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Sulfate-Reducing Bacteria and Their Activities in Cyanobacterial Mats of Solar Lake (Sinai, Egypt)

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The sulfate-reducing bacteria within the surface layer of the hypersaline cyanobacterial mat of Solar Lake (Sinai, Egypt) were investigated with combined microbiological, molecular, and biogeochemical approaches. The diurnally oxic surface layer contained between 10^6 and 10^7 cultivable sulfate-reducing bacteria ml^{-1} and showed sulfate reduction rates between 1,000 and 2,200 nmol ml^{-1} day^{-1}, both in the same range as and sometimes higher than those in anaerobic deeper mat layers. In the oxic surface layer and in the mat layers below, filamentous sulfate-reducing *Desulfonema* bacteria were found in variable densities of 10^4 to 10^6 cells ml^{-1}. A *Desulfonema*-related, diurnally migrating bacterium was detected with PCR and denaturing gradient gel electrophoresis within and below the oxic surface layer. Facultative aerobic respiration, filamentous morphology, motility, diurnal migration, and aggregate formation were the most conspicuous adaptations of Solar Lake sulfate-reducing bacteria to the mat matrix and to diurnal oxygen stress. A comparison of sulfate reduction rates within the mat and previously published photosynthesis rates showed that CO_2 from sulfate reduction in the upper 5 mm accounted for 7 to 8% of the total photosynthetic CO_2 demand of the mat.

Sulfate-reducing bacteria are universally distributed in marine sediments and microbial mats. Sulfate reduction is the dominant anaerobic biomineralization pathway in marine sediments, quantitatively equivalent to or exceeding aerobic respiration (44). In organic-carbon-depleted marine sediments of the deep-sea abyssal plain, the zone of sulfate reduction extends over a scale of many meters but shows a low biomass of sulfate-reducing bacteria and low activities (8, 40). With an increasing supply of organic nutrients by primary production and sedimentation, population densities and activities of sulfate-reducing bacteria increase. Since easily degradable, energy-rich substrates are rapidly consumed before reaching deeper sediment layers, numbers and activity of sulfate-reducing bacteria generally increase towards higher sediment layers (44), including the oxic surface layer (42). In marine sediments of the Kattegat (Denmark), high numbers of sulfate-reducing bacteria and high sulfate reduction rates were found in oxic sediment layers close to the sediment surface (46). In freshwater sediments of Lake Constance, the highest sulfate reduction rates were found in the upper two 1-cm layers of the sediment, coinciding with high population densities of sulfate-reducing bacteria (5, 6). In sediments of the oligotrophic freshwater lake Stechlinsee (Germany), conspicuous population and activity maxima were located at the oxic-anoxic interface (69, 70).

Cyanobacterial mats provide the most extreme examples of sulfate reduction coexisting with oxic conditions. Here, photosynthetic oxygen synthesis, sulfide production from sulfate reduction, and microbial sulfide oxidation overlap and create steep, opposing gradients of oxygen and sulfide, which fluctuate in the rythm of daylight and night, with significant modulation by cloud cover and season (25, 75, 77). Oxygen-tolerant sulfate reduction has been demonstrated in cyanobacterial mats of temperate climates: in a cyanobacterial mat on the Frisian island of Texel, the densest populations of sulfate-reducing bacteria (10^8 cells ml^{-1}) occurred in the top 5-mm layer, which showed the highest organic matter content (76, 77). The sulfate reducer peak coincided with population peaks of phototrophic and nonphototrophic sulfur-oxidizing bacteria in the surface layer (76, 77). Under oxic conditions, the surface mat layer retained a sulfate reduction rate of 123 nmol ml^{-1} day^{-1}, compared to 567 nmol ml^{-1} day^{-1} under anoxia (76, 77). Hypersaline cyanobacterial mats in Mediterranean and subtropical areas with dry, sunny climates show very high sulfate reduction rates, above 1,000 nmol of SO_4^{2-} ml^{-1} day^{-1}, in their oxic surface layers (13, 31, 71). In hypersaline cyanobacterial mats of Solar Lake (Sinai, Egypt), a maximal sulfate reduction rate of 5,400 nmol of SO_4^{2-} ml^{-1} day^{-1} in the 0- to 5-mm surface layer coincided with a maximal cell density of 2.5×10^6 cells ml^{-1} (47). Sulfate reduction maxima above 10,000 nmol of SO_4^{2-} ml^{-1} day^{-1} have been found in the oxic surface layers of hypersaline cyanobacterial mats in Guerrero Negro, Mexico (9–11). These sulfate reduction rates, measured under oxic conditions during daytime, often exceed those observed at night under anoxia (9, 10). This phenomenon is explained in part by elevated temperatures during the day, since sulfate reduction rates generally show an Arrhenius-like temperature dependence with habitat- and substrate-related differences in apparent activation energies (45, 79). Ultimately, the high sulfate reduction rates in hypersaline cyanobacterial mats are driven by cyanobacterial photosynthetic production in situ (14, 15).

The benthic cyanobacterial mats of Solar Lake, a shallow, hypersaline, meromictic desert lake in the Sinai (Egypt), show oxygen-tolerant, photosynthesis-coupled sulfate reduction (15,

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TABLE 1. 16S rRNA primers and probes used in this study

Name	Position	Sequence	Comments (references)
Forward primer GM5 with 5′-GC-clamp[a]	341–357	5′-GC-clamp-CCTACGGGAGGCAGCAG-3′	Bacterial primer for 16S rRNA gene DGGE fragment amplification (60, 61)
Forward primer 385 with 5′-GC-clamp[a]	385–402	5′-GC-clamp-CCTGACGCAGC(G/A)ACGCCG-3′	To be used instead of GM5; selective but not specific for delta proteobacterial sulfate reducers (3, 63)
Reverse primer 907	926–907	5′-CCGTCAATTC(A/C)TTTGAGTTT-3′	Bacterial primer for 16S rRNA gene DGGE fragment amplification (61, 62)
Probe 657	657–676	5′-TTCCG(C/T)TTCCCTCTCCCATA-3′	16S rRNA probe for *Desulfonema* spp. (33)

[a] GC-clamp: 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3′ (61).

47). Sulfate-reducing bacteria in the surface layer have to tolerate oxygen exposure during daylight hours. In this study, we investigated the sulfate-reducing bacteria of Solar Lake cyanobacterial mats and their relation to photosynthetic oxygenation of the mat surface layer with microbiological, molecular, and biogeochemical approaches.

MATERIALS AND METHODS

Sampling for MPN counts and sulfate reduction rates. In November 1994, pieces of a laminated, undisturbed mat (approximately 50 by 70 cm, 10 cm thick) were harvested by hand from a depth of 0.6 to 0.7 m on the eastern bank of Solar Lake, in water with a salinity of 85‰. This mat resembled the deep, flat mat type of Solar Lake (48). The mat pieces were transported to the nearby H. Steinitz Marine Biology Laboratory of Eilat, Israel, and stored for the duration of the experiments (2 to 3 days) in a hypersaline pond with 90‰ salinity at a depth of 0.4 to 0.5 m. These mat samples were used for most-probable-number (MPN) counts of sulfate-reducing bacteria and for sulfate reduction rate measurements. The extent of the oxic zone in these Solar Lake mats and the mat slicing scheme were determined as follows. The steepest oxygen and sulfide gradients, and the maximal depth of the oxic zone, occur in Solar Lake mats between 10 am and 2 pm, centered at around 12 am (49, 65). Noontime light intensities on submerged Solar Lake mats reached 1,200 to 1,300 microeinsteins m^{-2} s^{-1} under low water levels of 10 to 20 cm in late summer and approximately 900 microeinsteins m^{-2} s^{-1} under a high water level of 80 cm in spring (49). Therefore, oxygen profiles were determined in the laboratory under steady-state conditions with a light intensity of 1,000 microeinsteins m^{-2} s^{-1}, as a close approximation to the noontime in situ light intensity at the sampling site at a depth of 60 to 70 cm. In two separate measurements, maximal oxygen concentrations of 500 to 600% air saturation occurred at a depth of 0.5 mm (100% = 158 μM O_2 at 22°C and 10‰ salinity). Oxygen decreased to 100% air saturation at 1.5 mm, to 10 to 30% at 2 mm, and to 0 at a depth of 2.2 to 2.3 mm (54). Thus, the upper 2-mm mat layer corresponded to the diurnally oxygenated zone at noon, the 2- to 4-mm layer included the oxycline, whereas the deeper layers (4 to 7 mm, 7 to 10, and 10 to 13 mm) remained permanently anaerobic. Mat cores for MPN counts were obtained with plastic corers (2-cm diameter) at noon and midnight, pushed out with a millimeter-graduated piston, and sliced with dissection blades into the following layers: 0 to 2, 2 to 4, 4 to 7, 7 to 10, and 10 to 13 mm. The rubber-like mat slices were homogenized in 9 volumes of Solar Lake water for 10 min with a 10-ml Potter-Elvehjem homogenizer with a Teflon-coated piston. In the absence of empirically optimized homogenization procedures (77) for the Solar Lake mat, homogenization was checked by microscopy: the cyanobacterial filaments which form the matrix of the Solar Lake mat were broken up to release single bacterial cells previously attached to the matrix. *Synechococcus* cells from the surface layer remained visible and intact. Triplicate MPN dilution series were inoculated with 1 ml of a 10-fold-diluted mat homogenate, equivalent to 0.1 ml of mat material, and subsequently diluted in eight 1:10 dilution steps. Standard MPN evaluation tables and 95% confidence intervals were used (4). MPN tubes were incubated in the dark at 20 to 25°C for a year to determine the final scores of extremely slow-growing *Desulfonema* bacteria. MPNs were scored positive when microbial growth, either as a turbid suspension or as bacterial clumps or filaments on the culture tube wall, coincided with sulfide production, as determined with the $CuSO_4$ test (81).

Sulfate reduction rate determinations. In parallel with the MPN samples, mat cores were harvested at the same time from the same mats for sulfate reduction rate measurements (37, 38). As for MPN determinations, mat pieces with a homogeneous, smooth surface were used and occasional blisters or ripples on the mat surface were avoided. Triplicate cores were injected vertically with a ^{35}S-sulfate radiotracer, sealed, and returned to the pond for 30 min of incubation under in situ temperature and light conditions. Subsequently, the cores were sliced in the same way as for the MPN determinations. Samples were fixed in zinc acetate and deep frozen before analysis (12).

Microbiological media. The salt base for MPN count media contained the following constituents per liter of distilled water: 40 g of NaCl, 5.67 g of $MgCl_2 \cdot 6H_2O$, 6.8 g of $MgSO_4 \cdot 7H_2O$, 1.47 g of $CaCl_2 \cdot 2H_2O$, 0.19 g of $NaHCO_3$, 0.66 g of KCl, and 0.09 g of KBr. Compared to normal artificial seawater, which contains 26.37 g of NaCl $liter^{-1}$, the NaCl concentration was increased to 40 g $liter^{-1}$, corresponding to the winter chlorinity (4%) of Solar Lake surface water (16). Medium for sulfate-reducing bacteria contained, per liter of this artificial Solar Lake water, the following: 1 ml of nonchelated trace element mixture no. 1, 1 ml of selenite-tungstate solution, 30 ml of a 1 M $NaHCO_3$ solution, 1 ml of a vitamin mixture, 1 ml of a thiamine solution, 1 ml of a vitamin B_{12} solution, and 7.5 ml of an Na_2S solution (81). The salt concentrations of the Solar Lake medium are higher than those of many standard brackish or marine sulfate reducer media and enabled the growth of *Desulfonema* bacteria, which require elevated Ca^{2+} and Mg^{2+} concentrations (81). Media were prepared anaerobically in a pressure-proof modified Erlenmeyer flask (81) and supplemented with either 20 mM lactate, 20 mM acetate, or 10 mM formate plus 2 mM acetate to account for nonautotrophic bacteria. These concentrations allow the growth of most lactate- and acetate-utilizing sulfate-reducing bacteria (81). Media were dispensed into glass culture tubes. Headspaces were gassed with a mixture of 90% (vol/vol) N_2 and 10% (vol/vol) CO_2 by using a gassing syringe in accordance with the Hungate technique, and the tubes were sealed with butyl rubber stoppers.

Sampling for nucleic acid extraction and total cell counts. Another sample set from May 1994, not identical to the November 1994 samples used for MPN and sulfate reduction rate determinations, was used for DNA extraction and analysis by denaturing gradient gel electrophoresis (DGGE). Solar Lake mat cores were taken in May 1994 from an undisturbed flat mat (approximately 0.4-m depth) from the eastern bank of Solar Lake (48). Mat pieces were transported to the nearby H. Steinitz Marine Biology Laboratory, Eilat, Israel, and stored for the duration of the experiments (2 to 3 days) in a hypersaline artificial pond near the laboratory. Oxygen profiles were determined under natural light with a Clark-type oxygen microsensor (64), and mat samples for 4,6-diamidino-2-phenylindole total cell counts were harvested and formaldehyde fixed as previously described (63). Three sample sets were taken over a diurnal course to record the changing day-night regimens of the mat: at 5 am, during night anoxia of the surface layer, and at 12 noon and 5 pm, during daytime oxygenation of the surface layer. Mats were sampled with a small metal core. A tightly fitting piston pushed the mat out at the head of the core, and a motile blade mounted on the head of the core sliced the mat into 1-mm layers. These samples were used for nucleic acid extraction and subsequent PCR and DGGE. The upper 5 mm of the 5 am mat and the upper 1 cm of the 5 pm mat were sampled in duplicates.

Nucleic acid extraction. Nucleic acids were extracted from the Solar Lake mat by hot phenol extraction. Mat slices (0.2 ml) were homogenized and mixed with the same volume of ice-cold AE buffer (20 mM Na-acetate, 1 mM EDTA, pH 5.5) and kept on ice. To each sample, 500 μl of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol; pH 5) and 5 μg of 25% sodium dodecyl sulfate were added. Phenolic and aqueous phases were vortexed for 1 min. After 5 min of incubation at 60°C in a water bath, the samples were cooled on ice and then centrifuged for 5 min at 4,000 × *g*. The aqueous phases were transferred to new vials containing 25 μl of a 2 M sodium acetate solution, pH 5.2. Contaminating proteins and lipids were removed by subsequent twofold extraction of the aequeous phase with 500 μl of phenol-chloroform-isoamyl alcohol. Nucleic acids were precipitated with 2.5 volumes of 96% (vol/vol) ethanol overnight at −70°C, followed by 10 min of centrifugation at 4,000 × *g*. The resulting white pellets were overlaid with 100 μl of fresh ethanol and stored at −70°C, with a 12 h interval at −20 to 0°C during the return trip to the laboratory in Bremen. All chemicals and buffers used for the isolation of nucleic acids from the Solar Lake mat, except the phenol-chloroform-isoamyl alcohol mixture, were treated with diethyl pyrocarbonate to remove DNase and RNase activities (68).

PCR amplification of 16S rRNA gene fragments. For PCR amplification of 16S rRNA gene fragments from mat samples, two different primer combinations were used: GM5 (with GC-clamp) and 907R, which amplified a 550-bp fragment of the 16S rRNA gene, and 385 (with GC-clamp) and 907R, which amplified a 520-bp fragment of the 16S rRNA gene (Table 1). Both fragments are suitable for subsequent DGGE analysis, membrane hybridization, sequencing, and identification of the phylotype (62, 74). The primer sequences, except 385 (3, 63); their locations on the 16S rRNA gene; and the PCR conditions have been described by Muyzer et al. (60, 61). Touchdown PCR with a hot start was performed to increase the specificity of the amplification and to reduce the

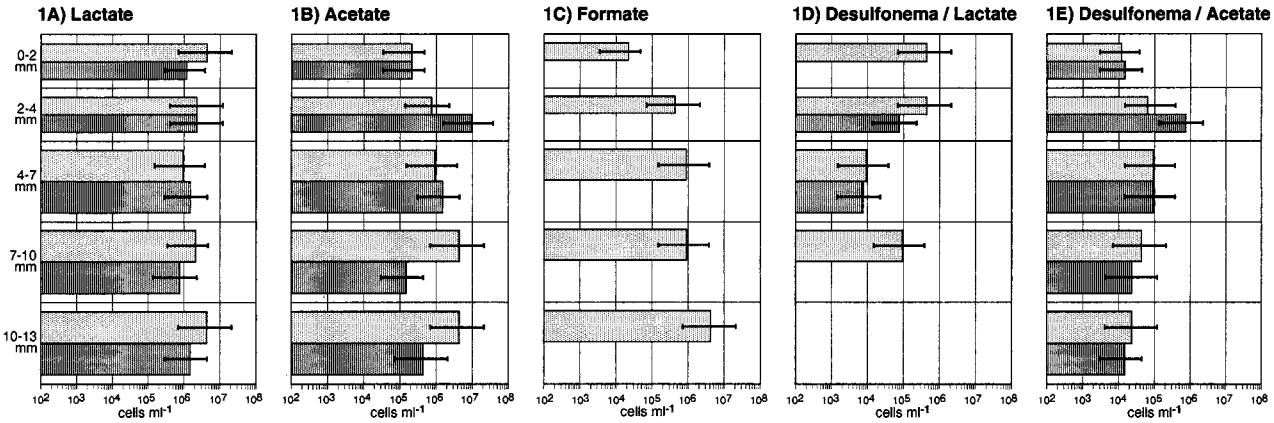

FIG. 1. MPN counts of sulfate-reducing bacteria from Solar Lake cyanobacterial mat layers at depths of 0 to 2, 2 to 4, 4 to 7, 7 to 10, and 10 to 13 mm (November 1994 samples). Cell densities are plotted on a logarithmic scale. Dark bars correspond to MPN counts of samples taken at midnight, bright bars correspond to MPN counts of samples taken at noon, and 95% confidence intervals are shown. Panels: A, 20 mM lactate; B, 20 mM acetate; C, 10 mM formate plus 2 mM acetate; D, *Desulfonema* occurrence in 20 mM lactate; E, *Desulfonema* occurrence in 20 mM acetate.

formation of spurious by-products (27). Approximately 50 ng of DNA was used as the PCR template.

DGGE. PCR products were analyzed by DGGE, followed by hybridization and sequencing. DGGE was performed with a Protean II system (Bio-Rad Laboratories, Hercules, Calif.). A 30 to 70% denaturing gradient was used for all experiments. One hundred percent corresponds to 7 M urea and 40% (vol/vol) formamide (61). Electrophoresis was continued for 8 h at a constant voltage of 100 V and a temperature of 60°C. After electrophoresis, the gels were stained in an aqueous ethidium bromide solution (0.5 μg liter^{-1}) and photographed on a UV (302 nm) transillumination table with a Cybertech CS1 digital camera (Cybertech, Berlin, Germany). Negative images were used in Fig. 4A to D for better contrast. Small pieces of selected DGGE bands were excised from the DGGE gel, eluted, and reamplified with the same primers but without GC-clamp. DGGE patterns were electroblotted onto Hybond-N+ membranes (Amersham, Amersham, United Kingdom) with a Trans-Blot SD Semi Dry Transfer Cell (Bio-Rad), followed by UV cross-linking of the DNA to the membrane (61).

The DGGE pattern was analyzed by hybridization with rRNA-targeted probes (Table 1). The 16S rRNA region amplified by primers GM5F and 907R or 385 and 907R, respectively, includes several target sites for general and genus- and species-specific oligonucleotide probes for sulfate-reducing bacteria (24, 33). Probes were purchased with 5′-digoxigenin label (Biometra, Göttingen, Germany) or were terminal transferase labeled with digoxigenin-ddUTP by using the DIG Oligo labeling kit (Boehringer, Mannheim, Germany). Digoxigenin-labeled probes were detected by an antibody coupled to alkaline phosphatase, which gives a chemiluminescent reaction with CSPD (Tropix, Bedford, Mass.). Hybridization was performed as described by Muyzer et al. (61). Probe 657 was hybridized and washed at 47°C.

Phylogenetic identification. 16S rRNA sequences were aligned with those of other bacteria obtained from the Ribosomal Database Project (56). The SIMILARITY_RANK tool of the Ribosomal Database Project was used to search for close evolutionary relatives. Sequence alignments were prepared with the sequence alignment editor SeqPup (35). Jukes-Cantor distances were calculated with DNADIST, and phylogenetic trees were inferred with FITCH as implemented in the software package PHYLIP Version 3.5 (28). Bootstrap testing of the branching pattern was performed in 100 resamplings with DNABOOT as included in PHYLIP Version 3.5.

Nucleotide sequence accession number. The DGGE main band sequence has GenBank accession no. AF035425.

RESULTS

Numbers and activity of sulfate-reducing bacteria. Numbers of sulfate-reducing bacteria were determined by MPN counts in relation to the oxic-anoxic zonation of a Solar Lake cyanobacterial mat in November 1994 for the 0- to 2-, 2- to 4-, 4- to 7-, 7- to 10-, and 10- to 13-mm mat layers. The distribution patterns of sulfate-reducing bacteria within the mat are shown in Fig. 1.

The lactate MPN estimates were in the range of 0.9×10^6 to 4.6×10^6 cells ml^{-1} for all layers (Fig. 1A). Since the 95% confidence intervals overlapped to a large extent in all cases, minor differences, such as the slightly elevated daytime counts in the oxic surface layer, are of limited significance. Lactate-utilizing sulfate reducing bacteria occur in the diurnally oxic surface layer (0 to 2 mm) at densities similar to those found in deeper, permanently anaerobic layers. The acetate MPN counts of bacteria which were inoculated with the same homogenate as the samples used for lactate MPN counts are less homogeneous than the lactate bacterial MPNs. During the day and at night, approximately 2×10^5 cells ml^{-1} were found in the 0- to 2-mm surface layer (Fig. 1B). This is a conservative estimate: in the day and in the night MPN counts of the surface layer, two of three dilution series in this triplicate MPN sample reached 10^7 cells ml^{-1}, but gaps in the third dilution series enforced significantly lower estimates. Day and night acetate counts showed different patterns: during the day, the numbers of acetate-oxidizing sulfate reducers increased towards deeper mat layers and reached 4.3×10^6 cells ml^{-1} at depths of 7 to 10 and 10 to 13 mm. On the other hand, the night acetate MPN showed a pronounced maximum, 0.9×10^7 cells ml^{-1}, in the 2- to 4-mm layer and was unusually low, 1.5×10^5 cells ml^{-1}, in the 7- to 10-mm layer (Fig. 1B). The daytime formate MPN showed a pattern similar to that of the daytime acetate MPN, although the numbers in the surface layer were much lower (Fig. 1C). The formate medium contained 2 mM acetate for nonautotrophic strains. Therefore, the formate MPN count could have included acetate-utilizing sulfate reducers.

The combined daytime acetate and lactate MPN of the oxic surface layer (4.5×10^6 cells ml^{-1}) was higher than the daytime numbers of the 2- to 4- and 4- to 7-mm layers (1.9×10^6 to 3.0×10^6 cells ml^{-1}) and close to the average of all layers (4.9×10^6 cells ml^{-1}) (Table 2).

***Desulfonema* MPN.** After 6 months of storage in a dark cabinet at room temperature, filamentous bacteria had developed in many MPN dilution cultures and covered the glass walls of the culture vials. The filaments were identified as filamentous, sulfate-reducing *Desulfonema* spp. (82), based on morphology, gliding motility, sulfate-reducing ability, and growth of subcultures on the typical *Desulfonema* substrate isobutyrate. *Desulfonema* spp. from Solar Lake mats resemble *Desulfonema limicola* morphologically and have similar filament diameters of 2 to 2.5 μm (Fig. 2). The consistent pattern of *Desulfonema* growth in the acetate MPN series allowed MPN estimates for this bacterium (Fig. 1E). Approximately 10^4 cells ml^{-1} were found in the 0- to 2-mm surface layer during the day and at night. The highest numbers, 0.75×10^6 cells ml^{-1}, were

TABLE 2. Total MPN counts of sulfate-reducing bacteria, corresponding in situ sulfate reduction rates, and specific sulfate reduction rates per cell in Solar Lake mat

Time and layer (mm)	MPN (ml^{-1})[a]	SO_4^{2-} reduced (nmol ml^{-1} day^{-1})[b]; %	SO_4^{2-} reduced (nmol $cell^{-1}$ day^{-1})[c]
12 am			
0–2	4.5×10^6	2,207 ± 1,258; 57	4.70×10^{-4}
2–4	3.0×10^6	3,127 ± 1,004; 32	10.4×10^{-4}
4–7	1.9×10^6	2,780 ± 482; 17	14.9×10^{-4}
7–10	6.4×10^6	1,119 ± 257; 23	1.75×10^{-4}
10–13	8.6×10^6	547 ± 164; 30	0.64×10^{-4}
12 pm			
0–2	1.8×10^6	1,002 ± 315; 31	5.6×10^{-4}
2–4	11.6×10^6	1,842 ± 51; 3	1.6×10^{-4}
4–7	3.0×10^6	1,831 ± 452; 25	6.1×10^{-4}
7–10	0.9×10^6	761 ± 76; 10	8.5×10^{-4}
10–13	1.9×10^6	472 ± 82; 17	2.5×10^{-4}
Day[d], 0–5	2.0×10^6	Maximum rate, 5,400	27.0×10^{-4}

[a] Counts of incompletely (lactate) and completely (acetate) oxidizing sulfate-reducing bacteria combined. Overlaps between the two populations are possible principally because several acetate oxidizers, i.e., *Desulfococcus*, *Desulfosarcina*, and *Desulfonema* spp., are nutritionally versatile and utilize different compounds, including lactate (80, 82). However, *Desulfonema* bacteria appeared only after nonfilamentous sulfate reducers had already turned the highest MPN dilutions on lactate and acetate positive and rarely grew to the highest dilutions.

[b] Average measurements from three cores are shown along with standard deviations and with the percent values of the standard deviations to the average. In situ sulfate reduction rates of the upper two layers (0 to 2- and 2 to 4-mm) were lower than bag incubation rates (37), suggesting interference of H_2S reoxidation.

[c] Specific sulfate reduction rates for pure cultures are in the range of 0.5×10^{-4} to 0.002×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1} (43).

[d] Literature data (47).

found in the 2- to 4-mm layer at night. Approximately 10^5 cells ml^{-1} were obtained in the 4- to 7-mm layer during the day and at night. The numbers decreased gradually towards deeper layers, down to 2×10^4 to 3×10^4 cells ml^{-1} in the 10- to 13-mm layer. A noon lactate MPN estimate of 4.3×10^5 *Desulfonema* cells ml^{-1} was obtained for the 0- to 2- and 2- to 4-mm layers (Fig. 1D), 1 order of magnitude below the nonfilamentous counts. *Desulfonema* occurrence in the lactate MPN samples was obscured by frequent blank samples in which *Desulfonema* bacteria were probably outcompeted by faster-growing sulfate reducers. In these cases, no MPN data are given (Fig. 1D). The *Desulfonema* occurrence pattern of all samples, including the scattered positives which did not allow calculation of MPN data, confirmed that *Desulfonema* occurrence decreased in deeper mat layers: *Desulfonema* filaments were never found at dilutions higher than 10^5 in mat layers below 4 mm; above 4 mm, they also occurred in several 10^6 and 10^7 dilutions.

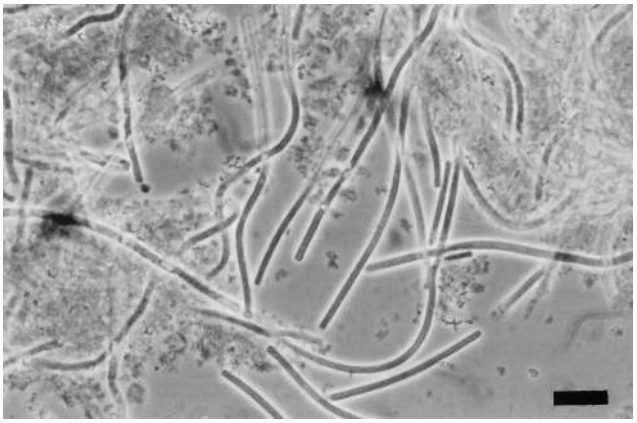

FIG. 2. *Desulfonema* filaments from the 0- to 2-mm oxic surface layer of a Solar Lake cyanobacterial mat sample harvested at noon. The *Desulfonema* enrichment grew in a 10^6 MPN dilution with lactate. The scale bar corresponds to 10 μm.

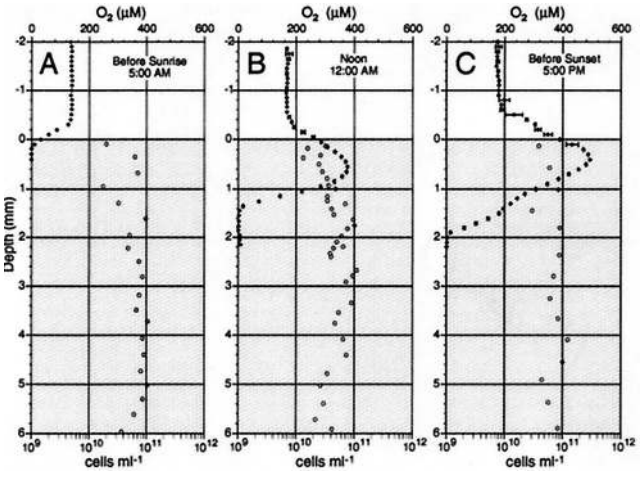

FIG. 3. Total bacterial counts (○) and shifting oxygen gradients (●) in Solar Lake mats at different time points during a diurnal cycle (May 1994 samples). One-millimeter layers of this mat were used for DGGE.

The MPN method has probably systematically underestimated the *Desulfonema* population density. This sulfate reducer grows not in single, easily dispersible cells but in filaments of approximately 10 to several hundred cells (82). Since the inoculum for the MPNs was characterized by broken and partially destroyed cyanobacterial filaments, *Desulfonema* filaments were probably also broken into smaller units, but to which degree is unknown.

Sulfate reduction rates. In situ sulfate reduction rates were determined in parallel with MPN counts and ranged from approximately 500 to 3,100 nmol of SO_4^{2-} ml^{-1} day^{-1}. The highest rates occurred in the upper three layers, 0 to 2, 2 to 4, and 4 to 7 mm (Table 2). In all mat layers, daytime rates were higher than night rates. This factor increased from lower to upper mat layers (1.16 to 2.2), suggesting enhanced sulfate reduction by photosynthetic primary production. Oxygen did not inhibit sulfate reduction significantly: the sulfate reduction rate in the oxic surface layer (2,200 nmol of SO_4^{2-} ml^{-1} day^{-1}) reached 70% of the peak rate of the mat (3,100 nmol of SO_4^{2-} ml^{-1} day^{-1}), which was located in the chemocline layer (Table 2). The in situ sulfate reduction rates in the oxic layer have probably been underestimated due to reoxidation of hydrogen sulfide (37, 38).

16S rRNA gene profiles in relation to oxygen. In May 1994, a separate set of Solar Lake mat samples was analyzed for total cell counts, oxygen profiles, and 16S rRNA gene profiles by DGGE. Total counts of 4,6-diamidino-2-phenylindole-stained cells in Solar Lake mat cryosections indicated 10^{10} to 10^{11} cells ml^{-1} in all layers, independent of shifting oxygen gradients over a diurnal course (Fig. 3). The oxygen profiles differed from the profiles in November 1994. At night, atmospheric oxygen diffused only 0.2 mm into the mat, where it was rapidly consumed. During the day, cyanobacterial oxygenic photosynthesis created oxygen-supersaturated conditions, with oxygen concentrations reaching 200 to 400 μM. Oxygen penetration

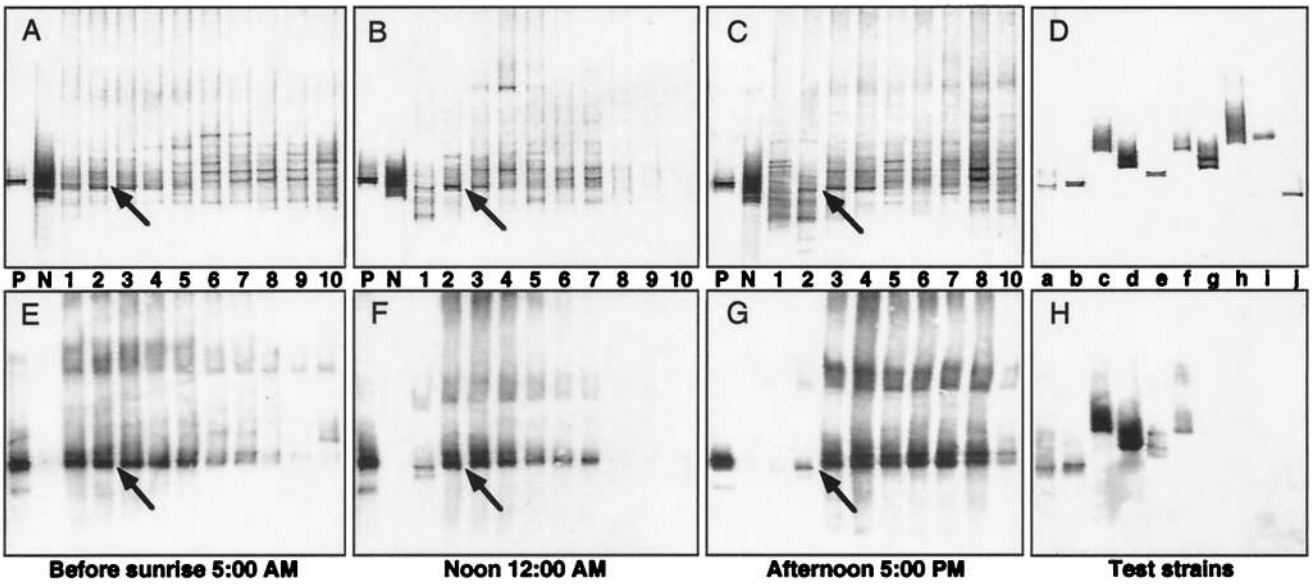

FIG. 4. DGGE patterns of PCR-amplified 16S rRNA genes from horizontal Solar Lake mat sections. DGGE patterns were stained with ethidium bromide (A to D [negative images]) and subsequently hybridized with probe 657 (E to H). DGGE patterns at 5 am (A and E), at noon (B and F), and at 5 pm (C and G) are shown. One-millimeter layers of the Solar Lake mat, 0 to 1 mm, 1 to 2 mm, etc., until 9 to 10 mm, are numbered 1 to 10. Lane P, *Desulfonema* sp. enrichment used as a positive control for probe 657. Lane N, *Desulfovibrio oxyclinae* used as a negative control. The arrows indicate the DGGE band which hybridized with probe 657 and are always positioned at the 1- to 2-mm layer. DGGE patterns D and H show the hybridization of probe 657 with enrichments and pure cultures of sulfate-reducing bacteria. Lanes: a, mat sample of the 3- to 4-mm layer at 5 pm; b, *Desulfonema* sp. enrichment, also used as a positive control with the mat samples; c, *Desulfonema limicola*; d, *Desulfonema magnum*; e, *Desulfococcus multivorans*; f, *Desulfosarcina variabilis*; g, *Desulfobotulus sapovorans*; h, *Desulfobacter postgatei*; i, *Desulfobacterium autotrophicum*; j, *Desulfovibrio salexigens*.

reached 1.5 mm at noon and 2 mm at 5 pm. For three time points (5 am, 12 am, and 5 pm), nucleic acids of the upper 10 1-mm layers were analyzed by PCR and DGGE. Two primer combinations were used (Table 1): reverse bacterial primer 907 in combination with forward bacterial primer GM5, and in combination with forward primer 385, which is selective, but not specific, for delta proteobacterial sulfate-reducing bacteria (3, 63). The DGGE pattern of the GM5-907 primer combination gave consistently negative results in hybridizations with probes for sulfate-reducing bacteria. The most conspicuous DGGE band in this pattern was excised, sequenced, and identified as a *Marinobacter* sequence, a genus of facultatively anaerobic, fermentative, heterotrophic bacteria (results not shown). General PCR primers missed 16S rRNA genes of sulfate-reducing bacteria. The second PCR primer combination introduced an amplification bias towards delta subdivision sulfate-reducing bacteria and resulted in a new set of DGGE patterns (Fig. 4). In all three sample sets from 5 am, 12 am, and 5 pm, a conspicuous band was found at a gel position similar to that of one of the hybridization controls, the DGGE band of a *Desulfonema* sp. enrichment from Solar Lake (Fig. 4A to C). The DGGE gels were blotted on a Hybond+ membrane and hybridized with probes to detect particular bands derived from sulfate-reducing bacteria and to follow them through this series of complex DGGE patterns. Detection and monitoring of particular bands, such as the conspicuous main band, by hybridization is more sensitive than by ethidium bromide staining, which can miss bands that do not exceed the background in staining intensity or are obscured by intense bands nearby (Fig. 4A to C and E to G).

The DGGE patterns were hybridized with 16S rRNA probe 657, which has been designed as a specific 16S rRNA probe for fluorescent in situ hybridization detection of the genus *Desulfonema*, encompassing the species *D. magnum*, *D. limicola*, and *D. ishimotoi* (33). The probe was used under semistringent conditions to detect the perfectly matching species *D. limicola* and *D. magnum* but also *Desulfonema*-related bacteria with one mismatch, such as *Desulfococcus multivorans* and *Desulfosarcina variabilis*. Two mismatches, as in *Desulfobotulus sapovorans*, prevented hybridization (Table 3; Fig. 4D and H). In all DGGE patterns, the conspicuous band in the ethidium bromide-stained gels hybridized with probe 657 (Fig. 4). DGGE bands from all three diurnal sample sets compared side by side on one gel showed identical positions. At a later stage, sequencing confirmed their identity. The DGGE band sequence showed one mismatch at the probe 657 target site (Table 3).

If the PCR amplification bias towards a target sequence remains constant throughout a sample series, the DGGE series

TABLE 3. 16S rRNA target sequences complementary to *Desulfonema* probe 657 (33) and to *Desulfococcus-Desulfosarcina-Desulfobotulus* probe 814 (24)[a]

Sequence or organism	Probe 657 3′-ATACCCTCTCCCTTYGCCTT-5′	Probe 814 3′-TTTGCAACTAGTGATCCA-5′
Complementary sequence	UAUGGGAGAGGGAARCGGAA	AAACGUUGAUCACUAGGU
D. magnum	UAUGGGAGAGGGAAGCGGAA	AAACG**G**UGAUCACUAGGU
D. limicola	UAUGGGAGAGGGAAGCGGAA	AAACGUUGAUCAC**C**AGGU
D. ishimotoi	UAUGGGAGAGGGAAACGGAA	AAACG**G**UGAUCACUAGGU
Solar Lake band	UAUGGGAGAGGGNAG**U**GGAA	AAACG**G**UGNUCACUAGGU
D. multivorans	UAUGGGAGAGGNNAG**U**GGNA	AAACGUUGAUCACUAGGU
D. variabilis	UAUGGGAGAGGGAAG**U**GGAA	AAACGUUGAUCACUAGGU
D. sapovorans	UAUGG**C**AGAGG**A**AAGCGGAA	AAACGUUGAUCACUAGGU
Strain HxD3	UAUGGGAGAGGGAAG**U**GGAA	AAACGUUGAUCACUAGGU
MMP91	UAUGGGAGAGGGAAG**U**GGAA	AAACGUUGA**A**CACUAGGU
D. autotrophicum	UA**C**GG**U**NGAGG**A**AAGGGGNA	AAACGUUG**UAU**ACUAGGU
D. toluolica	UA**C**GGGAGAGG**A**AAGCGGAA	AAACG**A**UG**UA**CACUAGGU
D. postgatei	UA**CC**G**U**AGAGG**AG**AG**A**GGAA	AAACGUUG**UA**CACU**C**GGU

[a] Underlining and boldface type indicate mismatches to the probe sequences.

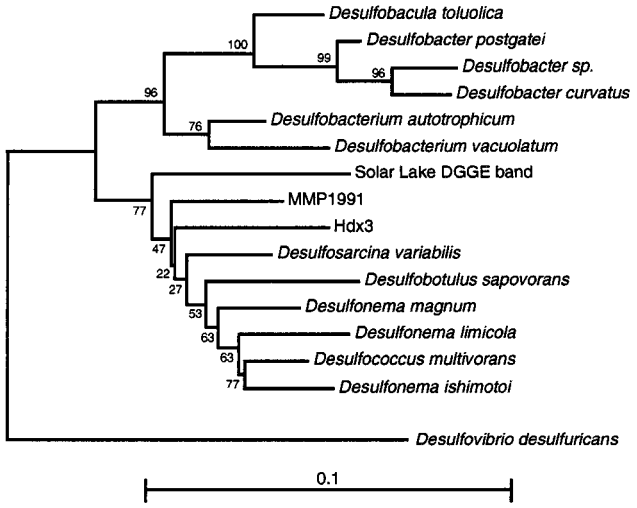

FIG. 5. 16S rRNA distance tree of predominantly acetate-oxidizing sulfate-reducing bacteria of the delta proteobacteria subdivision and the DGGE molecular isolate from the Solar Lake mat. The tree is rooted with *Desulfovibrio desulfuricans* as the outgroup and based on 16S rRNA sequence positions 481 to 906 (*Escherichia coli* numbering). The scale bar corresponds to 0.10 mutation per nucleotide position. The branching pattern was tested with 100 bootstrap resamplings.

monitors the relative increase and decrease of this particular microbial population against a background of constant cell numbers (Fig. 3). At the beginning of a diurnal course at 5 am, the *Desulfonema* probe-positive DGGE band was very conspicuous in the upper 5 mm of the mat and visible until a depth of 8 mm. At noon, the band appeared weaker in the surface 1-mm layer but remained fully visible between depths of 1 and 7 mm. At 5 pm, the band appeared very faint in the surface layer, became stronger in the second 1-mm layer of the mat, was fully visible between 2 and 8 mm, and was still detectable at a depth of 10 mm. This pattern was reproducible in replica samples of the upper 5 mm of the 5 am sample set and of the complete 5 pm sample set. The reduced intensity of the band in the upper two 1-mm layers of the mat was correlated to the gradual penetration of oxygen into the mat over a diurnal course (Fig. 3). Apparently, this bacterial population was migrating vertically to avoid the highest oxygen concentrations.

Phylogenetic position. The partial 16S rRNA sequence of the conspicuous molecular isolate from the Solar Lake mat surface layer was related to those of sulfate-reducing bacteria of the genera *Desulfococcus*, *Desulfosarcina*, *Desulfobotulus*, and *Desulfonema* (Fig. 5). *Desulfonema*, the genus of filamentous sulfate reducers, forms a cluster which includes the coccoid sulfate reducer *Desulfococcus multivorans* (33). A multicelled magnetotactic prokaryote (20) and the alkane-oxidizing sulfate-reducing bacterium Hdx3 (1) are other members of this phylogenetic branch. The limited sequence basis for this analysis, i.e., 16S rRNA positions 481 to 906 of the DGGE band, reversed the branching order of *Desulfosarcina variabilis* and *Desulfobotulus sapovorans* compared to the full sequence phylogeny (23). The instability of this branching pattern was indicated by low bootstrap values (Fig. 5). The affiliation of the Solar Lake sequence with the *Desulfonema-Desulfosarcina-Desulfococcus-Desulfobotulus* group was confirmed by 77 of 100 bootstrap test runs. *Desulfonema magnum* showed the highest individual sequence similarity to the Solar Lake sequence (0.087 Jukes-Cantor distance). The Solar Lake sequence was also compared to *Desulfosarcina*- and *Desulfonema*-related partial sequences of molecular isolates 4D19 and AO1 from *Spartina* roots (67). The overlapping portion of the three sequences, approximately 300 nucleotides, indicated no close phylogenetic affiliation of the Solar Lake molecular isolate to 4D19 and AO1 (Jukes-Cantor distances of approximately 0.10). The 16S rRNA sequence motifs for the group-specific *Desulfococcus-Desulfosarcina-Desulfobotulus* (24) and *Desulfonema* (33) probes occur in a similar form in the Solar Lake molecular isolate, although altered by at least one mismatch each (Table 3).

DISCUSSION

Evaluation of MPN counts. MPN counts indicated a large population of sulfate-reducing bacteria during daytime in the oxic surface layer of the Solar Lake mat, estimated as 4.5×10^6 cells ml^{-1}. This cell density was in the same range as previous counts of the upper 5-mm interval (47). These values may underestimate the actual population density of active sulfate-reducing bacteria by at least 1 order of magnitude: specific sulfate reduction rates (nanomoles of SO_4^{2-} per cell per day), calculated from Solar Lake mat sulfate reduction rates and from cell numbers per milliliter, were on the order of 1×10^{-4} to 10×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1} and averaged 5.7×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1} over the upper 13 mm of the mat (Table 2). The specific rate in the oxic zone at noon was 4.7×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1}. These calculated specific activities were at least 10 times higher than the range of specific activities determined for sulfate-reducing bacteria in pure culture, 0.5×10^{-4} to 0.002×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1} (43). Probably MPN counts have underestimated the actual population density of sulfate-reducing bacteria by this factor. When optimal homogenization conditions resulted in representative MPNs, specific sulfate reduction rates were found within the expected range. Cyanobacterial mats on the Frisian island of Texel showed specific sulfate reduction rates of 0.051×10^{-4} to 0.164×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1} (76), and the surface layers of a marine sediment in the Danish Kattegat showed 0.03×10^{-4} to 0.05×10^{-4} nmol of SO_4^{2-} $cell^{-1}$ day^{-1} (46).

rRNA quantifications from cyanobacterial mats of Guerrero Negro (Baja California, Mexico) also suggest higher cell densities (66). These mats resemble the shallow and deep, flat mat type of Solar Lake (48) in environmental setting, ultrastructure, biogeochemical cycling, and sulfate-reducing activity (9, 10, 18). By calibration of the sulfate-reducing bacterial rRNA yield with the cellular rRNA content of *Desulfovibrio* cultures, total sulfate-reducing bacterial numbers in the Guerrero Negro mat surface layer were estimated to be 10^7 to 10^8 cells per g of mat (66).

Adaptations to oxic conditions. The dense and highly active populations of sulfate-reducing bacteria in the oxic surface layer suggest that these bacteria have developed adaptive strategies to deal with oxygen, which include oxygen respiration, motility, and coculture and aggregate formation.

Many *Desulfovibrio* species, but also other sulfate-reducing bacteria, such as *Desulfobacterium autotrophicum*, *Desulfobulbus propionicus*, and *Desulfococcus multivorans*, can switch from sulfate reduction to aerobic respiration as soon as they are exposed to low concentrations of oxygen (19, 26, 51, 52, 58). Full aeration is survived by *Desulfovibrio* species for several hours, whereas *Desulfobacter postgatei* appears to be more sensitive and *Desulfotomaculum* species and *Desulfococcus multivorans* do not tolerate aeration at all (17). *Desulfovibrio* bacteria are highly motile and position themselves as microaerophilic gradient bacteria within oxic-anoxic gradients (41, 53), where they respire aerobically and even show aerobic growth (41, 53). The highly motile oxygen-respiring species

Desulfovibrio oxyclinae, isolated from the Solar Lake-derived Interuniversity Institute cyanobacterial mats in Eilat, showed the highest oxygen respiration rate among marine sulfate-reducing bacteria (52). *Desulfovibrio oxyclinae* was subsequently detected in Solar Lake mats by PCR and DGGE of [NiFe] hydrogenase gene fragments (78) and was reisolated from 10^5 and 10^6 dilutions of the upper 3-mm surface layer of the Solar Lake mat (50, 53). Migrations of oxygen-tolerant *Desulfovibrio* bacteria were strongly suggested by rRNA hybridization studies (59). *Desulfovibrio* populations accumulated in the oxygen-supersaturated upper mat later between 0 and 0.8 mm and at the mat oxycline between 1.6 and 2.4 mm, possibly due to the availability of photosynthetic carbon compounds (59). These peaks disappeared at night (59). The lactate MPN counts in the oxic 0- to 2-cm layer, which appear slightly higher at noon than at night, are consistent with this distribution (Fig. 1A).

Only a part of the sulfate-reducing bacterial population in the oxic surface layer could possibly switch from sulfate reduction to aerobic respiration, since sulfate reduction continues at high rates in the oxic mat surface layer. These sulfate-reducing bacteria were either intrinsically oxygen insensitive or shielded from full oxygen exposure and subsequent damage. Oxygen-insensitive sulfate-reducing bacteria, which do not stop sulfate reduction under full oxygen exposure, are not known. Therefore, it is likely that sulfate-reducing bacteria protect themselves from full oxygen exposure by forming cocultures, particle associations, or aggregates. Physiologically different bacteria can form stable consortia by mutual recycling of metabolic intermediates between the partners (7). Solar Lake *Desulfovibrio* spp. and other sulfate reducers have been shown to form stable cocultures with oxygen-scavenging facultatively aerobic bacteria (36, 73). As a second strategy, aggregation and particle association increase the viability of sulfate-reducing bacteria under oxygen stress (34). Clump formation has been observed with *Desulfovibrio oxyclinae* under oxygen stress (52). The growth patterns of marine acetate-oxidizing sulfate reducers of the genera *Desulfobacter*, *Desulfosarcina*, and *Desulfonema* are conducive to aggregate formation (80, 82). *Desulfonema* filaments can glide through the mat matrix, attach themselves to particles and surfaces, and aggregate into bundles of filaments (33, 82). These traits probably allow *Desulfonema* bacteria to grow in the oxic mat zone.

Mat microheterogeneities. Microelectrode surveys of different mat locations of Solar Lake at different times have not found anaerobic microniches (42, 48, 49, 65). Aggregate formation and patchy distribution patterns of sulfate-reducing bacteria are suggested by other approaches. Aggregation patterns of sulfate-reducing bacteria in the range of approximately 100 μm were directly visualized in a photosynthetic biofilm (64). There is evidence for uneven distribution patterns of sulfate-reducing bacteria and activity in the surface layer of the Solar Lake mat on a millimeter scale: millimeter- and even centimeter-sized spots and streaks of increased H_2S formation have been visualized by Ag_2S formation on silver foils inserted into the oxic mat layer (14, 55). Sulfate reduction rate measurements on three Solar Lake mat cores sliced and processed in parallel with those used for MPN counts yielded the highest core-to-core variations in the surface layer at noon and at midnight (Table 2). Sulfate reduction rates in Lake Constance sediments also showed the highest variations in the surface layer (5). The Guerrero Negro mats showed the most variable relative proportions of dominant sulfate-reducing bacterial populations, *Desulfovibrio* spp. and the *Desulfosarcina-Desulfococcus-Desulfonema* group, in their oxic surface layer (66). This recurring variability in surface layer activities and cell densities might also be a consequence of coarse slicing, which possibly cuts through activity peaks at the oxic-anoxic interface at the bottom of the oxic zone. Sampling strategies with finer spatial resolution are required.

Significance of *Desulfonema* bacteria. The Solar Lake results (Fig. 1D and E) confirm the new view of the genus *Desulfonema* as an important microbial mat component and oxic-anoxic interface organisms: *Desulfonema* filaments have been detected by fluorescent in situ hybridization in freshwater and marine mats (33). *Desulfonema* filaments grew as epibionts on the polysaccharide sheaths of marine sulfur-oxidizing *Thioploca* bacteria, possibly supplying the host bacteria with sulfide (33), preferentially on fresh sheaths towards the surface layer of the *Thioploca* mat (72). *Desulfonema*-related bacteria of the AO1 clone type contributed 5 to 10% of the total 16S rRNA abundance within the partially oxygenated rhizosphere of *Spartina alterniflora* (67). Quantitative rRNA hybridization data of Guerrero Negro cyanobacterial mats indicated that *Desulfonema* or *Desulfonema*-related bacteria are among the dominant sulfate reducers in the oxic mat layer (66). In two determinations, 0.4 and 5.2% of the total prokaryotic rRNA from this mat layer hybridized with probe 814 (66). This probe matches *Desulfococcus*, *Desulfosarcina*, and *Desulfobotulus* spp. (24), has one mismatch to *Desulfonema limicola* and *Desulfonema ishimotoi*, and has two mismatches to *Desulfonema magnum* (Table 3). Since single-mismatch discrimination in membrane blotting requires the use of competitive probes (57), the hybridization signal obtained with probe 814 could originate partly from *Desulfonema* rRNA. 16S rRNA probes specific for *Desulfonema* spp. and other acetate-oxidizing sulfate reducers could quantify the relative proportions of *Desulfonema* spp. and related sulfate reducers in more detail.

The question of whether the conspicuous DGGE molecular isolate is a *Desulfonema* sp. could not be answered without direct morphological evidence. The sequence showed the lowest Jukes-Cantor distance to *Desulfonema magnum*, but the phylogenetic analysis placed it at the root of the *Desulfonema-Desulfococcus-Desulfosarcina* branch of the sulfate-reducing bacterial tree. The phylogeny of this organism gave no clear information about its metabolism: phylogenetic relatives included the alkane-oxidizing sulfate-reducing bacterium HxD3 (1) and the fatty acid-oxidizing species *Desulfobotulus sapovorans* (23), which cannot oxidize acetate, and an uncultured multicelled magnetotactic prokaryote of the iron sulfide type (20). The conspicuous migration of this organism over a distance of several millimeters, reminiscent of similar migrations found for *Desulfovibrio* bacteria (59), suggested that it is actively motile.

Carbohydrate substrates. Sulfate reduction in the oxic surface layer of a Solar Lake-derived mat in the experimental ponds at the Interuniversity Institute, Eliat, Israel, was stimulated by acetate and glycolate (31). The acetate-stimulated populations could include *Desulfonema* bacteria. However, *Desulfonema* species can also use a wide range of fatty acids, organic acids, and intermediates of the tricarboxylic acid cycle (80, 82). All Solar Lake *Desulfonema* enrichments, from acetate and lactate, could be transferred and grown in isobutyrate agar shakes (32). Lactate and ethanol, the typical *Desulfovibrio* substrates, had no measurable stimulating effect within the surface layer (31). Glycolate, a dominant photorespiration and dark fermentation product of cyanobacteria (39), had the highest stimulatory effect on sulfate reduction in the mat surface layer (31). A glycolate-oxidizing, sulfate-reducing bacterium has been isolated from marine anoxic sediment (29). This new genus and species, *Desulfofustis glycolicus*, was phylogenetically distinct from all other sulfate-reducing bacteria (30) and did not match 16S rRNA probes 814 and 657. Therefore, Solar

Lake could harbor significant glycolate-oxidizing sulfate-reducing bacterial populations which have so far eluded molecular detection, as well as cultivation, attempts.

Sulfate reduction and CO_2 recycling. Sulfate-reducing bacterial populations provide considerable quantities of CO_2 within or near the photosynthetically active mat layer, as shown by the following calculations. The photosynthetic O_2 production and the corresponding CO_2 demand of the Solar Lake flat mat, integrated over the depth of the photosynthetic zone, were previously determined to be 13.3 to 15.2 mmol of CO_2 m^{-2} h^{-1} (48, 65). In this study, depth-integrated sulfate reduction rates from the upper 5 mm of the mat amounted to 1,340 nmol of SO_4^{2-} cm^{-2} day^{-1} or 0.56 mmol of SO_4^{2-} m^{-2} h^{-1}. This sulfate reduction rate provided 1.12 mmol of CO_2 m^{-2} h^{-1}, corresponding to 7.4 to 8.4% of the photosynthetic CO_2 demand of the deep flat mat (48, 65). Higher contributions were suggested by a previous study (47). Of the total depth-integrated sulfate reduction activity of 2.8 mmol of SO_4^{2-} m^{-2} h^{-1}, 50% or 1.4 mmol of SO_4^{2-} m^{-2} h^{-1} occurred within the upper 5 mm of the mat (47). This rate corresponds to 2.8 mmol of CO_2 m^{-2} h^{-1} produced by sulfate reduction within the upper 5 mm, equivalent to 18.4 to 21.0% of the photosynthetic CO_2 demand (48, 65). The actual CO_2 contribution from sulfate reduction could be higher, since sulfide reoxidation in the oxic layer leads to underestimated sulfate reduction rates (37); on the other hand, CO_2 in the upper mat layers has to be shared among cyanobacteria and sulfur-oxidizing, chemolithotrophic, and anoxygenic, phototrophic bacteria (47). Similar sulfate reduction rates and CO_2 contributions were found in the hypersaline cyanobacterial mats of Guerrero Negro with high seasonal variability (9–11).

Unusually heavy $\partial^{13}C$ values of organic mat carbon in Solar Lake (2), Guerrero Negro (22), and other hypersaline cyanobacterial mats (21) are interpreted as consequences of reduced isotopic discrimination of cyanobacterial photosynthesis under inorganic carbon limitation (22). In highly productive hypersaline cyanobacterial mats, carbon remineralization by sulfate reduction alleviates CO_2 limitations and, together with aerobic carbon remineralization, recycles inorganic carbon for cyanobacterial photosynthesis.

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