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ORIGINAL PAPER

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Sulfolobus tokodaii sp. nov. (f. *Sulfolobus* sp. strain 7), a new member of the genus *Sulfolobus* isolated from Beppu Hot Springs, Japan

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Abstract The taxonomic position of a thermoacidophilic crenarchaeote *Sulfolobus* sp. strain 7, previously isolated from the Beppu Hot Springs in the geothermal area of Kyushu Island, Japan, was investigated by cloning and sequencing, by phylogenetic analysis of the 16S rRNA gene sequence, by DNA–DNA homology with similar species, and by biochemical characterization of the isolate. This isolate is an obligate aerobe and grows optimally at 80°C and pH 2.5–3 under aerobic and chemoheterotrophic growth conditions by aerobic respiration rather than simple fermentation. In conjunction with the phenotypic properties, the present phylogenetic analysis based on the 16S rRNA gene sequence and DNA–DNA hybridization experiments indicate that this isolate is related to the described *Sulfolobus* taxon and should be considered a novel species of the genus. We propose that this isolate is a novel species of the genus *Sulfolobus* that we name *Sulfolobus tokodaii* sp. nov. The type strain is strain 7 (JCM 10545).

Key words *Sulfolobus tokodaii* sp. nov. · Archaea · Thermoacidophile · Crenarchaeote · 16S rDNA · Phylogeny

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Introduction

The archaeal order *Sulfolobales* within the Crenarchaeota kingdom of the Archaea domain (Woese et al. 1990) is composed mainly of the genera *Sulfolobus*, *Acidianus*, *Metallosphaera*, *Stygiolobus*, *Sulfurisphaera*, and *Sulfurococcus* (Stetter et al. 1990). These thermoacidophilic cocci have been isolated from continental solfataric fields and smoldering mining refuse piles and phylogenetically characterized (Huber et al. 1989; Segerer et al. 1991; Fuchs et al. 1996; Trevisanato et al. 1996; Burggraf et al. 1997; Jan et al. 1999). *Sulfolobus* and *Metallosphaera* are obligate aerobes, while *Acidianus* and *Sulfurisphaera* are facultative anaerobes. *Stygiolobus* is an obligate anaerobe. The present taxonomy of the order *Sulfolobales* based on the phenotypic properties is confusing and does not always coincide with the inferences derived from the phylogenetic analysis of their 16S rDNA sequences (Zillig 1993; Fuchs et al. 1996; Kurosawa et al. 1998). Hence, the intra- and intergeneric relationships among these organisms must be determined by biochemical and phylogenetic analyses.

The thermoacidophilic archaeon, *Sulfolobus* sp. strain 7^T sp. nov. (formerly called *S. acidocaldarius* strain 7; Inatomi et al. 1983), was isolated from an acidic spa in Beppu Hot Springs, Kyushu, Japan in the early 1980s by Oshima and colleagues and initially characterized by its phenotypic properties. This isolate is an obligate aerobe that grows optimally at pH 2–3 and at 75°–80°C, preferably under chemoheterotrophic growth conditions (Inatomi et al. 1983; Kondo et al. 1991; Iwasaki et al. 1995). A number of key enzymes involved in the cognate central metabolic pathways and the bioenergetic system have been subjected to extensive biochemical and molecular biological investigation (Denda et al. 1990; Iwasaki et al. 1995; Iwasaki and Oshima 2001). Sequence analysis of V_oV_1-type (also called A_oA_1-type) ATP synthase subunits from the isolate strain 7^T

has provided a clue to the putative evolution of eukaryal species (Gogarten et al. 1989; Denda et al. 1990). Phylogenetic relationships with other organisms on the basis of some of these enzymes indicated that this isolate most likely represents a distinct species different from either *Sulfolobus acidocaldarius* or *S. solfataricus* (Hochstein and Stan-Lotter 1992; Schäfer et al. 1994; Iwasaki et al. 1995; Cosper et al. 1999). However, descriptions of the isolate strain 7^T are confusing in recent literature (e.g., Schäfer et al. 1994), and should have awaited phylogenetic analysis based on its 16S rDNA sequence. In this paper, we characterize this isolate in terms of morphology, and on the basis of phylogenetic analyses based on the 16S rRNA gene sequence and DNA–DNA reassociation with similar species.

Materials and methods

Strains and media

The isolate *Sulfolobus* sp. strain 7^T [JCM 10545; Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-0198, Japan] was cultivated aerobically and heterotrophically at pH 3 and 80°C as described previously (Iwasaki et al. 1995) unless otherwise specified. Anaerobic cultivation was performed in minimal medium amended with a H_2/CO_2 (90 : 10) headspace, as described by others (Kurosawa et al. 1998). *Escherichia coli* JM109 was used as a host for DNA manipulation. Other chemicals mentioned in this study were of analytical grade.

Molecular cloning and sequencing of the 16S rRNA gene

Isolation of 16S rRNA and preparation of its hybridization probe labeled by [γ-^{32}P]ATP followed the method previously reported (Kondo et al. 1993). From the results of the previous southern hybridization experiments (Kondo et al. 1993), chromosomal DNA of the isolate strain 7^T was digested with *Xba*I and ligated into *Xba*I-digested pUC19, followed by transformation in *E. coli* JM109. The colony hybridization using the probe was carried out to isolate transformants harboring the 16S rRNA gene of strain 7^T, in accordance with the earlier finding that the archaeon has a set of 5S, 16S, and 23S rRNA genes in a 7.0-kbp *Xba*I region of the chromosome (Yamagishi and Oshima 1990). The nucleotide sequences were determined by the dideoxy chain termination method. The 2190-bp *Eco*RV–*Bam*HI region contained the complete 16S rRNA gene, comprising 1495-bp, and the 5′ region of the 23S rRNA gene, found 200 nucleotides downstream of the 3′ end of the 16S rRNA gene.

Phylogenetic analyses

The nucleotide sequences of the type strains of *Sulfolobus* used in the phylogenetic analyses were obtained from the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database. The 16S rRNA gene sequences were aligned using the program package Clustal W developed by D. G. Higgins and coworkers at the EMBL. Phylogenetic analyses were performed using the program package, Phylip 3.56c (Felsenstein 1985). For the calculation of the pairwise similarity matrix among 16S rRNA genes of the taxa, the positions present in the 1,242-bp nucleotide regions of 16S rRNA genes were used because of a shorter data set (1,242 bp) available for *Sulfolobus yangmingensis* strain YM1^T in the databases. Evolutionary distances of the 16S rRNA genes were estimated using the Kimura 2-parameter model program that weights transitions and transversions at 2:1 (Kimura 1980). A phylogenetic tree was reconstructed using the algorithm of the neighbor-joining method and the phylogenetic distance values. The statistical significance of the positions of some groups on the tree was reexamined by using the bootstrap method (Felsenstein 1985) with 1,000 replicates.

Nucleotide sequence accession number

The nucleotide sequence reported in this paper has been submitted to the DNA Data Bank of Japan (DDBJ), and will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number AB022438.

DNA–DNA hybridization

Genomic DNA for DNA–DNA hybridization experiments was isolated by the procedure described by Sambrook et al. (1989) from the following organisms: the isolate strain 7^T, *Sulfolobus shibatae* strain DSM5389 (kindly provided by Y. Ito, Soka University, Japan), *Sulfolobus yangmingensis* strain YM1^T (kindly provided by S.-D. Tsen, National Yang-Ming University, Republic of China), *Sulfurisphaera ohwakuensis* strain TA-1^T (kindly provided by Y. Ito, Soka University, Japan), and *Escherichia coli* strain DH5α. DNA was labeled with [α-^{32}P]dCTP by using the T7 QuickPrime Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). After removing free nucleotides by gel filtration, aliquots of labeled DNA (adjusted to 5×10^5 cpm ml^{-1} hybridization buffer) were then used as probes. Four sets of aliquots (containing 2 μg of each genomic DNA) were denatured and immobilized onto Hybond-N+ membranes (Amersham Pharmacia Biotech). DNA–DNA hybridization experiments were performed following the instructions described in the user's manual. Hybridization was carried out at 47.5°C for 18 h. Radioactivity that remained bound to the filter was then counted by using the BAS2000 system with imaging plate (Fuji Film, Tokyo, Japan).

Analytical procedures

The number of cells was determined by light microscopy, using a Thoma counting chamber (depth 0.02 mm). Very brief sonication was applied to dissociate the cells from the

sulfur powder to which the archaeon was attached, and, after 5 min to allow the sulfur sediment to settle to the bottom, the number of the cells in the supernatant fraction (10 µl) was counted.

The ability of the strain 7^T cells to fix added [^{14}C-U]-CO_2 was tested as follows. The cells were cultivated at 75°C in airtight 30-ml test tubes with 5 ml of the growth medium. [^{14}C-U]-CO_2 was added in the form of 0.1 µmol of sodium [^{14}C-U]-bicarbonate. The cells were cultivated for 18 h and harvested by passing through a 0.22-µm MF-Millipore membrane filter. The cells harvested on the filter were washed twice with 5 ml of the 50-mM phosphate buffer, pH 3, to remove excess media, and treated with HCl vapor for 5 min to remove free [^{14}C-U]-CO_2 adsorbed to the cells. The resultant filter was completely dried using an infrared lamp to reduce possible experimental errors by ensuring the removal of free [^{14}C-U]-CO_2, and the remaining ^{14}C fixed in the cells was measured with a liquid scintillation counter LSC-700 (Aloka, Tokyo, Japan).

Imaging of the strain 7^T cells was performed in the dynamic force mode (DFM) with a Nanopics atomic force microscope (Seiko Instruments, Matsudo-shi, Japan), using cantilevers, 120 µm in length, 50 µm in width, and 4–5 µm in thickness, with a force constant (calculation) of 30–40 N/m. The scan rate was typically about 3.33 Hz. For the surface analysis of the cells by atomic force microscopy, the cell suspension was appropriately diluted with distilled water (pH was adjusted to 3.0 with H_2SO_4), allowing single cells to be localized. A droplet of the diluted cells (about 5 µl) was spread onto a clean slide glass (washed with ethanol and dried prior to use), and dried for 14 h at room temperature in a clean room prior to the imaging.

The native membranes of strain 7^T were prepared, after disruption of the cells by a French press (Otake Works, Tokyo, Japan) as described previously (Iwasaki et al. 1995), in 60 mM potassium phosphate buffer, pH 6.8. Absorption spectra were recorded using a Hitachi U3210 spectrophotometer (Hitachi, Tokyo, Japan) equipped with a thermoelectric cell holder or a Beckman DU-7400 spectrophotometer (Palo Alto, CA, USA).

Results and discussion

Morphological and physiological characterization

Cells of strain 7^T (JCM 10545) give rise to pale tan, translucent, smooth, convex colonies with entire margins. Cells are irregular cocci and variable in size (usually 0.5–0.8 µm in diameter) and have flattened and uneven surfaces (Fig. 1). The cell envelope has been shown by electron microscopy to consist of a hexagonal *S*-layer lattice (spacing approximately 20 nm), which mainly consists of two glycoprotein subunits with apparent molecular masses of 40 and 100 kDa, respectively (Inatomi et al. 1983).

The isolate strain 7^T grows chemoheterotrophically under aerobic conditions in the *Sulfolobus* medium (modified Brock's basal salts mixture) defined by Brock et al. (1972)

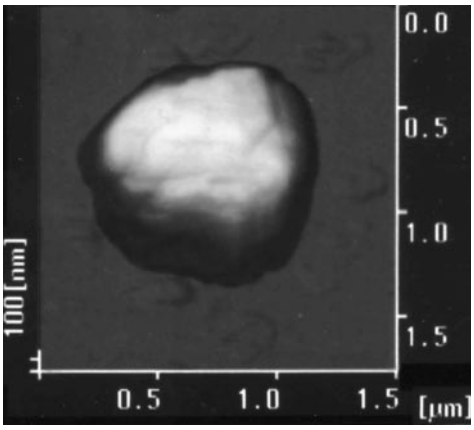

Fig. 1. Atomic force microscope image probing a typical cell shape and uneven surface of the isolate strain 7^T (= JCM 10545). The scale is indicated in the figure

(adjusted to pH 2.5 with H_2SO_4): 1.3 g/l $(NH_4)_2SO_4$, 0.28 g/l KH_2PO_4, 0.28 g/l $MgSO_4 \cdot 7H_2O$, 0.07 g/l $CaCl_2 \cdot 2H_2O$, 0.02 g/l $FeCl_3 \cdot 6H_2O$, 1.8 mg/l $MnCl_2 \cdot 4H_2O$, 4.5 mg/l $Na_2B_4O_7 \cdot 10H_2O$, 0.22 mg/l $ZnSO_4 \cdot 7H_2O$, 0.05 mg/l $CuCl_2 \cdot 2H_2O$, 0.03 mg/l $Na_2MoO_4 \cdot 2H_2O$, 0.03 mg/l $VOSO_4 \cdot 2H_2O$, and 0.01 m/g $CoSO_4$, supplied with 1 g/l trypton (Difco, Detroit, MI, USA), or amino acids such as 1 g/l L-alanine (Kondo et al. 1993). Best growth was observed in the shaking culture on the modified Brock's basal salts mixture at pH 2.5 and at 80°C, supplied with 1 g/l yeast extract (Difco), 1 g/l casamino acid (Difco), and 1 g/l glucose (Kondo et al. 1991; Iwasaki et al. 1995). Under the latter conditions, the main cytochromes are the membrane-bound cytochromes b_{562}, a_{583}, and aa_3, with the α bands at 562, 583, and 603 nm, respectively; they have been shown to be the constituents of a terminal caldariellaquinol oxidase supercomplex that can reconstitute the archaeal, succinate-oxidizing, and cyanide-sensitive aerobic respiratory chain together with the purified cognate respiratory complex II (succinate dehydrogenase complex) and caldariellaquinone in vitro (Iwasaki et al. 1995). No *c*-type, *d*-type, or soluble cytochromes could be detected spectrophotometrically (data not shown). The soluble fraction of strain 7^T contains zinc-containing ferredoxin and related oxidoreductases such as coenzyme A-acylating 2-oxoacid : ferredoxin oxidoreductase (Cosper et al. 1999; Iwasaki and Oshima 2001). These results indicate operation of an oxidative tricarboxylic acid cycle-linked aerobic respiratory chain under aerobic and chemoheterotrophic growth conditions (Iwasaki et al. 1995). The cytochrome and ferredoxin compositions did not change when the strain 7^T cells were grown without shaking at pH 2.5 and 80°C (Iwasaki et al. 1995; Iwasaki and Oshima 2001).

Although several isolates of *Sulfolobus* can grow autotrophically by oxidation of sulfur or sulfide (Stetter et al. 1990; Zillig 1993; Burggraf et al. 1997), no autotrophic growth of strain 7^T was observed in minimal media supplied with elemental sulfur (data not shown), unlike *S. ohwakuensis* strain TA-1^T (Kurosawa et al. 1998). After cultivation for at least three months while gradually reducing the content of the carbon source in the medium, very slow growth

(approximately 1×10^8 cells/ml in 18 h cultivation) was reproducibly observed in the presence of elemental sulfur supplied with alanine (10 mg/l) or polypeptone (0.1 g/l). The whole-cell labeling experiments using [^{14}C-U]-CO_2 indicated that these cells incorporated about 20% of the total amount of ^{14}C label added to the medium. No significant incorporation of the ^{14}C label was observed in the absence of elemental sulfur. The native membrane prepared from such cells was found to contain cytochromes a_{583} and aa_3 but only a trace amount of cytochrome b_{562}, suggesting the presence of an alternative *a*-type respiratory terminal oxidase (data not shown). Thus, strain 7^T can grow facultatively chemolithotrophically in the presence of elemental sulfur, but not autotrophically.

Strain 7^T grew at temperatures from 70° to 85°C and at pH values between 2 and 5 (data not shown). The optimal temperature and pH for growth were 80°C and pH 2.5–3 under aerobic and chemoheterotrophic growth conditions; the latter property distinguishes strain 7^T from *S. yangmingensis* strain YM1^T which grows at an optimal pH of 4.0 (Jan et al. 1999) (see below).

Phylogenetic analysis

The nucleotide sequence of the 16S rRNA gene of strain 7^T was aligned with those of the representatives (mainly Crenarchaeota) of the Archaea domain. Table 1 shows the pairwise 16S rDNA similarity and evolutionary distance matrices among the 15 members of the order *Sulfolobales*. The pairwise similarity matrix indicates that the 16S rRNA gene sequence of strain 7^T is most closely related to those of *S. yangmingensis, S. solfataricus*, *S. shibatae*, *S. acidocaldarius*, *Sulfolobus* sp. strain B6/2, and *Stygiolobus azoricus* (Table 1). This was confirmed by the pairwise evolutionary distance matrix analysis (1.0% evolutionary distance with *S. yangmingensis*, 5.5% with *S. acidocaldarius*, 7.3% with *Sulfolobus shibatae*, 7.4% with *Sulfolobus solfataricus*, 6.3% with *Sulfolobus* sp. strain B6/2, and 4.6% with *S. azoricus*; by comparison, the pairwise evolutionary distances range from 0.2% to 31.7% for all species of the thermoacidophilic archaea to the other representatives of the Crenarchaeota kingdom (not shown), and from 0.2% to 15.7% among the 15 members of the order *Sulfolobales,* shown in Table 1).

The topology of a phylogenetic tree constructed from the evolutionary distances by the neighbor-joining method (Fig. 2) is in good agreement with the results of previously reported phylogenetic analyses on the basis of the 16S rRNA genes (Fuchs et al. 1996; Kurosawa et al. 1998; Jan et al. 1999) and 23S rRNA genes (Trevisanato et al. 1996). The order *Sulfolobales* represents a monophyletic group within the Crenarchaeota kingdom of the Archaea domain in the tree, as indicated by a high bootstrap value of 1,000 on the *Sulfolobales* cluster (Fig. 2). The taxa of the order *Sulfolobales* can be classified into two major clusters, namely, the *Sulfolobus/Stygiolobus* cluster and the *Acidianus/Metallosphaera* cluster. In the phylogenetic tree, the 16S rDNA group of the genus *Sulfolobus* is very broad and phylogenetically incoherent; as pointed out by Fuchs et al. (1996), rearrangement of the order *Sulfolobales* must await further biochemical and molecular biological evidence that justifiably determines the splitting of the genus.

Within the *Sulfolobus/Stygiolobus* cluster, the isolate strain 7^T was clustered with *S. yangmingensis* with a high bootstrap value of 1,000 (Fig. 2). The phylogenetic distance values and the strictly aerobic mode of the growth of strain 7^T are consistent with it belonging to the genus *Sulfolobus*, but not to *S. acidianus*.

DNA–DNA hybridization experiments also showed the low relatedness of strain 7^T with *S. shibatae* strain DSM5389

Table 1. 16S rDNA similarity values and pairwise evolutionary distances of the members of the order *Sulfolobales*

		Species	Evolutionary distance													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Similarity (%)	1	*A. brierleyi*		11.1	9.2	9.2	8.1	8.1	13.6	14.0	11.2	12.9	11.4	12.1	12.3	11.9	12.0
	2	*S. hakonensis*	91.5		3.6	3.4	9.4	9.4	15.7	15.7	13.1	14.7	13.4	13.6	14.2	14.0	14.1
	3	*M. sedula*	91.3	98.3		0.2	8.1	8.0	13.8	14.0	11.6	13.0	11.7	11.9	12.4	11.8	11.9
	4	*M. prunae*	91.4	98.5	99.8		8.0	7.9	13.7	13.9	11.5	12.9	11.6	11.8	12.3	11.7	11.8
	5	*A. ambivalens*	92.4	93.0	92.4	92.4		0.3	12.5	13.2	9.9	10.4	9.1	9.5	10.0	8.5	8.7
	6	*A. infernus*	92.4	93.0	92.4	92.5	99.7		12.2	12.8	9.7	10.2	8.7	9.4	9.8	8.2	8.5
	7	*S. metallicus*	87.6	87.7	87.4	87.5	88.5	88.8		2.0	13.1	14.0	12.0	11.9	12.7	12.1	12.2
	8	*S.* strain LM	87.4	87.8	87.4	87.5	88.1	88.4	98.2		13.2	13.5	12.2	11.9	12.2	12.2	12.3
	9	*S. acidocaldarius*	89.6	89.8	89.3	89.4	90.7	90.9	88.0	88.1		5.9	4.5	5.5	6.1	8.7	8.8
	10	*S.* strain B6/2	88.4	88.7	88.3	88.4	90.6	90.7	87.5	88.1	94.6		2.3	6.3	6.5	9.7	9.8
	11	*S. azoricus*	89.5	89.5	89.2	89.3	91.5	91.8	89.0	89.0	95.7	98.0		4.6	5.4	8.0	8.1
	12	*S. tokodaii* strain 7	88.9	89.4	89.0	89.1	91.1	91.2	89.0	89.2	94.7	94.2	95.6		1.0	7.3	7.4
	13	*S. yangmingensis*	88.6	88.9	88.6	88.6	90.7	90.8	88.3	89.0	94.1	94.0	94.8	99.0		8.1	8.2
	14	*S. shibatae*	89.0	89.0	89.1	89.2	92.0	92.3	88.9	89.0	91.8	91.1	92.4	93.1	92.4		0.2
	15	*S. solfataricus*	89.0	89.0	89.0	89.1	91.8	92.0	88.8	88.9	91.7	91.1	92.4	93.0	92.3	99.8	

The values on the lower left show pairwise percent similarity of 1,242 nucleotide regions of 16S rRNA genes, and the values on the upper right show pairwise evolutionary distance calculated by using the Kimura 2-parameter model that weights transitions and transversions at 2:1 (Kimura, 1980)

A., Acidianus; *S.*, *Sulfolobus*, except *S. azoricus* = *Stygiolobus azoricus*; *M.*, *Metallosphaera*

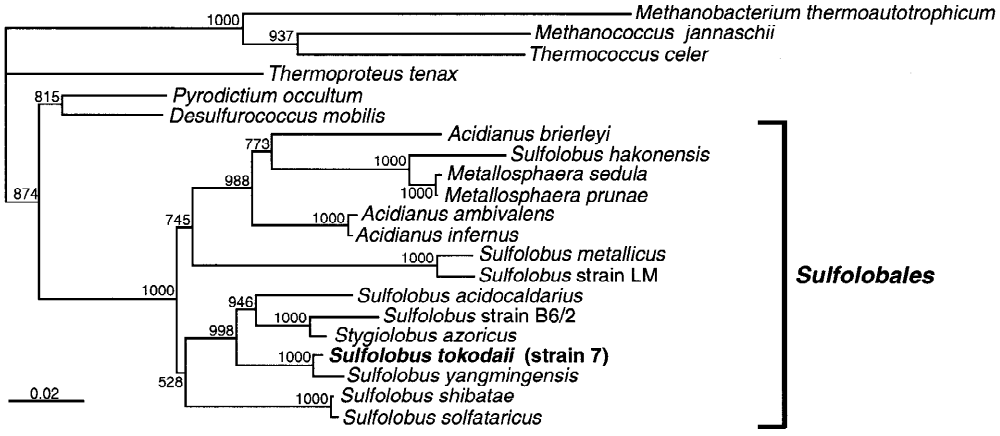

Fig. 2. Phylogenetic tree for the *Sulfolobales*. The tree is derived from the multiple nucleotide sequence alignments of the 16S rRNA genes of the representatives of selected archaea including *S. tokodaii* strain 7^T (JCM 10545) by the neighbor-joining method. The corresponding genes from the euryarchaeotes *Thermococcus celer* and *Methanobacterium thermoautotrophicum* are used as the outgroups in the phylogenetic analysis. *Numbers* on selected nodes indicate the bootstrap values

(Table 2). Higher relatedness was observed between strain 7^T and *S. yangmingensis* strain YM1^T, the closest relative in the phylogenetic tree (Fig. 2) and in the pairwise evolutionary distance matrices (Table 1). However, the pairwise percent DNA–DNA homologies among the three relatives, i.e., strain 7^T, *S. yangmingensis* strain YM1^T, and *S. ohwakuensis* strain TA-1^T, have similar values, all of which are lower than 70% (see Wayne et al. 1987) (Table 2). In addition, strain TA-1^T has tentatively been classified as a member of the genus *Sulfurisphaera* on the basis of its facultatively anaerobic growth in the presence of H_2/CO_2 of 90 : 10 (Kurosawa et al. 1998), which did not occur with strain 7^T or *S. yangmingensis* strain YM1^T (Jan et al. 1999). Although rearrangement of the order *Sulfolobales* has been the subject of debate (Zillig 1993; Trevisanato et al. 1996; Kurosawa et al. 1998) and splitting of the genus should await further strong and reliable biochemical support, which is presently unavailable (Fuchs et al. 1996), the molecular phylogenetic properties of strain 7^T when compared with those of other reported species of *Sulfolobus* distinguish it as a novel species of the genus *Sulfolobus* (Tables 1 and 2 ; Fig. 2).

In conclusion, its phenotypic characterization, the phylogenetic analysis of the 16S rRNA gene (Table 1 and Fig. 2), and the DNA–DNA homologies (Table 2) indicate that strain 7^T, isolated in the early 1980s from the Beppu Hot Springs, Kyushu, Japan, by Oshima and colleagues (Inatomi et al. 1983), is related to the described *Sulfolobus* taxon and should be considered a novel species of the genus. We name this novel species *Sulfolobus tokodaii* (tou.kou.dye. M. L. gen. adj. *tokodaii*, pertaining to the Japanese name of the Tokyo Institute of Technology, where the crenarchaeote was initially characterized). The type strain is strain 7^T (JCM 10545). This primary conclusion clarifies its position to prevent further confusion in the literature (e.g., Schäfer et al. 1994).

Table 2. Percentage DNA homology as determined by DNA–DNA hybridization

Source of filter-bound DNA	Percentage (means ± SD) of labeled probe DNA associated with filter-bound DNA	
	strain 7^T	*Sulfurisphaera ohwakuensis* strain TA-1^T
Strain 7^T	100	45 ± 8
Sulfolobus yangmingensis strain YM1^T	61 ± 7	61 ± 1
Sulfurisphaera ohwakuensis strain TA-1^T	46 ± 7	100
Sulfolobus shibatae DSM5389	8 ± 1	7 ± 3
Escherichia coli strain DH5α	<1	<1

Description of *Sulfolobus tokodaii* sp. nov.

Sulfolobus tokodaii (tou.kou.dye. M. L. gen. adj. *tokodaii,* pertaining to the Japanese name of the Tokyo Institute of Technology, where this isolate was initially characterized). Cells are irregular cocci and variable in size (usually 0.5–0.8 μm in diameter) and have some flattened and uneven surfaces. The cell envelope consists of a hexagonal *S*-layer lattice (spacing approximately 20 nm). Colonies are pale tan, translucent, smooth, and convex, with entire margins.

Cells grow chemoheterotrophically on a modified Brock's basal salts mixture supplied with trypton, yeast extract, casaminoic acid, or amino acids under obligatory aerobic conditions. Best growth occurs on a modified Brock's basal salts mixture supplied with yeast extract (1 g/l), casaminoic acid (1 g/l), and glucose (1 g/l). Supplements of elemental sulfur are not required for the chemoheterotrophic growth. Poor growth occurs under facultatively chemolithotrophic conditions in the presence of elemental sulfur when supplied with alanine (10 mg/l) or polypeptone (0.1 g/l). No growth occurs in minimal medium amended with a H_2/CO_2 (90 : 10) headspace. Growth occurs at 70° to 85°C; optimal growth is at 80°C under aerobic and chemoheterotrophic growth conditions. No growth was detected at 65° and 90°C. Growth occurs at pH 2 to 5; optimal growth is at pH 2.5–3. No growth was detected at pH 1 or pH 6. It is an obligately aerobic and facultatively chemoheterotrophic thermoacidophile that grows by aerobic respiration rather than by simple fermentation. Cells contain membrane-bound cytochromes b_{562}, a_{583}, and aa_3, with the α bands at 562 nm, 583 nm, and 603 nm, respec-

tively, as constituents of its aerobic respiratory terminal oxidase supercomplex and a soluble zinc-containing ferredoxin as a major cytosolic electron transport protein. No *c*- and *d*-type cytochromes are detected spectrophotometrically.

The complete genomic DNA sequence of *Sulfolobus tokodaii* strain 7^T has been analyzed by Kawarabayasi et al. (2001). The G + C content of the genomic DNA is 32.8 mol%, as calculated from its whole genomic DNA sequence. The phylogenetic analysis of the 16S rRNA gene sequence and the DNA–DNA homologies with similar species distinguish it as a new species belonging to the genus *Sulfolobus*.

The type strain is *Sulfolobus tokodaii* sp. strain 7^T, which was isolated from the Beppu Hot Springs in the continental acidic geothermal area of Kyushu Island, Japan, and it has been deposited in the Japan Collection of Microorganisms as strain JCM 10545.

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