Desulfatirhabdium butyrativorans gen. nov., sp. nov., a butyrate-oxidizing, sulfate-reducing bacterium isolated from an anaerobic bioreactor

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A novel sulfate-reducing bacterium, strain HB1^T, was isolated from an upflow anaerobic sludge blanket (UASB) reactor treating paper-mill wastewater operated at 37 °C. Cells of strain HB1^T were oval to rod-shaped, 1–1.3 μm wide and 2.6–3.5 μm long and Gram-negative. The optimum temperature for growth was 28–30 °C. In the presence of sulfate, the isolate was able to grow on H_2/acetate, formate, ethanol, propionate, fumarate, succinate, butyrate, crotonate, catechol, benzoate, 4-hydroxybenzoate, palmitate and stearate. The isolate only grew on H_2 when acetate was added as a carbon source; when grown on formate, acetate was not required. Growth was also possible on pyruvate and crotonate without an electron acceptor. The isolate showed very poor growth on acetate. Thiosulfate and sulfate were used as electron acceptors. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain HB1^T represents a novel lineage within the *Deltaproteobacteria*; sequence similarities between strain HB1^T and members of other related genera were less than 91 %. Strain HB1^T was also distinguished from members of related genera based on differences in several phenotypic characteristics. It is a member of the family *Desulfobacteraceae*. The major cellular fatty acids of strain HB1^T were $C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$ and $C_{14:0}$. β-Hydroxy fatty acids were also present in the range of $C_{14:0}$ to $C_{18:0}$, of which $C_{16:0}$ was the most abundant. The G+C content of the DNA was 55.1 mol%. Based on physiological, biochemical and chemotaxonomic traits together with results of comparative 16S rRNA gene sequence analysis, strain HB1^T is considered to represent a novel species in a new genus, for which the name *Desulfatirhabdium butyrativorans* gen. nov., sp. nov. is proposed. The type strain of *Desulfatirhabdium butyrativorans* is HB1^T (=DSM 18734^T =JCM 14470^T).

Dissimilatory sulfate reduction is the most important anaerobic process in many different environments (e.g. Jørgensen, 1982; Canfield *et al.*, 1993; Thamdrup & Canfield, 1996; Rysgaard *et al.*, 1998; Kostka *et al.*, 1999; Glud *et al.*, 2000; Rabus *et al.*, 2006). Bacterial sulfate-reducers fall into different branches, the *Deltaproteobacteria*, with more than 25 genera, the Gram-positive bacteria, including genera such as *Desulfotomaculum* (Campbell & Postgate, 1965) and *Thermodesulfobium* (Mori *et al.*, 2003), and Gram-negative sulfate-reducers such as the genera *Thermodesulfobacterium* (Zeikus *et al.*, 1983) and *Thermodesulfatator* (Moussard *et al.*, 2004) within the class *Thermodesulfobacteria* and *Thermodesulfovibrio* (Henry *et al.*, 1994) within the class '*Nitrospira*'.

Although the *Deltaproteobacteria* is not a large assemblage of genera, its members show considerable morphological and physiological diversity (Rabus *et al.*, 2006). Members of the family *Desulfobacteraceae* in the order *Desulfobacterales* are widely distributed in freshwater, marine, hypersaline and oil- or hydrocarbon-polluted sediments (Bak & Widdel, 1986; Brysch *et al.*, 1987; Szewzyk & Pfennig, 1987; Gogotova & Vainstein, 1989; Schnell *et al.*, 1989; Rees & Patel, 2001; Cravo-Laureau *et al.*, 2004; Kjeldsen *et al.*, 2007). These bacteria are anaerobic,

Abbreviation: UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HB1^T is DQ146482.

mesophilic, Gram-negative, oval to coccoid, slightly curved or rod-shaped and grow optimally at 20–35 °C. A wide variety of substrates including alcohols, fatty acids and aromatic and aliphatic compounds can be utilized by this group of organisms (Kuever *et al.*, 2005). Members of the family *Desulfobacteraceae* play an important role in the degradation of volatile fatty acids in anaerobic bioreactors treating sulfate-rich wastewaters, such as those from paper mills, tanneries or the food oil industry (Oude Elferink *et al.*, 1994; Colleran *et al.*, 1995; Roest *et al.*, 2005). Acetate and butyrate are important intermediates in the anaerobic degradation of wastewaters. Some bacteria in the family *Desulfobacteraceae* are able to oxidize butyrate either incompletely to acetate or completely to carbon dioxide with concomitant reduction of sulfate to sulfide (Stieb & Schink, 1989; Platen *et al.*, 1990; Brandt *et al.*, 1999).

The microbial community of an upflow anaerobic sludge blanket (UASB) reactor treating paper-mill wastewater (Industriewater, Eerbeek, The Netherlands) was investigated. In this reactor, both sulfate reduction and methanogenesis have been found to be important (Oude Elferink *et al.*, 1998). In a previous study, the dominant micro-organisms present in the anaerobic paper-mill wastewater treatment system were assessed by molecular techniques (Roest *et al.* 2005). As it was not clear from these earlier molecular studies which bacteria were involved in butyrate degradation with sulfate, in the present study, particular attention was paid to bacteria that are able to degrade butyrate with sulfate. Here, we report the taxonomic characterization of strain HB1^T, which was isolated from granular sludge of the full-scale mesophilic UASB reactor at Eerbeek. Detailed characteristics of the granular sludge were described previously by Oude Elferink *et al.* (1998). About 10 ml granular sludge was disintegrated by a Potter homogenizer (Tamson). Serial dilutions of the homogenized sample were prepared in a bicarbonate-buffered anaerobic medium containing sodium butyrate and sodium sulfate. Bacterial growth was evident at a dilution of 10^{-8} of the incubation at 37 °C within 30 days.

Dilution series were made in a basal bicarbonate-buffered medium containing 10 mM sodium butyrate with 20 mM sodium sulfate. The basal liquid culture medium contained (g l^{-1} unless indicated): NaCl (7), $NaHCO_3$ (4), Na_2SO_4 (2.8), $MgCl_2.6H_2O$ (1.2), KCl (0.5), NH_4Cl (0.3), KH_2PO_4 (0.2), $CaCl_2$ (0.15), $Na_2S.7$–$9H_2O$ (0.3), yeast extract (0.02), selenite/tungstate solution (1 ml l^{-1}) (Widdel & Bak, 1992) and a trace element solution (1 ml l^{-1}) containing (mg l^{-1} unless indicated): $FeCl_2.4H_2O$ (1500), $CoCl_2.2H_2O$ (190), $MnCl_2.4H_2O$ (100), $ZnCl_2$ (70), H_3BO_3 (62), $Na_2MoO_4.2H_2O$ (36), $NiCl_2.6H_2O$ (24), $CuCl_2.2H_2O$ (17), EDTA (500) and 37 % HCl (7 ml l^{-1}). In addition, vitamins were added from a concentrated stock solution according to Stams *et al.* (1983). The cultures were grown routinely in 117 ml serum vials with butyl rubber stoppers and aluminium crimp seals. The vials contained 50 ml basal medium and a gas phase of 1.7 bar N_2/CO_2 or H_2/CO_2 (80/20 %, v/v). Concentrated stock solutions of substrates were prepared anoxically, sterilized by filtration and added to the medium to final concentrations of 5–20 mM. Besides the substrates, vitamins, $CaCl_2$, $NaHCO_3$ and $Na_2S.7$–$9H_2O$ were added from stock solutions after sterilization of the medium. The pH of the medium was 7. Unless stated otherwise, all cultivations were carried out at 30 °C.

For isolation, the enriched culture was incubated in the presence of butyrate by using the soft-agar dilution method. This procedure was applied in 117 ml serum vials. Colonies, which were visible after 1 month of incubation, were picked with a sterile needle and subcultured in liquid medium containing butyrate and sulfate. The colonies were about 1 mm in diameter, lens-shaped and brownish. Serial agar and liquid dilutions were repeated until a pure culture was obtained.

Microscope observations were performed with a Leica DC250 photomicroscope. Cells of strain HB1^T were oval to rod-shaped, 1–1.3 μm wide and 2.6–3.5 μm long, depending on the growth phase (Fig. 1). The cells stained Gram-negative (Murray *et al.*, 1994). The cells were non-spore-forming and occurred singly, in pairs or in long chains.

The purity of the culture was checked by phase-contrast microscopy. Purity of the isolate was confirmed by incubations at different temperatures between 20 and 65 °C under anaerobic and aerobic conditions in media containing 10 g yeast extract l^{-1} or in anaerobic Wilkins–Chalgren broth (Oxoid).

Temperature limits for growth were determined by culture incubation from 10 to 65 °C in the basal medium. The pH limits for growth were determined in the same medium adjusted to pH values between 5 and 9 by changing the CO_2 content of the gas phase. The dependence of growth on NaCl concentration was determined in basal medium containing NaCl at concentrations ranging from 0 to 40 g

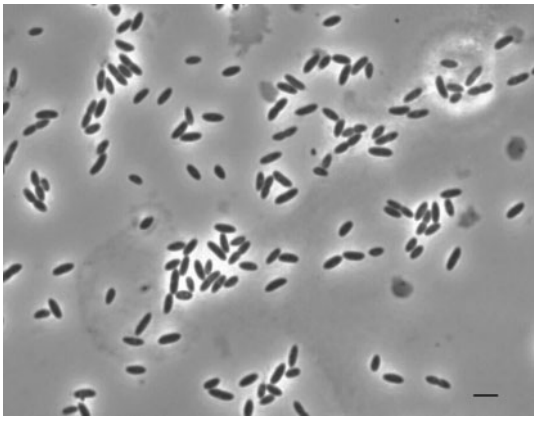

Fig. 1. Phase-contrast micrograph of cells of strain HB1^T growing on butyrate in the presence of sulfate. Bar, 2.5 μm.

l^{-1}. Under optimal conditions, the doubling time of the isolate in media with butyrate and sulfate was about 130 h (μ_{max}=0.0037 h^{-1}) and the final cell density (OD_{600}) was about 0.38.

Strain HB1^T was mesophilic. Growth occurred between 15 and 37 °C, with an optimum growth temperature of 28–30 °C. Growth was possible at pH 6.5–8.0, with an optimum at pH 7.0. Growth was observed in 0–15 g NaCl l^{-1}, with an optimum around 5 g NaCl l^{-1}.

The G+C content of the DNA was determined by using standard HPLC analysis (Mesbah *et al.*, 1989) at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). Genomic DNA was isolated according to the procedure of Cashion *et al.* (1977).

Organic compounds were measured by HPLC as described previously by Stams *et al.*, (1993). Gases and alcohols were measured by gas chromatography (Balk *et al.*, 2003; Henstra & Stams, 2004) and thiosulfate, nitrate and sulfate were analysed by an HPLC system equipped with an Ionpac AS9-SC column and an ED 40 electrochemical detector (Dionex) (Scholten & Stams, 1995). Sulfide was analysed by the method of Trüper & Schlegel (1964).

Strain HB1^T used the following substrates as electron donors and carbon sources (tested at 20 mM, unless indicated): H_2/acetate (1.7 bar/2 mM), formate, ethanol (5 mM), propionate, 1-propanol (5 mM), 1-butanol (5 mM), 2,3-butandiol (5 mM), fumarate, succinate, butyrate (10 mM), crotonate, catechol (0.5 mM), phenol (1 mM), benzoate (3 mM), 4-hydroxybenzoate (3 mM), palmitate (5 mM) and stearate (2 mM). Growth on ethanol, propionate, crotonate and benzoate was much faster than on butyrate. Growth on pyruvate and crotonate was possible without an electron acceptor. The strain was not able to grow on H_2/CO_2, methanol, acetone, lactate, malate, glucose or fructose. Slight growth was observed on acetate in the presence of sulfate. A maximum 2 mM acetate was utilized by strain HB1^T during 3 weeks of incubation and acetate was not fully oxidized, even after longer incubations. Aromatic compounds including catechol, phenol, benzoate and 4-hydroxybenzoate were oxidized completely by strain HB1^T.

Sulfate (20 mM) and thiosulfate (20 mM) were used as electron acceptors, but nitrate (10 mM), sulfite (5 mM) and elemental sulfur (0.5 g l^{-1}) were not. Thiosulfate and sulfite were not disproportionated.

Butyrate was oxidized completely by strain HB1^T. After 28 days of incubation in the presence of sulfate, no acetate was detected, and the molar ratio of the butyrate oxidized (7.2 mmol) to sulfide formed (16.0 mmol) of 1 : 2.23 was close to the expected theoretical values for the complete oxidation of butyrate according to the following reaction:

$$2CH_3CH_2CH_2COO^- + 5\,SO_4^{2-} \rightarrow 8\,HCO_3^- + 5\,HS^- + H^+$$

DNA was extracted as described by Zoetendal *et al.* (1998). PCR was performed with the bacterial primers 7f and 1510r (Lane, 1991) by using the *Taq* DNA polymerase kit (Life Technologies) to amplify the bacterial 16S rRNA gene. PCR products were purified with the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Sequencing of the complete 16S rRNA gene was performed at Westburg Genomics (http://genomics.westburg.nl). A total of 1535 nucleotides of the 16S rRNA gene were sequenced. The sequences were checked for reading errors with the alignment programs of the ARB package (Ludwig *et al.* 2004), and a rooted neighbour-joining tree was constructed using *Escherichia coli* ATCC 11775^T as an outgroup (Fig. 2). Online similarity analysis of the 16S rRNA gene sequences was performed with the BLAST program at the GenBank and EMBL databases. The closest phylogenetic relatives of strain HB1^T were *Desulfococcus multivorans* DSM 2059^T, *Desulfococcus biacutus* DSM 5651^T (sequence similarity of 91 % to both) and *Desulfobacterium indolicum* DSM 3383^T (90 %) (Fig. 2).

For lipid analyses, bacterial cultures of strain HB1^T grown on crotonate were harvested by centrifugation (14 500 ***g***, 20 min, 4 °C) and pellets were washed with distilled water. Lipids from lyophilized cells were extracted ultrasonically using a mixture of dichloromethane (DCM) and methanol (2 : 1, v/v) and this procedure was repeated four times. The residue was saponified with 1 M KOH in 96 % methanol by refluxing for 1 h and subsequently neutralized and extracted with DCM. After addition of internal standards, the free and bound lipid extracts were methylated and

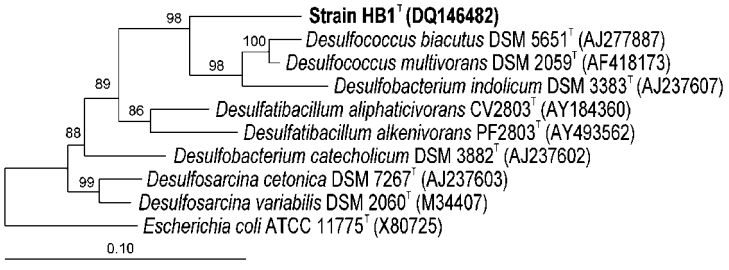

Fig. 2. Dendrogram showing the position of strain HB1^T among related bacteria. Phylogenetic analysis was based on 16S rRNA gene sequences available from GenBank (accession numbers in parentheses). The neighbour-joining tree was reconstructed from distance matrices; bootstrap values above 50 % are expressed at branching points. *Escherichia coli* ATCC 11775^T served as the outgroup. Bar, evolutionary distance of 0.10.

silylated and subsequently analysed by GC and GC-mass spectrometry (GC-MS). The most abundant fatty acids of strain HB1^T were $C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$ and $C_{14:0}$ (Table 1). After saponification of the residue, β-hydroxy fatty acids were also present in the range of $C_{14:0}$ to $C_{18:0}$, of which $C_{16:0}$ was the most abundant. The C_{35} lipid bacteriohopanetetrol was also present in small amounts (approx. 1 % of total lipids).

The presence of the fatty acids iso-$C_{15:0}$, anteiso-$C_{15:0}$ and $C_{16:0}$ as major components is also characteristic for *Desulfococcus multivorans* grown on benzoate (Kohring *et al.*, 1994), but the lipid profile of *Desulfococcus multivorans* differs from that of strain HB1^T by the predominance of anteiso-$C_{17:0}$. The predominant fatty acids of strain HB1^T were iso-$C_{15:0}$ and anteiso-$C_{15:0}$. This is not the case for *Desulfobacterium* species grown on benzoate (Kohring *et al.*, 1994). Bacteriohopanetetrol derivatives have recently been identified in sulfate reducers (Blumenberg *et al.* 2006); the presence of this lipid in strain HB1^T fits this pattern.

Based on morphological and physiological differences from members of the class *Deltaproteobacteria*, it is proposed that strain HB1^T represents a novel species in a new genus. Characteristics that differentiate strain HB1^T from phylogenetically related species are given in Table 2; other detailed characteristics determined are given in the species description below.

The 16S rRNA gene sequence, physiological and morphological characteristics of strain HB1^T were quite different from those of its phylogenetic neighbours. In addition, the closest similarity (91 %) of the 16S rRNA gene sequence of strain HB1^T with a recognized bacterium (the type strain of *Desulfococcus multivorans*) was much lower than the threshold level that is generally used to define a new genus (Ludwig *et al.*, 2004). Therefore, we propose that isolate HB1^T should be classified as representing a novel species in a new genus, *Desulfatirhabdium butyrativorans* gen. nov., sp. nov., within the family *Desulfobacteraceae*, class *Deltaproteobacteria*.

Table 1. Lipid composition of strain HB1^T

Values are percentages of total quantified lipids. –, Not detected.

Fatty acid	Free lipid fraction	Free and bound lipid fraction	Residual bound lipid fraction
iso-$C_{14:0}$	2	1	–
$C_{14:0}$	11	7	4
iso-$C_{15:0}$	17	14	17
anteiso-$C_{15:0}$	21	17	6
$C_{16:1}$	7	5	–
$C_{16:0}$	31	34	39
iso-C_{17}	1	1	3
$C_{17:1}$	2	2	–
$C_{18:1}$	1	1	–
$C_{18:0}$	6	16	12
β-OH-$C_{14:0}$	–	–	2
iso β-OH-$C_{15:0}$	–	–	1
β-OH-$C_{16:0}$	–	–	11
iso β-OH-$C_{17:0}$	–	–	1.5
β-OH-$C_{18:0}$	–	–	0.5

Description of *Desulfatirhabdium* gen. nov.

Desulfatirhabdium (De.sul.fa′ti.rhab′di.um. L. pref. *de-* from; N.L. masc. n. *sulfas -atis* sulfate; Gr. neut. n. *rhabdium* a little rod; N.L. neut. n. *Desulfatirhabdium* a sulfate-reducing small rod).

Cells are anaerobic, Gram-negative, non-spore-forming and oval to rod-shaped. Thiosulfate and sulfate are reduced to sulfide. Volatile fatty acids are oxidized completely. Predominant cellular fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:0}$ and $C_{14:0}$. Phylogenetically, the genus belongs to the family *Desulfobacteraceae* in the class *Deltaproteobacteria*. The type species is *Desulfatirhabdium butyrativorans*.

Description of *Desulfatirhabdium butyrativorans* sp. nov.

Desulfatirhabdium butyrativorans (bu.ty.ra.ti.vo′rans. N.L. n. *butyras -atis* butyrate; L. part. adj. *vorans* devouring; N.L. part. adj. *butyrativorans* butyrate-devouring).

Displays the following properties in addition to those described for the genus. Cells are 1–1.3 × 2.6–3.5 μm. Colonies are brownish, lens-shaped in the agar after about 1 month of incubation. Growth occurs at 15–37 °C (optimum 28–30 °C) and pH 6.5–8.0 (optimum pH 7.0). Growth occurs at NaCl concentrations of 0–15 g NaCl l^{-1}, with an optimum at 5 g NaCl l^{-1}. Sulfate and thiosulfate are used as electron acceptors. In the presence of an electron acceptor, growth occurs on H_2/acetate (1.7 bar/2 mM), formate (20 mM), ethanol (5 mM), pyruvate (20 mM), propionate (20 mM), propanol (5 mM), 1-butanol (5 mM), 2,3-butandiol (5 mM), fumarate (20 mM), succinate (20 mM), 1-butyrate (10 mM), crotonate (20 mM), catechol (0.5 mM), phenol (1 mM), benzoate (3 mM), 4-hydroxybenzoate (3 mM), palmitate (5 mM) and stearate (2 mM). Only slight growth is observed on acetate. Fermentative growth occurs on pyruvate and crotonate. Not able to grow on H_2/CO_2, methanol, acetone, lactate, malate, glucose or fructose. β-Hydroxy fatty acids are present in the range of $C_{14:0}$ to $C_{18:0}$, of which $C_{16:0}$ was the most abundant. The DNA G+C content of the type strain is 55.1 mol%.

The type strain, HB1^T (=DSM 18734^T =JCM 14470^T), was isolated from a UASB reactor treating paper-mill wastewater operated at 37 °C with butyrate as the energy source.

Table 2. Characteristics of strain HB1^T and related representatives of the genera *Desulfococcus* and *Desulfobacterium*

Data for reference species were obtained from Widdel (1980), Bak & Widdel (1986) and Platen *et al.* (1990). NR, Not reported; (+) weak growth. All taxa use ethanol and propionate as electron donors and sulfate as an electron acceptor.

Characteristic	Strain HB1^T	*Desulfococcus multivorans*	*Desulfococcus biacutus*	*Desulfobacterium indolicum*
Morphology	Oval to rod	Spherical	Lemon	Oval to rod
Origin	Anaerobic reactor	Sewage digester	Sewage digester	Marine sediment
DNA G+C content (mol%)	55.1	57	56.5	47.4
Optimum temperature (°C)	28–30	35	20–30	28
Optimum pH	7.0	NR	6.8–7.8	7.0
Optimum salinity (g NaCl l^{-1})	5	5	−	20
Electron donors				
H_2/CO_2	−	−	NR	−
Formate	+	+	NR	+
Acetate	(+)	(+)	(+)	+
Pyruvate	+	NR	+	+
Lactate	−	+	−	−
Acetone	−	+	+	NR
Propanol	+	NR	+	+
Fumarate	+	−	−	+
Succinate	+	−	−	+
Malate	−	−	−	+
Butanol	+	NR	+	+
Butyrate	+	+	+	−
Catechol	+	NR	NR	−
Benzoate	+	+	−	−
Palmitate	+	NR	NR	NR
Stearate	+	NR	NR	NR
Electron acceptors				
Thiosulfate	+	+	NR	+
Sulfite	−	+	+	NR
Nitrate	−	−	−	NR

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References

Bak, F. & Widdel, F. (1986). Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., sp. nov. *Arch Microbiol* **146**, 170–176.

Balk, M., Weijma, J., Friedrich, M. W. & Stams, A. J. M. (2003). Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. *Arch Microbiol* **179**, 315–320.

Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H. M., Oppermann, B. I., Seifert, R., Pape, T. & Michaelis, W. (2006). Biosynthesis of hopanoids by sulfate-reducing bacteria (genus *Desulfovibrio*). *Environ Microbiol* **8**, 1220–1227.

Brandt, K. K., Patel, B. K. C. & Ingvorsen, K. (1999). *Desulfocella halophila* gen. nov., sp. nov., a halophilic, fatty-acid-oxidizing, sulfate-reducing bacterium isolated from sediments of the Great Salt Lake. *Int J Syst Bacteriol* **49**, 193–200.

Brysch, K., Schneider, C., Fuchs, G. & Widdel, F. (1987). Lithoautotrophic growth of sulfate-reducing bacteria, and description of *Desulfobacterium autotrophicum*. *Arch Microbiol* **148**, 264–274.

Campbell, L. L. & Postgate, J. R. (1965). Classification of the spore-forming sulfate-reducing bacteria. *Bacteriol Rev* **29**, 359–363.

Canfield, D. E., Jorgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., Thamdrup, B., Hansen, J. W., Nielsen, L. P. & Hall, P. O. (1993). Pathways of organic carbon oxidation in three continental margin sediments. *Mar Geol* **113**, 27–40.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Colleran, E., Finnegan, S. & Lens, P. (1995). Anaerobic treatment of sulfate-containing waste streams. *Antonie van Leeuwenhoek* **67**, 29–46.

Cravo-Laureau, C., Matheron, R., Joulian, C., Cayol, J. L. & Hirschler-Réa, A. (2004). *Desulfatibacillum alkenivorans* sp. nov., a novel n-alkene-degrading, sulfate-reducing bacterium, and emended description of the genus *Desulfatibacillum*. *Int J Syst Evol Microbiol* **54**, 1639–1642.

Glud, R. N., Risgaard-Petersen, N., Thamdrup, B., Fossing, H. & Rysgaard, S. (2000). Benthic carbon mineralization in a high-Arctic sound (Young Sound, NE Greenland). *Mar Ecol Prog Ser* **206**, 59–71.

Gogotova, G. I. & Vainstein, M. B. (1989). Description of sulfate-reducing bacterium *Desulfobacterium macestii* sp. nov. capable of autotrophic growth. *Mikrobiologiia* **58**, 76–80 (in Russian).

Henry, E. A., Devereux, R., Maki, J. S., Gilmour, C. C., Woese, C. R., Mandelco, L., Schauder, R., Remsen, C. C. & Mitchell, R. (1994). Characterization of a new thermophilic sulfate-reducing bacterium *Thermodesulfovibrio yellowstonii*, gen. nov. and sp. nov.: its phylogenetic relationship to *Thermodesulfobacterium commune* and their origins deep within the bacterial domain. *Arch Microbiol* **161**, 62–69.

Henstra, A. M. & Stams, A. J. M. (2004). Novel physiological features of *Carboxydothermus hydrogenoformans* and *Thermoterrabacterium ferrireducens*. *Appl Environ Microbiol* **70**, 7236–7240.

Jørgensen, B. B. (1982). Mineralization of organic matter in the sea bed – the role of sulfate reduction. *Nature* **296**, 643–645.

Kjeldsen, K. U., Loy, A., Jakobsen, T. F., Thomsen, T. R., Wagner, M. & Ingvorsen, K. (2007). Diversity of sulfate-reducing bacteria from an extreme hypersaline sediment, Great Salt Lake (Utah). *FEMS Microbiol Ecol* **60**, 287–298.

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.

Kostka, J. E., Thamdrup, B., Glud, R. N. & Canfield, D. E. (1999). Rates and pathways of carbon oxidation in permanently cold Arctic sediments. *Mar Ecol Prog Ser* **180**, 7–21.

Kuever, J., Rainey, F. A. & Widdel, F. (2005). Family I. *Desulfobacteraceae* fam. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 959–960. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

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.

Mori, K., Kim, H., Kakegawa, T. & Hanada, S. (2003). A novel lineage of sulfate-reducing microorganisms: *Thermodesulfobiaceae* fam. nov., *Thermodesulfobium narugense*, gen. nov., sp. nov., a new thermophilic isolate from a hot spring. *Extremophiles* **7**, 283–290.

Moussard, H., L'Haridon, S., Tindall, B. J., Banta, A., Schumann, P., Stackebrandt, E., Reysenbach, A.-L. & Jeanthon, C. (2004). *Thermodesulfatator indicus* gen. nov., sp. nov., a novel thermophilic chemolithoautotrophic sulfate-reducing bacterium isolated from the Central Indian Ridge. *Int J Syst Evol Microbiol* **54**, 227–233.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods of General and Molecular Biology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Oude Elferink, S. J. W. H., Lens, P. N. L., Dijkema, C. & Stams, A. J. M. (1994). Sulfate reduction in methanogenic bioreactors. *FEMS Microbiol Lett* **142**, 237–241.

Oude Elferink, S. J. W. H., Boschker, H. T. S. & Stams, A. J. M. (1998). Identification of sulfate reducers and *Syntrophobacter* sp. in anaerobic granular sludge by fatty acid biomarkers and 16S rRNA probing. *Geomicrobiol J* **15**, 3–17.

Platen, H., Temmes, A. & Schink, B. (1990). Anaerobic degradation of acetone by *Desulfococcus biacutus* sp. nov. *Arch Microbiol* **154**, 355–361.

Rabus, R., Hansen, T. A. & Widdel, F. (2006). Dissimilatory sulfate- and sulfur-reducing prokaryotes. In *The Prokaryotes. A Handbook on the Biology of Bacteria*, 3rd edn, vol. 2, pp 659–768. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Rees, G. N. & Patel, B. K. (2001). *Desulforegula conservatrix* gen. nov., sp. nov., a long-chain fatty acid-oxidizing, sulfate-reducing bacterium isolated from sediments of a freshwater lake. *Int J Syst Evol Microbiol* **51**, 1911–1916.

Roest, K., Heilig, H. G., Smidt, H., de Vos, W. M., Stams, A. J. & Akkermans, A. D. (2005). Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. *Syst Appl Microbiol* **28**, 175–185.

Rysgaard, S., Thamdrup, B., Risgaard-Petersen, N., Fossing, H., Berg, P., Christensen, P. B. & Dalsgaard, T. (1998). Seasonal carbon and nutrient mineralization in a high-Arctic coastal marine sediment, Young Sound, Northeast Greenland. *Mar Ecol Prog Ser* **175**, 261–276.

Schnell, S., Bak, F. & Pfennig, N. (1989). Anaerobic degradation of aniline and dihydroxybenzenes by newly isolated sulfate-reducing bacteria and description of *Desulfobacterium anilini*. *Arch Microbiol* **152**, 556–563.

Scholten, J. C. & Stams, A. J. M. (1995). The effect of sulfate and nitrate on methane formation in a freshwater sediment. *Antonie van Leeuwenhoek* **68**, 309–315.

Stams, A. J. M., Veenhuis, M., Weenk, G. H. & Hansen, T. A. (1983). Occurrence of polyglucose as a storage polymer in *Desulfovibrio* species and *Desulfobulbus propionicus*. *Arch Microbiol* **136**, 54–59.

Stams, A. J. M., van Dijk, J. B., Dijkema, C. & Plugge, C. M. (1993). Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. *Appl Environ Microbiol* **59**, 1114–1119.

Stieb, M. & Schink, B. (1989). Anaerobic degradation of isobutyrate by methanogenic enrichment cultures and by a *Desulfococcus multivorans* strain. *Arch Microbiol* **151**, 126–132.

Szewzyk, R. & Pfennig, N. (1987). Complete oxidation of catechol by strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. *Arch Microbiol* **147**, 163–168.

Thamdrup, B. & Canfield, D. E. (1996). Pathways of carbon oxidation in continental margin sediments of central Chile. *Limnol Oceanogr* **41**, 1629–1650.

Trüper, H. G. & Schlegel, H. G. (1964). Sulphur metabolism in *Thiorhodaceae*. I. Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie van Leeuwenhoek* **30**, 225–238.

Widdel, F. (1980). *Anaerober Abbau von Fettsäuren und Benzoesäure durch neu Isolierte Arten Sulfat-reduzierender Bakterien*. PhD thesis, Göttingen University, Germany (in German).

Widdel, F. & Bak, F. (1992). Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn., vol. 4, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Zeikus, J. G., Dawson, M. A., Thompson, T. E., Ingvorsen, K. & Hatchikian, E. C. (1983). Microbial ecology of volcanic sulfidogenesis: isolation and characterization of *Thermodesulfobacterium commune* gen. nov. and sp. nov. *J Gen Microbiol* **129**, 1159–1169.

Zoetendal, E. G., Akkermans, A. D. L. & de Vos, W. M. (1998). Temperature gradient gel electrophoresis from human fecal samples reveals stable and host-specific communities of bacteria. *Appl Environ Microbiol* **64**, 3854–3859.