Desulfovibrio butyratiphilus sp. nov., a Gram-negative, butyrate-oxidizing, sulfate-reducing bacterium isolated from an anaerobic municipal sewage sludge digester

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Strictly anaerobic, mesophilic, sulfate-reducing bacterial strains were isolated from two anaerobic municipal sewage sludge digesters. One representative strain (BSY^T) was characterized phenotypically and phylogenetically. Cells were Gram-negative, motile by means of a single polar flagellum, non-spore-forming, curved rods. Cells had desulfoviridin and cytochrome type *c*. Catalase and oxidase activities were not detected. The optimum NaCl concentration for growth was 0.5 % (w/v). The optimum temperature was 35 °C and the optimum pH was 7.1. Strain BSY^T utilized butyrate, 2-methylbutyrate, valerate, pyruvate, lactate, ethanol, 1-propanol, butanol and H_2 as electron donors for sulfate reduction. This strain grew lithoautotrophically with H_2/CO_2 under sulfate-reducing conditions. Most organic electron donors were incompletely oxidized to mainly acetate, whereas 2-methylbutyrate and valerate were oxidized to equivalent amounts of acetate and propionate. Strain BSY^T utilized thiosulfate as an electron acceptor, and grew with pyruvate in the absence of electron acceptors. The genomic DNA G+C content of strain BSY^T was 63.3 mol%. Menaquinone MK-6(H_2) was the major respiratory quinone. Major cellular fatty acids were $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7$ and $C_{18:1}\omega7$. Phylogenetic analyses based on 16S rRNA and dissimilatory sulfite-reductase β-subunit gene sequences assigned strain BSY^T to the genus *Desulfovibrio* in the family *Desulfovibrionaceae* within the class *Deltaproteobacteria*. Its closest recognized relative based on 16S rRNA gene sequences was the type strain of *Desulfovibrio putealis* (95.3 % similarity). On the basis of significant differences in 16S rRNA gene sequences and phenotypic characteristics, the sewage sludge strains are considered to represent a single novel species of the genus *Desulfovibrio*, for which the name *Desulfovibrio butyratiphilus* sp. nov. is proposed. The type strain is BSY^T (=JCM 15519^T=DSM 21556^T).

Butyrate is an important intermediate in the anaerobic degradation of organic matter in various anaerobic ecosystems. Because the oxidation of butyrate is usually thermodynamically unfavourable under anaerobic conditions, it is generally degraded by syntrophic interactions between H_2-producing acetogenic bacteria and H_2-utilizing methanogens in methanogenic conditions (Stams, 1994; Schink, 1997; Sekiguchi *et al.*, 2000; Zhang *et al.*, 2004). However, in the presence of sulfate as an electron acceptor, some sulfate-reducing bacterial species oxidize butyrate either completely to CO_2 or incompletely to acetate (Rabus *et al.*, 2000). These sulfate-reducing bacterial species belong to the families *Desulfobacteraceae* (Cravo-Laureau *et al.*, 2004; Kuever *et al.*, 2005; Balk *et al.*, 2008; Suzuki *et al.*, 2008), *Desulfohalobiaceae* (Belyakova *et al.*, 2006) and *Syntrophobacteraceae* (Beeder *et al.*, 1995; Sievert & Kuever, 2000; Tanaka *et al.*, 2000) in the class *Deltaproteobacteria* or to the genus *Desulfotomaculum* in the phylum *Firmicutes* (Daumas *et al.*, 1988; Tasaki *et al.*, 1991; Fardeau *et al.*, 1995; Kuever *et al.*, 1999; Vandieken *et al.*, 2006).

In the present study, we isolated five sulfate-reducing bacterial strains (BSY^T, BSY-C, BST, BST-B and BST-C) from two anaerobic municipal sewage sludge digesters via enrichment cultures. All strains reduced sulfate with

Abbreviation: DSR, dissimilatory sulfite-reductase.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains BST, BST-B, BST-C, BSY^T and BSY-C are AB303302, AB303303, AB303304, AB303305 and AB303306, respectively. The accession number for the dissimilatory sulfite-reductase β-subunit gene sequence of strain BSY^T is AB490775.

A table comparing the cellular fatty acid compositions of strain BSY^T and *Desulfovibrio sulfodismutans* ThAc01^T and *Desulfovibrio carbinolicus* EDK82^T is available as supplementary material with the online version of this paper.

butyrate as an electron donor and were closely related to species in the genus *Desulfovibrio* based on initial 16S rRNA gene sequence analysis. Strain BSY^T was selected as a representative and was further characterized comprehensively. Butyrate-oxidizing *Desulfovibrio* species have not previously been described, and differences in phylogenetic and phenotypic characteristics between strain BSY^T and related *Desulfovibrio* species supported the proposal that it represents a novel species of the genus *Desulfovibrio*.

Samples obtained from two anaerobic digesters treating municipal sewage sludge (Yokohama and Tsuruoka in Japan) were used for isolation of bacterial strains. Each sewage sludge sample was inoculated (0.5 ml each) into defined liquid medium (9.5 ml) containing 20 mM sodium butyrate as described below under the flow of O_2-free gas (95 : 5 N_2/CO_2). Cultures (0.1 ml) showing sulfate reduction were transferred to the same fresh medium (10 ml). After several subcultures, sulfate-reducing bacteria were isolated from the cultures by using the anaerobic roll tube method (Hungate, 1966). Black colonies that appeared in the agar were picked and five isolates (strains BSY^T and BSY-C from Yokohama and strains BST, BST-B and BST-C from Tsuruoka) were obtained after purification procedures. The novel strains displayed high levels of 16S rRNA gene sequence similarity (about 99–100 %) and showed almost identical phenotypic characteristics, including utilization of both electron donors and acceptors (see further below). Thus, strain BSY^T was selected as a representative for further characterization.

The following defined medium was used for the enrichment culture, isolation and the general physiological characterization of the strains (per litre distilled water): 0.5 g KH_2PO_4, 1.0 g NH_4Cl, 2.5 g $MgSO_4 . 7H_2O$, 0.1 g $CaCl_2 . 2H_2O$, 1 mg sodium resazurin, 10 ml trace element solution (Widdel *et al.*, 1983), 1.0 g NaCl and 0.5 g L-cysteine . HCl . H_2O with appropriate electron donors (Ueki *et al.*, 1980; Widdel & Bak, 1992). pH was adjusted to 7.4–7.5 with 1 M NaOH. Agar (Difco) (1.5 %, w/v) medium with sodium butyrate (20 mM) was used for the anaerobic roll tube method for isolation as well as slant cultures for maintenance of isolates. Cultivation and transfer of the enrichment cultures and isolates were carried out under an O_2-free N_2/CO_2 (95 : 5) atmosphere. Cultivation was at 30 °C unless stated otherwise.

The Gram reaction and cell morphology were confirmed by using light microscopy. Cell motility was examined by using phase-contrast microscopy. Staining of flagella was carried out according to Blenden & Goldberg (1965). Physiological tests were performed according to the methods described by Suzuki *et al.* (2007a, b, c). Utilization of electron donors by the isolates was determined by using the defined medium containing each compound at a final concentration of 20 mM. Utilization of H_2 as an electron donor was determined in the presence or absence of acetate (5 mM) as an organic carbon source under an H_2/CO_2 (90 : 10) atmosphere. Utilization of electron acceptors other than sulfate was determined in sulfate-free medium containing the same concentrations of chloride in place of sulfate in the defined medium (Suzuki *et al.*, 2007a, b, c). Substrate utilization in the absence of electron acceptors was determined in sulfate-free medium (Suzuki *et al.*, 2007a, b, c). Fatty acids and amino acids were used in the form of sodium salts and were added to the medium from sterilized stock solutions. Utilization of each electron donor or acceptor was determined by comparing growth in the presence or absence of each compound as well as measurement of the concentration in the medium after cultivation. Growth was monitored by direct measurement of the optical density at 660 nm (OD_{660}) of the culture tubes with a spectrophotometer.

Volatile fatty acids, non-volatile fatty acids, alcohols, gases, sulfate, sulfite, thiosulfate and nitrate were analysed as described by Akasaka *et al.* (2003a), Nakamoto *et al.* (1996) and Ueki *et al.* (1986). The presence of desulfoviridin in cells was determined according to the method of Postgate (1959). The presence and type of cytochrome were determined by measuring an air-oxidized/dithionite-reduced difference spectrum of cell-free extract (U-2010 spectrophotometer; Hitachi). Genomic DNA extracted according to the method described by Akasaka *et al.* (2003b) was digested with P1 nuclease by using a YAMASA GC kit (Yamasa Shoyu) and its G+C content was measured by HPLC (L-7400; Hitachi), the chromatograph being equipped with a μBondapak C18 column (3.9 × 300 mm; Waters). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by using a mass spectrometer (JMS-SX102A; JEOL). Whole-cell fatty acids were converted to methyl esters according to the method of Miller (1982). Methyl esters of cellular fatty acids were analysed with a gas chromatograph (Hewlett Packard Hp6890 or Hitachi G-3000) equipped with an Ultra 2 column (Hewlett Packard). Fatty acids were identified by equivalent chain-length (Miyagawa *et al.*, 1979; Ueki & Suto, 1979) according to the protocol of TechnoSuruga Co., Ltd based on the MIDI microbial identification system (Microbial ID) of Moore *et al.* (1994).

Extraction of DNA and PCR amplification of the 16S rRNA genes of the novel strains were carried out according to the methods described by Akasaka *et al.* (2003b). The PCR-amplified 16S rRNA gene (by using primer set 27F and 1492R) was sequenced via a Thermo Sequenase Primer Cycle Sequencing kit (Amersham Biosciences) and a model of 4000L DNA sequencer (Li-COR). Multiple alignments of the sequence with reference sequences in GenBank/EMBL/DDBJ were performed with the BLAST program (Altschul *et al.*, 1997). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) by using the CLUSTAL W program (Thompson *et al.*, 1994) as well as the maximum-likelihood program (DNAML) of the PHYLIP 3.66 package (Felsenstein, 2006). All gaps and unidentified base positions in the alignments were excluded before assemblage.

A partial sequence of the gene encoding the α- and β-subunits of dissimilatory sulfite-reductase (DSR) was amplified by using primer set P94-F and P93-R (Karkhoff-Schweizer *et al.*, 1995), with DNA extracted from cells of strain BSY^T. The PCR product was cloned by using the pGEM-T Easy vector (Promega) and was recovered from each colony by PCR with primers T7W and SP6W (as a primer set for the pGEM-T Easy vector sequence flanking the insertion) (Watanabe *et al.*, 2000). The partial sequence of the gene encoding the β-subunit of DSR within the PCR product was sequenced, and phylogenetic analysis was performed according to the methods described above for the 16S rRNA gene. Amplification of the DSR gene sequence with primer set DSR1F/DSR4R (Wagner *et al.*, 1998) was unsuccessful.

Cells of strain BSY^T were Gram-negative, relatively large curved rods with rounded ends, 0.8–0.9 μm wide and 2.4–5.6 μm long. Cells usually occurred singly and spore formation was not observed (Fig. 1). Cells were motile by means of a single polar flagellum. Cells formed greyish, thin colonies on agar slant medium. Strain BSY^T did not grow aerobically. Desulfoviridin was detected in the cell extract. A difference absorption spectrum of air-oxidized/dithionite-reduced cell extract showed peaks at 418 and 553 nm, which indicated the presence of cytochrome type *c*. Catalase and oxidase activities were not detected.

Strain BSY^T did not grow in the absence of added electron donors and required carbonate or bicarbonate for growth in the defined medium. Table 1 gives details of consumption of electron donors and compounds produced by strain BSY^T by sulfate reduction as well as the growth rate with each electron donor. In the presence of sulfate as an electron acceptor, strain BSY^T utilized butyrate, 2-methylbutyrate, valerate, pyruvate, lactate, ethanol, 1-propanol, butanol and H_2. It showed weak lithoautotrophic growth with H_2/CO_2 in the absence of acetate under sulfate-reducing conditions.

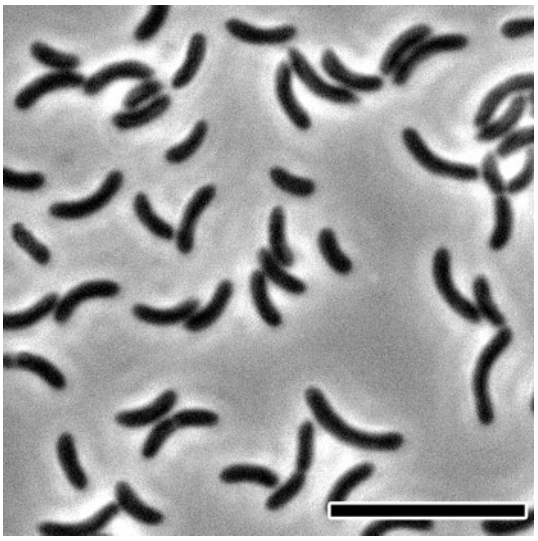

Fig. 1. Phase-contrast photomicrograph of cells of strain BSY^T grown anaerobically in defined medium. Bar, 10 μm.

Butyrate, pyruvate, lactate, ethanol and butanol were oxidized to acetate, whereas almost equivalent amounts of acetate and propionate were produced during anaerobic growth with 2-methylbutyrate and valerate. When the time-courses of consumption of the latter two electron donors were examined, the same amounts of acetate and propionate accumulated simultaneously in the medium accompanied by a decrease in the concentration of each electron donor as well as sulfate. 1-Propanol was oxidized to propionate. Thus, strain BSY^T had an incomplete-type oxidation profile of organic substrates. The stoichiometric ratio of butyrate oxidation (butyrate oxidized/sulfate reduced/acetate produced) was about 2 : 1 : 4. The ratios for 2-methylbutyrate and valerate (2-methylbutyrate or valerate/sulfate/acetate/propionate) were about 2 : 1 : 2 : 2. These ratios were consistent with theoretical values for the incomplete oxidation of the substrates through the β-oxidation pathway. When cells of strain BSY^T were cultivated with butanol as an electron donor, acetate was detected as a major product (butanol/sulfate/acetate, 1 : 1 : 2). During the time-course of sulfate reduction with butanol, a small amount of butyrate was produced after sulfate was almost exhausted. When strain BSY^T was cultivated with H_2/CO_2 + acetate, a trace amount of butyrate was also detected.

Strain BSY^T grew with butyrate as the preferred electron donor without a significant lag period after inoculation of cells to the medium. In contrast, relatively long lag periods were observed for growth with 2-methylbutyrate (5–6 days after inoculation) and valerate (7–8 days). Lag periods were not significantly shortened even after successive subcultures in the medium containing the same electron donors. After beginning of growth as observed by an increase in turbidity of the culture, however, strain BSY^T grew rapidly at almost the same growth rates as that with butyrate.

No growth was observed with the following electron donors for sulfate reduction: formate, acetate, propionate, isobutyrate, isovalerate, caprylate, crotonate, fumarate, malate, succinate, methanol, 2-propanol, glycerol, glycine, L-alanine, L-serine, L-aspartate, L-glutamate, D-glucose, D-fructose and yeast extract (0.05 %, w/v).

Strain BSY^T utilized thiosulfate as an electron acceptor with butyrate as an electron donor. The growth rate was almost the same as that with sulfate. The stoichiometric ratio (butyrate/thiosulfate/acetate) was about 2 : 1 : 4. The strain did not use sulfite, nitrate or fumarate as electron acceptors. In the absence of electron acceptors, pyruvate supported weak growth of strain BSY^T. The strain produced acetate (0.9 mmol l^{-1}), butyrate (1.4 mmol l^{-1}), CO_2 (1.4 mmol l^{-1}) and a trace amount of H_2 (1.4 mmol l^{-1}) by pyruvate oxidation. It did not oxidize butyrate, lactate, fumarate or malate in the absence of electron acceptors.

Table 1. Utilization of substrates as electron donors and compounds produced by strain BSY^T by sulfate reduction

ND, Not determined; −, absent.

Electron donor	Electron donors consumed (mmol l^{-1})	Sulfate reduced (mmol l^{-1})	Compounds produced (mmol l^{-1})		Specific growth rate (h^{-1})
			Acetate	Others	
No addition	ND	ND	0.2	−	ND
Butyrate	20.0	9.3	41.2	−	0.058
2-Methylbutyrate	16.3	8.7	16.7	Propionate (16.3)	0.053
Valerate	15.7	7.1	16.4	Propionate (15.6)	0.050
Pyruvate	7.8	2.3	10.4	−	0.013
Lactate	4.6	2.2	4.5	−	0.013
Ethanol	17.9	7.9	19.6	−	0.025
1-Propanol	18.3	7.6	–	Propionate (16.7)	0.025
Butanol	8.3	8.5	15.4	Butyrate (1.6)	0.031
H_2/CO_2 + acetate	ND	9.4	−	Butyrate (0.3)	0.063
H_2/CO_2	ND	3.5	−	−	0.012

In the presence of butyrate as an electron donor, the NaCl concentration range for growth of strain BSY^T was 0–2.0 % (w/v) with an optimum at 0.5 % (w/v), temperature range for growth was 25–40 °C with an optimum at 35 °C, and pH range for growth was 6.2–8.0 with an optimum at pH 7.1.

The G + C content of the genomic DNA of strain BSY^T was 63.3 mol%. The major respiratory quinone was menaquinone MK-6(H_2). The strain had $C_{18:1}\omega7$ (32.1 %), $C_{16:1}\omega7$ (25.5 %), $C_{14:0}$ (24.1 %) and $C_{16:0}$ (10.2 %) as major cellular fatty acids, with $C_{12:0}$, $C_{15:0}$, $C_{18:0}$, $C_{16:1}\omega5$, $C_{18:1}\omega9$, $C_{18:1}\omega5$, iso-$C_{12:0}$, anteiso-$C_{15:0}$, $C_{16:0}$ 2-OH, $C_{14:0}$ dimethylacetal, $C_{16:0}$ dimethylacetal, $C_{17:0}$ cyclopropane and C_{19} cyclopropane as minor or trace components.

An almost full-length 16S rRNA gene sequence (1450 bp) of strain BSY^T was determined. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BSY^T was affiliated with the class *Deltaproteobacteria* and was related to members of the genus *Desulfovibrio* in the family *Desulfovibrionaceae* (Fig. 2). Its closest relative was 'Uncultured deltaproteobacterium clone MBNTA bac-1' (95.4 % 16S rRNA gene sequence similarity). The closest recognized relative of strain BSY^T was the type strain of *Desulfovibrio putealis* (95.3 % 16S rRNA gene sequence similarity); the next closest relatives were the type strains of *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* (with much lower sequence similarities of 90.6 and 90.5 %, respectively). Together with the other strains (BSY-C, BST, BST-B and BST-C) isolated in this study, strain BSY^T formed a distinct cluster within the *Desulfovibrio* clade (Fig. 2). Strain BSY^T was distantly related to the type strain of the type species of the genus *Desulfovibrio*, *Desulfovibrio desulfuricans* (86.7 % 16S rRNA gene sequence similarity). The tree topology evaluated by using the maximum-likelihood method was almost the same as that obtained with the neighbour-joining method.

The partial sequence (730 bp) of the β-subunit of the DSR gene of strain BSY^T was determined. Phylogenetic analysis based on DSR gene sequences showed that strain BSY^T was related most closely to 'Uncultured sulfate-reducing bacterium clone GranDSR12' (83.5 % sequence similarity). Its closest recognized relatives were the type strains of *Desulfovibrio alkalitolerans* and *Desulfovibrio aminophilus* (73.4 % DSR gene sequence similarity). Thus, on the basis of DSR gene sequence analysis, strain BSY^T was also closely related to species of the genus *Desulfovibrio*. The level of DSR gene sequence similarity between strain BSY^T and the type strain of *Desulfovibrio carbinolicus* was 66.7 %. The sequences of *Desulfovibrio putealis* and *Desulfovibrio sulfodismutans* were not available.

Strain BSY^T shared major characteristics with *Desulfovibrio* species such as cell morphology, presence of cytochrome type *c* and desulfoviridin as a sulfite-reductase, incomplete oxidation of electron donors, and mesophilic growth (Kuever *et al.*, 2005). Strain BSY^T contained MK-6(H_2) as the major respiratory quinone, consistent with data for the genus *Desulfovibrio* (Collins & Widdel, 1986). However, strain BSY^T could be distinguished from recognized *Desulfovibrio* species based on utilization of electron donors, namely utilization of butyrate, 2-methylbutyrate and valerate as electron donors for sulfate reduction. Some sulfate-reducing bacterial species belonging to the families *Desulfobacteraceae* and *Syntrophobacteraceae* utilize butyrate as well as longer-chain fatty acids (Beeder *et al.*, 1995; Sievert & Kuever, 2000; Tanaka *et al.*, 2000; Cravo-Laureau *et al.*, 2004; Kuever *et al.*, 2005; Balk *et al.*, 2008), although 2-methylbutyrate is utilized by only a few sulfate-reducing bacterial species belonging to the genera *Desulfobacterium*, *Desulfococcus*, *Desulfonema* and *Desulfosarcina* (Kuever *et al.*, 2005). Given that sulfate-reducing bacterial strains with the same properties as strain BSY^T were enriched and isolated from two digesters located distantly from each other, it appears that such bacteria are widely distributed in anaerobic municipal sewage sludge digesters.

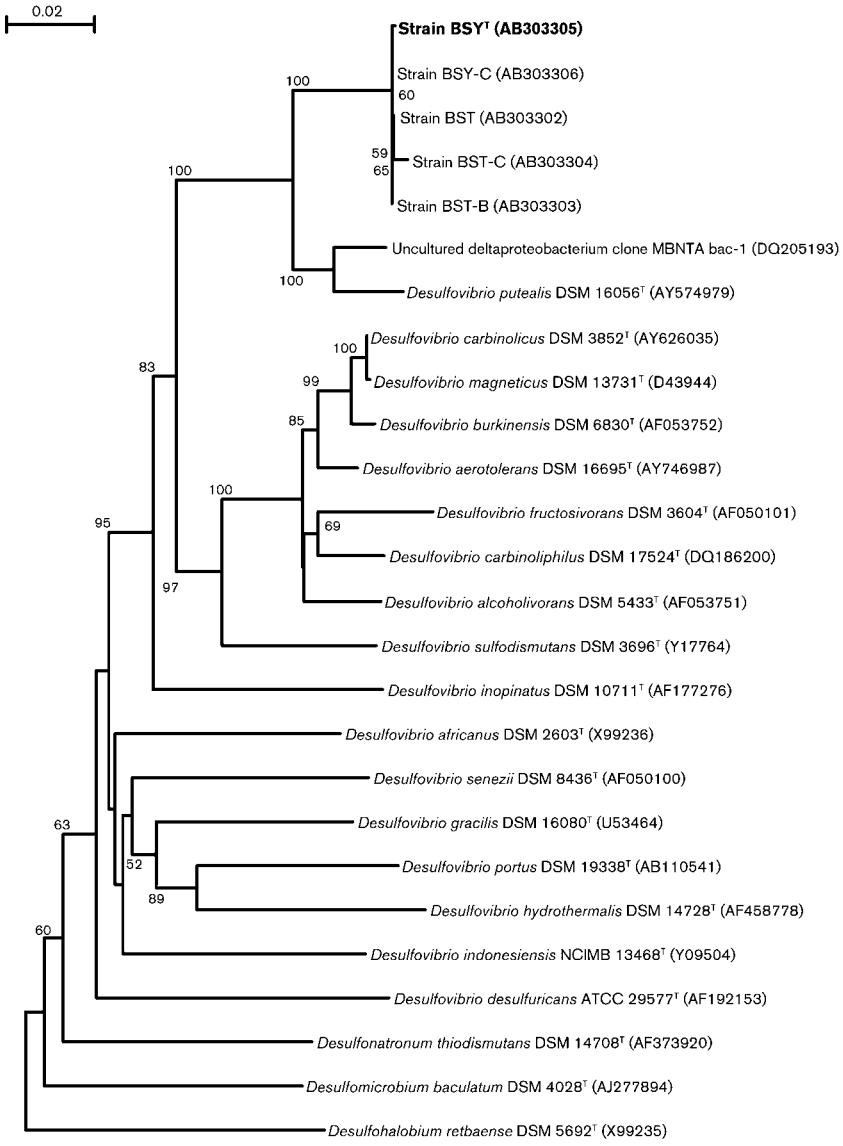

Fig. 2. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain BSY^T and related members of the order *Desulfovibrionales*. Bootstrap values (expressed as percentages of 1000 replications) above 50 % are shown at branch nodes. *Desulfohalobium retbaense* DSM 5692^T was used as an outgroup. Bar, 2 % estimated sequence divergence.

The physiological characteristics of strain BSY^T were compared with those of the type strains of *Desulfovibrio putealis*, *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* (Table 2). In addition to data for butyrate, 2-methylbutyrate and valerate, the range of electron donor utilization (such as formate, fumarate, malate, succinate and propanol) of strain BSY^T was not consistent with these three species. Strain BSY^T was unable to utilize sulfite or fumarate as electron acceptors, whereas *Desulfovibrio putealis*, *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* were able to utilize at least one of them. In the absence of electron acceptors, strain BSY^T and *Desulfovibrio sulfodismutans* did not utilize fumarate or malate, whereas *Desulfovibrio putealis* and *Desulfovibrio carbinolicus* utilize both.

The cellular fatty acid profiles of strain BSY^T and the type strains of *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* are compared in Supplementary Table S1 (available in IJSEM Online); the profile for *Desulfovibrio putealis* has not been reported. Most species of the genus *Desulfovibrio* have branched-chain fatty acids such as iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ or iso-$C_{17:1}$ as major or dominant components (Ueki & Suto, 1979; Kohring *et al.*, 1994; Vainshtein *et al.*, 1992). *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* have branched-chain fatty acids (anteiso-$C_{15:0}$, iso-$C_{16:0}$ or anteiso-$C_{17:0}$) as major components; by contrast, strain BSY^T had saturated and unsaturated straight-chain fatty acids as major components but only trace amounts of branched-chain fatty acids. The DNA G+C content of strain BSY^T was almost the same as those of the type strains of *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* (Table 2).

Thus, in addition to differences in 16S rRNA and DSR β-subunit gene sequences, the characteristics of strain BSY^T

Table 2. Differential characteristics between strain BSY^T and the type strains of related *Desulfovibrio* species

Strains: 1, BSY^T (*D. butyratiphilus* sp. nov.); 2, *Desulfovibrio putealis* B7-43^T (data from Basso *et al.*, 2005); 3, *Desulfovibrio sulfodismutans* ThAc01^T (Bak & Pfennig, 1987); 4, *Desulfovibrio carbinolicus* EDK82^T (Nanninga & Gottschal, 1987). +, Used; −, not used.

Characteristic	1	2	3	4
Source	Anaerobic municipal sewage sludge	Deep subsurface water	Anoxic freshwater mud	Anaerobic purification plant
Cell shape	Curved rods	Vibrio	Curved rods	Rods
Motility	Motile	Motile	Motile	Non-motile
Utilization of electron donors				
Formate	−	−	−	+
Butyrate	+	−	−	−
Fumarate	−	+	−	+
Malate	−	+	−	+
Succinate	−	−	−	+
Propanol	+	−	+	+
Utilization of electron acceptors				
Sulfite	−	+	+	+
Fumarate	−	+	−	−
Utilization of substrates in the absence of electron acceptors				
Pyruvate	+	+	−	+
Fumarate	−	+	−	+
Malate	−	+	−	+
DNA G+C content (mol%)	63.3	57.8	64.1	65.0

were significantly different from those of recognized *Desulfovibrio* species especially with respect to utilization of electron donors and cellular fatty acid profiles. The sewage sludge strains are thus considered to represent a single novel species of the genus *Desulfovibrio*, for which the name *Desulfovibrio butyratiphilus* sp. nov. is proposed.

Description of *Desulfovibrio butyratiphilus* sp. nov.

Desulfovibrio butyratiphilus (bu.ty.ra.ti′phi.lus. N.L. n. *butyras -atis* butyrate; N.L. masc. adj. *philus* from Gr. adj. *philos* friendly to, loving; N.L. masc. adj. *butyratiphilus* butyrate-loving).

Cells are relatively large curved rods, 0.8–0.9 μm wide and 2.4–5.6 μm long. Strictly anaerobic. Gram-negative. Motile by means of a single polar flagellum. Non-spore-forming. Colonies are greyish, thin and spread on agar slants. Contains desulfoviridin and cytochrome type *c*. Catalase- and oxidase-negative. Requires carbonate or bicarbonate in the growth medium. The NaCl concentration range for growth is 0–2.0 % (w/v) with an optimum at 0.5 % (w/v). The temperature range for growth is 25–40 °C with an optimum at 35 °C. The pH range for growth is 6.2–8.0 with an optimum at pH 7.1. Utilizes butyrate, 2-methylbutyrate, valerate, pyruvate, lactate, 1-propanol, butanol and H_2 as electron donors for sulfate reduction. Almost all organic electron donors are incompletely oxidized to acetate; 2-methylbutyrate and valerate are oxidized to both acetate and propionate. Weak lithoautotrophic growth occurs with H_2/CO_2. Does not grow with formate, acetate, propionate, isobutyrate, isovalerate, caprylate, crotonate, fumarate, malate, succinate, methanol, 2-propanol, glycerol, glycine, L-alanine, L-serine, L-aspartate, L-glutamate, D-glucose, D-fructose or yeast extract under sulfate-reducing conditions. Sulfate and thiosulfate serve as electron acceptors, but not sulfite, nitrate or fumarate. Pyruvate supports weak growth in the absence of electron acceptors, but butyrate, lactate, fumarate and malate do not. The genomic DNA G+C content of the type strain is 63.3 mol%. The major respiratory quinone is menaquinone MK-6(H_2). Major cellular fatty acids are $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7$ and $C_{18:1}\omega7$.

The type strain, BSY^T (=JCM 15519^T=DSM 21556^T), was isolated from an anaerobic sewage sludge digester in Yokohama, Japan. Reference strain BST (=JCM 15520) was isolated from a digester in Tsuruoka, Japan. BSY-C, BST-B and BST-C are other strains of the species.

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