

Salinisphaera hydrothermalis sp. nov., a mesophilic, halotolerant, facultatively autotrophic, thiosulfate-oxidizing gammaproteobacterium from deep-sea hydrothermal vents, and emended description of the genus *Salinisphaera*

Melitza Crespo-Medina,^{1,2} Aspasia Chatziefthimiou,¹
Ramaydalis Cruz-Matos,^{1,2} Ileana Pérez-Rodríguez,^{1,2} Tamar Barkay,¹
Richard A. Lutz,² Valentin Starovoytov³ and Costantino Vetriani^{1,2}

Correspondence
Costantino Vetriani
vetriani@marine.rutgers.edu

¹Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08901, USA

²Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08901, USA

³Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ 08854, USA

A mesophilic, aerobic, facultatively chemolithoautotrophic bacterium, designated strain *EPR70*^T, was isolated from hydrothermal fluids from diffuse-flow vents on the East Pacific Rise at 9° 50' N 104° 17' W. Cells were Gram-negative rods, approximately 0.8–1.0 µm long and 0.3–0.5 µm wide. Strain *EPR70*^T grew at 20–40 °C (optimum 30–35 °C), 1–25% NaCl (optimum 2.5%) and pH 5.0–7.5 (optimum pH 5.5). The shortest generation time observed for strain *EPR70*^T was 42 min. Growth occurred under aerobic chemolithoautotrophic conditions in the presence of thiosulfate and CO₂. Strain *EPR70*^T grew heterotrophically with acetate or n-alkanes as sole carbon and energy sources, and in complex artificial seawater medium. Nitrate was not used as an electron acceptor. The G + C content of the genomic DNA was 64 mol%. Phylogenetic analysis of the 16S rRNA gene indicated that this organism is a member of the class *Gammaproteobacteria*, with *Salinisphaera shabanensis* E1L3A^T as its closest relative (94% sequence similarity). On the basis of phylogenetic analyses based on 16S rRNA, *rbcL* and *alkB* genes and physiological analysis, it is proposed that the organism represents a novel species within the genus *Salinisphaera*, for which the name *Salinisphaera hydrothermalis* sp. nov. is proposed. The type strain is *EPR70*^T (=DSM 21483^T =JCM 15514^T).

Deep-sea hydrothermal vents are located in tectonically active areas of the sea floor and release either high-temperature fluids (focused-flow vents) or moderate-temperature fluids (diffuse-flow vents). At deep-sea vents,

seawater interacts with volcanic rocks at high temperature and becomes enriched in a variety of reduced chemical species, which represent a constant flux of electron donors for microbial oxidations. In the absence of light, chemosynthetic micro-organisms oxidize these reduced chemical species (predominantly sulfur compounds) and mediate the primary production of organic carbon (Jannasch, 1995; McCollom & Shock, 1997).

Abbreviations: EPR, East Pacific Rise; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains *EPR70*^T, *EPR72* and *EPR71* are EU740416–EU740418. The accession numbers for the partial *rbcL* gene sequences from strains *EPR70*^T, *EPR71* and *EPR72* and *S. shabanensis* DSM 14853^T are EU740422–EU740425 and those for the partial *alkB* gene sequences of strain *EPR70*^T, *S. shabanensis* DSM 14853^T and strain *EPR71* are EU740419–EU740421.

Graphs showing the effects of temperature, pH and NaCl concentration on growth of strain *EPR70*^T and neighbour-joining trees based on amino acid sequences of RuBisCO form I and AlkB are available as supplementary material with the online version of this paper.

Typically, representatives of the genera *Thiomicrospira* and *Thiobacillus* have been isolated when diffuse-flow hydrothermal fluids were inoculated in culture media for the enrichment of chemosynthetic, aerobic, sulfide-, sulfur- and thiosulfate-oxidizing bacteria (Ruby *et al.*, 1981; Ruby & Jannasch, 1982; Jannasch *et al.*, 1985; Durand *et al.*, 1993; Teske *et al.*, 2000; Takai *et al.*, 2004). Heterotrophic bacteria of the genera *Marinobacter*, *Vibrio*, *Pseudoalteromonas* and *Halomonas*, among others, have also been isolated routinely

from hydrothermal vent samples (Raguénès *et al.*, 1997; Teske *et al.*, 2000; Kaye *et al.*, 2004; Vetriani *et al.*, 2005; Simon-Colin *et al.*, 2008) and their abundance in vent fluids collected from the Pacific Ocean was estimated to be up to 28% of the total micro-organisms (Kaye & Baross, 2000).

Here, we report the isolation of three strains of mesophilic, halotolerant, facultatively chemolithoautotrophic, thiosulfate-oxidizing gammaproteobacteria from deep-sea hydrothermal vents located on the East Pacific Rise (EPR) at 9° N and the characterization of one of these strains.

Hydrothermal fluid samples from diffuse-flow vents were collected from the EPR (9° 50' N 104° 17' W) at a depth of approximately 2500 m during an oceanographic expedition aboard the R/V *Atlantis* in April 2004. The fluids were collected using titanium samplers operated by the manipulator of the DSV *Alvin*, immediately above the venting source and 1 m above the source. On the surface, samples were transferred promptly to the ship's laboratory and subsamples were stored at 4 °C. Primary enrichment cultures were initiated immediately after sample collection in growth medium designed to enrich for chemolithoautotrophic, thiosulfate-oxidizing bacteria. The medium used was a modification of medium 142 (<http://www.dsmz.de>), referred to as 142-A, which was composed of (l⁻¹): NaCl, 25.0 g; (NH₄)₂SO₄, 1.0 g; MgSO₄·7H₂O, 1.5 g; CaCl₂·2H₂O, 0.42 g; KCl, 0.64 g; NaHCO₃, 0.046 g; K₂HPO₄, 0.05 g. Two millilitres of a 0.5% phenol red solution was added to 1 l medium as a pH indicator. Following sterilization, the medium was supplemented with 20 mM Na₂S₂O₃, 1 µM vitamin B₁₂, 1 ml mixed vitamin solution 141 (<http://www.dsmz.de>) and 1 ml trace-element solution SL10 (<http://www.dsmz.de>). For isolation of single colonies, liquid cultures were inoculated on Petri dishes containing medium 142-A solidified with 15 g Noble agar l⁻¹ (Sigma). Stocks for long-term storage were prepared by adding 150 µl sterile glycerol (Fisher Scientific) to 850 µl culture grown overnight, and were stored at -80 °C.

Heterotrophic growth was determined by transferring 100 µl of an overnight culture from medium 142-A to medium 142+A, which was depleted of NaHCO₃ and supplemented with 10 mM sodium acetate (Sigma). Growth was also tested in artificial seawater (ASW) medium (l⁻¹: NaCl, 24 g; KCl, 0.7 g; MgCl₂, 7.0 g; yeast extract, 3.0 g; peptone, 2.5 g), in low-strength ASW (LS ASW) medium (modified ASW containing 0.1 g yeast extract and 0.5 g peptone l⁻¹) and in ASW minimal medium (ASW MM) (l⁻¹: NaCl, 23.6 g; KCl, 0.64 g; MgCl₂·6H₂O, 4.53 g; MgSO₄·7H₂O, 5.94 g; CaCl₂·2H₂O, 1.3 g) Na₂HPO₄·7H₂O, 43.0 mg; NaNO₃, 0.22 g; NH₄Cl, 0.65 g) supplemented with dodecane (C₁₂H₂₆) in the vapour phase as the only carbon and energy source. After autoclaving, ASW MM was supplemented with 1 µM vitamin B₁₂, 1 ml trace-element solution SL-10 and 1 ml mixed vitamin solution 141.

Growth rates (µ; h⁻¹) were estimated as described previously (Vetriani *et al.*, 2004). Unless specified otherwise, growth

ranges and optimal growth conditions were determined in LS ASW medium. To determine the optimal growth temperature of the new isolate, cultures were incubated between 10 and 45 °C (at 5 °C intervals). All other experiments were carried out at 35 °C, the optimal growth temperature. To determine optimal salt requirements, the concentration of NaCl was varied between 1.0 and 25% (w/v). The influence of pH on growth was determined between pH 4.5 and 8.0 by using the following buffers at a concentration of 10 mM: acetate at pH 4.5 and 5.0; MES at pH 5.5 and 6.0; PIPES at pH 6.5, 7.0 and 7.5; and Tris at pH 8.0. Anaerobic growth with nitrate as an electron acceptor was tested in ASW medium supplemented with 7.3 mM KNO₃ under a N₂ atmosphere.

Catalase activity was determined as described previously (Vetriani *et al.*, 2004) and the presence of cytochrome *c*, a component of the cytochrome oxidase system, was determined according to the protocol described by Kovacs (1956). *Escherichia coli* K-12 and *Pseudomonas aeruginosa* were used as negative and positive controls for the cytochrome oxidase test, respectively.

A Biolog GN2 MicroPlate test panel was used to compare the carbon utilization/oxidation profiles of the new isolate with that of the reference strain, *Salinisphaera shabanensis* DSM 14853^T. Confluent growth of both strains was obtained on solid ASW medium overnight. Cells were collected using a sterile cotton swab and resuspended in 15 ml salt solution (l⁻¹: NaCl, 23.5 g; MgCl₂·6H₂O, 10.6 g). The cell suspension was adjusted to an OD₆₀₀ of 0.3 ± 0.05, supplemented with 5 mM sodium thioglycolate and dispensed (in 150 µl aliquots) to each well of two Biolog GN2 MicroPlates, which were incubated at 35 °C. A change in colour, indicative of the oxidation of the substrate, was monitored for 48 h.

For direct counts, cells were stained routinely with 0.1% acridine orange and visualized with an Olympus BX 60 microscope with an oil-immersion objective lens (UplanF1 × 100/1.3). Cells for ultrathin sections and for platinum shadowing were prepared as described previously (Vetriani *et al.*, 2004). Motility was determined by phase-contrast microscopy. Gram staining was performed as described elsewhere (Holt *et al.*, 1994).

Genomic DNA was extracted from cells collected by centrifugation using the UltraClean Microbial DNA isolation kit, according to the manufacturer's instructions (MoBio Laboratories). The full-length sequence of the 16S rRNA gene was selectively amplified from the genomic DNA by PCR, cloned, sequenced and subjected to phylogenetic analysis as described previously (Vetriani *et al.*, 2004; Voordeckers *et al.*, 2005).

The genes encoding the enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase form I and II (RuBisCO) and alkane hydroxylase (AlkB, involved in the oxidation of hydrocarbons) were amplified from the genomic DNA of the novel strains and *S. shabanensis* DSM 14853^T. A 500 bp

fragment of the gene encoding form I RuBisCO (*rbcL/cbbL*) from *S. shabanensis* DSM 14853^T was amplified as described previously (Nanba *et al.*, 2004), and its sequence was determined. Internal primers ss *rbcLF* (5'-GGTCTATGAAAGCGCTCAAGG-3') and ss *rbcLR* (5'-ATCCATTTTCGAGATCACGCGG-3') were designed based on the *rbcL* sequence from *S. shabanensis* DSM 14853^T (using the IDT OligoAnalyser 3.1 program; <http://www.idtdna.com>), and were used to amplify a 400 bp fragment of the *rbcL* gene from the three novel strains. The PCR protocol used to amplify the *rbcL* genes was 5 min at 94 °C followed by 30 cycles of 45 s at 94 °C, 1 min at 50 °C and 45 s at 72 °C, ending with a final extension of 20 min at 72 °C. The PCR for the amplification of a 1040 bp fragment of the gene encoding the form II RuBisCO (*cbbM*) was carried out as described previously (Elsaied *et al.*, 2007). A 550 bp fragment of the gene encoding alkane hydroxylase (*alkB*) was amplified selectively from the genomic DNA of *S. shabanensis* DSM 14853^T and of two of the novel strains by PCR, as described previously (Smits *et al.*, 1999). The sequences for *rbcL* and *alkB* were determined for both strands and were translated into the respective amino acid sequences using EMBOSS Transeq (<http://www.ebi.ac.uk/emboss/transeq>). The amino acid sequences were aligned with CLUSTAL_X v. 1.8 (Thompson *et al.*, 1997) and adjusted manually using SeaView (Galtier *et al.*, 1996). Phylogenetic distances were calculated using the Observed Divergence matrix and the neighbour-joining method was used to evaluate tree topologies. PHYLO_WIN was used to plot tree topologies (Galtier *et al.*, 1996) and their robustness was tested by bootstrap analysis with 1000 resamplings. The determination of total DNA base composition (mol% G+C) was carried out by HPLC (Mesbah *et al.*, 1989).

Enrichment cultures for mesophilic, chemolithoautotrophic, thiosulfate-oxidizing bacteria were obtained by inoculating 10 ml medium 142-A with 1 ml fluid from three different samples collected on the EPR. The temperature of the fluids at the time of collection was 2.5, 6 and 13 °C, respectively. Cultures were incubated at 30 °C. A change in colour of the pH indicator present in the medium, suggesting growth, was observed after 1 or 2 days from the beginning of the incubation and then confirmed by direct cell counting. Three independent cultures showed consistent growth after repeated transfers and were purified by successive isolations of single colonies on solidified medium. The resulting pure cultures were designated strains EPR70^T, EPR71 and EPR72. Preliminary phylogenetic analysis of the 16S rRNA gene sequence indicated that the three strains were closely related (sequence identity 99%). Strain EPR70^T was selected for further characterization.

Cells of EPR70^T were short rods, 0.8–1.0 µm long and 0.3–0.5 µm wide, and divided by constriction (Fig. 1a). Cells stained Gram-negative. The cell envelope of EPR70^T included a cytoplasmic membrane surrounded by a periplasmic space and an outer membrane (Fig. 1b). The

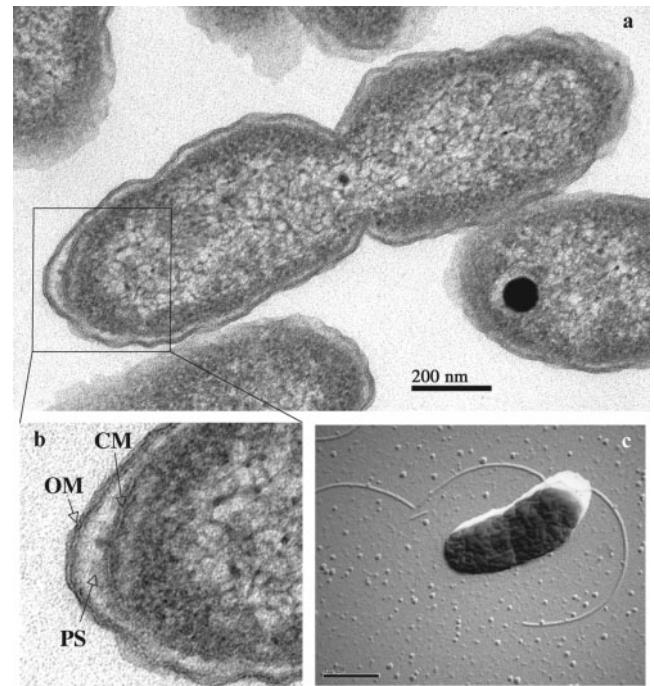


Fig. 1. (a) Electron micrograph of a thin section of cells of strain EPR70^T. (b) Details of the cell envelope of strain EPR70^T. OM, Outer membrane; PS, periplasmic space; CM, cytoplasmic membrane. (c) Electron micrograph of a platinum-shadowed cell of strain EPR70^T showing the presence of flagella. Bars, 200 nm (a) and 0.5 µm (c).

organism was motile and possessed one or more flagella, which were observed in electron micrographs of platinum-shadowed cells (Fig. 1c). The presence of endospores was not observed.

Under strictly autotrophic conditions, EPR70^T, EPR71 and EPR72 oxidized Na₂S₂O₃ and acidified the culture medium. Heterotrophic growth occurred when medium 142 was depleted of Na₂S₂O₃ and NaHCO₃ and supplemented with 10 mM acetate; under these conditions, strain EPR70^T alkalified the culture medium. EPR70^T, EPR71 and EPR72 also grew well heterotrophically in ASW, LS ASW and ASW MM supplemented with dodecane as the sole carbon and energy source. Strain EPR70^T grew at temperatures between 20 and 40 °C, with optimal growth at 35 °C. No growth was detected at 10 or 45 °C. EPR70^T grew at NaCl concentrations between 1.0 and 25.0% (w/v) with an optimum at 2.5% (w/v). Growth occurred between pH 5.0 and 7.5 with an optimum at pH 5.5. No growth was observed at pH 4.5 or 8.0. The shortest generation time of strain EPR70^T in LS ASW was 42 min (Supplementary Fig. S1, available in IJSEM Online). For comparison, the generation time of strain EPR70^T grown under optimal conditions of temperature, salinity and pH and with CO₂ as the sole carbon source (in medium 142) was 231 min. Strain EPR70^T was a fully aerobic organism,

and growth did not occur in medium 142 supplemented with 5% oxygen (v/v) or in ASW medium with 7.3 mM nitrate.

Phylogenetic analysis of the 16S rRNA gene sequence, carried out using the neighbour-joining method, placed EPR70^T, EPR71 and EPR72 (98–99% sequence identity between each sequence) in a unique cluster within the class *Gammaproteobacteria* (Fig. 2). The closest relatives of these strains were *S. shabanensis* E1L3A^T, a moderate halophile which was isolated from the brine–seawater interface in the Shaban Deep in the Red Sea (94% sequence identity to the 16S rRNA gene of strain EPR70^T) and which is the only formally described member of this genus (Antunes *et al.*, 2003), *Salinisphaera* sp. strain ARD M17, isolated from deep-sea water from the Knipovich Ridge, in the Arctic Ocean (94% sequence identity to strain EPR70^T) (T. Okamoto and T. Naganuma, unpublished; GenBank accession no. AB167073), and ‘*Salinisphaera dokdoensis*’ CL-ES53, isolated from the East Sea of Korea (95% sequence identity to strain EPR70^T) (B. C. Cho and others, unpublished; EF988634). While the type species of the genus *Salinisphaera*, *S. shabanensis*, was originally described as a heterotroph (Antunes *et al.*, 2003), our study demonstrated that this organism also grew chemo-lithoautotrophically by thiosulfate oxidation and with n-alkanes as the sole carbon and energy source (Table 1). Interestingly, 16S rRNA gene sequences related to *Salinisphaera* species were retrieved from the microbial community attached to hydrocarbon-contaminated rocks along the Spanish shoreline, represented in Fig. 2 by clones Rc23, Rc32 and Rc58 (J. Alonso-Gutierrez and others, unpublished).

High bootstrap values supported the branching topology of the EPR strains relative to the other strains (Fig. 2). The G+C content of the genomic DNA of strain EPR70^T,

determined by HPLC analysis of deoxyribonucleosides, was 64.0 mol%, while that of *S. shabanensis* E1L3A^T was 61.8 mol% (Antunes *et al.*, 2003).

Comparative analyses of strain EPR70^T and *S. shabanensis* DSM 14853^T revealed morphological (rods and cocci, respectively) and physiological differences (Table 1). In particular, EPR70^T had lower salinity and pH optima than *S. shabanensis* DSM 14853^T (2.5 vs 10% NaCl and pH 5.5 vs ~pH 7.0, respectively), suggesting specific adaptations to the slightly acidic vent fluids, and strain EPR70^T could not grow anaerobically in the presence of nitrate as a terminal electron acceptor (Table 1). The metabolic fingerprints of strain EPR70^T and *S. shabanensis* DSM 14853^T, determined using a Biolog assay on a GN2 MicroPlate, showed that both bacteria were able to oxidize a wide range of carbon sources, but some differences were evident (Table 2). For instance, EPR70^T oxidized sugars and sugar alcohols preferentially, while *S. shabanensis* preferred amino acid derivatives.

In order to investigate the carbon-fixation pathway in the EPR strains and in *S. shabanensis* DSM 14853^T, we carried out PCR amplification of the genes encoding both RuBisCO forms I (*rbcL/cbbL*) and II (*cbbM*) for all strains, and a product was obtained only for the *rbcL/cbbL* gene (encoding RuBisCO form I). The amino acid identity among the RuBisCO sequences of the three EPR strains was 98–99%, while the sequence identity between these enzymes and the RuBisCO from *S. shabanensis* DSM 14853^T ranged from 94 to 95%. Phylogenetic analysis of the amino acid sequence of the form I RuBisCO of strains EPR70^T, EPR71 and EPR72 and *S. shabanensis* DSM 14853^T, carried out using the neighbour-joining method, showed that these sequences formed a unique cluster related to other form I, type C enzymes (Supplementary Fig. S2; Xu & Tabita, 1996). The closest relatives of the

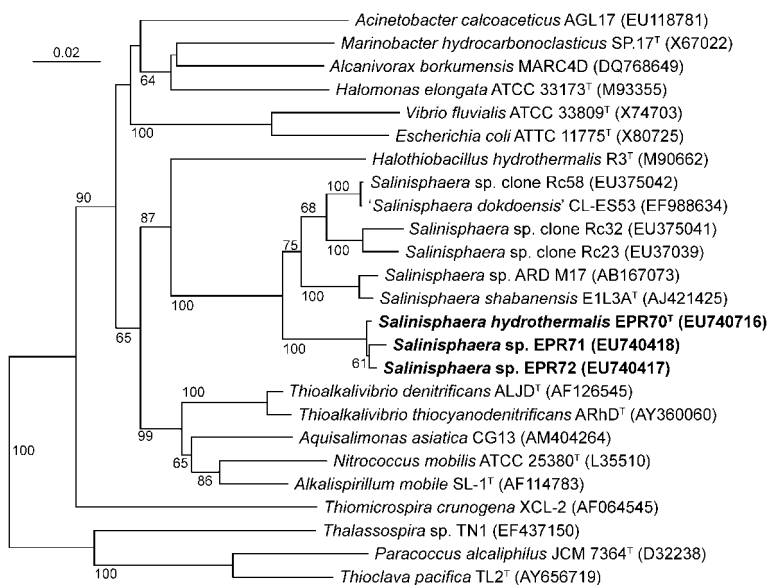


Fig. 2. Phylogenetic position of strains EPR70^T, EPR71 and EPR72, according to their 16S rRNA gene sequences. The neighbour-joining tree was constructed using PHYLO_WIN. Bootstrap values higher than 50% are indicated. Bar, 2% estimated base substitutions.

Table 1. Differentiating features of strain EPR70^T and *S. shabanensis* E1L3A^T

Both strains are catalase-positive. Data for *S. shabanensis* E1L3A^T were taken from Antunes *et al.* (2003) unless indicated.

Feature	Strain EPR70 ^T	<i>S. shabanensis</i> E1L3A ^T
Morphology	Small rods	Cocci
Cell size (µm)	0.8–1.0 × 0.3–0.5	0.7–1.2 (diameter)
Oxidase	Negative	Positive
Temperature for growth (°C)		
Range	20–40	5–42
Optimum	30–35	30–37
Salinity for growth (% NaCl)		
Range	1–25	1–28
Optimum	2.5	10
pH for growth		
Range	5.0–7.5	4.0–8.0
Optimum	5.5	6.5–7.5
Chemolithoautotrophic growth	Yes	Yes*
Growth on dodecane	Yes	Yes*
Anaerobic growth in ASW plus nitrate	No	Yes
DNA G + C content (mol%)	64.0	61.8

*Data for *S. shabanensis* DSM 14853^T obtained in this study.

RuBisCO from the EPR strains and *S. shabanensis* DSM 14853^T were the enzymes of the methylotrophic bacterium *Methylobium petroleiphilum* PM1^T and of the ammonia-oxidizing bacteria *Nitrosospira multiformis* ATCC 25196^T and *Nitrosococcus oceani* ATCC 19707^T (Supplementary Fig. S2). It is worth noting that the RuBisCO enzymes from the EPR strains were also related to a group of sequences retrieved from natural microbial communities associated with the plumes of black smokers located in the Western Pacific arc hydrothermal vent system, represented in Supplementary Fig. S2 by clones ICS1 and ICP1 (Elsaied *et al.*, 2007).

Since both the EPR strains and *S. shabanensis* DSM 14853^T can use n-alkanes as their sole carbon and energy source, we investigated the presence in these organisms of the *alkB* gene, which encodes alkane hydroxylase (AlkB), an enzyme that catalyses the first step in the oxidation of hydrocarbons. Phylogenetic analysis of the amino acid sequence of the AlkB enzyme from strains EPR70^T, EPR71 and *S. shabanensis* DSM 14853^T placed these enzymes in a discrete cluster related to the alkane hydroxylase of *Nocardia farcinia* IFM 10152 (Supplementary Fig. S3). The identity between the AlkB amino acid sequences of EPR70^T and EPR71 was 90 %, while the sequence identities of the enzymes from the two EPR strains and the alkane hydroxylase from *S. shabanensis* DSM 14853^T were 62 and 63 %, respectively.

Physiological and phylogenetic analyses indicated that strain EPR70^T and *S. shabanensis* DSM 14853^T are not related at the species level, and therefore EPR70^T represents a new species within the genus *Salinisphaera*, for which we propose the name *Salinisphaera hydrothermalis* sp. nov.

At deep-sea hydrothermal vents, micro-organisms must adapt to highly dynamic environmental conditions, where

there are fluctuations in temperature, salinity and nutrient availability (Karl, 1995). The presence of the *rbcL/cbbL* gene, which encodes RuBisCO form I, in all the *Salinisphaera* strains suggests strongly that these bacteria use the Calvin–Benson–Bassham cycle to fix CO₂. The metabolic versatility of *S. hydrothermalis*, which can fix CO₂ and use a wide range of organic carbon sources, may be an advantage for its growth and survival in these environments. In particular, the ability of *S. hydrothermalis* to grow autotrophically, to oxidize n-alkanes (which are enriched in hydrothermal fluids; Brault *et al.*, 1988) and to grow optimally at a slightly acidic pH suggests that this bacterium is particularly well suited to thrive in the moderate-temperature fluids that are typically emitted by diffuse-flow deep-sea hydrothermal vents.

Emended description of the genus *Salinisphaera* Antunes *et al.* 2003

Cells are Gram-negative cocci or short rods, possessing one or more flagella. Mesophilic, halotolerant and catalase-positive. Aerobic or facultatively anaerobic. Growth occurs chemolithoautotrophically with thiosulfate as an electron donor and oxygen as an electron acceptor, heterotrophically on n-alkanes (dodecane) as the sole carbon and energy source or on complex media. Known strains have been found in marine environments. The type species is *Salinisphaera shabanensis* Antunes *et al.* 2003.

Description of *Salinisphaera hydrothermalis* sp. nov.

Salinisphaera hydrothermalis (hy.dro.ther.ma'lis. N.L. fem. adj. *hydrothermalis* hydrothermal, pertaining to a hydrothermal vent).

Table 2. Comparative metabolic profiles of strain EPR70^T and *S. shabanensis* DSM 14853^T

Both strains oxidized Tweens 20 and 80, D-mannitol, D-mannose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, γ -hydroxybutyric acid, DL-lactic acid, succinic acid and L-alanine. Data were obtained in this study using the Biolog GN2 MicroPlate.

Carbon source	Strain EPR70 ^T	<i>S. shabanensis</i> DSM 14853 ^T
Glycogen	–	+
Adonitol	+	–
D-Arabitol	+	–
i-Erythritol	+	–
D-Fructose	+	–
D-Galactose	+	–
α -D-Glucose	+	–
L-Rhamnose	+	–
D-Sorbitol	+	–
cis-Aconitic acid	–	+
Formic acid	–	+
α -Ketobutyric acid	+	–
α -Ketoglutaric acid	–	+
Bromosuccinic acid	–	+
Succinamic acid	+	–
L-Alanyl glycine	–	+
L-Asparagine	–	+
L-Aspartic acid	–	+
L-Glutamic acid	–	+
Glycyl L-aspartic acid	–	+
Glycyl L-glutamic acid	–	+
L-Proline	+	–
γ -Aminobutyric acid	+	–
Glycerol	+	–

Cells are small rods (0.8–1.0 μ m long, 0.3–0.5 μ m wide), which are motile by means of one or more flagella. Obligate aerobe. Oxidase-negative. Growth occurs at 20–40 °C (optimum 30–35 °C), 1–25 % NaCl (optimum 2.5 %) and pH 5.0–7.5 (optimum pH 5.5). The shortest generation time observed is 42 min. Growth occurs under aerobic, chemolithoautotrophic conditions in the presence of thiosulfate and CO₂. Heterotrophic growth occurs with acetate or n-alkanes as sole carbon and energy sources and in complex ASW medium. Nitrate is not used as an electron acceptor. The DNA G+C content of the type strain is 64.0 mol% as determined by the HPLC method.

The type strain is EPR70^T (=DSM 21483^T =JCM 15514^T), which was isolated from diffuse-flow hydrothermal vent fluids collected from the East Pacific Rise at 9° 50' N.

Acknowledgements

We wish to thank Amber G. Jensen for excellent technical assistance. We thank the crew of the R/V *Atlantis* and the crew and pilots of the

DSV *Alvin* for their skilled operation at sea. This research was supported by NSF grant OCE 03-27353 to C.V. and R.A.L., NSF grant MCB 04-56676 to C.V., NSF grant EAR 04-33793 to T.B., and by NIH R25GM-58389 and an NSF Graduate Research Fellowship to M.C.-M.

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