

# Linkage of High Rates of Sulfate Reduction in Yellowstone Hot Springs to Unique Sequence Types in the Dissimilatory Sulfate Respiration Pathway

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**Diversity, habitat range, and activities of sulfate-reducing prokaryotes within hot springs in Yellowstone National Park were characterized using endogenous activity measurements, molecular characterization, and enrichment. Five major phylogenetic groups were identified using PCR amplification of the dissimilatory sulfite reductase genes (*dsrAB*) from springs demonstrating significant sulfate reduction rates, including a warm, acidic (pH 2.5) stream and several nearly neutral hot springs with temperatures reaching 89°C. Three of these sequence groups were unrelated to named lineages, suggesting that the diversity and habitat range of sulfate-reducing prokaryotes exceeds that now represented in culture.**

Sulfate-reducing prokaryotes (SRP) are widely distributed in the environment. Their habitat range includes freshwater, marine, and hypersaline aquatic systems, cold oceanic sediments, the deep subsurface, hydrothermal vents, and hot springs (7, 13, 14, 21, 22, 24). Although cultivated SRP are phylogenetically and physiologically diverse, they are restricted to four divisions within the *Bacteria* and one within the *Archaea* (25). Over 90% of described species are affiliated with the delta subdivision of the *Proteobacteria* (ca. 14 genera and 50 species) or the *Fermicutes* (*Desulfotomaculum* spp.). Those few that do not associate with these two divisions are affiliated with the thermophilic bacterial genera *Thermodesulfobacterium* and *Thermodesulfobivrio* or a single archaeal genus, *Archaeoglobus*. We now know their natural diversity is much greater than represented in culture, as revealed by endogenous activity measurements and more recent use of explicit molecular criteria (4–6, 8, 9, 17, 19). The report of sulfate respiration at 110°C in hydrothermal marine sediments is well beyond the maximum growth temperature of any isolate (16). Direct environmental surveys based on comparative sequencing of genes encoding the dissimilatory sulfite reductase (*dsrAB*) enzyme have identified novel clades in different environments, including a hypersaline microbial mat community, a uranium tailing site, a coastal fjord, and a hydrothermal vent worm (5, 6, 17, 19).

A more complete census of the environmental diversity of SRP has considerable importance for resolving their varied environmental roles, not necessarily restricted to sulfate reduction, and developing a better understanding of their origins and evolution. Since the genes in the pathway for sulfate respiration are sufficiently conserved to be recovered by PCR amplification, and since virtually all recognized genera have

been characterized by comparative sequencing of genes encoding the 16S rRNA and the dissimilatory sulfite reductase (*dsrAB*) (18), these sequences now provide a framework to explicitly identify environmental populations. In the present study, we used a threefold approach, incorporating endogenous activity measurements, molecular characterization, and enrichment to characterize the diversity and activity of SRP in various thermal springs in Yellowstone National Park, Wyo.

**Site descriptions and analytical methods.** The sediments of different geothermal pools and microbial mats in shallow run-off streams presented a wide range of temperatures (ca. 40 to 90°C), pH (ca. 2.5 to 6.6), and sulfate concentrations (ca. 0.5 to 90 mM) (Table 1). Using a combined temperature-pH probe (WTW, Ft. Myers, Fla.) (pH 330), temperature and pH were measured on site. Sulfate concentrations were determined by capillary electrophoresis following the return to the lab (Quanta 2000 capillary ion analyzer; Waters Corp., Milford, Mass.). Using replicate mat (3 cm deep) or sediment ( $\leq 10$  cm deep) samples collected in 5-ml syringe corers, endogenous measurements of sulfate-reduction rates (SRR) were initiated on site. A 25- $\mu$ l Hamilton syringe was used to evenly distribute 10  $\mu$ l (185 kBq) of carrier-free  $\text{Na}_2[^{35}\text{SO}_4]$  immediately before sealing each sample with a butyl rubber stopper and incubating in site water for 30 min, a time during which we have shown uptake of sulfate to be linear at one of the most active sites (New Pit Spring; data not shown). However, since linearity was not determined for all sites, reported values should be treated as conservative rates. Reactions were terminated (and sulfide trapped) by expelling syringe contents into 10 ml of a 20% zinc acetate solution. Sodium molybdate (6.6 mM) was added to a control sample to inhibit microbially mediated sulfate reduction. An additional control was immediately quenched with zinc acetate following  $\text{Na}_2[^{35}\text{SO}_4]$  addition. Samples were processed following return to the laboratory using the single-step chromium reduction method of Fossing and Jørgensen (12) and rates calculated by the method of Jørgensen (15).

Triplicate  $\sim 0.5$ -ml samples from selected sites were frozen on dry ice and stored at  $-80^\circ\text{C}$  for later determination (using

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TABLE 1. Physical, chemical and biological characterization of Yellowstone hot spring sampling sites

Study site, and specific location	Sample type	pH	Temp (°C)	SO <sub>4</sub> concn (mM)	SRR (nmol of SO <sub>4</sub> cm <sup>-3</sup> day <sup>-1</sup> )	Phospholipid content (nmol of P g <sup>-1</sup> )	Enrichments obtained	No. of clades found with DSR PCR amplification of samples from <sup>f</sup> :	
								Environment	Enrichments
Mud Volcano area, Obsidian Pool	Sediment	6.3	89	0.6	104 (144) <sup>b</sup>	9.7 (3.7)	— <sup>e</sup>	1	
Nymph Creek area									
Nymph Creek	Scraped Mat	2.5	38–47	4.7	212 (110)	n.a. <sup>a</sup>	—	2	
Black Sediment Pool	Sediment	6.6	69	0.6	87 (97)	n.a.	—	3	
Mammoth Springs									
New Pit Spring	Mat	6.1	56	31.6	11,111 (1,497)	n.a.	+	0	1
Bath Lake Vista	Mat	6.5	54	6.3	n.a.	n.a.	+	2	1
Roland's Well	Mat	6.5	52	n.a.	n.a.	n.a.	+	1	1
Norris Geyser Basin									
Site C	Sediment	2.3	88	87.5	704 <sup>c</sup>	n.a.	—	0	
Site D	Sediment	2.2	42	6.5	1.6 (1.4)	n.a.	—	0	
Site E	Sediment	4.1	91	0.3	b.d. <sup>d</sup>	3.9 (3.1)	—		
Cinder Pool	Sediment	2.5	91	0.5	2 <sup>c</sup>	2.4 (1.2)	—	0	
Black Spring	Sediment	3.0	80	2.6	5.6 <sup>c</sup>	1.8 (0.6)	—	0	
Mt. Washburn area									
Site A	Sediment	2.2	46	54.0	b.d.	25.8 (1.8)			
Site B	Sediment	6.1	81	39.1	b.d.	n.a.			
Acid Inkpot Spring	Sediment	2.8	77	38.2	b.d.	6.9 (5.1)			
Inkpot Spring	Sediment	6.2	86	24.1	b.d.	1.3 (0.1)			

<sup>a</sup> n.a., data not available.

<sup>b</sup> Values represent means (standard deviations). *n* = 3 for all samples except those from New Pit, for which *n* = 2.

<sup>c</sup> The rate was determined from one core; the other two cores gave results below the detection limit.

<sup>d</sup> b.d., below detection limit.

<sup>e</sup> —, enrichment was attempted with no success.

<sup>f</sup> Values represent the number of clades present. Zeros represent unsuccessful attempts at amplification. DSR, dissimilatory sulfite reductase.

the Bligh and Dryer technique [2] as modified by Findlay et al. [10, 11] of total phospholipid phosphate levels.

**Enrichment cultures.** Using 20% filtered source water and either 3 mM acetate or H<sub>2</sub> (5 lb/in<sup>2</sup>) plus 1.5 mM acetate (Table 1) as described by Boone et al. (3), dilution series enrichment cultures were developed from selected sites under a N<sub>2</sub>/CO<sub>2</sub> atmosphere. Cultures were incubated at 55°C and transferred three to four times over a 4-month period prior to molecular characterization.

**Molecular characterization.** Site material was frozen immediately following collection by placement on dry ice. Using a high salt-sodium dodecyl sulfate-heat method (27), total DNA was later extracted from approximately 2 to 5 g of sediment or mat from sites demonstrating significant SRR (>10 nmol of SO<sub>4</sub> cm<sup>-3</sup> day<sup>-1</sup>) and from 10 ml of enrichment culture. Using 0.04 to 0.4 ng of DNA template/ml and 10 pmol each of DSR1F and DSR4R primers (23) in a total volume of 20 μl, an approximately 1,900-bp fragment of the genes encoding the dissimilatory sulfite reductase (*dsrAB*) was amplified by PCR following the instructions for Platinum *Taq* DNA polymerase (Gibco/Life Technologies, Rockville, Md.). Amplification was carried out using a gradient thermal cycler (ThermoHybaid, Franklin, Mass.) as follows: 5 min at 95°C, 30 cycles of 95°C for 30 s, a gradient of annealing temperatures from 50°C to 65°C for 15 s, and elongation at 72°C for 30 s, with final elongation at 72°C for 10 min. Amplified products were cloned following the instructions for the TOPO TA cloning kit for sequencing (Invitrogen/Life Technologies, Carlsbad, Calif.). Using M13F (–29) and M13 reverse primers, inserts were reamplified directly from the plated colonies. Partial nucleotide sequences were determined directly from PCR products following a stan-

dard cycle sequencing protocol and using Sequitherm EXCEL II DNA sequencing kits (Epicentre Technologies, Madison, Wis.). Infrared dye-labeled DSR1F and DSR4R sequencing primers (Li-Cor, Lincoln, Nebr.) were used to determine sequence from both ends of the amplified *dsrAB* fragment. Full-length *dsrAB* sequences were determined for select clones by using an EZ:TN transposon insertion kit (Invitrogen/Life Technologies) and TET primers provided by the kit or by sequencing (using the internal primer DSRMIDR [5'-CCAV CCCTGRGTGTG-3'], DSRNCF [5'-ACTGCATMAATAAG ATGCC-3'], or DSRNCR [5'-GGCATCTTATTKATGCAG T-3']) directly from PCR products. These primers correspond to *Desulfovibrio vulgaris* positions 1591 to 1576, 737 to 756, and 756 to 737, respectively.

Phylogenetic trees were constructed using an alignment of all available sequences, excluding regions of uncertain alignment or missing data. The ARB software package (<http://www.arb-home.de>) was used to insert new sequences into an established alignment (alignment tool), followed by manual inspection and refinement. A backbone tree was first generated using 483 amino acid positions of the Dsr from SRP grown in cultures (18) and at least one fully sequenced member from each unique environmental clade. Backbone trees were constructed using the neighbor-joining and parsimony methods implemented in PAUP\* v. 4.0b10 software (D.L. Swofford; Sinauer Associates, Sunderland, Mass.) and the maximum-likelihood method implemented in Tree-Puzzle v. 5.0 software (<http://www.tree-puzzle.de>). Subtrees were constructed using 162 to 273 residues of environmental or enrichment clones with reference to one or two closely related, fully sequenced relatives and then added manually to the backbone tree using the fully

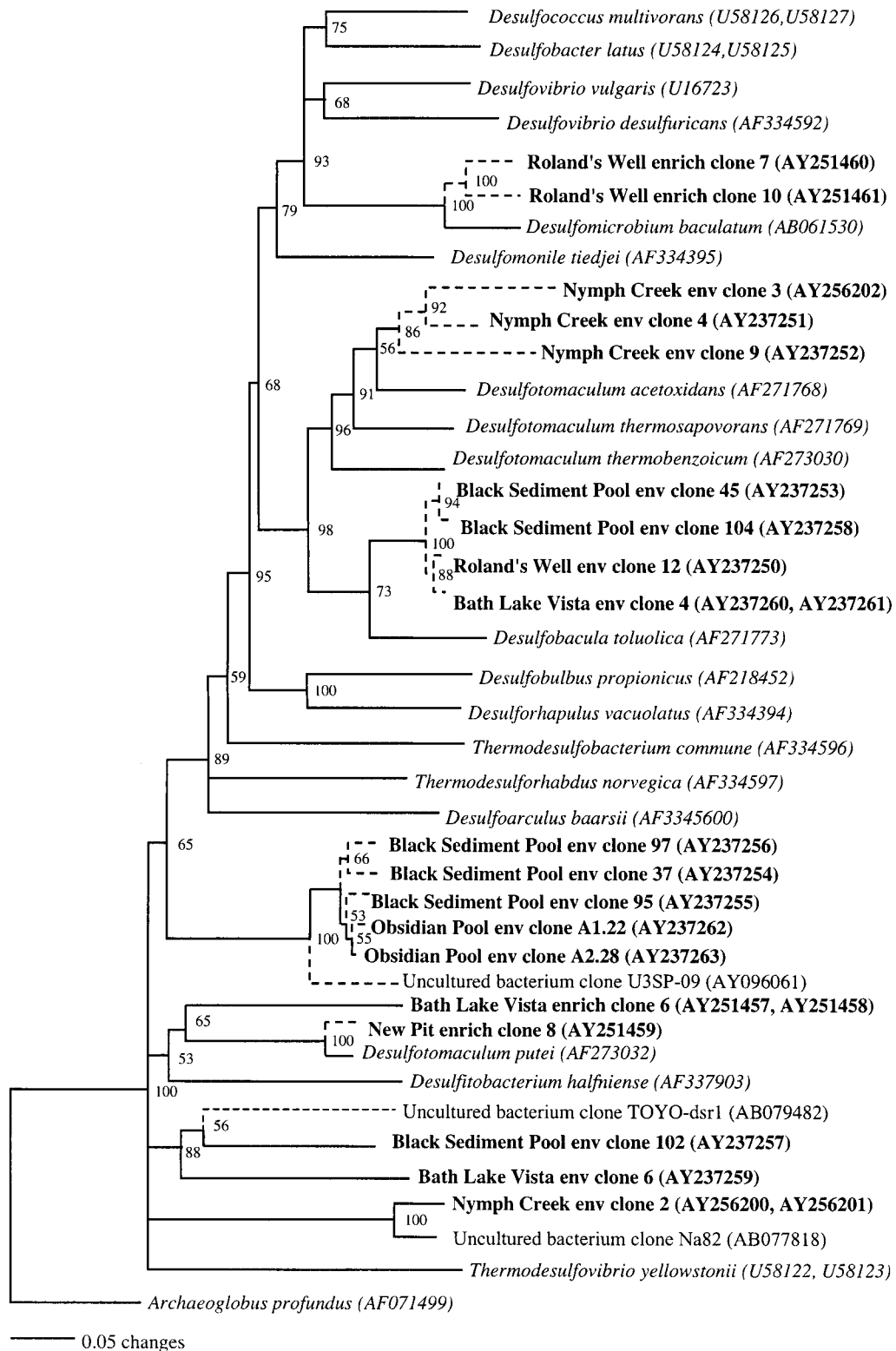


FIG. 1. Unrooted dendrogram depicting relationships among *dsrAB* gene sequences. A mask of 483 amino acid positions (gaps and ambiguous residues were excluded) was created with ARB software. The backbone tree was constructed using the neighbor-joining algorithm in PAUP\*. Previously published (18) sequences from SRP grown in cultures are shown in italic characters; sequences from environmental (env) and enrichment culture (enrich) clone libraries are shown in boldface. For groups for which no member grown in culture is known, the closest sequences from the GenBank database that are representative of strains not grown in culture were included in plain characters for reference. Subtrees were constructed similarly using partial sequences (162 to 273 residues) (shown with dashed lines) and added manually to the backbone tree by using a fully sequenced member as an anchor lineage. Values at nodes are bootstrap confidence values expressed as percentages of 1,000 replications. Nodes without values indicate those unsupported by a majority of tree reconstructions. *Archaeoglobus profundus* was used as an outgroup in all trees. Accession numbers for all sequences are in parentheses.

sequenced members as reference lineages. In cases in which sequences were not closely related to sequences from cultivated SRP, the nearest relative sequences from BLAST (1) searches of the GenBank *dsrAB* database were added to the tree for reference. Bootstrap resampling of the neighbor-joining and parsimony trees was performed using 1,000 and 100 replicates, respectively. For likelihood analysis using quartet puzzling, at least 1,000 replicates were performed. Phylogenetic trees and subtrees inferred from neighbor-joining, parsimony, and maximum-likelihood analyses of aligned *dsrAB* sequences all showed similar topologies, with the only major differences observed in the deeply branching nodes not resolved by bootstrap analysis. These trees were in general agreement with previously published phylogenies of strains grown in cultures (18).

Mean SRR varied from below detectable levels to over 11,000 nmol cm<sup>-3</sup> day<sup>-1</sup> among the sites investigated. SRR values for controls were less than 10% of measured rates at the most active sites (>10 nmol of SO<sub>4</sub> cm<sup>-3</sup> day<sup>-1</sup>) and also significantly less than mean values for lower activity sites (<50%). Values shown in Table 1 have been corrected by subtracting the control values. The high rates observed at Nymph Creek (212 nmol of SO<sub>4</sub> cm<sup>-3</sup> day<sup>-1</sup>) and New Pit Spring (11,111 nmol of SO<sub>4</sub> cm<sup>-3</sup> day<sup>-1</sup>) likely reflect input of endogenous electron donors from associated photosynthetic microbial mat communities. Nymph Creek, an acidic 38- to 47°C stream, contains a mat dominated by the alga *Cyanidium caldarium*, while New Pit Spring, a more alkaline 47°C spring, contains a mat composed primarily of photosynthetic bacteria. However, significant SRR were also observed in a nonphotosynthetic high-temperature (89°C) near-neutral spring (Obsidian Pool [OP]) of relatively low biomass (Table 1). The SRP in such low-biomass springs may be using non-biologically derived electron donors (such as H<sub>2</sub> or CO) such as are present in many geothermal environments.

The low-pH (2.2 to 3.0) springs in Norris Geyser Basin exhibited a range of temperatures (42 to 91°C) and SRR values. With the exception of one replicate in Norris (site C), rates were usually low (<10 nmol cm<sup>-3</sup> day<sup>-1</sup>) or below detection. Rates at all sites near Mt. Washburn, including both low-pH (ca. 2) and near-neutral-pH (ca. 6) springs, were below detection. However, the high sulfate concentrations in this region (>21 mM SO<sub>4</sub>; Table 1) significantly reduce the sensitivity of our analysis. Thus, longer incubation times, or higher isotope concentrations, may be needed for detection. Although Zeikus et al. (26) reported detectable SRR at Inkpot Spring in the Mt. Washburn area, measurable rates were only observed for samples incubated at the cooler margin of the spring. Reduction rates in samples incubated in higher-temperature water, as was done in this study, were close to the lower limit of detection.

We attempted to amplify *dsrAB* sequences from all sites demonstrating appreciable sulfate reduction (>10 nmol of SO<sub>4</sub> cm<sup>-3</sup> day<sup>-1</sup>) and from stable enrichments. With the exception of New Pit Spring and Site C in Norris Geyser Basin, successful amplification generally corresponded with higher activity sites. Environmental sequences affiliated with five major clades (Fig. 1); three were novel and two were represented by named species. For the latter, sequences from three near-neutral springs grouped with *Desulfobacula toluolica*, while those from

Nymph Creek were most closely related to gram-positive *Desulfotomaculum* spp. Another Nymph Creek *dsrAB* sequence belonged to a novel, deeply branching clade (Fig. 1) with strong nucleotide identity (85%) to another environmental clone recovered from a hot spring in Japan (K. Mori and S. Hanada, unpublished data). The Nymph Creek data suggest that SRP can inhabit highly acidic environments, although no sulfate-reducing microorganism has been reported that is capable of growth at such low pH. Like this Nymph Creek sequence, sequences from the two other novel clades were not closely related to any SRP grown in cultures and, in these cases, the sequences were only modestly related (<80% nucleotide identity) to environmental clones from the database (4, 20) (Fig. 1). Most of the Black Sediment Pool (BSP) sequences and all of the OP sequences form a closely related assemblage within a novel clade. The three BSP sequences in this clade are representative of 12 closely related sequences differing by <1% at the nucleotide level. The close relationship between sequence types recovered from BSP and OP suggests some similarity of habitat. Although the temperatures of these springs differ by 20°C, they share similar chemistry and their SRR are statistically indistinguishable ( $t = 0.16$ ;  $P = 0.8772$ ) (Table 1). Thus, their affiliation may be more closely linked to chemical parameters than to temperature. Also, apparent sequence diversity in the lower-temperature spring was greater than for OP. Two additional sequence types recovered from BSP were related to sequences from lower temperature (ca. 50 to 55°C) sites in the Mammoth Springs area (Bath Lake Vista and Roland's Well), possibly suggesting that SRP diversity is restricted at higher temperatures.

Our limited inspection of sites by enrichment was intended primarily to provide some perspective on the types of SRP recoverable (using standard enrichment methods) from these sites. As has been commonly observed for other such comparisons, there was no congruence between environmental and enrichment sequences. All *dsrAB* sequences derived from enrichments were closely affiliated with representatives grown in cultures (Fig. 1).

In an early publication of investigations using comparative 16S rRNA sequencing to define relationships among SRP, the patchy distribution of sulfate-respiring lineages within the bacterial tree was also noted (8). At that time, we offered the suggestion that diversity was not fully represented in the available culture collection. The results of this study provide more direct evidence that the environmental diversity of SRP greatly exceeds that represented by organisms grown in cultures and includes deeply diverging lineages not closely related to described sulfate-reducing groups. These findings underscore the importance of further investigation of SRP in these and other environments. A more complete understanding of the diversity and habitat distribution of SRP is an essential precursor to determining the contribution of members of this important functional group to biogeochemical cycling and of understanding their origins and evolution.

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## REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Bligh, E. G., and W. Dryer. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**:911–917.
- Boone, D. R., R. L. Johnson, and Y. Liu. 1989. Diffusion of the interspecies electron carriers H<sub>2</sub> and formate in methanogenic ecosystems and its implications in the measurements of Km for H<sub>2</sub> or formate uptake. *Appl. Environ. Microbiol.* **55**:1735–1741.
- Castro, H., K. R. Reddy, and A. Ogram. 2002. Composition and function of sulfate-reducing prokaryotes in eutrophic and pristine areas of the Florida Everglades. *Appl. Environ. Microbiol.* **68**:6129–6137.
- Chang, Y.-J., A. D. Peacock, P. E. Long, J. R. Stephen, J. P. McKinley, S. J. Macnaughton, A. K. M. A. Hussain, A. M. Saxton, and D. C. White. 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. *Appl. Environ. Microbiol.* **67**:3149–3160.
- Cottrell, M. T., and S. C. Cary. 1999. Diversity of dissimilatory bisulfite reductase genes of bacteria associated with the deep-sea hydrothermal vent polychaete annelid *Alvinella pompejana*. *Appl. Environ. Microbiol.* **65**:1127–1132.
- Detmers, J., U. Schulte, H. Strauss, and J. Keuver. 2001. Sulfate reduction at a lignite seam: microbial abundance and activity. *Microb. Ecol.* **42**:238–247.
- Devereux, R., M. Delaney, F. Widdel, and D. A. Stahl. 1989. Natural relationships among sulfate-reducing bacteria. *J. Bacteriol.* **171**:6689–6695.
- Devereux, R., M. E. Hines, and D. A. Stahl. 1996. S cycling: characterization of natural communities of sulfate-reducing bacteria by 16S rRNA sequence comparisons. *Microb. Ecol.* **32**:283–292.
- Findlay, R. H. 1996. The use of phospholipid fatty acids to determine microbial community structure, p. 4.1–4.17. *In* A. K. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn (ed.), *Molecular microbial ecology manual*. Kluwer, Dordrecht, The Netherlands.
- Findlay, R. H., G. M. King, and L. Watling. 1989. Efficiency of phospholipid analysis in determining microbial biomass in sediments. *Appl. Environ. Microbiol.* **55**:2888–2893.
- Fossing, H., and B. B. Jørgensen. 1989. Measurement of bacterial sulfate reduction in sediments: evaluation of a single step chromium reduction method. *Biogeochemistry* **8**:205–222.
- Fry, N., J. Fredrickson, S. Fishbain, M. Wagner, and D. Stahl. 1997. Population structure of microbial communities associated with two deep, anaerobic, alkaline aquifers. *Appl. Environ. Microbiol.* **63**:1498–1504.
- Henry, E. A., R. Devereux, J. S. Maki, C. C. Gilmour, C. R. Woese, L. Mandelco, R. Schauder, C. C. Remsen, and R. Mitchell. 1994. Characterization of a new thermophilic sulfate-reducing bacterium *Thermodesulfobacterium yellowstonii*, gen. nov. and sp. nov.: its phylogenetic relationship to *Thermodesulfobacterium commune* and their origins deep within the bacterial domain. *Arch. Microbiol.* **161**:62–69.
- Jørgensen, B. B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. III. Estimation from chemical and bacteriological field data. *Geomicrobiol. J.* **1**:49–64.
- Jørgensen, B. B., M. F. Isaksen, and H. W. Jannasch. 1992. Bacterial sulfate reduction above 100°C in deep-sea hydrothermal vent sediments. *Science* **258**:1756–1757.
- Joulian, C., N. B. Ramsing, and K. Ingvorsen. 2001. Congruent phylogenies of most common small-subunit rRNA and dissimilatory sulfite reductase gene sequences retrieved from estuarine sediments. *Appl. Environ. Microbiol.* **67**:3314–3318.
- Klein, M., M. Friedrich, A. J. Roger, P. Hugenholtz, S. Fishbain, H. Abicht, L. L. Blackall, D. A. Stahl, and M. Wagner. 2001. Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. *J. Bacteriol.* **183**:6028–6035.
- Minz, D., J. L. Flax, S. J. Green, G. Muyzer, Y. Cohen, M. Wagner, B. E. Rittmann, and D. A. Stahl. 1999. Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. *Appl. Environ. Microbiol.* **65**:4666–4671.
- Nakagawa, T., S. Hanada, A. Murayama, K. Marumo, T. Urabe, and M. Fukui. 2002. Distribution and diversity of thermophilic sulfate-reducing bacteria within a Cu-Pb-Zn mine (Toyoha, Japan). *FEMS Microbiol. Ecol.* **41**:199–209.
- Ollivier, B., P. Caumette, J. L. Garcia, and R. A. Mah. 1994. Anaerobic bacteria from hypersaline environments. *Microbiol. Rev.* **58**:27–38.
- Stetter, K. O., G. Lauerer, M. Thomm, and A. Neuner. 1987. Isolation of extremely thermophilic sulfate reducers: evidence for a novel branch of *Archaeobacteria*. *Science* **236**:822–824.
- Wagner, M., A. J. Roger, J. L. Flax, G. A. Brusseau, and D. A. Stahl. 1998. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J. Bacteriol.* **180**:2975–2982.
- Widdel, F. 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria, p. 469–575. *In* A. J. B. Zehnder (ed.), *Biology of anaerobic microorganisms*. Wiley & Sons, New York, N.Y.
- Widdel, F., and T. A. Hansen. 1992. The dissimilatory sulfate- and sulfur-reducing bacteria, p. 583–624. *In* A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes*. Springer Verlag, New York, N.Y.
- Zeikus, J. G., M. A. Dawson, T. E. Thompson, K. Ingvorsen, and E. C. Hatchikian. 1983. Microbial ecology of volcanic sulfidogenesis. Isolation and characterization of *Thermodesulfobacterium commune* gen. nov. and sp. nov. *J. Gen. Microbiol.* **129**:1159–1169.
- Zhou, J., M. Bruns, and J. Tiedje. 1996. DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol.* **62**:316–322.