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ORIGINAL PAPER

Temperature and nutrient induced responses of Lake Fryxell sulfate-reducing prokaryotes and description of *Desulfovibrio lacusfryxellense*, sp. nov., a pervasive, cold-active, sulfate-reducing bacterium from Lake Fryxell, Antarctica

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Abstract The effects of temperature and carbon substrate availability on the stimulation of sulfate reduction by indigenous populations of sulfate-reducing prokaryotes (SRP) in permanently ice-covered Lake Fryxell, Antarctica were investigated. Psychrophilic and halotolerant, lactate-degrading SRP showed significant metabolic activity throughout all sampled depths of the water column, suggesting that such organisms, possibly of marine origin, may be key contributors to carbon and sulfur cycling in Lake Fryxell. Planktonic and benthic strains of lactate-oxidizing sulfate-reducing bacteria (SRB) were isolated from samples of various depths of the anoxic water column and from surficial sediments. Phylogenetic analyses of 16S rRNA gene sequences placed the Fryxell sulfate-reducer (FSR) strains within the *Deltaproteobacteria* and showed them to be most closely related to the Arctic marine species of SRB *Desulfovibrio frigidus* and *Desulfovibrio ferrireducens*. Based on phylogenetic and phenotypic differences between the Antarctic FSR strains and related species of the genus *Desulfovibrio*, strain FSRs^T (=DSM 23315^T =ATCC BAA-2083^T) is proposed as the type strain of a novel species of cold-active SRB, *Desulfovibrio lacusfryxellense*, sp. nov.

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Abbreviations

dsrA	Dissimilatory sulfite reductase alpha subunit
MOPS	4-Morpholinepropanesulfonic acid
SR	Sulfate reduction
SRP	Sulfate-reducing prokaryotes
SRB	Sulfate-reducing bacteria

Introduction

Dissimilatory sulfate reduction by sulfate-reducing bacteria (SRB) is of major significance to biogeochemical sulfur cycling on a global scale, and sulfate reduction is typically the dominant anaerobic respiration observed in anoxic environments containing sulfate (Widdel and Bak 1992). SRB are often isolated from marine sediments, where sulfate is abundant (Widdel and Bak 1992). Thus, several studies of SRB have focused on Arctic marine sediments, where isolated species have shown a psychrophilic or psychrotolerant phenotype (Isaksen and Teske 1996; Knoblauch et al. 1999; Sahm et al. 1999; Tarpgaard et al. 2005; Vandieken et al. 2006b). Studies of Antarctic SRB have been fewer by comparison, but recent data suggest that SRB are prevalent in these aquatic systems, as well (Purdy et al. 2003; Karr et al. 2005).

SRB have been isolated from lakes and fjords of the Vestfold Hills of eastern Antarctica (Franzmann et al. 1988; Bowman et al. 2000), but to our knowledge, only two species of Antarctic SRB have been described. *Desulfotomaculum antarcticum* was isolated from pond sediment and had an optimum growth temperature of 20–30°C (Iizuka et al. 1969). Curiously, the authors did not test for growth of this

species at colder temperatures, and unfortunately, cultures of the organism have since been lost (Stackebrandt et al. 1997). An undefined species of *Desulfovibrio* was later isolated from sediments of Lake Fryxell, a meromictic lake located in the McMurdo Dry Valleys of East Antarctica (Rees et al. 1986). Despite in situ temperatures near 1°C in Lake Fryxell, the bacterium reportedly grew at temperatures as high as 42°C and, like *Desulfotomaculum antarcticum*, grew optimally at 25°C (Rees et al. 1986). The growth response of this organism to colder temperatures also was not described.

Lake Fryxell is an exclusively microbial ecosystem in which significant biogenic sulfur cycling occurs at constantly cold temperatures, and it is the only lake in the Taylor Valley that supports significant sulfidogenesis and methanogenesis (Howes and Smith 1990; Smith et al. 1993; Karr et al. 2005). The permanent ice cover (5–6 m), lack of wind mixing, and salinity gradient (freshwater to nearly 1% NaCl at the sediment surface) of Lake Fryxell maintain a highly stable stratification of its 18-m water column (Fig. 1a, b) (Howes et al. 1992; Smith et al. 1993; Sattley and Madigan 2006). Moreover, organic carbon inputs from seasonal streams are trivial due to the nutrient- and plant-deficient soils that surround the lake (Aiken et al. 1996). Primary productivity in Lake Fryxell is due mainly to the activity of phytoplankton that inhabit the euphotic mixolimnion, immediately beneath the ice cover (Vincent 1981; Priscu et al. 1987). The mixolimnion, present beneath the ice cover to 8.5 m, is supersaturated with dissolved oxygen (Aiken et al. 1996), and opposing oxygen/sulfide gradients occur from 9 to 10 m (Fig. 1b) (Sattley and Madigan 2006). The water is anoxic below 10 m due to the upward diffusion of sulfide, and sulfide concentrations increase with depth to nearly 1.3 mM just above the sediments (Fig. 1b) (Sattley and Madigan 2006).

In this study, we investigated the effects of temperature and substrate availability on sulfidogenesis by indigenous populations of sulfate-reducing prokaryotes (SRP) in Lake Fryxell. We also describe a novel, pervasive, lactate-degrading and psychrophilic SRB from the anoxic water column and sediments of this unusual lake. Complementary to the cold-adapted, chemolithotrophic, sulfur-oxidizing bacteria described in our previous work (Sattley and Madigan 2006), the SRB described in this study likely contribute to a closed, microbially driven sulfur cycle within the nearly freezing water column of Lake Fryxell.

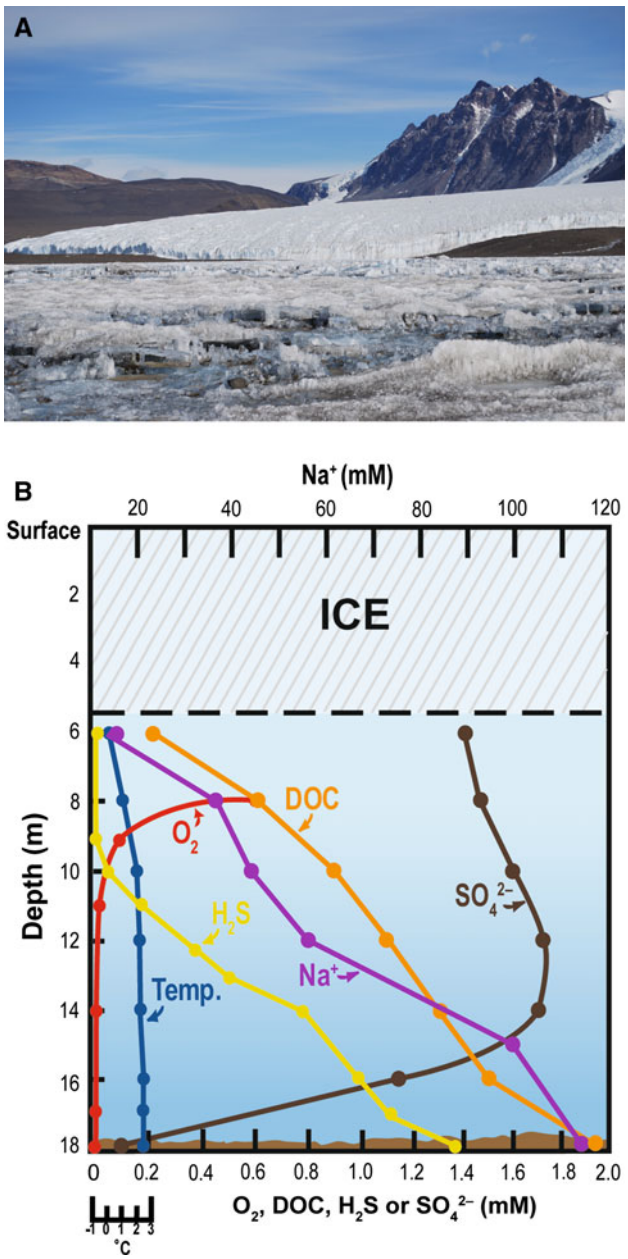

Fig. 1 Geomorphology and physiochemical parameters of Lake Fryxell, Taylor Valley, Antarctica. **a** Photograph showing a westward view from the surface of Lake Fryxell, taken in November 2008. The permanent ice cover ranges from 4.5 to over 6 m in thickness. The surface has become rougher in recent years due to solar melting beneath windblown soil deposits. Ice ridges rise to nearly 1 m above the lake surface, and the Canada Glacier is visible about 2 km in the distance. **b** Physiochemical profile of Lake Fryxell showing temperature and concentrations of dissolved oxygen, sulfide, sulfate, sodium (all adapted from Karr et al. 2005), and dissolved organic carbon (DOC; adapted from Takacs et al. 2001)

Materials and methods

Field study site and sample collection

Water samples were collected from Lake Fryxell, Antarctica during November 2003 and 2005 at global positioning system coordinates 77°36.604′S, 163°08.853′E and 77°36.630′S, 163°08.826′E, respectively. The sampling holes were drilled and melted as previously described (Karr et al. 2003). Water samples from the 2003 field season

were obtained using a Niskin bottle limnological sampling device (Karr et al. 2003), while water obtained in 2005 was retrieved using a peristaltic pump fitted with 1/4-in. (ID) vacuum tubing and a custom-made acrylic sampling device. This device consisted of two 6-in. square pieces of 3/4-in. thick clear acrylic plastic positioned atop one another and fastened at each corner with a nylon bolt fitted with a 1-cm tall spacer to separate the pieces of acrylic. A hole was drilled and tapped into the center of the top piece of acrylic, and a nylon fitting was threaded into it to allow for connection of the vacuum tubing. A plastic-coated lead weight was fixed to the sampling device to add ballast and ensure proper vertical positioning in the water column. Lake water entered the vacuum tubing via the 1-cm space between the two pieces of acrylic (i.e., water entered the sampler horizontally from all sides), allowing for a very fine sampling resolution with minimal disruption to the water column.

Water samples for enrichment of SRP were collected in completely filled, sterile, 150 ml crimp-top serum vials that were sealed immediately upon retrieval. Slow peristaltic pump rates ($\leq$20 rpm) were used at all times to minimize dissipation of volatile gases (e.g., H_2S and CH_4) as sample containers were filled. Approximately twice the volume of lake water in the tubing (1–2 l) was pumped and discarded between samplings. Samples were kept cold but unfrozen (approximately 4°C) for transport to the laboratory and during all laboratory manipulations.

Physiochemical analyses

Sulfide was measured with a maximum error of $\pm$2 μg/ml using the methylene blue colorimetric method described by Trüper and Schlegel (1964), with slight modifications for our purposes described in Karr et al. (2003). Sulfate was measured turbidimetrically as barium sulfate, as previously described (Karr et al. 2005). Temperature and dissolved oxygen were measured using a Yellow Springs Instrument Company model 57 precalibrated probe. Sodium ion concentrations were retrieved from the McMurdo Dry Valleys Long Term Ecological Research website (http://www.mcmlter.org/index.html), with measurements obtained in December 2005 by McMurdo LTER personnel using a Dionex DX-120 ion chromatography dual column system.

Culture conditions and measurements of sulfidogenesis

The temperature response of Lake Fryxell SRP was measured in duplicate 150 ml serum vials containing lake water collected at 17 m amended with 1 mM lactate and 5 mM sodium sulfate (final concentrations). Incubation temperatures included 4, 12, 18, and 35°C. A killed-cell control was established by injecting a final concentration of 5% (w/v) zinc acetate into the vial prior to incubation at 18°C; zinc acetate has been shown to inhibit growth of SRB (Utgikar et al. 2002). Sulfate reduction at different temperatures was measured as sulfide accumulation (from a time zero lake water sulfide measurement of 1.15 mM) in sealed vials over a period of 10 days.

Stimulation of Lake Fryxell SRP by specific organic substrates used as carbon and energy sources was tested by the addition of sodium lactate, acetate, or pyruvate to a final concentration of 1 mM and sodium sulfate to a final concentration of 5 mM to duplicate 150 ml serum vials containing 10-, 14-, or 17-m lake water. Culture assays for substrate utilization by SRP included both an unamended control and a killed-cell control containing 5% (w/v) zinc acetate. All culture incubations for substrate-specific stimulation of sulfate reduction were at 18°C for 12 days. Carbon substrate stimulation of sulfate respiration was measured as the accumulation of sulfide over time. Time zero lake water sulfide concentrations were 0.10 mM (10 m), 0.83 mM (14 m), and 1.15 mM (17 m).

Enrichment cultures for the isolation of lactate-oxidizing SRP were established by aseptically placing 1 ml of lake water from depths of either 12, 14, or 17 m or 0.5 g lake sediment into a 1.4 mM sulfide-reduced, MOPS-buffered (10 mM) minimal medium containing sodium (D, L) lactate (20 mM) and sodium sulfate (70 mM; later reduced to 21 mM for maintenance of isolated strains) as previously described (Karr et al. 2005; Sattley and Madigan 2007). Enrichment cultures were incubated at 4°C. The basal medium contained (per liter deionized water): NaCl, 2.0 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; $CaCl_2{\cdot}2H_2O$, 0.08 g; NH_4Cl, 1.0 g; KCl, 0.5 g; KH_2PO_4, 1.0 g; and trace elements (Wahlund et al. 1991), 1 ml. Dilute yeast extract was added at a final concentration of 0.003% (w/v) to all samples to meet possible vitamin requirements of the organisms.

Purification of positive enrichments was carried out at 10°C as previously described (Sattley and Madigan 2007) using the modified agar tube dilution method of Isaksen and Teske (1996). Culture purity was verified by microscopic examination and by a lack of growth in anoxic complex media, including tryptic soy broth and nutrient broth. Routine transfers of pure cultures were performed every 3–5 weeks and were maintained at 4, 10, or 18°C. Growth rates of pure cultures were obtained from optical density measurements (OD_{540}) averaged from triplicate samples. Temperature, pH, and NaCl ranges and optima were assessed from triplicate cultures in which optical density and microscopic observation were used to verify growth under a given condition.

Carbon substrate utilization by isolated sulfate-reducing strains was tested by the addition of a single carbon source (3–20 mM, depending on the substrate) to triplicate cultures containing an anoxic mineral salts medium with

21 mM sodium sulfate. Growth using hydrogen or formate as electron donor was assessed in the presence and absence of acetate (5 mM), added as a carbon source. Incubations for assessing carbon substrate utilization took place at 18°C for 32 days.

Primary enrichment cultures for SRP were also established using lake water of various depths amended with 10 mM acetate or pyruvate as carbon source and electron donor. Although these cultures showed evidence of sulfidogenesis (as accumulation of black FeS in the medium), they could not be successfully transferred in artificial growth media. We thus focused our attention on lactate-oxidizing SRP.

Microscopy

Mid-exponential phase cells were fixed in 2% glutaraldehyde followed by post-fixation in OsO_4 and ethanol dehydration. Embedded cells were stained with uranyl acetate followed by lead citrate before examination at 20,000× magnification using a Hitachi H500H transmission electron microscope. For SEM, cells fixed in 1% glutaraldehyde followed by OsO4 were dehydrated in ethanol and subjected to critical point drying before examination at 15,000× magnification in a Hitachi S570 scanning electron microscope operating at 20 kV.

Molecular analyses

Genomic DNA extractions and polymerase chain reaction (PCR) amplification of 16S rRNA genes were performed as previously described (Sattley and Madigan 2006). Amplified DNA was purified using the QIAquick PCR purification kit according to the manufacturer's instructions (Qiagen Sciences, Germantown, MD, USA). Sequencing of 16S rRNA gene products was performed at the Genome Sequencing Center, Washington University, St. Louis, MO, USA. Sequenced fragments were assembled using the CAP3 sequence assembly program (Huang and Madan 1999), and related species were identified using both the BlastN function of the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) and the Sequence Match function of the Ribosomal Database Project (Cole et al. 2009). A phylogenetic tree was constructed from a multiple alignment using MacVector v7.2.2 (Accelrys, Inc., 2004). Details of the phylogenetic tree construction are described in the corresponding figure legend.

Database and culture information

Sequences of the 16S rRNA genes from Antarctic sulfate-reducing strains isolated in this study were deposited into the GenBank database under accession numbers DQ767883–DQ767889, as indicated in the phylogenetic tree. Strain $FSRs^T$ (GenBank DQ767889) has been deposited in the American Type Culture Collection as ATCC BAA-2083 and in the German Collection of Microorganisms and Cell Cultures (DSMZ) as DSM 23315.

Results

Effects of temperature on sulfate reduction by natural populations of Lake Fryxell SRP

Sulfate reduction as a function of temperature was measured in anoxic lake water samples from a depth of 17 m amended with 1 mM lactate and 5 mM sulfate (lactate was chosen as electron donor for these experiments for reasons to be discussed later). Sulfide concentrations in lake water enrichment cultures incubated at 4 and 12°C for 10 days increased from an initial concentration of 1.15 to 1.26 mM and 1.41 mM, respectively (Table 1). Although in situ lake temperatures are constant at about 1°C, the metabolic activity of Lake Fryxell SRP was highest at 18°C, where sulfide concentrations increased from 1.15 to 1.59 mM after 10 days. No sulfate reduction occurred at 35°C. From these experiments, a cold-active phenotype could be predicted for natural populations of Lake Fryxell SRP. These data are consistent with the growth response of pure cultures of Lake Fryxell SRB to varying temperatures, as will be discussed below.

Carbon substrate utilization by natural populations of Lake Fryxell SRP

Based on molecular evidence indicating that a diverse but uneven distribution of genera of SRB is present in the Lake Fryxell water column (Karr et al. 2005), single carbon sources were added to lake water from different depths to test the hypothesis that a vertically stratified substrate bias for SR may exist in the lake. Sampled depths included water from immediately below the oxycline (10 m), the central anoxic zone (14 m) where sulfate concentrations are highest, and the benthic zone (17 m) where sulfate is nearly depleted (Fig. 1b). To ensure that sulfate was not limiting in these experiments, all vials were supplemented with sodium sulfate (5 mM).

The addition of lactate stimulated sulfate reduction in all three zones. In absolute terms, the greatest sulfide production occurred at 14 m, followed closely by that at 17 m. As expected, sulfidogenesis at 10 m was much lower since water at this depth lies right at the chemocline where varying concentrations of dissolved oxygen are present; however, even here, lactate was clearly the preferred substrate (Table 1). In contrast to this pervasive nature of lactate stimulation of SR, pyruvate- and acetate-driven SR was localized to specific depths. Pyruvate stimulated sulfate reduction in lake water samples from a depth of 10 m, while acetate did not.

Table 1 Effects of temperature and carbon substrate availability to sulfate reduction by indigenous populations of sulfate-reducing prokaryotes in the Lake Fryxell water column

Sulfide production in 17-m Lake Fryxell water vs. temperature[a]				
Temp. (°C)	Sulfide (mM)[b]		Δ (mM)	% Δ
	0 h	240 h		
4	1.15	1.26	0.11	+9.6
12	1.15	1.41	0.26	+23
18	1.15	1.59	0.44	+38
35	1.15	1.07	−0.08	−7.0
Killed control[c]	1.15	0.70[d]	−0.45	−39
Sulfide production at 10, 14, and 17 m vs. carbon substrate availability[a]				
	Sulfide (mM)[b]		Δ (mM)	% Δ
	0 h	288 h		
10 m				
Acetate	0.10	0.07	−0.03	−30
Pyruvate	0.10	0.21	0.11	+110
Lactate	0.10	0.30	0.20	+200
Unamended	0.10	0.03	−0.07	−70
Killed control[c]	0.10	0.08	−0.02	−20
14 m				
Acetate	0.83	0.92	0.09	+11
Pyruvate	0.83	0.72	−0.11	−13
Lactate	0.83	1.38	0.55	+66
Unamended	0.83	0.86	0.03	+3.6
Killed control[c]	0.83	0.37	−0.46	−55
17 m				
Acetate	1.15	1.19	0.04	+3.5
Pyruvate	1.15	0.97	−0.18	−16
Lactate	1.15	1.67	0.52	+45
Unamended	1.15	1.16	0.01	+0.9
Killed control[c]	1.15	0.45	−0.70	−61

[a] See "Materials and methods" for details of the experiment

[b] Mean values for duplicates agreeing within ±0.08 mM are given

[c] The decrease of sulfide in the killed-cell controls is likely due to the addition of 5% (f.c.) Zn-acetate, which could lead to underestimated sulfide concentrations since aggregates of insoluble ZnS would not remain evenly distributed in solution during sampling

Conversely, acetate stimulated sulfate reduction in lake water from 14 and 17 m, depths at which pyruvate had no effect. However, based on the amount of sulfide generated, neither acetate nor pyruvate stimulated SR to the same degree as lactate for any of the depths sampled (Table 1).

Morphology and culture characteristics of isolated SRB

Six strains of SRB (designated strains FSR12A, FSR12B, FSR14A, FSR14B, FSR17A, and FSR17B) were isolated from Lake Fryxell water samples. The number in each strain designation corresponds to the depth (in meters) from which the isolate was obtained. In addition, a seventh strain, FSRs, was isolated from a Lake Fryxell sediment sample. All strains showed similar, but not identical, morphological characteristics. Individual cells of all FSR strains were short to medium rods (0.5–0.8 × 0.8–2.5 μm) displaying varying degrees of an ovoid or elliptical shape with bluntly pointed ends, as typified by strain FSRs (Fig. 2).

All FSR strains were obligately anaerobic and motile, stained Gram-negatively, and existed as single cells or cell pairs in culture. Regardless of the age of the culture, cells of the FSR strains often clumped together and settled to the bottom of the vessel during incubation. However, cells could easily be resuspended to a uniform turbidity by mixing. Cells of the FSR strains remained viable even after extended incubation. For example, cultures grown and maintained at 18°C could be successfully transferred as much as 3 weeks after entering stationary phase.

Phylogeny of FSR strains

Comparative phylogenetic analyses employing 16S rRNA gene sequencing were performed on all seven strains of Lake Fryxell SRB. Distance analysis placed the strains within the *Deltaproteobacteria*, specifically, within a clade containing species of *Desulfovibrio* (Fig. 3). The FSR strains showed anywhere from 99.5 to 100% 16S rRNA gene sequence identity with each other over the 1,488 bases included in the analysis. The most closely related described species to the FSR strains included *Desulfovibrio frigidus* (97.9%) and *Desulfovibrio ferrireducens* (97.6%), psychrotolerant SRB isolated from permanently cold Arctic fjord sediments off the west coast of Svalbard (Vandieken et al. 2006b). Other fairly closely related *Desulfovibrio* species included *D. hydrothermalis* (95.2%), isolated from a deep-sea hydrothermal vent (Alazard et al. 2003), and *D. zosterae* (93.8%), an organism isolated from within the roots of a benthic marine seagrass (Fig. 3) (Nielsen et al. 1999).

Physiology of FSR strains: Electron donors and culture conditions

The pattern of carbon source/electron donor usage was identical among the FSR strains and is summarized in Table 2. Best growth occurred in a minimal medium containing lactate; such cultures became turbid (OD_{540} >0.175) within about 2 weeks at 18°C. Carbon sources that supported moderate to weak growth compared with lactate were fumarate, pyruvate, ethanol, and malate. Formate or H_2/CO_2 also supported growth but only if acetate was also present as a carbon source; acetate did not support growth as an electron

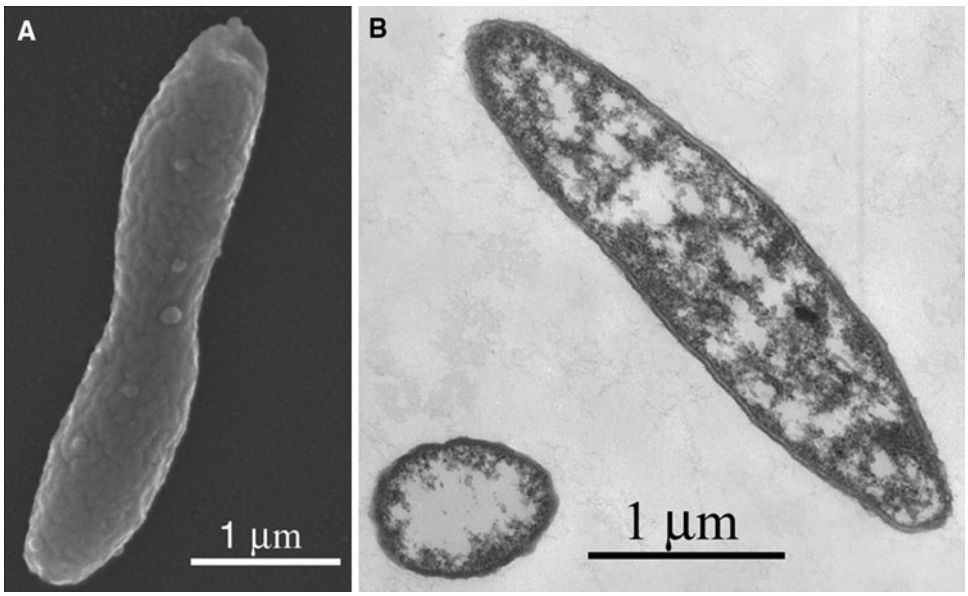

Fig. 2 Cell morphology of sulfate-reducing strain FSRs, isolated from the sediment of Lake Fryxell, Antarctica. **a** Scanning electron micrograph showing dividing cells. **b** Transmission electron micrograph showing longitudinal and cross-sectioned cells

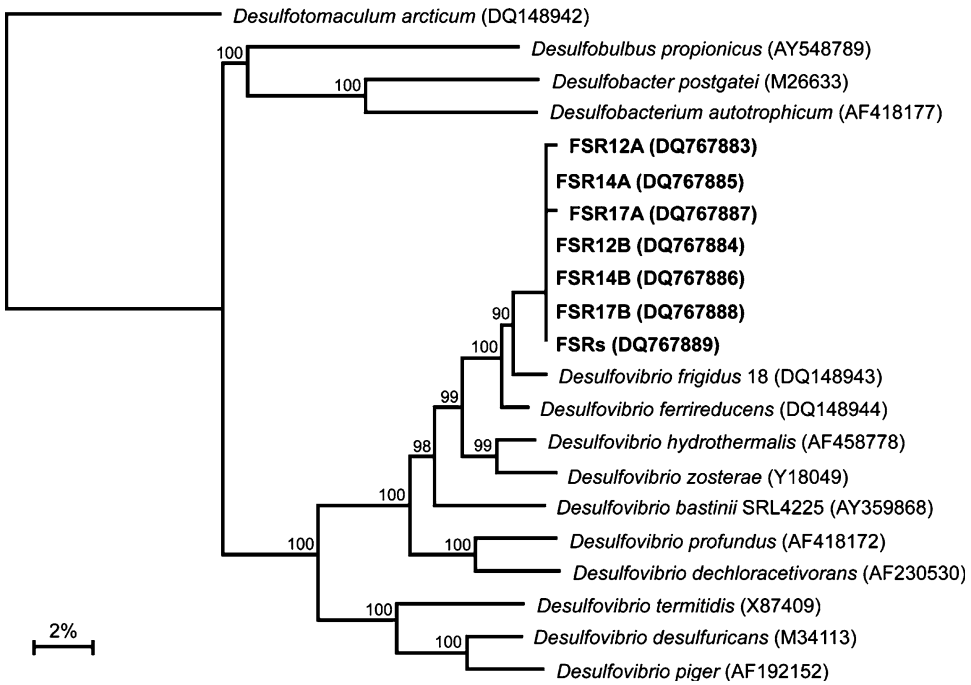

Fig. 3 Phylogenetic tree generated from 1488 nucleotide positions of the 16S rRNA gene using the Kimura 2-parameter distance algorithm in a heuristic search. Only isolated strains were included in the analysis. GenBank accession numbers for bacterial species used in the analysis are shown in parentheses. All organisms are members of the *Deltaproteobacteria* except the outgroup organism, *Desulfotomaculum arcticum*, an endospore-forming sulfate-reducing bacterium belonging to the *Firmicutes* (Vandieken et al. 2006a). Tree topology was maintained using Jukes-Cantor correction, and bootstrap values from 1000 replicates are shown at the branching points

donor (Table 2). No growth of the FSR strains occurred for any carbon source tested when sulfate was absent from the minimal medium. Therefore, the FSR strains appear incapable of fermentation. Growth on sugars did not occur, regardless of the presence of sulfate. Moreover, no growth of any strain occurred in complex liquid media, including tryptic soy broth or nutrient broth.

As reported for close relatives *D. frigidus* and *D. ferrireducens* (Table 2), the pH range supporting growth of the FSR strains was narrow (data not shown). No growth occurred below pH 6.5, and the maximum pH tolerated was pH 7.5. Optimal growth of the FSR strains in lactate-containing minimal medium occurred at pH 7. The pH of 10 mM MOPS-buffered lactate cultures increased from pH 7.1 to pH 7.3 after reaching stationary phase.

Unlike *D. frigidus* and *D. ferrireducens*, none of the FSR strains required NaCl for growth. However, all FSR strains were moderately halotolerant, with 1–2.5% (w/v) NaCl providing optimal growth (Table 2). Interestingly, salt tolerance of the FSR strains varied with respect to the lake depth from which each strain was isolated. Salt tolerance increased slightly in strains isolated from deeper in the water column, which is consistent with the salinity gradient present in Lake Fryxell (Fig. 1b). Strains isolated from

Table 2 Morphological and physiological characteristics of Lake Fryxell SRB (FSR strains) and related *Desulfovibrio* species

	FSR strains	*D. frigidus*[b]	*D. ferrireducens*[b]	*D. hydrothermalis*[c]	*D. zosterae*[d]
Source	Lake Fryxell, Antarctica	Arctic fjord sediments	Arctic fjord sediments	Deep-sea hydrothermal vent	Seagrass root tissue
Cell shape	Elliptical rods	Vibrioid or rods	Vibrioid or sigmoid	Vibrioid or sigmoid	Sigmoid or curved rods
Cell size (l × w, μm)	0.8–2.5 × 0.5–0.8	3.5–4.5 × 0.5–0.7	2.5–5.5 × 0.5–0.7	1–2 × 0.5–1	3.0 × 0.5
Temperature range (°C)	0–25	−2 to 25	−2 to 30	20–40	5–34.5
Optimum temp. (°C)	19–21	20–23	23	35	32.5–34.5
pH range	6.5–7.5	6.9–7.5	6.3–7.5	6.2-8.4	5.5–7.5
pH optimum	7–7.3	6.9–7.2	7.1–7.5	7.8	6.8–7.3
NaCl (w/v) range	0 to up to 4.6%	2–3.5%	0.7–4%	>0–4%	0–3.5%
NaCl (w/v) optimum	1–2.5%	2–3%	1–2.5%	2.5%	1.2%
Electron donors[a]					
H_2/CO_2 (+acetate)	+	+	+	+	+
Formate (+acetate)	(+)	+	+	+	(+)
Lactate	++	+	+	+	+
Pyruvate	(+)	−	−	+	+
Fumarate	+	+	+	(+)	+
Malate	(+)	−	−	+	+
Glucose	−	−	−	−	−
Fructose	−	−	−	−	+
Ethanol	(+)	+	+	+	+
Propanol	−	+	+	−	−

All species included in the table are motile and do not use acetate as electron donor. Reference strains were chosen based on high 16S rRNA gene sequence identities to the FSR strains. All FSR strains showed the same physiological profiles, except for slight variations in maximum NaCl tolerance (3.8–4.6%)

[a] Growth of the FSR strains was measured as OD_{540}. Time zero OD_{540}, 0.045–0.06; ++, OD_{540} >0.2; +, OD_{540} 0.1–0.2; (+), OD_{540} 0.06–0.099; −, OD_{540} <0.06. Growth of other species is indicated as +, positive; (+), weak; or −, negative. Final concentrations of carbon sources included in the table are: H_2:CO_2, 80:20 (v/v); formate, 20 mM; all others, 10 mM. Additional substrates that did not support growth of the FSR strains included acetate, propionate, butyrate, succinate, benzoate, citrate, valerate, caproate, betaine, trimethylamine, and a variety of sugars and alcohols

[b] Vandieken et al. (2006b)

[c] Alazard et al. (2003)

[d] Nielsen et al. (1999)

12-, 14-, and 17-m lake water had maximum NaCl tolerances of 3.8, 4.2, and 4.6% (w/v), respectively. The salt tolerance of the sediment isolate (strain FSRs) was the same as that of strains isolated from lake water at a depth of 17 m.

Physiology of FSR strains: temperature relationships

The FSR isolates showed a cold-active phenotype, and the data closely paralleled the activity experiments performed on lake water samples (Table 1). The temperature range for growth of all FSR strains was 0–25°C, and the optimum was 19–21°C; thus the strains are psychrophilic. Cultures grown at 21°C reached stationary phase within 1 week (Fig. 4, with strain FSRs being representative of all FSR strains), and a mean generation time of approximately 30 h was calculated for strain FSRs at this temperature. Decreasing the incubation temperature to 5°C increased the mean generation time to about 7 days, with cultures reaching stationary phase after nearly 4 weeks (Fig. 4). Growth of the FSR strains at in situ temperatures (1–2°C, Fig. 1a) was measurable but slow, requiring more than 6 weeks for cultures to reach stationary phase at 1°C (Fig. 4). Growth at 25°C was apparent over the first 6 days of incubation (Fig. 4). However, final cell densities were well below those observed at lower temperatures, and cell growth was clearly compromised at this temperature.

Discussion

The biogeochemical profile of Lake Fryxell makes it an excellent model for studying major nutrient cycling

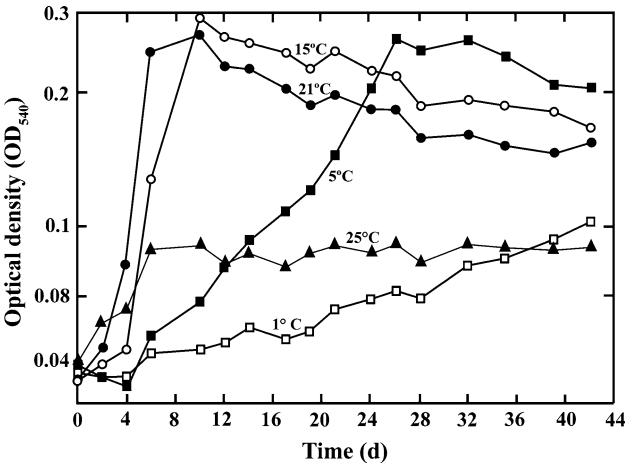

Fig. 4 Growth (OD_{540}) versus incubation temperature for the sulfate-reducing *Desulfovibrio* strain FSRs over a 42-day incubation period in a lactate-containing minimal medium. The growth response to temperature was nearly identical among all FSR strains, and thus, only data from strain FSRs are shown

processes in permanently cold, exclusively microbial ecosystems. These processes include sulfidogenesis (sulfate reduction), chemolithotrophic sulfur oxidation, oxygenic and anoxygenic photosynthesis, methanogenesis, anaerobic methane oxidation, acetogenesis, and organic acid fermentation (Vincent 1981; Smith et al. 1993; Madigan et al. 2000; Karr et al. 2003, 2005, 2006; Sattley and Madigan 2006, 2007; Sattley et al. 2008). Because sulfate is the most abundant electron acceptor present, much of the anaerobic carbon degradation in Lake Fryxell is likely linked to SR. While planktonic sulfur-oxidizing chemolithotrophic bacteria contribute to autotrophic activities, particularly at the oxycline (Howes et al. 1992; Sattley and Madigan 2006), heterotrophic SRB mineralize dissolved organic carbon within the water column and sediments, with the concomitant production of sulfide.

The pattern of carbon substrate stimulation of sulfidogenesis observed in this study suggests that diverse populations of SRP may exist in Lake Fryxell and that they may be localized as to depth. Sulfate reduction just below the oxycline was highly stimulated by lactate. By contrast, activity of acetate-oxidizing SRP in Lake Fryxell was only detected deeper in the water column, suggesting that the physiochemical conditions associated with deeper waters, such as increased salinity (Fig. 1b), may be more favorable or even required for the proliferation of indigenous completely oxidizing SRP. Of the carbon sources tested, lactate stimulated sulfate reduction the best at all depths, pointing to the likely importance of *Desulfovibrio*-like SRB to the biogeochemistry of Lake Fryxell. This conclusion is consistent with a molecular sampling study in which both phylogenetic (genus-specific 16S rRNA gene) and metabolic (*dsrA*, which encodes the α subunit of dissimilatory sulfite reductase) primer sets showed that phylotypes of SRB most closely related to *Desulfovibrio* are present throughout the water column and sediments of Lake Fryxell (Karr et al. 2005). Thus, *Desulfovibrio*-like strains related to marine species may be the dominant SRB in this lake.

Several lines of evidence suggest a marine origin for Lake Fryxell SRB. The proximity of Lake Fryxell to the Ross Sea, a distance of only about 7 km, and the lake water column itself, containing a salinity gradient and significant quantities of sulfate (Fig. 1b), indicate that Lake Fryxell was once contiguous with marine waters. In fact, it is thought that Taylor Valley lakes are the remnants of a much larger proglacial lake, Lake Washburn, which abutted the Ross Ice Sheet during the Last Glacial Maximum (Wagner et al. 2006). The salt tolerance (up to 4.6%) of the FSR strains is typical of marine bacteria, and it is in accordance with the brackish hypolimnion of Lake Fryxell. Moreover, the salt tolerance of the FSR strains is approximately equal to or exceeds that of their closest phylogenetic relatives, the marine SRB *D. frigidus* and *D. ferrireducens*. Other closely related species of *Desulfovibrio*, including *D. hydrothermalis* and *D. zosterae*, also were isolated from marine environments. Finally, activity of acetate-oxidizing SRP was only apparent in deeper waters of Lake Fryxell. Because acetate oxidation by SRB is a process usually associated with marine environments (Widdel and Bak 1992), the observed limited activity of acetate-oxidizing SRB to only brackish waters of Lake Fryxell may indicate an evolutionary link to marine species.

While both this study and that of Karr et al. (2005) provide strong evidence for the presence of completely oxidizing SRB in Lake Fryxell, the extent of their activity and contribution to nutrient cycling within the lake ecosystem remains unresolved. Cold-active acetogenic and acetate-producing, strictly fermentative bacteria are present in Lake Fryxell (Sattley and Madigan 2007; Sattley et al. 2008), and therefore a sustainable supply of acetate should be available. The reason for the failure of acetate-oxidizing SRB to develop in our enrichment cultures is unclear, but it is likely due to nutritional deficiencies in our growth medium. Since enrichment cultures were incubated for more than 1 year, it is unlikely that acetate-oxidizing species, even very slowly growing species, would have been missed otherwise.

The Lake Fryxell FSR strains isolated in this study are moderately psychrophilic. They share a growth temperature range of 0–25°C and an optimum growth temperature of 19–21°C. Good growth of these strains occurred well below 10°C, and only weak growth was observed at 25°C (Fig. 4). Because the maximum temperature of these strains is lower than the temperature optimum of nearly all

mesophilic SRB (Widdel and Bak 1992), the FSR strains show clear cold temperature adaptation and are more accurately described as psychrophilic than psychrotolerant.

The distribution and phylogeny of the *Desulfovibrio* FSR strains, coupled with our in situ experiments on temperature and substrates, support the hypothesis that lactate-oxidizing SRB are pervasive in Lake Fryxell and thus may be major contributors to anaerobic carbon cycling in this polar lake. Together with the sulfur-oxidizing chemolithotrophic bacteria that inhabit Lake Fryxell described previously (Sattley and Madigan 2006), the SRB described here may also be important contributors to sulfur cycling activities within this permanently ice-covered Antarctic lake. To recognize the basic biology of these SRB strains, we propose the creation of a new taxon within the genus *Desulfovibrio*; *Desulfovibrio lacusfryxellense*, a SRB from Lake Fryxell.

Description of *Desulfovibrio lacusfryxellense* sp. nov.

Desulfovibrio lacusfryxellense (la'cus.fry.xel.len'se. N.L. neut. adj. *lacusfryxellense*, of Lake Fryxell, the permanently ice-covered Antarctic lake from which the type strain was isolated).

Cells are short to medium rods (0.5–0.8 × 0.8–2.5 μm) displaying varying degrees of an ovoid shape with bluntly pointed ends. Cells occur singly or in pairs and show swimming motility. Stain Gram-negative. Obligate anaerobe. Lactate or fumarate support good growth with sulfate reduction. Weak to moderate growth is supported by pyruvate, ethanol, malate, or either formate or H_2/CO_2 plus acetate (as carbon source). Sugars do not support growth. Moderately halotolerant, with optimum NaCl concentration from 1 to 2.5%. NaCl is not required for growth, but a small amount of salt is growth stimulatory. No growth factor requirements. Optimum pH is 7, with growth occurring over a narrow pH range of 6.5–7.5. Psychrophilic. Temperature optimum 19–21°C, with growth occurring from 0–25°C.

Strain FSRs^T (=DSM 23315^T = ATCC BAA-2083^T) is the type strain, isolated from surficial sediments of permanently ice-covered, meromictic Lake Fryxell, McMurdo Dry Valleys, Antarctica.

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