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Desulfuromonas palmitatis sp. nov., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids

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Abstract Studies on the microorganisms living in hydrocarbon-contaminated sediments in San Diego Bay, California led to the isolation of a novel Fe(III)-reducing microorganism. This organism, designated strain SDBY1, was an obligately anaerobic, non-motile, non-flagellated, gram-negative rod. Strain SDBY1 conserves energy to support growth from the oxidation of acetate, lactate, succinate, fumarate, laurate, palmitate, or stearate. H_2 was also oxidized with the reduction of Fe(III), but growth with H_2 as the sole electron donor was not observed. In addition to various forms of soluble and insoluble Fe(III), strain SDBY1 also coupled growth to the reduction of fumarate, Mn(IV), or S^0. Air-oxidized *minus* dithionite-reduced difference spectra exhibited peaks at 552.8, 523.6, and 422.8 nm, indicative of *c*-type cytochrome(s). Strain SDBY1 shares physiological characteristics with organisms in the genera *Geobacter, Pelobacter*, and *Desulfuromonas*. Detailed analysis of the 16S rRNA sequence indicated that strain SDBY1 should be placed in the genus *Desulfuromonas*. The new species name *Desulfuromonas palmitatis* is proposed. *D. palmitatis* is only the second marine organism found (after *D. acetoxidans*) to oxidize multicarbon organic compounds completely to carbon dioxide with Fe(III) as an electron acceptor and provides the first pure culture model for the oxidation of long-chain fatty acids coupled to Fe(III) reduction.

Key words *Desulfuromonas* · Fe(III) reduction · Sulfur reduction · Marine sediments · Palmitate

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Introduction

Fe(III) and Mn(IV) reduction are important and are sometimes the predominant processes for the decomposition of naturally occurring organic matter in a variety of marine and estuarine sediments (Sørensen 1982; Aller et al. 1986; Lovley and Phillips 1986b; Canfield 1989; Aller 1990; Hines et al. 1991; Canfield et al. 1993a, b). For example, in detailed studies on the anaerobic processes at three sites off the coast of Denmark, Fe(III) and Mn(IV) reduction were found to account for 21–100% of the anaerobic decomposition in the upper 10 cm (Canfield et al. 1993a). Even when oxygen respiration was considered in a subsequent study, Fe(III) and Mn(IV) reduction accounted for as much as 30–90% of the organic matter decomposition at these sites (Canfield et al. 1993b). Most of the organic matter oxidation coupled to Fe(III) and Mn(IV) reduction in marine sediments and other sedimentary environments can be attributed to the activity of dissimilatory Fe(III)- and Mn(IV)-reducing microorganisms that are capable of coupling the oxidation of organic compounds to carbon dioxide with Fe(III) or Mn(IV) serving as the sole electron acceptor (Lovley 1991, 1994).

In addition to playing a role in the diagenesis of naturally occurring organic matter, there is also the potential that Fe(III) reducers may be involved in the degradation of organic contaminants in marine sediments. Fe(III) reduction is an important process for the removal of organic contaminants from polluted aquifers (Lovley et al. 1989; Lyngkilde and Christensen 1992; Baedecker et al. 1993), and degradation of organic contaminants coupled to Fe(III) reduction has been demonstrated in pure cultures and enrichments (Lovley et al. 1989, 1994; Lovley and Lonergan 1990; Lonergan and Lovley 1991). Microbial Fe(III) reduction may also be involved in other processes in marine sediments, such as release of trace metals bound to the natural Fe(III) oxides and magnetization of the sediments (Lovley 1991).

The organisms responsible for Fe(III) and Mn(IV) reduction in marine sediments have yet to be elucidated.

None of the marine organisms available in pure culture that are known to be Fe(III) reducers were isolated directly with Fe(III) as the sole electron acceptor. Most are H_2-oxidizing Fe(III) reducers. These include *Shewanella alga* (formerly strain BrY; Caccavo et al. 1992), several *Desulfovibrio* species (Coleman et al. 1993; Lovley et al. 1993b), and *Pelobacter carbinolicus* (Lovley et al. 1995b). In addition to H_2, these organisms can also metabolize some multicarbon organic compounds, such as lactate (*S. alga*, *D. desulfuricans*) or ethanol (*P. carbinolicus*). However, these substrates are incompletely oxidized to acetate.

Organisms that can completely oxidize organic acids to carbon dioxide with Fe(III) serving as the sole electron acceptor are likely to be responsible for most of the organic matter oxidation coupled to Fe(III) reduction in sedimentary environments (Lovley 1991, 1993, 1994). One reason for this is that short-chain fatty acids, most notably acetate, are important intermediates in the metabolism of fermentable compounds in Fe(III)-reducing environments (Lovley and Phillips 1989). The only marine organism that has been found to oxidize multicarbon compounds completely to carbon dioxide with Fe(III) or Mn(IV) as the electron acceptor is *Desulfuromonas acetoxidans* (Roden and Lovley 1993). This organism was originally isolated as an acetate-oxidizing sulfur reducer. Its ability to reduce Fe(III) was only evaluated after it was recognized that it was closely related to the freshwater organism, *Geobacter metallireducens*, which also grows by acetate oxidation coupled to Fe(III) reduction (Lovley et al. 1987, 1993a; Lovley and Phillips 1988).

In addition to short-chain fatty acids, which can be important fermentation products, long-chain fatty acids are also a source of organic carbon in anoxic environments (Mackie et al. 1991). Studies with enrichment cultures have suggested that long-chain fatty acids can be oxidized with the reduction of Fe(III) (Lovley 1991), but a pure culture that can serve as a model for this metabolism has not previously been available.

As part of a study on the role of Fe(III) reduction in the decomposition of petroleum contaminants in sediments of San Diego Bay (Lovley et al. 1995a), Fe(III)-reducing microorganisms were isolated from the sediments using a solid medium in which Fe(III) was provided as the sole electron acceptor. Here we report on one of these isolates, which is unique in being the first organism found to oxidize long-chain fatty acids with the reduction of Fe(III).

Materials and methods

Source of sediments and organisms

Sediments were collected in San Diego Bay, Calif. near Palleta Creek with a gravity corer as described previously (Lovley et al. 1995a). The sediment was extruded from the core on shipboard, and the top 2.5 cm from three cores were combined and stored under N_2 in a canning jar. In the laboratory, 1.0-g aliquots were inoculated into 10 ml of the acetate/Fe(III) oxide medium described below.

Desulfuromonas acetexigens (DSM 1397) was purchased from the German Collection of Microorganisms (DSM; Braunschweig, Germany).

Media and culturing techniques

Standard anaerobic culturing techniques were used throughout (Hungate 1969; Miller and Wolin 1974). All incubations were in anaerobic pressure tubes or serum bottles capped with thick butyl rubber stoppers. Anoxic medium was prepared by boiling under N_2-CO_2 (80:20, v/v) to remove dissolved O_2 and dispensed under N_2-CO_2 into anaerobic pressure tubes or serum bottles that were then capped with thick butyl rubber stoppers.

For the initial enrichment and isolation, the previously described defined freshwater acetate/Fe(III) oxide medium (Lovley and Phillips 1988) was modified to marine salinities by adding NaCl (18 g/l) and $MgCl_2 \cdot 6H_2O$ (4.24 g/l) from sterile anoxic stocks solutions. The incubation temperature was 25° C.

In order to obtain isolated colonies from the enrichment, dilutions of the enrichment were mixed in a Coy (Ann Arbor, Michigan) anaerobic chamber with the same medium containing molten agar (held at 50 ° C) that contained acetate (10 mM) as the electron donor and Fe(III) pyrophosphate (25 mM) as the electron acceptor. After gentle mixing, the contents of the tubes were poured into petri dishes and left to solidify for 15 min. The agar plates were incubated under a positive pressure (101 kPa) of N_2 CO_2 (80:20, v/v) in sealed chambers similar to those described previously (Balch et al. 1979). Incubation of plates and subsequent incubations of the isolate were at 30° C.

For characterization of the organism, APW medium was used. This medium was designed based on an analysis of the composition of the San Diego Bay water by emission spectroscopy (DCP) and ion chromatography. The basal APW medium contained (g/l): NaCl, 20; KCl, 0.67; $NaHCO_3$, 2.5; NH_4Cl, 0.1; KH_2PO_4, 0.01; $MgSO_4 \cdot 7H_2O$, 0.02; resazurin, 0.001, and vitamin and mineral mixtures (Lovley and Phillips 1988). After autoclaving, the APW medium was amended from sterile anoxic stocks with 50 ml/l of salt solution (which contained $MgCl_2 \cdot 6H_2O$ (212 g/l) and $CaCl_2 \cdot 2H_2O$ (30.4 g/l)), and 10 ml/l of a $FeCl_2 \cdot 4H_2O$ stock solution (51.7 g/l) as a reductant.

Electron donors were added from sterile anoxic stocks. Hydrophobic hydrocarbon electron donors (toluene, benzene, cyclohexane, eicosane, hexadecane, phenanthrene) were prepared in degassed mineral oil at 2% (v/v) as outlined previously (Rabus et al. 1993). Hydrophobic long-chain fatty acids (0.1 ml) were added to clean, dry medium tubes from 100 mM stocks prepared in ethanol. The ethanol was evaporated under a constant stream of N_2/CO_2 for 20 min prior to the addition of 9 ml of prepared anoxic APW medium. H_2 was added at 100 kPa.

Soluble Fe(III) (50 mM) was supplied as Fe(III) citrate (Lovley and Phillips 1988), Fe(III) pyrophosphate (Caccavo et al. 1994), or Fe(III) nitrilotriacetic acid (NTA) (Roden and Lovley 1993). Poorly crystalline Fe(III) oxide (100 mM) was prepared as described previously (Lovley and Phillips 1986a). In order to evaluate the potential for Mn(IV) reduction, synthetic MnO_2 (Lovley and Phillips 1988) was added to basal medium to provide 10–30 mmol of Mn(IV) per liter. When noted, alternative electron acceptors in the form of sodium salts of nitrate, thiosulfate, sulfite, sulfate, fumarate, malate, selenate, or selenite were added to the basal medium from anoxic sterile stocks to give a final concentration of 10 mM. Collodial S^0, prepared as described previously (Blumentals et al. 1990), was provided at 10 mM.

D. acetexigens was grown in the medium suggested for culturing this organism by the DSM (DSM medium no. 148) with fumarate as the electron acceptor. To determine the ability to grow by Fe(III) reduction, the fumarate was replaced with poorly crystalline Fe(III) oxide (100 mM).

Analytical techniques

Concentrations of HCl-extractable Fe(II) were determined by the ferrozine assay (Lovley and Phillips 1988), cell numbers were counted using epifluorescent microscopy (Hobbie et al. 1977), and sulfide was determined with the methylene blue assay (Cline 1969). Growth of cultures on soluble electron acceptors was mon-

itored by directly inserting the culture tubes into a Spectronic 20 spectrophotometer and measuring the OD_{600}. Short-chain fatty acids were analyzed by HPLC (Dionex 4000i) using an HPICE-AS1 column and a conductivity detector. The eluent was 2% 2-propanol in 1 mM octane sulfonic acid at a flow rate of 0.8 ml min^{-1}. The regenerant was 5 mM tetrabutylammonium hydroxide at a flow rate of 2 ml min^{-1}. Scanning electron photomicrographs were prepared of cells of strain SDBY1 grown on acetate and Fe(III) citrate. Cells were 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.

Cytochromes

Dithionite-reduced *minus* air-oxidized difference spectra were obtained with washed cell suspensions of acetate/fumarate-grown cells in bicarbonate buffer as described previously (Lovley et al. 1993a), amended with NaCl (20 g/l) and $MgCl_2 \cdot 6H_20$ (4.24 g/l). The ability of Fe(III) citrate to oxidize *c*-type cytochrome(s) in whole-cell suspensions that were reduced by the addition of H_2 (Lovley et al. 1993a) was also evaluated.

16S rRNA gene sequencing and phylogenetic analysis

DNA was isolated, amplified, and sequenced as described previously (Lovley et al. 1995b). The partial 16S rRNA gene of strain SDBY1 was amplified using the eubacterial primer 8F and the universal primer 1492R (Eden et al. 1991; Weisburg et al. 1991), and the partial 16S rRNA gene of *D. acetexigens* was amplified using the primer 50F (Lane et al. 1988) and the universal primer 1492R (Eden et al. 1991; Weisburg et al. 1991). Sequencing was performed at the Michigan State University Sequencing Facility (East Lansing, Mich., USA). Sequences were obtained for both strands of each partial 16S rRNA sequence.

The nearly complete 16S rRNA sequences of strain SDBY1 and *D. acetexigens* were aligned manually against aligned sequences of closely related bacteria obtained from the Ribosomal Database Project (Larson et al. 1993). A distance tree was constructed using the least-squares algorithm (De Soete 1983) with evolutionary distances computed by the method described by Jukes and Cantor (1969).

The Genbank and EMBL accession numbers for sequences used in the phylogenetic analysis are as follows: *Clostridium pasterianum*, M23930; *Aeromonas hydrophila*, X74676; *Desulfovibrio desulfuricans*, M34113; *Desulfovibrio vulgaris*, M34399; *Geobacter hydrogenophilus*, U28173; *Geobacter metallireducens*, L07834; *Geobacter sulfurreducens*, U13928; *Desulfuromonas acetoxidans*, M26634; *Pelobacter carbinolicus*, U23141; and *Pelobacter acetylenicus*, X70955. The sequences of *Magnetospirillum magnetotacticum* (Mag.magne2) and *Magnetospirillum gryphiwandense* strain MSR-1 (Mag.gryphi) were obtained from the Ribosomal Database Project (Larson et al. 1993). The aligned sequence of *Shewanella putrefaciens* was kindly provided by E. Delong and D. Franks (University of California-Santa Barbara, USA).

Results and discussion

Enrichment and isolation

Within 18 days, the initial acetate enrichment had reduced all of the poorly crystalline Fe(III) oxide. The enrichment was transferred two more times (10% inoculum) and then diluted to extinction in the acetate/Fe(III) oxide medium. The final dilution in which Fe(III) was reduced was plated on acetate/Fe(III)-pyrophosphate agar dilution plates. After 1 month, isolated colonies were picked and inoculated into liquid acetate/Fe(III)-pyrophosphate medium. Positive tubes were restreaked onto acetate/Fe(III)-pyrophosphate agar plates. Strain SDBY1 was isolated from one of these plates.

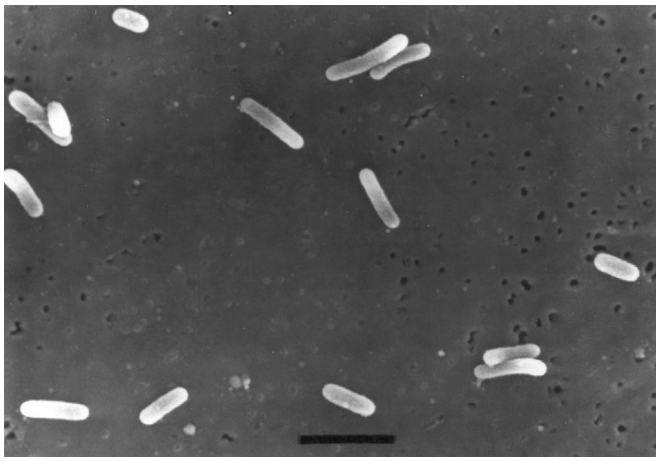

Fig. 1 Scanning electron micrograph of cells of strain SDBY1. *Bar* 2 μm

Cell and colony morphology

Cells of strain SDBY1 were gram-negative, non-motile, non-sporeforming, non-flagellated rods (Fig. 1), 0.3 μm × 1-2 μm. Colonies grown on acetate/Fe(III)-pyrophosphate agar medium were white, domed, and typically less than 1 mm in diameter. When grown on agar medium with acetate as the electron donor and Fe(III) citrate or fumarate as the electron acceptor, the colonies were red, domed, smooth, and wet. These colony characteristics are similar to those observed previously for *Geobacter metallireducens* (Lovley et al. 1993a) and *Geobacter hydrogenophilus* (J. D. Coates, unpublished) during growth on similar medium.

Electron donors and acceptors

Strain SDBY1 grew in defined medium with acetate as the sole electron donor and Fe(III) as the electron acceptor (Fig. 2). Strain SDBY1 also reduced Fe(III) with H_2 (Fig. 3), but did not grow in H_2/Fe(III) medium even when carbon sources such as casamino acids or yeast extract were provided. Strain SDBY1 grew with lactate, succinate, or fumarate as the sole electron donor and Fe(III) as the sole electron acceptor (Table 1). This pattern of electron donors that support growth on Fe(III) is unlike that of any previously described for a dissimilatory Fe(III) reducer. A wide variety of other electron donors was not used (Table 1).

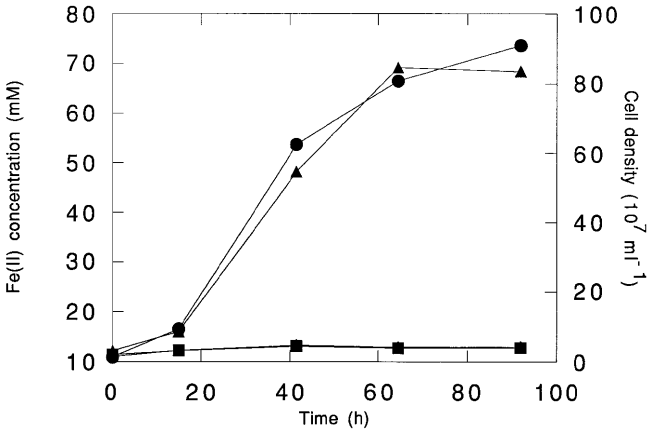

Fig. 2 Growth of strain SDBY1 with Fe(III) citrate (100 mM) as electron acceptor and acetate (10 mM) as electron donor. *Filled circles* Fe(II) production, 10 mM acetate; *filled diamonds* Fe(II) production, no acetate; *filled triangles* cell density, 10 mM acetate; *filled squares* cell density, no acetate. Results depicted are of one representative of triplicate cultures

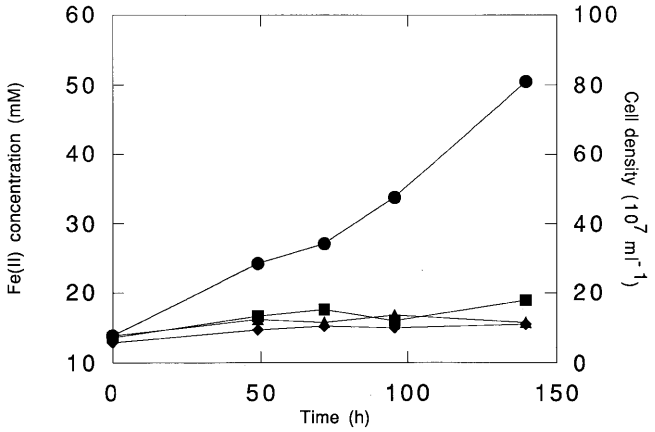

Fig. 3 Fe(III) reduction with H_2 as the electron donor in medium amended with yeast extract (0.2 g l^{-1}) as a carbon source. *Filled circles* Fe(II) production, 101kPa H_2; filled diamonds Fe(II) production, no H_2; *filled triangles* cell density, 101kPa H_2; *filled squares* cell density, no H_2. Results shown are of one representative of triplicate cultures

In contrast to all previously isolated Fe(III)-reducing organisms, strain SDBY1 conserved energy to support growth, coupling the oxidation of palmitate to the reduction of Fe(III) (Fig. 4). There was no accumulation of short-chain fatty acids in the medium during growth on palmitate, suggesting that palmitate was directly oxidized to carbon dioxide. When strain SDBY1 was grown on Fe(III) citrate with 0.25 mM palmitate, there was a net production of 19.21 ± 2.86 mM Fe(II) (mean ± SD, $n = 3$), giving a stoichiometry of Fe(III) reduced per mole of palmitate oxidized of 76.4 which suggests that strain SDBY1 oxidizes palmitate according to

$$C_{16}H_{32}O_2 + 92[Fe^{3+}] + 30H_2O \rightarrow 16CO_2 + 92[Fe^{2+}] + 92H^+$$

Strain SDBY1 also used both laurate and stearate, but did not use caprylate (data not shown).

Table 1 Compounds tested as electron donors in the presence of Fe(III) citrate (50 mM) or tested as electron acceptors in the presence of acetate (10 mM). Concentrations (mM) of the respective electron donors/acceptors are given in parentheses

Electron donors

Utilized
Acetate (10), lactate (10), laurate (1), palmitate (1), stearate (1), fumarate (10), succinate (10), hydrogen (101 kPa)

Tested, but not utilized
Formate (10), propionate (10), butyrate (10), caprylate (1), ethanol (10), methanol (10), benzoate (5), phenol (0.5), toluene (1), benzene (1), cyclohexane (1), octane (1), eicosane (0.35), catechol (1), phenanthrene (0.6), glucose (10), citrate (10), pyruvate (10), nitriloacetic acid (4), methane (101 kPa), casamino acids (1 g/l), yeast extract (1 g/l)

Electron acceptors

Utilized
Fe(III) citrate (50), Fe(III) NTA (10), Fe(III) pyrophosphate (10), poorly crystalline Fe(III) oxide (100), MnO_2 (20), fumarate (50), S^0 (10).

Tested, but not utilized
Nitrate (10), sulfate (10), sulfite (10), thiosulfate (10), selenate (10), selenite (10), malate (10)

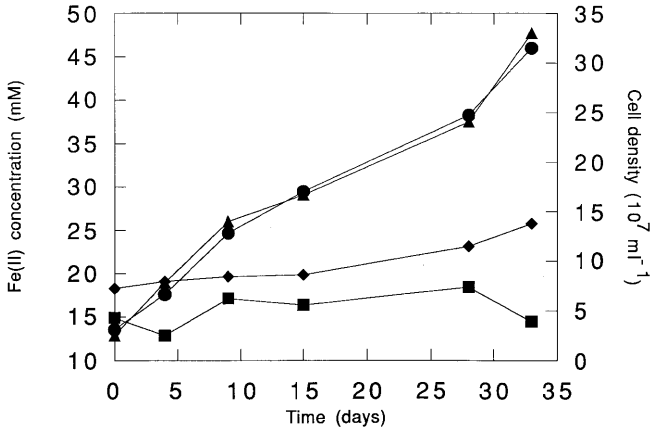

Fig. 4 Growth of strain SDBY1 with Fe(III) citrate (50 mM) and palmitate (1 mM) as electron acceptor and donor, respectively. *Filled circles* Fe(II) production, 1 mM palmitate; *filled diamonds* Fe(II) production, no palmitate; *filled triangles* cell density, 1 mM palmitate; *filled squares* cell density, no palmitate. Results are of one representative of triplicate cultures

In addition to both the soluble and insoluble forms of Fe(III) tested (Table 1), strain SDBY1 also grew with Mn(IV), fumarate, (data not shown) or elemental sulfur as electron acceptors. When growing with S^o as the electron acceptor and acetate as an electron donor, strain SDBY1 produced an excess of 6 mM sulfide in 30 h (negligiable sulfide production in the control), which is comparable to *Desulfuromonas acetoxidans* (Pfennig and Biebl 1976) and three times faster than *Geobacter sulfurreducens* (Caccavo et al. 1994). A wide variety of other electron acceptors tested was not used (Table 1).

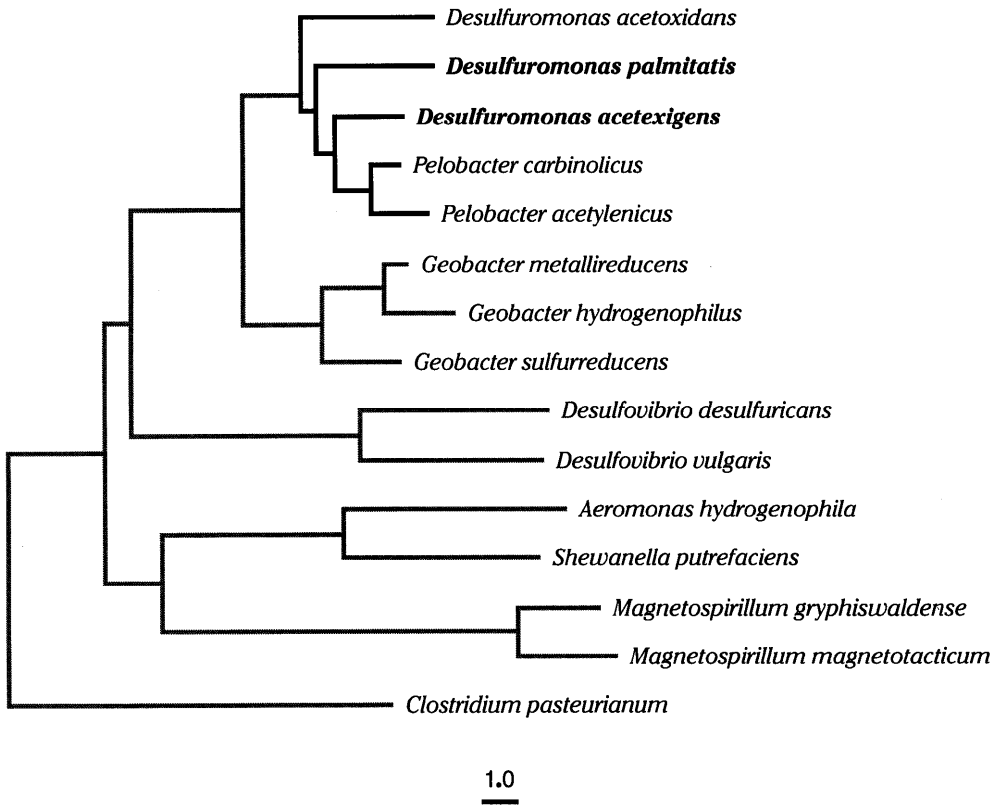

Fig. 5 Phylogenetic tree inferred from 16S rRNA sequences. The length of the bar represents one evolutionary distance unit

Temperature optimum

Strain SDBY1 had a temperature optimum of 40° C (data not shown), which is higher than values observed for other known Fe(III)-reducing *Desulfuromonas* or *Geobacter* species (Pfennig and Biebl 1976; Lovley et al. 1993a; Caccavo et al. 1994; Finster et al. 1994; J. D. Coates, unpublished).

Phylogenetic and physiological relationships to other dissimilatory Fe(III) reducers

The 16S rRNA sequence of strain SDBY1 (GenBank accession number U28172) places it among a group of microorganisms in the delta Proteobacteria, which are known Fe(III) and/or sulfur reducers (Fig. 5). These include organisms in the genera *Geobacter, Desulfuromonas, Pelobacter,* and *Desulfuromusa*. The closest known relatives of strain SDBY1 are *Desulfuromonas acetexigens* (94.2%; 1311 positions compared), *Desulfuromonas acetoxidans* (92.1%; 1294 positions compared), *Pelobacter carbinolicus* (94.7%; 1310 positions compared), and *Pelobacter acetylenicus* (94.7%; 1311 positions compared). Detailed analysis of the partial 16S rDNA sequence of strain SDBY1 indicated the presence of nucleotides and higher order structures characteristic of several *Desulfuromonas* and *Pelobacter* species (D. J. Lonergan, unpublished)

Strain SDBY1 shares many physiological characteristics with its two closest *Desulfuromonas* relatives, the marine species *D. acetoxidans* (Pfennig and Biebl 1976) and the freshwater species *D. acetexigens* (Finster et al. 1994). Previous studies have demonstrated that *D. acetoxidans* can grow by oxidizing acetate with S^0 (Pfennig and Biebl 1976) or Fe(III) (Roden and Lovley 1993) as terminal electron acceptor. In a similar manner, *D. acetexigens* has been previously shown to grow via sulfur reduction (Finster et al. 1994), and it also conserved energy to support growth from acetate oxidation coupled to Fe(III) reduc-

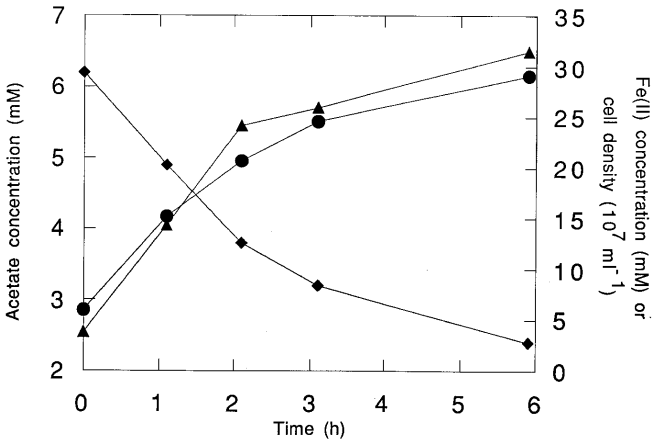

Fig. 6 Growth of *Desulfuromonas acetexigens* with acetate as electron donor and poorly crystalline Fe(III) oxide (100 mM) as electron acceptor. *Filled circles* Fe(II) production; *filled triangles* cell density; *filled diamonds* acetate consumption. The results are of one representative of duplicate cultures

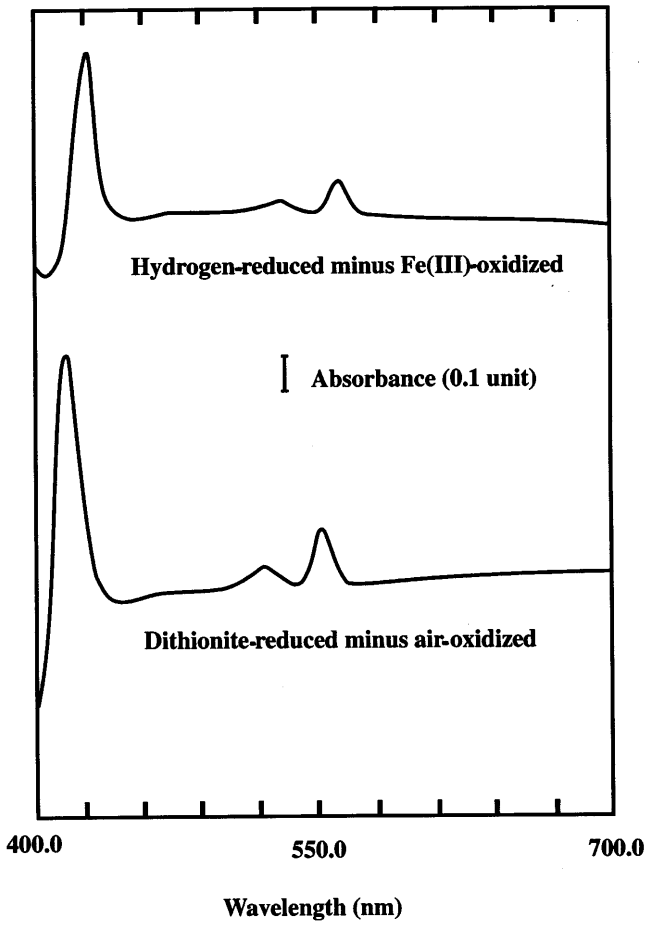

Fig. 7 Difference spectra of dithionite-reduced *minus* air-oxidized cell suspensions of strain SDBY1 and H_2-reduced [Fe(III) citrate added as electron acceptor] *minus* H_2-reduced cell suspensions of strain SDBY1. *Scale bar* 0.1 absorbance units

tion (Fig. 6). The electron acceptors used by strain SDBY1 and the two *Desulfuromonas* species are similar with the exception of the inability of strain SDBY1 to use malate. Strain SDBY1 can use a broader range of electron donors than *D. acetoxidans* and *D. acetexigens*.

Strain SDBY1 is also closely related to the three *Pelobacter* species (*P. carbinolicus*, *P. acetylenicus*, and *P. venetianus*) that are known Fe(III) reducers (Lovley et al. 1995b). However, these organisms are very different physiologically in that they cannot use acetate as an electron donor and can only incompletely oxidize multicarbon organic compounds with Fe(III) as the electron acceptor (D. J. Lonergan, unpublished; Lovley et al. 1995b). Furthermore, unlike strain SDBY1, these organisms can grow on Fe(III) with H_2 as the electron donor.

A major distinction between organisms in the genus *Pelobacter* and those in the genera *Desulfuromonas* and *Geobacter* is the presence of *c*-type cytochromes in the *Desulfuromonas* and *Geobacter* species (Pfennig and Biebl 1976; Lovley et al. 1993a; Roden and Lovley 1993; Caccavo et al. 1994; J. D. Coates, unpublished) and a lack of cytochromes in *Pelobacter* species (Schink 1992). Dithionite-reduced *minus* air-oxidized spectra of whole cell suspensions of strain SDBY1 had absorbance maxima at 552.8, 523.6, and 422.8 nm, indicative of *c*-type cytochrome(s) (Fig. 7). In a manner similar to that described previously for *Geobacter* and *Desulfuromonas* species, the cytochrome(s) were oxidized with Fe(III) (Fig. 7), suggesting that the *c*-type cytochromes might be involved in electron transport to Fe(III).

Environmental significance

As reviewed in the Introduction, it is becoming increasingly apparent that Fe(III) and Mn(IV) reduction are important processes for organic matter decomposition in marine environments. Strain SDBY1 represents the first direct isolation of a dissimilatory Fe(III)-reducing organism from a marine sediment using Fe(III) as the electron acceptor. It is interesting that this isolation technique yielded an organism very similar to *D. acetoxidans*, which is also a marine Fe(III) reducer (Roden and Lovley 1993), but was initially isolated as a sulfur reducer (Pfennig and Biebl 1976). The isolation of these two closely related Fe(III) reducers from different sites with different isolation techniques demonstrates that organisms of this type are present in diverse marine environments. The ability to reduce S^0, as well as Fe(III) and Mn(IV), is likely to be a useful characteristic in the Fe(III) and Mn(IV) reduction zone of marine sediments as this is often a zone of S^0 accumulation (Sørensen and Jørgensen 1987).

Acetate is expected to be a key intermediate in the metabolism of organic matter in anaerobic marine sediments (Sørensen et al. 1981; Christensen 1984), and thus acetate-oxidizing Fe(III) reducers with a metabolism like that of strain SDBY1 and *D. acetoxidans* probably account for much of the Fe(III) reduction in these environments. Although long-chain fatty acids are unlikely to be as important as acetate in the overall carbon and electron flow in anaerobic sediments, their input into anaerobic environments can be substantial (Mackie et al. 1991). Sulfate-reducing (Widdel 1988; Mackie et al. 1991) and H_2-producing syntrophic bacteria (Mackie et al. 1991) that can metabolize long-chain fatty acids have been described previously. The ability of strain SDBY1 to oxidize long-chain fatty acids with the reduction of Fe(III) demonstrates that these compounds may also be degraded in the Fe(III)-reduction zone of marine sediments.

In summary, strain SDBY1 provides a useful pure culture model for Fe(III) reduction in marine sediments. Its unique physiological characteristics and its 16S rRNA sequence demonstrate that strain SDBY1 represents a new species in the genus *Desulfuromonas*. The name *Desulfuromonas palmitatis* is proposed.

Description of *Desulfuromonas palmitatis* sp. nov.

Desulfuromonas palmitatis (pal.mi.ta'tis) sp. nov. N. L. chem. n. *palmitas* palmitate; N. L. gen. n. *palmitatis* of

palmitate, because it oxidizes palmitate. *D. palmitatis* is a strictly anaerobic chemoorganotroph with rod-shaped cells, 0.3 μm × 1-2 μm. Cells are gram-negative, nonmotile, and nonspore-forming. *D. palmitatis* grows by oxidizing acetate, lactate, succinate, fumarate, laurate, palmitate, or stearate with the concomittant reduction of Fe(III). H_2 is oxidized, but H_2 oxidation does not support growth. *D. palmitatis* can also conserve energy for growth by the reduction of Mn(IV), S^0, or fumarate. Other forms of sulfur or nitrogen were not used as electron acceptors. Cells of *D. palmitatis* contain *c*-type cytochrome(s). It has a temperature optimum of 40° C, and can grow in medium of marine salinity, but not in freshwater medium.

The type strain of *Desulfuromonas palmitatis*, strain SDBY1, was enriched from marine sediment samples taken from Palleta Creek in San Diego Bay, San Diego, Calif. with acetate as an electron donor and poorly crystalline Fe(III) oxide as the electron acceptor.

The strain has been deposited in the American Type Culture Collection (ATCC 51701). The 16S rRNA sequence has been deposited in GenBank (accession number U28172).

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