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Geothrix fermentans gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer

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In an attempt to understand better the micro-organisms involved in anaerobic degradation of aromatic hydrocarbons in the Fe(III)-reducing zone of petroleum-contaminated aquifers, Fe(III)-reducing micro-organisms were isolated from contaminated aquifer material that had been adapted for rapid oxidation of toluene coupled to Fe(III) reduction. One of these organisms, strain H-5^T, was enriched and isolated on acetate/Fe(III) medium. Strain H-5^T is a Gram-negative strict anaerobe that grows with various simple organic acids such as acetate, propionate, lactate and fumarate as alternative electron donors with Fe(III) as the electron acceptor. In addition, strain H-5^T also oxidizes long-chain fatty acids such as palmitate with Fe(III) as the sole electron acceptor. Strain H-5^T can also grow by fermentation of citrate or fumarate in the absence of an alternative electron acceptor. The primary end-products of citrate fermentation are acetate and succinate. In addition to various forms of soluble and insoluble Fe(III), strain H-5^T grows with nitrate, Mn(IV), fumarate and the humic acid analogue 2,6-anthraquinone disulfonate as alternative electron acceptors. As with other organisms that can oxidize organic compounds completely with the reduction of Fe(III), cell suspensions of strain H-5^T have absorbance maxima indicative of a *c*-type cytochrome(s). It is proposed that strain H-5^T represents a novel genus in the *Holophaga–Acidobacterium* phylum and that it should be named *Geothrix fermentans* sp. nov., gen. nov.

Keywords: Fe(III) reduction, humic acid reduction, toluene degradation, *Acidobacterium*, *Holophaga*

INTRODUCTION

Dissimilatory Fe(III)-reducing bacteria (DIRB) conserve energy to support growth by coupling the oxidation of organic compounds and/or H_2 to the reduction of ferric iron (Lovley *et al.*, 1997). Dissimilatory Fe(III) reduction may have been one of the first globally significant processes for the oxidation of organic matter to CO_2 (Walker, 1987; Lovley, 1991; Vargas *et al.*, 1998) and has a significant role in modern sedimentary environments, not only in the oxidation of organic matter (Lovley, 1995), but also in the dissolution of iron(III) oxides and the formation of geologically significant minerals such as magnetite and siderite (Lovley *et al.*, 1987; Coleman *et al.*, 1993; Lovley, 1995). DIRB have been recovered from a wide variety of sedimentary environments including freshwater and marine aquatic sediments, the deep terrestrial subsurface, petroleum-contaminated aquifers, soils and hydrothermal zones (Lovley *et al.*, 1997; Vargas *et al.*, 1998).

Fe(III)-reducing bacteria are both phenotypically and taxonomically diverse (Lovley *et al.*, 1997; Vargas *et al.*, 1998). They fall into two basic physiological categories, those that oxidize multicarbon compounds completely to CO_2 and those that oxidize multicarbon compounds incompletely to acetate. The complete

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Abbreviations: AQDS, 2,6-anthraquinoline disulfonate; DIRB, dissimilatory Fe(III)-reducing bacteria; NTA, nitrilotriacetic acid.

The GenBank accession number for the 16S rDNA sequence of *Geothrix fermentans* strain H-5^T is U41563.

oxidizers include members of the family *Geobacteraceae* (Lonergan *et al*., 1996) in the δ-subclass of the *Proteobacteria*, such as species from the genera *Geobacter* (Lovley *et al*., 1993; Caccavo *et al*., 1994; Coates *et al*., 1996), *Desulfuromonas* (Roden & Lovley, 1993; Coates *et al*., 1995) and *Desulfuromusa* (Finster & Bak, 1993; Lonergan *et al*., 1996), as well as phylogenetically distinct genera such as *Geovibrio* (Caccavo *et al*., 1996) and the recently described *Deferribacter* (Greene *et al*., 1997). The incomplete oxidizers include organisms in the genera *Shewanella* (Lovley *et al*., 1989b; Caccavo *et al*., 1992; Rossello-Mora *et al*., 1994; Coates *et al*., 1998b), *Ferrimonas* (Rossello-Mora *et al*., 1995), *Pelobacter* (Lovley *et al*., 1995) and *Thermoterrabacterium* (Slobodkin *et al*., 1997), as well as the organism known as strain SES-3 (Laverman *et al*., 1995). All of the incompletely oxidizing, but only a few of the completely oxidizing, DIRB can use H_2 (Lovley *et al*., 1997). All of the Fe(III)-reducing hyperthermophilic *Archaea* and *Bacteria* that have been examined to date also have the ability to oxidize H_2 with Fe(III) (Vargas *et al*., 1998).

In order to learn more about the diversity and metabolic capabilities of DIRB, we investigated DIRB populations in the Fe(III)-reducing zone of petroleum-contaminated aquifers. Fe(III) reduction has previously been shown to be an important terminal electron-accepting process in such environments (Lovley *et al*., 1989a; Lovley, 1997), but little is know about the DIRB populations that inhabit these systems. The most readily isolated Fe(III)-reducers from mesophilic environments belong to the family *Geobacteraceae* in the δ-subclass of the *Proteobacteria* (Lonergan *et al*., 1996) and are usually species of the genus *Geobacter* (Coates *et al*., 1996). However, a recent study (Anderson *et al*., 1998), using density-gradient gel electrophoresis coupled to most-probable-number counts, on the Fe(III)-reducing population of a hydrocarbon-contaminated aquifer indicated that another, as yet undescribed, class of DIRB was dominant in the pristine portion of the aquifer and equivalent in numbers to the *Geobacter* species in the contaminated Fe(III)-reducing zone of the aquifer. Here, we report on the first isolate of this new group of DIRB. This organism was isolated from the Fe(III)-reducing zone of a petroleum-contaminated aquifer. This organism has a physiology distinct from all other known DIRB and is a member of the recently recognized *Holophaga–Acidobacterium* phylum.

METHODS

Source of organism. Strain H-5^T was isolated from sediments that had been used to study the effects of nitrilotriacetic acid (NTA) on aromatic hydrocarbon degradation in petroleum-contaminated aquifers (Lovley *et al*., 1994b). The sediments had been collected from a petroleum-contaminated aquifer at the Defense Fuel Supply Center in Hanahan, SC, USA. The sediments were from site MW 20, which is within a zone in which Fe(III) reduction is the terminal electron-accepting process (Lovley *et al*., 1994a, b). In anoxic laboratory incubations, the sediments were amended with NTA (about 2 mmol NTA kg^{-1} sediment) and toluene (about 10 μM) as described previously (Lovley *et al*., 1994b). The sediments were refed with toluene when it was depleted. When sediments were well adapted for high rates of toluene 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 iron(III) NTA as the electron acceptor.

Table 1. Compounds tested as electron donors

Compounds were tested in the presence of iron(III) NTA (10 mM) at the concentrations given in parentheses.

Utilized	Not utilized
Acetate (10 mM)	Formate (10 mM)
Propionate (5 mM)	H_2 (101 kPa)
Palmitate (1·0 mM)	Methanol (10 mM)
Succinate (1·0 mM)	Ethanol (10 mM)
Fumarate (10 mM)	Phenol (0·5 mM)
Lactate (10 mM)	Toluene (1·0 mM)
Yeast extract (1 g l^{-1})	Benzene (1·0 mM)
	Hexadecane (1·0 mM)
	Benzoate (0·5 mM)
	Glucose (10 mM)
	Glycerol (10 mM)

Culturing on Fe(III). Standard anaerobic culturing techniques were used throughout (Hungate, 1969; Miller & Wolin, 1974; Balch *et al*., 1979). 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, capped with thick butyl-rubber stoppers and sterilized by autoclaving. The basal medium was the bicarbonate-buffered freshwater medium that had been used previously to isolate *Geobacter* species (Coates *et al*., 1996). Unless otherwise noted, acetate (10 mM) was the electron donor. Soluble Fe(III) (10 mM) was supplied as iron(III) citrate (Lovley & Phillips, 1988b), iron(III) pyrophosphate (Caccavo *et al*., 1994; Coates *et al*., 1996) or iron(III) NTA (Roden & Lovley, 1993). Poorly crystalline iron(III) oxide was prepared as described previously (Lovley & Phillips, 1986) and provided at 100 mmol l^{-1} medium.

Purified agar (2% w/v) was included to prepare agar plates, which 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 aluminium chambers similar to those described previously (Balch *et al*., 1979). All incubations were at 30 °C unless otherwise noted.

Alternative electron donors and acceptors. Alternative electron donors were added from sterile, anoxic stock solutions at concentrations listed in Table 1, as described previously (Coates *et al*., 1996). H_2 was added directly to the headspace of sealed vials by syringe to give a final partial pressure of 101 kPa. When noted, alternative electron acceptors to Fe(III) were added to basal medium from sterile, anoxic stock solutions in the form of sodium salts of nitrate, thiosulfate, sulfate, selenate, fumarate or malate at concentrations outlined in Table 1. Elemental sulfur was added in the form of polysulfide from a sterile, anoxic stock as

described previously (Coates *et al.*, 1998a). Basal medium containing the humic acid analogue 2,6 anthraquinone disulfonate (AQDS) (5 mM) as the sole electron acceptor was prepared as described previously (Coates *et al.*, 1998a) in order to evaluate the ability of strain H-5^T to couple the oxidation of carbon to the reduction of humic substances (Lovley *et al.*, 1996, 1998; Coates *et al.*, 1998a).

Cytochrome content. As a preliminary investigation of the cytochrome content of strain H-5^T, dithionite-reduced versus air-oxidized difference spectra were obtained on washed cell suspensions of lactate/fumarate-grown cells in bicarbonate buffer as described previously (Lovley *et al.*, 1993; Coates *et al.*, 1996).

Scanning electron microscopy. Cells of strain H-5^T for scanning electron microscopy were grown on lactate/fumarate medium and filtered onto 0·2 μm pore-size filters. The samples were fixed for 2 h in 2% (v/v) glutaraldehyde and washed three times, for 20 min per wash, in 0·1 M sodium cacodylate. The fixed samples were successively dehydrated with ethanol and stored overnight at 4 °C in 100% ethanol. These samples were dried by critical-point drying, coated with gold/palladium and examined with a Cambridge 250 MK3 scanning electron microscope at 21 kV.

16S rRNA gene sequencing and analysis. Nucleic acids were isolated from a cell pellet of strain H-5^T and the nearly complete 16S rDNA was amplified and purified, as described previously (Lonergan *et al.*, 1996). Sequencing of both strands of the 16S rDNA was performed at the Michigan State University Sequencing Facility using eubacterial 16S rDNA sequencing primers on a 373A DNA sequencing system (Applied Biosystems).

The H-5^T 16S rDNA and other sequences used in the phylogenetic analysis were aligned manually to sequences obtained from the Ribosomal Database Project (Maidak *et al.*, 1997). Evolutionary distances, computed as described previously (Jukes & Cantor, 1969), were used to construct a distance tree by the De Soete least-squares algorithm (De Soete, 1983).

The GenBank and EMBL accession numbers for sequences used in the phylogenetic analysis were: *Acidobacterium capsulatum*, D26171; *Aeromonas hydrophila*, M59148; *Clostridium pasteurianum*, M23930; *Deferribacter thermophilus*, U75602; '*Desulfuromonas palmitatis*', U28172; *Desulfuromusa bakii*, X79412; *Ferrimonas balearica*, X93021; *Geobacter metallireducens*, L07834; '*Geovibrio ferrireducens*', X95744; *Holophaga foetida*, X77215; *Pelobacter carbinolicus*, U23141; *Shewanella alga*, X81622; *Shewanella putrefaciens*, X81623; strain SES-3, U41564; *Thermoterrabacterium ferrireducens*, U76363; and *Wolinella succinogenes*, M88159.

Analytical techniques. As described previously, Fe(III) reduction was monitored spectrophotometrically by ferrozine assay of HCl-extractable Fe(II) (Lovley & Phillips, 1986) or soluble Fe(II) (Lovley & Phillips, 1988a). Direct cell counts were done by epifluorescent microscopy of acridine-orange-stained samples (Hobbie *et al.*, 1977). Growth of cultures on soluble electron acceptors was monitored by inserting the culture tubes directly into a Spectronic 20 spectrophotometer (Spectronic Instruments) and measuring optical density at 600 nm. Sulfide was analysed colorimetrically with the methylene blue method as described previously (Cline, 1969). Concentrations of organic acids in culture broths were determined by HPLC as described previously (Lovley & Phillips, 1989) with UV detection at 210 nm. The concentration of the reduced product of AQDS, 2,6-anthrahydroquinone disulfonate, was determined spectrophotometrically at 450 nm as described previously (Lovley *et al.*, 1996).

RESULTS

Enrichment and isolation

After three successive transfers (10% inoculum) of the acetate-oxidizing, Fe(III)-reducing enrichment culture, the third transfer was streaked onto agar plates of acetate/iron(III) pyrophosphate medium (Caccavo *et al.*, 1994; Coates *et al.*, 1996). Fe(III)-reducing colonies were recognized easily, as their growth resulted in clearing zones around the colonies in the green-coloured iron(III) pyrophosphate medium

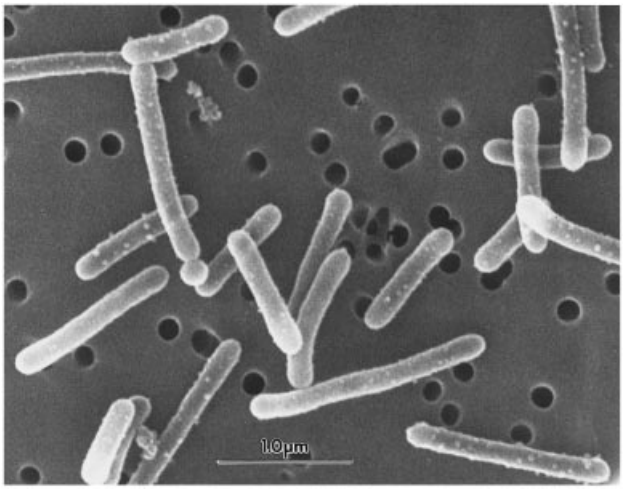

Fig. 1. Scanning electron micrograph of cells of strain H-5^T. Bar, 1 μm.

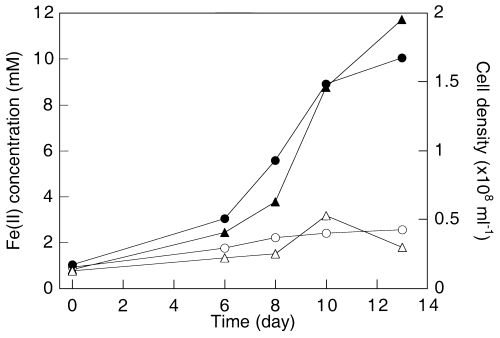

Fig. 2. Growth and Fe(III) reduction by strain H-5^T with palmitate as the electron donor. (●) Fe(II) formed while growing on 0·5 mM palmitate; (○) Fe(II) formed in the absence of palmitate; (▲) increase in cell density while growing on 0·5 mM palmitate; (△) increase in cell density in the absence of palmitate. Data presented are representative of triplicate determinations.

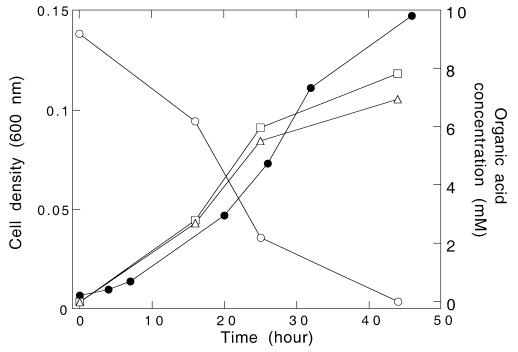

Fig. 3. Growth, citrate removal and acetate and succinate production by strain H-5^T during fermentation of citrate. (●) Optical density at 600 nm; (○) citrate concentration during growth; (□) acetate production during fermentation of citrate; (△) succinate production during fermentation of citrate. Data presented are representative of triplicate determinations.

(Coates *et al.*, 1996). However, growth of fermentative micro-organisms is also stimulated on iron(III) pyrophosphate because of the organic compounds included by the manufacturer as chelating agents (Coates *et al.*, 1996). Individual Fe(III)-reducing colonies that were small (about 0·5 mm diameter) were picked and transferred to fresh basal medium with acetate and iron(III) pyrophosphate as the electron donor and acceptor, respectively. Fully grown cultures of these picked colonies were restreaked onto agar plates of acetate/iron(III) pyrophosphate medium, from which several morphologically identical isolates were obtained. One of these isolates, strain H-5^T, was selected for further characterization.

Cell and colony morphology

Cells of strain H-5^T were non-motile, non-spore-forming rods, 1–2 × 0·1 μm (Fig. 1). Flagella were not detected. Colonies grown on iron(III) pyrophosphate medium were typically less than 1 mm in diameter. Colonies were white and domed and appeared to be coated with an Fe(II) mineral, presumably vivianite [$Fe_3(PO_4)_2.8H_2O$], similar to that observed for *Geobacter* species grown on the same medium (Coates *et al.*, 1996). When growing on fumarate medium, the colonies were red, domed, entire, smooth and wet, which is also similar to colonies of *Geobacter* species grown on the same medium (J. D. Coates, unpublished). In liquid medium with Fe(III), strain H-5^T appeared as single cells or short chains of two to three cells each. In liquid medium with fumarate as the sole electron acceptor, strain H-5^T grew in long, intertwined, hair-like chains of several hundred cells.

Electron donors and acceptors and growth conditions

In addition to acetate, strain H-5^T grew with a variety of organic acids including propionate, lactate, succinate and fumarate as alternative electron donors with Fe(III) as the sole electron acceptor (Table 1). Strain H-5^T also coupled growth to the oxidation of palmitate with Fe(III) serving as the sole electron acceptor (Fig. 2). No organic acids were detected when

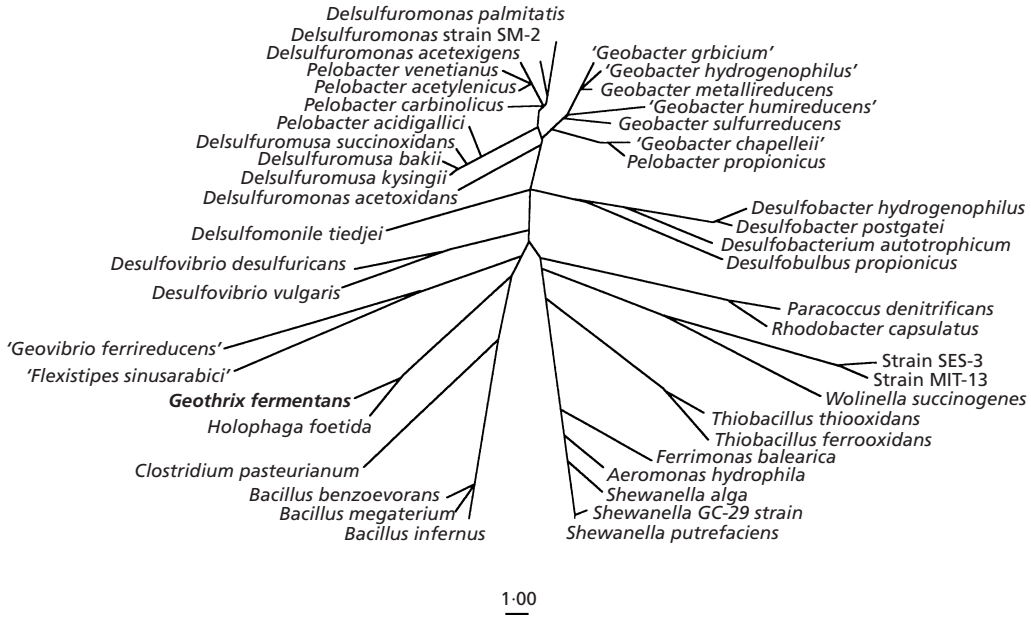

Fig. 4. Phylogenetic tree inferred from 16S rRNA sequences showing the placement of *Geothrix fermentans* strain H-5^T. The tree was inferred by the distance-matrix method and 1125 positions were considered. *Clostridium pasteurianum* is the outgroup. The bar represents one evolutionary distance unit.

culture broths of strain H-5^T grown on palmitate/Fe(III) medium were analysed by HPLC, suggesting that palmitate was completely mineralized to CO_2. Yeast extract also served as an electron donor for Fe(III) reduction by strain H-5^T. A variety of other potential electron donors were not utilized (Table 1).

In addition to iron(III) pyrophosphate, strain H-5^T could grow with other forms of Fe(III) including iron(III) NTA, iron(III) citrate and poorly crystalline iron(III) oxide. Other electron acceptors used by strain H-5^T with lactate as the electron donor included NO_3, Mn(IV), the humic acid analogue AQDS and fumarate. No growth or reduction was observed with elemental sulfur or any of the sulfur anions tested (Table 1). Similarly, strain H-5^T did not grow aerobically, even under low partial pressures of O_2 (100 Pa).

In addition to anaerobic respiration, strain H-5^T could also grow fermentatively on organic acids such as citrate or fumarate. Acetate and succinate were the primary end-products of citrate fermentation (Fig. 3).

Strain H-5^T grew optimally between 35 and 40 °C (data not shown); no growth was observed at 25 °C or lower.

Cytochrome content

Oxidized versus reduced spectra of washed whole-cell suspensions of strain H-5^T that had been grown in lactate/fumarate medium indicated absorbance maxima at 552, 524 and 423 nm, which are indicative of a *c*-type cytochrome(s).

Phylogeny of strain H-5^T

Phylogenetic analysis of the almost complete 16S rDNA sequence of strain H-5^T indicated that it belongs to a distinct, novel line of descent within the domain *Bacteria*, and places it within the recently described *Holophaga–Acidobacterium* phylum (Ludwig *et al*., 1997) (Fig. 4). The closest known cultured relative of strain H-5^T, *Holophaga foetida*, has 94·3 % sequence identity to strain H-5^T over 1432 unambiguously aligned positions (Fig. 4).

DISCUSSION

Strain H-5^T is phylogenetically unique among micro-organisms that have the ability to conserve energy for growth from the reduction of Fe(III). It is only the second dissimilatory Fe(III)-reducing micro-organism that does not fall within the family *Geobacteraceae* and has been shown to oxidize multicarbon organic compounds completely to CO_2. The only other complete oxidizer described outside the *Geobacteraceae* is '*Geovibrio ferrireducens*', which, along with '*Flexistippes sinusarabici*', is part of novel lineage within the *Bacteria* (Caccavo *et al*., 1996).

Strain H-5^T and closely related organisms represent a recently recognized phylum among the known major bacterial lines of descent (Ludwig *et al*., 1997). Numerous 16S rDNA sequences recovered from a variety of soils fall within this lineage, but to date the anaerobic acetogen *Holophaga foetida* (Bak *et al*., 1992; Liesack & Finster, 1994) and the aerobic *Acidobacterium capsulatum* (Hiraishi *et al*., 1995) are the only organisms other than strain H-5^T that are available in culture. Attempts to grow *Holophaga foetida* and *Acidobacterium capsulatum* with Fe(III) as the electron acceptor have not been successful.

The phylogenetic and physiological differences between the dissimilatory Fe(III) reducer strain H-5^T and the acetogen *Holophaga foetida* support the placement of strain H-5^T in a new genus and species within the *Holophaga–Acidobacterium* phylum. The name *Geothrix fermentans* gen. nov., sp. nov. is proposed.

Comparison with other DIRB

The ability of strain H-5^T to oxidize simple organic acids completely to CO_2 with Fe(III) serving as the sole electron acceptor distinguishes it from a variety of other mesophilic Fe(III) reducers, such as species of *Shewanella*, *Ferrimonas*, *Aeromonas* and *Bacillus* (Lovley *et al*., 1997), that oxidize organic compounds only incompletely to acetate. Strain H-5^T is similar to species of *Geobacter* (Lovley *et al*., 1993; Caccavo *et al*., 1994; Coates *et al*., 1996, 1998a), *Desulfuromonas* (Roden & Lovley, 1993; Coates *et al*., 1995) and *Desulfuromusa* (Liesack *et al*., 1994) of the *Geobacteraceae*, as well as '*Geovibrio ferrireducens*' (Caccavo *et al*., 1996), in its ability to conserve energy for growth by the oxidation of acetate with the reduction of Fe(III). Strain H-5^T can also conserve energy for growth by the oxidation of palmitate, which is similar to '*Desulfuromonas palmitatis*' (Coates *et al*., 1995) but contrasts with all other known Fe(III) reducers (Lovley *et al*., 1997). Strain H-5^T is the first freshwater example of a micro-organism that can grow by the oxidation of a long-chain fatty acid with Fe(III) as the electron acceptor.

In contrast to many dissimilatory Fe(III) reducers, including *Geobacter sulfurreducens* (Caccavo *et al*., 1994), *Desulfuromonas* species (Pfennig & Biebl, 1976; Coates *et al*., 1995), *Desulfuromusa* species (Liesack *et al*., 1994), *Shewanella putrefaciens* (Moser & Nealson, 1996) and many of the hyperthermophilic Fe(III) reducers (Vargas *et al*., 1998), strain H-5^T did not couple growth to the reduction of elemental sulfur. However, other DIRB such as *Geobacter metallireducens* (Lovley *et al*., 1993), '*Geobacter hydrogenophilus*' and '*Geobacter chapelleii*' (Coates *et al*., 1996) also couple acetate oxidation to the reduction of elemental sulfur, but do not grow well in media with acetate as the sole electron donor and elemental sulfur as the sole electron acceptor.

Similarly to the *Desulfuromusa* species (Finster & Bak, 1993; Liesack *et al*., 1994), strain H-5^T can also grow

by the fermentation of simple organic acids such as citrate or fumarate in the absence of a suitable electron acceptor. Although it has been reported that *Desulfuromonas* species can similarly grow by fermentation of fumarate or malate, this metabolism will only occur at low bicarbonate/CO_2 concentrations (Widdel, 1988).

Like most known mesophilic DIRB, strain H-5^T contains a *c*-type cytochrome(s). Previous studies have indicated the involvement of *c*-type cytochromes in the transfer of electrons to Fe(III) in *Geobacter*, *Desulfuromonas* and *Shewanella* species (Lovley *et al*., 1997, and references therein). However, the discovery that *Pelobacter* species, which do not contain cytochromes, can grow by the reduction of Fe(III) (Lovley *et al*., 1995) indicates that the presence of *c*-type cytochromes is not necessary for dissimilatory Fe(III) reduction.

Environmental and evolutionary significance

Although strain H-5^T was recovered from aquifer sediments in which Fe(III) reduction was stimulated with the addition of toluene and NTA, dissimilatory Fe(III)-reducing organisms closely related to H-5^T have been recovered from freshly collected sediments from the Fe(III)-reduction zone of another petroleum-contaminated aquifer (Anderson *et al*., 1998). In studies in which acetate-oxidizing DIRB were enumerated via culturing, organisms closely related to strain H-5^T were as numerous in the Fe(III)-reducing zone of the aquifer as *Geobacter* species (Anderson *et al*., 1998). Furthermore, in a nearby uncontaminated portion of the aquifer, organisms closely related to H-5^T were the dominant acetate-oxidizing Fe(III) reducers recovered. These results contrast with previous studies on aquatic sediments and aquifers (Coates *et al*., 1996), which suggested that organisms in the *Geobacteraceae* are the most common acetate-oxidizing Fe(III) reducers that can be recovered in culture. Further studies that use molecular approaches that avoid culture bias will be necessary in order to determine the true relative distribution of *Geobacteraceae* and organisms related to strain H-5^T in a variety of sedimentary environments.

Geological evidence, as well as the fact that a variety of hyperthermophilic *Archaea* and *Bacteria* have the ability to reduce Fe(III), suggests that Fe(III) reduction was one of the earliest forms of microbial respiration (Caccavo *et al*., 1996; Vargas *et al*., 1998). If so, it might be expected that the capacity for Fe(III) reduction would be widely distributed throughout the *Bacteria*. Although initial studies on DIRB indicated that the micro-organisms that could conserve energy to support growth from Fe(III) reduction were all *Proteobacteria* (Lovley *et al*., 1997), continued study of the diversity of dissimilatory Fe(III) reducers has now indicated that the capacity for Fe(III) reduction is in fact widespread among phylogenetically diverse organisms (Lovley *et al*., 1989b, 1997; Rossello-Mora *et al*., 1994; Boone *et al*., 1995; Caccavo *et al*., 1996; Greene *et al*., 1997; Slobodkin *et al*., 1997; Coates *et al*., 1998b). The discovery of dissimilatory Fe(III) reducers in the new *Holophaga–Acidobacterium* phylum continues this trend.

Description of *Geothrix* gen. nov.

Geothrix (Ge′o.thrix. Gr. n. *gea* Earth; Gr. fem. n. *thrix* hair; M.L. fem. n. *Geothrix* hair-like cell from the earth).

Rod-shaped cells, 1–2 × 0·1 μm, non-motile, non-spore-forming. Cells occur singly or in chains. Strictly anaerobic chemo-organotroph that oxidizes acetate with Fe(III) serving as the sole electron acceptor. Cells grown with Fe(III) or fumarate as an electron acceptor contain a *c*-type cytochrome(s).

Description of *Geothrix fermentans* sp. nov.

Geothrix fermentans (fer.men′tans. L. part. adj. *fermentans* fermenting).

Geothrix fermentans can use Mn(IV), nitrate, 2,6-anthraquinone disulfonate or fumarate as alternative electron acceptors. Propionate, palmitate, lactate, fumarate or succinate serve as alternative electron donors with Fe(III) as the electron acceptor. In the absence of a suitable electron acceptor, growth is possible by fermentation of citrate or fumarate. Acetate and succinate are the primary end-products of citrate fermentation. Optimum growth temperature is 35 °C. The type strain of *Geothrix fermentans*, strain H-5^T, has been deposited in the American Type Culture Collection as ATCC 700665^T. *Geothrix fermentans* strain H-5^T was isolated from sediment samples from a petroleum-contaminated aquifer at Hanahan, SC, USA, that had been enriched for anaerobic toluene oxidation.

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