Desulfobaculum xiamenensis gen. nov., sp. nov., a member of the family *Desulfovibrionaceae* isolated from marine mangrove sediment

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A taxonomic study was carried out on strain P1^T, which was isolated from mangrove sediment samples collected from Qinglan Port (Hainan, China). Cells were curved rods, that were motile, with a single polar flagellum. The strain was non-spore-forming with a cell size of 0.6×1.5–2.2 µm. Catalase and oxidase activities were not detected. Growth was observed in the temperature range 22–44 °C (optimum, 35–40 °C) and pH range 5.5–8.5 (optimum, pH 7.0). NaCl was required for growth and tolerated at up to 3.5 % (w/v) (optimum, 0.5 %). Strain P1^T utilized hydrogen, succinate, L-malate, citrate, oxalate, DL-lactate, pyruvate, or cysteine as electron donors, and sulfate or sulfite as electron acceptors. Fermentation products from pyruvate were acetate, H₂ and CO₂. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain P1^T formed a distinct evolutionary lineage within the family *Desulfovibrionaceae*. Strain P1^T was most closely related to members of the genera *Desulfovibrio* (92.0–94.3 % 16S rRNA gene sequence similarity), *Desulfocurvus* (91.1 %), *Bilophila* (87.9 %) and *Lawsonia* (86.0 %) of the family *Desulfovibrionaceae*. The DNA G+C content of strain P1^T was 64.5 mol% and the major cellular fatty acids were iso-C_{15:0} (18.8 %), anteiso-C_{15:0} (5.0 %), C_{16:0} (14.2 %) and iso-C_{17:1ω9c} (24.4 %). The predominant menaquinone was MK-7 (97 %). Major polar lipids were phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol. Strain P1^T was distinguishable from members of phylogenetically related genera by differences in several phenotypic properties. On the basis of the phenotypic and phylogenetic data, strain P1^T represents a novel species of a new genus, for which the name *Desulfobaculum xiamenensis* gen. nov., sp. nov. is proposed. The type strain of *Desulfobaculum xiamenensis* is P1^T (=CGMCC 1.5166^T=DSM 24233^T).

In anaerobic environments, sulfate-reducing bacteria obtain their energy by oxidizing organic compounds or molecular hydrogen while reducing sulfates to sulfides, especially to hydrogen sulfide (Peck, 1993; Widdel & Hansen, 1992). Bacterial sulfate reduction is an important process of mineralization of organic matter in anoxic environments, especially in marine and hypersaline systems (Ollivier *et al.*,

1994). Mangrove ecosystems play an important role in maintaining and improving biological environments, and are home to a large, unexplored microbial diversity (Chang *et al.*, 2009; Li & Lee, 1997; Liao *et al.*, 2009). In an investigation of a cellulolytic consortium, we isolated a novel sulfate-reducing strain, designated strain P1^T, from typical mangrove forest sediment samples. Comparative 16S rRNA gene sequence analysis indicated that strain P1^T formed a deep branch within the family *Desulfovibrionaceae*. The family *Desulfovibrionaceae* was first proposed by Kuever *et al.* (2005) based on phylogenetic analysis of 16S rRNA gene sequences, and contains four genera: *Desulfovibrio*, *Desulfocurvus*, *Bilophila* and *Lawsonia*. In this study the exact taxonomic position of strain P1^T was determined by a polyphasic approach.

Abbreviations: ASW, artificial sea water; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and *dsr* gene sequence of strain P1^T are HQ697913 and JF742988, respectively.

Three supplementary figures are available with the online version of this paper.

The mangrove sediment samples were collected from Qinglan Port area (Hainan, China). Strain P1^T was isolated from a culture enriched with filter paper as the sole carbon source under a gas phase of CO₂/N₂. The streak-plate procedure was repeated several times before strain P1^T was obtained. Defined YTBC medium was used for the enrichment culture, isolation and general physiological characterization of the strain, and contained [per litre artificial seawater (ASW)]: yeast extract 1.0 g, tryptone 5.0 g, casein acid 5.0 g, beef extract 5.0 g, K₂HPO₄ 1.0 g, NH₄NO₃ 1.0 g, Na₂WO₄·2H₂O 0.1 mg, Na₂SeO₃·5H₂O 0.1 mg, NiCl₂·6H₂O 7.5 mg, resazurin 1.0 mg (for anaerobic), cysteine HCl 0.25 g (for anaerobic), Na₂S·9H₂O 0.25 g (for anaerobic). ASW contained (per litre distilled water): NaCl 5 g, MgCl₂·6H₂O 0.75 g, MgSO₄·7H₂O 6.0 g, NaHCO₃ 0.05 g, CaCl₂·2H₂O 0.1 g, KCl 0.1 g, KH₂PO₄ 0.1 g. The pH of YTBC medium was adjusted to pH 7.0–7.5 with 2 M NaOH. The reference strain *Desulfovibrio senexii* DSM 8436^T was obtained from the DSMZ (Braunschweig, Germany) and incubated as described previously (Tsu *et al.*, 1998).

Gram staining was carried out according to Cowan & Steel (1993). Cell morphology was examined by light microscopy (TE2000 S/U; Nikon) and transmission electron microscopy (JEM-1230; JEOL) after cultivation at 35 °C for 48 h. Catalase and oxidase activities were determined by using an Oxidase Reagent kit (bioMérieux) according to the manufacturer's instructions. The physiological characterization of strain P1^T included determination of the temperature, pH and salinity ranges that allowed bacterial growth. Substrate utilization was investigated by growing strain P1^T in YTBC medium containing 1% (w/v) of each of the following substrates: cellobiose, D-glucose, xylose, arabinose, maltose, mannose, mannitol, trehalose, sorbitol, lactose, fructose, starch, raffinose, rhamnose, sucrose, galactose and glycerol. Oxygen was tested as the electron acceptor in liquid and solid YTBC media. Fumarate, nitrate, sulfate, sulfite, thiosulfate (20 mM each) and elemental sulfur (20 mg ml⁻¹) were tested as alternate electron acceptors by replacing sulfate in the growth medium. Utilization of H₂ as an electron donor was determined in the presence or absence of acetate (5 mM) as an organic carbon source under an H₂/CO₂ atmosphere. Utilization of electron donors by the isolates was investigated by using acetate, DL-lactate, pyruvate, Casamino acids, cysteine, yeast extract, succinate, L-malate, citrate, oxalate, formate, propionate, butyrate, D-glucose, methanol and ethanol at a final concentration of 20 mM each. Fermentative growth was tested in the YTBC medium. These results are given in the genus and species description, Table 1 and Fig. S1 (available in IJSEM Online).

Escherichia coli DH5α, used as the host strain for recombinant plasmids, was grown in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) at 37 °C. pMD18-T (TaKaRa Biotechnology) was used as a cloning vector for PCR products. The 16S rRNA gene fragment was amplified by using universal 16S rRNA primers 27F and 1492R

(Weisburg *et al.*, 1991). The purified PCR product was cloned into pMD18-T and sequenced with M13 primers by Invitrogen Biotechnology. The almost complete 16S rRNA gene sequence (1471 nt) of strain P1^T was obtained and analysed using BLAST searches against the GenBank and EzTaxon databases (Chun *et al.*, 2007). The 16S rRNA gene sequences of closely related taxa obtained from the EzTaxon database were aligned. The phylogenetic tree was constructed by using the neighbour-joining method with MEGA 4.0 software (Tamura *et al.*, 2007). The topology of the tree was evaluated by performing a bootstrap analysis using 1000 replications. Phylogenetic analysis showed strain P1^T formed a distinct evolutionary lineage within the family *Desulfovibrionaceae* and placed it in a phylogenetic position most closely related to the genera *Desulfovibrio* and *Desulfocurvus* (Fig. 1). The closest relatives included *Desulfovibrio senexii* DSM 8436^T, *Desulfovibrio bizertensis* MB3^T and *Desulfocurvus vexinensis* VNs36^T with gene sequence similarities of 94.3%, 92.0% and 91.1%, respectively. Although the branching of strain P1^T with species of the genus *Desulfovibrio* in the phylogenetic tree was not supported strongly by bootstrap analysis, the trees generated with the distance matrix by using maximum-likelihood methods showed the same topology (data not shown). Of the sequences from recognized species, strain P1^T was most closely related to those from the genera *Desulfovibrio* (92.0–94.3% 16S rRNA gene sequence similarity), *Desulfocurvus* (91.1%), *Bilophila* (87.9%) and *Lawsonia* (86.0%), of the family *Desulfovibrionaceae*. All of the 16S rRNA gene sequence divergences between strain P1^T and recognized species were greater than 5.7%, and the distinct phylogenetic relationships revealed that strain P1^T could not be assigned to any of the recognized genera. These relatively low levels of 16S rRNA gene sequence similarity with closely related genera suggested that strain P1^T may represent a novel species of a new genus.

For further phylogenetic analysis of strain P1^T, the dissimilatory sulfite reductase gene (*dsr*) of the strain was sequenced using the method described by Karkhoff-Schweizer *et al.* (1995). The sequences were aligned using CLUSTAL W version 1.8 (Macintosh). Phylogenetic analyses were performed by the maximum-likelihood method by MOLPHY version 2.3b (UNIX) (Adachi & Hasegawa, 1996). The local bootstrap probabilities were calculated as the statistical confidences of the tree branches (Hasegawa & Kishino, 1994). The partial sequence of the *dsr* gene of strain P1^T was determined. Phylogenetic analysis based on *dsr* gene sequences showed that strain P1^T was related most closely to 'Bacterium AR1201' (87% sequence similarity). Its closest recognized relatives were *Desulfovibrio aminophilus*, DSM 12245^T, and *Desulfovibrio longus*, SEBR 2582^T, (79% and 78% *dsr* gene sequence similarity, respectively). The *dsr* gene sequence similarity between strain P1^T and the type strain of *Desulfovibrio senexii*, DSM 8436^T, was 87.7% in this study (data not shown). On the basis of *Dsr* amino acid sequence analysis, strain P1^T was also closely related to species of the genus *Desulfovibrio* (Fig. S2).

Table 1. Characteristics that differentiate strain P1^T from related genera of the family *Desulfovibrionaceae*

Taxa: 1, *Desulfobaculum xiamenensis* gen. nov., sp. nov. P1^T; 2, *Desulfovibrio* (data from Devereux *et al.*, 1990; Dzierzewicz *et al.*, 1996; Gebhart *et al.*, 1993; Kuever *et al.*, 2005; Postgate & Campbell, 1966); 3, *Desulfocurvus* (Klouche *et al.*, 2009); 4, *Bilophila* (Baron *et al.*, 1989; Kuever *et al.*, 2005; McOrist *et al.*, 2001); 5, *Lawsonia* (Kuever *et al.*, 2005; McOrist *et al.*, 1995). NR, Not reported; +, present, observed in all species; –, not present, not observed in all species; ±, variable; w, weakly present; SP, single polar flagellum; LO, lophotrichous flagellum; DV, desulfovibrin; SA, strictly anaerobic; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol

Characteristic	1	2	3	4	5
Morphology:					
Rod-shaped	+	–	–	+	–
Vibrio-shaped	–	+	+	–	+
Flagellum	SP	SP/LO	SP	–	–
Cell motility	+	+	+	–	–
Catalase	–	–	–	+	NR
O ₂ metabolism	SA	SA	SA	SA	Microaerophilic
Sulfite reductase	DV	DV	–	W	NR
Major menaquinone	MK-7	MK-6 or MK-6(H ₂)	NR	NR	NR
Size (µm)	0.6 × 2.0–2.2	0.2–1.5 × 2.5–10.0	0.5 × 3.0–5.0	0.7–1.1 × 1–10	0.3–0.4 × 1.5–2.0
Growth conditions:					
NaCl requirement	+	±	–	–	–
Optimum temperature (°C)	35–40	30–37	37	35	35–37
pH range	5.0–8.5	7.2	5.0–9.0	NR	NR
Electron donors and carbon sources:					
Hydrogen	+	+	–	NR	NR
Formate	–	±	+	+	–
Acetate	+	±	–	+	–
Ethanol	–	±	–	+	NR
Lactate	+	+	+	+	–
Pyruvate	+	+	+	+	–
Fumarate	+	+	–	NR	NR
Succinate	+	–	–	NR	NR
Malate	+	+	–	NR	NR
Electron acceptors:*					
Sulfate	+	+	+	–	
Sulfite	+	+	+	+	
Sulfur	–	–	–	NR	
Thiosulfate	–	+	+	+	
Nitrate	–	±	–	+	
Major fatty acids	iso-C _{15:0} , C _{16:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{17:0} , iso-C _{17:1} ω9c	NR	iso-C _{15:0} , C _{16:0} , C _{18:1} ω9c, C _{19:0} cyclo ω9c	NR
Major polar lipids	PC, PE, PG	PE, PG	NR	NR	NR
DNA G + C content (mol%)	64.5	47–60	67.2	59.2	34
Isolation source	Mangrove sediments	Freshwater and marine	Deep saline aquifer	Freshwater or clinical isolates	Porcine

*Members of the genus *Lawsonia* exhibit no sulfate reduction.

Genomic DNA was extracted and purified using the method described by Syn & Swarup (2000). The DNA G + C content was determined with HPLC of the deoxyribonucleosides as described by Mesbah & Whitman (1989). The G + C content of the genomic DNA of strain P1^T was 64.5 ± 1.0 mol%, which was similar to values reported for the genera *Desulfovibrio* (46–60 mol%) and *Desulfocurvus* (67.2 mol%), but differed by more than 5 mol% from values reported for the genera *Bilophila* (59.2 mol%) and *Lawsonia* (34 mol%).

Fatty acids of 0.2 g freeze-dried whole cells grown in YTBC liquid medium at 35 °C for 48 h were extracted, saponified and esterified, followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). The major fatty acids of strain P1^T were iso-C_{15:0} (18.8%), anteiso-C_{15:0} (5.0%), C_{16:0} (14.2%) and iso-C_{17:1}ω9c (24.4%). Minor amounts of C_{14:0} (4.3%), C_{16:1}ω7c and/or C_{16:1}ω6c (4.3%), iso-C_{17:0} (4.2%), anteiso-C_{17:1}ω9c (2.9%), C_{18:0} (1.9%), C_{18:1}ω9c (3.4%) and C_{18:1}ω7c (4.1%) were also found in strain

Fig. 1. Unrooted neighbour-joining tree of strain P1^T and related genera based on 16S rRNA gene sequences. Numbers at nodes represented the levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. GenBank accession numbers of 16S rRNA gene sequences are given in the parentheses. Bar, 0.02 nt substitution rate (K_{nc}) units.

P1^T. These data differentiated strain P1^T from members of the genera *Desulfovibrio* and *Bilophila*. The major cellular fatty acids of members of the genus *Desulfovibrio* are iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1}ω9c (Kohring *et al.*, 1994; Taylor & Parkes, 1983; Vainshtein *et al.*, 1992). Fatty acid methyl ester analysis of the genus *Bilophila* revealed a unique series of whole-cell fatty acids, which included iso-C_{15:0}, C_{16:0}, C_{18:1}ω9c and C_{19:0} cyclo ω9c (Baron *et al.*, 1989).

The presence of cytochromes and sulfite reductase (desulfoviridin) was measured according to the method of Badziong *et al.* (1978). Visible absorption spectra of a cell-free extract of strain P1^T showed the presence of redox potential cytochrome *c*₃ with absorption peaks at 522 and 550 in the dithionite reduced form. The characteristic absorption band of desulfoviridin (the bisulfite reductase characteristic of the genus *Desulfovibrio*) at about 630 nm was also detected in the cell-free extract of strain P1^T. The characteristic absorption band of desulfoviridin was not detected in the cell-free extract of *Desulfocurvus vexinensis* VNs36^T (Klouche *et al.*, 2009).

Analysis of respiratory quinones was carried out by the Identification Service and Dr Brian Tindall, DSMZ, Braunschweig, Germany. For polar lipid analysis, cell biomass of strain P1^T and *Desulfovibrio senezii* DSM 8436^T was obtained from cultures grown in YTBC medium at 35 °C for 48 h. Cellular polar lipids were extracted according to procedures described by Yassin *et al.* (1993), separated on silica gel 60 plates (Merck) by using TLC, and identified according to their reactions with the phosphate spray reagent of Dittmer and Lester (Dittmer & Lester, 1964). The predominant respiratory quinone of P1^T was MK-7 (97%), and a minor amount of MK-6 (3%) was

detected. However, all recognized strains of the genus *Desulfovibrio* contain MK-6 or MK-6(H₂) as the major menaquinone (Collins & Weddel, 1986). The polar lipid profiles of strain P1^T mainly consisted of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). The major phospholipids of *Desulfovibrio senezii* DSM 8436^T were PE and PG. The obvious difference between strain P1^T and *Desulfovibrio senezii* DSM 8436^T was the presence of PC in strain P1^T (Fig. S3). Other species of the genus *Desulfovibrio* exhibited a phospholipid profile with PE or PG as the major phospholipid (Makula & Finnerty, 1974). The type strain of the type species of the genus *Desulfovibrio* (*Desulfovibrio desulfuricans* ATCC 29577^T) exhibited a phospholipid pattern typical of Gram-negative bacteria with PE comprising the major phospholipid (Makula & Finnerty, 1974).

A comparison of the main characteristics between strain P1^T and the type strains of related species is given in Table 1. Although strain P1^T is most closely related to members of the genus *Desulfovibrio*, with the highest similarity to *Desulfovibrio senezii* DSM 8436^T (94.3%), it cannot be affiliated to the genus *Desulfovibrio* due to the obvious differences of major menaquinone, major fatty acids and polar lipid components between strain P1^T and members of the genus *Desulfovibrio*. In addition, strain P1^T could be distinguished from the genus *Desulfocurvus* based on electron donors used and the presence of sulfite reductase. Cell motility by means of a single polar flagellum, negative catalase activity, and the presence of sulfite reductase differentiated strain P1^T from members of the genera *Bilophila* and *Lawsonia*. Overall, the low 16S rRNA gene sequence similarity, together with differences in chemotaxonomic, physiological

and biochemical properties, indicated that strain P1^T represented a novel species of a new genus, for which the name *Desulfobaculum xiamenensis* gen. nov., sp. nov. is proposed.

Description of *Desulfobaculum* gen. nov.

Desulfobaculum (De.sul.fo.ba'cu.lum. L. pref. *de* from; L. n. *sulfur* sulfur; L. masc. n. *baculum*, a stick or rod; N.L. masc. n. *Desulfobaculum* a rod sulfate-reducing bacterium).

Cells are Gram-stain-negative rods. Oxidase- and catalase-negative. Obligately anaerobic, mesophilic and moderately halophilic bacteria with growth at pH 5.0–8.5. The predominant fatty acids are iso-C_{15:0}, C_{16:0} and iso-C_{17:1}ω9c. The predominant respiratory quinone is MK-7. The genus is assigned phylogenetically to the family *Desulfovibrionaceae* in the order *Desulfovibrionales*. The only currently known strain was isolated from offshore mangrove sediment. The type species is *Desulfobaculum xiamenensis*.

Description of *Desulfobaculum xiamenensis* sp. nov.

Desulfobaculum xiamenensis (xi.a.men.en'sis. L. masc. adj. *xiamenensis* of or belonging to Xiamen, the place of isolation).

Shows the following characteristics in addition to those given in the genus description above. Cells are short rods (1.5–2.2 µm long and 0.6 µm wide), non-spore-forming, and motile by means of a single polar flagellum. Grows at temperatures of 22–44 °C (optimum, 35–40 °C) and at pH 5.5–8.5 (optimum, pH 7.0). Grows in the presence of 0.2–3.5 % (w/v) NaCl (optimum, 0.5 %). Utilizes cellobiose and galactose, but not D-glucose, xylose, arabinose, maltose, mannose, mannitol, trehalose, sorbitol, lactose, fructose, starch, raffinose, rhamnose, sucrose or glycerol. Grows on sulfate or sulfite as electron acceptors, but does not grow on elemental sulfur, thiosulfate, nitrate, or nitrite as electron acceptors. Grows on acetate, succinate, L-malate, citrate, oxalate, DL-lactate, pyruvate, cysteine, Casamino acids and yeast extract with sulfate or sulfite as electron acceptor. Does not use formate, ethanol, propionate, butyrate or methanol as electron donors. Ferments acetate, H₂ and CO₂ from pyruvate. Desulfovibrin and c-type cytochromes are present. Major phospholipid species are PC, PE and PG.

The type strain, P1^T (=CGMCC 1.5166^T=DSM 24233^T), was isolated from mangrove sediment samples from Qinglan Port in Hainan Island, China. The DNA G+C content of the type strain is 64.5 mol%.

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