Short Communication

Iron reduction by psychrotrophic enrichment cultures

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Abstract

Psychrotrophic ($< 20°C$) enrichment cultures from deep Pacific marine sediments and Alaskan tundra permafrost reduced ferric iron when using organic acids or H_2 as electron donors. The representative culture W3-7 from the Pacific sediments grew fastest at 10°C, which was 5-fold faster than at 25°C and more than 40-fold faster than at 4°C. Fe(III) reduction was also the fastest at 10°C, which was 2-fold faster than at 25°C and 12-fold faster than at 4°C. Overall, about 80% of the enrichment cultures exhibited microbial Fe(III) reduction under psychrotrophic conditions. These results indicated that microbial iron reduction is likely widespread in cold natural environments and may play important roles in cycling of iron and organic matter over geological times. © 1999 Published by Elsevier Science B.V. All rights reserved.

Microbial iron reduction plays an important role in the cycling of iron and organic matter in natural environments [1–3]. Many iron-reducing bacteria can also reduce other metals, including manganese, chromium, cobalt and uranium [2,4–6], as well as degrade petroleum hydrocarbons [7,8]. Thus, this process is important for bioremediation of contaminated environments. Furthermore, microbial iron reduction is considered to be an early form of respiration and may have implications in the evolution of life on Earth [1,3,9,10].

Dissimilatory Fe(III) reduction by mesophilic (20–35°C) bacteria has been extensively studied [1,4,11–15]. For example, *Geobacter* and *Shewanella* species were isolated from diverse sedimentary environments including marine and freshwater aquatic sediments, pristine deep aquifers and a petroleum-contaminated shallow aquifer [3,11–14]. Phylogenetic analysis showed that most of the mesophilic iron-reducing bacteria belong to the delta and gamma subdivisions of the *Proteobacteria* [15–17].

Recently, thermophilic ($> 45°C$) iron-reducing bacteria have been reported from geothermal environments such as the deep subsurface [18–20] and continental hot springs ([21]; Reysenbach et al., 1998, abstract for the 1998 American Geophysical Union Meeting, San Francisco, CA, USA). Vargas et al. [10] reported that several hyperthermophilic archaeal bacterial species could reduce Fe(III) to Fe(II) and conserve energy to support growth. Fur-

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thermore, thermophilic iron-reducing bacteria obtained from the deep subsurface produced magnetite particles that were distinct from those formed by mesophilic iron-reducing bacteria [22]. This finding may have significant implications for potential microbial contributions to paleomagnetism in geothermal environments. Studies of Fe(III) reduction under psychrotrophic ($< 20°C$) conditions, however, are rare [23]. The objective of this study was to examine psychrotrophic iron reduction in enrichment cultures obtained from the deep Pacific marine sediments and the Alaskan tundra permafrost.

A modified medium from Zhang et al. [6] was used for the enrichment and cultivation of psychrotrophic iron-reducing bacteria. The medium contained NaCl (1 g l^- for permafrost samples, 30 g l^- for marine sediments), $MgCl_2 \cdot 6H_2O$ (0.2 g l^- for permafrost samples, 5 g l^- for marine sediments), $NaHCO_3$ (2.5 g l^-), $CaCl_2 \cdot 2H_2O$ (0.1 g l^-), NH_4Cl (1.0 g l^-), yeast extract (0.0 or 0.5 g l^-), trace minerals and vitamins [6]. Fe(III) was provided as filter-sterilized Fe(III)-citrate or Fe(III)-EDTA (~ 20 mM final concentration) or autoclave-sterilized amorphous ferric oxyhydroxide (70 mM final concentration). These iron forms were used to examine how they would affect the growth of iron-reducing bacteria and the rate of iron reduction. H_2 (balanced with 20% CO_2) and the organic acids pyruvate, acetate and formate (all 10 mM final concentrations) were used as the electron donors.

All enrichment cultures were grown in 26-ml pressure tubes containing about 10 ml media. Initial inoculation was made in an anaerobic chamber (N_2/H_2, 95:5, v:v) by transferring 1-g samples into tubes using sterile spatulas. Tubes were capped with rubber septa and gassed with N_2/CO_2 for pyruvate, acetate or formate-grown cultures and gassed with H_2/CO_2 for hydrogen-grown cultures. Incubations were performed in the dark at 1.5, 4, 10 and 25°C. Control experiments included (1) ferric citrate plus organic acids or hydrogen for abiotic reduction of iron in the absence of bacteria, (2) enrichment culture without organic acids or hydrogen for iron reduction via bacterial fermentation of yeast extract that was provided in the medium, and (3) enrichment culture plus only ferric citrate for iron reduction via bacterial fermentation of citrate that was chelated with the iron. Bacterial growth and iron reduction

was initially determined by the disappearance of the brownish color of Fe(III) and by increases in cell density indicated by turbidity. Subsequent transfers of the cultures into fresh media were made using sterile needles and syringes. At least three transfers were made to verify the presence of iron reducers in each sample. Cell numbers were enumerated using
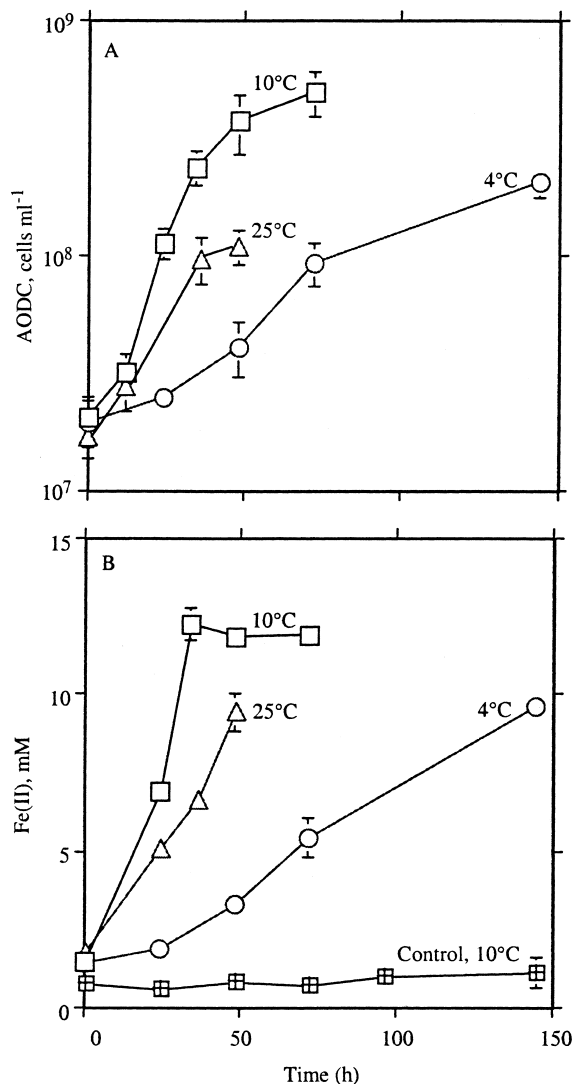

Fig. 1. Time-course analysis of cell abundance (A) and Fe(II) production (B) in W3-7 enrichment cultures at different temperatures using pyruvate (10 mM) as the electron donor and Fe(III)-citrate (20 mM) as the electron acceptor. Values are the mean ± S.D. of two replicate samples.

epifluorescence microscopy as previously described [6].

The rate and extent of Fe(III) reduction was determined using a method modified from Zhang et al. [6]. In this method, 0.1–0.3 ml-liquid sample was withdrawn from the tube and added directly to 3 ml ferrozine solution pH 7. The sample was mixed, filtered through a Whatman syringe filter (13-mm filter diameter, 0.2-μm pore diameter) and measured for absorbance at 562 nm.

Initial screening showed that enrichment cultures from the two Alaskan samples and from 16 of the 20 Pacific marine samples were able to reduce Fe(III) at 10°C when grown on organic acids or hydrogen in the presence of 0.05% yeast extract. The disappearance of brownish color of Fe(III)-citrate usually occurred first in pyruvate or H_2/CO_2-amended cultures and last in formate or acetate-amended cultures. Without the organic acids or hydrogen, however, the biomass was low and the color did not disappear, suggesting that iron reduction was insignificant by the enrichment culture grown only on the yeast extract. Iron reduction was not detected if (1) ferric citrate plus organic acids or hydrogen were added, but the enrichment cultures were not (Fig. 1B, control, 10°C), or (2) ferric citrate plus enrichment cultures were added, but organic acids or hydrogen was not (data not shown). These results suggested that iron reduction was not occurring abiotically and the enrichment cultures could not ferment citrate for iron reduction.

Time-course experiments performed at 4, 10 and 25°C were shown in Fig. 1 for the W3-7 enrichment culture. Among these temperatures, the 10°C experiment showed the fastest rates of biomass production and iron reduction. The rate of biomass production at 10°C was about 47-fold faster than at 4°C and 5-fold faster than at 25°C. The rate of Fe(II) pro-

duction at 10°C was about 12-fold faster than at 4°C and 2-fold faster than at 25°C (Table 1). Calculations of biomass production based on per mmol Fe(II) produced also indicated that more cells were produced at 10°C than at the other two temperatures (Table 1). Note that in Fig. 1B, the final Fe(II) concentrations at 4°C (9.64 mM, 144 h) and at 25°C (9.42 mM, 48 h) were lower than the final concentration at 10°C (11.9 mM, 72 h). This may be due to a slower reduction rate and not enough incubation time at these temperatures. Additional experiments at 1.5°C showed a 2-fold increase in biomass $(8.6 \times 10^7 \pm 8.6 \times 10^6$ cells ml^{-1} at 0 h and $1.8 \times 10^8 \pm 1.4 \times 10^6$ cells ml^{-1} at 336 h) and 5-fold increase in Fe(II) $(3.1 \pm 0.14$ mM at 0 h and 14.5 ± 0.1 mM at 336 h), after 2 weeks of incubation.

W3-7 could also reduce Fe(III)-EDTA and amorphous ferric oxyhydroxide. The rate of amorphous Fe(III) reduction, however, was much slower than the reduction of Fe(III)-citrate. This is perhaps because the reduction of a solid iron form requires a physical contact between the bacteria and the solid material [24]. Because soluble iron is more available to the bacteria, organic ligands such as nitrilotriacetic acid have been used to bind to insoluble iron oxide to increase the bioavailability of the iron in environmental samples [25].

This study demonstrated that Fe(III) was significantly reduced at low temperatures (< 15°C) by bacterial growth on organic acids and hydrogen (Fig. 1). The lack of growth and iron reduction using yeast extract as the sole carbon source suggested that cellular growth in the enrichments was mainly a result of iron reduction coupled to the oxidation of the organic acids and hydrogen. However, because we were using a mixed culture, other bacteria may be present and grow using the byproducts of the iron reducers. For example, the biomass in the pyruvate-

Table 1
Rates of biomass production and Fe(III) reduction in W3-7 cultures within the first 24 h of incubation at 4, 10 and 25°C

Temperature (°C)	Biomass production (cells ml^{-1} h^{-1})	Fe(II) production (mM h^{-1})	Cells mmol^{-1} Fe(II)$^{24 h}$	Cells mmol^{-1} Fe(II)$^{48 h}$
4	0.2×10^6	0.02	2.33×10^8	1.86×10^{10}
10	9.3×10^6	0.23	3.89×10^9	3.44×10^{10}
25	1.9×10^6	0.14	Not available	1.23×10^{10}

Also calculated were the biomass productions per mmol Fe(II) produced at 24 and 48-h incubations. Fe(III)-citrate (20 mM) was used as the electron acceptor and pyruvate (10 mM) was used as the electron donor.

amended culture continued to increase when Fe(III) reduction had ceased (Fig. 1). Further study using pure isolates is needed to fully characterize the process of iron reduction at low temperatures.

Acknowledgements

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