	–	–	7.10	–	–	–	8.96	–	–	–	15.7	–	–	–	2.59	–	
	10	–	–	6.95	–	–	–	10.8	–	–	–	17.8	–	–	–	3.19	–	
	21	–	–	7.52	–	–	–	14.9	–	–	–	24.2	–	–	–	3.89	–	

^a Averages of 1 (exp. #1), 1 (exp. #2), 1 (exp. #3), or 2–3 (exp. #4) replicates from experiments as described in text. Dashes indicate conditions were not used in a particular experiment, or data was not obtained for the indicated time point.

^b Abbreviations: HB - hornblende; DFAM - desferrioxamine mesylate.

^c Error in concentrations estimated at ± 2 –5%.

guard LC-18-DB guard column (Waters Inc.) by using a Waters M45 solvent system pump. The column was washed with 0.1% trifluoroacetic acid (TFA) for 5 min at a pump rate of 1.0 mL/min, at which point the siderophore was eluted with 0.1% TFA in acetonitrile for 15 min. This fraction was evaporated by blowing nitrogen over it; it was then put in the freezer and subsequently freeze-dried. Fast atom bombardment mass spectrometry (FABMS) analysis was conducted on a Kratos MS-50 mass spectrometer using a 3-nitrobenzyl alcohol matrix.

3. RESULTS

3.1. Identification of Soil Microorganism

An Adirondack soils isolate that exhibited continued growth in the hornblende-enriched medium was chosen for these studies. Genomic DNA was extracted from a 5d culture of the isolate using the Puregene Genomic DNA Extraction kit (Gentra Systems, Inc.), and amplified by polymerase chain reaction

(PCR) in a Progene thermal cycler (Techne, Inc., Princeton, NJ). Partial sequencing of the 16S rRNA gene (Nucleic Acid Facility, Life Sciences Consortium, The Pennsylvania State University) indicated that this species is a streptomyces not yet identified in the Ribosomal Database Project (RDP) database (Michigan State University; Maidak et al., 1999). The species is most closely related to *Streptomyces lividans*, based on a similarity rank of 0.796 and a % complemented sequence of 0.725 (1.0 indicates a perfect match).

3.2. Dissolution Rates

3.2.1. Unbuffered experiments

Experiments using unbuffered growth medium revealed significant pH changes in cultures containing the isolate and

Table 3. Fe, Si, and Al release rates^a in buffered hornblende dissolution experiments.

Sample name ^b	Fe release rate ($\times 10^{-13}$ mol m s)			Si release rate ($\times 10^{-13}$ mol m s)			Al release rate ($\times 10^{-13}$ mol m s)		
	Rate #2	Rate #3	Rate #4	Rate #2	Rate #3	Rate #4	Rate #2	Rate #3	Rate #4
HB only	6 ± 2	6 ± 1	6 ± 2	40 ± 10	80 ± 30	60 ± 10	5 ± 2	6 ± 2	2 ± 3
HB + Stm	–	30 ± 7	40 ± 9	–	80 ± 20	70 ± 20	–	8 ± 2	1 ± 2
HB + 24 μ M DFAM	40 ± 7	40 ± 10	30 ± 8	160 ± 30	180 ± 50	140 ± 40	30 ± 6	40 ± 10	30 ± 8
HB + 24 μ M DFAM + Stm	100 ± 10	70 ± 20	80 ± 20	50 ± 5	110 ± 40	120 ± 30	30 ± 20	30 ± 10	20 ± 6
HB + 120 μ M DFAM	–	80 ± 20	60 ± 10	–	270 ± 70	170 ± 40	–	60 ± 20	40 ± 10
HB + 120 μ M DFAM + Stm	–	170 ± 40	210 ± 50	–	270 ± 80	190 ± 40	–	60 ± 20	40 ± 10
Hb + 240 μ M DFAM	100 ± 20	110 ± 30	110 ± 40	290 ± 60	310 ± 80	180 ± 40	70 ± 10	80 ± 20	50 ± 10
HB + 240 μ M DFAM + Stm	200 ± 40	240 ± 50	220 ± 50	200 ± 40	290 ± 80	230 ± 50	60 ± 10	70 ± 20	50 ± 10

^a Initial rates were calculated from linear regressions of $d(CV)/dt$ from samples of 1–2 (exp. #2), 1 (exp. #3), or 2–3 (exp. #4) replicates collected over the first 5–6 days in each experiment. Error estimates assume 1 standard error in the slope, and do not incorporate error in surface area. These error estimates were made strictly to compare experiment sets. Rates for experiment #1 are not included, as data was not collected until day 7. Dashes indicate conditions were not used in a particular experiment.

^b Abbreviations: HB - hornblende; DFAM - desferrioxamine mesylate; Stm - *Streptomyces* sp.

hornblende, from 7.2 to 5.4 over 21 days (Δ pH = -1.8) and a subsequent increase to 8.7 between 21 and 56 days (Δ pH = $+3.3$; data not shown). These pH changes were not reproduced in the control (bacteria-free) cultures; therefore, element release rates were not interpreted. The observed pH change in cultures containing hornblende only was -0.75 at 21 d, and $+0.17$ at 56 d, whereas in cultures containing the isolate only, Δ pH was -1.84 at 21 d, and -0.19 at 56 days (changes are expressed with respect to previous pH observation; data not shown).

After removal from the inoculated cultures and cleaning with ultrapure acetone and ultraviolet ozone (Vig, 1992; Zazzera and Evans, 1993), the hornblende discs were viewed by differential interference contrast microscopy (DIC) (Fig. 1). Residual dendritic colonies, characteristic of streptomycete mycelial growth,

were still visible on the discs. Subsequent attempts to remove this material by 400°C ashing resulted in the removal of some, but not all of the material from the hornblende surface. Treatment of the discs with the enzyme lysozyme (10 mg/ml^{-1}) for 60 min in an ultrasonicator removed approximately 70% of the residual material.

3.2.2. Buffered experiments

The pH of the phosphate-buffered medium changed only slightly for most experiments (Δ pH = ± 0.2) over 21 days (Tables 1 and 2). However, the concentration of Fe in solution was increased by the presence of *Streptomyces* sp. (Fig. 2, Tables 1 and 2). The presence of *Streptomyces* sp. also in-

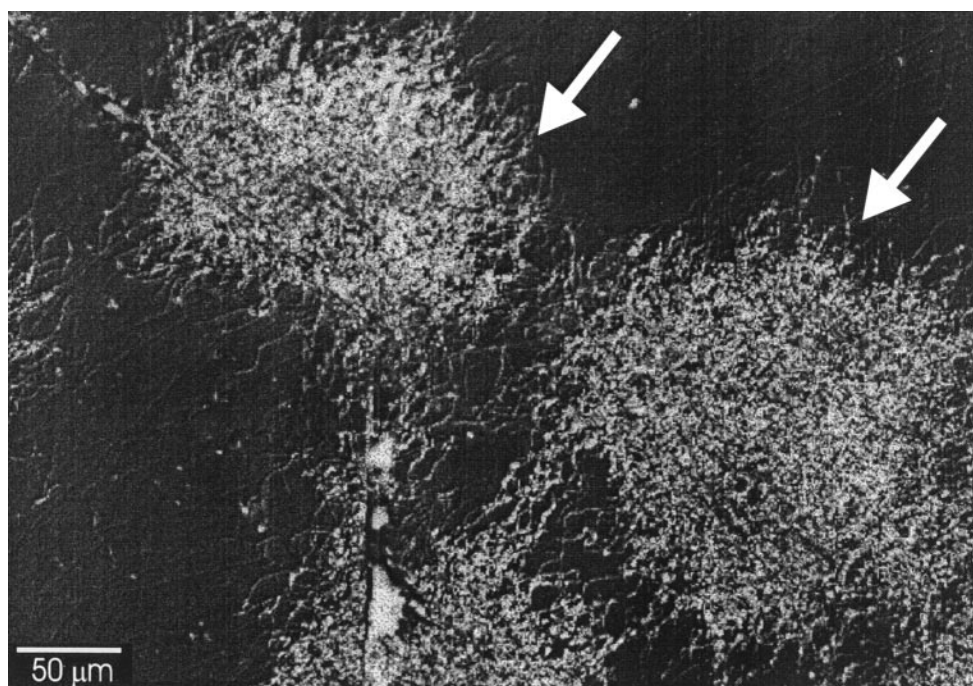


Fig. 1. DIC images of hornblende disk covered with colonies of *Streptomyces* sp. after acetone cleaning and ultraviolet ozone cleaning.

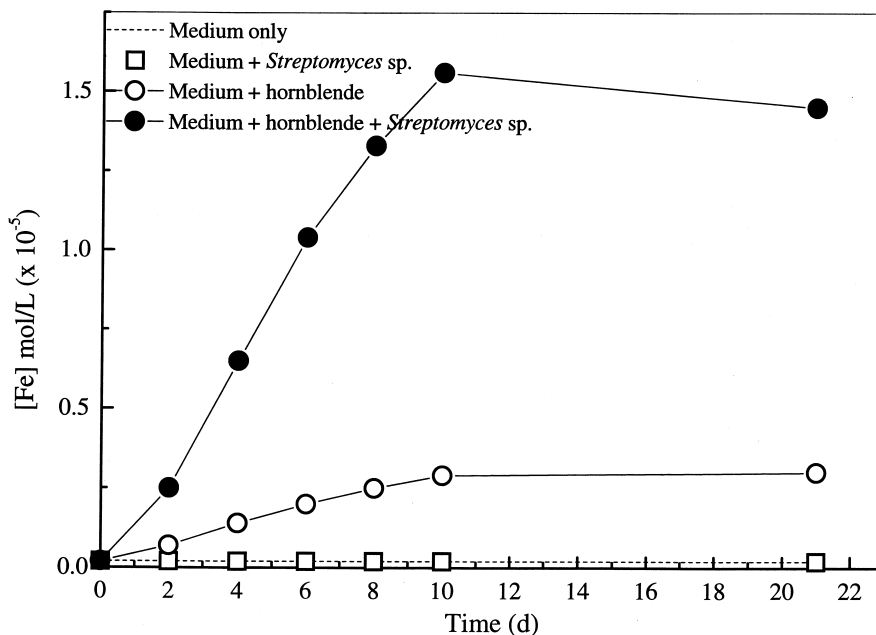


Fig. 2. Comparison of Fe concentration (mol/L) as a function of time between buffered cultures with or without soil isolate *Streptomyces* sp. in a representative hornblende dissolution experiment (Experiment #3; Tables 1 and 2).

creased the initial rate of Fe release over the uninoculated solutions (Table 3).

The hydroxamate siderophore DFAM also increased Fe release into the medium, with greater concentrations of DFAM

resulting in greater increases in Fe concentration (Fig. 3, Table 1). Given that DFAM is capable of binding Fe^{3+} as a 1:1 chelate complex ($K_{ass} = 10^{30.6}$ M), dashed lines on Figure 3 indicate the theoretical Fe^{3+} binding capacity of DFAM, which

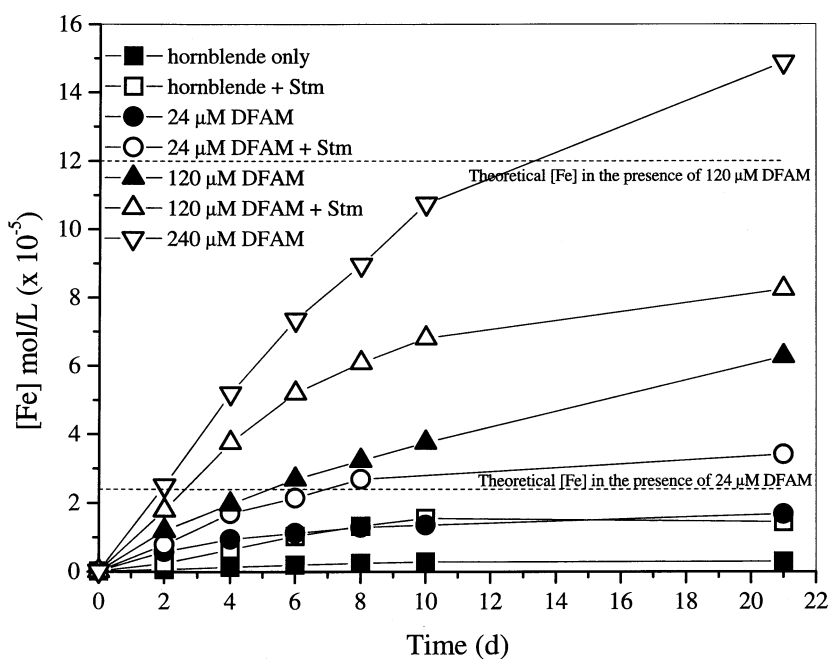


Fig. 3. Comparison of Fe concentration (mol/l) as a function of time between buffered cultures with different concentrations of added DFAM, with and without isolate, in representative hornblende dissolution experiments (Experiment #3; Tables 1 and 2). Culture with 240 μ M DFAM became contaminated after 4 d, and is not included. Abbreviations: DFAM, desferrioxamine mesylate; Stm, *Streptomyces* sp. Dashed lines indicate theoretical Fe binding capacities of 24 μ M DFAM and 120 μ M DFAM, assuming a 1 : 1 chelate complex; actual Fe binding is approximately 70% of theoretical capacity.

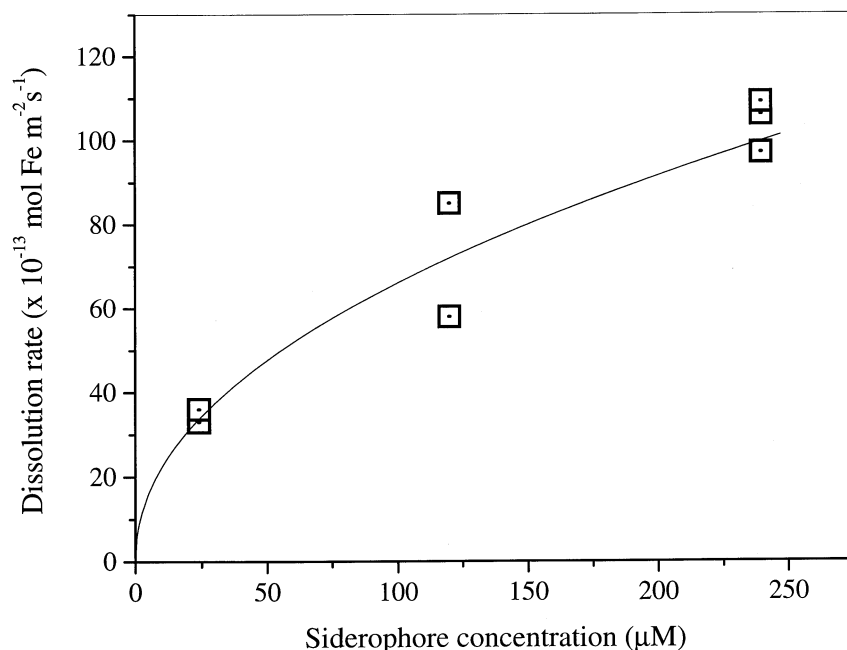


Fig. 4. Release rate of Fe (mol Fe m/s) vs. siderophore concentration, expressed by the equation $R = kC^n$ (see text for explanation of variables).

is equal to the molar concentration of DFAM. The actual binding capacity observed in these studies was approximately 70% of the theoretical capacity. When *Streptomyces* sp. was added to cultures in addition to the siderophore, an even greater enhancement of Fe release was detected (Fig. 3; Table 2).

Initial release rates of Fe in these experiments were calculated using Eqn. 1:

$$R = \left(\frac{d(C_{Fe}V)}{dt} - C_{ave} \frac{dV}{dt} \right) \frac{1}{Am} \quad (1)$$

where R is the release rate (mol Fe/m²s), C_{Fe} is the molar concentration of Fe in solution sampled at time t , V is the volume in the flask at time t before sampling, A is specific surface area (m²/g, measured by Kr BET adsorption), and m is the mass of hornblende powder. The second term in the equation corrects the apparent release rate for changes in concentration due to the decrease in volume of solution during sampling of the experiment. The surface area of the hornblende powder was 0.17 m²/g, as measured by a Micromeritics 2010 ASAP surface analyzer.

The rate of Fe release decreased significantly under all experimental conditions after approximately 10 days (Fig. 3; Tables 1 and 2). Only initial rates ($t = 5-6$ d) are calculated and presented because of the decreasing rate with time. Fe release rates measured for different sets of experiments (hornblende, hornblende + DFAM, or hornblende + DFAM + *Streptomyces* sp.) were identical within each set within one standard error (Table 3).

Comparison of average initial dissolution rates over a 6d period (Table 3) shows that *Streptomyces* sp. causes an approximately five to sixfold increase in the Fe release rate compared

to cultures with hornblende only; the release rate is similar to the rate observed in the presence of 24 μM DFAM without the isolate present. Increased concentrations of DFAM increased the dissolution rate nonlinearly (Fig. 4). However, when DFAM was added along with the isolate at all concentrations, a two to threefold rate increase over the rate in DFAM alone, regardless of DFAM concentration, was observed (Table 3).

Analyses of Si (Fig. 5) and Al (Fig. 6) release showed that the *Streptomyces* sp. did not enhance release of either element; however, DFAM enhanced release of both. Also, release of Al and Si were less reproducible than release of Fe (Table 3).

Without *Streptomyces* sp. present, Fe/Si and Al/Si release rate ratios, abbreviated here as RRRs (avg. 0.11, 0.08, respectively), were lower than similar ratios in the hornblende starting material (0.21, 0.30, respectively). The hornblende therefore dissolved nonstoichiometrically in these experiments, presumably forming an Fe- and Al-enriched surface layer. In contrast, in the presence of *Streptomyces*, the Fe/Si RRR was increased (avg. 0.48) whereas the Al/Si RRR remained relatively unchanged (avg. 0.06). Thus, the surface layer formed in the presence of the *Streptomyces* sp. became Fe-depleted and Al-enriched (using Si as a conservative element). In the presence of very low (24 μM) concentrations of DFAM, Fe/Si RRRs were roughly stoichiometric (avg. 0.23) whereas the Al/Si RRR (avg. 0.21) was still suppressed with respect to the bulk hornblende. For all other concentrations of DFAM with and without *Streptomyces*, the Fe/Si RRRs were greater than the hornblende values and Al/Si RRRs were roughly constant at 0.2 to 0.3.

3.3. Growth of Microorganisms

Analysis of glucose concentration (gram/liter) of Experiment #4 samples from Days 1, 3, and 5 indicated that glucose was

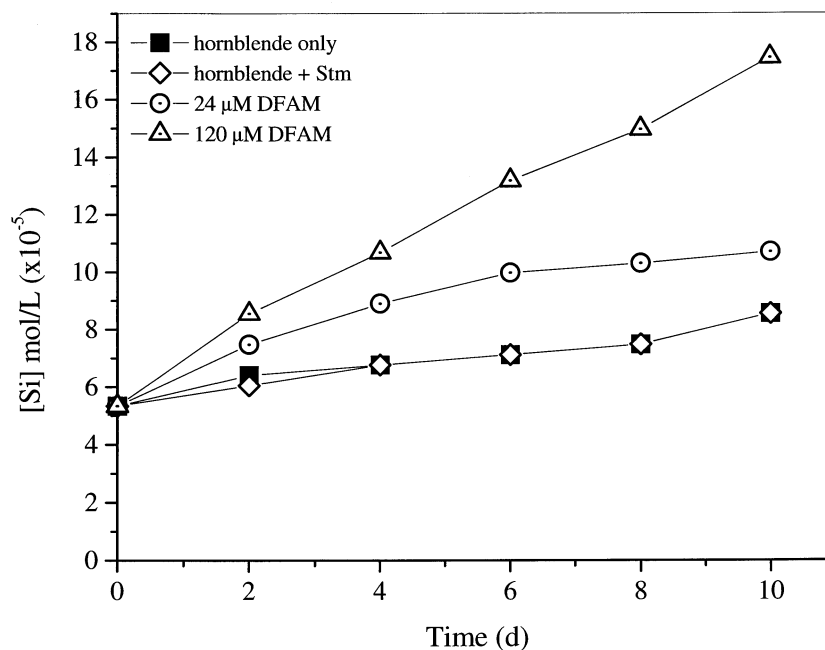


Fig. 5. Si concentration (mol/L) as a function of time in representative buffered hornblende dissolution experiment (Experiment #3; Tables 1 and 2). For abbreviations, see Figure 3.

consumed in all inoculated flasks, but not in uninoculated controls (Fig. 7). In fact, the glucose concentration increased slightly (~10%) from day 1 to day 5 in control cultures, most likely due to evaporation of H₂O. Presumably evaporation occurred in inoculated cultures as well, but glucose levels still dropped due to consumption by the growing organisms. How-

ever, there was not a significant difference in the amount of glucose consumed among the inoculated cultures, with the possible exception of 240 μM DFAM + streptomyces at Day 5 (Fig. 7).

Dry cell mass data produced similar results; whereas most cultures containing hornblende +/- siderophore had nearly

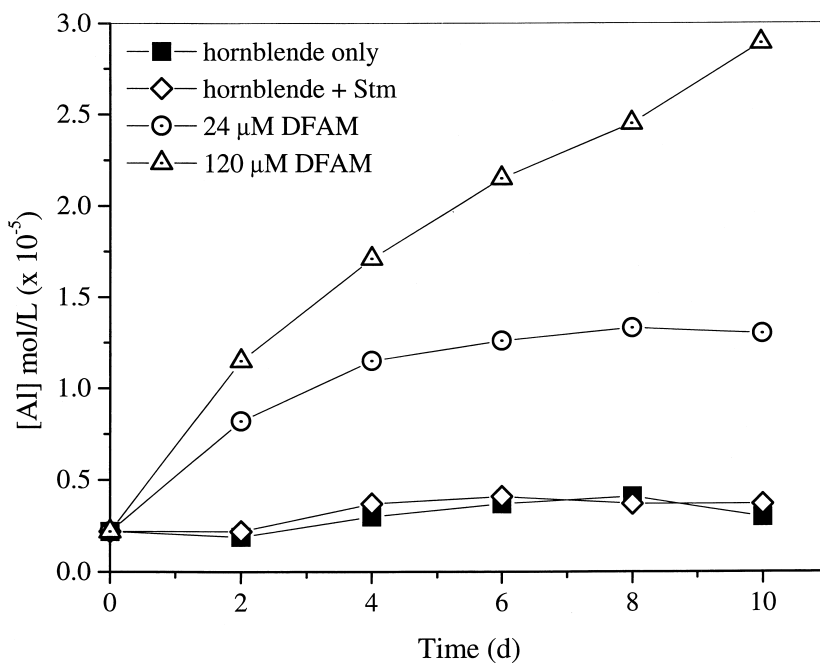


Fig. 6. Al concentration (mol/L) as a function of time in representative buffered hornblende dissolution experiment (Experiment #3; Tables 1 and 2). For abbreviations, see Figure 3.

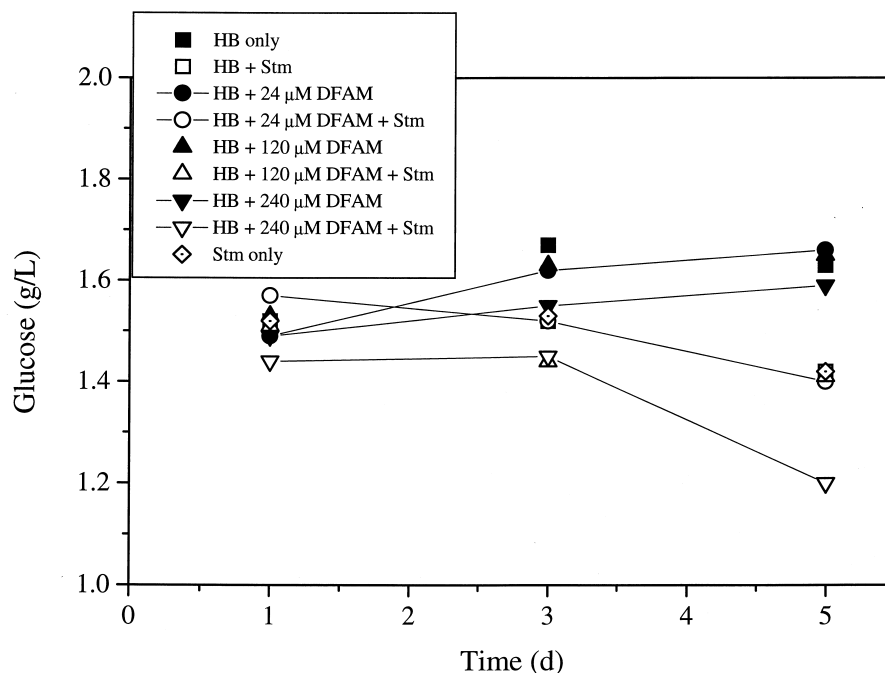


Fig. 7. Comparison of average glucose concentration as a function of time between abiotic and inoculated cultures, +/- hornblende, and DFAM. Samples were from Days 1, 3, and 5 from Experiment #4 (see text). Error in glucose measurements was $\pm 2\%$. For abbreviations, see Figure 3.

twice the cell mass as the culture with the isolate only (~ 0.01 g vs. ~ 0.02 g) there was not a significant difference in mass between cultures with different concentrations of siderophore, or with hornblende but no siderophore, during the time course of this experiment (data not shown). However, in a separate study of *Streptomyces* sp. in the presence and absence of hornblende for 30 d, increased cell mass ($\sim 300\%$) was observed for *Streptomyces* sp. (data not shown).

3.4. Siderophore Analysis

Cultures of *Streptomyces* sp. in low-Fe medium typically turned a brownish color by approximately 2 weeks, an indication of the production of catecholate siderophores (Hughes and Poole, 1989). A universal assay based on removal of Fe from a CAS-Fe dye complex was used to assay for the presence of siderophore in 2 to 3 week cultures. Cultures without added hornblende tested positive, whereas cultures with hornblende or hornblende and DFAM tested negative. For the latter results, assuming that the isolate produced a siderophore, we infer that because the assay relies on the presence of free siderophore to remove iron from the Fe-dye complex, siderophore complexed with Fe from the hornblende will not be detected in the assay.

As streptomycetes typically produce hydroxamate siderophores, a hydroxamate-specific assay (Arnold and Viswanatha, 1983) was used to test culture supernatants. If a hydroxamate siderophore is present in the culture fluid, a loss of color due to removal of Fe(III) from the dye complex can be detected at 532 nm in this assay. However, when 2 to 4 week cultures without hornblende were tested by this method, no loss of absorbance was detected as compared to DFAM standards,

which indicates that this streptomycete does not produce a hydroxamate siderophore.

Culture supernatants were tested for the presence of a catecholate class siderophore by a modified Arnow method (Arnow, 1937; Neilands and Nakamura, 1991). In a positive reaction, compounds with 2 or 3 phenolic OH groups, characteristic of catechols, change color from yellow to red upon alkalization, giving an absorbance value at 515 nm similar to a catechol standard. When analyzed by this assay, culture supernatants tested positive, indicating the presence of a catecholate siderophore.

HPLC analysis of culture supernatants showed that the retention time of the isolate siderophore at 226 nm was approximately the same as desferrioxamine B using this chromatographic system; however, the shapes of the peaks were slightly different. Although DFAM gave a peak with a long tailing, regardless of the concentration, the siderophore excreted from the isolate gave a sharp peak without tailing when acidified to $\text{pH} < 1$. In addition, the DFAM showed more of a narrow peak with higher concentration of acetonitrile in the mobile phase. These differences imply that the siderophore produced by *Streptomyces* sp. has different properties than DFAM and thus is a different compound.

An acidified sample from a culture with only *Streptomyces* sp. in the medium produced a well separated peak at 6.95 min, with a flow rate of 1 mL/min (Fig. 8a). This peak was not visible at 430 nm, whereas a peak at 2.83 min had a much higher intensity than at 226 nm, implying that some of the siderophore had chelated Fe released from the chromatographic system. Another sample with *Streptomyces* sp. and hornblende was also analyzed (Fig. 8b). No free siderophore was observed

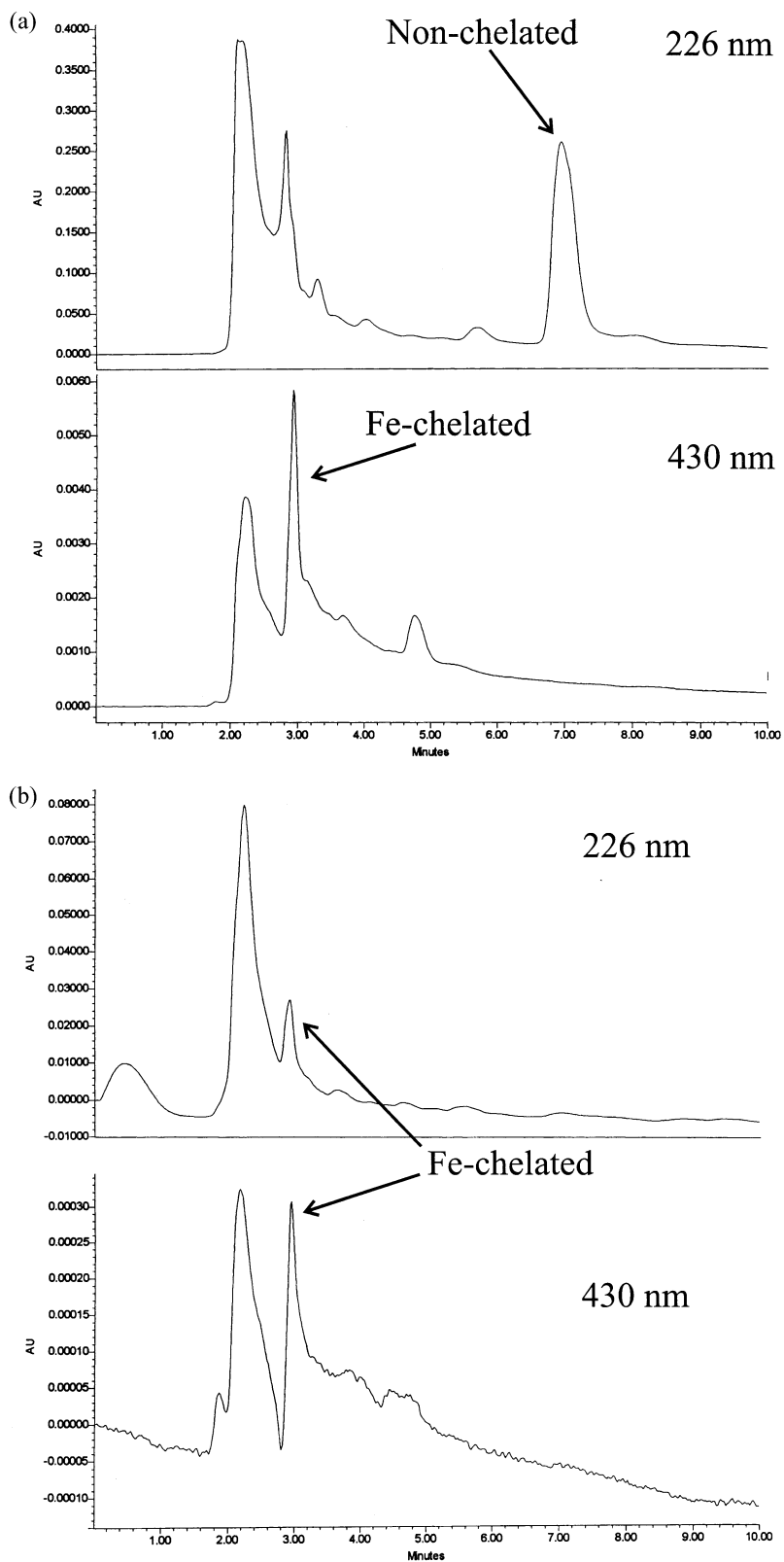


Fig. 8. HPLC chromatograms showing Fe-chelated and nonchelated siderophore profiles: (a) from buffered culture with *Streptomyces* sp. only; top profile measured at 226 nm shows non-chelated peak at 6.95 min, bottom profile at 430 nm shows Fe-chelated peak at 2.83 min; (b) from buffered culture with *Streptomyces* sp. and hornblende; top profile at 226 nm shows no peak at 6.95 min, indicating that all siderophore in this sample is Fe-chelated, as expected, bottom profile at 430 nm shows Fe-chelated peak at 2.83 min.

in the chromatogram at 226 nm, indicating that all of the siderophore was chelating Fe. A strong absorption peak was observed after 2.83 min at 430 nm (Cramer et al., 1984). Mass spectrometry revealed that the molecular mass to charge ratio was 463.4 for the unknown siderophore, compared to 561.3 for the known desferrioxamine. (This mass/charge ratio excludes the mesylate component).

4. DISCUSSION

4.1. Bacterial Adherence

Adherence of *Streptomyces* sp. to the hornblende surface is consistent with mycelial fungus-like growth, in which hyphae may penetrate the mineral surface (Fig. 1). Treatment with the enzyme lysozyme, which cleaves the β -glycosidic bond between sugar residues in gram-positive bacterial cell walls, removed only 70% of the bacterial colonies, and caused thinning of those that remained.

Such penetration of mycelia into the hornblende surface could either increase weathering of this mineral by increasing exposed surface area or could block access to the surface for other potential weathering agents. However, if bacterial coverage of the surface were to have decreased the effect of other reagents, we might have anticipated a decreased effect of DFAM in the presence of the streptomycete. Our observations (Tables 1 and 2), in which Fe release is enhanced in the presence of DFAM + isolate as compared to DFAM alone, are therefore consistent with no inhibition due to bacterial coverage of the surface.

Whether contact with the hornblende surface is necessary for enhanced Fe release in the presence of this *Streptomyces* sp. is, however, still unclear. If the Fe release is due only to siderophore action, it would not be necessary for the isolate to be in direct contact with the mineral.

4.2. pH Effects

In unbuffered experiments with hornblende + isolate, the pH of the medium dropped by 2 units over 21 d. Such a decrease in pH is commonly observed in bacterial cultures, and is usually attributed to production of small organic acids during metabolism. The increase in pH observed at 56 days in the same experiments may be due to metabolic production of ammonia from nitrogen-containing compounds, as observed with various other species of microorganisms (Aristovskaya and Kutuzova, 1968; Williams and Mayfield, 1971; Kutuzova, 1973). Alternately, the increase in pH after the first several weeks may be related to the enhanced rate of bacterial death over growth during nutrient depletion, with the release of basic components upon cell death.

Several authors have documented increasing dissolution rate of hornblende with decreasing pH (Brantley and Chen, 1995; Zhang et al., 1996; Frogner and Schweda, 1998). The observed increase in pH in the presence of *Streptomyces* sp.—regardless of the cause—could generate, under natural conditions, a decrease in observed weathering rate of hornblende. The importance of increases in pH due to microbial activity has generally been underemphasized in most microbe–mineral studies, and is deserving of further study.

4.3. Fe Release Rates

4.3.1. Effects of *Streptomyces* sp.

Initial Fe release rates from hornblende in buffered solutions over a 6 d period were rapid, whereas after 10 days the release rates decreased. Decrease in Fe release rate could have been related to formation of Fe hydroxide precipitates as saturation was reached. At pH 7.0, Fe hydroxide precipitation occurs at Fe^{3+} concentrations on the order of 10^{-9} M in the absence of any chelators (Stumm and Morgan, 1981). Obviously, even in the abiotic experiments without DFAM, this level of Fe concentration was superseded, and may have partially explained the slowing of release rate in those experiments. However, in the presence of chelators, higher concentrations of Fe are possible. For example, in the presence of DFAM, the calculated Fe concentration expected at pH 7 is much larger than that observed in any experiment reported, suggesting that oxyhydroxide precipitation cannot explain the slowing in rates for those experiments.

However, as the growth rate of a population of microorganisms in a static culture decreases and death rate increases due to nutrient depletion, production of siderophores would decrease, as would the number of organisms capable of using siderophores. In our longer-term experiments, cultures began to show characteristics of decreased growth after 10 d, as demonstrated by the formation of sporulating aerial mycelia, appearing as a gray, powdery mat on the surface of the medium. It is thus consistent to conclude that death of organisms may have contributed somewhat to the decrease in Fe release rates over time in the biotic experiments.

Alternatively, if initial Fe release from hornblende (as siderophore–Fe complex) was occurring faster than the net siderophore production rate (production of siderophore minus uptake of siderophore–Fe complex) by the organism, release rates would slow with time, as all siderophore molecules in solution would be complexed with Fe and unable to chelate more Fe. Slow uptake of the siderophore–Fe complex by the bacteria is consistent with observations by Imbert et al. (1995) and Muller and Raymond (1984) that uptake of the siderophore–Fe complex is energy-dependent.

4.3.2. Effects of DFAM

Iron release rates also decreased after several days in the presence of DFAM alone. In these abiotic experiments, the slowing in release rate cannot be caused by bacterial death or slow uptake of siderophore–Fe complexes. Perhaps, as the DFAM molecules became complexed with $\text{Fe}^{3+}(\text{aq})$, further surface complexation reactions and concomitant dissolution became unlikely. Similarity between biotic and abiotic experiments may suggest that this effect is also important in the biotic experiments.

Increasing concentrations of the synthetic siderophore DFAM increased Fe release rates nonlinearly. Fitting our data to a rate law yields the following equation:

$$R = kC^n \quad (2)$$

where k is the rate constant, C is the concentration of siderophore, and n is an experimentally obtained order of reaction determined with respect to the siderophore (Fig. 4). Using this

rate law with $k = 7.6 \times 10^{-13}$ (mol Fe) L^{0.47} m⁻² s⁻¹ (mol DFAM)^{-0.47} and $n = 0.47$ shows that inoculation with *Streptomyces* sp. caused Fe release rates from hornblende that were consistent with dissolution in the presence of approximately 20 to 30 μ m DFAM.

The form of this rate equation is characteristic of dissolution rate laws related to ligand complexation at the mineral surface, where some effect causes saturation of surface complexation at high ligand concentration. For example, if the Fe release rate from hornblende in the presence of DFAM were linearly proportional to the concentration of adsorbed DFA (Γ , mol cm⁻²), and if adsorption of DFA to the hornblende surface followed a Freundlich isotherm (Stumm, 1992) such that $\Gamma \propto [C]^{0.5}$, then Eqn. 2 would be the expected rate equation. Saturation at the surface may be related to the finite number of surface sites, or, as perhaps may be the case here, related to steric hindrance between the large siderophore molecules complexed at the mineral surface.

4.3.3. Effects of *Streptomyces* sp. + DFAM

The reasons for rate enhancement in the presence of both *Streptomyces* sp. and DFAM are unclear. It is unlikely that increased Fe release seen with streptomyces + DFAM is due to increased growth of the isolate in these cultures, as glucose consumption and dry mass data do not support significantly increased growth rates during the time course of the rate calculations. As siderophore production by microorganisms is highly regulated by iron availability in the surrounding environment under conditions of readily available siderophore, further production of siderophore should be wasteful from an energy standpoint. Furthermore, isotopic labeling and Fe uptake studies with ⁵⁵Fe have shown that species of streptomyces are capable of incorporating siderophores produced by other streptomycetes, and that uptake rates are dependent on the siderophore, not the strain of bacteria (Imbert et al., 1995). Therefore, increases in Fe release rate in the presence of both bacteria and DFAM could be explained in several different ways: 1) the *Streptomyces* sp. uses the DFAM (not surprising given that many streptomycetes produce hydroxamates), and recycles it; 2) the *Streptomyces* sp. both uses the DFAM and produces an additional siderophore; 3) the *Streptomyces* sp. cannot use the DFAM and produces a different siderophore, so that both siderophores extract Fe.

If the isolate were unable to use the DFAM, Fe release rates for 120 μ m and 240 μ m siderophore + isolate would be expected to equal the predicted sum of the rates of siderophore alone and isolate alone. However, in both cases the rates for siderophore + isolate were approximately double the rates of the siderophore alone (well above the predicted rates). It is possible that increasing concentrations of DFAM resulted in more Fe being complexed in solution and thus less available to the isolate, which would then need to produce more siderophore to obtain sufficient Fe (hypothesis 3, above). Alternatively, if this isolate were able to use the siderophore provided, it would suggest the DFAM was recycled with a cycling time approximately one half the experiment duration of 5 to 6 days (hypothesis 1). Although we did not observe differences in growth rates, it is possible that the isolate is capable of storing Fe internally after release from either siderophore, such that

free siderophore would still be released for further Fe complexation.

Some siderophores, including DFAM, bind Al as well as Fe, though with lower affinity (Hughes and Poole, 1989; Dionis et al., 1991; Page, 1993). Al complexation is consistent with enhanced release of Al in cultures with added DFAM, which could also account for the 70% effective Fe³⁺ chelate ability, as indicated in Figure 3. The fact that *Streptomyces* sp. did not accelerate Al release on its own suggests that the catechol class siderophore produced by this species is a poor complexing agent for Al³⁺ as compared to Fe³⁺. The Al release rates did not increase with addition of both DFAM and *Streptomyces* sp., indicating that the isolate does not utilize Al from DFAM-Al³⁺ complexes to a significant extent and does not armor the surface against dissolution.

4.4. Growth Rates

The isolate grew faster in the presence of hornblende or hornblende + DFAM, in comparison to medium only. However, there was no evidence for a substantial increase in the growth rate of the isolate in the presence of hornblende + DFAM, as opposed to hornblende only, during the short time course (<1 week) of most of these experiments. This is in contrast to results obtained by Watteau and Berthelin (1994) in an experiment with the fungus *S. granulatus* incubated with goethite, wherein fungal biomass increased much faster than Fe release over a 7d period, in a richer nutrient medium. Siderophore production by the fungi in these studies did not increase significantly until between 21 and 32 d, with a concomitant significant increase in mobilized Fe at that time. These researchers also incubated goethite with the siderophore desferal (another name for DFAM), organic acids, and HCl abiotically, and found that Fe release was higher in the presence of desferal than in the presence of either organic acids or HCl. Siderophores have also been shown to enhance Fe release compared to dissolution in the presence of organic acids, HCl, or aqueous solutions without ligand, from hematite (Hersman et al., 1995) and goethite (Holmen and Casey, 1996) in abiotic studies. These correlations between the presence of siderophore and mobilized Fe suggest that Fe release in our studies containing DFAM was due primarily to the presence of DFAM. Because release rates were obviously affected by the presence of the isolate when it was added along with the DFAM, we may also conclude that the isolate is interacting with the DFAM, as discussed in the previous section. Because all inoculated cultures exhibited a similar growth rate in our experiments, differential growth rates can be eliminated as a significant factor in increased Fe release rates seen with increasing concentrations of siderophore in the presence of *Streptomyces* sp.

As mentioned previously, in a separate study in this lab, cultures of soil isolates, including *Streptomyces* sp., were grown for a period of 30 days in MM9 medium in both the presence and absence of hornblende. These studies indicate that dry cell mass does increase substantially (more than 300% for *Streptomyces* sp., data not shown) in the presence of hornblende during a longer time period than was used in experiments reported here.

4.5. Siderophore Identification

A spectrophotometric assay designed for universal siderophore detection and HPLC results indicated that a siderophore was present in low-iron cultures inoculated with the isolate. HPLC and MS data indicated that the siderophore produced by this streptomycete differs from desferrioxamine B, from which DFAM is derived. Differences in behavior of the two siderophores were observed by HPLC; for example, the siderophore from the isolate could not be detected without acidifying, whereas DFAM could. Wavelength scanning between 200 and 300 nm revealed that the unknown siderophore did not have its maximum absorbance at 226 nm, but slightly below 220 nm. Catechol compounds have benzene groups in contrast to the linear or branched hydroxamate compounds. Benzene absorbs strongly at 184 nm and 202 nm and has a series of absorption bands between 230 to 270 nm, (e.g., Fessenden and Fessenden, 1986). The mass/charge ratios of the two molecules also differed significantly. A hydroxamate-specific assay indicated that the siderophore produced by this isolate was not a hydroxamate, whereas a catechol-specific assay yielded a positive result.

Although our experiments did not yield direct evidence that the identified catechol class molecule is responsible for enhanced Fe release in the presence of the streptomycete, the similar effect of enhanced Fe release in the presence of DFAM, also produced by a streptomycete, is indirect confirmation. In addition, although the association constants for siderophores vary widely, one of the highest constants reported (10^{52} , Neilands, 1981; Hughes and Poole, 1989; Matzanke, 1991) has been reported for a catechol class siderophore, enterobactin, produced by enteric bacteria. Finally, literature reports suggest that catechols have higher association constants than hydroxamates for Fe^{3+} in general (Bergeron and McManis, 1991; Page, 1993). We therefore conclude that it is likely that siderophore production by the streptomycete isolate causes enhanced Fe release from hornblende.

4.6. Implications for Field vs. Laboratory Weathering

Most researchers investigating mineral weathering have noticed that the rate of weathering measured in the field is 1 to 5 orders of magnitude slower than the comparable rate measured in the laboratory. Of the many reasons suggested for this discrepancy (see, e.g., White, 1995), one possibility is that biologic effects in field settings cause slower dissolution rates. However, as indicated in the introduction, most previous researchers have pointed out that biologic effects tend to increase the rate of dissolution rather than decrease it. Several observations made during the course of this study indicate that this view may be too simplistic.

First, in the early unbuffered experiments, the isolate first decreased the pH, and then, after 21 days of growth it also increased the pH of the solution in the presence of hornblende. This increase in pH, attributed to formation of ammonia or to bacterial death, would decrease the overall weathering rate of a mineral such as hornblende (Brantley and Chen, 1995). Second, because the *Streptomyces* adhere to the mineral surface, any increase in pH could be localized at the hornblende surface, and would be undetectable in the bulk porewater solution. Thus, an

experiment in the laboratory mimicking a soil-weathering environment would use a solution of lower pH than actually occurred at the soil mineral surface. Finally, although both studied siderophores promote Fe release from hornblende, these experiments show that Al and Si release in the presence of the isolate is not affected (Table 3). Thus, the effects of powerful chelation may not always serve to enhance overall mineral weathering rates although they serve to enhance metal release.

The complex interactions observed in experiments with both DFAM and the streptomycete emphasize that nonlinear interactions are to be expected in biotic experiments. Such interactions will be difficult to analyze or to extrapolate to natural systems with unknown numbers of bacterial species.

5. CONCLUSIONS

Few studies have been completed in which both the rates and mechanisms of bacterial influence on mineral dissolution have been measured. We hypothesized that some bacteria in hornblende-rich soils should be capable of causing Fe transport under aerobic conditions at neutral pH. One such bacteria was isolated and identified as a species of *Streptomyces* using standard culturing and isolation procedures with an Adirondack soil. Cultures of these bacteria in the presence of hornblende could both increase or decrease solution pH in unbuffered media, suggesting that hornblende weathering could be either enhanced or inhibited by this species. Growth of the species was adherent to the mineral surface, revealing growth of mycelia into cracks and pits at the surface. In buffered experiments, Fe release was enhanced by a factor of 5 in the presence of *Streptomyces* sp., and similarly in the presence of 24 μM DFAM, a commercially available siderophore produced by this genus. Such levels of siderophore concentration are well within the range expected for siderophore concentrations in soil solutions.

The siderophore released by *Streptomyces* sp. was identified as a catecholamide, which is unusual for streptomycetes. We have found no reference to any catechol class siderophores being produced by streptomycetes; however, there are known mixed ligand siderophores produced by actinomycetes (catechol-hydroxamate) (Matzanke, 1991; Hofte, 1993), and many new siderophores are being discovered and characterized (Neilands and Nakamura, 1991; Hofte, 1993). The major functional difference between hydroxamate siderophores and the catecholamides is related to environmental iron concentration. The hydroxamates are generated by the microorganism in a higher iron environment, whereas the catecholamide works as a "back-up" system when the iron concentrations are lower (Bergeron and McManis, 1991). Whereas hornblende dissolution (release of Si and Al) was enhanced in the presence of the hydroxamate, no such increase in Si and Al release was observed in the presence of the catecholate. When both molecules were potentially present (in the presence of the isolate), Fe release was enhanced above that predicted for either molecule alone. Thus, in soils with approximately 10^8 to 10^{10} bacteria/g soil mass (Alexander, 1977), the rates of weathering will be complex nonlinear interactions among many species, and will not be easily estimated from simple monoculture experiments.

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