JOURNAL OF BACTERIOLOGY, Dec. 1989, p. 6689–6695
0021-9193/89/126689-07$02.00/0

Vol. 171, No. 12

Natural Relationships among Sulfate-Reducing Eubacteria

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Received 17 May 1989/Accepted 15 September 1989

Phylogenetic relationships among 20 nonsporeforming and two endospore-forming species of sulfate-reducing eubacteria were inferred from comparative 16S rRNA sequencing. All genera of mesophilic sulfate-reducing eubacteria except the new genus *Desulfomicrobium* and the gliding *Desulfonema* species were included. The sporeforming species *Desulfotomaculum ruminis* and *Desulfotomaculum orientis* were found to be gram-positive organisms sharing 83% 16S rRNA sequence similarity, indicating that this genus is diverse. The gram-negative nonsporeforming species could be divided into seven natural groups: group 1, *Desulfovibrio desulfuricans* and other species of this genus that do not degrade fatty acids (this group also included "*Desulfomonas*" *pigra*); group 2, the fatty acid-degrading "*Desulfovibrio*" *sapovorans*; group 3, *Desulfobulbus* species; group 4, *Desulfobacter* species; group 5, *Desulfobacterium* species and "*Desulfococcus*" *niacini*; group 6, *Desulfococcus multivorans* and *Desulfosarcina variabilis*; and group 7, the fatty acid-oxidizing "*Desulfovibrio*" *baarsii*. (The quotation marks are used to indicate the need for taxonomic revision.) Groups 1 to 3 are incomplete oxidizers that form acetate as an end product; groups 4 to 7 are complete oxidizers. The data were consistent with and refined relationships previously inferred by oligonucleotide catalogs of 16S rRNA. Although the determined relationships are generally consistent with the existing classification based on physiology and other characteristics, the need for some taxonomic revision is indicated.

The obligately anaerobic sulfate-reducing bacteria obtain energy for growth by the oxidation of organic compounds or hydrogen and reduction of sulfate, the terminal electron acceptor, to sulfide (16, 21, 23, 33). The first genera described, *Desulfotomaculum* (5) and *Desulfovibrio* (24), comprised mesophilic or moderately thermophilic endospore-forming and nonsporeforming bacteria, respectively. These organisms are nutritionally similar; both incompletely oxidize a number of simple organic compounds (e.g., lactate to acetate), and for that reason the sulfate-reducing bacteria were long thought to comprise a small and nutritionally limited group. However, later isolations revealed much greater morphological and nutritional diversity among these bacteria, including several species capable of completely oxidizing acetate or other organic compounds (18, 22, 33, 35, 38). All isolates were eubacteria. However, the recent isolation of the extremely thermophilic sulfate-reducing archaebacterium *Archaeoglobus fulgidus* (1, 30) has shown that the capacity for dissimilatory sulfate reduction is not restricted to the eubacteria.

Classification of the mesophilic or moderately thermophilic sulfate-reducing bacteria was based on nutrition and morphology and was supported by chemical data such as the G+C content of genomic DNA and the presence of certain types of pigments (23, 33). By these criteria, 11 genera have been established (Table 1).

16S rRNA sequence similarity is now a generally accepted measure of phylogenetic relationships among bacteria and among bacterial groups (9, 39). The initial 16S rRNA-sequencing study of sulfate-reducing eubacteria was based on comparisons of oligonucleotide catalogs (8). That analysis, although lacking the detail of complete sequence comparisons, was on the whole consistent with the existing taxonomic profile based on phenotypic characteristics. *Desulfotomaculum acetoxidans* and *Desulfotomaculum nigrificans* were more closely related to the gram-positive eubacteria than to the nonsporeforming gram-negative sulfate-reducing bacteria. Among the latter, *Desulfovibrio gigas* and *Desulfovibrio desulfuricans* were specifically related and were distinct from the other sulfate-reducing bacteria examined. *Desulfosarcina variabilis* and *Desulfonema limicola* were also closely related, whereas no specific relationship could be established between *Desulfococcus niacini*, *Desulfobulbus propionicus*, and *Desulfobacter postgatei*. Sulfur-reducing bacteria of the genus *Desulfuromonas*, which cannot reduce sulfate, formed a distinct, coherent group within the gram-negative sulfate-reducing bacteria.

16S rRNA cataloging established a specific (but distant) relationship among sulfate-reducing bacteria, myxobacteria, and bdellovibrios (8, 12, 40). More extensive 16S rRNA sequence comparisons assigned the sulfate-reducing bacteria, sulfur-reducing bacteria, myxobacteria, and bdellovibrios to one subdivision (delta) of the four so far defined within the purple photosynthetic bacteria (20, 39). This study describes a more comprehensive phylogenetic analysis of dissimilatory sulfate-reducing bacteria based on near-complete 16S rRNA sequence comparisons.

MATERIALS AND METHODS

Source and growth of strains. The species and sources of sulfate-reducing bacteria examined as well as the cultivation media used are shown in Table 2. All species were grown in anaerobic medium previously described in detail (6). Cells for analyses were grown in 8-liter batch cultures.

Reverse transcriptase 16S rRNA sequencing. Near-complete sequences of 16S rRNAs were determined by the dideoxynucleotide-chain termination procedure, with reverse transcriptase and 16S rRNA as the template (14). Sequencing reactions were primed with synthetic oligonucleotides complementary to conserved 16S rRNA tracts. Primers complementary to the following regions (*Escherichia coli* numbering) were used (primer sequences are in

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TABLE 1. Classified sulfate-reducing eubacteria, including some strains without species designations[a]

Species[b]	16S rRNA group[c]	Form	Mol% G+C	Desulfoviridin[d]	Motility[e]	Major menaquinone[f]	Oxidation[g]	Electron donor[h]								
								Hydrogen	Formate	Lactate	Ethanol	Acetate	Fatty acids (carbon atoms)	Fumarate	Malate	Benzoate
Desulfovibrio																
desulfuricans	1	Vibrio	59	+	+	MK-6	i	+	+	+	+	−	−	+	+	−
vulgaris	1	Vibrio	65	+	+	MK-6	i	+	+	+	(+)	−	−	−	−	−
gigas	1	Large vibrio	65	+	+	MK-6	i	+	+	+	(+)	−	−	+	+	−
africanus		Vibrio	65	+	+	MK-6 (H_2)	i	+	+	+	+	−	−	NR	+	−
salexigens	1	Vibrio	49	+	+	MK-6 (H_2)	i	+	+	+	+	−	−	NR	+	−
sulfodismutans		Vibrio	64	+	+	NR[i]	i	(+)	−	+	+	−	−	−	−	−
carbinolicus		Rod	65	+	−	NR	i	+	+	+	+	−	−	+	+	NR
"*Desulfomonas*" *pigra*	1	Rod	66	+	−	MK-6	i	+	−	+	+	−	−	−	NR	NR
Desulfomicrobium																
baculatum		Short rod	57	−	+	NR	i	(+)	+	+	−	−	−	−	+	NR
apsheronum		Rod	52	−	+	NR	i	+	+	+	−	−	−	+	+	NR
"*Desulfovibrio*" *sapovorans*	2	Vibrio	53	−	+	MK-7	i	−	−	+	−	−	4–16	−	−	−
Desulfobulbus																
propionicus	3	Oval or onion	60	−	±	MK-5 (H_2)	i	+	−	+	+	−	3	−	−	NR
sp. strain 3pr10	3	Ellipsoid	NR	−	+	MK-5 (H_2)	i	+	+	+	+	−	3	NR	NR	NR
elongatus		Rod	59	−	+	MK-5 (H_2)	i	+	−	+	+	−	3	−	NR	NR
Desulfobacter																
postgatei	4	Oval rod	46	−	±	MK-7	c	−	−	−	−	+	−	−	−	−
hydrogenophilus	4	Rod	45	−	−	MK-7 (H_2)	c	+	−	−	(+)	+	−	−	−	−
latus	4	Large oval rod	44	−	±	MK-7	c	−	−	−	−	+	−	−	−	−
curvatus	4	Vibrio	46	−	+	MK-7 (H_2)	c	+	−	−	+	+	−	−	−	−
sp. strain 3ac10	4	Rod	NR	−	±	MK-7 (H_2)	c	−	−	(+)	+	+	−	NR	NR	NR
sp. strain 4ac11	4	Rod	NR	−	+	MK-7	c	−	−	(+)	−	+	−	NR	NR	NR
Desulfococcus multivorans	6	Sphere	57	+	−	MK-7	c	−	+	+	+	(+)	3–16	−	−	+
Desulfosarcina variabilis	6	Oval rod, packages	51	−	±	MK-7	c	+	+	+	+	(+)	3–14	+	−	+
Desulfobacterium																
autotrophicum	5	Oval rod	48	−	+	MK-7	c	+	+	+	+	(+)	(3)–16	+	+	−
vacuolatum	5	Oval rod or sphere	45	−	−	MK-7 (H_2)	c	+	+	+	(+)	(+)	(3)–16	+	+	−
phenolicum		Oval rod	41	−	+	MK-7 (H_2)	c	−	(+)	−	(+)	(+)	(4)	(+)	(+)	+
indolicum		Oval rod	47	−	+	MK-7 (H_2)	c	−	(+)	−	(+)	(+)	(3)	(+)	(+)	−
catecholicum		Lemon shape	52	−	−	NR	c	(+)	(+)	(+)	(+)	(+)	(3–20)	(+)	(+)	+
niacini (formerly *Desulfococcus*)	5	Irregular sphere	46	−	+	MK-7	c	+	+	−	+	(+)	(3)–16	+	+	−
Desulfonema																
limicola		Filament	35	+	g	MK-7	c	+	+	+	−	(+)	3–14	+	−	−
magnum		Filament	42	−	g	MK-9	c	−	+	−	−	(+)	3–10	+	(+)	+
"*Desulfovibrio*" *baarsii*	7	Vibrio	66	−	+	MK-7 (H_2)	c	−	+	−	−	+	(3)–18	−	−	−
Desulfotomaculum																
nigrificans		Rod	49	−	+	MK-7	i	+	+	+	+	−	−	−	−	−
orientis	Gr+	Slightly curved rod	45	−	+	MK-7	i	+	+	+	+	−	−	−	−	−
ruminis	Gr+	Rod	49	−	+	MK-7	i	+	+	+	+	−	−	−	−	−
antarticum		Rod	NR	−	+	NR	i	NR	−	+	NR	−	−	NR	NR	NR
acetoxidans		Slightly curved rod	38	−	+	MK-7	c	−	−	−	+	+	4–5	−	−	−
guttoideum		Rod	52	−	+	NR	i	+	−	+	−	−	NR	NR	−	NR
sapomandens		Rod	48	−	+	NR	c	NR	+	+	+	(+)	4–18	(+)	(+)	+
kuznetsovii		Rod	49	−	+	NR	c	+	+	+	+	+	3–18	+	+	−
Thermodesulfobacterium																
commune		Rod	34	−	−	MK-7	i	+	NR	+	−	−	−	NR	−	NR
mobilis		Rod	38	−	+	MK-7	i	+	+	+	−	−	−	NR	NR	NR

[a] For references, see text and references 22, 23, 33, and 38. (Recent classifications or reclassifications are given in references 18, 25, and 26.)
[b] Rearrangements in the classification as inferred from this study are included. Because "*Desulfovibrio*" *sapovorans* and "*Desulfovibrio*" *baarsii* represent separate lineages, they were removed from the genus *Desulfovibrio*. "*Desulfococcus*" *niacini* is affiliated with the genus *Desulfobacterium*.
[c] Groups 1 to 7 are defined in text. Gr+, affiliated with gram-positive eubacteria.
[d] +, Present; −, absent.
[e] +, Motile; −, nonmotile; g, gliding motility.
[f] After reference 6; (H_2) indicates a terminal saturation in the isoprenoid side chain.
[g] c, Complete; i, incomplete.
[h] +, Utilized; (+), poorly or slowly utilized; −, not utilized.
[i] NR, Not reported.

TABLE 2. Strains of sulfate-reducing bacteria used and conditions of cultivation[a]

Species	Source[b]	Growth substrate	Medium
Desulfovibrio			
vulgaris Hildenborough	DSM644	Lactate	Freshwater
gigas	ATCC 19364	Lactate	Freshwater
salexigens	ATCC 14822	Lactate	Marine
sapovorans 1pa3	ATCC 33892	Lactate	Freshwater
baarsii 2st14	DSM 2075	Butyrate + caproate	Freshwater
Desulfomonas pigra	ATCC 29098	Lactate	Freshwater
Desulfococcus multivorans	ATCC 33890	Lactate	Freshwater
Desulfobacterium ("*Desulfococcus*") *niacini*	DSM 2650	Ethanol	Marine
Desulfosarcina variabilis	DSM 2060	Lactate	Marine
Desulfobacterium			
vacuolatum	DSM 3385	Ethanol	Marine
autotrophicum	DSM 3382	Malate	Marine
Desulfobulbus			
propionicus 1pr3	ATCC 33891	Lactate	Freshwater
sp. strain 3pr10	DSM 2058	Lactate	Marine
Desulfobacter			
hydrogenophilus	DSM 3380	Acetate	Marine
curvatus	DSM 3379	Ethanol	Marine
lactus	DSM 3381	Acetate	Marine
sp. strain 3ac10	DSM 2035	Acetate	Marine
sp. strain 4ac11	DSM 2057	Acetate	Marine
Desulfotomaculum			
orientis Singapore I	NCIMB 8382	Lactate	Freshwater
ruminis DL	NCIMB 8452	Lactate	Freshwater

[a] Cells were grown anaerobically in the presence of sulfate as described in detail previously (6). Concentrations of NaCl and $MgCl_2 \cdot 6H_2O$ in freshwater and marine medium were 1.0 and 0.5 g/liter, respectively, in freshwater medium and 20.0 and 3.0 g/liter, respectively, in marine medium.

[b] DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Federal Republic of Germany; ATCC, American Type Culture Collection, Rockville, Md.; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

parentheses; brackets indicate mixed position): 350 (CTG CTGC[GC][CT]CCCGTAG), 536 (ACCGCGGC[GT]GCTG GC), 691 (GAT[AC]TCTACG[GA]ATTTCAC), 915 (GCC CCC[TC]CAATTCCT), 1110 (AGGGTTGCGCTCGTTG), and 1400 (ACGGGCGGTGTGT[GA]C). The 691-region primer was modified from an earlier primer, 690 (TCTACGC ATTTCACC), and is generally applicable to sequencing 16S rRNAs of delta-subdivision bacteria. The sequences are available through GenBank or may be requested from one of the authors.

Construction of phylogenetic trees. Percent similarities and evolutionary distances were calculated between pairs of aligned sequences as previously described (19). Nucleotide positions for which any sequence had an ambiguous or undetermined base were eliminated from the calculations. Trees were constructed from distance matrices by the least-squares method (7).

RESULTS AND DISCUSSION

Phylogenetic analyses. Similarities among pairs of 16S rRNA sequences from sulfate-reducing bacteria are shown in Table 3. The lower portion of the table was obtained by using about 650 comparable positions, and the upper portion was obtained by using about 1,050 comparable positions. The reverse transcriptase-generated transcripts of 16S rRNAs from species related to *Desulfovibrio desulfuricans* terminated at nucleotide position 972 (*E. coli* numbering). This was likely due to a modified nucleotide in this region of the 16S rRNA template. Similarly, a sequencing gap occurred between positions 510 and 675 of the *Desulfococcus niacini* sequence. Elimination of the truncated sequences and several others adds some 400 comparable nucleotide positions to the calculation of similarity among the remaining sequences and therefore better estimates phylogenetic relationships. Nevertheless, similarity values between the two calculations generally differed by no more than 2%.

Evolutionary distance estimates were calculated from the values given in the lower portion of Table 3. An optimized (19) phylogenetic tree constructed from these evolutionary distances is shown in Fig. 1. Use of the more extensive sequence comparisons (upper portion of Table 3) did not alter the inferred relationships of gram-negative sulfate-reducing bacteria. The branching point of *Myxococcus xanthus* fell within the radiation of sulfate-reducing bacteria in both instances. However, given this short segment length, the position of the myxobacteria relative to that of the sulfate-reducing bacteria should be considered uncertain.

Natural groupings and phenotypic relationships. For convenience of discussion, the sulfate-reducing bacteria are divided into sporeforming and nonsporeforming types and then further grouped according to the major physiological characteristics that conform to the emerging phylogeny. At present, however, these divisions should be treated as an organizational scheme and not as a formal determinative hierarchy. The reader is referred to the phylogenetic tree (Fig. 1) and the morphological, nutritional, and biochemical characteristics of sulfate-reducing bacteria (Table 1).

Endospore-forming sulfate-reducing bacteria. The 16S rRNA sequences of *Desulfotomaculum ruminis* and *Desulfotomaculum orientis* grouped with those from gram-positive eubacteria represented in the tree by *Bacillus subtilis* (Fig. 1). This relationship had been established by the earlier cataloging study (8). Although other *Desulfotomaculum* species are clearly affiliated with the gram-positive eubacteria (given the rRNA similarity and their characteristic trait of spore formation), the genus is likely to be very diverse; *D. ruminis* and *D. orientis* shared but 83% sequence similarity. Indeed, the inferred relationship is one of independent lineages within the gram-positive eubacteria and not one of sister groups with a common origin. Whether there is more than one point of origin for endospore-forming sulfate-reducing bacteria within the gram-positive eubacteria will be clarified when additional 16S rRNA sequences of *Desulfotomaculum* species and gram-positive bacteria become available. Diversity among *Desulfotomaculum* species was previously indicated by comparative 16S rRNA cataloging of *Desulfotomaculum acetoxidans* and *Desulfotomaculum nigrificans* (8) and by the relatively wide range of G+C values (38 to 52 mol%) among *Desulfotomaculum* species (Table 1).

Gram-negative sulfate-reducing bacteria. The nonsporeforming sulfate-reducing bacteria were gram-negative organisms that could be assigned to seven natural groups (lines of descent). These groups were defined with consideration of phenotypic traits and the emerging phylogeny. Therefore, they are independent of any a priori assigned value of sequence similarity as a group delimiter. This classification is in accordance with the recommendation by the International Committee on Systematic Bacteriology that "any phylogenetically based taxonomic schemes that result must also show phenotypic consistency" (31).

TABLE 3. 16S rRNA sequence similarities[a]

Organism	1	2	3	4	5	6	7	8	9	10	11
1. *Escherichia coli*		0.782	0.803					0.792	0.802	0.791	
2. *Myxococcus xanthus*	0.786		0.810					0.815	0.832	0.831	
3. *Desulfovibrio desulfuricans*	0.794	0.824						0.827	0.829	0.825	
4. "*Desulfomonas*" *pigra*	0.784	0.808	0.945								
5. *Desulfovibrio vulgaris*	0.794	0.816	0.913	0.906							
6. *D. salexigens*	0.777	0.804	0.879	0.868	0.897						
7. *D. gigas*	0.790	0.808	0.881	0.877	0.872	0.872					
8. "*Desulfovibrio*" *sapovorans*	0.785	0.829	0.836	0.811	0.838	0.815	0.820		0.851	0.837	
9. "*Desulfovibrio*" *baarsii*	0.792	0.836	0.833	0.811	0.850	0.824	0.835	0.863		0.844	
10. *Desulfobulbus propionicus*	0.776	0.831	0.827	0.816	0.827	0.823	0.834	0.844	0.847		
11. *Desulfobulbus* sp. strain 3pr10	0.780	0.832	0.815	0.816	0.822	0.822	0.838	0.829	0.837	0.918	
12. *Desulfosarcina variabilis*	0.785	0.832	0.836	0.824	0.841	0.825	0.836	0.884	0.860	0.853	0.858
13. *Desulfococcus multivorans*	0.795	0.833	0.837	0.827	0.828	0.818	0.826	0.898	0.866	0.832	0.849
14. *Desulfobacterium niacini*	0.772	0.820	0.821	0.819	0.820	0.823	0.819	0.878	0.827	0.833	0.848
15. *D. vacuolatum*	0.771	0.827	0.828	0.822	0.823	0.826	0.821	0.879	0.834	0.835	0.850
16. *D. autotrophicum*	0.793	0.839	0.840	0.826	0.835	0.828	0.832	0.893	0.845	0.846	0.859
17. *Desulfobacter postgatei*	0.791	0.830	0.833	0.822	0.815	0.823	0.830	0.871	0.839	0.855	0.856
18. *D. latus*	0.790	0.817	0.832	0.825	0.816	0.819	0.822	0.867	0.842	0.845	0.855
19. *D. curvatus*	0.787	0.806	0.826	0.816	0.811	0.808	0.812	0.856	0.827	0.838	0.850
20. *D. hydrogenophilus*	0.786	0.825	0.837	0.825	0.820	0.822	0.824	0.871	0.840	0.851	0.856
21. *Desulfobacter* sp. strain 3ac10	0.782	0.822	0.834	0.820	0.816	0.817	0.819	0.866	0.838	0.849	0.854
22. *Desulfobacter* sp. strain 4ac11	0.780	0.809	0.826	0.822	0.814	0.817	0.818	0.861	0.834	0.847	0.859
23. *Desulfotomaculum ruminis*	0.765	0.801	0.801	0.793	0.790	0.792	0.804	0.793	0.784	0.784	0.793
24. *D. orientis*	0.773	0.804	0.808	0.795	0.799	0.798	0.794	0.800	0.792	0.793	0.809
25. *Bacillus subtilis*	0.758	0.793	0.791	0.785	0.801	0.785	0.801	0.797	0.790	0.787	0.790

[a] Values were determined from about 650 (lower left) and 1,050 (upper right) unambiguous positions.
[b] References for published sequences: *E. coli* (3), *M. xanthus* (20), *D. desulfuricans* (20), *B. subtilis* (11).

Each line is composed of only either incompletely or completely oxidizing species. The lines of incomplete oxidizers are (quotations indicate those genera requiring taxonomic revision): group 1, *Desulfovibrio desulfuricans* and related species; group 2, "*Desulfovibrio*" *sapovorans*; and group 3, two *Desulfobulbus* species. The completely oxidizing lineages are: group 4, six species of *Desulfobacter*; group 5, *Desulfobacterium* species and "*Desulfococcus*" *niacini*; group 6, *Desulfococcus multivorans* and *Desulfosarcina variabilis*; and group 7, "*Desulfovibrio*" *baarsii*.

Each of the three groups of incompletely oxidizing species contains a unique major menaquinone. *Desulfovibrio desul-*

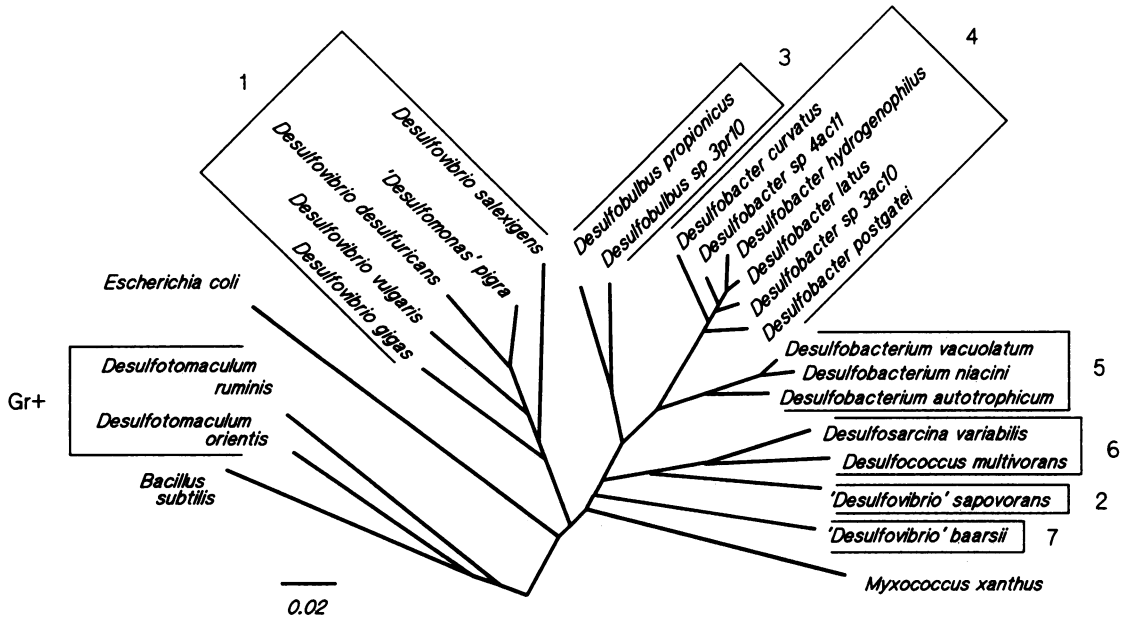

FIG. 1. 16S rRNA phylogenetic tree of sulfate-reducing eubacteria. The tree was constructed by the least-squares method (7), using distance measurements determined from the values in the lower portion of Table 3. Enclosed species comprise the indicate line of descent as discussed in the text. The 16S rRNA sequence of the archaebacterium *Halococcus morrhuae* (15) was used as the outgroup to root the tree. The scale bar is in units of fixed nucleotide substitutions per sequence position.

TABLE 3—*Continued*

12	13	14	15	16	17	18	19	20	21	22	23	24	25
0.799	0.803		0.772	0.792	0.788		0.780	0.784	0.779	0.774			0.769
0.825	0.824		0.815	0.828	0.816		0.796	0.811	0.805	0.795			0.784
0.828	0.825		0.825	0.832	0.823		0.813	0.825	0.821	0.819			0.788
0.879	0.880		0.870	0.881	0.857		0.843	0.861	0.851	0.851			0.789
0.857	0.863		0.825	0.831	0.824		0.809	0.825	0.816	0.817			0.796
0.862	0.834		0.828	0.843	0.846		0.829	0.841	0.837	0.835			0.790
	0.924		0.881	0.893	0.872		0.854	0.869	0.859	0.854			0.802
0.923			0.877	0.880	0.863		0.849	0.860	0.855	0.853			0.797
0.890	0.887												
0.890	0.892	0.979		0.944	0.889		0.881	0.893	0.888	0.886			0.781
0.899	0.900	0.947	0.950		0.896		0.889	0.909	0.898	0.896			0.784
0.883	0.878	0.897	0.901	0.911			0.948	0.959	0.969	0.948			0.782
0.874	0.877	0.898	0.900	0.913	0.963								
0.865	0.868	0.891	0.894	0.902	0.954	0.957		0.951	0.952	0.945			0.762
0.879	0.876	0.903	0.901	0.917	0.962	0.979	0.950		0.974	0.972			0.766
0.875	0.875	0.894	0.899	0.911	0.971	0.976	0.959	0.977		0.966			0.762
0.865	0.871	0.889	0.891	0.902	0.951	0.982	0.951	0.974	0.967				0.760
0.791	0.791	0.781	0.783	0.794	0.784	0.780	0.778	0.781	0.778	0.775			
0.804	0.812	0.790	0.793	0.802	0.792	0.787	0.776	0.891	0.789	0.782	0.831		
0.805	0.794	0.774	0.775	0.779	0.777	0.758	0.757	0.766	0.760	0.755	0.813	0.839	

furicans and related species contain menaquinone MK-6, *Desulfobulbus* species contain menaquinone MK-5, and "*Desulfovibrio*" *sapovorans* has menaquinone MK-7 (6). The major menaquinone in the lines of completely oxidizing species is MK-7 (6). On a deeper level of relationship, "*Desulfovibrio*" *sapovorans* groups with the complete oxidizers (Fig. 1), to which it also appears biochemically related by the presence of MK-7.

The thermophilic eubacterium *Thermodesulfobacterium commune* (41) was not included in this study. Partial sequencing data suggest that this species, though metabolically similar to *Desulfovibrio desulfuricans*, is a separate, deep branch within the eubacteria and not specifically related to other groups of sulfate-reducing bacteria (C. R. Woese, personal communication). *Desulfovibrio thermophilus* (nutritionally and phylogenetically resembling *T. commune*) has been recently reclassified as *Thermodesulfobacterium mobile* (26).

Notable aspects of phenotypic and phylogenetic relationships among the sulfate-reducing bacteria characterized are addressed below.

***Desulfovibrio* species.** Species determined to be related to *Desulfovibrio desulfuricans* were *Desulfovibrio gigas*, *Desulfovibrio salexigens*, *Desulfovibrio vulgaris*, and the physiologically very similar "*Desulfomonas*" *pigra* (17). This lineage was found to be phylogenetically more diverse than the other lineages of sulfate-reducing bacteria characterized (minimum similarity value, 87%; Table 3). Similarity values within the group may be as low as those separating other genera of sulfate-reducing bacteria, yet the lineage is coherent and its members are phenotypically similar (Table 1). Earlier 16S rRNA cataloging data already had suggested that some *Desulfovibrio* species, defined by our studies as those closely related to *D. desulfuricans*, form a coherent and exclusive assemblage of sulfate-reducing bacteria (8).

The "*Desulfovibrio*" *baarsii* and "*Desulfovibrio*" *sapovorans* lineages arise outside the *Desulfovibrio desulfuricans* group. This phylogenetic diversity is also consistent with the metabolic features of these separate lines (Table 1). Because they cannot be readily affiliated with existing genera of sulfate-reducing bacteria, the establishment of new genera is indicated. However, taxonomic revision should include the phylogenetic analysis of additional, similar fatty acid-degrading isolates. Thus, for the present, we will use the old genus names in quotation marks to indicate that both "*Desulfovibrio*" *sapovorans* and "*Desulfovibrio*" *baarsii* represent lines of descent distinct from other *Desulfovibrio* species and are designated for future reclassification.

The remaining groups of gram-negative sulfate-reducing bacteria, as defined here, formed more closely related clusters than does the *Desulfovibrio desulfuricans* group. Organisms within these groups shared at least 90% 16S rRNA sequence similarity. Similarity values among members of the seven groups ranged from 81 to 90% (Table 3).

***Desulfobulbus* species.** The genus *Desulfobulbus* was established for oval- or lemon-shaped sulfate-reducing bacteria that incompletely oxidize propionate as the characteristic substrate on which they are enriched (37). The two isolates characterized by 16S rRNA sequence analysis, *Desulfobulbus propionicus* and *Desulfobulbus* sp. strain 3pr10, shared 92% sequence similarity and no more than 86% sequence similarity with any member of other groups (Table 3). The relatively low sequence similarity between the 16S rRNAs of the two *Desulfobulbus* species indicates that this phenotypically homogeneous group may be phylogenetically more diverse than is either the genus *Desulfobacterium* (95% minimum similarity) or the genus *Desulfobacter* (95% minimum similarity).

***Desulfobacter* species.** The six representatives of the *Desulfobacter* genus were found to constitute a closely related group, with members sharing at least 95% sequence similarity (Table 3). This result is in good agreement with the original classification. *Desulfobacter* species are defined by their ability to grow well on acetate and limited (or no) ability to use other organic substrates (32, 36). They all have rather similar G+C contents (Table 1). *Desulfobacter* species so far

investigated oxidize acetyl coenzyme A via the citric acid cycle (10, 28). In contrast, completely oxidizing sulfate-reducing bacteria of other genera degrade acetyl coenzyme A via a cleavage by carbon monoxide dehydrogenase and oxidation of the methyl group bound to tetrahydropterine (27).

***Desulfobacterium* species and "*Desulfococcus*" niacini.** *Desulfobacterium vacuolatum* and "*Desulfococcus*" *niacini* were found to be very closely related (98% sequence similarity; Table 3). "*D.*" *niacini* was placed within the genus *Desulfococcus* (*D. multivorans* is the only other described species of *Desulfococcus*) primarily on consideration of morphology and substrate versatility some years before the genus *Desulfobacterium* was established (2, 4, 13). However, with the data obtained in this study, reclassification of "*Desulfococcus*" *niacini* as *Desulfobacterium niacini* is appropriate. This classification would also agree with the G+C values; the G+C value for *D. niacini* is within the values for *Desulfobacterium* species but differs from the value for *D. multivorans* (Table 3). Morphology, as previously observed for many other genera, is not a reliable trait for establishing relationships among the sulfate-reducing bacteria (8, 9).

Desulfobacterium autotrophicum 16S rRNA shared 95% sequence similarity with those from *D. vacuolatum* and *D. niacini* (Table 3). Thus, the comparison of these three species suggests that the genus *Desulfobacterium* represents a coherent assemblage.

Desulfobacterium and *Desulfobacter* species together constituted a larger, phylogenetically coherent assemblage (Fig. 1 and Table 3). The two groups of organisms radiated from a common line and were related by as much as 90% sequence similarity. The moles percent G+C contents among species of the two groups was also quite similar. However, *Desulfobacterium* species were phenotypically distinguished from *Desulfobacter* species in possessing a more versatile nutritional capability, particularly an ability to utilize formate, fumarate, malate, butyrate, and higher-carbon-chain fatty acids (Table 1).

Conclusions. The nutritional versatility of the sulfate-reducing bacteria is now well appreciated. The phylogenetic diversity yet coherence of sulfate-reducing bacteria was first revealed by 16S rRNA cataloging (8, 38). This study has established relationships among the genera of sulfate-reducing bacteria by using the precision of near-complete 16S rRNA sequence comparisons. The relationships inferred by oligonucleotide cataloging (8) were substantiated.

The genealogical relationships are, on the whole, consistent with groups established by physiological and biochemical criteria. In particular, there is a correlation between the grouping by 16S rRNA sequencing and the menaquinone composition (Table 3 and Fig. 1). Nevertheless, some unexplained phenotypic and phylogenetic incongruencies remain. For example, the bisulfite reductase desulfoviridin occurs both in the *Desulfovibrio desulfuricans* group and in the distantly related, metabolically distinct *Desulfococcus multivorans* and *Desulfonema limicola* (8, 22, 33, 34, 38). A phylogenetic association that awaits clarification of metabolic evolution is the relationship between sulfate-reducing bacteria and the aerobic *Myxococcus* and *Bdellovibrio* species (8, 20, 39).

The phylogenetic analysis has also pointed up candidates for reclassification. These are "*Desulfovibrio*" *baarsii* and "*Desulfovibrio*" *sapovorans* (as new genera), "*Desulfomonas*" *pigra* (as *Desulfovibrio*), and "*Desulfococcus*" *niacini* (as *Desulfobacterium*). The suggested reclassifications are consistent with nutritional features and also, in the case of the latter species, with the G+C content (Table 1).

Dissimilatory sulfate reduction is not limited to the eubacteria or to one assemblage within the eubacteria. Nevertheless, although phylogenetically diverse, the trait is of relatively restricted occurrence. Among mesophilic gram-negative sulfate-reducing species, this life style is restricted to a remarkably coherent assemblage within the delta subdivision of the purple bacteria (proteobacteria). As a contrasting analogy, dissimilatory nitrate reduction is widespread. Although this feature is suggestive of a complex and highly adapted life style, better understanding of the limited phylogenetic distribution of dissimilatory sulfate reduction will likely await more detailed biochemical, comparative, and environmental studies.

This study also serves to address environmental diversity. 16S rRNA targeted oligonucleotide hybridization probes, circumscribing phylogenetically defined bacterial groups, have proven useful in characterizing natural populations of microorganisms, in particular those difficult to isolate and identify (29). The 16S rRNA sequences compiled during this study provide a data base for defining rRNA sequences unique to individual species and groups of sulfate-reducing bacteria (R. Devereux and D. A. Stahl, manuscript in preparation). A natural classification based on explicit nucleic acid sequence characters should serve as a foundation for the continued exploration of the ecological, phylogenetic, and metabolic diversity of these organisms.

ACKNOWLEDGMENTS

We thank C. R. Woese for providing the 16S rRNA sequence of *D. postgatei*.

This work was supported by grant EPA-CR-812496 from the U.S. Environmental Protection Agency and in part by grant DE-FG02-86ER 13651 to R. S. Wolfe. F. Widdel was a Feodor Lynen Fellow of the Alexander von Humbolt-Stiftung during a portion of this study.

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