A novel species of thermoacidophilic archaeon, *Sulfolobus yangmingensis* sp. nov.

Ren-Long Jan,¹ Jeffrey Wu,² Shu-Miaw Chaw,³ Chien-Wei Tsai¹ and Suh-Der Tsen¹

Author for correspondence: Suh-Der Tsen. Tel: +886 2 28267108. Fax: +886 2 28212880.
e-mail: nymutsen@ym.edu.tw

Institute of Microbiology and Immunology, National Yang-Ming University, Shih-Pai¹, Taipei American School² and Institute of Botany, Academia Sinica³, Taipei, Taiwan, ROC

A novel microbe was isolated from a geothermal vent in Yang-Ming National Park in northern Taiwan. This spherical microbe with mean cell diameter of $1.1 \pm 0.2 \mu\text{m}$ is a facultatively chemolithoautotrophic archaeon that grows on elemental sulfur and reduced sulfur compounds. The optimal pH and temperature for growth are 4.0 (pH range 2.0–6.0) and 80 °C (temperature range 65–95 °C). Its membranes contain the lipids calditoglycerocaldarchaeol and caldarchaeol, which are common to other members of the *Sulfolobaceae*. Like *Sulfolobus acidocaldarius*, *Sulfolobus shibatae* and *Sulfolobus solfataricus*, the new isolate utilizes sugars and amino acids effectively as sole carbon sources. The G+C content of the genomic DNA was 42 mol%. DNA of the isolate hybridized weakly to the DNA of other *Sulfolobus* species. Phylogenetic analysis of the 16S rRNA indicated that the new isolate represents a deep branch within the genus *Sulfolobus*. On the basis of these properties, the new isolate appears to represent a new species of *Sulfolobus*, for which the name *Sulfolobus yangmingensis* sp. nov. is proposed. The type strain is strain YM1^T.

Keywords: thermoacidophile, archaeon, *Sulfolobus*

INTRODUCTION

Members of the genus *Sulfolobus*, the first reported genus of the family *Sulfolobaceae* (Brock *et al.*, 1972; Segerer *et al.*, 1986), are characterized by aerobic growth at high temperatures and low pH in the presence of elemental sulfur. Members of the genus are usually found in habitats such as acidic hot springs and mud holes that contain elemental sulfur. Among the described species of *Sulfolobus*, *Sulfolobus acidocaldarius*, originally discovered in Yellowstone National Park, USA, and *Sulfolobus solfataricus*, originally discovered in Pisciarelli, Italy, have been used widely in research. Both species are distributed worldwide. *Sulfolobus hakonensis* was isolated in Hakone, Japan (Takayanagi *et al.*, 1996).

Taiwan has many active geothermal vents. A new microbial strain was isolated from an acidic, muddy, hot spring in Longfong valley at the southern Yang-

Ming National Park in northern Taiwan. The temperature of this hot spring can reach 105 °C and the pH is as low as 2. In this report, we have characterized this novel strain in terms of morphology, nutritional requirements, DNA homology with similar species and phylogenetic analysis of its 16S rRNA sequence.

METHODS

Microbial strains. *S. acidocaldarius* DSM 639^T, *S. hakonensis* DSM 7519^T, *Sulfolobus shibatae* DSM 5389^T and *S. solfataricus* DSM 1616^T were obtained from the DSMZ, Braunschweig, Germany.

Initial isolation. Muddy water was collected from a geothermal vent in Longfong valley, stored in sterile 50 ml centrifuge tubes and brought back to the laboratory. The temperature of the gas bubbling from the vent was 105 °C. The temperature of the surrounding water ranged from 70 to 100 °C. The vent water was at pH 2.2 and contained the following ions and minerals (in mg l⁻¹): Na⁺, 44; K⁺, 7.5; Ca²⁺, 111; Mg²⁺, 34; Fe²⁺, 14.5; Al³⁺, 60; Cl⁻, 342; SO₄²⁻, 1325; SiO₂, 198 (Chen, 1989). Vent water was supplemented with 0.1% yeast extract and 0.1% glucose and then autoclaved. The pH was around 3.0 and was not adjusted further. A fresh sample (0.5 ml) from the geothermal vent was inoculated into 35 ml sterilized, supplemented vent

Abbreviations: MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank accession number for the 16S rDNA sequence of strain YM1^T is AB010957.

water in a 125 ml Erlenmeyer flask and incubated at 70 °C with shaking (200 r.p.m.). As the cultures became turbid (OD_{520} greater than 0.3 after about 3 d), 0.1 ml culture suspension was diluted 100-fold with sterile water. An aliquot (0.1 ml) of the liquid was then mixed with 30 ml modified Allen's medium containing Gelrite (0.4%) and poured into a Petri dish in order to isolate individual colonies (Brock *et al.*, 1972). After incubation at 70 °C for 2 weeks, large colonies appeared both in the gel and on the surface. They were isolated and subcultured.

Microbial growth and growth media. In order to examine cell growth, the isolate was inoculated in modified Allen's medium (pH 4.0), prepared according to Brock *et al.* (1972) under aerobic conditions. In order to determine the optimal temperature for cell growth, the inoculum was incubated at various temperatures from 60 to 95 °C in 5 °C increments. The cell density of the growing culture was measured every 4 h by monitoring the OD_{520} using a spectrophotometer (model DU 7000; Beckman). In order to examine the optimal pH for growth, the new isolate was inoculated in modified Allen's medium with different pH values ranging from 1.0 to 7.0 at 1.0 increments and then incubated at 80 °C with shaking. The pH was adjusted using either 1 M HCl or 1 M NaOH. The growth of cells was measured as described above.

Nutritional requirements. In order to examine the ability of the new isolate to utilize sulfur compounds, sugars and amino acids, cells growing heterotrophically on yeast extract and glucose were harvested by centrifugation (6000 r.p.m. for 15 min at room temperature). Cell pellets were washed twice with sterile distilled water in order to avoid carrying over residual nutrients. Cells were then cultivated in modified Allen's medium without glucose and yeast extract, but in the presence of each sugar or amino acid at a concentration of 0.1%. In order to test for autotrophic growth on sulfur-containing inorganic compounds, washed cells were placed in flasks containing modified Allen's medium without any organic compounds, but with specific sulfur compounds in suspension (0.1% w/v). The flasks were streamed with compressed air containing 5% (v/v) CO_2 in order to provide inorganic carbon and oxygen. The growth criteria are described in legend of Table 1. The criteria for autotrophic growth were based on both the increase in cell number and acid production.

Microscopy. Growth was observed by phase-contrast microscopy (Nikon Fluophot) with an oil-immersion objective lens and by fluorescence microscopy (Olympus BX50). For fluorescence microscopy, samples were stained with Hoechst 33258 dye (Molecular Probes) and embedded in low-melting-point agarose. Samples for scanning electron microscopy and negative staining were prepared by the methods of Kellenberger *et al.* (1958) and Kurr *et al.* (1991).

Determination of the DNA G + C content. DNA of the new strain was isolated according to the method of Silhavy *et al.* (1984). Its melting temperature was measured using the method of Marmur & Doty (1962) with slight modifications. The following procedure was used: 50 µg DNA was dissolved in the solution of 0.15 M NaCl and 0.015 M sodium citrate (pH 7.0). This DNA solution was then placed in a cuvette and heated from 60 to 95 °C in increments of 2 °C. At each temperature, the A_{260} was measured using a spectrophotometer (Hitachi U-3410). The G + C content was computed using the formula $T_m = 69.3 + 0.41 \times (G + C)$. For comparison and accuracy, the T_m of DNA from *Escherichia coli* and *S. hakonensis* were determined by the

same method to be 90 and 84.7 °C, respectively. These values are consistent with measurements reported previously.

Lipid analysis. Total lipids were extracted by the methods of Takayanagi *et al.* (1996). Lipids were then degraded by acid methanolysis as described by Furuya *et al.* (1980) and separated by TLC on silica gel plates (type HPTLC, catalogue no. 1.05631; Merck). The first solvent system was chloroform/methanol (4:1). After the solvent front had ascended 3 cm, the plate was taken out and dried. The second solvent system was hexane/diethyl ether/acetic acid (30:20:1). The plate was taken out when the front had reached the top of the plate. The spots were visualized by spraying the TLC plates with 50% (v/v) sulfuric acid and then heating them to 150 °C for 5 min.

DNA-DNA hybridization. DNA was isolated according to the method of Silhavy *et al.* (1984), sonicated and denatured by heating in a 95 °C dry bath. DNA was labelled with [α - ^{32}P]dCTP by using the Rediprime DNA labelling system (Amersham) and was then mixed with unlabelled DNA (a total of 200 ng DNA) for use as a probe. Probes were adjusted to $1-2 \times 10^6$ c.p.m. ml⁻¹ hybridization buffer. Six micrograms of single-stranded, unlabelled DNA was immobilized on nitrocellulose paper (grade BA85, pore size 0.45 µm, diameter 25 mm; Schleicher & Schuell) by using a UV cross-linker. DNA-DNA hybridization experiments were performed following the instructions described in the ExpressHyb hybridization solution user's manual (Clontech). Hybridization was performed at 68 °C for 1 h. Radioactivity that remained bound to the filter was then counted.

16S rRNA sequencing. In order to avoid amplifying pseudogenes (or non-functional genes) (Chaw *et al.*, 1995), total RNA was extracted from fresh cells using the modified method of Raha *et al.* (1990), in which genomic DNA was removed by DNase I treatment (Boehringer Mannheim). The method of Goodman & MacDonald (1979) was then used to synthesize first-strand cDNA with the primer R1 (5'-GAGGTGATCCAGCCGAGG-3') to prime the 3' end (Takayanagi *et al.*, 1996) and AMV reverse transcriptase (Promega). The reverse transcription reaction contained (µl⁻¹): 20 ng RNA template, 0.5 U AMV reverse transcriptase, 1 pmol primer, 5 nmol $MgCl_2$ and 1 nmol dNTPs. The reaction was incubated at 42 °C for 30 min. Three independent cDNA products were synthesized and used as templates for the PCR. PCR amplification followed the method of Chaw *et al.* (1995) except that primers F1 (5'-TCCGGTTGATCCTGCCGGA-3'; Takayanagi *et al.*, 1996) and R1 were used. The PCR products were purified by using the GENEIII Kit (Bio 101) and subcloned into pGEM-T Easy vector (Promega). One or more clones from each of the three independent PCRs were sequenced on a PE Applied Biosystems ABI 373 DNA sequencer. The protocol and reagent kit recommended by PE Applied Biosystems were utilized. In addition to the commercial T7 and SP6 primers, which are specific to the cloning site of the vector, the following internal primers were used for sequencing: 5'-GTGTCAGCCGCCGCGGTAATAC-3', 5'-GTATTACCGCGGCGGCTGACAC-3' (for reverse strand), 5'-GCGGAGAGGAGGTGCATG-3' and 5'-GACGGCCATGCACCTCTC-3' (for reverse strand). They are located at positions 515-536, 536-515, 1039-1056 and 1062-1045, respectively (based on the 16S rRNA sequence of *E. coli*).

Phylogenetic analysis. To date, 14 archaeal 16S rRNA gene sequences are available in GenBank. Their accession numbers are: strain YM1^T, AB010957; *Sulfolobus thur-*

Table 1. Utilization of sulfur compounds, sugars, amino acids and yeast extract for growth

Microbial strains were incubated at 80 °C for 1 week. To test for autotrophic growth, compressed air with 5% CO₂ was streamed into flasks (details described in Methods). All chemicals tested were at concentrations of 0.1 %. The amount of growth is expressed as the ratio of the OD₅₂₀ after 1 week of incubation to the OD₅₂₀ before incubation (which was adjusted to 0.05), as follows: —, ratio 1 or less; ±, ratio between 1 and 2; +, ratio greater than 2 (positive growth usually gave a ratio greater than 5). Data were taken from De Rosa *et al.* (1975) and Takayanagi *et al.* (1996) (*S. acidocaldarius*, *S. solfataricus* and *S. hakonensis*), Grogan *et al.* (1990) (*S. shibatae*) and Huber & Stetter (1991) (*S. metallicus* and amino acid utilization data). NA, Data not available.

Nutrient source	Strain YM1 ^T	<i>S. acidocaldarius</i> DSM 639 ^T	<i>S. solfataricus</i> DSM 1616 ^T	<i>S. shibatae</i> DSM 5389 ^T	<i>S. hakonensis</i> DSM 7519 ^T	<i>S. metallicus</i> DSM 6482 ^T
Sulfur compounds:						
Elemental S	+	+	+	+	+	+
FeS	+	+	+	NA	+	—
Tetrathionate	+	+	+	NA	+	NA
H ₂ S	NA	+	+	NA	+	NA
Na ₂ S	—	NA	NA	NA	NA	NA
Sugars:						
D-Arabinose	+	±	±	+	—	—
D-Ribose	±	—	±	NA	—	—
D-Xylose	+	—	±	NA	—	—
D-Fructose	+	±	±	NA	—	—
D-Galactose	+	—	+	+	—	—
D-Glucose	+	+	+	+	—	—
D-Mannose	+	+	+	+	—	—
L-Rhamnose	+	—	—	NA	—	NA
Lactose	+	—	+	+	—	—
Maltose	+	+	+	+	±	NA
Sucrose	+	+	+	+	—	—
Raffinose	+	+	+	+	—	—
Sorbitol	+	NA	NA	NA	NA	NA
Cellobiose	+	NA	NA	NA	NA	NA
Trehalose	+	NA	NA	NA	NA	NA
Amino acids:*						
L-Aspartic acid	+	±	±	NA	—	NA
L-Glutamic acid	+	+	±	NA	±	—
L-Tryptophan	+	+	—	NA	±	NA
Yeast extract	+	+	+	+	+	—

* Utilization of all 20 common amino acids by strain YM1^T was tested; all were used except cysteine.

ingiensis, X90485; *S. acidocaldarius*, D14876; *S. solfataricus*, D26490; *S. shibatae*, M32504; *Sulfolobus* strain LM, U40813; *Sulfolobus* strain B6, U38360; *S. hakonensis*, D14052; *Desulfurococcus mobilis*, M36474; *Pyrodictium occultum*, M21807; *Thermophilum pedens*, X14835; *Thermoproteus tenax*, M35966; *Thermococcus celer*, M21529; and *Methanobacterium formicicum*, M36508. We did not include the sequence of *Sulfolobus metallicus* (accession number X90479) in this study because there are still many undetermined sites. These sequences were aligned using the PILEUP and LINEUP programs of the Genetics Computer Group sequence analysis software.

Excluding the PCR primer positions, the length of the aligned sequences was 1445 bp (positions 40–1444), including gaps. After eliminating three regions where alignment was difficult (positions 178–198, 1111–1112 and 1390–1406), 1405 positions were available for comparison. Of these, 794 characters were constant, 227 variable characters were parsimony-uninformative and 384 were parsimony-informa-

tive. The aligned sequence data (not shown) were analysed using distance and parsimony methods embodied in the software PAUP*4.0 (D. Swofford, personal communication). When using the distance method, pairwise distances were first determined by the two-parameter model of Kimura (1980). This distance matrix was then used to reconstruct a phylogenetic tree by the neighbour-joining (NJ) method (Saitou & Nei, 1987).

The maximum-parsimony (MP) trees were derived by the heuristic search with the tree-bisection-reconnection branch-swapping algorithm and MULPARS option. These searches were run on Power Macintosh 9600/233 computers. *Thermococcus celer* and *Methanobacterium formicicum* were used as outgroups as both belong to orders other than the *Sulfolobales*. To obtain an estimate of the strength of support for the topology of the resultant NJ and MP trees, the bootstrap method (Felsenstein, 1985) with the heuristic search was also applied to the two tree-reconstruction methods. In both trees, 1000 replicates were conducted.

RESULTS AND DISCUSSION

Growth

Cells of strain YM1^T formed large colonies on the solid Gelrite surface. Since cells from a single colony were quite uniform in morphology when examined under a phase-contrast microscope, this colony appeared to be pure and was designated YM1^T.

Although strain YM1^T grew slowly in autoclaved water from the geothermal vent, it grew well in modified Allen's medium supplemented with glucose and yeast extract under aerobic conditions at low pH and high temperature (Fig. 1). Strain YM1^T did not grow in the same medium when supplied with an H₂/CO₂ gas mixture. Nor did it grow anaerobically when sulfur was added to the Allen's medium. However, when elemental sulfur, FeS or tetrathionate was supplied to the Allen's medium, strain YM1^T grew well aerobically. Thus, strain YM1^T seemed to be able to grow chemolithoautotrophically by oxidizing sulfur.

Fig. 1. The growth rate of strain YM1^T in relation to medium temperature and pH. The optimal growth temperature is 80 °C and optimal pH is 4.

Fig. 2. Electron micrographs of strain YM1^T. (a) Scanning electron micrograph of strain YM1^T. The arrowhead denotes a surface protrusion, which are often present. (b) Transmission electron micrograph of a fixed whole-mount cell negatively stained with uranyl acetate. Bars, 1 µm.

Strain YM1^T grew at temperatures from 65 to 90 °C and at pH values between 2.5 and 6.0 (Fig. 1). The optimal temperature and pH for growth were 80 °C and pH 4.0. This indicated that the new isolate was an acidophilic thermophile.

Morphology

Under the phase-contrast microscope, YM1^T cells did not move actively. In actively growing cell cultures, most cells had a dark cytoplasm without granules. When cells were in the stationary phase, the cytoplasm in many cells became transparent and irregularly shaped black granules appeared. While conducting an ultrastructural study of *S. solfataricus*, Millonig *et al.* (1975) discovered that old cultures had many dead cell ghosts with transparent cytoplasm and coagulated cytoplasmic granules. Since we observed many apparently similar cells in freshly sampled hot-spring water, we wondered whether these were dead cells. By using the DNA dye Hoechst 33258 to stain the cells, we observed that the black granules became fluorescent under UV illumination. Presumably, the black

Table 2. Morphological, physiological and biophysical properties of strain YM1^T and other *Sulfolobus* strains

Data were taken from De Rosa *et al.* (1975) and Takayanagi *et al.* (1996) (*S. acidocaldarius*, *S. solfataricus* and *S. hakonensis*), Grogan *et al.* (1990) (*S. shibatae*) and Huber & Stetter (1991) (*S. metallicus*). NA, Data not available.

Characteristic	Strain YM1 ^T	<i>S. acidocaldarius</i> DSM 639 ^T	<i>S. solfataricus</i> DSM 1616 ^T	<i>S. shibatae</i> DSM 5389 ^T	<i>S. hakonensis</i> DSM 7519 ^T	<i>S. metallicus</i> DSM 6482 ^T
Colony morphology:						
Colour	Dark-yellow	Dark-yellow	Greyish	Pale-tan	Dark-yellow	NA
Shape	Smooth, flat	Smooth, flat	Smooth, flat	NA	Smooth, flat	NA
Diameter (mm)	1–2	0.5–1.0	0.5–1.0	NA	0.5–1.0	NA
Cell shape	Lobed	Lobed	Lobed	NA	Lobed	Irregular
Cell diameter (µm)	0.8–1.5	0.8–1.0	0.8–2.0	0.7–1.5	0.9–1.1	1.5
Flagella	—	—	—	NA	NA	NA
Motility	—	—	—	Weak	NA	NA
Cell wall	NA	+	+	+	+	+
Temperature for growth (°C):						
Range	65–90	55–85	55–85	NA	50–80	50–75
Optimum	80	70	70	81	70	65
pH for growth:						
Range	2.0–6.0	2.0–4.0	2.0–4.0	NA	1.0–4.0	1.0–3.5
Optimum	4.0	2.5	4.5	3.0	3.0	NA
G + C content (mol %)	42 ± 1.5*	38.2 ± 1.5	36†	34.6	38.4 ± 1.5	37

* Mean ± SD from three experiments.

† Value taken from Zillig *et al.* (1980).

granules were condensed nucleic acids and the transparent cells were quiescent or dead cells.

Scanning electron microscope images revealed that cells of strain YM1^T were spherical or irregularly lobed polyhedrons with diameters of 1.1 ± 0.2 µm (Fig. 2). There were lobes or protrusions present on the surface. No pili, flagella or fimbriae were observed by negative staining (Fig. 2b). Such structures have not been observed in other *Sulfolobus* species (Millonig *et al.*, 1975).

Lipid analysis

The membranes of thermoacidophilic archaea are monolayers based on two core lipids. Both are cyclic tetraethers, in which two polyols are linked through two C₄₀ isoprenoid chains (De Rosa *et al.*, 1980). The first type, caldarchaeol, has two glycerol moieties as its hydrophilic portion. The second type, calditoglycerocaldarchaeol, has glycerol and calditol as its hydrophilic portion. An analysis of the lipid core of strain YM1^T and four other *Sulfolobus* species by TLC revealed that their patterns and content were identical. Two major spots were prominent in the chromatograms of all strains. The slower-migrating spot corresponded to calditoglycerocaldarchaeol and the faster-migrating spot corresponded to caldarchaeol. Mixing lipid cores of strain YM1^T with those of *S. hakonensis* yielded exactly the same pattern, demonstrating the identity of their lipid cores. Since calditoglycerocald-

archaeol is found only in members of the *Sulfolobales* (De Rosa *et al.*, 1983; De Rosa & Gambacorta, 1988; Lo *et al.*, 1989), strain YM1^T might properly be classified in the same taxon.

The characteristics of strain YM1^T, including its cellular morphology, its thermoacidophilic mode of life, its aerobic chemolithoautotrophic growth by utilizing sulfur and its lipid core contents, suggest that strain YM1^T belongs to the genus *Sulfolobus*.

DNA analysis

The melting temperature of DNA of strain YM1^T was 86.4 °C. The G + C content of strain YM1^T was determined to be 42 ± 1.5 mol %. This is slightly higher than the reported G + C contents of five other *Sulfolobus* species (34.6 – 38.4 ± 1.5 mol %; Table 2). However, this value is still distinct from the G + C contents of other genera in the family *Sulfolobaceae*, such as those of *Acidianus* (30–33 mol %) and *Metallosphaera* (45–47 mol %) (Huber *et al.*, 1989).

Comparison of nutrient utilization

Table 1 shows a comparison of utilization of different nutrients by strain YM1^T and the five *Sulfolobus* species for which data are available. Although these species resemble each other in morphology, in their affinities for hot and acidic niches and in their genomic G + C contents, their nutritional requirements differ.

Table 3. Percentage DNA homology as determined by DNA–DNA hybridizationValues are means $\pm$ SD from three experiments.

Source of filter-bound DNA	Percentage of labelled probe DNA associated with filter-bound DNA	
	Strain YM1 ^T	<i>S. hakonensis</i> DSM 7519 ^T
Strain YM1 ^T	100	6 $\pm$ 3
<i>S. acidocaldarius</i> DSM 639 ^T	16 $\pm$ 6	9 $\pm$ 5
<i>S. solfataricus</i> DSM 1616 ^T	20 $\pm$ 5	9 $\pm$ 4
<i>S. shibatae</i> DSM 5389 ^T	6 $\pm$ 2	5 $\pm$ 2
<i>S. hakonensis</i> DSM 7519 ^T	4 $\pm$ 3	100
<i>E. coli</i>	< 1	< 1

Fig. 3. Phylogenetic tree inferred from the 16S rRNA sequence data. The NJ and MP methods gave almost the same topology. The arrow indicates the node that was not present in one of the two equally most-parsimonious trees (see text for details). Branch lengths were computed by the NJ method; the bar represents one substitution per 100 nucleotides. The upper number at each node denotes the proportions of bootstrap replicates that supported the monophyly of the taxa in the subset designated by the node. The lower italicized numbers at each node refer to the number of substitutions inferred on the two equally most-parsimonious trees.

Strain YM1^T is quite versatile in using a wide range of sugars and amino acids for growth. This makes it closer to *S. acidocaldarius* and *S. solfataricus* than to *S. hakonensis*, which cannot grow on many sugars. We found that neither of the former two species, however, were able to utilize L-rhamnose, while strain YM1^T utilized this sugar.

Of the twenty amino acids tested, only cysteine was not utilized by strain YM1^T. On the basis of its different abilities to utilize sulfur compounds, sugars and amino acids (Table 1), strain YM1^T is readily distinguishable

from other *Sulfolobus* species. Furthermore, as indicated by the low hybridization rate compared with other *Sulfolobus* species (Table 3), strain YM1^T is clearly a distinct species within the genus *Sulfolobus*.

Phylogenetic analysis

The nucleotide sequences of 16S rRNA from 14 thermophilic archaea were compared using the LINEUP and PILEUP programs of the GCG package. A similarity matrix was then constructed by using the

program PAUP*4.0. The 16S rRNA sequence of strain YM1^T was most similar to the sequences of *S. acidocaldarius* and *S. thuringiensis* and differed from them by 6.95 and 6.96%, respectively. The sequences of the latter two species are exactly the same after elimination of the gaps. After these two species, the next three most similar sequences were from *S. solfataricus*, *S. shibatae* and *Sulfolobus* strain B6. Within the genus *Sulfolobus*, the highest divergence, which occurs between *Sulfolobus* strain B6 and *S. hakonensis*, was 15.46%. Fig. 3 shows the phylogenetic tree inferred by the NJ and MP methods. The numbers above each node denote the bootstrap values (Felsenstein, 1985). Our heuristic search for the MP trees resulted in two equally most-parsimonious trees of 1308 substitutions or steps. One was identical to the NJ tree (Fig. 3) in its branching pattern; the other (not shown) differed from the NJ tree in the position of the clade consisting of *S. solfataricus* and *S. shibatae*. These taxa formed a sister group with the clade consisting of *Sulfolobus* strain LM and *S. hakonensis*. As can be seen from Fig. 3, there is fairly strong evidence to support the clade consisting of strain YM1^T–*S. thuringiensis*–*S. acidocaldarius*–*Sulfolobus* strain B6, as 42 (or 33) substitutions (numbers beneath the branch) separated these taxa from the rest of *Sulfolobus*.

There is even stronger evidence for the distinctness of strain YM1^T, as 30 (or 36) substitutions separated it from the other three *Sulfolobus* species. Indeed, regardless of whether the phylogenetic tree was constructed by the distance or parsimony method, the genus *Sulfolobus* constituted a monophyletic group and strain YM1^T was nested within the genus and also formed a monophyletic group with *Sulfolobus* strain B6, *S. thuringiensis* and *S. acidocaldarius*. In addition, these monophylyies were well supported by 100% of bootstrap replicates.

In conclusion, lipid analysis and 16S rRNA sequencing clearly indicate that strain YM1^T is related to the described *Sulfolobus* taxon and should be considered as a member of the genus. Furthermore, its remarkable physiological properties, G+C content, DNA homology and phylogenetic position distinguish it as a new species.

Description of *Sulfolobus yangmingensis* sp. nov.

Sulfolobus yangmingensis (yang.ming.en'sis. M.L. adj. *yangmingensis* pertaining to the Yang-Ming National Park, Taiwan, Republic of China, from where the organism was isolated).

Sulfolobus yangmingensis is a lobed, spherical, thermoacidophilic archaeon with a cell diameter of $1.1 \pm 0.2 \mu\text{m}$. The optimal pH and temperature for growth are 4.0 (range 2.0–6.0) and 80 °C (range 65–95 °C). It is versatile in its nutritional requirements. The G+C content of the genomic DNA is 42 mol%. The major cellular lipids are calditoglycerocald-archaeol and caldarchaeol.

The level of DNA homology and the computer analysis of 16S rRNA sequence distinguish it as a new species. The type strain of *Sulfolobus yangmingensis* is YM1^T, which was isolated from an acidic, muddy hot spring.

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