Current Microbiology Vol. 48 (2004), pp. 159–163
DOI: 10.1007/s00284-003-4155-9

Current Microbiology
An International Journal

Acidianus tengchongensis sp. nov., a New Species of Acidothermophilic Archaeon Isolated from an Acidothermal Spring

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Received: 20 May 2003 / Accepted: 23 June 2003

Abstract. A new thermoacidophilic, obligately chemolithotrophic, facultatively aerobic archaeon *Acidianus* S5^T, was isolated from a Tengchong acidothermal spring in southwestern China. It is a Gram-negative, nonmotile, irregular coccoid organism with a cell diameter of 1.2 μm. The optimal pH and temperature for growth are 2.5 and 70°C, respectively. Under anaerobic conditions, the organism reduces elemental sulfur with molecular hydrogen, producing hydrogen sulfide. Under aerobic conditions, it oxidizes elemental sulfur and produces sulfuric acid. No growth occurs when it is cultivated in an iron medium, indicating that ferrous iron cannot serve as an energy source. The G+C content is 38% (mol/mol), which is much different from that of other *Acidianus* species (31%–32.7%). The phylogenetic distances, based on 16S rDNA sequences, to *A. brierleyi*, *A. infernus*, and *A. ambivalens* were 0.2, 2.6, and 2.5%, respectively. DNA-DNA hybridization rates of strain S5^T to *A. brierleyi*, *A. infernus*, and *A. ambivalens* are 44, 22, and 23%, respectively. Thus, a new name, *Acidianus tengchongensis* sp. nov., is proposed for this strain S5^T.

The order *Sulfolobales* is a collection of extremely thermophilic, chemolithotrophic, sulfur-metabolizing archaea [15, 18]. Among the *Sulfolobales*, the genus *Acidianus* is characterized by facultatively anaerobic growth at high temperature and low pH in the presence of elemental sulfur [20], and it includes three current species: *A. brierleyi* [22], *A. infernus* [14], *A. ambivalens* [14].

Sulfur oxidizers of the bacteria and archaea are widely distributed in natural sites like hot springs, solfatara areas, etc. The ecology, biotopes, and strategies to obtain hyperthermophiles including thermophilic sulfur-oxidizing species have been reviewed recently [9]. In this paper, we describe a new species of acidothermophilic archaeon, *A. tengchongensis* sp. nov., which was isolated from an acidic thermal spring in Tengchong, a county of Yunnan province located in southwestern China.

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Materials and Methods

Isolation, media, and cultural conditions. Samples were obtained from a Tengchong acidothermal spring in Yunnan of southwestern China. Acidothermophilic, sulfur-oxidizing archaeon strain S5^T was isolated by the method of Jan et al. [10] and grown at 70°C in modified Allen medium with 2% elemental S as the only energy source [1, 3]. Anaerobic media were prepared by using the technique of Segerer et al. [14]. *A. brierleyi* (DSM1651), *A. infernus* (DSM3191), *A. ambivalens* (DSM3772), *Sulfolobus acidocaldarius* DSM639 (D14876), and *Sulfolobus metallicus* DSM6482 (X90479) were obtained from the Japan Collection of Microorganisms (JCM, Rikan, Japan). Cultivation of these archaeal strains was in modified Allen medium with 2% elemental sulfur [1, 3].

Bacterial growth and metabolism. The optimal temperature, optimal pH, and cell concentrations were determined by the methods of Jan et al. [10]. In order to determine the substrate of the new isolates, some organic and inorganic substances were assayed as described earlier [10]. In order to determine whether iron can be used as an energy source by S5^T, the medium was prepared according to Brierleyi et al. [2], and the S5^T was inoculated in the medium at 55°C and 70°C.

Each of the following organic substances was added to the modified Allen mineral medium in concentrations of 0.2, 0.5, and 1 g/L in the presence (2 g/L) and absence of S under either aerobic or anaerobic culture conditions: D (−) ribose, L (+) arabinose, D (+) xylose, D (+) glucose, L (−) glucose, D (+) galactose, maltose, lactose, sucrose, melibiose, raffinose, cellulose, starch, mannitol (all Merck), 20 differ-

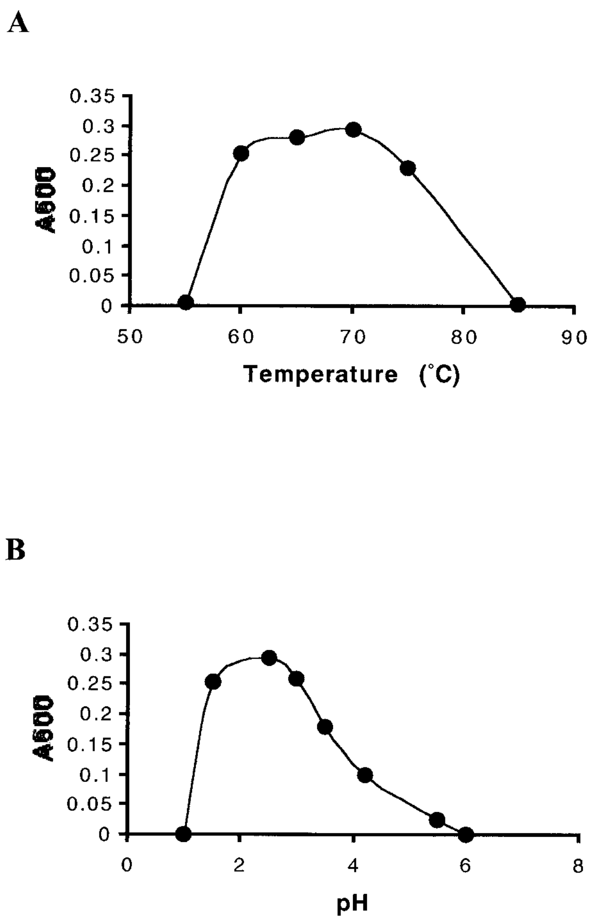

Fig. 1. Effect of different temperatures and pHs on microbial growth. (A) Temperature range of strain S5^T growth; (B) pH value range of strain S5^T growth.

ent L-amino acids (Sigma Chemical Co.), yeast extract, tryptone, peptone, and beef extract (Merck).

H_2S was analyzed as described previously [16]; SO_4^{2-} was determined gravimetrically after precipitation of $BaCl_2$ by the method of Zhong et al. [21] and Li et al. [13].

DNA-DNA hybridization and 16S rDNA sequence. The genomic DNA of strain S5^T and of other *Acidianus* species were isolated as described previously [8]. DNA-DNA hybridization was done according to Xu et al. [19]. The 16S rDNA fragment of strain S5^T was amplified by primers F: 5′-TTCCG CTT GAT CCY GCC CGG-3′, and R: 5′-AAG GAG GTG WTC CAR CC-3′ [4, 19] and corresponded to position 3–29 and 1541–1525 respectively in the 16S rRNA gene (*E. coli* numbering). The derived sequences were aligned against a represenstive collection of archaeal 16S rRNA gene sequence (Ribosomal Database Project). Pairwise evolutionary distances were computed from percentage similarity by the method of Xu et al. [19].

Phylogenetic analysis. The 16S rDNA sequence of *Acidianus* S5^T was deposited in GenBank (AF22698). The accession numbers of 16S rDNA sequence of the test strains are listed in Fig. 2 (in parentheses). The tree was constructed by application of the electronic tools of the software package PHYLIP Version 3.5C [5].

Electron miscroscopy. Scanning and thin sections were prepared and electron microscopy was carried out according to Zhong et al. [21].

Results

Collection of samples and isolation of the new strain. Nine aerobic samples were collected from water and mudholes of Tengchong acidothermal spring. The original temperatures were between 45°C and 95°C and pH between 1.5 and 5.0. All samples were carried to the laboratory without pH and temperature control and were stored there at 4°C. One mL of each of the original samples was transferred into 30 mL of modified Allen medium. After 1 week of incubation at 70°C with shaking (150 rpm), coccoid organisms, resembling *Sulfolobus* in shape, became visible. Pure cultures were obtained by an isolated colony, which was transferred into broth. Strain S5^T was isolated from the samples, and its G+C (mol/mol) composition is 38% (Table 1).

Characterization and description of *Acidianus tengchongensis* sp. nov. Growth of strain S5^T in Allen modified medium was obtained between 55°C and 80°C with an optimum around 70°C (Fig. 1A) and pH range between 1.0 and 5.5, with an optimum around 2.5 (Fig. 1B).

In the electron microscope, cells of the isolate S5^T appeared as coccoid about 1.2 μm in diameter. The cells appeared immotile and occurred singly or in pairs (Fig. 3). S5^T was able to grow facultatively aerobically by means of two contrary modes of chemolithotrophy. Under extremely anaerobic conditions, the S5^T grew autotrophically, forming H_2S. Growth depended strictly on H_2 and S, and H_2 could not be replaced by the organic compounds listed in Materials and Methods. Under aerobic conditions, in the presence of S, the S5^T was able to grow by oxidation of S, forming sulfuric acid, like the *Sulfolobus* type strains. S5^T was unable to grow without S by oxidizing organic compounds and was, therefore, strictly chemolithotrophic. Comparison of this strain with other species of *Acidianus* is listed in Table 2. G+C contents of strain S5^T was 38% and was clearly different from other *Acidianus* species in G+C contents (between 31 and 33%) (Table 1). It was further distinguished from *A. brierleyi* by being incapable of using ferrous iron as an energy source. As we recently discovered, strain S5^T takes thiosulfate as the sole energy source [17]. This property has not been described for other species of *Acidianus.*

Phylogenetic analysis based on 16S rDNA sequences revealed that strain S5^T is closest to *A. brierleyi* (phylogenetic distance is 0.2%; see Fig. 2). However, DNA-DNA hybridization of strain S5^T to *A. brierleyi*, *A.*

Table 1. Comparison of strain S5^T with other species of the genus *Acidianus*

Species and/or strains	Strain S5^T	*A. brierleyi* DSM1651	*A. infernus* DSM3191	*A. ambivalens* DSM3992
Cell shape	coccoid	coccoid	coccoid	coccoid
Cell diameter (μm)	1.2	1.0–1.5	1.0–1.5	NA[a]
Temp. for growth (°C):				
Ranges	55–80	45–75	65–96	NA–87
Optima	70	70	90	80
pH for growth:				
Ranges	1.0–5.0	1.0–6.0	1.0–5.5	1.0–3.5
Optima	2.5	1.5–2.0	2.0	2.5
G+C content (mol%)	38	31	31	32.7
Ability to use Fe^{2+} as energy source	−	+	−	−
	1[b]	1 or 2	1	1

[a] NA, not available. [b] 1, Obligate chemolithotrophy; 2, organotrophy.

Table 2. DNA-DNA similarity between S5 and other species of the genus *Acidianus*

Filter-bound DNA	α-^{32}P-labeled DNA from *A. brierleyi* DSM 1651	*A. infernus* DSM 3191	*A. ambivalens* DSM 3772	*E. coli*	S5
A. brierleyi DSM 1651	100	24	19	<1	44
A. infernus DSM 3191	21	100	52	<1	22
A. ambivalens DSM 3772	19	55	100	<1	23
S. acidocaldarius DSM 639	ND[a]	7	ND	ND	12
S. metallicus DSM6482	ND	8	ND	ND	7
E. coli	ND	ND	ND	100	<1
S5	43	23	22	<1	100

[a] ND, not determined.

infernus, and *A. ambivalens* (the hybridization rates are 44, 22, and 23%, respectively) (Table 2) clearly distinguished strain S5^T from other *Acidianus* species and supports the establishment of a new species of the genus *Acidianus*. Thus, a new name, *Acidianus tengchongensis* sp. nov., is proposed for this strain S5^T.

Description of *Acidianus tengchongensis* sp. nov. *Acidianus tengchongensis* (teng-chen-gen-sis. N.L. masc. adj.) is from Tengchong, the place where this strain was isolated. Cells are Gram-negative, irregular coccoi, with diameter of 1.2 μm. Thermoacidophilic, facultatively anaerobic. Obligate chemolithotrophy, utilizing CO_2 as carbon source with either S^0+O_2 (yielding sulfuric acid) or S^0+H_2 (yielding H_2S) as energy sources. Thiosulfate is also used for energy. The optimal pH and temperature for growth are 2.5 (range 1.0–5.5) and 70°C (range 55°C–80°C), respectively. Isolated from an acidothermal spring in Tengchong of Yunnan province of China. The type strain is *Acidianus tengchongensis* S5^T, deposited in the China General Microbiological Culture Collection Center (Collection number: AS 1.3347), Beijing.

Discussion

The archaea- and bacteria-mediated oxidation of sulfur to sulfuric acid is one of the major reactions of the global sulfur cycles. These microorganisms widespread in sulfur-rich environments like thermal vents [2, 10], soda lakes, and other solfatara areas [6]. China, especially in the southwest parts, is abundant of geothermal vents and biodiversities of these sites have not been well studied. Only till recently did a biological survey on the biodiversity of hot spring in Tengchong lead to the discovery

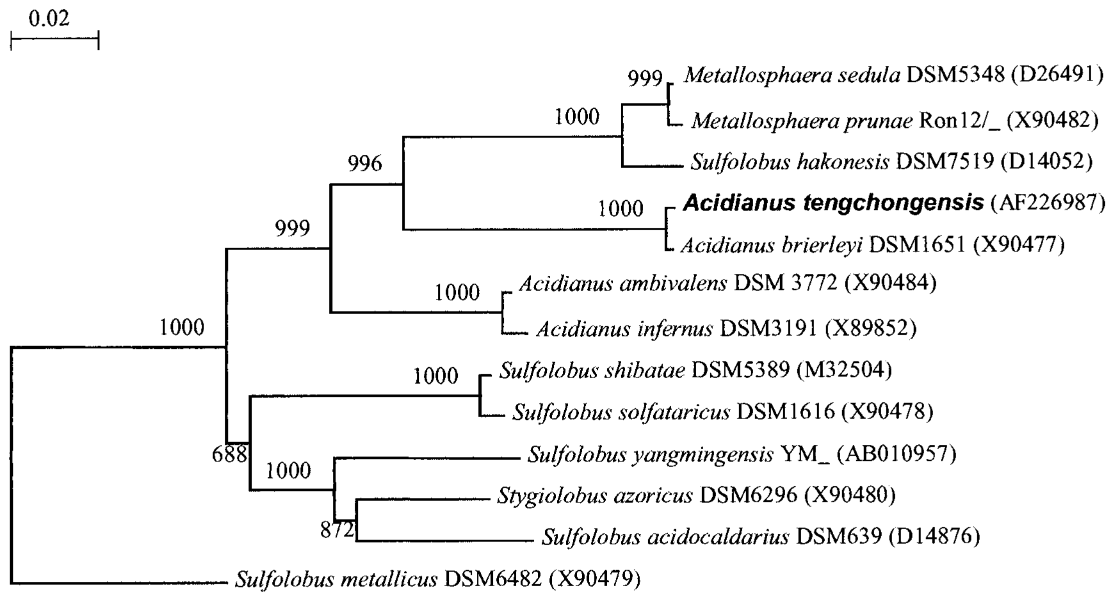

Fig. 2. Phylogenetic position of strain S5^T within *Sulfolobales* based on 16S rDNA sequence similarities. Pairwise evolutionary distances were computed from percentage similarity [19], and the phylogenetic tree was constructed by application of the electronic tools of the software package PHYLIP Version 3.5C [5]. Significant bootstrap values are indicated as percentages at the branching points. The bar indicates two nucleotide substitutions per 100 nucleotides. The accession numbers of the sequences are given in brackets.

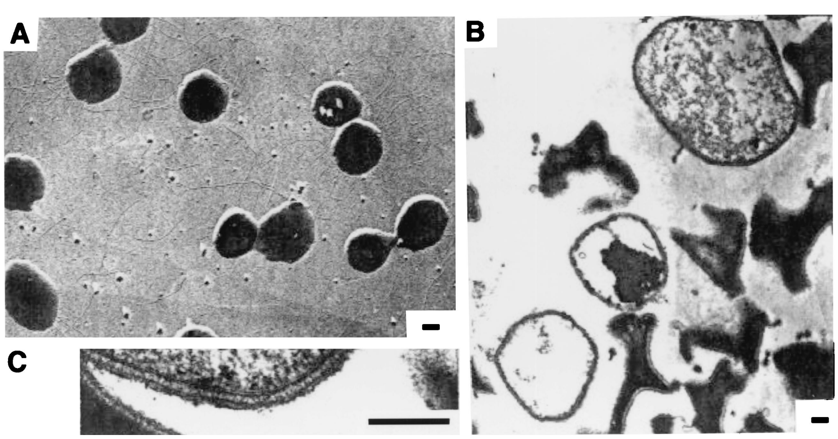

Fig. 3. Electron micrographs of strain S5^T. (A) Scanning electromicrograph; (B) thin-section electron micrograph; (C) the peripheral region of the cell. Bar = 0.2 μm.

of previously unknown species, *Thermoanaerobacter tengchongensis,* which is a heterotrophic, obligate anaerobe, able to reduce thiosulfate and sulfur to sulfite [20] and was obtained under the effort to find thermophilic cellulase producers [20]. This species reduced thiosulfate and sulfur to sulfide, but it did not account for the oxidation of sulfur in the O_2-rich zones of the thermal vents. Different from *T. tengchongensis,* strain S5^T is obligately chemolithotrophic, sulfur oxidizing. Thus, it could fill such position as it could reduce sulfur to sulfuric acid and sulfide under aerobic or anaerobic conditions, respectively. The identification of *A. tengchongensis* as a new species of the genus *Acidianus* is fully supported by this study and also by previous studies. Besides the physiological and DNA molecular differences, significant differences of the chromosal DNA fragments harboring the SOR genes were found in *A. tengchongensis* [8] and *A. ambivalens* [7, 11, 12]. Neither the organization of the ORFs nor the ORFs themselves near the SOR gene exhibited any similarities.

ACKNOWLEDGMENTS

We are grateful to Prof. Shuangjiang Liu for critically reading and revising the manuscript. We thank Prof. Peijing Zhou for many helpful suggestions and discussions. This work was supported by a grant from National Natural Science Foundation of China.

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