Pseudodesulfovibrio Alkaliphilus, Sp. Nov., An Alkaliphilic Sulfate-Reducing Bacterium Isolated From a Terrestrial Mud Volcano

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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-1^T) from a terrestrial mud volcano located at the Taman peninsula, Russia. Cells of strain F-1^T 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-1^T 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, H_2S and CO_2. Lactate and pyruvate could also be fermented. The major product of lactate fermentation was acetate. The main cellular fatty acids were anteiso-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$, and iso-$C_{17:1}\omega8$. Phylogenetic analysis revealed that strain F-1^T was most closely related to *Pseudodesulfovibrio aespoeensis* (98.05% similarity). The total size of the genome of the novel isolate was 3.23Mb and the genomic DNA G + C content was 61.93 mol%. The genome contained all genes essential for dissimilatoty sulfate reduction. We propose to assign strain F-1^T to the genus *Pseudodesulfovibrio*, as a new species, *Pseudodesulfovibrio alkaliphilus* sp. nov. The type strain is F-1^T (= KCTC 15918^T = VKM B-3405^T).

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 belongs to the phylum *Desulfobacterota* among which the class *Desulfovibrionia* is one of the largest (Waite et al. 2020). The first strain of *Desulfovibrio* was isolated by Beijerink in 1895 and since then more than 100 species of *Desulfovibrio* have been described (Parte et al., 2020 -https://lpsn.dsmz.de/genus/pseudodesulfovibrio). 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 nine species with validly published names: *P. indicus*, *P. hydrargyri*, *P. profundus*, *P. aespoeensis*, *P. portus* and *P. piezophilus P. halophilus*, *P. oxyclinae*, 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, 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 surface 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-1^T from a terrestrial mud volcano and describe its physiological, metabolic and genomic properties. Our data suggest that strain F-1^T belongs to the genus *Pseudodesulfovibrio*, but differs from other species of this genus. Thus we propose to assign strain F-1^T to a new species, *Pseudodesulfovibrio alkaliphilus* sp. nov.

Materials And Methods

Origin of the strain

Strain F-1^T was isolated from a sample of mud collected from the active gryphon of terrestrial mud volcano Gnilaya 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 SO_4^{2-}. Samples were taken anaerobically in plastic tightly stoppered bottles and transported to the laboratory.

Media and cultivation

Strain F-1^T 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 KH_2PO_4, 0.33 g NH_4Cl, 0.33 g KCl, 0.33 g $CaCl_2$*$6H_2O$, 2.00 g $NaHCO_3$, 0.33 g $MgCl_2$*$6H_2O$, 10.00 g NaCl, 0.63 g Na_2S*$9H_2O$, 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 N_2 flow, and then the reducing agent (Na_2S*$9H_2O$) 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 N_2. The pH of 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-1^T was cultivated in the same media used for isolation unless noticed otherwise. The effects of temperature, pH and salinity on growth were examined at 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 ^oC with 5 ^oC intervals. The cell-wall structure was examined using the Gram method (Beveridge et al. 2014). Cell morphology and motility were examined in a liquid medium after 48 h of incubation using a Zeiss Primo Star microscope. 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, while insoluble substrates were added directly into each test tube with liquid medium prior to sterilization. Elemental sulfur was added in each Hungate tube with liquid medium. Determination of gaseous products of metabolism was performed by GC equipped with a HayeSep N 80/100 mesh column at 40 °C and flow rates of 20 ml min^{-1} (argon 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-3apT was grown in the same media used for isolation; the cells were harvested in the late exponential phase of growth (48 h). Major fatty acids were anteiso-$C_{15:0}$ (13.8%), $C_{16:0}$ (12.5%), $C_{18:0}$ (11.8%), and iso-$C_{17:1}\omega8$ (12.0). Other branched saturated and monounsaturated fatty acids were detected in fewer amounts (Supplementary Table S1). The fatty acids profile is similar to that of *Pseudodesulfovibrio indicus*J2^T (Cao et al. 2016).

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 were 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.ezbio cloud.net) to identify its 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-1^T is MN601397.

The genome of strain F-3ap^T was sequenced using MiSeq system (Illumina, San Diego, California, USA. Whole-genome sequence allowed us to specify the taxonomic position of strain F-1^T 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 accession WODC01000000.

Results

Enrichment and isolation

For the initial enrichment, the mud sample was inoculated (10% w/v) into sterile anaerobic liquid medium with pyruvate as the growth substrate. After 2 days of incubation at 30 °C, microbial growth was observed. After three subsequent transfers and following serial 10-fold 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-1^T 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 µm in length and 0.5 µm 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 (Fig. 1b).

The temperature range for growth of strain F-1^T was 6–37 °C, with an optimum at 24 °C. No growth was detected at 4 °C or below and 42 °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 bellow 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^{-1}. 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_2S (5 mM) and CO_2 (0.5 mM). Pyruvate (10 mM) , malate (5mM), 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 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), CO_2 (0.11 mM) and hydrogen (0.06 mM) were also produced. Fumarate, glucose and cellobiose were not fermented. Strain F-1^T demonstrated a weak ($5*10^6$ 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 (H_2/N_2; 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 (5mM), selenate (5 mM) or ferrihydrite (poorly

crystalline Fe(III) oxide, 90 mmol Fe(III) l^{-1}) were not utilized. Strain F-1^T 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-$C_{15:0}$ (13.8%), $C_{16:0}$ (12.5%), $C_{18:0}$ (11.8%), and iso-$C_{17:1}\omega8$ (12.0). Other branched saturated and monounsaturated fatty acids were detected in fewer amounts (Supplementary Table S1 and Figure S1).

Phylogeny

The 16S rRNA gene sequences of strain F-1^T obtained by amplification with universal bacterial primers and retrieved from whole-genomic data were identical. A comparison of 1541 nucleotides of 16S rRNA gene sequences of strain F-1^T 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 10631^T (98.05 %) and *Pseudodesulfovibrio indicus* J2^T (96.00 %). The 16S rRNA gene phylogenetic tree reconstruction revealed that the strain F-1^T constituted a monophyletic branch clearly separated from the most closely related species (Fig. 2).

Pairwise ANI value of the genome of the strain F-1^T and the genome of the closest relative organism, *P. aespoeensis* (DSM 10631^T) was 82.07%. The *in silico* DDH value predicted between strain F-1^T and *P. aespoeensis* (DSM 10631^T) 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-1^T has a total length of 3227153 bp and N50 value of 302886 bp within 29 contigs and the genomic DNA G+C content was 61.93 mol%. The genome of F-1^T was predicted to contain 3061 protein-coding sequences and 54 RNA genes. 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 (WP_155932275), manganese-dependent inorganic pyrophosphatase (WP_155934818), APS reductase subunits AprA (WP_155932273) and AprB (WP_155932274), the subunits of dissimilatory sulfite reductase DsrABCD (WP_155934369 - WP_155934373), and electron transfer complexes DsrMKJOP (WP_155932960 - WP_155932964) and QmoABC (WP_155932272 - WP_155932270).

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

Strain F-1^T 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 (WP_155934624), aconitase (WP_155932278), isocitrate dehydrogenase (WP_155932020), succinyl-CoA synthetase (WP_155935188), fumarase (WP_155932566, WP_155932568), succinate dehydrogenase/fumarate reductase (WP_155932561 - WP_155932564), fumarate hydratase (WP_155932566, WP_155932568) and malate dehydrogenase (WP_155934516). Therefore, it can be hypothesized that in strain F-1^T CO_2 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 (WP_155931905, WP_155931907) which has a bifunctional activity and is required either for the uptake of molecular hydrogen or for H_2 release during fermentation of organic substances. The [Ni-Fe] hydrogenase maturation system HypABCDEF in the genome of strain F-1^T is encoded in WP_155931624, WP_155931626, WP_155931901, WP_155935612, WP_196772909 and WP_155931785.

The genome of strain F-1^T contains two copies of gene of arsenate reductase, *arsC* (WP_155934701, 155934643). 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) (WP_155932561, WP_155932563, WP_155932564), as it was previously reported for *Desulfovibrio vulgaris and Desulfovibrio desulfuricans* (Zaunmüller et al., 2006).

The genome of strain F-1^T contains genes– of the nitrogenase complex *nifDHK,* which is required for nitrogen fixation. Two components the iron protein (WP_155934608) and the molybdenum-iron protein (WP_155934611, WP_155934612), as well as two genes of P-II family nitrogen regulators (WP_155934609, WP_155934610) are present

Discussion

Strain F-1^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 SO_4^{2-}, providing an electron acceptor for the energy metabolism of the new isolate. Strain F-1^T has pH, temperature and salinity ranges for growth consistent with environmental parameters of its habitat that suggests an indigenous nature of the strain.

Phylogenetic analysis based on 16S rRNA gene revealed that strain F-1^T belongs to the genus *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^T to a new species. It is the first representative of the genus *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 *Pseudodesulfovibrio* strain F-1^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 *Pseudodesulfovibrio* species described so far, are neutrophilic bacteria optimally growing at pH around 7.0. Strain F-1^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^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^T to fix dinitrogen.

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

Description of *Pseudodesulfovibrio alkaliphilus* sp. nov.

Pseudodesulfovibrio alkaliphilus (al.ka.li.phi`lus M.L. n. *alkali* soda ash; N.L. masc. adj. *philus* (from Gr. masc. adj. philos) loving; N.L. musc. adj. *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 ^oC (optimum 24 ^oC). 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, H_2S and 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-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$, and iso-$C_{17:1\omega8}$.

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^T (=KCTC 15918^T =VKM B-3405^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^T is MN601397, the Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WODC01000000.

Declarations

Data availability statement

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 WODC01000000.

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.

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Ethical statement

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

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Tables

Table 1. Differential characteristics of strain F-1^T and species of the genus *Pseudodesulfovibrio*

Taxa: 1, *Pseudodesulfovibrio alkaliphilus,* sp. nov. F-1^T; 2, *P. aespoeensis* (Motamedi and Petersen, 1998; Fichtel et al. 2012); 3, *P. indicus* (Cao et al. 2016); 4, *P. piezophilus* (Khelaifia et al. 2011); 5, *P. profundus* (Bale et al., 1997); 6, *P. portus* (Suzuki et al. 2009); 7, *P. hydrargyri* (Ranchou-Peyruse et al. 2018); 8. *P. halophilus* (Caumette et al. 1991); 9. *P. oxyclinae* (Krekeler et al. 1997); 10. *P. tunisiensis* (Ben Ali Gam et al. 2009). Characteristics are scored as: +, positive; -, negative; w, weakly positive; ND – not determined.

Characteristics	1	2	3	4	5	6	7	8	9	10
Growth conditions										
Optimum temperature (^{o}C)	24	25 – 30	30 – 35	30	25	35	30	35	35	37
Optimum pH	9.5	7.5	6.5 – 7.0	7.3	7.0	6.5	6.0 – 7.4	5.5 – 8.5	ND	7.0
Optimum NaCl (%)	0.5 – 1.0	0.7	2.5	2.5	0.6 – 8.0	2.0	1.5	10	5.0 – 10.0	4
Electron donors										
Ethanol	-	-	-	+	-	+	W	+	+	-
Fumarate	+	-	-	+	-	+	+	-	-	+
Malate	-	-	+	+	-	+	-	-	+	-
Electron acceptors										
Sulfur	+	+	-	-	-	ND	-	+	+	+
Nitrate	-	-	W	-	+	ND	-	-	-	-
Fumarate	+	ND	W	-	+	-	-	-	+	-
Fermentation										
Fumarate	-	ND	-	+	-	+	-	ND	ND	+
Lactate	+	ND	+	-	+	-	-	+	+	+
Pyruvate	+	+	+	+	+	+	+	-	+	+
Major fatty acids	anteiso-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$, and iso-$C_{17:1\omega8}$	ND	iso-$C_{15:0}$, anteiso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl), iso-$C_{17:0}$	$C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$	$C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:0}$, iso-$C_{15:0}$, iso-$C_{17:1\omega}9c$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$, iso-$C_{17:1}\omega9c$	$C_{18:0}$, anteiso-$C_{15:0}$, $C_{16:0}$, $C_{18:1}\omega7$	ND	ND	ND
DNA G+C content (mol%)	61.93	61.0	63.5	50.0	53.0	62.1	62.6	60.7	59.1*	59.6
Isolation source	Terrestrial mud volcano	Deep groundwater	Deep-sea hydrothermal vent	Deep marine wood falls	Marine sediments	Estuarine sediment	Brackish lagoon sediment	Hypersaline lake	Hypersaline cianobacterial mat	Exhaust water

*Data from GenBank

Figures

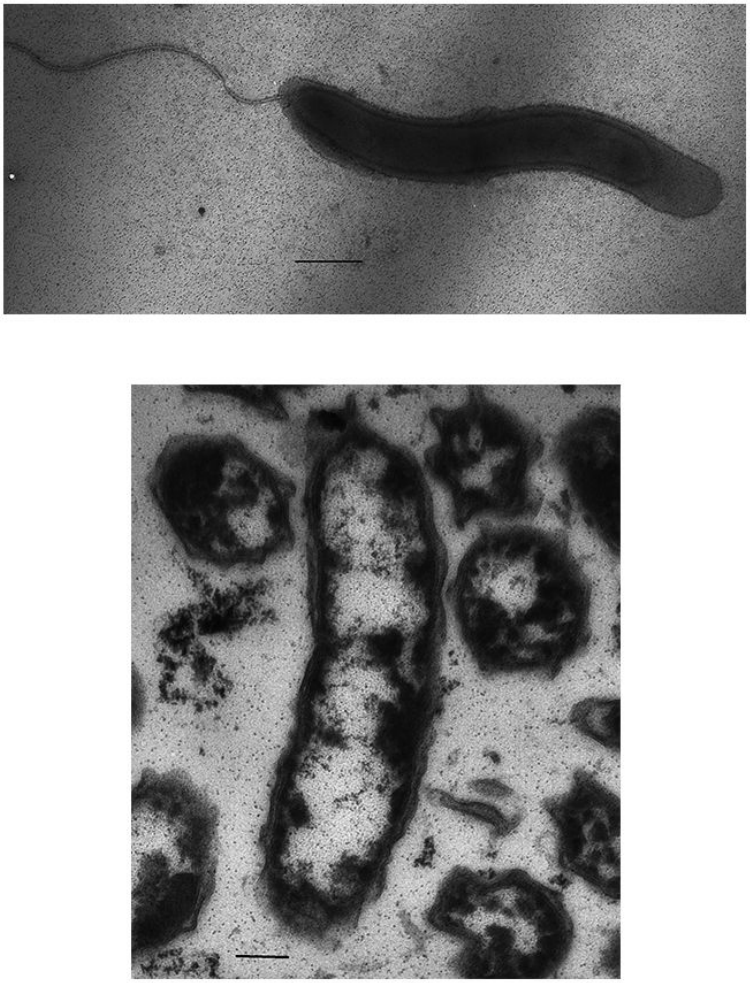

Figure 1

Electron micrographs of cells of strain F-1T. (a) A negatively stained cells. Bar, 0.6 μm. (b) Thin section of a cell. Bar, 0.5 μm.

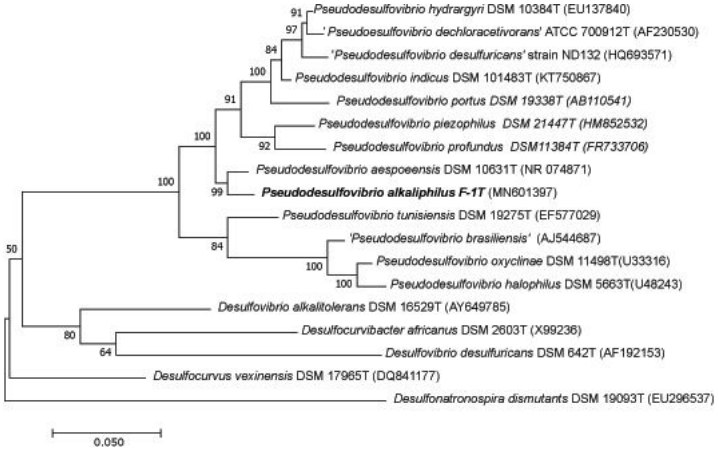

Figure 2

Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain F-1T and the related microorganisms. The tree was reconstructed using the maximum-likelihood method. Trees reconstructed by the neighbor-joining and minimum-evolution algorithms displayed the same topology. Each number indicates the bootstrap value from 1000 trials. Bar, 0.05 substitutions per nucleotide position. The GenBank accession numbers are given in parentheses.

Supplementary Files

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