Pseudodesulfovibrio sediminis sp. nov., a Mesophilic and Neutrophilic Sulfate-reducing Bacterium Isolated From Sediment of a Brackish Lake

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

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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 formate, lactate, ethanol and glucose as growth substrate. Chemolithoautotrophic growth on H₂ was also observed. 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 from 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.

Introduction

The genus *Pseudodesulfovibrio* encompasses species of Gram-stain-negative sulfate-reducing bacteria with rod-shaped motile cells (Galushko and Kuever, 2019). The type species is *P. indicus* (Cao et al., 2016). According to the List of Prokaryotic Names with Standing in Nomenclature (LPSN), there are 10 species with validly published names in this genus, as of the end of 2021. They include 7 species which were originally described as *Desulfovibrio* species, i.e., *D. halophilus* (Caumette et al. 1991), *D. profundus* (Bale et al., 1997), *D. aespoeensis* (Motamedi and Pedersen 1998), *D. tunisiensis* (Ben Ali Gam et al., 2009), *D. portus* (Suzuki et al., 2009), *D. piezophilus* (Khelaifia et al., 2011), *D. senegalensis* (Thioye et al., 2017). In contrast to these species transferred to the genus *Pseudodesulfovibrio* in subsequent works (Cao et al., 2016; Galushko and Kuever 2019; Waite et al., 2020), *P. hydrargyri* (Ranchou-Peyruse et al., 2018) and *P. mercurii* (Gilmour et al., 2021) were described as novel species of *Pseudodesulfovibrio*, although their type strains had been classified in the genus *Desulfovibrio* in the past. In addition to the 10 species mentioned above, some other species have been described in this genus. As the newest species of the genus, '*P. alkaliphilus*' (Frolova et al., 2021) and '*P. cashew*' (Zheng et al., 2021) were recently proposed, while they have not been included in the validation list yet. It has also been indicated that *D. oxyclinae* (Kreler et al., 1997), '*D.*

dechloracetivorans' (Sun et al., 2000) and '*Desulfovibrio brasiliensis*' (Warthmann et al., 2005) should be reclassified into the genus *Pseudodesulfovibrio* (Galushko and Kuever 2019; Waite et al., 2020). Although *D. oxyclinae* is validly published name, proposed name for its reclassification, '*P. oxyclinae*', has not been validated because its type strain is only available in one culture collection (Waite et al., 2020).

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

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

Phylogenetic analysis based on the 16S rRNA gene

Nearly full length of the 16S rRNA gene was amplified by PCR with primer pair of 27F and 1492R (Lane, 1991). The PCR product was directly sequenced, and the resulting sequence was subjected to blastn search to identify the closest relatives. Phylogenetic analysis was conducted using MEGA version 11 (Tamura et al., 2021), as described below. The 16S rRNA gene sequence of strain SF6^T was aligned with those of type strains in the genus *Pseudodesulfovibrio*, using the MUSCLE algorithm. With the resulting alignment, models for genetic distance calculation were evaluated by using the model selection tool

in MEGA. With the best model giving the lowest Bayesian Information Criterion (BIC) score, genetic distances were calculated by excluding positions with gaps.

Phenotypic characterization

In all experiments for phenotypic characterizations, strain SF6^T was cultured at 28°C in the basal medium supplemented with 5 mM lactate, unless otherwise specified.

Effect of temperature on growth was examined by culturing at 5, 8, 10, 13, 15, 18, 22, 25, 28, 30, 32, 35, 37, 42 and 45°C. Effect of salinity on growth was examined by altering 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 and 6.5% (w/v). To investigate effect of pH on growth, composition of the medium was modified by replacing bicarbonate with 20 mM MES, MOPS, or TAPS. The MES-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 adjusting the pH with NaOH. In the same way, MOPS-buffered medium was used for pH 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, 8.4, 8.6, 8.8 and 9.0.

Utilization of growth substrates was tested in the basal medium, supplemented with one of the following substrates (mM; unless otherwise specified); formate (5), acetate (5), propionate (2), lactate (5), butyrate (5), isobutyrate (5), malate (5), succinate (5), fumarate

(5), benzoate (2), pyruvate (5), citrate (5), methanol (5), ethanol (5), glucose (5) and yeast extract (0.05% w/v). Hydrogen-dependent autotrophic growth was tested under a gas mixture of N₂, H₂ and CO₂ (50:40:10 v/v/v, 200 kPa total pressure). Fermentative growth and utilization of electron acceptors were tested with a modified version of the basal medium which contained no sulfate. In the test of fermentation, the sulfate-free medium was supplemented with ethanol (5), pyruvate (10), lactate (5), succinate (5), malate (5) or fumarate (5). As electron acceptors, thiosulfate (10), elemental sulfur (0.5 % w/v), sulfite (1 and 5), nitrate (10) and tetrathionate (5) were tested in the presence of 5 mM lactate.

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

Genomic characterization

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

As genome relatedness indices between SF6^T and its close relatives, values

of average nucleotide identity (ANI) and average amino acid identity (AAI) were calculated by using tools provided by Kostas lab (<http://enve-omics.ce.gatech.edu/>).

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

Results and Discussion

Physiological and chemotaxonomic characteristics

The fundamental characteristics of strain SF6^T are summarized in Table 1 and presented in the species description. Cells of strain SF6^T were motile, rod-shaped, 0.6–0.9 µm in width, 1.2–2.5 µm in length. Under the sulfate-reducing conditions, strain SF6^T grew at 10–37°C with optimum growth at 28°C, and grew at pH range of 5.8–8.2 with the optimum pH of 7.0. The NaCl range for growth was 0.6–6.0 %, with optimum growth at 2.0%.

In the presence of sulfate, formate, lactate, ethanol and glucose supported heterotrophic growth of SF6^T accompanying sulfide production. Among the substrate

tested, only pyruvate supported fermentative growth of the strain. The pyruvate-dependent growth was also observed in the presence of sulfate, but sulfide was not detected even in this case. This means that strain SF6^T grows by fermentation of pyruvate, but does not use it as electron donor for sulfate reduction. This pattern of pyruvate utilization was previously reported in by *P. alkaliphilus* F-1^T (Frolova et al., 2021). Chemolithoautotrophic growth was observed in the presence of H₂ gas. In addition to sulfate, sulfite, thiosulfate and nitrate were used as electron acceptor for lactate oxidation.

In the cellular fatty acid profile of cells grown on lactate, anteiso-C_{15:0} was predominant, accounting for 21% of total. Other major components (>10% of total) were summed feature 10 (C_{18:1}ω7c and/or unknown 17.834; 13.3%), C_{18:0} (11.7%), C_{16:1}ω7c (11.6%) and C_{16:0} (10.1%). All fatty acids detected are shown in Table S1.

Genomic features

The complete genome of strain SF6^T was reconstructed by assembling 3,394,816 DNBSEQ reads and 126,221 GridION reads, with coverage of 330-fold. It consists of a single circular chromosome with size of chromosome 3,764,150 bp and G+C content of 54.0% (Table 1). In the genome, 3527 protein-coding sequences, 9

RNA genes and 57 tRNA genes were predicted. Three copies of the 16S rRNA gene had identical sequence. The encoded proteins include those involved in glycolysis via Embden-Meyerhof pathway, membrane transport of monosaccharides, respiratory nitrate reduction to nitrite and nitrogen fixation.

Taxonomic assignment

In the blastn analysis of the 16S rRNA gene sequence, high sequence identities were observed between strain SF6^T and type strains of *Pseudodesulfovibrio* species (Table 1). Among them, *P. indicus* J2^T showed the highest identity of 97.4%. By constructing phylogenetic tree of the 16S rRNA gene, it was indicated that strain SF6^T belongs to the genus *Pseudodesulfovibrio* (Fig. 1). The tree also indicated that strain SF6^T phylogenetically distinct from existing species.

The analysis of 16S rRNA gene suggested that strain SF6^T represents a novel species in the genus *Pseudodesulfovibrio*, and it was supported by some genomic characteristics. The G + C content of strain SF6^T is distinct from those of other type strains of *Pseudodesulfovibrio* species (except for *P. profundus*), with differences greater than 4% (Table 1). In general, differences between genomic G + C contents of

strains from the same species are 1 % or smaller (Meier-Kolthoff et al., 2014). The values of ANI and AAI between strain SF6^T and the type strains of *Pseudodesulfovibrio* species are shown in Table 1. All these values are lower than threshold for species delineation. Further, the genome of strain SF6^T was subjected to phylogenomic analysis with the GTDB-tk. By phylogenetic analysis based on 120 conserved proteins (Parks et al., 2018), strain SF6^T was classified into a novel species in the genus *Pseudodesulfovibrio*.

The analyses of genetic information consistently indicated that a novel species should be created in the genus *Pseudodesulfovibrio*, to accommodate strain SF6^T. The creation of new species is supported by some phenotypic characteristics which differentiate strain SF6^T from other species (Table 1). For the species represented by strain SF6^T, the name *Pseudodesulfovibrio sediminis* sp. nov. is proposed here.

Description of *Pseudodesulfovibrio sediminis* sp. nov.

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

Cells and rod shaped, 1.2–2.5 µm in length and 0.6–0.9 µm in width. Grows at 10–37°C with an optimum growth at 28°C. The pH range for growth is 5.8–8.2, with an

optimum pH of 7.0. Grows with 0.6–6.0% NaCl (optimum 2.0%). Predominant fatty acid is anteiso-C₁₅:0. Under sulfate-reducing conditions, grows on formate, lactate, ethanol and glucose. Acetate, propionate, butyrate, isobutyrate, malate, succinate, fumarate, benzoate, pyruvate, citrate, methanol and yeast extract are not utilized as growth substrate. Ferments pyruvate but does not use it as electron donor for sulfate reduction. Does not ferment malate and fumarate. Grows chemolithoautotrophically on H₂ and CO₂. Uses sulfate, sulfite, thiosulfate and nitrate as electron acceptor. G + C content of genomic DNA of the type strain is 54.0 mol%. 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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218 **Reference**

- 219 Bale SJ, Goodman K, Rochelle PA, Marchesi JR, Fry JC, Weightman AJ, Parkes RJ
220 (1997) *Desulfovibrio profundus* sp. nov., a novel barophilic sulfate-reducing
221 bacterium from deep sediment layers in the Japan Sea. Int J Syst Evol Microbiol 47:
222 515–521. <https://doi.org/10.1099/00207713-47-2-515>
- 223 Ben Ali Gam Z, Oueslati R, Abdelkafi S, Casalo L, Tholozan JL, Labat M (2009)
224 *Desulfovibrio tunisiensis* sp. nov., a novel weakly halotolerant, sulfate-reducing
225 bacterium isolated from exhaust water of a Tunisian oil refinery. Int J Syst Evol
226 Microbiol 59: 1059–1063. <https://doi.org/10.1099/ijms.0.000943-0>
- 227 Cao J, Gayet N, Zeng X, Shao Z, Jebbar M, Alain K (2016) *Pseudodesulfovibrio indicus*
228 gen. nov., sp. nov., a piezophilic sulfate-reducing bacterium from the Indian Ocean
229 and reclassification of four species of the genus *Desulfovibrio*. Int J Syst Evol
230 Microbiol 66: 3904–3911. <https://doi.org/10.1099/ijsem.0.001286>
- 231 Caumette P, Cohen Y, Matheron R (1991) Isolation and characterization of *Desulfovibrio*
232 *halophilus* sp. nov., a halophilic sulfate-reducing bacterium isolated from Solar Lake
233 (Sinai). Syst Appl Microbiol 14:33–38 <https://doi.org/10.1016/S0723->
234 2020(11)80358-9

235 Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH (2020) GTDB-Tk: a toolkit to classify
236 genomes with the genome taxonomy database. *Bioinformatics* 36:1925–1927.
237 <https://doi.org/10.1093/bioinformatics/btz848>

238 Frolova A, Merkel AY, Kuchierskaya AA, Bonch-Osmolovskaya EA, Slobodkin AI
239 (2021) *Pseudodesulfovibrio alkaliphilus*, sp. nov., an alkaliphilic sulfate-reducing
240 bacterium isolated from a terrestrial mud volcano. *Ant van Leeuw* 114:1387–1397.
241 <https://doi.org/10.1007/s10482-021-01608-5>

242 Galushko A, Kuever M (2019) *Pseudodesulfovibrio* In: *Bergey's Manual of Systematics*
243 *of Archaea and Bacteria*. <https://doi.org/10.1002/9781118960608.gbm01574>

244 Gilmour CC, Soren AB, Gionfriddo CM, Podar M, Wall JD, Brown SD et al. (2021)
245 *Pseudodesulfovibrio mercurii* sp. nov., a mercury-methylating bacterium isolated
246 from sediment. *Int J Syst Evol Microbiology* 71:3
247 <https://doi.org/10.1099/ijsem.0.004697>

248 Khelaifia S, Fardeau ML, Pradel N, Aussignargues C, Garel M, Tamburini C, et al. (2011)
249 *Desulfovibrio piezophilus* sp. nov., a piezophilic, sulfate-reducing bacterium isolated
250 from wood falls in the Mediterranean Sea. *Int J Syst Evol Microbiol* 61:2706–2711.
251 <https://doi.org/10.1099/ijms.0.028670-0>

252 Krekeler D, Sigalevich P, Teske A, Cypionka H, Cohen Y (1997) A sulfate-reducing
 253 bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai),
 254 *Desulfovibrio oxyclinae* sp. nov. Arch Microbiol 167:369–375.
 255 <https://doi.org/10.1007/s002030050457>

256 Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M., (Eds.),
 257 Nucleic acid techniques in bacterial systematics, John Wiley & Sons, Ltd, New York,
 258 pp. 115–175

259 Meier-Kolthoff JP, Göker M, Klenk H-P (2014) Taxonomic use of DNA G+C content and
 260 DNA-DNA hybridization in the genomic age. Int J Syst Evol Microbiol 64:352–356.
 261 <https://doi.org/10.1099/ij.s.0.056994-0>

262 Motamedi M, Pedersen K (1998) *Desulfovibrio aespoeensis* sp. nov., a mesophilic
 263 sulfate-reducing bacterium from deep groundwater at äspö hard rock laboratory,
 264 Sweden. Int J Syst Bacteriol 48:311–315. <https://doi.org/10.1099/00207713-48-1-311>

265 Parks DH, Chuvpochina M, Waite DW, Rinke C, Skarszewski A, Chaumeil PA,
 266 Hugenholtz P (2018) A standardized bacterial taxonomy based on genome phylogeny
 267 substantially revises the tree of life. Nat Biotechnol 36:996–1004.
 268 <https://doi.org/10.1038/nbt.4229>

269 Ranchou-Peyruse M, Goni-Urriza M, Guignard M, Goas M, Ranchou-Peyruse A,
 270 Guyoneaud R (2018) *Pseudodesulfovibrio hydrargyri* sp. nov., a mercury-methylating
 271 bacterium isolated from a brackish sediment. Int J Syst Evol Microbiol 68: 1461–
 272 1466. <https://doi.org/10.1099/ijsem.0.002173>

273 Sun B, Cole JR, Sanford RA, Tiedje JM (2000) Isolation and characterization of
 274 *Desulfovibrio dechloracetivorans* sp. nov., a marine dechlorinating bacterium
 275 growing by coupling the oxidation of acetate to the reductive dechlorination of 2-
 276 chlorophenol. Appl Environ Microbiol 66:2408–2413.
 277 <https://doi.org/10.1128/AEM.66.6.2408-2413.2000>

278 Suzuki D, Ueki A, Amaishi A, Ueki K (2009) *Desulfovibrio portus* sp. nov., a novel
 279 sulfate-reducing bacterium in the class *Deltaproteobacteria* isolated from an estuarine
 280 sediment. J Gen Appl Microbiol 55:125–133. <https://doi.org/10.2323/jgam.55.125>

281 Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics
 282 Analysis version 11. Mol Biol Evol 38:3022–3027.
 283 <https://doi.org/10.1093/molbev/msab120>

284 Tanizawa Y, Fujisawa T, Nakamura Y (2018) DFAST: a flexible prokaryotic genome
 285 annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039.

286 <https://doi.org/10.1093/bioinformatics/btx713>

287 Thioye A, Gam ZBA, Mbengue M, Cayol JL, Joseph-Bartoli M, Toure-Kane C et al.

288 (2017) *Desulfovibrio senegalensis* sp. nov., a mesophilic sulfate reducer isolated from

289 marine sediment. Int J Syst Evol Microbiol 67: 3162–3166.

290 <https://doi.org/10.1099/ijms.0.028670-0>

291 Waite DW, Chuvochina M, Pelikan C, Parks, DH, Yilmaz P, Wagner M et al. (2020)

292 Proposal to reclassify the proteobacterial classes *Deltaproteobacteria* and *Oligoflexia*,

293 and the phylum *Thermodesulfobacteria* into four phyla reflecting major functional

294 capabilities. Int J Syst Evol Microbiol 70:5972–6016.

295 <https://doi.org/10.1099/ijsem.0.004213>

296 Warthmann R, Vasconcelos C, Sass H, McKenzie JA (2005) *Desulfovibrio brasiliensis*

297 sp. nov., a moderate halophilic sulfate-reducing bacterium from Lagoa Vermelha

298 (Brazil) mediating dolomite formation. Extremophiles 9: 255–261.

299 <https://doi.org/10.1007/s00792-005-0441-8>

300 Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: The

301 prokaryotes. Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K. (ed).

302 Springer, pp. 3352–3378

Zheng R, Wu S, Sun C (2021) *Pseudodesulfovibrio cashew* sp. nov., a novel deep-sea sulfate-reducing bacterium, linking heavy metal resistance and sulfur cycle. Microorganisms 9:429. <https://doi.org/10.3390/microorganisms9020429>

Statements and Declarations

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Figure legend

Fig. 1. Phylogenetic position of strain SF6^T within the genus *Pseudodesulfovibrio*, based on the 16S rRNA gene sequences. The phylogenetic tree was inferred by using the maximum likelihood method and Kimura 2-parameter model. A discrete gamma distribution was used to model evolutionary rate differences among sites, allowing some sites to be invariable. All positions containing gaps and missing data were eliminated, leaving a total of 1346 positions in the final dataset. Numbers on nodes represent percentage values of 1000 bootstrap resampling.

Table 1. Differential characteristics of strain SF6^T and type strains of *Pseudodesulfovibrio* species. Strains: 1, SF6^T; 2, *P. indicus* J2^T (Cao et al., 2016); 3, *P. halophilus* SL 8903^T (Caumette et al. 1991); 4, *P. profundus* 500-1^T (Bale et al., 1997); 5, *P. aespoensis* Aspo-2^T (Motamedi and Pedersen 1998); 6, *P. tunisiensis* RB22^T (Ben Ali Gam et al., 2009); 7, *P. portus* MSL79^T (Suzuki et al., 2009); 8, *P. piezophilus* C1TLV30^T (Khelaifia et al., 2011); 9, *P. senegalensis* BLaC1^T (Thioye et al., 2017); 10, *P. hydrargyri* BerOc1^T (Ranchou-Peyruse et al., 2018); 11, *P. mercurii* ND132^T (Gilmour et al., 2021); 12, *D. oxycliniae* (= '*P. oxycliniae*') P1B^T (Kreler et al., 1997); 13, '*P. cashew*' SRB007^T (Zheng et al., 2021); 14, '*P. alkaliphilus*' F-1^T (Frolova et al., 2021). Data were retrieved from respective references except for genomic features of some strains.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimum growth conditions														
NaCl (%)	2.0	2.5	6.0-7.0	0.6-8	0.7	4.0	2.0	2.5	3.0	1.5	2.0	5-10	5.0	0.5-1.0
Temperature (°C)	28	30-35	35	25	25-30	37	35	30	40	30	32	35	30	24
pH	7.0	6.5-7.0	7.0	7.0	7.5	7.0	6.5	7.3	7.5	ND	7.8	7.4	7.0	9.5
Growth														
at 42°C or higher	—	—	+	+	—	+	—	+	+	—	+	ND	+	+
at 10°C or lower	+	+	—	—	+	—	+	—	—	—	—	ND	+	+
at 9 or higher pH	—	—	—	+	—	+	—	—	—	—	—	—	—	+
Electron donor														
Formate	+	+	+	—	—	+	+	+	+	—	+	ND	+	—
Acetate	—	—	—	+	—	—	—	—	+	—	—	—	+	—
Succinate	—	ND	—	ND	—	+	+	—	ND	—	—	—	+	ND
Fumarate	—	—	—	—	—	+	+	+	—	+	+	—	+	+
Malate	—	+	—	—	—	—	+	+	—	—	—	+	+	—
Methanol	—	ND	ND	ND	—	+	—	ND	—	—	ND	—	+	ND
Ethanol	+	—	+	—	—	—	+	+	—	+	—	+	+	—
Glucose	+	ND	ND	ND	ND	ND	ND	ND	ND	—	ND	—	ND	+
Electron acceptor														
Elemental sulfur	—	—	+	—	+	+	ND	—	—	—	ND	+	—	+
Nitrate	+	+	—	+	—	—	ND	—	—	—	—	—	+	—
Fermentation														
Lactate	—	+	ND	+	ND	ND	—	—	+	—	—	—	ND	+
Fumarate	—	—	—	ND	—	+	+	+	—	—	+	ND	ND	—
Genome size (Mb)	3.76	3.96	3.34	4.22	3.63	ND	ND	3.65	3.37	4.08	3.86	3.32	3.91	3.23
G+C content (mol %)	54.0	63.5	60.7	53.0	62.6	59.6	62.1	49.6	58.1	63.8	65.2	59.1	59.9	61.9
Relatedness to strain SF6 ^T														
Identity of 16S (%)	100	97.43	91.21	94.92	95.73	91.76	95.25	95.73	92.57	96.37	96.82	90.79	95.19	95.33
ANI (%)	100	78.63	75.77	78.11	77.5	ND	ND	78.29	76.11	78.42	78.3	75.42	78.86	77.15
AAI (%)	100	73.87	60.29	70.4	69.56	ND	ND	70.52	61.9	73.37	72.2	60.62	71.88	68.81

Table S1. Cellular fatty acid profile of SF6^T grown on lactate at 28°C. Summed features consist of fatty acids that could not be separated, as follows: Summed feature 6, anteiso-C_{15:0} 3OH and/or C_{16:1}ω9*c* DMA; Summed feature 8, C_{17:1}ω8*c* and/or C_{17:2} at 16.801; Summed feature 10, C_{18:1}ω7*c* and/or unknown 17.834; Summed feature 12, unknown 18.622 and/or iso-C_{19:0}.

Fatty acid	%
iso-C _{14:0}	3.66
C _{14:0}	1.31
iso-C _{15:0}	3.97
anteiso-C _{15:0}	20.65
C _{15:1} ω6 <i>c</i>	0.16
C _{15:0}	0.31
iso-C _{15:0} DMA	0.13
iso-C _{16:0}	3.72
C _{16:1} ω7 <i>c</i>	11.62
C _{16:1} ω5 <i>c</i>	0.15
C _{16:00}	10.06
iso-C _{15:0} 3OH	0.54
iso-C _{17:0}	2.47
anteiso-C _{17:0}	3.24
C _{17:1} ω6 <i>c</i>	1.22
C _{17:00}	2.6
C _{17:0} DMA	2.96
iso-C _{18:0}	2.87
C _{18:1} ω9 <i>c</i>	0.2
C _{18:1} ω5 <i>c</i>	0.21
C _{18:0}	11.72
C _{17:0} 3OH	0.63
anteiso C _{19:0}	0.4
C ₁₉ cycloprop. 9,10	0.25
C _{19:0}	0.21
C _{20:0}	0.11
Summed feature 6	0.37
Summed feature 8	0.78
Summed feature10	13.27
Summed feature 12	0.21