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

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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 GenBank/EMBL/DDBJ accession number for the dissimilatory sulfite-reductase β-subunit gene sequence of strain BSY^T is AB490775.

ABSTRACT

A strictly anaerobic, mesophilic, sulfate-reducing bacterial strain (BSY^T) isolated from an anaerobic municipal sewage sludge digester was characterized phenotypically and phylogenetically. Cells were Gram-negative, motile with a polar flagellum, non-spore-forming, curved rods. Cells had desulfoviridin and *c*-type cytochrome. Catalase and oxidase activities were not detected. The optimum NaCl concentration for growth was 0.5% (w/v). The optimum temperature was 35^{o}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. The strain grew lithoautotrophically with H_2/CO_2 under the sulfate-reducing condition. Most organic electron donors were incompletely oxidized to mainly acetate, while 2-methylbutyrate and valerate were oxidized to equivalent amounts of acetate and propionate, respectively. 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 was 63.3 mol% and 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 the 16S rRNA gene and dissimilatory sulfite-reductase β-subunit gene sequences assigned the strain to the genus *Desulfovibrio* in the family *Desulfovibrionaceae* within the class *Deltaproteobacteria*. The closest described relative based on the 16S rRNA gene sequences was *Desulfovibrio putealis* (sequence similarity of 95.3%). On the basis of significant differences in the 16S rRNA gene sequences and the phenotypic characteristics between strain BSY^T and each of the closely related species, *Desulfovibrio butyratiphilus* sp. nov. was proposed. The type strain is BSY^T (= JCM 15519^T = DSM 21556^T).

MAIN TEXT

Butyrate is an important intermediate in anaerobic degradation of organic matter in various anaerobic ecosystems. Because the oxidation of butyrate is usually thermodynamically unfavorable 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 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 this 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 through enrichment cultures. All strains reduced sulfate with butyrate as an electron donor and were closely related to species in the genus *Desulfovibrio* based on the 16S rRNA gene sequence analysis. Strain BSY^T was selected as a representative strain and further characterized comprehensively. Butyrate-oxidizing *Desulfovibrio* species have not yet been reported, and the differences in phylogenetic and phenotypic characteristics between strain BSY^T and related *Desulfovibrio* species supported the proposal of a novel species of *Desulfovibrio* with strain BSY^T as the type strain.

Samples obtained from two anaerobic digesters treating municipal sewage sludge (Yokohama and Tsuruoka in Japan) were used for isolation of the strains. The sewage sludge samples were inoculated (0.5

ml each) into defined liquid medium (9.5 ml) containing 20 mM of sodium butyrate as described below under the flow of O_2-free gas (N_2/CO_2 = 95%/5%), respectively. 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 using the anaerobic roll tube method (Hungate, 1966). Black colonies that appeared in the agar were picked and a total of five isolates (strains BSY^T and BSY-C from Yokohama and the other three strains from Tsuruoka) were finally obtained after purification procedures. All strains displayed high similarities (about 99-100%) of 16S rRNA gene sequences and showed almost the same phenotypic characteristics including utilization of both electron donors and acceptors as shown below. Thus, strain BSY^T was selected for further characterization.

The following defined medium was used for the enrichment culture, isolation and the general physiological characterization of the strains, (l^{-1}): 0.5 g KH_2PO_4, 1.0 g NH_4Cl, 2.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 1 mg sodium resazurin, 10 ml of trace element solution (Widdel *et al*., 1983), 1.0 g NaCl and 0.5 g L-cysteineHCl·H_2O with appropriate electron donors (Ueki *et al*., 1980; Widdel & Bak, 1992). The pH was adjusted to 7.4-7.5 with 1 N NaOH. The 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 the isolates. Cultivation and transfer of the enrichment cultures and the isolates were carried out under an O_2-free N_2/CO_2 (95%/5%) atmosphere. Cultivation temperature was 30ºC, unless stated otherwise.

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

Volatile fatty acids, non-volatile fatty acids, alcohols, gases, sulfate, sulfite, thiosulfate and nitrate were analyzed as described previously (Akasaka *et al*., 2003a; Nakamoto *et al*., 1996; Ueki *et al*., 1986). The presence of desulfoviridin in cells was determined according to the method of Postgate (1959). The presence and the type of cytochrome were determined by measuring an air-oxidized/dithionite-reduced

difference spectrum of cell-free extract with a spectrophotometer (HITACHI U-2010). Genomic DNA extracted according to the method as 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 (HITACHI L-7400) equipped with a µBondapak C18 column (3.9 × 300 mm; Waters). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analyzed 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 CFAs were analyzed with a gas-chromatograph (Hewlett-Packard Hp6890 or Hitachi G-3000) equipped with a HP Ultra 2 column. CFAs were identified by equivalent chain-length (ECL) (Miyagawa *et al*., 1979; Ueki & Suto, 1979) according to the protocol of TechnoSuruga Co., Ltd (Shimidu, Japan) based on the MIDI microbial identification system (Microbial ID) of Moore (Moore *et al*., 1994).

Extraction of DNA and PCR-amplification of 16S rRNA gene of the strains were carried out according to the method described by Akasaka *et al*. (2003b). The PCR-amplified 16S rRNA gene using a primer set, 27f and 1492r, was sequenced by using 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 assemblages.

A partial sequence of the gene encoding α and β-subunits of dissimilatory sulfite-reductase (DSR) was amplified by using a primer set, P94-F and P93-R (Karkhoff-Schweizer *et al*., 1995), with DNA extracted from the cells of the strain. The PCR product was cloned using the pGEM-T Easy vector (Promega) and 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 the phylogenetic analysis was performed according to the method as described above for the 16S rRNA gene. We also tried to amplify the DSR gene sequence with a primer set, DSR1F/DSR4R (Wagner *et al*., 1998), however, we did not succeed.

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 a single polar flagellum. Strain BSY^T made grayish and thin colonies on agar slant medium. The strain did not grow aerobically. Desulfoviridin was detected in cell extract. A difference absorption spectrum of dithionite-reduced minus air-oxidized cell extract showed peaks at 418 and 553 nm, which indicated the presence of *c*-type cytochrome in the cells. 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 shows consumption of electron donors and compounds produced 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. The strain weakly grew lithoautotrophically with H_2/CO_2 in the absence of acetate under the sulfate-reducing condition.

Butyrate, pyruvate, lactate, ethanol and butanol were oxidized to acetate, while 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 were simultaneously accumulated in the medium along with the decrease in the concentration of each electron donor as well as sulfate. 1-Propanol was oxidized to propionate. Thus, the strain had an incomplete type of oxidation 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, respectively. These ratios were almost consistent with the theoretical values for incomplete oxidation of the substrates through the pathway of β-oxidation, respectively. When the 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). In the time course of sulfate reduction with butanol, a small amount of butyrate was produced after sulfate was almost exhausted. When the strain was cultivated with H_2/CO_2

+ acetate, a trace amount of butyrate was also detected.

The strain grew with butyrate as the most preferable electron donor without a significant lag period after inoculation of cells to the medium. In contrast, rather long lag periods were observed for growth with 2-methylbutyrate (5-6 days after inoculation) and valerate (7-8 days). The 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 the increase in turbidity of the culture, however, the strain 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 and 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 by pyruvate oxidation (1.4 mmol l^{-1}). The strain did not oxidize butyrate, lactate, fumarate and malate in the absence of electron

acceptors.

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

The G+C content of genomic DNA of strain BSY^T was 63.3 mol%. The major respiratory quinone of the strain 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 CFAs, while $C_{12:0}$, $C_{15:0}$, $C_{18:0}$, $C_{16:1}\omega5$, $C_{18:1}\omega9$, $C_{18:1}\omega5$, 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 were detected as minor or trace compounds.

Almost full-length of 16S rRNA gene sequence (1450 bp) was determined for strain BSY^T. Based on the 16S rRNA gene phylogenetic analysis, the strain was affiliated with the class *Deltaproteobacteria* and related to the members of the genus *Desulfovibrio* in the family *Desulfovibrionaceae* (Fig. 2). The most closely related sequence of the strain on the database was “Uncultured delta proteobacterium clone MBNTA bac-1” with sequence similarity of 95.4%. The closest described species of strain BSY^T was *Desulfovibrio putealis* with sequence similarity of 95.3%. The next closely related species were *Desulfovibrio sulfodismutans* and *Desulfovibrio carbinolicus* with much lower sequence similarities (90.6% and 90.5%), respectively. Together with the strains (BSY-C, BST, BST-B and BST-C) which were

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 species of the genus *Desulfovibrio*, *Desulfovibrio desulfuricans*, with sequence similarity of 86.7%.

The partial sequence (730 bp) of β-subunit of DSR gene of strain BSY^T was determined. Based on the phylogenetic analysis of the DSR gene sequence, the most closely related sequence of the strain on the database was “Uncultured sulfate-reducing bacterium clone GranDSR12” with sequence similarity of 83.5%. The most closely related described species to strain BSY^T were *Desulfovibrio alkalitolerans* and *Desulfovibrio aminophilus* with sequence similarity of 73.4% each. Thus, on the basis of the DSR gene phylogenetic analysis, strain BSY^T was also closely related to the species in the genus *Desulfovibrio*. The similarity of the DSR gene sequence of BSY^T to that of *D. carbinolicus* was 66.7%. The sequences of *D. putealis* and *D. sulfodismutans* were not available.

The strain shared major characteristics with *Desulfovibrio* species such as morphology, presences of *c*-type cytochrome and desulfoviridin as a sulfite-reductase, incomplete oxidation of electron donors and the mesophilic property (Kuever *et al.*, 2005). Strain BSY^T contained MK-6(H_2), which is known as one of major menaquinones in *Desulfovibrio* species (Collins & Widdel, 1986). In spite of these common features, strain BSY^T had a significantly different property for utilization of electron donors from the known *Desulfovibrio* species, since no *Desulfovibrio* species are known to utilize butyrate, 2-methylbutyrate and valerate as electron donors for sulfate reduction. As shown above, 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), however, 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). Since sulfate-reducing bacterial strains with the same properties as strain BSY^T were enriched and isolated from the two digesters distantly located each other, it seems that the bacterial groups are widely distributed in anaerobic municipal sewage sludge digesters.

Physiological characteristics of strain BSY^T were compared with those of three related species, *D. putealis*, *D. sulfodismutans* and *D. carbinolicus* (Table 2). In addition to butyrate, 2-methylbutyrate and valerate, the range of electron donor utilization (such as formate, fumarate, malate, succinate and propanol) of strain BSY^T is not consistent with any of these relatives. Strain BSY^T utilizes neither sulfite nor fumarate as electron acceptors, but the three relatives utilize at least one of them. In the absence of electron acceptors, strain BSY^T as well as *D. sulfodismutans* does not utilize fumarate and malate, but *D. putealis* and *D. carbinolicus* utilize both.

The CFAs profile of strain BSY^T is compared with those of relatives, *D. sulfodismutans* and *D. carbinolicus* (Table 3). The CFAs composition of *D. putealis* has not been reported. Most species in 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 CFAs (Ueki & Suto, 1979; Kohring *et al.*, 1994; Vainshtein *et al.*, 1992). *D. sulfodismutans* and *D. carbinolicus* also have branched-chain fatty acids (anteiso-$C_{15:0}$, iso-$C_{16:0}$ or anteiso-$C_{17:0}$) as major CFAs. In contrast, branched chain fatty acids were only trace compounds in the CFAs of strain BSY^T and major CFAs of strain BSY^T were even and straight chain fatty acids. The G+C content of strain BSY^T was almost the same with those of *D. sulfodismutans* and *D. carbinolicus* (Table 2).

Thus, in addition to the differences of 16S rRNA gene and β-subunit of DSR gene sequences, the characteristics of strain BSY^T were significantly different from the known *Desulfovibrio* species especially with respect to the utilization of electron donors and CFAs profiles. Thus, the strain should be classified as a novel species in the genus *Desulfovibrio*. We propose strain BSY^T as the type strain of *Desulfovibrio butyratiphilus* sp. nov..

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 a single polar flagellum. Non-spore-forming. Colonies are grayish and thin, and spread on agar slants. Contains desulfoviridin and cytochrome of the *c*-type. Catalase and oxidase activities are absent. 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^{o}C with an optimum at 35^{o}C. The pH range for growth is 6.2-8.0 with an optimum at 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, while 2-methylbutyrate and valerate are oxidized to both acetate and propionate. Weak lithoautotrophic growth 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 and yeast extract under sulfate-reducing conditions. Sulfate and thiosulfate serve as electron acceptors, but not sulfite, nitrate and fumarate. Pyruvate supports weak growth in the absence of electron acceptors, but not butyrate, lactate, fumarate and malate. The genomic DNA G+C content 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 is strain BSY^{T} (= JCM 15519^{T} = DSM 21556^{T}), which was isolated from an anaerobic sewage sludge digester in Yokohama, Japan. Reference strain BST (= JCM 15520) was isolated from a digester in Tsuruoka, Japan.

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REFERENCES

Akasaka, H., Izawa, T., Ueki, K. & Ueki, A. (2003a). Phylogeny of numerically abundant culturable anaerobic bacteria associated with degradation of rice plant residue in Japanese paddy field soil. *FEMS Microbiol Ecol* **43**, 149-161.

Akasaka, H., Ueki, A., Hanada, S., Kamagata, Y. & Ueki, K. (2003b). *Propionicimonas paludicola* gen. nov., sp. nov., a novel facultatively anaerobic, Gram-positive, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil. *Int J Syst Evol Microbiol* **53**, 1991-1998.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389-3402.

Bak, F. & Pfennig, N. (1987). Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. *Arch Microbiol* **147**, 184-189.

Balk, M., Altinbas, M., Irene, Rijpstra, W. I. C., Damsté, J. S. S. & Stams, A. J. M. (2008). *Desulfatirhabdium butyrativorans* gen. nov., sp. nov., a butyrate-oxidizing, sulfate-reducing bacterium isolated from an anaerobic bioreactor. *Int J Syst Evol Microbiol* **58**, 110-115.

Basso, O., Caumette, P. & Magot, M. (2005). *Desulfovibrio putealis* sp. nov., a novel sulfate-reducing bacterium isolated from a deep subsurface aquifer. *Int J Syst Evol Microbiol* **55**, 101-104.

Beeder, J., Torsvik, T. & Lien, T. (1995). *Thermodesulforhabdus norvegicus* gen. nov., sp. nov., a novel thermophilic sulfate-reducing bacterium from oil field water. *Arch Microbiol* **164**, 331-336.

Belyakova, E. V., Rozanova, E. P., Borzenkov, I. A., Tourova, T. P., Pusheva, M. A., Lysenko, A. M. & Kolganova, T. V. (2006). The new facultatively chemolithoautotrophic, moderately halophilic, sulfate-reducing bacterium *Desulfovermiculus halophilus* gen. nov., sp. nov., isolated from an oil field. *Microbiology* (English translation of *Microbiologiya*) **75**, 161-171.

Blenden, D. C. & Goldberg, H. S. (1965). Silver impregnation stain for *Leptospira* and flagella. *J Bacteriol* **89**, 899-900.

Collins, M. D. & Widdel, F. (1986). Respiratory quinones of sulphate-reducing and sulphur-reducing bacteria: a systematic investigation. *Syst Appl Microbiol* **8**, 8–18.

Cravo-Laureau, C. Matheron, R., Cayol, J.-L., Joulian, C. & Hirschler-Réa, A. (2004). *Desulfaibacillum aliphaticivorans* gen. nov., sp. nov., an n-alkane- and n-alkene-degrading,

sulfate-reducing bacterium. *Int J Syst Evol Microbiol* **54**, 77-83.

Daumas, S., Cord-Ruwisch, R. & Garcia, J. L. (1988). *Desulfotomaculum geothermicum* sp. nov., a thermophilic, fatty acid-degrading, sulfate-reducing bacterium isolated with H_2 from geothermal ground water. *Antonie van Leeuwenhoek* **54**, 165-178.

Fardeau, M.-L., Ollivier, B., Patel, B. K. C., Dwivedi, P., Ragot, M. & Garcia J.-L. (1995). Isolation and characterization of a thermophilic sulfate-reducing bacterium, *Desulfotomaculum thermosapovorans* sp. nov. *Int J Syst Bacteriol* **45**, 218-221.

Felsenstein, J. (2006). PHYLIP (Phylogeny Inference Package), Ver 3.66. Department of Genetics, University of Washington, Seattle.

Hungate, R. E. (1966). *The Rumen and its Microbes*. New York: Academic Press.

Karkhoff-Schweizer, R. R., Huber, D. P. W. & Voordouw, G. (1995). Conservation of the genes for dissimilatory sulfite reductase from *Desulfovibrio vulgaris* and *Archaeoglobus fulgidus* allows their detection by PCR. *Appl Environ Microbiol* **61**, 290-296.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods*

Microbiol **19**, 161-207.

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.

Kuever, J., Rainey, F. A. & Hippe, H. (1999). Description of *Desulfotomaculum* sp. Groll as *Desulfotomaculum gibsoniae* sp. nov. *Int J Syst Bacteriol* **49**, 1801-1808.

Kuever, J., Rainey, F. A. & Widdel, F. (2005). Class IV. *Deltaproteobacteria* class nov. In *Bergey's Manual of Systematic Bacteriology*, Vol. 2, Part C, 2nd edn, pp. 922-1144. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584-586.

Miyagawa, E., Azuma, R. & Suto, E. (1979). Cellular fatty acid composition in Gram-negative obligately anaerobic rods. *J Gen Appl Microbiol* **25**, 41-51.

Moore, L. V. H., Bourne, D. M. & Moore, W. E. C. (1994). Comparative distribution and taxonomic value of cellular fatty acids in thirty-three genera of anaerobic gram-negative bacilli. *Int J Syst Bacteriol* **44**, 338-347.

Nakamoto, M., Ueki, A. & Ueki, K. (1996). Physiological properties of a sulfate-reducing bacterium isolated from municipal sewage sludge and its possible role as a syntrophic acidogen in the ecosystem. *J Gen Appl Microbiol* **42**, 109-120.

Nanninga, H. J. & Gottschal, J. C. (1987). Properties of *Desulfovibrio carbinolicus* sp. nov. and other sulfate-reducing bacteria isolated from an anaerobic-purification plant. *Appl Environ Microbiol* **53**, 802-809.

Postgate, J. R. (1959). A diagnostic reaction of *Desulphovibrio desulphuricans*. *Nature* **183**, 481-482.

Rabus, R., Hansen, T. & Widdel, F. (2000). Dissimilatory sulfate- and sulfur-reducing prokaryotes. In *The Prokaryotes*: An Evolving Electronic Resource for the Microbiological Community, 3rd ed. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer-Verlag.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Bio Rev* **61**, 262-280.

Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A. & Harada, H. (2000). *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. *Int J Syst Evol Microbiol* **50**, 771-779.

Sievert, S. M. & Kuever, J. (2000). *Desulfacinum hydrothermale* sp. nov., a thermophilic, sulfate-reducing bacterium from geothermally heated sediments near Milos Island (Greece). *Int J Syst Evol Microbiol* **50**, 1239-1246.

Stams, A. J. M. (1994). Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek* **66**, 271-294.

Suzuki, D., Ueki, A., Amaishi, A. & Ueki, K. (2007a). *Desulfopila aestuari*i gen. nov., sp. nov., a novel, Gram-negative, rod-like, sulfate-reducing bacterium isolated from an estuarine sediment in Japan. *Int J Syst Evol Microbiol* **57**, 520-526.

Suzuki, D., Ueki, A., Amaishi, A. & Ueki, K. (2007b). *Desulfobulbus japonicus* sp. nov., a novel,

Gram-negative, propionate-oxidizing, sulfate-reducing bacterium isolated from an estuarine sediment in Japan. *Int J Syst Evol Microbiol* **57**, 849-855.

Suzuki, D., Ueki, A., Amaishi, A. & Ueki, K. (2007c). Diversity of substrate utilization and growth characteristics of sulfate-reducing bacteria isolated from estuarine sediment in Japan. *J Gen Appl Microbiol* **53**, 119-132.

Suzuki, D., Ueki, A., Amaishi, A. & Ueki, K. (2008). *Desulfoluna butyratoxydans* gen. nov., sp. nov., a novel, Gram-negative, butyrate-oxidizing, sulfate-reducing bacterium isolated from an estuarine sediment in Japan. *Int J Syst Evol Microbiol* **58**, 826-832.

Tanaka, K., Stackebrandt, E., Tohyama, S. & Eguchi T. (2000). *Desulfovirga adipica* gen. nov., sp. nov., an adipate-degrading, Gram-negative, sulfate-reducing bacterium. *Int J Syst Evol Microbiol* **50**, 639-644.

Tasaki, M., Kamagata, Y., Nakamura, K. & Mikami, E. (1991). Isolation and characterization of a thermophilic benzoate-degrading, sulfate-reducing bacterium, *Desulfotomaculum thermobenzoicum* sp. nov. *Arch Microbiol* **155**, 348-352.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of

progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673-4680.

Ueki, A. & Suto, T. (1979). Cellular fatty acid composition of sulfate-reducing bacteria. *J Gen Appl Microbiol* **25**, 185-196.

Ueki, A., Minato, H., Azuma, R. & Suto, T. (1980). Enumeration and isolation of anaerobic bacteria in sewage digester fluids: Enumeration of sulfate-reducers by the anaerobic roll tube method. *J Gen Appl Microbiol* **26**, 25-35.

Ueki, A., Matsuda, K. & Ohtsuki, C. (1986). Sulfate reduction in the anaerobic digestion of animal waste. *J Gen Appl Microbiol* **32**, 111-123.

Vainshtein, M. B., 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.

Vandieken, V., Knoblauch, C. & Jørgensen, B. B. (2006). *Desulfotomaculum arcticum* sp. nov., a novel spore-forming, moderately thermophilic, sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard. *Int J Syst Evol Microbiol* **56**, 687-690.

Wagner, M., Roger, A. J., Flax, J. L., Brusseau, G. A. & Stahl, D. A. (1998). Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J Bacteriol* **180**, 2975-2982.

Watanabe, K., Watanabe, K., Kodama, Y., Syutsubo, K. & Harayama, S. (2000). Molecular characterization of bacterial populations in petroleum-contaminated groundwater discharged from underground crude-oil-storage cavities. *Appl Environ Microbiol* **66**, 4803-4809.

Widdel, F., Kohring, G. W. & Mayer, F. (1983). Studies of dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. and sp. nov. and *Desulfonema magnum* sp. nov. *Arch Microbiol* **134**, 286-294.

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

Zhang, C., Liu, X. & Dong, X. (2004). *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens. *Int J Syst Evol Microbiol* **54**, 969-973.

LEGENDS FOR FIGURES

Fig. 1.

A phase-contrast photomicrograph of cells of strain BSY^T grown anaerbically in the defined medium. Bar, 10 μm.

Fig. 2.

Neighbour-joining tree, based on the 16S rRNA gene sequences, showing the phylogenetic relationship of strain BSY^T and related species in the order *Desulfovibrionales*. Bootstrap values (expressed as percentages of 1000 replications) above 50% are shown at branch nodes. Bar, 2% estimated difference in nucleotide sequence position. *Desulfohalobium retbaense* DSM 5692^T was used as the outgroup. The tree topology evaluated by the maximum-likelihood method was almost the same as that obtained with the neighbour-joining method.

Fig. 1.

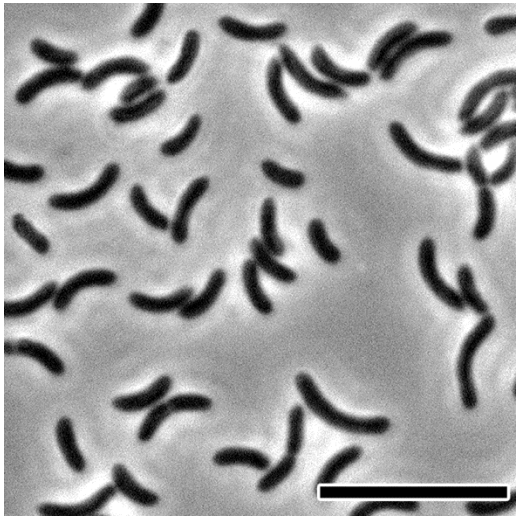

Fig. 2.

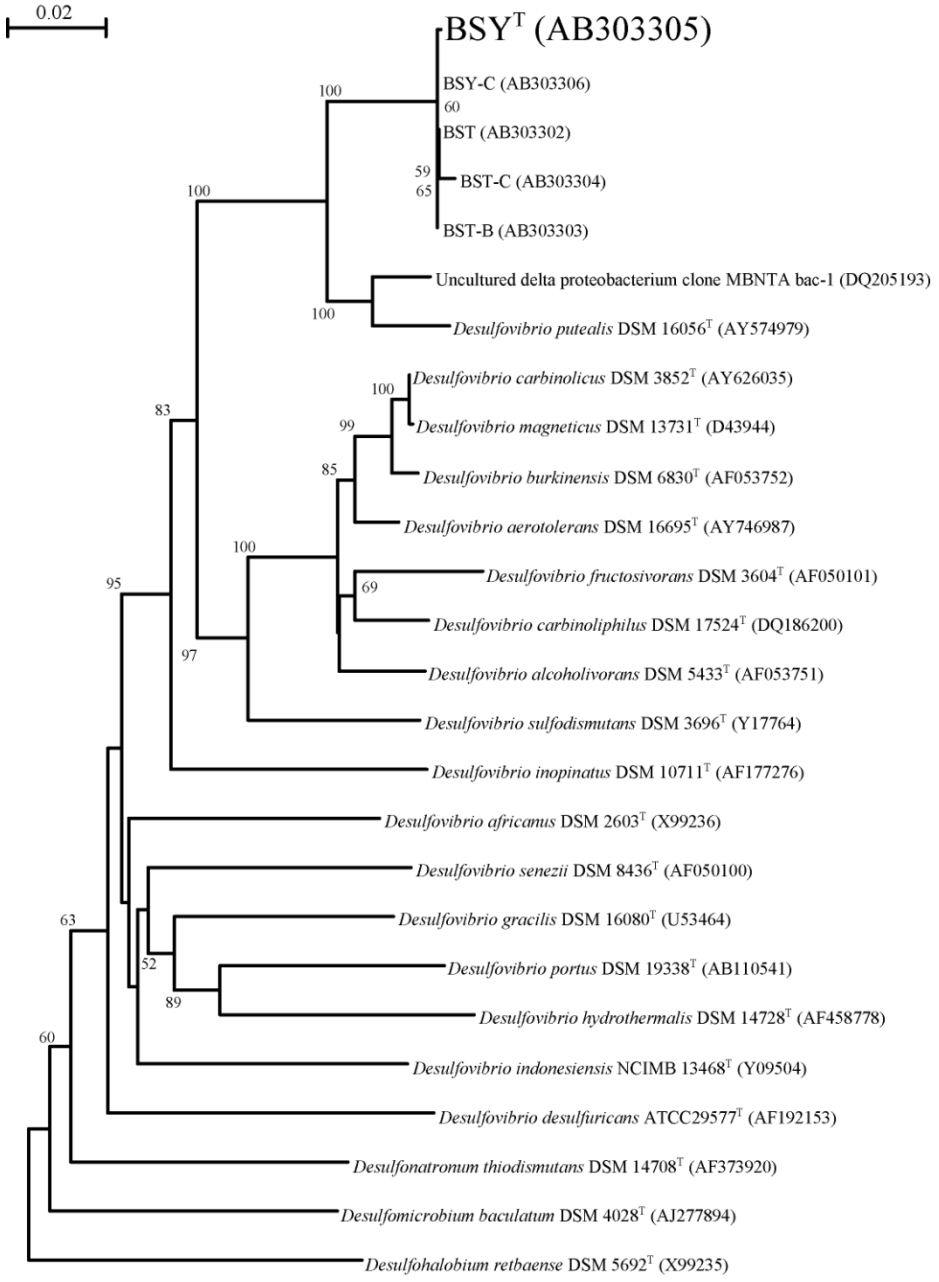
0.02
BSY^T (AB303305)
BSY-C (AB303306)
BST (AB303302)
BST-C (AB303304)
BST-B (AB303303)
Uncultured delta proteobacterium clone MBNTA bac-1 (DQ205193)
Desulfovibrio putealis DSM 16056^T (AY574979)
Desulfovibrio carbinolicus DSM 3852^T (AY626035)
Desulfovibrio magneticus DSM 13731^T (D43944)
Desulfovibrio burkinensis DSM 6830^T (AF053752)
Desulfovibrio aerotolerans DSM 16695^T (AY746987)
Desulfovibrio fructosivorans DSM 3604^T (AF050101)
Desulfovibrio carbinoliphilus DSM 17524^T (DQ186200)
Desulfovibrio alcoholivorans DSM 5433^T (AF053751)
Desulfovibrio sulfodismutans DSM 3696^T (Y17764)
Desulfovibrio inopinatus DSM 10711^T (AF177276)
Desulfovibrio africanus DSM 2603^T (X99236)
Desulfovibrio senezii DSM 8436^T (AF050100)
Desulfovibrio gracilis DSM 16080^T (U53464)
Desulfovibrio portus DSM 19338^T (AB110541)
Desulfovibrio hydrothermalis DSM 14728^T (AF458778)
Desulfovibrio indonesiensis NCIMB 13468^T (Y09504)
Desulfovibrio desulfuricans ATCC29577^T (AF192153)
Desulfonatronum thiodismutans DSM 14708^T (AF373920)
Desulfomicrobium baculatum DSM 4028^T (AJ277894)
Desulfohalobium retbaense DSM 5692^T (X99235)
100
60
59
65
100
100
100
83
99
85
100
69
95
97
63
52
89
60

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

Electron donors[a]	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	n.d.	n.d.	0.2	-	n.d.
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	n.d.	9.4	-	Butyrate (0.3)	0.063
H_2/CO_2	n.d.	3.5	-	-	0.012

[a] Substrates tested as electron donors were formate, acetate, propionate, butyrate, isobutyrate, 2-methylbutyrate, valerate, isovalerate, caprylate, crotonate, pyruvate, lactate, fumarate, malate, succinate, methanol, ethanol, 1-propanol, 2-propanol, butanol, glycerol, glycine, L-alanine, L-serine, L-aspartate, L-glutamate, D-glucose, D-fructose, H_2/CO_2 + acetate and H_2/CO_2. Electron donors tested but not utilized were not shown.

-, not detected; n.d., not determined.

Table 2. Characteristics of strain BSY^T and related *Desulfovibrio* species.

Strains: 1, BSY^T; 2, *Desulfovibrio putealis* B7-43^T (Basso *et al*., 2005); 3, *Desulfovibrio sulfodismutans* ThAc01^T (Bak & Pfennig, 1987);

4, *Desulfovibrio carbinolicus* EDK82^T (Nanninga & Gottschal, 1995).

+, used; -, not used.

	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	-	+	-	+
G+C content (%)	63.3	57.8	64.1	65.0

Table 3. Cellular fatty acid composition (%) of strain BSY^T and related *Desulfovibrio* species.

Strains: 1, BSY^T; 2, *Desulfovibrio sulfodismutans* ThAc01^T (Vainshtein *et al*., 1992);

3, *Desulfovibrio carbinolicus* EDK82^T (Vainshtein *et al*., 1992).

-, not detected.

Fatty acids	1	2	3
Saturated straight-chain:			
$C_{14:0}$	24.1	1.3	0.8
$C_{16:0}$	10.2	10.6	6.6
Unsaturated straight-chain:			
$C_{16:1}\omega 7$	25.5	3.4	1.0
$C_{18:1}\omega 7$	32.1	0.8	-
Saturated branched-chain:			
iso-$C_{15:0}$	-	3.2	3.0
anteiso-$C_{15:0}$	0.1	30.5	54.3
iso-$C_{16:0}$	-	10.7	1.2
iso-$C_{17:0}$	-	2.6	1.3
anteiso-$C_{17:0}$	-	2.4	16.6
Unsaturated branched-chain:			
iso-$C_{16:1}$	-	2.8	0.7
iso-$C_{17:1}$	-	2.9	2.5
anteiso-$C_{17:1}$	-	1.8	5.7