View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Yamagata University Academic Repository

COVER SHEET

This is the author-version of article published as:

Daisuke Suzuki, Atsuko Ueki, Aya Amaishi, Katsuji Ueki (2007) *Desulfopila aestuarii* gen. nov., sp. nov., a novel, Gram-negative, rod-like sulfate-reducing bacterium isolated from an estuarine sediment in Japan. *International Journal of Systematic and Evolutionary Microbiology* 57(3): pp.520-526.

Accessed from http://repo.lib.yamagata-u.ac.jp/

・YOU Campus Repository System is built to collect, archive and offer electronically academic documents produced by University Consortium Yamagata.
・The copyright and related rights of the article are held by authors or publishers.
・You must require the permission of the copyright owner if you want to use over the curtailment of copyrights.
・Author-version manuscripts may have some differences in layouts and wordings from publisher version.

・ゆうキャンパスリポジトリは、大学コンソーシアムやまがた参加機関で生産された学術文献を電子的に収集・保存・発信するシステムです。
・論文の著作権は、著者または出版社が保持しています。著作権法で定める権利制限規定を超える利用については、著作権者に許諾を得てください。
・著者版原稿(Author-version)は出版社版（Publisher-version）との間に、レイアウト、字句校正レベルの異同がある場合があります。予めご了承下さい。

Desulfopila aestuarii gen. nov., sp. nov., a novel, Gram-negative, rod-like 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: *Desulfopila aestuarii* gen. nov., sp. nov.

Key words: *Desulfopila aestuarii*, *Deltaproteobacteria*, anaerobic Gram-negative rods, sulfate-reducers, estuarine sediment

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MSL86^T is AB110542.

Abstract

A strictly anaerobic, mesophilic sulfate-reducing bacterial strain (MSL86^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 rods. Catalase was not detected. The optimum NaCl concentration for growth was 0·1 % (w/v) and the optimum temperature was 35 °C. Strain MSL86^T was slightly alkaliphilic with an optimum pH at 7·2–7·6. Organic electron donors were incompletely oxidized to mainly acetate. Strain MSL86^T utilized formate, pyruvate, lactate, fumarate, ethanol, propanol, butanol and

glycerol as electron donors for sulfate reduction and did not use acetate, propionate, butyrate, succinate, malate, methanol, glycine, alanine, serine, aspartate, glutamate and H_2. Sulfite, thiosulfate and fumarate were used as electron acceptors with lactate as an electron donor. Without electron acceptors, the strain fermented pyruvate and fumarate. The genomic DNA G + C content was 54·4 mol%. Menaquinone MK-8(H_4) was the major respiratory quinone. Major cellular fatty acids were $C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$ and $C_{17:1\omega6}$. Phylogenetic analysis based on the 16S rRNA gene sequence placed the strain in the class *Deltaproteobacteria*. The recognized closest relative of strain MSL86^T was '*Desulfobacterium catecholicum*' (sequence similarity of 94·4%) and the next closest recognized species were *Desulfotalea psychrophila* (94·2%) and *Desulfotalea arctica* (93·7%). Since the physiological and chemotaxonomic characteristics were distinctly different from those of any related species, a new genus and species *Desulfopila aestuarii* gen. nov., sp. nov. is proposed to accommodate the strain. The type strain is MSL86^T (= JCM 14042^T = DSM 18488^T).

Main text

Sulfate-reducing bacteria (SRBs) include phylogenetically diverse anaerobic bacterial species (Castro *et al.*, 2000; Kuever *et al.*, 2005). Since sulfate is sufficiently supplied in marine environments, it has been reported that SRBs are responsible for up to 50% of the organic carbon mineralization in marine sediments (Jørgensen, 1982). Various novel species of SRBs have been recently isolated from a wide range of marine sediments (Jeanthon *et al.*, 2002; Sass *et al.*, 2002; Audiffrin *et al.*, 2003; Cravo-Laureau *et al.*, 2004; Moussard *et al.*, 2004; Kuever *et al.*, 2005). In addition, many studies on diversity of SRBs in natural environments have been performed using cultivation-independent molecular techniques based on PCR amplification of the 16S rRNA or dissimilatory sulfite reductase gene (Devereux & Mundfrom, 1994; Voordouw *et al.*, 1996; Wagner *et al.*,

1998; Joulian *et al*., 2001; Klein *et al*., 2001; Purdy *et al*., 2002; Dhillon *et al*., 2003; Leloup *et al*., 2006), and these studies have revealed that phylogenetically diverse lineages of un-cultivated SRBs are still present in natural ecosystems.

In the course of investigation on SRBs in an estuarine sediment of Japanese island, we isolated various strains of SRBs from the sediment. The phylogenetical, physiological and chemotaxonomic characteristics of one of the strains, MSL86^T, supported the proposal of a novel genus and species in the class *Deltaproteobacteria* to affiliate the strain. Since the strain was isolated from estuarine sediment by the dilution colony counting method using a sample diluted to 10^{-4}, it was thought that a SRB group relating the strain should be present at a rather high population in the sediment.

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, which is located on the side of the Sea of Japan in Japanese islands (38°54.5′N, 139°50.6′E), on 12th of November 2000. The sediment sample was diluted by consecutive 10-fold dilutions with 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 SRBs were determined by the anaerobic roll tube method (Hungate, 1966). Several strains of SRBs were obtained by picking up black colonies of SRBs that appeared on the roll tube agar after incubation for about a month. Strain MSL86^T was 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. The seawater medium contained (l^{-1} of seawater): 0·5 g of KH_2PO_4, 0·3 g of NH_4Cl, 0·1 g of yeast extract, 1 mg of sodium resazurin, 10 ml of the trace element solution (l^{-1}: 10 ml of 25% (v/v) HCl, 1·5 g of $FeCl_2.4H_2O$, 0·19 g of $CoCl_2.6H_2O$, 0·1 g of $MnCl_2.4H_2O$, 0·07 g of $ZnCl_2$, 0·062 g of H_3BO_3, 0·036 g of $Na_2MoO_4.2H_2O$, 0·024 g of $NiCl_2.6H_2O$, and 0·017 g of $CuCl_2.2H_2O$), 1 ml of the vitamin solution (l^{-1}: 10 µg of biotin, 40 µg of *p*-aminobenzoic acid, and 100 µg of niacin), and 0·5 g of 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 lactate 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 strain, contained (l^{-1}): 0·5 g of KH_2PO_4, 1·0 g of NH_4Cl, 1·0 g of Na_2SO_4, 2·0 g of $MgSO_4.7H_2O$, 0·1 g of $CaCl_2.2H_2O$, 0·5 g of yeast extract, 1 mg of sodium resazurin, 10 ml of the trace element solution, 1 ml of the vitamin solution, 15 g of NaCl, and 0·5 g of 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. Cultivation and transfer of the strain were performed under an O_2-free N_2 (100%) atmosphere. The strain was cultivated at 30°C, unless otherwise stated. The strain was maintained in slant cultures of the seawater medium or the defined medium with lactate as an electron donor. The pH of both media after autoclaving was around pH 6·3. Each electron donor was added at a final concentration of 20 mM.

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). 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. Effects of temperature on growth were examined using the seawater medium with sodium lactate as an electron donor. Growth of the strain was monitored by measurement of OD_{660} with a spectrophotometer (HITACHI U-1000).

Utilization of electron donors by the strain 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. Utilization of electron acceptors was determined with a sulfate-free medium, which contained 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 possible electron acceptors. 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 (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). 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 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).

16S rRNA gene of the strain was extracted according to the method described by Akasaka *et al*. (2003a,b) and amplified by PCR 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.

Cells of strain MSL86^T were Gram-negative rods, 0·7 -1·2 µm wide and 1·9-3·8 µm long with rounded ends. 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. Cells of strain MSL86^T aggregated during growth in the liquid medium and deposited to the bottom of test tubes. Spore formation was not observed.

Strain MSL86^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. The strain grew in the presence of NaCl up to 0·5 (w/v) and the optimum NaCl concentration for growth was 0·1% (w/v). The strain grew even in the absence of NaCl added in the defined medium. Temperature range for growth was 10-40ºC with an optimum at 35ºC. The strain was slightly alkaliphilic and had a pH optimum at 7·7 -7·6 with pH range of 6·3-8·5 for growth. It could not grow at initial pH 5.6.

Strain MSL86^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. Strain MSL86^T utilized formate, pyruvate, fumarate, ethanol, propanol, butanol and glycerol as well as lactate as electron donors for sulfate reduction. Although all organic electron donors were usually oxidized to mainly acetate, succinate (2·9 mM) was produced in addition to acetate (8·3

mM) with fumarate as an electron donor. Propanol and butanol were oxidized to their corresponding carboxylic acids. Strain MSL86^T did not utilize acetate, propionate, butyrate, malate, succinate, methanol, glycine, alanine, serine, aspartate, glutamate and H_2 as electron donors.

Strain MSL86^T utilized sulfite, thiosulfate and fumarate in addition to sulfate as electron acceptors using lactate as an electron donor. When compared in the presence of 1% (w/v) of NaCl in the defined medium, the growth rate with fumarate as an electron acceptor (μ = 0·960 h^{-1}) was slightly faster than those with sulfate (μ = 0·085 h^{-1}), sulfite (μ = 0·052 h^{-1}) or thiosulfate (μ = 0·038 h^{-1}). When fumarate was utilized as an electron acceptor, succinate (3·2 mM) and propionate (1·4 mM) were produced as well as acetate (0·3 mM) in accordance with oxidation of lactate (7·6 mM). In the absence of electron acceptors, strain MSL86^T oxidized pyruvate (10·4 mM) and produced acetate (7·8 mM) and propionate (2·2 mM). The strain also oxidized fumarate (13·0 mM) without electron acceptors and produced acetate (9·5 mM) and succinate (7·0 mM). The strain did not use lactate and malate in the absence of electron acceptors.

The G + C content of genomic DNA of strain MSL86^T was 54·4 ± 0·2 mol%. The major respiratory quinone was MK-8(H_4). The predominant CFAs of strain MSL86^T were $C_{16:0}$ (33·6%), $C_{16:1\omega7}$ (6·0%), $C_{16:1\omega5}$ (17·1%) and $C_{17:1\omega6}$ (13·7%). About 66% of the total CFAs were even-numbered straight-chain fatty acids. Lower amounts of hydroxy fatty acids, iso-$C_{11:0}$ 3-OH (1·1 %), $C_{14:0}$ 3-OH (1·8 %) and $C_{16:0}$ 3-OH (1·6 %), and branched $C_{17:1}$ (4·6%) were also detected.

Based on the almost full-length 16S rRNA gene sequence (1471 bp), strain MSL86^T was affiliated with the

class *Deltaproteobacteria*. The closest relative of the strain on the database was *Desulfotalea* sp. SFA4 isolated from intertidal flat with sequence similarity of 98%. The closest known relative of strain MSL86 ^{T} was a SRB '*Desulfobacterium catecholicum*' with sequence similarity of 94·4% (Szewzyk & Pfennig, 1987). The next closely related recognized species were *Desulfotalea psychrophila* (similarity of 94·2%) and *Desulfotalea arctica* (93·7%) in the *Desulfobulbaceae* (Kuever *et al*., 2005). Strain MSL86^{T} formed a distinct cluster with strain MSL53, which was isolated from the same sediment as used for isolation of strain MSL86^{T} (Fig. 2). The sequence similarity between strain MSL86^{T} and strain MSL53 was 986%.

It has been proposed that '*D. catecholicum*' is no longer included in the genus *Desulfobacterium*, since the 16S rRNA gene sequence analysis performed after the original description revealed the need for reclassification of the species as a member of a new genus within the *Desulfobulbaceae* (Kuever *et al*., 2005). Cells of '*D. catecholicum*' are non-motile and oval to lemon-shaped, while strain MSL86^{T} has motile, typically rod-shaped cells. Furthermore, '*D. catecholicum*' is a completely-oxidizing type of SRB (Szewzyk & Pfennig, 1987), while strain MSL86^{T} is an incompletely-oxidizing type. Since other important physiological characteristics of strain MSL86^{T} including utilization of propionate and butyrate as electron donors for sulfate reduction are also different from those of '*D. catecholicum*' (Table 1), we thought that strain MSL86^{T} should not be affiliated with the novel genus to accommodate '*D. catecholicum*'.

Some physiological and chemotaxonomic characteristics of strain MSL86^{T} and the two closest *Desulfotalea* species are compared in Table 1. Both *Desulfotalea* species isolated from permanently cold Arctic marine sediments are psychrophilic SRBs and the optimum growth temperatures of *D. psychrophila* and *D. arctica*

are 10°C and 18°C, respectively (Knoblauch *et al*., 1999), while strain MSL86^T is mesophilic and the optimum growth temperature is 35°C. Both the closest *Desulfotalea* species are also incompletely-oxidizing SRBs, however, the range of electron donors used by strain MSL86^T is different from that of these species. Strain MSL86^T utilizes glycerol, but not malate, while *D. psychrophila* utilizes malate, but not glycerol. Strain MSL86^T utilizes fumarate, propanol and butanol as an electron donor, however, *D. arctica* dose not. Strain MSL86^T does not use amino acids and H_2 (with acetate as a carbon source), however, both *Desulfotalea* species utilize. The range of electron acceptors used by strain MSL86^T is similar to that of *D. psychrophila*, but it is different from that of *D. arctica*. *D. arctica* does not utilize sulfite and thiosulfate, however strain MSL86^T utilizes both. In the absence of electron acceptors, strain MSL86^T and *D. psychrophila* utilize fumarate, while *D. arctica* does not. Strain MSL86^T produced a small amount of propionate together with acetate from pyruvate in the absence of electron acceptors. Furthermore, propionate was also produced when fumarate was served as an electron acceptor with lactate as an electron donor. Although some SRBs such as *Desulfobulbus* and *Desulfosarcina* species also produce propionate by fermentation of lactate or pyruvate, these species utilize propionate conversely as an electron donor for sulfate reduction (Kuever *et al*., 2005). Since strain MSL86^T does not utilize propionate as an electron donor, the propionate production seems to give one of distinct physiological characteristics to the strain.

The G + C content of the genomic DNA of strain MSL86^T (54·4 mol%) is significantly different from those of *D. psychrophila* (46·8 mol%) and *D. arctica* (41·8 mol%) (Knoblauch *et al*., 1999). The respiratory quinones of *Desulfotalea* species are menaquinone MK-6(H_2) or MK-6 (Knoblauch *et al*., 1999), while strain MSL86^T has MK-8(H_4). Many of SRBs in the class *Deltaproteobacteria* including *Desulfovibrio* species

have menaquinone MK-6 or MK-6(H_2) and some species in the *Desulfobacteraceae* have menaquinone MK-7 or MK-7(H_2) (Kuever *et al*., 2005). Although it is known that *Desulfofaba gelida* in the *Desulfobacteraceae,* a psychrophilic SRB, and sulfur-reducing *Desulfuromonas* species in the *Desulfuromonaceae* have MK-8, MK-8 is a relatively rare menaquinone in SRBs or related organisms (Kuever *et al*., 2005). The CFA profiles of strain MSL86^T and related *Desulfotalea* species are compared in Table 2. Even-numbered unsaturated fatty acids, $C_{16:1\omega7}$ and $C_{16:1\omega5}$, are the main CFAs found in the *Desulfotalea* species. The high percentages of unsaturated fatty acids (about 90%) in CFAs of *Desulfotalea* species should be thought to be an adaptation to low temperature (Knoblauch *et al*., 1999). Strain MSL86^T has much higher amounts of saturated fatty acids in CFAs than the related *Desulfotalea* species and the strain has an odd-numbered unsaturated fatty acid ($C_{17:1}$) as one of main CFAs which is absent or present as only a minor CFAs in the *Desulfotalea* species. Thus, strain MSL86^T has distinctly different chemotaxonomic characteristics from those of closely related *Desulfotalea* species.

Based on the phylogenetic, physiological and chemotaxonomic characteristics shown above, we propose here a novel genus and species in the class *Deltaproteobacteria* to accommodate the strain with strain MSL86^T as the type species.

Description of *Desulfopila* gen., nov.

Desulfopila (De.sul.fo.pi'la. L. pref. *de* from; L. n. *sulfur* sulfur; L. fem. n. *pila* pillar; N.L. fem. n. *Desulfopila* a sulfate-reducing pillar).

Mesophilic. Strictly anaerobic. Cells are Gram-negative, non-spore-forming rods. Sulfate, other inorganic sulfur compounds and fumarate serve as electron acceptors. Organic electron donors are incompletely oxidized to mainly acetate. Type species is *Desulfopila aestuarii*.

Description of *Desulfopila aestuarii* sp. nov.

Desulfopila aestuarii (*ae.stu.a'ri.i.* L. n.; *aestus* tides; L. gen. n. *aestuarii* of an estuary).

Has the following characteristics in addition to those described for the genus. Cells are rod-shaped with rounded ends, 0·7 -1·2 µm wide and 1·9-3·8 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 0-5·0 (w/v) with an optimum at 1·0% (w/v). The temperature range for growth is 10-40^{o}C with an optimum at 35^{o}C. Slightly alkaliphilic and the pH range for growth is 6·3 -8·5 with an optimum at 7·5 -7·6. Utilizes formate, pyruvate, lactate, fumarate, ethanol, propanol, butanol, and glycerol as electron donors for sulfate reduction. Does not use acetate, propionate, butyrate, succinate, malate, methanol, glycine, alanine, serine, aspartate, glutamate and H_2. Sulfate, sulfite, thiosulfate and fumarate serve as electron acceptors. Propionate is produced together with succinate with fumarate as an electron acceptor using lactate as an electron donor. Pyruvate and fumarate are fermented in the absence of electron acceptors. The genomic DNA G + C content is 54·4 mol%. Major cellular fatty acids are $C_{16:0}$, $C_{16:1\omega7}$, $C_{16:1\omega5}$ and $C_{17:1\omega6}$. The major respiratory quinone is MK-8(H_4). Isolated from an estuarine sediment located on the side of the Sea of Japan of the Japanese islands. Type strain is MSL86^{T} (= JCM 14042^{T} = DSM 18488^{T}).

Acknowlegements

We are grateful to Drs. T. Murayama and K. Takahashi for their technical teaching for analysis of isoprenoid quinones.

References

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

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

Audiffrin, C., Cayol, J.-L., Joulian, C., Casalot, L., Thomas, P., Garcia, J.-L. & Ollivier, B. (2003). *Desulfonauticus submarinus* gen. nov., sp. nov., a novel sulfate-reducing bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **53**, 1585-1590.

Blenden, 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.

Cravo-Laureau, C., Matheron, R., Cayol, J.-L., Joulian, C. & Hirschler-Réa, A. (2004). *Desulfatibacillum aliphaticivorans* gen. nov., sp. nov., an n-alkane- and n-alkene-degrading, sulfate-reducing bacterium. *Int J Syst Evol Microbiol* **54**, 77-83.

Devereux, R. & Mundfrom, G. W. (1994). A phylogenetic tree of 16S rRNA sequences from sulfate-reducing bacteria in a sandy marine sediment. *Appl Environ Microbiol* **60**, 3437-3439.

Dhillon, A., Teske, A., Dillon, J., Stahl, D. A. & Sogin, M. L. (2003). Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. *Appl Environ Microbiol* **69**, 2765-2772.

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

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

J**eanthon, C., L'Haridon, S., Cueff, V., Banta, A., Reysenbach, A.-L. & Prieur, D. (2002).** *Thermodesulfobacterium hydrogeniphilum* sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a deep-sea hydrothermal vent at Guaymas Basin, and emendation of the genus *Thermodesulfobacterium*. *Int J Syst Evol Microbiol* **52**, 765-772.

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

Joulian, C., Ramsing, N. B. & Ingvorsen, K. (2001). Congruent phylogenies of most common small-subunit rRNA and dissimilatory sulfite reductase gene sequences retrieved from estuarine sediments. *Appl Environ Microbiol* **67**, 3314-3318.

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

Klein, M., Friedrich, M., Roger, A. J., Hugenholtz, P., Fishbain, S., Abicht, H., Blackall, L. L., Stahl, D. A. & Wagner, M. (2001). Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. *J Bacteriol* **183**, 6028–6035.

Knoblauch, C., Sahm, K. & Jørgensen, B. B. (1999). Psychrophilic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments, description of *Desulfofrigus oceanense* gen. nov., sp. nov.,

Desulfofrigus fragile sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov. and *Desulfotalea arctica* sp. nov. *Int J Syst Bacteriol* **49**, 1631-1643.

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 Edition, pp. 922-1144. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Leloup J., Quillet L., Berthe T. & Petit F. (2006). Diversity of the *dsrAB* (dissimilatory sulfite reductase) gene sequences retrieved from two contrasting mudflats of the Seine estuary, France. *FEMS Microbiol Ecol* **55**, 230-238.

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.

Moussard, H., L'Haridon, S., Tindall, B. J., Banta, A., Schumann, P., Stackebrandt, E., Reysenbach, A.-L. & Jeanthon, C. (2004). *Thermodesulfatator indicus* gen. nov., sp. nov., a novel thermophilic chemolithoautotrophic sulfate-reducing bacterium isolated from the Central Indian Ridge. *Int J Syst Evol Microbiol* **54**, 227-233.

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.

Purdy, K. J., Embley, T. M. & Nedwell, D. B. (2002). The distribution and activity of sulphate reducing bacteria in estuarine and coastal marine sediments. *Antonie Leewenhoek* **81**, 181-187.

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

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.

Szewzyk, R. & Pfennig, N. (1987). Complete oxidation of catechol by the strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. *Arch Microbiol* **147**,163-168.

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.

Voordouw, G., Armstrong, S. M., Reimer, M. F., Fouts, B., Telang, A. J., Shen, Y. & Gevertz, D. (1996). Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. *Appl Environ Microbiol* **62**, 1623-1629.

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

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.

Legends for figures

Fig. 1.

A phase-contrast photomicrograph of cells of strain MSL86^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 MSL86^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.

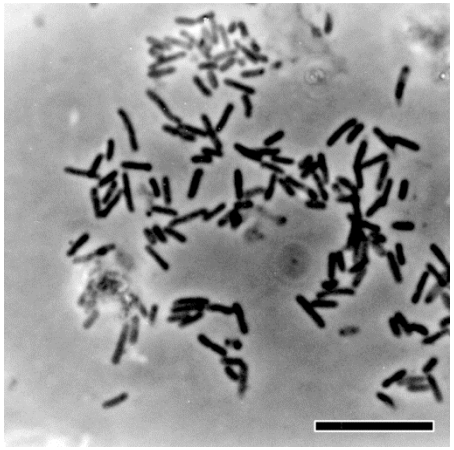

Fig.2.

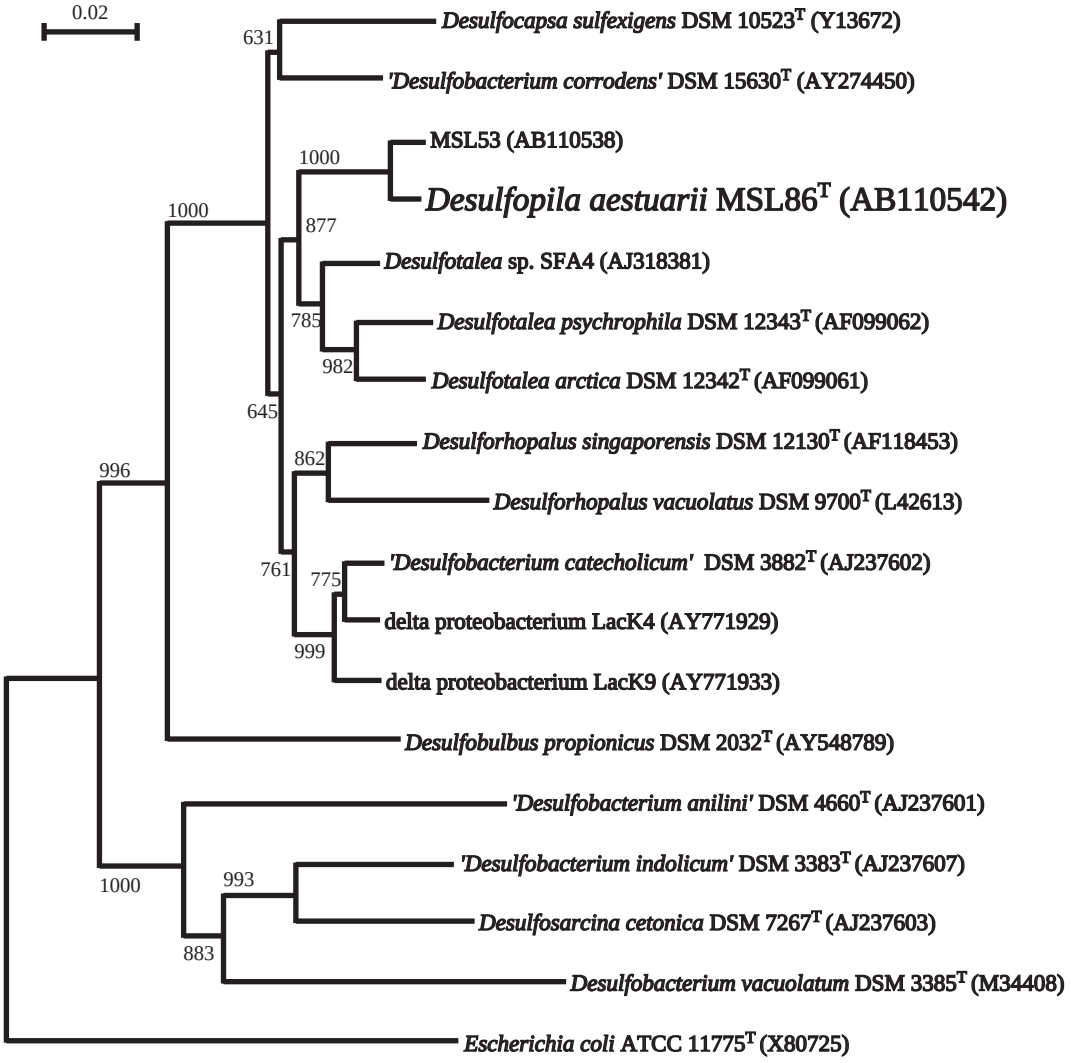
0.02
631
Desulfocapsa sulfexigens DSM 10523T (Y13672)
'Desulfobacterium corrodens' DSM 15630T (AY274450)
1000
MSL53 (AB110538)
Desulfopila aestuarii MSL86T (AB110542)
1000
877
Desulfotalea sp. SFA4 (AJ318381)
785
Desulfotalea psychrophila DSM 12343T (AF099062)
982
Desulfotalea arctica DSM 12342T (AF099061)
645
Desulforhopalus singaporensis DSM 12130T (AF118453)
862
996
Desulforhopalus vacuolatus DSM 9700T (L42613)
761
'Desulfobacterium catecholicum' DSM 3882T (AJ237602)
775
delta proteobacterium LacK4 (AY771929)
999
delta proteobacterium LacK9 (AY771933)
Desulfobulbus propionicus DSM 2032T (AY548789)
'Desulfobacterium anilini' DSM 4660T (AJ237601)
'Desulfobacterium indolicum' DSM 3383T (AJ237607)
1000
993
Desulfosarcina cetonica DSM 7267T (AJ237603)
883
Desulfobacterium vacuolatum DSM 3385T (M34408)
Escherichia coli ATCC 11775T (X80725)

Table 1. Some characteristics to differentiate strain MSL86^T from related species

Strains: 1, MSL86^T; 2, *Desulfotalea psychrophila* LSv54^T (Knoblauch *et al*., 1999);

3, *Desulfotalea arctica* LSv514^T (Knoblauch *et al*., 1999); 4, '*Desulfobacterium catecholicum*' NZva20^T (Szewzyk & Pfennig, 1987).

n.d., not determined; +, used; -, not used.

	1	**2**	**3**	**4**
Isolation source	Estuarine sediment	Arctic marine sediment	Arctic marine sediment	Anoxic mud of a bay
Cell shape	Rods	Rods	Rods	Oval to lemon
Motility	Motile	Motile	Motile	Non-motile
Optimum growth condition				
NaCl (%, w/v)	1·0	1·0	1·9-2·5	0·1*
Temperature (^{o}C)	35	10	18	28
pH	7·5-7·6	7·3-7·6	7·2-7·9	6·9-7·1
Utilization of electron donor				
Acetate	-	-	-	+
Propionate	-	-	-	+
Butyrate	-	-	-	+
Fumarate	+	+	-	+
Malate	-	+	-	+
Methanol	-	-	-	+
Propanol	+	+	-	+
Butanol	+	+	-	+
Glycerol	+	-	+	n.d.
Glycine	-	+	-	n.d.
Alanine	-	+	-	n.d.
Serine	-	+	+	n.d.
Glutamate	-	n.d.	n.d.	+
H_2	-	+	+	+
Utilization of electron acceptor				
Sulfite	+	+	-	+
Thiosulfate	+	+	-	+
Utilization of substrate in the absence of electron acceptor				
Fumarate	+	+	-	+
G+C content (mol%)	54·4	46·8	41·8	52·4
Isoprenoide quinone	MK-8(H_4)	MK-6(H_2)	MK-6	n.d.

*Strain NZva20^T grows best in fresh water medium containing 0·1% (w/v) NaCl and does not grow in medium with NaCl concentrations exceeding 85 mmol/l (about 0·5%, w/v).

Table 2. Cellular fatty acid composition (%) of strain MSL86^T and related species

Strains: 1, MSL86^T; 2, *Desulfotalea psychrophila* LSv54^T (Knoblauch *et al* ., 1999);

3, *Desulfotalea arctica* LSv514^T (Knoblauch *et al* ., 1999).

-, not detected.

Fatty acid	1	2	3
Saturated straight-chain			
$C_{14:0}$	1·4	1·4	-
$C_{15:0}$	-	0·4	-
$C_{16:0}$	33·6	6·5	8·3
$C_{17:0}$	3·4	-	-
$C_{18:0}$	2·5	-	-
Unsaturated straight-chain			
$C_{14:1\omega 5}$	-	1·1	1·5
$C_{15:1\omega 6}$	1·1	1·7	-
$C_{16:1\omega 9}$	-	1·5	3·6
$C_{16:1\omega 7}$	6·0	55·0	53·9
$C_{16:1\omega 5}$	17·1	25·5	29·4
$C_{17:1\omega 6}$	13·7	2·4	-
$C_{18:1\omega 9}$	1·3	-	-
$C_{18:1\omega 7}$	1·7	1·5	1·5
$C_{18:1\omega 5}$	2·7	0·7	1·0
Hydroxy acids			
iso-$C_{11:0}$ 3-OH	1·1	-	-
$C_{14:0}$ 3-OH	1·8	-	-
$C_{16:0}$ 3-OH	1·6	-	-
Unsaturated branched-chain			
Branched $C_{17:1}$	4·6	-	-