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Geobacter sulfurreducens sp. nov., a Hydrogen- and Acetate-Oxidizing Dissimilatory Metal-Reducing Microorganism

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A dissimilatory metal- and sulfur-reducing microorganism was isolated from surface sediments of a hydrocarbon-contaminated ditch in Norman, Okla. The isolate, which was designated strain PCA, was an obligately anaerobic, nonfermentative, nonmotile, gram-negative rod. PCA grew in a defined medium with acetate as an electron donor and ferric PP_i, ferric oxyhydroxide, ferric citrate, elemental sulfur, Co(III)-EDTA, fumarate, or malate as the sole electron acceptor. PCA also coupled the oxidation of hydrogen to the reduction of Fe(III) but did not reduce Fe(III) with sulfur, glucose, lactate, fumarate, propionate, butyrate, isobutryate, isovalerate, succinate, yeast extract, phenol, benzoate, ethanol, propanol, or butanol as an electron donor. PCA did not reduce oxygen, Mn(IV), U(VI), nitrate, sulfate, sulfite, or thiosulfate with acetate as the electron donor. Cell suspensions of PCA exhibited dithionite-reduced minus air-oxidized difference spectra which were characteristic of *c*-type cytochromes. Phylogenetic analysis of the 16S rRNA sequence placed PCA in the delta subgroup of the proteobacteria. Its closest known relative is *Geobacter metallireducens*. The ability to utilize either hydrogen or acetate as the sole electron donor for Fe(III) reduction makes strain PCA a unique addition to the relatively small group of respiratory metal-reducing microorganisms available in pure culture. A new species name, *Geobacter sulfurreducens*, is proposed.

Dissimilatory iron-reducing microorganisms can gain energy to support growth by coupling the oxidation of organic acids, alcohols, H_2, or aromatic compounds to the reduction of a variety of metals (26). Recent studies with both pure cultures and sediments demonstrate that sulfur- and sulfate-reducing bacteria also have the potential to enzymatically reduce Fe(III) (11). The marine, sulfur-reducing microorganism *Desulfuromonas acetoxidans* is closely related to *Geobacter metallireducens* phylogenetically and conserves energy for growth by coupling the oxidation of organic acids and alcohols to the reduction of Fe(III) (41). Several sulfate-reducing bacteria can enzymatically reduce Fe(III) and U(IV) but are unable to grow with these metals as a terminal electron acceptor (34, 35). A c_3 cytochrome purified from *Desulfovibrio vulgaris* reduces U(VI) and amorphous iron oxide (30). Lipid analysis of a salt marsh, siderite, concretion formation found that organisms similar to *G. metallireducens* and *Shewanella putrefaciens* were not abundant in the Fe(III)-reducing zone, while organisms similar to *Desulfovibrio* species were enriched in the concretions (11). Sulfate-reducing bacteria are abundant in Fe(III)-reducing zones of deep aquifers in the Atlantic coastal plain (27).

Previous studies of freshwater and marine dissimilatory Fe(III)- and Mn(IV)-reducing bacteria have generated a trophic model that describes the complete mineralization of complex organic matter with Fe(III) as the sole electron acceptor (26). In this model, the primary fermentation products are acetate and H_2. Acetate is oxidized to CO_2 by Fe(III) reducers with a metabolism like that of *G. metallireducens* (28, 29), strain 172 (27), or *D. acetoxidans* (41). The reduction of Fe(III) can be coupled to H_2 or lactate oxidation by organisms similar to *S. putrefaciens* (33), *Pseudomonas* sp. (3), or strain BrY (9). To date, no organism which can couple the oxidation of both acetate and hydrogen to the reduction of Fe(III) has been described.

This study reports the isolation of a dissimilatory iron-, cobalt- and sulfur-reducing microorganism that can use both acetate and hydrogen as electron donors. The unique physiology of strain PCA expands our understanding of the trophic groups present in Fe(III)-reducing environments and further substantiates the interrelationship between the biogeochemical cycles of iron and sulfur in both freshwater and saline anaerobic environments.

MATERIALS AND METHODS

Source of the organism. Inocula for enrichments were collected by taking cores of a hydrocarbon-contaminated ditch near Norman, Okla. The top 3 cm of sediment was extruded into glass tubes, stoppered, and immediately transported back to the laboratory, where it was placed in an anaerobic chamber.

Media and cultivation. Standard anaerobic techniques were used throughout the study (5, 7). All anaerobic media were boiled and cooled under a constant stream of 80% N_2–20% CO_2, dispensed into aluminum-sealed culture tubes under the same gas phase, capped with butyl rubber stoppers, and sterilized by autoclaving (121°C, 20 min). Additions to sterile media, inoculation, and sampling were done by using syringes and needles (5). All incubations were at 35°C in the dark.

The basal medium contained the following (in grams per liter of deionized H_2O): $NaHCO_3$, 2.5; NH_4Cl, 1.5; KH_2PO_4, 0.6; KCl, 0.1; vitamins, 10 ml; and trace minerals, 10 ml (29). For enrichment of iron-reducing bacteria, sodium acetate (10 mM) was added as the electron donor and soluble ferric PP_i (3 g/liter) was added as the electron acceptor. The enrichment was initiated by adding 1.0 g (wet weight) of anaerobic sediment to sterile tubes containing the acetate-Fe(III) basal

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medium (10 ml) inside the anaerobic chamber. The headspaces of enrichment tubes were evacuated and replaced with 80% N_2–20% CO_2 immediately after inoculation.

The basal medium was modified to test for the use of different electron donors or acceptors. The use of different electron acceptors was tested with sodium acetate (10 to 30 mM) as the sole electron donor. Duplicate determinations were done for each electron acceptor. The experiment was repeated if replicates differed from the mean by more than 10 to 15%. Negative controls for the use of alternate electron acceptors did not contain acetate. Inocula (10%) for electron acceptor experiments were taken from cultures grown with basal medium, acetate, and ferric citrate. The use of poorly crystalline Fe(III) oxide (ca. 100 mM) and Fe(III) citrate (20 mM) as alternative reducible forms of Fe(III) was determined by monitoring the increase in cell number and Fe(II) over time. Synthetic MnO_2 (30 mM) (29) was used to evaluate the potential for Mn(IV) reduction, and the production of Mn(II) was monitored over time. Sodium nitrate, sodium thiosulfate, sodium sulfite, sodium sulfate, fumaric acid, or malic acid was added to the basal medium from anaerobic stock solutions to provide 20 mM. Growth on these acceptors was monitored by measuring the increase in cell density at an A_{540}. U(VI) was provided as uranyl chloride (0.5 mM); the loss of visible color in the medium and the accumulation of a black precipitate of reduced uranium (uraninite) were monitored over time as evidence of U(VI) reduction. Elemental sulfur was added aseptically as sublimed sulfur flowers (1.0 g/liter) to sterile medium under a stream of sterile 80% N_2–20% CO_2. The production of sulfide and an increase in cell numbers were monitored over time. Co(III)-EDTA medium was prepared as described previously (18). The decrease of Co(III) and increase in cell numbers over time were monitored as indicators of reduction and growth, respectively.

The use of different electron donors was tested with the basal medium without acetate and with ferric citrate as the electron acceptor. The electron donors were added to the basal medium from sterile anaerobic stocks to give a final concentration of 10 to 30 mM. Ten milliliters of a 90% H_2–10% CO_2 mixture was added to the gas phase to test for the use of H_2 as an electron donor. Inocula (10%) for electron donor experiments were from acetate-ferric citrate cultures that were transferred and grown in basal medium with ferric citrate so that any acetate which was transferred was used before inoculation into experimental tubes. The use of a compound as an electron donor was determined by comparing the production of Fe(II) to that by negative controls that lacked the compound and to positive controls that contained 30 mM sodium acetate. Duplicate determinations were done for each donor. Stoichiometries for the extent of Fe(III) reduction during acetate or H_2 oxidation were determined by measuring Fe(II) production when acetate (4 mM) or H_2 (15 kPa) was provided as the sole electron donor.

The optimum temperature for Fe(III) reduction by PCA was determined by growing PCA in basal medium with acetate and ferric citrate at different temperatures and by monitoring the production of Fe(II) over time. Strain PCA's ability to reduce Fe(III) under saline conditions was determined by growing it in basal medium with acetate, ferric citrate, and no NaCl or $MgCl_2$ (FW), with 10 g of NaCl and 5 g of $MgCl_2$ per liter (1/2 SW), or with 20 g of NaCl and 10 g of $MgCl_2$ per liter (SW) and by determining the concentration of Fe(II) produced after 2 and 5 days.

16S rRNA gene sequencing. Cells from an actively growing culture of PCA were harvested by centrifugation and stored at −70°C. The method devised by Murray and Thompson (37), as described by Ausubel et al. (2), was used to isolate DNA from the frozen cell pellet. The DNA was treated with RNase and diluted 10-fold prior to amplification. The 16S rDNA was amplified using primer 50F (5′-AACACATGCAAGTCGAA CG-3′) (22) and eubacterial primer 1492R (5′-GGTTACCT TGTTACGACTT-3′) (13, 44). The 16S rDNA PCR product was resuspended in water after purification with a Wizard PCR Prep System (Promega Corp., Madison, Ohio). Automated dye dideoxy terminator sequencing of both strands was performed on a model 373A DNA sequencing system (Applied Biosystems, Foster City, Calif.) by the Michigan State University Sequencing Facility. Oligonucleotides complementary to the conserved regions of the eubacterial 16S rDNA were chosen to prime the sequencing reactions and were synthesized on either a model 394 DNA-RNA synthesizer or a model 380B DNA synthesizer (Applied Biosystems). Sequence alignments were either performed manually or were obtained from the Ribosomal Data Base Project (23). Evolutionary distances were computed as described previously (21) and were used to construct a distance tree by the least-squares algorithm described by DeSoete (12). The topology of the phylogenetic analysis was confirmed by the maximum likelihood method (14, 23, 39). The 16S rRNA sequence of *Geobacter sulfurreducens* PCA has been deposited in GenBank.

Cytochrome content. Cytochrome analysis was done with cells grown in basal medium with acetate (30 mM) and Fe(III) citrate. A dithionite-reduced minus air-oxidized difference spectrum of whole cells was obtained on a Shimadzu 2101PC spectrophotometer. Cells (3.51 mg of protein per ml) were resuspended in 20 mM piperazine-*N,N′*-bis(2-ethanesulfonic acid) (PIPES) buffer at pH 7.0.

Electron microscopy. Cells grown in basal medium with acetate and Fe(III) citrate were initially fixed by the addition of glutaraldehyde directly into the anaerobic tubes (final concentration, 2.5%). The cells were then transferred to evacuated centrifuge tubes anoxically and were spun at 4,000 × *g* for 20 min. The spent medium was carefully removed to avoid precipitation of soluble iron. The cell pellet was then washed three times in 0.1 M cacodylate buffer and was treated with 1% osmium tetroxide. After dehydration in an ethanol series and propylene oxide, the cells were embedded in Spurr's low-viscosity medium. Thin sections were stained with uranyl acetate and lead citrate and observed on a Philips 201 transmission electron microscope at 60 kV.

Analytical techniques. Fe(III) reduction was monitored by measuring the accumulation of Fe(II) over time. The amount of Fe(II) solubilized after 15 min in 0.5 N HCl was determined with ferrozine as previously described (29). Co(III)-EDTA reduction was determined by measuring the loss of Co(III) over time spectrophotometrically at 535 nm. Sulfide was measured colorimetrically (42). Cell numbers in cultures growing with Fe(III) or Co(III) were determined by a modification of the epifluorescence microscopy technique (20) as previously described (29). Cell densities in cultures without metals were monitored spectrophotometrically at 540 nm. Acetate was quantified by high-pressure liquid chromatography (1). H_2 was measured by gas chromatography (33).

RESULTS

Isolation. The primary enrichment was transferred three times (10% inoculum) into the basal medium with sodium acetate and ferric PP_i. The Fe(III) in the initial enrichment tube was completely reduced within 3 days. Fe(III) reduction was observed as a change in the medium color from yellow to green to a clearing of the medium and the formation of a white

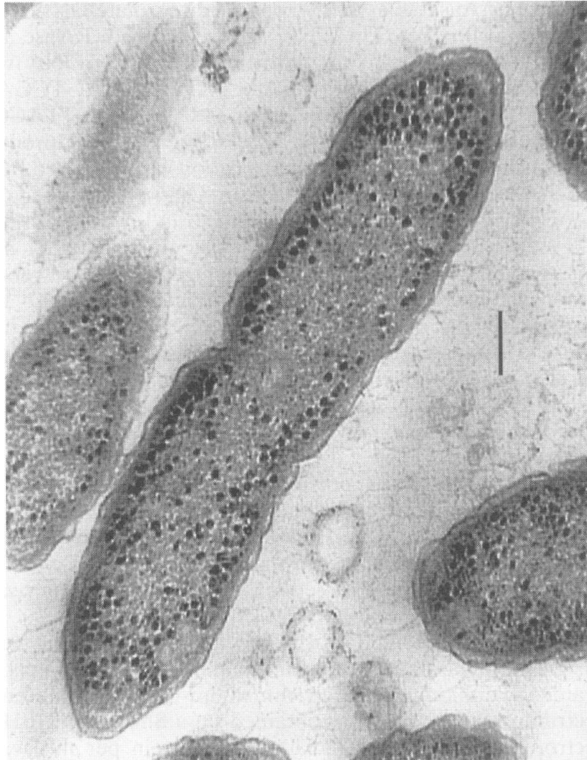

FIG. 1. Transmission electron micrograph of a thin section of strain PCA. Bar, 0.2 μm.

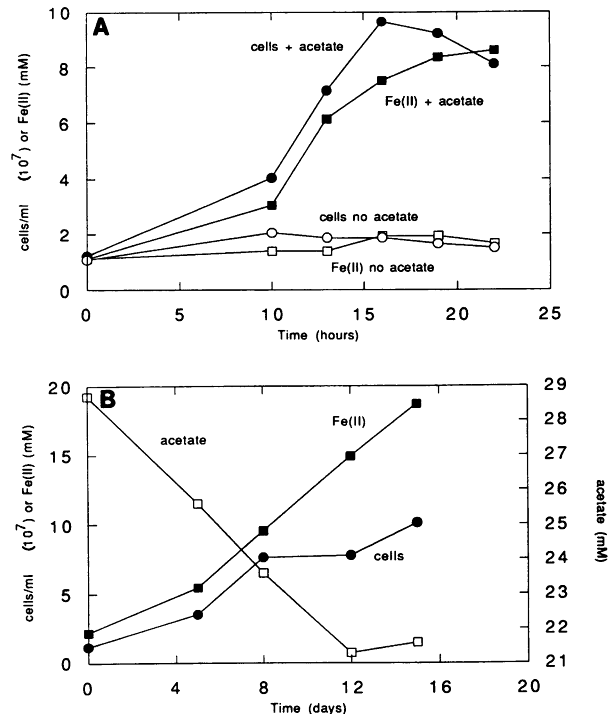

FIG. 2. Growth of PCA in basal medium with acetate as an electron donor and ferric PP_i (A) or poorly crystalline ferric oxide (B) as an electron acceptor.

precipitate. After the third transfer, the enrichment was serially diluted to extinction in this medium. Subsequent transfers became completely reduced within 1 day. The highest dilution which was positive for Fe(III) reduction (10^{-8}) was again serially diluted in this medium. The highest dilution which was positive for Fe(III) reduction (10^{-9}) completely reduced the Fe(III) within 7 days and was then streaked for isolation on anaerobic ferric PP_i agar slants, which were made by the addition of a final concentration of 1.5% (wt/vol) purified agar to the basal medium with acetate and ferric PP_i. A pure culture was obtained by repeatedly restreaking isolated colonies onto agar slants until an isolate of uniform colony and cell morphology was obtained. Colonies formed on agar slants within 7 days. Colonies were pinpoint and turned white along with the surrounding agar when the Fe(III) in the medium was reduced. Cells from isolated colonies were single straight rods 2 to 3 μm in length by 0.5 μm in width (Fig. 1). They were nonmotile and stained gram negative. No spores were observed in wet mounts by phase-contrast microscopy. The obligately anaerobic microorganism was designated strain PCA. The culture was maintained on basal medium with acetate and ferric citrate.

Electron donors and acceptors. Strain PCA grew by the oxidation of acetate coupled to the reduction of Fe(III) PP_i (Fig. 2A), poorly crystalline Fe(III) oxide (Fig. 2B), or Fe(III) citrate (data not shown). The increase in cell numbers coincided with the production of Fe(II) in the presence of acetate and each of the electron acceptors. No Fe(III) reduction or cell growth occurred in the absence of acetate. With Fe(III) citrate as the electron acceptor, the ratio of Fe(II) produced to acetate consumed was 6.8 ± 0.4 (mean ± standard deviation for five cultures). Considering that some acetate is likely to have been incorporated for cell synthesis, these results suggest that strain PCA oxidizes acetate according to the following equation: $CH_3COO^- + 8Fe(III) + 4H_2O \rightarrow 2HCO_3^- + 8Fe(II) + 9H^+$. Growth of PCA on ferric oxyhydroxide resulted in the formation of a black, magnetic precipitate which was presumably magnetite.

Strain PCA also coupled the oxidation of hydrogen to the reduction of Fe(III) citrate (Fig. 3A and B). The addition of Casamino Acids (0.25 g/liter) slightly enhanced growth and Fe(II) production. No growth or Fe(III) reduction occurred with Casamino Acids alone or in controls which lacked H_2. For each mole of H_2 consumed, 1.7 ± 0.2 mol (mean ± standard deviation for five cultures) of Fe(II) was produced. This suggests that H_2 was oxidized according to the following equation: $H_2 + 2Fe(II) \rightarrow 2H^+ + 2Fe(II)$.

Strain PCA did not use sulfur, glucose, lactate, fumarate, propionate, butyrate, isobutryate, isovalerate, succinate, yeast extract, phenol, benzoate, ethanol, propanol, or butanol with Fe(III) as an electron donor (Table 1). The small amounts of Fe(II) produced in cultures with glucose, lactate, malate, propanol, methanol, and yeast extract were similar to those found in cultures without an electron donor and probably represent the amounts of Fe(II) produced from the small amounts of acetate in the inocula. No Fe(II) was produced with many of the electron donors tested, suggesting that these compounds inhibited the ability of PCA to use the small amount of acetate in the medium.

Strain PCA coupled the oxidation of acetate to the reduction of Co(III)-EDTA (Fig. 4). An increase in cell numbers over time was followed by a concomitant decrease in the concentration of Co(III). Growth and reduction of Co(III) were electron donor dependent since there was not an increase in cell numbers or a decrease in Co(III) in the absence of acetate.

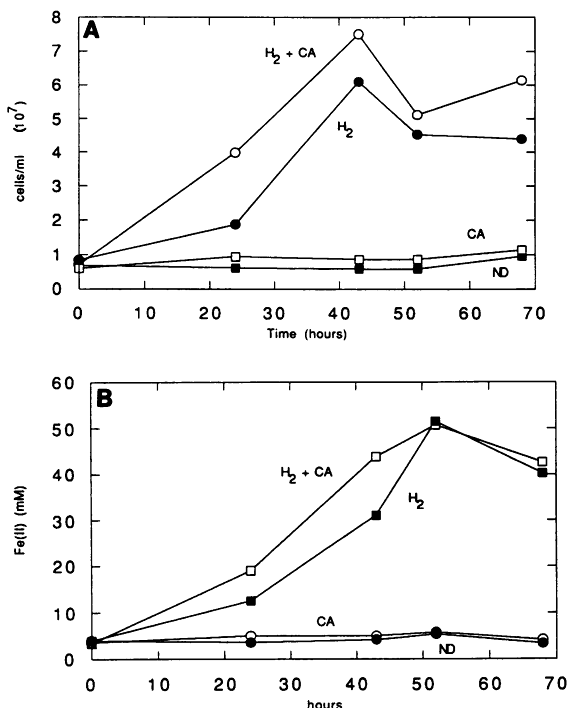

FIG. 3. Growth (A) and Fe(II) production (B) by PCA with H_2. PCA was grown with Fe(III) citrate as an electron acceptor and no donor (ND), hydrogen (H_2), Casamino Acids (CA), or hydrogen plus Casamino Acids (H_2 + CA) as an electron donor.

Strain PCA did not use Mn(IV) or U(VI) as electron acceptors.

Strain PCA was capable of growth with acetate as an electron donor and elemental sulfur as the sole electron acceptor (Fig. 5). Cell numbers and sulfide concentration increased over time only when acetate was present in the medium. Strain PCA grew with malate or fumarate (Fig. 6) as

TABLE 1. Electron donors used for Fe(III) reduction by PCA

Donor	Donor concn (mM)	Fe(II) produced after 5 days (mM)
None		2.1
Acetate	30	53
Hydrogen		13
Glucose	30	1
Lactate	30	2.7
Fumarate	10	0.0
Malate	10	1.5
Propionate	10	0.0
Formate	30	0.0
Butyrate	30	0.0
Isobutyrate	30	0.0
Isovalerate	30	0.0
Pyruvate	30	0.0
Succinate	30	0.0
Phenol	0.5	0.0
Benzoate	0.5	0.0
Ethanol	30	0.0
Butanol	30	0.0
Propanol	30	0.5
Methanol	4	0.75
Yeast extract	0.1 g/liter	2.6

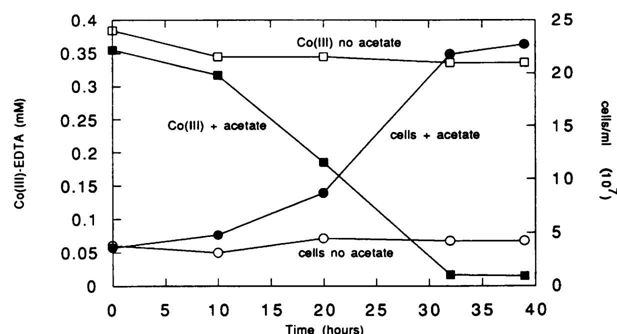

FIG. 4. Growth and Co(III)-EDTA reduction by PCA with acetate as an electron donor.

electron acceptors. No growth occurred in the absence of acetate. PCA was not capable of growth with oxygen, nitrate, sulfate, sulfite, or thiosulfate with acetate as an electron donor. There was no increase in absorbance for any of these acceptors with or without acetate.

The optimum temperature for Fe(III) reduction was 35°C. No Fe(II) production was observed at 4 or 50°C. Strain PCA reduced Fe(III) after 2 days in FW medium. Fe(III) reduction was observed after 5 days in 1/2 SW medium but not in SW medium.

Cytochromes. The difference spectra of whole cells showed the presence of *c*-type cytochromes (Fig. 7). The reduced cytochromes had absorbance peaks at 552, 522, and 419 nm.

Phylogeny. Comparison of the 16S rRNA sequence of strain PCA with the 16S rRNA sequences of selected representatives of the proteobacteria places it in the delta subdivision of the proteobacteria (Fig. 8). Analysis of the secondary structure and signature nucleotides confirmed the phylogenetic placement. Strain PCA shares a 95% similarity with *G. metallireducens* and an 89% similarity with *D. acetoxidans.*

DISCUSSION

Strain PCA is the first bacterium described for pure culture that couples the oxidation of acetate or hydrogen to the reduction of Fe(III). These results suggest that Fe(III)-reducing bacteria cannot be broadly distinguished phylogenetically or metabolically as acetate oxidizing (delta proteobacteria [*Geobacter* species]) or hydrogen oxidizing (gamma proteobacteria [*Shewanella* species]) as in previous models (26). The use of either acetate or hydrogen as an electron donor by a single

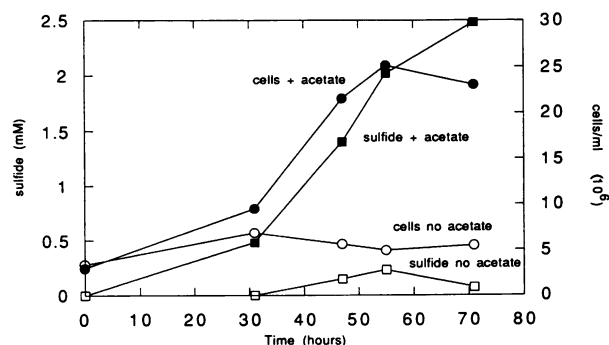

FIG. 5. Growth and H_2S production by PCA with acetate as an electron donor and S^0 as an electron acceptor.

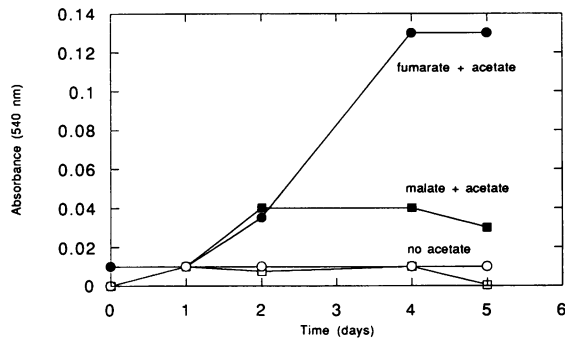

FIG. 6. Growth of PCA with acetate as an electron donor and fumarate or malate as an electron acceptor.

species has been previously demonstrated in the sulfate-reducing (45) and methanogenic (4) bacteria. It remains to be determined whether, under Fe(III)-reducing conditions, PCA can compete with *G. metallireducens* for acetate or with organisms such as *Shewanella* species for H_2. Since these experiments were done using Fe(III) citrate as the electron acceptor, it is not known if PCA can use CO_2 as the sole carbon source and thus grow autotrophically with H_2 and Fe(III).

Fe(III) and sulfur reduction. The ability of strain PCA to couple the oxidation of acetate to the reduction of either Fe(III) or S^0 provides further evidence for the interrelationship between the microbial iron and sulfur cycles in anaerobic environments. Previous studies have shown that the metal-reducing microorganisms *S. putrefaciens* (40) and strain BrY (9) reduce thiosulfate in the absence of a metal. The sulfur-reducing microorganism *D. acetoxidans* was recently shown to grow by dissimilatory reduction of Fe(III) and Mn(IV) (41). Reduced sulfur compounds can be microbiologically reoxidized using iron oxide or manganese as terminal electron acceptors, and certain species may be involved in the cycling of both elements (32, 43). Sulfate-reducing bacteria have been shown to enzymatically reduce, but not grow with, Fe(III), U(VI) (34, 35), and Cr(VI) (31) as the terminal electron acceptor. The abundance of sulfate-reducing bacteria in the Fe(III)-reducing zone of deep aquifers in the Atlantic coastal

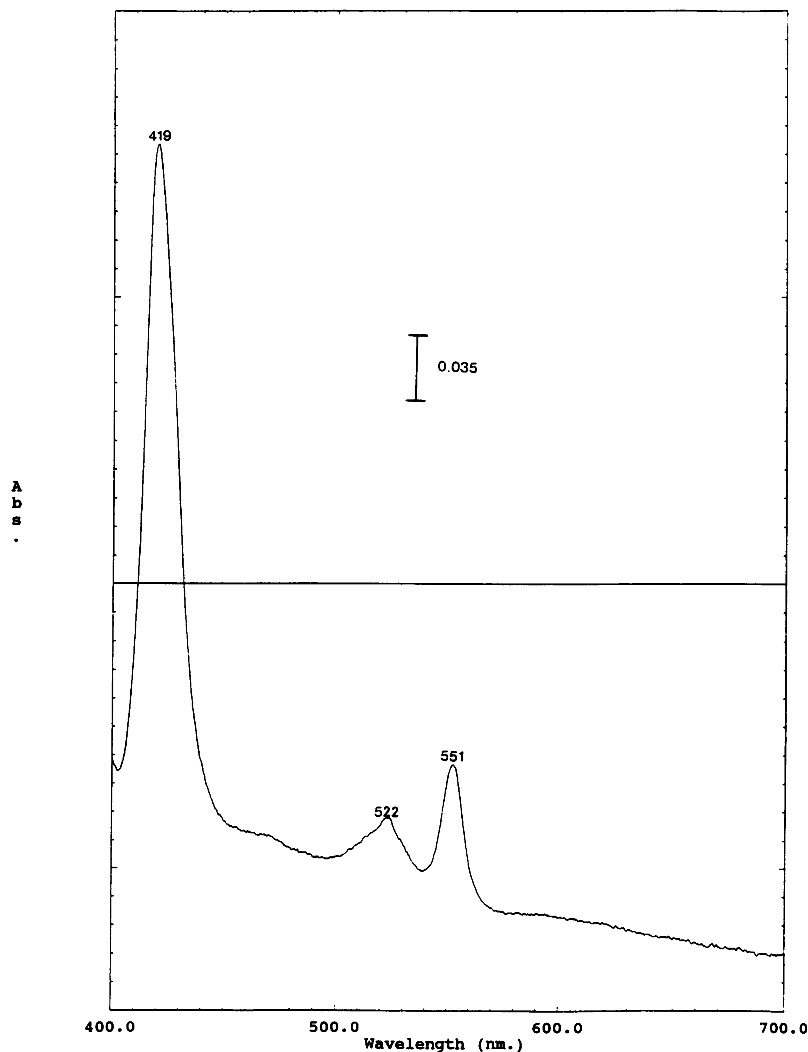

FIG. 7. Difference spectrum of whole cells of *G. sulfurreducens*. Abs, absorbance.

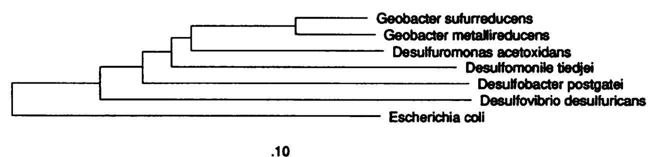

FIG. 8. Phylogenetic tree showing the relationship between *G. sulfurreducens* and representative delta proteobacteria. The sequence of *Escherichia coli* was used as the outgroup. The tree was inferred. The bar represents a 0.1-nucleotide change per position, i.e., 0.1 evolutionary distance unit.

plain and in salt marsh siderite concretions in which Fe(III)-reducing bacteria similar to *G. metallireducens* and *S. putrefaciens* were not detected suggests an in situ correlation between microbially mediated iron and sulfur geochemistry. These observations suggest that metal reduction may be an important ecological function of sulfur- and sulfate-reducing bacteria and that microorganisms physiologically defined as dissimilatory metal-reducing bacteria such as *Geobacter* species, *Shewanella* species, and strain BrY (26) are certainly not the only, and possibly not even the dominant, metal-reducing bacteria in some anaerobic environments. The evidence presented above enables us to expand the Fe(III)-reducing food chain model to include metal- and sulfur-reducing microorganisms like strain PCA and *D. acetoxidans*, as well as sulfate-reducing microorganisms which can enzymatically reduce metals.

Co(III) reduction. ^{60}Co(III) is a nuclear by-product that is often codisposed with the synthetic chelator EDTA. The Co(III)-EDTA complex is extremely stable to chemical reduction and exchange reactions and is thus highly mobile in saturated subsurface environments. The dissimilatory reduction of Co(III)-EDTA by strain PCA represents a novel mode of microbial metabolism. The fact that growth is coupled to cobalt reduction suggests that the reduction of cobalt is an energy-yielding process. Biologically, cobalt is found in vitamin B_{12} and other corrin ring molecules. The biologically active forms of these molecules function in hydrogen rearrangement and methyl transfer reactions (25). Cobalt was not previously thought to undergo biologically mediated oxidation-reduction reactions, although such a role for cobalt has been proposed in the formation of methane (6) and acetate (19). Further studies on the dissimilatory reduction of Co(III)-EDTA will be published elsewhere (18). In addition to finding a new biologically important role for cobalt, this discovery also has important biotechnological implications. Microbial reduction of Co(III)-EDTA may limit the far-field migration of ^{60}Co in contaminated subsurface environments. Co(II)-EDTA strongly adsorbs to aluminum oxides in soils and subsoils, while Co(III)-EDTA does not (17). Microbial reduction of Co(III)-EDTA will increase the likelihood that ^{60}Co will adsorb to subsurface materials and prevent the far-field migration of this contaminant.

Isolation. Previously, Fe(III)-reducing bacteria were enriched for and isolated with poorly crystalline Fe(III) oxide serving as the sole terminal electron acceptor (9, 27, 29). However, this approach presents several problems. Growth on Fe(III) oxide is slow, and the enrichment process is lengthy. Since iron oxide is insoluble, it is difficult to perform physiological studies on isolates in this medium, and nitrate is used as the electron acceptor to obtain isolates. This technique thus selects for metal-reducing bacteria which are also capable of reducing nitrate. The use of Fe(III) PP_i facilitated the enrichment and isolation of the Fe(III)-reducing bacterium PCA. Fe(III) PP_i is a soluble form of ferric iron that contains both citrate and phosphate as chelating agents. The soluble Fe(III) is more quickly reduced than insoluble iron oxide (Fig. 2A and B) (36). Strain PCA was isolated from sediment in approximately 40 days, while another organism was concurrently enriched and isolated on acetate and iron oxide in approximately 90 days (8). Interestingly, PCA was isolated with Fe(III) PP_i as the sole electron acceptor and was unable to reduce nitrate, whereas other Fe(III) reducers, such as *G. metallireducens*, used nitrate. Although Fe(III) PP_i was used successfully in the primary enrichments from which strain PCA was isolated, similar enrichments with phenol or palmitate as the sole electron donor yielded fermentative isolates that presumably fermented the citrate chelator and reduced the Fe(III) in a nondissimilatory manner (8). Fe(III) PP_i would therefore be most useful as a soluble source of Fe(III) in solid isolation media or in secondary enrichments.

Taxonomic and phylogenetic relationship to *Geobacter* species and *Desulfuromonas* species. The physiological characteristics and phylogeny of strain PCA suggest a relationship to both *Geobacter* species and *Desulfuromonas* species. All three organisms are members of the delta proteobacteria (28, 46) and are obligately anaerobic, gram-negative rods which contain *c*-type cytochromes and couple the oxidation of acetate to the reduction of Fe(III) (15, 24, 28, 38, 41). It is not yet known whether PCA oxidizes acetate via the citric acid cycle, as do *G. metallireducens* (10) and *D. acetoxidans* (16). Like *G. metallireducens* (29), PCA is nonmotile, whereas *Desulfuromonas* species are motile. Strain PCA is similar to *D. acetoxidans* in that it is capable of using Fe(III), S^0, fumarate, and malate but not U(VI) as terminal electron acceptors with acetate as the electron donor. *Desulfuromonas* species do not use H_2 as an electron donor, as does PCA, and several *Desulfuromonas* species use other organic electron donors, while PCA does not (41). These phenotypic differences and phylogenetic analyses preclude the inclusion of strain PCA in the genus *Desulfuromonas*.

Phylogenetic analysis of the 16S rRNA sequence of strain PCA indicated that it is most closely related to *G. metallireducens*. Both species are capable of growth by coupling the oxidation of acetate to the reduction of Fe(III) and Co(III). However, the two organisms differ in many respects as well. PCA differs from all previously described metal-reducing bacteria in its inability to reduce Mn(IV). PCA is able to use H_2 as an electron donor for Fe(III) reduction. PCA does not use nitrate as an electron acceptor, while *G. metallireducens* does (29). *G. metallireducens* is able to use several alcohols and aromatic compounds as electron donors for Fe(III) reduction (29), while PCA cannot. Strain PCA reduced Fe(III) in medium which contained up to one-half the NaCl concentration found in seawater, while *G. metallireducens* can grow only in freshwater medium (29). On the basis of phenotypic and phylogenetic characteristics, we propose that strain PCA be established as the type strain of a new species, *Geobacter sulfurreducens*.

Description of *Geobacter sulfurreducens* sp. nov. *Geobacter sulfurreducens* (sul'fer.re.du'cens. L. n. *sulfur*, sulfur; L. part. adj. *reducens*, converting to a different state; N.L. adj. *sulfurreducens*, reducing sulfur). Rod-shaped, gram-negative cells 2 to 3 by 0.5 μm, nonmotile, with no spore formation. Strict anaerobic chemoorganotroph which oxidizes acetate with Fe(III), S^0, Co(III), fumarate, or malate as the electron acceptor. Hydrogen is also used as an electron donor for Fe(III) reduction, whereas other carboxylic acids, sugars, alcohols, amino acids, yeast extract, phenol, and benzoate are not. Temperature optimum is 30 to 35°C. Cells contain *c*-type

cytochromes. Grows in up to one-half the NaCl concentration of seawater.

Habitat. *G. sulfurreducens* was enriched from surface sediments of a ditch in Norman, Okla., with acetate as the electron donor and ferric PP_i as the electron acceptor.

Type strain. The type strain of *G. sulfurreducens* is PCA.

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