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Isolation of *Geobacter* Species from Diverse Sedimentary Environments

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In an attempt to better understand the microorganisms responsible for Fe(III) reduction in sedimentary environments, Fe(III)-reducing microorganisms were enriched for and isolated from freshwater aquatic sediments, a pristine deep aquifer, and a petroleum-contaminated shallow aquifer. Enrichments were initiated with acetate or toluene as the electron donor and Fe(III) as the electron acceptor. Isolations were made with acetate or benzoate. Five new strains which could obtain energy for growth by dissimilatory Fe(III) reduction were isolated. All five isolates are gram-negative strict anaerobes which grow with acetate as the electron donor and Fe(III) as the electron acceptor. Analysis of the 16S rRNA sequence of the isolated organisms demonstrated that they all belonged to the genus *Geobacter* in the delta subdivision of the *Proteobacteria*. Unlike the type strain, *Geobacter metallireducens*, three of the five isolates could use H_2 as an electron donor for Fe(III) reduction. The deep subsurface isolate is the first Fe(III) reducer shown to completely oxidize lactate to carbon dioxide, while one of the freshwater sediment isolates is only the second Fe(III) reducer known that can oxidize toluene. The isolation of these organisms demonstrates that *Geobacter* species are widely distributed in a diversity of sedimentary environments in which Fe(III) reduction is an important process.

Fe(III) reduction is an important process for the degradation of natural and contaminant organics in many aquifers and aquatic and marine sediments (1, 7–9, 22, 24, 29, 39, 40). Although many existing isolates from various genera have recently been found to grow by dissimilatory Fe(III) reduction, only a few Fe(III)-reducing bacteria have been isolated from sediments through enrichment with Fe(III) as the sole electron acceptor (4–6, 11, 26, 44).

Organisms which conserve energy to support growth via Fe(III) reduction fall into two categories, those that completely oxidize multicarbon compounds to carbon dioxide and those that incompletely oxidize multicarbon organics to acetate. The complete oxidizers include species from the *Geobacter* (4, 26), *Desulfuromonas* (11), *Desulfuromusa* (13, 18), and the recently described *Geovibrio* (5) genera, while the incomplete oxidizers include *Shewanella* (6, 36, 44) and *Pelobacter* (37) species as well as the organism known as strain SES-3 (17).

Fe(III) reducers which can oxidize acetate with the reduction of Fe(III) are of interest because much of the carbon and electron flow in pristine Fe(III)-reducing environments proceeds through acetate (19, 20, 36). Fe(III) reducers which can oxidize aromatic hydrocarbons are likely to play an important role in Fe(III) reduction in anoxic petroleum-contaminated aquifers in which aromatic hydrocarbons are an important electron donor (22, 28).

Thus, in an effort to better understand what organisms might be responsible for the oxidation of organic compounds in Fe(III)-reducing environments, Fe(III) reducers capable of oxidizing acetate or toluene were isolated from freshwater aquatic sediments and from the Fe(III) reduction zones of a deep pristine aquifer and a shallow petroleum-contaminated aquifer. Despite the diversity of sediments examined and differences in the enrichment techniques employed, all five of the isolates characterized were found to be *Geobacter* species. These results suggest that *Geobacter* species may be important components of the Fe(III)-reducing community in many sedimentary environments.

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MATERIALS AND METHODS

Source of organisms. *Geobacter metallireducens* GS15 was obtained from our laboratory culture collection and was grown on acetate-Fe(III) citrate medium, as previously described (26).

Strains H-2 and H-4 were isolated from sediments that were used to study the effects of nitrilotriacetic acid (NTA) on aromatic hydrocarbon degradation in petroleum-contaminated aquifers (39). The sediments were collected from a petroleum-contaminated water table aquifer at the Defense Fuel Supply Center in Hanahan, S.C. The sediments were from site MW-20, which is within a zone in which Fe(III) reduction is the terminal electron-accepting process (25, 39). In anoxic laboratory incubations at 25°C, the sediments were amended with NTA (ca. 2 mmol/kg of sediment), toluene (ca. 10 μM), or benzene (ca. 3 μM), as previously described (39). The sediments were refed with toluene or benzene when they were depleted. When sediments were well adapted for high rates of toluene or benzene oxidation coupled to Fe(III) reduction, a sample of the sediments (1 g) was used to establish enrichment cultures with acetate as the sole electron donor and Fe(III)-NTA (10 mM) as the electron acceptor. Strains H-2 and H-4 came from enrichments initiated with toluene- or benzene-adapted sediments. The enrichments were plated onto anaerobic agar plates with acetate (10 mM) as the electron donor and soluble Fe(III) [Fe(III)-NTA or Fe(III)-pyrophosphate for strains H-2 and H-4, respectively] at a concentration of 10 mM as the electron acceptor, and the plates were incubated at 30°C.

Strain 172 was isolated from enrichments initiated from subsamples taken from deep aquifer sediments of the Atlantic Coastal Plain in South Carolina (24). The subsamples were taken from the sediment core at a depth of 52 m, as outlined previously (24). A highly enriched culture was obtained by continual transfer over a 5-year period (10% inoculum) into fresh medium with acetate (10 mM) and Fe(III)-oxide (50 mM) and incubation at 25°C. The final two transfers were into acetate medium with Fe(III)-NTA (10 mM) as the electron acceptor. A pure culture of an acetate-oxidizing, Fe(III)-reducing microorganism was obtained by sequential transfer of an active culture from liquid medium to

anaerobic agar plates with acetate (10 mM) as the electron donor and Fe(III)-NTA (10 mM) as the electron acceptor.

Strains TACP-2 and TACP-5 were isolated from freshwater aquatic sediment taken from the Potomac River Estuary, Virginia, at the same site that previously yielded *G. metallireducens* (32). Enrichments were initiated with this sediment by amending 9 ml of Fe(III) oxide medium with 1.0 g of sediment. Toluene (5 μM) was added as the electron donor. A highly enriched culture was obtained by sequential transfer over a 5-year period at 25°C. Two isolates were obtained by the standard agar shake tube method, outlined previously (45), at 30°C, using either acetate (strain TACP-2) or benzoate (strain TACP-5) as the electron donor at a concentration of 10 or 0.5 mM, respectively, and Fe(III)-NTA (10 mM) as the electron acceptor.

Culturing on Fe(III). Standard anaerobic culturing techniques were used throughout the study (2, 16, 41). The medium was boiled under N_2-CO_2 (80:20) to remove dissolved O_2 and then dispensed into anaerobic pressure tubes or serum bottles under N_2-CO_2; the tubes or bottles were capped with thick butyl rubber stoppers and sterilized by autoclaving. The basal medium was the bicarbonate-buffered freshwater medium that had previously been used for culturing *G. metallireducens* (32). Unless otherwise noted, acetate (10 mM) was the electron donor. Soluble Fe(III) was supplied as Fe(III)-citrate (50 mM) (32), Fe(III)-pyrophosphate (10 mM) (4), or Fe(III)-NTA (10 mM) (43). Poorly crystalline Fe(III) oxide was prepared as previously described (30) and provided at 100 mmol/liter of medium.

Purified agar (2%, wt/vol) was included to prepare agar plates or shake tubes. Agar plates were poured under an atmosphere of N_2-CO_2-H_2 (85:5:10) in an anaerobic chamber. Plates were incubated under a positive pressure (100 kPa) of N_2-CO_2 (80:20) in sealed aluminum chambers similar to those previously described (2).

For studies with strain H-2 in which sediment Fe(III) oxides were provided as the electron acceptor, sediments from the MW-20 site were suspended in anoxic basal medium (1/1, vol/vol) under N_2-CO_2. Aliquots (10 ml) were dispensed into pressure tubes, which were sealed under N_2-CO_2 and autoclaved at 121°C for 20 min. The tubes were amended with a mixture of 10 mM acetate and either 4 mM NTA or 1 mM EDTA from sterile stocks. Strain 172 was incubated at 25°C; all other incubations were at 30°C unless otherwise noted.

Alternative electron acceptors and donors. In order to evaluate the potential for Mn(IV) reduction, synthetic MnO_2 (32) was added to basal medium to provide 10 to 30 mmol of Mn(IV) per liter. When noted, alternative electron acceptors in the form of sodium salts of nitrate, thiosulfate, sulfite, sulfate, fumarate, malate, selenate, or selenite were added to the basal medium from anoxic sterile stocks to give a final concentration of 10 mM. Colloidal elemental sulfur (S^0) prepared as previously described (3) was provided at a concentration of 10 mM. Alternative electron donors were also added from sterile anoxic stocks at the concentrations given in Results. Hydrophobic hydrocarbon electron donors (toluene, benzene, cyclohexane, eicosane, and phenanthrene) were prepared in degassed mineral oil at 2% (vol/vol), as previously outlined (42).

The ability of washed cell suspensions in bicarbonate buffer to reduce U(VI) was determined as previously outlined (34, 35), with uranyl chloride as the electron acceptor and hydrogen as the electron donor.

Cytochromes. As a preliminary investigation into the cytochrome content of strains H-2 and 172, dithionite-reduced versus air-oxidized difference spectra were obtained on washed cell suspensions of acetate-Fe(III)-grown cells in bicarbonate buffer, as previously described (26).

The ability of potential electron acceptors to oxidize the type *c* cytochromes was determined as previously described (26). Briefly, cell suspensions (2 ml) were placed into two sealed quartz cuvettes under N_2-CO_2. The suspensions were bubbled with H_2-CO_2 (80:20) for 2 min to reduce the cytochromes and then bubbled with N_2-CO_2 for 1 min. An aliquot (0.5 ml) of an anoxic 2.5 mM stock solution of the potential electron acceptor in bicarbonate buffer was added to one cuvette, and 0.5 ml of the anoxic bicarbonate buffer was added to the second cuvette. Difference absorbance spectra for the two treatments were recorded with a scanning spectrophotometer.

Analytical techniques. As previously described, Fe(III) reduction was monitored by using ferrozine to measure the production of HCl-extractable Fe(II) (30) or soluble Fe(II) (31) in studies with cultures and aquifer material, respectively. Cells were counted with epifluorescence microscopy (15). Growth of cultures on soluble electron acceptors was monitored by directly inserting the culture tubes into a Spectronic 20 spectrophotometer and measuring A_{600}. Sulfide was analyzed colorimetrically by the methylene blue method (10). Acetate, lactate, and benzoate concentrations were determined with high-pressure liquid chromatography (HPLC), as previously described (22, 33), with a UV detector set at 210 nm. H_2 was quantified with gas chromatography and a reduction gas analyzer (27). U(VI) was measured under anaerobic conditions with a Kinetic Phosphorescence Analyzer (KPA-10; Chemchek Instruments) (35).

16S rRNA gene sequencing and phylogenetic analysis. Nucleic acids were isolated from frozen cell pellets of strains TACP-2, H-2, and H-4 as previously described (37). The 16S rRNA gene of TACP-5 was amplified directly from a colony suspended in 10 mM Tris, pH 8.0. The partial 16S rDNAs were amplified and sequenced as previously described (37), using eubacterial primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) in place of primer 50F for strains TACP-5, H-2, and H-4 and primers 519F (5′-CCAGCAGCCGCGGTAATAC-3′) and 907R [5′-CCGTCAATTC(C/A)TTT(G/A)AGTTT-3′] for strain TACP-2. Only one strand of the 16S rDNA gene of TACP-2 was sequenced.

Nucleotide sequence accession numbers. The partial 16S rDNA sequences of strains TACP-2, TACP-5, H-2, and H-4 have been deposited in GenBank. The sequence accession numbers are as follows: strain H-2, U28173; strain H-4, U46860; strain 172, U41561; strain TACP-2, U46862; strain TACP-5, U46861.

TABLE 1. Growth of strain 172 with acetate or lactate as the electron donor and Fe(III)-NTA (10 mM) as the electron acceptor

Electron donor	Incubation time (h)	Net Fe(II) production (mM)	Net cell no. increase (10^7)
Acetate (2 mM)	72	8.46	9.02
Lactate (1.5 mM)	98	8.09	7.14

RESULTS

Cell and colony morphology. Cells of strains H-2, H-4, 172, TACP-2, and TACP-5 were morphologically identical. Cells were nonmotile, non-spore-forming, gram-negative rods, 1 to 2 by 0.6 μm. Dividing cells formed a central constriction between daughter cells. Flagella were not detected.

Colonies grown on Fe(III)-pyrophosphate medium were typically less than 1 mm in diameter. They were white and domed and appeared to be coated with an Fe(II) mineral, presumably vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$]. When grown on Fe(III)-citrate medium, the colonies were red, domed, entire, smooth, and wet, similar to colonies of *G. metallireducens* grown on the same medium (26).

Acetate and H_2 oxidation coupled to Fe(III) reduction. With acetate as the electron donor, strains H-2, H-4, and TACP-2 could be grown with the three forms of soluble Fe(III) that were evaluated as well as poorly crystalline Fe(III) oxide. Strains 172 and TACP-5 did not grow with Fe(III)-citrate.

Reduction of Fe(III) by all strains was accompanied by an increase in cell numbers (Table 1 and data not shown), indicating that energy to support growth was conserved from this metabolism. No growth was noted in the absence of Fe(III) (data not shown). Stoichiometric values of acetate oxidation coupled to Fe(III) reduction were not determined for strains H-4, TACP-2, and TACP-5; however, when strain H-2 was grown with Fe(III)-citrate as the electron acceptor, the ratio of Fe(II) produced to acetate consumed was 7.35 ± 0.12 (mean ± standard deviation; $n = 3$). For strain 172, the ratio was 6.98. When it is considered that some acetate was probably incorporated into cell biomass, these results suggest that acetate was oxidized according to the following reaction: $CH_3COO^- + 8Fe(III) + 4H_2O \rightarrow 2HCO_3^- + 8Fe(II) + 9H^+$.

Highly crystalline Fe(III) forms were not evaluated. Poorly crystalline Fe(III) oxide was reduced to a black magnetic precipitate that was presumably composed of magnetite (38). Strain H-2 reduced naturally occurring Fe(III) oxides in the Hanahan aquifer sediment (Fig. 1).

H_2 could also serve as an electron donor for Fe(III) reduction in strains H-2, H-4, and TACP-5. Strain H-2 can obtain energy for growth by this metabolism (Fig. 2). The stoichiometry of Fe(II) produced per H_2 consumed was 1.73 ± 0.04 (mean ± standard deviation; $n = 3$), which suggests that strain H-2 oxidized H_2 according to the following reaction: $H_2 + 2Fe(III) \rightarrow 2H^+ + 2Fe(II)$.

Growth of strain H-2 on H_2 could not be maintained during consecutive transfers in Fe(III)-NTA medium but could be maintained with Fe(III)-citrate. As citrate was not used as an electron donor, these results suggested that citrate served as a carbon source during growth on H_2. Yeast extract (0.2 g/liter)

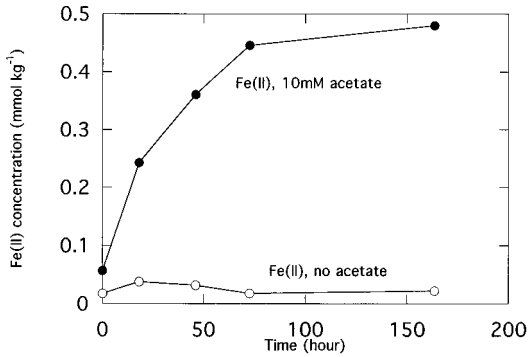

FIG. 1. Reduction of indigenous Fe(III) oxide in MW-20 aquifer sediment by strain H-2. Comparative tubes without acetate were used as controls. The results are the means of triplicate cultures.

stimulated growth on H_2 with Fe(III)-citrate. Strain H-2 metabolized H_2 down to a minimum threshold of 0.092 ± 0.007 Pa (mean ± standard deviation; $n = 3$).

All isolates grew at 30°C. Strains H-2 and H-4 had a temperature optimum of 35°C, while strain 172 grew optimally at 25°C.

Other electron acceptors and donors. The isolates varied in the electron donors utilized (Table 2). All isolates could oxidize ethanol. Ethanol supported rates of Fe(III) reduction similar to those observed with acetate for all isolates. Strains H-2, H-4, and TACP-5 slowly reduced Fe(III) with formate, propionate, butyrate, benzoate, or pyruvate as the electron donor. The stoichiometry of benzoate oxidized to Fe(III) reduced for strain H-2 was 25.98 ± 2.99 (mean ± standard deviation; $n = 5$) which, allowing for carbon incorporation, is consistent with the following theoretical metabolism: $C_7H_5O_2^- + 30Fe(III) + 19H_2O \rightarrow 7HCO_3^- + 30Fe(II) + 36H^+$.

Strain 172 could obtain energy for growth by the oxidation of lactate coupled to the reduction of Fe(III) (Table 1). No organic acids were formed (HPLC determination) in the culture broth during growth of strain 172, and the stoichiometry of lactate utilized to Fe(III) produced is 11.37 ± 2.79 (mean ± standard deviation). When carbon uptake for biomass is taken into account, the stoichiometry of lactate utilized to Fe(II) produced is consistent with the complete oxidation of the lactate to CO_2 via the reaction: $C_3H_5O_3^- + 12Fe(III) + 6H_2O \rightarrow 3HCO_3^- + 12Fe(II) + 14H^+$.

A wide variety of other electron donors did not support

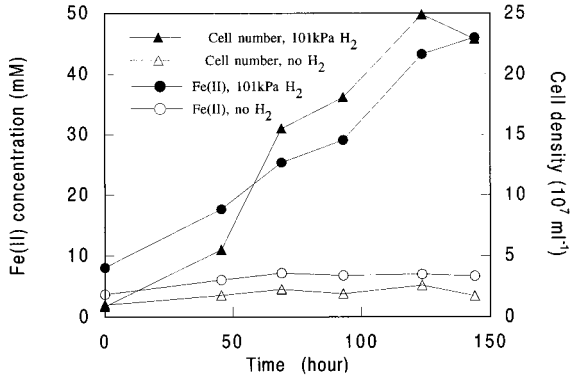

FIG. 2. Growth of strain H-2 and Fe(III) reduction with H_2 as the electron donor and Fe(III)-citrate as the electron acceptor in medium also containing 0.2 g of yeast extract per liter. The results are the means of triplicate cultures.

TABLE 2. Compounds tested as electron donors for each of the isolates with soluble Fe(III) as the electron acceptor

Electron donor (mM)	Utilization[a] by strain:				
	H-2	H-4	172	TACP-2	TACP-5
Acetate (10)	+	+	+	+	+
Propionate (5)	+	+	−	+	+
Butyrate (5)	+	+	−	+	+
Formate (10)	+	+	+	+	+
Palmitate (1)	−	−	−	−	−
Methanol (10)	−	−	−	−	−
Ethanol (10)	+	+	+	+	+
Phenol (0.5)	−	−	−	−	−
Toluene (1)	−	−	−	−	+
Benzene (1)	−	−	−	−	−
Cyclohexane (1)	−	−	−	−	−
Octane (1)	−	−	−	−	−
Eicosane (0.35)	−	−	−	−	−
Benzoate (0.5)	+	+	−	−	+
Phenanthrene (0.6)	−	−	−	−	−
Glucose (10)	−	−	−	−	−
Citrate (10)	−	−	−	−	−
NTA (4)	−	−	−	−	−
Lactate (10)	−	−	+	−	−
Casamino Acids (1 g/liter)	−	−	−	−	−
Yeast extract (1 g/liter)	−	−	−	−	−
Succinate (5)	+	+	−	−	−
Fumarate (5)	ND[b]	ND	ND	−	−
CH_4 (101 kPa)	−	−	−	−	−
H_2 (101 kPa)	+	+	−	−	+
Pyruvate (10)	+	+	ND	+	+

[a] +, utilization of electron donor; −, no utilization.
[b] ND, not determined.

Fe(III) reduction for the isolates (Table 2). None of the isolates tested grew by fermentation in complex, organic compound-rich medium in which the basal medium was amended with yeast extract (5 g/liter), Bacto Peptone (1.0 g/liter), and glucose (1.8 g/liter). Strain H-2, when tested, did not require the vitamin mixture for growth on acetate and Fe(III) but did require the trace metal mixture. This was not evaluated for the other isolates.

Only strains H-2, H-4, and 172 were studied in detail for the ability to grow on alternative electron acceptors. Unlike most Fe(III) reducers (19), strains H-2 and H-4 did not reduce Mn (IV) when only Mn(IV) was provided. However, the Mn(IV) was not toxic, as it was reduced when a small amount of Fe(III) was also added. Mn(IV) reduction in the presence of Fe(III) was presumably due to the reduction of the Fe(III) to Fe(II), with the subsequent chemical reduction of Mn(IV) by Fe(II) (19). With acetate as the electron donor, the following potential electron acceptors did not support growth for strains H-2, H-4, and 172 when provided at 10 mM: nitrate, selenite, selenate, sulfate, sulfite, thiosulfate, and malate. Similarly to the type strain *G. metallireducens* GS15, S^0 was reduced by strains H-2, H-4, and 172, but S^0 reduction did not support growth for strain H-2 (data not shown). Strains H-2, H-4, and 172 did grow with acetate as the electron donor and fumarate as the electron acceptor. When tested, washed cell suspensions of both strains H-2 and 172 also reduced U(VI) in the presence of H_2 but whether these organisms conserve energy to support growth from this metabolism has not been determined.

Cytochrome content and oxidation by potential electron acceptors. Oxidized versus reduced spectra of whole-cell suspensions of strains H-2, H-4, and 172 were similar to that of *G. metallireducens*. The absorbance maxima at 552, 524, and 424

TABLE 3. Potential electron acceptors tested for the ability to reoxidize type *c* cytochrome(s) on hydrogen-reduced cell suspensions of strains H-2 and 172

Potential electron donor	Reoxidation[a] by strain:	
	H-2	172
Fe(III)	+	+
Chromate(VI)	+	+
Nitrate	−	−
Sulfate	−	−
Gold(III)	+	+
Silver(I)	+	+
Mercury(II)	+	+
Tungstate(VI)	+	−
Uranium(VI)	+	ND[b]
Vanadate(V)	+	+
Molybdate(VI)	+	−

[a] +, positive reoxidation; −, negative reoxidation.
[b] ND, not determined.

nm were indicative of type *c* cytochrome(s). Analysis of cell extracts with polyacrylamide gel electrophoresis and heme staining (14) has suggested that these strains contain at least four different type *c* cytochromes (21). Hydrogen-reduced type *c* cytochrome(s) in whole-cell suspensions was reoxidized by metals known to act as electron acceptors for these strains (Table 3). Nitrate did not oxidize the cytochrome(s). Molybdate and tungstate oxidized the type *c* cytochrome(s) of strain H-2 but not that of strain 172. The ability of these metals to support growth of strain H-2 was not determined.

Phylogenetic analysis. Comparison of the 16S rRNA sequences of strains TACP-2, TACP-5, H-2, H-4, and 172 with other eubacterial 16S rRNA sequences places the isolates within the delta subdivision of the *Proteobacteria* (18). The 16S rRNA sequences of strains H-2 and H-4 are 100.0% similar to each other when unambiguously determined positions are considered and share a 99.0% similarity to *G. metallireducens* (1,385 and 1,449 positions considered, respectively). Strain TACP-2 is 99.6% similar to *G. metallireducens* (277 positions of one strand considered), and strain TACP-5 is 99.0% similar to *G. metallireducens* and 98.4% similar to strain H-4 (613 positions of both strands considered). The closest known relatives of strain 172 in the *Geobacter* genus are *G. metallireducens* and *G. sulfurreducens* (92.9% similarity to each; 1,449 and 1,408 nucleotides considered, respectively). Thus, all of the isolates can be considered *Geobacter* strains.

DISCUSSION

These results demonstrate that *Geobacter* species are readily isolated from a variety of sedimentary environments in which Fe(III) reduction is a significant process. Previous studies have found that *Geobacter* species were the organisms that could be enriched for and isolated from a freshwater aquatic sediment (38) and a submerged soil (4). In this study, two *Geobacter* strains were again recovered from the same freshwater sediment that yielded *G. metallireducens* even though the electron donor for enrichment (toluene) and the electron acceptor for isolation [Fe(III)] were different from those used in isolating *G. metallireducens. Geobacter* strains H-2 and H-4 represent the first examples of dissimilatory Fe(III) reducers isolated from a petroleum-contaminated aquifer, while *Geobacter* strain 172 is the first acetate-oxidizing Fe(III) reducer isolated from the deep subsurface.

Comparison with other dissimilatory Fe(III) reducers. More detailed analysis, including DNA-DNA hybridization, will be required before each *Geobacter* strain isolated in this study can be assigned to an appropriate species. Of the most environmental relevance are the metabolic capabilities of the isolates. All of the *Geobacter* strains recovered in this study are similar to *G. metallireducens* in conserving energy to support growth by coupling the oxidation of acetate to the reduction of Fe(III). Strains H-2, H-4, TACP-2, and 172 are significantly distinct from *G. metallireducens* in the range of electron donors and acceptors that they utilize. Strain 172 is also the first dissimilatory Fe(III) reducer shown to be capable of completely oxidizing lactate to CO_2 with Fe(III) as the sole electron acceptor. Although *Desulfuromonas palmitatis* can also oxidize lactate with the concomitant reduction of Fe(III), it was not demonstrated whether or not *D. palmitatis* completely oxidizes lactate (11). Other lactate-oxidizing Fe(III) reducers such as *Shewanella putrefaciens* and *Shewanella alga* only incompletely oxidize lactate to acetate and carbon dioxide (6, 23, 36).

Strains H-2 and H-4 only slowly oxidized benzoate and could not use the other aromatic compounds evaluated. This is in contrast to *G. metallireducens*, which oxidizes a variety of monoaromatic compounds including the aromatic hydrocarbon toluene (22, 28). Strain TACP-5 is phenotypically more similar to *G. metallireducens* than strain TACP-2, as strain TACP-5 can also oxidize monoaromatic compounds, including toluene. However, in contrast to *G. metallireducens*, strain TACP-5 can use H_2 as an electron donor. This ability to oxidize both aromatic hydrocarbons and H_2 makes strain TACP-5 unique among the known Fe(III) reducers.

Although early models suggested that H_2 and acetate were oxidized by distinct populations of Fe(III) reducers (19, 20), it is now clear that *Geobacter* species, and the closely related *Desulfuromonas* species, can use both electron donors. This was first observed in *G. sulfurreducens* (4) and subsequently in *D. palmitatis* (11). When the organisms isolated in this study are considered, the majority of *Geobacter* strains have the capacity to use H_2 as well as acetate.

Previous studies have suggested that type *c* cytochromes are involved in electron transport to Fe(III) and some other metals in *G. metallireducens* (26) and the closely related *Desulfuromonas acetoxidans* (43) and *D. palmitatis* (11). The studies reported here suggest that strains H-2, H-4, and 172 contain type *c* cytochromes similar to those in *G. metallireducens* that also appear to be involved in electron transport to metals. The finding that, in contrast to *G. metallireducens*, the type *c* cytochrome(s) present in strain H-2 is oxidized by tungstate, vanadate, and molybdate, while that of 172 is oxidized by vanadate but not tungstate or molybdate, and the finding that the cytochromes of neither of the new isolates were oxidized by nitrate implies that the reductases in these organisms are different and are specific for certain metals and other electron acceptors. The oxidation of the type *c* cytochromes by Cr(VI), Au(III), Ag(I), and Hg(II), as well as the other metals outlined above, suggests that strains H-2 and 172 may also be able to reduce these metals, although toxicity to the organisms could be a factor limiting growth with these electron acceptors.

Environmental significance. H_2 and acetate are expected to be the two major extracellular intermediates in the oxidation of fermentable organics in sediments in which Fe(III) reduction is the terminal electron-accepting process (19, 20, 33). Thus, organisms such as *Geobacter* species, which can metabolize these intermediates, could be important catalysts of Fe(III) reduction in sedimentary environments. The minimum threshold for H_2 uptake by strain H-2 reported here is in the middle of the range reported for other H_2-oxidizing Fe(III) reducers (6, 12,

36). These results suggest that strain H-2 should be competitive with other H_2-oxidizing Fe(III) reducers in sediments. Strain H-2 might have the advantage over H_2-oxidizing *Shewanella* and *Desulfovibrio* species in that it can also use acetate, which is likely to be the most important electron donor for Fe(III) reduction in sediments (19, 20).

Further evidence that the isolated strains are well adapted to life in sediments is the ability of strains H-2 and 172 to use the naturally occurring Fe(III) oxides present in the respective aquifer materials from which they were isolated. The studies reported here demonstrate that strain H-2 reduced Fe(III) in sediments from the petroleum-contaminated aquifer, and previous studies with the deep subsurface isolate, strain 172 (24), demonstrated that it could reduce the Fe(III) oxides present in a deep pristine aquifer.

Toluene is expected to be an important electron donor for Fe(III) reduction in petroleum-contaminated aquifers (22, 28, 39). This and previous studies (22) have demonstrated that toluene-oxidizing Fe(III) reducers can be recovered by isolation with acetate as the electron donor. However, this isolation strategy failed to yield toluene oxidizers from the petroleum-contaminated aquifer. In the future, direct isolation with toluene as the electron donor will be attempted in order to isolate toluene-oxidizing Fe(III) reducers from this environment.

In summary, the combination of the results of this and previous studies (4, 32) demonstrates that *Geobacter* species are the most easily and consistently isolated species of acetate-oxidizing Fe(III) reducers from a variety of Fe(III)-reducing sediments. However, it remains to be determined, with techniques that do not require cultivation, whether these readily isolated acetate-oxidizing Fe(III) reducers represent the predominant populations of acetate-oxidizing Fe(III) reducers in sedimentary environments.

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