

# *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals

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**Abstract.** The gram-negative metal-reducing microorganism, previously known as strain GS-15, was further characterized. This strict anaerobe oxidizes several short-chain fatty acids, alcohols, and monoaromatic compounds with Fe(III) as the sole electron acceptor. Furthermore, acetate is also oxidized with the reduction of Mn(IV), U(VI), and nitrate. In whole cell suspensions, the *c*-type cytochrome(s) of this organism was oxidized by physiological electron acceptors and also by gold, silver, mercury, and chromate. Menaquinone was recovered in concentrations comparable to those previously found in gram-negative sulfate reducers. Profiles of the phospholipid ester-linked fatty acids indicated that both the anaerobic desaturase and the branched pathways for fatty acid biosynthesis were operative. The organism contained three lipopolysaccharide hydroxy fatty acids which have not been previously reported in microorganisms, but have been observed in anaerobic freshwater sediments. The 16S rRNA sequence indicated that this organism belongs in the delta proteobacteria. Its closest known relative is *Desulfuromonas acetoxidans*. The name *Geobacter metallireducens* is proposed.

**Key words:** Iron – Uranium – Manganese – Nitrate – Anaerobic sediments – Delta proteobacteria – Aromatics – Heavy metals

The reduction of Fe(III) to Fe(II) is one of the most significant chemical changes that takes place during the development of anaerobic soils and sediments (Ponnamperuma 1972). In many environments, dissimilatory Fe(III)-reducing microorganisms enzymatically catalyze most of the Fe(III) reduction (Lovley et al. 1991b). Microbial Fe(III) reduction is considered to play an important role in a variety of processes of environmental concern, such as: the oxidation of natural and contaminant organic matter, the release of phosphate and trace metals into water supplies, the release of undesirably high concentrations of dissolved iron into ground waters, soil

gleying, the magnetization of aquatic sediments, and the inhibition of methane production in shallow freshwater environments (Lovley 1991a). Furthermore, microbial Fe(III) reduction may have been the first globally significant mechanism for the oxidation of organic matter to carbon dioxide (Lovley 1991a). The dissimilatory reduction of other metals with multiple redox states, such as Mn(IV) and U(VI) are also of environmental significance (Lovley 1991a; Lovley et al. 1991a).

Studies with pure cultures and with natural mixed populations of microorganisms living in Fe(II)-reducing aquatic sediments and subsurface soils have suggested that Fe(III)-reducing microorganisms which can completely oxidize fermentation acids and aromatic compounds to carbon dioxide are responsible for much of the Fe(III) reduction in sedimentary environments (Lovley and Phillips 1989; Lovley 1991a). Only two microorganisms capable of completely oxidizing multi-carbon organic compounds to carbon dioxide with Fe(III) as the sole electron acceptor have been described. One, designated strain 172, was recovered from deep subsurface sediments of the Atlantic Coastal Plain (Lovley et al. 1990). Other than its ability to completely oxidize acetate with Fe(III) as the electron acceptor, strain 172 has not been well characterized. The other isolate, known as strain GS-15, has been studied in more detail (Lovley et al. 1987; Lovley and Phillips 1988a; Lovley and Phillips 1988b; Lovley et al. 1989a; Lovley and Lonergan 1990; Champine and Goodwin 1991; Gorby and Lovley 1991; Lovley et al. 1991a; Gorby and Lovley 1992). The physiological characteristics of GS-15 do not suggest a close relationship to any previously described taxa (Lovley and Phillips 1988b). Here, we report additional characteristics, including 16S rRNA sequence analysis, which indicate that GS-15 is unique from previously described genera. The name *Geobacter metallireducens* is proposed.

## Materials and methods

### *Source of organism and culturing procedures*

As previously described (Lovley and Phillips 1988b), *Geobacter metallireducens* was isolated from an anaerobic enrichment culture

that contained acetate and yeast extract as potential electron donors and poorly crystalline Fe(III) oxide and carbon dioxide as potential electron acceptors. The enrichment culture was established with surficial bottom sediments collected from a previously described freshwater site (Lovley and Phillips 1986) in the Potomac River, Maryland.

*G. metallireducens* was routinely cultured under strict anaerobic conditions as previously described (Lovley and Phillips 1988b). The medium contained the following (g/l): NaHCO<sub>3</sub>, 2.5; CaCl<sub>2</sub> · 2 H<sub>2</sub>O, 0.1; KCl, 0.1; NH<sub>4</sub>Cl 1.5; NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O, 0.6 as well as vitamin and trace element mixtures. Unless otherwise noted, acetate (6.8 g/l) was the carbon source. Poorly crystalline Fe(III) oxide (ca. 100 mmol/l) was provided as the Fe(III) source (Lovley and Phillips 1988b). For growth with Fe(III)-citrate (50 mM) or nitrate (5–20 mM) as the electron acceptor the CaCl<sub>2</sub> · 2H<sub>2</sub>O and the Fe(III) oxide were omitted. The gas phase was N<sub>2</sub>–CO<sub>2</sub> (80:20). For growth on solid media, the medium was amended with 1.5% agar and either nitrate (20 mM) or Fe(III) citrate (50 mM). The ability of *G. metallireducens* to utilize various electron donors other than acetate was determined in liquid media with poorly crystalline Fe(III) oxide as the electron acceptor.

As previously described (Lovley and Phillips 1988b), media was dispensed into anaerobic pressure tubes (10 ml) or 160 ml serum bottles (100 ml), bubbled to remove dissolved oxygen and capped with thick butyl rubber stoppers, and sterilized by autoclaving. The final pH was ca. 6.7. All incubations were at 33 °C in the dark. Fe(II) production from Fe(III) reduction was quantified as previously described (Lovley and Phillips 1988b).

### Electron microscopy

For electron microscopy, cells of Fe(III) citrate-grown *G. metallireducens* were washed twice in 10 mM cacodylate buffer (pH 6.8) containing 10 mM MgCl<sub>2</sub>. Cells were fixed for 1 h with glutaraldehyde (5% v/v), washed three times and treated with osmium tetroxide (1% v/v). Samples were successively dehydrated with ethanol and propylene oxide and embedded in EPON 812-Araldite resin. Thin sections were stained with 5% uranyl acetate and 0.4% lead citrate (Reynolds 1963) and were examined with a Hitachi H600 STEM at 80 kV in the TEM mode.

### Cytochrome content and oxidation by terminal electron acceptors

Fe(III)-citrate-grown *G. metallireducens* was examined for the presence of cytochromes by obtaining difference spectra for dithionite-reduced minus air-oxidized cell suspensions. In order to localize the cytochromes, whole cell suspensions of Fe(III) citrate-grown *G. metallireducens* were broken by 3 passes through a French pressure cell at 100000 kPa, unbroken cells were removed with low speed centrifugation (12100 × g, 20 min, 4 °C), and then the soluble and membrane fractions were separated with ultracentrifugation (200000 × g, 2h, 4 °C).

In order to investigate the possible reoxidation of cytochromes by various potential electron acceptors, Fe(III) citrate-grown *G. metallireducens* were washed and resuspended in anaerobic bicarbonate buffer (2.5 g/l) in order to provide ca. 500 µg of cell protein per ml. Cell suspensions (2.0 ml) were placed in sealed quartz cuvettes under H<sub>2</sub>. Potential electron acceptors in bicarbonate buffer (500 µM; 0.5 ml) were added to one cuvette of the cell suspension and buffer (0.5 ml) without electron acceptor was added to an equivalent cell suspension. The difference spectra of cell suspensions with the added electron acceptors minus cell suspensions without added electron acceptor was determined. Potential electron acceptors were added as their sodium or chloride salts with the exception of chromate and silver which were added as the potassium and sulfate salts respectively.

### Detection of menaquinone

Lipophilic extracts of *G. metallireducens* were prepared as described previously (Krivankova and Dadak 1980). The presence of quinones in the extract was analyzed as borohydride-reduced minus air-oxidized difference spectra (Kroger 1978). Menaquinone extracted from *Bacillus subtilis* and ubiquinone from *Pseudomonas aeruginosa* were used for comparison. For quantitative determinations, menaquinone was purified on silica gel G plates, and then extracted with three washes of 95% ethanol. The concentration of menaquinone in each wash was calculated from the extinction coefficient of 11.3 mM<sup>-1</sup> for the A<sub>280</sub> – A<sub>270</sub> after reduction with borohydride (Kroger 1978).

### Lipid analysis

Approximately 20–25 mg dry weight of bacterial cells were extracted in a Bligh and Dyer (Bligh and Dyer 1959) single phase solvent system modified to include phosphate buffer (50 mM PO<sub>4</sub>, pH = 7.4) (White et al. 1979). As previously recommended (Guckert et al. 1985), care was taken to prevent artifacts and contamination. The bacteria were extracted for 3 h at room temperature in 142.5 ml chloroform: phosphate buffer (1:2:0.8, v:v:v). Then 37.5 ml each of chloroform and distilled water were added to separate the aqueous (upper) and organic (lower) phases overnight. The organic phase (containing the bacterial lipids) was collected after filtration through a pre-extracted fluted Whatman 2V filter and the solvent was removed with a rotary evaporator at 37 °C. The total lipid extract was fractionated on silicic acid columns into neutral, glyco- and phospholipids (Parker et al. 1982). The polar lipid fraction, which was recovered in methanol, was transesterified with mild alkaline methanolysis (White et al. 1979). The resulting methyl esters were separated, quantified, and tentatively identified with capillary gas chromatography (GC) as previously described (Ringelberg et al. 1988). The phospholipid esterlinked fatty acids (PLFA) structures were verified using GC/mass spectrometry (Ringelberg et al. 1988). Mono and dienolic PLFA double bond positions and conformation were determined by GC/MS analysis of the dimethyl disulfide adducts (Nichols et al. 1986). Cyclopropyl PLFA ring positions were determined by GC/MS after hydrogenation (Guckert et al. 1985). Equivalent chain length (ECL) was calculated as previously described (Christie 1989).

The lipopolysaccharide (LPS) hydroxy fatty acids were recovered from the upper aqueous phase of the Bligh and Dyer extraction (Edlund et al. 1985; Nichols et al. 1985). The upper phase was removed in a rotary evaporator in vacuo and hydrolyzed in 1 N HCl for 2–4 h at 100 °C. The residue was re-extracted for 24 h with two portions of methanol and three of chloroform so that the final volume contained chloroform:methanol:1M HCl (5:2:3, v:v:v). After centrifugation, the chloroform phase was recovered, evaporated to dryness and methylated with "magic" methanol (methanol:chloroform:concentrated HCl, 10:1:1) (W. Mayberry, personal communication), at 100 °C for 1 h. The methylated hydroxy fatty acids (OHFA) were recovered in hexane:chloroform (4:1, v:v) with three washes in the upper phase. The volatile solvent was removed with a stream of nitrogen and the OHFA were purified on thin layer plates that had been cleaned with hexane: ethylether, (1:1 v:v). The plates were developed in the same solvent. The OHFA band was localized by simultaneously running a standard. The hydroxyl groups were derivatized with N, O-bis (Trimethylsilyl) trifluoroacetamide (Pierce, Rockford, Ill., USA) and the derivatized OHFAs were analyzed with GC/MS.

Fatty acids were designated as follows- the total number of carbon atoms: number of double bonds, with the position of the double bond closest to the methyl end (w) of the molecule. Configuration of the double bonds is indicated as *cis* (c) or *trans* (t). For example, 16:1w7c is a PLFA with 16 total carbons with one double bond 7 carbons from the methyl end in the *cis* configuration. Branched fatty acids are designated as *iso* (i) or *anteiso* (a) if the methyl branch is one or two carbons from the w

end (115:0). Otherwise, the position from the methyl end of the molecule is designated (10Me16:0). Cyclopropyl (cy) fatty acids are designated by the total number of carbons (cy17:0) and the position of the hydroxyl from the carboxyl end of the fatty acid is indicated as a prefix (3 OH16:0).

### 16S rRNA Gene cloning and sequencing

The methods used to clone, sequence and analyze the 16S rRNA gene are described in detail elsewhere (Lane et al. 1985; Britschgi and Giovannoni 1991; Giovannoni 1991). Briefly, nucleic acids were prepared from a frozen cell pellet by enzymatic lysis, extraction with phenol, and isopycnic centrifugation in cesium trifluoroacetate. A partial sequence of the 16S rRNA was determined with reverse transcriptase (Lane et al. 1985). The gene was amplified with general eubacteria primers, cloned into the phagemid vector pBluescript (Stratagene, La Jolla, Calif., USA) and sequenced with Sequenase (US Biochemical, Cleveland, Ohio, USA).

### G + C Content

Nucleic acid was extracted from Fe(III)-citrate grown cells using a modification of previously described methods (Marmur and Doty 1962). The G + C content was determined by the thermal denaturation method (Marmur and Doty 1962). The thermal denaturation temperature for *Escherichia coli* CSH27 was determined as a calibration standard.

## Results

### Cell and colony morphology

As previously reported (Lovley and Phillips 1988b) *Geobacter metallireducens* is a nonmotile, nonspore-forming, gram-negative rod that is 2 to 4 by 0.5  $\mu\text{m}$ . Transmission electron microscopy of thin sections indicated that *G. metallireducens* displayed a typical gram-negative cell wall with an outer membrane separated from an inner membrane by a periplasmic region (Fig. 1). Dividing cells formed a central constriction between daughter cells which is typical for gram-negative bacteria

(Conti and Gettner 1962; Steed and Murray 1966). Neither cellular inclusions (e.g. PHB or volutin granules) nor flagella were detected.

Colonies on agar medium with nitrate or Fe(III) citrate as the electron acceptor are typically 1 mm or less and are red, domed, entire, smooth and wet. The colonies hold fast to the medium. During growth on medium containing Fe(III) citrate as the electron acceptor the area around the colony changes from light brown to dark green within several days and eventually turns light green or clear.

### Electron acceptors and donors and nutrition

Electron acceptors which could support growth of *G. metallireducens* with acetate as the electron donor are summarized in Table 1. Some of these have been reported previously (Lovley and Phillips 1988b; Lovley et al. 1991a). Washed cell suspensions of *G. metallireducens* can also reduce Co(III)-EDTA to Co(II)-EDTA (Y. Gorby, unpublished data). Although potential sulfur-containing electron acceptors did not support growth (Table 1), the ability of cell suspensions to reduce sulfate,

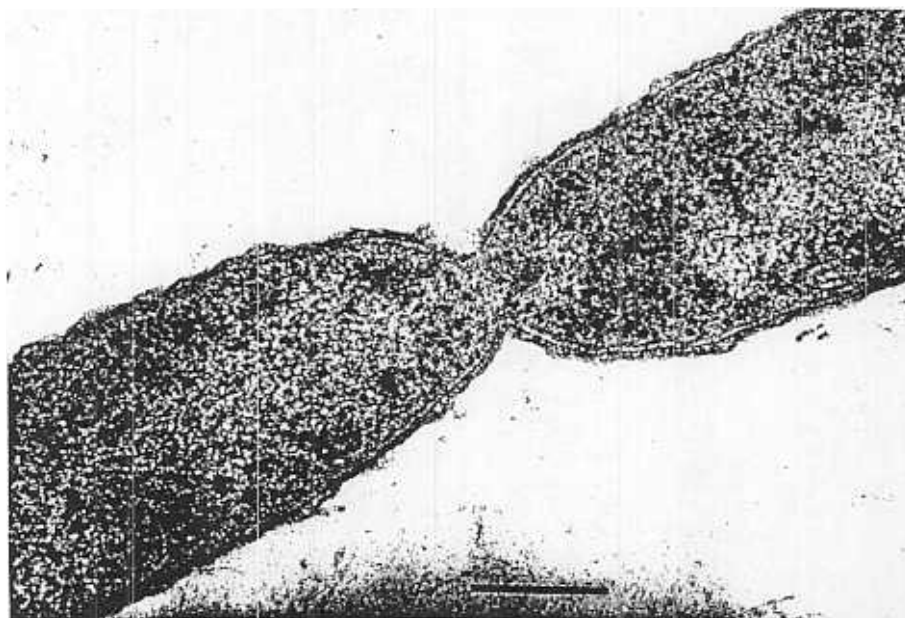
**Table 1.** Compounds tested as electron acceptors for acetate oxidation. Concentrations (mmol/l) of electron acceptors are given in parentheses

#### Utilized

Synthetic poorly crystalline Fe(III) oxide (100–250), natural Fe(III) oxide in a sand and gravel aquifer (16), natural oxide resulting from oxidation of Fe(II) in groundwater (200), Fe(III)-citrate (20–50), Mn(IV) oxide (birnessite) (15), nitrate (5–20), and U(VI) (8)

#### Tested but not utilized

Oxygen (atmospheric and 1%), fumarate (20 mM) sulfate (10 mM), elemental sulfur (ca. 1 g/l), sulfite (10), thiosulfate (10), selenate (10), selenite (10), trimethylamine N oxide (10)



**Fig. 1.** Transmission electron micrograph of thin section of dividing cells of *Geobacter metallireducens*. The horizontal bar represents 0.2  $\mu\text{m}$

sulfite, and thiosulfate was also tested in resting cell suspensions because of the close relationship between *G. metallireducens* and dissimilatory sulfur- and sulfate-reducers (see below). Cell suspensions of *G. metallireducens* did not reduce any of the sulfur compounds with acetate as the electron donor. Indirect evidence suggests that some metals, other than those listed in Table 1, may also serve as electron acceptors (see next section).

Electron donors for Fe(III) oxide reduction, some of which have been reported in other studies (Lovley and Phillips 1988b; Lovley et al. 1989a; Lovley and Lonergan 1990) are summarized in Table 2. Previous studies have shown that *G. metallireducens* does not grow fermentatively in complex, organic-rich anaerobic media (Lovley and Phillips 1988b) and it does not grow fermentatively with pyruvate as the substrate.

*G. metallireducens* did not require the vitamin mixture for growth but did require the trace metal mix.

#### Cytochrome content and oxidation by terminal electron acceptors

Reduced minus oxidized spectra of whole cell suspensions gave adsorption maxima at 553, 523, and 423, suggesting that *G. metallireducens* contains *c*-type cytochrome(s) (Fig. 2). Subsequent detailed studies have demonstrated that *G. metallireducens* contains several *c*-type cytochromes and no *b*-type cytochromes (R. R. Naik, F. M. Murrillo, and J. F. Stolz, FEMS Letters, in press).

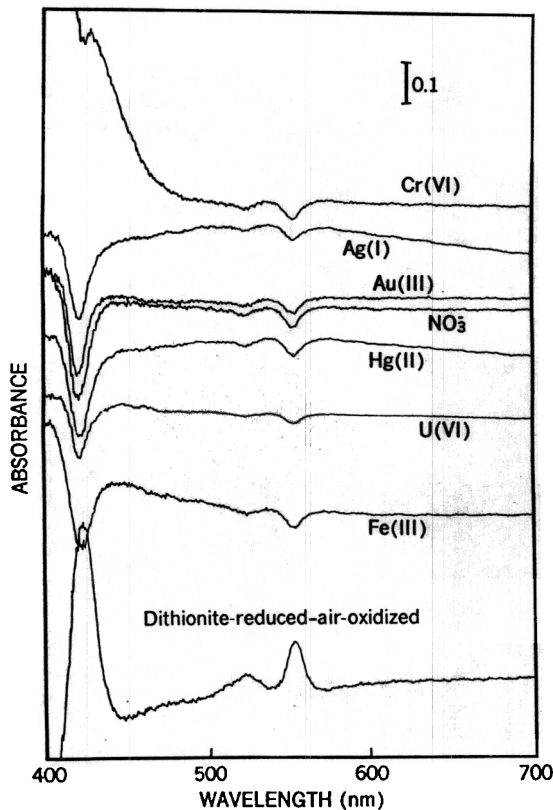


Fig. 2. Difference spectra for dithionite-reduced minus air-oxidized cells and  $H_2$ -reduced (electron acceptor added) minus  $H_2$ -reduced cells

Table 2. Compounds tested as electron donors in the presence of poorly crystalline Fe(III) oxide. Concentrations (mM) of electron donors are given in parentheses

#### Utilized

Acetate (10–50), ethanol (20), propionate (20), butyrate (20), isobutyrate (10), valerate (10), isovalerate (10), pyruvate (10), propanol (10), butanol (10), toluene (1–10), benzoate (1), benzaldehyde (0.5), benzylalcohol (0.5), *p*-hydroxybenzoate (0.5), *p*-hydroxybenzaldehyde (0.5), *p*-hydroxybenzylalcohol (0.5), phenol (0.5), *p*-cresol (0.5)

#### Tested but not utilized

Glucose (20), malate (20), fumarate (20), caproate (10), methanol (20), glycerol (20), trimethylamine (20), formate (20), lactate (20), hydrogen (130 kPa), fructose (20), Casamino Acid mixture (0.5 g/l), glutamine (10), serine (10), arginine (10), leucine (10), proline (10), glutamate (10), tryptophane (10), tyrosine (0.5), tert-butanol (10), isopropanol (10), benzene (0.5), *o*-xylene (0.5), *m*-xylene (0.5), *p*-xylene (0.5), ethylbenzene (0.5), *o*-cresol (0.5), *m*-cresol (0.5), *o*-hydroxybenzoate (0.5), *m*-hydroxybenzoate (0.5), nicotinate (0.5), syringate (0.5), ferulate (0.5), phenylacetate (0.5), *o*-phthalate (0.5), *m*-phthalate (0.5), *p*-phthalate (0.5), *o*-toluate (0.5), *m*-toluate (0.5), *p*-toluate (0.5), naphthalene (0.5), elemental sulfur (ca. 1 g/l)

The *c*-type cytochrome(s) were oxidized when Fe(III), U(VI), or nitrate (electron acceptors which are known to support growth of *G. metallireducens*) were added to whole cell suspensions (Fig. 2). The *c*-type cytochrome(s) were also oxidized if gold, silver, mercury or chromate was provided as a potential electron acceptor (Fig. 2). Whether the reduction of these other metals can function to support growth has not been determined. Metals that were tested but did not result in the oxidation of the *c*-type cytochrome(s) were vanadate, molybdate, and tungstate.

#### Menaquinone

Difference spectra of lipophilic extracts indicated that *G. metallireducens* had a similar spectrum to the menaqui-

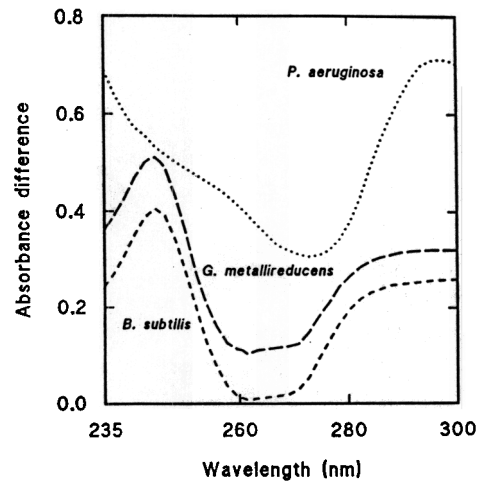


Fig. 3. Difference spectra for borohydride-reduced minus air-oxidized lipophilic extracts of *Geobacter metallireducens*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*

none preparation from *Bacillus subtilis* (Fig. 3). The concentrations of menaquinone in nitrate and Fe(III)-citrate grown cells was 3.4 and 2.0  $\mu\text{mol}$  per gram of cell protein, respectively. There was no evidence for the presence of ubiquinone.

#### Oxygen sensitivity

Previous studies have indicated that addition of air to Fe(III)-, Mn(IV)-, or nitrate-reducing medium inhibited growth (Lovley and Phillips 1988b). Tests for catalase activity were negative and no carotenoids were detected in acetone:methanol (7:2, v:v) extracts. These results suggest that *G. metallireducens* is a strict anaerobe which does not possess typical means for abating oxygen toxicity.

#### Lipids

The phospholipid ester-linked fatty acid (PFLA), and LPS hydroxy fatty acid (LPS OH-FA) profiles of *G. metallireducens* were comparable whether it was grown with Fe(III) citrate or nitrate as the electron acceptor (Table 3). In the PFLA profile, i15:0, 16:1w7c, and 16:0 account for 83–87 mol% of the PFLA. This indicates that two major fatty acid biosynthetic pathways are required. The i15:0 indicates the presence of the branched pathway whereas the 16:1w7c is synthesized by the anaerobic desaturase pathway (White 1988; Vestal and White 1989).

The amide and ester linked hydroxy fatty acids from the LPS (LPS OH-FA) of *G. metallireducens* are distinctly different from those of other bacteria. Hydroxy 16:0 comprise over 80% of the total and the 9-, 10-, and 11-OH16:0 comprise 23% of the total. These mid-chain LPS OH-FA have not previously been reported in microorganisms (Wilkinson 1988).

#### G + C Content

The mol% G + C of *G. metallireducens* was 56.6.

#### Phylogeny

The 16S rRNA sequence of *G. metallireducens* has been deposited in GenBank (LO 7834). The aligned 16S rRNA sequence of *G. metallireducens* was compared to about 400 sequences from diverse prokaryotes. The highest sequence similarities emerging from this analysis (0.93 to *Desulfuromonas acetoxidans*) clearly place *G. metallireducens* among the delta subdivision of the proteobacteria. This phylogenetic placement was confirmed by an analysis of higher order structure and signature nucleotides. An extended stem and loop structure of eleven base pairs between positions 184 and 193, and another stem and loop structure of eight base pairs between positions 198 and 219 (relative to *Escherichia coli* 16S rRNA; (Brosius et al. 1981)) are significant features shared in common by *G. metallireducens* and other delta proteobacteria. Furthermore, *G. metallireducens* has the signature nucleotides described by Woese for the delta proteobacteria, including those at positions

**Table 3.** Phospholipid fatty acids and lipopolysaccharide hydroxy fatty acids in *Geobacter metallireducens* grown with either Fe(III) or nitrate as the electron acceptor

	$\mu\text{mol/g}$ of dry cells		Mol%	
	Fe(III)-grown	Nitrate-grown	Fe(III)-grown	Nitrate-grown
<b>Phospholipid fatty acid (methyl ester)</b>				
14:1w7	227	259	0.22	0.32
14:0	3760	4908	3.66	6.02
unknown	103	148	0.10	0.18
i15:0	10775	14589	10.50	17.88
A15:0/15:1w8	135	108	0.13	0.13
15:0	42	38	0.04	0.05
16:1w7C	46324	33210	45.13	40.70
16:1w5C	1509	1133	1.47	1.39
16:0	33067	21454	32.21	26.30
I17:1w8	261	274	0.25	0.34
10ME16:0	764	561	0.74	0.69
I17:0	878	796	0.86	0.98
CY17.0	31	802	0.03	0.98
unknown	77	100	0.08	0.12
18:1w7C	3835	2701	3.74	3.31
18:1w5C	198	125	0.19	0.15
18:0	663	382	0.65	0.47
TOTAL	102649	81587	100.00	100.00
<b>Hydroxy fatty acid (trimethyl silyl derivative):</b>				
ALC14	31	43	1.55	1.99
unknown	46	39	2.34	1.83
3-OH14:0	139	248	7.06	11.48
ALC16	54	65	2.77	3.01
3-OH15:0	21	68	1.05	3.14
3-OH16:0	1131	1140	57.44	52.82
9-OH16:0	231	219	11.72	10.17
10-OH16:0	242	223	12.27	10.33
11-OH16:0	15	13	0.78	0.59
ALC18	46	81	2.35	3.78
3-OH17:0	13	18	0.68	0.85
TOTAL	1970	2158	100.00	100.00

44, 398, and 469, which set the delta proteobacteria apart from other proteobacteria (Woese 1987). A phylogenetic tree comparing *G. metallireducens* to representative eubacteria is shown in Fig. 4.

#### Discussion

##### *Phylogeny of Geobacter metallireducens-comparison with sulfur reducers*

The analysis of the 16S rRNA sequence of *Geobacter metallireducens* indicates that the closest known relatives of *G. metallireducens* are gram-negative sulfur- and sulfate-reducers. There are also a number of physiological similarities between these organisms and *G. metallireducens*. For example, *G. metallireducens* and its closest known relative, *Desulfuromonas acetoxidans*, oxidize acetate to carbon dioxide via the citric acid cycle whereas most strict anaerobic microorganisms that oxidize acetate do not use this pathway (Thauer et al. 1989; Champine and Goodwin 1991).

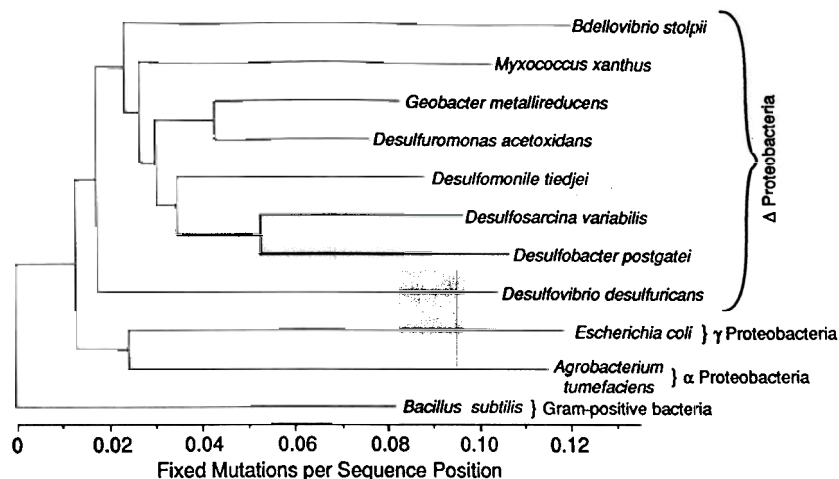


Fig. 4. Phylogenetic tree inferred from 16S rRNA sequence divergence. The tree was inferred by a distance matrix method (Fitch and Margoliash 1967) with a software package kindly provided by Dr. Gary Olsen of the University of Illinois. Evolutionary distances were calculated by the method of Jukes and Cantor (Jukes and Cantor 1969). Positions of uncertain homology in regions containing insertions and deletions were omitted from the analysis, which included about 1200 positions. The sequence of *Synechococcus* PCC 6301 was used to position the root of the tree. Evolutionary distances are indicated by the sum of horizontal branch lengths

*G. metallireducens* joins sulfate- and sulfur-reducers as the only gram-negative, obligately anaerobic respiratory microorganisms which have menaquinone as an electron carrier (Collins and Jones 1981). *G. metallireducens* contains menaquinone in quantities similar to those previously found in sulfate-reducers (Brandis-Heep et al. 1983; Schmitz et al. 1990).

Cytochromes of the *c*-type predominate in gram-negative sulfate- and sulfur-reducers and are considered to be integral components of the respiratory metabolism of these organisms (LeGall and Fauque 1988). The results presented here and in another recent study (R. R. Naik, F. M. Murillo, and J. F. Stolz, FEMS Letters, in press) indicate that *c*-type cytochrome(s) also predominate in *G. metallireducens*. This finding is in contrast to a previous report (Gorby and Lovley 1991), which indicated that *G. metallireducens* primarily contained *b*-type rather than a *c*-type cytochrome(s). That previous report was in error as the result of a miscalibrated spectrophotometer. The finding that the *c*-type cytochrome(s) of *G. metallireducens* is oxidized in the presence of Fe(III), U(VI), or nitrate suggests that *c*-type cytochrome(s) may be a component of the electron transport chain(s) leading to these terminal electron acceptors. The oxidation of the *c*-type cytochrome(s) in the presence of Cr(VI), Hg(II), Ag(I), or Au(III) suggests that *G. metallireducens* may be able to reduce these metals as well.

*G. metallireducens* and sulfate-reducers are similar in that those sulfate-reducers which use nitrate as a terminal electron acceptor reduce nitrate to ammonia (Widdel 1988). *G. metallireducens* and *Desulfobacterium catecholicum* (Szewzyk and Pfennig 1987) are the only organisms known to completely oxidize organic compounds to carbon dioxide with the concurrent reduction of nitrate to ammonia. *G. metallireducens* is capable of fixing molecular nitrogen to support growth (D. A. Bazylinski and D. R. Lovley, unpublished data) as are a number of gram-negative sulfate-reducing microorganisms (Widdel 1988).

An important contrast between *G. metallireducens* and its nearest phylogenetic relatives is that, at least under current culturing conditions, *G. metallireducens* is unable to reduce sulfur or sulfate. One sulfate reducer, *Desul-*

*fovibrio desulfuricans* can reduce Fe(III) (Jones et al. 1984) and U(VI) (Lovley and Phillips 1991) but this metabolism does not support growth (Lovley and Phillips 1991). The ability of other gram-negative, sulfate- and sulfur-reducing microorganisms to use metals as a terminal electron acceptor is currently under investigation.

#### Phylogeny of *G. metallireducens*-Comparison with other metal-reducers and magnetogens

The 16S rRNA analysis indicates that *G. metallireducens* is not closely related to the other organisms that conserve energy from metal reduction. *Shewanella* (formerly *Alteromonas*) *putrefaciens* grows with Fe(III), Mn(IV), or U(VI) as the terminal electron acceptor and hydrogen, formate, lactate, or pyruvate as the electron donor (Myers and Nealson 1988; Lovley et al. 1989b; Lovley et al. 1991a). Lactate and pyruvate are incompletely oxidized to acetate and carbon dioxide. *S. putrefaciens* is not a near relative of *G. metallireducens* as *S. putrefaciens* is a member of the gamma proteobacteria (MacDonnell and Colwell 1985). A dissimilatory Fe(III)-reducer which is metabolically similar to *S. putrefaciens* has been classified as *Pseudomonas* sp. (Balashova and Zavarzin 1980) and another, strain BrY, (Caccavo F Jr et al. 1992) also does not appear closely related to *G. metallireducens*.

Microorganisms which produce intracellular or extracellular nm-sized magnetic particles have been termed magnetogens (Blakemore and Frankel 1989). It has been hypothesized that there may be a common genetic basis for magnetogenesis (Eden et al. 1991). However, *Aquaspirillum magnetotacticum* which produces intracellular chains of ultrafine-grain magnetite (Blakemore 1982) has recently been classified in the alpha group of the proteobacteria (Eden et al. 1991) and thus, it is not closely related to *G. metallireducens* which produces extracellular ultrafine-grain magnetite during reduction of poorly crystalline Fe(III) oxide (Lovley et al. 1987; Lovley 1991b).

#### Lipids

*G. metallireducens* is the first bacterium known to contain 9-, 10-, and 11-OH16:0 LPS OH-FA. However, these

have previously been recovered from anaerobic freshwater sediments and waste digester samples (Ringelberg and White, unpublished observations). Midchain hydroxy fatty acids are rarely found in nature. They have only been found previously in the eukaryotes, in the sphorolipids of yeasts and in plant glycolipids (Tulloch 1990).

Although the presence of both the branched and the desaturase pathways for synthesis of PFLA in a single bacterium is somewhat unusual, this double pattern is typical for the gram-negative sulfate-reducer, *Desulfovibrio* and, to a lesser extent, *Desulfobacter* (Edlund et al. 1985; Dowling et al. 1986). However, these sulfate-reducers have significant proportions of specific unusual fatty acids, i17:1w7 in the *Desulfovibrio* (Edlund et al. 1985) and 10Me16:0 in the *Desulfobacter* (Dowling et al. 1986), that are not found in *G. metallireducens*. In contrast, *Desulfobulbus*, another gram-negative sulfate-reducer, does not contain branched fatty acids but has the unusual 17:1w6 as a major component (Parkes and Calder 1985).

The dissimilatory Fe(III)-reducing microorganism, *S. putrefaciens* is similar to *G. metallireducens* in that *S. putrefaciens* also contains i15:0, 16:1w7c, and 16:0 as major PLFA (Ringelberg DB, Neelson KH, and White DC, unpublished data). However, the LPS-OHFA of *S. putrefaciens* are clearly different from *G. metallireducens* in containing 3-OH12:0, Br-3-OH13:0 and 3-OH14:0 as the major LPS-OHFA with no mid chain hydroxy LPS-OHFA.

#### Significance for ecological studies

The metabolism of *G. metallireducens* serves as a model for a variety of environmental phenomena associated with Fe(III) reduction (Lovley 1991a). However, due to the relatively few studies on dissimilatory Fe(III)-reducing microorganisms, there is no information on whether *G. metallireducens* or other, as yet undescribed, organisms are the numerically dominant organisms involved in oxidizing organic compounds in various sedimentary environments in which Fe(III) reduction is the predominant terminal electron accepting process. The nucleotide sequence of the 16S rRNA of *G. metallireducens* as well as the finding of unique LPS OH-FA in this organism indicate that it should be possible to develop probes to determine whether *G. metallireducens* or closely related organisms play an important role in Fe(III) reduction.

#### Description

*Geobacter* (gen nov), Ge.o.bac'ter) Gr. n. ge the earth; Gr. hyp. masc. n. \*bacter a rod; N. L. masc. n. *Geobacter* a rod from the earth.

Rod-shaped, gram-negative cells 2–4 by 0.5 µm, non-motile, with no spore formation. Strict anaerobic chemorganotroph which completely oxidizes acetate, ethanol, propionate, butyrate, isobutyrate, valerate, isovalerate, pyruvate, propanol, butanol, toluene, benzoate,

benzaldehyde, benzylalcohol, *p*-hydroxybenzoate, *p*-hydroxybenzaldehyde, *p*-hydroxybenzylalcohol, phenol, or *p*-cresol to carbon dioxide with Fe(III) as the electron acceptor. Reduction of Fe(III) oxide can result in copious accumulations of ultrafine-grained magnetite. Other electron acceptors for acetate oxidation are Mn(IV), nitrate, and U(VI). Nitrate is reduced to ammonia. Capable of fixing molecular nitrogen as its sole source of nitrogen to support growth. Temperature optimum 30–35 °C. Cells contain *c*-type cytochromes and menaquinone. The lipopolysaccharide fraction has three unique 16 carbon hydroxyfatty acids with a hydroxyl group on the 9th, 10th, or 11th carbon from the carboxyl carbon. The G + C content of the DNA is 56.6 mol%.

#### The type species-*Geobacter metallireducens*

*Geobacter metallireducens* (sp. nov.), me.ta.li.re.du'cens L. n. *metallum* metal; L. part. adj. *reducens* converting to a different state; N. L. adj. *metallireducens* reducing metal.

The description is the same as for the genus.

The type strain of *Geobacter metallireducens* is strain GS-15. It was enriched from freshwater sediments of the Potomac River, Maryland with acetate as the electron donor and poorly crystalline Fe(III) as the electron acceptor.

The strain has been deposited in the American Type Culture Collection (ATCC 53774) and the Deutsche Sammlung von Mikroorganismen (DSM 7121).

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