Desulfosoma caldarium gen. nov., sp. nov., a thermophilic sulfate-reducing bacterium from a terrestrial hot spring

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A thermophilic, sulfate-reducing bacterium, designated strain USBA-053^T, was isolated from a terrestrial hot spring located at a height of 2500 m in the Colombian Andes (5° 45' 33.29" N 73° 6' 49.89" W), Colombia. Cells of strain USBA-053^T were oval- to rod-shaped, Gram-negative and motile by means of a single polar flagellum. The strain grew autotrophically with H₂ as the electron donor and heterotrophically on formate, propionate, butyrate, valerate, isovalerate, lactate, pyruvate, ethanol, glycerol, serine and hexadecanoic acid in the presence of sulfate as the terminal electron acceptor. The main end products from lactate degradation, in the presence of sulfate, were acetate, CO₂ and H₂S. Strain USBA-053^T fermented pyruvate in the absence of sulfate and grew optimally at 57 °C (growth temperature ranged from 50 °C to 62 °C) and pH 6.8 (growth pH ranged from 5.7 to 7.7). The novel strain was slightly halophilic and grew in NaCl concentrations ranging from 5 to 30 g l⁻¹, with an optimum at 25 g l⁻¹ NaCl. Sulfate, thiosulfate and sulfite were used as electron acceptors, but not elemental sulfur, nitrate or nitrite. The G + C content of the genomic DNA was 56 ± 1 mol%. 16S rRNA gene sequence analysis indicated that strain USBA-053^T was a member of the class *Deltaproteobacteria*, with *Desulfacinum hydrothermale* MT-96^T as the closest relative (93 % gene sequence similarity). On the basis of physiological characteristics and phylogenetic analysis, it is suggested that strain USBA-053^T represents a new genus and novel species for which the name *Desulfosoma caldarium* gen. nov., sp. nov. is proposed. The type strain of the type species is USBA-053^T (=KCTC 5670^T=DSM 22027^T).

Colombian Andean hot springs provide multiple habitats and micro niches (Thevenieau *et al.*, 2007), which can potentially be inhabited by highly metabolically diverse micro-organisms. Several of these springs associated with volcanic activity have been recorded at elevations of up to 2500 m in the Colombian central Andes, comprising the Western, Central and Eastern mountain ranges, located in the tropical zone, with their climate determined by their high altitude. The springs have different physical and geochemical characteristics with temperatures ranging from 20 °C to 75 °C, salinity from 0.4 to 55 g l⁻¹ and pH from 3.7 to 7.2. Sulfate, chloride and bicarbonate (in decreasing order) are the dominant anions (Alfaro, 2002). As sulfate is the dominant ion in these Andean hot springs,

and sulfate-reducing micro-organisms play a key role in the degradation of organic matter in many anoxic ecosystems, our research group has focussed its attention on studies of thermophilic sulfate-reducing microbes from these hot springs. Thermophilic sulfate-reducing bacteria are currently classified into several families including the *Thermodesulfobacteriaceae*, *Desulfobacteriaceae*, *Desulfomicrobiaceae*, *Syntrophobacteriaceae*, *Peptococcaceae* and *Thermodesulfobiaceae* (Rogosa, 1971; Hatchikian *et al.*, 2002; Mori *et al.*, 2003; Kuever *et al.*, 2006a, b, c). In this report, the isolation and characterization of a novel thermophilic sulfate-reducing member of the family *Syntrophobacteriaceae* is described.

Sediment and water samples were collected from three hot springs (identified as sites P3, P4 and P6) located in the Andean region at 5° 45' 33.29" N 73° 6' 49.89" W, 5° 45' 30.69" N 73° 6' 50.61" W and 5° 43' 37.29" N 73° 7' 9.19" W, respectively. Samples were collected in

Abbreviation: BM, basal medium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain USBA 53^T is FJ491989.

sterile glass containers that were filled to the brim, capped and transported to the laboratory. The temperatures at the sampling points ranged between 53 °C and 68 °C, the pH was 7.0 and the conductivity ranged from 43 to 52 mS cm⁻¹. The sulfate concentration varied between 8750 (P6) and 19 250 mg l⁻¹ (P3 and P4) and the total dissolved solids varied between 39 g l⁻¹ (P3 and P4) and 41.8 g l⁻¹ (P6). The sodium content was 12.5 g l⁻¹ for sites P3 and P4 and 5.5 g l⁻¹ for site P6.

Enrichments were initiated by inoculating 1 ml samples from each of the three springs into Hungate tubes containing 5 ml pre-reduced anaerobic basal medium (BM) supplemented with sodium lactate (20 mM), anhydrous sodium sulfate (20 mM), 1 g yeast extract l⁻¹ and 3 g MgCl₂ l⁻¹. BM contained (l⁻¹ deionized water): 0.3 g KH₂PO₄; 0.3 g K₂HPO₄; 1.0 g NH₄Cl; 23 g NaCl; 0.1 g KCl; 3.0 g MgCl₂·6H₂O; 0.1 g CaCl₂·2H₂O; 0.5 g cysteine-HCl; 10 ml trace mineral element solution (Balch *et al.*, 1979) and 1 ml stock solution of resazurin (1 g l⁻¹). The headspace gas for cultivation was N₂/CO₂ (80:20). Prior to use, 0.05 ml Na₂S·9H₂O (20 g l⁻¹) and 0.1 ml NaHCO₃ (100 g l⁻¹) were injected into each tube. Increasing turbidity and H₂S production from sulfate reduction were observed in enrichments from all three samples after 6 days incubation at 55 °C. Microscopic examination revealed the presence of oval- to short rod-shaped cells which were present singly or in pairs. After repeated transfers, stable cultures with the same dominant cellular morphology and sulfide production were obtained from all enrichment cultures. The enrichments at this point were diluted serially and inoculated into growth medium amended with 20 g l⁻¹ Noble agar (Sigma) using the roll-tube technique (Hungate, 1969). After 5 days incubation, white to brown circular convex colonies developed in the roll-tubes. Single well-isolated colonies were transferred into the growth medium and the procedure repeated at least twice before the cultures were regarded as pure. Purity was verified by inoculating the cultures in basal medium containing 1 g yeast extract l⁻¹ and 20 mM glucose. An absence of growth in this glucose-containing medium was used as an indicator of culture purity. Five morphologically similar cultures were isolated on basal medium containing lactate and sulfate. All strains were found to stain Gram-negative and were motile, anaerobic, thermophilic, non-spore-forming and reduced sulfate to sulfide. 16S rRNA genes of the isolates were sequenced and showed 100 % similarity to each other. Consequently, only one isolate, designated strain USBA-053^T from the P3 hot spring, was selected for further characterization.

Morphology was determined by phase-contrast microscopy (Eclipse 50; Nikon) and electron microscopy as described by Patel *et al.* (1985). Cells of strain USBA-053^T stained Gram-negative, were oval- to rod-shaped (1.0–1.5 µm × 2.0 µm), often occurred in pairs and were motile by means of a single polar flagellum (Fig. 1). Oval-shaped cells were frequently observed in the exponential growth phase, whereas late cultures exhibited slighter longer rod-shaped cells. Spores

were not observed under light or electron microscopy. In addition, cultures incubated at 80 °C for up to 20 min followed by subculturing into fresh growth medium failed to grow, suggesting the absence of heat-resistant bodies such as spores.

Characterization studies were performed in BM supplemented with 20 mM sodium lactate, 20 mM anhydrous sodium sulfate, 1 g yeast extract l⁻¹ and 3 g MgCl₂ l⁻¹. Cell growth was monitored photometrically at 580 nm and all tests were performed in triplicate with the strain subcultured at least once under the same experimental conditions before use. The pH of the basal medium was adjusted to between 4.0 and 9.0 with stock solutions of NaHCO₃ (100 g l⁻¹) and Na₂CO₃ (100 g l⁻¹). Strain USBA-053^T grew optimally at pH 6.8 (growth pH range between pH 5.7 and 7.7). The optimum temperature for growth was 57 °C (growth temperature range, 50–62 °C) and the sodium chloride optimum was 25 g l⁻¹ (growth range was 5–30 g NaCl l⁻¹). Unless indicated otherwise, all subsequent growth experiments were conducted using these optimal conditions.

Utilization of organic carbon sources was tested in basal medium containing 1 g yeast extract l⁻¹. Strain USBA-053^T grew heterotrophically on formate, propionate, butyrate, valerate, isovalerate, lactate, pyruvate, ethanol, glycerol, serine and hexadecanoic acid at 10 mM using sulfate as the

Fig. 1. Transmission electron micrograph of a negatively stained cell of strain USBA-053^T showing a polar flagellum. Bar, 0.5 µm.

terminal electron acceptor. The isolate did not require peptides or vitamins for growth although 0.25 g yeast extract l^{-1} and 0.5 g Bio-Trypticase l^{-1} (Sigma) enhanced growth. To examine the utilization of potential electron acceptors, thiosulfate, sulfate and nitrate at 20 mM, sulfite at 2 mM and elemental sulfur at 10 g l^{-1} were used in BM containing 20 mM lactate, formate or pyruvate. Sulfide production was determined photometrically using the method of Cord-Ruwisch (1985). After 2 weeks of incubation at 55 °C, end products were measured by HPLC (Shimadzu) equipped with a reversed-phase Ultra Aqueous C18 column (Restek; 150 mm $\times$ 4.6 mm ID), with a diode array detector (SPD-M20A, Shimadzu) at 210 nm. The main end products resulting from lactate were acetate (6.0 mM), CO_2 and H_2S in the presence of sulfate. Fermentation of 10 mM lactate, pyruvate, serine or 1 g yeast extract l^{-1} was tested in BM, but strain USBA-053^T fermented only pyruvate to acetate. Growth was not observed on glucose, galactose, sucrose, acetate, fumarate, succinate, malate, citrate, ethanol, methanol, peptidase or Casamino acids, in the presence or absence of sulfate. Sulfate, thiosulfate and sulfite were utilized as electron acceptors, but not elemental sulfur, ferric iron or nitrate. Autotrophic growth was determined using BM supplemented with sodium sulfate at 20 mM and H_2/CO_2 (80:20, v/v) at a 2 bar atmosphere and strain USBA-053^T grew autotrophically under these conditions. Sensitivity to chloramphenicol and penicillin G was determined at a final concentration of 50 and 100 $\mu g\ ml^{-1}$, respectively. Strain USBA-053^T was inhibited by 50 μg chloramphenicol ml^{-1} and 100 μg penicillin G ml^{-1} at 57 °C.

The DNA of strain USBA-053^T was prepared by using the whole genome amplification method (Ogg & Patel, 2009). The G+C content of this amplified DNA was determined by the thermal denaturation (T_m) method (Marmur & Doty, 1962) and calculated to be $56 \pm 1\ mol\%$.

The methods for 16S rRNA gene amplification and sequencing have been reported previously (Andrews & Patel, 1996). Sequences generated were assembled into a single contig and the consensus sequence of 1479 nt was corrected manually for errors using BioEdit v5.0.1 (Hall, 1999). The most closely related sequences in the GenBank (version 152) and the Ribosomal Database Project II (release 10) identified using BLAST (Altschul *et al.*, 1997) were extracted, aligned and manually adjusted according to the 16S rRNA secondary structure using BioEdit. Nucleotide ambiguities were omitted and evolutionary distances calculated using the Jukes and Cantor option (Jukes & Cantor, 1969) in TREECON (Van de Peer *et al.*, 1997). Phylogenetic trees were reconstructed from evolutionary distances using the neighbour-joining method (Saitou & Nei, 1987). Tree topology was re-examined by the bootstrap method of resampling (1000 replications) (Felsenstein, 1985).

16S rRNA gene sequence analysis (1479 bp) consistently placed strain USBA-053^T in the vicinity of the genus *Desulfacinum*, family *Syntrophobacteraceae*, class *Deltaproteobacteria* (Fig. 2). The closest phylogenetic relatives to the novel strain were members of the genus *Desulfacinum*, with *Desulfacinum hydrothermale* MT-96^T and *Desulfacinum infernum* BxG1^T sharing 93 % gene sequence similarity. The novel strain was distantly related to *Desulfoglaeba alkanexedens* ALDC^T and *Thermodesulforhabdus norvegica* A8444^T with gene sequence similarities of 92 % and 89 %, respectively.

Strain USBA-053^T shared a number of phenotypic and physiological properties with *Desulfacinum hydrothermale* and *Desulfacinum infernum* (Table 1). All three strains were oval to short rods and were thermophilic, halophilic and sulfate-reducing bacteria that were able to reduce thiosulfate and sulfite and grow autotrophically with H_2

Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain USBA-053^T and representatives of related taxa. Bootstrap values are shown as percentages (based on 1000 replications) and only values >95 % are shown. *Desulfococcus biacutus* was used as an outgroup. GenBank accession numbers are given in parentheses. Bar, 10 substitutions per 100 nt positions.

Table 1. Characteristics of *Desulfacinum infernum*, *Desulfacinum hydrothermale* and strain USBA-053^T

Taxa: 1, strain USBA-053^T (data from this study); 2, *Desulfacinum infernum* BzG1^T (Rees *et al.*, 1995); 3, *Desulfacinum hydrothermale* MT-96^T (Sievert & Kuever, 2000). All strains are able to ferment pyruvate. +, Positive; –, negative.

Characteristic	1	2	3
Cell width (µm)	1.0–1.5	1.0	0.8–1.0
Cell length (µm)	2.0	2.5–3.0	1.5–2.5
Motility	+	–	+
Optimal temperature/range (°C)	57 (50–62)	60 (40–65)	60 (37–64)
Optimal NaCl content/range (g l ⁻¹)	25 (5–30)	10 (0–50)	32–36 (15–78)
Electron donors:			
Acetate	–	+	+
Alanine	–	+	+
Fumarate	–	+	–
Glycerol	+	+	–
Malate	–	+	–
Succinate	–	+	–
Growth factor requirement	None	Vitamins	None
Source	Terrestrial hot spring	Petroleum reservoir	Hydrothermal vent
DNA G + C content (mol%)	56.1 (T _m)	64 (T _m)	59.5 (HPLC)

and on short chain fatty acids. In addition, they used formate, propionate, butyrate, valerate, isovalerate, ethanol, pyruvate, lactate and H₂/CO₂, but not sucrose or citrate.

However, strain USBA-053^T differed from *Desulfacinum hydrothermale* MT-96^T and *Desulfacinum infernum* BzG1^T in its narrow temperature range for growth and utilization pattern of electron donors; strain USBA-053^T did not oxidize acetate, succinate, malate, fumarate or alanine. Because strain USBA-053^T was both phenotypically and phylogenetically distinct from *Desulfacinum hydrothermale* MT-96^T and *Desulfacinum infernum* BzG1^T and the G + C content of the genomic DNA of strain USBA-053^T also differed from these species, it is concluded that strain USBA-053^T represents a new genus and novel species for which the name *Desulfosoma caldarium* gen. nov., sp. nov. is proposed.

Description of *Desulfosoma* gen. nov.

Desulfosoma (De.sul.fo.so'ma. L. pref. *de* from; L. n. *sulfur* sulfur; N.L. pref. *desulfo*- prefix used to characterize a dissimilatory sulfate-reducing prokaryote; Gr. neut. n. *soma* body; N.L. neut. n. *Desulfosoma* sulfate-reducing body).

Cells are oval- to rod-shaped. Obligately anaerobic. Sulphate-reducing and thermophilic. Do not form spores. Gram-reaction is negative. The type species is *Desulfosoma caldarium*.

Description of *Desulfosoma caldarium* sp. nov.

Desulfosoma caldarium (cal.da'ri.um. L. neut adj. *caldarium* pertaining to warmth, warming).

Exhibits the following properties in addition to those given the genus description. Cells are 1.0–1.5 µm × 2.0 µm, often occur in pairs and are motile by means of a single polar flagellum. Growth occurs between 50 °C and 62 °C (optimum, 57 °C). The pH range for growth is pH 5.7–7.7 (optimum, pH 6.8). Slightly halophilic and grows in NaCl concentrations ranging from 5 to 30 g l⁻¹, with an optimum of 25 g NaCl l⁻¹. Sulfate, thiosulfate and sulfite are used as electron acceptors, but not elemental sulfur, nitrate or nitrite. Electron donors utilized in the presence of sulfate are formate, propionate, butyrate, valerate, isovalerate, lactate, pyruvate, ethanol, glycerol, serine and hexadecanoic acid. The main end products resulting from lactate degradation, in the presence of sulfate, are acetate, CO₂ and H₂S. The type strain ferments pyruvate. Grows autotrophically on hydrogen. Sensitive to chloramphenicol and penicillin.

The type strain, USBA-053^T (=KCTC 5670^T=DSM 22027^T), was isolated from terrestrial hot springs (Paipa, Colombia). The G + C content of the genomic DNA of the type strain is 56 ± 1 mol% (T_m).

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