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Siderophore production by an aerobic *Pseudomonas mendocina* bacterium in the presence of kaolinite

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Abstract

The purpose of this study was to quantify siderophore production by the aerobic bacterium, *Pseudomonas mendocina*, under Fe-limited conditions as a function of Fe source: supplied in dissolved form (as 30 μM Fe-EDTA), as natural Fe-containing kaolinite, and a no-Fe-added control. Siderophores are Fe(III)-specific organic ligands produced by microorganisms under conditions of Fe stress. Siderophore production was compared with previous experiments wherein Fe was supplied as hematite [Geomicrobiol. J. 17 (2000) 1]. Microbial growth increased in the order: no-added-Fe control < kaolinite < Fe-EDTA. Production of siderophore on a per cell basis decreased in the order: no-Fe-added control > kaolinite > hematite ≥ Fe-EDTA. Thus, the bacterium was less Fe stressed in the presence of kaolinite than in the no-added-Fe control, confirming that kaolinite serves as a source of Fe to the bacterium. Although more siderophore per cell was produced in the most stressed system (no-Fe-added), more total siderophore was produced in the least stressed system (Fe-EDTA). This is due to the presence of a larger total population size in the least stressed system. Hence, the ability of a microbial population to produce siderophores as a means to access mineral-bound Fe is a complex function of both Fe stress and population size.

Keywords: Siderophore; *Pseudomonas mendocina*; Kaolinite

1. Introduction

Although Fe is an essential nutrient for almost all microorganisms, the bioavailability of Fe in aerobic circumneutral environments tends to be limited by Fe(III)(hydr)oxide solubility (e.g., Russel et al., 1974; Schwertmann, 1991). One of the principal means used by microorganisms to acquire Fe in such environments is through the use of extracellular Fe(III) binding ligands, especially siderophores (Neilands, 1981). Siderophores are microbially produced metal-complexing ligands with strong binding affinities for Fe(III). Siderophore–Fe complex formation constants are on the order of 10^{23} to 10^{52} (Neilands, 1981; Yoshido et al., 1983; Hider, 1984; Raymond et al., 1984; Hughes and Poole, 1989; Winkelmann, 1991; Albrecht-Gary and Crumbliss, 1998). Siderophore–Fe binding constants are much higher than those of low molecular weight organic acids such as oxalic acid, which has an Fe binding constant of $10^{+7.6}$ (Perrin, 1979). Siderophores also can bind a variety of other trace metals, including Al^{3+} (e.g., Yoshido et al.,

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1983; Evers et al., 1989; Hernlem et al., 1996). Several studies have shown that siderophores can enhance the dissolution rates of Fe(III)(hydr)oxides (e.g., Watteau and Berthelin, 1994; Hersman et al., 1995, 2000; Holmén and Casey 1996; Holmén et al., 1999; Kraemer et al., 1999; Cocozza et al., 2002) and aluminosilicates (Liermann et al., 2000; Kalinowski et al., 2000a,b; Rosenberg and Maurice, submitted for publication).

The focus of this research is siderophore production by an aerobic *Pseudomonas mendocina* bacterium as a function of different amounts of Fe stress; i.e., when Fe is added in the dissolved form, as an Fe oxide, or as a trace constituent of the aluminosilicate clay mineral, kaolinite. *P. mendocina* is a true aerobe that is unable to use Fe(III) as a terminal electron acceptor for oxidative phosphorylation, but still requires Fe as an essential nutrient for metabolic processes (Hersman et al., 1996; Forsythe et al., 1998). Therefore, *P. mendocina* only requires μM concentrations of Fe (e.g., Hersman et al., 1996, 2000; Forsythe et al., 1998; Maurice et al., 2000, 2001a,b; Ams et al., 2000). This is in contrast to high Fe-requiring bacteria, such as dissimilatory Fe-reducing bacteria (DIRB), which require mM concentrations (e.g., Arnold et al., 1988, 1990; Lovley, 1991; Lovley and Phillips, 1986, 1988; Nealson and Myers, 1992; Roden and Zachara, 1996; Zachara et al., 1998; Grantham et al., 1997). Like many microorganisms (e.g., Neilands, 1981), *P. mendocina* produces siderophores when under Fe stress as a means of acquiring Fe (Hersman et al., 1996, 2000). Hersman et al. (2000) showed that siderophore production by *P. mendocina* increased on a per cell basis as the availability of Fe decreased, in the order: Fe-EDTA < Fe(III)(hydr)oxides < no-Fe-added control. Hersman et al. (1995) also showed that the siderophore produced by *P. mendocina* enhanced Fe(III)(hydr)oxide dissolution.

Recently, Maurice et al. (2001a,b) demonstrated that *P. mendocina* can acquire Fe that is present in trace quantities in the aluminosilicate clay mineral, kaolinite, and that dissolution of Al from kaolinite is enhanced in the presence of the bacterium. The role of siderophores in the enhanced Al release and/or Fe acquisition process(es) is currently unknown.

As a first step towards understanding the potential role of bacterial siderophores in Fe acquisition from clays, the research reported herein focused on amount of microbial growth and siderophore production by *P. mendocina* in the presence of natural kaolinites. Results were compared to similar and/or simultaneous experiments wherein Fe was added as Fe-EDTA, hematite, and no-Fe-added controls. We hypothesized that if, as suggested by Maurice et al. (2001a,b), bacteria are able to acquire Fe from kaolinite, then siderophore production on a per cell basis in the presence of kaolinite would be less than in no-Fe-added controls. This is because the *P. mendocina* would be less Fe-stressed when kaolinite was present as a source of Fe. We further hypothesized that microbial growth would decrease in the order Fe-EDTA ≫ kaolinite > no-Fe-added controls with increasing Fe stress, and that siderophore production on a per cell basis would decrease in the order: no-Fe-added controls > kaolinite > hematite > Fe-EDTA.

2. Materials and methods

2.1. Kaolinite

Kaolinite was chosen for this study because: (1) it is one of the most abundant and ubiquitous clay minerals on the surface of the Earth (Chamley, 1989; Moore and Reynolds, 1989); (2) it is one of the simplest aluminosilicate clay minerals, exhibiting a 1:1 layer structure with no interlayer cations (Moore and Reynolds, 1989); (3) it has a relatively stable composition, $Al_2Si_2O_5(OH)_4$, with little variability, yielding a very minor permanent structural charge; (4) bulk Fe contents in kaolinite can range from 0.1% up to 3% (Weaver and Pollard, 1973; Schroeder and Pruett, 1996; Schroeder et al., 1998); (5) kaolinite surface properties and dissolution kinetics have been studied extensively by many researchers (e.g., Carroll-Webb and Walther, 1988; Carroll and Walther, 1990; Nagy et al., 1991; Chin and Mills, 1991; Brady and Walther, 1992; Wieland and Stumm, 1992; Xie and Walther, 1992; Ganor et al., 1995; Brady et al., 1996; Schroth and Sposito, 1997; Ward and Brady, 1998; Huertas et al., 1998, 1999); and (6) *P. mendocina* can acquire Fe from kaolinite and enhance kaolinite dissolution (Maurice et al., 2001a,b; Ams et al., 2000).

The kaolinite samples used in this study were Clay Minerals Society Source Clay kaolinites KGa-1b and

KGa-2. These kaolinites were characterized by Sutheimer et al. (1999). In brief, KGa-1b is a well ordered kaolinite from Washington County, GA; KGa-2 is a poorly ordered kaolinite from Warren County, GA. X-ray diffraction analysis (XRD) showed trace amounts of halloysite present in KGa-1b and traces of anatase in KGa-2. The point of zero net proton condition ($pH_{p.z.n.p.c.}$) value for cleaned KGa-1b and KGa-2 is 5.1 ± 0.2 and 4.9 ± 0.2, and the mean ratio of edge-to-total surface area is ~ 0.2 and ~ 0.19, respectively. Bulk chemical analyses of both kaolinites (Maurice et al., 2001a) show trace amounts of Fe (as Fe_2O_3, 0.04 wt.% for KGa-1b; 0.94 wt.% for KGa-2); however, it is not known how the Fe is distributed. Schroeder and Pruett (1996) have suggested that Fe impurities in kaolinite may exist as accessory minerals, surface precipitates, and/or as structural substitution for Al.

Clay samples were weakly cleaned in a 1 M NaCl solution adjusted to pH 3 with HCl and rinsed prior to dissolution, to remove the most labile forms of Fe and surface contaminants such as some adsorbed organics or amorphous Al oxyhydroxides. The procedure was a modification of the method of Schroth and Sposito (1997) and is described in detail by Sutheimer et al. (1999) and Maurice et al. (2001a,b).

2.2. Bacterial strain

The bacterium used in this study, a *P. mendocina* strain, was isolated from sediment in a surface holding pond of a drilling operation at the Nevada Test Site, NV, USA. It is rod-shaped, ~ 2 μm long, and 0.5–1.0 μm in diameter with a single polar flagellum. Through comparisons of total rRNA sequence analysis, this bacterium was identified as *P. mendocina* (at 0.78% confidence level; MIDI, DE) (Hersman et al., 2001). It tested positive for catalase, oxidase, and nitrate reduction (assimilatory) and negative for fermentation and iron and sulfate reduction. Therefore, it is inferred that this microbe is a strict aerobe unable to use Fe as a terminal electron acceptor for oxidative phosphorylation but still requires Fe in μM concentrations as an essential trace nutrient for metabolic processes (Hersman et al., 1996; Forsythe et al., 1998). The *P. mendocina* has, however, been shown to reduce Fe during growth on hematite under overall aerobic conditions (Hersman et al., 1996, 2001), perhaps doing so as part of its Fe acquisition strategy. This bacterium was chosen because it dissolves Fe from Fe(III)(hydr)oxides and Fe-containing aluminosilicates for metabolic uses only (Hersman et al., 1996; Maurice et al., 2001a,b) and only requires μM concentrations of Fe for growth.

2.3. Medium

An Fe-deficient growth medium was prepared in the same manner as Hersman et al. (2001). In brief, the following analytical grade chemicals (Fluka Chemie, Switzerland) were added to 1 l of distilled deionized water: 0.5 g of K_2HPO_4; 1.0 g of NH_4Cl; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.05 g of $CaCl_2$; 5.0 g of succinic acid disodium salt anhydrous ($C_4H_4Na_2O_4$), and 0.125 ml of trace elements (5 mg of $MnSO_4 \cdot H_2O$; 6.5 mg of $CoSO_4 \cdot 7H_2O$; 2.3 mg of $CuSO_4$; 3.3 mg of $ZnSO_4$; and 2.4 mg of MoO_3 per 100 ml of distilled deionized water). The growth medium purposely contained no added dissolved Fe, except in the case of added dissolved Fe-EDTA. Therefore, in order for population densities to reach levels greater than non-kaolinite containing controls in growth experiments, the bacterium had to obtain Fe from the solid kaolinite phase. A small amount of growth in bacterial controls (no-Fe-added) observed in the experiments described herein and previously by our group indicates that there is likely a trace of Fe present in the growth medium, but below detection (i.e., <ppb) by graphite-furnace atomic absorption spectrophotometry (Perkin Elmer 5100 PC, GFAAS).

2.4. Bacterial growth curves

Microbial growth experiments were performed in the presence of weakly cleaned KGa-1b, KGa-2, 30 μM Fe-EDTA, 30 μM Fe-EDTA + KGa-1b, and no-added-Fe as a control. The methods used were similar to those described by Maurice et al. (2000, 2001a,b). Acid-washed, 150-ml Teflon flasks were used as reaction vessels. Approximately 0.0571 g of KGa-1b and 0.0324 g of KGa-2 were added to the appropriate flasks in order to normalize the BET surface areas of the kaolinites (KGa-1b = 12.6; KGa-2 = 22.2 $m^2\ g^{-1}$) (Sutheimer et al., 1999) to 24 $m^2\ l^{-1}$. Ten milliliters of growth medium with no added Fe or containing 90 μM Fe-EDTA were added, and the vessels were then

sterilized by autoclave. Twenty milliliters of inoculum was added to each flask (inoculum prepared as described below), and samples (now 30 ml each) were placed on a shaker table in the dark at 22 °C. At various reaction times, subsamples were removed by sterile pipet for analysis of microbial population size by absorbance at 600 nm. All experiments were run in triplicate. At the end of the experiments (4 days), flasks were centrifuged and filtered (0.1 μm Nuclepore polycarbonate membrane filters), and solutions were analyzed by ICP-OES (optical ICP).

2.5. Siderophore production

The experimental design for the siderophore production experiments is shown schematically in Fig. 1. Siderophore production experiments were performed in the presence of 30 μM Fe-EDTA, KGa-2, and a no-Fe containing control. Glass reaction flasks (250 ml; BELLCO) were acid-washed with trace-metal-grade nitric acid and rinsed three times with distilled deionized water (18 MΩ cm^{-1}). Similar to growth experiments, ~ 0.0324 g of KGa-2 was added to each of nine flasks in order to normalize the BET surface area of the kaolinite to 24 $m^2 l^{-1}$. Ten milliliters of sterile growth medium was added to each of the KGa-2-containing flasks and nine additional flasks without kaolinite (i.e., no-Fe-added control). Ten milliliters of a 90-μM Fe-EDTA solution prepared in growth medium was added to nine more flasks without kaolinite. All flasks were then sterilized by autoclave. Previous experiments showed that autoclaving resulted in a small amount of Si release from kaolinite, but no detectable Al or Fe release from the kaolinite

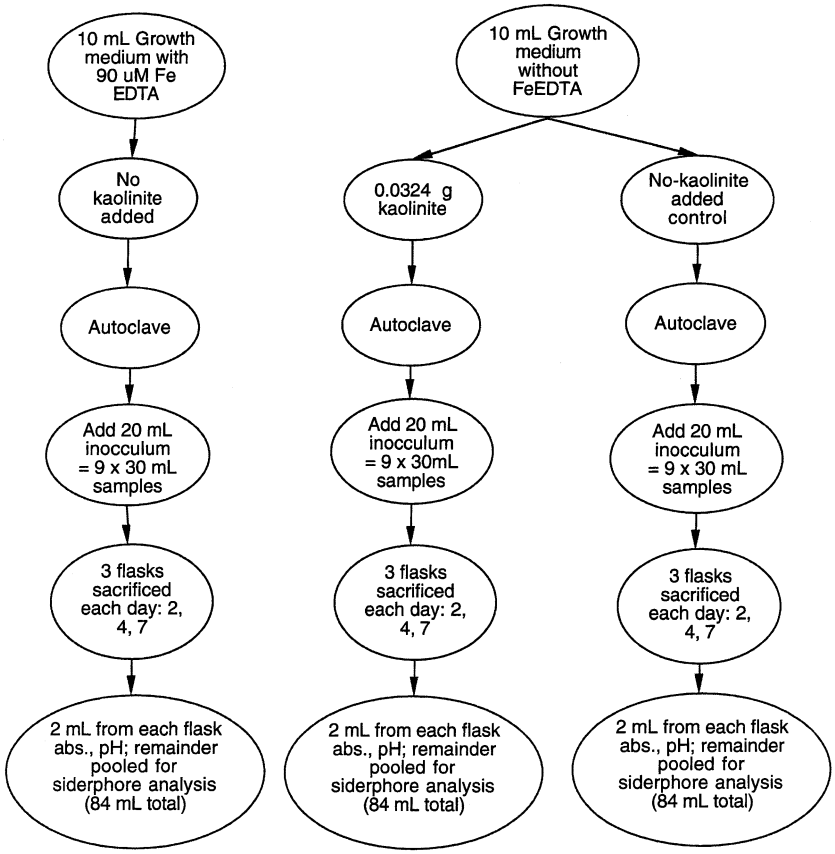

Fig. 1. Schematic illustration of the experimental design for the measurements of bacterial siderophore production.

(Maurice et al., 2001b; Ams and Maurice, unpublished data). Maurice et al. (2001b) showed that when bacteria were grown in medium that had been autoclaved containing kaolinite, centrifuged and filtered to remove the kaolinite, and reautoclaved prior to inoculation, the *P. mendocina* grew to approximately the same population size, or slightly less than controls that had not been exposed to kaolinite. This indicated negligible mobilization of Fe from kaolinite by the autoclave procedure.

A stock solution of inoculum was prepared by adding a calculated proportion of a starter culture (grown in Fe-deficient medium as described above) to previously prepared sterile growth medium such that the concentration of bacteria was 2.25×10^6 CFU ml^{-1}. The proportion of starter culture added was determined through correlation of absorbance with # cells ml^{-1} (as in Hersman et al., 1996; Forsythe et al., 1998). Twenty milliliters of the stock inoculum solution was then added to each of the sterilized reaction flasks bringing the total volume in each flask to 30 ml, thus normalizing the solutions to 1.5×10^6 CFU ml^{-1} in all experiments; and 30 μM Fe-EDTA and 24 $m^2\ l^{-1}$ in appropriate experiments. All flasks were left in an incubator at 28 °C (the lowest temperature permitted by the incubator), in the dark, on a shaker table (50 rpm). We chose this temperature because it was only slightly above room temperature, and facilitated relatively rapid and controlled microbial growth.

At each sampling time (days 2, 4, and 7), three flasks from each experiment (no-added-Fe control, 30 μM Fe-EDTA, and KGa-2) were sacrificed randomly for analysis. From each sacrificed flask, 1 ml of sample was used for absorbance readings at 600 nm to monitor cell growth, and a second 1 ml was used for pH measurements. The growth data were used to normalize siderophore production on a per cell basis. The remaining solution from each time interval, for any three replicate flasks, was then combined to a total of 84 ml for siderophore analysis.

The siderophore purification procedure has been described previously by Hersman et al. (2000). In brief, after pooling samples, the cells were removed by centrifugation (5000 rpm for 1 h). The supernatant was decanted into a graduated cylinder and the volume was noted. The contents were then transferred into a separatory funnel followed by an equal volume of phenol/chloroform (1:1 ratio) and the funnel was shaken with ample venting. The contents were allowed to separate in the dark. Following separation, the organic phase was saved and the aqueous phase was discarded. Twice the volume of ether/water (1:1 ratio) was added to the organic phase, followed by shaking, venting and separation in the dark. After separation, the organic phase was discarded and an equal volume of ether was added to the remaining aqueous phase. The funnel was shaken, vented, and allowed to separate. The aqueous phase was washed continuously with ether in the same way until separation occurred instantly. The aqueous phase was then decreased to ~ 10 ml by rotoevaporation. In order to remove excess salts, the sample was eluted with 0.1 M pyridine through a Sephadex C-25 column ($\sim 25 \times 350$ mm); absorbance was monitored at 280 nm as fractions were collected. Once the siderophore fractions were identified, they were pooled and lyophilized. The samples were then brought back into solution by adding 1 ml of distilled deionized water (18 $M\Omega\ cm^{-1}$) and injected into a high pressure liquid chromatograph (HPLC) for analysis. For HPLC, a C-4 analytical column was eluted with a gradient of 99.9% water/0.1% trifluoroacetic acid (TFA) to 99.9% acetonitrile/0.1% TFA over 200 min. The absorbance was monitored at 230 nm.

Elution locations of siderophore within the HPLC chromatogram have been previously identified (Hersman et al., 1993) with the chrome azural assay of Schwyn and Neilands (1987). *P. mendocina* was shown to produce five distinct HPLC peaks that test positive for siderophore (Hersman et al., 1993, 2000). All of the peaks have identical molecular masses (929 Da) and identical amino acid content (Hersman et al., 1993), as determined by the Waters Pico-Tag method (Bidglingmeyer et al., 1984). In the current study, the peaks were clearly identified with different elution times than determined in the literature because the HPLC set-up (i.e., the analytical column) was a slight modification of previous studies. Because purified *P. mendocina* siderophore of known concentration was not available as a standard for measuring absolute concentrations, the relative concentrations of siderophore were estimated through correlation of experimental HPLC chromatogram peak areas with peak areas of DFAM as a standard (data not shown here).

3. Results and discussion

3.1. Microbial growth

Fig. 2 shows the results of microbial growth experiments comparing changes in population size over time in the presence of Fe-EDTA, Fe-EDTA + KGa-1b, KGa-1b, KGa-2, and no-Fe-added controls. In agreement with previous observations (Maurice et al., 2001a,b), *P. mendocina* was able to grow to a substantial population size, relative to that observed in no-Fe-added controls, when kaolinite was the sole source of Fe. Because the bacteria were Fe-limited, growth beyond that of controls could only occur if Fe were obtained from the solid phase.

A gradual increase in population size was observed for the kaolinite only experiments to ~2.5 days, followed by a gradual decrease in population size; the decrease is likely due at least in part to accumulation of toxic byproducts of bacterial growth. The growth experiment containing Fe-EDTA alone, exhibited a sharp increase in population size through day 1, followed by a decrease. Initially, the growth curve for Fe-EDTA + KGa-1b showed a similar trend to that of the Fe-EDTA only experiment. However, at the end of day 1, when the population size of the Fe-EDTA only experiment was declining, the Fe-EDTA + KGa-1b experiment exhibited a continued but more gradual growth, resembling growth exhibited in the kaolinite only experiments. This suggests that the bacteria were accessing Fe from the kaolinite as well as from Fe-EDTA. There was very little growth observed in the no-Fe-added control over the entire course of the experiment.

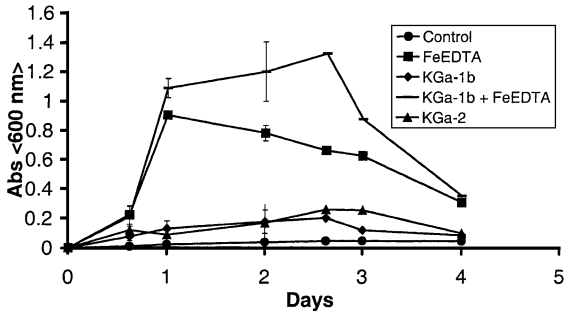

Fig. 2. Changes in microbial population size over time for no-Fe-added control, Fe-EDTA, KGa-1b, KGa-1b + Fe-EDTA, and KGa-2. Each point represents the mean of three samples. Microbial population size measured by absorbance at 600 nm.

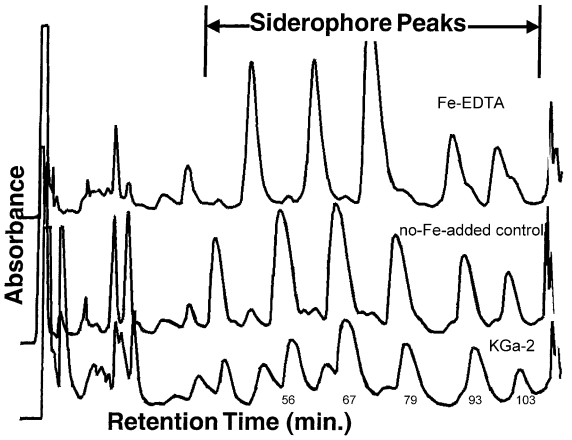

Fig. 3. HPLC chromatograms of samples from the no-Fe-added control, KGa-2, and 30 μM Fe–EDTA experiments at day 2 (28 °C). *X*-axis depicts column retention time in minutes; *Y*-axis depicts absorbance at 230 nm. Data show decrease in siderophore production in the order: Fe–EDTA>no-Fe-added control>KGa-2.

As shown in Fig. 2, the maximum population size was greater and the time to maximum population size lesser when Fe was added as highly available Fe-EDTA (peak at $\sim 1.09 \times 10^9$ CFU at ~day 1) than when Fe was added as a trace constituent of kaolinite (peak at $\sim 2.84 \times 10^8$ CFU at ~2.5 days). As previously determined by the dilution pour plate method, 1.0 A equals $\sim 1.4 \times 10^9$ CFU (colony forming units) ml^{-1} (Hersman et al., 2000). Growth was least in the no-Fe-added control (maximum at $\sim 6.67 \times 10^7$ CFU at ~2.5 days). The overall maximum population size was observed when Fe was added as a combination of Fe-EDTA and kaolinite KGa-1b (peak at $\sim 1.85 \times 10^9$ CFU at ~2.5 days). In agreement with results of similar studies (Ams et al., 2000; Maurice et al., 2001a,b), a change in initial pH from ~8 to a final pH of ~9.5 was observed when the bacteria were in the presence of added Fe (as either form). A decrease in pH from ~8 to ~6.5 was observed in the no-Fe-added control, in agreement with previous observations in our laboratories. Also in agreement with Maurice et al.'s (2001b) previous experiments, Al was released from the kaolinites in the presence of bacteria (4 days' reaction time KGa-1b: 245 ± 6 ppb here vs. 303 ± 10 observed previ-

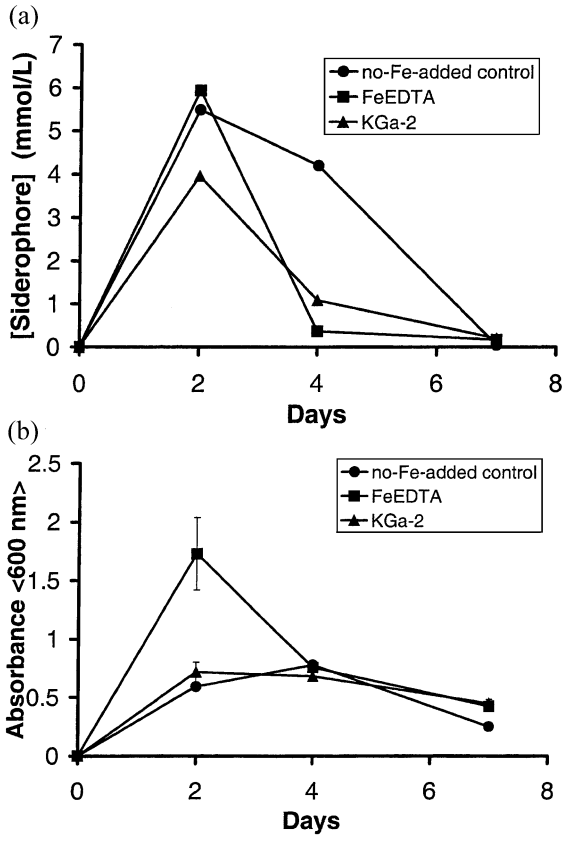

Fig. 4. (a) Comparison of total siderophore concentration (in millimoles per liter) in reaction vessels at each day for Fe–EDTA, KGa-2, and no-Fe-added control experiments. (b) Changes in microbial population size over time for no-Fe-added control, Fe–EDTA, and KGa-2 experiments (corresponding to data in (a)). Each point represents the mean of three samples. Experiments were run at a higher temperature (28 vs. 22 °C) than those shown in Fig. 2.

ously; KGa-2: 279 ± 3 ppb here vs. 333 ± 9 observed previously).

3.2. Siderophore production

Fig. 3 shows HPLC chromatograms of the no-Fe-added control, KGa-2, and 30 μM Fe-EDTA experiments for day 2. As previously mentioned and defined in more detail by Hersman et al. (1993), the five dominant peaks near the middle of the chromatograms (Fig. 3) represent siderophores produced by *P. mendocina*. Figs. 3 and 4a show that the total siderophore present in the reaction vessels at day 2 decreased in the order: Fe-EDTA>no-Fe-control≫KGa-2. The pH changes were similar to those described above.

Siderophore production normalized to population size (Fig. 5) reveals additional information regarding siderophore production vs. Fe stress. As shown in Fig. 5, the amount of siderophore present on a per cell basis reached a peak at ~ day 2, with decreasing siderophore present as the experiment progressed. This latter decrease in siderophore content suggests that the siderophore may not be recycled, and that it appears to be consumed or destroyed as the population wanes. Although total siderophore production (Fig. 4a) was greatest when Fe was present as Fe-EDTA, normalization to siderophore production per cell (Fig. 5) yielded the least amount of siderophore produced per cell in the Fe-EDTA only experiment. In the no-Fe-added control, large concentrations of total siderophore were present (Figs. 3 and 4a), but at low cell counts, which resulted in large concentrations of siderophore per unit cell (Fig. 5). When the only added source of Fe was KGa-2, total siderophore production (Figs. 3 and 4a) was low because of the lower population size in this system resulting from the low concentration of readily available Fe (Fig. 4b). However, the siderophore production per cell was high relative to that observed in the Fe-EDTA only experiment.

Siderophore production in the presence of kaolinite can be compared with results of previous experiments by Hersman et al. (2000) using hematite. Hersman et al. (2000) determined that siderophore production on

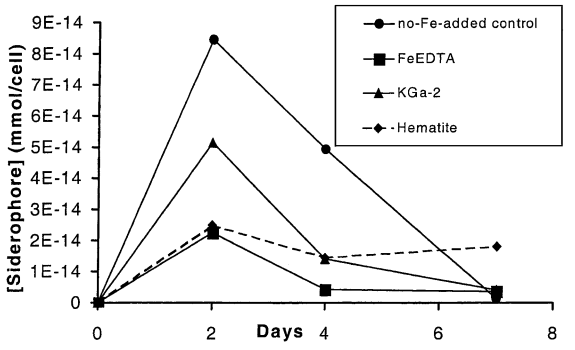

Fig. 5. Comparison of siderophore production per cell in Fe–EDTA, KGa-2, and no-Fe-added control experiments, compared with previous data from a hematite-containing experiment (see text). Siderophore concentration per cell decreased with decreasing Fe stress in the order: no-Fe-added control>KGa-2>hematite = Fe–EDTA.

a per cell basis by *P. mendocina* under similar conditions as this experiment, decreased with decreasing Fe stress: no-Fe-added control>hematite>Fe-EDTA, as the availability of Fe increased. This is in agreement with findings by Neilands (1981) and Neilands and Ratledge (1982) who suggested that siderophore production increased with greater Fe deficiencies in a system. Hersman et al. (2000) calculated daily siderophore production from the concentration of total siderophore present in solution at each time interval minus the total from the previous time interval. In this study, total or cumulative siderophore present at each day is plotted (Fig. 5). Combination of Hersman et al.'s (2000) raw data (i.e., cumulative concentration) with the current data set yields a general trend of decreasing siderophore production on a per cell basis in the following order: no-Fe-added control>kaolinite> hematite ≥ Fe-EDTA (Fig. 5). Considering that Fe is present only as a trace constituent of kaolinite but as a primary constituent of hematite, kaolinite predictably has less readily available Fe than hematite, resulting in enhanced siderophore production.

3.3. Implications for siderophore-mediated dissolution

Hersman et al. (1996) showed that the siderophore produced by *P. mendocina* enhanced hematite dissolution. A 28 mM solution of siderophore at pH 3 solubilized ~ 6 μmol l^{-1} Fe in 24 h, about 2 μmol l^{-1} above an inorganic control. As shown in Fig. 4a, above, the *P. mendocina* produced millimolar concentrations of siderophore, which could potentially have a significant effect on mineral dissolution. Considering that siderophore production varied with the source of Fe to the system, the potential for mineral dissolution would also vary. On the one hand, when Fe is more readily available and the bacteria are less Fe stressed, siderophore production per unit cell is low but total production is relatively high because of the large bacterial population. On the other hand, when Fe is less readily available and the bacteria are more Fe stressed, siderophore production per cell is higher but total siderophore production somewhat lower because of the small population size. This suggests that the ability of a monoculture bacterial population to effect the weathering of Fe-containing minerals via siderophore production is dependent on both the ability of a given population to reach a significant size and the degree of Fe deficiency within the system. Therefore, although siderophore production on a per cell basis is suppressed when Fe is readily available, total siderophore production and hence potential for siderophore-promoted mineral dissolution is relatively high. Hence, the ability of a microbial population to produce siderophores as a means to access mineral-bound Fe is a complex function of both Fe stress and population size.

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