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EXPERIMENTAL ARTICLES

Distribution, Diversity, and Activity of Sulfate-Reducing Bacteria in the Water Column in Gek-Gel Lake, Azerbaijan

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Abstract—The distribution and activity of sulfate-reducing bacteria (SRB) in the water column of the alpine meromictic Gek-Gel lake were studied. Apart from traditional microbiological methods based on cultivation and on measuring the process rates with radioactive labels, in situ fluorescent hybridization (FISH) was used, which enables identification and quantification without cultivating organisms. The peak rate of sulfate reduction, 0.486 μg S l^{-1} day^{-1}, was found in the chemocline at 33 m. The peak SRB number of 2.5×10^6 cells/ml, as determined by the most probable number method on selective media, was found at the same depth. The phylogenetic affiliation of the SRB, as determined by FISH, revealed the predominance of the *Desulfovibrio* spp., *Desulfobulbus* spp., and *Desulfoarculus* spp./*Desulfomonile* spp. groups. The numbers of spore-forming *Desulfotomaculum* spp. increased with depth. The low measured rates of sulfate reduction accompanied by high SRB numbers and the predominance of the groups capable of reducing a wide range of substrates permit us to assume utilization of electron acceptors other than sulfate as the main activity of the SRB in the water column.

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Key words: meromictic lakes, Gek-Gel lake, sulfate-reducing bacteria, fluorescent in situ hybridization, *Desulfovibrio, Desulfotomaculum.*

Gek-Gel Lake is of interest for microbiologists due to its low trophicity combined with the meromictic nature of its water column and its significant depth [1–3]. The last investigation of the microbiological processes in the water column and in the sediments of the lake was performed in 1972 [3]. The development of new molecular methods for microbial ecology provided new possibilities for studying the distribution and composition of microbial communities in situ, including unculturable organisms. Fluorescent in situ hybridization (FISH) is one of these methods; it enables determination of the phylogenetic position of the microorganisms from natural samples by binding whole cell rRNA with fluorescent labeled oligonucleotide probes specific for certain taxonomic groups of different hierarchical levels.

A set of two probes, SRB385 and SRB385Db, is often used in ecological research for determination of sulfate-reducing bacteria (SRB) by FISH. The SBR385 probe is specific for the SRB of the family *Desulfovibrionaceae* [4], and the SRB385Db probe determines representatives of the family *Desulfobacteriaceae* [5]. During the development of the SRB385Db probe, however, it became evident that it did not hybridize with bacteria of the genus *Desulfobulbus* [5] and gave a positive signal with a number of non-sulfate-reducing bacteria [5]. Moreover, the probes SRB385 and SRB385Db differed only in position 396 [6] and were therefore subject to nonspecific binding (the creators of the probes reported a weak signal in the course of hybridization with nonspecific groups).

The capability for dissimilatory sulfate reduction is polyphyletic and is known to be present in four orders of the domain *Bacteria* and one order of *Archaea* [7]. In spite of creation of the so-called PhyloChip based on 132 bacterial oligonucleotide probes and encompassing most sulfate-reducing prokaryotes (SRPs) [8], oligonucleotide probes specific to archaeal and thermophilic bacterial SRB are still not available for FISH. New probes and hybridization conditions were recently developed for determination by FISH of the sulfate-reducing representatives of *Firmicutes* (with low G+C content) [9]. Until recently, however, their use has been limited to investigation of SRB distribution in soil, human feces, animal colic samples, and anaerobic bioreactors [9].

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In our research of the distribution, numbers, and activity of the SRB of the water column of Gek-Gel lake, both traditional methods of microbial ecology based on the use of radioactive isotopes to determine the process rate under the conditions closest to in situ, and of selective media to determine the numbers of cultivable SRB, and FISH analysis to identify and determine the organisms without cultivation were used. Molecular monitoring of the SRB enrichments obtained under different culture conditions was also performed with FISH. The sequences specific for the major genera and monophyletic groups of mesophilic δ-*Proteobacteria* and of spore-forming, low G+C *Firmicutes* were chosen as oligonucleotide probes.

MATERIALS AND METHODS

The samples of Gek-Gel Lake water were collected in September 2003 from a boat by a 1-l glass bathometer. The sampling horizons were chosen based on preliminary measurements of hydrogen sulfide and oxygen profiles with Merck field kits (Germany) and on the readings of the pH and Eh electrodes of a pH 320 field ion meter (WTW, Germany).

The amounts of sulfate-reducing bacteria were determined by the most probable number (MPN) method on Widdel liquid freshwater medium [10] with lactate, acetate, ethanol, or formate as electron donors. Penicillin vials were filled completely with the medium. Iron paper clips were used as a minor iron source; it also helped to maintain the low redox potential via production of cathode hydrogen and served as a nucleation site in the course of iron sulfide formation. The MPN dilution series were incubated at 28 and 4°C. SRB growth was assessed by darkening of the medium due to iron sulfide formation and by accumulation of hydrogen sulfide. All MPN dilution series were performed in three replicas, and the most probable number was calculated by McCready tables.

Enrichment cultures of SRB were obtained by transfers into Widdel liquid medium from the MPN dilution series used for enumeration of bacteria. Lactate, acetate, ethanol, and formate were used as growth substrates; incubation was performed either at 28°C or at 4°C.

The rate of sulfate reduction was determined by the radioisotope method with $Na_2{}^{35}SO_4$. Labeled sulfate (0.2 ml) with a total radioactivity of 10 μCi was introduced into penicillin vials. After injection of labeled sulfate, the vials were submerged on nylon strings to the respective depths to maintain natural conditions of temperature and illumination. The samples were incubated in the lake for 48 h. After incubation, the samples were fixed with 1 ml of the mixture (1 : 1) of 2 N KOH and 10% zinc acetate. The subsequent treatment of the samples was performed in the laboratory. Sulfide was extracted by acidic distillation; this process, together with the measurement of radioactivity and the calculation of sulfate reduction rates, was described in detail previously [11]. Sulfate concentration in the water samples was determined by ion chromatography (Eppendorf Biotronik GmbH, Hamburg, Germany).

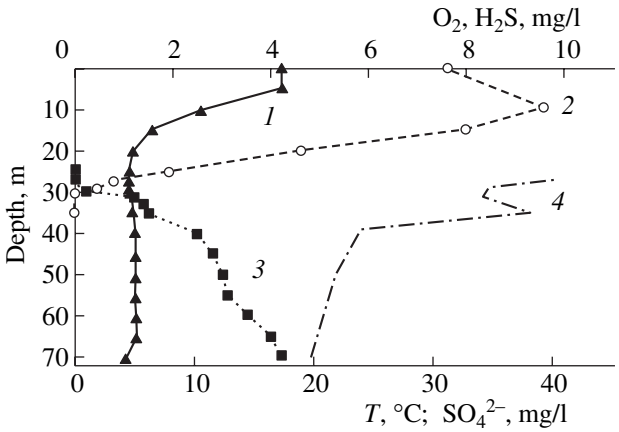

Fig. 1. Changes in temperature (*1*) and concentrations of oxygen (*2*), hydrogen sulfide (*3*), and sulfate (*4*) with depth in the water column of Gek-Gel Lake in September 2003.

The samples for FISH analysis were fixed with 96% ethanol (1 : 1) and stored under refrigeration prior to the analysis. The fixed cells were precipitated by centrifugation and washed twice with the PBS buffer (10 mM sodium phosphate, 130 mM NaCl, pH 7.2). The cells were resuspended in the PBS buffer and placed in the wells on gelatin-covered microscope slides (Gerhard Menzel Glasbearbeitungswerk GmbH & Co. KG, Brauschweig, Germany). The procedures for hybridization and cell count were described in detail previously [12]. The genus- and group-specific 16S rRNA oligonucleotide probes (MedProbe Eurogentech, Serain, Belgium) used in this investigation are listed in Table 1.

RESULTS

Physicochemical parameters of the Gek-Gel Lake water column affecting the distribution and activity of SRB. In September 2003, the water column of the lake exhibited pronounced temperature stratification (Fig. 1). Sharp changes in temperature occurred in the metalimnion, at 10–20 m. At 29 m, the redox zone of the concomitant presence of oxygen and hydrogen sulfide was found; traces of H_2S (<0.1 mg/l) were present, and the concentration of dissolved oxygen was 0.45 mg/l. The concentration of hydrogen sulfide increased with depth and reached 4.2 mg/l at 70 m. Sulfate concentration changed in the opposite direction, increasing from 19.7 mg/l at 70 m, near the bottom, to the thermocline, with the peak of 40.6 mg/l at 27 m. It should be mentioned, however, that sulfate concentrations of the epilimnion (above 27 m) were not measured. At 29–31 m, sulfate concentration decreased in comparison to the upper and lower layers to 34.3 mg/l.

Table 1. Oligonucleotide probes used in the present work

Probe	Target organisms	Sequence (5' → 3')	Target 16S rRNA fragment	Formamide concentration, %	Reference*
NON338	Negative control	ACTCCTACGGGAGGCAGC	338–355	10	[13]
DSR651	*Desulforhopalus*, *Desulfocapsa*, *Desulfofustis*	CCCCCTCCAGTACTCAAG	651–668	35	[4]
DSS658	*Desulfosarcina*, *Desulfofaba*, *Desulfococcus*, *Desulfomusa*, *Desulfofrigus* spp.	TCCACTTCCCTCTCCCAT	658–685	60	[4]
DSV698	*Desulfovibrio* spp., *Bilophila wadsworthia*, *Lawsonia intracellularis*	GTTCCTCCAGATATCTACGG	698–717	35	[4]
DSV1292	*Desulfovibrio* spp., *Bilophila wadsworthia*	CAATCCGGACTGGGACGC	1292–1309	35	[4]
DSV214	*Desulfomicrobium* spp.	CATCCTCGGACGAATGC	214–230	10	[4]
DSMA488	"*Desulfoarculus*", *Desulfomonile*, and *Syntrophus* spp.	GCCGGTGCTTCCTTTGGCGG	488–507	60	[4]
Sval428	*Desulfotalea*, *Desulfofustis*	CCATCTGACAGGATTTTAC	428–446	25	[14]
660	*Desulfobulbus* spp.	GAATTCCACTTTCCCCTCTG	660–679	60	[15]
221	*Desulfobacterium* spp.	TGCGCGGACTCATCTTCAAA	221–240	35	[15]
Dtm229	*Desulfotomaculum* spp.	AATGGGACGCGGATCCAT	229–247	15	[9]

* Probe specificity was ascertained according to the current condition of the Ribosomal Database Project 9.0 database.

Distribution and activity of SRB in the water column. The peak SRB number determined by the MPN method was 2.5×10^6 cells/ml at 31 m (Fig. 2). Culturable SRB were detected in the oxic zone of the lake as well; two peaks were present, above the redox zone at 27 m (15×10^3 cells/ml), and at 10 m (1.5×10^2 cells/ml). In the anaerobic zone, at 55 m depth, the second SRB maximum was found (2.0×10^6 cells/ml). Compared to lactate, ethanol, and formate, the amount of SRB on acetate-containing media was the highest (Figs. 3a, 3b). There was no growth on lactate- and formate-containing media inoculated with the samples from the aerobic zone. The SRB numbers obtained by the MPN method at 28°C were generally higher than those obtained at 4°C (data not shown). In the 33 m sample with acetate, however, 11.5×10^3 cells/ml were found after incubation at decreased temperature, and only 200 cells/ml in the dilution series incubated at 28°C. In the sample from 55 m depth, the SRB numbers determined by the terminal dilutions method at 4°C on all substrates were higher than those obtained at 28°C (Fig. 4).

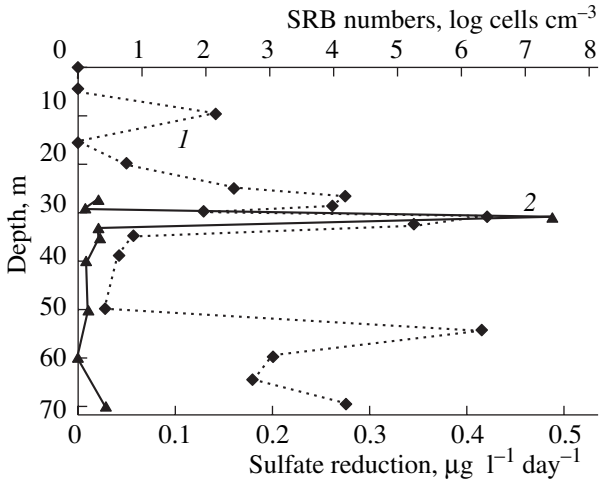

Fig. 2. Maximal SRB numbers as determined by the MPN method (*1*), and sulfate reduction rate as determined by the radioisotope method (*2*) in the water of Gek-Gel Lake.

The peak of SRB activity coincided with the highest cell number determined by the MPN method; it was located at 31 m, directly under the redox zone (Fig. 2). Sulfate reduction rate did not exceed 0.486 μg S l^{-1} day^{-1}. Sulfate reduction was low near the bottom, at the highest H_2S level; the value of 0.029 μg S l^{-1} day^{-1} was close to the sensitivity of the method. In the oxic epilimnion at 27 m, a small amount of the label (0.022 μg S l^{-1} day^{-1}) was found in reduced sulfur compounds. Sulfate reduction in the rest of the anaerobic hypolimnion was very low, in the range of 10–20 ng of reduced sulfur per day.

FISH analysis of the phylogenetic affiliation of the SRB from the water samples. Hybridization was performed with the samples from 10, 31, 33, and 70 m (Table 2). At 31 m, in the zone of the highest sulfate reduction, the representatives of the order *Desulfovibrionales* were the predominant group. Of all the DAPI-stained cells, about 14% hybridized with the *Desulfovibrio*-specific probes DSV698 and DSV1292;

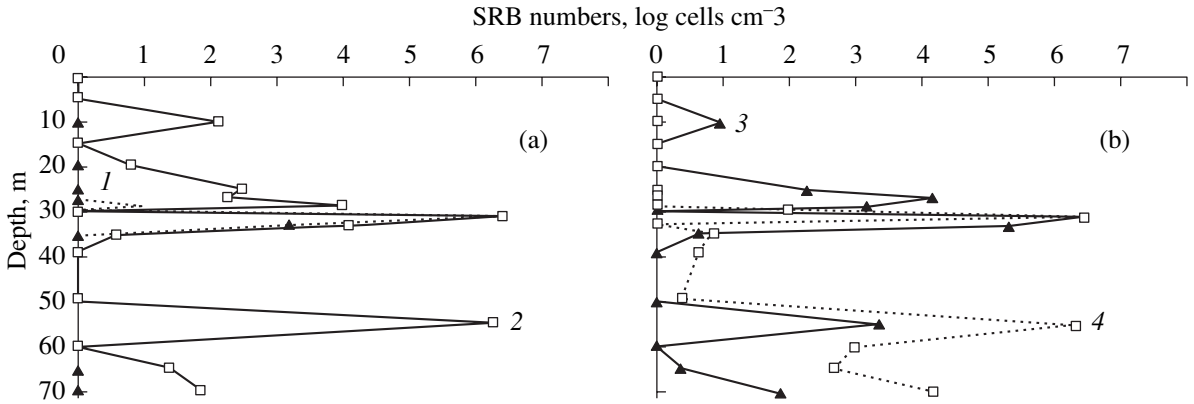

Fig. 3. SRB numbers as determined by the terminal dilutions method on media with lactate (*1*), and acetate (*2*) (a), and with ethanol (*3*) and formate (*4*) (b).

7.6% of the cells hybridized with the DSV214 probe specific for *Desulfomicrobium* spp.; *Desulfoarculus* spp. and *Desulfomonile* spp. that hybridized with the DSMA448 probe were also found in the zone of the highest SRB growth, together with the δ-proteobacterial compete oxidizers of the *Desulfosarcina-Desulfococcus* spp. group (probe DSS658). Spore-forming *Desulfotomaculum* spp. hybridizing with the Dtm229 probe were also found at 31 m. The number of cells hybridizing with the *Desulfotomaculum* spp. probe increased with depth; at 70 m it reached 15.5% of all DAPI-stained cells. Bacteria hybridizing with the *Desulfobulbus*-specific 660 probe also constituted a significant portion of the sulfate-reducing community of the anaerobic zone of the lake. At 33 m they constituted 19.3% of all DAPI-stained cells. The positive signal with the Sval428 probe specific for the psychrophilic *Desulfotalea* and for the mesophilic *Desulfofustis* spp. was unexpected. They were found in the oxic zone at 10 m, and at 31 m. In the samples from 70 m depth, the highest number of the cells (3.8%) hybridized with the DSR651 probe specific for the psychrotolerant *Desulforhopalus* spp. The cells hybridizing with the Sval428 probe constituted the majority of the SRB of the aerobic zone at a depth of 10 m. *Desulfovibrio*-specific probes also showed positive signals at this depth. Only for this water sample was a positive signal obtained with the *Desulfobacterium*-specific probe 221.

Phylogenetic affiliation of the culturable SRB. As expected, the diversity of the SRB types determined by FISH in enrichment cultures was less than that found in water samples (Table 3). The cells giving a positive signal with the *Desulfomicrobium* spp.-specific DSV214 probe predominated in enrichment cultures with formate and acetate from the 25, 33, and 70 m water samples. The cells hybridizing with the *Desulfovibrio* DSV1292 probe and psychrophilic *Desulfotalea* and *Desulfofustis* (probe Sval428) were also found.

DISCUSSION

SRB activity in the hypolimnion. The measured hydrogen sulfide concentration in the water column was close to the level determined during the 1960s–1970s [2, 3]; this finding indicates the stable character of the microbiological processes of sulfur and carbon cycles in Gek-Gel Lake. The rate of sulfate reduction was within the range characteristic for oligotrophic, low-sulfate water bodies. The maximal sulfate reduction in the chemocline zone is a phenomenon known not only for oligotrophic lakes, but for high-trophicity water bodies as well. Such localization of the maximum indicates limitation in sulfate reduction due to the concentration of organic matter in conditions of sulfate saturation. At relatively low sulfate concentrations in the lake, sulfur reoxidation in the chemocline must occur at sufficiently high rates. A decrease in sulfate concentra-

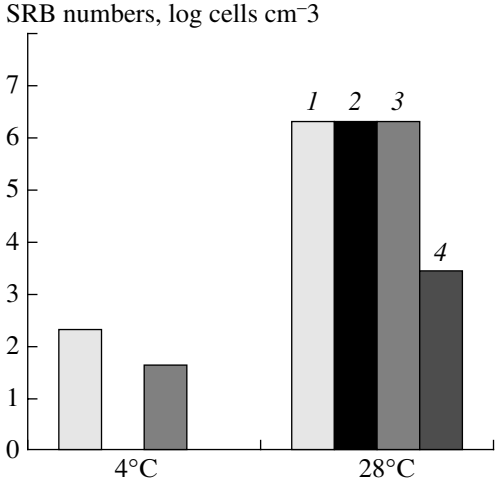

Fig. 4. SRB numbers as determined by the MPN method on media with different substrates at different incubation temperatures: (*1*), lactate; (*2*), acetate; (*3*), formate; (*4*), ethanol.

Table 2. The distribution of SRB genera and groups in the water column of Gek-Gel Lake at different depths as determined by FISH. The number of hybridized cells is given as percentages of the total number of DAPI-stained cells

SRB genera and groups; the respective oligonucleotide probes	Sampling depth, m			
	10	31	33	70
Desulfovibrio spp./DSV698	0.4	13.6	0	0
Desulfovibrio spp./DSV1292	5.8	14.4	20.5	9.9
Desulfomicrobium spp./DSV214	0	7.6	11.6	0.9
Desulfobacterium spp./221	2.2	0	0	0
Desulfobulbus spp./660	0	0	19.3	3.3
Desulfosarcina and *Desulfococcus* spp./DSS658	0	6.3	0	2.8
Desulfoarculus and *Desulfomonile* spp./DSMA448	n.d.	8.2	22.5	23.1
Desulforhopalus spp./DSR651	0	0	0	3.8
Desulfotalea and *Desulfofustis* spp./Sval428	7.8	2.3	0	0
Desulfotomaculum spp./Dtm229	0	6.6	13.3	15.5
Microbial numbers (direct count with DAPI), $\times 10^6$ cells/ml	1.1	12.0	6.1	9.0

Table 3. Generic and group affiliation of SRB in enrichment cultures obtained under different conditions from water samples, Gek-Gel Lake. The number of hybridized cells is given as percentages of the total number of DAPI-stained cells

SRB genera and groups; the respective oligonucleotide probes	Sampling depth, m; growth substrate			
	25 m, acetate + H_2	33 m, acetate + H_2	70 m, acetate + H_2	70 m, formate
Desulfovibrio spp./DSV698	0	0	0	0
Desulfovibrio spp./DSV1292	5.1	6.7	0	0
Desulfovibrio spp./DSV407	0	0	0	0
Desulfomicrobium spp./DSV214	12.1	10.1	9.5	9.2
Desulfobacterium spp./221	0	0.7	7.7	0
Desulfobulbus spp./660	0	0	0	0
Desulfosarcina and *Desulfococcus* spp./DSS658	0	0	0	0
Desulfoarculus and *Desulfomonile* spp./DSMA448	0	0	0	0
Desulforhopalus spp. / DSR651	0	0	0	0
Desulfotalea and *Desulfofustis* spp./Sval428	0	0	11.5	6.3
Desulfotomaculum spp./Dtm229	0	0	0	0
Sum of the total cell number (DAPI)	17.2	17.5	28.7	15.5

tion from 40 mg/l at 27 m to 34 mg/l at 31 m is probably the result of SO_4^{2-} consumption via sulfate respiration. Decreased sulfate concentration near the bottom of the lake is possibly caused by decreased rates of sulfur reoxidation. The absence of a peak in sulfate reduction in the water near the bottom of the lake where sedimentary organic matter accumulates is unusual for meromictic lakes. An electron acceptor other than sulfate is possibly dominant in the oxidation of organic matter in the deep region of the epilimnion; alternatively, most of the organic matter is oxidized directly in the chemocline.

In this work, the peak of SRB numbers determined by the MPN method was in a good correlation with the process rate determined with the radioactive label. Many instances are known of such a correlation not revealed by the studies of sulfate reduction in various ecosystems. The SRB number as determined by inoculation of selective media was relatively high compared to the numbers usually obtained by this method for samples from oligotrophic environments. These high SRB numbers were possibly the result of the use of several substrates for dilutions series cultures. The highest SRB numbers were found on the media with acetate and formate rather than on media with lactate, which is

often used as the sole substrate for cultivation of the SRB from natural samples. The SRB numbers as determined by FISH were in good correlation with the results obtained on selective media. The use of traditional culture-based techniques was previously demonstrated to enable the isolation of a physiologically and genetically diverse SRB community from the sediments of the oligotrophic Lake Stechlin [16].

Sulfate reduction in the oxic zone. A fact that deserves special attention is that sulfate reduction rates determined in the near-bottom anaerobic zone and at 27 m, where the ambient oxygen concentration was 0.8 mg/l, were of the same order of magnitude. Recent studies revealed that the distribution of SRB in the environment is not necessarily limited by the presence of oxygen [7]. Modern works on SRB ecology provide abundant evidence of the wide distribution of SRB in the aerobic zone and of the oxidation of various organic substrates with oxygen as the electron acceptor. We have determined relatively high sulfate reduction rates in oxidized wetland sediments (+340 mV) impacted by mine drainage waters [12].

The traditional radioisotope method of measuring sulfate reduction rates implies determination of the amount of labeled sulfate sulfur transformed to reduced compounds. Sulfate reduction products can undergo rapid oxidation in the oxic samples. The rate of the process as determined by laboratory measurement of radioactive sulfur can be therefore underestimated. Substantial amounts of radioactive sulfur were recovered as thiosulfate in our previous measurements of sulfate reduction rates in slightly oxic sediments and in the near-bottom water in the shallow zones of shelves of the Black Sea, the Sea of Okhotsk, the Caribbean, and the Sea of Japan [17]. Rapid chemical and/or biological reoxidation of sulfur produced in the course of sulfate reduction was a possible source of $S_2O_3^{2-}$ in the samples.

Unfortunately, no measurements of sulfate reduction rates in the oxic zone above 27 m were performed in the present investigation. However, the presence of SRB cells in the oxic zone was demonstrated both by analysis of culturable SRB and by FISH, which enabled the estimation of uncultured organisms. Their role in carbon and sulfur cycles requires further investigation.

Phylogenetic diversity of SRB. The set of oligonucleotide probes used in this work enabled the determination of the major genera and monophyletic groups of sulfate-reducing δ-*Proteobacteria* and low G+C *Firmicutes*. The ecological characteristics of the water column of the alpine oligotrophic lake provide no indication that sulfate-reducing *Archaea* and thermophilic eubacteria can be the dominant members of the dissimilatory sulfate-reducing community in this environment. The set of probes used in the present work is therefore adequate for evaluating the phylogenetic diversity of the SRB of the water column in the lake.

According to our findings on the SRB diversity in the Gek-Gel Lake water column, *Desulfovibrionales* were an important group both in the epilimnion and in the hypolimnion. The use both of traditional, culture-based microbiological techniques, and of modern molecular methods, which allow determination of unculturable organisms, led to the conclusion that *Desulfovibrio* are widespread in a variety of ecosystems, and their presence is not restricted by the sulfate availability in the system [7]. The strains phylogenetically related to *Desulfovibrio* constituted the majority of the culturable bacteria isolated from the oxic sediments of the oligotrophic Stechlin Lake in Germany [16]. The authors also remarked that oxygen was probably the main stress factor for SRB, in spite of the higher resistance of these isolates to O_2, the broader spectrum of substrates oxidized by oxygen, and higher catalase activity compared to the strains isolated from the anaerobic zone.

In the analysis of our data, the fact that *Desulfovibrio* probes used in the present work were selected in order to facilitate determination of the greatest number of representatives of the genus must be taken into account. The investigators who proposed this probe set demonstrated that some of them (e.g. DSV698 and DSV1292) can partially "overlap," being specific for the same species [6]. The simple calculation of the sum of all hybridized cells can therefore lead to overestimates due to the counting of one cell several times. The specificity of certain probes to non-sulfate-reducing phylogenetically related bacteria can also lead to overestimates. The probe DSMA488 (*Desulfoarculus–Desulfomonile*) shows, for example, 100% homology with two *Syntrophus* species [4].

Desulfobulbus spp., which was present in significant numbers at 33 m, is also a physiologically highly flexible sulfate-reducer incompletely oxidizing organic substrates. In estuarine sediments, *Desulfobulbus* was demonstrated to specialize in sulfate-independent H_2 oxidation [18]. This group is known to actively utilize such alternative electron acceptors as nitrate or nitrite, to ferment various organic compounds, to grow mixotrophically with H_2 and organic compounds, and to reduce ethanol and acetate to propionate with hydrogen [10].

In the oxic zone at 10 m, the highest number of cells was found by growth on media with acetate. The results of FISH demonstrated that *Desulfobacterium* spp. was the main group of SRB complete oxidizers at this depth.

The predominance of the SRB hybridizing with the DSM448 probe in the aerobic zone is in accordance with the known ecology of this group. In a number of studies, this group of halogen-reducing SRB was detected in ecosystems containing high concentrations of halogen-substituted organic compounds, e.g., in activated sludge, although most of the taxonomically distinct species of this group were isolated from marine sediments. Bacteria designated as "morphotype R" and characterized by 95% homology with *Desulfomonile*

constituted up to 30% of all DAPI-staining cells in the monimolimnion of meromictic Lake Cadagno; they numbered 1.5×10^6 cells/ml [19]. In the chemocline zone of Lake Cadagno, the *Desulfovibrio* group predominated, similar to Gek-Gel Lake. The cells of "morphotype T5," subsequently described as *Desulfomonile tiedjei* and *D. limimaris*, predominated in the hypolimnion of Lake Pluzee [20] and constituted 60% of all bacteria in the hypolimnion of lakes Wintergreen and Burke in Michigan [21].

The probes specific to *Desulfotomaculum* spp. were previously not used in studies on the phylogenetic diversity of SRB in lakes. Reports of the isolation of spore-forming SRB from lakes or of their detection by molecular techniques are scarce. We are not aware of published studies that report on the finding of *Desulfotomaculum* in lake water. Recent investigations confirm that gram-positive SRB are possibly the main SRB group in underground sulfidogenic communities [7]. Our data provide evidence of the possible important role of *Desulfotomaculum* in the anaerobic zone of water bodies. However, in the present work, only direct FISH analysis of water samples revealed cells related to *Desulfotomaculum*. No positive hybridization with Dtm229 probe was found in enrichment cultures.

Our results demonstrate that sulfate reduction in the water column is most active at 31 m, directly below the redox zone. A phylogenetically diverse SRB community is present in the zone of active sulfate reduction, as well as in the oxic zone. In the water column of the lake, the SRB performing incomplete oxidation of organic substrates prevail. The genus *Desulfovibrio*, endowed by numerous physiological and adaptive abilities, is prominent among the incomplete oxidizers. *Desulfobacterium* is prominent among complete oxidizers; this genus was found in the oxic zone of the lake together with *Desulfovibrio* spp. and *Desulfotalea–Desulfofustis*. The spore-forming *Desulfotomaculum* have been found for the first time in lake water. The low measured rates of sulfate reduction and high numbers of SRB, together with their systematic composition indicating the dominance of groups capable of reducing a wide range of substrates, allow us to suggest that SRB in lake water are possibly specialized in utilization of electron acceptors other than SO_4^{2-}.

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