INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Pérez-Bernal *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:3219–3225

DOI 10.1099/ijsem.0.004159

MICROBIOLOGY SOCIETY

Desulfobotulus mexicanus sp. nov., a novel sulfate-reducing bacterium isolated from the sediment of an alkaline crater lake in Mexico

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Abstract

A novel Gram-negative, non-spore-forming, vibrio-shaped, anaerobic, alkaliphilic, sulfate-reducing bacterium, designated strain PAR22N^T, was isolated from sediment samples collected at an alkaline crater lake in Guanajuato (Mexico). Strain PAR22N^T grew at temperatures between 15 and 37 °C (optimum, 32 °C), at pH between pH 8.3 and 10.1 (optimum, pH 9.0–9.6), and in the presence of NaCl up to 10%. Pyruvate, 2-methylbutyrate and fatty acids (4–18 carbon atoms) were used as electron donors in the presence of sulfate as a terminal electron acceptor and were incompletely oxidized to acetate and CO_2. Besides sulfate, both sulfite and elemental sulfur were also used as terminal electron acceptors and were reduced to sulfide. The predominant fatty acids were summed feature 10 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$), $C_{18:1}\omega9c$ and $C_{16:0}$. The genome size of strain PAR22N^T was 3.8 Mb including 3391 predicted genes. The genomic DNA G+C content was 49.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that it belongs to the genus *Desulfobotulus* within the class *Deltaproteobacteria*. Its closest phylogenetic relatives are *Desulfobotulus alkaliphilus* (98.4% similarity) and *Desulfobotulus sapovorans* (97.9% similarity). Based on phylogenetic, phenotypic and chemotaxonomic characteristics, we propose that the isolate represents a novel species of the genus *Desulfobotulus* with the name *Desulfobotulus mexicanus* sp. nov. The type strain is PAR22N^T (=DSM 105758^T=JCM 32146^T).

Despite the harsh conditions that prevail in soda lakes, several studies have shown that biological oxido-reductive reactions within the sulfur cycle are of ecological significance [1, 2]. Bacteria participating in reductive activities include sulfate-reducing bacteria (SRB), which have been reported to inhabit these extreme environments [3]. In such environments, sulfate reduction into sulfide is performed by obligate haloalkaliphilic SRB belonging to three orders within the *Deltaproteobacteria*, namely *Desulfobacterales*, *Desulfovibrionales* and *Syntrophobacterales* [2, 4, 5]. Regarding the *Desulfobacterales*, members of three genera including *Desulfobotulus*, *Desulfonatronobacter* (both genera belonging to the family *Desulfobacteraceae*) and *Desulfobulbus* (family *Desulfobulbaceae*) are recognized to inhabit these saline alkaline habitats.

At the time of writing, the genus *Desulfobotulus* comprises only two species with validly published names [6]: *Desulfobotulus sapovorans* [7], an alkali-tolerant bacterium isolated from freshwater mud in Germany, and *Desulfobotulus alkaliphilus* [8], an haloalkaliphilic bacterium isolated from sediments of a hypersaline soda lake in Russia. Members of this genus are Gram-negative, strictly anaerobic, mesophilic, chemoorganotrophic bacteria. They oxidize incompletely fatty acids and pyruvate to acetate and CO_2, using sulfate or sulfite as terminal electron acceptors. Both *Desulfobotulus* species of this genus are able to ferment pyruvate.

Here we report on the characterization of a novel haloalkaliphilic *Desulfobotulus* species, designated PAR22N^T, isolated from sediment sampled at an alkaline crater lake in Mexico. In contrast to the two other haloalkaliphilic *Desulfobotulus*

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Keywords: alkaliphilic anaerobic bacterium; sulfate reduction; *Desulfobotulus*.
Abbreviations: PHA, polyhydroxyalkanoates; SRB, sulfate-reducing bacteria.
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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence for strain PAR22N^T is KY523541. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number NZ_VDMB00000000. The version described in this paper is version NZ_VDMB00000000.1.

species reported so far, strain PAR22N^T can grow with elemental sulfur as a terminal electron acceptor.

Sediment samples were collected at the shoreline of a soda lake (pH 10.7; NaCl 16 g l^{-1}), located in Rincón de Parangueo, a maar of phreatomagmatic origin (i.e. a volcanic crater resulting from an explosive eruption with interaction between magma and water), at the north-west of the city of Valle de Santiago in the state of Guanajuato, Mexico (20° 25′ N; 101° 12.1′ W), in September 2013. Samples of the upper 3–5 cm sediment were transferred in serum bottles which were completely filled with water from the site and sealed with butyl stoppers. The culture medium used for enrichment of SRB was the same as previously reported by Pérez Bernal *et al.* [9]. This medium was prepared with 50% filter-sterilized site water and contained sodium acetate, sodium lactate, sodium pyruvate together with glycerol as growth substrates, and sulfate and thiosulfate as terminal electron acceptors. However, no pure culture was obtained by liquid serial dilutions using this culture medium with the four substrates. Supplementary liquid serial dilutions were performed using the following culture medium containing (per litre of distilled water): NaCl, 15 g; KH_2PO_4, 0.2 g; Na_2CO_3, 3.5 g; NH_4Cl, 0.25 g; $MgCl_2{\cdot}6H_2O$, 0.1 g; KCl, 0.2 g; $FeSO_4{\cdot}7H_2O$, 1.42 mg; $Na_2WO_4{\cdot}2H_2O$, 38 µg; $Na_2SeO_3{\cdot}5H_2O$, 3 µg; yeast extract, 0.2 g; trace element solution, 1 ml [10]. The pH was adjusted to pH 9. The culture medium was dispensed into Hungate tubes under N_2 atmosphere. Once autoclaved and prior to inoculation, the following compounds were added from sterile stock solutions: vitamin V7 solution [11], 1 ml l^{-1} and $Na_2S{\cdot}9H_2O$, (0.4 g l^{-1}). Sodium butyrate (10 mM) or sodium octanoate (5 mM) were added as electron donors in the presence of sulfate (20 mM) as an electron acceptor. These fatty acids were chosen based on the disclosure of a partial 16S rRNA gene sequence retrieved by molecular approaches (see below for procedure) indicating the presence of a potential fatty acid-degrading bacterium belonging to the genus *Desulfobotulus* [8] in the previous liquid serial dilutions. Finally, two successive roll-tube dilution series [12] permitted to obtain a pure culture, namely strain PAR22N^T, when using octanoate and sulfate as electron donor and electron acceptor, respectively. $FeSO_4{\cdot}7H_2O$ (1 mM) was added to the solid medium to monitor sulfidogenic activity and facilitate the location of SRB colonies by the appearance of a black halo resulting from the precipitation of oxidized iron and sulfide into FeS. Purity of the strain was checked microscopically and by inoculation in a medium supplemented with peptone, yeast extract and glucose (1 g l^{-1} each).

The genomic DNA was extracted with the Wizard Genomic DNA Purification kit, according to the recommendations of the manufacturer (Promega). The PCR products, obtained with the primers Rd1 and Fd1 [13], were sequenced by the Sanger method at GATC Biotech (Konstanz, Germany). The nearly complete 16S rRNA gene sequence of strain PAR22N^T was achieved (1540 bp). According to the minimal standards proposed by Chun *et al.* [14], the whole genome was sequenced. Thus, the genomic DNA was extracted and purified according to the method described by Marteinsson *et al.* [15]. Analysis was performed at Molecular Research LP (MRDNA, Texas). Briefly, the library was prepared using the Nextera DNA Flex library preparation kit (Illumina), sequenced paired end for 300 cycles using the HiSeq system (Illumina), assembled with NGEN version 15 and annotated using the NCBI Prokaryotic Genome Annotation Pipeline [16]. The genome size of strain PAR22N^T was 3.8 Mb and it consisted of 69 contigs. This draft genome had a coverage depth >100×, had an N50 length of 139203 and 3391 predicted genes of which there were 70 RNA genes and 71 pseudogenes.

In order to validate the genome results, the full-length 16S rRNA gene sequence (1563 bp) was extracted from the draft genome sequence and then compared with the 16S rRNA gene sequence obtained previously by the Sanger method. The sequences showed an alignment of 99.55%, validating the authenticity of the genome data.

This full-length 16S rRNA gene sequence of strain PAR22N^T was aligned using the CLUSTAL_W program with closely related sequences obtained from the GenBank database of the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov/), using the BLAST algorithm. The positions of sequences with alignment uncertainties were omitted, and 1325 unambiguous aligned base-pairs were used to reconstruct the phylogenetic tree with MEGA 7 [17], using the neighbour-joining [18], maximum-parsimony [19] and maximum-likelihood [20] methods, with a bootstrap of 1000 replications. The phylogenetic results revealed that strain PAR22N^T was affiliated to the genus *Desulfobotulus* (order *Desulfobacterales*), being most closely related to *D. alkaliphilus* and *D. sapovorans*, with 98.49 and 98.07% 16S rRNA gene gene similarity, respectively. The sequence similarities are below the 98.7% threshold value proposed by Chun *et al.* in 2018 [14] for determining a new bacterial species. The phylogenetic relationships between strain PAR22N^T, *Desulfobotulus* species and neighbouring genera within the family *Desulfobacteraceae* are shown in Fig. 1.

Considering the proposed threshold of 98.7 %, no overall genome related index is required. However since DNA–DNA hybridizations between strain PAR22N^T, *D. alkaliphilus* and *D. sapovorans* were already preformed in June 2017 at the DSMZ [21, 22], it is noteworthy to report the low values that were obtained (54.9 and 28.6% respectively with *D. alkaliphilus* and *D. sapovorans*), confirming that strain PAR22N^T represents a novel species.

The genomic DNA G+C content of strain PAR22N^T was 49.0 mol%, a value quite lower than those of *D. alkaliphilus* and *D. sapovorans* (51.3 and 53 mol%, respectively).

Morphological characteristics of cells of strain PAR22N^T were examined using a Nikon Eclipse 600 phase-contrast microscope and by transmission electron microscopy of negatively stained cells (uranyl acetate 2%). The Gram type of cell wall was determined with the Gram Staining Kit from Sigma-Aldrich, and by electron microscopy as described previously [9]. Microphotographs were taken with an FEI Tecnai G2 electron microscope. Cells were vibrio-shaped,

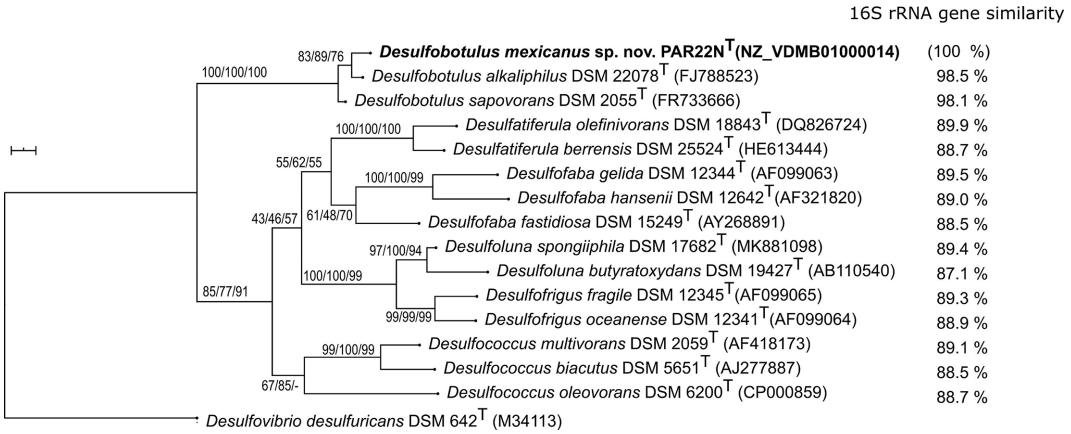

Fig. 1. Maximum-likelihood tree based on the alignment of 1325 bp of 16S rRNA gene sequences, showing the phylogenetic position of strain PAR22N^T among the genus *Desulfobotulus* and other *Desulfobacteraceae* SRB. Bootstrap values are shown from left to right for maximum-likelihood, neighbour-joining and maximum-parsimony trees obtained with the same alignment dataset. Accession numbers of the sequences are given in parentheses. Percentage values next to the species names indicate 16S rRNA gene similarity to strain PAR22N^T. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bar, 0.01 substitutions per nucleotide position.

non-spore-forming, 2–3.5 µm long and 0.8–1 µm wide (Fig. 2a). They stained Gram-negative, with the corresponding cell-wall structure demonstrated in Fig. 2b. Cells were motile by a single polar flagellum (Fig. 2c). They occurred singly or in pairs, accumulating polyhydroxyalkanoates granules (Fig. 2b).

Growth experiments were performed in duplicate using the culture medium mentioned above in the presence of octanoate (5 mM) as energy source and sodium sulfate (20 mM) as electron acceptor. Strain PAR22N^T was subcultured at least once under the same experimental conditions prior to determination of growth rates. Turbidity (600 nm) was used to assess growth. Determination of the temperature range for growth was performed at 10, 15, 20, 25, 28, 32, 35, 37 and 40 °C. The isolate grew at 15–37 °C (optimum, 32 °C). Salt tolerance was tested at 30 °C at different NaCl concentrations (0, 1, 5, 10, 15, 20, 30, 40, 60, 70, 80, 90, 100 and 110 g l^{-1}). The strain grew in a range of 0–100 g NaCl l^{-1}, with an optimum of 15 g NaCl l^{-1} and therefore should be considered as halotolerant. The pH range for growth was tested using the optimized culture medium without Na_2CO_3, and different pH values (pH 8.0, 8.3, 8.8, 9, 9.9, 9.8, 10.1, 10.5, 10.8 and 11) were obtained by adding sodium carbonate and/or sodium bicarbonate from sterile stock solutions. Growth occurred at pH 8.3–10.1 with an optimum at pH 9.0–9.6 thus confirming the alkaliphilic feature of the novel isolate.

Further additional physiological tests were performed under those above defined optimal growth conditions.

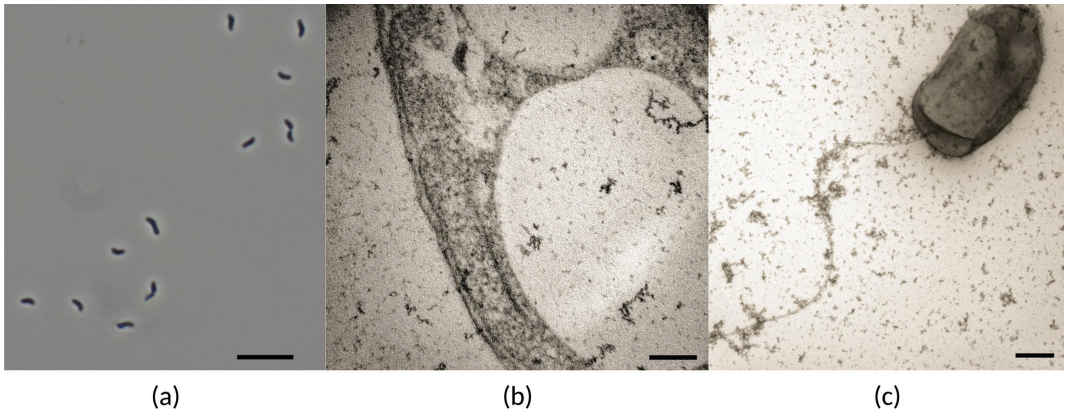

Fig. 2. (a) Phase contrast microscopy image of strain PAR22N^T grown with octanoate as substrate and sulfate as electron acceptor at 30 °C. Bar, 10 µm. (b) Ultrathin section of a strain PAR22N^T cell showing the Gram-negative cell-wall structure and PHA granules. Bar, 0.1 µm. (c) Transmission electron microscopy image of negatively stained cells of strain PAR22N^T showing the location of the flagellum. Bar, 0.5 µm.

The ability to use electron donors and acceptors were monitored by turbidimetry and by chemical analyses. The end products of substrate oxidation (e.g. acetate) were determined by HPLC using an Aminex HPX-87H (Bio-Rad) column with 2.5 mM H_2SO_4 as mobile phase. Sulfate, sulfite and thiosulfate concentrations were determined by ion chromatography using a Metrosep Anion Supp 1 column (Metrohm). Sulfide was assayed either using the rapid method of Cord-Ruwisch [23] or the method of Cline [24] when elemental sulfur was tested as an electron acceptor.

In the presence of sulfate, strain PAR22N^T oxidized pyruvate (20 mM), 2-methylbutyrate (10 mM) and straight-chain fatty acids from C4 to C18: butyrate (10 mM), valerate (10 mM), caproate (5 mM), octanoate (5 mM), nonaoate (1 mM), caprate (1 mM), myristate (1 mM), palmitate (1 mM) and stearate (1 mM). No growth was obtained on the following substrates (20 mM, except where stated) despite the presence of sulfate: acetate, formate, formate/acetate as carbon source (2 mM), lactate, propionate, succinate, fumarate, malate, methanol, ethanol, butanol, isobutanol, glycerol, ribose, glucose, fructose, casamino acids (2 g l^{-1}), yeast extract (1 g l^{-1}), H_2 (1 bar) with or without acetate as carbon source (2 mM). Pyruvate (20 mM) was not fermented by the isolate within a month of incubation.

Sulfate (20 mM), thiosulfate (10 mM), elemental sulfur (0.1 % w/v), sulfite (2 mM), fumarate (10 mM), dimethyl sulfoxide (10 mM) and selenite (5 mM) were assayed as potential electron acceptors in the presence of octanoate (5 mM) as an energy source. To test ferric iron [$Fe(OH)_3$, 90 mM] as terminal electron acceptor, sodium sulfide was omitted, and the culture medium was mildly reduced with cysteine–HCl (44 mg l^{-1}). The use of manganese dioxide (15 mM), arsenate (2 mM), Fe–citrate (50 mM), nitrate (10 mM), nitrite (2.5 mM) and dioxygen (1.5%) as terminal electron acceptors, was tested in the absence of a reducing agent in the culture medium.

Among the electron acceptors tested, strain PAR22N^T was able to use only sulfate, sulfite and elemental sulfur as electron acceptors with octanoate as an electron donor. Furthermore, thiosulfate (10 mM) was also assayed as an electron acceptor in the presence of pyruvate as an energy source. Under these conditions, strain PAR22N^T was not able to use thiosulfate.

Yeast extract (0.2 g l^{-1}) was required for growth. The addition of vitamins did not stimulate growth.

Fatty acid analyses of strain PAR22N^T and *D. alkaliphilus* DSM 22078^T were carried out at the DSMZ. Both bacteria were grown in the same medium with octanoate as substrate and sulfate as electron acceptors. Fatty acids were extracted using the method of Miller [25], modified by Kuykendall *et al.* [26]. The profile of cellular fatty acids was analysed by GC using the Microbial Identification System (MIDI; Sherlock version 6.1; database, TSBA40; GC model 6890 N, Agilent Technologies).

The main fatty acids of strain PAR22N^T were summed feature 10 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω9*t* and/or$C_{18:1}$ ω12*t*, 46.4 %), $C_{18:1}$ ω9*c* (20.7 %) and $C_{16:0}$ (20.5 %). The fatty acids are listed in the Table 1. In the case of *D. alkaliphilus*, the predominant fatty acid was $C_{18:1}$ ω9*c* (32 %).

Table 1. Cellular fatty acid content (%) of strain PAR22N^T and *Desulfobotulus alkaliphilus* DSM 22078^T

Strains: 1, PAR22N^T; 2, *Desulfobotulus alkaliphilus* DSM 22078^T. All data were taken from this study after growth with octanoate and sulfate at 30 °C. TR, trace amount (<0.5%); –, not detected

Fatty acid	1	2
$C_{10:0}$	TR	1.2
$C_{14:0}$	1	1.6
$C_{16:1}$ ω9*c*	1.4	2.3
$C_{16:1}$ ω7*c*	5.6	2.7
$C_{16:0}$	20.5	23.0
$C_{16:0}$ 3-OH	2.2	2.3
$C_{18:2}$ ω9,6*c*	–	2.8
$C_{18:1}$ ω9*c*	20.7	32
$C_{18:0}$	0.7	5.2
$C_{20:1}$ ω9*c*	TR	–
Summed features:*		
1	TR	–
5	1.1	1.2
10	46.4	25.6
12	TR	–

*Summed features contain fatty acids that could not be separated by GLC using the Microbial Identification System (MIDI). Summed feature 1 comprised $C_{13:1}$ ω1*c* and/or $C_{14:0}$ ALDE. Summed feature 5 contained $C_{15:0}$ DMA and/or $C_{14:0}$ 3-OH. Summed feature 10 comprised $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω9*t* and/or $C_{18:1}$ ω12*t*. Summed feature 12 contained iso-$C_{19:0}$ and/or an unknown compound 18.622.

Analysis of respiratory quinones was also carried out at the DSMZ. Following their extraction, using the method described by Tindall [27, 28], they were separated by thin-layer chromatography on silica gel and further analysed by HPLC. The predominant menaquinone was MK7 (97%), with MK6 (3%) as a minor component.

Strain PAR22N^T was tested for production of cytochrome *c* and desulfoviridin. Washed cells were suspended in Tris–HCl buffer (10 mM pH 7.6) and disrupted by sonication. Cell-free extract was obtained by centrifugation and examined with a Varian Cary 50 UV/Vis spectrophotometer between 300 and 700 nm. The soluble extract exhibited the peak at 409 nm, and when reduced with sodium dithionite, the characteristic peaks were at 419, 523 and 553 nm, thus highlighting the presence of cytochrome c_3 [29]. Desulfoviridin was not present.

Strain PAR22N^T is a mesophilic, anaerobic, sulfate-reducing, obligate alkaliphilic bacterium, growing optimally on octanoate as electron donor and sulfate as electron acceptor. Within 5 days of growth at pH 9, 17 mM sulfide was produced. Under optimal growth conditions, the growth rate of strain PAR22N^T was 0.028 h^{-1}. With pyruvate as an electron donor and sulfate as an electron acceptor, strain PAR22N^T grew more slowly (growth rate, 0.003 h^{-1}). With octanoate as substrate and sulfite as electron acceptors, 2.3 mM sulfide was produced after 6 days of incubation and growth was also slow with a growth rate of 0.008 h^{-1}. In the presence of octanoate as substrate and elemental sulfur as an electron acceptor, strain PAR22N^T produced in a subculture 7–10 mM sulfide after 6 days incubation. This is thus the first report of a haloalkaliphilic SRB with the ability to grow with elemental sulfur as a terminal electron acceptor.

According to the genome annotation, strain PAR22N^T was predicted to produce Na^+/H^+ antiporters and a Na^+ coupled solute uptake system (symporter). Presence of these transporters is one of the well-known ways in which alkaliphiles adapt to alkaline conditions [30]. Regarding adaptation to osmotic stress, accumulation of compatible solutes (e.g. glycine betaine, ectoine) is one of the common strategies used by halophiles [30], a strategy likely used by our isolate because of the presence of genes related to the transport and biosynthesis of compatible compounds in its genome. Otherwise, genes involved in sulfite reductase, long-chain fatty-acid transporter and flagella structure were also present, thus supporting our physiological studies and microscopic examinations.

Fatty acid (from C4 to C18) oxidation has been already reported for SRB [31]; however, little is known about SRB performing such metabolisms under alkaline conditions. To our knowledge, this concerns three alkaliphilic bacteria originating from alkaline environments, *Desulfonatronobacter acidivorans* [4], *Desulfonatronobacter acetoxydans* [32] and *Desulfobotulus alkaliphilus* [8], and one alkali-tolerant bacterium isolated from freshwater mud, *Desulfobotulus sapovorans* [7]. While *D. acidivorans*, *D. acetoxydans* and *D. alkaliphilus* are able to oxidize fatty acids from C4 to C9, strain PAR22N^T and *D. sapovorans* are the only bacteria with the ability to oxidize longer chain fatty acids up to C18. However, in contrast to *D. sapovorans*, strain PAR22N^T does not use lactate, does not ferment pyruvate, displays a different optimum pH for growth and has a lower G+C content in the DNA (Table 2). In this respect, strain PAR22N^T can be distinguished from its two closest phylogenetic relatives belonging to the genus *Desulfobotulus*.

Table 2. Comparison between strain PAR22N^T and the type strains of the species of the genus *Desulfobotulus*

Strains: 1, PAR22N^T; 2, *Desulfobotulus alkaliphilus* ASO4-4^T [8]; 3, *Desulfobotulus sapovorans* 1pa3^T [7]. All strains are Gram-negative vibrios, non-spore-forming and motile, and accumulate PHA granules. They all use sulfate and sulfite as an electron acceptor, and are able to respire pyruvate with sulfate. +, Positive; –, negative; ND, not determined.

Characteristics	1	2	3
Size (μm)	0.8–1.0×2.0–3.5	1×3–6	2×3–5
Temperature range (°C) for growth (optimum)	15–37 (32)	ND–40 (32)	15–38 (34)
pH range for growth (optimum)	8.3–10.1 (9.0–9.6)	8.7–10.7 (9.9–10.1)	6.5–9.3 (7.7)
Salt tolerance, M Na^+ (optimum)	0.1–1.8 (0.4)	0.1–1.75 (0.6)	<0.5
Electron donors with SO_4^{2-}:			
Straight-chain fatty acids	C4–C6, C8–C10, C14, C16, C18	C4, C6, C8, C9	C4–C16 (C18)
2-Methyl butyrate	+	–	+
Lactate	–	–	+
Electron acceptors:			
S_2O_3 with pyruvate	–	+	ND
S° with fatty acid	+	+*	ND
Fermentation of:			
Pyruvate	–	+	+
Quinones	MK7 (major)	ND	MK7 (major)
DNA G+C (mol%)	49.0 (WGS)	51.3 (T_m)	53 (T_m)
Habitat	Soda lake	Soda lake	Freshwater

*In cell suspension with butyrate as reductant.

Finally, based on phenotypic, phylogenetic and chemotaxonomic differences observed between strain PAR22N^T and *D. alkaliphilus* and *D. sapovorans*, we propose to assign strain PAR22N^T as representing a novel species of the genus *Desulfobotulus* called *Desulfobotulus mexicanus* sp. nov.

DESCRIPTION OF *DESULFOBOTULUS MEXICANUS* SP. NOV.

Desulfobotulus mexicanus (me.xi.ca'nus. N.L. masc. adj. *mexicanus*, from Mexico, from where this bacterial strain was isolated).

Cells are Gram-negative, vibrio-shaped (2–3.5 µm × 0.8–1 µm) and motile by means of a single polar flagellum. No spores are detected. Accumulates PHA granules. Able to grow in solid medium. Mesophilic, with growth at 15–37 °C (optimum, 32 °C). Obligately alkaliphilic, with growth at pH 8.3–10.1 (optimum, pH 9.0–9.6). Halotolerant, with growth in the range of 0–100 g l^{-1} NaCl (optimum, 15 g l^{-1}). Oxidizes 2-methylbutyrate, fatty acids up to C18, and pyruvate in the presence of sulfate as terminal electron acceptor. Acetate, formate, formate/acetate (carbon source), lactate, propionate, succinate, fumarate, malate, methanol, ethanol, butanol, isobutanol, glycerol, ribose, glucose, fructose, casamino acids, yeast extract and H_2 are not used. Requires yeast extract for growth. Organic substrates are incompletely oxidized to acetate and CO_2. Pyruvate is not fermented. Sulfate is the preferred electron acceptor over sulfite and elemental sulfur. Thiosulfate, fumarate, dimethylsulfoxide, selenite, arsenate, ferric iron, Fe–citrate, nitrate, nitrite, manganese dioxide and dioxygen are not used as terminal electron acceptors. The major fatty acids are summed feature 10 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω9*t* and/or $C_{18:1}$ ω12*t*), $C_{18:1}$ ω9*c* and $C_{16:0}$. The predominant quinone is MK7 (97 %). Cytochrome c_3 is present and desulfoviridin is absent.

The type strain, PAR22N^T (=DSM 105758^T=JCM 32146^T), was isolated from sediment of a crater soda lake (Rincón de Parangueo, Mexico). The genome of the type strain is about 3.8 Mb and has a G+C content of 49.0 mol%. The GenBank accession numbers of the 16S rRNA gene sequence and the genome sequence are KY523541 and NZ_VDMB00000000, respectively.

Funding information
This research was supported by the project BIOMETAL (ANR-12-ISV7-0006-02). M.F. P.-B. was supported by a grant from the CONACyT (Mexico) and an ARTS grant from the IRD (France).

Acknowledgements
The authors wish to thank Léa Sylvi for technical help with DNA extraction. The laboratories of the MIO, Aix-Marseille University, where most of the experiments were performed, are in compliance with ISO9001-2015.

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

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