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Desulfonatronum parangueonense sp. nov., a sulfate-reducing bacterium isolated from sediment of an alkaline crater lake

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Abstract

Novel Gram-stain-negative, non-spore-forming, vibrio-shaped, anaerobic, alkaliphilic, sulfate-reducing bacteria, designated strains PAR180^T and PAR190, were isolated from sediments collected at an alkaline crater lake in Guanajuato (Mexico). Strain PAR180^T grew at temperatures between 15 and 40 °C (optimum 35 °C), and at pH between 8.3 and 10.4 (optimum 9). It was halotolerant, growing with up to 8 % (w/v) NaCl. Lactate, formate, pyruvate and ethanol were used as electron donors in the presence of sulfate and were incompletely oxidized to acetate and CO_2. The isolate was able to grow with hydrogen and with CO_2 as a carbon source. Beside sulfate, sulfite and thiosulfate were used as terminal electron acceptors. The isolate was able to grow by disproportionation of sulfite and thiosulfate, but not elemental sulfur, using acetate as a carbon source. The predominant fatty acids were $C_{16:0}$, $C_{16:1}\omega7c$ and summed feature 10 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$). The DNA G+C content was 56.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that it belongs to the genus *Desulfonatronum*, class *Deltaproteobacteria*. Its closest relative is *Desulfonatronum thiosulfatophilum* (98.7 % 16S rRNA gene sequence similarity). The DNA–DNA relatedness value between strain PAR180^T and the type strain of *D. thiosulfatophilum* was 37.1±2.5 %. On the basis of phylogenetic, phenotypic and chemotaxonomic characteristics, the isolates is considered to represent a novel species of the genus *Desulfonatronum*, for which the name *Desulfonatronum parangueonense* sp. nov. is proposed. The type strain is PAR180^T (=DSM 103602^T=JCM 31598^T).

Sediments from alkaline environments are of interest for the bioprospection of extremely alkaliphilic microorganisms that may be used in biotechnological processes [1]. Among anaerobes, sulfate-reducing bacteria (SRB) recognized as potential candidates for bioremediation of heavy metals/metalloids [2] are known to inhabit such extreme environments and to significantly contribute to the sulfur and carbon geochemical cycle [3, 4]. Obligate alkaliphilic SRB belong to four families within the *Deltaproteobacteria*, namely *Desulfobacteraceae* [5], *Desulfobulbaceae* [6], *Desulfohalobiaceae* [7] and *Desulfonatronaceae* [8, 9]. The last comprises a single genus *Desulfonatronum*, which consists of, at the time of writing, eight species with validly published names: *Desulfonatronum lacustre*, the type species [10], *D. thiodismutans* [11], *D. cooperativum* [12], *D. thioautotrophicum* and *D. thiosulfatophilum* [13], *D. buryatense* [14], *D. alkalitolerans* [15] and *D. zhilinae* [16]. They were all isolated from hypersaline and hyposaline soda lakes, with the exception of *D. alkalitolerans* which was isolated from a microbial consortium of a bioreactor in the Netherlands operating at alkaline conditions for removal of H_2S [15]. Members of this genus are anaerobic, mesophilic and alkaliphilic, requiring sodium and carbonate for their growth [9]. They all utilize sulfate, sulfite and thiosulfate as terminal electron acceptors to be reduced to sulfide.

Here we focused on the bioprospection of SRB from sediments of an alkaline crater lake in Mexico and report on the isolation and characterization of a novel *Desulfonatronum* species.

Sediments were collected at the shoreline of a soda lake (pH 10.7; salinity 16 g l^{-1}) known for its high content of carbonates [17], located in a maar of phreatomagmatic origin (Rincón de Parangueo), 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. Sediments were transferred to

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Abbreviation: SRB, sulfate-reducing bacteria.
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One supplementary table and two supplementary figures are available with the online Supplementary Material.

serum bottles. The bottles were completely filled with water of the site and sealed with butyl stoppers. To ensure enrichment of SRB, both sulfate and thiosulfate recognized as suitable terminal electron acceptors for substrate oxidation by these bacteria were added to the culture medium. Specifically, the medium was prepared in a 1 : 1 (v/v) proportion with filtered water of the sampling site and distilled water containing (per litre): Na_2SO_4, 4 g; $Na_2S_2O_3$, 0.79 g; NH_4Cl, 0.25 g; KH_2PO_4, 0.2 g; NaCl, 15 g; yeast extract, 0.1 g; HEPES, 10 mM; selenite tungstate solution [18], 1 ml; trace element SL12 solution [19], 1 ml; resazurin solution 0.1 % (w/v), 1 ml. The pH was adjusted to 9. After sterilization, the culture medium was immediately flushed with nitrogen gas. Prior to use, the following components were added from anoxic sterile solutions: vitamin V7 solution [20], 1 ml; $FeSO_4{\cdot}7H_2O$, 1 mM, to monitor sulfidogenic activity (removal of sulfide by production of FeS); and $Na_2S_2O_4$, 0.2 mM, as reductant. A mixture of four substrates, namely sodium acetate, sodium lactate, sodium pyruvate and glycerol (5 mM each), was added to serum bottles containing 50 ml of culture medium. These bottles were inoculated with around 1 g of the collected sediment, and incubated for 3–4 weeks at room temperature (≈30 °C) until a black colour linked to sulfide production (FeS) appeared, thus demonstrating sulfate-reducing activity. After two or three successful subcultures of the enrichment culture, the isolation process started. Isolations were performed in several steps. In the first step, a high-throughput dilution-to-extinction procedure in 384-well microplates [21] was applied using the previous culture medium containing the four substrates. Microplates were inoculated and incubated for 3–5 weeks at room temperature (27–33 °C) in anaerobic bags (BD GasPakTM EZ Gas Generating Pouch Systems), until positive wells were detected by the black iron sulfide precipitate. As this first isolation step was not successful in obtaining pure cultures, supplementary liquid serial dilutions were performed from the positive cultures of two wells, using the following modified 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 [22], 1 ml. The pH was adjusted to 9. The culture medium was dispensed into Hungate tubes under N_2 atmosphere. Prior to inoculation, the following compounds were added from sterile stock solutions: vitamin V7 solution, 1 ml l^{-1}; $Na_2S{\cdot}9H_2O$, 0.4 g l^{-1}. Different electron donor/electron acceptor combinations were assayed: lactate (20 mM)/sulfate or thiosulfate (20 mM); formate (80 mM)/sulfate or thiosulfate (20 mM); and ethanol (20 mM)/sulfite (10 mM). Disproportionation was also assayed using thiosulfate as an electron donor and electron acceptor (10 mM) with or without acetate (2 mM) as a carbon source. The combinations of electron donors and electron acceptors was chosen following the disclosure of the partial 16S rRNA gene (see below for experimental details) and taking into account information reported by Sorokin *et al.* [13] regarding the difficulties encountered in obtaining pure cultures of alkaliphilic SRB. To obtain axenic cultures, two successive roll-tube dilution series [23] made under the same culture conditions were performed. Two strains were obtained, namely PAR180^T and PAR190, when using ethanol/sulfite and lactate/sulfate as electron donor/electron acceptor combinations, respectively. The purity of the two strains 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 primers Rd1 and Fd1 [24] were sequenced by the Sanger method at GATC Biotech. The nearly complete 16S rRNA gene sequences of strains PAR180^T and PAR190 were determined (1518 and 1522 bp, respectively). The alignment of these two sequences revealed 99.8 % similarity. The rRNA gene sequences of strains PAR180^T and PAR190 were aligned via the CLUSTAL W program with closely related sequences obtained from the GenBank database of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/), using the BLAST algorithm. The positions of sequences with alignment uncertainties were omitted, and 1396 unambiguous aligned base pairs were used to reconstruct the phylogenetic tree with MEGA 6 [25], via the neighbour-joining algorithm of Saitou and Nei [26] with 1000 bootstrap replicates. Phylogenetic analysis revealed that both strains were affiliated to the genus *Desulfonatronum* (order *Desulfovibrionales*, family *Desulfonatronaceae*), being most closely related to the type strain of *D. thiosulfatophilum* (98.7 % 16S rRNA gene sequence similarity). The phylogenetic relationships between strains PAR180^T and PAR190 and *Desulfonatronum* species are shown in Fig. 1. Strain PAR180^T was chosen for further characterization. The G+C content of the DNA was determined at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) by HPLC with conditions adapted from those of Tamaoka and Komagata [27], and was calculated according to the method of Mesbah *et al.* [28]. The DNA G+C content of strain PAR180^T was 56.1 mol%, a value within the range quoted for other members of the genus *Desulfonatronum* (Table 1).

DNA–DNA hybridization between strain PAR180^T and *D. thiosulfatophilum* was carried out at the DSMZ as described by De Ley *et al.* [29] under consideration of the modifications described by Huss *et al.* [30]. The DNA–DNA hybridization value obtained was 37.1±2.5 %, thus confirming that strain PAR180^T represents a novel species within the genus *Desulfonatronum* [31].

The morphological characteristics of cells of strain PAR180^T were examined using a Nikon Eclipse 600 phase-contrast microscope and by transmission electron microscopy of negatively stained cells (uranyl acetate, 2 % w/v). The Gram type of the cell wall was determined with the Gram Staining Kit of Sigma-Aldrich, and by electron microscopy. Briefly, to prepare thin sections, exponentially grown cells were centrifuged, and fixed for 20 and 30 min in 10 % (v/v)

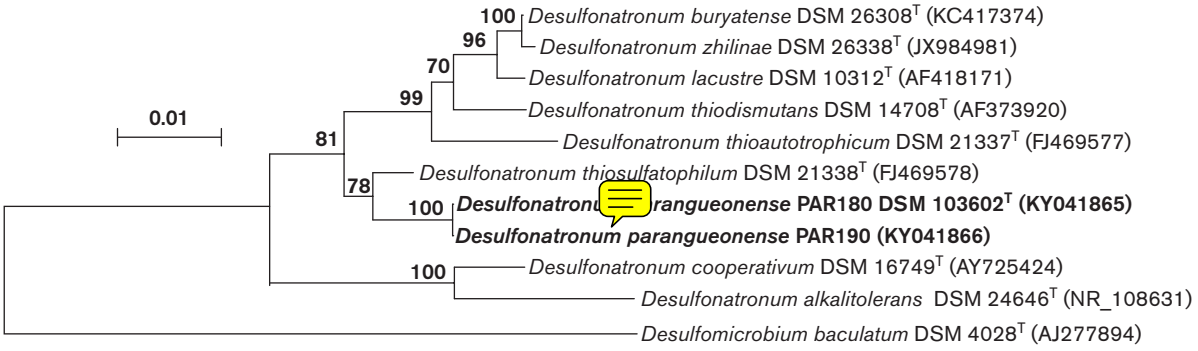

Fig. 1. Neighbour-joining phylogenetic tree based on an alignment of 1396 bp of 16S rRNA gene sequences, showing the positions of strains PAR180^T and PAR190 among the genus *Desulfonatronum*. Accession numbers of the sequences are given in parentheses. Bar, 0.01 substitutions per nucleotide position.

glutaraldehyde and 2 % (v/v) osmium tetroxide, respectively. Cells were then washed, embedded in 2.5 % agarose, dehydrated and embedded in Epon. Thin sections (90 nm) were stained with 2 % (w/v) uranyl acetate for 4 min and with 2 % (w/v) lead citrate for 1.5 min. Photomicrographs were taken with an FEI Tecnai G2 electron microscope. Cells were vibrio-shaped, non-spore-forming, 1.2–2.3 µm long and 0.7–0.9 µm wide. They stained Gram-negative with the corresponding cell-wall ultrastructure, as demonstrated in Fig. S1 (available in the online Supplementary Material). Cells were motile by a single polar flagellum (Fig. S2). They occurred singly, in pairs or as short spirilla.

Growth experiments were performed in duplicate with the modified medium with lactate (20 mM) as an energy source and sodium sulfate (4 g l^{-1}) as an electron acceptor. After growth optimization, the NaCl content of the medium was decreased to 5 g l^{-1}. Unless stated, strain PAR180^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, 30, 35, 37, 40 and 45 °C. The isolate grew at 15–40 °C (optimum 35 °C). Salt tolerance was tested at 35 °C at different NaCl concentrations (0, 1, 5, 10, 15, 20, 25, 35, 40, 45, 50, 60, 80 and 90 g l^{-1}). Strain PAR180^T grew over a range of 0–80 g NaCl l^{-1}, with an optimum of 5 g NaCl l^{-1}. The requirement of Na^+ and carbonates for growth was tested in triplicate with inoculum washed twice in sodium- and carbonate-free media, respectively. Analysis of Na^+ requirements was performed by substituting NaCl by KCl (5 g l^{-1}). Other sodium-containing compounds were also substituted by potassium-containing compounds. During this experiment ethanol (20 mM), to replace sodium lactate, and L-cysteine hydrochloride (0.25 g l^{-1}) were used as an energy source and reductive agent, respectively. Carbonate requirements for growth were determined by replacing them with equimolar amounts of Na_2SO_4 and maintaining the pH with 20 mM CAPS. Growth was obtained in the absence of NaCl but not in the absence of Na^+ or without carbonates. The pH range for growth was tested with the optimized culture medium without Na_2CO_3 buffer. Different pH values (7.5, 8, 8.3, 9, 9.5, 10, 10.5, 10.7 and 11) were assessed by adding a mixture of sodium bicarbonate/sodium carbonate or sodium bicarbonate only. Growth occurred at pH 8.3–10.4 with an optimum at pH 9.

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

The ability to use electron donors and acceptors was 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 the 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 turbidimetric method of Cord-Ruwisch [32] or using the colorimetric method of Cline [33] when elemental sulfur was present in the assays. Hydrogen was analysed by using a gas chromatograph equipped with a carbosphere SS 60/80 mesh column.

With sulfate as an electron acceptor, strain PAR180^T oxidized lactate (20 mM), pyruvate (20 mM), ethanol (20 mM) and formate (80 mM). Furthermore, strain PAR180^T grew lithotrophically on H_2. No growth of strain PAR180^T was observed when increasing yeast extract from 0.2 to 1 g l^{-1} in the absence of H_2, thus demonstrating that yeast extract was not used as a carbon and energy source. Accordingly, growth obtained with strain PAR180^T using H_2 as an energy source may only be the result of the use of carbonate/CO_2 as a carbon source. No growth was observed with the following substrates (20 mM, except where stated) and with sulfate (20 mM) as an electron acceptor: acetate, propionate, succinate, fumarate, malate, butyrate (10 mM), valerate (10 mM), octanoate (5 mM), methanol, glycerol, glucose, mannose, fructose, xylose, casamino acids (2 g l^{-1}), yeast

Table 1. Comparison between strain PAR180^T and the type strains of species of the genus *Desulfonatronum*

Strains: 1, PAR180^T; 2, *D. thiosulfatophilum* ASO4-2^T [13]; 3, *D. lacustre* Z-7951^T [10]; 4, *D. thiodismutans* MLF1^T [11]; 5, *D. cooperativum* Z-7999^T [12]; 6, *D. thioautotrophicum* ASO4-1^T [13]; 7, *D. buryatense* Ki5^T [14]; 8, *D. alkalitolerans* HSRB-L^T [15]; 9, *D. zhilinae* A1915-01^T [16]. All strains are Gram-strain-negative vibrios, non-spore-forming and motile. All strains are positive for the utilization of formate as a growth substrate, and of sulfate, sulfite and thiosulfate as an electron acceptor. +, Positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Cell size (μm)	0.7–0.9×1.2–2.3	0.4–0.5×1.5–4	0.7–0.9×2.5–3.0	0.6–0.7×1.2–2.7	0.4–0.5×1.0–2.5	0.5–0.6×2.0–4.0	0.5–0.7×2.0–2.5	0.5–0.6×1.5–2.5	0.4–0.5×1.2–2.3
Temperature range (°C)	15–40	Mesophilic	22–45	15–48	15–40	Mesophilic	20–40	Mesophilic	10–40
Temperature optimum (°C)	35	Max. 40	40	37	35–38	Max. 40	34	Max. 42	36
pH range	8.3–10.4	8.0–10.4	8.0–10.1	8.0–10.5	6.7–10.3	8.3–10.5	7.5–10.5	7.5–9.7	8.0–10.5
pH optimum	9.0	9.5	9.3–9.5	9.5	8.0–9.0	9.3–10.0	9.4	8.5–9.0	9.0
NaCl range (g l^{-1})	0–80	5–76	0–100	10–70	10–80	5–130	2–40	5–100	5–100
NaCl optimum (g l^{-1})	5	17	0	30	5–15	23–35	5	17–35	40
Electron donors with SO_4^{2-}									
Lactate	+	+	+*	−	+	+	+	+	+
Pyruvate	+	+	−	−	−	+	+	+	+
Ethanol	+	+	+	+	−	+	+	−	−
H_2/acetate	+	+	+	+	+	+	−	+	+
H_2/CO_2	+	−	−	+	−	+	−	−	−
Electron acceptors with lactate:									
S°	+†	+†	−	−	−	+†	+	−	−
Fermentation of:									
Pyruvate	−	+	−	−	−	+	+	+	ND
Growth by disproportionation	Thiosulfate, sulfite (with acetate)	Thiosulfate, sulfite (with acetate)	Thiosulfate (with acetate)	Thiosulfate, sulfite (with acetate)	ND	Thiosulfate, sulfite (autotrophic)	ND	−	ND
DNA G+C content (mol%)	56.1	57	57.3±1*	59.1±0.1	56.5±1	57.6	48.8*	55.8	58.3±1

*Data from Zakharyuk *et al.* [16].

†Elemental sulfur was reduced by resting cells (no growth was observed).

extract (1 g l^{-1}) and peptone (1 g l^{-1}). Lactate (20 mM) and pyruvate (20 mM) were not fermented. In contrast to *D. thiosulfatophilum*, strain PAR180^T did not ferment pyruvate, but was able to use CO_2 as a carbon source when growing with hydrogen.

Sulfate (20 mM), thiosulfate (10 mM), elemental sulfur (0.1 %, w/v), sulfite (2 mM), fumarate (10 mM), DMSO (10 mM) and selenite (5 mM) were assayed as potential electron acceptors using lactate (20 mM) as an energy source. To test ferric iron [$Fe(OH)_3$, 90 mM] as a 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 any reducing agent in the culture medium.

Strain PAR180^T was able to use sulfate, thiosulfate and sulfite as electron acceptors with a growth rate of 0.008, 0.005 and 0.0035 h^{-1}, respectively. In contrast to *D. thiosulfatophilum,* sulfate was preferred over thiosulfate. Although elemental sulfur did not permit growth on lactate, resting cells of strain PAR180^T were shown to reduce it to sulfide. None of the other electron acceptors tested were used by this strain. Nitrite was shown to inhibit growth. Disproportionation of sulfurous compounds [thiosulfate (10 mM), sulfite (5 mM), S° (≈ 10 mM)] was tested in triplicate in the presence and in the absence of acetate (2 mM) as carbon source, without sub-culturing, using an inoculum washed twice with sulfate- and lactate-free medium. The isolate was able to disproportionate sulfite and thiosulfate in the presence of acetate, producing sulfate and sulfite. Disproportionation of elemental sulfur was not observed.

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

Fatty acid analyses of strain PAR180^T and *D. thiosulfatophilum* DSM 21338^T were carried out at the DSMZ. Both bacteria were cultured in the same medium with formate as substrate and sulfate as an electron acceptor. Fatty acids were extracted using the method of Miller [34], as modified by Kuykendall *et al.* [35]. The profile of cellular fatty acids was analysed by GC using the Microbial Identification System (MIDI, Sherlock version 6.1; database, TSBA40; GC model 6890N, Agilent Technologies). The main fatty acids of strain PAR180^T were $C_{16:0}$ (19.1 %), $C_{16:1}\omega7c$ (15.9 %) and summed feature 10 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$, 15.5 %). The minor fatty acids are listed in Tables 2 and S1. In *D. thiosulfatophilum,* the predominant fatty acid was $C_{16:1}\omega7$c (35.8 %).

Analysis of respiratory quinones was also carried out at the DSMZ. Following their extraction, using the method described by Tindall [36, 37], they were separated by TLC on silica gel, and further analysed by HPLC. The predominant menaquinone was MK-6 (96 %), with MK-5 (4 %) as a minor component.

Table 2. Cellular fatty acid contents (%) of strain PAR180^T and *D. thiosulfatophilum*

Strains: 1, PAR180^T; 2, *D. thiosulfatophilum* DSM 21338^T. All data were taken from this study after growth with formate and sulfate at 30 °C. Fatty acids making up <1 % in both strains are not shown. –, Not detected.

Fatty acid	1	2
$C_{14:0}$	7.6	10.2
iso-$C_{15:0}$	1.6	0.9
anteiso-$C_{15:0}$	1.2	1.4
$C_{15:0}$	2.2	0.7
$C_{16:1}\omega9c$	2.4	–
$C_{16:1}\omega7c$	15.9	35.8
$C_{16:0}$	19.1	10.8
$C_{17:1}\omega6c$	1.9	–
$C_{17:0}$	1.6	0.5
$C_{18:2}\omega6,9c$	3.5	3.5
$C_{18:1}\omega9c$	9.8	9.1
$C_{18:0}$	9.1	11.2
Summed features*		
8	3.7	1.5
10	15.5	9.3

*Summed features contain fatty acids that could not be separated by GLC using the Microbial Identification System (MIDI). Summed feature 8 comprised $C_{17:1}\omega8$c and/or $C_{17:2}$. Summed feature 10 comprised $C_{18:1}\omega7c$ and/or $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$.

Strain PAR180^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 a peak at 409 nm, and when reduced with sodium dithionite, the characteristic peaks were at 418.5, 523 and 553 nm, highlighting the presence of cytochrome c_3 [38]. The spectrum also showed a strong absorption band at 630 nm characteristic of desulfoviridin.

Strain PAR180^T is a mesophilic, anaerobic, sulfate-reducing, alkaliphilic bacterium, growing optimally on lactate as an electron donor and sulfate as an electron acceptor in culture medium buffered with Na_2CO_3. After 12 days of growth at pH 9, 22 mM sulfide accumulated. Under defined optimal growth conditions (5 g NaCl l^{-1}, pH 9, 35 °C, lactate as electron donor and sulfate as electron acceptor), the growth rate of strain PAR180^T was 0.008 h^{-1}, corresponding to a doubling time of 3.6 days. The isolate utilized a narrow range of organic electron donors, namely lactate, formate, pyruvate and ethanol, and was able to oxidize hydrogen by reducing sulfate to sulfide. Another notable physiological property of strain PAR180^T is its ability to disproportionate thiosulfate and sulfite to sulfate and sulfide. Disproportionation of sulfurous compounds by *Desulfonatronum* species has already

been demonstrated for *D. thiosulfatophilum*, *D. lacustre*, *D. thiodismutans* and *D. thioautotrophicum*. Recent investigations of alkaline environments [39] have provided evidence that these oxido-reductive processes are also performed by non-SRB (e.g. *Desulfurivibrio* and *Dethiobacter* species), thus suggesting that they may be of geomicrobiological significance regarding the sulfur cycle in such extreme habitats.

Phenotypic, phylogenetic and genetic data demonstrate that strain PAR180^T belongs to the genus *Desulfonatronum*. Phenotypic and chemotaxonomic differences observed between strain PAR180^T and its closest phylogenetic relative, *D. thiosulfatophilum* (Table 1), as well as the low DNA–DNA hybridization value measured between these two bacteria, indicate that strain PAR180^T represents a novel species of the genus *Desulfonatronum*, for which the name *Desulfonatronum parangueonense* sp. nov. is proposed.

DESCRIPTION OF *DESULFONATRONUM PARANGUEONENSE* SP. NOV.

Desulfonatronum parangueonense (pa.ran.gueo.nen′se. N.L. neut. adj. *parangueonense* from Rincón de Parangueo, in the state of Guanajuato, Mexico, from where this microorganism was isolated).

Cells are Gram-stain-negative, vibrio-shaped (1.2–2.3×0.7–0.9 μm) and motile by means of a single polar flagellum. No spores are detected. Mesophilic, with growth at 15–40 °C (optimum 35 °C). Alkaliphilic, with growth at pH 8.3–10.4 (optimum pH 9). Halotolerant, with growth in the range 0–80 g NaCl l^{-1}. Obligately dependent on Na^+ and CO_3^{2-} ions for growth. Lactate, pyruvate, ethanol, formate and hydrogen are used as electron donors in the presence of sulfate, which was found as the preferred terminal electron acceptor over thiosulfate and sulfite. Organic substrates are incompletely oxidized to acetate and CO_2. Able to use CO_2 as a carbon source when growing lithotrophically with H_2. Grows by disproportionation of thiosulfate or sulfite to sulfate and sulfide in the presence of acetate as a carbon source. The major fatty acids are $C_{16:0}$, $C_{16:1}\omega7c$ and summed feature 10 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$). The predominant quinone is MK-6. Cytochrome c_3 and desulfoviridin are present.

The type strain, PAR180^T (=DSM 103602^T=JCM 31598^T), was isolated from sediments of a crater soda lake (Rincón de Parangueo, Mexico). The DNA G+C content of the type strain is 56.1 mol%. PAR190 is a second strain of the species.

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

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