JOURNAL OF BACTERIOLOGY, Jan. 2002, p. 278–289 Vol. 184, No. 1
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.1.278–289.2002

Phylogenetic Analysis Reveals Multiple Lateral Transfers of Adenosine-5′-Phosphosulfate Reductase Genes among Sulfate-Reducing Microorganisms

Michael W. Friedrich*

Max-Planck-Institute for Terrestrial Microbiology, D-35043 Marburg, Germany

Received 10 April 2001/Accepted 2 October 2001

Lateral gene transfer affects the evolutionary path of key genes involved in ancient metabolic traits, such as sulfate respiration, even more than previously expected. In this study, the phylogeny of the adenosine-5′-phosphosulfate (APS) reductase was analyzed. APS reductase is a key enzyme in sulfate respiration present in all sulfate-respiring prokaryotes. A newly developed PCR assay was used to amplify and sequence a fragment (~900 bp) of the APS reductase gene, *apsA*, from a taxonomically wide range of sulfate-reducing prokaryotes ($n = 60$). Comparative phylogenetic analysis of all obtained and available ApsA sequences indicated a high degree of sequence conservation in the region analyzed. However, a comparison of ApsA- and 16S rRNA-based phylogenetic trees revealed topological incongruences affecting seven members of the *Syntrophobacteraceae* and three members of the *Nitrospinaceae*, which were clearly monophyletic with gram-positive sulfate-reducing bacteria (SRB). In addition, *Thermodesulfovibrio islandicus* and *Thermodesulfobacterium thermophilum*, *Thermodesulfobacterium commune*, and *Thermodesulfobacterium hveragerdense* clearly branched off between the radiation of the δ-proteobacterial gram-negative SRB and the gram-positive SRB and not close to the root of the tree as expected from 16S rRNA phylogeny. The most parsimonious explanation for these discrepancies in tree topologies is lateral transfer of *apsA* genes across bacterial divisions. Similar patterns of insertions and deletions in ApsA sequences of donor and recipient lineages provide additional evidence for lateral gene transfer. From a subset of reference strains ($n = 25$), a fragment of the dissimilatory sulfite reductase genes (*dsrAB*), which have recently been proposed to have undergone multiple lateral gene transfers (M. Klein et al., J. Bacteriol. 183:6028–6035, 2001), was also amplified and sequenced. Phylogenetic comparison of DsrAB- and ApsA-based trees suggests a frequent involvement of gram-positive and thermophilic SRB in lateral gene transfer events among SRB.

One of the oldest types of biological energy conservation on Earth is sulfate respiration, which developed ~2.8 to 3.1 billion years ago (3, 37). The evolutionary diversification of sulfate-reducing prokaryotes (SRP) since then should, in principle, be reflected in the history of their genes. However, lateral gene transfer (LGT) (32), which appears to be an important and frequent event in evolution (26), even across domains (1), may blur the evolutionary conclusions about certain enzymes (8). There is mounting evidence that genes coding for key enzymes of sulfate respiration were transferred horizontally from gram-positive bacteria to archaeal *Archaeoglobus* spp. (14, 20, 41). More recently, Klein et al. (19) discovered the occurrence of multiple lateral transfers of genes coding for the dissimilatory sulfite reductase (*dsrAB*) among SRP. The phylogenetic tree based on comparative sequence analysis of DsrAB gene fragments of >30 reference strains was partially inconsistent with the corresponding 16S rRNA-based phylogenetic tree. It was concluded that *dsrAB* genes of several *Desulfotomaculum* spp. (low-G+C gram-positive division), *Thermodesulfobacterium* spp., and *Desulfobacula toluolica* had been laterally transferred from unidentified ancestors of sulfate-reducing bacteria (SRB) within the δ-proteobacteria.

Whereas dissimilatory sulfite reductase occurs also in non-sulfate-reducing, sulfite-respiring microorganisms, such as *Pyrobacculum islandicum* (29), *Desulfitobacterium* spp. (15, 19, 21), and *Bilophila wadsworthia* (24), other sulfate-respiring prokaryotes possess adenosine-5′-phosphosulfate (APS) reductase in addition to sulfite reductase (35). After activation of the chemically inert sulfate by ATP sulfurylase (9) the Fe-S flavoprotein APS reductase (EC 1.8.99.2) catalyzes the two-electron reduction of APS to sulfite and AMP ($E_0' = -60$ mV). It has been assumed that the same enzyme activity catalyzes also the inverse reaction in a variety of sulfur-oxidizing bacteria (for a review see reference 11); however, this "reverse" function has recently been questioned in connection with the phototroph *Allochromatium vinosum* (5). The genes for APS reductase, *apsBA* (*Desulfovibrio vulgaris*; GenBank accession no. Z69372) and *aprBA* (*Archaeoglobus fulgidus* and *Allochromatium vinosum* [14] and *Desulfovibrio desulfuricans* [12]) encode subunits that appear to form a 1:1 αβ heterodimer (12). Both subunits of the APS reductase are highly conserved (12), and the APS reductase genes have been proposed as a useful phylogenetic marker (14). However, it is still under debate whether the APS reductase genes of *Archaeoglobus fulgidus* were transferred from an ancestral donor within the domain *Bacteria* (14).

Recently, new assays for the PCR amplification of fragments from the *apsA* gene have been developed (7, 49) and utilized to study the diversity and distribution of SRB in gastrointestinal

* Mailing address: Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch Straße, D-35043 Marburg, Germany. Phone: 49-6421-178 830. Fax: 49-6421-178 809. E-mail: friedric@mailer.uni-marburg.de.

TABLE 1. PCR primers utilized for the amplification of *apsA* gene fragments

Primer	Sequence (5′→3′)[e]	Primer binding site[a]	Reference
APS-FW	TGG CAG ATM ATG ATY MAC GG	481–500	7[b]
APS-RV	GGG CCG TAA CCG TCC TTG AA	847–866	7
APS-uni-F	TGG CAG ATV ATG ATY MAC GG	481–500	This study[c]
APS7-F	GGG YCT KTC CGC YAT CAA YAC	206–236	This study
APS7a-F	GGG YCT SAG CGC YAT CAA Y	206–234	This study[d]
APS7b-F	GG YCT STC CGC YAT CAA Y	205–234	This study[d]
APS8-R	GCA CAT GTC GAG GAA GTC TTC	1139–1159	This study

[a] Positions of the *Desulfovibrio-vulgaris apsA* open reading frame.
[b] Published primer sequence contains an additional G at the 3′ end, which was a typing error (B. Deplancke, personal communication).
[c] Primer is a modification of primer APS-FW.
[d] Primer is a modification of primer APS7-F.
[e] Degenerate positions are in boldface.

tracts. However, the lack of a thorough phylogenetic framework of APS reductase from cultivated sulfate reducers still prevents a reliable assignment of molecular, environmental sequences to known taxa of sulfate reducers and thus prevents the use of the *apsA* gene as a functional marker gene for molecular ecology studies.

This study analyzed the evolutionary relationship of a wide taxonomic range of SRP based on the α-subunit of the APS reductase (ApsA). A new PCR assay targeting the *apsA* gene was developed, and *apsA* PCR products were directly sequenced and comparatively analyzed. Incongruences between phylogenetic trees of ApsA and 16S rRNA genes revealed evidence for the intradomain lateral transfer of the *apsA* gene among distantly related gram-positive SRB and distinct groups of δ-proteobacteria, comprising members of the *Syntrophobacteraceae* and the *Nitrospinaceae*. A comparison of DsrAB- and ApsA-based phylogenetic trees revealed patterns of LGT for key enzymes of SRP.

MATERIALS AND METHODS

Microorganisms. Reference strains of validly described sulfate-reducing microorganisms (see Table 2) were obtained from Kai Finster (Aarhus, Denmark), Alexander Galushko (Konstanz, Germany), Bernhard Schink (Konstanz, Germany), Hans Scholten (Marburg, Germany), and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) either as lyophilized cells or as actively growing cultures. *Archaeoglobus veneficus* (DSM11195) was isolated by K. O. Stetter (University of Regensburg, Regensburg, Germany).

DNA isolation. Cells of exponentially growing cultures (10 ml) were harvested by centrifugation and washed with 120 mM sodium phosphate buffer, pH 8.0, before DNA extraction. DNA from lyophilized cells of reference strains was directly extracted without further cultivation of the bacteria. Genomic DNA was extracted from reference strains using a direct-lysis protocol modified from that described by Moré et al. (30) as described previously (13). Briefly, cells were disrupted by bead beating (45 s at 6.5 m s^{-1}) in a sodium dodecyl sulfate solution. DNA was purified from the supernatant with ammonium acetate, isopropanol, and ethanol precipitation steps. The DNA extracts were further purified using a silica matrix-based purification protocol (EasyPure; Biozym, Hess. Oldendorf, Germany). Aliquots of DNA extracts were analyzed by standard gel electrophoresis to verify extraction.

PCR amplification of *apsA* gene fragments. The nomenclature of the APS reductase gene operon has not yet been resolved, and *aps* and *apr* have been used synonymously. In this paper, *apsA* is used to designate the APS reductase α-subunit gene.

An ~390-bp *apsA* segment was amplified by PCR from genomic DNA of pure cultures as described by Deplancke et al. (7) using primers APS-FW and APS-RV (Table 1). Longer *apsA* fragments were amplified using primers APS7-F (and its derivatives) and APS8-R (~900 bp; Table 1). The reaction mixture contained, in a total volume of 50 μl, 25 μl of 2× premix E (Epicentre Technologies, Madison, Wis.), a proprietary PCR premix (containing 400 μM deoxynucleoside triphosphates, 5 mM $MgCl_2$, and 4× betaine as a PCR enhancer), 2 μM primer APS7-F, 0.5 μM primer APS8-R, and 1.25 U of Ampli*Taq* DNA polymerase (Applied Biosystems, Weiterstadt, Germany). DNA from pure cultures (~20 ng of nucleic acids) was added as the template. All reaction mixtures were prepared at 4°C in 0.2-ml reaction tubes to avoid unspecific priming. Amplification was started by placing the reaction tubes into the preheated (94°C) block of a Gene Amp 9700 thermocycler (Applied Biosystems). The standard thermal profile for amplification was as follows: an initial denaturation step (3 min, 94°C) was followed by 30 to 35 cycles of denaturation (30 s, 94°C), annealing (55 s, 60°C), and extension (60 s, 72°C). After a terminal extension (7 min, 72°C), the samples were kept at 4°C until further analysis. For PCR screening of *apsA* gene fragments, the annealing temperature was altered in a range between 45 and 60°C as indicated in Table 2. Aliquots of the amplicons (5 μl) were analyzed by electrophoresis on 1% agarose gels and visualized after staining with ethidium bromide using a gel imaging system (MWG Biotech).

PCR amplification of *dsrAB* gene fragments. An ~1.9-kb fragment encompassing parts of the dissimilatory sulfite reductase genes *dsrA* and *dsrB* was amplified using primers DSR1-F and DSR4-R (41). Reaction mixtures contained, in a total volume of 50 μl, 25 μl of 2× premix E, 0.5 μM (each) primer, genomic DNA (~20 ng of nucleic acids), and 2.0 U of Ampli*Taq* DNA polymerase. The thermal profile for amplification was as follows: an initial denaturation step (2 min, 94°C) was followed by 38 cycles of denaturation (45 s, 94°C), annealing (45 s, 54°C), and extension (90 s, 72°C) and one terminal extension step (5 min, 72°C).

PCR amplification of 16S rRNA gene fragments. 16S rRNA genes were amplified from genomic DNA of pure cultures using primers 27F and 1492R (22) or the primer pair 27F (22) and 1542R (18). Reaction mixtures contained, in a volume of 50 μl, 10 μl of 10× PCR buffer, 0.5 μM (each) primer, 50 μM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, and 1.25 U of Ampli*Taq* DNA polymerase. DNA from pure cultures (~20 ng of nucleic acids) was added as the template. The thermal profile for amplification was as follows: an initial denaturation step (2 min, 94°C) was followed by 30 to 35 cycles of denaturation (30 s, 94°C), annealing (45 s, 55°C), and extension (60 s, 72°C) and one terminal extension step (5 min, 72°C).

Sequencing. PCR products of *apsA* genes were directly sequenced using primers APS-FW and APS-RV, APS-uni-F and APS-RV, APS7-F (and its derivatives), and APS8-R (Table 1), and *dsrAB* PCR products were sequenced using primers DSR1F and DSR4R and sequencing primers DSR6F (5′-ATC GGC AC**M** TGG AGA GAC-3′), DSR7F (5′-**K**CC ATC GC**B** CGT TCC GAC-3′), DSR8F (5′-GGC **M**AG AAC CG**Y** GAG CG**Y**-3′), DSR9F (5′-**M**CA ACC C**S**T A**Y**A TCT TCT-3′), and DSR10F (5′-GGA AGA **R**GG CAA **R**AA CCG-3′). 16S rDNA PCR products were sequenced using primers 27F, 533R, 907R (44), 1542R (18), 1114F, and 1368R (10). PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using the ABI BigDye terminator cycle sequencing kit (Applied Biosystems) with 100 ng of template DNA as specified by the manufacturer. Cycle sequencing products were purified from excess dye terminators and primers using Autoseq G-50 columns (Amersham-Pharmacia Biotech, Freiburg, Germany) and analyzed with an ABI 373 or 377 DNA sequencer (Applied Biosystems).

Sequence data analysis and phylogenetic placement. Raw sequence data were assembled and checked with the Lasergene software package (DNASTAR, Madison, Wis.). The data were phylogenetically analyzed (i.e., alignment, treeing) using the ARB (version 2.5b; O. Strunk and W. Ludwig, Technische Universität München, Munich, Germany [http://www.biol.chemie.tu-muenchen.de/pub/ARB/]), PHYLIP (J. Felsenstein, PHYLIP [phylogeny inference package], ver-

TABLE 2. PCR amplification of *apsA* gene fragments using genomic DNA of sulfate-reducing reference strains and selected characteristics

Species[a]	Strain[b]	Oxidation[c]	T_{opt}[d] (°C)	Genomic G+C content (mol %)	PCR product obtained with primer pair[e]:				GenBank accession no.		
					APS-FW, APS-RV[f] (~390 bp)	APS7-F, APS8-R (~900 bp)	APS7a-F, APS8-R (~900 bp)	APS7b-F, APS8-R (~900 bp)	*apsA*	16S rRNA	*dsrAB*
Archaea, Euryarchaeota											
Archaeoglobus fulgidus	DSM4304^T	C	83	46	+[g]	+[g]			AE000988	X05567, Y00275	M95624
Archaeoglobus profundus	DSM5631^T	C	82	41	+	+ (50)			**AF418134**	AF297529	AF071499
Archaeoglobus veneficus	DSM11195^T	C	75–80	45.4	+	+ (50)			**AF418132**	**AF418181**	
Bacteria											
Nitrospira phylum											
Thermodesulfovibrio islandicus	DSM12570^T	I	65	38	−	+ (48)			**AF418113**	X96726	AF334599
Thermodesulfovibrio yellowstonii	DSM11347^T	I	65	29.5	**−**	**− (45)**	**− (45)**	**− (45)**			
Thermodesulfobacteria phylum											
Thermodesulfobacterium commune	DSM2178^T	I	70	34	−	+ (48)			**AF418114**	**AF418169**	AF334596
Thermodesulfobacterium thermophilum	DSM1276^T	I	65	31	−	+ (48)			**AF418112**	AF334601	
Thermodesulfobacterium hveragerdense	DSM12571^T	I	70–74	40	−	+ (48)			**AF418119**	X96725	
***Firmicutes*, "*Clostridia*," Peptococcaceae**											
Desulfotomaculum kuznetsovii	DSM6115^T	C	60–65	49	+	− (45)	+ (59)	+ (59)	**AF418152**	Y11569	AF273031
Desulfotomaculum thermobenzoicum	DSM6193^T	C	62	53	+	− (45)	+ (59)	+ (59)	**AF418161**	L15628	AF273030
Desulfotomaculum geothermicum	DSM3669^T	C	54	50	+	+ (48)			**AF418115**	X80789	AF273029
Desulfotomaculum sapomandens	DSM3223^T	C	38	48	−	−	+ (45)	− (59)	**AF418150**	AF168365	
Desulfotomaculum alkaliphilum	DSM12257^T	C	50–55	41	−	+ (48)	−	− (59)	**AF418154**	AF097024	**AF418195**
Desulfotomaculum acetoxidans	DSM771^T	C	37	38	−	+ (48)	− (59)	− (59)	**AF418153**	Y11566	AF271768
Desulfotomaculum putei	DSM12395^T	I	50–65	47	+	− (45)	+ (59)	+ (59)	**AF418147**	AF053929	AF273032
Desulfotomaculum nigrificans	DSM574^T	I	55	45	−	−	+ (45)	− (59)	**AF418154**	U33455	
Desulfotomaculum ruminis	DSM2154^T	I	28	49	−	− (45)	1 (45)	1 (45)	**AF418164**	Y11572	
Desulfotomaculum aeronauticum	DSM10349^T	I	37	44	−	+ (45)	+ (45)	+ (45)	**AF418156**	X98407	AF273033
Desulfotomaculum halophilum	DSM11559^T	I	35	56.3	+	− (45)	− (45)	− (45)	**AF418167**		
Desulfosporosinus orientis	DSM8344	I	30	45	**−**	**− (45)**	**− (45)**	**− (45)**			
***Proteobacteria* delta subdivision**											
"*Desulfobacteraceae*"[a]											
Desulfobacter curvatus	DSM3379^T	C	28–32	46	−	+			**AF418107**	**AF418175**	**AF418199**
Desulfobacter postgatei	DSM2034^T	C	28–33	46	−	+ (45)	1 (45)	+ (45)	**AF418157**	**AF418180**	**AF418198**
Desulfospira joergensenii	DSM10085^T	C	26–30	49.9	1	+			**AF418116**	X99637	
Desulfotignum balticum	DSM7044	C	30	62.4	−	+			**AF418127**	**AF418176**	
Desulfobacula toluolica	DSM7467^T	C	28	42	1	+			**AF418128**	X70953	
Desulfobacterium autotrophicum	DSM3382^T	C	20–26	48	−	+			**AF418108**	**AF418177**	**AF418182**
"Desulfobacterium vacuolatum"	DSM3385^T	C	25–30	45	−	+			**AF418124**	**AF418178**	**AF418203**
Desulfocella halophila	DSM11763^T	I	34	35	−	+			**AF418117**	AF022936	**AF418200**
Desulfonema ishimotonii	DSM9680^T	C	30	55	+	+ (50)			**AF418135**	U45992	
Desulfonema magnum	DSM2077^T	C	32	41.6	−	+			**AF418122**	U45989	
Desulfofrigus oceanense	DSM12341^T	C	10	52.8	1	− (45)		+ (55)	**AF418145**	AF099064	
Desulfofaba gelida	DSM12344^T	I	7	52.5	+	+			**AF418118**	AF099063	AF334593
Desulfococcus biacutus	DSM5651^T	C	28–30	56.5	1	1 (50)			**AF418151**	AJ277887	
Desulfococcus multivorans	DSM2059^T	C	35	57.4	+	+ (50)			**AF418136**	**AF418173**	U58126/7
Desulfosarcina variabilis	DSM2060^T	C	33	51	−	+			**AF418121**	M34407	AF191907
Desulfobacterium indolicum	DSM3383^T	C	28	47	+	+			**AF418123**	AJ237607	
"Desulfobacterium oleovorans"	DSM6200^T	C	n.a.[i]	n.a.	+	+			**AF418126**	Y17698	**AF418201**
Desulfotalea psychrophila	DSM12343^T	I	10	46.8	**−**	**− (45)**	**− (45)**	**− (45)**			

"Desulfobulbaceae"[a]											
Desulfobulbus rhabdoformis	DSM8777[T]	I	31	50.6	+	+			**AF418110**	U12253	AJ250473
Desulfobulbus elongatus	DSM2908[T]	I	35	59	+	+		l (55)	**AF418146**	X95180	**AF418202**
Desulfocapsa sulfexigens	DSM10523[T]	A	30	47.2	+	+			**AF418131**	Y13672	
Desulfocapsa thiozymogenes	DSM7269[T]	I	30	50.7	+	+ (45)	+ (45)	+ (45)	**AF418166**		
Desulfofustis glycolicus	DSM9705[T]	I	28	56	l	+			**AF418130**	X99707	**AF418191**
Desulforhopalus sp. strain LsV 20	DSM13038	n.a.	<20	n.a.	−	l (45)		− (45)	**AF418160**	AF099057	
Desulforhopalus singaporensis	DSM12130[T]	I	31	50.6	−	+ (45)	− (45)	+ (45)	**AF418163**	AF118453	**AF418196**
"Desulfovibrionales"[a]											
Desulfovibrio desulfuricans subsp. *desulfuricans* Essex 6	DSM642[T]	I	30	59	+	+			AF226708	AF192153	AJ249777
Desulfovibrio intestinalis	DSM11275[T]	I	37	54.5–55.5	+	+			**AF418106**	Y12254	**AF418183**
Desulfomonas pigra	DSM749[T]	I	37	66	+	+			**AF418129**	AF192152	**AF418184**
Desulfovibrio termitidis	DSM5308[T]	I	35	67–68	+	+		+ (55)	**AF418142**	X87409	**AF418184**
Desulfovibrio vulgaris	DSM644[T]	I	30–36	65	+[g]	+[g]			Z69372	M34399	U16723
Desulfovibrio burkinensis	DSM6830[T]	I	37	67	+	+		+ (55)	**AF418143**	AF053752	**AF418186**
Desulfovibrio fructosovorans	DSM3604[T]	I	35	64	l	+			**AF418109**	AF050101	**AF418187**
Desulfovibrio africanus	DSM2603[T]	I	30–36	65	+	+ (50)		+ (55)	**AF418140**	X99236	AF271772
Desulfovibrio giganteus	DSM4370[T]	I	37	56	+	+ (50)		+ (55)	**AF418141**	**AF418170**	
Desulfovibrio profundus	DSM11384[T]	I	25	53	+	+ (50)			**AF418133**	**AF418172**	
Desulfomicrobium baculatum	DSM1743[T]	I	28–37	57	+	+			**AF418120**		
Desulfomicrobium apsheronum	DSM5918[T]	I	25–30	52	+	+			**AF420281**	U64865	**AF418188**
Desulfohalobium retbaense	DSM5692[T]	I	37–40	57.1	l	+ (50)			**AF418125**	X99235	**AF418190**
Desulfonatronum lacustre	DSM10312[T]	I	37–40	57.3	+	+ (50)			**AF418137**	**AF418171**	**AF418189**
Desulfonatronovibrio hydrogenovorans	DSM9292[T]	n.a.	37	48.6	+	+			**AF418111**	X99234	**AF418197**
"Syntrophobacteraceae"[a]											
Syntrophobacter wolinii	DSM2805M[T]	I	n.d.[j]	n.d.	+	+ (45)	+ (45)	+ (45)	**AF418165**	X70905	**AF418192**
Syntrophobacter pfennigii	DSM10092[T]	I	37	57.3	+	+ (45)	+ (45)	+ (45)	**AF418168**	X82875	
Syntrophobacter fumaroxidans	DSM10017[T]	I	37	60.6	+	+ (45)	+ (59)	+ (59)	**AF418138**	X82874	**AF418193**
Desulforhabdus amnigena	DSM10338[T]	C	37	52.5	+	+ (45)	+ (59)	+ (59)	**AF418139**	X83274	AF337901
Thermodesulforhabdus norvegica	DSM9990[T]	C	60	51	+	+ (45)	+ (45)	+ (59)	**AF418159**	U25627	AF334597
Desulfacinum infernum	DSM9756[T]	n.a.	60	64	+	+ (45)	+ (59)	+ (59)	**AF418144**	L27426	**AF418194**
Desulfacinum hydrothermale	DSM13146[T]	C	60	59.5	+	+ (45)		+ (50)	**AF418148**	AF170417	
"Nitrospinaceae"[a]											
Desulfomonile tiedjei	DSM6799[T]	C	37	49	+	+ (45)	+ (45)	+ (50)	**AF418162**	M26635	AF334595
"Desulfarculus baarsii"	DSM2075[T]	C	37	66	+	+ (45)	+ (45)	+ (55)	**AF418149**	**AF418174**	AF334600
"Desulfobacterium anilini"	DSM4660[T]	C	35	59.1	−	+ (45)	+ (45)	+ (45)	**AF418158**	AJ237601	
Desulfobacca acetoxidans	DSM11109[T]	C	37	51.5	−	**− (48)**	**− (45)**	**− (45)**			

[a] Phylum and family names according to the taxonomic outline of *Bergey's Manual of Systematic Bacteriology* (http://www.cme.msu.edu/Bergeys/april2001-genus.pdf).
[b] DSMZ strain numbers. T, type strain.
[c] C, complete oxidation of organic carbon substrates; I, incomplete oxidation; A, chemolithoautotrophic growth.
[d] T_{opt}, optimum growth temperature.
[e] PCR annealing temperatures in degrees Celsius are in parentheses. Boldface, no PCR product obtained. l, low yield.
[f] PCR annealing temperature 60°C according to Deplancke et al. (7).
[g] Binding of the primer pair deduced from sequence.
[h] Sequences from this study are in boldface.
[i] n.a., not available.
[j] n.d., not determined.

sion 3.6; Department of Genetics, University of Washington, Seattle), and PUZZLE (version 5.0) (39) software packages.

Briefly, deduced APS amino acid sequences were fitted manually into an alignment of APS sequences retrieved from public databases (2) using Genetic Data Environment (version 2.2) as implemented in the ARB software package. Regions of ambiguous homology and insertions or deletions (indels) not present in all sequences analyzed were excluded, yielding an amino acid data set with 252 positions. Trees were reconstructed from distance matrices using FITCH (PHYLIP), neighbor-joining (ARB and PHYLIP), parsimony (PROTPARS; PHYLIP), and maximum-likelihood (PROTML; Institute Pasteur [http://bioweb.pasteur.fr/seqanal/interfaces/molphy.html]; PUZZLE) methods. Distance matrices were calculated using PROTDIST with the Dayhoff PAM 001 matrix as the amino acid replacement model (6). FITCH trees were reconstructed using the global rearrangement and randomized species input order ("jumble," random number seed 7, seven times) options. Bootstrap analyses were performed using parsimony analysis (500 resamplings; randomized input order, "jumble" three times) or neighbor joining (100 resamplings) as implemented in the PHYLIP package. PUZZLE analysis was performed using tree reconstruction by Quartet Puzzling (QP; 10,000 or 25,000 puzzling steps), approximation of parameters using a neighbor-joining tree, and either JTT (Jones, Taylor, Thornton [17]), variable-time (31), or the WAG matrix (45) as amino acid replacement models (amino acid frequency was estimated from the data set) and assuming either a uniform rate of evolution or a gamma distribution. All trees were reconstructed as "unrooted."

Deduced *dsrAB* sequences were analyzed similarly. Regions of ambiguous homology and indels not present in all sequences analyzed were excluded, yielding an amino acid data set with 528 positions for the concatenated data set (19). Phylogenetic trees were reconstructed as described above.

16S rRNA gene sequences were phylogenetically analyzed using distance matrix (neighbor-joining and FITCH using the Kimura two-parameter model), parsimony (DNAPARS), and maximum-likelihood methods (fastDNAML [33]; PUZZLE) by including only nucleotide positions with >50 to 100% invariance. Statistical support for tree topologies was obtained by bootstrap resampling (parsimony, $n = 100$; neighbor joining, $n = 500$) or PUZZLE analysis (QP; nucleotide substitution model by Tamura and Nei [40]).

Nucleotide sequence accession numbers. Sequences of reference strains were deposited in the GenBank database under accession no. AF418106 to AF418168 (*apsA*), AF418182 to AF418203 (*dsrAB*), and AF418169 to AF418181 (16S rRNA genes) as specified in Table 2.

RESULTS

PCR amplification of *apsA* fragments from sulfate-reducing microorganisms. PCR amplicons of the *apsA* gene with the expected sizes (390 to 400 bp) were obtained from a wide phylogenetic range of sulfate-reducing microorganisms using primer combination APS-FW and APS-RV (Table 2). These included mostly δ-proteobacteria of the order "*Desulfovibrionales*" and of the families "*Desulfobulbaceae*," "*Syntrophobacteraceae*," and "*Nitrospinaceae*." However, we were unable to obtain an *apsA* amplicon using the standard PCR conditions (annealing temperature at 60°C [7]) for the thermophilic sulfate-reducing *Thermodesulfobacterium* spp. and *Thermodesulfovibrio* spp.; some gram-positive sulfate-reducing *Desulfotomaculum* spp.; some members of the *Desulfobacteraceae*; and *Desulfobulbaceae*, *Desulfobacca acetoxidans*, and *Desulfobacterium anilini* (Table 2). A new primer pair comprising APS7-F and APS8-R (Table 1) was developed based on the comparison of conserved sites in full-length *apsA*/*aprA* sequences of *Desulfovibrio vulgaris*, *Desulfovibrio desulfuricans, Allochromatium vinosum* (GenBank accession no. U84759), and *Archaeoglobus fulgidus*. Use of this primer pair allowed the amplification of an ~900-bp fragment of the *apsA* gene from a wide range of sulfate-reducing microorganisms, thereby providing considerably more information for phylogenetic analyses. Notably, *apsA* PCR products were obtained from the thermophilic sulfate-reducing *Thermodesulfobacterium* spp. and *Thermodesulfovibrio islandicus*, as well as members of the "*Desulfobacteraceae*" (Table 2). For some species, amplification required a considerable reduction of the annealing temperature to 45°C using primers APS7-F and APS8-R, e.g., *Desulfotomaculum* spp. Even at the lower annealing temperatures, PCR amplification using these primers failed for most members of the "*Syntrophobacteraceae*." Derivatives of primer APS7-F, e.g., APS7a-F and APS7b-F, with variations in degenerate codons (Table 1) were successfully used to obtain a PCR product also from members of the "*Syntrophobacteraceae*" and most *Desulfotomaculum* spp. We were unable to obtain amplification products using all primer pairs for only a few species, such as *Desulfosporosinus orientis*, *Thermodesulfovibrio yellowstonii*, *Desulfotalea psychrophila*, and *Desulfobacca acetoxidans* (Table 2); however, the 16S rRNA genes of these strains could be amplified, verifying the quality of the genomic DNA for amplification.

Phylogeny of APS reductase. *apsA* PCR fragments (primer pair APS7-F and APS8-R) were directly sequenced, yielding sequences varying in length between 860 and 950 bp. The absence of ambiguous nucleotides in sequences of all strains tested indicated that probably only one *apsA* gene copy is present in each strain. The deduced ApsA amino acid sequences ($n = 60$) of fragments >860 bp and the published complete AspA sequences of *Archaeoglobus fulgidus*, *Desulfovibrio vulgaris*, and *Desulfovibrio desulfuricans* were aligned. Amino acid positions that could not be aligned unambiguously or that included indels were excluded from phylogenetic analysis by using filters, yielding a data set with 252 amino acid positions (*Desulfovibrio vulgaris* ApsA positions 82 to 375) for phylogenetic analysis.

ApsA amino acid sequences analyzed were >53% similar, and intrafamily similarities were >80% (Table 3). Notably, sequence similarities among *Archaeoglobus* spp. were low (71 to 83%), which contrasts with the close similarity of the known species on the 16S rDNA level (93 to 98%). The ApsA amino acid sequence of *Archaeoglobus fulgidus* differed the most from those of *Archaeoglobus profundus* and *Archaeoglobus veneficus* (73 and 71%, respectively). Unexpectedly, the ApsA amino acid sequences of members of the *Syntrophobacteraceae* and *Nitrospinaceae* were more similar to those of gram-positive *Desulfotomaculum* spp. than to those of gram-negative δ-proteobacterial SRB.

The deduced ApsA amino acid sequences were phylogenetically analyzed and compared using distance matrix (FITCH, neighbor-joining), maximum-parsimony, and maximum-likelihood methods (PROTML, PUZZLE). The phylogeny of SRP based on ApsA sequence analyses was compared to the phylogeny based on 16S rRNA gene and DsrAB analyses. All treeing methods used (distance matrix, parsimony, and maximum-likelihood) for ApsA- and 16S rRNA-based phylogenies indicated similar relative branching orders of most taxa (Fig. 1). Both trees were rooted with the archaeal *Archaeoglobus* spp. Certain branch points of the gram-positive SRB lineages and of the "*Desulfobacteraceae*" branch of the δ-proteobacteria were resolved poorly, as indicated by low bootstrap values. These branch points were indicated as "multifurcations" (27) (Fig. 1), which were also directly shown by PUZZLE analysis (not shown).

TABLE 3. ApsA sequence similarities of selected sulfate-reducing microorganisms representing major lineages of sulfate-respiring prokaryotes[a]

Sulfate-reducing microorganism	% Amino acid sequence similarity to:																		
	D. curvatus	*D. postgatei*	*D. variabilis*	*D. rhabdoformis*	*D. glycolicus*	*D. vulgaris*	*D. fructosivorans*	***T. thermophilum***	***T. commune***	***T. islandicus***	***D. amnigena***	***S. wolinii***	***D. baarsii***	*D. ruminis*	*D. putei*	*D. kuznetsovii*	*A. veneficus*	*A. profundus*	*A. fulgidus*
Desulfobacter curvatus																			
Desulfobacter postgatei	95.5																		
Desulfosarcina variabilis	83.3	82.3																	
Desulfobulbus rhabdoformis	71.9	71.9	73.5																
Desulfofustis glycolicus	73.7	73.7	75.3	82.2															
Desulfovibrio vulgaris	65.4	65.0	68.2	67.5	66.3														
Desulfovibrio fructosovorans	65.5	65.5	68.3	70.0	68.9	87.7													
Thermodesulfobacterium thermophilum	67.3	66.5	66.2	62.4	63.7	64.5	66.1												
Thermodesulfobacterium commune	67.0	66.3	66.4	62.5	63.4	65.0	66.2	96.5											
Thermodesulfovibrio islandicus	62.9	63.3	62.0	60.7	64.7	63.7	66.8	64.6	64.6										
Desulforhabdus amnigena	60.1	60.5	58.6	57.0	57.1	56.5	59.7	59.5	60.0	59.6									
Syntrophobacter wolinii	62.0	62.4	60.5	57.2	58.0	57.1	61.0	62.0	62.9	59.8	90.4								
"Desulfoarculus baarsii"	57.7	58.5	55.2	54.4	53.6	55.3	58.9	57.0	58.0	58.5	78.7	79.5							
Desulfotomaculum ruminis	59.1	59.5	56.6	56.3	55.2	56.3	59.1	55.1	55.6	57.7	77.4	77.9	76.9						
Desulfotomaculum putei	59.8	60.6	58.1	57.7	57.0	56.6	59.8	56.1	56.6	58.8	78.9	77.1	76.4	93.6					
Desulfotomaculum kuznetsovii	60.5	60.1	59.4	57.3	57.9	59.7	62.5	62.0	62.1	60.2	79.2	79.3	74.4	74.3	75.8				
Archaeoglobus veneficus	60.2	62.2	57.0	58.0	58.2	57.8	60.2	59.5	59.2	57.3	61.8	60.4	60.3	60.9	61.9	63.5			
Archaeoglobus profundus	60.6	61.0	58.6	57.0	58.4	57.7	57.7	62.6	62.7	57.7	59.2	59.5	58.9	59.2	59.4	64.5	83.3		
Archaeoglobus fulgidus	61.0	61.4	59.3	56.4	57.8	54.6	57.0	58.7	59.6	56.5	61.8	61.2	59.1	59.4	60.9	61.6	71.0	73.4	

[a] Microorganisms with inferred xenologous *apsA* are in boldface.

ApsA- and 16S rRNA-based trees both indicated that *Archaeoglobus*, gram-positive SRB, and δ-proteobacterial SRB form monophyletic lineages. SRB belonging to the order "*Desulfovibrionales*" and to the families "*Desulfobacteraceae*" and "*Desulfobulbaceae*" were recovered as sublineages within the δ-proteobacteria. However, there were major differences in the relative branching order between the 16S rRNA- and ApsA-based trees for the thermophilic *Thermodesulfovibrio islandicus* and *Thermodesulfobacterium* spp., δ-*Proteobacteria* belonging to the "*Syntrophobacteraceae*," the "*Nitrospinaceae*" (*Desulfobacterium anilini*, *Desulfoarculus baarsii, Desulfomonile tiedjei*), and the desulfobacteraceal *Desulfocella halophila*. All "*Syntrophobacteraceae*" and "*Nitrospinaceae*" analyzed (Table 2) grouped well within the phylogenetic radiation of low-G+C gram-positive genus *Desulfotomaculum* (Fig. 1). *Desulfotomaculum halophilum*, for which only a short *apsA* amplification fragment was recovered (GenBank accession no. AF418167), was found to group with the low-G+C gram-positive SRB (not shown in Fig. 1).

Thermodesulfovibrio islandicus and *Thermodesulfobacterium* spp. each formed monophyletic lineages and branched off between the gram-positive SRB and the δ-proteobacterial SRB with high support values (bootstrapping: 100 [neighbor-joining], 99 [parsimony], and 93% [QP]). PUZZLE analysis indicated a weak association of *Thermodesulfovibrio islandicus* and *Thermodesulfobacterium* spp., but only with a low support value (QP support of <70%). In contrast, 16S rRNA gene analysis clearly showed that the two species represented lines of descent close to the root of the *Bacteria*.

The most parsimonious explanation for these significant topological differences between the ApsA- and 16S rRNA-based trees is the occurrence of multiple lateral transfers of *apsA* genes between SRP of the "*Syntrophobacteraceae*," the "*Nitrospinaceae*," and the thermophilic *Thermodesulfovibrio islandicus* and *Thermodesulfobacterium* spp. Another topological difference between the two trees was observed: the ApsA-based tree indicated that *Desulfocella halophila* is closely related to *Desulfofaba gelida* and *Desulfofrigus oceanense*, although only with low bootstrap support, whereas the 16S rRNA-based trees indicated a closer relationship of *Desulfocella halophila* to the *Desulfobacter* branch of the "*Desulfobacteraceae*," but again only with low bootstrap support.

We also compared ApsA- and DsrAB-based phylogenies from a subset of 42 sulfate-reducing microorganisms. This comparison was of particular interest because of the recently described occurrence of lateral *dsrAB* gene transfer events (19). Fragments of the *dsrAB* genes were amplified from reference strains ($n = 25$) using primer pair DSR1-F and DSR4-R (41) and sequenced directly, or sequences available in public databases were used for comparison (as indicated in Table 2). The overall tree topologies of ApsA- and DsrAB-derived analyses were similar (Fig. 2). Some of the differences in topologies were due to different occurrences of lateral transfer events affecting mostly a subset of gram-positive SRB and *Archaeoglobus* spp. for the *dsrAB* genes and members of the "*Syntrophobacteraceae*" and "*Nitrospinaceae*" for the *apsA* genes. The only SRB affected by a dual lateral transfer of both genes were *Thermodesulfobacterium* spp. Other topological differences included the relative branching order of the "*Desulfobulbaceae*" clade, which was closer to the "*Desulfobacteraceae*" in the ApsA-based trees (>80% bootstrap support); in DsrAB-based trees, the "*Desulfovibrionaceae*" clade was closer to the "*Desulfobacteraceae*." Although the "*Desulfovibrionales*" formed a consistent clade in 16S rRNA- and ApsA-based trees,

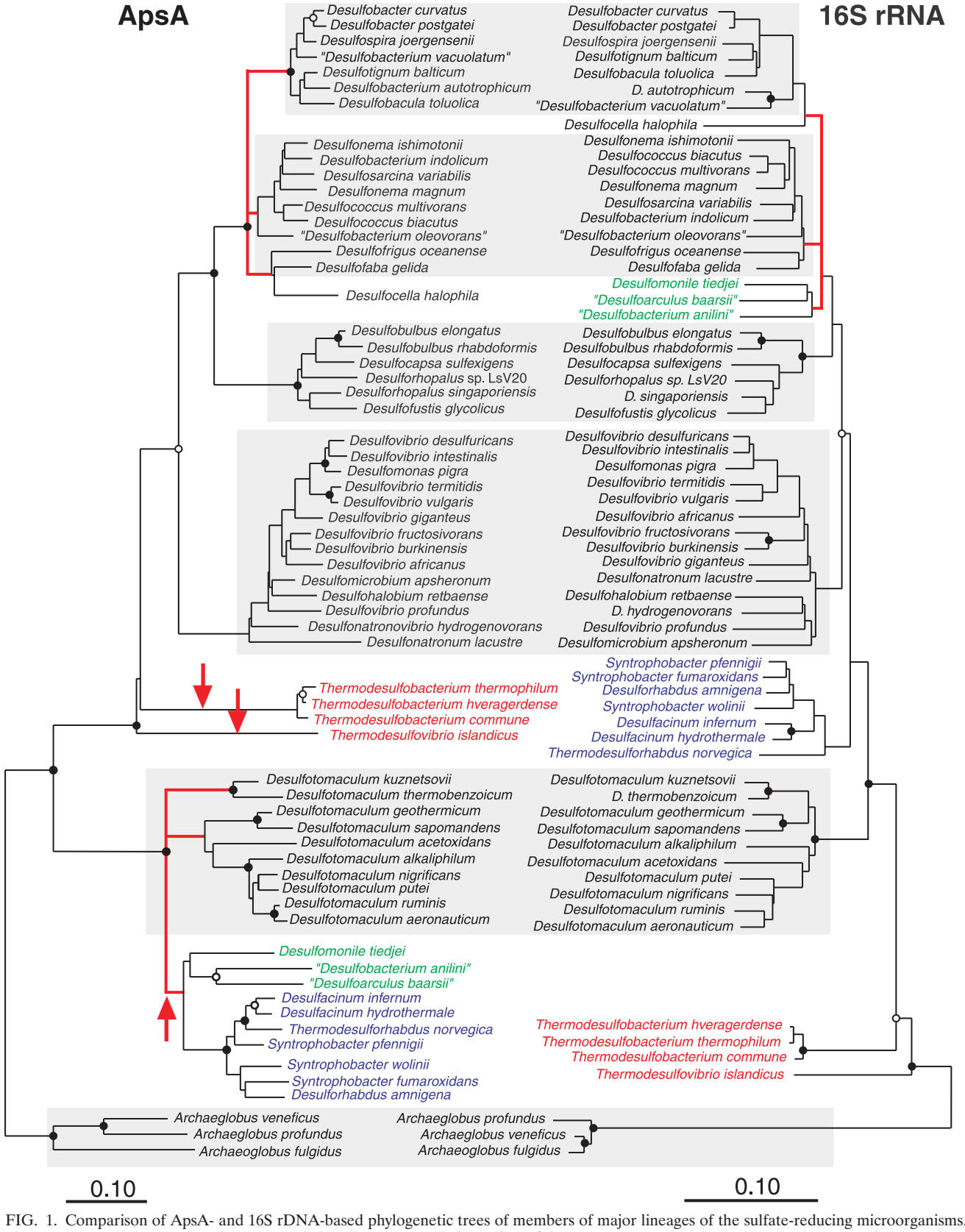

FIG. 1. Comparison of ApsA- and 16S rDNA-based phylogenetic trees of members of major lineages of the sulfate-reducing microorganisms. Both trees were calculated using distance matrix-based FITCH analysis. *Archaeoglobus* spp. were used as the outgroup reference. Microorganisms with inferred laterally transferred *apsA* genes are color coded: red, *Thermodesulfobacterium* spp. and *Thermodesulfovibrio islandicus*; blue, "*Syntrophobacteraceae*", green, "*Nitrospinaceae*." Red lines, multifurcations; red arrow, lineage with inferred lateral gene transfer. Bootstrap support values were obtained from neighbor-joining (100 resamplings) and parsimony analyses (500 resamplings). Solid circles, nodes with a bootstrap support and QP support of >80% for all analyses; open circles, nodes with support of >60 and <80%. Groups monophyletic in the two trees are shaded in gray. Bars, 0.1 changes per nucleotide or amino acid position.

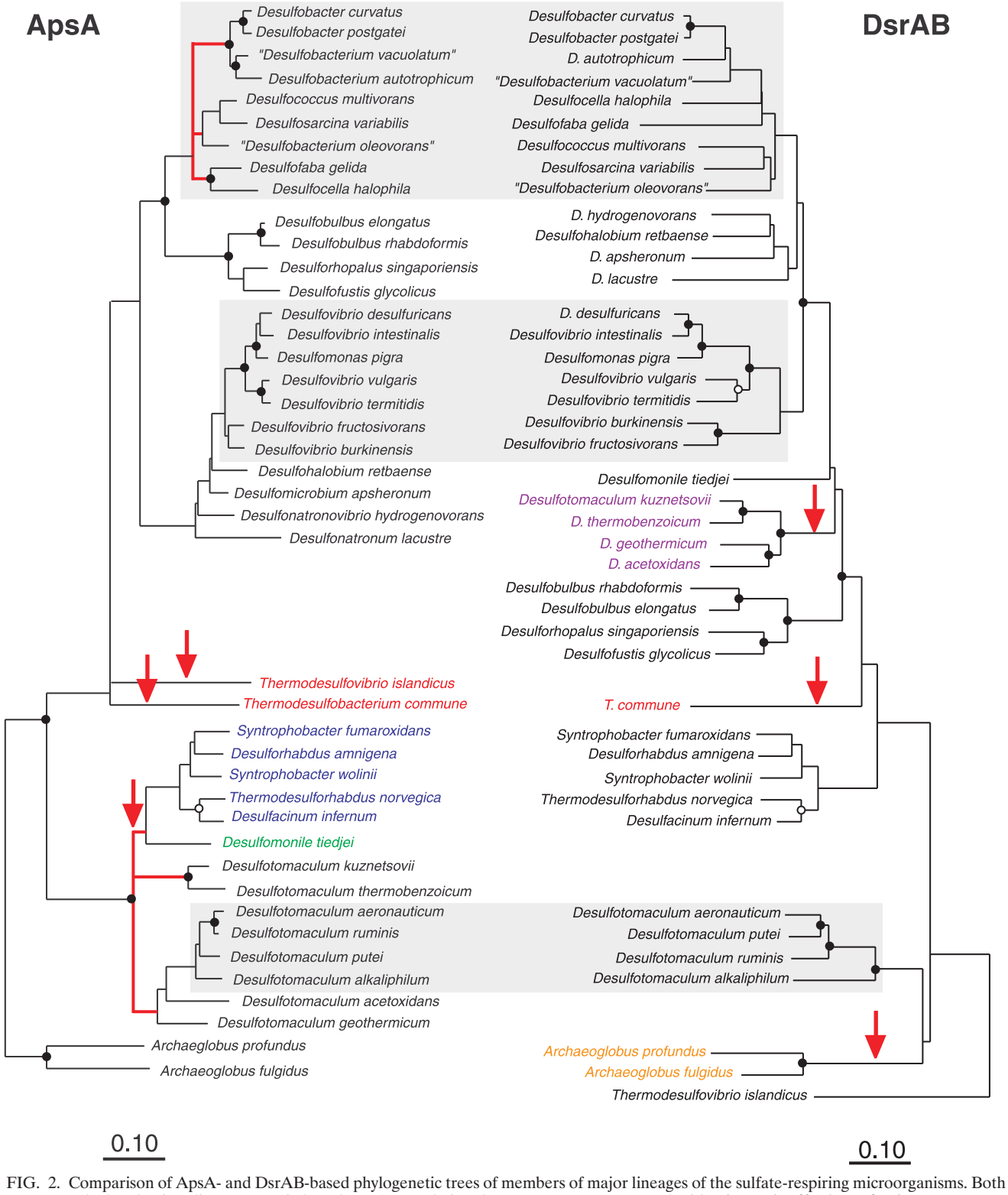

FIG. 2. Comparison of ApsA- and DsrAB-based phylogenetic trees of members of major lineages of the sulfate-respiring microorganisms. Both trees were calculated using distance matrix-based FITCH analysis. The DsrAB tree was rooted with *Thermodesulfovibrio islandicus*. Bootstrap analyses, node labeling, and color coding are as described in the legend of Fig. 1. Microorganisms with inferred laterally transferred *dsrAB* genes are color coded: purple, *Desulfotomaculum* spp.; orange, *Archaeoglobus* spp.; red, *Thermodesulfobacterium commune*. Bars, 0.1 changes per amino acid position.

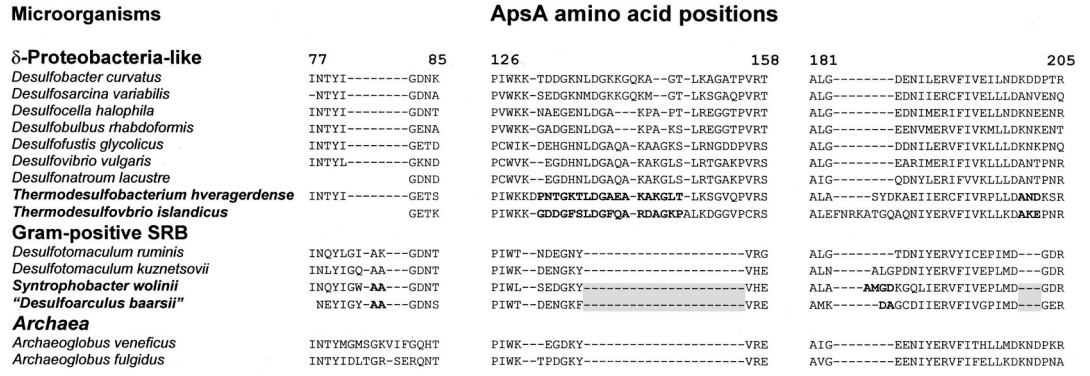

FIG. 3. Sequence alignments showing indels within ApsA among representatives of the major lineages of sulfate-respiring microorganisms. Note that δ-proteobacterial members of the *Syntrophobacteraceae* (i.e., *Syntrophobacter* spp.) and *Nitrospinaceae* (i.e., *Desulfoarculus baarsii*) carry insertions (boldface) and deletions (shaded in gray) characteristic of gram-positive *Desulfotomaculum* spp., whereas *Thermodesulfobacterium* spp. and *Thermodesulfovibrio islandicus* carry insertions (boldface) characteristic of δ-proteobacteria. Sulfate-reducing microorganisms with an inferred xenologous ApsA are in boldface. Amino acid positions are numbered according to ApsA of *Desulfovibrio vulgaris*.

they were recovered only in two separate branches: the "*Desulfomicrobiaceae*" and "*Desulfohalobiaceae*" grouped within the radiation of the "*Desulfobacteraceae*," whereas the "*Desulfovibrionaceae*" did not (>80% bootstrap support).

Indels present in ApsA. Additional evidence for lateral transfer of *apsA* genes among SRB arises from six regions of the ApsA sequence with minor and major indels (excluded from phylogenetic analyses) present in different lineages (three regions shown in Fig. 3). Archaea, gram-positive SRB, and those gram-negative SRB with a presumed xenologous *apsA* gene (i.e., "*Syntrophobacteraceae*" and "*Nitrospinaceae*") all have a major deletions between positions 137 and 156 of ApsA (numbering according to *Desulfovibrio vulgaris*; Fig. 3). On the other hand, certain insertions were not present in δ-proteobacterial SRB. For example, between positions 183 and 184 of the *Desulfovibrio vulgaris* ApsA are found two (*Desulfomonile tiedjei*, *Desulfobacterium anilini* [not shown]), three (*Thermodesulfobacterium* spp., *Desulfotomaculum kuznetsovii*, *Desulfotomaculum thermobenzoicum* [not shown]), four ("*Syntrophobacteraceae*"), or eight (*Thermodesulfovibrio islandicus*) additional amino acids.

DISCUSSION

This is the first comprehensive study to analyze the phylogeny of the APS reductase α-subunit (ApsA) from a taxonomically wide range of sulfate-reducing prokaryotes. Comparative analysis of ApsA- and 16S rRNA-based phylogenies revealed significant differences in tree topologies, which suggests that multiple lateral *apsA* gene transfers occurred among SRP.

Using the newly developed PCR primer set comprising APS7-F and APS8-R, *apsA* fragments with high sequence similarity to previously published *apsA*/*aprA* sequences were obtained from almost all SRP tested (Table 2). The *apsA* genes of certain strains were amplified only when a considerably lower annealing temperature was used, which indicates possible mismatches at the primer binding sites. Still, the new primer pair allowed us to obtain an amplified *apsA* DNA fragment considerably longer than those obtained by using the previously used primer pair APS-FW and APS-RV (7).

All ApsA sequences determined (including positions 73 to 386 of the *Desulfovibrio vulgaris* numbering) contained most of the core regions of the α-subunit of the APS reductase. Based on sequence comparisons with other flavin adenine dinucleotide-containing oxidoreductases, it has been suggested that this region contains the active site of APS reductase (14, 38). In general, the potential binding site of the substrate APS (residues 248 to 272) was highly conserved in all ApsA sequences analyzed. However, notably the gram-positive SRB and members of the "*Syntrophobacteraceae*" and of the "*Nitrospinaceae*" contained an additional aliphatic amino acid residue (between *Desulfovibrio vulgaris* positions 260 and 261) and a phenylalanine (between *Desulfovibrio vulgaris* positions 261 and 262) and had a deletion at position 265 (data not shown), which may affect APS binding. A more detailed comparison of structural features of APS reductase with the extensive AprA sequence data available will soon be possible since the three-dimensional structure of the APS reductase from *Archaeoglobus fulgidus* is being determined (36).

The universal phylogenetic tree based on rRNA, and the small-subunit (SSU) rRNA in particular, is still generally accepted to reflect the phylogeny of all organisms (8, 48). The SSU rRNA molecule is ubiquitous, exhibits functional constancy, and changes slowly in sequence, which makes it suitable as a phylogenetic marker. Most importantly, however, is the apparent lack of extensive LGTs affecting the rRNA genes (48). Only a few instances of a genus level transfer of 16S rRNA genes have been reported so far (43) but none across bacterial divisions. Based on the assumption that the 16S rRNA genes of SRP reflect their true evolutionary history, we compared both 16S rRNA- and ApsA-based trees to identify microorganisms which have been involved in LGT of *apsA* genes.

Major topological differences between the ApsA- and 16S rRNA-based trees suggest that seven species of the "*Syntrophobacteraceae*," three members of the "*Nitrospinaceae*," three *Thermodesulfobacterium* spp., and *Thermodesulfovibrio islandicus* carry xenologous *apsA* genes. The most parsimonious explanation for the discrepancies in tree topologies is the lateral transfer of *apsA* genes, which is supported by the following findings. (i) All treeing methods utilized for phylogenetic re-

construction agree on the tree topology, with strong statistical support for the decisive nodes (e.g., branching of gram-positive SRB relative to δ-proteobacterial gram-negative SRB; Fig. 1). The δ-proteobacterial members of the "*Syntrophobacteraceae*" and "*Nitrospinaceae*" were clearly recovered monophyletically with the gram-positive SRB. The thermophilic *Thermodesulfovibrio islandicus* and the *Thermodesulfobacterium* spp. branched off between the δ-proteobacterial SRB and the gram-positive SRB and not, as would be expected from 16S rRNA analysis, close to the root of the tree. (ii) The ApsA sequences are highly conserved (Table 2), which excludes treeing artifacts stemming from alignment errors. (iii) The patterns of indels of gram-positive SRB, the "*Syntrophobacteraceae*," and the "*Nitrospinaceae*" were similar (Fig. 3).

An alternative interpretation of the discrepancy in tree topologies is a series of gene duplications and losses. However, it is unlikely that the convergent evolution of paralogous ApsA sequences in members of the δ-proteobacterial gram-negative "*Syntrophobacteraceae*" and "*Nitrospinaceae*" resulted in sequences closely related to the ApsA sequences of the gram-positive SRB rather than to those of δ-proteobacterial ApsA. Furthermore, and a "*Syntrophobacteraceae*" and "*Nitrospinaceae*" are distantly related at the 16S rRNA level (Fig. 1). A gene duplication and a loss event cannot be ruled out completely, however, for the *apsA* genes of the thermophilic *Thermodesulfovibrio islandicus* and *Thermodesulfobacterium* spp. since an orthologous *apsA* gene of the donor lineage of a putative LGT is not present. Finally, a putative gene duplication event would require in all cases either that the orthologous *apsA* gene was lost or that the orthologous *apsA* gene copy was overlooked. The PCR products were directly sequenced, and no evidence for the presence of two or more gene copies (i.e., ambiguous sequence data) was obtained. In addition, PCR fragments obtained by using two independent PCR assays targeting different regions of the *apsA* gene (i.e., using primer pairs APS-FW and APS-RV and APS7-F and APS8-R) were sequenced, and phylogenetic placement of ApsA sequences was the same regardless of whether the trees were based on the shorter or longer PCR fragment (i.e., using primer pairs APS-FW and APS-RV or APS7-F and APS8-R, respectively) (data not shown). Thus the presence of different *apsA* sequences in the reference strains studied was not indicated. This issue can only be resolved by extensive Southern hybridization experiments, which were beyond the scope of this study.

A donor lineage for the LGT event inferred for the thermophilic *Thermodesulfovibrio islandicus* and *Thermodesulfobacterium* spp. is not apparent; in contrast the gram-positive SRB clearly represent the donor lineage for the LGT affecting members of the "*Syntrophobacteraceae*" and of the "*Nitrospinaceae*." Since the ApsA-based tree topology indicates only a weak association of the two families (Fig. 1 and 2), the possibility that both families received their xenologous *apsA* genes in two independent LGT events cannot be ruled out. A direct *apsA* gene donor, however, cannot be currently inferred since all analyzed gram-positive SRB form two separate clades (Fig. 1), which also indicates that the observed LGT was not a recent event. This is also supported by the absence of a conclusive difference in the G+C contents of xenologous *apsA* genes and orthologous *dsrAB* genes from within the same SRB (Fig. 2) (19) (G+C data not shown). Differences in G+C contents of host genomes and acquired genes have been used to detect recent LGT events (25). Thus, a recent LGT could have been detected by similar G+C contents and codon biases of the xenologous *apsA* gene of the recipient and the orthologous *apsA* of the putative donor SRB. However, codon biases and G+C contents of xenologous genes will be ameliorated to reflect the DNA composition the host genome over time since the same mutational processes affect all genes in the recipient genome.

Another lateral transfer of *apsA* genes probably affected the *Archaeoglobales* (Fig. 1); however, only limited support for this assumption is provided by our analyses. Such a LGT has been considered earlier, albeit one based on the phylogenetic analysis of a limited ApsA data set, including sequences of the sulfur-oxidizing phototroph *Allochromatium vinosum,* as well as *Desulfovibrio vulgaris* and *Archaeoglobus fulgidus* (14). Based on our analysis, the high degree of conservation of the ApsA sequences of *Archaeoglobus* spp. compared to those of the gram-positive *Desulfotomaculum* spp. and the relatively short length of the *Archaeoglobales* branch compared to the length in the 16S rRNA-based tree (Fig. 1) suggest that *Archaeoglobus* spp. possibly carry a xenologous *apsA* gene. Conversely, *Archaeoglobus* spp. appear to branch off deeply in the ApsA-based tree of the SRP analyzed, which justified the rooting of the tree with ApsA sequences of *Archaeoglobus* spp. Further support for xenologous *apsA* genes in *Archaeoglobus* spp. arises from phylogenetic analysis of DsrAB (Fig. 2) since there is increasing evidence that *Archaeoglobus* spp. carry xenologous *dsrAB* genes (19, 23, 29). Paralogous rooting of DsrA and DsrB indicated that *Thermodesulfovibrio* spp., and not *Archaeoglobus* spp. as expected from 16S rRNA-based trees (Fig. 1), are closest to the root (19). Thus, taking into consideration that *Archaeoglobus* spp. are currently the only recognized archaeal sulfate reducers, it appears likely that not only the *dsrAB* genes but also the *apsA* genes of *Archaeoglobus* spp. were laterally transferred. Interestingly, *Archaeoglobus veneficus* reduces only sulfite and thiosulfate, but not sulfate (16), even though this strain carries an *apsA* gene.

The high frequency of lateral transfers involving genes essential for sulfate reduction, such as the *dsrAB* genes (19) and the *apsA* genes, indicates that lateral transfer has been a frequent event affecting the evolutionary path of sulfate-respiring prokaryotes. As a possible explanation for the widespread lateral distribution of the *dsr* genes, it has been suggested that the *dsr* genes could be part of mobilizable metabolic islands (19). Several lines of evidence suggest that at least the APS reductase and the sulfite reductase genes do not form a genomic island in the genomes of SRP. (i) All lateral *apsA* transfers detected were not paralleled by a lateral transfer of the *dsrAB* genes, with the exception of those for *Thermodesulfobacterium* spp. and possibly the *Archaeoglobus* spp. (Fig. 2) (19). (ii) The only completed genome sequence of a sulfate-reducing microorganism, i.e., *Archaeoglobus fulgidus* (the sequencing of several others, i.e., those of *Desulfovibrio vulgaris*, *Desulfovibrio desulfuricans, Desulfotalea psychrophila*, and *Desulfobacterium autotrophicum*, is under way [http://wit.integratedgenomics.com/GOLD/prokaryagenomes.html]) reveals that *dsrAB* and *aprBA* genes are not present in one operon or physically close (nucleotide positions 381478 to 383834 and 1498455 to 1500855 of the *Archaeoglobus fulgidus* genome, respectively

[20]). However, the ATP sulfurylase gene, *sat*, is adjacent to *aprBA* within the same operon (20). Although this linkage of genes remains to be shown for other SRP, it makes sense from an ecophysiological point of view. Whereas *apsBA* and *sat* are required for sulfate respiration, sulfite respiration is an independent metabolic trait, and indeed several microorganisms cannot reduce sulfate but reduce sulfite instead, e.g., *Pyrobaculum islandicum* (29), *Desulfitobacterium* spp. (15), *Archaeoglobus veneficus* (16), and *Bilophila wadsworthia* (24).

Although sulfate and sulfite respiration appears to be vital for growth of these microorganisms, this is not the case, and several types of sulfate reducers are capable of fermentative growth in the absence of sulfate or other inorganic electron acceptors (46). This versatility of energy conservation could have facilitated the integration of newly acquired xenologous *aps* or *dsr* genes into the genetic framework of the recipients without the recipients becoming dependent on these genes before the genes became fully functional in the cell with respect to codon usage or regulation. In fact, *Syntrophobacter* spp., which carry xenologous *apsA* genes, grow fermentatively as syntrophic propionate oxidizers, and had been isolated as such, and their ability to reduce sulfate was detected only later (42).

Further patterns of LGTs in SRP exist; these may also be linked to their ecophysiology. Most recipients of xenologous *dsrAB* and *apsA* genes are thermophilic (Table 2; Fig. 1 and 2), which suggests a thermophilic lifestyle of sulfate-reducing prokaryotes involved in LGTs (19). Moreover, some of the spore-forming gram-positive SRB are *dsrAB* recipients (Fig. 2) (19), and gram-positive SRB represent the donor lineage for xenologous *apsA* genes in members of the "*Syntrophobacteraceae*" and "*Nitrospinaceae*" (Fig. 1). Spore-forming SRB have a selective advantage in environments with fluctuating water availability and oxygen stress (47). It may be a further advantage to acquire new genes under these conditions, which could explain the frequent involvement of spore-forming gram-positive SRB in LGT.

Functional markers for physiologically coherent groups of microorganisms (guilds) such as the *dsrAB* and *apsA* genes have been used for the characterization of sulfate-reducing populations in a variety of habitats (4, 7, 28, 34, 49); however, a thorough phylogenetic framework has not yet been available. Besides the LGT events detected, we could show that the overall phylogeny of the ApsA-based tree is rather similar to the rRNA-based tree for most of the larger taxa of recognized SRB, including "*Desulfovibrionales*," "*Desulfobacteraceae*," and "*Desulfobulbaceae*." In addition, we could add a substantial set of *dsrAB* reference sequences, including those of further members of the "*Desulfovibrionales*," "*Desulfobacteraceae*," "*Desulfobulbaceae*," and the "*Syntrophobacteraceae*." The phylogenetic analysis of DsrAB is in agreement with that established previously. Interestingly, some of the "*Desulfovibrionales*" (including *Desulfomicrobium* spp. *D. lacustre*, *D. hydrogenovorans*, and *D. retbaense* but not *D. pigra*) formed a cluster separate from members of the "*Desulfovibrionaceae*."

With the phylogenetic framework of the two gene markers provided here and previously (19) it will now be possible to link environmental sequences at least to most of the recognized lineages of SRP. Since we could demonstrate that LGT is a frequent event in the evolution of SRP, phylogenetic inferences in environmental diversity studies should be interpreted cautiously.

ACKNOWLEDGMENTS

This study was supported by the Max Planck Society, Munich.

I thank Ralf Conrad for continuing support and Thomas Gebhardt for Linux server maintenance, as well as Kai Finster, Alexander Galushko, Bernhard Schink, and Hans Scholten for providing sulfate-reducing strains, Werner Liesack for critically reading the manuscript, and Karen A. Brune for editing the manuscript. A special thank you goes to Bianca Wagner for her excellent technical assistance.

REFERENCES

1. **Aravind, L., R. L. Tatusov, Y. I. Wolf, D. R. Walker, and E. V. Koonin.** 1998. Evidence for massive gene exchange between archaeal and bacterial hyperthermophiles. Trends Genet. **14:**442–444. (Erratum, **15:**41, 1999.)
2. **Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, B. A. Rapp, and D. L. Wheeler.** 2000. GenBank. Nucleic Acids Res. **28:**15–18.
3. **Cameron, E. M.** 1982. Sulphate and sulphate reduction in early Precambrian oceans. Nature **296:**145–148.
4. **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.
5. **Dahl, C.** 1996. Insertional gene inactivation in a phototrophic sulphur bacterium: APS-reductase-deficient mutants of *Chromatium vinosum*. Microbiology **142:**3363–3372.
6. **Dayhoff, M. O., R. M. Schwartz, and B. C. Orcutt.** 1978. A model of evolutionary change in proteins, p. 345–352. *In* M. O. Dayhoff (ed.), Atlas of protein sequence structure. National Biomedical Research Foundation, Washington, DC.
7. **Deplancke, B., K. R. Hristova, H. A. Oakley, V. J. McCracken, R. Aminov, R. I. Mackie, and H. R. Gaskins.** 2000. Molecular ecological analysis of the succession and diversity of sulfate-reducing bacteria in the mouse gastrointestinal tract. Appl. Environ. Microbiol. **66:**2166–2174.
8. **Doolittle, W. F.** 1999. Phylogenetic classification and the universal tree. Science **284:**2124–2128.
9. **Fauque, G., J. Legall, and L. L. Barton.** 1991. Sulfate-reducing and sulfur-reducing bacteria, p. 271–337. *In* J. M. Shively and L. L. Barton (ed.), Variations in autotrophic life. Academic Press, London, United Kingdom.
10. **Ferris, M. J., and D. M. Ward.** 1997. Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **63:**1375–1381.
11. **Friedrich, C. G.** 1998. Physiology and genetics of sulfur-oxidizing bacteria. Adv. Microb. Physiol. **39:**235–289.
12. **Fritz, G., T. Buchert, H. Huber, K. O. Stetter, and P. M. H. Kroneck.** 2000. Adenylylsulfate reductases from archaea and bacteria are 1:1 alpha beta-heterodimeric iron-sulfur flavoenzymes—high similarity of molecular properties emphasizes their central role in sulfur metabolism. FEBS Lett. **473:**63–66.
13. **Henckel, T., M. Friedrich, and R. Conrad.** 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl. Environ. Microbiol. **65:**1980–1990.
14. **Hipp, W. M., A. S. Pott, S. Thum, I. Faath, C. Dahl, and H. G. Trüper.** 1997. Towards the phylogeny of APS reductases and sirohaem sulfite reductases in sulfate-reducing and sulfur-oxidizing prokaryotes. Microbiology **143:**2891–2902.
15. **Holliger, C., G. Wohlfarth, and G. Diekert.** 1998. Reductive dechlorination in the energy metabolism of anaerobic bacteria. FEMS Microbiol. Rev. **22:**383–398.
16. **Huber, H., H. Jannasch, R. Rachel, T. Fuchs, and K. O. Stetter.** 1997. *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers. Syst. Appl. Microbiol. **20:**374–380.
17. **Jones, D. T., W. R. Taylor, and J. M. Thornton.** 1992. The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. **8:**275–282.
18. **Joulian, C., B. Ollivier, B. C. Patel, and P. A. Roger.** 1998. Phenotypic and phylogenetic characterization of dominant culturable methanogens isolated from ricefield soils. FEMS Microbiol. Ecol. **25:**135–145.
19. **Klein, M., M. Friedrich, A. J. Roger, P. Hugenholtz, S. Fishbain, H. Abicht, L. L. Blackall, and D. A. Stahl.** 2001. Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. J. Bacteriol. **183:**6028–6035.
20. **Klenk, H. P., R. A. Clayton, J. F. Tomb, O. White, K. E. Nelson, K. A. Ketchum, R. J. Dodson, M. Gwinn, E. K. Hickey, J. D. Peterson, D. L. Richardson, A. R. Kerlavage, D. E. Graham, N. C. Kyrpides, R. D. Fleisch-**

mann, J. Quackenbush, N. H. Lee, G. G. Sutton, S. Gill, E. F. Kirkness, B. A. Dougherty, K. McKenny, M. D. Adams, B. Loftus, S. Peterson, C. Reich, L. K. McNeil, J. H. Badger, A. Glodek, L. Zhou, R. Overbeek, J. D. Gocayne, J. F. Weidman, L. Mcdonald, T. Utterback, M. D. Cotton, T. Spriggs, P. Artiach, B. Kaine, S. M. Sykes, P. W. Sadow, K. P. D'Andrea, C. Bowman, C. Fujii, S. Garland, T. M. Mason, G. J. Olsen, C. M. Fraser, H. O. Smith, C. R. Woese, and J. C. Venter. 1997. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature **390:**364–370.

21. **Lampreia, J., G. Fauque, N. Speich, C. Dahl, I. Moura, H. G. Trüper, and J. J. G. Moura.** 1991. Spectroscopic studies on APS reductase isolated from the hyperthermophilic sulfate-reducing archaebacterium *Archaeglobus fulgidus*. Biochem. Biophys. Res. Commun. **181:**342–347.
22. **Lane, D. J.** 1991. 16S/23S rRNA sequencing, p. 115–147. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acids techniques in bacterial systematics. John Wiley & Sons, Chichester, United Kingdom.
23. **Larsen, Ø., T. Lien, and N.-K. Birkeland.** 1999. Dissimilatory sulfite reductase from *Archaeoglobus profundus* and *Desulfotomaculum thermocisternum*: phylogenetic and structural implications from gene sequences. Extremophiles **3:**63–70.
24. **Laue, H., M. Friedrich, J. Ruff, and A. M. Cook.** 2001. Dissimilatory sulfite reductase (desulfoviridin) of the taurine-degrading, non-sulfate-reducing bacterium *Bilophila wadsworthia* RZATAU contains a fused DsrB-DsrD subunit. J. Bacteriol. **183:**1727–1733.
25. **Lawrence, J. G., and H. Ochman.** 1997. Amelioration of bacterial genomes: rates of change and exchange. J. Mol. Evol. **44:**383–397.
26. **Lawrence, J. G., and H. Ochman.** 1998. Molecular archaeology of the *Escherichia coli* genome. Proc. Natl. Acad. Sci. USA **95:**9413–9417.
27. **Ludwig, W., O. Strunk, S. Klugbauer, N. Klugbauer, M. Weizenegger, J. Neumaier, M. Bachleitner, and K. H. Schleifer.** 1998. Bacterial phylogeny based on comparative sequence analysis. Electrophoresis **19:**554–568.
28. **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.
29. **Molitor, M., C. Dahl, I. Molitor, U. Schaefer, N. Speich, R. Huber, R. Deutzmann, and H. G. Trüper.** 1998. A dissimilatory sirohaem-sulfite-reductase-type protein from the hyperthermophilic archaeon *Pyrobaculum islandicum*. Microbiology **144:**529–541.
30. **Moré, M. I., J. B. Herrick, M. C. Silva, W. C. Ghiorse, and E. L. Madsen.** 1994. Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. Appl. Environ. Microbiol. **60:**1572–1580.
31. **Müller, T., and M. Vingron.** 2000. Modeling amino acid replacement. J. Comp. Biol. **7:**761–776.
32. **Ochman, H., J. G. Lawrence, and E. A. Groisman.** 2000. Lateral gene transfer and the nature of bacterial innovation. Nature **405:**299–304.
33. **Olsen, G. J., H. Matsuda, R. Hagstrom, and R. Overbeek.** 1994. FastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput. Appl. Biosci. **10:**41–48.
34. **Power, M. E., J. C. Araujo, J. R. Van Der Meer, H. Harms, and O. Wanner.** 1999. Monitoring sulfate-reducing bacteria in heterotrophic biofilms. Water Sci. Technol. **39:**49–56.
35. **Rabus, R., T. A. Hansen, and F. Widdel.** 1999. Dissimilatory sulfate- and sulfur-reducing prokaryotes, p. 1–87. *In* M. Dworkin, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes: an evolving electronic database for the microbiological community. Springer, New York, N.Y.
36. **Roth, A., G. Fritz, T. Buchert, H. Huber, K. O. Stetter, U. Ermler, and P. M. Kroneck.** 2000. Crystallization and preliminary X-ray analysis of adenylylsulfate reductase from Archaeoglobus fulgidus. Acta Crystallogr. D **56:**1673–1675.
37. **Schidlowski, M.** 1979. Antiquity and evolutionary status of bacterial sulfate reduction: sulfur isotope evidence. Origins Life **9:**299–311.
38. **Speich, N., C. Dahl, P. Heisig, A. Klein, F. Lottspeich, K. O. Stetter, and H. G. Trüper.** 1994. Adenylylsulphate reductase from the sulphate-reducing archaeon *Archaeoglobus fulgidus*: cloning and characterization of the genes and comparison of the enzyme with other iron-sulphur flavoproteins. Microbiology **140:**1273–1284.
39. **Strimmer, K., and A. von Haeseler.** 1996. Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. Mol. Biol. Evol. **13:**964–969.
40. **Tamura, K., and M. Nei.** 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. **10:**512–526.
41. **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.
42. **Wallrabenstein, C., E. Hauschild, and B. Schink.** 1994. Pure culture and cytological properties of '*Syntrophobacter wolinii*.' FEMS Microbiol. Lett. **123:**249–254.
43. **Wang, Y., and Z. Zhang.** 2000. Comparative sequence analyses reveal frequent occurrence of short segments containing an abnormally high number of non-random base variations in bacterial rRNA genes. Microbiology **146:** 2845–2854.
44. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
45. **Whelan, S., and N. Goldman.** 2001. A new empirical model of amino acid evolution. Mol. Biol. Evol. **18:**691–699.
46. **Widdel, F.** 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria, p. 469–585. *In* A. J. B. Zehnder (ed.), Biology of anaerobic microorganisms. Wiley, New York, N.Y.
47. **Widdel, F.** 1999. The genus *Desulfotomaculum*, p. 1–6. *In* M. Dworkin, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes: an evolving electronic database for the microbiological community. Springer, New York, N.Y.
48. **Woese, C. R.** 2000. Interpreting the universal phylogenetic tree. Proc. Natl. Acad. Sci. USA **97:**8392–8396.
49. **Zinkevich, V., and I. B. Beech.** 2000. Screening of sulfate-reducing bacteria in colonoscopy samples from healthy and colitic human gut mucosa. FEMS Microbiol. Ecol. **34:**147–155.