Diversity of Sulfur Isotope Fractionations by Sulfate-Reducing Prokaryotes†

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Batch culture experiments were performed with 32 different sulfate-reducing prokaryotes to explore the diversity in sulfur isotope fractionation during dissimilatory sulfate reduction by pure cultures. The selected strains reflect the phylogenetic and physiologic diversity of presently known sulfate reducers and cover a broad range of natural marine and freshwater habitats. Experimental conditions were designed to achieve optimum growth conditions with respect to electron donors, salinity, temperature, and pH. Under these optimized conditions, experimental fractionation factors ranged from 2.0 to 42.0‰. Salinity, incubation temperature, pH, and phylogeny had no systematic effect on the sulfur isotope fractionation. There was no correlation between isotope fractionation and sulfate reduction rate. The type of dissimilatory bisulfite reductase also had no effect on fractionation. Sulfate reducers that oxidized the carbon source completely to CO_2 showed greater fractionations than sulfate reducers that released acetate as the final product of carbon oxidation. Different metabolic pathways and variable regulation of sulfate transport across the cell membrane all potentially affect isotope fractionation. Previous models that explained fractionation only in terms of sulfate reduction rates appear to be oversimplified. The species-specific physiology of each sulfate reducer thus needs to be taken into account to understand the regulation of sulfur isotope fractionation during dissimilatory sulfate reduction.

The stable sulfur isotope ratio between ^{32}S and ^{34}S of solid and dissolved sulfur compounds is widely used as a marker for bacterial sulfate reduction and bacterial processes associated with the recycling of sulfide (5, 8, 18). The reduction of sulfate by sulfate reducers is coupled to a pronounced enrichment of ^{32}S in the produced sulfide. However, the extent of the isotope enrichment remains a matter of ongoing debate. Results from batch-culture, continuous-culture, and resting-cell experiments suggested that the isotope enrichment is inversely proportional to sulfate reduction rates (9, 22, 23). Furthermore, below a threshold concentration of sulfate, the discrimination against ^{34}S apparently decreases (21). Previous experimental studies of the isotope fractionation were conducted with only a few selected species that were known at that time, mainly *Desulfovibrio* spp. and two *Desulfotomaculum* spp. (9, 15, 22, 28). Moreover, since most of these species were isolated from freshwater environments, they are not necessarily of ecological importance in marine environments.

The different electron donors used in these early pure-culture studies included ethanol, lactate, acetate, pyruvate, glucose, yeast extract, and hydrogen (22, 23). Today, a number of sulfate reducers are known that can metabolize a wide range of substrates including long-chain fatty acids, alcohols, and even aromatic compounds that represent relevant substrates for natural environments (33, 45, 47). Hydrogen, propionate, bu-

tyrate, and acetate appear to be the most important electron donors for sulfate reducers in natural marine environments (31, 40), but propionate and butyrate have never been used as electron donors in sulfur isotope fractionation experiments. There is a need to expand the existing database of sulfur isotope fractionations by sulfate reducers with organisms that are important in natural environments and to conduct experiments with additional relevant electron donors. For this reason, we included microorganisms that cover the total temperature range of environments from which sulfate reducers have been isolated. Furthermore, we used a variety of likely natural substrates and conducted experiments at different salinities and pHs to cover as broad a range of natural conditions as possible.

MATERIALS AND METHODS

Cultures, growth conditions, and sampling. The investigated microorganisms (Table 1) were obtained from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen [DSMZ], Braunschweig, Germany) or are recently isolated strains (*Desulfobacter* sp. ASv20; *Desulfovibrio* sp. strain X). The environmental sources for all organisms are listed in Table 1. In order to ensure reproducible growth conditions, all strains were transferred into fresh medium two times before an experiment was started. Cells were grown in strictly anoxic, carbonate-buffered mineral medium containing a single carbon source (see Table 2) and sodium sulfate in concentrations between 15 and 28 mM (46). Anoxic conditions were maintained by the addition of a 1 M sodium sulfide solution to a final concentration of 1 mM. The electron acceptor was not limiting. Strain-specific additives to the media (vitamins, trace metals, fatty acids) were prepared as described elsewhere in detail for each culture (DSMZ; http://www.dsmz.de/species/bacteria.htm). Growth experiments were performed in screw-cap glass bottles (56-ml volume) without headspace. To avoid cracking of the culture bottles from expanding hot media, the thermophilic strains were incubated in 125-ml butyl rubber-stoppered glass bottles containing 50 ml of growth media. The headspace was completely replaced by N_2-CO_2 (80/20 [vol/vol]). A similar incubation procedure was necessary for hydrogen-oxidizing *Desulfomicrobium autotrophicum*. For this

TABLE 1. List of investigated strains

Microorganism	DSMZ[a]	Nucleo-tide[b]	Incuba-tion (°C)	NaCl (g/liter)
Archaeoglobus fulgidus strain Z	4139	Y00275	80	18
"*Desulfarculus baarsii*"	2075	M34403	28	1
Desulfobacca acetoxidans	11109	AF002671	37	1
Desulfobacter sp. ASv20			20	20
Desulfobacterium autotrophicum	3382	M34409	28	20
Desulfobacula phenolica	3384	AJ237606	28	20
Desulfobacula toluolica	7467	X70953	28	20
"*Desulfobotulus sapovorans*"	2055	M34402	28	1
Desulfobulbus elongatus	2908	X95180	28	1
Desulfobulbus "marinus"	2058	M34411	28	20
Desulfocella halophila	11763	AF022936	28	40
Desulfococcus sp.	8541		28	20
Desulfofrigus oceanense	12341	AF099064	9	20
Desulfohalobium redbaense	5692	X99235	37	100
Desulfomicrobium baculatum	4028	M37311	28	1
Desulfonatronovibrio hydrogeno-vorans	9292	X99234	28	11
Desulfonatronum lacustre	10312	Y14594	28	1
Desulfonema magnum	2077	U45989	28	20
Desulfosarcina variabilis	2060	M34407	28	14
Desulfospira joergensenii	10085	X99637	28	20
Desulfotalea arctica	12342	AF099061	20	20
Desulfotalea psychrophila	12343	AF099062	9	20
Desulfotignum balticum	7044	AF233370	28	20
Desulfotomaculum geothermicum	3669	X80789	50	20
Desulfotomaculum gibsoniae	7213	Y11576	28	1
Desulfotomaculum thermo-cisternum	10259	U33455	60	21
Desulfovibrio halophilus	5663	U48243	28	70
"*Desulfovibrio oxyclinae*"	11498	U33316	28	70
Desulfovibrio profundus	11384	U90726	28	20
Desulfovibrio sp. strain X			28	20
Thermodesulfobacterium commune	2178	L10662	60	0
Thermodesulfovibrio yellowstonii	11347	L14619	60	0

[a] DSMZ strain number.
[b] Nucleotide sequence accession number.

culture, the headspace was replaced by H_2-CO_2 (80/20 [vol/vol]) at 10^5 Pa overpressure. The gas was replenished several times during the incubation. All strains were incubated in the dark without agitation. Bottles were shaken manually every second day for approximately 10 s to prevent biofilm formation.

For every experiment, a set of 10 bottles was inoculated with a culture grown to the mid-exponential growth phase. Measurements were made on individual culture bottles immediately after inoculation (T_0) and in the early exponential (T_1), mid-exponential (T_2), late exponential (T_3), and early stationary (T_4) phases. At each time point, a screw-cap bottle was opened to withdraw aliquots of the cultures to determine the concentrations of dissolved sulfate, dissolved sulfide, $\delta^{34}S_{sulfate}$, $\delta^{34}S_{sulfide}$, and cell numbers. The aliquots were withdrawn in less than 30 s to minimize loss or oxidation of hydrogen sulfide. For the serum vials, aliquots for the determination of cell numbers and sulfate/sulfide concentrations were withdrawn with a syringe through the septum. The remaining volume was used for sulfur isotope analysis.

Determination of sulfate and sulfide. A 50-μl aliquot of the culture was added to 300 μl of 20% zinc acetate to precipitate dissolved sulfide. This procedure guaranteed that loss or oxidation of dissolved sulfide was negligible. Sulfate was determined after further dilution by nonsuppressed anion chromatography and conductivity detection. The eluent was 1 mM isophthalic acid in 10% methanol adjusted to a pH of 4.7 with sodium tetraborate. The flow rate was 1 ml/min. Sulfide was determined spectrophotometrically by the methylene blue method (11).

csSRR. A 500-μl aliquot of each culture was used for cell counting using an Axioplan phase-contrast microscope (Carl Zeiss, Jena, Germany) and a modified Neubauer grid (0.0025 mm² by 0.02 mm). Cells were fixed in 2% glutaraldehyde and stained with 4′,6′-diamidino-2-phenylindole (32). Cell-specific sulfate reduction rates (csSRRs, in moles cell^{-1} day^{-1}) were calculated for the exponential phase using the change in concentration of sulfate and cell number (cn) between time points (T_1) and (T_2) according to the following equation:

$$csSRR = \frac{SO_4^{2-}{}_{(2)} - SO_4^{2-}{}_{(1)}}{\frac{(cn_{(1)} + cn_{(2)})}{2} \cdot (T_{(2)} - T_{(1)})}$$

We prefer to use this measure of metabolic activity because it can be directly compared to the change in isotopic composition of sulfate during cell growth. The equation is not valid for the lag and stationary phases.

Determination of stable sulfur isotopes. For the screw-cap bottles, 40 ml of the remaining culture was added to 10 ml of 20% zinc acetate to terminate microbial activity and to precipitate all dissolved sulfide. For the butyl rubber-stoppered serum vials, 10 ml of 20% zinc acetate was directly added though the septum. Dissolved sulfate and precipitated zinc sulfide were separated by filtration through 0.45-μm-pore-size Millipore filters. The filter was washed three times and the wash was added to the filtrate. Dissolved sulfate was precipitated as $BaSO_4$ with 1 M $BaCl_2$ at pH 4.0. For sulfur isotope determination, 300 to 400 μg of $BaSO_4$ was weighed into tin cups that contained a 10-fold excess of V_2O_5. The isotopic composition of $BaSO_4$ was determined by continuous-flow isotope-ratio-monitoring gas chromatography-mass spectrometry according to methods described elsewhere (16). The sulfur isotopic composition is expressed in the standard δ-notation given by $\delta^{34}S = (R_{sample}/R_{standard} - 1) \cdot 1,000$, where $R = {}^{34}S/{}^{32}S$. Values are expressed on a per mille (‰) basis using the VCDT scale (37). The mean and standard deviation for the international reference standard NBS 127 (20.0‰) was 20.0‰ ± 0.3‰ versus VCDT.

Determination of isotope fractionation factors. Microbial reduction of sulfate by the culture occurred in sealed serum vials without loss of product. These conditions are analogous to closed systems, allowing calculation of the isotope fractionation according to a Rayleigh fractionation model (27). Isotope fractionation factors (ε) were determined after non-linear regression to determine the function best reflecting the isotopic composition of dissolved sulfate ($\delta^{34}S$) at each time point (T_0 to T_4) on the basis of the isotopic composition of sulfate and the fraction of remaining sulfate (SO_4^{2-}), according to the following equation: $\delta^{34}S^{T1}{}_{SO_4} = -\varepsilon \ln{}_{(SO_4^{2-})} + \delta^{34}S^{T0}{}_{(SO_4)^{2-}}$. In dual experiments, the standard deviation of ε usually was smaller than 1‰.

Comparative analysis of 16S rRNA sequences. The sequences of the 16S rRNA genes were determined as described previously (29). Sequences that were not included in the 16S rRNA sequence database of the Technical University Munich in the program package ARB (41) were added from other databases. All sequences contained at least 1,200 bases. The tool ARB_ALIGN was used for sequence alignment. The alignment was checked visually and corrected manually. Tree topologies were evaluated by performing maximum parsimony, neighbor joining, and maximum likelihood analysis. Alignment positions at which less than 50% of the sequences of the entire data set shared the same residues were excluded from the calculations.

RESULTS

Variability of isotope fractionation. All of the 32 sulfate-reducing bacteria discriminated against ^{34}S during sulfate reduction. *Desulfonema magnum* showed the largest fractionation (ε = 42.0‰), and *Desulfovibrio halophilus* showed the smallest (ε = 2.0‰) (Table 2). Complete-oxidizing sulfate reducers fractionated sulfate between 15.0‰ (*Desulfosarcina variabilis*) and 42.0‰ (*Desulfonema magnum*), whereas the acetate-excreting incomplete oxidizers showed fractionations between 2.0‰ (*Desulfovibrio halophilus*) and 18.7‰ (*Desulfonatrunum lacustre*) (Table 2). The average isotope fractionation of the complete oxidizers (ε = 25‰) was more than 15‰ greater than that of the incomplete oxidizers (ε = 9.5‰). When the electron donor for *Desulfobacterium autotrophicum* was changed from butyrate to hydrogen, the fractionation decreased from 32.7 to 14.0‰. The oxidation of formate by *Desulfonatronovibrio hydrogenovorans* yielded a fractionation of 5.5‰.

Phylogeny. In order to cover the known phylogenetic diversity of sulfate reducers, we investigated Archaea (*Archaeoglobus fulgidus*), members of the deep-branching Thermodesulfovibrio (*T. yellowstonii*) and Nitrospira (*Thermodesulfobacterium commune*) subgroups, the low G+C subgroup (*Desulfoto-*

TABLE 2. Cell-specific sulfate reduction rates and fractionation factors of investigated sulfate-reducing prokaryotes

Microorganism[a]	Isolated from:	Electron donor (mM)	csSRR (fmol cell^{-1} day^{-1})	ε (‰)
Complete oxidizing				
Desulfonema magnum	Marine mud	Benzoate (3)	5.9	42.0
Desulfobacula phenolica	Marine mud	Benzoate (3)	125.0	36.7
Desulfobacterium autotrophicum	Marine mud	Butyrate (20)	64.8	32.7
Desulfobacula toluolica	Marine mud	Benzoate (3)	40.3	28.5
Desulfotomaculum gibsoniae	Freshwater mud	Butyrate (20)	13.0	27.8
Desulfospira joergensenii	Marine mud	Pyruvate (20)	0.9	25.7
"*Desulfarculus baarsii*"	Lake mud	Butyrate (20)	11.5	23.2
Desulfotignum balticum	Marine mud	Butyrate (20)	4.2	23.1
Desulfofrigus oceanense	Arctic sediment	Acetate (20)	7.6	22.0
Desulfobacter sp. ASv20	Arctic sediment	Acetate (20)	6.6	18.8
Desulfobacca acetoxidans	Anaerobic sludge	Acetate (20)	17.0	18.0
Desulfococcus sp.	Marine mud	Pyruvate (20)	41.8	16.1
Desulfosarcina variabilis	Marine mud	Benzoate (3)	11.1	15.0
Incomplete oxidizing				
Desulfonatronum lacustre∗	Alkaline lake mud	Ethanol (20)	16.2	18.7
Archaeoglobus fulgidus strain Z∗	Submarine hot spring	Lactate (20)	63.8	17.0
Thermodesulfovibrio yellowstonii	Thermal vent water	Lactate (20)	24.0	17.0
"*Desulfobotulus sapovorans*"	Freshwater mud	Lactate (20)	26.0	16.5
Desulfotomaculum thermocisternum∗	Oil reservoir	Lactate (20)	310.0	15.0
Desulfomicrobium baculatum	Manganese ore	Lactate (20)	4.8	12.7
Desulfotomaculum geothermicum∗	Aquifer	Lactate (20)	8.1	12.5
Desulfohalobium redbaense	Saline sediment	Lactate (20)	434.0	10.6
Desulfocella halophila	Great salt lake	Pyruvate (20)	10.2	8.1
Desulfobulbus "*marinus*"	Marine mud	Propionate (20)	28.9	6.8
Desulfotalea arctica	Arctic sediment	Lactate (20)	4.2	6.1
Desulfobulbus elongatus	Digester	Propionate (20)	35.3	5.5
Desulfovibrio sp. strain X	Hydrothermal vent	Lactate (20)	36.0	5.4
Thermodesulfobacterium commune	Thermal spring	Lactate (20)	45.4	5.0
"*Desulfovibrio oxyclinae*"	Hypersaline mat	Lactate (20)	69.1	4.5
Desulfotalea psychrophila	Arctic sediment	Lactate (20)	6.3	4.3
Desulfovibrio profundus	Deep sea sediment	Lactate (20)	17.0	4.1
Desulfovibrio halophilus	Hypersaline microbial mat	Lactate (20)	33.1	2.0
Hydrogen and formate				
Desulfobacterium autotrophicum	Marine mud	H$_2$ (10^5 Pa)	34.8	14.0
Desulfonatronovibrio hydrogenovorans	Lake mud	Formate (20)	12.7	5.5

[a] Under experimental conditions, strains followed by an asterisk are considered incomplete oxidizers.

maculum spp.), and the δ subclass of the *Proteobacteria* (δ-*Proteobacteria*) (Fig. 1). Strains of all three orders of the δ-*Proteobacteria* with dissimilatory sulfate-reducing activity (*Desulfovibrionales, Desulfobacterales*, and *Synthrophobacterales*) were selected for isotopic characterization. However, there was no relationship between the fractionation and the phylogeny of the investigated strains (Fig. 1). For example, the distant species *Desulfarculus baarsii* and *Desulfotignun balticum* yielded similar fractionations of 23.2 and 23.1‰, whereas *Desulfocella halophila* (8.1‰), which is closely related to *Desulfarculus baarsii*, showed a very different fractionation. On the other hand, very closely related strains such as *Desulfotalea arctica* and *Desulfotalea psychrophila* fractionated similarly (4.6 and 6.5‰, respectively).

Strain-specific factors. The sulfur isotope fractionation was independent of the sulfate reduction rates when the specific optimum growth conditions for each organism were used (Fig. 2). These rates can be considered as the maximum possible sulfate reduction rates for each organism under batch culture conditions. Nevertheless, the rates varied by more than 2 orders of magnitude. The scatter in Fig. 2 indicates that no uniform relationship exists between isotope fractionation and sulfate reduction rate that would be valid for all sulfate reduc-

ers. Furthermore, the lowest and highest rates measured, 0.9 fmol cell^{-1} day^{-1} for *Desulfospira joergensenii* and 4,340 fmol cell^{-1} day^{-1} for *Desulfohalobium redbaense*, yielded only intermediate fractionations of 25.7 and 10.6‰, respectively (Table 2). Conversely, despite similar sulfate reduction rates of 64.8 and 69.1 fmol cell^{-1} day^{-1} for *Desulfobacterium autotrophicum* and *Desulfovibrio oxyclinae*, the fractionations were very different, with values of 32.7‰ for *Desulfobacterium autotrophicum* when growing on butyrate and 4.5‰ for *Desulfovibrio oxyclinae* when growing on lactate.

We determined the isotope fractionation of 26 mesophilic sulfate reducers which had not been characterized previously with respect to their fractionation behavior. In addition, we also investigated psychrophilic sulfate reducers (*Desulfofrigus oceanense, Desulfotalea psychrophila*) and psychrotolerant (*Desulfotalea arctica*), thermophilic (*Desulfotomaculum geothermicum, Desulfotomaculum thermocisternum, Thermodesulfobacterium commune, Thermodesulfovibrio yellowstonii*), and hyperthermophilic (*Archeaoglobus fulgidus*) organisms. All organisms were incubated at or very close to their temperature optimum. A comparison of the incubation temperature and fractionation behavior also indicated no correlation (Tables 1 and 2).
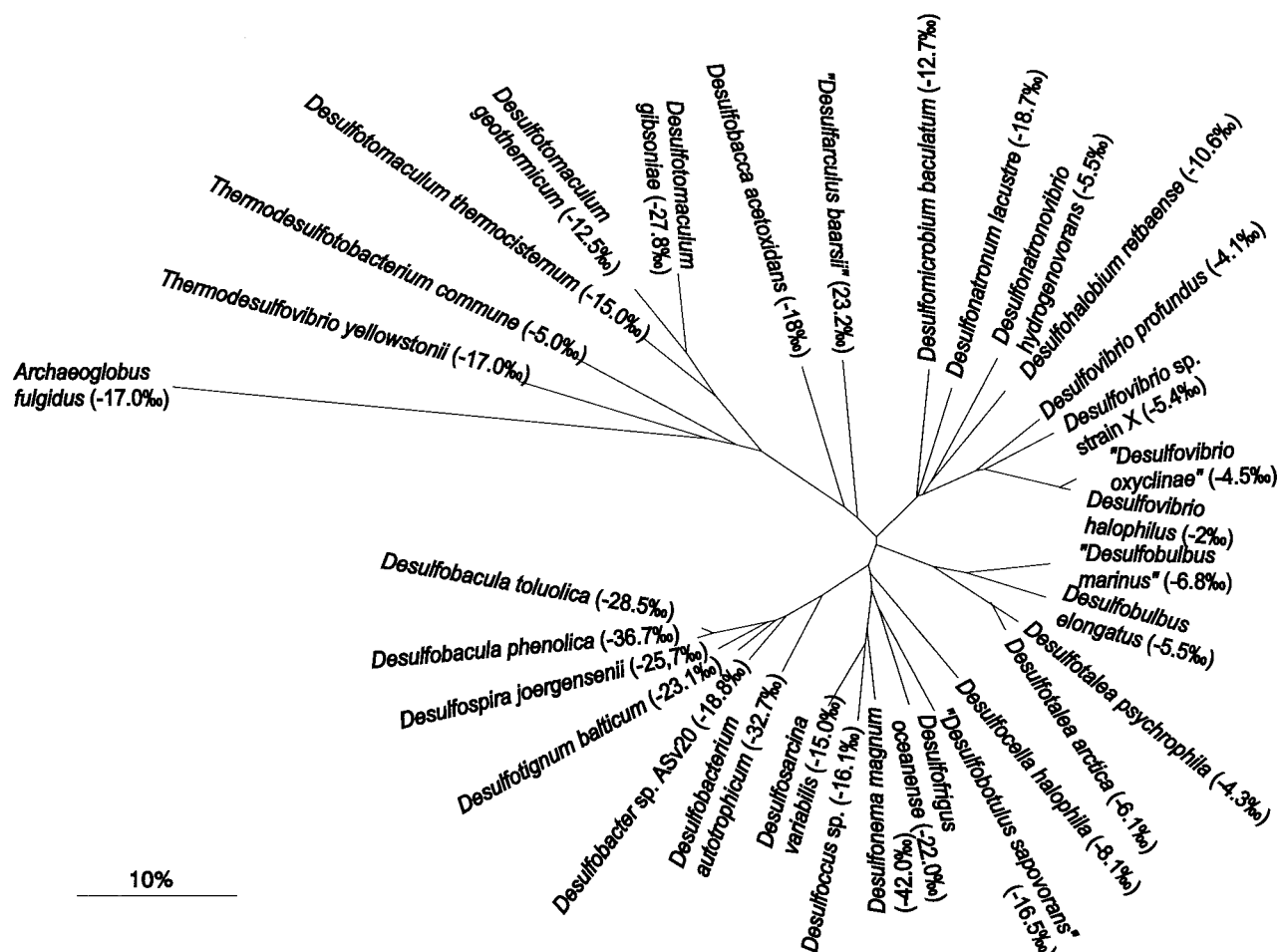
FIG. 1. Phylogenetic affiliation and sulfur isotope fractionation factors of investigated sulfate-reducing microorganisms. Neighbor-joining tree based on 1,308 positions of nearly full-length 16S rRNA sequences from 30 bacteria. *Archaeoglobus fulgidus* was taken to root the tree. Trees constructed with other tree reconstruction algorithms (maximum likelihood and parsimony) resulted in general in the same overall tree topology. The bar indicates 10% sequence divergence.

The strains were isolated from freshwater, brackish, marine, and hypersaline environments (Table 1), and their pH optima are between 6.7 (*Desulfotomaculum thermocisternum*) (30) and 9.6 (*Desulfonatronovibrio hydrogenovorans*) (48). All strains were grown under their optimal conditions with respect to salinity and pH. Comparison with the isotope fractionation also indicated no systematic relationship. Other strain-specific characteristics such as cell size, capability for spore formation, or oxygen sensitivity also did not affect the isotope fractionation.

DISCUSSION

Diversity of isotope fractionation. The overall range in sulfur isotope fractionation (ε = 2.0 to 42.0‰) for this diverse group of sulfate-reducing prokaryotes is very large and spans the full range of fractionations previously observed (4, 15, 22, 23, 28). In previous studies, very high fractionations (greater than 40‰) were obtained by growing cultures under physiologically stressed conditions, e.g., by determining fractionations with *Desulfovibrio desulfuricans* below the minimum

temperature for growth (22). In contrast, our experimental conditions were optimized for each strain to permit a comparison of isotope fractionations.

There is no relationship between phylogenetic distance on the basis of 16S rRNA sequences and differences in isotope fractionation (Fig. 1). This is particularly apparent for the Archaeon *Archaeoglobus fulgidus*, whose isotope fractionation (ε = 17.0‰) was similar to that for a variety of incomplete-oxidizing sulfate reducers from the δ-*Proteobacteria* subgroup (Table 2). Thus, different isotope fractionation patterns do not reflect 16S rRNA-based phylogenetic relationships. Phylogenetic trees based on gene sequences that encode the dissimilatory bisulfite reductase (DSR) are not significantly different from 16S rRNA-based trees (44). Although the presently available data set is small, a comparison of DSR gene-based relative sequence dissimilarity with differences in isotope fractionation yields results similar to the 16S rRNA-based comparison.

Various attempts have been made to develop models for the sulfur isotope fractionation during dissimilatory sulfate reduction (10, 21, 22, 36), but none of these models take the physiological diversity of sulfate reducers into account. While it is
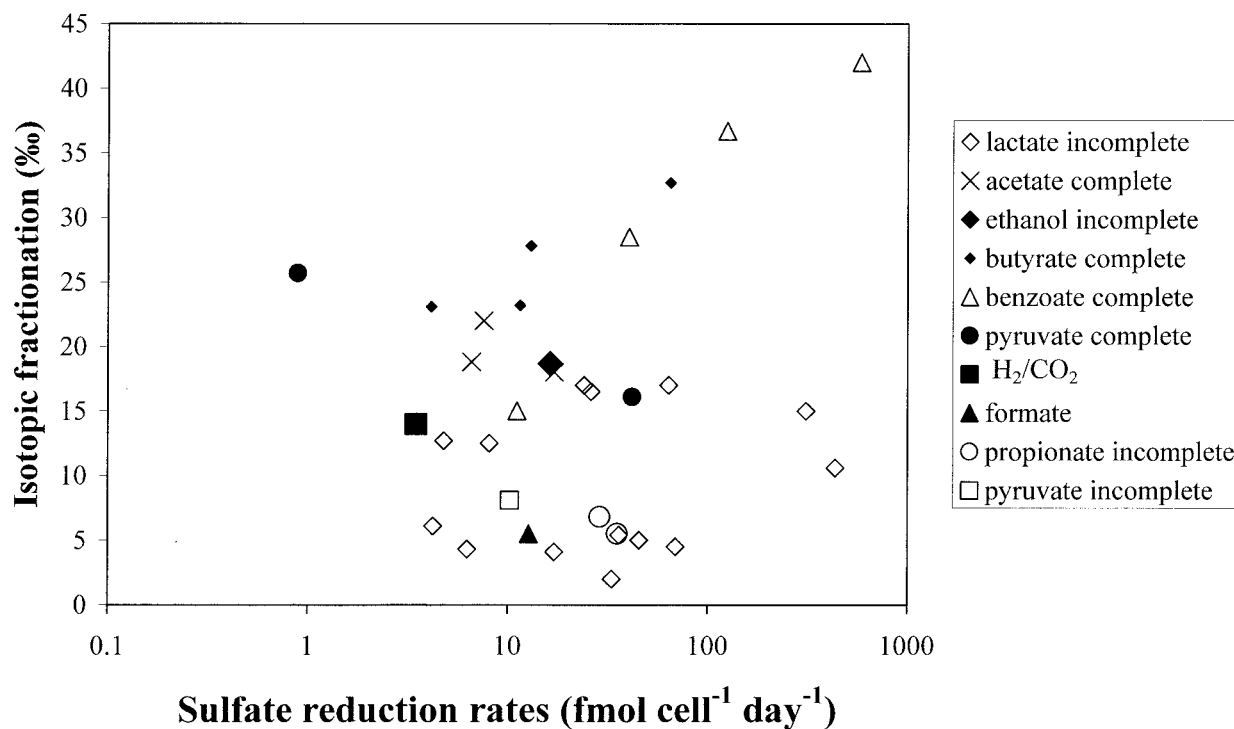
FIG. 2. Relationship between sulfate reduction rates in the mid-exponential growth phase (femtomoles of sulfate reduced per cell per day) and isotope fractionation. Each data point represents a different culture. The different substrates used are shown by the different symbols. Growth conditions were optimized for each culture so that the sulfate reduction rates were presumably close to the maximum potential rates for each organism.

clear that isotope fractionation most likely occurs when a sulfur-oxygen bond is broken, dissimilatory sulfate reduction proceeds in multiple steps (20). Isotope fractionation can occur at the adenosine phosphosulfate reductase (APSR) and the DSR (21, 22, 36), but too little is known about the structural differences between these enzymes among different sulfate reducers to assess their effect on isotope fractionation. It may be of interest, however, that the amino acid sequences important for gene function of the DSR are highly conserved (Bauer, personal communication). This may suggest a similarity in the structure of the reactive center and, possibly, similar isotope fractionation at these enzymes.

Sulfur isotope fractionation can also be modulated through a rate-limiting step that occurs either during sulfate uptake, at the APSR, or at the DSR (22, 23, 36). This rate-limiting step determines whether isotope fractionation can occur in successive reduction steps, but it may occur at different locations for the different sulfate reducers. Some sulfate reducers, in particular freshwater strains such as *Desulfobulbus propionicus*, have been shown to concentrate sulfate in their cells up to 2,500-fold (24). For these sulfate reducers, sulfate uptake is probably not the rate-limiting step. By contrast, marine species may not require a comparable preconcentration mechanism, and the regulation of sulfate uptake may take place by a different mechanism.

Complete versus incomplete electron donor oxidation are the only physiological characteristics that are consistently distinguished by sulfur isotope fractionation. In general, complete oxidizers fractionate more strongly (>15.0‰) than do incomplete oxidizers (<18.7‰). Only a small overlap exists between these two types. These results may be rationalized in terms of the energy conserved during electron donor oxidation for complete- and incomplete-oxidizing sulfate reducers (20, 47). In general, during incomplete oxidation of a substrate more energy is conserved per mole of sulfate reduced (Table 3). For example, the incomplete oxidation of lactate to acetate by sulfate yields more than three times as much energy as the complete oxidation of acetate to CO_2 (-160.1 versus -47.6 kJ mol^{-1} sulfate). All incomplete-lactate-oxidizing sulfate reducers fractionated between 2.0 and 17.0‰, whereas all examined acetate-oxidizing species fractionated between 18.0 and 22.0‰. The underlying causes for the correlation between a thermodynamic property such as energy yield and a kinetic property such as isotope fractionation remain unclear. A possible explanation could be that for a reaction yielding more free energy the redox potential difference ($\Delta E_0'$) is also higher. In this case, the reaction equilibria for the partial reactions during sulfate reduction are shifted toward the product side and the potential for isotope discrimination between APS at the APSR and bisulfite at the DSR is minimized. A test of this hypothesis requires determination of the redox potential of each enzyme participating in the electron transport chain during dissimilatory sulfate reduction.

Electron donor effects on isotope fractionation were already suggested by Kaplan and Rittenberg (22), who observed an increasing fractionation for *Desulfovibrio desulfuricans* in the sequence of lactate, acetate, and ethanol and significantly lower fractionations for autotrophic growth on H_2-CO_2. In these studies, the increase in fractionation always coincided with a decrease in sulfate reduction rates. Therefore, these two

TABLE 3. Free energy changes at standard state ($\Delta G^{0'}$) and corresponding range of isotope fractionations (ε) during dissimilatory sulfate reduction with various electron donors for complete and incomplete oxidation

Electron donor (type of oxidation)	Stoichiometry	$\Delta G^{0'}$ [kJ mol^{-1} $(SO_4)^{2-}$]^a	ε (‰)
Pyruvate (incomplete)	$4\ CH_3COCOO^- + 4\ H_2O + SO_4^{2-} \rightarrow 4\ CH_3COO^- + 4\ HCO_3^- + HS^- + 3\ H^+$	−340.9	8.1
Lactate (incomplete)	$2\ CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + HS^- + H^+$	−160.1	2.0–17.0
Hydrogen	$4\ H_2 + SO_4^{2-} + H^+ \rightarrow 4\ H_2O + HS^-$	−152.2	14.0
Formate	$4\ HCOO^- + SO_4^{2-} + H^+ \rightarrow 4\ HCO_3^- + HS^-$	−146.9	5.5
Ethanol (incomplete)	$2\ CH_3CH_2OH + SO_4^{2-} \rightarrow 2\ CH_3COO^- + HS^- + 2\ H_2O + H^+$	−146.6	18.7
Pyruvate (complete)	$4\ CH_3COCOO^- + 4\ H_2O + 5\ SO_4^{2-} \rightarrow 12\ HCO_3^- + 5\ HS^- + 3\ H^+$	−106.3	16.1; 25.7
Propionate (incomplete)	$4\ CH_3CH_2COO^- + 3\ SO_4^{2-} \rightarrow 4\ CH_3COO^- + 4\ HCO_3^- + 3\ HS^- + H^+$	−50.2	5.5; 6.8
Benzoate (complete)	$C_7H_5O_2^- + 3.75\ SO_4^{2-} + 4\ H_2O \rightarrow 7\ HCO_3^- + 3.75\ HS^- + 2.25\ H^+$	−49.7	15.0–42.0
Butyrate (complete)	$CH_3CH_2CH_2COO^- + 2.5\ SO_4^{2-} \rightarrow 4\ HCO_3^- + 2.5\ HS^- + 0.5\ H^+$	−49.2	23.1–32.7
Acetate (complete)	$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	−47.6	18.0–22.0

^{a} Calculated from the free energy change of formation using data from Hanselmann (19) and Thauer et al. (43).

authors postulated that changes in substrate affected isotope fractionation only insofar as they affected sulfate reduction rates. A correlation between sulfate reduction rates and fractionation is also supported by continuous culture experiments (9). Our data do not support a single relationship between the type of substrate, the sulfur isotope fractionation, and sulfate reduction rates because isotope fractionation was independent of the sulfate reduction rate (Fig. 2). The dependence of isotope fractionation on electron donor oxidation appears to be more important. Possibly, a correlation between sulfate reduction rate and isotope fractionation could be found if various substrates were tested for each organism. However, our data require a relationship between isotope fractionation and sulfate reduction rate that is characteristic for each organism. At the present time, we have sufficient information on the sulfate transport mechanisms, as well as the electron donor and electron acceptor pathways, for only a few sulfate reducers (12, 13, 20, 46, 47). This prevents us from relating the observed isotope fractionations to the specific physiology of each species. Further studies are required to understand sulfur isotope fractionation during dissimilatory sulfate reduction at the biochemical level.

Abundance of the investigated microorganisms in natural environments. Most of the reported isotope fractionations by sulfate reducers published before this study were derived from experiments with *Desulfovibrio* spp. and *Desulfotomaculum* spp. (9, 15, 22, 23, 28). Although *Desulfovibrio* spp. were detected in marine sediments (1, 2, 38) and *Desulfotomaculum* spp. were encountered in aquifers (3, 14), these sulfate reducers are not abundant in many other environmental settings. Therefore, overall fractionations in sulfate-reducing environments may often be influenced by organisms other than *Desulfovibrio* spp. and *Desulfotomaculum* spp. Furthermore, all of the previously investigated strains were incomplete-oxidizing sulfate reducers. In some marine sediments, however, complete-oxidizing species represent more than 70% of the identifiable sulfate reducers (34). Some of the sulfate reducers investigated here are of quantitative importance in their natural habitats. For example, *Desulfococcus* spp. and *Desulfotalea* spp. were the most abundant sulfate reducers in marine arctic sediments (34, 35). In near-shore sediments of the Wadden Sea in northern Germany and in hypersaline mats, *Desulfonema* spp. accounted for an important fraction of the total

bacterial biomass (26, 42). *Desulfobulbus* spp. were the most abundant sulfate reducers in a freshwater lake (25).

Biogeochemical implications for interpretation of the sulfur cycle from isotope abundances. The present study is the first one to demonstrate that sulfate-reducing prokaryotes can produce widely different sulfur isotope fractionations during sulfate reduction. However, not the phylogenetic differences between the organisms but the physiological differences appear to be decisive for the isotope fractionation. Natural environments commonly contain a mixture of sulfate-reducing prokaryotes (26, 34). Thus, the characteristic community in a particular marine habitat can affect the isotope fractionations during bacterial sulfate reduction. Which sulfate reducers are present in a particular environment and which specific substrates are utilized thus become relevant controlling parameters for the isotope fractionation. There is no general agreement about the dominant substrates for sulfate reducers in marine environments. Acetate is generally regarded as an important terminal substrate in marine environments, but hydrogen can be an important substrate in syntrophic bacterial communities (31). Since the composition of the organic matter varies from place to place it is likely that the anaerobic food chain and the microbial community of sulfate reducers in different habitats varies as well. There is now clear molecular genetic evidence for the presence of different sulfate-reducing communities in different marine habitats (26, 34, 35, 39). Consequently, the overall isotope fractionations by sulfate-reducing communities in different environments may vary because different sulfate reducers are present.

Isotopic differences between sulfate and sulfide in marine sediments and porewaters are generally much greater than the experimentally determined isotope fractionations for pure cultures (5, 6, 18). In the natural environment prokaryotes are generally limited by the availability of organic substrate (32). General substrate limitation may also increase the isotope fractionation. Furthermore, in the natural environment additional isotope effects exist in the oxidative part of the sedimentary sulfur cycle through disproportionation of thiosulfate, elemental sulfur, or sulfite (7, 17). Therefore, in addition to considering variations in the microbial community structure of sulfate reducers, interpretation of isotope signals preserved in sediments and porewaters also have to take into account isotope effects in the oxidative part of the sulfur cycle.

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