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Diversity and Distribution of Sulfate-Reducing Bacteria in Permanently Frozen Lake Fryxell, McMurdo Dry Valleys, Antarctica

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The permanently frozen freshwater Lake Fryxell, located in the Dry Valleys of Antarctica, exhibits an ideal geochemistry for microbial sulfate reduction. To investigate the population of sulfate-reducing bacteria in Lake Fryxell, both 16S rRNA gene and metabolic primer sets targeting the *dsrA* gene for the dissimilatory sulfite reductase alpha subunit were employed to analyze environmental DNA obtained from the water column and sediments of Lake Fryxell. In addition, enrichment cultures of sulfate-reducing bacteria established at 4°C from Lake Fryxell water were also screened using the *dsrA* primer set. The sequence information obtained showed that a diverse group of sulfate-reducing prokaryotes of the domain *Bacteria* inhabit Lake Fryxell. With one exception, the enrichment culture sequences were not represented within the environmental sequences. Sequence data were compared with the geochemical profile of Lake Fryxell to identify possible connections between the diversity of sulfate-reducing bacteria and limnological conditions. Several clone groups were highly localized with respect to lake depth and, therefore, experienced specific physiochemical conditions. However, all sulfate-reducing bacteria inhabiting Lake Fryxell must function under the constantly cold conditions characteristic of this extreme environment.

Lake Fryxell is a permanently ice-covered lake located in the McMurdo Dry Valleys, Antarctica. A perennial ice cover of 4 to 6 m and a small influx of glacial meltwater impose a stable chemical stratification of the water column (21). The upper portion of the water column of Lake Fryxell consists of freshwater supersaturated with dissolved oxygen, whereas the water becomes anoxic and increasingly sulfidic below 10 m, coinciding with a gradual increase in salinity that reaches a maximum of nearly 1% at the sediment-water interface (14) (see Fig. 3).

The biota of Lake Fryxell is strictly microbial (21), and the lake geochemistry lends itself to the development of vertically stratified microbial populations. For example, our previous work on anoxygenic phototrophic bacteria in Lake Fryxell showed a distinct vertical stratification of purple phototrophic bacteria (1, 11). Interestingly, however, and despite the high levels of sulfide present, enrichment cultures for purple bacteria in Lake Fryxell yielded only purple nonsulfur bacteria (10, 11). One such organism obtained in pure culture was shown to be extremely sulfide tolerant and to contain gas vesicles (10).

Because Lake Fryxell is highly sulfidic, it has been assumed that biological sulfate reduction was responsible. Sulfate-reducing bacteria (SRB) reside within the δ subdivision of the *Proteobacteria*, the gram-positive and *Thermodesulfobacterium* subdivisions of *Bacteria*, and the archaeal domain *Euryarchaeota* (26). SRB reduce sulfate to sulfide by using a variety of electron donors, including H_2, fatty acids, alcohols, and aromatic compounds (32). SRB are present in permanently cold environments, and several psychrophilic representatives have been isolated in recent years, all from anoxic marine environments (19). Sulfate reduction is a major process in marine sediments, an environment dominated by low temperatures, where SRB play an important role in the sulfur cycle as well as the carbon cycle (12, 22).

The enzyme dissimilatory sulfite reductase (DSR) catalyzes the final steps in sulfate reduction and is therefore an essential enzyme. As such, DSR is synthesized by all known SRB (30). The ubiquity of DSR and its high sequence conservation has made this enzyme ideal for assessing the biodiversity of SRB in anoxic environments (30). In this regard, genes encoding the α (*dsrA*) and β (*dsrB*) subunits of DSR have been employed in several environmental studies utilizing PCR to profile communities of SRB (5, 6, 17, 18). To explore the biodiversity of SRB in Lake Fryxell, we have performed *dsrA* analyses of water and sediments from this extreme environment by using a combination of PCR and denaturing gradient gel electrophoresis (DGGE) and have complemented these studies with 16S rRNA gene analyses and enrichment cultures. From our results, we conclude that a diverse group of SRB inhabit Lake Fryxell, including phylotypes only distantly related to known species of this group.

MATERIALS AND METHODS

Sample collection and processing. Water and sediment samples were collected from Lake Fryxell (Taylor Valley, McMurdo Dry Valleys, Southern Victoria Land, Antarctica) during November 2001 and November 2003 at global positioning system coordinates 77°36.570S, 163°08.969E and 77°36.604S, 163°08.853E, respectively. Samples were collected using a 5-liter Niskin bottle through a hole drilled and melted into the lake ice cover. Water depth was determined from the level to which the lake water rose within the sampling hole. Water samples were collected at depths of 8, 9, 11, 14, and 17 m. Surface sediment samples were collected with an Eckman dredge. All samples were transferred to

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Crary Lab, McMurdo Station, in sterile containers for immediate processing; samples were kept cold but unfrozen and shielded from light during transport.

Water chemistry and growth media. Sulfide, temperature, and oxygen measurements were performed as previously described (11). Sulfate concentrations were measured using a standard turbidimetric method in which sulfate is precipitated with barium at acidic pH to form barium sulfate (3). Following primary cultivation of SRB in sealed serum bottles containing lake water amended with 20 mM lactate and 50 mM Na_2SO_4 (4°C), subcultures were established in anoxic 10 mM MOPS (4-morpholinepropanesulfonic acid)-buffered medium (pH 7.2) containing lactate (20 mM) as a carbon source, Na_2SO_4 (70 mM) as an electron acceptor, and the following mineral salts (per liter of deionized water): NaCl, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; NH_4Cl, 1.0 g; KCl, 0.5 g; KH_2PO_4, 1.0 g; and trace elements (31), 1 ml. Yeast extract (0.005%, wt/vol) was added as a source of vitamins. Either sulfide (1.4 mM) or sodium ascorbate (7.6 mM) was added as a reducing agent, and resazurin (1 mg/liter) was added to confirm anoxic conditions. Cultures were maintained in crimp-sealed serum vials or completely filled 17-ml tubes and were incubated at 10°C in darkness. Pyruvate (5 mM final concentration) was added later to enhance growth of all cultures.

DNA extraction. To concentrate the biomass, 500-ml aliquots of lake water were filtered through sterile 0.2-μm Nalgene analytical test filters. The filters were aseptically cut into approximately 2-mm^2 pieces and transferred to a biopulverizer tube (Qbiogene, Carlsbad, CA) for extraction of total environmental DNA. Biopulverizer tubes containing lysing matrix E, 978 μl sodium phosphate buffer, and 122 μl MT buffer (Fast DNA Spin Kit for Soil; Qbiogene) and test filters were processed in a mini-bead beater (Biospec Products, Bartlesville, OK) for 30 seconds at 4,600 rpm. The remainder of the DNA extraction procedure was performed according to the manufacturer's instructions.

PCR and nested PCR. The SRB-specific 16S rRNA gene primer sets were used as previously described (7). The *dsr* primer set (1F [5′-ACSCACTGGAAGCAC G-3′] and 4R [5′-GTGTAGCAGTTACCGCA-3′]) (30) was used to amplify the *dsrAB* genes. PCR components (Promega, Madison, WI) consisted of 32.5 μl water, 5 μl 10× buffer, 1.25 μl bovine serum albumin (0.25 mg/ml) in initial amplifications, 3 μl 25 mM $MgCl_2$ (1.5 mM), 1 μl each deoxynucleoside triphosphate at 10 mM, 0.5 μl each primer at 125 μg/ml, 1 μl template DNA (30 to 300 ng), and 2.5 μl (2.5 units) *Taq* polymerase (Sigma, St. Louis, MO). Cycling parameters were as follows: an initial denaturation for 15 s at 94°C followed by 30 cycles of 15 s at 94°C, 20 s at 54°C, and 54 s at 72°C, concluding with a 1-min extension at 72°C. Amplification products were analyzed on a 0.7% agarose gel to ensure correct size (1.9 kb) and eluted from the agarose by using the GENECLEAN spin kit (Qbiogene) for use in nested PCR.

To obtain a fragment of the *dsrA* gene of a size suitable for DGGE analysis, a nested PCR approach was undertaken. The 1.9-kb product from the initial PCR amplification was diluted 1:15 and used as template DNA in a subsequent PCR (with parameters as described above) using the *dsr* 1F primer (see above) with the addition of a GC clamp on the 5′ end (5′-CGCCCGCCGCGCCCC GCGCCCGTCCCGCCGCCCCCGCCCC-3′) and the 5R primer (5′-TGCCGA GGAGAACGATGTC-3′) (30). Amplification products were analyzed on a 2% agarose gel to ensure correct size (243 bp) and eluted from the agarose by using the GENECLEAN spin kit (Qbiogene) for DGGE analysis.

DGGE and sequencing. Four gel-purified *dsrA* PCR mixtures were combined (for a total volume of 200 μl) and concentrated to 10 μl in a DNA SpeedVac (Savant, Farmingdale, NY) for loading onto a DGGE gel. Samples were electrophoresed on a 10% acrylamide-bisacrylamide gel prepared from 40% acrylamide-bisacrylamide (38.93 g acrylamide and 1.07 g bisacrylamide dissolved in 100 ml double-distilled water) with a 30% to 90% denaturant range (100% denaturant is 7 M urea and 40% [vol/vol] formamide) at 130 V for 8 h using a DCode universal mutation detection system (Bio-Rad, Hercules, CA). DGGE gels were stained using SYBRI-green (Sigma) and visualized on a UV transilluminator. Bands were excised from the gel and eluted in tissue culture water at 4°C overnight. The eluted DNA was then reamplified using the *dsrA* primer set (1F/5R) and cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA). Sequences of the *dsrA* inserts were generated using vector primers (M13F and M13R; Invitrogen, Carlsbad, CA) and the ThermoSequenase cycle sequencing kit (USB, Cleveland, OH).

Sequence analysis. Similarity to *dsrA* sequences from cultured SRB was determined using the BlastX function of BLAST (2). Lake Fryxell *dsrA* clone sequences were manually aligned to *dsrA* sequences from known SRB obtained from GenBank using the computer program SeqApp (8). The nucleotide alignment was used to create phylogenetic trees by using the PAUP* software package (27). Tree topology was determined using the distance optimality criterion with a neighbor-joining algorithm and the maximum likelihood option using 215 nucleotide positions. The overall tree topology was constrained to the topology of a backbone phylogenetic tree generated using the entire *dsrAB* gene sequences of cultured SRB. This technique ensured that branching patterns were more consistent with trees generated based on the entire *dsrAB* operon and that biases imposed from phylogenetic analyses of shorter sequences were less evident in the resulting tree.

GenBank accession numbers of sequences used in *dsr* alignment and phylogenetic analyses are as follows: *Archaeoglobus fulgidis*, ARFDSRAB; *Desulfomicrobium baculatum*, AB061530; *Desulfomicrobium escambiense*, AB061531; *Desulfobacter vibrioformis*, AJ250472; *Desulfovibrio termitidis*, AB061542; *Desulfovibrio longus*, AB061540; *Desulfovibrio* sp. strain TBP-1, AF327307; *Desulfotomaculum thermosapovorans*, AF271769; *Desulfosarcina variabilis*, AF360643; *Desulfosarcina* sp. strain CME1, AF360645; *Thermodesulforhabdus norvegica*, TN0277293; *Desulfovibrio piger*, AB061534; *Desulfovibrio desulfuricans*, AF273034; *Desulfovibrio cuneatus*, AB061537; *Desulfofaba gelida*, *Desulfomusa hansenii*, AF419393; *Desulfotignum balticum*, AF420287; *Desulfococcus multivorans*, DMU58126; *Desulfonema limicola*, U58128; *Desulfobotulus sapovorans*, DSU58120; *Desulfobulbus rhabdoformis*, DRH250473; *Desulfofustis glycolicus*, AF418191; *Desulforhopalus vacuolatus*, AF334594; *Desulfotomaculum putei*, AF273032; *Thermodesulfobacterium commune*, AF271771; *Desulfovirga adipica*, AF334591; *Desulforhopalus singaporensis*, AF418196; *Desulfobacterium autotrophicum*, AF418182; *Desulfitobacterium dehalogenans*, AF337903; *Thermodesulfovibrio yellowstonii*, U58122; and *Desulfotomaculum ruminis*, U58118.

Nucleotide sequence accession numbers. Sequences generated in this study were deposited in GenBank under accession numbers AY273266 to AY273292, AY642269 to AY642278, and AY703988.

RESULTS

Phylogenetic evidence for sulfate-reducing bacteria in Lake Fryxell from 16S rRNA gene analyses. In preliminary investigations of the SRB population in Lake Fryxell, genomic DNAs obtained from various lake depths (8, 9, 11, 14, and 17 m) as well as from a sediment grab sample were subjected to PCR amplification using 16S rRNA SRB group-specific primers (7). The primer set specific for the *Desulfobulbus* group yielded positive results in the 8-m and sediment samples (Fig. 1). By contrast, 16S primer sets specific for the *Desulfotomaculum* and *Desulfobacterium* groups yielded no positive results. The *Desulfobacter*-specific primer set yielded positive results in the 9-m, 11-m, 14-m, 17-m, and sediment samples, although the 14-m, 17-m, and sediment samples were only faintly positive (Fig. 1). The 16S primer sets targeting the *Desulfovibrio* group and the group that includes the genera *Desulfococcus*, *Desulfonema*, and *Desulfosarcina* yielded positive reactions in all the samples tested, although the 8-m positive reaction for the *Desulfovibrio* primer set was faint (Fig. 1). All positive reactions yielded the expected band size (7) for the primer set. Negative (no-DNA) controls were electrophoresed on a separate gel (not shown) and yielded no evidence of DNA contamination. From these data it was clear that several phylogenetic groups of SRB inhabit Lake Fryxell.

Evidence for sulfate-reducing bacteria in Lake Fryxell from the metabolic gene *dsrA*. To further analyze the SRB population in Lake Fryxell, genomic DNAs from the same five depths plus sediment were amplified using the *dsr* primer set 1F/4R (30). All samples tested yielded a positive result, with the 8-m reaction yielding only a faint positive, as also occurred with universal bacterial 16S rRNA gene amplification (data not shown). To obtain a gene fragment suitable for DGGE analysis, a 1.9-kbp *dsrAB* PCR product was used as a template in a PCR utilizing the *dsrA* primers 1F and 5R. As expected, this amplification yielded a 243-bp fragment (30). Subsequent DGGE analysis of these fragments revealed multiple *dsrA*

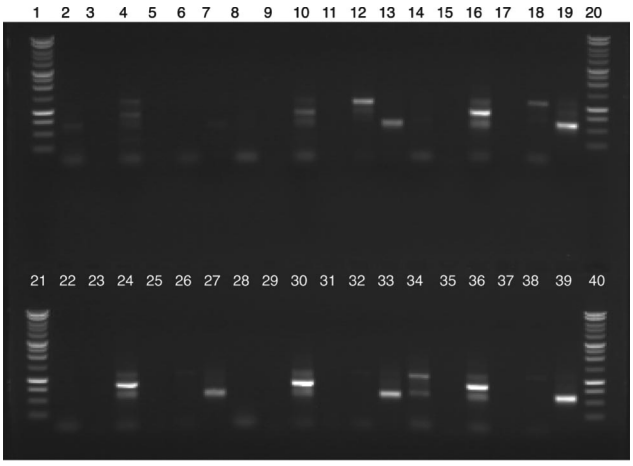

FIG. 1. PCR using SRB genus-specific 16S rRNA gene primer sets on Lake Fryxell genomic DNA samples. Lanes: 1, 1-kb ladder; 2, 8 m, *Desulfobulbus*; 3, 8 m, *Desulfobacterium*; 4, 8 m, *Desulfococcus-Desulfonema-Desulfosarcina* group; 5, 8 m, *Desulfotomaculum*; 6, 8 m, *Desulfobacter*; 7, 8 m, *Desulfovibrio*; 8, 9 m, *Desulfobulbus*; 9, 9 m, *Desulfobacterium*; 10, 9 m, *Desulfococcus-Desulfonema-Desulfosarcina* group; 11, 9 m, *Desulfotomaculum*; 12, 9 m, *Desulfobacter*; 13, 9 m, *Desulfovibrio*; 14, 11 m, *Desulfobulbus*; 15, 11 m, *Desulfobacterium*; 16, 11 m, *Desulfococcus-Desulfonema-Desulfosarcina* group; 17, 11 m, *Desulfotomaculum*; 18, 11 m, *Desulfobacter*; 19, 11 m, *Desulfovibrio*; 20, 1-kb ladder; 21, 1-kb ladder; 22, 14 m, *Desulfobulbus*; 23, 14 m, *Desulfobacterium*; 24, 14 m, *Desulfococcus-Desulfonema-Desulfosarcina* group; 25, 14 m, *Desulfotomaculum*; 26, 14 m, *Desulfobacter*; 27, 14 m, *Desulfovibrio*; 28, 17 m, *Desulfobulbus*; 29, 17 m, *Desulfobacterium*; 30, 17 m, *Desulfococcus-Desulfonema-Desulfosarcina* group; 31, 17 m, *Desulfotomaculum*; 32, 17 m, *Desulfobacter*; 33, 17 m, *Desulfovibrio*; 34, sediment, *Desulfobulbus*; 35, sediment, *Desulfobacterium*; 36, sediment, *Desulfococcus-Desulfonema-Desulfosarcina* group; 37, sediment, *Desulfotomaculum*; 38, sediment, *Desulfobacter*; 39, sediment, *Desulfovibrio*; 40, 1-kb ladder.

phylotypes at each depth in Lake Fryxell (data not shown). All DGGE bands were extracted and analyzed.

Sequence analysis of the retrieved *dsrA* DGGE bands resulted in seven *dsr* clone groups (groups A to G) (Fig. 2). Groups A and C were the most diverse, containing between them 18 of the 25 total phylotypes retrieved (Fig. 2). Group A clones exhibited *dsrA* sequence similarities ranging from 84.6 to 89% to known species of the genus *Desulfovibrio*, with *dsrA* from *Desulfovibrio termitidis* being the closest of cultured relatives (Fig. 2). Clone group C was only distantly related to the *Desulfococcus-Desulfonema-Desulfosarcina* group of SRB (Fig. 2), with sequence similarities ranging from 66.1 to 73.8%. Group D clones formed a small but distinct sister group to group C clones and exhibited distant sequence similarity (75.2 to 77.1%) to *dsrA* from *Desulfosarcina variabilis* (Fig. 2).

Clone group E showed low sequence similarity (67.6 to 69.7%) to *dsrA* from *Desulfobulbus rhabdoformis* (Fig. 2). Although group E clustered with the *Desulfobulbus* group on the *dsrA* tree (Fig. 2), the two group E sequences were slightly more similar in sequence (69.6 to 71%) to the *dsrA* gene of *Desulfovibrio longus*. However, despite employing multiple treeing options, including removal of the backbone constraint tree and/or removal and replacement of relevant taxa, the position of this group within the *dsrA* tree never changed.

Clone groups F and G contained deeply branching SRB. Group F clones showed distant sequence similarity (63.1 to 63.9%) to *dsrA* from *Thermodesulforhabdus norvegica*, while group G clones showed sequence similarity to various *dsrA* genes (Fig. 2). For example, clone LFdsrC11 showed 61.2% similarity to *dsrA* from *Desulfotomaculum putei*, a gram-positive sulfate-reducing bacterium, whereas clone LFdsrC24 showed 67.6% similarity to *dsrA* from *Desulfovibrio* sp. strain PT-2 and 69.5% similarity to *dsrA* from *Desulfovibrio desulfuricans*, genes that are scattered on the *dsr* tree (Fig. 2). Group B was represented by only a single *dsrA* sequence and showed distant sequence similarity to *Desulfomusa hansenii* (74.5%) and to *Desulfofaba gelida* (68.6%), a psychrophilic sulfate-reducing bacterium (12, 13) (Fig. 2).

Distribution of *dsrA* phylotypes with depth in Lake Fryxell. Lake Fryxell is highly stratified with respect to oxygen, sulfide, sulfate, and several other parameters (Fig. 3). Since genomic DNA was obtained from water taken at different depths, the distribution of *dsrA* phylotypes with respect to these physiochemical variables could be tracked. Some *dsrA* phylotypes, such as those in group A, were distributed throughout the water column, whereas others were found to be distinctly stratified (Fig. 3). Group A was the most widely distributed phylotype, being present at all depths tested, including 8 m, where no other phylotypes were detected. However, the highest diversity of group A phylotypes was at 14 m (Fig. 3). Groups D and F, although not highly diverse, were present both in oxic waters (9 m) and in the sediments; one group D sequence was obtained at 14 m as well, the peak of group A diversity (Fig. 3). Group G *dsrA* clones were present only in anoxic waters, but in both low-sulfide and high-sulfide regions of the water column (Fig. 3). The remaining clone groups (B, C, and E) were detected only in oxic to barely anoxic waters. Clone group B was the most extreme in this regard, appearing only in the 9-m sample. At this depth oxygen is readily detectable, E_h is still very positive, and sulfide is undetectable (Fig. 3).

Enrichment culture evidence for sulfate-reducing bacteria in Lake Fryxell. Water column samples collected from various depths of Lake Fryxell were sealed in completely filled 125-ml crimp-top serum bottles immediately upon retrieval and kept cold but unfrozen for transport from the field to Crary Lab at McMurdo. The bottles were then amended with 20 mM lactate and 50 mM Na_2SO_4 and incubated at 4°C. Visible turbidity was achieved with the 11-, 14-, and 17-m samples within 3 to 4 weeks. Growth was very weak in a 9-m enrichment established in the same way.

Subcultures from the initial enrichments were transferred into a 10 mM MOPS-buffered defined SRB medium (pH 7.2) containing lactate and sulfate. Although growth occurred initially, the cultures could not be repeatedly transferred in this medium. However, supplementing the medium with 5 mM pyruvate significantly improved growth and allowed the cultures to be transferred repeatedly. Microscopic observations of the enrichments showed that the 11- and 14-m cultures contained at least two morphologically distinct organisms, while the 17-m culture appeared more homogeneous (Fig. 4). Vibrio-shaped organisms dominated all cultures. The 11- and 14-m cultures contained both long and short vibrios (Fig. 4A

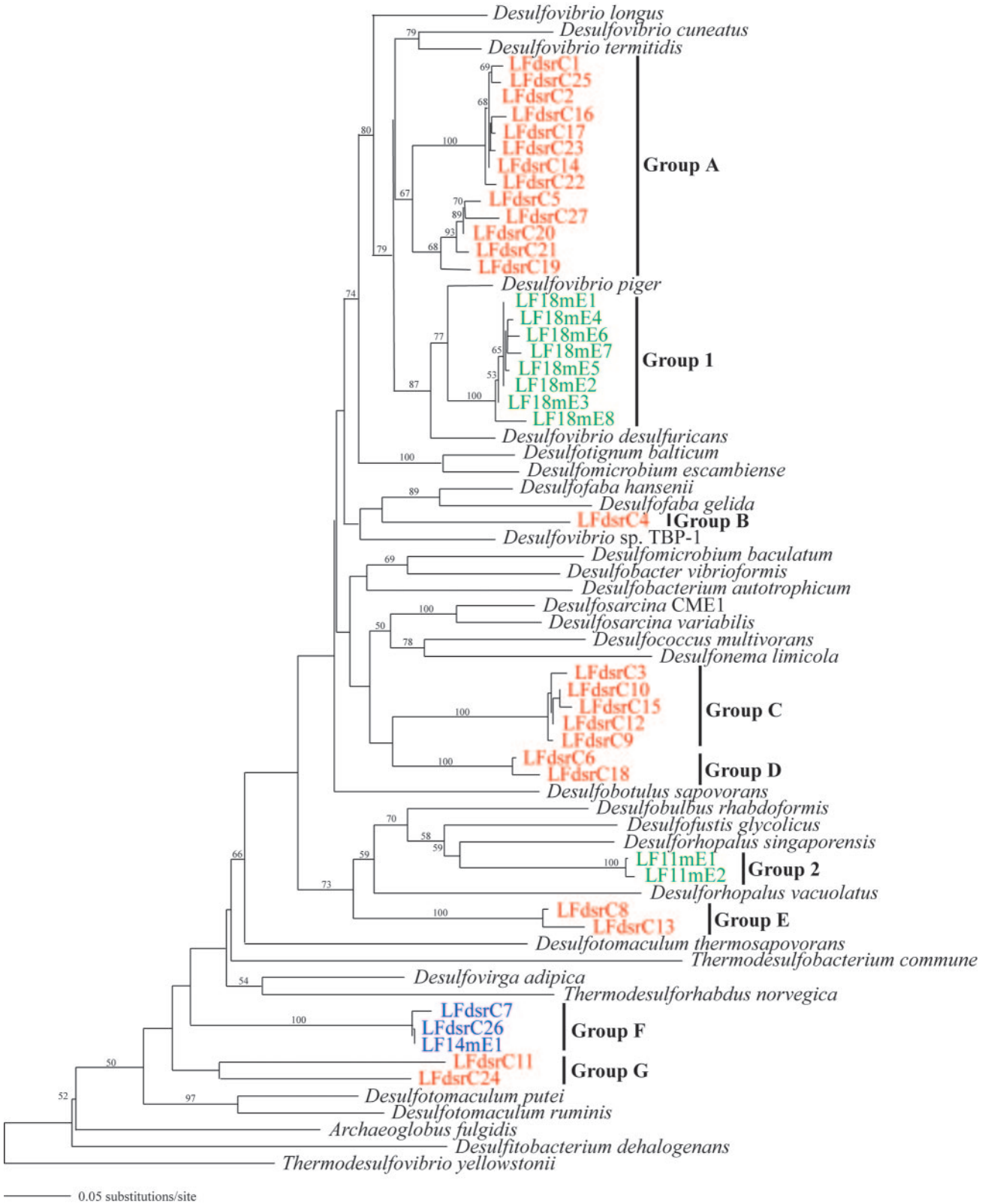

FIG. 2. Rooted neighbor-joining distance tree based on 215 nucleotide positions of the *dsrA* gene, using *Thermodesulfovibrio yellowstonii* as the outgroup. All designations beginning with LF are from Lake Fryxell. Environmental clone groups are represented in red, enrichment culture clones are represented in green, and the one group containing both enrichment and environmental clones is shown in blue. Bootstrap values of >50% are indicated.

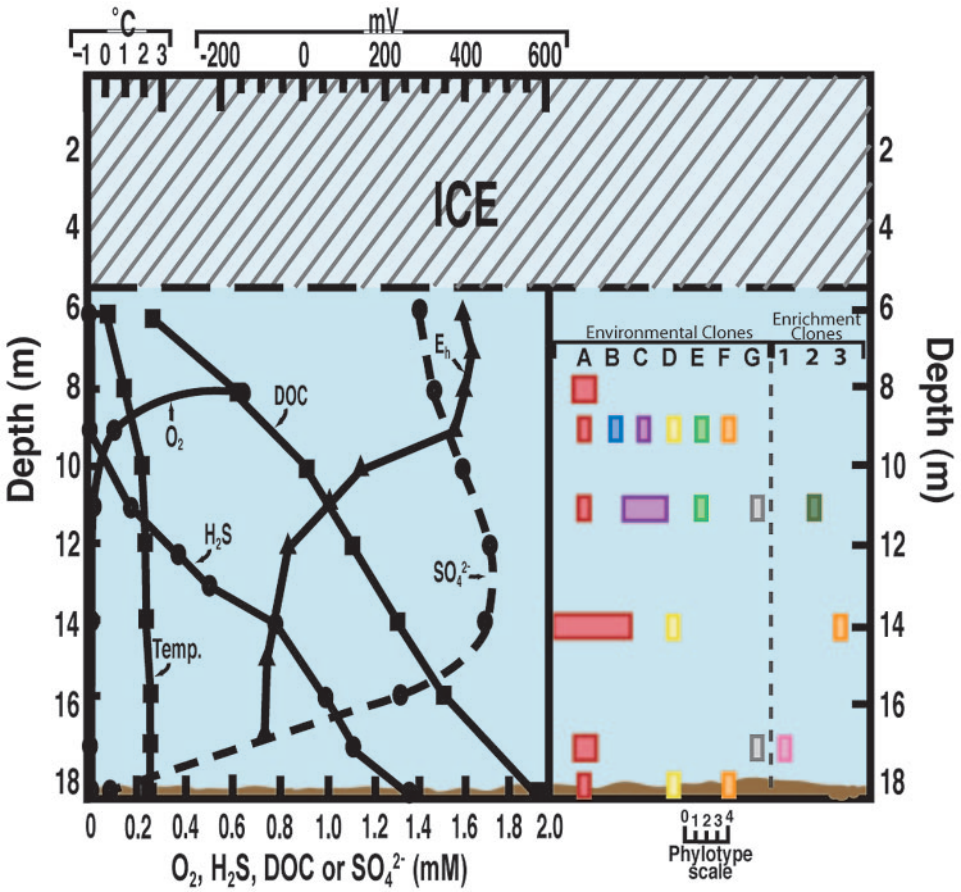

FIG. 3. Profile of Lake Fryxell, summarizing key physiochemical parameters (left) and the locations with depth of different groups of *dsrA* phylotypes in the water column (right). The widths of the colored boxes are proportional to the number of different phylotypes residing at a particular depth according to the scale bar on the bottom of the figure. Figure 2 shows the phylotypes in each group. The 14-m group 3 enrichment clone is identical in *dsrA* sequence to the group F environmental clone, LFdsrC26, found at 9 m and in the sediment. DOC concentrations are expressed as millimolar carbon (adapted from data in reference 29).

and B). In the 11-m enrichment, nearly equal numbers of a long, thin vibrio and a shorter, thicker vibrio were present (Fig. 4A). In the 14-m cultures, the latter organism was present in large numbers while the thinner organism was present in only low numbers (Fig. 4B). In contrast, the 17 m cultures appeared to be nearly axenic, as the shorter, thicker vibrio dominated the enrichments (Fig. 4C).

DNAs obtained from all enrichments except for that at 9 m yielded *dsrA* amplification products, and the products clustered into three groups. Two of the groups contained *dsrA* sequences that did not match any of the seven groups defined by community sampling (Fig. 2 and 3). Group 1 enrichment sequences were shotgun clones of *dsrA* PCR amplification products obtained from the 17-m enrichment. This group exhibited 91 to 93% similarity to the *dsrA* gene of *Desulfovibrio piger* (recent reclassification of *Desulfomonas pigra*) (16) (Fig. 2 and 3). Group 2 enrichment sequences from the 11-m sample yielded two clone groups showing ~75% similarity to the *dsrA* gene of the morphologically distinct *Desulforhopalus singaporensis*, a taurine-fermenting SRB that produces cell surface spinae (15). Finally, the single 14-m clone group matched an environmental sequence, being identical to LFdsrC26, a group F clone (Fig. 2 and 3). Although group F phylotypes were not specifically detected at 14 m by community sampling, they were detected in samples taken both above and below 14 m (Fig. 3). Thus, it is likely that this group is distributed throughout the water column of Lake Fryxell.

DISCUSSION

Biological sulfate reduction in Lake Fryxell has previously been suspected (9, 25), but these studies focused on the biogeochemistry of the process rather than the diversity of the organisms involved. In one isolation study, a strain of *Desulfovibrio*, designated strain FESu, was isolated from Lake Fryxell sediment and described (20). However, despite nearly freezing in situ temperatures, strain FESu was enriched at 25°C, grew optimally at the same temperature, and had an upper limit for growth of just above 40°C; the organism is therefore a mesophile (20).

In a more extensive exploration of the diversity of SRB in the water column and sediment of Lake Fryxell, we have employed sequence analysis of the *dsrA* gene from community DNA along with enrichment studies using in situ enrichment temperatures. In our study, initial evidence for the presence of SRB in Lake Fryxell came from community sampling of 16S

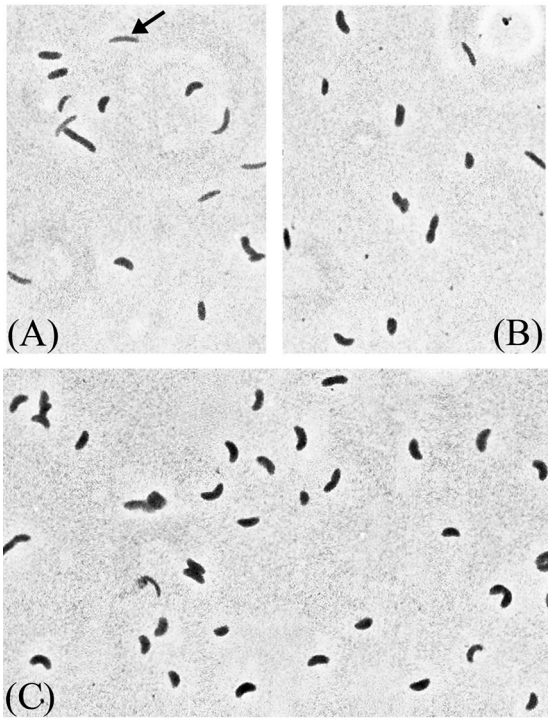

FIG. 4. Phase-contrast photomicrographs showing cells of SRB from enrichment cultures established with (A) 11-m, (B) 14-m, and (C) 17-m Lake Fryxell water. Note the gradual loss of the longer, thinner rod (arrow in panel A) with depth, possibly because of a higher sensitivity of this organism to sulfide. Figure 2 shows the phylogenetic positions of *dsrA* clones obtained from each enrichment, as follows: 11 m, group 2; 14 m, LF14mE1 (group F); 17 m, group 1.

rRNA genes. Phylogenetic analysis indicated that at least four of the six major phylogenetic groups of SRB (*Desulfovibrio*, *Desulfosarcina* group, *Desulfotomaculum*, *Desulfobulbus*, *Desulfobacter*, and *Desulfobacterium*) were present in Lake Fryxell. The only groups that could not be detected by 16S rRNA gene analysis were the *Desulfobacterium* and gram-positive *Desulfotomaculum* groups. This result was confirmed by *dsrA* analyses, except for a single sequence obtained that was distantly related to the gram-positive *Desulfotomaculum* group. However, none of the enrichment cultures showed *Desulfotomaculum*-like organisms.

Amplification of *dsrA* revealed the presence of SRB throughout the water column and in the surface sediments of Lake Fryxell. However, the *dsrA* sequences obtained exhibited only distant similarity to *dsrA* sequences from cultured SRB of the delta *Proteobacteria*. Moreover, some Lake Fryxell *dsrA* sequences showed sequence similarities to a deeply branching clade of SRB containing no cultured representatives. Collectively, the *dsrA* results thus suggest that novel lineages of SRB inhabit Lake Fryxell. However, in a caveat to our *dsr* work, we acknowledge the potential for lateral transfer of the *dsrAB* operon (26, 30); this constrains our use of *dsrA* to characterize natural communities of SRB in a robust phylogenetic framework. Despite this, in many cases our *dsrA* results showed such distant sequence similarity to cultured SRB that it would be indeed surprising if the sources of these *dsrA* genes were not themselves phylogenetically distinct. Also, considering the environmental conditions in Lake Fryxell, it is likely that at least some of these lineages will turn out to be cold-active species of SRB. Stable enrichment cultures for SRB established at 4°C support this conclusion.

Although *dsrA* sequences could be retrieved from all lake depths tested in this study, several clone groups clustered specifically at particular depths. Lake Fryxell displays sharp gradients of oxygen, E_h, sulfide, sulfate, and dissolved organic carbon (DOC) (Fig. 3). Oxygen levels are supersaturated in the upper depths because oxygen produced by phytoplankton and cyanobacteria becomes trapped under the ice. Conditions become anoxic at about 10 m, where detectable sulfide first appears, increasing to nearly 1.5 mM sulfide near the sediments (Fig. 3). DOC also increases with depth, while the temperature remains near 0°C throughout the water column (Fig. 3). The distribution of *dsr* phylotypes observed in our study may therefore be a function of the different physiochemical conditions present at different depths in Lake Fryxell, especially in regard to oxygen and sulfide. Group A phylotypes detected in the oxic zone included *Desulfovibrio* species, many of which show a higher tolerance for oxygen than do other SRB (26). In fact, at least one species of *Desulfovibrio*, *D. oxyclinae*, can actually respire O_2 (23). Therefore, it is distinctly possible that species of SRB present at 9-m depth or above in Lake Fryxell physiologically resemble *D. oxyclinae*. Group B organisms in particular should fit this pattern, since they were undetectable in the anoxic zones of the water column.

Sulfate reaches a maximum concentration of 1.72 mM just below the oxycline (13 m) of Lake Fryxell and falls off slowly with depth. However, sulfate decreases sharply from 1.17 mM at 17 m to just 0.34 mM at 17.5 m (Fig. 3). This suggests that maximum rates of sulfate reduction are occurring near the sediments. If this is true, representatives of groups A, D, F, and G are likely the most abundant and physiologically active of the SRB in Lake Fryxell. The high levels of DOC and low E_h in the deeper waters of Lake Fryxell likely stimulate sulfate reduction in this narrow zone near the sediments.

In contrast to group A phylotypes, which are present mainly in regions of the Lake Fryxell water column where sulfide levels are high, phylotypes in groups B, C, and E are present where sulfide is undetectable. This suggests that species in these groups may be quantitatively and/or physiologically insignificant in terms of overall sulfate reduction in Lake Fryxell. However, this conclusion must be tempered by the known presence of other sulfur-cycling organisms in the oxycline of Lake Fryxell. In particular, sulfur-oxidizing chemolithotrophic bacteria and purple phototrophic bacteria are present in the upper regions of the Lake Fryxell water column (10, 11; W. M. Sattley, E. A. Karr, L. A. Achenbach, and M. T. Madigan, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. I-129, 2003). Thus, it is possible that sulfide produced by organisms in groups B, C, and E is rapidly oxidized by sulfide-oxidizing prokaryotes coexisting with them in a sulfuretum. In situ activity measurements of sulfate reduction are needed to resolve this question and identify the most active zone(s) of sulfidogenesis in Lake Fryxell.

Enrichment cultures from several lake depths exhibited clear signs of sulfate reduction. These included (i) the ability to grow and produce sulfide in a lactate-pyruvate mineral medium containing only vitamin levels of yeast extract and (ii) the

amplification of *dsrA*. Cloning and sequencing of the *dsrA* amplification products from the enrichments yielded two groups of *dsrA* clones that were not detected in environmental clones, a phenomenon widely observed in microbial community profiles (4). However, of all the *dsrA* sequences generated in this study, group 1 enrichment clones yielded the highest similarity to the *dsrA* gene of a cultured SRB (>90% to *Desulfovibrio piger* [Fig. 2]). By contrast, group 2 clones obtained from water at 11 m were not represented by any of the environmental clones and showed only weak similarity (~75%) to cultured SRB. This suggests that the 11-m enrichments contain SRB that are not close relatives of known SRB. Finally, the sequence match to a group F environmental *dsrA* clone (Fig. 2) that emerged from the 14-m enrichment culture indicates that putatively deeply branching SRB can be cultured from Lake Fryxell.

In conclusion, it is clear from our combined molecular and cultural work that a phylogenetically and possibly physiologically diverse group of SRB inhabit Lake Fryxell. An understanding of the diversity and distribution of SRB in Lake Fryxell is an important prerequisite to understanding sulfur cycling in constantly cold freshwater lakes. Further investigations into the bacterial community of Lake Fryxell are also important for understanding ecosystems that are entirely microbial. Lake Fryxell is characterized by a permanent ice cover, an absence of wind mixing and higher organisms, and seasonal decoupling, with 5 months of darkness per year (24, 28). The microflora of Lake Fryxell has undoubtedly been shaped by these unusual limnological conditions, and the biodiversity of SRB that we report here exemplifies this.

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REFERENCES

1. **Achenbach, L. A., J. Carey, and M. T. Madigan.** 2001. Photosynthetic and phylogenetic primers for detection of anoxygenic phototrophs in natural environments. Appl. Environ. Microbiol. **67:**2922–2926.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **American Public Health Association.** 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Washington, D.C.
4. **Brambilla, E., H. Hippe, A. Hagelstein, B. J. Tindall, and E. Stackebrandt.** 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles **5:**23–33.
5. **Castro, H., K. R. Reddy, and A. Ogram.** 2002. Composition and function of sulfate-reducing prokaryotes in eutrophic and pristine areas of the Florida Everglades. Appl. Environ. Microbiol. **68:**6129–6137.
6. **Chang, Y.-J., A. D. Peacock, P. E. Long, J. R. Stephen, J. P. McKinley, S. J. Macnaughton, A. K. M. A. Hussain, A. M. Saxton, and D. C. White.** 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. Appl. Environ. Microbiol. **67:**3149–3160.
7. **Daly, K., R. J. Sharp, and A. J. McCarthy.** 2000. Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria. Microbiology **146:**1693–1705.
8. **Gilbert, D. G.** 1993. SeqApp 1.9a157 ed. Biocomputing Office, Biology Department, Indiana University, Bloomington.
9. **Howes, B. L., and R. L. Smith.** 1990. Sulfur cycling in a permanently ice covered amictic Antarctic lake, Lake Fryxell. Ant. J. U.S. **25:**230–233.
10. **Jung, D. O., L. A. Achenbach, E. A. Karr, S. Takaichi, and M. T. Madigan.** 2004. A gas vesiculate planktonic strain of the purple nonsulfur bacterium *Rhodoferax antarcticus* isolated from Lake Fryxell, Dry Valleys, Antarctica. Arch. Microbiol. **182:**236–243.
11. **Karr, E. A., W. M. Sattley, D. O. Jung, M. T. Madigan, and L. A. Achenbach.** 2003. Remarkable diversity of phototrophic purple bacteria in a permanently frozen Antarctic lake. Appl. Environ. Microbiol. **69:**4910–4914.
12. **Knoblauch, C., B. B. Jørgensen, and J. Harder.** 1999. Community size and specific sulfate reduction rates of psychrophilic sulfate-reducing bacteria in arctic sediments: evidence for high activity at low temperature. Appl. Environ. Microbiol. **65:**4230–4233.
13. **Knoblauch, C., K. Sahm, and B. B. Jørgensen.** 1999. Psychrophilic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov., and *Desulfotalea arctica* sp. nov. Intl. J. Syst. Bacteriol. **49:**1631–1643.
14. **Lawrence, M. J. F., and C. H. Hendy.** 1985. Water column and sediment characteristics of Lake Fryxell, Taylor Valley, Antarctica. New Zeal. J. Geol. Geophys. **28:**543–552.
15. **Lie, T. J., M. L. Clawson, W. Godchaux, and E. R. Leadbetter.** 1999. Sulfidogenesis from 2-aminoethanesulfonate (taurine) fermentation by a morphologically unusual sulfate-reducing bacterium, *Desulforhopalus singaporensis* sp. nov. Appl. Environ. Microbiol. **65:**3328–3334.
16. **Loubinoux, J., F. M. A. Valente, I. A. C. Pereira, A. Costa, P. A. D. Grimont, and A. E. Le Faou.** 2002. Reclassification of the only species of the genus *Desulfomonas*, *Desulfomonas pigra*, as *Desulfovibrio piger* comb. nov. Int. J. Syst. Evol. Microbiol. **52:**1305–1308.
17. **Minz, D., J. L. Flax, S. J. Green, G. Muyzer, Y. Cohen, M. Wagner, B. E. Rittmann, and D. A. Stahl.** 1999. Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl. Environ. Microbiol. **65:**4666–4671.
18. **Perez-Jimenez, J. R., and L. J. Kerkhof.** 2005. Phylogeography of sulfate-reducing bacteria among disturbed sediments, disclosed by analysis of the dissimilatory sulfite reductase genes (*dsrAB*). Appl. Environ. Microbiol. **71:**1004–1011.
19. **Ravenschlag, K., K. Sahm, C. Knoblauch, B. B. Jørgensen, and R. I. Amann.** 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine Arctic sediments. Appl. Environ. Microbiol. **66:**3592–3602.
20. **Rees, G. N., P. H. Janssen, and C. G. Harfoot.** 1986. An unusual strain of *Desulfovibrio* sp. from an Antarctic lake. FEMS Microbiol. Lett. **37:**363–366.
21. **Roberts, E. C., J. Laybourn-Parry, D. M. McKnight, and G. Novarinos.** 2000. Stratification and dynamics of microbial loop communities in Lake Fryxell, Antarctica. Freshwat. Biol. **44:**649–661.
22. **Sahm, K., C. Knoblauch, and R. Amann.** 1999. Phylogenetic affiliation and quantification of psychrophilic sulfate-reducing isolates in marine Arctic sediments. Appl. Environ. Microbiol. **65:**3976–3981.
23. **Sigalevich, P., and Y. Cohen.** 2000. Oxygen-dependent growth of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in coculture with *Marinobacter* sp. strain MB in an aerated sulfate-depleted chemostat. Appl. Environ. Microbiol. **66:**5019–5023.
24. **Smith, R. L., and B. L. Howes.** 1990. Bacterial biomass and heterotrophic activity in the water column of an amictic Antarctic lake. Ant. J. U.S. **25:**233–235.
25. **Smith, R. L., L. G. Miller, and B. L. Howes.** 1993. The geochemistry of methane in Lake Fryxell, an amictic, permanently ice-covered, Antarctic lake. Biogeochemistry **21:**95–115.
26. **Stahl, D. A., S. Fishbain, M. Klein, B. J. Baker, and M. Wagner.** 2002. Origins and diversification of sulfate-respiring microorganisms. Antonie Leeuwenhoek **81:**189–195.
27. **Swofford, D. L.** 1999. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods), version 4.0. Sinauer Associates, Inc., Sunderland, Mass.
28. **Takacs, C. D., and J. C. Priscu.** 1998. Bacterioplankton dynamics in the McMurdo Dry Valley lakes, Antarctica: production and biomass loss over four seasons. Microb. Ecol. **36:**239–250.
29. **Takacs, C. D., J. C. Priscu, and D. M. McKnight.** 2001. Bacterial dissolved organic carbon demand in McMurdo Dry Valley lakes, Antarctica. Limnol. Oceanogr. **46:**1189–1194.
30. **Wagner, M., A. J. Roger, J. L. Flax, G. A. Brusseau, and D. A. Stahl.** 1998. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J. Bacteriol. **180:**2975–2982.
31. **Wahlund, T. M., R. W. Castenholz, C. R. Woese, and M. T. Madigan.** 1991. A thermophilic green sulfur bacterium from New Zealand hot springs, *Chlorobium tepidum*, nov. sp. Arch. Microbiol. **156:**81–91.
32. **Widdel, F., and F. Bak.** 1992. Gram-negative mesophilic sulfate-reducing bacteria, p. 3352–3378. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.