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Desulfobulbus japonicus sp. nov., a novel, Gram-negative, propionate-oxidizing, sulfate-reducing bacterium isolated from an estuarine sediment in Japan

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Key words: *Desulfobulbus japonicus*, *Deltaproteobacteria*, anaerobic Gram-negative rods, sulfate-reducers, estuarine sediment

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strains Strains Pro1^T and Pro16 is AB110549 and AB110550, respectively.

Abstract

Strictly anaerobic, mesophilic sulfate-reducing bacterial strains Pro1^T and Pro16 isolated from an estuarine sediment in the Sea of Japan of the Japanese islands were phenotypically and phylogenetically characterized. Strains Pro1^T and Pro16 had almost the same physiological and chemotaxonomic characteristics. Cells of both strains were Gram-negative, motile, non-spore-forming rods. Catalase was

not detected. The optimum NaCl concentration for growth was 3·0% (w/v). The optimum temperature was 35°C and the optimum pH was at 6·7. Both strains used formate, propionate, pyruvate, lactate, fumarate, malate, ethanol, propanol, butanol, glycerol, alanine, glucose, fructose and H_2 as electron donors for sulfate reduction and did not use acetate, butyrate, succinate, methanol, glycine, serine, aspartate, glutamate, cellobiose and sucrose. Organic electron donors were incompletely oxidized to mainly acetate. Both strains used in addition thiosulfate as an electron acceptor. Without electron acceptors, both strains fermented pyruvate and lactate. The genomic DNA G + C contents of strains Pro1^T and Pro16 were 48·6 and 46·0 mol%, respectively. The major respiratory quinone of both strains was menaquinone MK-5(H_2). Major cellular fatty acids of both strains were $C_{15:0}$, $C_{16:0}$, $C_{17:1\omega6}$ and $C_{18:1\omega7}$. Phylogenetic analysis based on the 16S rRNA gene sequence placed both strains in the class *Deltaproteobacteria*. The recognized closest relative of both strains was *Desulfobulbus mediterraneus* (sequence similarities of 95·2 and 94·8%, respectively). Based on the phylogenetic, physiological and chemotaxonomic characteristics, strains Pro1^T (= JCM 14043^T = DSM 18378^T) and Pro16 (= JCM 14044 = DSM 18379) were affiliated with the genus *Desulfobulbus* and are described as a novel species *Desulfobulbus japonicus*.

Main text

Sulfate-reducing bacteria (SRB) include phylogenetically diverse anaerobic bacterial species and most of recognized species of SRB belong to the class *Deltaproteobacteria* and the family *Peptococcaceae* in the phylum *Firmicutes* (Castro *et al*., 2000). Major intermediates of anaerobic decomposition of organic

matter such as acetate, propionate, butyrate and H_2 serve as the most important electron donors for sulfate-reduction in environments, and thus SRB significantly contribute to mineralization of organic matter as well as the sulfur cycle on the earth (Jørgensen, 1982; Sørensen *et al*., 1981). It is known that SRB often take a principal role in oxidation of propionate in various anoxic environments (Parkes *et al*., 1993; Ueki *et al*., 1986). Out of propionate-oxidizing SRB from marine environments, species in the genera *Desulfobacterium*, *Desulfonema* and *Desulfacinum* oxidize propionate completely to carbon dioxide, whereas species in the genera *Desulfobulbus*, *Desulforhopalus* and *Desulfofaba* oxidize it incompletely to acetate (Kuever *et al*., 2005). In this study, strains of propionate-oxidizing SRB (Pro1^T and Pro16) isolated through enrichment culture with propionate as an electron donor from an estuarine sediment of the Japanese islands (Suzuki *et al*., in press) were subjected to a comprehensive characterization. .

Sediment cores were collected to a depth of 6 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, which is located on the side of the Sea of Japan in Japanese islands (38°54.5′N, 139°50.6′E) on the 24th of June 1999. Diluted (10^{-3}-10^{-6}) sediment samples (0.2 ml) were inoculated into liquid seawater medium containing 20 mM of sodium propionate as described below. Cultures showing sulfate-reduction by formation of a black precipitate in the medium were transferred to fresh medium containing the same electron donor. After two to four subcultures in the same manner, SRB were isolated from the enrichment culture using the anaerobic roll tube method (Hungate, 1966) with sodium propionate as an electron donor. Several strains of SRB were

obtained by picking up black colonies of SRB that appeared in agar roll tubes after about one month of incubation. Strains Pro1^{T} and Pro16 were finally obtained after several purification procedures through colony isolation by the anaerobic roll tube method.

Two basal media (seawater medium and defined medium) were used in this study as described previously (Suzuki *et al.*, in press). The seawater medium, which was used for enrichment culture, contained ($l^{-1}$of 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 (modified from SL-10 described by Widdel *et al.*, 1983), 1 ml the B-vitamin solution (Widdel & Bak, 1992), and 0·5 g L-cysteine.HCl.H_2O, as well as an appropriate electron donor. The pH was adjusted to 7·2-7·4 with 1 N 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 with sodium propionate as an electron donor. The following medium, which was designated as 'defined medium' in contrast with the seawater medium and used for the general physiological characterization of the strains, contained (l^{-1}): 0·5 g KH_2PO_4, 1·0 g NH_4Cl, 1·0 g Na_2SO_4, 2·0 g $MgSO_4.7H_2O$, 0·1 g $CaCl_2.2H_2O$, 0·5 g yeast extract, 1 mg sodium resazurin, 10 ml the trace element solution, 1 ml the B-vitamin solution, 30 g NaCl, and 0·5 g L-cysteine.HCl.H_2O (Nakamoto *et al.*, 1996; Ueki *et al.*, 1980; Widdel & Bak, 1992). The pH was adjusted to 7·2-7·4 with 1 N NaOH. Agar (Difco) (1·5%, w/v) was added to the medium and used for the slant cultures with propionate as an electron donor. The concentration of sulfate in the defined medium was about 15 mM. Cultivation and transfer of the strains were performed under an O_2-free N_2 (100%) atmosphere. The strains were cultivated at 30°C, unless stated otherwise.

The strains were maintained in slant cultures of the seawater medium or the defined medium with sodium propionate (20 mM) as an electron donor.

Flagella-staining was carried out according to Blenden & Goldberg (1965). Aerobic growth was examined in the presence of sodium propionate 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). Effects of NaCl concentration, temperature and pH on growth of the strains were examined in the presence of sodium propionate as an electron donor using the defined medium. Growth of the strains was monitored by measurement of OD_{660} with a spectrophotometer (HITACHI U-1000).

Utilization of electron donors by the strains was determined using the defined medium containing each compound at a final concentration of 20 mM (fatty acids, amino acids and alcohols) or 10 mM (carbohydrates). H_2 utilization was determined in the presence of sodium acetate (5 mM) with H_2 in the atmosphere. Utilization of electron acceptors was determined with sodium propionate (20 mM) as an electron donor in a sulfate-free medium, which contained the same concentrations of chloride in place of sulfate in the defined medium. Sodium sulfite (3 mM), sodium thiosulfate or disodium fumarate (20 mM each) was added to the sulfate-free medium as possible electron acceptors. Utilization of pyruvate, lactate, fumarate, malate (20 mM each), glucose and fructose (10 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 (OD_{660}) 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 (Hitachi G-5000 or 263-30), as described by Ueki *et al*. (1986). Non-volatile fatty acids and formate were analyzed by HPLC (Shimadzu LC-10AD) as described by Akasaka *et al*. (2003a). Reducing sugars were quantified by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Sulfate, sulfite and thiosulfate were analyzed with an ion chromatograph (Dionex 2000i) as described by Nakamoto *et al*. (1996). Genomic DNA was extracted according to the method described by Kamagata & Mikami (1991). Extracted DNA 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 µBondpack 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 (Hp6890, Hewlett-Packard or G-3000, Hitachi) 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*. (2003a, b) using a primer set, 27f and 1492r. PCR-amplified 16S rRNA gene 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.

Strains Pro1^T and Pro16 had almost the same physiological and chemotaxonomic characteristics. Cells of both strains were Gram-negative rods with rounded ends, 0·8-1·6 µm wide and 1·4-2·9 µm long. Cells usually occurred singly or in pairs (Fig. 1). Spore formation was not observed. Cells were motile by a single polar flagellum. Both strains made grayish and thin colonies on agar slants of the defined medium as well as the seawater medium.

Both strains reduced sulfate with propionate as an electron donor and produced acetate at a molar ratio of about 4 : 3 : 4 (propionate : sulfate : acetate) in the defined medium, which was almost consistent with the theoretical ratio. Thus, both strains had an incomplete type of oxidation of electron donors.

Growth rates of the strains were not affected by the presence or absence of the B-vitamin mixture in the defined medium containing yeast extract. Both strains did not grow aerobically in the defined liquid medium. Catalase and oxidase activities were not detected. NaCl concentration range for growth was 1·0-7·0% (w/v) with an optimum at 3·0% (w/v). Temperature range for growth was 15-35ºC with an optimum at 35ºC. pH range for growth was 6·1-7·5 with an optimum at 6·7. Both strains utilized formate, pyruvate, lactate, fumarate, malate, ethanol, propanol, butanol, glycerol, alanine, glucose, fructose and H_2 as well as propionate as electron donors for sulfate reduction. Both strains grew slightly faster with pyruvate or lactate (μ = about 0·16 h^{-1} for both substrates) than with propionate (μ = about 0·13 h^{-1}) as an electron donor. Although growth rates with glucose (μ = about 0·02 h^{-1}) and fructose (μ = about 0·07 h^{-1}) were significantly lower than that with propionate, final growth yields with these carbohydrates reached about twice (OD_{660} = about 2·0) as that with propionate (OD_{660} = 0·8-1·0). Although almost all organic electron donors were oxidized to mainly acetate, propanol and butanol were oxidized to their corresponding carboxylic acids. Propionate (about 20 mM) produced by oxidation of propanol (20 mM) was not further oxidized in spite of the presence of sulfate unreduced (about 5 mM) in the medium. Glucose and fructose were oxidized to acetate with molar ratios of about 1 : 1 : 1·6-1·7 (carbohydrates : sulfate : acetate), which were slightly different from the theoretical ratio (1 : 1 : 2). The strains did not utilize acetate, butyrate, succinate, methanol, glycine, serine, aspartate, glutamate, cellobiose and sucrose as electron donors. Strain Pro1^T did not use arabinose, ribose, xylose, galactose, mannose, rhamnose, sorbose, lactose and melibiose (utilization of these compounds of strain Pro16 was not determined). Strains Pro1^T and Pro16 utilized thiosulfate in addition to sulfate as an electron

acceptor with propionate as an electron donor. Both strains did not use sulfite and fumarate as electron acceptors. In the absence of electron acceptors, both strains oxidized pyruvate and lactate (about 5·5 mM each) and produced acetate (2·6-3·3 mM) and propionate (1·2-3·7 mM), while both did not oxidize fumarate and malate. In the absence of electron acceptors, strain Pro1^T oxidized small amounts of glucose and fructose (about 2 mM each) and produced trace amounts of acetate (0·2 mM and 0·5 mM, respectively), propionate (0·2 mM each) and H_2.

The G + C content of genomic DNA of strain Pro1^T was 48·6 mol% and that of strain Pro16 was 46·0 mol%. The major respiratory quinone of both strains was menaquinone MK-5(H_2). Strains Pro1^T and Pro16 had almost the same CFAs composition and $C_{15:0}$ (21·0 and 21·7%, respectively), $C_{16:0}$ (7·5 and 7·1%), $C_{17:1\omega6}$ (39·2 and 46·4%) and $C_{18:1\omega7}$ (12·4 and 11·4%) were major CFAs for both strains.

Almost full-length of 16S rRNA gene sequences were determined for both strains (Pro1^T, 1485 bp; Pro16, 1479 bp). The sequence similarity between both strains was 98·1%. Based on the 16S rRNA gene phylogenetic analysis, strains Pro1^T and Pro16 were affiliated with the class *Deltaproteobacteria* and formed a cluster in the family *Desulfobulbaceae* (Fig. 2). The closest relative of both strains on the database was *Desulfobulbus* sp. BG25 isolated from salt marsh sediment with sequence similarities of 98·5 and 98·4%, respectively. The closest recognized species of the strains was *Desulfobulbus mediterraneus* (Sass *et al*., 2002) with sequence similarities of 95·2 (sequence length compared, 1428 bp) and 94·8% (1429 bp), respectively, and those with *Desulfobulbus rhabdoformis* (Lien *et al*., 1998)

were 94·0 (1453 bp) and 93·7% (1457 bp), respectively. Sequence similarities with *Desulfobulbus marinus* (Widdel & Pfennig, 1982) were 94·1 and 94·5%, respectively, although the sequence length available for comparison was rather short (1211 bp).

Physiological and chemotaxonomic characteristics of both strains were compared with those of all described species in the genus *Desulfobulbus* (Table 1). The incomplete-oxidation of propionate as an electron donor for sulfate-reduction and production of acetate and propionate from lactate and pyruvate without electron acceptors are both specific characteristics for *Desulfobulbus* species. Thus, strains Pro1^T and Pro16 share several important features with those of *Desulfobulbus* species including cell morphology and the composition of major respiratory quinone.

The ranges of electron donors utilized by strains Pro1^T and Pro16 were not consistent with any of the *Desulfobulbus* species, especially for the electron donors such as formate, fumarate, malate, succinate, glycerol, alanine and glutamate (Table 1). Strains Pro1^T and Pro16 are the second group in the *Desulfobulbus* species reported to utilize carbohydrates as electron donors for sulfate reduction, although the range is rather limited as compared with that of *D. mediterraneus* (Sass *et al*., 2002). *D. mediterraneus* is reported to ferment glucose and fructose in the absence of electron acceptors, whereas strain Pro1^T only slightly consumed them and grew very weakly on them.

As shown in Table 1, the G + C contents of the genomic DNA of *Desulfobulbus* species seem to be

divided into two levels (47-50% and about 59%, respectively). The G + C contents of strains Pro1^T and Pro16 were almost consistent with those of the lower level. The CFAs profiles of our strains are compared with those of the closest relatives, *D. mediterraneus* and *D. rhabdoformis*, in Table 2. Strains Pro1^T and Pro16 have a common profile of overall CFAs compositions with those of the relatives. Although differences of cultivation conditions of the cells should be considered to compare the profiles, major differences among the compositions are shown in the percentages of CFAs such as $C_{14:0}$, $C_{15:0}$, $C_{16:1\omega7}$, $C_{17:1\omega6}$ and $C_{18:1\omega7}$.

Based on the phylogenetic, physiological and chemotaxonomic characteristics shown above, strains Pro1^T and Pro16 should be classified as one of the species in the genus *Desulfobulbus* and we propose here a novel species, *Desulfobulbus japonicus* sp. nov., to accommodate the strains with strain Pro1^T as the type strain.

Description of *Desulfobulbus japonicus* sp. nov.

Desulfobulbus japonicus (ja.po'ni.cus. N.L. masc. adj. *japonicus* pertaining to Japan, where the type strain was originally isolated).

Cells are rod-shaped, 0·8-1·1 µm wide and 1·4-2·9 µm long. Strictly anaerobic. Gram-negative. Motile by a single polar flagellum. Non-spore-forming. Catalase and oxidase activities are not detected. Colonies are grayish and thin and spread on slant media. The NaCl concentration range for growth is

1·0-7·0 (w/v) with an optimum at 3·0% (w/v). The temperature range for growth is 15-35°C with an optimum at 35°C. The pH range for growth is 6·1-7·5 with an optimum at 6·7. Utilizes formate, propionate, pyruvate, lactate, fumarate, malate, ethanol, propanol, butanol, glycerol, alanine, glucose, fructose and H_2 as electron donors for sulfate reduction. Does not use acetate, butyrate, succinate, methanol, glycine, serine, aspartate, glutamate, arabinose, ribose, xylose, galactose, mannose, rhamnose, sorbose, cellobiose, lactose, melibiose and sucrose. Almost all organic electron donors are incompletely oxidized to acetate. Sulfate and thiosulfate serve as electron acceptors. Does not use sulfite and fumarate. Pyruvate and lactate are fermented in the absence of electron acceptors to acetate and propionate. The genomic DNA G + C content is 46·0-48·6 mol%. Major cellular fatty acids are $C_{15:0}$, $C_{16:0}$, $C_{17:1\omega6}$ and $C_{18:1\omega7}$. The major respiratory quinone is menaquinone MK-5(H_2). Isolated from an estuarine sediment located on the side of the Sea of Japan of the Japanese islands. Strain Pro1^T is designated as the type strain of the species. Strains Pro1^T (= JCM 14043^T = DSM 18378^T) and Pro16 (= JCM 14044 = DSM 18379) are deposited with the Japan Collection of Microorganisms (JCM) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ).

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1 **Legends for figures**

2 **Fig. 1.**

3 A phase-contrast photomicrograph of cells of strain Pro1^T grown anaerbically on an agar slant of the

4 seawater medium. Bar, 10 μm.

5

6 **Fig. 2.**

7 Neighbour-joining tree showing the phylogenetic relationship of strains Pro1^T and Pro16 and related

8 species in the class *Deltaproteobacteria* based on the 16S rRNA gene sequences. Bootstrap values

9 shown are based on analysis of 1000 replicates. The scale bar represents an estimated difference of 2%

10 in nucleotide sequence positions. As the outgroup, *Escherichia coli* was used. Tree topology evaluated

11 by the maximum-likelihood method was almost the same as that obtained with the neighbour-joining

12 method.

13

Fig. 1.

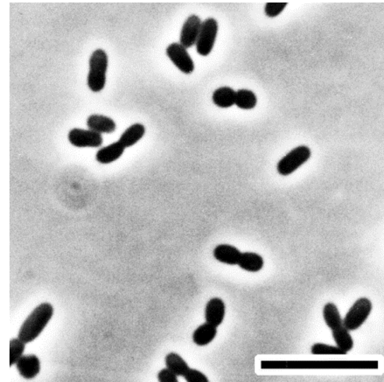

Fig.2.

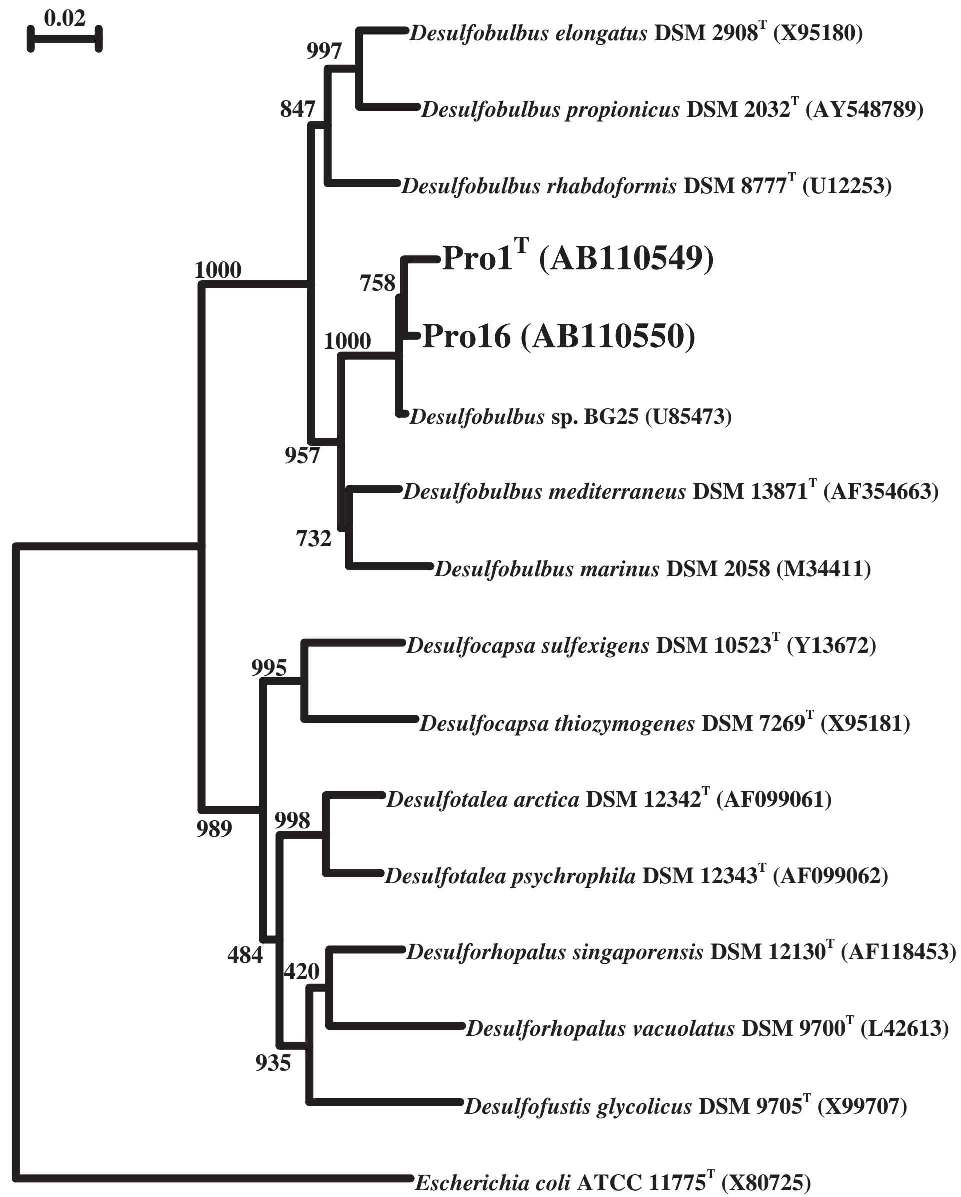

Table 1. Some characteristics of strains Pro1^T, Pro16 and *Desulfobulbus* species

Strains: 1, Pro1^T; 2, Pro16; 3, *Desulfobulbus mediterraneus* 86FS1^T (Sass *et al* ., 2002); 4, *Desulfobulbus rhabdoformis* M16^T (Lien *et al* ., 1998; Kuever *et al* ., 2005);

5, *Desulfobulbus marinus* 3pr10 (Widdel & Pfennig, 1982; Kuever*et al* ., 2005); 6, *Desulfobulbus elongatus* FP^T (Samain *et al* ., 1984); 7, *Desulfobulbus propionicus* 1pr3^T (Widdel & Pfennig, 1982).

+, used; , +^{w}, weakly used; -, not used; n.d., not determined or no data available.

	1	2	3	4	5	6	7
Source	Estuarine sediment	Estuarine sediment	Deep-sea sediment	Water-oil separation system	Anoxic mud flat	Anaerobic digester	Anoxic freshwater ditch
Cell shape	Rods	Rods	Ovoid	Rods	Oval	Rods	Oval or lemon
Motility	Motile	Motile	Motile	Non-motile	Motile	Motile	Non-motile
Optimum growth condition							
NaCl (%, w/v)	3·0	3·0	2·0	1·5-2·0	2·0	n.d.^{b}	n.d.^{b}
Temperature (^{o}C)	35	35	25	31	29	31	39
pH	6·7	6·7	n.d.^{a}	6·8-7·2	7·4	7·0	7·1-7·5
Utilization of electron donor							
Formate	+	+	-	-	+	-	-
Fumarate	+	+	+	+	-	-	-
Malate	+	+	+	+	-	-	-
Succinate	-	-	+	+	-	-	-
Glycerol	+	+	-	n.d.	n.d.	n.d.	n.d.
Alanine	+	+	+	n.d.	n.d.	n.d.	-
Glutamate	-	-	+	n.d.	n.d.	n.d.	n.d.
Fructose	+	+	+	n.d.	n.d.	-	-
Glucose	+	+	+	n.d.	n.d.	-	-
Ribose	-	n.d.	+	n.d.	n.d.	n.d.	n.d.
Galactose	-	n.d.	+	n.d.	n.d.	n.d.	n.d.
Cellobiose	-	-	+	n.d.	n.d.	n.d.	-
Lactose	-	n.d.	+	n.d.	n.d.	n.d.	n.d.
Sucrose	-	-	+	n.d.	n.d.	n.d.	n.d.
H_2	+	+	-	+	+	+	+
Utilization of electron acceptor							
Sulfite	-	-	+	+	+	+	+
Utilization of substrates in the absence of electron acceptors							
Fumarate	-	-	-	+	-	-	-
Malate	-	-	-	+	-	-	-
Glucose	+^{w}	n.d.	+	n.d.	n.d.	n.d.	n.d.
Fructose	+^{w}	n.d.	+	n.d.	n.d.	n.d.	n.d.
G+C content (mol%)	48·6	46·0	58·6	50·6	47·3	59·0	59·9
Isoprenoide quinone	MK-5(H_2)	MK-5(H_2)	n.d.	MK-5(H_2)	MK-5(H_2)	MK-5(H_2)	MK-5(H_2)

^{a} Strain 86FS1^T does not grow at pH values below 6·3 and above 8·0.

^{b} Strains FP^T and 1pr3^T do not require NaCl for growth.

Table 2. Cellular fatty acid composition (%) of strains Pro1^T, Pro16 and related species

Strains: 1, Pro1^T; 2, Pro16; 3, *Desulfobulbus mediterraneus* 86FS1^T (Sass *et al*., 2002);

4, *Desulfobulbus rhabdoformis* M16^T (Lien *et al*., 1998).

-, not detected.

Fatty acid	1	2	3	4
Saturated straight-chain:				
$C_{11:0}$	-	2·3	-	-
$C_{14:0}$	2·7	-	11·4	9·4
$C_{15:0}$	21·0	21·7	1·9	13·9
$C_{16:0}$	7·5	7·1	3·0	-
$C_{17:0}$	2·1	3·1	-	-
$C_{18:0}$	1·0	-	-	-
Unsaturated straight-chain:				
$C_{14:1\omega 5}$	-	-	0·5	-
$C_{15:1\omega 8}$	-	-	0·9	-
$C_{15:1\omega 6}$	1·4	-	2·4	-
$C_{16:1\omega 7}$	3·0	3·5	27·1	7·9
$C_{16:1\omega 5}$	2·3	2·1	9·9	4·4
$C_{17:1\omega 8}$	1·9	-	1·1	-
$C_{17:1\omega 6}$	39·2	46·4	11·1	24·1
$C_{18:1\omega 7}$	12·4	11·4	26·6	24·5
Unsaturated branched-chain:				
Branched $C_{17:1}$	2·6	2·4	-	-

Temperature for cultivation: Pro1^{T}and Pro16, 30°C; 86FS1^T, 20°C; M16^T, 30°C.