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MiniReview

Phylogeny of sulfate-reducing bacteria[1]

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Abstract

Sulfate-reducing bacteria (SRB) are a diverse group of prokaryotes that may be divided into four groups based on rRNA sequence analysis: Gram-negative mesophilic SRB; Gram-positive spore forming SRB; thermophilic bacterial SRB; and thermophilic archaeal SRB. In this review, we have assembled representative 16S rRNA sequences reported to date for SRB and have constructed phylogenetic trees from these sequences. Physiological characteristics particular to each of these groups are discussed, as is the availability of tested group-specific phylogenetic probes and PCR primers directed toward individual groups.

Keywords: Sulfate-reducing bacterium; Phylogeny; rRNA phylogeny

1. Introduction

Sulfate-reducing bacteria (SRB) constitute a diverse group of prokaryotes that contribute to a variety of essential functions in many anaerobic environments. In addition to their obvious importance to the sulfur cycle, SRB are important regulators of a variety of processes in wetland soils, including organic matter turnover, biodegradation of chlorinated aromatic pollutants in anaerobic soils and sediments, and mercury methylation [1,2]. Because of their importance to critical processes in ecosystem functioning and environmental remediation, interest in SRB has been increasing over the last 10 years. With the development of rRNA phylogenetic analysis, notable advances have been made in the taxonomy and phylogeny of this very diverse group [3].

Phylogenetic classification of SRB by rRNA sequence analysis has a variety of advantages, including providing insights into the evolutionary origins of sulfate reduction in distantly related species, and in facilitation of development of group-specific phylogenetic probes and PCR primers for use in ecological studies. A comprehensive review of SRB phylogeny is important at this time to assemble recent work from a variety of laboratories and clearly define the SRB genera belonging to each group. This MiniReview will review and compile recent advances in defining phylogenetic relationships between the various branches of this diverse functional group of microorganisms.

SRB are a complex physiological bacterial group, and various properties have been used in traditional classification schemes (Table 1). The most important of these properties were cell shape, motility, GC content of DNA, presence of desulfovirin and cytochromes, optimal temperature, and complete versus incomplete oxidation of acetate. For classification within a particular genus, different electron donors are tested. Analysis of rRNA sequences has allowed organization of the various SRB species into four distinct groups: Gram-negative mesophilic SRB; Gram-positive spore forming SRB; thermophilic bacterial SRB; and thermophilic archaeal SRB. All of these groups are characterized by their use of sulfate as a terminal electron acceptor during anaerobic respiration. Assignation of individual species into appropriate groups based on rRNA analysis is in general agreement with those obtained by traditional taxonomy, although some exceptions exist and will be discussed below.

All sequences used in our analyses were obtained from the latest version of GenBank (http://www.ncbi.nlm.nih.

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gov) and initially aligned by the Pileup function of GCG [4]. Final alignments were constructed by visual inspection in PAUP* 4.0b2a [5]. Trees were built by a maximum parsimony method using PAUP* 4.0b2a [5], and bootstrap support was obtained by heuristic searches of the reweighted trees with 1000 replicates.

2. Gram-negative mesophilic SRB

This group of SRB is located within the delta subdivision of the Proteobacteria (Figs. 1 and 2). At some point in their evolutionary history, the delta subdivision diverged from other Proteobacteria from a common ancestral phototroph, and members of the delta subdivision lost their photosynthetic ability and converted to heterotrophy [6].

The delta subdivision includes such non-SRB as sulfur-reducing bacteria (*Desulfurella*, *Desulfuromusa*, and *Desulfuromonas*), *Myxobacteria* and *Bdellovibrio*, and *Pelobacter* and *Geobacter* [7]. Phylogenetic relationships between SRB and other members of the delta subdivision remains unresolved [8], although it has been suggested that *Myxobacteria* and bdellovibrios may represent aerobic adaptations of an ancestral anaerobic sulfur-metabolizing phenotype [8,9]. Devereux et al. [10], however, reported that placement of the exact root of *Myxobacteria* (inside or outside the Gram-negative mesophilic SRB) is affected by the outgroup sequences used to construct the tree, suggesting that *Myxobacteria* may not have originated from within this group of SRB.

Pelobacter and *Geobacter* reduce Fe(III) to Fe(II), a metabolic characteristic partially shared with some SRB belonging to this group. However, to date no known Gram-negative SRB that reduce Fe(III) to Fe(II) are capable of growth with Fe(III) as a sole electron acceptor [11].

Two families of SRB have been proposed within the δ-Proteobacteria: the Desulfovibrionaceae (presented in detail in Fig. 1) and the Desulfobacteriaceae (presented in detail in Fig. 2) [10,12]. The Desulfovibrionaceae family includes the genera *Desulfovibrio* and *Desulfomicrobium*. It should be noted that a reclassification has been proposed for *Desulfomonas pigra* to *Desulfovibrio piger* based on 16S rRNA sequence analysis [13], although *D. piger* is different from other desulfovibrios in its motility (nonmotile) and shape (rod versus vibrioid). Two recently described genera, *Desulfohalobium* (represented by *D. retbaense*; GenBank accession number U48244) and *Desulfonatronum* (represented by *D. lacustre*; GenBank accession number Y14594), have not yet been officially placed within the family Desulfovibrionaceae, although they fall firmly within this family by our analyses.

The original Desulfobacteriaceae family (Fig. 2) included all SRB within the δ-Proteobacteria that were not part of the Desulfovibrionaceae [10,12]. This rather broad definition included species of the genera *Desulfobulbus*, *Desulfobacter*, *Desulfobacterium*, *Desulfococcus*, *Desulfosarcina*, *Desulfomonile*, *Desulfonema*, *Desulfobotulus*, and *Desulfoarculus*. Our analyses suggest that several newly proposed genera may fall within the Desulfobacteriaceae on the basis of rRNA sequence analysis. These newly

Table 1
Important characters in the classification of representative sulfate-reducing bacteria

	Shape	Motility	GC content of DNA (%)	Desulfovirin	Cytochromes	Oxidation of acetate	Growth temp. (°C)
Gram-negative mesophilic SRB							
Desulfobulbus	lemon to rod	−/+	59–60	−	b, c, c_3	I[a]	25–40
Desulfomicrobium	ovoid to rod	+/−	52–67	−	b, c	I	25–40
Desulfomonas	rod	−	66	+	c	I	30–40
Desulfovibrio	spiral to vibrioid	+	49–66	+/−	c_3, b, c	I	25–40
Desulfobacter	oval to rod	+/−	44–46	−		C[b]	20–33
Desulfobacterium	oval to rod	+/−	41–52	−	b, c	C	20–35
Desulfococcus	spherical or lemon	−/+	46–57	+/−	b, c	C	28–35
Desulfomonile	rod	−	49	+	c_3	C	37
Desulfonema	filaments	gliding	35–42	+/−	b, c	C	28–32
Desulfosarcina	oval rods or coccoid, packages	+/−	51	−	b, c	C	33
Gram-positive spore-forming SRB							
Desulfotomaculum	straight to curved rods	+	48–52	−	b, c	I/C	most 25–40, some 40–65
Bacterial thermophilic SRB							
Thermodesulfobacterium	vibrioid to rod	−/+	30–38	−	c_3, c	I	65–70
Archaeal thermophilic SRB							
Archaeoglobus	coccoid	+/−	41–46	−	n.r.[c]	I	64–92

[a] I, incomplete.
[b] C, complete.
[c] n.r., not reported.

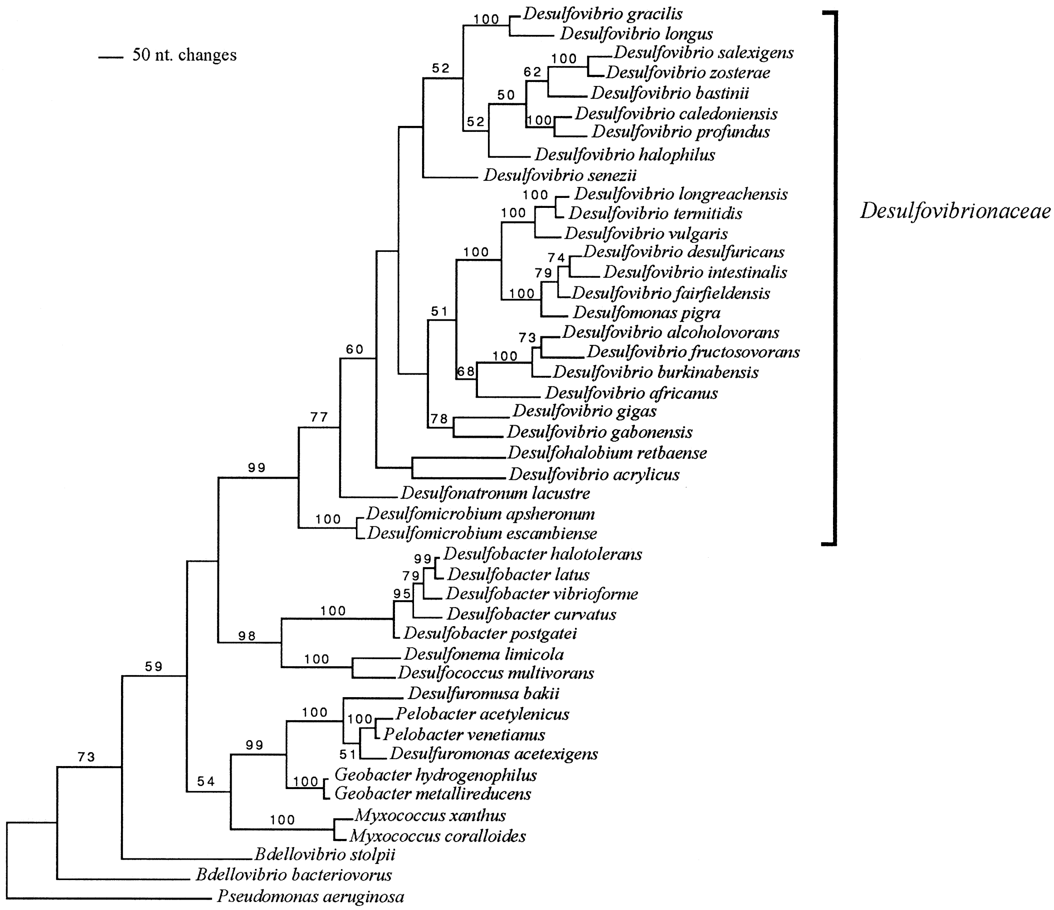

Fig. 1. Phylogenetic tree for the Gram-negative mesophilic SRB, with emphasis on the family Desulfovibrionaceae. Numbers before branch points represent percentages of bootstrap resampling based on 1000 trees. Bootstrap values below 50% are not presented.

added genera include *Desulfobacula*, *Desulfospira*, *Desulfocella*, *Desulfobacca*, *Desulfacinum*, *Thermodesulforhabdus*, *Desulforhabdus*, *Desulfocapsa*, *Desulforhopalus*, and *Desulfofustis*. Reclassification of the genera *Desulfobotulus* and *Desulfoarculus* to *Desulfovibrio sapovorans* as *Desulfobotulus sapovorans*, and *Desulfovibrio baarsii* as *Desulfoarculus baarsii* has recently been proposed [3,13], although no official reclassifications have been published to date concerning these proposals. Phylogenetic analyses suggest that these two species do not belong to the Desulfovibrionaceae family, a finding consistent with metabolic features (specifically electron donors) of these two species compared with the desulfovibrios [8]. The majority of members of this family are mesophilic; however, *Desulfacinum infernum* (GenBank accession number L27426) and *Thermodesulforhabdus norvegicus* [14] are thermophilic.

A precise definition of the family Desulfobacteriaceae is not possible by our analyses, nor is the accurate inclusion of certain genera in this family. This ambiguity is due to the lack of support provided by bootstrap resampling at critical branches within the proposed Desulfobacteriaceae (Fig. 2). The *Desulfobulbus* and *Desulfocapsa*/*Desulfofustis* clusters may represent a deeply branching group that may constitute a separate family, or they may be placed within the Desulfobacteriaceae. More physiological characterization and sequence data of related species are required to confirm or reject their placement within the Desulfobacteriaceae.

Interesting morphological aspects of the Desulfobacteriaceae family include formation of clumps, as is seen in *Desulfosarcina*, and the gliding motion of filamentous *Desulfonema*. Clump formation can provide protection against unfavorable changes in environmental redox potential, and the gliding motility of *Desulfonema* allows these bacteria to move against chemical gradients to reach areas of favorable nutrient concentration. Moreover, the formation of filaments by this SRB may provide resistance against phagocytosis by ciliates and amebas [15].

3. Gram-positive spore forming SRB

This general group is dominated by the genus *Desulfotomaculum*, and is placed within low GC Gram-positive bacteria (Fig. 3) such as *Bacillus* and *Clostridium*. These include the only SRB known to form heat-resistant endospores, a trait shared with many *Bacillus* and *Clostridium* species. In contrast with the mesophilic SRB, some species

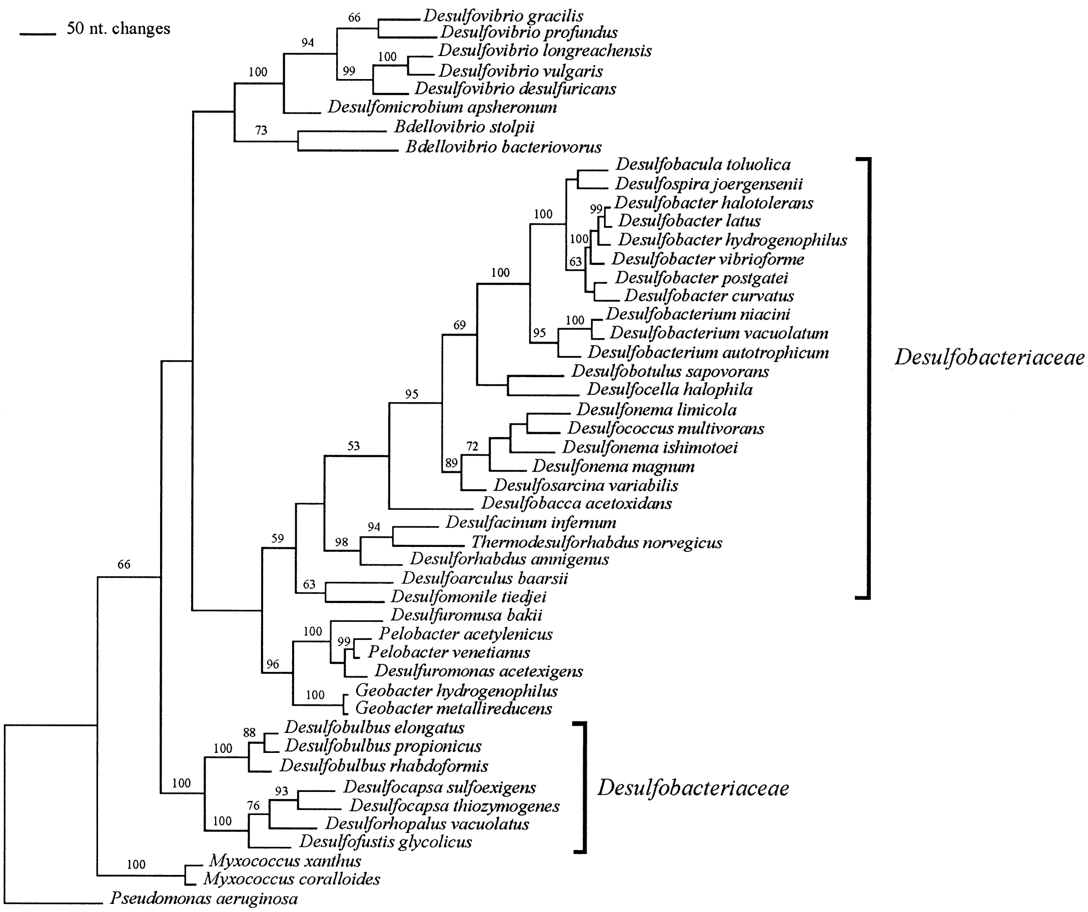

Fig. 2. Phylogenetic tree for the Gram-negative mesophilic SRB, with emphasis on the family Desulfobacteriaceae. Numbers before branch points represent percentages of bootstrap resampling based on 1000 trees. Bootstrap values below 50% are not presented.

of *Desulfotomaculum* are thermophilic, although their optimal growth temperatures are lower than those of thermophilic Gram-negative and archaeal sulfate reducers (Table 1).

To date, a single family of SRB has been proposed within the Gram-positive SRB. Changes within this family are currently being proposed, however, and these changes are supported by our analyses. These proposed changes include reclassification of *Desulfotomaculum guttoideum* to another genus, perhaps *Clostridium* [16]. The 16S rDNA analysis suggests that *D. guttoideum* is closely related to a cluster of *Clostridium*, and appears on a separate branch from the rest of the *Desulfotomaculum* species. A recently reclassified genus within this group is *Desulfotomaculum orientis*, now *Desulfosporosinus orientis* [16], adding a second genus to this family.

Different species within the genus *Desulfotomaculum* exhibit a great versatility in the type of electron donors they are capable of using for growth, and include acetate, aniline, succinate, catechol, indole, ethanol, nicotinate, phenol, acetone, stearate, and others. Depending on the species, organic substrates are oxidized incompletely to acetate or completely to CO_2 (Table 1) [1]. In contrast to δ-Proteobacteria SRB, the ability to use Fe(III) as sole terminal electron acceptor for growth has been described for some Gram-positive SRB, such as *Desulfotomaculum reducens* [17].

Although most spore forming SRB are found in similar environments to δ-Proteobacteria SRB, spore formation allows this group to survive for long periods of desiccation and oxic conditions. For example, *Desulfotomaculum* is the prevalent genus of SRB in rice paddies due to alternating oxic and anoxic conditions as a result of seasonal flooding [18].

4. Bacterial thermophilic SRB

The two most well characterized species in this group of SRB are *Thermodesulfobacterium commune* [19] and *Thermodesulfovibrio yellowstonii* (Fig. 4) [20]. The sequences available for analysis of both these species contain significant amounts of ambiguity (12% for *T. commune* and 20% for *T. yellowstonii*) that may effect accurate phylogenetic placement, although over 1 kb of readable sequence are available for each species. Both bacteria were isolated

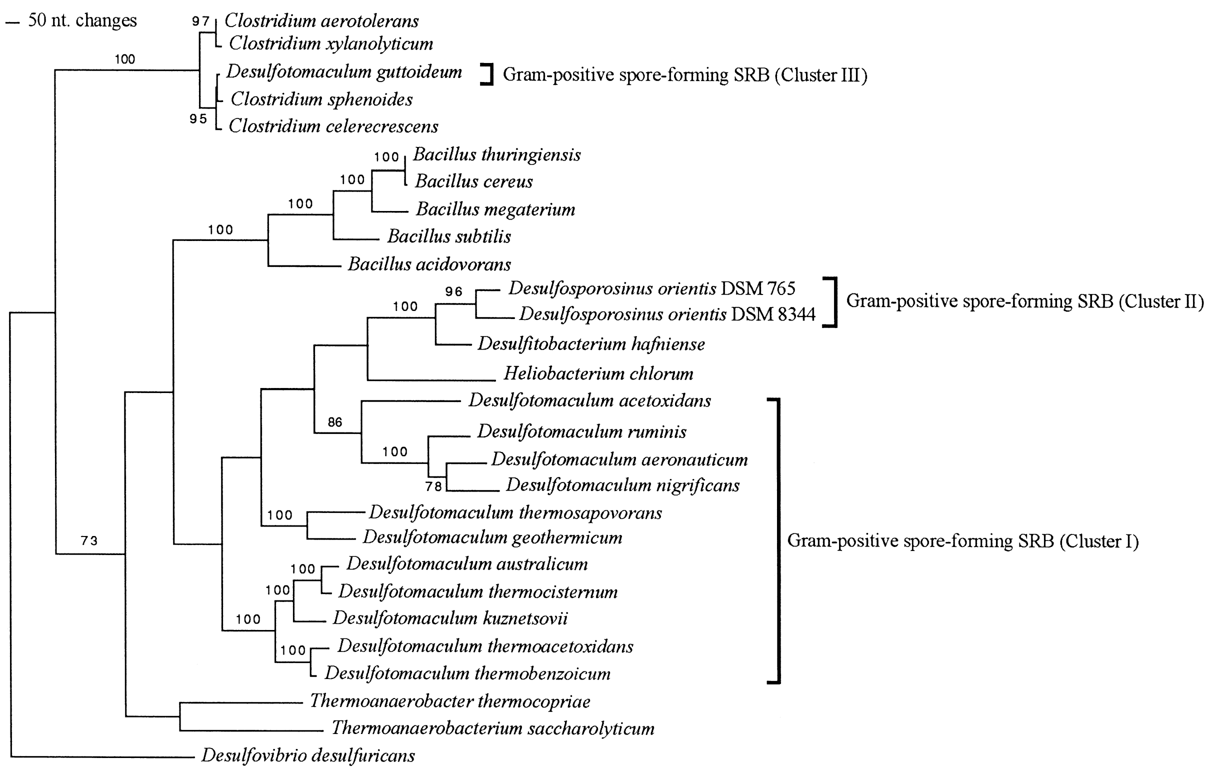

Fig. 3. Phylogenetic tree for the genus *Desulfotomaculum* within the cluster of low G+C content Gram-positive bacteria. Numbers before branch points represent percentages of bootstrap resampling based on 1000 trees. Bootstrap values below 50% are not presented.

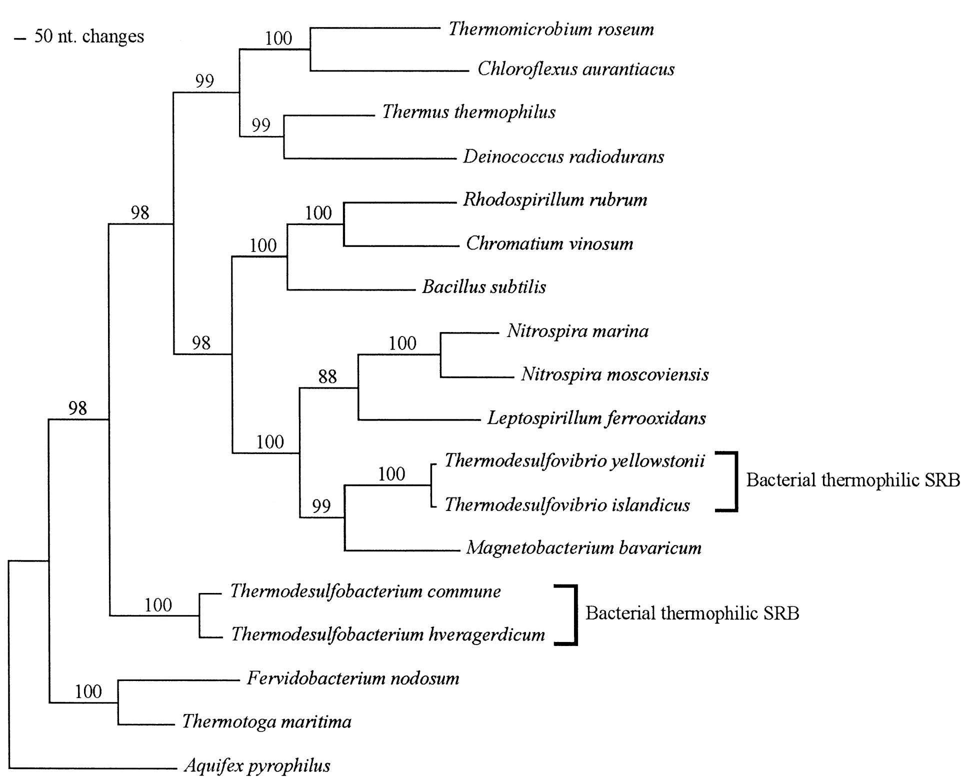

Fig. 4. Phylogenetic relationships of Gram-negative bacterial thermophilic SRB with other Bacterial and Archaeal groups. Numbers before branch points represent percentages of bootstrap resampling based on 1000 trees. Bootstrap values below 50% are not presented.

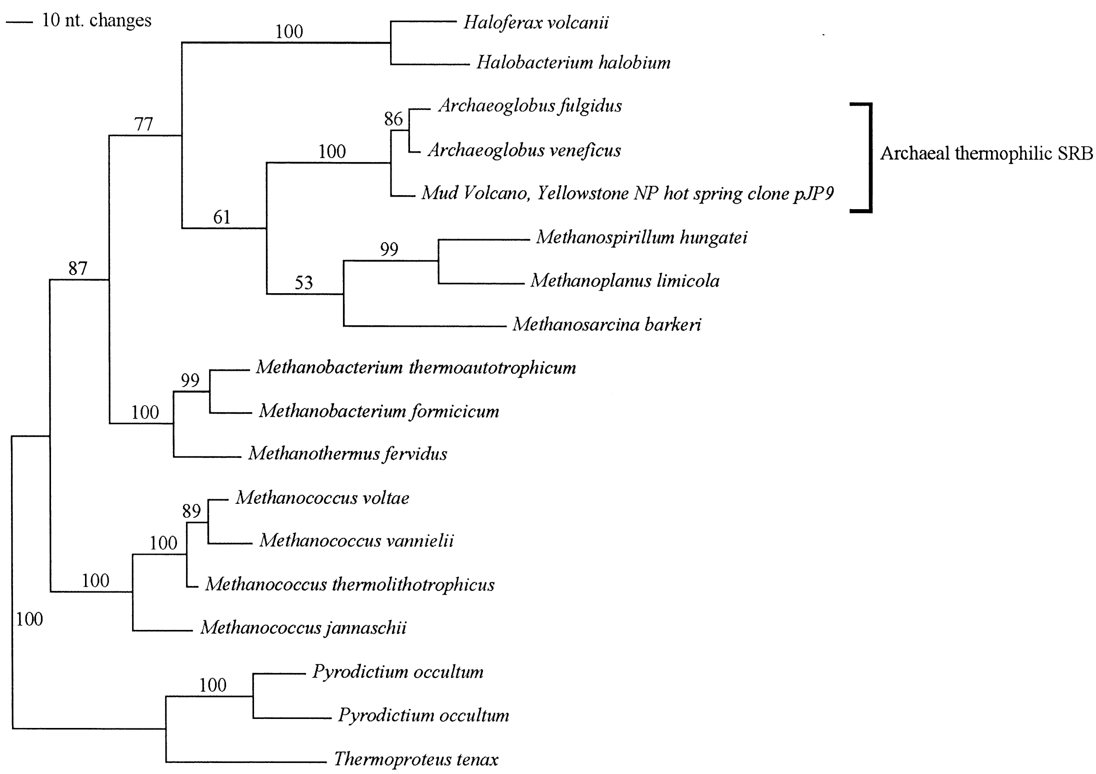

Fig. 5. Phylogenetic position of Archaeal thermophilic SRB. Numbers before branch points represent percentages of bootstrap resampling based on 1000 trees. Bootstrap values below 50% are not presented.

from hydrothermal vent waters in Yellowstone National Park, and their optimal growth temperatures are higher than those described for Gram-positive spore forming thermophilic SRB, but lower than those of the Archaeal SRB (Table 1). Although these two genera have similar physiological and phenotypic characteristics, they differ in shape (vibrio versus rod) and GC content (30% versus 34%) for *T. yellowstonii* and *T. commune*, respectively. Sequence analysis of 16S rRNA also suggests that they are phylogenetically distant (Fig. 4), confirming their placement in separate genera [20]. Henry et al. [20] suggested that categorization of thermophilic SRB (similar physiology but different phylogeny) is similar to the situation with the *Desulfovibrio* family: a group that shares physiological similarities but is phylogenetically diverse and is grouped within a family. Moreover, both thermophilic SRB and *Desulfovibrio* spp. exhibit incomplete oxidation of acetate and utilize a limited number of electrons donors; for these reasons, Henry et al. [20] proposed that thermophilic SRB and desulfovibrios play similar functional roles in their respective environments.

Phylogenies for thermophilic Gram-negative bacteria branch deeply in the Bacteria domain, in accordance with the theory of thermophilic origins of the Bacteria [21]. Both genera utilize H_2 as an electron acceptor if acetate is present, which in these extreme environments can be derived from thermophilic fermentations or geothermal reactions [22]. Although these genera have optimal growth temperatures between 65 and 70°C, they can survive at lower temperatures [23].

5. Archaeal thermophilic SRB

This group (Fig. 5) exhibits optimal growth temperatures above 80°C. Only two species have been described to date, both of which were isolated from marine hydrothermal systems: *Archaeoglobus fulgidus* [24] and *A. profundus* [25]. Major differences between the two species are that *A. fulgidus* possess flagella, are facultative chemolithoautotrophs, and produce small amounts of methane, while *A. profundus* do not possess flagella, are obligate chemolithoheterotrophs, and do not produce methane. No rRNA sequence is available at this time for *A. profundus*, and so it was not included in Fig. 5.

Using 16S and 23S rRNA sequence analysis, Woese et al. [26] indicated that *A. fulgidus* falls within the Methanomicrobiales and extreme halophiles cluster (kingdom Euryarchaeota), as is shown in Fig. 5. Woese et al. [26] proposed that sulfate-reducing activity did not arise as early as was proposed by Achenbach-Ritcher et al. [27], and today *A. fulgidus* is thought to have evolved from methanogenic ancestors. Microorganisms belonging to the Methanomicrobial branch (Fig. 5) of the Archaea are characterized by metabolic characteristics other than methanogenesis, as this group also includes extreme halo-

Table 2
16S rRNA oligonucleotides probes for sulfate-reducing bacteria

Oligonucleotide[a]	Original probe name	Sequence 5' to 3'	Position[b]	Specificity (Reference)
S-*-Dsb-0804-a-A-18	Probe 804	CAACGTTTACTGCGTGGA	804–821	*Desulfobacter* group [32]
S-*-Dscoc-0814-a-A-18	Probe 814	ACCTAGTGATCAACGTTT	814–831	*Desulfococcus* group [32]
S-*-Dsv-0636-a-A-19		ACTATGACGACACGAACTC	636–654	Phylogenetically coherent cluster of *Desulfovibrio*; A. Teske, unpublished; [33]
S-*-Dsv-0683-a-A-22		TCTACGGATTTCACTCCTACAC	683–705	*Desulfovibrionaceae*/metal reducers [34]
S-*-Srb-0385-a-A-18	SRB	CGGCGTCGCTGCGTCAGG	385–402	SRB of the δ-Proteobacteria [35], and other δ-Proteobacteria species and Gram-positive bacteria [36]
S-*-Srb-0385-a-S-18	SRB385-F (PCR primer)	CCTGACGCAGCIACGCCG	385–402	Sulfate reducers, also nontarget sequences, e.g., *Chlorobium*, *Campylobacter*, and *Clostridium* [37]
S-F-Dsv-0687-a-A-16	Probe 687	TACGGATTTCACTCCT	687–702	*Desulfovibrio* [32]
S-G-Dsb-0129-a-A-18	Probe 129	CAGGCTTGAAGGCAGATT	129–146	*Desulfobacter* [32]
S-G-Dsb-0220-a-A-20	Desulfobacter	T(C/A)CGCA(G/A)ACTCATCCCCAAA	220–239	*Desulfobacter* [32]
S-G-Dsbb-0660-a-A-20	Probe 660	GAATTCCACTTTCCCCTCTG	660–679	*Desulfobulbus* [32]
S-G-Dsbm-0221-a-A-20	Probe 221	TGCGCGGACTCATCTTCAAA	221–240	*Desulfobacterium* [32]
S-S-Dsm.sp-0453-a-A-19	*D. acetoxidans*-like	CTGATTAGCACCATGGCGG[c]	453–470	*Desulfuromonas acetoxidans*-like 16S rRNA sequence [37]
S-S-Dsm.sp-0647-a-A-19	Population type 1	TCTCCCGTATTCAAGTCTG	647–665	*Desulfuromonas acetoxidans* 16S rRNA sequence (96% similar) [37]
S-S-DSV.sp-0453-a-A-19	*D. vulgaris*-like	GGTATTAACCGACTATCAT[c]	453–470	*Desulfovibrio vulgaris*-like 16S rRNA sequence [37]
S-S-Dsv.sp-0647-a-A-19	Population type 2	TCTCCCGAACTCAAGTCCA	647–655	*Desulfovibrio vulgaris* 16S rRNA (98% similar) [37]
	A01-183	CCCCTAAGAAAATACGAT	183–201	Uncultivated clone, 89.1% similar to *Desulfococcus multivorans* [29]
	4D19-189	CCCTTGATCCAACATTCC	189–207	Uncultivated clone, 96.3% similar to *Desulfosarcina variabilis* [29]

[a]OPD nomenclature convention.
[b]*Escherichia coli* numbering.
[c]Sequences not shown in original paper; obtained from OPD [33].

philes (*Halobacterium halobium*) and thermo-acidophiles (*Thermoplasma acidophilum*) [26]. The question of how sulfate reduction in *Archaeoglobus* was acquired remains unresolved, although Wagner et al. [28] proposed that either a common ancestor of the Archaea and Bacteria domains possessed the enzyme, or the gene was laterally transferred into *Archaeoglobus* from a member of the Bacteria soon after divergence of the domains.

6. Group-specific probes and PCR primers

Due to the importance of SRB in microbial ecology, several oligonucleotide probes have been developed over the past 8 years. Most probes tested target Gram-negative mesophilic SRB, however, and probes for other phylogenetic groups have yet to be tested (Table 2). Rooney-Varga et al. [29] developed probes for uncultivated clones of SRB, but these clones were very similar to species of the *Desulfococcus* and *Desulfosarcina* (Gram-negative mesophilic SRB). Probes specifically targeting all groups would greatly facilitate our understanding of the role of each of these groups in the environment.

7. Existence of possible undescribed groups

Even though SRB are currently only divided into four phylogenetic groups, new divisions could be added as more information on the diversity of SRB in extreme environments becomes available. Jorgensen et al. [30] observed sulfate-reducing activity in sediments from Guaymas Basin in different ranges of temperatures than previously described for the four known groups of SRB. They also reported sulfate reduction between 100 and 110°C, temperatures from which no SRB have yet been isolated. These authors postulated that SRB may be present in those extreme thermophilic environments and that there may be some hyperthermophilic SRB still to be discovered.

SRB able to degrade complex high molecular mass aromatic hydrocarbons such as naphthalene and phenanthrene have not been isolated to date; however, Coates et al. [31] reported oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. Although SRB were not isolated, incubation of [^{14}C]naphthalene- or phenanthrene-spiked harbor sediments under sulfate-reducing conditions resulted in the production of $^{14}CO_2$. Moreover, addition of molybdate, a specific inhibitor of sulfate reduction, resulted in a complete inhibition of $^{14}CO_2$ evolution, suggesting that undescribed SRB may be present.

The assumption that greater than 99% of bacteria in soils remain uncultivated is another challenging area where phylogeny of SRB may play an important role. Rooney-Varga et al. [29], using oligonucleotides probes targeting novel uncultivated SRB, found that one of the uncultivated clones played an important role in a salt marsh sediment. Wagner et al. [28], using phylogenetic analyses of dissimilatory sulfite reductases, found different sequences from previously described sequences, also suggesting the possible presence of undescribed SRB.

The question of why sulfate reduction may be relatively restricted to certain phylogenetic groups remains without answer, especially when compared with other metabolic characteristics such as reduction of nitrate that is spread through different bacterial groups.

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