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COVER SHEET

Desulfoluna butyratoxydans gen. nov., sp. nov., a novel, Gram-negative, butyrate-oxidizing, sulfate-reducing bacterium isolated from an estuarine sediment in Japan

Daisuke Suzuki[1], Atsuko Ueki[1], Aya Amaishi[1, 2] and Katsuji Ueki[1]

[1]Faculty of Agriculture, Yamagata University, Wakaba-machi 1-23, Tsuruoka, Yamagata 997-8555, Japan

[2]Present address: Taisei Corporation, Naze-machi 344-1, Totsuka-ku, Yokohama, Kanagawa 245-0051, Japan

Author for correspondence: Atsuko Ueki. Tel: +81 235 28 2866. Fax: +81 235 28 2846.

e-mail: uatsuko@tds1.tr.yamagata-u.ac.jp

Short running title: *Desulfoluna butyratoxydans* gen. nov., sp. nov.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strains MSL71[T] is AB110540.

Abstract

A strictly anaerobic, mesophilic sulfate-reducing bacterial strain (MSL71[T]) isolated from an estuarine sediment in the Sea of Japan of the Japanese islands was characterized phenotypically and phylogenetically. Cells were Gram-negative, motile, non-spore-forming, slightly curved rods. Catalase and oxidase activities were not detected. The optimum NaCl concentration for growth was 2·0% (w/v).

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The optimum temperature was 30°C and the optimum pH was 6·3. Strain MSL71^T utilized formate, butyrate, pyruvate, lactate, malate, ethanol, propanol, butanol, glycerol and H$_2$ as electron donors for sulfate reduction. Organic electron donors used were incompletely oxidized to mainly acetate. The strain did not use acetate, propionate, fumarate, succinate, methanol, glycine, alanine, serine, aspartate and glutamate. Sulfite and thiosulfate were used as electron acceptors with lactate as an electron donor, while fumarate was not. Without electron acceptors, pyruvate and malate were fermented, while lactate and fumarate were not. The genomic DNA G + C content was 62·0 mol%. Menaquinone MK-8(H$_4$) was the major respiratory quinone. Major cellular fatty acids were C$_{14:0}$, C$_{16:0}$, C$_{16:1}\omega7$, C$_{18:1}\omega9$, C$_{18:1}\omega7$ and C$_{14:0}$ 3-OH. Phylogenetic analysis based on the 16S rRNA gene sequence placed the strain in the class *Deltaproteobacteria*. The recognized closest relative of strain MSL71^T was *Desulfofrigus fragile* (sequence similarity of 93·9%) and the next closest recognized species was *Desulfofrigus oceanense* (93·5%). Based on the significant differences in the 16S rRNA gene sequence and phenotypic characteristics between strain MSL71^T and each of related species, a novel genus and species *Desulfoluna butyratoxydans* gen. nov., sp. nov. is proposed to accommodate the strain. The type strain is MSL71^T (= JCM 14721^T = DSM 19427^T).

Main text

Sulfate-reducing bacteria (SRB) are capable of utilizing various compounds such as H$_2$, fatty acids, alcohols, amino acids and sugars, as well as aliphatic and aromatic hydrocarbons as electron donors for sulfate-reduction (Hansen, 1993; Castro *et al.*, 2000; Rabus *et al.*, 2000). Major intermediates of

anaerobic decomposition of organic matter such as formate, acetate, propionate, butyrate, lactate and H_2 serve as the most important electron donors for sulfate-reduction, and thus SRB significantly contribute to mineralization of organic matter and the sulfur cycle on the earth (Sørensen *et al.*, 1981; Jørgensen, 1982). In the course of investigation on SRB in an estuarine sediment of Japanese islands, we isolated various strains of phylogenetically diverse SRB including several novel lineages. It was shown from the comprehensive physiological investigation on these isolates that the SRB group composed of diverse lineages actually had abilities to oxidize all the major intermediates of anaerobic decomposition of organic matter and that each lineage living in the same sediment should occupy a respective and specific niche in the SRB group in relation to oxidation of electron donors (Suzuki *et al.*, 2007a, b, c).

Of the strains belonging to the novel lineages in our isolates, we have proposed one novel genus of SRB, *Desulfopila aestuarii* gen. nov., sp. nov. (Suzuki *et al.*, 2007a) and one novel species of SRB *Desulfobulbus japonicus* sp. nov. (Suzuki *et al.*, 2007b). In this paper, we will describe the characterization of another isolate (MSL71[T]) oxidizing butyrate as one of electron donors for sulfate-reduction (Suzuki *et al.*, 2007c). The phylogenetical, physiological and chemotaxonomic characteristics of strain MSL71[T] supported the proposal of a novel genus and species in the class *Deltaproteobacteria* to affiliate the strain.

Sediment cores were collected to a depth of 10 cm with a core sampler (5 cm in diameter) from sediment at a water depth of 2 m in Niida river estuary in Sakata harbor, Japan (Suzuki *et al.*, 2007c).

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The sediment sample was diluted by consecutive 10-fold dilutions with sterilized seawater bubbled with O_2-free N_2 gas. The diluted samples (0.2 ml) were inoculated into the seawater agar medium (10 ml) containing 20 mM of sodium lactate and viable colony counts of SRB were determined by the anaerobic roll-tube method (Hungate, 1966). Several strains of SRB were obtained by picking up black colonies of SRB that appeared on the roll-tube agar after incubation for about a month. Strain MSL71[T] was finally obtained after several purification procedures through colony isolation by the anaerobic roll-tube method (Suzuki et al., 2007c).

Two basal media (seawater medium and defined medium) were used in this study. The seawater medium contained (l^{-1}seawater): 0·5 g KH_2PO_4, 0·3 g NH_4Cl, 0·1 g yeast extract, 1 mg sodium resazurin, 10 ml the trace element solution (l^{-1}: 10 ml 25% (v/v) HCl, 1·5 g $FeCl_2 \cdot 4H_2O$, 0·19 g $CoCl_2 \cdot 6H_2O$, 0·1 g $MnCl_2 \cdot 4H_2O$, 0·07 g $ZnCl_2$, 0·062 g H_3BO_3, 0·036 g $Na_2MoO_4 \cdot 2H_2O$, 0·024 g $NiCl_2 \cdot 6H_2O$ and 0·017 g $CuCl_2 \cdot 2H_2O$) (Widdel et al., 1983) and 0·5 g L-cysteine·HCl·H_2O, as well as sodium lactate (20mM) as an electron donor. The pH was adjusted to 7·2-7·4 with 1 M NaOH. Agar (Difco) (1·5%, w/v) was added to the medium and used for the anaerobic roll-tube method for isolation and slant cultures. The following medium, which was designated the 'defined medium' in contrast to the seawater medium and used for the general physiological characterization of the strain, contained (l^{-1}): 0·5 g KH_2PO_4, 1·0 g NH_4Cl, 1·0 g Na_2SO_4, 2·0 g $MgSO_4 \cdot 7H_2O$, 0·1 g $CaCl_2 \cdot 2H_2O$, 0·5 g yeast extract, 1 mg sodium resazurin, 10 ml the trace element solution, 15 g NaCl and 0·5 g L-cysteine·HCl·H_2O with appropriate electron donors (Nakamoto et al., 1996; Ueki et al., 1980; Widdel & Bak, 1992). The pH was adjusted to 7·2-7·4

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with 1 M NaOH. Cultivation and transfer of the strain were performed under an O_2-free N_2 (100%) atmosphere. The strain was cultivated at 30°C. The strain was maintained in slant cultures of the seawater medium or the defined medium with lactate as an electron donor.

The Gram reaction and cellular morphology were confirmed by light microscopy. The motility of the cells was examined by phase-contrast microscopy and flagella-staining was carried out according to Blenden & Goldberg (1965). Growth of the strain under the aerobic condition was examined in the presence of sodium lactate as an electron donor using the defined medium without L-cysteine·HCl·H_2O and sodium resazurin. Catalase and oxidase activities of cells were tested as described by Akasaka *et al.* (2003a). The effects of NaCl concentration and pH on growth of the strain were examined in the presence of sodium lactate as an electron donor using the defined medium. The effects of temperature on growth were examined using the seawater medium. Growth of the strain was monitored by measurement of OD_{660} with a spectrophotometer (U-1000; Hitachi).

The utilization of electron donors was determined using the defined medium containing each compound at a final concentration of 20 mM. H_2 utilization was determined in the presence of acetate (5 mM) with H_2 in the atmosphere. The utilization of other electron acceptors than sulfate was determined with a sulfate-free medium, containing the same concentrations of chloride in place of sulfate in the defined medium, with sodium lactate (20 mM) as an electron donor. Sodium sulfite (3 mM), sodium thiosulfate (15 mM) or disodium fumarate (20 mM) was added to the sulfate-free medium as a possible electron

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acceptor. Utilization of pyruvate, lactate, fumarate or malate (20 mM each) in the absence of electron acceptors in the medium was also determined using the sulfate-free medium. 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.

Volatile fatty acids and alcohols were analyzed by gas-chromatography (G-5000 or 263-30; Hitachi), as described by Ueki *et al.* (1986). Non-volatile fatty acids and formate were analyzed by HPLC (LC-10AD; Shimadzu) as described by Akasaka *et al.* (2003a) and Ueki *et al.* (2006b). Sulfate, sulfite and thiosulfate were analyzed with an ion chromatograph (Dionex 2000i) as described by Nakamoto *et al.* (1996). Genomic DNA extracted 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 μBondapack C18 column (3.9 x 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 (CFAs) were converted to methyl esters by saponification, methylation and extraction 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

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identification system (Microbial ID) of Moore (Moore *et al.*, 1994).

Extraction of DNA and PCR-amplification of 16S rRNA gene of the strain were carried out according to the method described by Akasaka *et al.* (2003b) and Ueki *et al.* (2006a). 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 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 position in the alignments were excluded before assemblages.

Cells of strain MSL71^T were Gram-negative, slightly curved rods with rounded ends, 0·8-0·9 μm wide and 1·6-3·4 μm long. Cells usually occurred singly or in pairs (Fig. 1). Cells were motile by a single polar flagellum. The strain made grayish and thin colonies on agar slants of the defined medium as well as the seawater medium. Spore formation was not observed. Since cells of the strain showed relatively rapid lysis during the storage on slant cultures, the cultures were transferred to the fresh slant medium every two or three weeks.

Strain MSL71^T reduced sulfate with lactate as an electron donor and produced acetate at a molar ratio of

about 2 : 1 : 2 (lactate : sulfate : acetate) in the defined medium. Thus, the strain had an incomplete type

of oxidation of electron donors. Both catalase and oxidase activities were not detected. The strain could

not grow in air in the defined liquid medium. Strain MSL71[T] essentially required NaCl addition in the

defined medium and the NaCl concentration range for growth was 1·0-6·5% (w/v) with an optimum at

2.0% (w/v). The temperature range for growth was 10-35°C with an optimum at 30°C. The pH range for

growth was 5·6-8·5 with an optimum at pH 6·3.

Strain MSL71[T] grew even in the absence of electron donors added to the medium and reduced sulfate

with concomitant production of acetate, suggesting that yeast extract or L-cysteine·HCl·H_2O added to

the medium was used as an electron donor. The strain utilized formate, butyrate, pyruvate, malate,

ethanol, propanol, butanol, glycerol and H_2 as well as lactate as electron donors for sulfate reduction.

Almost all organic electron donors used were oxidized to mainly acetate. The strain had higher growth

rates with lactate, pyruvate and glycerol (μ = 0·175 h^{-1}, 0·197 h^{-1} and 0·157 h^{-1}, respectively) in the

electron donors used. Although the growth rate with butyrate was significantly lower (μ = 0·081 h^{-1})

than those with these preferred electron donors and the molar ratio (butyrate : sulfate : acetate) was

slightly different from the theoretical ratio (2 : 1 : 4), a substantial amount of butyrate (18·8 mM) was

oxidized to acetate (34·6 mM) with sulfate reduction (10·8 mM) during about four days of cultivation.

Propanol was oxidized to propionate, while butanol (5·7 mM) was oxidized to acetate (6·1 mM) with a

trace amount of butyrate (0·3 mM). Butyrate produced through oxidation of butanol might be further

converted to acetate. The strain did not utilize acetate, propionate, fumarate, succinate, methanol,

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glycine, alanine, serine, aspartate and glutamate as electron donors.

The strain utilized sulfite and thiosulfate in addition to sulfate as electron acceptors with lactate as an electron donor. The strain did not utilize fumarate as an electron acceptor. In the absence of electron acceptors, strain MSL71^T oxidized pyruvate (18·8 mM) and produced acetate (18·2 mM) and butyrate (1·8 mM). The strain also oxidized a small amount of malate and produced acetate. The strain did not use lactate and fumarate in the absence of electron acceptors.

The G + C content of genomic DNA of strain MSL71^T was 62.0 ± 0.7 mol%. The major respiratory quinone was MK-8(H$_4$). Almost all CFAs of strain MSL71^T were even-numbered straight-chain fatty acids with C$_{14:0}$ (11·4%), C$_{16:0}$ (27·0%), C$_{16:1}\omega$7 (12·9%), C$_{18:1}\omega$9 (7·5%), C$_{18:1}\omega$7 (17·8%) and C$_{14:0}$ 3-OH (8·9%) as predominant ones.

Based on the almost full-length 16S rRNA gene sequence (1498 bp), strain MSL71^T was affiliated with the class *Deltaproteobacteria*. The closest relative of the strain on the database was strain Delta proteobacterium LacK5 isolated from marine subsurface sediment with sequence similarity of 96·2% (sequence length compared, 1399 bp). The closest known relative of strain MSL71^T was a SRB *Desulfofrigus fragile* with sequence similarity of 93·9% (Knoblauch *et al.*, 1999) (sequence length compared, 1475 bp). The next closely related recognized species was *Desulfofrigus oceanense* with similarity of 93·5% (sequence length compared, 1477 bp) (Knoblauch *et al.*, 1999). Strain MSL71^T

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formed a separate branch from these related species in the phylogenetic tree (Fig. 2).

Some physiological and chemotaxonomic characteristics of strain MSL71^T and the two closest *Desulfofrigus* species are compared in Table 1. Cells of strain MSL71^T and *D. fragile* are slightly curved rods, while those of *D. oceanense* are thick rods. Cells of *D. fragile* are reported to show rapid lysis in the stationary phase of growth (Knoblauch *et al.*, 1999), and thus cells of strain MSL71^T have a similar property.

Both *Desulfofrigus* species were isolated from permanently cold Arctic marine sediments. The optimum growth temperatures of *D. fragile* and *D. oceanense* are 18°C and 10°C, respectively, and the temperature ranges for growth are -1·8-27°C and -1·8-16°C, respectively (Knoblauch *et al.*, 1999). In contrast, the optimum growth temperature of strain MSL71^T isolated from temperate estuarine sediment is 30°C and the range is 10-35°C. Both *Desulfofrigus* species have pH optima at the neutral range, however, that of MSL71^T is slightly acidic.

The oxidation type of electron donors is heterogeneous in the *Desulfofrigus* species, that is, *D. fragile* has an incomplete type, while *D. oceanense* has a complete type (Knoblauch *et al.*, 1999). Thus, the oxidation type of strain MSL71^T is consistent with that of *D. fragile*. Strain MSL71^T and both *Desulfofrigus* species commonly utilize butyrate as an electron donor, however, the utilization range of electron donors of strain MSL71^T is different from any of *Desulfofrigus* species. Strain MSL71^T utilizes

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H_2, but not fumarate, while *D. fragile* utilizes fumarate, but not H_2. Both *Desulfofrigus* species utilize amino acids, but strain MSL71[T] does not.

The range of electron acceptors used by strain MSL71[T] is similar to that of *D. oceanense*, but it is different from that of *D. fragile*. Strain MSL71[T] utilizes both sulfite and thiosulfate, however, *D. fragile* does not. In the absence of electron acceptors, strain MSL71[T] and *D. fragile* do not utilize lactate, while *D. oceanense* utilizes it. Strain MSL71[T] produces a small amount of butyrate together with acetate from pyruvate in the absence of electron acceptors. To our knowledge, strain MSL71[T] is the first species in SRB described to produce butyrate by fermentation of pyruvate.

The G + C content of the genomic DNA of strain MSL71[T] (62.0 mol%) is significantly different from those of *Desulfofrigus* species (52·1 and 52·8 mol%, respectively). Strain MSL71[T] has MK-8(H_4) as a respiratory quinone, while both *Desulfofrigus* species has menaquinone MK-9. The presence of menaquinones such as MK-5(H_2) (*Desulfobulbus* species), MK-6 or MK-6(H_2) (*Desulfovibrio* species and *Desulfotalea* species) and MK-7 or MK-7(H_2) (some species in the *Desulfobacteraceae*) are relatively common in SRB species belonging to the class *Deltaproteobacteria* (Kuever *et al.*, 2005). Although menaquinones MK-8 and MK-8(H_4) have been found in *Desulfofaba gelida* in the *Desulfobacteraceae,* a psychrophilic species of SRB (Knoblauch *et al.*, 1999), and *Desulfopila aestuarii* in *Desulfobulbaceae* (Suzuki *et al.*, 2007a), respectively, the presence menaquinone MK-8 including the hydrogenated types is rather rare in SRB species.

The CFA profiles of strain MSL71^T and both *Desulfofrigus* species are compared in Table 2. The overall CFA profile of strain MSL71^T is similar to those of both *Desulfofrigus* species, however, the percentages of even-numbered unsaturated fatty acids of *Desulfofrigus* species are much higher (about 62-74%) than that of strain MSL71^T (about 38%). Strain MSL71^T has $C_{14:0}$ 3-OH as one of major fatty acids which is not detected in the *Desulfofrigus* species.

Of known butyrate-oxidizing SRB isolated from marine environments, many species in the genera such as *Desulfobacterium*, *Desulfosarcina*, *Desulfobacula*, *Desulfotignum*, *Desulfonema*, *Desulfothermus*, *Thermodesulforhabdus* and *Desulfacinum* oxidize butyrate completely to CO_2 (Kuever *et al.*, 2005). In contrast, incompletely-oxidizing species are restricted to a few species such as *Desulforhopalus singaporensis*, *D. gelida* and *D. fragile* (Kuever *et al.*, 2005). Thus, strain MSL71^T is valuable as a novel incompletely butyrate-oxidizing SRB isolated from marine environments. Furthermore, it is noteworthy that although almost all known butyrate-oxidizing SRB species have been isolated using enrichment cultures with various electron donors (Kuever *et al.*, 2005), strain MSL71^T was isolated directly by picking up a colony using the agar roll-tube method with lactate as an electron donor (Suzuki *et al.*, 2007c).

Based on the physiological and chemotaxonomic characteristics in addition to the significant differences in the 16S rRNA gene sequences (similarities of 93·5-93·9%) between strain MSL71^T and

the related *Desulfofrigus* species, we propose here a novel genus and species, *Desulfoluna*

butyratoxydans gen. nov., sp. nov., in the class *Deltaproteobacteria* to accommodate the strain with

strain MSL71^T as the type strain.

Description of *Desulfoluna* gen. nov.

Desulfoluna (De.sul.fo.lu′na. L. pref. *de* from; L. n. *sulfur* sulfur; L. fem. n. *luna* the figure of a half

moon, a crescent, lune; N.L. fem. n. *Desulfoluna* a sulfate-reducing crescent).

Mesophilic. Strictly anaerobic. Cells are Gram-negative, non-spore-forming, curved rods. Sulfate

serves as an electron acceptor. Organic electron donors are incompletely oxidized. Type species is

Desulfoluna butyratoxydans.

Description of *Desulfoluna butyratoxydans* sp. nov.

Desulfoluna butyratoxydans (bu.ty.rat.o′xy.dans. N.L. n. *butyras -atis* butyrate; N.L. part. adj. *oxydans*

oxidizing; N.L. part. adj. *butyratoxydans* butyrate-oxidizing).

Has the following characteristics in addition to those described for the genus. Cells are slightly curved

rod-shaped with rounded ends, 0·8-0·9 μm wide and 1·6-3·4 μm long. Motile by a single polar flagellum.

Catalase and oxidase activities are negative. Colonies are grayish and thin and spread on slant media.

The NaCl concentration range for growth is 1·0-6·5% (w/v) with an optimum at 2·0% (w/v). The

temperature range for growth is 10-35°C with an optimum at 30°C. The pH range for growth is 5·6-8·5 with an optimum at 6·3. Utilizes formate, butyrate, pyruvate, lactate, malate, ethanol, propanol, butanol, glycerol and H_2 as electron donors for sulfate reduction. Organic electron donors are oxidized to mainly acetate. Does not utilize acetate, propionate, fumarate, succinate, methanol, glycine, alanine, serine, aspartate and glutamate. Utilizes sulfite and thiosulfate in addition to sulfate as electron acceptors, but not fumarate. Ferments pyruvate and malate in the absence of electron acceptors. Butyrate together with acetate is produced by fermentation of pyruvate. Does not ferment lactate and fumarate. The genomic DNA G + C content is 62·0 mol%. The major respiratory quinone is MK-8(H_4). Major cellular fatty acids are $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7$, $C_{18:1}\omega9$, $C_{18:1}\omega7$ and $C_{14:0}$ 3-OH. Isolated from an estuarine sediment located on the side of the Sea of Japan of the Japanese islands. Type strain is MSL71[T] (= JCM 14721[T] = DSM 19427[T]).

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Legends for figures

Fig. 1.

A phase-contrast photomicrograph of cells of strain MSL71^T grown anaerbically on an agar slant of the seawater medium. Bar, 10 μm.

Fig. 2.

Neighbour-joining tree showing the phylogenetic relationship of strain MSL71^T and related species in the class *Deltaproteobacteria* based on the 16S rRNA gene sequences. Bootstrap values shown are based on analysis of 1000 replicates. The scale bar represents an estimated difference of 2% in nucleotide sequence positions. As the outgroup, *Escherichia coli* was used. Tree topology evaluated by the maximum-likelihood method was almost the same as that obtained with the neighbour-joining method.
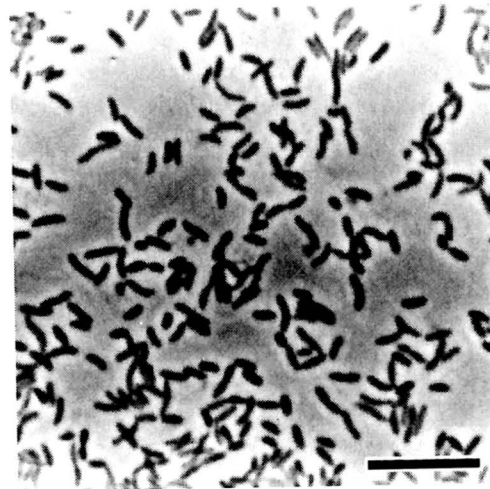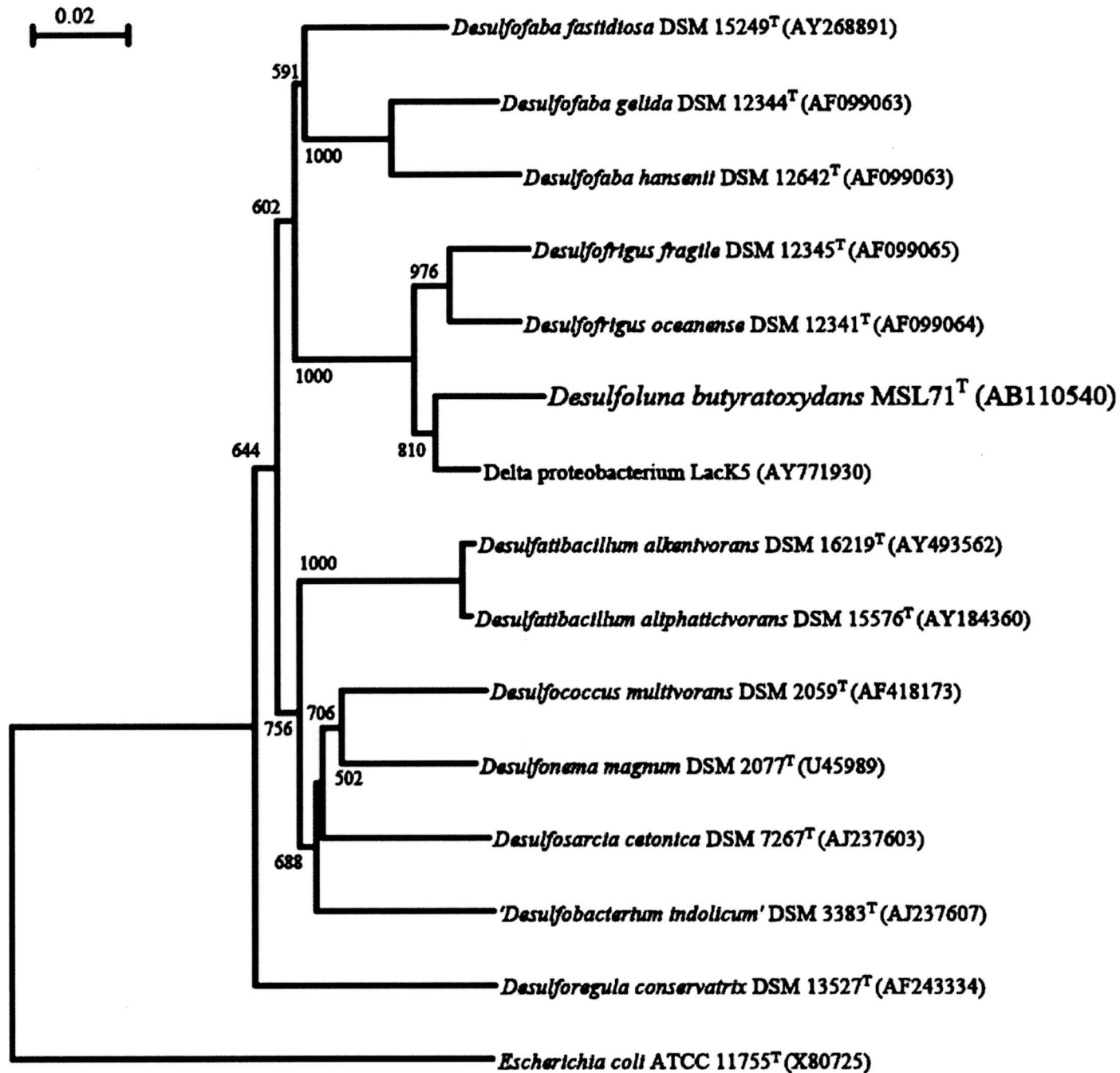
Fig. 1.

Fig. 2.

Table 1. Characteristics useful for differentiating strain MSL71^T from related species

Strains: 1, MSL71^T; 2, *Desulfofrigus fragile* LSv21^T (Knoblauch *et al*., 1999);

3, *Desulfofrigus oceanense* ASv26^T (Knoblauch *et al*., 1999).

+, used; -, not used.

Characteristic	1	2	3
Source	Estuarine sediment	Arctic marine sediment	Arctic marine sediment
Cell shape	Curved rods	Curved rods	Rods
Optimum growth condition			
NaCl (%, w/v)	2·0	1·0-2·5	1·5-2·5
Temperature (°C)	30	18	10
pH	6·3	7·0-7·4	7·0-7·5
Utilization of electron donor			
Acetate	-	-	+
Fumarate	-	+	-
Glycine	-	-	+
Alanine	-	+	-
Serine	-	+	+
H$_2$	+	-	+
Utilization of electron acceptor			
Sulfite	+	-	+
Thiosulfate	+	-	+
Utilization of substrate in the absence of electron acceptor			
Lactate	-	-	+
G+C content (mol%)	62·0	52·1	52·8
Isoprenoide quinone	MK-8(H$_4$)	MK-9	MK-9

Table 2. Cellular fatty acid compositions (%) of strain MSL71[T] and related species

Strains: 1, MSL71; 2, *Desulfofrigus fragile* LSv21[T] (Knoblauch *et al*., 1999); 3, *Desulfofrigus oceanense* ASv26[T] (Knoblauch *et al*., 1999).

-, Not detected.

Fatty acid	1	2	3
Saturated straight-chain:			
$C_{10:0}$	-	2·5	-
$C_{12:0}$	-	0·6	-
$C_{14:0}$	11·4	5·0	6·7
$C_{16:0}$	27·0	21·7	9·3
$C_{18:0}$	2·1	0·7	0·6
Unsaturated straight-chain:			
$C_{14:1}\omega 5$	-	-	0·9
$C_{16:1}\omega 9$	-	3·0	0·7
$C_{16:1}\omega 7$	12·9	30·6	43·7
$C_{16:1}\omega 5$	-	0·5	2·2
$C_{18:1}\omega 9$	7·5	8·6	2·8
$C_{18:1}\omega 7$	17·8	18·5	23·0
$C_{18:1}\omega 5$	-	-	0·4
$C_{20:1}\omega 7$	-	0·3	-
Hydroxy acids:			
$C_{14:0}$ 3-OH	8·9	-	-
Unsaturated branched-chain:			
Branched $C_{17:1}$	1·5	-	-