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Pseudodesulfovibrio sediminis sp. nov., a mesophilic and neutrophilic sulfate-reducing bacterium isolated from sediment of a brackish lake

Ayaka Takahashi[1,2], Hisaya Kojima[2*], Miho Watanabe[3] and Manabu Fukui[2]

1. Graduate School of Environmental Science, Hokkaido University, Kita-10, Nishi-5, Kita-ku, Sapporo 060-0810, Japan

2. The Institute of Low Temperature Science, Hokkaido University. Kita-19, Nishi-8, Kita-ku, Sapporo 060-0819, Japan

3. Department of Biological Environment, Faculty of Bioresource Sciences, Akita Prefectural University. Shimo-Shinjyo Nakano, Akita 010-0195, Japan.

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*Corresponding author

Tel/fax number: +81 11 706 5460

E-mail: kojimah@lowtem.hokudai.ac.jp

The Institute of Low Temperature Science, Hokkaido University. Kita-19, Nishi-8, Kita-ku, Sapporo 060-0819, Japan

Abstract

A novel mesophilic and neutrophilic sulfate-reducing bacterium, strain SF6^T, was isolated from sediment of a brackish lake in Japan. Cells of strain SF6^T were motile and rod-shaped with length of 1.2–2.5 μm and width of 0.6–0.9 μm. Growth was observed at 10–37°C with an optimum growth temperature of 28°C. The pH range for growth was 5.8–8.2 with an optimum pH of 7.0. The most predominant fatty acid was anteiso-$C_{15:0}$. Under sulfate-reducing conditions, strain SF6^T utilized lactate, ethanol and glucose as growth substrate. Chemolithoautotrophic growth on H_2 was not observed, although H_2 was used as electron donor. Fermentative growth occurred on pyruvate. As electron acceptor, sulfate, sulfite, thiosulfate and nitrate supported heterotrophic growth of the strain. The complete genome of strain SF6^T is composed of a circular chromosome with length of 3.8 Mbp and G + C content of 54 mol%. Analyses of the 16S rRNA gene and whole genome sequence indicated that strain SF6^T belongs to the genus *Pseudodesulfovibrio* but distinct form all existing species in the genus. On the basis of its genomic and phenotypic properties, strain SF6^T (= DSM111931^T = NBRC 114895^T) is proposed as the type strain of a new species, with name of *Pseudodesulfovibrio sediminis* sp. nov.

40 **Introduction**

41 The genus *Pseudodesulfovibrio* encompasses species of Gram-stain-negative sulfate-
42 reducing bacteria with rod-shaped motile cells (Galushko and Kuever, 2019). The type
43 species is *P. indicus* (Cao et al., 2016). According to the List of Prokaryotic Names with
44 Standing in Nomenclature (LPSN), there are 10 species with validly published names in
45 this genus, as of the end of February 2022. They include 7 species which were originally
46 described as *Desulfovibrio* species, i.e., *D. halophilus* (Caumette *et al.* 1991), *D.*
47 *profundus* (Bale et al., 1997), *D. aespoeensis* (Motamedi and Pedersen 1998), *D.*
48 *tunisiensis* (Ben Ali Gam et al., 2009), *D. portus* (Suzuki et al., 2009), *D. piezophilus*
49 (Khelaifia et al., 2011), *D. senegalensis* (Thioye et al., 2017). These species were
50 transferred to the genus *Pseudodesulfovibrio* in subsequent works (Cao et al., 2016;
51 Galushko and Kuever 2019; Waite et al., 2020). *P. hydrargyri* (Ranchou-Peyruse et al.,
52 2018) and *P. mercurii* (Gilmour et al., 2021) were described as novel species of
53 *Pseudodesulfovibrio*, although their type strains had been classified in the genus
54 *Desulfovibrio* in the past. '*P. alkaliphilus*' (Frolova et al., 2021) and '*P. cashew*' (Zheng
55 et al., 2021) were recently proposed, while they have not been included in the validation
56 list yet. It has also been indicated that *D. oxyclinae* (Kreler et al., 1997), '*D.*
57 *dechloracetivorans*' (Sun et al., 2000) and '*Desulfovibrio brasiliensis*' (Warthmann et al.,

58 2005) should be reclassified into the genus *Pseudodesulfovibrio* (Galushko and Kuever
59 2019; Waite et al., 2020). Although *D. oxyclinae* is validly published name, proposed
60 name for its reclassification, '*P. oxyclinae*', has not been validated because its type strain
61 is only available in one culture collection (Waite et al., 2020). '*D. dechloracetivorans*'
62 cannot be validated or renamed, as its type strain is not available in culture collections at
63 present. On the other hand, the type strain of '*D. brasiliensis*' is currently available in two
64 culture collections (as DSM 15816 and JCM 12178). It was also indicated that
65 '*Paradesulfovibrio onnuriensis*' is the closest relative of *P. senegalensis* (Kim et al.,
66 2020), and belongs to a lineage in the *Pseudodesulfovibrio*.

67 Phylogenetic analysis based on the 16S rRNA gene indicated that there are two
68 distinct phylogenetic groups within the genus *Pseudodesulfovibrio* (Galushko and Kuever,
69 2019). The divergence between the groups (referred to as "cluster 1" and "cluster 2",
70 respectively) is large enough to separate them into different genera. In other words,
71 reclassification of cluster 2 as a separate genus is to be expected (Galushko and Kuever,
72 2019).

73 In this study, a novel sulfate-reducing bacterium isolated and characterized, as a
74 representative of a new species in the genus *Pseudodesulfovibrio*.

75

Materials and methods

Enrichment and isolation

The novel isolate, strain SF6^T was isolated from sediment of a brackish lake, Lake Akkeshi in Japan. Water depth of the sampling site (43.05° N 144.89° E) was 1.6 m. At the time of sampling, temperature and of pH of overlying water were 22.3℃ and 8.0 respectively. Throughout this study, a bicarbonate-buffered and sulfide-reduced defined medium was used as basal medium. The basal medium for marine sulfate-reducing bacteria was prepared as described previously (Widdel & Bak, 1992), and headspace of culture bottles was filled with N_2 /CO_2 (80 : 20, v/v). To establish the first enrichment, 0.2 g of the sediment was taken from 5–6 cm layer and inoculated into the basal medium supplemented with 5 mM formate. The culture bottle was incubated at 18°C in the dark. The grown culture was transferred to the same medium three times. The resulting enrichment culture was subjected to agar shake dilution. A black colony was picked up in the same medium and incubated at 18°C. After growth became visible, grown culture was transferred to the basal medium supplemented with 5 mM lactate, and incubation temperature was changed to 28°C. Finally, pure culture of strain SF6^T was obtained from the culture grown on lactate, by agar shake dilution. Purity of the resulting culture was

95 confirmed by microscopic observation with a phase-contrast microscope (Axioplan 2;
96 Zeiss) and repeated sequencing of the 16S rRNA gene fragments.

97

98 Phylogenetic analysis based on the 16S rRNA gene

99 Nearly full length of the 16S rRNA gene was amplified by PCR with primer pair of
100 27F and 1492R (Lane, 1991). The PCR product was directly sequenced, and the resulting
101 sequence was subjected to blastn search to identify the closest relatives. Phylogenetic
102 analysis was conducted using MEGA version 11 (Tamura et al., 2021), as described below.
103 The 16S rRNA gene sequence of strain SF6^T was aligned with those of type strains in the
104 genus *Pseudodesulfovibri*o, using the MUSCLE algorithm. With the resulting alignment,
105 models for genetic distance calculation were evaluated by using the model selection tool
106 in MEGA. With the best model giving the lowest Bayesian Information Criterion (BIC)
107 score, genetic distances were calculated by excluding positions with gaps.

108

109 Phenotypic characterization

110 In all experiments for phenotypic characterizations, strain SF6^T was cultured at 28°C
111 in the basal medium supplemented with 5 mM lactate, unless otherwise specified. Its
112 growth was monitored as turbidity of cultures.

113 Effect of temperature on growth was examined by culturing at 5, 8, 10, 13, 15, 18, 22,
114 25, 28, 30, 32, 35, 37, 42 and 45°C. Effect of salinity on growth was examined by altering
115 NaCl concentration to 0.1, 0.6, 1.1, 1.6, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and
116 7.0% (w/v). To investigate effect of pH on growth, composition of the medium was
117 modified by replacing bicarbonate with 20 mM MES, MOPS, or TAPS. The MES-
118 buffered medium was used to test growth at pH 5.3, 5.5, 5.8, 6.0, 6.2, 6.4, 6.6 and 6.8, by
119 adjusting the pH with NaOH. In the same way, MOPS-buffered medium was used for pH
120 6.5, 7.0, 7.3, 7.5, 7.8. The pH of TAPS-buffered medium was adjusted to 7.8, 8.0, 8.2,
121 8.4, 8.6, 8.8 and 9.0.

122 Utilization of growth substrates was tested in the basal medium, supplemented with
123 one of the following substrates (mM; unless otherwise specified); formate (5), acetate (5),
124 propionate (2), lactate (5), butyrate (5), isobutyrate (5), malate (5), succinate (5), fumarate
125 (5), benzoate (2), pyruvate (5), citrate (5), methanol (5), ethanol (5), glucose (5) and yeast
126 extract (0.05% w/v). Hydrogen-dependent autotrophic growth was tested under a gas
127 mixture of N_2, H_2 and CO_2 (50:40:10 v/v/v, 200 kPa total pressure). For formate and
128 hydrogen, growth was also assessed in the presence of acetate (1 mM) as carbon source.
129 Sulfide production was assessed by mixing the culture with sulfide detection reagent
130 consisting of 5 mM $CuCl_2$ and 50 mM HCl (Cord-Ruwisch, 1985). Fermentative growth

131 and utilization of electron acceptors were tested with a modified version of the basal
132 medium which contained no sulfate. In the test of fermentation, the sulfate-free medium
133 was supplemented with ethanol (5), pyruvate (10), lactate (5), succinate (5), malate (5) or
134 fumarate (5). As electron accepters, thiosulfate (10), elemental sulfur (0.5 % w/v), sulfite
135 (1 and 5), nitrate (10) and tetrathionate (5) were tested in the presence of 5 mM lactate.

136 For cellular fatty acid analysis, strain SF6 was grown in the basal medium supplemented
137 with 20 mM lactate. The fatty acid profile was obtained with the Sherlock Microbial
138 Identification System (MIDI) version 6.0 (database; MOORE6).

139

140 Genomic characterization

141 Whole genome sequencing was performed using the platforms of Illumina NextSeq
142 and Nanopore GridION. Short and long reads from the platforms were subjected to hybrid
143 assembly using Unicycler (Ver 0.4.7). The assembled genome sequence was annotated
144 with DFAST (Tanizawa et al., 2018).

145 As genome relatedness indices between SF6^T and its close relatives, values
146 of average nucleotide identity (ANI) and average amino acid identity (AAI) were
147 calculated by using tools provided by Kostas lab (http://enve-omics.ce.gatech.edu/). The
148 Genome-to-Genome Distance Calculator provided by DSMZ were used to calculate

149 digital DNA–DNA hybridization (dDDH) values, by applying the formula 2 (Meier-
150 Kolthoff et al., 2013).

151 A genome-based taxonomic classification was carried out with the Genome
152 Taxonomy Database (GTDB) (Parks et al., 2018). Taxonomic position of the strain SF6^T
153 in the GTDB (release 95) was identified using GTDB-Tk (Chaumeil et al., 2020).

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155

156 **Results and Discussion**

157

158 Physiological and chemotaxonomic characteristics

159 The fundamental characteristics of strain SF6^T are summarized in Table 1 and
160 presented in the species description. Cells of strain SF6^T were motile, rod-shaped, 0.6–
161 0.9 μm in width, 1.2–2.5 μm in length. Under the sulfate-reducing conditions, strain
162 SF6^T grew at 10–37°C with optimum growth at 28°C, and grew at pH range of 5.8–8.2
163 with the optimum pH of 7.0. The NaCl range for growth was 0.6–6.5 %, with optimum
164 growth at 2.0%.

165 In the presence of sulfate, lactate, ethanol and glucose supported heterotrophic
166 growth of SF6^T accompanying sulfide production. The molar ratio of generated sulfide

167 to consumed lactate never exceeded 0.8. This upper limit is clearly lower than expected
168 ratio for complete oxidation of lactate (1.5), suggesting incomplete lactate oxidation by
169 strain SF6^T. Chemolithotrophic growth on hydrogen was not observed. Formate and
170 hydrogen were utilized as electron donor, but acetate was required as carbon source for
171 growth. Among the substrate tested, only pyruvate supported fermentative growth of the
172 strain. The pyruvate-dependent growth was also observed in the presence of sulfate, but
173 sulfide was not detected in this case. This means that strain SF6^T grows by fermentation
174 of pyruvate, but does not use it as electron donor for sulfate reduction. This pattern of
175 pyruvate utilization was previously reported in *P. alkaliphilus* F-1^T (Frolova et al.,
176 2021). In addition to sulfate, sulfite, thiosulfate and nitrate were used as electron
177 acceptor for lactate oxidation.

178 In the cellular fatty acid profile of cells grown on lactate, anteiso-$C_{15:0}$ was
179 predominant, accounting for 21% of total. Other major components (>10% of total)
180 were summed feature 10 ($C_{18:1}\omega7c$ and/or unknown 17.834; 13.3%), $C_{18:0}$ (11.7%), $C_{16:1}\omega7c$ (11.6%) and $C_{16:0}$ (10.1%). All fatty acids detected are shown in Table S1.
181
182
183 Genomic features
184 The complete genome of strain SF6^T was reconstructed by assembling

185 3,394,816 DNBSEQ reads and 126,221 GridION reads, with coverage of 330-fold. It
186 consists of a single circular chromosome with size of chromosome 3,764,150 bp and
187 G+C content of 54.0% (Table 1). In the genome, 3527 protein-coding sequences, 9
188 RNA genes and 57 tRNA genes were predicted. Three copies of the 16S rRNA gene had
189 identical sequence. The encoded proteins include those involved in glycolysis via
190 Embden-Meyerhof pathway, membrane transport of monosaccharides, respiratory
191 nitrate reduction to nitrite and nitrogen fixation.

192 Some genes encoding key enzymes for inorganic carbon fixation by sulfate
193 reducers were not identified in the genome of strain SF6^{T}. The genome lacks the *fhs* and
194 *acsB* genes, encoding and formate-tetrahydrofolate ligase and carbon monoxide
195 dehydrogenase/acetyl-CoA synthase, respectively. These enzymes are key components
196 of the Wood–Ljungdahl pathway. In addition, formate-tetrahydrofolate ligase also plays
197 a critical role in carbon fixation via reductive glycine pathway (Sánchez-Andrea et al.,
198 2020).

199

200 Taxonomic assignment

201 In the blastn analysis of the 16S rRNA gene sequence, high sequence identities were

202 observed between strain SF6^T and type strains of *Pseudodesulfovibrio* species (Table 1).
203 Among them, *P. indicus* J2^T showed the highest identity of 97.4%. By constructing
204 phylogenetic tree of the 16S rRNA gene, it was indicated that strain SF6^T belongs to the
205 genus *Pseudodesulfovibrio* (Fig. 1). The tree also indicated that strain SF6^T is
206 phylogenetically distinct from existing species, and belongs to the cluster 1 defined in
207 the previous study (Galushko and Kuever, 2019).

208 Some genomic characteristics are consistent with the results of 16S rRNA gene
209 analysis which suggested that strain SF6^T represents a novel species. The G + C content
210 of strain SF6^T is distinct from those of other type strains of *Pseudodesulfovibri*o species
211 (except for *P. profundus*), with differences greater than 4% (Table 1). In general,
212 differences between genomic G + C contents of strains from the same species are 1% or
213 smaller (Meier-Kolthoff et al., 2014). The values of ANI, AAI and dDDH between
214 strain SF6^T and the type strains of *Pseudodesulfovibri*o species are shown in Table 1.
215 All these values are lower than threshold for species delineation. Further, the genome of
216 strain SF6^T was subjected to phylogenomic analysis with the GTDB-tk. By
217 phylogenetic analysis based on 120 conserved proteins (Parks et al., 2018), strain SF6^T
218 was classified as a novel species in the genus *Pseudodesulfovibrio*.

219 The creation of new species, suggested by the phylogenetic analyses, is

220 supported by some phenotypic characteristics which differentiate strain SF6^T from other

221 species (Table 1). For the species represented by strain SF6^T, the name

222 *Pseudodesulfovibrio sediminis* sp. nov. is proposed here.

223

224 **Description of *Pseudodesulfovibrio sediminis* sp. nov.**

225 *Pseudodesulfovibrio sediminis* (se.di'mi.nis. L. gen. n. *sediminis,* of sediment).

226 Cells and rod shaped, 1.2–2.5 μm in length and 0.6–0.9 μm in width. Grows at 10–

227 37°C with an optimum growth at 28°C. The pH range for growth is 5.8–8.2, with an

228 optimum pH of 7.0. Grows with 0.6–6.5% NaCl (optimum 2.0%). Predominant fatty acid

229 is anteiso-$C_{15:0}$. Under sulfate-reducing conditions, grows on lactate, ethanol and glucose.

230 Acetate, propionate, butyrate, isobutyrate, malate, succinate, fumarate, benzoate,

231 pyruvate, citrate, methanol and yeast extract are not utilized as growth substrate. Formate

232 and hydrogen are utilized as electron donor for growth with acetate as carbon source.

233 Ferments pyruvate but does not use it as electron doner for sulfate reduction. Does not

234 ferment malate and fumarate. Uses sulfate, sulfite, thiosulfate and nitrate as electron

235 acceptor. G + C content of genomic DNA of the type strain is 54.0 mol%.

236 The type strain SF6^T (= DSM111931^T = NBRC 114895^T) was isolated from sediment

of a brackish lake in Japan.

The GenBank/EMBL/DDBJ accession number for the complete genome of strain SF6^T is AP024485.

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Statements and Declarations

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356 interests to disclose.

357

358 **Figure legend**

359 Fig. 1. Phylogenetic position of strain SF6^{T} within the genus *Pseudodesulfovibrio*, based

360 on the 16S rRNA gene sequences. The phylogenetic tree was inferred by using the

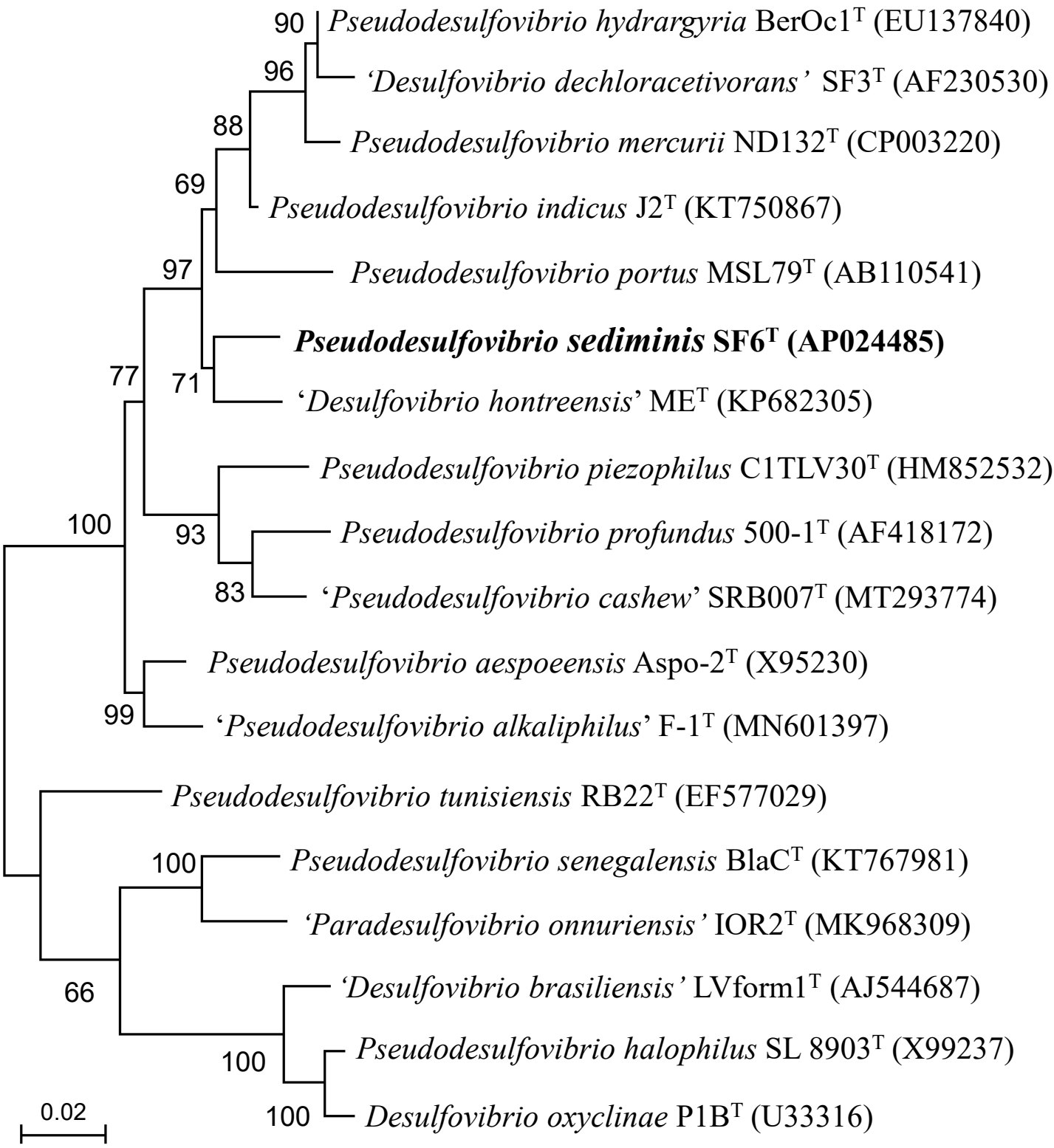
361 maximum likelihood method and Kimura 2-parameter model. A discrete gamma

362 distribution was used to model evolutionary rate differences among sites, allowing some

363 sites to be invariable. All positions containing gaps and missing data were eliminated,

364 leaving a total of 1340 positions in the final dataset. Numbers on nodes represent

365 percentage values of 1000 bootstrap resampling.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Pseudodesulfovibrio hydrargyria BerOc1T (EU137840)
'Desulfovibrio dechloracetivorans' SF3T (AF230530)
Pseudodesulfovibrio mercurii ND132T (CP003220)
Pseudodesulfovibrio indicus J2T (KT750867)
Pseudodesulfovibrio portus MSL79T (AB110541)
Pseudodesulfovibrio sediminis SF6T (AP024485)
'Desulfovibrio hontreensis' METT (KP682305)
Pseudodesulfovibrio piezophilus C1TLV30T (HM852532)
Pseudodesulfovibrio profundus 500-1T (AF418172)
'Pseudodesulfovibrio cashew' SRB007T (MT293774)
Pseudodesulfovibrio aespoeensis Aspo-2T (X95230)
'Pseudodesulfovibrio alkaliphilus' F-1T (MN601397)
Pseudodesulfovibrio tunisiensis RB22T (EF577029)
Pseudodesulfovibrio senegalensis BlaCT (KT767981)
'Paradesulfovibrio onnuriensis' IOR2T (MK968309)
'Desulfovibrio brasiliensis' LVform1T (AJ544687)
Pseudodesulfovibrio halophilus SL 8903T (X99237)
Desulfovibrio oxyclinae P1BT (U33316)