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# Desulfovibrio portus sp. nov., a novel sulfate-reducing bacterium in the class Deltaproteobacteria isolated from an estuarine sediment

Daisuke Suzuki, Atsuko Ueki,\* Aya Amaishi,† and Katsuji Ueki

Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan

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A strictly anaerobic, mesophilic, sulfate-reducing bacterial strain (MSL79<sup>T</sup>) isolated from an estuarine sediment in the Sea of Japan of the Japanese islands 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 2.0% (wt/vol). The optimum temperature was 35°C and the optimum pH was 6.5. Strain MSL79<sup>T</sup> utilized H<sub>2</sub>, formate, pyruvate, lactate, fumarate, malate, succinate, ethanol, propanol and butanol as electron donors for sulfate reduction. The organic electron donors were incompletely oxidized to mainly acetate. Sulfite and thiosulfate were used as electron acceptors with lactate as an electron donor. Without electron acceptors, pyruvate, fumarate and malate supported the growth. The genomic DNA G + C content was 62.1 mol%. Menaquinone MK-6(H<sub>2</sub>) was the major respiratory quinone. Major cellular fatty acids were  $C_{16:0}$ , iso- $C_{15:0}$ , anteiso- $C_{15:0}$ , iso- $C_{17:0}$ , anteiso- $C_{17:0}$  and iso- $C_{17:1}\omega_9$ . Phylogenetic analysis based on the 16S rRNA gene sequence as well as the α-subunit of dissimilatory sulfite reductase gene sequence assigned the strain to the family Desulfovibrionaceae within the class Deltaproteobacteria. The closest validly described species based on the 16S rRNA gene sequences were Desulfovibrio aespoeensis (sequence similarity; 95.0%) and Desulfovibrio profundus (94.3%). On the basis of the significant differences in the 16S rRNA gene sequences and the phenotypic characteristics between strain MSL79<sup>T</sup> and each of the most closely related species, *Desulfovibrio portus* sp. nov. is proposed. The type strain is MSL79<sup>T</sup> (= JCM  $14722^{T} = DSM 19338^{T}$ ).

**Key Words**—*Deltaproteobacteria*; *Desulfovibrio*; *Desulfovibrio portus* sp. nov.; dissimilatory sulfite reductase gene; estuarine sediment; 16S rRNA gene; sulfate-reducing bacteria

#### Introduction

Sulfate-reducing bacteria (SRB), which commonly reduce oxidized sulfur compounds to sulfide, include

<sup>\*</sup> Address reprint requests to: Dr. Atsuko Ueki, Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka, Yamagata 997-8555, Japan.

Tel: 81-235-28-2866 Fax: 81-235-28-2846

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

phylogenetically diverse bacterial species (Castro et al., 2000; Kuever et al., 2005; Rabus et al., 2000). During the last two decades, various novel SRB species have been isolated from a wide range of anoxic environments such as freshwater sediment, marine sediments (estuarine, coastal and deep sea), deep subterranean groundwater, oil fields, hot springs and rice field soil (Garrity and Holt, 2001; Kuever et al., 2005; Mori et al., 2003; Moussard et al., 2004).

In the course of investigation on SRB in an estuarine sediment of the Japanese islands, we isolated various strains of phylogenetically diverse SRB including seve-

<sup>&</sup>lt;sup>†</sup> Present address: Taisei Corporation, 344-1 Naze-machi, Totsuka-ku, Yokohama, Kanagawa 245-0051, Japan.

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ral novel lineages within the class Deltaproteobacteria (Suzuki et al., 2007c). Of the strains belonging to the novel lineages in our isolates, we have proposed two novel genera, that is, Desulfopila gen. nov., with Desulfopila aestuarii sp. nov. as the type species (Suzuki et al., 2007a) and Desulfoluna gen. nov., with Desulfoluna butyratoxydans sp. nov. as the type species (Suzuki et al., 2008), and one novel species; Desulfobulbus japonicus sp. nov. (Suzuki et al., 2007b). In this paper, we will describe the characterization of strain MSL79<sup>T</sup> representing another novel lineage in the family Desulfovibrionaceae. The strain was classified in the genus Desulfovibrio based on the 16S rRNA gene sequence, and the differences in phylogenetic and phenotypic characteristics between the strain and related Desulfovibrio species supported the proposal of a novel species of *Desulfovibrio* with strain MSL79<sup>T</sup> as the type strain.

# Materials and Methods

Source of the organism. Strain MSL79<sup>T</sup> was isolated from sediment collected to a depth of 10 cm with a core sampler (5 cm in diameter) at a water depth of 2 m in Niida river estuary in Sakata Harbor, Japan, on 12 November 2000 (Suzuki et al., 2007c).

Media. Two basal media (seawater medium and defined medium) were used. The seawater medium contained ( $L^{-1}$  seawater): 0.5 g KH<sub>2</sub>PO<sub>4</sub>, 0.3 g NH<sub>4</sub>Cl, 0.1 g yeast extract, 1 mg sodium resazurin, 10 ml of trace element solution (Widdel et al., 1983) and 0.5 g L-cysteine · HCI · H<sub>2</sub>O, as well as sodium lactate (20 mM) as an electron donor. The pH was adjusted to 7.2-7.4 with 1 M NaOH. Agar (Difco) (1.5%, wt/vol) 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<sub>2</sub>PO<sub>4</sub>, 1.0 g NH<sub>4</sub>Cl, 1.0 g Na<sub>2</sub>SO<sub>4</sub>, 2.0 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.1 g CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.5 g yeast extract, 1 mg sodium resazurin, 10 ml of trace element solution, 15 g NaCl and 0.5 g L-cysteine. HCI·H<sub>2</sub>O with appropriate electron donors (Nakamoto et al., 1996; Ueki et al., 1980; Widdel and Bak, 1992). The pH was adjusted to 7.2-7.4.

Isolation and cultivation. The sediment sample was diluted by consecutive 10-fold dilutions in sterilized anoxic seawater purged with  $O_2$ -free  $N_2$  gas. The  $10^{-3}$ -

fold diluted samples (0.2 ml) were inoculated into the seawater agar medium (10 ml) containing 20 mM of sodium lactate by the anaerobic roll-tube method (Hungate, 1966). Several strains of SRB including strain MSL79<sup>T</sup> were obtained by picking black colonies of SRB that appeared on the roll-tube agar after incubation for about a month. Strain MSL79<sup>T</sup> was finally obtained through colony isolation for purification by the anaerobic roll-tube method. On the phylogenetic tree based on the 16S rRNA gene sequences, the strain formed a cluster with one of the other isolates, strain MSL80, with a sequence similarity of 98.7% (Suzuki et al., 2007c). Since both strains reduced sulfate with lactate as an electron donor and showed almost the same phenotypic characteristics in the preliminary examinations, strain MSL79<sup>T</sup> was selected for further characterization.

Cultivation and transfer of the strain were performed under  $O_2$ -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.

Phenotypic characterization. The Gram reaction and cellular morphology were confirmed by light microscopy. The motility of the cells was examined by phase-contrast microscopy, and the flagella of the cells were examined by transmission electron microscopy (Beveridge et al., 1994). All physiological tests were performed according to the methods as described previously (Suzuki et al., 2007a, b, c). The utilization of electron donors was determined using the defined medium. 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. Substrates utilization in the absence of electron acceptors was also determined in 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.

Analytical methods. Volatile fatty acids, non-volatile fatty acids, alcohols,  $H_2$ , sulfate, sulfite and thiosulfate 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 from cells 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  $\mu$ Bondapack C18 column (3.9  $\times$  300 mm; Waters) (Kamagata and Mikami, 1991). Isoprenoid quinones were extracted as described by Komagata and Suzuki (1987) and analyzed by using a mass spectrometer (JMS-SX102A; JEOL). Whole-cell fatty acids (CFAs) 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 and 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).

16S rRNA gene sequencing and phylogenetic analysis. 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). The PCR-amplified 16S rRNA gene using a primer set, 27f and 1492r (Weisburg et al., 1991), 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 neighbor-joining method (Saitou and 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.

Dissimilatory sulfite reductase gene sequencing and phylogenetic analysis. A partial sequence covering approximately 1,900 bp of the gene encoding  $\alpha$  and  $\beta$ -subunits of dissimilatory sulfite reductase (DSR) was PCR-amplified using a primer set, DSR-1F and DSR-4R (Wagner et al., 1998), from DNA extracted from the cells of the strain. The partial sequence of the gene encoding the  $\alpha$ -subunit of DSR within the PCR product was sequenced by using a primer DSR-1F and the phylogenetic analysis of the gene sequence was performed according to the method as described above for the 16S rRNA gene.

Nucleotide sequence accession numbers. Accession numbers of the 16S rRNA and DSR gene sequences determined in this study are AB110541 and AB373125, respectively.

# Results

### General characteristics

Cells of strain MSL79<sup>T</sup> were Gram-negative curved rods with rounded ends,  $0.7-1.0 \mu m$  wide and 1.8-2.3  $\mu m$  long. Cells usually occurred singly (Fig. 1a) and were motile by a single polar flagellum (Fig. 1b). Spore formation was not observed. The strain made grayish and thin colonies on agar slants of the defined medium as well as the seawater medium. The strain required yeast extract for growth and did not grow aerobically. Desulfoviridin was detected in cell extracts. A difference absorption spectrum of dithionite-reduced minus air-oxidized cell extract showed peaks at 418, 523 and 553 nm, which indicated the presence of *c*type cytochrome in the cells. Catalase and oxidase activities were not detected.

# Growth conditions

Strain MSL79<sup>T</sup> reduced sulfate with lactate as an electron donor and produced acetate approximately at a theoretical molar ratio of about 2 : 1 : 2 (lactate :



Fig. 1. A phase-contrast photomicrograph (a) and a transmission electron micrograph (b) of cells of strain  $MSL79^{T}$ . Bars: (a) 10 µm, (b) 1 µm. sulfate : acetate) in the defined medium. Thus, the strain had an incomplete type of oxidation of electron donors. The optimum NaCl concentration for growth was 2.0% (wt/vol). The strain did not grow in the absence of NaCl in the defined medium and the NaCl concentration for growth ranged from about 0.15% to 6.5%. The temperature range was 10-40°C with an optimum at 35°C. The pH range for growth was 5.7-8.5 with an optimum at 6.5.

# Substrate spectra

Strain MSL79<sup>T</sup> did not grow in the absence of added electron donors in the defined medium, indicating that yeast extract did not serve as an electron donor for sulfate reduction. The strain utilized H<sub>2</sub>, formate, pyruvate, fumarate, malate, succinate, ethanol, propanol and butanol as well as lactate as electron donors for sulfate reduction. The strain grew relatively fast with H<sub>2</sub>  $(\mu = 0.159 h^{-1})$  as well as lactate  $(\mu = 0.171 h^{-1})$  as an electron donor as compared with other electron donors. The growth rates with three dicarboxylates (fumarate, malate and succinate) were 0.120  $h^{-1}$ , 0.121 h<sup>-1</sup> and 0.052 h<sup>-1</sup>, respectively. The molar ratios for sulfate reduction with these substrates (substrate : sulfate : acetate) were almost consistent with the theoretical ratios (2:1:2 for fumarate and malate; 4: 3:4 for succinate). Although almost all organic electron donors were oxidized to mainly acetate, propanol and butanol were oxidized to their corresponding carboxylates. The strain did not utilize acetate, propionate, butyrate, methanol, glycerol, glycine, alanine, serine, aspartate or glutamate as an electron donor. The strain utilized sulfite ( $\mu = 0.145 \text{ h}^{-1}$ ) and thiosulfate ( $\mu = 0.136 \text{ h}^{-1}$ ) as electron acceptors with lactate as an electron donor. Growth was not supported in the presence of lactate or H<sub>2</sub> with fumarate as the electron acceptor. When the cells of the strain were inoculated to the medium containing lactate and fumarate (as an electron donor and acceptor, respectively), a small amount of H<sub>2</sub> was detected in the headspace. The strain oxidized pyruvate (6.9 mM) and produced acetate (7.5 mm) in the absence of electron acceptors. The strain also utilized fumarate (18.2 mm) without electron acceptors and produced acetate (4.6 mM), malate (4.2 mm) and succinate (6.8 mm). The strain also utilized malate (7.6 mm) without electron acceptors and produced small amounts of acetate (1.5 mM), fumarate (1.8 mm) and succinate (2.0 mm). The growth rates in the three substrates (pyruvate, fumarate and

malate) without electron acceptors were 0.057  $h^{-1}$ , 0.057  $h^{-1}$  and 0.034  $h^{-1}$ , respectively. Although growth of the strain was not observed with lactate in the absence of electron acceptors, trace amounts of H<sub>2</sub> and acetate were generated during the incubation.

# Genomic DNA G + C content, respiratory quinone and cellular fatty acids

The G + C content of genomic DNA of strain MSL79<sup>T</sup> was 62.1 mol%. The major respiratory quinone of the strain was menaquinone MK-6(H<sub>2</sub>). Major CFAs of the strain were  $C_{16:0}$  (7.3%), iso- $C_{15:0}$  (12.0%), anteiso- $C_{15:0}$  (12.4%), iso- $C_{17:0}$  (11.8%), anteiso- $C_{17:0}$  (8.8%) and iso- $C_{17:1}\omega$ 9 (15.8%). In addition,  $C_{18:0}$  (4.2%),  $C_{16:1}\omega$ 7 (1.4%),  $C_{18:1}\omega$ 7 (4.5%), iso- $C_{16:0}$  (2.1%), iso- $C_{16:1}$  (1.4%), anteiso- $C_{17:1}\omega$ 9 (4.5%), branched  $C_{17:1}$  (2.0%), iso- $C_{15:0}$  3-OH (2.1%) and iso- $C_{17:0}$  3-OH (1.6%) were detected as minor CFAs.

#### Phylogenetic affiliation

Almost a full-length 16S rRNA gene sequence (1,425 bp) was determined for strain MSL79<sup>T</sup>. 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 closest relative of the strain in the database was Desulfovibrio sp. HS2 (Dinh et al., 2004) isolated from marine sediment with sequence similarity of 98.7%. The next closest relative was 'Desulfovibrio caledoniensis' with sequence similarity of 96.2%, while characteristics of the organism have not been reported and only its 16S rRNA gene sequence is known. The closest described species of strain MSL79<sup>T</sup> was 'Desulfovibrio dechloracetivorans' (Sun et al., 2000) with a sequence similarity of 96.1%; however, it is not validly described yet. The next closely related species were Desulfovibrio aespoeensis (Motamedi and Pedersen, 1998), Desulfovibrio profundus (Bale et al., 1997; Friedrich, 2002) and Desulfovibrio brasiliensis (Warthmann et al., 2005) with sequence similarities of 95.0%, 94.3% and 91.7% to respective type strains. Strain MSL79<sup>T</sup> formed a distinct cluster from the recognized Desulfovibrio species together with Desulfovibrio sp. HS2 and strain MSL80, which was isolated from the same sediment at the same time as strain MSL79<sup>T</sup> (Suzuki et al., 2007c) (Fig. 2). Strain MSL79<sup>T</sup> was only distantly related to the type species of the genus Desulfovibrio, Desulfovibrio desulfuricans, with a sequence similarity of 85.4%.

Desulfovibrio portus sp. nov.



Fig. 2. Neighbor-joining tree showing the phylogenetic relationship of strain MSL79<sup>1</sup> and related species in the genus *Desulfovibrio* based on the 16S rRNA gene sequences. Bootstrap values shown are based on analysis of 1,000 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 neighbor-joining method.

The partial sequence (625 bp) of  $\alpha$ -subunit of DSR gene was determined for strain MSL79<sup>T</sup>. Based on the phylogenetic analysis of the DSR gene sequence, the closest recognized species of the strain in the database was *Desulfovibrio oxyclinae* with a sequence similarity of 84.5%. The next closely related species were *D. aespoeensis* (84.2%) and *Desulfovibrio halophilus* (83.7%). Thus, on the basis of the DSR gene phylogenetic analysis, the strain was also assigned to the genus *Desulfovibrio* and placed close to the similar members as shown by the 16S rRNA gene sequence analysis (Fig. 2). The DSR gene sequences of other related species, *D. profundus* and *D. brasiliensis*, are

not available. The similarity of the DSR gene sequence of MSL79<sup>T</sup> to that of *D. desulfuricans* was 80.3%.

# Discussion

The *Desulfovibrio* species have been isolated from a wide variety of environments such as freshwater to marine sediment, hypersaline water and subsurface habitats (Kuever et al., 2005; Voordouw, 1995). Currently, the genus contains about 45 species showing phylogenetic and physiological diversities. Based on the phylogenetic analysis of both 16S rRNA and DSR gene sequences, strain MSL79<sup>T</sup> was affiliated with the

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genus *Desulfovibrio*; however, the strain was distantly related to the known relatives.

Of the closest relatives, 'D. dechloracetivorans' is not yet validly published as based on Rules 27(3) and 30 of the Bacteriological Code (De Vos and Trüper, 2000; Euzéby and Tindall, 2004). 'D. dechloracetivorans' was isolated from bay sediment and reported to have a specific ability to conduct reductive dechlorination of 2-chlorophenol (Sun et al., 2000). Although the physiology of reductive dechlorination by the bacterium was studied in detail, the species was poorly characterized as a sulfate-reducing bacterium. Thus, various phenotypic features of 'D. dechloracetivorans' including utilization ranges of electron donors and fermentative substrates as well as chemotaxonomic characteristics have not been reported. Since the 16S rRNA gene sequence similarity between strain MSL79<sup>T</sup> and 'D. dechloracetivorans' is 96.1%, indicating that it is unlikely that these organisms have more than 70% identity in the DNA-DNA hybridization and belong to the same species (Stackebrandt and Goebel, 1994), we did not examine the ability of reductive dechlorination of strain MSL79<sup>T</sup> to compare with the phenotypic characteristics of 'D. dechloracetivorans'.

Both closely related recognized species, Desulfovi-

*brio aespoeensis* (sequence similarity, 95.0%) and *Desulfovibrio profundus* (94.3%), were isolated from rather unusual environments: deep subterranean groundwater and deep marine sediment (Bale et al., 1997; Motamedi and Pedersen, 1998), respectively. The next closely related species of strain MSL79<sup>T</sup>, *D. brasiliensis* (91.7%), was also isolated from an extreme environment, a hypersaline lagoon (Warthmann et al., 2005). Thus, strain MSL79<sup>T</sup> was assigned to a unique branch in the genus *Desulfovibrio* consisting of species isolated from uncommon or extreme environments.

Strain MSL79<sup>T</sup> has various characteristics commonly found in *Desulfovibrio* species such as morphology, motility with a polar flagellum, incomplete type oxidation of electron donors, presence of desulfoviridin and *c*-type cytochrome, the CFA profile and the type of respiratory quinone. Strain MSL79<sup>T</sup>, however, has some distinctly different features from the closest relatives (Table 1). The range of electron donors utilized by strain MSL79<sup>T</sup> was not consistent with those of the two closely related species, especially for formate, fumarate, malate and ethanol. Strain MSL79<sup>T</sup> as well as *D*. *aespoeensis* did not utilize fumarate as an electron acceptor, but *D. profundus* utilizes it. Furthermore, the

	1	2	3
Source	Estuarine sediment	Deep subterranean granitic groundwater	Deep marine sediment
Cell shape	Curved rods	Vibrio	Vibrio
Optimum growth condition			
Optimum NaCl (%, w/v)	2.0	0.7	0.6-8.0
Temperature (°C)	35	30	25
Utilization of electron donor			
Formate	+	_	-
Fumarate	+	-	
Malate	+	-	-
Ethanol	+	-	-
Utilization of electron acceptor			
Fumarate	a	-	÷
Utilization of substrates in the abse	ence of electron acceptors		
Lactate	-	n.d.	. +
Fumarate	+	-	_
Malate	+	-	-
DNA G+C content (mol%)	62.1	61.0	53.0

Table 1. Some characteristics of strain MSL79<sup>T</sup> and related *Desulfovibrio* species.

The strains listed are as follows: 1, MSL79<sup>T</sup>; 2, *Desulfovibrio aespoeensis* Aspo-2<sup>T</sup>(Motamedi and Pedersen, 1998); 3, *Desulfovibrio profundus* 500-1<sup>T</sup> (Bale et al., 1997).

Symbols: +, used; -, not used; n.d., no date available.

<sup>a</sup>Determined with lactate or H<sub>2</sub> as a sole electron donor.

substrate utilization profile (lactate, fumarate and malate) in the absence of electron acceptors of strain MSL79<sup>T</sup> was different from any of related species.

The G + C contents of the genomic DNA of most *Desulfovibrio* species are reported in the range from about 45 to 67 mol% (Kuever et al., 2005). The G + C content of strain MSL79<sup>T</sup> was within the range as well as that of *D. aespoeensis*, which was significantly higher than that of *D. profundus*.

Strain MSL79<sup>T</sup> contained MK-6(H<sub>2</sub>) as a major respiratory quinone, which is known as one of the major menaquinones in Desulfovibrio species (Collins and Widdel, 1986). Most species in the genus Desulfovibrio have iso- $C_{15:0}$ , anteiso- $C_{15:0}$ , iso- $C_{17:0}$  or iso- $C_{17:1}$ as major or dominant CFAs (Kohring et al., 1994; Kuever et al., 2005; Ueki and Suto, 1979; Vainshtein et al., 1992). Strain MSL79<sup>T</sup> also has these CFAs as major ones; however, the CFA profile is significantly different from any of the Desulfovibrio species and the presence of these CFAs at almost equal amounts (12-15%) is one of the unique features of the profile. Strain MSL79<sup>T</sup> has anteiso-C<sub>17:0</sub> (8.8%) at a rather high amount, which is not so common in the Desulfovibrio species. D. profundus is reported (without detailed percentages) to have saturated and monounsaturated C16 and C18 fatty acids as dominant CFAs and also to contain branched-chain fatty acids (iso-C15:0 and iso- $C_{17:1}\omega$ 7) (Bale et al., 1997). The profile is distinctly different from that of strain MSL79<sup>T</sup>.

Based on the phylogenetic analysis of both 16S rRNA and DSR gene sequences and phenotypic characteristics shown above, strain MSL79<sup>T</sup> should be classified as a novel species in the genus *Desulfovibrio* and we propose *Desulfovibrio portus* sp. nov. to accommodate the strain with strain MSL79<sup>T</sup> as the type strain.

### Description of Desulfovibrio portus sp. nov.

Desulfovibrio portus (por'tus. L. gen. n. portus of harbour, port, estuary of a river, pertaining to the original habitat of the type strain).

Cells are curved rods, 0.7–1.0  $\mu$ m wide and 1.8– 2.3  $\mu$ m long. Strictly anaerobic. Gram-negative. Motile by a single polar flagellum. Non-spore-forming, Colonies are grayish and thin and spread on slant media. Contains desulfoviridin and cytochrome of the *c*-type. Catalase and oxidase activities are not detected. The NaCl concentration range for growth is 0.15–6.5% (wt/ vol) with an optimum at 2.0% (wt/vol). The temperature range for growth is 10-40°C with an optimum at 35°C. The pH range for growth is 5.7-8.5 with an optimum at 6.5. Requires yeast extract for growth. Utilizes H<sub>2</sub>, formate, pyruvate, lactate, fumarate, malate, succinate, ethanol, propanol and butanol as electron donors for sulfate reduction. Does not use acetate, propionate, butyrate, methanol, glycerol, glycine, alanine, serine, aspartate or glutamate. Almost all organic electron donors are incompletely oxidized to acetate. Sulfate, sulfite and thiosulfate serve as electron acceptors, but not fumarate. Pyruvate, fumarate and malate are utilized in the absence of electron acceptors, but not lactate. The genomic DNA G + C content is 62.1 mol%. The major respiratory guinone is menaguinone MK-6(H<sub>2</sub>). Major cellular fatty acids are C<sub>16:0</sub>, iso-C<sub>15:0</sub>, anteiso- $C_{15;0}$ , iso- $C_{17;0}$ , anteiso- $C_{17;0}$  and iso- $C_{17;1}\omega 9$ . Isolated from estuarine sediment located on the side of the Sea of Japan of the Japanese islands. Strain MSL79<sup>T</sup> is the type strain of the species. Strain MSL79<sup>T</sup> is deposited with the Japan Collection of Microorganisms (JCM) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) (= JCM  $14722^{T} = \text{DSM } 19338^{T}$ ).

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#### References

- Akasaka, H., Izawa, T., Ueki, K., and 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., and Ueki, K. (2003b) Propionicimonas paludicola gen. nov., sp. nov., a novel facultatively anaerobic, Gram-positive, propionateproducing 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., and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.
- Bale, S. J., Goodman, K., Rochelle, P. A., Marchesi, J. R., Fry, J.
  C., Weightman, A. J., and Parkes, R. J. (1997) *Desulfovibrio* profundus sp. nov., a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan Sea. *Int.*

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J. Syst. Bacteriol., 47, 515-521.

- Beveridge, T. J., Popkin, T. J., and Cole, R. M. (1994) Electron microscopy. *In* Methods for General and Molecular Bacteriology, ed. by Gerhardt, P., Murray, R. G. E., Wood, W. A., and Krieg, N. R., American Society for Microbiology, Washington, DC, pp. 42–71.
- Castro, H. F., Williams, N. H., and Ogram, A. (2000) Phylogeny of sulfate-reducing bacteria. *FEMS Microbiol. Ecol.*, **31**, 1–9.
- Collins, M. D. and Widdel, F. (1986) Respiratory quinones of sulphate-reducing and sulphur-reducing bacteria: A systematic investigation. *Syst. Appl. Microbiol.*, **8**, 8–18.
- De Vos, P. and Trüper, H. G. (2000) Judicial Commission of the International Committee on Systematic Bacteriology. IXth International (IUMS) Congress of Bacteriology and Applied Microbiology. Minutes of the Meetings, 14, 15 and 18 August 1999, Sydney, Australia. *Int. J. Syst. Evol. Microbiol.*, 50, 2239-2244.
- Dinh, H. T., Kuever, J., Mussmann, M., Hassel, A. W., Stratmann, M., and Widdel, F. (2004) Iron corrosion by novel anaerobic microorganisms. *Nature*, **427**, 829–832.
- Euzéby, J. P. and Tindall, B. J. (2004) Status of strains that contravene Rules 27(3) and 30 of the Bacteriological Code. Request for an Opinion. *Int. J. Syst. Evol. Microbiol.*, **54**, 293-301.
- Felsenstein, J. (2006) PHYLIP (Phylogeny Inference Package), ver 3.66. Department of Genetics, University of Washington, Seattle.
- Friedrich, M. W. (2002) Phylogenetic analysis reveals multiple lateral transfers of adenosine-5'-phosphosulfate reductase genes among sulfate-reducing microorganisms. *J. Bacteriol.*, **184**, 278–289.
- Garrity, G. M. and Holt, J. G. (2001) Phylum BIII. *Thermodesul-fobacteria* phy. nov. *In* Bergey's Manual of Systematic Bacteriology, Vol. 1, 2nd ed., ed. by Boone, D. R., Castenholz, R. W., and Garrity, G. M., Springer, New York, pp. 389–393.
- Hungate, R. E. (1966) The Rumen and Its Microbes. Academic Press, New York.
- Kamagata, Y. and Mikami, E. (1991) Isolation and characterization of a novel thermophilic *Methanosaeta* strain. *Int. J. Syst. Bacteriol.*, **41**, 191–196.
- Kohring, L. L., Ringelberg, D. B., Devereux, R., Stahl, D. A., Mittelman, M. W., and 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.
- Komagata, K. and Suzuki, K. (1987) Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.*, **19**, 161–207.
- Kuever, J., Rainey, F. A., and Widdel, F. (2005) Class IV. Deltaproteobacteria class nov. In Bergey's Manual of Systematic Bacteriology, Vol. 2, Part C, 2nd ed., ed. by Brenner, D. J., Krieg, N. R., Staley, J. T., and Garrity, G. M., Springer,

New York, pp. 922-1144.

- 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., and 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., and 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.
- Mori, K., Kim, H., Kakegawa, T., and Hanada, S. (2003) A novel lineage of sulfate-reducing microorganisms: *Thermodesulfobiaceae* fam. nov., *Thermodesulfobium narugense*, gen. nov., sp. nov., a new thermophilic isolate from a hot spring. *Extremophiles*, **7**, 283–290.
- Motamedi, M. and Pedersen, K. (1998) *Desulfovibrio aespoeensis* sp. nov., a mesophilic sulfate-reducing bacterium from deep groundwater at Äspö hard rock laboratory, Sweden. *Int. J. Syst. Bacteriol.*, **48**, 311–315.
- Moussard, H., L'Haridon, S., Tindall, B. J., Banta, A., Schumann, P., Stackebrandt, E., Reysenbach, A.-L., and Jeanthon, C. (2004) *Thermodesulfatator indicus* gen. nov., sp. nov., a novel thermophilic chemolithoautotrophic sulfatereducing bacterium isolated from the Central Indian Ridge. *Int. J. Syst. Evol. Microbiol.*, **54**, 227–233.
- Nakamoto, M., Ueki, A., and 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.
- Postgate, J. R. (1959) A diagnostic reaction of *Desulphovibrio* desulphuricans. Nature, **183**, 481–482.
- Rabus, R., Hansen, T., and Widdel, F. (2000) Dissimilatory sulfate- and sulfur-reducing prokaryotes. *In* The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community, 3rd ed., ed. by Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E., Springer-Verlag, New York.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406-425.
- Stackebrandt, E. and Goebel, B. M. (1994) Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.*, **44**, 846–849.
- Sun, B., Cole, J. R., Sanford, R. A., and Tiedje, J. M. (2000) Isolation and characterization of *Desulfovibrio dechloracetivorans* sp. nov., a marine dechlorinating bacterium growing by coupling the oxidation of acetate to the reductive dechlorination of 2-chlorophenol. *Appl. Environ. Microbiol.*, **66**, 2408-2413.
- Suzuki, D., Ueki, A., Amaishi, A., and Ueki, K. (2007a) *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.

- Suzuki, D., Ueki, A., Amaishi, A., and 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., and Ueki, K. (2007c) Diversity of substrate utilization and growth characteristics of sulfatereducing bacteria isolated from estuarine sediment in Japan. J. Gen. Appl. Microbiol., 53, 119–132.
- Suzuki, D., Ueki, A., Amaishi, A., and 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.
- Thompson, J. D., Higgins, D. G., and 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., Matsuda, K., and Ohtsuki, C. (1986) Sulfate reduction in the anaerobic digestion of animal waste. *J. Gen. Appl. Microbiol.*, **32**, 111–123.
- Ueki, A., Minato, H., Azuma, R., and 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. and Suto, T. (1979) Cellular fatty acid composition of

sulfate-reducing bacteria. J. Gen. Appl. Microbiol., 25, 185-196.

- Vainshtein, M. B., Hippe, H., and 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.
- Voordouw, G. (1995) The genus *Desulfovibrio*. The centennial. *Appl. Environ. Microbiol.*, **61**, 2813–2819.
- Wagner, M., Roger, A., Flax, J., Brusseau, G., and Stahl, D. A. (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J. Bacteriol.*, 180, 2975–2982.
- Warthmann, R., Vasconcelos, C., Sass, H., and McKenzie, J. A. (2005) *Desulfovibrio brasiliensis* sp. nov., a moderate halophilic sulfate-reducing bacterium from Lagoa Vermelha (Brazil) mediating dolomite formation. *Extremophiles*, 9, 225–261.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. (1991) 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol., 173, 697–703.
- Widdel, F. and Bak, F. (1992) Gram-negative mesophilic sulfatereducing bacteria. *In* The Prokaryotes, 2nd ed., ed. by Balows, A., Trüper, H. G., Dworkin, M., Harder, W., and Schleifer, K.-H., Springer-Verlag, New York, pp. 3352–3378.
- Widdel, F., Kohring, G.-W., and Mayer, F. (1983) Studies on 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.