Multiple Lateral Transfers of Dissimilatory Sulfite Reductase Genes between Major Lineages of Sulfate-Reducing Prokaryotes

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A large fragment of the dissimilatory sulfite reductase genes (*dsrAB*) was PCR amplified and fully sequenced from 30 reference strains representing all recognized lineages of sulfate-reducing bacteria. In addition, the sequence of the *dsrAB* gene homologs of the sulfite reducer *Desulfitobacterium dehalogenans* was determined. In contrast to previous reports, comparative analysis of all available DsrAB sequences produced a tree topology partially inconsistent with the corresponding 16S rRNA phylogeny. For example, the DsrAB sequences of several *Desulfotomaculum* species (low G+C gram-positive division) and two members of the genus *Thermodesulfobacterium* (a separate bacterial division) were monophyletic with δ-proteobacterial DsrAB sequences. The most parsimonious interpretation of these data is that *dsrAB* genes from ancestors of as-yet-unrecognized sulfate reducers within the δ-*Proteobacteria* were laterally transferred across divisions. A number of insertions and deletions in the DsrAB alignment independently support these inferred lateral acquisitions of *dsrAB* genes. Evidence for a *dsrAB* lateral gene transfer event also was found within the δ-*Proteobacteria*, affecting *Desulfobacula toluolica*. The root of the *dsr* tree was inferred to be within the *Thermodesulfovibrio* lineage by paralogous rooting of the alpha and beta subunits. This rooting suggests that the *dsrAB* genes in *Archaeoglobus* species also are the result of an ancient lateral transfer from a bacterial donor. Although these findings complicate the use of *dsrAB* genes to infer phylogenetic relationships among sulfate reducers in molecular diversity studies, they establish a framework to resolve the origins and diversification of this ancient respiratory lifestyle among organisms mediating a key step in the biogeochemical cycling of sulfur.

Siroheme dissimilatory sulfite reductases (EC 1.8.99.3) catalyze the reduction of sulfite to sulfide, an essential step in the anaerobic sulfate-respiration pathway. Consequently, this enzyme has been found in all dissimilatory sulfate-reducing prokaryotes (SRPs) investigated so far. Furthermore, siroheme dissimilatory sulfite reductase-like enzymes have been detected in the hyperthermophilic archaeon *Pyrobaculum islandicum* capable of using sulfite as terminal electron acceptor (23), the phototrophic bacterium *Allochromatium vinosum* (10, 12), and the obligate chemolithotrophic species *Thiobacillus denitrificans* (32). In the latter two organisms the dissimilatory sulfite reductase has a proposed function in sulfide oxidation.

Siroheme sulfite reductases consist of at least two different polypeptides in an $\alpha_2\beta_2$ structure. The genes encoding the two subunits are found adjacent to each other in the respective genomes (see, for example, references 3, 15, 17, 18, and 35) and probably arose from duplication of an ancestral gene (3). Comparative amino acid sequence analysis of the dissimilatory sulfite reductase genes (*dsrAB*) has recently been used to in-

vestigate the evolutionary history of anaerobic sulfate (sulfite) respiration (10, 17, 18, 35). The presence of *dsrAB* homologs in at least five highly divergent prokaryotic lineages and overall phylogenetic congruence of the *dsrAB* tree with the 16S rRNA gene tree suggested that the dissimilatory sulfite reductases of extant SRPs evolved vertically from common ancestral protogenotic genes (35). The remarkable degree of conservation of the *dsrAB* genes also provided a basis for culture-independent molecular diversity studies of natural sulfate-reducing assemblages with the use of PCR primers broadly specific for a large fragment of all known *dsrAB* genes (1, 22). However, one contradiction between the *dsrAB* and 16S rRNA phylogenies was recently recognized in that the *dsrAB* sequences of *Desulfotomaculum thermocisternum* (17) and *Desulfotomaculum ruminis* are not monophyletic (18). This finding could indicate that, in addition to vertical transmission, lateral gene transfer is involved in the evolution of SRPs.

In the present study we investigated this question further by phylogenetic analysis of the *dsrAB* genes from a wide range of cultivated SRPs. We found a clear case for multiple lateral transfer events of the *dsrAB* genes between major lineages of *Bacteria* and likely between the domains *Bacteria* and *Archaea*, suggesting that genes involved in primary metabolic functions, such as sulfate respiration, may be more prone to lateral transfer than previously thought.

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TABLE 1. Physiological and biochemical properties of the sulfite- and sulfate-reducing prokaryotes investigated in this study

Species[a]	Strain no.	Oxidation[b]	T_{opt} (°C)[c]	G+C content (mol%)[d]					Accession no.	
				Genome'	dsrAB	Genome'/dsrAB	dsrAB3rd'	Genome'/dsrAB3rd'	dsrAB	16S rDNA
Archaea										
Euryarchaeota										
*Archaeoglobus profundus**	DSM5631[T]	C	82	41	47	0.87	50	0.82	AF071499	AF297529
*Archaeoglobus fulgidus**	DSM4304[T]	C	83	46	50	0.92	59	0.78	M95624	X05567, Y00275
Bacteria										
Nitrospira division										
Thermodesulfovibrio yellowstonii	DSM11347[T]	I	65	30	39	0.77	28	1.07	U58122/3	L14619
Thermodesulfovibrio islandicus	DSM12570[T]	I	65	38	39	0.97	29	1.31	AF334599	X96726
Thermodesulfobacterium division										
*Thermodesulfobacterium commune**	DSM2178[T]	I	70	34	41	0.83	33	1.03	AF334596	L10662
*Thermodesulfobacterium mobile**	DSM1276[T]	I	65	31	41	0.76	34	0.91	AF334598	AF334601
Firmicutes: Bacillus/Clostridium group										
Desulfotomaculum ruminis	DSM2154[T]	I	28	49	46	1.07	45	1.09	U58118/9	Y11572
Desulfotomaculum aeronauticum	DSM10349[T]	I	37	44	48	0.92	52	0.85	AF273033	X98407
Desulfotomaculum putei	DSM12395[T]	I	50–65	47	52	0.90	61	0.77	AF273032	AF053929
*Desulfotomaculum geothermicum**	DSM3669[T]	C	54	50	53	0.94	65	0.77	AF273029	X80789
*Desulfotomaculum thermosapovorans**	DSM6562[T]	I	50	51	52	0.98	62	0.82	AF271769	Y11575
*Desulfotomaculum kuznetsovii**	DSM6115[T]	C	60–65	49	56	0.88	74	0.66	AF273031	Y11569
*Desulfotomaculum thermocisternum**	DSM10259[T]	I	62	57	55	1.04	67	0.85	AF074396	U33455
*Desulfotomaculum thermobenzoicum**	DSM6193[T]	C	62	53	56	0.95	74	0.72	AF273030	L15628
*Desulfotomaculum thermoacetoxidans**	DSM5813[T]	C	55–60	50	56	0.89	73	0.68	AF271770	Y11573
*Desulfotomaculum acetoxidans**	DSM771[T]	C	37	38	45	0.84	41	0.93	AF271768	Y11566
Desulfosporosinus orientis	DSM765[T]	I	30	45	42	1.07	30	1.50	AF271767	Y11570
Desulfitobacterium dehalogenans	DSM9161[T]	I	37	45	47	0.96	46	0.98	AF337903	L28946
Desulfitobacterium hafniense	DSM10664[T]	I	37	47	48	0.98	47	1.00	ND	X94975
Proteobacteria delta subdivision										
Desulfobacter vibrioformis	DSM8776[T]	C	33	47	49	0.96	55	0.85	AJ250472	U12254
Desulfobacter latus	DSM3381[T]	C	29–32	44	49	0.90	54	0.81	U58124/5	M34414
*Desulfobacula toluolica**	DSM7467[T]	C	28	42	53	0.79	66	0.64	AF271773	X70953
Desulfofaba gelida	DSM12344[T]	I	7	53	52	1.02	61	0.87	AF334593	AF099063
Desulfobotulus sapovorans	DSM2055[T]	I	34	53	52	1.02	59	0.90	U58120/1	M34402
Desulfosarcina variabilis	DSM2060[T]	C	33	51	55	0.93	68	0.75	AF191907	M34407
Desulfococcus multivorans	DSM2059[T]	C	35	57	56	1.02	72	0.79	U58126/7	M34405
Desulfovibrio vulgaris	DSM644[T]	I	30–36	65	61	1.07	82	0.79	U16723	M34399
Desulfovibrio sp. strain PT-2	ATCC 49975	I	30	65	62	1.05	86	0.76	U58114/5	M98496
Desulfovibrio desulfuricans Essex 6	DSM642[T]	I	30	59	60	0.98	79	0.75	AJ249777	AF192153
Desulfovibrio africanus	DSM2603[T]	I	30–36	65	62	1.05	86	0.76	AF271772	X99236
Desulfovibrio desulfuricans El Agheila Z	DSM1926	ND[e]	30	ND	56	ND	72	ND	AF334592	M37316
Desulfoarculus baarsii	DSM2075[T]	C	37	66	62	1.06	85	0.78	AF334600	M34403
Desulfomonile tiedjei	DSM6799[T]	C	37	49	53	0.92	63	0.78	AF334595	M26635
Desulfobulbus rhabdoformis	DSM8777[T]	I	31	51	52	0.98	55	0.93	AJ250473	U12253
Desulfobulbus propionicus	DSM2032[T]	I	39	60	57	1.05	72	0.83	AF218452	M34410
Desulfobulbus sp. strain 3pr10	DSM2058[T]	I	29	ND	48	ND	44	ND	AF337902	M34411
Desulforhopalus vacuolatus	DSM9700[T]	I	18	48	47	1.02	46	1.04	AF334594	L42613
Desulfovirga adipica	DSM12016[T]	C	35	60	57	1.05	75	0.80	AF334591	AJ237605
Desulforhabdus amnigena	DSM10338[T]	C	37	53	52	1.02	57	0.93	AF337901	X83274
Thermodesulforhabdus norvegica	DSM9990[T]	C	60	51	53	0.96	63	0.81	AF334597	U25627

[a] SRPs with a putative xenologous DsrAB are indicated by an asterisk.
[b] C, complete; I, incomplete.
[c] T_{opt}, optimum growth temperature.
[d] Genome/dsrAB and genome/dsrAB 3rd, quotient of genomic and dsrAB G+C content and quotient of genomic and dsrAB third position G+C content, respectively. The accuracy of the genomic G+C values may vary due to the different determination methods used.
[e] ND, no data available.

MATERIALS AND METHODS

Bacterial strains. The investigated reference strains of sulfate- and sulfite-reducing bacteria are listed in Table 1. If necessary, strains were cultured as recommended by the DSMZ type culture collection (Braunschweig, Germany).
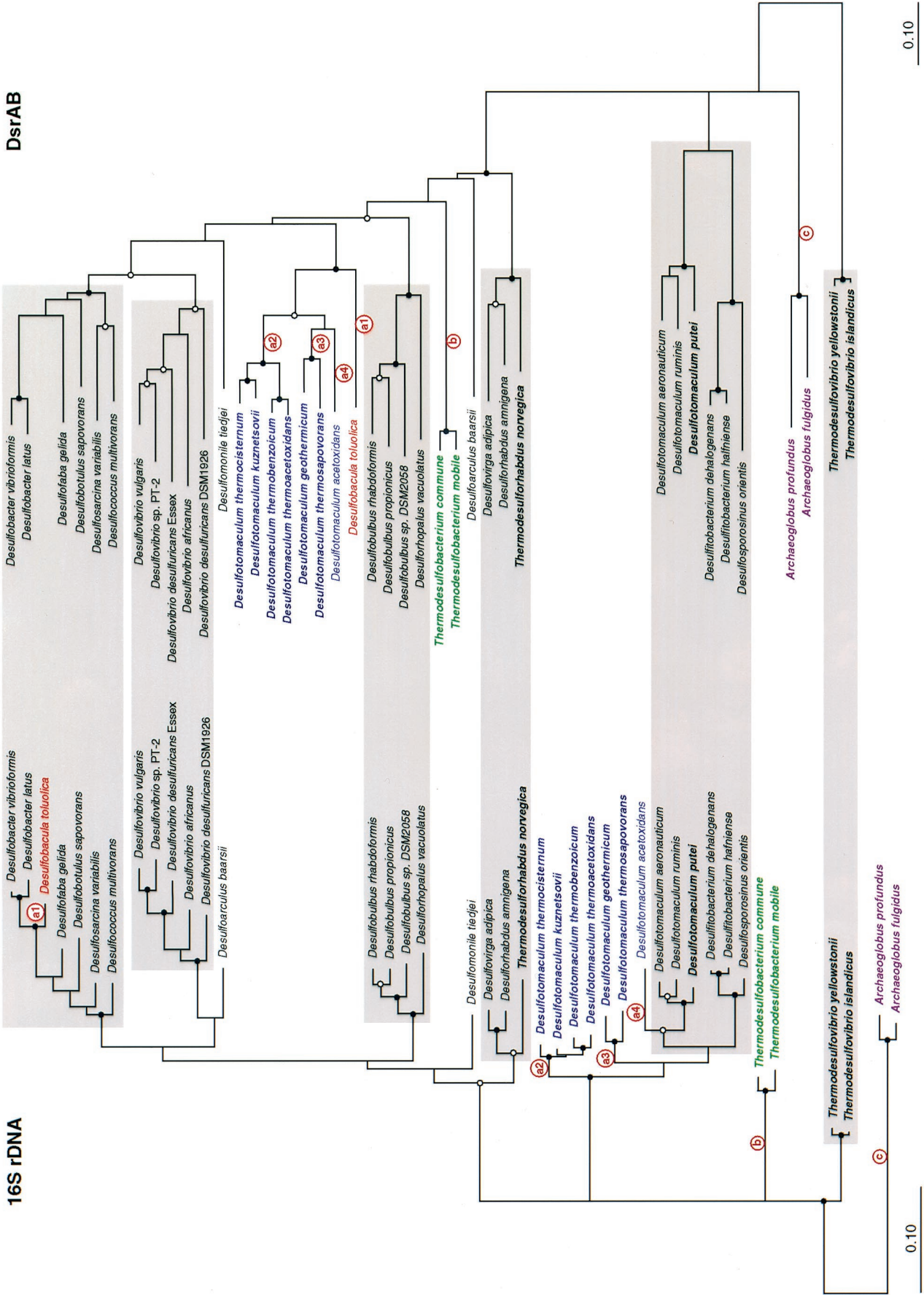
DNA isolation and PCR amplification. Genomic DNA of the reference organisms investigated was obtained from logarithmically growing or lyophilized cells by either using the FastPrep FP120 bead beater and the FastDNA Kit MH (Bio-101, Inc., La Jolla, Calif.) or another direct lysis technique (24) modified as described previously (9). An approximately 1.9-kb *dsrAB* segment was PCR amplified as described previously (35). Since amplification of the *dsrAB* gene fragment was not possible for all investigated reference strains, additional degeneracies were introduced in the previously published primers DSR1F and DSR4R (DSR1Fdeg, 5′-ACSCAYTGGAARCACG-3′; DSR4Rdeg, 5′-GTGTA

RCAGTTDCCRCA-3′), making them fully complementary to the respective target sites of recently published *dsrAB* sequences (17, 18). However, it should be noted that many "non-*dsrAB*" amplicates of ca. 1.9 kb were obtained using the degenerated primers.

Cloning and sequencing of *dsrAB* gene fragments. If not mentioned otherwise *dsrAB* PCR products of the sulfite- and sulfate-reducing reference strains were ligated into pCR2.1-TOPO or pCR-XL-TOPO vectors (Invitrogen). Clones with approximate 1.9-kb inserts were recovered with the QIAprep spin kit (Qiagen, Hilden, Germany) and sequenced with a 4200L automated Li-Cor Long Reader DNA Sequencer (MWG, Ebersberg, Germany). *dsrAB* PCR products of the *Desulfotomaculum* species *D. aeronauticum*, *D. putei*, *D. geothermicum*, *D. kuznetsovii*, and *D. thermobenzoicum* were directly sequenced. In addition, *dsr* sequences of *Desulforhabdus amnigena*, *Desulfobulbus* sp., and *Desulfitobacterium*

DsrAB

0.10

Desulfobacter vibrioformis
Desulfobacter latus
Desulfobacula toluolica
Desulfofaba gelida
Desulfobotulus sapovorans
Desulfosarcina variabilis
Desulfococcus multivorans

Desulfovibrio vulgaris
Desulfovibrio sp. PT-2
Desulfovibrio desulfuricans Essex
Desulfovibrio africanus
Desulfovibrio desulfuricans DSM1926

Desulfomonile tiedjei
Desulfotomaculum thermocisternum
Desulfotomaculum kuznetsovii
Desulfotomaculum thermobenzoicum
Desulfotomaculum thermoacetoxidans
Desulfotomaculum geothermicum
Desulfotomaculum thermosapovorans
Desulfotomaculum acetoxidans
Desulfobacula toluolica

Desulfobulbus rhabdoformis
Desulfobulbus propionicus
Desulfobulbus sp. DSM2058
Desulforhopalus vacuolatus

Thermodesulfobacterium commune
Thermodesulfobacterium mobile

Desulfoarculus baarsii
Desulfovirga adipica
Desulforhabdus amnigena
Thermodesulforhabdus norvegica

Desulfotomaculum aeronauticum
Desulfotomaculum ruminis
Desulfotomaculum putei
Desulfitobacterium dehalogenans
Desulfitobacterium hafniense
Desulfosporosinus orientis

Archaeoglobus profundus
Archaeoglobus fulgidus

Thermodesulfovibrio yellowstonii
Thermodesulfovibrio islandicus

16S rDNA

Desulfobacter vibrioformis
Desulfobacter latus
Desulfobacula toluolica
Desulfofaba gelida
Desulfobotulus sapovorans
Desulfosarcina variabilis
Desulfococcus multivorans

Desulfovibrio vulgaris
Desulfovibrio sp. PT-2
Desulfovibrio desulfuricans Essex
Desulfovibrio africanus
Desulfovibrio desulfuricans DSM1926
Desulfoarculus baarsii

Desulfobulbus rhabdoformis
Desulfobulbus propionicus
Desulfobulbus sp. DSM2058
Desulforhopalus vacuolatus

Desulfomonile tiedjei
Desulfovirga adipica
Desulforhabdus amnigena
Thermodesulforhabdus norvegica

Desulfotomaculum thermocisternum
Desulfotomaculum kuznetsovii
Desulfotomaculum thermobenzoicum
Desulfotomaculum thermoacetoxidans
Desulfotomaculum geothermicum
Desulfotomaculum thermosapovorans
Desulfotomaculum acetoxidans
Desulfotomaculum aeronauticum
Desulfotomaculum ruminis
Desulfotomaculum putei
Desulfitobacterium dehalogenans
Desulfitobacterium hafniense
Desulfosporosinus orientis

Thermodesulfobacterium commune
Thermodesulfobacterium mobile

Thermodesulfovibrio yellowstonii
Thermodesulfovibrio islandicus

Archaeoglobus profundus
Archaeoglobus fulgidus

0.10

dehalogenans were determined by direct sequencing as well as sequencing of the cloned PCR product. Previously published (35) partial *dsrAB* sequences of *Desulfotomaculum ruminis*, *Thermodesulfovibrio yellowstonii*, *Desulfobacter latus* sp., *Desulfobotulus sapovorans*, *Desulfococcus multivorans*, and *Desulfovibrio* sp. strain PT-2 were completed by resequencing of the original clones.

16S rRNA of *Thermodesulfobacterium mobile*. The 16S rRNA gene sequence of *Thermodesulfobacterium mobile* (=*T. thermophilum*) was obtained as described previously (14).

Phylogeny inference. Phylogenetic analyses were performed on alignments of the 16S ribosomal DNA (rDNA) nucleotide and the inferred amino acid sequences of the *dsrAB* genes. Regions of ambiguous positional homology were removed from the 16S rDNA data set using the Lane mask (16) and a DsrAB amino acid alignment mask prepared in ARB (http://www.arb-home.de). A total of 1,335 nucleotides and 543 amino acid positions (alpha subunit, 327; beta subunit, 216) were used in 16S rDNA and DsrAB analyses, respectively. For paralogous rooting DsrA sequences were aligned against DsrB and trees were calculated based on 173 amino acid positions, including positions with insertions and deletions. Phylogenetic analyses were performed with PAUP* version 4.0b2a (D. L. Swofford, Sinauer Associates, Sunderland, Mass.), ARB, or PHYLIP version 3.57c (J. Felsenstein, University of Washington, Seattle). Evolutionary distance (ED) analyses were conducted on the 16S rDNA data set using the Kimura 2 parameter and general time-reversible substitution model corrections with and without rate correction. Rate heterogeneities were corrected using a gamma distribution model (the shape parameter, alpha, was estimated to be 0.52 using a parsimony-based approximation in PAUP*). ED analysis of the DsrAB data set was performed by using a Dayhoff PAM correction and neighbor joining. Maximum parsimony (MP) trees were constructed for both data sets using the default settings in PAUP*. Maximum likelihood (ML) analysis of the 16S rDNA data set was performed in the ARB package using the fastDNAml program (28). Bootstrap resampling of the ED and MP trees was performed for all analyses to provide confidence estimates for the inferred topologies. A total of 1,000 or 2,000 replicates was used in all cases, with the exception of the ED analysis of the DsrAB data set, wherein 100 replicates were calculated.

RESULTS

Dissimilatory sulfite reductase phylogeny. A DNA fragment ca. 1.9 kb in size, encompassing most of the alpha and beta subunit genes of the dissimilatory sulfite reductase, was amplified from 30 sulfite- and sulfate-reducing bacteria (Table 1). Complete sequences of the PCR products were obtained. Compiled sequences were entered into the *dsrAB* database, translated into amino acids, and manually aligned. Previously published partial length *dsr* sequences of *Desulfovibrio oxyclinae* (U58116/7 [35]), *Desulfovibrio simplex* (U78738 [10]), *Desulfovibrio gigas* (U80961), *Desulfonema limicola* (U58128/9 [35]), and *Desulfobacterium autotrophicum* (Y15478) were not included to avoid resolution loss in phylogenetic analyses. Comparative sequence analyses were performed based on each subunit and both subunits combined. No major differences were noted between the individual and combined subunit tree topologies regardless of the inference method used, indicating a shared evolutionary history for the alpha and beta subunits. Consistent with these findings, the G+C contents of *dsrA* and *dsrB* were almost identical for each organism (data not shown). Consequently, detailed phylogenetic analyses were performed on a combined (DsrAB) data set in order to include the maximum number of 543 comparable amino acid positions. For

comparison, trees were calculated from the 16S rRNA genes of the identical set of organisms to avoid sampling artifacts (Fig. 1). Since the 16S rDNA sequence of *Thermodesulfobacterium mobile* was not available, it was determined in this study (1,520 nucleotides). In Fig. 1, the *Archaeoglobus* sequences were used as the outgroup for the 16S rRNA tree since they are the only representatives of the archaeal domain in an otherwise bacterial tree. In contrast, the *Thermodesulfovibrio* sequences (bacterial *Nitrospira* division) were used as the outgroup in the DsrAB analyses since paralogous outgrouping of the alpha and beta subunits suggests that the root of the Dsr tree is along the *Thermodesulfovibrio* line of descent (Fig. 2). Therefore, it appears likely that the dissimilatory sulfite reductases of the *Archaeoglobales* have a bacterial origin (see Discussion).

Overall, highly similar orderings of taxa, shaded gray in Fig. 1, were found between the 16S rRNA and DsrAB trees with all treeing methods. However, major incongruencies were found between DsrAB- and 16S rRNA-based analysis for seven members of the genus *Desulfotomaculum*, for both species of the genus *Thermodesulfobacterium*, and for the δ-proteobacterium *Desulfobacula toluolica* (color coded; Fig. 1). In contrast to relationships inferred using the rRNA, the genus *Desulfotomaculum*, a member of the low G+C gram-positive division (33), is not monophyletic in the DsrAB tree. *Desulfotomaculum aeronauticum*, *D. ruminis*, and *D. putei* form a clearly separated grouping, together with *Desulfosporosinus orientis*, based on their DsrAB sequences, while the other seven *Desulfotomaculum* species cluster together with *Desulfobacula toluolica* within the δ-proteobacterial radiation. Similarly, *Thermodesulfobacterium commune* and *T. mobile* comprise a division level lineage by rRNA analysis but branch within the δ-*Proteobacteria* according to their DsrAB sequences. A final discrepancy recognized is the inconsistent branching point of *Desulfobacula toluolica*. By 16S rRNA comparison, this species is closely related to *Desulfobacter latus* and *Desulfobacter vibrioformis*, while its DsrAB sequence is robustly associated with the *Desulfotomaculum* group in the δ-*Proteobacteria* (Fig. 1). The most parsimonious interpretation is that these significant topological conflicts reflect lateral transfer of the DsrAB genes (see Discussion). Points of inferred lateral gene transfer (LGT) are indicated in Fig. 1 by circled letters on the 16S rRNA tree.

Additional evidence for lateral transfer of dissimilatory sulfite reductase. Insertions and deletions within the DsrAB amino acid sequences (excluded in the phylogenetic analyses) were investigated as additional signposts of the deduced evolutionary relationships, particularly with respect to inferred LGT events. In total, three insertions were unique to the δ-*Proteobacteria*: one in the alpha subunit and two in the beta subunit (Fig. 3). These insertions were also found in the δ-proteobacterium-like DsrAB sequences of the seven *Desulfo-*

FIG. 1. Comparison of 16S rRNA (ML) and DsrAB (ED) trees for the sulfate- and sulfite-reducing prokaryotes investigated. Branch points supported by phylogenetic analysis (bootstrap support values of >90% in all ED and MP methods) are indicated by filled circles. Open circles at branch points indicate >75% bootstrap support in most or all analyses, while branch points without circles were not resolved (bootstrap values of <75%) as specific groups in the different analyses. Both trees are collapsed back at the division level. Thermophilic prokaryotes are in boldface. Consistent monophyletic groups between both trees are shaded gray. Microorganisms affected by putative LGT events of the *dsrAB* genes are color coded. *dsrAB* recipient or donor lineages are indicated by circled letters (a to c) located above or below the branch, respectively. The bars represent 0.1 changes per nucleotide or amino acid, respectively.
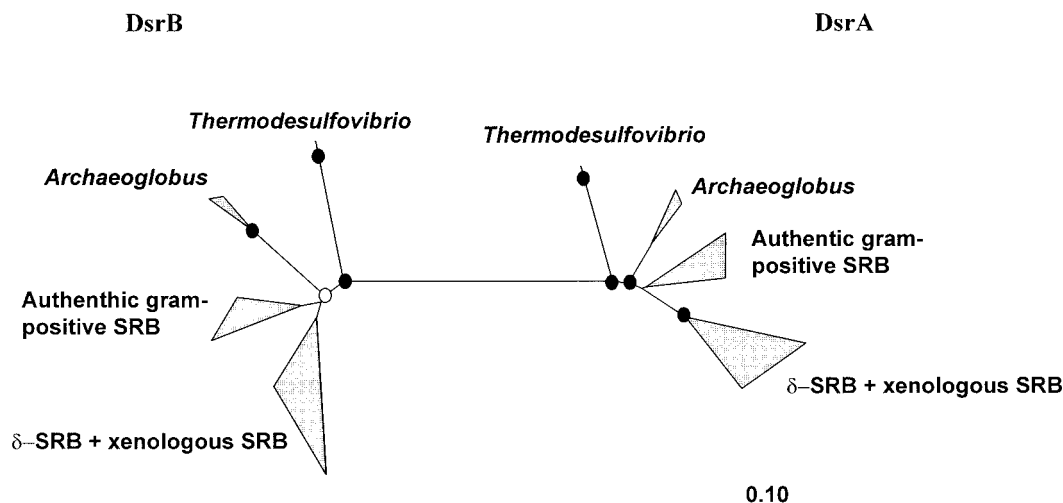
DsrB

DsrA

FIG. 2. Unrooted amino acid tree (ED) based on an alignment of DsrA to DsrB. The dissimilatory sulfite reductases of *Allochromatium vinosum* and *Pyrobaculum islandicum* were excluded from the analysis since they likely are members of different enzyme families (9, 22). The bar represents 0.1 changes per amino acid. Bootstrap analyses were performed using the PHYLIP parsimony method with 100 resamplings (Felsenstein, University of Washington). Branch points with parsimony bootstrap support of >85% are indicated by filled circles. Open circles at branch points indicate >50% bootstrap support, while branch points without circles either have parsimony bootstrap values of <50% (authentic gram-positive sulfate-reducing bacteria [SRB] DsrB; δ-SRB + xenologous SRB DsrB) or are not obtained with the parsimony method (*Archaeoglobus* and authentic gram-positive sulfate-reducing bacteria DsrA sequences form a monophyletic cluster in the parsimony method).

tomaculum species, and two *Thermodesulfobacterium* species, thus independently supporting the suggested LGT events.

Sizable differences in G+C content of the host genomes and acquired genes have been used to infer recent LGT events (20). A variation of more than 10% between the *dsrAB* G+C content and the respective genomic G+C content was found in *Thermodesulfobacterium mobile, Thermodesulfobacterium commune, Thermodesulfovibrio yellowstonii, Desulfobacula toluolica* sp., *Desulfotomaculum acetoxidans, Desulfotomaculum kuznetsovii, Desulfotomaculum thermoacetoxidans,* and *Archaeoglobus profundus* (Table 1). In seven of these eight organisms, LGT of *dsrAB* was predicted by comparison of tree topologies (Fig. 1). We attempted but failed to refine this analysis using the approach of Lawrence and Ochman (20) to identify atypical sequence characteristics (data not shown) since this method produces unreliable estimates for samples containing fewer than 1,500 codons as described previously (20).

Dissimilatory sulfite reductase homolog of *Desulfitobacterium dehalogenans*. The conserved *dsrAB* primers also amplified a fragment of the expected length from *Desulfitobacterium dehalogenans*, a bacterium capable of sulfite reduction but not of sulfate reduction (34). Comparative sequence analysis of the amplicon demonstrated a specific relationship to the dissimilatory sulfite reductase of *Desulfosporosinus orientis*, a finding consistent with their 16S rRNA-based relationship (Fig. 1). Furthermore, the recently completed genome sequence of *Desulfitobacterium hafniense* (http://www.jgi.doe.gov) contains a *dsrAB* sequence highly similar to the one of *Desulfitobacterium dehalogenans* (97.0% amino acid identity). As expected from the close relationship of both species by 16S rDNA comparison (96.7% similarity), their DsrAB sequences group together independent of the treeing methods applied.

DISCUSSION

In this study we investigated the phylogeny of the dissimilatory sulfite reductase from a study set of reference species encompassing all described lineages of SRPs in order to clarify whether *dsr* genes, in addition to undergoing vertical transmission, have also been laterally transferred. Using degenerated PCR primers, DNA fragments with strong sequence similarities over their entire length to previously published *dsrAB*

Organism	Amino acid positions (according to *Desulfovibrio vulgaris*)		
	256 DsrA 276	119 DsrB 131	238 DsrB 249
δ-*Proteobacteria*-like			
D. propionicus	ANDSEYPSNAGAHKGRDWGKFDI	KSH-----GTTFP	PAKAINSAGEEVK
D. amnigena	GGE--IVPHAGAG-GTEKRAFDI	RSR-----GNWFP	PKKVDD-----KK
T. norvegica	AGE--LVPNGGAH-GPEKRPLDI	KSR-----GNWFP	PKKVPI-DGKFYK
D. vulgaris	AGE--FKPNAGAHSGRDWGKFDI	ASRKFDGGSLKFP	PTKLEIGD-KKVN
D. variabilis	GGE--FAPNGGAHSGRNWGAFDI	ESRKFDGGSFKFP	PAKVTVGD-KELK
D. vibrioformis	ENDPAYPANAGAHKGKDWGPFDI	ASRKFPGGSLKFP	PAKVTLPNGTEVK
*D. toluolica	GGE--IAPNAGAHARKDWGAFDI	NSRKHVTGSYKFP	PTKTES----GKK
*D. acetoxidans	NGE--YAPNAGAHAGKDWGKFDI	NSRKFVSGSFKFP	PDKTAD----GKK
*D. thermosapovorans	AGD--VVPNGGAHKGRDWGKFDI	ESRKFVSGSYKFP	PDKTPE----GGK
*D. geothermicum	AGE--YVPNAGAHAGRDWGKFP	QSRKFVGGSYKFP	PDKTPE----GKK
*D. thermocisternum	AGD--IVPRGGAHRGRDWGKFDT	WSRKFVSGSYKFP	PDKTPE----GRR
*D. kuznetsovii	AGD--IVPRGGSHKGRDWGKFDI	WSRKFVSGSYKFP	PDKTPE----GRR
*D. thermobenzoicum	ACD--IVPRGGAQKGRDWGKFDI	AGRKFASGSYKFP	PDKTPE----GRR
*D. thermoacetoxidans	AGD--IVPRGGARR-QDWGKFDI	AGRKFASGSYKFP	PDKTPE----GRR
*T. commune	RGE--LKPNAGAFSDRDWGPFDI	KNRKHPNGSYKFS	PA-TAEVGGKKKK
*T. mobile	RGE--LKPNAGAFSDRDWGPFDI	KNRKHPNGSYKFP	PA-TAEVDGKKKK
Low G+C Gram positives			
D. orientis	SEG----------------FDI	DAK-------GLR	PDP-------KNK
D. aeronauticum	ADG----------------LDI	KAM-------GVP	PNP-------XLK
D. putei	AEG----------------FDI	NAM-------GVP	PNP-------KLK
D. ruminis	AAG----------------FDI	QER-------GTP	PNP-------KLK
D. hafniense	AEG----------------LNI	GAK-------GLP	PNP-------KEK
D. dehalogenans	AEV----------------XNI	GAK-------GLP	PNP-------KEN
Nitrospira division			
T. yellowstonii	KKG----------------MNI	KAK-------KYM	PDP-------AKK
T. islandicus	KKG----------------MNI	KAK-------KYM	PDP-------AKK
Archaea			
A. profundus	KAC----------------VDI	QEK------VGFP	PDM-------KRK
A. fulgidus	SW-----------------MDI	QER------VGFP	PDM-------KNK

FIG. 3. Amino acid alignment of DsrA and DsrB showing insertions supporting the δ-proteobacterial origin of the putative laterally transferred sulfite reductases (labeled with an asterisk). It should be noted that the presumably xenologous DsrA and DsrB of *Archaeoglobus* do not show the typical δ-proteobacterial insertions.

sequences were obtained from all investigated SRPs and from the sulfite reducer *Desulfitobacterium dehalogenans*.

DSR sequence motifs. The newly determined *dsrAB*-like sequences contain the essential cluster-binding residues typical for dissimilatory sulfite reductases. In particular, all alpha-subunit sequences contain the complete (Cys-X_5-Cys)-X_n-(Cys-X_3-Cys) motif required for coupling of the [Fe_4S_4]-siroheme cofactor (2). As for other dissimilatory sulfite reductases (10), this Cys motif is truncated in the beta subunit of the newly determined DsrAB sequences. In contrast to the prediction of Dahl et al. (3) the DsrB subunit of *Thermodesulfobacterium mobile* and *Thermodesulfobacterium commune* (4, 8) does not contain a complete siroheme-[Fe_4S_4] binding site that could explain the measured binding of four sirohemes per $\alpha_2\beta_2$ molecule (versus two sirohemes for typical sulfite reductases). Furthermore, all DsrA sequences possess the Cys-Pro and Cys-X_2-Cys-X_2-Cys motif required for linking [Fe_4S_4] clusters (3). Since the reverse PCR primers used for amplification target part of the [Fe_4S_4] cluster binding motif of DsrB only the Cys-Pro signature is present in all deduced DsrB sequences. The absolute conservation of functionally important protein sequences and the absence of frameshift or nonsense mutations suggests that the characterized genes are transcribed and translated and function as dissimilatory sulfite reductases. The sequenced dissimilatory sulfite reductase genes of *Thermodesulfobacterium mobile* are most likely functionally expressed since the highly variable N-terminal sequence of the beta-subunit is identical to the one determined by Edman degradation (4). Comparison of the 10 N-terminal amino acids of the beta-subunit determined by Edman degradation of the dissimilatory sulfite reductase of *Thermodesulfobacterium commune* (8) to the sequence deduced in our study revealed a single amino acid difference at position 1 (Thr/Ser predicted by Edman and Gly [GGA codon] found in our study). This inconsistency is either caused by an experimental artifact (mistake in the Edman degradation determination or at least two *Taq*-induced mutations in the *dsrAB* clone of *Thermodesulfobacterium commune*) or by the presence of more than one type of *dsrAB* genes in this organism. Differences between the deduced N-terminal sequence and that determined by N-terminal polypeptide sequencing were also reported for the DsrB protein of *Desulfovibrio desulfuricans* (25).

DSR homologs. Additional homologs to the investigated *dsrAB* genes may exist in some of the analyzed strains. This is not the case for *Desulfitobacterium hafniense* and *Archaeoglobus fulgidus*, since no additional *dsrAB* homologs are present in their complete genome sequences. Under the assumption that the PCR primers applied would amplify all putative *dsr* copies, we have indirect evidence that the *Desulfotomaculum* species, *D. aeronauticum*, *D. putei*, *D. geothermicum*, *D. kuznetsovii*, and *D. thermobenzoicum* do not contain multiple *dsrAB* copies which differ in sequence since the respective *dsrAB* PCR amplificates could be sequenced directly. For the other analyzed SRPs, knowledge of the copy number of *dsrAB*-like genes must await an extensive Southern hybridization or complete genome sequence analysis which was beyond the scope of this study.

DSR phylogeny and lateral transfer. The core of our study was a direct comparison between 16S rRNA and DsrAB trees of the respective SRPs (Fig. 1). In this analysis it is an explicit assumption that the 16S rRNA phylogeny reflects the organ-

ismal phylogeny (36), that is, that these highly conserved genes have undergone no lateral transfer in the organisms studied. Based on this supposition, seven *Desulfotomaculum* species, two *Thermodesulfobacterium* species, and *Desulfobacula toluolica* possess nonorthologous *dsrAB* genes as demonstrated by major inconsistencies between the DsrAB and 16S rRNA trees. These inconsistencies most likely reflect lateral transfer of *dsrAB* genes rather than the occurrence of *dsrAB* paralogs which diverged after an initial *dsr* operon duplication since all nonorthologous *dsrAB* genes are phylogenetically affiliated with the (presumably orthologous) *dsrAB* genes of the δ-*Proteobacteria* (Fig. 1). Furthermore, organisms distantly related by 16S rRNA sequence relationship, such as *Desulfobacula toluolica* and several *Desulfotomaculum* species, contain similar nonorthologous *dsrAB* genes. This close relatedness of *dsrAB* genes between species belonging to different bacterial divisions is unlikely to be the product of convergent evolution and can more reasonably be explained by multiple lateral acquisitions from a common donor lineage within the δ-*Proteobacteria*. Consistent with this inference, all putative xenologous *dsrAB* sequences have insertions typical for the δ-*Proteobacteria* (Fig. 3).

Five independent LGT events (the red circles in Fig. 1) of *dsrAB* genes have been postulated to explain the observed discrepancies between the 16S rDNA and DsrAB topologies. It should be noted that for SRPs (i) which do not have close phylogenetic relatives in the current *dsrAB* data set or (ii) whose positions in the deduced phylogenetic trees vary dependent upon the treeing method used, our analysis cannot rule out that their characterized *dsrAB* sequences are xenologs. Within the δ-*Proteobacteria* these limitations apply to *Desulfoarculus baarsii* and *Desulfomonile tiedjei*. Furthermore, the characterized DsrAB sequences of *Archaeoglobus* and *Thermodesulfovibrio* species and the "authentic" *Desulfotomaculum* and *Desulfitobacterium* species possibly could originate from a progenitor of the δ-*Proteobacteria* or from other as-yet-unidentified SRPs. In fact, it seems likely that the genus *Archaeoglobus* inherited *dsrAB* genes from a bacterial donor (i) because the evolutionary distance between *Archaeoglobus* species and the bacterial sulfate reducers is much shorter in the DsrAB tree than in the 16S rRNA tree and (ii) because the sulfate-reducing phenotype is currently restricted to the genus *Archaeoglobus* within the archaeal domain. Further support for a lateral transfer of the *dsrAB* genes to the *Archaeoglobales* was obtained by a phylogenetic analysis on an alignment of the alpha- against the beta-subunit amino acid sequences. Such analysis can be used to root the Dsr subunit trees (6, 13) (Fig. 2), since the subunits are paralogs arising from an ancestral *dsr* gene duplication (3). Independent of the treeing method used, the root was consistently indicated between the DsrAB of the *Thermodesulfovibrio* species and the DsrABs of all other analyzed SRPs, including the *Archaeoglobales*. This is inconsistent with the 16S rRNA phylogeny and points to a bacterial origin of the *Archaeoglobales dsrAB* genes (ⓒ in the DsrAB tree; Fig. 1). However, the results from the paralogous rooting should not be overemphasized since the alignment of the Dsr subunits against each other (i) is relatively short (173 amino acid positions) and (ii) contains several regions which cannot unambiguously be aligned (caused by the relatively low sequence similarities of the subunits to each other). Furthermore, no

evidence for lateral transfer of the *Archaeoglobus fulgidus dsrAB* genes was indicated by atypical sequence characteristic analysis (27; J. Lawrence, unpublished data), suggesting that, if the genes are xenologs, they have completely ameliorated toward their host genome and were the result of an ancient LGT event.

DSR donor lineages. The *dsrAB* gene donors were members of at least two distinct evolutionary lineages within the δ-*Proteobacteria* (ⓐ1 to ⓐ4 and ⓑ in the DsrAB tree; Fig. 1). Donor lineage a contributed *dsrAB* genes to two phylogenetically remote groups of bacteria, *Desulfobacula toluolica* (δ-*Proteobacteria*) and several *Desulfotomaculum* species (low G+C gram positives) (ⓐ1 to ⓐ4 in the 16S rDNA tree; Fig. 1), suggesting that this lineage is particularly adept at donating *dsrAB* and possibly other genes. The specific identities of the donor lineages is unknown based on the current data, since no orthologous *dsrAB* genes were identified within the putative xenolog groups. It is however striking that *Desulfobacula toluolica* and all but two *Desulfotomaculum* species which received the xenologous *dsrAB* are oxidizing their characteristic substrates completely to CO_2 while the "authentic" *Desulfotomaculum* and *Desulfitobacterium* species are exclusively incomplete oxidizers. One possible explanation for this feature is that the *dsrAB* donor was a complete oxidizer which bestowed this metabolic capability to the *Desulfotomaculum* and *Desulfobacula* species, which subsequently was lost in *Desulfotomaculum thermosapovorans* and *Desulfotomaculum thermocisternum* (Table 1). Furthermore, most of the recipients of xenologous *dsrAB* are thermophilic (Table 1) which could indicate a thermophilic lifestyle of the donor species.

DSR recipient lineages. *Desulfobacula toluolica* is the recipient of the most recent putative LGT event thus far identified (ⓐ1; Fig. 1) since its close relatives, *Desulfobacter latus* and *Desulfobacter vibrioformis,* contain orthologous *dsrAB* genes. This is also supported by no identifiable amelioration of the third codon position G+C content of the xenologous *dsr* genes toward the mean G+C content of the host *D. toluolica* genome (Table 1). The evolution of the genus *Desulfotomaculum* was also affected by LGT events of *dsrAB* genes. The number of LGT events within this genus is difficult to predict since the subclustering of its members is not always well supported in the 16S rDNA tree (Fig. 1). On the basis of the presented 16S rDNA tree, it is most parsimonious to postulate at least three LGT events within this genus (ⓐ2 to ⓐ4 in the 16S rDNA tree of Fig. 1). Alternatively, one could hypothesize that a single lateral *dsrAB* gene transfer event occurred to the common ancestor of the genera *Desulfotomaculum, Desulfosporosinus,* and *Desulfitobacterium* (which did not displace the orthologous *dsr* genes), followed by a subsequent xenolog gene loss on at least two independent occasions from the ancestors of the "authentic" *Desulfotomaculum* and *Desulfosporosinus-Desulfitobacterium* species, respectively.

In conclusion, this study demonstrates that the genes encoding the dissimilatory sulfite reductase are subject to frequent LGT events within and across bacterial divisions and possibly even between the bacterial and archaeal domains. This finding was unexpected since the dissimilatory sulfite reductase represents an essential enzyme for anaerobic sulfate and sulfite respiration which acts (at least for the SRPs) in concert with other enzymes. One possible explanation for the observed widespread lateral distribution of dissimilatory sulfite reductases could be that the genes encoding this enzyme are part of a mobilizable metabolic island similar to the genes required for anaerobic nitrate respiration of *Thermus thermophilus* (30). More generally, our findings add to the accumulating evidence that lateral gene transfer is a potent mechanism shaping the composition of prokaryotic genomes (see, for example, references 5, 7, 19, 20, 21, 26, and 31). On the other hand, our data also demonstrate that the DsrAB phylogeny of most SRPs analyzed is still consistent with the 16S rRNA phylogeny. This observation and the paralogous rooting of the Dsr tree still support an early and thermophilic origin of sulfate respiration.

The use of functional genes, including *dsrAB* as molecular markers for defined physiological groups of bacteria has become increasingly popular in investigations of complex microbial communities (see, for example, references 1, 22, 29, and 37). If the functional genes are exploited for phylogenetic analysis of the respective bacteria, lateral gene transfer can complicate the interpretation. This has previously been demonstrated for the *nifH* gene encoding the nitrogenase reductase of nitrogen-fixing bacteria (11) and was shown here to also hold true for the dissimilatory sulfite reductase genes. Therefore, the phylogenetic DsrAB framework established in our study provides an essential basis for better interpreting environmental diversity surveys of SRPs based on comparative DsrAB sequence analysis.

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