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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\textsuperscript{T}, was isolated from sediment of a brackish lake in Japan. Cells of strain SF6\textsuperscript{T} were motile and rod-shaped with length of 1.2–2.5 \mu m and width of 0.6–0.9 \mu m. 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\textsubscript{15}:0. Under sulfate-reducing conditions, strain SF6\textsuperscript{T} utilized lactate, ethanol and glucose as growth substrate. Chemolithoautotrophic growth on H\textsubscript{2} was not observed, although H\textsubscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{T} (= DSM111931\textsuperscript{T} = NBRC 114895\textsuperscript{T}) is proposed as the type strain of a new species, with name of *Pseudodesulfovibrio sediminis* sp. nov.
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 February 2022. 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). These species were 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. ‘*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.,...
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). ‘*D. dechloracetivorans*’ cannot be validated or renamed, as its type strain is not available in culture collections at present. On the other hand, the type strain of ‘*D. brasiliensis*’ is currently available in two culture collections (as DSM 15816 and JCM 12178). It was also indicated that ‘*Paradesulfovibrio onnuriensis*’ is the closest relative of *P. senegalensis* (Kim et al., 2020), and belongs to a lineage in the *Pseudodesulfovibrio*.

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

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\textsuperscript{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), and headspace of culture bottles was filled with N\textsubscript{2}/CO\textsubscript{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\textsuperscript{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 SF6T 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 SF6T was cultured at 28°C in the basal medium supplemented with 5 mM lactate, unless otherwise specified. Its growth was monitored as turbidity of cultures.
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, 6.5 and 7.0% (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). For formate and hydrogen, growth was also assessed in the presence of acetate (1 mM) as carbon source. Sulfide production was assessed by mixing the culture with sulfide detection reagent consisting of 5 mM CuCl₂ and 50 mM HCl (Cord-Ruwisch, 1985). 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 accepters, 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 SF6T 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/). The Genome-to-Genome Distance Calculator provided by DSMZ were used to calculate
digital DNA–DNA hybridization (dDDH) values, by applying the formula 2 (Meier-Kolthoff et al., 2013).

A genome-based taxonomic classification was carried out with the Genome Taxonomy Database (GTDB) (Parks et al., 2018). Taxonomic position of the strain SF6\textsuperscript{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\textsuperscript{T} are summarized in Table 1 and presented in the species description. Cells of strain SF6\textsuperscript{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\textsuperscript{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 0.6–6.5 %, with optimum growth at 2.0%.

In the presence of sulfate, lactate, ethanol and glucose supported heterotrophic growth of SF6\textsuperscript{T} accompanying sulfide production. The molar ratio of generated sulfide
consumed lactate never exceeded 0.8. This upper limit is clearly lower than expected ratio for complete oxidation of lactate (1.5), suggesting incomplete lactate oxidation by strain SF6\textsuperscript{T}. Chemolithotrophic growth on hydrogen was not observed. Formate and hydrogen were utilized as electron donor, but acetate was required as carbon source for growth. 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 in this case. This means that strain SF6\textsuperscript{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 \textit{P. alkaliphilus} F-1\textsuperscript{T} (Frolova et al., 2021). 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\textsubscript{15}:0 was predominant, accounting for 21% of total. Other major components (>10% of total) were summed feature 10 (C\textsubscript{18:1}ω\textsubscript{7c} and/or unknown 17.834; 13.3%), C\textsubscript{18:0} (11.7%), C\textsubscript{16:1}ω\textsubscript{7c} (11.6%) and C\textsubscript{16:0} (10.1%). All fatty acids detected are shown in Table S1.

Genomic features

The complete genome of strain SF6\textsuperscript{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.

Some genes encoding key enzymes for inorganic carbon fixation by sulfate reducers were not identified in the genome of strain SF6T. The genome lacks the $fhs$ and $acsB$ genes, encoding and formate-tetrahydrofolate ligase and carbon monoxide dehydrogenase/acetyl-CoA synthase, respectively. These enzymes are key components of the Wood–Ljungdahl pathway. In addition, formate-tetrahydrofolate ligase also plays a critical role in carbon fixation via reductive glycine pathway (Sánchez-Andrea et al., 2020).

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$^T$ is phylogenetically distinct from existing species, and belongs to the cluster 1 defined in the previous study (Galushko and Kuever, 2019).

Some genomic characteristics are consistent with the results of 16S rRNA gene analysis which suggested that strain SF6$^T$ represents a novel species. 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, AAI and dDDH 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 as a novel species in the genus *Pseudodesulfovibrio*.

The creation of new species, suggested by the phylogenetic analyses, 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.**


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 0.6–6.5% NaCl (optimum 2.0%). Predominant fatty acid is anteiso-C$_{15}$:0. Under sulfate-reducing conditions, grows on lactate, ethanol and glucose. Acetate, propionate, butyrate, isobutyrate, malate, succinate, fumarate, benzoate, pyruvate, citrate, methanol and yeast extract are not utilized as growth substrate. Formate and hydrogen are utilized as electron donor for growth with acetate as carbon source. Ferments pyruvate but does not use it as electron doner for sulfate reduction. Does not ferment malate and fumarate. 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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**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 1340 positions in the final dataset. Numbers on nodes represent percentage values of 1000 bootstrap resampling.