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 Desulfotobacterium 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 the Proteobacteria DsrAB sequences. The most parsimonious interpretation of these data is that dsrAB genes from ancestors of as-yet-unrecognized sulfate reducers within the 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 dsrA lateral gene transfer event also was found within the 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 archaean Pyrolobus 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 α2β2 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 investigate 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 protogentic 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.
The accuracy of the genomic G+C content and quotient of genomic and dsrAB third position G+C content may vary due to the different determination methods used.

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 bacteria. The investigated reference strains of sulfate- and sulfite-reducing bacteria were lyophilized (17, 18). However, it should be noted that many “non-dsrAB” amplifications 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 sulfate- and sulfite-reducing reference strains were ligated into pCR2.1-TOPO or pCR-XL-TOPO vectors (Invitrogen). Clones with dsrAB target sites of recently published sequences (17, 18) were directly sequenced. In addition, additional degeneracy were introduced in the previously published primers DSR1F and DSR1R (DSR1Fdeg, 5′-ACSCAYTGGAARCCG-3′; DSR1Rdeg, 5′-GTGTA RCAGTDCCCRCA-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” amplicons were obtained using the degenerated primers.

**Cloning and sequencing of dsrAB gene fragments.** If not mentioned otherwise, dsrAB PCR products of the sulfate- and sulfite-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. pueti, D. geothermicum, D. kuznetsovii, and D. thermobenzoicum were directly sequenced. In addition, dsrAB gene fragments were sequenced of Desulfotomaculum ammonia, Desulfobulbus sp., and Desulfitobacterium.
replicates was used in all cases, with the exception of the ED analysis of the inferred topologies. A total of 1,000 or 2,000 data set was performed in the ARB package using the fastDNAml program (28). Maximum likelihood (ML) analysis of the 16S rDNA data set was performed by using a Dayhoff PAM correction and neighbor joining. ED analysis of the DsrAB gamma distribution model (the shape parameter, alpha, was estimated to be 0.52) 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 RNA 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 RNA and DsrAB trees with all treeing methods. However, major incongruencies were found between DsrAB- and 16S RNA-based analysis for seven members of the genus Desulfoformicimonas, for both species of the genus Thermodesulfobacterium, and for the δ-proteobacterium Desulfohaliobaculum toluolica (color coded; Fig. 1). In contrast to relationships inferred using the rRNA, the genus Desulfoformicimonas, a member of the low G+C gram-positive division (33), is not monophyletic in the DsrAB tree. Desulfohaliobaculum aeronauticum, D. ruminis, and D. putei form a clearly separated grouping, together with Desulfospirillum orientis, based on their DsrAB sequences, while the other seven Desulfoformicimonas species cluster together with Desulfohaliobaculum 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 Desulfohaliobaculum toluolica. By 16S RNA comparison, this species is closely related to Desulfofberger latus and Desulfofberger vibrioformis, while its DsrAB sequence is robustly associated with the Desulfoformicimonas group in the δ-Proteobacteria (Fig. 1).

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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-
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).

**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 were previously published dsrAB

![Fig. 2. Unrooted amino acid tree (ED) based on an alignment of DsrA to DsrB. The dissimilatory sulfite reductases of Allochromatium vinosum and Pyrococcus 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).](http://jb.asm.org/)

![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.](http://jb.asm.org/)

![Organism | Amino acid positions (according to Desulfovibrio vulgaris)](http://jb.asm.org/)
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$_7$-Cys)-X$_6$- (Cys-X$_7$-Cys) motif required for coupling of the [Fe$S_6$]-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$S_6$] 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$_7$-Cys-X$_7$-Cys motif required for linking [Fe$S_6$] clusters (3). Since the reverse PCR primers used for amplification target part of the [Fe$S_6$] 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 Desulfotobacterium 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. aeronticum, D. putei, D. geothermicum, D. kuenziitovii, 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 organismal 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 Desulfobaccula 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 $\delta$-Proteobacteria (Fig. 1). Furthermore, organisms distantly related by 16S rRNA sequence relationship, such as Desulfobaccula 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 $\delta$-Proteobacteria. Consistent with this inference, all putative xenologous dsrAB sequences have insertions typical for the $\delta$-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 $\delta$-Proteobacteria these limitations apply to Desulforaculcus baarsii and Desulfomonile tiedjei. Furthermore, the characterized DsrAB sequences of Archaeoglobus and Thermodesulfovibrio species and the “authentic” Desulfotomaculum and Desulfobacterium species possibly could originate from a progenitor of the $\delta$-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 (6) 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 atypal 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 β-Proteobacteria (4) to (6) and (3) in the DsrAB tree; Fig. 1). Donor lineage a contributed dsrAB genes to two phylogenetically remote groups of bacteria, Desulfovibaculacola toluolicola (β-Proteobacteria) and several Desulfotomaculum species (low G+C gram positives) (7) 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 Desulfovibaculacola toluolicola and all but two Desulfotomaculum species which received the xenologous dsrAB are oxidizing their characteristic substrates completely to CO₂ 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 Desulfovibaculacola species, which subsequently was lost in Desulfotomaculum thermosapovorans and Desulfotomaculum thermocitrium (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.** Desulfovibaculacola toluolicola is the recipient of the most recent putative LGT event thus far identified (5) since its close relatives, Desulfovibacter latus and Desulfofacter 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 (6) to (8) 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 sulfate 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 sulfate 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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