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

Maria Fernanda Pérez Bernal,1,2,3 Elcia Margareth Souza Brito,1,2 Manon Bartoli,1 Johanne Aubé,3 Marie-Laure Fardeau,1 German Cuevas Rodriguez,2 Bernard Ollivier,1 Rémy Guyoneaud3 and Agnès Hirschler-Réa1,*

Abstract

Novel Gram-stain-negative, non-spore-forming, vibrio-shaped, anaerobic, alkaliphilic, sulfate-reducing bacteria, designated strains PAR180T and PAR190, were isolated from sediments collected at an alkaline crater lake in Guanajuato (Mexico). Strain PAR180T 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 CO₂. The isolate was able to grow with hydrogen and with CO₂ as a carbon source. Beside sulfate, sulfate 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 C₁₈:₀, C₁₆:₁ω7c and summed feature 10 (C₁₈:₁ω7c and/or C₁₈:₁ω9t and/or C₁₈:₁ω12t). 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 PAR180T 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 PAR180T (=DSM 103602T=JCM 31598T).

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 Desulfovibacteraeaceae [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 hypersaline 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 H₂S [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

Author affiliations: ¹Aix Marseille Université, Université Toulon, CNRS, IRD, MIO UJF 110, Mediterranean Institute of Oceanography, Marseille, France; ²Laboratory of Sanitary and Environmental Engineering, Engineering Division, Campus de Guanajuato, University of Guanajuato, Guanajuato, Mexico; ³Environmental Microbiology group, IPREM UMR CNRS 5254, Université de Pau et des Pays de l’Adour, IBEAS, Pau, France.

*Correspondence: Agnès Hirschler-Réa, agnes.hirschler@univ-amu.fr

Keywords: alkaliphilic bacterium; anaerobic; sulfate-reduction; disproportionation; Desulfonatronum.

Abbreviation: SRB, sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PAR180T and PAR190 are KY041865 and KY041866, respectively.

One supplementary table and two supplementary figures are available with the online version of this article.
Microplates were inoculated and incubated for 3

the previous culture medium containing the four substrates. A

procedure in 384-well microplates [21] was applied using

strating sulfate-reducing activity. After two or three success-

linked to sulfide production (FeS) appeared, thus demon-

following compounds were added from sterile stock solu-

were detected by the black iron sulfide precipitate. As

gate tubes under N

serum bottles. The bottles were completely filled with water

of the site and sealed with butyl stoppers. To ensure enrich-

ment of SRB, both sulfate and thiosulfate recognized as suit-

able terminal electron acceptors for substrate oxidation by

these bacteria were added to the culture medium. Specific-

ally, the medium was prepared in a 1 : 1 (v/v) proportion

with filtered water of the sampling site and distilled water

containing (per litre): Na₂SO₄, 4 g; Na₂S₂O₃, 0.79 g; NH₄Cl,

0.25 g; KH₂PO₄, 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; FeSO₄·7H₂O, 1 mM, to monitor sulfidogenic activity

(removal of sulfide by production of FeS); and Na₂S₂O₃,

0.2 mM, as reductant. A mixture of four substrates, namely

sodium acetate, sodium lactate, sodium pyruvate and glyc-

erol (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 demon-

strating sulfate-reducing activity. After two or three success-

ful 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 Gas-

PakTM 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 per-

formed from the positive cultures of two wells, using the fol-

lowing modified culture medium containing (per litre of

distilled water): NaCl, 15 g; KH₂PO₄, 0.2 g; Na₂CO₃, 3.5 g;

NH₄Cl, 0.25 g; MgCl₂·6H₂O, 0.1 g; KCl, 0.2 g; FeSO₄·7H₂O,

1.42 mg; Na₂WO₄·2H₂O, 38 µg; Na₂SeO₃·5H₂O, 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 Hun-

gate tubes under N₂ atmosphere. Prior to inoculation, the

following compounds were added from sterile stock solu-

tions: vitamin V7 solution, 1 ml l⁻¹; Na₂S·9H₂O, 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 PAR180ᵀ 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 inocula-

tion 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 PAR180ᵀ and PAR190 were

determined (1518 and 1522 bp, respectively). The align-
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-spor-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 version of this article). 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 PAR180ᵀ 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, 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 PAR180ᵀ 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 Na₂SO₄ 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 Na₂CO₃ 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 H₂SO₄ 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 PAR180ᵀ oxidized lactate (20 mM), pyruvate (20 mM), ethanol (20 mM) and formate (80 mM). Furthermore, strain PAR180ᵀ grew lithotrophically on H₂. No growth of strain PAR180ᵀ was observed when increasing yeast extract from 0.2 to 1 g l⁻¹ in the absence of H₂, thus demonstrating that yeast extract was not used as a carbon and energy source. Accordingly, growth obtained with strain PAR180ᵀ using H₂ as an energy source may only be the result of the use of carbonate/CO₂ 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (µm)</td>
<td>0.7–0.9×1.2–2.3</td>
<td>0.4–0.5×1.5–4</td>
<td>0.7–0.9×2.5–3</td>
<td>0.6–0.7×1.2–2.7</td>
<td>0.4–0.5×1.0–2.5</td>
<td>0.5–0.6×2.0–4.0</td>
<td>0.5–0.7×2.0–2.5</td>
<td>0.5–0.6×1.5–2.5</td>
<td>0.4–0.5×1.2–2.3</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>15–40</td>
<td>Mesophilic</td>
<td>22–45</td>
<td>15–48</td>
<td>15–40</td>
<td>Mesophilic</td>
<td>20–40</td>
<td>Mesophilic</td>
<td>10–40</td>
</tr>
<tr>
<td>Temperature optimum (°C)</td>
<td>35</td>
<td>Max. 40</td>
<td>40</td>
<td>37</td>
<td>35–38</td>
<td>Max. 40</td>
<td>34</td>
<td>Max. 42</td>
<td>36</td>
</tr>
<tr>
<td>pH range</td>
<td>8.3–10.4</td>
<td>8.0–10.4</td>
<td>8.0–10.1</td>
<td>8.0–10.5</td>
<td>6.7–10.3</td>
<td>8.3–10.5</td>
<td>7.5–10.5</td>
<td>7.5–9.7</td>
<td>8.0–10.5</td>
</tr>
<tr>
<td>pH optimum</td>
<td>9.0</td>
<td>9.5</td>
<td>9.3–9.5</td>
<td>9.5</td>
<td>8.0–9.0</td>
<td>9.3–10.0</td>
<td>9.4</td>
<td>8.5–9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>NaCl range (g l(^{-1}))</td>
<td>0–80</td>
<td>5–76</td>
<td>0–100</td>
<td>10–70</td>
<td>10–80</td>
<td>5–130</td>
<td>2–40</td>
<td>5–100</td>
<td>5–100</td>
</tr>
<tr>
<td>NaCl optimum (g l(^{-1}))</td>
<td>5</td>
<td>17</td>
<td>0</td>
<td>30</td>
<td>5–15</td>
<td>23–35</td>
<td>5</td>
<td>17–35</td>
<td>40</td>
</tr>
<tr>
<td>Electron donors with SO(_4^{2–})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>+</td>
<td>+</td>
<td>+*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H(_2)/acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H(_2)/CO(_2)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Electron acceptors with lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S(^{-})</td>
<td>+†</td>
<td>+†</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+†</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fermentation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>56.1</td>
<td>57</td>
<td>57.3±1</td>
<td>59.1±0.1</td>
<td>56.5±1</td>
<td>57.6</td>
<td>48.8*</td>
<td>55.8</td>
<td>58.3±1</td>
</tr>
</tbody>
</table>

*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), sulfitite (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 sulfate 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 sulfur compounds [thiosulfate (10 mM), sulfate (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$_{16:1,ω7c}$ (15.9 %) and summed feature 10 (C$_{16:1,ω7c}$ and/or C$_{18:1,ω9t}$ and/or C$_{18:1,ω7c}$, 15.5 %). The minor fatty acids are listed in Tables 2 and S1. In *D. thiosulfatophilum*, the predominant fatty acid was C$_{16:1,ω7c}$ (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.

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$_2$CO$_3$. 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 $^\circ$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 sulfite to sulfate. Another notable physiological property of strain PAR180$^{T}$ is its ability to disproportionate thiosulfate and sulfite to sulfate and sulfide. Disproportionation of sulfur compounds by *Desulfonatronum* species has already

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{14:0}$</td>
<td>7.6</td>
<td>10.2</td>
</tr>
<tr>
<td>iso-C$_{15:0}$</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td>anteiso-C$_{15:0}$</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>C$_{16:0}$</td>
<td>2.2</td>
<td>0.7</td>
</tr>
<tr>
<td>C$_{16:1,ω9c}$</td>
<td>2.4</td>
<td>–</td>
</tr>
<tr>
<td>C$_{16:1,ω7c}$</td>
<td>15.9</td>
<td>35.8</td>
</tr>
<tr>
<td>C$_{16:0}$</td>
<td>19.1</td>
<td>10.8</td>
</tr>
<tr>
<td>C$_{17:0,ω6c}$</td>
<td>1.9</td>
<td>–</td>
</tr>
<tr>
<td>C$_{17:0}$</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>C$_{18:1,ω6,9c}$</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>C$_{18:1,ω9c}$</td>
<td>9.8</td>
<td>9.1</td>
</tr>
<tr>
<td>C$_{18:1,ω12t}$</td>
<td>9.1</td>
<td>11.2</td>
</tr>
</tbody>
</table>

*Summed features contain fatty acids that could not be separated by GLC using the Microbial Identification System (MIDI). Summed feature 8 comprised C$_{17:1,ω8c}$ and/or C$_{17:1,ω12t}$; Summed feature 10 comprised C$_{18:1,ω7c}$ and/or C$_{18:1,ω9t}$ and/or C$_{18:1,ω12t}$. 

---

---
been demonstrated for *D. thiosulfatophilum*, *D. lacustrum*, *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 PARANGUEONSEN SP. NOV.**

*Desulfonatronum parangueonense* (pa.ran.gue.o.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\(_{16:1}\)ω7c and summed feature 10 (C\(_{18:1}\)ω7c and/or C\(_{18:1}\)ω9t and/or C\(_{18:1}\)ω12t). 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.

**References**

19. Armienta MA, Vilaclaara G, de la Cruz-Reyna S, Ramos S, Ceniceros N et al. Water chemistry of lakes related to active and


Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.