Description of *Desulfotomaculum* sp. Groll as *Desulfotomaculum gibsoniae* sp. nov.

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Strain Groll^T, isolated from fresh water, is a mesophilic, spore-forming, sulfate-reducing bacterium that uses a large variety of substrates as electron donors ranging from simple organic compounds to long-chain fatty acids and several aromatic compounds. Sulfate, thiosulfate and sulfite are used as electron acceptors. Homoacetogenic growth occurs under sulfate-free conditions. Substrate oxidation is usually complete, leading to CO₂, but acetate or other fatty acids can accumulate at high substrate concentrations. The G+C content of the DNA is 54.8 mol%. Strain Groll^T was found to be phenotypically and phylogenetically different from known members of the genus *Desulfotomaculum*. 16S rRNA gene sequence analyses show that this organism falls within the radiation of the genus *Desulfotomaculum* cluster and has <96% sequence similarity to previously described species. The name *Desulfotomaculum gibsoniae* sp. nov. is proposed for this strain; the type strain is Groll^T (= DSM 7213^T).

Keywords: *Desulfotomaculum*, sulfate-reducing bacterium, anaerobic degradation of aromatic compounds

INTRODUCTION

All spore-forming, dissimilatory sulfate-reducing bacteria were originally classified in the genus *Desulfotomaculum* (Campbell & Singleton, 1986; Widdel, 1992). Phylogenetically, they are members of the *Clostridium*–*Bacillus* subphylum of the Gram-positive bacteria (Stackebrandt *et al.*, 1997) and comprise mesophilic and moderately thermophilic species that show either complete or incomplete oxidation of organic substrates with concomitant reduction of sulfate and other sulfur oxyanions to sulfide (Widdel & Pfennig, 1977, 1981; Nazina *et al.*, 1989; Min & Zinder, 1990). Recent detailed phylogenetic analysis of the majority of the described species of the genus *Desulfotomaculum* and additional strains has demonstrated that the genus is not monophyletic but rather contains two different branches (Stackebrandt *et al.*, 1997). The majority of the species and isolates form one phylogenetically coherent cluster, whereas the strains of *Desulfotomaculum orientis* represent an additional lineage closely related to the genus *Desulfitobacterium*. These strains have therefore been described as a novel genus, *Desulfosporosinus*. While the

taxonomic status of the *Desulfotomaculum guttoideum* is unclear, this strain shows high sequence similarity to a previously described *Clostridium* species (Stackebrandt *et al.*, 1997). Since the phylogenetic survey of the genus was presented (Stackebrandt *et al.*, 1997), four additional species have been added to the genus: *Desulfotomaculum putei* (Liu *et al.*, 1997), *Desulfotomaculum luciae* (Liu *et al.*, 1997), ‘*Desulfotomaculum auripigmentum*’ (Newman *et al.*, 1997) and *Desulfotomaculum halophilum* (Tardy-Jacquenod *et al.*, 1998). With the exception of ‘*Desulfotomaculum auripigmentum*’, which shows highest sequence similarity to the genus *Desulfosporosinus*, these newly described species add further phylogenetic diversity to the genus *Desulfotomaculum*.

Members of these genera seem to be well adapted to changing redox conditions, facilitated by the formation of spores. They can be found in marine and freshwater ecosystems, including thermally heated groundwaters and oilfield brines, and were even isolated from corroded aluminium alloys (Widdel & Pfennig, 1981; Cord-Ruwisch & Garcia, 1985; Daumas *et al.*, 1988; Nazina *et al.*, 1989; Hagenauer *et al.*, 1997; Hippe *et al.*, 1997; Tardy-Jacquenod *et al.*, 1998). The increase of new descriptions of spore-forming, sulfate-reducing bacteria in the last few years demonstrates the abundance and ecological import-

The EMBL accession number for the 16S rRNA gene sequence of strain Groll^T is Y11576.

ance of this specialized group (Min & Zinder, 1990; Tasaki *et al.*, 1991; Kuever *et al.*, 1993; Vainshtein *et al.*, 1995; Liu *et al.*, 1997; Love *et al.*, 1993; Nilsen *et al.*, 1996). The broad substrate spectrum of organisms of this group, ranging from simple organic compounds to long-chain fatty acids and several different aromatic compounds, implies that they must play a major role in the breakdown of organic matter under anoxic conditions.

As reported by several authors, some spore-forming, sulfate-reducing bacteria show homoacetogenic growth under sulfate-free conditions, which gives them the ability to survive even under sulfate-limiting conditions, as found typically in freshwater sediments or aquifers (Klempers *et al.*, 1985; Tasaki *et al.*, 1991; Kuever *et al.*, 1993; Hanselmann *et al.*, 1995). Here, we describe an additional new species of the genus *Desulfotomaculum* isolated from a freshwater mud sample.

METHODS

Source of organism. Strain Groll^T was isolated from an enrichment culture inoculated with a black-mud sample from a small freshwater ditch in Bremen, northern Germany. Benzoate was used as the only carbon source and electron donor. A pure culture was obtained by repeated application of the agar dilution method. The strain was deposited with DSMZ under the accession number DSM 7213^T.

Culture methods and media. The organism was cultivated as described previously (Kuever *et al.*, 1993).

Chemical and biochemical characterization. The presence of desulfovirodin was tested as described by Postgate (1956). The whole-cell fatty acid composition was determined by means of capillary GC using cell material of strains grown under identical conditions (Vainshtein *et al.*, 1992).

Phylogenetic analyses of 16S rRNA gene sequences. 16S rRNA gene sequences available from the public databases were aligned using the ae2 editor (Maidak *et al.*, 1996). The strain designations and nucleotide sequence accession numbers are as follows: *Desulfotomaculum acetoxidans* DSM 771^T, Y11566; *Desulfotomaculum aeronauticum* DSM 10349^T, X98407; *Desulfotomaculum australicum* ACM 3917^T, M96665; *Desulfotomaculum geothermicum* DSM 3669^T, Y11567; *Desulfotomaculum halophilum* DSM 11559^T, U88891; *Desulfotomaculum kuznetsovii* DSM 6115^T, Y11569; *Desulfotomaculum luciae* SMCC W644^T, AF069293; *Desulfotomaculum nigrificans* NCIMB 8395^T, X62176; *Desulfotomaculum putei* SMCC W459^T, AF053929; *Desulfotomaculum ruminis* DSM 2154^T, Y11572; *Desulfotomaculum sapomandens* DSM 3223^T, AF168365; *Desulfotomaculum thermoacetoxidans* DSM 5813^T, Y11573; *Desulfotomaculum thermobenzoicum* DSM 6193^T, Y11574; *Desulfotomaculum thermocisternum* DSM 10259^T, U33455; *Desulfotomaculum thermosapovorans* DSM 6562^T, Y11575; *Desulfotomaculum* sp. DSM 7213, Y11576; *Desulfotomaculum* sp. DSM 7440, Y11579; *Desulfotomaculum* sp. DSM 7474, Y11577; *Desulfotomaculum* sp. DSM 7475, Y11580; *Desulfotomaculum* sp. DSM 7476, Y11578; and *Desulfosporosinus orientis* DSM 765^T, Y11570. After removal of the 5' end of each sequence because of variations in sequence length and alignment uncertainty, the alignment used in the phylogenetic analyses comprised 1100 nucleotide positions between positions 109 and 1387 (*Escherichia coli*

numbering). The programs of the PHYLIP package, including DNADIST and NEIGHBOUR, were used for the phylogenetic analyses (Felsenstein, 1993). The tree topology was re-analysed using 500 bootstrapped data sets and the programs SEQBOOT, DNADIST and CONSENSE of the PHYLIP package (Felsenstein, 1993).

RESULTS

Enrichment and isolation

Media containing benzoate as the only carbon source were inoculated with black, anaerobic mud from a freshwater ditch. After 2 weeks incubation, formation of sulfide and growth of large spore-forming rods showing slow motility were observed. After several transfers into liquid medium, a pure culture was obtained by repeated agar dilution series. Additional experiments showed that benzyl alcohol was a very selective substrate for enrichment of *Desulfotomaculum* strains similar to strain Groll^T. *Desulfococcus multivorans* strains, which are also common benzoate-utilizing, sulfate-reducing bacteria in freshwater habitats, were unable to use this aromatic compound for growth.

Cell morphology

Vegetative cells of strain Groll^T grown with butyrate and sulfate were straight or slightly curved rods measuring 1.0–2.5 × 4–7 µm with pointed ends. The spores were spherical and located in the centre of the cells, causing swelling. Vegetative cells were actively motile, but motility was lost during cultivation. The arrangement of flagella therefore could not be determined.

DNA G + C content

The determination of the DNA base ratio yielded a G + C content of 54.8 mol% (Kuever *et al.*, 1993).

Physiological properties

Desulfovirodin was absent in strain Groll^T. The optimum growth temperature of strain Groll^T was determined to be 35–37 °C. No growth was observed at temperatures greater than 40 °C or lower than 20 °C. The pH range for growth was between 6.0 and 8.0 with an optimum at pH 7.0–7.4. Vitamins were not essential for growth. After transfer to fresh medium, the isolate required sodium dithionite (100 µM) as additional reductant to initiate growth.

Strain Groll^T used the following compounds as electron donors and carbon sources in the presence of 10 mM sulfate (concentrations of added substrates, in mM except where indicated, are given in parentheses) (Kuever *et al.*, 1993; Londrey *et al.*, 1997): H₂ plus CO₂ (80:20; v/v), formate (20), propionate (10), butyrate (5), valerate (5), caproate (3), caprylate (2), caprylate (2), isobutyrate (5), 2-methylbutyrate (5), 3-methylbutyrate (5), crotonate (8), ethanol (10), 1-propanol (5), 1-butanol (5), 1-hexanol (5), 1-octanol

Table 1. Stoichiometric measurements with strain Groll^T after growth on butanol as electron donor and carbon source and sulfate as electron acceptor

Substrate added (mM)	Acetate (butyrate) excreted (mM)	Cell dry weight formed (mg ml ⁻¹)	Substrate consumed for cell material* (mM)	Substrate oxidized by sulfate reduction (mM)	Sulfide produced per mol substrate oxidized (mol)	Growth yield (g dry weight mol ⁻¹ substrate oxidized)
0.5	0	13.8	0.10	0.42	3.33	32.9
0.9	0	21.0	0.14	0.76	3.16	27.6
1.3	1.1	23.9	0.16	1.14	2.02	23.0
1.6	2.0	28.3	0.20	1.40	1.86	20.2
2.1	4.3	29.2	0.20	1.90	1.26	15.4
3.9	7.9	47.2	0.32	3.58	1.17	13.2
8.5	10.2 (2.9)	68.5	0.47	8.03	0.81	8.5
13.5	14.6 (6.2)	81.8	0.56	12.94	0.80	6.3
19.0	16.6 (9.6)	93.4	0.64	18.36	0.73	5.1

*Substrate consumed for cell material was calculated by the following equation: $17\text{C}_4\text{H}_{10}\text{O} + 28\text{HCO}_3^- \rightarrow 24(\text{C}_4\text{H}_7\text{O}_2) + 28\text{OH}^- + \text{H}_2\text{O}$; thus 0.0069 mmol 1-butanol is required for 1.0 mg cell dry weight.

(2), 2-methyl-1-propanol (5), 1,3-propanediol (5), pyruvate (10), malate (5), fumarate (5), succinate (5), maleate (5), benzoate (2), 3-hydroxybenzoate (2), 4-hydroxybenzoate (2), 2-aminobenzoate (2), 2-fluorobenzoate (0.5), 4-fluorobenzoate (0.5), 3-methylbenzoate (0.5), phenol (2), catechol (2), 4-methylcatechol (0.5), *m*-cresol (1), *p*-cresol (1), 4-hydroxybenzyl alcohol (1), 3-hydroxybenzyl alcohol (0.5), benzyl alcohol (2), 4-hydroxybenzaldehyde (1), 3-hydroxybenzaldehyde (0.5), benzaldehyde (2), 3,4-dihydroxybenzaldehyde (1), 3,4-dihydroxybenzoate (2), phenylacetate (2), phenylpropionate (2), phenylbutyrate (2), vanillyl alcohol (1), vanillin (2), vanillate (2), anisol (1), cinnamate (2) and ferulate (2).

Substrates tested but not utilized were: fructose (2), glucose (2), xylose (2), maltose (2), galactose (2), cellobiose (2), methanol (20), methanol (20) plus acetate (2), 2-propanol (5), 2-butanol (5), glycerol (5), cyclohexanol (1), cyclohexanone (1), cyclohexanecarboxylate (2), acetate (10), undecanate (1), laurinate (0.5), myristate (1), palmitate (1.5), stearate (1.5), cysteine (2), glycine (5), aspartate (2), lactate (10), citrate (5), tartrate (5), oxalate (10), malonate (10), adipate (3), 2-hydroxybenzoate (2), phthalate (2), gallate (2), 3-methoxybenzoate (2), 4-methoxybenzoate (2), 2,4-dihydroxybenzoate (2), 2,5-dihydroxybenzoate (2), 3,5-dihydroxybenzoate (2), 4-aminobenzoate (2), resorcinol (1), hydroquinone (1), pyrogallol (1), phloroglucinol (1), hydroxyhydroquinone (1), 2-, 3- and 4-aminophenol (each at 0.5), 2,4-diaminophenol (0.5), anilin (0.5), 4-nitrophenol (0.5), toluene (0.5), 4-hydroxybiphenyl (0.5), 2-hydroxybiphenyl (0.5), nicotinate (2) and phenylalanine (2).

Sulfate, sulfite and thiosulfate were used as electron acceptors. Sulfur and nitrate did not serve as electron acceptors. Pyruvate, crotonate, formate, hydrogen plus carbon dioxide (80:20, v/v) and methoxylated

aromatic compounds allowed slow growth by homo-acetogenesis when yeast extract was supplied as a supplement.

Fastest growth occurred with butyrate and 1-butanol, with a minimum doubling time of 12–14 h. Increased substrate concentrations resulted in the production of acetate or other fatty acids, depending on the substrate (Table 1). Furthermore, high concentrations of 1-butanol led to butyrate and acetate production. Some aromatic compounds were transformed by *O*-demethylation or decarboxylation prior to complete oxidation. Sodium chloride was not required for growth, although the isolate tolerated salt concentrations up to 35 g l⁻¹.

Cellular fatty acid composition

The fatty acid compositions of strain Groll^T and its closest phylogenetically related species are listed in Table 2. Significant amounts of unidentified fatty acids (ECL 11.86, ECL 13.05, ECL 13.26) were found in strain Groll^T (19.2%), *Desulfotomaculum geothermicum* (13%) and *Desulfotomaculum thermosapovorans* (8.5%).

Phylogenetic analyses

The phylogenetic position of strain Groll^T has been shown previously and its novel species status within the genus *Desulfotomaculum* has been demonstrated (Stackebrandt *et al.*, 1997). Comparative phylogenetic analyses, which included four additional recently described *Desulfotomaculum* species, showed strain Groll^T to represent a distinct lineage within subcluster Ib and not to be closely related to any of these newly described taxa (Fig. 1). The 16S rRNA gene sequence of strain Groll^T shared 94.0–95.5% similarity with the 16S rRNA gene sequences of the three other *Desulfotomaculum* species that comprise cluster Ib, namely

Table 2. Fatty acid composition of *Desulfotomaculum gibsoniae* and phylogenetically related *Desulfotomaculum* species

Percentages of total fatty acids are shown. Fatty acids present in all strains at less than 1 % are not listed. ECL, Equivalent chain-length; dma, dimethyl acetal (equivalent to aldehyde); c, *cis*; i, *iso*; ai, *anteiso*; ald, aldehyde; cyc, cyclopropane.

ECL	Fatty acid methyl ester	<i>D. gibsoniae</i> strain Groll ^T	<i>D. geothermicum</i> DSM 3669 ^T	<i>D. thermosapovorans</i> DSM 6562 ^T	<i>D. sapomandens</i> DSM 3223 ^T
11:00	11:0	1.9	0.9	0.5	—
13:60	i14:0	1.6	—	1.4	—
14:00	14:0	2.6	7.9	2.1	1.0
14:41	i15:1	—	6.5	1.0	0.5
14:62	i15:0	5.4	32.8	23.1	2.0
14:71	ai15:0	—	1.4	—	—
14:79	15:1 c7	2.7	—	—	0.6
14:95	16:0 ald	2.2	—	0.6	—
15:00	15:0	—	1.5	1.4	0.8
15:11	i15:0 dma	—	2.2	1.2	—
15:46	15:0 dma	1.7	—	—	5.4
15:63	i16:0	—	1.6	2.6	3.5
15:77	16:1 c7	3.3	—	0.7	6.5
15:82	16:1 c9	15.1	4.0	3.6	21.3
15:91	16:1 c11	3.6	—	—	6.2
16:00	16:0	9.6	10.1	15.7	10.2
16:29	16:1 c9 dma	4.6	—	1.1	4.2
16:42	i17:1 c7	4.8	7.2	5.0	4.6
16:48	16:0 dma	6.7	5.0	3.9	1.8
16:63	i17:0	—	2.4	8.9	0.7
16:80	17:1 c9	—	—	—	1.0
16:87	17:1 c11	—	—	—	1.9
16:89	17:0 cyc	2.7	3.7	5.8	—
17:00	17:0	—	—	1.4	0.5
17:10	i17:0 dma	—	—	7.5	—
17:47	17:0 dma	—	—	—	1.7
17:77	18:1 c9	2.0	—	—	3.4
17:82	18:1 c11	3.3	—	0.9	6.9
17:92	18:1 c13	2.8	—	—	2.5
18:00	18:0	5.6	—	3.2	4.4
18:29	18:1 c11 dma	1.5	—	—	1.4
Total unidentified		19.2	13.0	8.5	1.7
Saturated, even-numbered		26.7	23.0	25.5	7.2
Saturated, odd-numbered		3.6	2.4	3.3	8.4
Branched, <i>iso</i>		11.8	52.7	50.7	11.3
Branched, <i>anteiso</i>		0.0	1.4	0.0	0.0
Unsaturated		43.7	17.7	12.3	61.8
Dimethyl acetals		14.5	7.2	13.7	15.4
Cyclopropane fatty acids		2.7	3.7	5.8	2.8

Desulfotomaculum geothermicum (94.0%), *Desulfotomaculum thermosapovorans* (94.9%) and *Desulfotomaculum sapomandens* (95.5%). The recently described *Desulfotomaculum* species all fell within the different clusters defined by Stackebrandt *et al.* (1997). *Desulfotomaculum putei* grouped with *Desulfotomaculum* sp. strain DSM 7740 in subcluster 1a (Fig. 1), the 16S rRNA gene sequences of these two taxa sharing 98.0% similarity and the sequence of *Desulfotom-*

aculum putei having all of the signature nucleotides designated for this subcluster (Stackebrandt *et al.*, 1997). The 16S rRNA gene sequence of *Desulfotomaculum luciae*, with a few exceptions, contains most of the signature nucleotides designated for subcluster 1c. These exceptions include A–U (compared with G–U) at positions 293–304 and G–U (compared with G–C) at positions 662–743. The thermophilic species *Desulfotomaculum luciae* showed highest 16S rRNA

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequence comparisons of all validly described species and additional strains of the genus *Desulfotomaculum*. The position of the root was determined by the inclusion of *Desulfosporosinus orientis* as the outgroup organism. The neighbour-joining tree was reconstructed from distance matrices. Bootstrap values from the analyses of 500 data sets (expressed as percentages) are shown at the branching points. The scale bar represents 10 nucleotide substitutions per 100 nucleotides. The phylogenetic subclusters of cluster I as previously defined (Stackebrandt *et al.*, 1997) and the newly defined subcluster If are indicated.

gene sequence similarity to another thermophile, *Desulfotomaculum kuznetsovii* (99.4%). The species status of *Desulfotomaculum luciae* requires further clarification at the DNA–DNA homology level considering this high 16S rRNA gene sequence similarity, although there are significant phenotypic differences between these two species. The recently described halophilic species *Desulfotomaculum halophilum* represents a distinct lineage within cluster I, with 16S rRNA gene sequence similarity values to the other members of cluster I in the range 86.8–88.6%. Although it clustered with *Desulfotomaculum acetoxidans* (Fig. 1), the bootstrap value of 71% indicates a less than significant branching order. Comparison of the nucleotide signatures defined for subcluster Ie (Stackebrandt *et al.*, 1997) (of which *Desulfotomaculum acetoxidans* is the only member) with the 16S rRNA gene sequence of *Desulfotomaculum halophilum* showed that only 20 of the 33 signatures defined are present. This finding, in combination with the low bootstrap and sequence similarity values, indicates that *Desulfotomaculum halophilum* should be assigned to an additional single species cluster or lineage, designated cluster If. The fourth recently described *Desulfotomaculum* species, '*Desulfotomaculum auripigmentum*', clustered with the *Desulfosporosinus orientis* strains, showing 97.5 and 98.3% 16S rRNA gene sequence similarity, respectively, to strains DSM 765^T and DSM 8344 (data not shown). Further phenotypic and physiological comparisons are required before '*Desulfotomaculum auripigmentum*' can be reclassified to the genus *Desulfosporosinus*.

DISCUSSION

A general feature of members of the genus *Desulfotomaculum* seems to be that increasing substrate concentrations can lead to incomplete oxidation of the organic substrates, although complete oxidation was shown for low substrate concentrations (Daumas *et al.*, 1988; Fardeau *et al.*, 1995). At low substrate concentrations, strain Groll^T showed complete oxidation of 1-butanol to CO₂ (see equation 1). Above a concentration of 1 mM, a stoichiometric release of acetate from butanol was observed (see equation 2), which might be a result of a CoA transferase reaction, as proposed for *Desulfobacterium autotrophicum* (Schauder *et al.*, 1986). In this reaction, one of the two acetyl CoA molecules resulting from the β -oxidation of butyrate is used for activation of the next butyrate to butyryl-CoA. Butyrate should be an intermediate in the oxidation of 1-butanol. At 1-butanol concentrations above 4 mM, oxidation became incomplete (see equation 3). When the substrate concentration reached 8.5 mM, butyrate was excreted into the medium in addition to acetate (see equation 4).

Table 3. Characteristics of the closest relatives of *Desulfotomaculum gibsoniae* strain Groll^T

Data were taken from Cord-Ruwisch & Garcia (1985) (*D. sapomandens*), Daumas *et al.* (1988) (*D. geothermicum*), Kuever *et al.* (1993) (strain Groll^T) and Fardeau *et al.* (1995) (*D. thermosapovorans*). ND, Not determined. Where appropriate, characters are shown as: +, positive; (+), weak; or –, negative.

Characteristic	<i>D. gibsoniae</i> strain Groll ^T	<i>D. geothermicum</i> DSM 3669 ^T	<i>D. thermosapovorans</i> DSM 6562 ^T	<i>D. sapomandens</i> DSM 3223 ^T
Cell shape	Rod	Rod	Rod	Rod
Cell size (µm)	1.5–2.5 × 4–7	0.5–0.8 × 2.3	1.5–2.0 × 5–8	1.2–2.0 × 5–7
Motility	+*	+	+	+
G + C content (mol %)	54.8	50.4	51.2	48
Spore shape	Spherical	Spherical	Spherical	Spherical
Gas vacuoles	–	+	–	+
Optimal growth temperature (°C)	35–37	54	50	38
NaCl requirement	–	–†	–	–
Growth factors required	None	None	Vitamins	Vitamins
Oxidation of organic substrates‡	Complete	Complete	Complete	Complete
Electron donors:				
H ₂ /CO ₂	+	+	+	–
Formate	+	+	+	(+)
Acetate	–	–	–	+
Propionate	+	+	–	–
Butyrate	+	+	+	+
C12–C18 fatty acids	–	+	+	+
Methanol	–	–	+	ND
Ethanol	+	+	+	+
Fumarate	+	–	+	(+)
Malate	+	ND	+	(+)
Benzoate	+	–	–	+
Hydroxylated benzoates	+	–	–	+
Phenol	+	ND	ND	–
Catechol	+	ND	ND	–
Methoxy groups of aromatic compounds	+	ND	ND	–
Pyruvate	+	ND	+	+
Lactate	–	+	+	–
Fructose	–	+	–	–
Electron acceptors:				
Sulfate	+	+	+	+
Thiosulfate	+	ND	+	+
Sulfite	+	+	+	+
Elemental sulfur	–	–	–	+
Nitrate	–	–	–	–
Fermentative growth with pyruvate	+	ND	+	–
Homoacetogenic growth without sulfate	+	ND	ND	ND

* Motility was lost during cultivation.

† NaCl is not essential; optimal growth in medium with 15 g NaCl l⁻¹.

‡ High substrate concentrations can lead to acetate excretion into the medium.

Spore-forming, sulfate-reducing bacteria like the one described in this paper might be important for the breakdown of organic material in freshwater habitats under varying redox conditions. They can use a large variety of organic compounds, including several aromatic compounds, and can grow even under sulfate-free conditions by homoacetogenesis.

The 16S rRNA gene sequence analyses presented previously (Stackebrandt *et al.*, 1997) and in this study demonstrate that strain Groll^T is a member of the genus *Desulfotomaculum* and represents a distinct lineage not closely related to any of the previously described species, all of which have been included in the analyses described here. The results of the cellular

fatty acid composition (Table 2) and the physiological properties (Table 3), in combination with the results of the 16S rRNA gene sequence comparison, demonstrate that strain Groll^T represents a novel species belonging to phylogenetic subcluster Ib of the genus *Desulfotomaculum*. Both of the thermophilic species of subcluster Ib, namely *Desulfotomaculum geothermicum* and *Desulfotomaculum thermosapovorans*, share relatively low (<95%) 16S rRNA gene sequence similarity with strain Groll^T, have much higher optimal growth temperatures and are not able to grow using aromatic compounds as sole carbon sources. Strain Groll^T differs from *Desulfotomaculum sapomandens* in its ability to grow autotrophically and to grow on a large variety of aromatic compounds. On the basis of the distinct phylogenetic position of strain Groll^T, the demonstrated lack of 16S rRNA gene sequence similarity with all previously described *Desulfotomaculum* species and the differences in physiological and chemotaxonomic characteristics when compared with its closest relatives, we propose the name *Desulfotomaculum gibsoniae* sp. nov. for strain Groll^T.

Description of *Desulfotomaculum gibsoniae* sp. nov.

Desulfotomaculum gibsoniae (gib.so'ni.æ M.L. gen. n. *gibsoniae* of Gibson; named after Jane Gibson, a British-American microbiologist and biochemist, who made important contributions to the field of anaerobic degradation of aromatic compounds).

Straight or slightly curved rods, 1.5–2.5 × 4–7 µm, with pointed ends. Spores spherical and central, causing swelling. No gas vesicles are observed. Cells are motile, but motility was lost during cultivation. Gram-stain reaction of cells in the exponential phase of growth is negative. Strict anaerobe. Growth on H₂/CO₂, formate, propionate, butyrate, valerate, capronate, caprylate, *iso*-butyrate, 2-methylbutyrate, 3-methylbutyrate, crotonate, ethanol, 1-propanol, 1-butanol, hexanol, octanol, 2-methyl-1-propanol, 1,3-propanediol, pyruvate, malate, fumarate, succinate, maleinate, benzoate, 3- and 4-hydroxybenzoate, 2-aminobenzoate, phenol, catechol, 3,4-dihydroxybenzoate, phenylacetate, phenylpropionate, aromatic alcohols and aldehydes and methoxylated aromatic compounds. Substrate oxidation is usually complete, leading to CO₂, but acetate or other fatty acids can accumulate at high substrate concentrations. Electron acceptors used are sulfate, sulfite and thiosulfate. Nitrate and sulfur are not used. Slow homoacetogenic growth on some compounds. Addition of NaCl is not necessary. NaCl is tolerated up to 35 g l⁻¹. Vitamins are not required for growth. Temperature requirements: T_{min}, 20 °C; T_{opt}, 35–37 °C; T_{max}, 42 °C. The pH range for growth is 6–8; pH optimum at 6.9–7.2. The G+C content of the DNA is 54.8 mol%. The EMBL accession number for the 16S rRNA gene sequence is Y11576. Phylogenetically a member of subcluster Ib of the genus *Desulfotomaculum*. The type strain of *Desulfotomaculum gibsoniae* is Groll^T (= DSM 7213^T).

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