International Journal Of Systematic And Evolutionary Microbiology
October 2016, Volume 66 Pages 3904-3911
http://dx.doi.org/10.1099/ijsem.0.001286
http://archimer.ifremer.fr/doc/00359/47018/

Pseudodesulfovibrio indicus gen. nov., sp nov., a piezophilic sulfate-reducing bacterium from the Indian Ocean and reclassification of four species of the genus *Desulfovibrio*

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Abstract :

A novel sulfate-reducing bacterium, strain J2T, was isolated from a serpentinized peridotite sample from the Indian Ocean. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain J2T clustered with the genus *Desulfovibrio* within the family *Desulfovibrionaceae* , but it showed low similarity (87.95 %) to the type species *Desulfovibrio desulfuricans* DSM 642T. It was most closely related to *Desulfovibrio portus* MSL79T (96.96 %), followed by *Desulfovibrio aespoeensis* Aspo-2T (96.11 %), *Desulfovibrio piezophilus* C1TLV30T (96.04 %) and *Desulfovibrio profundus* DSM 11384T (95.17 %). Other available sequences shared less than 93.33 % 16S rRNA gene sequence similarity. Cells were Gram-staining-negative, anaerobic, motile vibrios (2–6×0.4–0.6 µm). Growth was observed at salinities ranging from 0.2 to 6 % (optimum 2.5 %), from pH 5 to 8 (optimum pH 6.5–7) and at temperatures between 9 and 40 °C (optimum 30–35 °C). J2T was piezophilic, growing optimally at 10 MPa (range 0–30 MPa). J2T used lactate, malate, pyruvate, formate and hydrogen as energy sources. Sulfate, thiosulfate, sulfite, fumarate and nitrate were used as terminal electron acceptors. Lactate and pyruvate were fermented. The main fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl) and iso-$C_{17:0}$. The DNA G+C content of strain J2T was 63.5 mol%. The combined genotypic and phenotypic data show that strain J2T represents a novel species of a novel genus in the family *Desulfovibrionaceae* , for which the name *Pseudodesulfovibrio indicus* gen.

nov., sp. nov. is proposed, with the type strain J2T (=MCCC 1A01867T = DSM 101483T). We also propose the reclassification of *D. piezophilus* as *Pseudodesulfovibrio piezophilus* comb. nov., *D. profundus* as *Pseudodesulfovibrio profundus* comb. nov., *D. portus* as *Pseudodesulfovibrio portus* comb. nov. and *D. aespoeensis* as *Pseudodesulfovibrio aespoeensis* comb. nov.

Abbreviation:

SRP	sulfate-reducing prokaryotes

Keywords: sulfate-reducing bacterium, Pseudodesulfovibrio indicus

The GenBank[/EMBL/DDBJ accession number for the 16S rRNA sequence of *Pseudodesulfovibrio indicus* J2T is KT750867 and the GenBank accession number for the genome sequence is CP014206.

Two supplementary figures are available with the online Supplementary Material.

Sulfate-reducing prokaryotes (SRP) are anaerobic prokaryotes, using sulfate as a terminal electron acceptor for respiration and hydrogen or various organic acids as energy sources (Heidelberg *et al.*, 2004). Members of the bacterial genus *Desulfovibrio* are SRP of geomicrobiological significance (Heidelberg *et al.*, 2004; Khelaifia *et al.*, 2011). Most representatives of this genus oxidize simple organic compounds incompletely, leading to acetate as an end-product. At the time of writing, the genus *Desulfovibrio*, within the family *Desulfovibrionaceae*, comprises 66 species, which have been isolated ubiquitously in nature, mainly from freshwater and marine habitats (Khelaifia *et al.*, 2011; Thabet *et al.*, 2011). One of its species, *D. dechloracetivorans* ($SF3^T$ = DSM 16853^T = ATCC 700912^T) (Sun *et al.*, 2000; Sun *et al.*, 2001), has been recognized by the International Committee on Systematics of Prokaryotes but is not available anymore in public collections. The broad genus *Desulfovibrio* might deserve rearrangements, as some species currently affiliated to this genus are phylogenetically distant. Among *Desulfovibrio* isolates, only few strains were reported to be piezophilic; they include *Desulfovibrio profundus* (Bale *et al.*, 1997), *Desulfovibrio hydrothermalis* (Alazard *et al.*, 2003) and *Desulfovibrio piezophilus* (Khelaifia *et al.*, 2011).

In this paper, a novel mesophilic, piezophilic SRP, strain $J2^T$, is described. It is phylogenetically related to some members of the genus *Desulfovibrio* but forms a separate group in the family *Desulfovibrionaceae*. Phenotypic and 16S ribosomal RNA gene

phylogenetic studies were carried out to investigate the taxonomic position of this isolate.

Strain J2^T was isolated from a deep-sea serpentinized peridotite sample collected at a depth of 3173 m in a hydrothermal area of the Indian Ocean (27° 88' S, 63° 53' E; site 30I-TVG05) in December 2013, during the cruise DY30/I of *Da Yang Yi Hao*. The sample was collected using a grabber and anaerobically preserved in sterilized seawater onboard. Once in the lab, a subsample was used to inoculate a SO4PNsalts medium (Alain *et al.*, 2010), prepared with a gas phase of H_2/CO_2 (80/20, v/v, 200 kPa) and incubated at 30 °C. After 5 days of incubation, populations of highly motile vibrioid cells were observed, subcultured under the same conditions, and then purified by repeated dilutions-to-extinction series. One isolate, strain J2^T, is described in this study. The purity of this isolate was confirmed routinely by microscopic examination (including observations of cultures on rich media) and by repeated partial sequencing of the 16S rRNA gene using 4 different primers. Stock cultures were stored at -80°C with 5% (v/v) DMSO.

Genomic DNA was extracted with the QIAGEN Genomic-tip 20/G (QIAGEN, Düsseldorf, Germany) kit following the manufacturer's standard protocol and the 16S rRNA gene was amplified by PCR using primers Bac8F and U1492R that have been described previously (Liu & Shao, 2005). The 16S rRNA gene was sequenced by Sanger method using the primers Bac8F (5'-AGA GTT TGA TCA TGG CTC AG-3'), S8dir (5'-GTA GCG GTG AAA TGC GTA GA-3'), U1492R (5'-GGT TAC CTT GTT ACG ACT T-3') and W34 (5'-TTA CCG

CGG CTG CTG GCA C-3') (Zhang *et al.*, 2013). A nearly full-length 16S rRNA gene sequence (1491 bp) of strain J2^T was obtained. Pairwise 16S rRNA sequence similarity was calculated using global alignment algorithm implemented at the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; (Kim *et al.*, 2012). Phylogenetic analysis was performed using the software MEGA version 5.0 (Tamura *et al.*, 2011). Distances were calculated using the Kimura two-parameters model and clustering was performed with the neighbor-joining algorithm (Saitou & Nei, 1987). The robustness of the inferred topology was assessed by bootstrap analyses based on 1000 bootstrap resamplings.

The 16S rRNA gene-based analysis located the novel isolate within the class *Deltaproteobacteria*, family *Desulfovibrionaceae*, in the bacterial domain. Comparative 16S rRNA gene sequence analysis showed that strain J2^T clustered robustly with four members of the genus *Desulfovibrio* still available in public collections (Fig. 1) (the species *D. dechloracetivorans* SF3^T, sharing 98.05% 16S rRNA gene sequence similarity with strain J2^T, belongs also to the same cluster but is not available anymore in public collections). The node that grouped all these species had a bootstrap percentage value of 95%, which indicates a strong stability supporting the topology of this branch. Strain J2^T shared the highest sequence similarity of 96.96% with *D. portus* MSL79^T, followed by *D. aespoeensis* Aspo-2^T (96.11 %), *D. piezophilus* C1TLV30^T (96.04 %) and *D. profundus* DSM11384^T (95.17%). It shared low 16S rRNA gene similarity (<93.33%) with other species of the genus *Desulfovibrio*. Among *Bacteria*, the genus-level is even more difficult to delineate than the species-level since

phylogenetic divergence is not necessarily supported by phenotypic and chemotaxonomic properties, and since no clear-cut genus definition is available. Nevertheless, genera are generally described as agglomerates of nodal species and internodal strains (Gillis *et al.*, 2001), for which similarity values around 94.5-95% are commonly used for genus differentiation (Ludwig *et al.*, 1998; Yarza *et al.*, 2014). Strain J2^T showed low similarity (87.95%) with the type species *D. desulfuricans* DSM 642^T and formed a phylogenetically coherent group with with *D. portus* MSL79^T, *D. aespoeensis* Aspo-2^T, *D. piezophilus* C1TLV30^T and *D. profundus* DSM11384^T. Based on these criteria, strain J2^T might represent a novel genus in the family *Desulfovibrionaceae*, together with *D. portus*, *D. aespoeensis*, *D. piezophilus* and *D. profundus*. Phylogenetic analyses also showed that members of the genus *Desulfovibrio* were grouped into more than 10 separated clusters too distantly related to belong to only one genus (Fig. 1). Indeed, with the exception of the strains belonging to the cluster of the type species, strains from the other clusters displayed all 16S rRNA gene sequence similarity below 92.23% with the type species of the genus *D. desulfuricans* DSM 642^T. They might represent different novel genera. Their reclassification should be considered in the future.

The whole genome of strain J2^T was sequenced using the PacBio technology and annotated as described elsewhere (Cao *et al.*, 2016). The chromosomal DNA G+C content of strain J2^T was 63.5 mol%, based on the whole genome sequence. The sulfite reductase present in the genome was very closely related to the one of *D. desulfuricans* (94% to 100% amino acid

sequence similarity with the α, β and γ subunits of the desulfoviridin-type sulfite reductase of *D. desulfuricans*).

Morphological characteristics of cells of strain J2^T were determined by using light microscopy (Olympus BX60 and CX40) and scanning electron microscopy (FEI Quanta 200). Cells were motile, vibrios (2-6 μm in length and 0.4-0.6 μm in width, *n*=10, Supplementary Fig. S1) that appeared singly. They stained Gram-negative.

Unless stated otherwise, physiological characterization was carried out anaerobically in TRM medium reduced with sodium sulfide (Zeng *et al.*, 2009), in duplicate, using sulfate as a terminal electron acceptor. This medium was selected because it allowed efficient growth of the strain, notably when this medium was supplemented with 20 mM lactate. Growth experiments were generally carried out as described elsewhere (Khelaifia *et al.*, 2011). Growth was routinely monitored by direct cell counting by using a modified Thoma chamber (depth 10 μm) and growth rates were calculated using linear regression analysis of logarithmically transformed growth curves. Salt tolerance was tested at 35°C in TRM medium prepared with various concentrations of NaCl (0, 0.2, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0 %, w/v). Strain J2^T required NaCl for growth and growth was observed at 0.2–6% NaCl (optimum: 2.5%). The pH range for growth was tested from pH 4.0 to pH 9.0 (initial pH at 20°C) with increments of 0.5 units. Growth was observed at pH 5.0–8.0, the

optimum being around 6.5-7.0. Determination of the temperature range for growth was tested at 4, 9, 16, 20, 25, 30, 35, 37, 40, 45 and 50°C. The isolate was mesophilic and grew at 9–40 °C (optimum 30-35 °C). Determination of the hydrostatic pressure range for growth was tested at 0.1, 10, 20, 30 and 40 MPa, using a procedure and an equipment described previously (Alain *et al.*, 2002). The novel isolate $J2^T$ was piezophilic and grew within a pressure range of 0.1 to 30 MPa, with an optimum at 10 MPa (Supplementary Fig. S2).

Strain $J2^T$ was a strictly anaerobic bacterium that used lactate and sulfate as primary electron donor and acceptor, respectively. Its ability to use alternative electron acceptors was tested on the mineral base of the SO4PNsalts medium depleted of sulfate, but supplemented with a gas phase of H_2/CO_2 (80/20, v/v, 200 kPa). In order to ensure an adequate electron donor and carbon source, elemental sulfur (12 g L^{-1}), fumarate (20 mM) sulfite (2 mM), thiosulfate (20 mM), nitrate (10 mM), nitrite (2 mM) or oxygen (1% v/v) were added. Hydrogen sulfide production was determined as described elsewhere (Cord-Ruwisch, 1985). Sulfate, thiosulfate, sulfite, fumarate, and nitrate were used as terminal electron acceptors, but not elemental sulfur nor nitrite. When using sulfate as a terminal electron acceptor, strain $J2^T$ grew on hydrogen, malate, formate, pyruvate and lactate. The ability of the strain to grow by fermentation was tested on sulfate-depleted mineral base of the SO4PNsalts medium, after addition of lactate (20 mM), yeast extract (1 g L^{-1}), tryptone (4 g L^{-1}), fumarate (20 mM), formate (20 mM), malate (20 mM), and pyruvate (20 mM). Lactate and pyruvate were fermented.

The whole-cell fatty acid content of strain J2^T was analyzed on late exponential phase of growth cultures grown anaerobically in TRM medium supplemented with 20 mM lactate. Fatty acids in whole cells were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analyzed by GC (Agilent Technologies 6850) and identified by using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). The predominant fatty acids of strain J2^T were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:1}$ $\omega 9c$ and/or $C_{16:0}$ 10-methyl (Summed feature 9) and iso-$C_{17:0}$ (Table 1), which were in the same range than the values got for *D. piezophilus* and *D. portus* (Khelaifia *et al.*, 2011; Suzuki *et al.*, 2009).

Strain J2^T exhibited phylogenetic, phenotypic and chemotaxonomic differences with its closest relatives. These differences in phenotypic characteristics with (i) its closely related species, and (ii) members of other genera in the family *Desulfovibrionaceae*, are given in Tables 2 and 3. Strain J2^T cannot be affiliated to the genus *Desulfovibrio* due to the obvious differences in the genomic DNA G+C content, the electron donors used and the low 16S rRNA gene sequence similarity. Strain J2^T can also be distinguished from the other genera in the family *Desulfovibrionaceae*, based notably on its NaCl requirement, major fatty acids, genomic DNA G+C content and 16S rRNA gene sequence similarity. Therefore, from the polyphasic evidence, strain J2^T represents a novel species of a novel genus for which the name *Pseudodesulfovibrio indicus* gen. nov., sp. nov. is proposed. In view of the data presented in this study, we also propose the reclassification of *D. piezophilus* as

Pseudodesulfovibrio piezophilus comb. nov., *D. profundus* as *P. profundus* comb. nov., *D. portus* as *P. portus* comb. nov. and *D. aespoeensis* as *P. aespoeensis* comb. nov.

Description of *Pseudodesulfovibrio* gen. nov.

Pseudodesulfovibrio (Pseu.do.de.sul.fo.vi'bri.o. Gr. adj. *pseudês* false; N.L. masc. n. *Desulfovibrio* a bacterial genus; N.L. masc. n. *Pseudodesulfovibrio* like *Desulfovibrio*, referring to the close relationship to this genus).

Cells are Gram-negative, anaerobic, motile vibrios (1-6×0.4-1 μm). Sulfate, thiosulfate, and sulfite are used as terminal electron acceptors. The G+C content of the chromosomal DNA is 50-63.5 mol%. The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}$ *ω*9*c* and/or $C_{16:0}$ 10-methyl (Summed feature 9) and iso-$C_{17:0}$.

The type species is *Pseudodesulfovibrio indicus*.

Description of *Pseudodesulfovibrio indicus* sp. nov.

Pseudodesulfovibrio indicus (in'di.cus. L. masc. adj. indicus. Indian, referring to the Indian Ocean, from where the type strain was isolated).

Cells are Gram-negative, anaerobic, motile vibrios (2-6×0.5 μm). Growth is observed at salinities from 0.2 to 6% (optimum 2.5%), from pH 5 to 8 (optimum 6.5-7), and at temperatures between 9 and 40 °C (optimum 30-35 °C) in TRM medium. Strain $J2^T$ uses lactate, malate, pyruvate, formate and hydrogen as energy sources. Sulfate, thiosulfate, sulfite,

fumarate, and nitrate are used as terminal electron acceptors, but not elemental sulfur nor nitrite. Lactate and pyruvate are fermented. The predominant fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:1}$ $\omega 9c$ and/or $C_{16:0}$ 10-methyl (Summed feature 9) and iso-$C_{17:0}$. Strain J2^T is piezophilic, growing optimally at 10 MPa (range 0–30 MPa). Sulfite reductase is present. The G+C content of the chromosomal DNA is 63.5 mol%.

The type strain J2^T (=MCCC 1A01867^T =DSM 101483^T) was isolated from a deep-sea serpentinized peridotite sample collected at a depth of 3173 m in a hydrothermal area of the Indian Ocean (27° 88' S, 63° 53' E; site 30I-TVG05).

Description of *Pseudodesulfovibrio portus* comb. nov.

Basonym: *Desulfovibrio portus* (Suzuki *et al.*, 2010)

The description is identical to that of Suzuki *et al.* (2009). The type strain is strain MSL79^T (=JCM 14722^T =DSM 19338^T).

Description of *Pseudodesulfovibrio aespoeensis* comb. nov.

Basonym: *Desulfovibrio aespoeensis* (Motamedi & Pedersen, 1998)

The description is identical to that of Motamedi and Pedersen (1998). The type strain is Aspo-2^T (=ATCC 700646^T =DSM 10631^T).

Description of *Pseudodesulfovibrio piezophilus* comb. nov.

Basonym: *Desulfovibrio piezophilus* (Khelaifia *et al.*, 2011)

The description is identical to that of Khelaifia *et al.* (2011). The type strain is C1TLV30^T (=DSM 21447^T =JCM 15486^T).

Description of *Pseudodesulfovibrio profundus* comb. nov.

Basonym: *Desulfovibrio profundus* (Bale *et al.*, 1997)

The description is identical to that of Bale *et al.* (1997). The type strain is strain 500-1^T (=MCCC 1A01905^T =DSM 11384^T).

Acknowledgements

We are very grateful to the R/V *Da Yang Yi Hao* operation teams for helping us to collect the hydrothermal samples. We thank Loïs Maignien for the assembly of the genome. This work was supported by the European Union Seventh Framework Program (FP7/2007-2013), Project *MaCuMBA* under grant agreement n° 311975, the "Laboratoire d'Excellence" *LabexMER* (ANR-10-LABX-19) co-funded by a grant from the French government under the program "Investissements d'Avenir", the PICS-InEE *Phypress*, the PHC Cai Yuanpei *Pandore* (N° 30412WG), the PHC Cai Yuanpei *Provirvent* (N° 34634WE), the National Program on Key Basic Research Project (973 Program, No.2012CB417304) and the National Natural Science Foundation of China (41411130113).

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Table 1. Whole-cell fatty acid profile of strain J2^T. Values are percentages of total fatty acids.

Fatty acid	**Value (%)**
iso-$C_{15:0}$	25.6
anteiso-$C_{15:0}$	8.3
Summed feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH)	3.6
iso-$C_{16:0}$	3.2
Summed feature 3 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH)	2.4
$C_{16:0}$	4.8
Summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or $C_{16:0}$ 10-methyl)	15.6
Summed feature 4 (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B)	4.5
anteiso-$C_{17:1}$ ω9*c*	1.3
iso-$C_{17:0}$	14.8
anteiso-$C_{17:0}$	2.3
iso-$C_{18:0}$ H	1.0
Summed feature 8 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*)	3.6
$C_{18:0}$	3.3

Table 2. Differentiating characteristics between strain J2^T and related species reclassified in the novel genus *Pseudodesulfovibrio*.

Taxa: 1, *Pseudodesulfovibrio indicus* gen. nov., sp. nov. J2^T; 2, *D. piezophilus* (Khelaifia *et al.*, 2011); 3, *D. profundus* (Bale *et al.*, 1997); 4, *D. portus* (Suzuki *et al.*, 2009); 5, *D. aespoeensis* (Motamedi & Pedersen, 1998). Characteristics are scored as: +, positive; –, negative; w, weakly positive; ND, not determined; SA, strictly anaerobic; DV, desulfoviridin.

[§]Information deduced from the genome sequence.

Characteristics	**1**	**2**	**3**	**4**	**5**
Morphology					
Rod-shaped	–	–	–	–	–
Vibrio-shaped	+	+	+	+	+
Cell motility	+	+	+	+	+
Size (μm)	2.0-6.0×0.4-0.6	2.0-4.0×0.5	1.0-2.0×0.5-1.0	1.8-2.3×0.7-1.0	1.7-2.5×0.5
O_2 metabolism	SA	SA	SA	SA	SA
Sulfite reductase	+[§]	DV	DV	DV	DV
Growth conditions					
Optimum temperature (°C)	30-35	30	25	35	25-30
Optimum pH	6.5-7	7.3	7	6.5	7.5
NaCl requirement	+	+	+	+	–
Optimum pressure (MPa)	10	10	15	ND	ND
Electron donors					
Fumarate	–	+	–	+	–
Acetate	–	–	–	–	–
Alanine	–	ND	–	–	–
Propionate	–	–	–	–	–
Ethanol	–	+	–	+	–
Formate	+	+	–	+	–
Malate	+	+	–	+	–
Hydrogen	+	+	+	+	+
Succinate	ND	–	ND	+	–
Electron acceptors					
Sulfate	+	+	+	+	+
Sulfite	+	+	+	+	ND
Sulfur	–	–	–	ND	+
Thiosulfate	+	+	+	+	+
Nitrate	w	–	+	ND	–
Fumarate	w	–	+	–	ND
Fermentation					
Malate	–	–	–	+	ND
Fumarate	–	+	–	+	ND
Lactate	+	–	+	–	ND
Major fatty acids	iso-$C_{15:0}$, anteiso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl), iso-$C_{17:0}$	$C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{17:1}$, $C_{18:0}$ and $C_{18:1}$	$C_{16:0}$, $C_{16:1}$, $C_{18:0,}$ $C_{18:1,}$ iso-$C_{15:0}$, iso-$C_{17:1}$ $\omega7$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$, iso-$C_{17:1}\omega9$	ND

DNA G+C content (mol%)	63.5	50.0	53.0	53.0	61.0
Isolation source	Deep hydro-thermal vent	Deep ma-rine wood falls	Marine sed-iments	Estuarine sediment	Deep groundwater rock

Table 3. Differentiating features between the novel genus *Pseudodesulfovibrio* and related genera in the family *Desulfovibrionaceae*.

Taxa: 1, *Pseudodesulfovibrio* (this study and Bale *et al.*, 1997; Motamedi & Pedersen, 1998; Suzuki *et al.*, 2009; Khelaifia *et al.*, 2011) ; 2, *Desulfovibrio* (Devereux *et al.*, 1990; Postgate & Campbell, 1966; Zhao *et al.*, 2012); 7, *Desulfobaculum* (Zhao *et al.*, 2012); 8, *Desulfocurvus* (Hamdi *et al.*, 2013); Klouche *et al.* (2009); 9, *Bilophila* (Baron *et al.*, 1989); 10, *Lawsonia* (McOrist *et al.*, 1995). Characteristics are scored as: +, positive; -, negative; w, weakly positive; ND, not determined; SA, strictly anaerobic; DV, desulfoviridin.

Characteristics	**1**	**6**	**7**	**8**	**9**	**10**
Morphology						
Rod-shaped	–	–	+	–	+	–
Vibrio-shaped	+	+	–	+	–	+
Cell motility	+	+	+	+	–	–
O_2 metabolism	SA	SA	SA	SA	SA	Microaer ophilic
Sulfite reductase	+, DV	DV	DV	–	W	ND
Growth conditions						
Optimum temperature (°C)	25-35	30-37	35-40	37-40	35	35-37
Optimum pH	6.5-7.5	7.2	7	6.9-7.1	ND	ND
NaCl requirement	±	±	+	–	–	–
Electron donors						
Fumarate	±	+	+	–	–	ND
Acetate	–	–	+	–	+	–
Alanine	–	ND	ND	ND	ND	ND
Propionate	–	ND	–	–	ND	ND
Ethanol	±	+	–	–	+	ND
Formate	±	+	–	+	+	–

Malate	±	+	+	–	ND	ND
Hydrogen	+	+	+	–	ND	ND
Succinate	±	–	+	–	ND	ND
Electron acceptors						
Sulfate	+	+	+	+	+	–
Sulfite	+	+	+	+	+	ND
Sulfur	±	–	–	–	ND	ND
Thiosulfate	+	+	–	+	+	ND
Nitrate	±	+	–	–	+	ND
Fumarate	±	ND	ND	ND	ND	ND
Fermentation						
Malate	±	ND	ND	ND	ND	ND
Fumarate	±	ND	ND	ND	ND	ND
Lactate	±	ND	ND	+	ND	ND
Major fatty acids	iso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl), iso-$C_{17:0}$	iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{17:1}\omega9c$	iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:1}\omega9c$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:0}$	iso-$C_{15:0}$, $C_{16:0}$, $C_{18:1}\omega9c$, $C_{19:0}$ cyclo $\omega9c$	ND
DNA G+C content (mol%)	50-63.5	47.0-60.0	64.5	67.2-70.0	59.2	34.0
Isolation source	Deep-sea hydrothermal vent, marine wood falls, marine or estuarine sediments, deep groundwater rocks	Soil	Mangrove sediments	Deep saline aquifer or wastewater digestor	Freshwater or clinical isolate	Porcine

Fig. 1. Neighbor-joining tree showing the phylogenetic positions of strain J2^T and representatives of some other related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch nodes. Bar, 0.01 nucleotide substitution rate (K_{nuc}) units.