**Pseudodesulfovibrio alkaliphilus**, sp. nov., an alkaliphilic sulfate-reducing bacterium isolated from a terrestrial mud volcano

A. A. Frolova · A. Y. Merkel · A. A. Kuchierskaya · E. A. Bonch-Osmolovskaya · A. I. Slobodkin

Received: 21 March 2021 / Accepted: 23 June 2021 / Published online: 1 July 2021
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

**Abstract** The diversity of anaerobic microorganisms in terrestrial mud volcanoes is largely unexplored. Here we report the isolation of a novel sulfate-reducing alkaliphilic bacterium (strain F-1T) from a terrestrial mud volcano located at the Taman peninsula, Russia. Cells of strain F-1T were Gram-negative motile vibrios with a single polar flagellum; 2.0–4.0 μm in length and 0.5 μm in diameter. The temperature range for growth was 6–37 °C, with an optimum at 24 °C. The pH range for growth was 7.0–10.5, with an optimum at pH 9.5. Strain F-1T utilized lactate, pyruvate, and molecular hydrogen as electron donors and sulfate, sulfite, thiosulfate, elemental sulfur, fumarate or arsenate as electron acceptors. In the presence of sulfate, the end products of lactate oxidation were acetate, H₂S and CO₂. Lactate and pyruvate could also be fermented. The major product of lactate fermentation was acetate. The main cellular fatty acids were anteiso-C₁₅:₀, C₁₆:₀, C₁₈:₀, and iso-C₁₇:₁₀₈. Phylogenetic analysis revealed that strain F-1T was most closely related to *Pseudodesulfovibrio aespoeensis* (98.05% similarity). The total size of the genome of the novel isolate was 3.23 Mb and the genomic DNA G + C content was 61.93 mol%. The genome contained all genes essential for dissimilatory sulfate reduction. We propose to assign strain F-1T to the genus *Pseudodesulfovibrio*, as a new species, *Pseudodesulfovibrio alkaliphilus* sp. nov. The type strain is F-1T (= KCTC 15918T = VKM B-3405T).

**Keywords** Alkaliphile · Sulfate reduction · Mud volcano · Sulfur metabolism

**Introduction**

Dissimilatory sulfate-reducing bacteria are widespread in nature and play a significant role in the global cycling of carbon and sulfur (Rabus et al. 2015). Majority of the cultivated sulfate-reducers belong to the phylum *Desulfo bacterota* among which the class *Desulfovibrionia* is one of the largest (Waite et al. 2020). The first strain of *Desulfovibrio* was isolated by Beijerinck in 1895 and since then more than 100 species of *Desulfovibrio* have been described (Parte et al. 2020—https://lpsn.dsmz.de/genus/desulfovibrio). In 2016 four species of *Desulfovibrio* were reclassified into the new genus—
**Pseudodesulfovibrio**, mainly according to 16S rRNA gene phylogeny (Cao et al. 2016). In 2020, *Desulfovibrio* species were subdivided into 13 genera based on the analysis of 120 conserved single-copy marker genes (Waite et al. 2020).

Currently, the genus *Pseudodesulfovibrio* comprises ten species with validly published names: *P. indicus*, *P. hydrargyri*, *P. profundus*, *P. aespoeensis*, *P. portus*, *P. piezophilus*, *P. halophilus*, *P. oxyclinae*, *P. mercurii* and *P. tunisiensis* (Bale et al. 1997; Ben Ali Gam et al. 2009; Cao et al. 2016; Caumette et al. 1991; Khelaifia et al. 2011; Krekeler et al. 1997; Motamedi and Pedersen 1998; Ranchou-Peyruse et al. 2018; Gilmour et al. 2019; Suzuki et al. 2009).

*Pseudodesulfovibrio* species have been isolated from various habitats such as deep and shallow marine and brackish sediments, hypersaline ecosystems and deep granitic groundwater. There are no reports on the detection of *Pseudodesulfovibrio* in subsurface terrestrial environments, including terrestrial mud volcanoes, which have significant implications in hydrocarbon exploration, seismicity, and atmospheric budget of methane (Mazzini and Etiope 2017).

In this study, we report the isolation of an alkaliphilic sulfate-reducing strain F-1T from a terrestrial mud volcano and describe its physiological, metabolic and genomic properties. Our data suggest that strain F-1T belongs to the genus *Pseudodesulfovibrio*, but differs from other species of this genus. Thus we propose to assign strain F-1T to a new species, *Pseudodesulfovibrio alkaliphilus* sp. nov.

### Materials and methods

#### Origin of the strain

Strain F-1T was isolated from a sample of mud collected from the active gryphon of terrestrial mud volcano Gnilyaya Gora, Taman Peninsula, Krasnodarsky Krai, Russia. Coordinates of the sampling point were 45.251°N, 37.436°E. Samples were collected in May 2017, from the upper 20 cm of mud, pH 8.5, temperature 21 °C, 15.7 mM Cl⁻, 5.3 mM SO₄²⁻. Samples were taken anaerobically in plastic tightly stoppered bottles and transported to the laboratory.

#### Media and cultivation

Strain F-1T was isolated in pure culture after successive cultivations, using anaerobically prepared, bicarbonate-buffered liquid medium of the following composition (per liter distilled water): 0.33 g KH₂PO₄, 0.33 g NH₄Cl, 0.33 g KCl, 0.33 g CaCl₂·6H₂O, 2.00 g NaHCO₃, 0.33 g MgCl₂·6H₂O, 10.00 g NaCl, 0.63 g Na₂S·9H₂O, 0.001 g resazurin, 1 mL of a vitamin solution (Wolin et al. 1963) and 1 mL of a trace element solution (Slobodkin et al. 2012). The medium was prepared by boiling and cooling it under N₂ flow, and then the reducing agent (0.66 g Na₂S·9H₂O) was added. The medium was dispensed in 10 mL portions into 17 mL Hungate tubes and autoclaved at 121 °C for 60 min; the headspace was filled with N₂. The pH of the sterile medium was 9.0. Magnesium sulfate (14 mM) and sodium lactate (10 mM) were added from the sterile stock solutions before the inoculation of the sample.

#### Phenotypic characterization

Growth experiments were performed in triplicate. For morphological, physiological, and metabolic characterization, strain F-1T was cultivated in the same media used for isolation unless noticed otherwise. The effects of temperature, pH and salinity on growth were examined in the reduced medium with magnesium sulfate and sodium lactate. The range of NaCl concentrations for growth was evaluated at 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 5%, 6%, 7% (w/v) NaCl concentrations. The range of pH for growth was determined at 6.0–12.0 with 0.5 intervals and the range of temperature from 4 to 50 °C with 5 °C intervals. The cell-wall structure was examined using the Gram method (Beveridge et al. 2014). The growth of bacteria was determined by direct counting of the cells in the aliquot of a liquid culture with a phase contrast microscope (Zeiss Primo Star) and a counting chamber. Transmission electron microscopy was performed with a model JEM-100 electron microscope (JEOL) as described previously (Bonch-Osmolovskaya et al. 1990). Soluble substrates for growth were added from sterile anaerobic stock solutions before inoculation. Elemental sulfur was added in each Hungate tube with liquid medium. Medium with poorly crystalline Fe(III) oxide
(ferrihydrite) was prepared as described previously (Slobodkin et al. 1999). Determination of gaseous products of metabolism was performed by Gas Cromatography equipped with a HayeSep N 80/100 mesh column at 40 °C and flow rates of 20 ml min⁻¹ (argon was used as a carrier gas). Sulfide was measured colorimetrically with dimethyl-p-phenylenediamine (Trüper and Schlegel 1964). The ability of the strain to grow aerobically was tested in 50 ml bottles sealed with a rubber stopper and aluminum screw cap containing 10 ml aerobically prepared medium (100% air in the gas phase). For checking microaerobic growth, various amounts of air were injected in the headspace of bottles containing anaerobically prepared non-reduced medium.

Chemotaxonomic characterization

For chemotaxonomic analyses strain F-1ᵀ was grown in the same media used for isolation; the cells were harvested in the late exponential phase of growth (48 h) collected by centrifugation, and freeze-dried. Cellular fatty acids were converted to methyl esters by the direct methylation with HCl/MeOH, extracted, and analyzed with GC–MS as described elsewhere (Slobodkina et al. 2020).

16S rRNA gene analysis and genome sequencing, assembly, annotation and comparison

DNA for the 16S rRNA gene and complete genome sequencing was obtained using the FastDNA Spin Kit (MP Bio) following the manufacturer’s protocol. The 16S rRNA gene was amplified using universal primers for bacteria 27F, 357F, 530F, 1114F, 342R, 519R and 1492R (Weisburg et al. 1991). Sequencing of PCR products was carried out using the Sanger method. The 16S rRNA gene sequence of the isolate was compared with other sequences in GenBank (Benson et al. 1999) using the BLAST program (Altschul et al. 1990) and by means of the EzBio-Cloud server (Yoon et al. 2017; http://www.ezbiocloud.net) to identify it closest relatives. Alignment with a representative set of related 16S rRNA gene sequences was carried out using the ClustalW program implemented in the phylogenetic analysis package MEGA version 7.0 (Kumar et al. 2016). Bootstrap consensus trees were inferred from 1000 replicates (Felsenstein 1985) using the maximum-likelihood method based on the Tamura–Nei model, as well as the neighbor-joining and the minimum evolution methods (Rzhetsky and Nei 1992; Hazkani-Covo and Graur 2007) provided by MEGA version 7.0.

The GenBank/EMBL accession number of 16S rRNA gene sequence of the strain F-1ᵀ is MN601397.

The genome of strain F-1ᵀ was sequenced using MiSeq system (Illumina, San Diego, California, USA. Whole-genome sequence allowed us to specify the taxonomic position of strain F-1ᵀ using two methods: Average Nucleotide Identity (ANI) provided by EzBioCloud ANI calculator (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017) and the genome-to-genome distance method (GGDC) with the GGDC 2.0 BLAST + model provided by Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de) (Meier-Kolthoff et al. 2013). Gene search and annotation were performed using the RAST server (Brettin et al. 2015). SEED viewer was used for the assignment of the predicted genes to subsystem categories (Overbeek et al. 2014). Additionally, the Integrated Microbial Genomes non-redundant database, Pfam, KEGG and COG databases were used for genome analysis.

The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accessionWODC00000000.

Results

Enrichment and isolation

For the initial enrichment, the mud sample was inoculated (10% w/v) into sterile anaerobic liquid medium with lactate and sulfate as the growth substrate. After 2 days of incubation at 30 °C, microbial growth was observed expressing in media turbidity. After three subsequent transfers and following serial tenfold dilutions in the same medium, only one morphological type was observed in the highest positive dilution (10⁻⁹). Attempts to obtain separate colonies either anaerobically in agar blocks or aerobically on the surface of the medium with 1.5% of agar were unsuccessful. The purity of strain F-1ᵀ was assessed by routine microscopic examination and
confirmed by results of 16S rRNA gene and complete genome sequencing.

**Phenotypic and chemotaxonomic characteristics**

Mid-exponential-phase cells of strain F-1\(^T\) grown on sulfate and lactate were motile vibrios with a single polar flagellum, 2.0–4.0 \(\mu\)m in length and 0.5 \(\mu\)m in diameter (Fig. 1a). Cells stained Gram-negative in both the exponential and the stationary growth phases. The formation of endospores was not observed in the cultures grown under optimal or suboptimal conditions. Ultrathin sections of the strain F-1\(^T\) revealed a Gram-stain-negative cell wall type with an outer membrane (Fig. 1b). No intracellular membranes were observed.

The temperature range for growth of strain F-1\(^T\) was 6–37 \(^\circ\)C, with an optimum at 24 \(^\circ\)C. No growth was detected at 4 \(^\circ\)C or below and 42 \(^\circ\)C or above after incubation for a month. The pH range for growth was 7.0–10.5, with an optimum at pH 9.5. No growth was observed at pH values 6.5 or below or 11.0 or above. Growth of strain F-1\(^T\) was observed at NaCl concentrations from 0.3 to 3.0% (w/v) with an optimum at 0.5–1.0%, no growth was evident at 3.5% (w/v) NaCl or above. The doubling time on lactate/SO\(_4^{2-}\) under optimal growth conditions was 1.47 h. Addition of yeast extract (0.1 g/l) did not stimulate growth.

Strain F-1\(^T\) grew with sulfate (14 mM) as an electron acceptor and lactate (20 mM), fumarate (20 mM), D-glucose (5 mM), D-cellobiose (20 mM) or molecular hydrogen (H\(_2\)/N\(_2\); 80/20; v/v in the gas phase) as an electron donor. In the presence of sulfate the end products from lactate oxidation were acetate (13 mM), propionate (0.8 mM), H\(_2\)S (5 mM) and CO\(_2\) (0.5 mM). Pyruvate (10 mM), malate (5 mM), formate (20 mM), acetate (5 mM), butyrate (5 mM), ethanol (10 mM), propanol (10 mM), and arabinose (10 mM) were not used as electron donors with sulfate.

![Fig. 1](image-url)  
**Fig. 1** Electron micrographs of cells of strain F-1\(^T\). a A negatively stained cells. Bar, 0.6 \(\mu\)m. b Ultrathin section of a cell wall structure. CM cytoplasmic membrane, OM outer membrane. Bar, 0.5 \(\mu\)m
as an electron acceptor. In the absence of sulfate, lactate and pyruvate were fermented and supported growth. The major product of lactate fermentation was acetate (1.6 mM); trace amounts of propionate (0.4 mM), CO₂ (0.11 mM) and hydrogen (0.06 mM) were also produced. Fumarate, glucose and cellobiose were not fermented. Strain F-1ᵀ demonstrated a weak (5 × 10⁶ cells ml⁻¹), but sustainable (at least 5 consequent 5% (v/v) transfers) autotrophic growth with sulfate, sulfite, thiosulfate, elemental sulfur, fumarate or arsenate as an electron acceptors and molecular hydrogen (H₂/N₂; 80/20; v/v in the gas phase) as an electron donor. Addition of acetate (10 mM) as a carbon source did not have any effect on autotrophic growth. With lactate as an electron donor sulfate (14 mM), sulfite (2 mM), thiosulfate (20 mM), elemental sulfur (5 g/l), fumarate (20 mM) or arsenate (5 mM) were used as an electron acceptor for growth, but nitrate (20 mM), nitrite (5 mM), selenate (5 mM) or ferrihydrite (poorly crystalline Fe(III) oxide, 90 mmol Fe(III) l⁻¹) were not utilized. Strain F-1ᵀ was not able to grow by disproportionation of sulfite (5 mM), thiosulfate (10 mM) and elemental sulfur (5 g/l).

Major fatty acids were anteiso-C₁₅:₀ (13.8%), C₁₆:₀ (12.5%), C₁₈:₀ (11.8%), and iso-C₁₇:₁ω₈ (12.0). Other branched saturated and monounsaturated fatty acids were detected in fewer amounts (Supplementary Table S1 and Figure S1). The strain F-1ᵀ differs from *Pseudodesulfovibrio portus* MS(T) (Suzuki et al. 2009) and *Pseudodesulfovibrio indicus* J2ᵀ (Cao et al. 2016) by the lower amount of saturated branched fatty acids and higher amounts of saturated straight-chain and unsaturated straight-chain fatty acids.

**Phylogeny**

The 16S rRNA gene sequences of strain F-1ᵀ obtained by amplification with universal bacterial primers and retrieved from whole-genome data were identical. A comparison of 1541 nucleotides of 16S rRNA gene sequences of strain F-1ᵀ with those available in GenBank (Benson et al. 1999) and EzBioCloud (Yoon et al. 2017) databases showed that the novel isolate belongs to the genus *Pseudodesulfovibrio* and had the highest sequence similarity to *Pseudodesulfovibrio aespoeensis* DSM 10631ᵀ (98.05%) and *Pseudodesulfovibrio indicus* J2ᵀ (96.00%). The 16S rRNA gene phylogenetic tree reconstruction revealed that the strain F-1ᵀ constituted a monophyletic branch clearly separated from the most closely related species (Fig. 2).

Pairwise ANI value of the genome of the strain F-1ᵀ and the genome of the closest relative organism, *P. aespoeensis* DSM 10631ᵀ was 82.07%. The in silico DDH value predicted between strain F-1ᵀ and *P. aespoeensis* DSM 10631ᵀ by the recommended formula 2, was 24.50%. Both these values are much lower than the threshold for prokaryotic species delineation proposed to be 95–96% (ANI) and 70% (DDH) (Meier-Kolthoff et al. 2013, Rodriguez-R and Konstantinidis 2016).

**Genome analysis**

The draft genome assembly of strain F-1ᵀ has a total length of 3,227,153 bp and N50 value of 302,886 bp within 29 contigs and the genomic DNA G + C content was 61.93 mol%. The genome of F-1ᵀ was predicted to contain 3061 protein-coding sequences and 54 RNA genes. A total of 1914 coding sequences were assigned to non hypothetical and 1147 to hypothetical proteins. Most of the annotated genes were responsible for the synthesis of amino acids and derivatives (167), protein metabolism (155), cofactors, vitamins, prosthetic groups and pigment formation (86) (Supplementary Table S2 and Figure S2).

The genome of strain F-1ᵀ contains a full set of genes required for dissimilatory sulfate reduction (Pereira et al. 2011) including sulfate adenylyltransferase, manganese-dependent inorganic pyrophosphatase, APS reductase subunits AprA and AprB, the subunits of dissimilatory sulfite reductase DsrABCD, and electron transfer complexes DsrMKJOP and QmoABC (hereinafter see references in Supplementary Table S3).

The genome of strain F-1ᵀ possessed all genes for dissimilatory sulfate reduction (Pereira et al. 2011) including sulfate adenylyltransferase, manganese-dependent inorganic pyrophosphatase, APS reductase subunits AprA and AprB, the subunits of dissimilatory sulfite reductase DsrABCD, and electron transfer complexes DsrMKJOP and QmoABC (hereinafter see references in Supplementary Table S3).

The genome of strain F-1ᵀ possessed all genes for glycolysis via the Embden-Meierhoff-Parnas pathway. Surprisingly, the reductive pentose phosphate pathway in the genome of strain F-1ᵀ was absent, although ribulose biphosphate carboxylase, key enzyme of rPP, was present in the proteomes of several *Pseudodesulfovibrio* strains (Bell et al. 2018).

Strain F-1ᵀ can grow autotrophically, but its genome does not harbor the genes encoding the key enzymes of six well-characterized microbial carbon
fixation pathways, viz. ribulose 1,5-bisphosphate carboxylase (Calvin-Benson cycle), carbon monoxide dehydrogenase/acetyl-CoA synthase complex (reductive acetyl-CoA pathway), ATP-citrate lyase and citryl-CoA lyase (two variants of the reductive tricarboxylic acid cycle), 4-hydroxybutyryl-CoA dehydratase (3-hydroxypropionate/4-hydroxybutyrate and dicarboxylate/4-hydroxybutyrate cycles) or malonyl-CoA reductase (3-hydroxypropionate bi-cycle). A recently described reductive glycine pathway (Sánchez-Andrea et al. 2020; Song et al. 2020) is incomplete in the genome of strain F-1 T. However, the genome of strain F-1 T contains all enzymes of the TCA cycle, including citrate synthase, aconitase, isocitrate dehydrogenase, succinyl-CoA synthetase, fumarase, succinate dehydrogenase/fumarate reductase, fumarate hydratase and malate dehydrogenase. Therefore, it can be hypothesized that in strain F-1 T CO2 fixation can occur via “reversed oxidative TCA cycle” (Mall et al. 2018; Nunoura et al. 2018).

Strain F-1 T is capable of utilizing molecular hydrogen as an energy source. The genome of strain F-1 T encodes two subunits of periplasmic HynAB hydrogenase which has a bifunctional activity and is required either for the uptake of molecular hydrogen or for H2 release during fermentation of organic substances. The [Ni–Fe] hydrogenase maturation system HypABCDEF is encoded in the genome of strain F-1 T.

The genome of strain F-1 T contains two copies of arsenate reductase gene, arsC. The presence of arsC is the common feature through the genus Pseudodesulfovibrio. However, there are no published data on the ability of the members of Pseudodesulfovibrio to grow with arsenate as an electron acceptor.

In contrast to the canonical fumarate reductase/succinate dehydrogenase consisting of four subunits (frdABCD), the genome of strain F-1 T contains genes only for three subunits (frdABC), as it was previously reported for Desulfovibrio vulgaris and Desulfovibrio desulfuricans (Zaunmüller et al. 2006).
The genome of strain F-1\textsuperscript{T} contains genes of the nitrogenase complex \textit{nifDHK}, which is required for \textit{N}_2 fixation. Two components the iron protein and the molybdenum-iron protein, as well as two genes of P-II family nitrogen regulators are present.

**Discussion**

Strain F-1\textsuperscript{T} represents an alkaliphilic, mesophilic, sulfate-reducing bacterium isolated from a terrestrial mud volcano where it could participate in sulfur and carbon cycling. Mud fluids of Gnilaya Gora volcano contain up to 5 mM of \textit{SO}_4^{2-}, providing an electron acceptor for the energy metabolism of the new isolate. The ranges of pH, temperature and salinity for growth of strain F-1\textsuperscript{T} are consistent with the environmental parameters of its habitat, suggesting an indigenous origin of the strain. Phylogenetic analysis based on 16S rRNA gene revealed that strain F-1\textsuperscript{T} belongs to the genus \textit{Pseudodesulfovibrio}, where it forms a separate lineage of the species rank. ANI and in silico DDH data also support the assignment of strain F-1\textsuperscript{T} to a new species. It is the first representative of the genus \textit{Pseudodesulfovibrio} isolated from a surface terrestrial environment whereas all known species of the genus were recovered from marine-related or subsurface habitats.

As all members of \textit{Pseudodesulfovibrio} strain F-1\textsuperscript{T} is anaerobic mesophilic sulfate-reducing vibrio, but it differs in temperature, pH and salinity ranges and optima for growth and in the electron donors and acceptors utilized (Table 1). The most notable distinction is the growth pH. All \textit{Pseudodesulfovibrio} species described so far, are neutrophilic bacteria optimally growing at pH around 7.0. Strain F-1\textsuperscript{T} has the pH optimum at 9.5 and is unable to grow bellow 7.0; thus, it could be considered as obligate alkaliphile.

Metabolic potential encoded in the genome of strain F-1\textsuperscript{T} is consistent with the phenotypic data. The central carbon metabolism is based on Embden-Meierhoff-Parnas pathway. Sulfate respiration is ensured by the canonical set of genes for dissimilatory sulfate reduction. The reduction of elemental sulfur, fumarate and arsenate is provided by the respective reductases encoded in genome. The presence of gene cluster encoding all enzymes of nitrogenase complex indicates the ability of strain F-1\textsuperscript{T} to fix \textit{N}_2.

Therefore, based on phylogenetic position, phenotypic and physiological properties of strain F-1\textsuperscript{T} we propose to assign it to the genus \textit{Pseudodesulfovibrio} as a new species, \textit{P. alkaliphilus}.

**Description of \textit{Pseudodesulfovibrio alkaliphilus} sp. nov.**

\textit{Pseudodesulfovibrio alkaliphilus} (al.ka.li.philus M.L. n. \textit{alkali} soda ash; N.L. masc. adj. \textit{philus} (from Gr. masc. adj. \textit{philos}) loving; N.L. musc. adj. \textit{alkaliphilus} loving alkaline conditions).

Cells are motile vibrios 2.0–4.0 μm in length and 0.5 μm in diameter with a polar flagellum. Growth is observed at NaCl concentrations from 0.3 to 3% (w/v) (optimum 0.5–1%, w/v), in pH range 7.0–10.5 (optimum 9.5), and at temperatures between 6 and 37 °C (optimum 24 °C). Grows with sulfate as an electron acceptor and lactate, fumarate, D-glucose, D-cellobiose or molecular hydrogen as electron donors. In the presence of sulfate the end products of lactate oxidation are acetate, propionate, \textit{H}_2\text{S} and \textit{CO}_2. Sulfite, thiosulfate, elemental sulfur, fumarate or arsenate are used as electron acceptors for growth with lactate as an electron donor, but nitrate, nitrite, selenate or ferrihydrite are not utilized. Pyruvate, malate, formate, acetate, butyrate, ethanol, propanol, and arabinose are not used as electron donors with sulfate as an electron acceptor. In the absence of sulfate lactate and pyruvate are fermented and support growth. The major product of lactate fermentation is acetate. Fumarate, D-glucose and D-cellobiose are not fermented. Capable of weak but sustainable autotrophic growth with sulfate, sulfite, thiosulfate, elemental sulfur, fumarate or arsenate as electron acceptors and molecular hydrogen as an electron donor. Not able to grow by disproportionation of sulfite, thiosulfate and elemental sulfur. The predominant fatty acids are anteiso-C\textsubscript{15:0}, C\textsubscript{16:0}, C\textsubscript{18:0}, and iso-C\textsubscript{17:1}\text{ω8}.

The genome of the type strain is characterized by a size of 3.23 Mb and a G + C content of 61.93 mol%. The type strain F-1\textsuperscript{T} (= KCTC 15918\textsuperscript{T} = VKM B-3405\textsuperscript{T}) was isolated from a terrestrial mud volcano in the Taman peninsula, Russia.

The GenBank/EMBL accession number of 16S rRNA gene sequence of the strain F-1\textsuperscript{T} is MN601397, the Whole Genome Shotgun project has been
Table 1  Differential characteristics of strain F-1T and species of the genus *Pseudodesulfovibrio*

| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|-----------------|---|---|---|---|---|---|---|---|---|----|----|
| Growth conditions |   |   |   |   |   |   |   |   |   |    |    |
| Optimum temperature (°C) | 24 | 25–30 | 30–35 | 30 | 25 | 35 | 30 | 35 | 35 | 37 | 32 |
| Optimum pH | 8.5–9.5 | 7.5 | 6.5–7.0 | 7.3 | 7 | 6.5 | 6.0–7.4 | 5.5–8.5 | ND | 7 | 7.8 |
| NaCl requirement | – | – | + | + | + | + | + | + | + | – | – |
| Electron donors |   |   |   |   |   |   |   |   |   |    |    |
| Ethanol | – | – | – | + | – | + | W | + | + | – | – |
| Fumarate | + | + | – | + | – | + | + | – | – | + | + |
| Malate | – | – | – | + | – | – | + | – | – | + | – |
| Electron acceptors |   |   |   |   |   |   |   |   |   |    |    |
| Sulfur | + | + | – | – | – | ND | – | + | + | + | ND |
| Nitrate | – | – | – | W | – | + | ND | – | – | – | – |
| Fumarate | + | ND | W | – | – | + | – | – | – | + | + |
| Fumarate fermentation | – | ND | – | – | + | – | + | – | ND | ND | + | + |
| Lactate | + | ND | + | – | + | – | W | – | – | + | + |
| Pyruvate | + | + | + | + | + | + | + | + | + | – | + |
| Major fatty acids | Anteiso-C15:0, C16:0, C18:0, and iso-C17:1ω8 | ND | Iso-C15:0, anteiso-C15:0, summed feature 9 (iso-C17:1ω9c and/or C16:0 10- methyl), iso-C17:0 | C15:0, C16:0, C18:0, C17:1 | C15:0, C16:0, C18:0, C17:1 | C16:0, C18:0, iso-C15:0, iso-C17:0, iso-C17:1ω9c, anteiso-C15:0, anteiso-C16:0, anteiso-C17:0, iso-C17:1ω9c | C18:0, anteiso-C15:0, anteiso-C16:0, anteiso-C17:0, iso-C17:1ω9c | ND | ND | ND | ND |
| DNA G + C content (mol%) | 61.93 | 61 | 63.5 | 50 | 53 | 62.1 | 62.6 | 60.7 | 59.1* | 59.6 | 65.2 |
deposited at DDBJ/ENA/GenBank under the accession WODC00000000.

Authors contribution  AF conceived, planned and carried out experiments and wrote the original draft. AF and AS wrote the paper. AM performed genomic sequencing, assembly and annotation. AK performed lipid analysis. EB-O allowed funding acquisition. All authors provided critical feedback for the review of the manuscript.

Funding  The work on isolation, physiological and taxonomic studies was supported by the Russian Science Foundation, project no. 17–74-30025. The work on genome sequencing, assembly and annotation was supported by the Ministry of Science and Higher Education of the Russian Federation. The work of A. A. K. (lipid analysis) was supported by the State Assignment 0768–2020-0007 in scientific activities in Gubkin University.

Data availability  The GenBank/EMBL accession number of 16S rRNA gene sequence of the strain F-1 T is MN601397, the Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WODC00000000.

Declarations  

Conflict of interest  The authors declare that there are no conflicts of interest.

Ethical statement  This article does not contain any studies with human participants or animals performed by any of the authors.

References

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bale SJ, Goodman K, Rochelle PA et al (1997) Desulfovibrio profundus sp. nov., a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan sea. Int J Syst Bacteriol 47:515–521. https://doi.org/10.1099/00207713-47-2-515

Bell E, Lamminmäki T, Alneberg J et al (2018) Biogeochemical cycling by a low-diversity microbial community in deep groundwater. Front Microbiol 9:2129. https://doi.org/10.3389/fmicb.2018.02129

Benson DA, Boguski MS, Lipman DJ et al (1999) GenBank. Nucleic Acids Res 27:12–17. https://doi.org/10.1093/nar/27.1.12

Beveridge TJ, Lawrence JR, Murray RGE (2014) Sampling and staining for light microscopy. In: Methods for general and molecular microbiology. Ed. 3. Ch. 2. pp 19–33 https://doi.org/10.1128/9781555817497.ch2
Bonch-Osmolovskaya EA, Sokolova TG, Kostrikina NA et al (1990) Desulfurella acetivorans gen. nov. and sp. nov. -a new thermophilic sulfur-reducing eubacterium. Arch Microbiol 153:151–155. https://doi.org/10.1007/BF00247813

Brettin T, Davis JJ, Disz T et al (2015) RASTik: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365

Cao J, Gayet N, Zeng X et al (2016) Pseudodesulfovibrio indicus gen. nov., sp. nov., a piezophilic sulfate-reducing bacterium from the Indian ocean and reclassification of four species of the genus Desulfovibrio. Int J Syst Evol Microbiol 66:3904–3911. https://doi.org/10.1099/ijsem.0.01286

Caumette P, Cohen Y, Matheron R (1991) Isolation and characterization of Desulfovibrio halophilus sp. nov., a halophilic sulfate-reducing bacterium isolated from solar lake (Sain). Syst Appl Microbiol 14:33–38. https://doi.org/10.1016/S0723-2020(11)80358-9

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the Bootstrap. Evolution (n Y) 39(4):783–791. https://doi.org/10.2307/2408678

Fichtel K, Mathes F, Königke M et al (2012) Isolation of sulfate-reducing bacteria from sediments above the deep subseafloor aquifer. Front Microbiol 3:65. https://doi.org/10.3389/fmicb.2012.00065

Gam ZBA, Oueslati R, Abdelkafi S et al (2009) Desulfovibrio tunisiensis sp. nov., a novel weakly halotolerant, sulfate-reducing bacterium isolated from exhaust water of a Tunisian oil refinery. Int J Syst Evol Microbiol 59:1059–1063. https://doi.org/10.1099/ijs.0.000943-0

Gilmour CC, Soren AB, Gionfriddo CM et al (2019) Pseudodesulfovibrio mercari sp. nov., a mercury-methylating bacterium isolated from sediment. Int J Syst Evol Microbiol 71:3. https://doi.org/10.1099/ijsem.0.004697

Hazkani-Covo E, Graur D (2007) A comparative analysis of numt evolution in human and chimpanzee. Mol Biol Evol 24:13–18. https://doi.org/10.1093/molbev/msl149

Khelaïfi S, Fardeau ML, Pradel N et al (2011) Desulfovibrio piezophilus sp. nov., a piezophilic, sulfate-reducing bacterium isolated from wood falls in the Mediterranean Sea. Int J Syst Evol Microbiol 61:2706–2711. https://doi.org/10.1099/ijsem.0.028670-0

Krekeler D, Sigalevich P, Teske A et al (1997) A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai) Desulfovibrio Oxyclinae Sp Nov. Arch Microbiol 167:369–375. https://doi.org/10.1007/s002030050457

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Mall A, Sobotta J, Huber C et al (2018) Reversibility of citrate synthase allows autotrophic growth of a thermophilic bacterium. Science 359:563–567. https://doi.org/10.1126/science.aao2410

Mazzini A, Etiópe G (2017) Mud volcanism: An updated review. Earth-Science Rev 168:81–112. https://doi.org/10.1016/j.earscirev.2017.03

Meier-Kolthoff JP, Auch AF, Klenk HP et al (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471-2105-14-60

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

Nunoura T, Chikaraishi Y, Izaki R et al (2018) A primordial and reversible TCA cycle in a facultatively chemolithoautotrophic thermophile. Science 359:559–563. https://doi.org/10.1126/science.aao3407

Oren A, Garrity GM (2021) Validation list no. 197. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 71:1–6

Overbeek R, Olson R, Pusch GD et al (2014) The SEED and the rapid annotation of microbial genomes using Subsystems technology (RAST). Nucleic Acids Res 42:206–214. https://doi.org/10.1093/nar/gkt1226

Parte AC, Carbas JS, Meier-Kolthoff JP et al (2020) List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol 70:5607–5612. https://doi.org/10.1099/ijs.0.004332

Pereira IAC, Ramos AR, Greim F et al (2011) A comparative genomic analysis of energy metabolism in sulfate reducing bacteria and archaea. Front Microbiol 2:69. https://doi.org/10.3389/fmicb.2011.00069

Rabus R, Venceslau SS, Wohlbrand L et al (2015) A post-genomic view of the ecophysiology, catabolism and biotechnological relevance of sulphate-reducing prokaryotes. Adv Microb Physiol 66:55–321. https://doi.org/10.1016/bs.ampbs.2015.05.002

Ranchou-Peyruse M, Goni-Urriza M, Guignard M et al (2018) Pseudodesulfovibrio hydrargyrri sp. nov., a mercury-methylating bacterium isolated from a brackish sediment. Int J Syst Evol Microbiol 68:1461–1466. https://doi.org/10.1099/ijsem.0.002173

Rodriguez-R L, Konstantinidis K (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Prepr. https://doi.org/10.7287/peerj.preprints.1900

Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum-evolution trees. Mol Biol Evol 9:945–967. https://doi.org/10.1093/oxfordjournals.molbev.a040771

Sánchez-Andrea I, Guedes IA, Hornung B et al (2020) The reductive glycine pathway allows autotrophic growth of Desulfovibrio desulfuricans. Nat Commun 11:5090. https://doi.org/10.1038/s41467-020-18906-7

Slobodkin AI, Tourouva TP, Kuznetsov BB et al (1999) Thermoanaerobacter siderophilus sp. nov., a novel dissimilatory Fe(III)-reducing anaerobic thermophilic bacterium. Int J Syst Bacteriol 49:1471–1478. https://doi.org/10.1099/00207713-49-4-1471

Slobodkin AI, Reysenbach AL, Slobodkina GB et al (2012) Thermosulfilimonas dismutans gen. nov., sp. nov., an extremely thermophilic sulfur-disproportionating bacterium from a deep-sea hydrothermal vent. Int J Syst Evol Microbiol 62:2565–2571. https://doi.org/10.1099/ijse.0.034397-0
Slobodkina GB, Merkel AY, Novikov AA et al (2020) Pelomicrobium methylotrophicum gen. nov., sp. nov. a moderately thermophilic, facultatively anaerobic, lithoautotrophic and methylotrophic bacterium isolated from a terrestrial mud volcano. Extremophiles 24:177–185. https://doi.org/10.1007/s00792-019-01145-0

Song Y, Lee JS, Shin J et al (2020) Functional cooperation of the glycine synthasereductase and Wood-Ljungdahl pathways for autotrophic growth of Clostridium drakei. Proc Natl Acad Sci USA 117:7516–7523. https://doi.org/10.1073/pnas.1912289117

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

Trüper HG, Schlegel HG (1964) Sulphur metabolism in Thiorhodaceae I. Quantitative measurements on growing cells of Chromatium okenii. Antonie van Leeuwenhoek 30:225–238. https://doi.org/10.1007/BF02046728

Waite DW, Chuvochina M, Pelikan C et al (2020) Proposal to reclassify the proteobacterial classes Deltaproteobacteria and Oligoflexia, and the phylum Thermodesulfobacteria into four phyla reflecting major functional capabilities. Int J Syst Evol Microbiol 70:5972–6016. https://doi.org/10.1099/ijsem.0.004213

Weisburg WG, Barns SM, Pelletier DA et al (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Wolin EA, Wolin MJ, Wolfe RS (1963) Formation of methane. J Franklin Inst 176:737. https://doi.org/10.1016/s0016-0032(13)90081-8

Yoon SH, Ha S, min, Lim J, et al (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek, Int J Gen Mol Microbiol 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4

Zaunmüller T, Kelly DJ, Glöckner FO, Unden G (2006) Succinate dehydrogenase functioning by a reverse redox loop mechanism and fumarate reductase in sulphate-reducing bacteria. Microbiology 152:2443–2453. https://doi.org/10.1099/mic.0.28849-0

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.