Desulfosalsimonas propionica gen. nov., sp. nov., a halophilic, sulfate-reducing member of the family *Desulfobacteraceae* isolated from a salt-lake sediment

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A novel halophilic Gram-negative sulfate-reducing bacterium affiliated with the deltaproteobacterial family *Desulfobacteraceae*, strain PropA^T, was isolated from the extreme hypersaline sediment of the northern arm of Great Salt Lake, Utah, USA. Comparative 16S rRNA gene sequence analysis showed that strain PropA^T is the first cultured representative of a clade of phylotypes that have been retrieved from a range of geographically and ecologically distinct hypersaline environments. Strain PropA^T shared ≤90 % 16S rRNA gene sequence identity with cultured strains within the family *Desulfobacteraceae*. Cells of strain PropA^T were rod-shaped and sometimes motile. The strain required NaCl for growth and grew at salinities up to 200 g NaCl l⁻¹ (optimum 60 g l⁻¹). Growth was observed at 15–40 °C, optimum growth occurred at about 40 °C, while growth was absent at 10 and 45 °C. The pH range for growth was pH 6.0–8.3. Yeast extract (0.1 g l⁻¹) was required for growth. C_{2–4} alcohols, C_{3–4} carboxylic acids, yeast extract and H₂/acetate supported growth with sulfate as electron acceptor. Sulfate, thiosulfate and sulfite served as electron acceptors, but not elemental sulfur, nitrate or fumarate. The DNA G + C content of strain PropA^T was 54.1 mol%. Based on the genotypic and physiological properties, we propose that strain PropA^T represents a novel species within a novel genus, *Desulfosalsimonas propionica* gen. nov., sp. nov. The type strain of *Desulfosalsimonas propionica* is PropA^T (=DSM 17721^T =VKM B-2385^T).

Aquatic systems such as solar salterns, inland lakes and deep-sea brines are characterized by salinities far exceeding 100 g NaCl l⁻¹ (Oren, 2002). Sulfate reduction is often an important anoxic mineralization process in these hypersaline environments (Brandt *et al.*, 2001; Daffonchio *et al.*, 2006; Foti *et al.*, 2007), yet relatively few sulfate-reducing bacteria

(SRB) that are able to grow at such extreme salinities are known. Halophilic SRB primarily comprise members of the families *Desulfohalobiaceae* (Kuever *et al.*, 2005a) and *Desulfonatronaceae* (Kuever *et al.*, 2005b) along with certain members of the genus *Desulfovibrio* of the family *Desulfovibrionaceae* (e.g., Sass & Cypionka, 2004; Krekeler *et al.*, 1997). Members of the family *Desulfohalobiaceae* exhibit the highest known *in vitro* halotolerance and are able to grow in the presence of up to 240 g NaCl l⁻¹ (Beliakova *et al.*, 2006; Jakobsen *et al.*, 2006; Ollivier *et al.*, 1991). Here, we describe a novel halophilic SRB, designated strain PropA^T, which is affiliated with the deltaproteobacterial family *Desulfobacteraceae* (Kuever *et al.*, 2005c). Only three recognized members of the family *Desulfobacteraceae* are capable of growing at NaCl concentrations exceeding 100 g l⁻¹, namely *Desulfocella halophila* (Brandt *et al.*, 1999), *Desulfobacter halotolerans* (Brandt & Ingvorsen, 1997) and *Desulfotignum balticum* (Drzyzga *et al.*, 1993; Kuever *et al.*, 2001) (Table 1). The first two species, like strain

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Abbreviations: SPME-GC-MS, solid-phase micro-extraction gas chromatography mass spectrometry; SRB, sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and *dsrAB* sequences of strain PropA^T are DQ067422 and DQ386237, respectively.

Transmission electron micrographs of cells of strain PropA^T and details of sequences shown as genus groups and sequences constituting outgroups in Fig. 2 are available as supplementary material with the online version of this paper.

Table 1. Differential phenotypic characteristics between strain PropA^T and the three other members of the family Desulfobacteraceae that grow at salinities ≥ 100 g NaCl l⁻¹

Strains: 1, *Desulfosalsimonas propionica* gen. nov., sp. nov. PropA^T; 2, *Desulfobacter halotolerans* DSM 11383^T; 3, *Desulfocella halophila* DSM 11763^T; 4, *Desulfotignum balticum* DSM 7044^T. Data for reference strains were taken from Brandt & Ingvorsen (1997), Brandt *et al.* (1999), Drzyzga *et al.* (1993) and Kuever *et al.* (2001). +, Positive; -, negative; ND, no data available.

Characteristic	1	2	3	4
NaCl concentration for growth (g l ⁻¹)				
Range	10–200	5–130	20–200	10–110
Optimum	60	10–20	40–50	20
Temperature for growth (°C)				
Range	15–40	7–38	14–37	10–42
Optimum	~40	33	34	28–32
pH for growth				
Range	6.0–8.3	6.2–8.1	5.8–7.6	6.5–8.2
Optimum	~7.0	6.2–7.4	6.5–7.3	7.3
Oxidation of:				
Acetate	–	+	–	+
Propionate	+	–	–	–
Lactate	+	–	–	+
Butyrate	+	–	+	+
Isobutyrate	+	–	–	–
Formate	–	–	–	+
Fumarate	+	ND	–	+
Succinate	–	–	–	+
Malate	–	–	–	+
Ethanol	+	+	–	–
Propanol	+	–	ND	–
Butanol	+	ND	–	–
Benzoate	–	–	–	+
Glucose	–	ND	–	–
H ₂ /CO ₂	–	–	–	+
H ₂ /acetate	+	+	ND	+
Yeast extract	+	–	+	ND
Fermentation	–	ND	–	+
Reduction of:				
Sulfite	+	+	–	+
Thiosulfate	+	+	–	+
Elemental sulfur	–	–	–	ND
Autotrophic growth	–	–	–	+
Complete oxidation of carbon compounds	+/-	+	–	+
DNA G+C content (mol%)	54	49	35	53

PropA^T, were isolated from the hypersaline Great Salt Lake (Utah, USA), which is characterized by salinities of 120 and 270 g NaCl l⁻¹ in its southern and northern parts, respectively (Brandt *et al.*, 2001; Oren, 2002). In contrast, the aromatic compound-degrading species *Desulfotignum balticum*, which exhibits the lowest halotolerance of the three above-mentioned species, was isolated from a marine coastal sediment. Being able to grow at salinities up to 200 g NaCl l⁻¹, strain PropA^T is, together with *Desulfocella halophila*, the most halotolerant member of the family Desulfobacteraceae described so far. Notably, the 16S rRNA gene sequence of strain PropA^T clusters within a clade of uncultured phylotypes derived from various hypersaline

environments. Thus, strain PropA^T provides the first insights into the physiology of this distinct group of unknown members of the Desulfobacteraceae.

Strain PropA^T was isolated from hypersaline (270 g NaCl l⁻¹) sediment of the northern arm of Great Salt Lake as part of an investigation of the diversity of SRB in this extreme habitat (Kjeldsen *et al.*, 2007). Strain PropA^T was enriched, isolated and routinely cultivated in anoxic basal medium containing 100 g NaCl l⁻¹, prepared as described previously (Jakobsen *et al.*, 2006). Propionate served as the electron donor for enrichment and isolation. All incubations were carried out in the dark at 30 °C,

unless otherwise noted. Growth experiments and transmission electron microscopy were performed essentially as described previously (Jakobsen *et al.*, 2006). Lactate served as the electron donor for NaCl, pH and temperature experiments, whereas propionate was used as the electron donor for electron acceptor experiments.

Cells of strain PropA^T were rod shaped, and swimming motility was sometimes observed. Cells were 0.8–1.0 µm wide and 2–4 µm long and sometimes formed short filaments under routine growth conditions (Supplementary Fig. S1, available in IJSEM Online). The cells stained Gram-negative and did not form endospores. Growth was tested at 13 different salinities, ranging from 0 to 250 g NaCl l⁻¹, and was found to occur at 10–200 g NaCl l⁻¹, with an optimum growth rate at 60 g NaCl l⁻¹ (Fig. 1). Thus, according to the definitions of Larsen (1986), strain PropA^T is a moderate halophile. The observed upper *in vitro* salinity limit for growth indicates that strain PropA^T may be severely salt stressed in the sediment of the northern part of Great Salt Lake (270 g NaCl l⁻¹) from where it was isolated. Perhaps the strain is more active in the southern part of the lake, in which the salinity is only 120 g NaCl l⁻¹, or perhaps the sediment in the northern part contains niches of lower salinity, possibly arising from freshwater seeps, as previously speculated (Kjeldsen *et al.*, 2007). Discrepancies between the *in vitro* halotolerance of halophilic SRB and the *in situ* salinity of their habitat are, however, not unprecedented (Jakobsen *et al.*, 2006; Ollivier *et al.*, 1991; Sass & Cypionka, 2004). The pH dependence of growth was determined at eight different values ranging from pH 5.0 to 8.5. Growth was observed between pH 6.0 and 8.3, with an optimum at about pH 7.0. The temperature dependence of growth was tested at 10, 15, 20, 30, 40, 45, 50 and 60 °C. Growth was observed at 15–40 °C, with the fastest growth occurring at 40 °C. No growth was observed at 45 °C.

Strain PropA^T utilized short-chain carboxylic acids (propionate, pyruvate, lactate, butyrate, isobutyrate and fumarate) and alcohols (ethanol, 1-propanol, 1-butanol),

Fig. 1. Effect of NaCl on the specific growth rate of strain PropA^T, when grown at 30 °C at pH 7.0 on lactate and sulfate.

H₂/acetate and yeast extract (2 g l⁻¹) as electron donors. The strain required yeast extract (0.1 g l⁻¹) for growth irrespective of the type of electron donor provided, including H₂/acetate. This requirement was evident despite the routine amendment of the growth medium with the following defined trace element and vitamin solutions (Widdel & Bak, 1992): non-chelated trace element mixture, selenite–tungstate solution, thiamine solution, vitamin mixture (modified by including 30 mg folic acid l⁻¹) and vitamin B₁₂ solution. The following substrates did not support growth: D-glucose, D-galactose, trehalose, D-xylose, D-arabinose, L-rhamnose, sucrose, D-fructose, maltose, D-mannose, methanol, 1-pentanol, acetone, formate, acetate, citrate, succinate, DL-malate, glycine, glycerol, choline, benzoate, caprylate and Casamino acids. Besides sulfate, strain PropA^T grew with sulfite and thiosulfate as electron acceptors, but not nitrate, elemental sulfur or fumarate. Growth was absent in the presence of oxygen. Fermentative growth was absent on pyruvate, fumarate and lactate. By comparing the substrate spectrum of strain PropA^T with those of *Desulfobacter halotolerans* and *Desulfocella halophila*, the two other halophilic members of the family *Desulfobacteraceae* previously isolated from Great Salt Lake, it appears that strain PropA^T is metabolically the most versatile of the three (Table 1). This versatility might explain how several strains closely related to strain PropA^T seemingly coexist in the Great Salt Lake sediment (Kjeldsen *et al.*, 2007).

The capacity of strain PropA^T to oxidize lactate, propionate, ethanol and propanol completely to CO₂ was tested in triplicate growth experiments in which the concentration of acetate was measured at the beginning and end of the experiments along with the concentration of sulfate. Sulfate concentrations were determined using a BioLC ion chromatograph (Dionex) equipped with a 4 × 250 mm IonPac AS18 column (Dionex) at an eluent (23 mM KOH) flow rate of 1 ml h⁻¹. Acetate was measured by automated solid-phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS) directly from the growth medium (Glastrup *et al.*, 2006). In contrast to ion chromatography-based measurements of acetate, SPME-GC-MS measurements are not affected by the high concentration of chloride in the growth medium. Acetate did not accumulate (detection limit 5 µM) with growth on lactate or propionate, despite the consumption of 4–8 mM sulfate, suggesting that these carboxylic acids were oxidized completely. In contrast, the alcohols ethanol and propanol were oxidized incompletely, resulting in the accumulation of approximately stoichiometric amounts of acetate and propionate, respectively (quantified by SPME-GC-MS). Even though propionate and lactate were oxidized completely by strain PropA^T, acetate did not support growth, possibly due to the low energy yield gained from the oxidation of this substrate and the high energetic cost of maintaining the osmotic balance in a saline growth medium (Oren, 1999). Similar observations have been made, for example, for the completely oxidizing SRB

Desulfatibacillum alkenivorans (Cravo-Laureau *et al.*, 2004), *Desulfobacula toluolica* (Rabus *et al.*, 1993) and *Desulfospira joergensenii* (Finster *et al.*, 1997), which also belong to the family *Desulfobacteraceae*.

The sequences for *dsrAB*, which encodes the two major subunits of dissimilatory (bi)sulfite reductase (Wagner *et al.*, 2005), and the 16S rRNA gene (1946 and 1469 nt, respectively) of strain PropA^T were retrieved as described previously (Kjeldsen *et al.*, 2007). The 16S rRNA gene sequence was added to the alignment of the Silva SSURef version 95 ARB database (Pruesse *et al.*, 2007) containing >4500 high-quality deltaproteobacterial 16S rRNA gene sequences. A few additional short or recently published sequences that were found to be related to the 16S rRNA gene sequence of strain PropA^T by BLAST searches of the GenBank database were added manually to the ARB database. From this database, 51 sequences affiliated to the *Desulfobacteraceae* were subsequently selected for phylogenetic analyses. Furthermore, 14 sequences representing other major deltaproteobacterial families were included as an outgroup. The phylogenetic position of strain PropA^T among the selected taxa was inferred from Bayesian analysis using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) with sampling of 1395 unambiguously aligned sequence positions. Tree searches were performed with the general time reversible evolutionary model with gamma-distributed rate variation across sites, with a proportion of the sites being kept invariant. Program default priors on model parameters as well as default settings for the Metropolis-coupled Markov chain Monte Carlo runs were applied. Searches included 1 million generations with a sampling of trees for every 100. Likelihood values converged after approximately 100 000 generations. A consensus tree was constructed after removing the first 2500 trees as 'burn-in'. Distance-matrix-based bootstrap values were calculated in PAUP* version 4.0b10 (Swofford, 2003) using Jukes–Cantor distance correction and 1000 resamplings. Phylogenetic analysis of a dataset consisting of 50 DsrAB amino acid sequences (deduced from nucleotide sequences) with 563 unambiguously aligned sequence positions were performed using the TreePuzzle (quartet puzzling using a JTT amino acid substitution model) algorithm as implemented in the ARB program package (Ludwig *et al.*, 2004). Some taxa were grouped or removed from the trees presented in Fig. 2 in order to enhance clarity (details of grouped and removed sequences are given in Supplementary Table S1).

The 16S rRNA gene sequence of strain PropA^T shared ≤90 % similarity with sequences originating from named isolates. Its closest cultured relatives (89–90 % identity) were various members of the deltaproteobacterial family *Desulfobacteraceae*, particularly members of the genera *Desulfatibacillum*, *Desulfobacterium*, *Desulfococcus*, *Desulfofaba*, *Desulfonema* and *Desulfosarcina*, which are mostly associated with marine habitats (Cravo-Laureau *et al.*, 2004; Kuever *et al.*, 2005c). The 16S rRNA gene and DsrAB amino acid sequence-based phylogenetic analyses clearly placed the strain within the family *Desulfobacteraceae*

(Fig. 2). This family mainly includes SRB that, like strain PropA^T, possess the capacity to oxidize carbon compounds completely (Kuever *et al.*, 2005c). As seen from Fig. 2, neither the 16S rRNA gene sequence nor the DsrAB amino acid sequence of strain PropA^T clustered with any recognized member of the family *Desulfobacteraceae*. Rather, the 16S rRNA gene sequence of strain PropA^T formed a clade with a range of environmentally derived sequences that, interestingly, all originate from hypersaline environments, representing different habitat types such as sediments, microbial mats and anoxic basins (Fig. 2a). Thus, strain PropA^T is the first cultured representative of what seems to be a geographically and ecologically widely distributed lineage of putative halophilic SRB.

The DNA G + C content of strain PropA^T was 54.1 mol%, as determined by HPLC analysis by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The DNA G + C content varies considerably, from 35 to 62 mol%, among members of the family *Desulfobacteraceae* (Kuever *et al.*, 2005c). The mean ± SD DNA G + C content for recognized members of the family is 49.6 ± 6.3 mol% (41 species representing 16 genera; results not shown) and, consequently, the value of 54.1 mol% determined for strain PropA^T is in good agreement with G + C contents reported for the family *Desulfobacteraceae*.

In conclusion, we propose that strain PropA^T should be classified in a novel genus and species as *Desulfosalsimonas propionica* gen. nov., sp. nov. within the family *Desulfobacteraceae* because of its distant phylogenetic relatedness to recognized members of this family and its unique halotolerance.

Description of *Desulfosalsimonas* gen. nov.

Desulfosalsimonas [De.sul'fo.sal.si.mo'nas. L. pref. *de* from; L. n. *sulfur* sulfur; L. adj. *salsus* salty, saline; L. fem. n. *monas* unit, monad; N.L. fem. n. *Desulfosalsimonas* a sulfate-reducing monad that thrives in (hyper)saline environments].

Obligately anaerobic, sulfate-reducing chemoheterotrophs. Cells are rod-shaped, stain Gram-negative and do not produce endospores. Short-chain carboxylic acids, primary C_{2–4} alcohols and H₂ serve as electron donors. Optimal growth occurs in the presence of elevated concentrations of NaCl at neutral pH and at mesophilic temperatures. Phylogenetically, the genus belongs to the deltaproteobacterial family *Desulfobacteraceae*, as can be recognized by both *dsrAB* and 16S rRNA gene sequence analyses. The type species is *Desulfosalsimonas propionica*.

Description of *Desulfosalsimonas propionica* sp. nov.

Desulfosalsimonas propionica (pro.pi.o.ni.ci'ca. N.L. n. *acidum propionicum* propionic acid; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *propionica* belonging to propionic acid).

Fig. 2. Trees showing the inferred phylogenetic position of strain PropA^T among members of the family Desulfobacteraceae based on comparative analyses of 16S rRNA gene sequences (a) and DsrAB amino acid sequences (b). For the 16S rRNA gene sequence tree, bootstrap percentage values and Bayesian posterior probability percentages (>50%) are shown above and below branch nodes, respectively. For the DsrAB amino acid sequence tree, percentages at nodes are levels of bootstrap support. Numbers in square brackets indicate the number of taxa constituting the group. #, Short sequences subsequently added to the tree without changing the overall tree topology using parsimony criteria. Bars, 10% sequence divergence. Details of sequences represented as genus groups and outgroups are available in Supplementary Table S1.

The species has the following characteristics in addition to those listed for the genus. Cells are 0.8–1.0 µm wide and 2–4 µm long, sometimes forming filaments. Utilizes (with sulfate as electron acceptor) propionate, pyruvate, lactate, butyrate, isobutyrate, fumarate, ethanol, 1-propanol, 1-butanol, yeast extract and H₂/acetate. Oxidizes lactate and propionate completely and ethanol and propanol incompletely. Acetate does not support growth. Yeast extract is required for growth. In addition to sulfate, thiosulfate and sulfite serve as electron acceptors. NaCl is required for growth and up to 200 g NaCl l⁻¹ is tolerated; the optimum concentration for growth is 60 g l⁻¹. Growth occurs within the range pH 6.0–8.3. Growth occurs at 15–40 °C (optimum about 40 °C); growth is absent at 10 and 45 °C. The DNA G+C content of the type strain is 54.1 mol%.

The type strain, PropA^T (=DSM 17721^T =VKM B-2385^T), was isolated from the extreme hypersaline sediment of the northern arm of Great Salt Lake, Utah, USA.

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