

***Desulfocella halophila* gen. nov., sp. nov., a halophilic, fatty-acid-oxidizing, sulfate-reducing bacterium isolated from sediments of the Great Salt Lake**

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A new halophilic sulfate-reducing bacterium, strain GSL-But2^T, was isolated from surface sediment of the Southern arm of the Great Salt Lake, UT, USA. The organism grew with a number of straight-chain fatty acids (C₄–C₁₆), 2-methylbutyrate, L-alanine and pyruvate as electron donors. Butyrate was oxidized incompletely to acetate. Sulfate, but not sulfite or thiosulfate, served as an electron acceptor. Growth was observed between 2 and 19% (w/v) NaCl with an optimum at 4–5% (w/v) NaCl. The optimal temperature and pH for growth were around 34 °C and pH 6.5–7.3, respectively. The generation time under optimal conditions in defined medium was around 28 h, compared to 20 h in complex medium containing yeast extract. The G+C content was 35.0 mol%. 16S rRNA gene sequence analysis revealed that strain GSL-But2^T belongs to the family *Desulfobacteriaceae* within the delta-subclass of the *Proteobacteria* and suggested that strain GSL-But2^T represents a member of a new genus. The name *Desulfocella halophila* gen. nov., sp. nov. is proposed for this organism. The type strain of *D. halophila* is strain GSL-But2^T (= DSM 11763^T = ATCC 700426^T).

Keywords: *Desulfocella halophila*, halophilic sulfate-reducing bacteria, long-chain fatty acids, taxonomy

INTRODUCTION

The occurrence of dissimilatory sulfate-reduction in hypersaline environments has been known for several decades (ZoBell, 1958). More recent studies indicate that bacterial sulfate reduction is the most important anaerobic mineralization process in hypersaline microbial mats and sediments (Canfield & Des Marais, 1993; Caumette *et al.*, 1994; Jørgensen & Cohen, 1977; Skyring, 1987). However, not until recently have the first dissimilatory sulfate-reducing bacteria (SRB) from a wide range of hypersaline habitats been isolated and phylogenetically described (Brandt & Ingvorsen, 1997; Caumette *et al.*, 1991; Krekeler *et al.*, 1997; Nga *et al.*, 1996; Ollivier *et al.*, 1991; Tardy-Jacquenod *et al.*, 1996, 1998; Zhilina *et al.*, 1997). With the exception

of the acetate-oxidizing *Desulfobacter halotolerans* (Brandt & Ingvorsen, 1997), all these strains share the ability to oxidize lactate, molecular hydrogen and other substrates typically metabolized by members of the genus *Desulfovibrio*. Other types of SRB capable of growing on higher fatty acids and benzoate have been reported from hypersaline oilfields (Cord-Ruwisch *et al.*, 1987). However, these species were not sufficiently described to allow for a taxonomic affiliation with existing SRB. Hence nothing is known about the physiology and phylogeny of long-chain fatty acid-oxidizing SRB in hypersaline environments.

As a part of our investigations on the ecology and diversity of SRB in hypersaline environments, here we provide the characterization of a new butyrate- and higher fatty-acid-oxidizing, sulfate-reducing bacterium isolated from the Great Salt Lake, UT, USA. Phylogenetic analysis as well as other characteristics suggest that this strain, designated GSL-But2^T, is a representative of a new genus and a new species for

Abbreviation: SRB, sulfate-reducing bacteria.

The GenBank accession number for the 16S rDNA sequence of strain GSL-But2^T is AF022936.

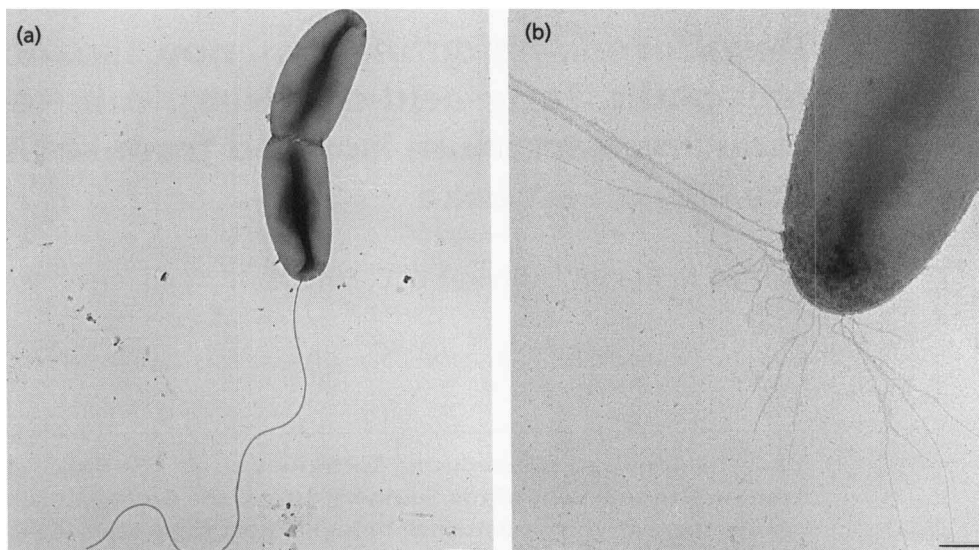


Fig. 1. Transmission electron micrographs of *Desulfocella halophila* strain GSL-But2^T after negative staining. (a) Dividing cell showing the polar flagellum; bar, 0.5 µm. (b) The polar region showing polar appendages; bar, 0.2 µm.

which we propose the name *Desulfocella halophila* gen. nov., sp. nov.

METHODS

Source of strains. Strain GSL-But2^T and physiologically similar strains were isolated from surface sediment collected from the Southern arm of the Great Salt Lake at 8–10 m depth (station AS2; Utah Geological Survey station code).

Isolation and media. Strains of SRB were initially enriched in Medium E (Brandt & Ingvorsen, 1997) containing 100 g NaCl l⁻¹ and a mixture of acetate (10 mM), propionate (10 mM) and butyrate (5 mM). A modified defined medium, Medium I (Brandt & Ingvorsen, 1997), containing 100 g NaCl l⁻¹ and butyrate (15 mM) as the sole electron donor was employed for all subsequent enrichments/isolations. Pure cultures were obtained by repeated use of deep agar dilution series (Widdel & Bak, 1992). If not stated otherwise, growth experiments were performed in Medium I (50 g NaCl l⁻¹) with butyrate and sulfate as growth substrates (27 or 30 °C, pH 6.9–7.3).

Morphology. Gram staining was performed as described by Dussault (1955). Exponentially growing cells were negatively stained with 1% (w/v) uranyl acetate and subsequently viewed with a JEOL 1200EX transmission electron microscope.

Analytical techniques. Hydrogen sulfide was analysed spectrophotometrically (Cord-Ruwisch, 1985). Fatty acids were quantified by HPLC analysis using an Aminex HPX-87H ion exclusion column (Bio-Rad) and UV detection as previously described (Brandt & Ingvorsen, 1997).

Lipid analysis and fatty acid nomenclature. Strain GSL-But2^T was cultivated in the standard medium of Vainshtein *et al.* (1992) supplemented with 50 g NaCl l⁻¹ and 20 mM butyrate. Cells were harvested by centrifugation (16000 g), freeze-dried and analysed according to Vainshtein *et al.*

(1992). The fatty acid analysis was performed by Professor Dr Reiner M. Kroppenstedt at DSMZ (Braunschweig, Germany) and the fatty acid nomenclature of Vainshtein *et al.* (1992) was used.

G+C content of genomic DNA and characterization of pigments. The G+C content of genomic DNA was determined at the Identification Service of the DSMZ (Braunschweig, Germany) by Dr Jutta Burghardt. Genomic DNA from strain GSL-But2^T was isolated and purified according to Visuvanathan *et al.* (1989) and the G+C content was determined by HPLC analysis (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). The presence of desulfovibrin was investigated in cell extracts as described previously by Widdel & Pfennig (1981).

16S rRNA gene sequencing and phylogenetic analysis. Genomic DNA from strain GSL-But2^T was isolated by the method of Porteous *et al.* (1994). A primer pair, designated Fd1 (5' CAGAGTTTGATCCTGGCTCAG 3') and Rd1 (5' AAGGAGGTGATCCAGCC 3') was used for PCR amplification of the 16S rRNA gene. The PCR product was purified by using QIAEX (QIAGEN) as described by the manufacturer. The purified PCR product was subsequently sequenced at Griffith University with an Applied Biosystems model 373A automated DNA sequencer by using a Prism dideoxy terminator cycle sequencing kit and by following the recommended protocol of the manufacturer (Applied Biosystems). The primers used for sequencing were Fd1, F1 (5' CTCCTACGGGAGGCAGCAG 3'), F3 (5' AAAGGGAATTGACGG 3'), F4 (5' TGTACACACCGCCGT 3'), R1 (5' CTGCTGCCCTCCCGTAG 3'), R2 (5' GTATTACCGCGGCTGCTG 3'), R4 (5' CCGTCAATTCCTTTGAGTTT 3'), R5 (5' GGGGTTGCGCTCGTTG 3'), R6 (5' TACGGTTACCTTGTTACGAC 3') and Rd1.

The 16S rRNA gene sequence was manually aligned against representative sequences from the delta-subclass of the *Proteobacteria* using the alignment editor ae2 (Maidak *et al.*, 1997). Pairwise evolutionary distances were calculated ac-

Table 1. Morphological and physiological characteristics of *Desulfocella halophila* strain GSL-But2^T as compared to the closest relatives among the dissimilatory sulfate-reducing bacteria

ND, Not done. Final concentrations (mM) of the electron donors/acceptors tested are given in parentheses. The sulfate concentration of the medium was 40.6 mM. Saturated unbranched fatty acids were added at concentrations recommended by Widdel & Bak (1992). The following electron donors were not utilized by strain GSL-But2^T: L-arginine (7.5), L-asparagine (5), betaine (10), 1-butanol (10), capric acid (10), citrate (10), L-cysteine (10), fructose (10), glucose (10), glutamate (10), glycerol (10), glycine (10), L-isoleucine (5), L-leucine (5), L-methionine (5), L-serine (10), thioglycolate (2.5), L-threonine (7.5), L-tryptophan (2) or xylose (10). Fe(III)-citrate (20), nitrate (10), elemental sulfur or fumarate (10) were not used as electron acceptors by strain GSL-But2^T. Symbols used: +, good growth; + (auto), autotrophic growth; (+), slow growth; −, no growth.

	<i>Desulfocella halophila</i>	' <i>Desulfobotulus sapovorans</i> '**	<i>Desulfococcus multivorans</i> *	<i>Desulfosarcina variabilis</i> *	<i>Desulfobacterium autotrophicum</i> †
Cell shape	Vibrio	Vibrio	Spherical	Oval (form aggregates)	Oval rod
Width (µm)	0.5–0.7	1.5	1.5–2.2	1–1.5	1–1.5
Length (µm)	2–4	3–5.5	–	1.5–2.5	1.5–2.5
Vitamin requirement	None	None	4-Aminobenzoate, biotin, thiamin	None	4-Aminobenzoate, biotin, nicotinate
Motility:	+ (polar flagellum)	+ (polar flagellum)	–	+ / – (polar flagellum)	+ (polar flagellum)
Electron donors:					
H ₂	–	–	–	+ (auto)	+ (auto)
Formate (10)	–	–	+ (auto)	+ (auto)	+ (auto)
Acetate (10)	–	–	(+)	(+)	(+)
Propionate (10)	–	–	+	+	(+)
Fatty acids (C-atoms)	4–6, 8, 12, 16	4–16	4–16	4–14	4–16
Isobutyrate (10)	–	–	+	–	+
2-Methylbutyrate (10)	+	+	+	(+)	+
3-Methylbutyrate (10)	–	–	+	(+)	–
lactate (10)	–	+	+	+	+
Ethanol (20)	–	–	+	+	+
Pyruvate (10)	+	+	+	+	+
Benzoate (2)	–	–	+	+	–
Succinate (10)	–	–	–	+	+
Fumarate (10)	–	–	–	+	+
Malate (10)	–	–	–	–	+
L-Alanine (10)	+	ND	ND	ND	ND
Oxidation of energy substrate	Incomplete	Incomplete	Complete	Complete	Complete
Electron acceptors:					
Sulfate	+	+	+	+	+
Sulfite (2–5)	–	+	+	+	–
Thiosulfate (5–10)	–	–	+	+	+
Shortest doubling time (h) on butyrate + sulfate	28 (20)‡	15	–	–	–
Optimum temperature (°C)	34	34	35	33	25–28
Optimum salinity (% NaCl, w/v)	4–5	0.1–0.7	0.7–2.0	1.3	2
Optimum pH	7.0	7.7	7.3	7.4	6.7
Desulfoviridin	–	–	+	–	–
G + C content of DNA (mol%)	35	53	57	51	48

* Widdel (1980).

† Brysch *et al.* (1987).

‡ Value in parentheses refers to growth in complex medium containing 1 g yeast extract l⁻¹.

ording to the method of Jukes & Cantor (1969) by using 992 unambiguous nucleotides. Dendrograms were constructed by the neighbour-joining method (Saitou & Nei, 1987) using part of the PHYLIP software package (Felsenstein, 1993). The topology of the tree was confirmed by bootstrap analysis. Sequences for phylogenetic analysis were retrieved from the Ribosomal Database Project (Maidak *et al.*, 1997) or from GenBank.

Culture collections. Strain GSL-But2^T has been deposited at the DSMZ (Braunschweig, Germany) as DSM 11763 and at the ATCC (Manassas, VA, USA) as ATCC 700426.

RESULTS

Enrichment and isolation

After 4 weeks incubation in liquid Medium E enrichment, cultures consisting of different cell morphologies showed intensive sulfide production. Subsequent transfers (5% v/v) in Medium I with butyrate as the only electron donor resulted in cultures with a predominance of 0.5–0.7 µm thick and 2–4 µm long vibrios. Thicker *Desulfobacter*-like rods with rounded ends were also present. Several pure cultures of the predominant vibrios were obtained after repeated serial dilutions in agar-containing medium. One isolate designated GSL-But2^T, showing only a weak tendency to clump during growth, was chosen for further studies.

Morphology

Cells of strain GSL-But2^T consisted of 0.5–0.7 µm thick and 2–4 µm long vibrios (Fig. 1a). Cells were motile by means of a single polar flagellum and possessed polar filamentous appendages (Fig. 1b). Spores were never observed. Strain GSL-But2^T stained Gram-negative.

Metabolism and growth characteristics

The growth characteristics of strain GSL-But2^T and its closest relatives are listed in Table 1. Strain GSL-But2^T grew well on butyrate and most higher fatty acids up to palmitate in the presence of sulfate. The branched fatty acid 2-methylbutyrate also yielded good growth. L-alanine and pyruvate were also good substrates albeit with a low production of sulfide (around 1 mM). The reason for the low production of sulfide during growth on alanine and pyruvate is not known. There was no growth with molecular hydrogen or formate as electron donors in Medium I supplemented with 2 mM acetate as a carbon source. Of the electron acceptors tested (Table 1), sulfate was the only one that supported growth with butyrate as the electron donor. Butyrate, L-alanine or pyruvate did not support growth of strain GSL-But2^T in sulfate-free medium. In the presence of sulfate, butyrate was incompletely oxidized to acetate. From 1 mol butyrate, 1.94 mol acetate was excreted into the medium along with 0.47 mol hydrogen sulfide. The molar ratios of bu-

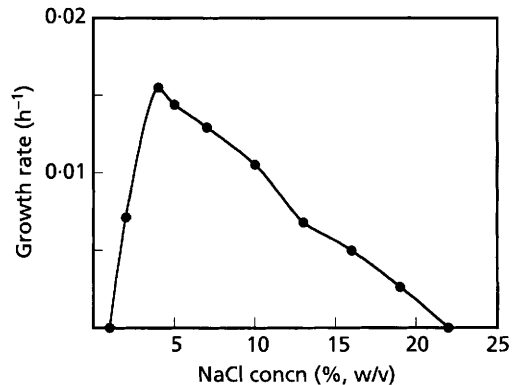
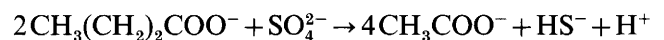


Fig. 2. Effect of NaCl concentration on growth rate of *Desulfocella halophila* strain GSL-But2^T grown on butyrate and sulfate at 27 °C.

tyrate dissimilated and acetate and sulfide excreted thus agreed well with the following equation:



Strain GSL-But2^T grew between 14 and 37 °C with an optimum at around 34 °C. Growth occurred between 2 and 19% (w/v) NaCl with an optimum at 4–5% (w/v) NaCl (Fig. 2). Strain GSL-But2^T grew between pH 5.8 and 7.6 but not at pH 5.5 or 8.2, with maximal growth rates at pH 6.5–7.3. The optimal magnesium concentration for growth was 2–100 mM although at 620 mM Mg²⁺, growth still occurred at about one-third of the maximal growth rate. Yeast extract stimulated growth considerably, reducing the doubling time from 28 to 20 h when grown at 30 °C. This growth stimulation was dose-independent from 0.25 to 2 g yeast extract l⁻¹.

Phylogenetic analysis

A total of 1547 bases from position 8 to position 1547 (*Escherichia coli* numbering) of the 16S rRNA gene of strain GSL-But2^T were sequenced. The phylogenetic analysis clearly showed that strain GSL-But2^T belongs to the delta-subclass of the *Proteobacteria* (Fig. 3; Table 2). Strain GSL-But2^T was only distantly related to known species of SRB and was found to belong to a new branch within the proposed family *Desulfobacteriaceae*. Bootstrap analysis confirmed the topology of the neighbour-joining-based dendrogram indicating that strain GSL-But2^T might form a monophyletic group with '*Desulfobotulus sapovorans*'. However, the relationship between the two strains was not robust (57% bootstrap).

G + C content of genomic DNA and characterization of pigments

The G + C content of the genome of strain GSL-But2^T was 35.0 mol%. The cells did not contain desulfoviridin.

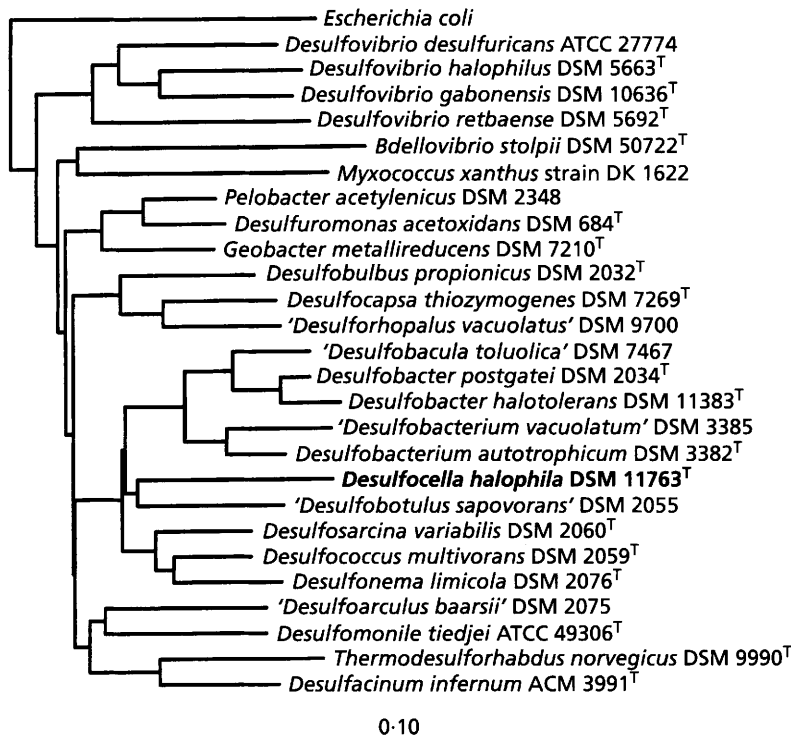


Fig. 3. Dendrogram showing the phylogenetic position of *Desulfocella halophila* strain GSL-But2^T compared to some of the closest relatives among the delta-subclass of the *Proteobacteria*. *Escherichia coli* was used as the outgroup.

Table 2. 16S rDNA similarity values between *Desulfocella halophila* strain GSL-But2^T and related taxa within the delta-subclass of the *Proteobacteria*

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 <i>Desulfocella halophila</i>	–															
2 ' <i>Desulfobotulus sapovorans</i> '	87.5	–														
3 <i>Desulfosarcina variabilis</i>	87.2	88.9	–													
4 <i>Desulfococcus multivorans</i>	87.5	90.1	93.4	–												
5 <i>Desulfonema limicola</i>	85.5	89.5	91.5	93.2	–											
6 ' <i>Desulfobacula toluolica</i> '	85.0	86.8	88.0	86.7	85.7	–										
7 ' <i>Desulfobacterium vacuolatum</i> '	86.3	87.1	88.9	87.4	86.4	90.4	–									
8 <i>Desulfobacterium autotrophicum</i>	86.4	89.0	90.4	89.0	88.7	92.1	84.0	–								
9 <i>Desulfobacter postgatei</i>	85.6	86.3	88.5	87.1	85.5	93.9	89.6	90.7	–							
10 <i>Desulfobacter halotolerans</i>	84.2	85.5	86.7	86.3	85.6	93.7	89.3	90.4	96.7	–						
11 <i>Desulfobulbus propionicus</i>	83.7	84.5	87.4	85.0	85.0	86.1	83.6	85.3	86.1	84.5	–					
12 ' <i>Desulforhopalus vacuolatus</i> '	84.4	85.0	85.4	85.3	84.1	85.0	83.1	84.7	84.2	82.7	89.3	–				
13 <i>Desulfocapsa thiozymogenes</i>	84.5	84.2	86.1	86.2	86.3	83.7	81.9	83.7	83.6	82.7	89.2	91.6	–			
14 ' <i>Desulfoarculus baarsii</i> '	83.3	86.3	86.8	88.1	86.4	84.1	82.9	83.6	83.9	82.7	85.4	84.4	83.1	–		
15 <i>Pelobacter acetylenicus</i>	84.5	86.1	87.1	86.6	85.5	85.2	83.9	86.3	86.0	83.7	86.7	86.5	86.6	86.8	–	
16 <i>Desulfovibrio halophilus</i>	80.5	80.1	82.5	82.8	80.7	79.8	79.3	79.5	79.6	77.8	82.0	82.4	81.5	85.1	83.1	–

Lipid analysis and fatty acid nomenclature

Strain GSL-But2^T contained mainly unbranched saturated and unsaturated fatty acids (Table 3). In addition, a small amount of β-hydroxy-myristic acid (14:0 3-OH) was present. The fatty acid composition of strain GSL-But2^T comprised 14:0 (2.3%), 14:0 3-OH + 15:0 dma (6.5%), 16:1 c7 (2.1%), 16:1 c9 (5.1%), 16:1 c11 (1.3%), 16:0 (36.5%), 18:1 c9 (25.8%), 18:1 c11 (16.7%), 18:0 (1.1%), and 20:1 c11 (2.6%). 14:0 3-OH and 15:0 dma fell into the same retention time window and are therefore listed together.

DISCUSSION

Strain GSL-But2^T is the first very halotolerant sulfate reducer described which is able to use long-chain fatty acids and L-alanine as electron donors. The bottom waters of the Southern arm of the Great Salt Lake are moderately hypersaline with a NaCl and Mg²⁺ content of approximately 130 g l⁻¹ (2.2 M) and 4.5 g l⁻¹ (185 mM), respectively (W. Gwynn, personal communication). Strain GSL-But2^T thus seems to be relatively well adapted to its natural habitat, although it prefers lower salinities of 40–50 g l⁻¹. The growth response of strain GSL-But2^T to NaCl hence displayed

Table 3. Cellular fatty acid composition (%) of *Desulfocella halophila* strain GSL-But2^T from this study and previously published data on its closest relatives among the Gram-negative SRB

Fatty acid	<i>Desulfocella halophila</i>	' <i>Desulfobotulus sapovorans</i> '		<i>Desulfococcus multivorans</i> †	<i>Desulfosarcina variabilis</i> †	<i>Desulfobacterium autotrophicum</i> *
		*	†			
Saturated unbranched						
even numbered	39.8	32.3	31.2	25.2	29.6	17.5
odd numbered	0.0	0.0	0.0	4.0	4.0	8.2
Branched						
iso	0.0	5.1	0.0	5.5	4.3	0.0
anteiso	0.0	28.8	0.0	35.8	39.6	0.0
Unsaturated	53.7	30.6	61.5	21.2	18.3	54.7

* From Vainshtein *et al.* (1992). Same culture conditions and analysis protocol as used for our study.

† From Kohring *et al.* (1994).

the same pattern as observed for other sulfate reducers isolated from hypersaline habitats. These strains, when tested *in vitro*, all exhibited salinity optima for growth significantly below the *in situ* salinities of their respective habitats (Brandt & Ingvorsen, 1997; Krekeler *et al.*, 1997; Ollivier *et al.*, 1994).

Phylogenetic analysis of the 16S rRNA gene showed that strain GSL-But2^T formed a new lineage within the proposed family *Desulfobacteriaceae*, the closest relatives being members of the genera '*Desulfobotulus*', *Desulfococcus*, *Desulfosarcina* and *Desulfobacterium* (Table 2; Fig. 3). We therefore propose that strain GSL-But2^T should be recognized as a member of a new genus, *Desulfocella* gen. nov. However, we did retrieve one 16S rDNA sequence from an unpublished strain tentatively named '*Desulfobotulus* BG14' (GenBank accession no. U85470), which was more closely related to strain GSL-But2^T (94.9% similarity) than the others tested. The creation of a new genus to include strain GSL-But2^T is further supported by the very low G+C content (35 mol%) of the genomic DNA from strain GSL-But2^T as compared to the much higher G+C content of its closest described relatives (Table 1).

The fatty acid profile of strain GSL-But2^T supported the results of the phylogenetic analysis (Table 3). The absence of branched-chain fatty acids thus clearly separates GSL-But2^T from members of the genus *Desulfovibrio* (Vainshtein *et al.*, 1992) and from other related genera (Ollivier *et al.*, 1991; Zhilina *et al.*, 1997). Rather, the low level of branching fatty acids and the high level of unsaturated fatty acids suggests a closer relationship to *Desulfospira joergensenii* (Finster *et al.*, 1997), several *Desulfobacterium* species (Kohring *et al.*, 1994; Vainshtein *et al.*, 1992) and '*Desulfobotulus sapovorans*' (Kohring *et al.*, 1994). The fatty acid profile of '*Desulfobotulus sapovorans*' has been found to be strongly influenced by culture conditions and/or techniques for analysis of cellular fatty acids

(Table 3). Strain GSL-But2^T thus differed significantly from '*Desulfobotulus sapovorans*', when the two strains were grown and analysed under similar conditions (Table 3). However, in another study, using somewhat different culture conditions and analysis protocols (Kohring *et al.*, 1994), the fatty acid profile obtained for '*Desulfobotulus sapovorans*' was found to be more similar to that of strain GSL-But2^T (Table 3).

Physiologically and morphologically, strain GSL-But2^T differs markedly from most of its closest relatives (Table 1; Widdel & Bak, 1992). The energy metabolism of strain GSL-But2^T, however, resembles that of '*Desulfobotulus sapovorans*', in that both species oxidize butyrate and higher fatty acids incompletely with acetate as the end product. Strain GSL-But2^T can be distinguished from '*Desulfobotulus sapovorans*' by being unable to use lactate and sulfite as the electron donor and electron acceptor, respectively. More importantly, strain GSL-But2^T differs significantly from the freshwater isolate '*Desulfobotulus sapovorans*' with respect to halotolerance and halophily (Table 1).

We here propose that strain GSL-But2^T should be placed in a new genus, *Desulfocella*, as a new species, *Desulfocella halophila*.

Description of *Desulfocella* gen. nov.

Desulfocella (De.sul.fo.cel'la. L. pref. *de* from; L. n. *sulfur* sulfur; L. fem. n. *cella* small room, cell; M.L. fem. n. *Desulfocella* sulfate-reducing cell).

Gram-negative, non-spore forming vibrios, motile by a single polar flagellum. Obligatory anaerobic chemoorganotroph; grows with sulfate as electron acceptor and produces hydrogen sulfide. A wide range of fatty acids (C₄-C₁₆) serve as electron donors and are incompletely oxidized. Does not grow by fermentation. Mesophilic and neutrophilic with a requirement for NaCl. Vitamins are not required. Yeast extract

stimulates growth. The G+C content of the DNA is around 35 mol%. Desulfovriodin not present. Phylogenetically a member of the family *Desulfobacteriaceae* within the delta-subclass of the *Proteobacteria*. The type species is *Desulfocella halophila*.

Description of *Desulfocella halophila* sp. nov.

Desulfocella halophila (ha.lo'phi.la. Gr. n. *hals* salt; Gr. adj. *philos* friendly to; M.L. fem. adj. *halophila* salt-loving).

Vibrio-shaped cells, 0.5–0.7 × 2–4 µm. Motile by means of one polar flagellum. Reduces sulfate, but not thiosulfate, sulfite, elemental sulfur, ferric iron, nitrate or fumarate. Pyruvate, L-alanine, 2-methylbutyrate and many straight-chain saturated fatty acids with 4–16 carbon atoms serve as electron donors. Does not grow by fermentation of pyruvate, L-alanine or butyrate when sulfate is absent. Vitamins are not required, but yeast extract stimulates growth. Temperature range associated with growth, 14–37 °C; optimum around 34 °C. Optimum salinity, 4–5% (w/v) NaCl; halotolerance, 2–19% (w/v) NaCl. Grows optimally in the presence of 2–100 mM Mg²⁺ and tolerates up to 620 mM Mg²⁺. The pH range for growth is 5.8–7.6 with optimum at pH 6.5–7.3. The G+C content of the DNA is 35.0 mol%. The type strain, *GSL-But2*^T, was isolated from hypersaline, thalassohaline sediment of the Great Salt Lake (UT, USA) and has been deposited at the DSMZ (Braunschweig, Germany) as DSM 11763^T and at the ATCC (Manassas, VA, USA) as ATCC 700426^T.

ACKNOWLEDGEMENTS

We are grateful to Dr Deborah Stenzel from Queensland University of Technology (Brisbane, Australia) for technical assistance (transmission electron microscopy) and for preparing TEM micrographs for publication. We thank Dr Wallace Gwynn from the Utah Geological Survey (Salt Lake City, UT, USA) for providing geochemical data about the Great Salt Lake.

REFERENCES

- Brandt, K. K. & Ingvorsen, K. (1997). *Desulfobacter halotolerans* sp. nov., a halotolerant acetate-oxidizing sulfate-reducing bacterium isolated from sediments of Great Salt Lake, Utah. *Syst Appl Microbiol* **20**, 366–373.
- Brysch, K., Schneider, C., Fuchs, G. & Widdel, F. (1987). Lithoautotrophic growth of sulfate-reducing bacteria, and description of *Desulfobacterium autotrophicum* gen. nov., sp. nov. *Arch Microbiol* **148**, 264–274.
- Canfield, D. E. & Des Marais, D. J. (1993). Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. *Geochim Cosmochim Acta* **57**, 3971–3984.
- Caumette, P., Cohen, Y. & Matheron, R. (1991). Isolation and characterization of *Desulfovibrio halophilus* sp. nov., a halophilic sulfate-reducing bacterium isolated from Solar Lake (Sinai). *Syst Appl Microbiol* **14**, 33–38.

- Caumette, P., Matheron, R., Raymond, N. & Relexans, J.-C. (1994). Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). *FEMS Microbiol Ecol* **13**, 273–286.
- Cord-Ruwisch, R. (1985). A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* **4**, 33–36.
- Cord-Ruwisch, R., Kleinitz, W. & Widdel, F. (1987). Sulfate-reducing bacteria and their activities in oil production. *J Petrol Technol* **1**, 97–106.
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5.1. Seattle: Department of Genetics, University of Washington.
- Finster, K., Liesack, W. & Tindall, B. J. (1997). *Desulfospira joergensenii*, gen. nov., sp. nov., a new sulfate-reducing bacterium isolated from marine surface sediment. *Syst Appl Microbiol* **20**, 201–208.
- Jørgensen, B. B. & Cohen, Y. (1977). Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mats. *Limnol Oceanogr* **22**, 657–666.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kohring, L. L., Ringelberg, D. B., Devereux, R., Stahl, D. A., Mittelman, M. W. & White, D. C. (1994). Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. *FEMS Microbiol Lett* **119**, 303–308.
- Krekeler, D., Sigalevich, P., Teske, A., Cypionka, H. & Cohen, Y. (1997). A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai), *Desulfovibrio oxyclineae* sp. nov. *Arch Microbiol* **167**, 369–375.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1997). The ribosomal database project (RDP). *Nucleic Acids Res* **25**, 109–111.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nga, D. P., Ha, D. T. C., Hien, L. T. & Stan-Lotter, H. (1996). *Desulfovibrio vietnamensis* sp. nov., a halophilic sulfate-reducing bacterium from Vietnamese oil fields. *Anaerobe* **2**, 385–392.
- Ollivier, B., Hatchikian, C. E., Prensier, G., Guezennec, J. & Garcia, J.-L. (1991). *Desulfohalobium retbaense* gen. nov., sp. nov., a halophilic sulfate-reducing bacterium from sediments of a hypersaline lake in Senegal. *Int J Syst Bacteriol* **41**, 74–81.
- Ollivier, B., Caumette, P., Garcia, J.-L. & Mah, R. A. (1994). Anaerobic bacteria from hypersaline environments. *Microbiol Rev* **58**, 27–38.
- Porteous, L. A., Armstrong, J. L., Sadler, R. J. & Watrud, L. S. (1994). An effective method to extract DNA from environmental samples for polymerase chain reaction amplification and DNA fingerprint analysis. *Curr Microbiol* **29**, 301–307.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Skyring, G. W. (1987). Sulfate reduction in coastal environments. *Geomicrobiol J* **5**, 295–374.

- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tardy-Jacquenod, C., Magot, M., Laigret, F., Kaghad, M., Patel, B. K. C., Guezennec, J., Matheron, R. & Caumette, P. (1996).** *Desulfovibrio gabonensis* sp. nov., a new moderately halophilic sulfate-reducing bacterium isolated from an oil pipeline. *Int J Syst Bacteriol* **46**, 710–715.
- Tardy-Jacquenod, C., Magot, M., Patel, B. K. C., Matheron, R. & Caumette, P. (1998).** *Desulfotomaculum halophilum* sp. nov., a new halophilic sulfate-reducing bacterium isolated from oil production facilities. *Int J Syst Bacteriol* **48**, 333–338.
- Vainshtein, M., Hippe, H. & Kroppenstedt, R. M. (1992).** Cellular fatty acid composition of *Desulfovibrio* species and its use in classification of sulfate-reducing bacteria. *Syst Appl Microbiol* **15**, 554–566.
- Visuvanathan, S., Moss, M. T., Stanford, J. L., Hermon-Taylor, J. & McFadden, J. J. (1989).** Simple enzymatic method for isolation of DNA from diverse bacteria. *J Microbiol Methods* **10**, 59–64.
- Widdel, F. (1980).** *Anaerober Abbau von Fettsäuren und Benzoessäure durch neu isolierte Arten sulfatreduzierender Bakterien*. PhD thesis, Göttingen University.
- Widdel, F. & Bak, F. (1992).** Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes: a Handbook of the Biology of Bacteria; Ecophysiology, Isolation, Identification, Applications*, 2nd edn, pp. 3353–3378. Edited by A. Balows and others. New York: Springer.
- Widdel, F. & Pfennig, N. (1981).** Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. *Arch Microbiol* **129**, 395–400.
- Zhilina, T. N., Zavarzin, G. A., Rainey, F. A., Pikuta, E. N., Osipov, G. A. & Kostrikina, N. A. (1997).** *Desulfonatronovibrio hydrogenovorans* gen. nov., sp. nov., an alkaliphilic, sulfate-reducing bacterium. *Int J Syst Bacteriol* **47**, 144–149.
- ZoBell, C. E. (1958).** Ecology of sulfate-reducing bacteria. *Producers' Monthly* **22**, 12–29.