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High genetic and physiological diversity of sulfate-reducing bacteria isolated from an oligotrophic lake sediment

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Abstract The community structure of sulfate-reducing bacteria in littoral and profundal sediments of the oligotrophic Lake Stechlin (Germany) was investigated. A collection of 32 strains was isolated from the highest positive dilutions of most-probable-number series, and their partial 16S rRNA gene sequences and genomic fingerprints based on ERIC (enterobacterial repetitive intergenic consensus)-PCR were analyzed. The strains fell into eight distinct phylogenetic lineages, and the majority (70%) showed a close affiliation to the genus *Desulfovibrio*. Most of the remaining strains (22%) were related to the gram-positive *Sporomusa* and *Desulfotomaculum* groups. A high redundancy of 16S rRNA gene sequences was found within several of the phylogenetic lineages. This low phylogenetic diversity was most pronounced for the subset of strains isolated from oxic sediment layers. ERIC-PCR revealed that most of the strains with identical 16S rRNA gene sequences were genetically different. Since strains with identical 16S rRNA gene sequences but different genomic fingerprints also differed considerably with respect to their physiological capabilities, the high diversity detected in the present work is very likely of ecological relevance. Our results indicate that a high diversity of sulfate-reducing bacterial strains can be recovered from the natural environment using the established cultivation media.

Key words Sulfate-reducing bacteria · Molecular fingerprinting · Bacterial diversity · Culturability

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Abbreviations *DGGE* Denaturing gradient gel electrophoresis · *ERIC* Enterobacterial repetitive intergenic consensus · *MPN* Most probable numbers

Introduction

Sulfate-reducing bacteria are of great significance for the carbon and sulfur cycles in sediment ecosystems. In marine sediments these bacteria can catalyze more than 50% of the total carbon oxidation (Jørgensen 1982; Howarth and Giblin 1983). High rates of sulfate reduction (Bak and Pfennig 1991 a) and high numbers of viable sulfate-reducing bacteria (Bak and Pfennig 1991 b; Rothfuß et al. 1997) have also been detected in freshwater sediments, where sulfate reduction may contribute more than 20% to total anaerobic mineralization (Ingvorsen and Brock 1982).

Recent molecular ecology studies of marine sediments have indicated differences in the community structure of sulfate-reducing bacteria both in a horizontal and in a vertical dimension. The communities differed markedly between vegetated and unvegetated sites in a salt marsh (Devereux et al. 1996 a). In a microbial mat, different phylogenetic groups of sulfate-reducing bacteria have been restricted to nonoverlapping depth intervals (Risatti et al. 1994).

To date, 16S rRNA gene sequences have been used to assess the diversity of natural populations of sulfate-reducing bacteria. The nucleotide sequences of 16S rRNA genes are conservative and change much more slowly than the whole bacterial genome and phenotypic traits. It can therefore be expected that different strains of sulfate-reducing bacteria with identical or nearly identical 16S rRNA gene sequences but with different physiological capacities coexist in aquatic sediments. Only little information is currently available on this subspecies diversity of sulfate-reducing bacteria in the natural habitat.

Diversity on the subspecies scale can be assessed either by phenotypic characterization or by molecular fingerprinting methods such as randomly amplified polymorphic DNA (RAPD), repetitive extragenic palindromic

(REP)-PRC, or enterobacterial repetitive intergenic consensus (ERIC)-PCR) (Versalovic et al. 1991). Both approaches rely on the availability of pure cultures. ERIC- and REP-PCR patterns have been mostly applied to assess the diversity of culturable strains of various soil bacteria or pathogens (e.g., De Bruijn 1992; Dunbar et al. 1997).

We studied the physiological and genetic differences in a collection of strains of sulfate-reducing bacteria isolated from the highest positive dilutions of most-probable-number (MPN) series. The collection represents the most frequent culturable sulfate-reducing bacteria of the natural bacterial community. In parallel, the diversity of 16S rRNA gene sequences within the culture collection was investigated, and it was tested whether the 16S rRNA gene fragments of the different strains yield distinct molecular fingerprints in denaturing gradient gel electrophoresis (DGGE; Muyzer et al. 1993). An oligotrophic freshwater sediment was chosen as the study site because vertical gradients of oxygen and sulfide are less steep in nutrient-poor sediments, and different ecological niches should be spatially well-separated in the vertical dimension under these conditions.

Materials and methods

Study-site and sampling procedures

Lake Stechlin is a dimictic, oligotrophic freshwater lake located approximately 100 km north of Berlin (Germany). The lake has a maximum depth of 68 m and a surface area of 4.25 km^2. In October and December 1993, sediment samples were collected from littoral (water depth, 0.5 m at a distance of 15 m from the shore) and profundal (32-m depth) sites (Sass et al. 1997). At the littoral sampling site, sediment cores were obtained manually with plexiglas corers. A Jenkin sediment sampler (Collins 1977) was employed for profundal sediments. After collection, sediment samples were sliced into 5- or 10-mm-thick segments and were processed immediately. Sediment temperatures were 10°C in the littoral sampling site and 5°C in the profundal one. In the following, "oxic" sediment layers denote those layers that contained detectable concentrations of molecular oxygen (≥ 2 μM) as measured by oxygen needle electrodes (Sass et al. 1997).

Isolation and phenotypic characterization of sulfate-reducing bacteria

The 32 pure cultures used in the present study had originally been isolated from the highest dilution steps of MPN dilution series set up in five parallels with sediment from ten consecutive depths between 0 and 8 cm (Sass et al. 1997). Three different growth media were used in that previous study. Lactate-utilizing strains were obtained on carbonate-buffered and dithionite-reduced mineral medium containing 20 mM lactate and 10 mM sulfate (Widdel and Bak 1992). Cultures were incubated for 5 weeks at 28°C. For the growth of acetate-utilizing strains, the same mineral medium was used, but with 20 mM acetate replacing the lactate. Thiosulfate-disproportionating bacteria were counted with 15 mM thiosulfate plus 2 mM acetate as a carbon source. MPN tubes for the latter two groups of bacteria were incubated for 3 months. From each depth, at least two isolates were obtained by repeated deep-agar dilution series, each originating from a different MPN tube.

A detailed description of the methods used for the physiological characterization of the 32 different strains has already been published (Sass et al. 1996, 1997). In the present study, we used these data to estimate the phenotypic diversity within our culture collection. The results were then compared with the estimates of diversity as obtained by molecular biological methods.

In the following, strains isolated from littoral sediment are designated STL, whereas those obtained from the profundal sediment are designated STP.

Numerical taxonomy

A matrix was constructed with a binary code for the presence (1) or absence (0) of each phenotypic trait of all the isolates. The simple matching coefficient (SM) for all pairs of strains was calculated using the SIMQUAL similarity program for qualitative data of the NTSYS-pc Numerical Taxonomy Computer Package developed by Rohlf (1993).

Sequencing of the 16S rRNA gene

Genomic DNA was extracted with hot phenol and was purified using the FMC SpinBind Kit (Overmann and Tuschak 1997). The 16S rRNA gene sequences were amplified, and the products were purified as decribed previously (Overmann and Tuschak 1997) except that a step-down protocol (10 thermal cycles with the annealing temperature set to 59°C, followed by 20 cycles at an annealing temperature of 53°C) was employed during PCR.

In the present study, the phylogenetic analysis of the isolated strains of sulfate-reducing bacteria was limited to a 626-bp fragment of the 16S rRNA gene. The rationale for this approach was threefold:

1. Phylogenetic assignments as obtained from partial and full-length sequences are very similar (Lane et al. 1985; Schmidt et al. 1991).
2. The fragment length of 626 bp represents the maximum to be separated by DGGE and has been used previously for a culture-independent analysis of a community of sulfate-reducing bacteria (Teske et al. 1996).
3. Partial sequencing is a much faster and less-expensive approach for routine purposes such as analysis of complex bacterial communities.

The 16S rRNA gene fragment was sequenced directly with the SequiTherm Long-Read Kit (Epicentre; Madison, Wis., USA) using an automated infrared laser fluorescence sequencer (Li-Cor Model 4000 DNA sequencer). The sequencing primers employed were 338f and 907r (Lane 1991; numbers refer to 5′-positions relative to *Escherichia coli* 16S rRNA). Five 16S rRNA gene sequences (STL1, STL4, STL6, STL8, and STL10) were already available in the EMBL database (Rodriguez-Tomé et al. 1996).

Distance matrix and phylogenetic trees

Alignment, calculation of phylogenetic distances, and construction of phylogenetic trees were preformed as previously described (Overmann and Tuschak 1997) except that both the gap open penalty and the gap extension penalty were set to a value of 2 during alignment.

Based on their 16S rRNA gene sequences, the isolated strains of sulfate-reducing bacteria were classified by comparison with all sequences available in the Ribosomal Database Project [RDP; Maidak et al. (1997)] using the SIMILARITY RANK option. The phylogenetically most closely related 16S rRNA sequences were obtained with the SUBALIGNMENT program of the database and were used in subsequent sequence alignments.

Selective amplification and separation of 16 S rRNA gene sequences of sulfate-reducing bacteria

A PCR protocol was used that allowed amplification of 580-bp DNA fragments of the 16S rRNA gene selectively from the ge-

nomic DNA of δ-proteobacteria. These fragments were subsequently separated by DGGE. We used primer 385f (5′-CCTGACGCAGCGACGCCG-3′; Amann et al. 1990) containing a 40-bp GC-clamp (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGC CCCCGCCCC-3′) at the 5′-end, which resulted in a stable melting behavior of the DNA fragments during the subsequent DGGE. The second primer was 907r (5′-CCGTCAATTCCTTTGAGTTT-3′; Lane 1991). The DNA was amplified as described above, but at an annealing temperature of 61°C for the first 10 thermal cycles, followed by 20 cycles with an annealing temperature of 56°C.

DNA fragments were separated by DGGE in the BioRad D Gene System (BioRad, Munich, Germany). PCR samples were applied directly onto 6% (w/v) polyacrylamide gels [acrylamide/N,N′-methylene bisacrylamide ratio, 37.5 : 1 (w/w) in 1 × TAE (40 mM Tris-acetate, 1 mM EDTA) buffer (pH 7.4)]. Gels contained a linear gradient of 30–70% denaturant [100% denaturant = 7 M urea plus 40% (v/v) formamide]. Electrophoresis proceeded for 5 h at 200 V and 60°C. Afterwards, gels were stained for 20 min with ethidium bromide and were photographed.

Distance coefficients based on genomic fingerprinting

Specific patterns of differently sized DNA fragments were produced by PCR using primers complementary to ERIC sequences (Versalovic et al. 1991) and the PCR conditions described previously (Overmann and Tuschak 1997). Amplification products were visualized by gel electrophoresis on 1.5% (w/v) agarose gels in 0.5 × TBE buffer [45 mM Tris-borate (pH 8.0) and 1 mM EDTA] using a 100-bp ladder standard for length calibration. Gels were stained with ethidium bromide (0.5 μg ml^{-1}), and digital images were captured under UV illumination using a CCD black-and-white video camera (CF 8/1 RCC; Kappa, Gleichen, Germany) and a personal computer image analysis system (ImageP2; H & K, Berlin, Germany).

The generated DNA band patterns were analyzed with the aid of the ONE-Dscan electrophoresis analysis software (Scanalytics; Billerica, Mass., USA). The lengths of all DNA fragments of each strain were determined with reference to the internal 100-bp standard. Relative intensities of all DNA bands in one lane (i.e., for each strain) were determined using the ONE-Dscan program. The relative density values of the fragments were incorporated in a matrix, and pairwise distance values were calculated with the SIMGEND program of the NTSYS package employing the Rogers (1972) distance coefficient as modified by Wright (Wright 1978).

Evaluation of the diversity of microbial communities

Our analysis yielded three types of pairwise distance or similarity coefficients: K_{nuc} values (i.e., substitutions per base of the 16S rRNA gene sequence), Rogers distance coefficients based on ERIC-PCR fingerprints, and similarity indices based on numerical taxonomy. The MXCOMP program of the NTSYS package allowed a direct comparison of the three coefficients for each pair of bacterial strains.

The diversity of culturable sulfate-reducing bacteria was assessed further based on species diversity indices. The Shannon-Weaver index (H′ and the standardized equitability J) was used to describe the apportionment of the various strains within the culturable fraction of the microbial community. These indices were calculated separately for oxic and anoxic sediment layers, or for littoral and profundal sediments.

The calculation of diversity indices was based on the clustering of 16S rRNA gene sequences or ERIC-PCR fingerprint patterns. For each habitat, the number of strains n_i with the same 16S rRNA gene sequence (i.e., a $K_{nuc} < 0.005$) or the same ERIC-PCR band pattern was calculated, and the total number of strains N per habitat was noted. The indices calculated were as follows (Atlas and Bartha 1993; Odum 1973):

Shannon-Weaver index of diversity: $H' = -\Sigma\,(n_i/N)\ln(n_i/N)$

and equitability: $J = H'/H_{max}$

where $H_{max} = -\ln(1/N)$. Equitability was chosen to account for the varying number of strains in different habitats.

Accession numbers

The 16S rRNA gene sequences obtained in the present study were deposited in the EMBL database under accession nos. X99501–X99505 and AJ006599–AJ006620.

Results

Phenotypic characterization

The results of the previous physiological characterization (Sass et al. 1997) were rearranged in a binary matrix suitable for a numerical taxonomy analysis (Fig. 1). Based on 34 different phenotypic properties, all 32 strains isolated from Lake Stechlin are different. Desulfoviridin-containing strains clearly dominated in littoral sediments. Gram-positive strains capable of endospore formation were exclusively recovered from profundal sediment samples. These strains exhibited a higher metabolic flexibility with regard to the spectrum of electron-donating substrates that could be utilized. Butyrate, methanol, alanine, and nicotinate were used only by strains from this group. All the gram-positive strains fermented lactate, but none showed catalase activity or could respire hydrogen, formate, lactate, ethanol, or reduced sulfur compounds with molecular oxygen.

Phylogenetic analysis and 16S rRNA gene fingerprints

Partial 16S rRNA gene sequences were obtained for all 32 strains of sulfate-reducing bacteria from Lake Stechlin. Five of the 16S rRNA gene sequences, which contained ambiguous base positions, were discarded.

According to our phylogenetic analysis, the isolated strains fall into eight distinct phylogenetic clusters (Fig. 2; Table 1). Two (the cluster STP 2/3/11/13/14, and STP12) are phylogenetically closely related to members of the gram-positive *Sporomusa* or *Desulfotomaculum* groups and were isolated exclusively from profundal sediments (Sass et al. 1997). To our knowledge, the newly isolated strains STP 2/3/11/13/14 to date represent the only members of the *Sporomusa* group that are capable of sulfate reduction. The remaining lineages represent members of the *Desulfovibrio* or *Desulfobulbus* assemblages of the δ-proteobacteria subdivision (Table 1). Out of 27 strains sequenced, 19 were members of the *Desulfovibrio* group, whereas only 2 fell into the *Desulfobulbus* assemblage. Interestingly, strain STP34 grouped with propionate-utilizing species but could not use this substrate. Because *Syntrophobacter* strains are known to grow extremely slowly on propionate plus sulfate (doubling time, 11 days; Wallrabenstein et al. 1994) as compared to *Desulfobulbus* strains (10 h; Widdel and Pfennig 1982), we used incubation times of ≥ 1 month to confirm that strain STP34 did not oxidize propionate. Only members of the *Desulfovib-*

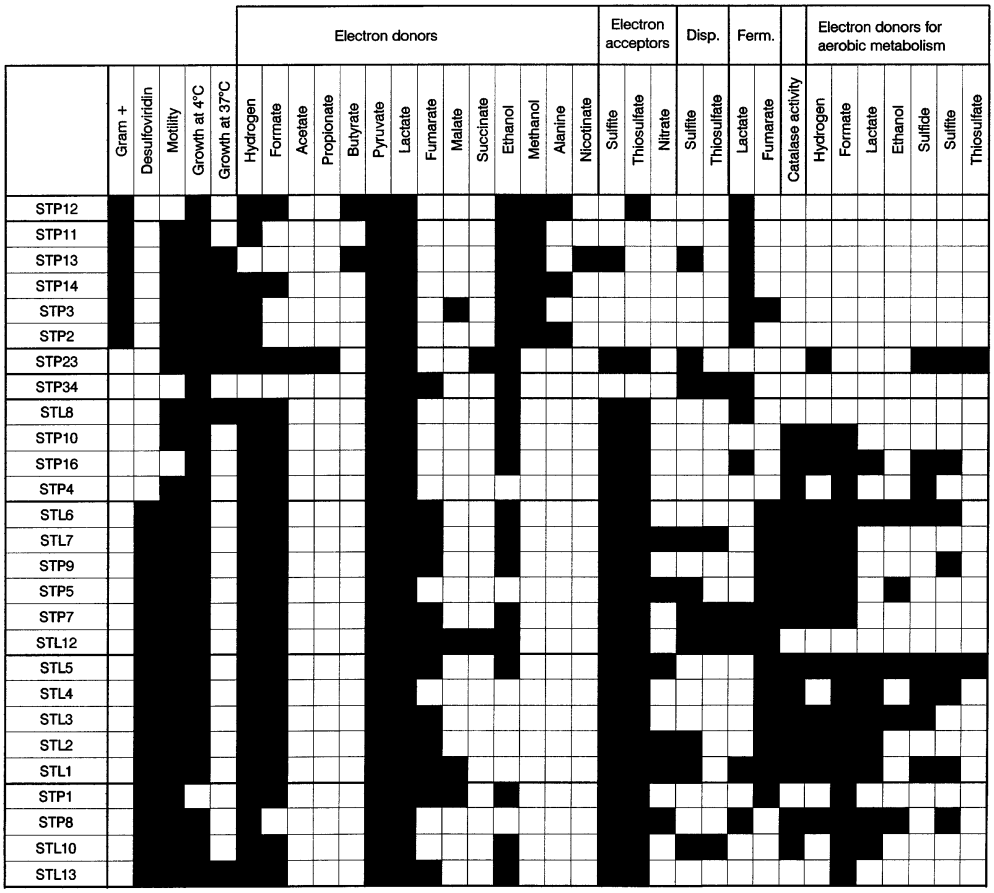

Fig. 1 Phenotypic characterization of sulfate-reducing bacteria from Lake Stechlin. The presence (■) and absence (□) of individual phenotypic traits is indicated. Only the 27 strains included in the phylogenetic tree are shown to facilitate comparison with Fig. 2 (*Disp*. disproportionation, *Ferm*. fermentation)

Table 1 Phylogenetic affiliation of the 27 strains isolated from sediments of Lake Stechlin (*RDP* Ribosomal Database Project)

Strains	Closest relative in RDP	% Similarity
Desulfotomaculum group		
STP12	*Desulfitobacterium dehalogenans* DSM 9161	94.2
Sporomusa subgroup		
STP2, 3, 11, 13, 14	*Sporomusa termitida* JSN-2	94.6–94.9
Desulfobulbus assemblage		
STP23	*Desulfobulbus* sp. DSM 2058	89.3
STP34	*Syntrophobacter wolinii*	95.4
Desulfovibrio group		
STL6, 7, 12; STP5, 7, 9	*Desulfovibrio desulfuricans* ATCC 27774	88.7–89.9
STL10, 13; STP1, 8	*Desulfovibrio desulfuricans* ATCC 27774	90.3–91.9
STL1, 2, 3, 4, 5	*Desulfovibrio longreachii* 16910a	90.1–90.4
STL8; STP4, 10, 16	*Desulfomicrobium escambium* ATCC 51164^T	96.8–98.4

rio lineage were isolated from oxic sediment layers and from littoral sediments as a whole.

The species composition of natural bacterial assemblages can be analyzed by DGGE (Muyzer et al. 1993), which separates 16S rRNA gene fragments according to their different base sequences. It was investigated whether the 16S rRNA gene sequences of the 26 gram-negative isolates (out of the complete collection of 34 strains) were sufficiently different to allow their separation by DGGE (Fig. 3).

Amplification of the 16S rRNA gene fragment with the primer pair GC385f and 907r yielded unspecific products in the case of four strains (not loaded on the DGGE gel of Fig. 3). The DNA fragments amplified from four additional strains did not exhibit a stable melting behavior during DGGE (two of them, strains STL13 and STP23, are depicted in Fig. 3). Thus, a total of 31% of all the gram-negative strains we isolated would not be detected at all by this cultivation-independent molecular approach. In general, 16S rRNA gene fragments amplified from closely related strains could not be separated by DGGE (compare the position of bands for strains STP4, STP16, STP10, and STL8 in Fig. 3).

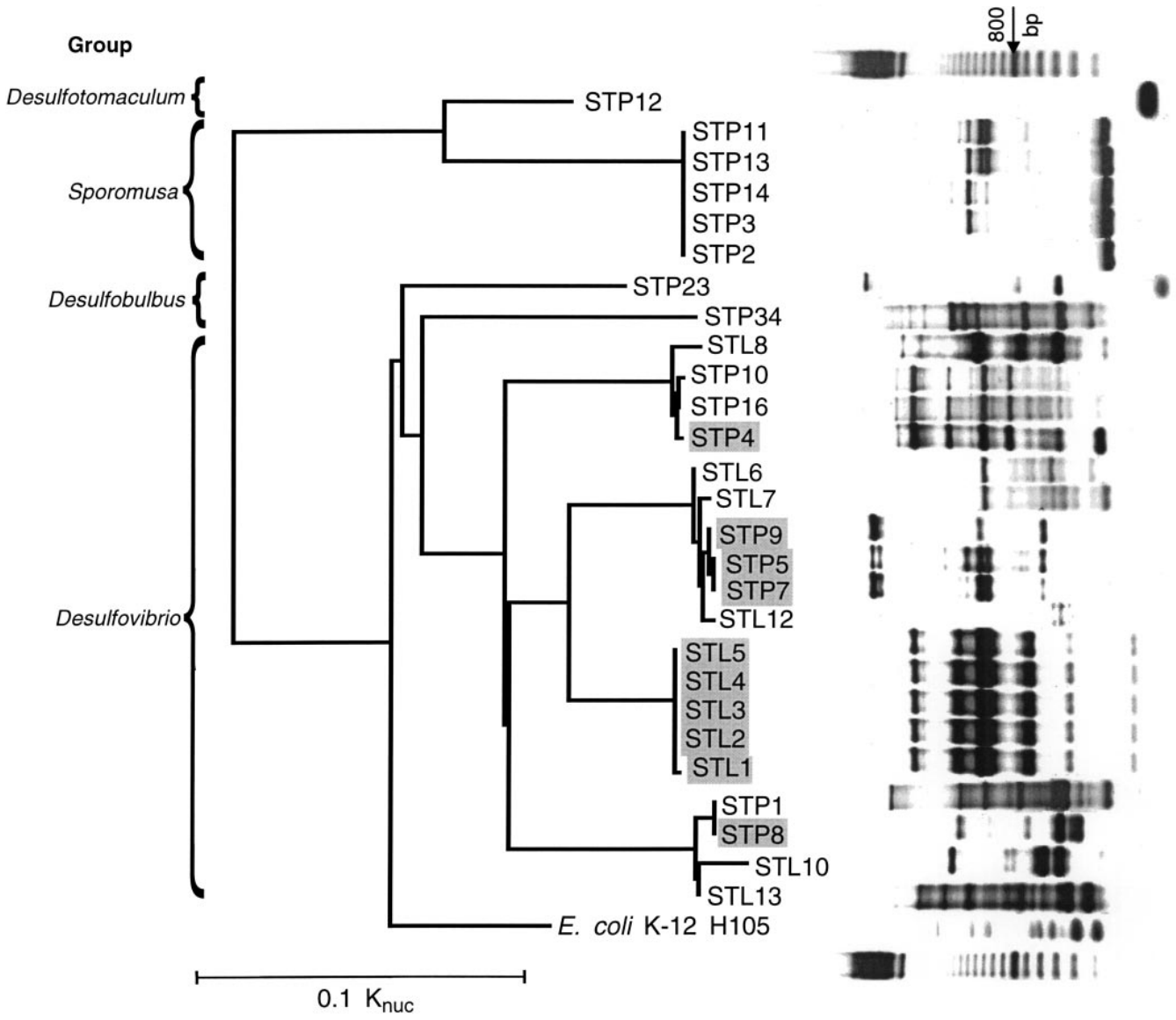

Fig. 2 Phylogenetic tree of 27 strains of sulfate-reducing bacteria isolated from sediments of Lake Stechlin. Strains isolated from oxic sediment layers (≥ 3 µM O_2) are indicated by *shaded boxes*. *On the right*, genomic fingerprints of the strains that were generated by enterobacterial repetitive intergenic consensus (ERIC)-PCR are depicted as a negative image of an ethidium-bromide-stained gel. Flanking lanes contain molecular size standards (100-bp DNA ladder)

Genomic fingerprinting

Most of the closely related strains within one phylogenetic cluster could be distinguished by their distinct ERIC-PCR band patterns. With the latter technique, the genetic diversity in our culture collection could be resolved to a much higher degree as compared to the 16S rRNA approach. Strains that had identical partial 16S rRNA gene sequences (strains STP1/STP8, STP5/STP7, or STP2/STP3/STP13) could be distinguished by their ERIC-PCR banding patterns (Fig. 2). Only the strains of the phylogenetic cluster STL1–5 were also identical with respect to their genomic fingerprints.

Different distance coefficients as a measure of diversity

Phenotypic properties, 16S rRNA gene sequences, and genomic fingerprints were used to calculate the phenotypic similarity coefficient, phylogenetic distance (K_{nuc}), and Rogers distance coefficient for each pair of strains of the collection. These different coefficients were then compared to each other (Fig. 4) in order to evaluate their potential as a measure of diversity.

Phenotypic similarity was significantly correlated with phylogenetic distance of the sulfate-reducing bacteria (Fig. 4 A; $r = -0.723$, $p < 0.01$). Consequently, phylogenetically closely related strains also tend to be phenotypically similar. However, the considerable scatter of data points in Fig. 4 A indicates that only part of the variance

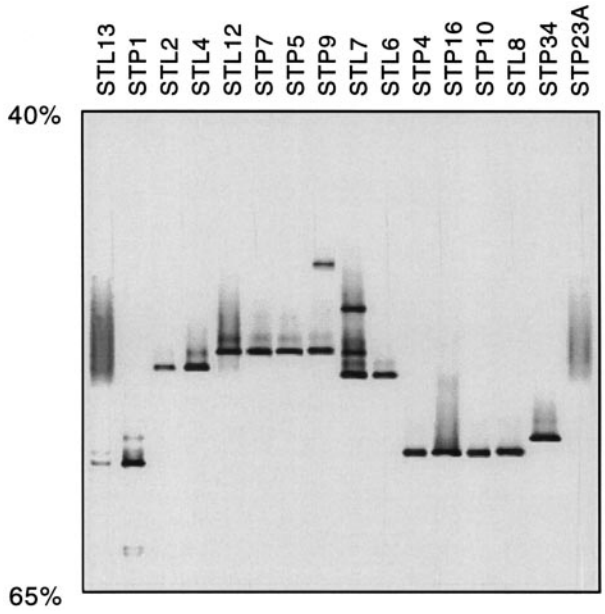

Fig. 3 Separation of 16S rRNA gene fragments by denaturing gradient gel electrophoresis (DGGE). Percent values *on the left* indicate concentration of denaturant. A negative image of an ethidium-bromide-stained gel is shown. Only PCR samples containing specific amplification products were applied to the DGGE gel. Since strains STL1–STL5 were indistinguishable by their partial 16S rRNA gene sequence and genomic fingerprints, only strains STL2 and STL4 were included in this analysis

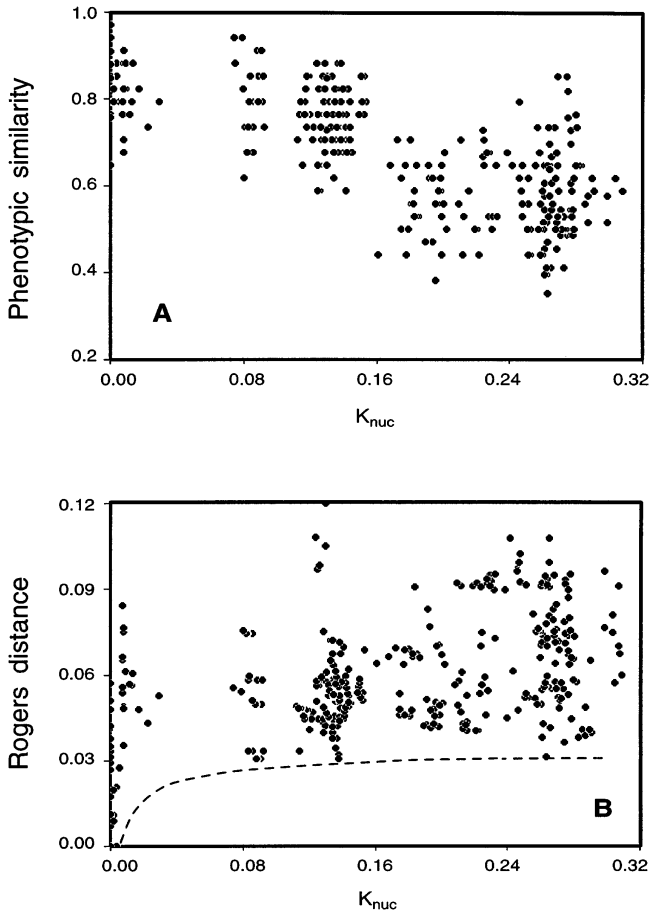

Fig. 4 **A** Correlation of similarity coefficients based on numerical taxonomy with the K_{nuc} values of the molecular phylogeny analysis. Each dot represents a pair of strains. **B** Comparison of Rogers distance coefficients based on molecular fingerprints with the K_{nuc} value. The *broken line* indicates the lower limit of Rogers distance coefficients observed for the set of strains from Lake Stechlin

(52%, as $r^2 = 0.523$) between both coefficients is explained by this correlation.

As in other groups of bacteria, fingerprinting by ERIC-PCR is suitable for distinguishing strains of sulfate-reducing bacteria with very similar or even identical 16S rRNA gene sequences. In our culture collection, 20 pairs of strains existed in which both strains had identical partial 16S rRNA gene sequences but exhibited a considerably different phenotype (e.g., strains STP13 and STP14; cf. Figs. 1 and 2). Of these, 80% could be distinguished by their different ERIC-PCR band pattern. According to our analyses (Fig. 4B), two strains with a Rogers distance coefficient of < 0.025 (and thus similar ERIC band patterns) invariably belong to the same species (defining a species by a 16S rRNA gene sequence similarity ≥ 98% and a $K_{nuc} < 0.02$; Stackebrandt and Goebel 1994). We never observed similar band patterns for phylogenetically distant strains (empty area below the broken line in Fig. 4B). On the other hand, a different genomic fingerprint does not indicate a high phylogenetic distance (compare the scatter of the Rogers distance coefficient at $K_{nuc} = 0.00$).

Diversity of sulfate-reducing bacteria recoverable by cultivation

The diversity of 16S rRNA gene sequences and ERIC band patterns observed within our culture collection was used to estimate the number of different strains that could possibly be obtained with our cultivation techniques. The 27 strains of sulfate-reducing bacteria fell into 13 clusters with identical 16S rRNA gene sequences. In a similar way, strains were divided into groups with identical genomic fingerprints. As a consequence of the higher resolving power of the fingerprinting method, a total of 21 groups were found (26 groups, if all 32 strains were considered). The cumulative curve of the 16S rRNA gene sequence types (triangles in Fig. 5) indicates that only a few strains of sulfate-reducing bacteria with new partial 16S rRNA gene sequences will be recovered in additional cultivation attempts with the mineral medium employed. As judged from the cumulative curve of ERIC band patterns in Fig. 5, however, further isolation attempts would be expected to yield a high number of genetically (and physiologically; see Discussion) new strains.

Based on the number of phylogenetic or phenetic groups and the total number of strains, diversity indices were calculated for the different sediment habitats. The values indicated that the diversity of sulfate-reducing bacteria was lower in oxic sediment layers as compared to the anoxic habitat, and that profundal sediments harbored a

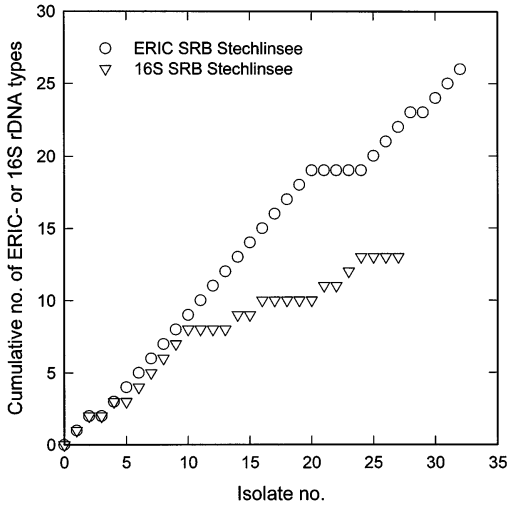

Fig. 5 Comparison of the diversity in the culture collection obtained from Lake Stechlin. Random numbers were generated and assigned to the strains (isolate number), and the sequential detection of cumulative enterobacterial repetitive intergenic consensus (ERIC)-band patterns (○) and 16S rRNA gene sequences (▽) plotted

Table 2 Shannon-Weaver index of diversity (*H′*) and equitability (*J*) of sulfate-reducing bacteria isolated from different habitats of Lake Stechlin (*ERIC* enterobacterial repetitive intergenic consensus)

Habitat	Phylotype[a]		ERIC patterns[b]	
	H′	J	H′	J
Oxic	1.17	0.507	1.83	0.797
Anoxic	2.20	0.775	2.59	0.914
Littoral	1.54	0.642	1.97	0.822
Profundal	1.77	0.639	2.51	0.901

[a] Indices calculated on the basis of 16S rDNA sequence types
[b] Indices calculated on the basis of DNA band patterns generated by ERIC-PCR

more diverse community of culturable sulfate-reducing bacteria than did the littoral sediment (Table 2). By comparison, the method of genomic fingerprinting was clearly superior to that of the phylogenetic analysis of 16S rRNA gene sequences with respect to the evaluation of the full genomic diversity.

Discussion

Diversity of the isolated sulfate-reducing bacteria

In the present study, the diversity of sulfate-reducing bacteria in freshwater sediments was analyzed by four different approaches: partial 16S rRNA gene sequencing, DGGE, genomic fingerprinting, and physiological characterization. All strains were obtained from the highest positive dilutions of MPN series and thus represent the most frequent culturable strains of the microbial community.

Out of the 27 strains sequenced, 70% belonged to the *Desulfovibrio* group. A very similar dominance of the *Desulfovibrio* group has been found in culture collections from another freshwater sediment (Bak and Pfennig 1991 b), from an oil well (Voordouw et al. 1996), and in enrichment cultures from several wastewater and soil sites (Telang et al. 1994). By means of culture-independent methods, *Desulfovibrionaceae* have also been found to be dominant in an estuarine sediment (Devereux et al. 1996 b) and in the upper layers of a hypersaline microbial mat (Risatti et al. 1994).

To date, mostly 16S rRNA gene sequences have been used for the assessment of the diversity of sulfate-reducing bacteria in natural habitats (Risatti et al. 1994; Devereux et al. 1996 a). Similar to our results, a high redundancy of 16S rRNA gene sequences in bacterial communities as a whole has been observed by several other investigators either by direct cloning and sequencing (Ward et al. 1990; Moyer et al. 1994) or by using collections of isolated strains (Dunbar et al. 1997). In other cases, a much higher diversity has been found (Bornemann et al. 1996; Godon et al. 1997). A high diversity of *Desulfovibrio* species has also been detected in a microbial mat using DGGE separation of [NiFe]hydrogenase gene fragments as a fingerprinting method (Wawer and Muyzer 1995).

For our culture collection, DNA band patterns generated by ERIC-PCR yielded a significantly higher estimate of genetic diversity than did 16S rRNA phylogeny. At least some of the diversity of sulfate-reducing bacteria thus resides in clusters of phylogenetically very tightly related strains. This diversity will not be resolved by DGGE or even by an extensive cloning/sequencing strategy targeting the 16S rRNA gene.

From an ecological point of view, however, an analysis of the full genetic diversity would be superfluous if all the closely related, but genetically different strains of sulfate-reducing bacteria in Lake Stechlin had an identical phenotype. We found two clusters of strains with identical partial 16S rRNA gene sequences: STL1-STL5 and STP2/3/11/13/14. Strains STL1-STL5 differ in as many as ten physiological properties (Fig. 1). Many of these properties (e.g., nitrate reduction, disproportionation of sulfite, fermentation of lactate, or the capacity for oxidation of H_2 and sulfide with molecular oxygen) are of concern with respect to the biogeochemical cycles in sediments. Thus, genetic diversity of the isolated sulfate-reducing bacteria is of ecological significance. Furthermore, the genomic and phenotypic diversity on the subspecies level of sulfate-reducing bacteria demands caution if the physiology of an organism is inferred from its 16S rRNA sequence alone [e.g., Kane et al. (1993)].

It is likely that our culture collection represents only a minor fraction of the total community of sulfate-reducing bacteria in Lake Stechlin sediments. This fraction can be estimated based on a comparison of the specific rate of sulfate reduction [31.2 fmol SO_4^{-2} (culturable cell)$^{-1}$ day^{-1}, calculated from MPN and sulfate reduction rates reported by Sass et al. (1997)] with published values of pure cultures. Considering the low in situ temperature (4–10°C)

and the Q_{10} of sulfate reduction (2.25; Bak and Pfennig 1991 b), the value calculated for culturable cells in Lake Stechlin is in the order of 100–200 fmol $cell^{-1}$ day^{-1}. For pure cultures, a maximum rate of 7.94 fmol $cell^{-1}$ day^{-1} was determined (Jørgensen 1982), indicating that between 1 and 10% of the physiologically active sulfate-reducing bacteria were recovered in our cultivation media.

The analysis of the genetic diversity of our culture collection strongly indicates that despite its limitations, the classic culture medium would yield even more genetically different strains upon further isolation attempts. The existing cultivation methods for sulfate-reducing bacteria already appear to be well-suited for the isolation of a high number of physiologically and genetically different strains. It remains to be elucidated whether (and which of) the isolates dominate the natural community of sulfate-reducing bacteria.

Diversity in different habitats of the sediment

Earlier studies (Canfield and DesMarais 1991; Fründ and Cohen 1992) have demonstrated high rates of sulfate reduction in oxic sediment layers. Our molecular biological analysis of diversity indicates that the oxic sediment layers in Lake Stechlin harbor a distinct but probably less diverse (Table 2) assemblage of culturable sulfate-reducing bacteria as compared to anoxic sediment layers. At the same time, the most probable numbers of sulfate-reducing bacteria in the oxic sediment layers were as high as those in the anoxic layers below (Sass et al. 1997). A similar vertical distribution was found for the sulfate reduction rates. Consequently, it appears that lower culturability is not the reason for the lower diversity of sulfate-reducing bacteria in oxic sediment layers. Instead, only a limited number of specialized strains seem to be adapted to the environmental conditions in this habitat.

The strains isolated from the oxic zone of Lake Stechlin sediment exhibited higher oxygen tolerance, a broader spectrum of substrates oxidized with O_2, and a higher catalase activity than did strains from anoxic habitats. However, as shown in previously published experiments (Sass et al. 1996), the growth of even these strains is inhibited by oxygen. Therefore, molecular oxygen appears to be a major environmental stress factor affecting sulfate-reducing bacteria in upper sediment layers. Our results are consistent with the general ecological theory that environments exposed to frequent stress harbor communities of lower diversity (Atlas and Bartha 1993).

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