Phylogenetic Positions of Desulfofustis glycolicus gen. nov., sp. nov., and Syntrophobotulus glycolicus gen. nov., sp. nov., Two New Strict Anaerobes Growing with Glycolic Acid

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The glycolate-oxidizing, sulfate-reducing bacterium strain PerGlyS and the syntrophically glycolate-oxidizing bacterium strain FlGlyR were studied with respect to their phylogenetic relationships on the basis of in vitro amplification and direct sequencing of 16S rRNA-encoding DNA. Strain PerGlyS clustered with representatives of the δ subclass of the class Proteobacteria, close to "Desulfurhopalus vacuolatus" but sufficiently distinct to preclude its assignment to this genus. These organisms, together with Desulfobulbus propionicus, represent a phylogenetic subgroup among members of the δ subclass of Proteobacteria. Strain FlGlyR was found to cluster with the gram-positive bacteria with low-G+C DNA, and Desulfitobacterium dehalogenans and Desulfofustis orientis are its closest relatives. Other species of the genus Desulfofustis are phylogenetically moderately closely related to these organisms. These results necessitate the establishment of new genera and species for these two strains. Strain PerGlyS was designated the type strain of Desulfofustis glycolicus gen. nov., sp. nov., and strain FlGlyR was designated the type strain of Syntrophobotulus glycolicus gen. nov., sp. nov.

Glycolate is an important constituent of fruits and sugar cane (15, 23) and is excreted by algae and autotrophic prokaryotes under conditions of carbon dioxide limitation and excess oxygen (1–3). Aerobic degradation of glycolate has been studied in detail; its anaerobic degradation has aroused interest only recently (9, 12). Defined cocultures of fermenting bacteria with homoacetogenic or methanogenic bacteria were isolated which converted glycolate completely to carbon dioxide and hydrogen, with concomitant reduction of carbon dioxide to either acetate or methane (9). The primary fermenting partner in these cocultures was isolated later in pure culture with glyoxylate as the substrate (strain FlGlyR; 11). This strain has been studied in detail with respect to its biochemistry; glycolate oxidation to glyoxylate and vice versa is coupled to a membrane-bound electron transport system that catalyzes either a proton potential-driven reversed electron transport from glycolate to hydrogen or a hydrogen-dependent glyoxylate reductase coupled to ATP synthesis by electron transport phosphorylation (10, 11). Also a glycolate-oxidizing sulfate-reducing bacterium, strain PerGlyS, was isolated that deserves attention because of its rather high desulforubidin content and the fact that only some part of acetyl intermediates is oxidized to CO2 (12).

The present communication reports on the phylogenetic positions of these two strains and is based on sequence data obtained by direct sequencing of 16S rRNA-encoding DNA.

MATERIALS AND METHODS

Pure cultures of strains FlGlyR (DSM 8271) and PerGlyS (DSM 9705) were taken from our laboratory collection. Both have been deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, under the reference numbers indicated. Strain FlGlyR was originally isolated from anoxic sewage sludge, and strain PerGlyS was from marine sediment obtained from a channel in Venice, Italy.

Both strains were cultivated in a bicarbonate-buffered, sulfide- or cysteine-reduced mineral medium (21, 30) which contained trace element solution SL10 (29), selenite-tungstate solution (29), and seven-vitamin solution (30). Details of cultivation and characterization are given in the original descriptions (9, 12).

In vitro amplification and direct sequencing of 16S rRNA encoding DNA were performed as described earlier (24). The new sequences were added to an alignment of about 5,000 complete or partial 16S rRNA primary structures from bacteria (17, 27) by using the alignment tool of the ARB program package (25). Phylogenetic analyses were performed by applying the distance matrix (ARB, PHYLP; 8), maximum-parsimony (ARB, PHYLP), and maximum-likelihood (fastDNAm1; 17) methods to different sets of data. Distance matrix and maximum-parsimony analyses were performed with sets of data comprising all available 16S rRNA primary structures from gram-positive bacteria with low-G+C DNA (about 850 sequences) and the δ subclass of the class Proteobacteria (66 sequences), as well as selected reference sequences from each of the remaining major phylogenetic groups of bacteria. Maximum-likelihood methods were used to analyze subsets of about 50 sequences comprising the homologous sequences of the nearest neighbors of the organisms studied here and selected outgroup references from other phylogenetic groups. The sets of data varied with respect to the reference sequences, as well as the alignment positions included. The variabilities of the individual alignment positions were determined by using the respective tool of the ARB program package and used as a criterion to successively remove highly variable positions from the set of data. This was done to recognize and minimize treeing artifacts resulting from alignment errors, database inconsistencies, and false identities (multiple base changes) in highly variable regions.

Nucleotide sequence accession numbers. The 16S rRNA-encoding DNA sequences of strains PerGlyS and FlGlyR were entered into the Ribosomal Database under accession numbers 99707 and 99706, respectively.

RESULTS AND DISCUSSION

The 16S rRNA gene sequences from glycolate-utilizing strains PerGlyS and FlGlyR were determined by direct sequencing of in vitro-amplified rRNA-encoding DNA. The moderate overall similarity (78%) of the two sequences indicates that the strains represent different lines of descent. The isolates PerGlyS and FlGlyR could unambiguously be assigned to the major bacterial phylogenetic groups comprising the δ subclass of Proteobacteria and the gram-positive bacteria with low-G+C DNA, respectively (18).

The phylogenetic relationships of strains PerGlyS and FlGlyR and a selection of related bacteria are shown in Fig. 1. The tree is based on the results obtained by performing a distance matrix analysis of a set of data comprising all currently
available 16S rRNA sequences from the (phylogenetically) gram-positive bacteria with low-G+C DNA and the 8 subclass of *Proteobacteria*. Homologous primary structures from selected representatives of all major phylogenetic groups (17, 32) were included as references. Only sequence positions which contain identical residues in at least 50% of all available 16S rRNA sequences from the 8 subclass of *Proteobacteria* and gram-positive bacteria with DNA with low G+C content, respectively. The triangles indicate groups of phylogenetically related species or genera. The bar indicates 10% estimated sequence divergence. 1, *Desulfobacter* spp. plus *Desfobacterium, Desulfobactera, Desulfobulbus, Desulfococcus, and Desulfurocorina* spp.; 2, *Syntrophobacter* spp. plus *Desulfobulbus, Desulfobaca, and Desulfobula* spp.; 3, *Pelobacter* spp. plus *Desulfitobacterium* and *Geobacter* spp.; 4, *Desulfovibrio* spp. plus *Bilophila* and *Desulforhabdus* spp.; 5, *Selenomonas* spp. plus *Acidaminococcus, Acomonema, Dialister, Megaphaera, Pectinatus, Phascolarctobacterium, Selenomonas, Veillonella, and Zymophilus* spp.

Multifurcations in the tree of Fig. 1 indicate branchings for which a relative order could not unambiguously be determined or was not supported by (at least the majority of) the analyses performed by applying different treeing methods. *Escherichia coli* and *Bacillus subtilis* were included in Fig. 1 to indicate the phylogenetic depths of *Proteobacteria* and gram-positive bacteria with low-G+C DNA, respectively.

Among the *Proteobacteria* of the 8 subclass, strain PerGlyS, "Desulfotrophus" sp. (14), and *Desulfobulbus* sp. (6) represent a phylogenetic cluster as also indicated by similarity values of 90.6 and 86.5%, respectively (Table 1). The corresponding values shared by strain PerGlyS and the other representatives of the 8 subclass are 80.6 and 85.4%. No closer relationship between this cluster and *Syntrophobacter* (13), *Syntrophus* (28), or *Desulfobulbus* spp. was supported by results obtained by applying different treeing analyses.

Within the phylogenetic group of gram-positive bacteria with low-G+C DNA, *Desulfitobacterium dehalogenans* (26) and *Desulfotomaculum orientis* (6) share 91.2% and 88.6% 16S
we propose the names Desulfotomaculum geothemicum; mobenzoicum; Syntrophospora bryantii; mophilus natus, Phascolarctobacterium, Selenomonas, Veillonella. Swf, botulus glycolicus, references 9 and 12. Desulfoarculum groups and the Desulfuromusa, values are given for phylogenetic groups. Abbreviations: Dba, such a grouping is low, as indicated by the short internode distance of the branch containing the former organisms or other members of the major phylogenetic group of gram-positive bacteria with DNA with a low G+C content are slightly rRNA sequence similarity, respectively, with strain FlGlyR (Table 2). The corresponding values for these organisms and species He1, Sbr, Dth, Dge, Dru, Dau, Dge, Dth, Sbr, SWf, Ddh, Dor, He1, Sel, Dni, Dru, Dau, Dge, Dth, Sbr, SWf. The values indicate the fraction of identical residues within the respective sequence pairs calculated by including only positions present in both sequences. Mean Description of Desulfofustis gen. nov. Desulfofustis (De.sul .fo.fus'tis. L. prefix de, off, L. n. sulfur, sulfur, fustis, club). M. L. m. n. Desulfofustis, a sulfate-reducing club. The description of this new genus is identical to that of its only species, D. glycolicus sp. nov. Description of Desulfofustis glycolicus sp. nov. glycolicus (gly-co'li.cus. M. L. n. acidum glycolicum, glycolic acid), glycolicus, referring to glycolic acid as the key substrate of this species. Gram-negative, rod-shaped bacteria, straight to slightly curved, 2.0 to 4.5 by 0.55 μm in size, occurring typically as single cells. Motile by one subterminally inserted flagellum, no spore formation. Strictly anaerobic bacteria, growing chemotrophically by oxidation of glycolate, glyoxylate, L-lactate, L-malate, fumarate, succinate, yeast extract, or hydrogen (in the presence of carbon dioxide and traces of acetate), with sulfate as an electron acceptor. Other substrates (formate, acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, caproate, 3-methylbutyrate, fructose, glucose, oxalate, pimelate, glutarate, glycolal-

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* The values indicate the fraction of identical residues within the respective sequence pairs calculated by including only positions present in both sequences. Mean values are given for phylogenetic groups. Abbreviations: Dba, Desulfobacter, Desulfobacterium, Desulfobulbus, Desulfobulbus, Desulfovibrio, and Desulfoisarcina spp.; Des, Desulfuviobrio spp.; Dpr, Desulfofusconis propionicus; Dva, Desulfofusconis vacuolatus; Eco, Escherichia coli; Mba, myxobacteria; Pba, Pelobacter, Desulfuromonas, Desulfovibrio, and Desulfofusconis spp.; Gpl, gram-positive bacteria with low G+C content; PGS, PerGlyS; Sba, Syntrophobacter, Desulfuromonas, and Desulfofusconis spp.; Sbu, Syntrophus buswellii; Sge, Syntrophospora gentianae.

TABLE 2. Overall 16s rRNA sequence similarities for strain PerGlyS and related reference organisms

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choline, cysteine, triethanolamine, glycine, indol, and benzo-
marine medium with 340 mM NaCl-14 mM MgCl,. Growth
completely oxidized, but part
Cells contain menaquinone-5(H2), desulforubidin, and cyto-
molecules.

**Description of Syntrophobutus spp.**

- **Syntrophobutus bakeri**
  - Growth in marine medium with 340 mM NaCl-14 mM MgCl2.
  - Growth possible also in brackish-water medium.
  - Cells contain menaquinone-7-10, DNA base ratio, 46.7 ± 0.1 mol% G+C (determined by high-pressure liquid chromatography analysis).

**Acknowledgments**

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