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

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Two strictly anaerobic, mesophilic, sulfate-reducing bacterial strains, Pro1^T and Pro16, were isolated from an estuarine sediment in the Sea of Japan of the Japanese islands and were characterized by phenotypic and phylogenetic methods. 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 activity was not detected. The optimum NaCl concentration for growth was 3.0% (w/v). The optimum temperature for growth was 35 °C and the optimum pH was 6.7. Both strains used formate, propionate, pyruvate, lactate, fumarate, malate, ethanol, propanol, butanol, glycerol, alanine, glucose, fructose and H₂ as electron donors for sulfate reduction and did not use acetate, butyrate, succinate, methanol, glycine, serine, aspartate, glutamate, cellobiose or sucrose. Organic electron donors were incompletely oxidized mainly to acetate. Both strains also used 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(H₂). Major cellular fatty acids of both strains were C_{15:0}, C_{16:0}, C_{17:1ω6} and C_{18:1ω7}. Phylogenetic analysis based on 16S rRNA gene sequences placed both strains in the class *Deltaproteobacteria*. The closest recognized relative of strains Pro1^T and Pro16 was *Desulfobulbus mediterraneus* with sequence similarities of 95.2 and 94.8%, respectively. Based on phylogenetic, physiological and chemotaxonomic characteristics, strains Pro1^T and Pro16 represent a novel species of the genus *Desulfobulbus*, for which the name *Desulfobulbus japonicus* is proposed. The type strain is Pro1^T (= JCM 14043^T = DSM 18378^T) and strain Pro16 (= JCM 14044 = DSM 18379) is a reference strain.

Sulfate-reducing bacteria (SRB) include phylogenetically diverse anaerobic bacterial species and most of the 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 H₂ serve as the most important electron donors for sulfate-reduction in environments and thus SRB contribute significantly to the mineralization of organic matter as well as to the Earth's sulfur cycle (Jørgensen, 1982;

Sørensen *et al.*, 1981). It is known that SRB often take a principal role in the oxidation of propionate in various anoxic environments (Parkes *et al.*, 1993; Ueki *et al.*, 1986). Of the 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, two strains of propionate-oxidizing SRB, Pro1^T and Pro16, that were isolated through enrichment culture with propionate as an electron donor from an estuarine sediment from the Japanese islands (Suzuki *et al.*, 2007) 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 the Niida river estuary in Sakata harbour, which is

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Abbreviations: CFA, whole-cell fatty acids; SRB, sulfate-reducing bacteria.

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located on the coast of the Sea of Japan in the Japanese islands (38° 54.5' N 139° 50.6' E) on the 24 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 performed 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.*, 2007). 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 trace element solution (modified from SL-10, described by Widdel *et al.*, 1983), 1 ml B-vitamin solution (Widdel & Bak, 1992) and 0.5 g L-cysteine.HCl.H₂O, as well as an appropriate electron donor. The pH was adjusted to 7.2–7.4 with 1 M NaOH. Agar (1.5 %, w/v; Difco) 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 'defined medium' in contrast to 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 $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.5 g yeast extract, 1 mg sodium resazurin, 10 ml trace element solution, 1 ml B-vitamin solution, 30 g NaCl and 0.5 g L-cysteine.HCl.H₂O (Nakamoto *et al.*, 1996; Ueki *et al.*, 1980; Widdel & Bak, 1992). 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 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 O₂-free N₂ (100 %) atmosphere. The strains were cultivated at 30 °C, unless stated otherwise. The strains were maintained in slant cultures of seawater medium or 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.H₂O and sodium resazurin. Catalase and oxidase activities of cells were tested as described by Akasaka *et al.* (2003a). The 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 OD₆₆₀ with a spectrophotometer (U-1000; Hitachi).

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). H₂ utilization was determined in the presence of sodium acetate (5 mM) with H₂ 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) were 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 (OD₆₆₀) in the presence or absence of each compound as well as by measurement of the concentration in the medium after cultivation.

Volatile fatty acids and alcohols were analysed by GC (G-5000 or 263-30; Hitachi), as described by Ueki *et al.* (1986). Non-volatile fatty acids and formate were analysed by HPLC (LC-10AD; Shimadzu) as described by Akasaka *et al.* (2003a). Reducing sugars were quantified by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Sulfate, sulfite and thiosulfate were analysed with an ion chromatograph (2000i; Dionex) 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 the G + C content was measured by HPLC (L-7400; Hitachi) equipped with a μ Bondpack C18 column (3.9 × 300 mm; Waters). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analysed 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 analysed by GC (HP6890, Hewlett Packard or G-3000, Hitachi) equipped with a HP Ultra2 column. CFAs 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 gene of the novel strains were carried out according to the method described by Akasaka *et al.* (2003a, b) using a primer set, 27f and 1492r. The PCR-amplified 16S rRNA gene was sequenced by using a Thermo Sequenase Primer Cycle

sequencing kit (Amersham Biosciences) and a DNA sequencer (4000L; 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 positions 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 greyish 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. Neither strain grew aerobically in the defined liquid medium. Catalase and oxidase activities were not detected. The NaCl concentration range for growth was 1.0–7.0 % (w/v), with an optimum at 3.0 % (w/v). The temperature range for growth was 15–35 °C, with an optimum at 35 °C. The pH range for growth was 6.1–7.5, with an optimum at pH 6.7. Both strains utilized formate, pyruvate, lactate, fumarate, malate, ethanol, propanol, butanol, glycerol, alanine, glucose,

fructose and H₂ as well as propionate as electron donors for sulfate reduction. Both strains grew slightly faster with pyruvate or lactate ($\mu \approx 0.16 \text{ h}^{-1}$ for both substrates) than with propionate ($\mu \approx 0.13 \text{ h}^{-1}$) as an electron donor. Although growth rates with glucose ($\mu \approx 0.02 \text{ h}^{-1}$) and fructose ($\mu \approx 0.07 \text{ h}^{-1}$) were significantly lower than that found with propionate, final growth yields with these carbohydrates reached about twice ($\text{OD}_{660} \approx 2.0$) those with propionate ($\text{OD}_{660} = 0.8\text{--}1.0$). 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 unreduced sulfate (about 5 mM) in the medium. Glucose and fructose were oxidized to acetate with molar ratios of about 1 : 1.6–1.7 (carbohydrates : sulfate : acetate), which were slightly different from the theoretical ratio (1 : 1 : 2). The novel strains did not utilize acetate, butyrate, succinate, methanol, glycine, serine, aspartate, glutamate, cellobiose or sucrose as electron donors. Strain Pro1^T did not use arabinose, ribose, xylose, galactose, mannose, rhamnose, sorbose, lactose or melibiose (utilization of these compounds was not determined for strain Pro16). Strains Pro1^T and Pro16 utilized thiosulfate in addition to sulfate as an electron acceptor with propionate as an electron donor. Neither strain used sulfite or 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 neither oxidized fumarate or 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 H₂.

The G + C contents of the genomic DNA 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(H₂). Strains Pro1^T and Pro16 had almost the same content of CFAs with C_{15:0} (21.0 and 21.7 %, respectively), C_{16:0} (7.5 and 7.1 %), C_{17:1ω6} (39.2 and 46.4 %) and C_{18:1ω7} (12.4 and 11.4 %) as major CFAs for both strains.

Almost full-length 16S rRNA gene sequences were determined for both strains (Pro1^T, 1485 bp; Pro16, 1479 bp). The sequence similarity between the two strains was 98.1 %. Based on phylogenetic analysis of the 16S rRNA gene sequence, 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 found on the database was *Desulfobulbus* sp. BG25, isolated from salt marsh sediment, with gene sequence similarities of 98.5 and 98.4 % to strains Pro1^T and Pro16, respectively. The closest recognized species to the novel strains were *Desulfobulbus mediterraneus* (Sass *et al.*, 2002), with sequence similarities of 95.2 (sequence length compared, 1428 bp) and 94.8 % (1429 bp), respectively, and *Desulfobulbus rhabdiformis* (Lien *et al.*, 1998) with sequence

Fig. 1. A phase-contrast photomicrograph of cells of strain Pro1^T grown anaerobically on an agar slant of the seawater medium. Bar, 10 µm.

Fig. 2. Neighbour-joining tree showing the phylogenetic relationship of strains Pro1^T and Pro16 and related species in the class *Deltaproteobacteria* based on 16S rRNA gene sequences. Bootstrap values shown are based on analysis of 1000 replicates. *Escherichia coli* was used as the outgroup. Tree topology evaluated by the maximum-likelihood method was almost the same as that obtained with the neighbour-joining method. Bar, estimated difference of 2% in nucleotide sequence positions.

similarities of 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 species of the genus *Desulfobulbus*, 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 recognized species of the genus *Desulfobulbus*, 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 genus *Desulfobulbus* that have been reported to utilize carbohydrates as electron donors for sulfate reduction, although the range of carbohydrates

utilized is rather limited 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 DNA G+C contents of *Desulfobulbus* species seem to be divided into two levels (47–50% and about 59%, respectively). The DNA G+C contents of strains Pro1^T and Pro16 were almost consistent with those of the lower level. The CFA profiles of the novel strains are compared with those of the closest relatives, *D. mediterraneus* and *D. rhabdoformis*, in Table 2. Strains Pro1^T and Pro16 shared a common profile of overall CFA composition with these phylogenetic relatives. Although differences in the cultivation conditions of cells should be considered when comparing CFA profiles, major differences between the species were found for CFAs such as C_{14:0}, C_{15:0}, C_{16:1ω7}, C_{17:1ω6} and C_{18:1ω7}.

Based on the phylogenetic, physiological and chemotaxonomic characteristics described above, strains Pro1^T and Pro16 should be classified as members of the genus *Desulfobulbus* and we propose that they represent a novel species, *Desulfobulbus japonicus* sp. nov., with strain Pro1^T as the type strain.

Table 1. Some characteristics of strains Pro1^T, Pro16 and other *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; ND, not determined or no data available.

Characteristic	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 conditions:							
NaCl (% w/v)	3.0	3.0	2.0	1.5–2.0	2.0	ND*	ND*
Temperature (°C)	35	35	25	31	29	31	39
pH	6.7	6.7	ND†	6.8–7.2	7.4	7.0	7.1–7.5
Utilization of electron donors:							
Alanine	+	+	+	ND	ND	ND	–
Cellobiose	–	–	+	ND	ND	ND	–
Formate	+	+	–	–	+	–	–
Fructose	+	+	+	ND	ND	–	–
Fumarate	+	+	+	+	–	–	–
Galactose	–	ND	+	ND	ND	ND	ND
Glucose	+	+	+	ND	ND	–	–
Glutamate	–	–	+	ND	ND	ND	ND
Glycerol	+	+	–	ND	ND	ND	ND
H ₂	+	+	–	+	+	+	+
Lactose	–	ND	+	ND	ND	ND	ND
Malate	+	+	+	+	–	–	–
Ribose	–	ND	+	ND	ND	ND	ND
Succinate	–	–	+	+	–	–	–
Sucrose	–	–	+	ND	ND	ND	ND
Utilization of electron acceptors:							
Sulfite	–	–	+	+	+	+	+
Utilization of substrates in the absence of electron acceptors:							
Fructose	w	ND	+	ND	ND	ND	ND
Fumarate	–	–	–	+	–	–	–
Glucose	w	ND	+	ND	ND	ND	ND
Malate	–	–	–	+	–	–	–
DNA G + C content (mol%)	48.6	46.0	58.6	50.6	47.3	59.0	59.9
Isoprenoid quinone	MK-5(H ₂)	MK-5(H ₂)	ND	MK-5(H ₂)	MK-5(H ₂)	MK-5(H ₂)	MK-5(H ₂)

*Strains FP^T and 1pr3^T do not require NaCl for growth.

†Strain 86FS1^T does not grow at pH values below 6.3 and above 8.0.

Description of *Desulfobulbus japonicus* sp. nov.

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

Cells are rod-shaped, 0.8–1.6 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 greyish 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 pH 6.7. Utilizes formate, propionate, pyruvate, lactate, fumarate, malate, ethanol, propanol, butanol, glycerol, alanine, glucose, fructose and H₂ 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 or sucrose.

Table 2. Cellular fatty acid contents (%) 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. The following temperatures were used for cultivation: Pro1^T, Pro16 and M16^T, 30 °C; 86FS1^T, 20 °C.

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 _{14:1ω5}	–	–	0.5	–
C _{15:1ω8}	–	–	0.9	–
C _{15:1ω6}	1.4	–	2.4	–
C _{16:1ω7}	3.0	3.5	27.1	7.9
C _{16:1ω5}	2.3	2.1	9.9	4.4
C _{17:1ω8}	1.9	–	1.1	–
C _{17:1ω6}	39.2	46.4	11.1	24.1
C _{18:1ω7}	12.4	11.4	26.6	24.5
Unsaturated branched-chain:				
Branched C _{17:1}	2.6	2.4	–	–

Almost all organic electron donors are incompletely oxidized to acetate. Sulfate and thiosulfate serve as electron acceptors. Does not use sulfite or 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_{17:1ω6} and C_{18:1ω7}. The major respiratory quinone is menaquinone MK-5(H₂).

The type strain, Pro1^T (=JCM 14043^T=DSM 18378^T), was isolated from an estuarine sediment located on the side of the Sea of Japan of the Japanese islands. Strain Pro16 (=JCM 14044=DSM 18379) is a reference strain.

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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). *Propionimonas 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.

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

Castro, H. F., Williams, N. H. & Ogram, A. (2000). Phylogeny of sulfate-reducing bacteria. *FEMS Microbiol Ecol* **31**, 1–9.

Felsenstein, J. (2006). PHYLIP (Phylogeny Inference Package), v3.66. Department of Genome Sciences, University of Washington, Seattle.

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

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

Kamagata, Y. & Mikami, E. (1991). Isolation and characterization of a novel thermophilic *Methanosaeta* strain. *Int J Syst Bacteriol* **41**, 191–196.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

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.

Lien, T., Madsen, M., Steen, I. H. & Gjerdevik, K. (1998). *Desulfobulbus rhabdoformis* sp. nov., a sulfate reducer from a water-oil separation system. *Int J Syst Bacteriol* **48**, 469–474.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* **31**, 426–428.

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.

Parkes, R. J., Dowling, N. J. E., White, D. C., Herbert, R. A. & Gibson, G. R. (1993). Characterization of sulfate-reducing bacterial-populations within marine and estuarine sediments with different rates of sulfate reduction. *FEMS Microbiol Ecol* **102**, 235–250.

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

Samain, E., Dubourguier, H. C. & Albagnac, G. (1984). Isolation and characterization of *Desulfobulbus elongatus* sp. nov. from a mesophilic industrial digester. *Syst Appl Microbiol* **5**, 391–401.

Sass, A., Rütters, H., Cypionka, H. & Sass, H. (2002). *Desulfobulbus mediterraneus* sp. nov., a sulfate-reducing bacterium growing on mono- and disaccharides. *Arch Microbiol* **177**, 468–474.

Sørensen, J., Christensen, D. & Jørgensen, B. B. (1981). Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. *Appl Environ Microbiol* **42**, 5–11.

- Suzuki, D., Ueki, A., Amaishi, A. & Ueki, K. (2007).** *Desulfopila aestuarii* 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.
- 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.
- Widdel, F. & Pfennig, N. (1982).** Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch Microbiol* **131**, 360–365.
- 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.
- 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.