Phylogenetic Analysis of Five Strains of Gram-Negative, Obligately Anaerobic, Sulfur-Reducing Bacteria and Description of *Desulfuromusa* gen. nov., Including *Desulfuromusa kysingii* sp. nov., *Desulfuromusa bakii* sp. nov., and *Desulfuromusa succinoxidans* sp. nov.

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Gram-negative, mesophilic, obligately anaerobic strains Kysw2^T (T = type strain), Kyval, and Kyprop isolated from an anoxic mud sample obtained from the Kysing Fjord south of Århus (Denmark) and strains Gyprop^T and Gylac^T isolated from the Guayamas Basin (Gulf of California) all exhibit complete oxidation of a wide range of electron donors (e. g., dicarboxylic acids and amino acids) linked to stoichiometric reduction of elemental sulfur to hydrogen sulfide. A comparative 16S ribosomal DNA sequence analysis revealed that these five strains, together with a previously described isolate (strain Gö11), constitute a coherent cluster of descent. This cluster belongs phylogenetically to a branch of the delta subclass of the *Proteobacteria* and is characterized by members of the genera *Pelobacter* and *Desulfuromonas*. Within the cluster strains Kysw2^T and Kyval, strains Gyprop^T and Kyprop, and strains Gylac^T and Gö11 have identical 16S ribosomal DNA sequences. The levels of DNA-DNA relatedness were 89% between strains Kysw2^T and Kyval, 98% between strains Gyprop^T and Kyprop, and 73% between strains Gylac^T and Gö11. The levels of DNA-DNA relatedness between members of the three DNA relatedness groups were less than 30%. On the basis of genomic data and phenotypic characteristics, a new genus, *Desulfuromusa*, is described; this new genus includes three new species, for which the names *Desulfuromusa kysingii*, *Desulfuromusa bakii*, and *Desulfuromusa succinoxidans* are proposed. The type species of the genus *Desulfuromusa* is *D. kysingii*. Strains Kysw2 (= DSM 7343), Gyprop (= DSM 7345), and Gylac (= DSM 8270) are the type strains of *D. kysingii*, *D. bakii*, and *D. succinoxidans*, respectively.

A number of microorganisms belonging to various genera exhibit growth-linked sulfur respiration. Sulfur reducers that are affiliated with the genus *Campylobacter* (27), *Sulfurospirillum deleyianum* (27, 33), *Shewanella putrefaciens* (19), *Pseudomonas mendocina* (1), and the sulfur-utilizing sulfate-reducing members of the genus *Desulfomicrobium* (2) are bacteria that incompletely oxidize substrates and form acetate as an end product. Members of the genus *Desulfurella* are thermophilic sulfur-reducing bacteria that are capable of complete oxidation of substrates (3, 18, 23). The only known mesophilic sulfur reducers that completely oxidize substrates belong to the genus *Desulfuromonas* (10, 21, 31). In a recent study Finster and Bak described the isolation of five strains of gram-negative, mesophilic, obligately anaerobic bacteria from anoxic mud samples collected at the shallow Kysing Fjord south of Århus (Denmark) (strains Kysw2^T [T = type strain], Kyval, and Kyprop) and at the Guayamas Basin (Gulf of California) from a depth of about 2,000 m (strains Gyprop^T and Gylac^T) (9). These isolates all completely oxidize acetate, propionate, and a range of dicarboxylic acids and amino acids (e. g., succinate, maleate, alanine, and aspartate) and substrate oxidation is coupled to concomitant stoichiometric reduction of elemental sulfur to hydrogen sulfide. Compared with the five new isolates, *Desulfuromonas* spp. use a rather limited range of substrates; acetate, ethanol, propanol, butanol, betaine, and pyruvate are

utilized by some strains as electron donors. To date, *Desulfuromonas acetoxidans* and *Desulfuromonas acetexigens* are the only validly described species of this genus (10, 21). More species of this genus have been isolated, but these bacteria have only been mentioned with provisional names in review papers (for example, “*Desulfuromonas thiophila*” and “*Desulfuromonas succinoxidans*”) (22, 30, 31).

In this paper we describe the results of a comparative 16S ribosomal DNA (rDNA) sequence analysis performed to elucidate the phylogenetic affiliation of the five new isolates, as well as the results of DNA-DNA relatedness studies. In addition, the 16S rDNA sequences of “*Desulfuromonas succinoxidans*” Gö11 and *Pelobacter carbinolicus* GraBd1^T (24) were determined for reference purposes. On the basis of phylogenetic data and phenotypic characteristics the new genus *Desulfuromusa* is described below. The results of our DNA-DNA relatedness studies indicated that the five new isolates and strain Gö11 are members of three genomic species belonging to the genus *Desulfuromusa*, for which we propose the names *Desulfuromusa kysingii*, *Desulfuromusa bakii*, and *Desulfuromusa succinoxidans*.

MATERIALS AND METHODS

Bacterial strains. Strains Kysw2^T (= DSM 7343^T) (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), Kyval (= DSM 7344), Gyprop^T (= DSM 7345^T), Kyprop, and Gylac^T (= DSM 8270^T) were isolated and cultivated as described by Finster and Bak (9). Strain Gö11 was obtained from N. Pfennig,

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TABLE 1. Similarity matrix based on 16S rRNA sequence data

Species	% Similarity or % difference ^a																					
	<i>Desulfuromusa kysingii</i>	<i>Desulfuromusa bakii</i>	<i>Desulfuromusa succinoxidans</i>	<i>Pelobacter acidigallici</i>	<i>Pelobacter acetylenicus</i>	<i>Pelobacter carbinolicus</i>	<i>Desulfuromonas acetoxidans</i>	<i>Geobacter metallireducens</i>	<i>Myxococcus xanthus</i>	<i>Desulfobacter postgatei</i>	<i>Desulfobacterium vacuolatum</i>	<i>Desulfococcus multivorans</i>	<i>Desulfosarcina variabilis</i>	<i>Desulfomicrobium baculatum</i>	<i>Desulfovibrio desulfuricans</i>	<i>Brucella abortus</i>	<i>Escherichia coli</i>	<i>Pseudomonas testosteroni</i>	<i>Desulfurella acetivorans</i>	<i>Wolinella succinogenes</i>	<i>Bacillus subtilis</i>	
<i>Desulfuromusa kysingii</i>		99.5	98.7	96.2	94.7	94.2	93.2	93.0	87.3	87.3	86.6	88.4	89.0	88.0	87.2	85.9	86.0	83.2	84.1	82.6	83.6	
<i>Desulfuromusa bakii</i>	0.5		98.7	96.2	94.6	94.2	93.0	93.0	87.2	87.5	86.7	88.2	88.7	88.1	87.2	86.0	85.7	83.1	84.1	82.7	83.6	
<i>Desulfuromusa succinoxidans</i>	1.3	1.3		96.3	94.4	94.0	93.6	92.8	87.2	87.3	86.9	88.3	89.4	88.1	87.0	86.1	85.8	83.5	84.6	82.8	84.0	
<i>Pelobacter acidigallici</i>	3.9	3.9	3.7		94.4	94.2	93.1	93.2	87.1	87.5	86.9	88.5	89.7	88.2	86.6	86.5	85.6	82.4	84.1	83.1	82.9	
<i>Pelobacter acetylenicus</i>	5.5	5.6	5.9	5.9		98.4	94.8	93.7	88.6	88.6	86.6	89.3	89.0	88.7	87.5	85.8	85.8	82.5	84.4	81.5	84.9	
<i>Pelobacter carbinolicus</i>	6.1	6.1	6.3	6.1	1.6		94.8	93.5	88.6	88.2	86.6	88.6	88.6	88.5	87.1	85.8	85.8	82.4	83.7	81.2	85.5	
<i>Desulfuromonas acetoxidans</i>	7.1	7.3	6.6	7.2	5.4	5.4		93.5	88.5	88.1	86.1	88.1	88.3	87.8	87.0	84.6	85.5	82.6	84.3	82.3	85.6	
<i>Geobacter metallireducens</i>	7.3	7.3	7.6	7.1	6.5	6.8	6.8		87.6	87.8	86.3	89.6	89.3	87.9	86.0	84.2	85.4	83.1	83.5	82.5	84.8	
<i>Myxococcus xanthus</i>	13.9	14.1	14.1	14.2	12.3	12.3	12.4	13.6		84.4	82.5	84.9	85.2	84.5	83.7	82.6	83.1	81.3	81.5	80.7	82.9	
<i>Desulfobacter postgatei</i>	13.9	13.7	13.9	13.7	12.3	12.8	12.9	13.3	17.5		91.1	89.0	90.3	86.8	85.1	81.5	82.5	81.7	82.0	80.3	80.8	
<i>Desulfobacterium vacuolatum</i>	14.8	14.7	14.4	14.4	14.8	14.8	15.3	15.1	19.9	9.4		89.3	90.0	85.4	84.2	81.8	81.7	79.8	81.2	80.4	80.0	
<i>Desulfococcus multivorans</i>	12.6	12.8	12.7	12.4	11.6	12.3	12.9	11.2	16.9	11.9	11.6		94.6	87.3	85.3	83.1	83.7	79.8	82.4	81.0	82.0	
<i>Desulfosarcina variabilis</i>	11.9	12.2	11.5	11.1	11.9	12.3	12.7	11.6	16.5	10.4	10.7	5.6		87.1	84.7	83.6	83.7	80.8	82.0	81.3	82.2	
<i>Desulfomicrobium baculatum</i>	13.1	12.9	12.9	12.8	12.2	12.4	13.3	13.2	17.4	14.6	16.2	13.9	14.2		90.6	85.0	84.4	82.2	83.5	82.7	82.5	
<i>Desulfovibrio desulfuricans</i>	14.1	14.1	14.3	14.8	13.7	14.2	14.3	15.4	18.3	16.6	17.8	16.4	17.1	10.0		83.1	83.3	80.3	83.2	83.3	82.8	
<i>Brucella abortus</i>	15.6	15.4	15.3	14.9	15.7	15.7	17.3	17.8	19.8	21.3	20.9	19.1	18.5	16.7	19.1		84.0	82.1	80.6	80.2	81.9	
<i>Escherichia coli</i>	15.4	15.8	15.7	16.0	15.7	15.7	16.1	16.2	19.1	19.9	21.0	18.3	18.3	17.5	18.9	18.1		84.0	79.4	81.0	81.6	
<i>Pseudomonas testosteroni</i>	19.0	19.1	18.6	20.1	19.9	20.1	19.8	19.1	21.4	21.0	23.6	23.6	22.1	20.3	22.8	20.5	18.1		80.2	80.3	79.9	
<i>Desulfurella acetivorans</i>	17.9	17.9	17.3	17.9	17.5	18.3	17.7	18.6	21.3	20.6	21.6	20.1	20.6	18.6	19.0	22.4	24.1	23.0		81.2	79.9	
<i>Wolinella succinogenes</i>	19.8	19.7	19.5	19.1	21.3	21.6	20.2	19.9	22.3	22.8	22.7	21.9	21.4	19.7	18.9	23.0	21.9	22.8	21.6		80.0	
<i>Bacillus subtilis</i>	18.5	18.5	18.1	19.4	16.9	16.1	16.0	17.0	19.4	22.1	23.3	20.6	20.3	19.9	19.5	20.8	21.2	23.4	23.4	23.3		

^a The values on the upper right are uncorrected percentages of similarity, and the values on the lower left are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor (16).

University of Konstanz; this strain was originally isolated by Bache and Pfennig from sediment obtained from the Jadebusen (North Sea, near Wilhelmshaven, Germany) (11, 31; unpublished data) and cultivated as described above. *Pelobacter carbinolicus* GraBd1^T (= DSM 2380^T) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; the lyophilized cell material which was delivered was used directly for isolation of genomic DNA (32).

DNA isolation, PCR amplification, and sequencing analysis. Cells were lysed by incubating them in 2.5% (wt/vol) sodium dodecyl sulfate for 20 min at 60°C. DNA was extracted from each crude lysate with a Prep-A-Gene kit as described in the instructions supplied by the manufacturer (BioRad, Munich, Germany). The PCR was performed by using 16S rDNA primers (17) which amplify the 16S rRNA genes from position 28 to position 1491 (International Union of Biochemistry [IUB] nomenclature for *Escherichia coli*) of a broad range of microorganisms belonging to the domain *Bacteria*. The thermal profile used for amplification included 29 cycles of primer annealing at 48°C for 1 min, primer extension at 72°C for 2 min, and denaturation at 94°C for 0.5 min. The aqueous phases obtained from three PCR were combined, and the volume was brought to 2.5 ml with sterilized distilled water. The PCR products were purified for subsequent sequence analysis with Centricon 100 columns as described by the manufacturer (Amicon, Witten, Germany). Sequencing reactions were performed nonradioactively by using a *Taq* Dye Cycle sequencing kit in accordance with the instructions of the manufacturer (Applied Biosystems, Weiterstadt, Germany), and the reaction products were separated by electrophoresis with an automatic sequence analyser (model 370; Applied Biosystems). Primers for the target regions from positions 343 to 357, 519 to 536, 907 to 926, 1100 to 1115, and 1385 to 1401 (IUB nomenclature for

E. coli) were used for sequencing (17). Sequences were obtained for both strands of each 16S rDNA.

Phylogenetic analysis. The 16S rDNA sequences of strains KySW2^T, Kyval, Gyprop^T, Kyprop, Gylac^T, and Gö11 and of *Pelobacter carbinolicus* GraBd1^T were aligned manually with reference sequences of various members of the *Proteobacteria* (6, 20). Levels of sequence similarity were calculated by comparing only those positions at which bases could be unambiguously aligned (960 nucleotides). Evolutionary distances between pairs of organisms were determined by using the Jukes-Cantor equation (16) and the DNADIST program of the PHYLIP version 3.5 package (Table 1) (8). A tree which estimated the phylogenetic relationships was derived by using the FITCH program of the same package, random-order input of sequences, and the global rearrangement option.

DNA-DNA hybridizations. DNA-DNA hybridization experiments were carried out by workers at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. Genomic DNAs were isolated by chromatography on hydroxyapatite, using the procedure of Cashion et al. (4). The DNA-DNA hybridization experiments were performed by the spectroscopic method of De Ley et al. (5), with the modifications described by Huß et al. (14), using a Gilford System 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and a plotter. The DNA concentration was 50 µg/ml. The optimal temperature of renaturation (70°C) was calculated from the average G+C content of the samples by the formula of Gillis et al. (12). The DNA reassociation rate was calculated by regression analysis between 10 and 30 min after start of the reaction, using the computer program TRANSFER.BAS (15). The homology values were calculated with the formula of De Ley et al. (5). Each measurement was repeated three times.

FIG. 1. Phylogenetic tree based on 16S rDNA sequences determined in this study for *Desulfuromusa kysingii*, *Desulfuromusa bakii*, *Desulfuromusa succinoxidans* and 18 reference organisms. Bar = 5% difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting two species.

Nucleotide sequence accession numbers. The 16S rDNA sequences of strains Gyprop^T and Kyprop were identical, as were the 16S rDNA sequences of strains Kysw2^T and Kyval and the 16S rDNA sequences of strains Gylac^T and Gö11; the 16S rDNA sequences of these organisms and of *Pelobacter carbinolicus* GraBd1^T have been deposited in the EMBL data bank under accession numbers X79412 to X79415.

The EMBL and GenBank database accession numbers for the sequences used as reference sequences for phylogenetic tree construction (Table 1 and Fig. 1) are as follows: *Bacillus subtilis*, K00637; *Brucella abortus*, X13695; *Desulfobacter postgatei*, M26633; *Desulfobacterium vacuolatum*, M34408; *Desulfococcus multivorans*, M34405; *Desulfomicrobium baculatum*, M37311; *Desulfosarcina variabilis*, M26632; *Desulfovibrio desulfuricans*, M34113; *Desulfurella acetivorans*, X72768; *Desulfuromonas acetoxidans*, M26634; *E. coli*, J01695; *Geobacter metallireducens*, LO7834; *Myxococcus xanthus*, M34114; *Pelobacter acidigallici*, X77216; *Pelobacter acetylenicus*, X70955; *Pseudomonas testosteroni*, M11224; and *Wolinella succinogenes*, M26636.

RESULTS AND DISCUSSION

Our comparative sequence analysis of the almost complete 16S rRNA genes of strains Kysw2^T, Kyval, Gyprop^T, Kyprop, and Gylac^T revealed that these isolates, together with the previously not validly described organism strain Gö11, represent a coherent cluster of descent. Within this cluster the strains can be separated into three closely related but distinct rRNA groups, the Kysw2^T-Kyval group, the Gyprop^T-Kyprop group, and the Gylac^T-Gö11 group; the members of each group have identical 16S rDNA sequences. The overall levels of intergroup similarity for the almost completely analyzed 16S rDNA sequences range from 98 to 99% for the three groups. Our phylogenetic treeing analysis confirmed the previous classification of strain Gö11 based on oligonucleotide cataloging data (11); we found that this organism belongs to a separate branch of the delta subgroup of the *Proteobacteria* which is characterized by species belonging to the genera *Desulfuromonas* and *Pelobacter* (Table 1 and Fig. 1). The members of these two genera exhibit fundamental differences

in their biochemical and physiological properties. *Desulfuromonas* spp. are sulfur-respiring microorganisms that are capable of complete oxidation of substrates, whereas *Pelobacter* spp. ferment a rather limited number of substrates (24–26). Our phylogenetic tree showed that members of these two genera not only have a common ancestor but also are phylogenetically intertwined, as described previously (7, 28). On the one hand, the five new isolates and strain Gö11 are more closely related to *Pelobacter acidigallici* than to the type species of the genus *Desulfuromonas*, *Desulfuromonas acetoxidans*. On the other hand, the next most closely related phylogenetic neighbors of *Desulfuromonas acetoxidans* are the species *Pelobacter acetylenicus* and *Pelobacter carbinolicus* (Fig. 1). The five new isolates, strain Gö11, and *Desulfuromonas acetoxidans* completely oxidize acetate to CO₂ with concomitant stoichiometric reduction of elemental sulfur to hydrogen sulfide. However, there are several differences among these organisms in the range of substrates utilized as electron donors (Table 2). In contrast to *Desulfuromonas acetoxidans*, neither the five new isolates nor strain Gö11 can utilize alcohols, while *Desulfuromonas acetoxidans* lacks the ability to oxidize dicarboxylic acids and amino acids. The five new isolates grow with fumarate or malate as the sole substrate, whereas *Desulfuromonas acetoxidans* can use these compounds only as electron acceptors. The G+C contents of the five new strains and strain Gö11 are 46 to 47 mol%, whereas *Desulfuromonas acetoxidans* strains have G+C contents of 50 to 52 mol%. Valid description of the five new isolates and strain Gö11 as members of the genus *Desulfuromonas* would require that the description of the genus *Desulfuromonas* be amended (21). Such a description would mean that the genus characteristics would have to be reduced to the main properties of the sulfur-reducing bacteria of the delta subclass of the *Proteobacteria*. The recently described organism *Desulfuromonas acetexigens* is phylogenetically intertwined with *Desulfuromonas acetoxidans* and strain Gö11, as determined by oligonucleotide cataloging (11). *Desulfuromonas acetexigens* has a genomic DNA G+C content of 58.6 to 62.5 mol% (10), meaning that the genus *Desulfuromonas* includes sulfur-reducing bacteria that have DNA base compositions ranging from 51 to 62.5 mol%. Description of the five new isolates and strain Gö11 as members of the genus

TABLE 2. Major characteristics of the three species belonging to the genus *Desulfuromusa* and the type species of the genus *Desulfuromonas*, *Desulfuromonas acetoxidans*

Characteristic	<i>Desulfuromusa kysingii</i> ^a	<i>Desulfuromusa bakii</i> ^a	<i>Desulfuromusa succinoxidans</i> ^a	<i>Desulfuromonas acetoxidans</i> ^b
Morphology	Curved rods	Curved rods	Curved rods	Rods
Width (µm)	0.5–0.6	0.5–0.6	0.8–1.0	0.4–0.7
Length (µm)	2–5	2–4	4–6	1–4
Motility ^c	+	+	+	+
G+C content of DNA (mol%)	47	46	46	51
Optimum pH	6.5–7.9	6.7–7.4	6.5–7.9	7.2–7.5
Optimum temp (°C)	30–35	25–30	30–35	30
Anaerobic oxidation of organic substrates	Complete	Complete	Complete	Complete
Electron donors used for sulfur reduction ^d				
Acetate	+	+	+	+
Propionate	+	+	+	–
Valerate	(+)	–	–	–
Citrate	+	+	–	–
Ethanol	–	–	–	+
Propanol	–	–	–	+
Electron acceptors ^b				
Sulfur	+	+	+	+
Fumarate or malate	+	+	+	+
Nitrate	+	–	–	–
Dimethyl sulfoxide	+	–	–	–
Fe(III)-citrate	+	–	–	–
Disproportionation				
Fumarate, malate	+	+	+	–
Aspartate, maleate	+	–	+	–
Habitat(s)	Anoxic marine or estuarine mud	Anoxic marine or estuarine mud	Anoxic marine mud	Anoxic marine or estuarine mud
Strain(s)	DSM 7343 ^T (= Kysw2 ^T), DSM 7344 (= Kyval)	DSM 7345 ^T (= Gyprop ^T)	DSM 8270 (= Gylac ^T)	DSM 684

^a Data from reference 9.^b Data from reference 21.^c The flagellation pattern is indicated in parentheses (sl, single, lateral; sp, single, polar).^d +, utilized; (+), utilized by some strains; –, not utilized. Other electron donors utilized by all *Desulfuromusa* species are succinate, fumarate, maleate, malate, oxalacetate, glutamate, aspartate, alanine, lactate, pyruvate, and yeast extract.

Desulfuromonas would mean that the range of the DNA base compositions of members of this genus would be 46 to 62.5 mol%. This would be contrary to the now generally accepted opinion that species differing in their G+C contents by more than 10 mol% should not be placed in the same genus (13). In conclusion, the phenotypic characteristics of the five new isolates and strain Gö11 and the results of our phylogenetic analysis suggest that the sulfur-reducing bacteria under discussion should be placed into two different genera.

The levels of DNA-DNA relatedness of the five new isolates and strain Gö11 confirmed the comparative rDNA analysis results. The two strains of each of the three groups that have identical 16S rDNA sequences (strains Kysw2^T and Kyval, strains Gyprop^T and Kyprop, and strains Gylac^T and Gö11) exhibited levels of DNA relatedness above the 70% threshold (29). However, the levels of DNA relatedness between the

three rRNA groups were less than 30% (Table 3). Our results indicate that the five new isolates and strain Gö11 are members of three closely related but distinct genomic species. Phenotypically, the three species can be differentiated by differences in the following properties: use of nitrate, dimethyl sulfoxide, and Fe(III)-citrate as electron acceptors, use of citrate as an electron donor, and growth with aspartate or maleate as the sole substrate (Table 2).

Description of *Desulfuromusa* gen. nov. *Desulfuromusa* (De.sul.fu.ro.mu'sa. L. pref. *de*, from; L. n. *sulfur*, sulfur; M.L.n. *musa*, banana; M.L.f. n. *Desulfuromusa*, a banana-shaped bacterium that reduces sulfur). Cells are motile and slightly curved or rod shaped; they stain gram negative and are obligately anaerobic. They do not form spores. Elemental sulfur is used as an electron acceptor and is reduced to hydrogen sulfide. No growth occurs when either sulfate, sulfite,

TABLE 3. Levels of DNA relatedness for *Desulfuromusa kysingii* Kysw2^T and Kyval, *Desulfuromusa bakii* Gyprop^T and Kyprop, and *Desulfuromusa succinoxidans* Gylac^T and Gö11

Source of chromosomal DNA	G+C content (mol %)	% Reassociation with DNA from ^a :					
		Strain Kysw2 ^T	Strain Kyval	Strain Gyprop ^T	Strain Kyprop	Strain Gylac ^T	Strain Gö11
<i>Desulfuromusa kysingii</i> Kysw2 ^T	47	100	89	24	19	21	ND ^b
<i>Desulfuromusa bakii</i> Gyprop ^T	46	24	ND	100	98	25	ND
<i>Desulfuromusa succinoxidans</i> Gylac ^T	46	21	ND	25	ND	100	73

^a The levels of reassociation were determined as described in Materials and Methods.^b ND, level of DNA relatedness not determined.

thiosulfate, tetrathionate, trimethylammonium oxide, cystine, oxidized glutathione, N_2O , or oxygen is added as an electron acceptor. Growth occurs with fumarate or malate as the sole substrate. Short-chain fatty acids, dicarboxylic acids, and amino acids are used as electron donors and are oxidized completely to CO_2 . Alcohols and sugars are not utilized. Growth occurs with elemental sulfur; in liquid medium the bacteria are closely attached to elemental sulfur, which is used as an electron acceptor. In agar medium the strains form whitish filamentous colonies. Growth with fumarate as the sole substrate: the generation times with fumarate are generally shorter than the generation times with elemental sulfur. In agar medium the peach-colored colonies are lens shaped and have entire edges. Phylogenetically, the genus belongs to a lineage of the delta subclass of the *Proteobacteria* characterized by members of the genera *Desulfuromonas* and *Pelobacter*. The G+C content of the DNA of the type species is 47 mol%. The type species is *Desulfuromusa kysingii*.

On the basis of DNA-DNA relatedness data and phenotypic characteristics, three different species of the genus *Desulfuromusa* are described below.

Description of *Desulfuromusa kysingii* sp. nov. *Desulfuromusa kysingii* (ky. sin'gi.i. M. L. gen. n. *kysingii*, of kysing, named after the Fjord south of Århus [Jutland, Denmark] from which the organism was isolated). Cells are 0.5 to 0.6 μm wide and 2 to 4 μm long when they are grown on elemental sulfur as the electron acceptor. Type strain Kysw2 cells are motile by means of a single, subpolarly inserted flagellum. Nitrate, dimethyl sulfoxide, and Fe(III)-citrate are utilized as electron acceptors and are reduced to ammonia, dimethyl sulfide, and dissolved Fe(II), respectively. Cells grow with aspartate or maleate without additional electron acceptors, but growth is considerably slower than growth in the presence of elemental sulfur. Citrate, acetate, pyruvate, lactate, propionate, oxalacetate, succinate, maleate, alanine, glutamate, aspartate, and yeast extract are used as electron donors. Strains can be cultivated in defined medium without addition of complex nutrients like yeast extract or peptone. Cells grow in the presence of NaCl and $MgCl_2$ concentrations of 114 and 3.3 mM, respectively. Oxygen (2%) is tolerated, but no growth occurs in the presence of this compound. Strain Kysw2^T cells are able to grow diazotrophically and require *p*-aminobenzoate. The optimum pH for growth is between 6.5 and 7.9; the minimum and maximum pH values for growth are 5.8 and 8.25, respectively. The minimum temperature for growth is 4°C; the maximum temperature is 35°C; and the optimum temperature is around 32°C.

Desulfuromusa kysingii Kysw2 (= DSM 7343) is the type strain; the G+C content of its DNA is 47 mol%.

The habitat of *Desulfuromusa kysingii* Kysw2^T and Kyval is the anoxic mud of the Kysing Fjord south of Århus, Denmark.

Description of *Desulfuromusa bakii* sp. nov. *Desulfuromusa bakii* (ba'ki.i. M. L. gen. n. *bakii*, of Bak, named in memory of Friedhelm Bak, who made important contributions to our knowledge of sulfur- and sulfate-reducing bacteria). Cells are 0.5 to 0.6 μm wide and 2 to 4 μm long when they are grown with elemental sulfur as the electron acceptor. Nitrate, dimethyl sulfoxide and Fe(III)-citrate are not utilized as electron acceptors. No growth occurs with aspartate or maleate without additional electron acceptors. Citrate, acetate, pyruvate, lactate, propionate, oxalacetate, succinate, maleate, alanine, glutamate, aspartate, and yeast extract are used as electron donors. Strains can be cultivated in defined medium without addition of complex nutrients like yeast extract or peptone. No growth occurs in the presence of NaCl and $MgCl_2$ concentrations of 114 and 3.3 mM, respectively. The optimum pH for

growth is between 6.7 and 7.4, and the minimum and maximum pH values for growth are 6.2 and 7.8, respectively. The minimum temperature for growth is 8°C; the maximum temperature for growth is 32°C; and the optimum temperature is between 25 and 30°C.

The type strain of *Desulfuromusa bakii* is strain Gyprop (= DSM 7345); the G+C content of its DNA is 46 mol%.

Desulfuromusa bakii Gyprop^T was isolated from an anoxic mud sample collected from Guayamas Basin (Gulf of California) at a depth of about 2,000 m. The habitat of *Desulfuromusa bakii* Gyprop is the anoxic mud of the shallow Kysing Fjord south of Århus, Denmark.

Description of *Desulfuromusa succinoxidans* sp. nov. *Desulfuromusa succinoxidans* (suc.cin.ox'i.dans. L. n. *succinum*, amber; M.L. n. *acidum succinicum*, succinic acid [derived from amber]; M.L. v. *oxido*, make acid, oxidize; M.L. part.adj. *succinoxidans*, oxidizing succinate). Cells are 0.8 to 1.0 μm wide and 4 to 6 μm long when they are grown on elemental sulfur as the electron acceptor. Nitrate, dimethyl sulfoxide, and Fe(III)-citrate are not utilized. Cells grow with aspartate or maleate without additional electron acceptors, but growth is considerably slower than growth in the presence of elemental sulfur. Acetate, pyruvate, lactate, propionate, oxalacetate, succinate, maleate, alanine, glutamate, aspartate, and yeast extract are used as electron donors. Citrate is not utilized as an electron donor. Strains can be cultivated in defined medium without addition of complex nutrients like yeast extract or peptone. Cells grow in the presence of NaCl and $MgCl_2$ concentrations of 114 and 3.3 mM, respectively. The optimum pH for growth is between 6.5 and 7.9; minimum and maximum pH values for growth are 5.8 and 8.25, respectively. The minimum temperature for growth is 4°C; the maximum temperature is 35°C; and the optimum temperature is around 32°C.

The type strain of *Desulfuromusa succinoxidans* is strain Gylac (= DSM 8270); the G+C content of its DNA is 46 mol%.

Desulfuromusa succinoxidans Gylac^T was isolated from an anoxic mud sample collected at a depth of about 2,000 m from the Guayamas Basin (Gulf of California).

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