Caldisphaera lagunensis gen. nov., sp. nov., a novel thermoacidophilic crenarchaeote isolated from a hot spring at Mt Maquiling, Philippines

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Four novel, thermoacidophilic, crenarchaeotic cocci that grew anaerobically and heterotrophically were isolated from an acidic hot spring in the Philippines; two representative strains were characterized in detail. Most cells were regular cocci, 0.8–1.1 μm in width, which occurred singly or in pairs. They were non-motile and grew at 45–80 °C (optimum 70–75 °C) and pH 2.3–5.4 (optimum 3.5–4.0). They utilized starch, glycogen, gelatin, beef extract, yeast extract and peptone as carbon and energy sources. Growth was stimulated by the presence of sulfur as an electron acceptor. The lipid fraction contained cyclic and acyclic tetraether core lipids. The DNA G+C content was 31 mol%; phylogenetic analysis based on 16S rDNA sequences showed that the novel cocci represent an independent lineage in the phylum Crenarchaeota, distantly related to Acidilobus aceticus and an allied strain, NC12. Caldisphaera lagunensis gen. nov., sp. nov. is proposed to accommodate the four strains. The type strain is IC-154T (=JCM 11604T =MCC-UPLB 1331T =ANMR 0165T).

INTRODUCTION

In the domain Archaea, hyperthermophilic and extremely thermophilic archaea are distributed widely over the phyla Crenarchaeota and Euryarchaeota (Stetter, 1998). Until now, all Crenarchaeota species that have been isolated are either hyperthermophilic or extremely thermophilic, although the existence of mesophilic and psychrophilic Crenarchaeota is suggested by culture-independent molecular phylogenetic analyses (Hershberger et al., 1996). The phylum Crenarchaeota currently comprises the orders Sulfolobales, Thermoproteales and Desulfurococcales, which are well-supported by 16S rDNA sequence data and by phenotypic properties, such as cell morphology and lipid composition (Burggraf et al., 1997; Reysenbach, 2001). Members of the order Desulfurococcales are coccoidal or disc-shaped, strictly anaerobic [except for Aeropyrum pernix (Sako et al., 1996)] and neutrophilic or weakly acidophilic, growing optimally at pH 5.5–7.5 (Huber & Stetter, 2001). Two families, Desulfurococccaceae and Pyrodictiaceae, are known in the order Desulfurococcales. On the 16S rDNA-based phylogenetic tree, members of the family Pyrodictiaceae (with optimal growth at ≥100 °C) form a coherent cluster, whereas members of the family Desulfurococccaceae, with optimal growth at 85–95 °C, are more diverse. At present, there are three and seven genera with validly published names, respectively, in these families [i.e. Pyrodictium, Hyperthermus and Pyrolobus in the family Pyrodictiaceae, and Desulfurococcus, Aeropyrum, Ignicoccus, Staphylothermus, Stetteria, Sulfothermus and Thermoplasma in the family Desulfurococccaceae (Huber & Stetter, 2001)]. In addition, the recently described species Acidilobus aceticus (Prokofeva et al., 2000) and crenarchaeote strain NC12 (‘Caldisphaera noboribetii’; Aoshima et al., 1996) seem to be distinctly affiliated to the order Desulfurococcales.

During the course of a search for novel thermophilic archaea from a hot spring named ‘Mud Spring’ on the side of Mt Maquiling, Philippines, we have isolated a number of...
thermophilic Archaea (unpublished data). Among the four rod-shaped isolates, two strains were identified as *Caldivirga maquilingensis*, a member of the family Thermoproteaceae (Itoh et al., 1999). In the present study, we characterize another group of the archaeal isolates, which are cocci distantly related to the genus *Acidilobus* and strain NC12 (based on phylogenetic analysis of 16S rDNA), and propose the name *Caldisphaera lagunensis* gen. nov., sp. nov.

**METHODS**

**Isolation and culture conditions.** Samples of hot spring water, mud and soil, collected from a hot spring called 'Mud Spring' on the side of Mt Maquiling, Laguna, Philippines (Itoh et al., 1999), were incubated in an enrichment medium under the following growth conditions. Gas phase: air, N₂ or H₂/CO₂ (4:1, v/v; 100 kPa); temperature, 70 or 85 °C; pH, 2-5 or 5-0 (adjusted at room temperature). The enrichment medium was composed of *Sulfolobus* medium (Brock et al., 1972) supplemented with yeast extract (Difco) (1-0 g l⁻¹ for aerobes and 0-5 g l⁻¹ for anaerobes) and 10 g sulfur l⁻¹. For the isolation of anaerobic strains in N₂ or H₂/CO₂ (4:1, v/v; 100 kPa), the enrichment medium was further supplemented with 1-0 mg resazurin l⁻¹ and reduced with 0-5 g Na₂S.9H₂O l⁻¹. After 1 week of cultivation, cultures were diluted serially (1:10) and incubated under the same conditions as for the enrichment cultivation. Following the serial dilution–cultivation method (three times), the highest dilution that showed growth was again diluted serially (1:10) and each dilution was divided equally into five tubes/vials and cultivated. This step was repeated for one grown culture at the highest dilution. Finally, one grown culture at the highest dilution was assigned an IC number, as shown in Table 1. Two isolates (IC-154 and IC-163) were cultivated routinely in modified TCD medium (Itoh et al., 1999) in an atmosphere of N₂ at 75 °C.

**Phenotypic and genetic studies.** Morphology, growth characteristics, utilization of carbon sources, possible electron acceptors, antibiotic sensitivity, lipid composition and DNA base composition were determined as described previously (Itoh et al., 1998). Unless otherwise stated, modified TCD medium (Itoh et al., 1999) was used as the basal medium for phenotypic studies, and cultures were incubated at 75 °C in an atmosphere of N₂. For electron microscopy, cells were placed on a collodion-coated grid, shadowed with platinum-palladium and examined with a transmission electron microscope (H-300; Hitachi). To determine the pH range for growth, the pH was adjusted at room temperature; it was almost the same at 75 °C in the presence of 10 mM trisodium citrate as buffer. To test tolerance of O₂, the strains were inoculated into sulfur-free, non-reduced TCD medium in an atmosphere of N₂ that contained various levels of air, and incubated on a reciprocal shaker (80 r.p.m.). Possible electron acceptors were identified by using test medium (Itoh et al., 1998) reduced with 0-5 g Na₂S.9H₂O l⁻¹ in an atmosphere of N₂. Utilization of O₂ as an electron acceptor was examined in the same test medium, but not reduced, in an atmosphere of N₂ that contained 1% O₂. Growth was estimated by fluorescence intensity after treatment with NanoOrange dye (Molecular Probes) following the supplier’s protocol, or by direct counting with a Petroff–Hauser counting chamber (0-02 mm in depth). A good correlation between the two methods was obtained. Metabolic products were detected by using a gas chromatograph (GC-7A, Shimadzu) equipped with a 2 m glass column (FAL-M 25%, 80/100 mesh) and a flame-ionization detector. Likewise, H₂ and CO₂ were detected by GLC with a thermal conductivity detector [column: H₂ with MS-5A, and CO₂ with PoraPak Q (both from Supelco)]. 16S rDNA was amplified with primers A-20F (5'-TCCGTTGATCCTGCGG-3', corresponding to positions 8–24 in the *Escherichia coli* numbering system) and A-1530R (5'-GGAGGTGATCCAGCCGGC-3', positions 1540–1525). Initially, partial 16S rDNA sequences of all isolates were determined by using a sequencing primer, A-520R (5'-GTATTACCGCGGCGGCTG-3', positions 536–519), and almost whole 16S rDNA sequences of the representative strains (IC-154 and IC-163) were determined as described previously (Itoh et al., 1999). The 16S rDNA sequences were first aligned with the CLUSTAL X program (Thompson et al., 1997) and edited manually with the aid of the SSU rRNA database (Van de Peer et al., 2000). Evolutionary distances were calculated after gaps, ambiguous bases and unalignable regions had been eliminated. The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) and was evaluated by bootstrap resampling (Felsenstein, 1985).

**Table 1. Profile of isolated cocci**

<table>
<thead>
<tr>
<th>Isolate(s)</th>
<th>Isolation conditions</th>
<th>Most closely related species</th>
<th>Sequence similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas phase</td>
<td>Temp (°C)</td>
<td>pH</td>
</tr>
<tr>
<td>IC-147</td>
<td>Air</td>
<td>70</td>
<td>2-5</td>
</tr>
<tr>
<td>IC-144, IC-145, IC-148</td>
<td>Air</td>
<td>70</td>
<td>5-0</td>
</tr>
<tr>
<td>IC-151</td>
<td>Air</td>
<td>85</td>
<td>2-5</td>
</tr>
<tr>
<td>IC-146, IC-153</td>
<td>Air</td>
<td>85</td>
<td>5-0</td>
</tr>
<tr>
<td>IC-163, IC-165</td>
<td>N₂</td>
<td>70</td>
<td>5-0</td>
</tr>
<tr>
<td>IC-156</td>
<td>H₂/CO₂</td>
<td>70</td>
<td>5-0</td>
</tr>
<tr>
<td>IC-154†, IC-158</td>
<td>H₂/CO₂</td>
<td>85</td>
<td>2-5</td>
</tr>
<tr>
<td>IC-155, IC-157, IC-160</td>
<td>H₂/CO₂</td>
<td>85</td>
<td>5-0</td>
</tr>
<tr>
<td>IC-159, IC-161</td>
<td>H₂/CO₂</td>
<td>85</td>
<td>5-0</td>
</tr>
</tbody>
</table>

*Also affiliated to *Sulfinariophaga ohwakuensis* and *Sulfolobus tokodaii* with 98-9 and 98-6% sequence similarity, respectively.
†Also affiliated to *Caldoccocus noboribetus* NC12 with 88-4% sequence similarity.
RESULTS AND DISCUSSION

Isolation

Seventeen strains with coccoid cells were obtained and tentatively assigned to one of three crenarchaeotic species, based on partial 16S rDNA sequence similarities, as shown in Table 1. Three Metallosphaera strains were obtained under one set of growth parameters (air, 70 °C, pH 5-0) and ten strains related to Sulfolobus yammingensis, Sulfurisphaera ohwakuensis and Sulfolobus tokodaii were obtained under several sets of growth conditions [i.e. air or H2/CO2 (4:1, v/v; 100 kPa), 70 or 85 °C, pH 2:5 or 5:0]. On the other hand, four strains (IC-154, IC-158, IC-163 and IC-165), isolated under N2 or H2/CO2 (4:1, v/v; 100 kPa), at 70 °C, pH 5-0, showed low similarity values to Acidilobus aceticus (Prokofeva et al., 2000) and strain NC12 [representing ‘Caldococcus noboribetus’ (Aoshima et al., 1996)], suggesting that they represent a novel taxon related to the genus Acidilobus. Of these four isolates, strains IC-154 and IC-163, isolated under H2/CO2 (4:1, v/v; 100 kPa) and N2, respectively, were selected for further characterization.

Morphology and phenotypic characteristics

Cells of strains IC-154T and IC-163 were mostly regular cocci, 0.8–1.1 μm in diameter (Fig. 1). Cells 2.0–2.5 μm in diameter were occasionally observed. They usually occurred singly or in pairs, and sometimes in aggregates of several cells. Of the two strains characterized, only IC-163 had pili attached to its cells (up to three pili). The two strains were non-motile and grew under strictly anaerobic conditions with N2, N2/CO2 (4:1, v/v; 100 kPa) or H2/CO2 (4:1, v/v; 100 kPa) as the gas phase. They also grew in a low-oxygen atmosphere (up to 2% O2), but not in 5% O2 or higher. They grew at 45–80 °C, and at pH 2:3–5:4 in the presence of 10 mM trisodium citrate as buffer. No growth was observed at 40 or 82 °C (at pH 4:0), or at pH 2:0 or 6:0 (at 75 °C). In buffered medium, strain IC-154T grew optimally at 70–78 °C and at pH 3:5–4:0. The doubling time under optimal conditions (75 °C, pH 3:7) was 5 h and, at stationary phase, the cultures contained approximately 1×10^7 cells ml−1. The two strains did not grow under autotrophic conditions: medium with no yeast extract under H2/CO2 (4:1, v/v; 100 kPa). They utilized starch, glycojen, gelatin, beef extract, yeast extract and peptone as carbon and energy sources, but not D-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, mannose, D-ribose, sucrose, D-xylene, acetate, butyrate, citrate, formate, fumarate, lactate, L-malate, propionate, pyruvate, succinate, methanol, formamide, methylamine or trimethylamine (final concentrations: 0.05% for proteinaceous substances, 0.5% for sugars and 0.2% for other compounds). Only strain IC-154T could utilize casamino acids (weakly). Addition of a vitamin mixture (Balch et al., 1979) to the medium weakly promoted growth of the two strains. Both strains tolerated up to 1.5% NaCl in the growth medium (no growth occurred at 1.75% NaCl). Growth was significantly enhanced by the presence of sulfur as an electron acceptor in the medium. Likewise, growth was slightly promoted by the presence of fumarate, sulfate (only for IC-163) and O2 (1%, only for IC-154T), but not by thiosulfate, cystine, oxidized glutathione, malate, nitrate or FeCl3. Hydrogen sulfide was detected from cultures grown with sulfur. Acetate, propionate, isobutyrate, butyrate and isovalerate, as well as H2 and CO2, were detected as metabolic products from cultures grown with or without sulfur. Both strains were sensitive to erythromycin, novobiocin and rifampicin, but resistant to ampicillin, chloramphenicol, kanamycin, oleandomycin, streptomycin and vancomycin (100 μg ml−1). The core lipid fractions contained cyclic and acyclic tetraethers.

DNA base composition and 16S rDNA analysis

The DNA G+C contents of strains IC-154T and IC-163 were 30.7 and 30.9 mol%, respectively, as determined by the HPLC method of Tamaoka (1994). The almost-entire 16S rDNA sequences (1466 bases) determined for the two strains were identical. The G+C ratio was 61.8%. The 16S rDNA sequence of IC-154T was free from chimeric artefacts, according to the CHECK_CHIMERA program of the Ribosomal Database Project (Maidak et al., 2001). It contained all of the small-subunit rRNA signature bases that define the crenarchaeotes (Woese et al., 1993), except for two bases at positions 34 and 1335 (see Table 2). Phylogenetic analysis was conducted with other
crenarchaeote species by comparing 1066 bases in order to construct a phylogenetic tree (Fig. 2). The analysis revealed that the two strains were most closely associated with the order Desulfurococcales within the phylum Crenarchaeota. However, they formed an independent lineage that was related distantly to Acidilobus aceticus and strain NC12, which is tentatively named 'Caldococcus noboribetus' (sequence similarity, 91.5–92.6%). Sequence similarity to members of the orders Desulfurococcales (except for Acidilobus aceticus and strain NC12), Sulfolobales and Thermoproteales was 88.8–91.4, 84.8–86.9 and 86.2–88.1%, respectively. On the phylogenetic tree, strain IC-154T, Acidilobus aceticus and strain NC12 clustered together with a relatively high bootstrap value (94%), thereby this group of strains is tentatively named the Acidilobus group. As shown in Table 2, signature sequence analysis revealed that the Acidilobus group had five sequence positions (i.e. positions 34, 321:332, 1308:1329, 1335 and 1393) that are unique to this group among the phylum Crenarchaeota, and only two positions (i.e. positions 784:798 and 1364) that are shared with the remaining members of the order Desulfurococcales. In addition to the phylogenetic analysis, members of the Acidilobus group share certain phenotypic properties: all are acidophilic cocci (optimal pH, 3.0–4.0), are strictly anaerobic and organotrophic, use sulfur as an electron acceptor and inhabit terrestrial acidic hot springs. It is noteworthy that all remaining species of the order Desulfurococcales, including the terrestrial genera Desulfurococcus (Zillig et al., 1982; Bonch-Osmolovskaya et al., 1988), Sulfothrobacillus (Hensel et al., 1997) and

Proposal of a novel genus and species

Strains IC-154T and IC-163 are strictly anaerobic cocci that thrive at high temperatures (70–80°C) and acidic pH (3.0–4.0) and grow organotrophically with facultative reduction of sulfur. In addition to these phenotypic properties, lipid composition and 16S rDNA sequence-based phylogenetic analysis indicated that the two strains are related to the order Desulfurococcales of the phylum Crenarchaeota (Burggraf et al., 1997; Huber & Stetter, 2001). Furthermore, 16S rDNA sequence analysis revealed that the two strains are most closely related to Acidilobus aceticus (Prokofeva et al., 2000) and strain NC12, a representative of 'Caldococcus noboribetus' (Aoshima et al., 1996), and form a cluster named the Acidilobus group, as described above. In addition to the phylogenetic analysis, members of the Acidilobus group share certain phenotypic properties: all are acidophilic cocci (optimal pH, 3.0–4.0), are strictly anaerobic and organotrophic, use sulfur as an electron acceptor and inhabit terrestrial acidic hot springs. It is noteworthy that all remaining species of the order Desulfurococcales, including the terrestrial genera Desulfurococcus (Zillig et al., 1982; Bonch-Osmolovskaya et al., 1988), Sulfothrobacillus (Hensel et al., 1997) and

Table 2. 16S rRNA sequence signatures that distinguish the Acidilobus group and/or Desulfurococcales in the phylum Crenarchaeota

<table>
<thead>
<tr>
<th>Sequence position*</th>
<th>Acidilobus group</th>
<th>Desulfurococcales</th>
<th>Sulfolobales</th>
<th>Thermoproteales–Sulfolobales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific for Acidilobus group</td>
<td>Acidilobus group</td>
<td>Desulfurococcales</td>
<td>Sulfolobales</td>
<td>Thermoproteales–Sulfolobales</td>
</tr>
<tr>
<td>34</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>321:332</td>
<td>C:G</td>
<td>A:G</td>
<td>A:G</td>
<td></td>
</tr>
<tr>
<td>605:633</td>
<td>T:G</td>
<td>C:G</td>
<td>T:G</td>
<td></td>
</tr>
<tr>
<td>1308:1329</td>
<td>T:A</td>
<td>C:G</td>
<td>C:G</td>
<td></td>
</tr>
<tr>
<td>1335</td>
<td>C</td>
<td>G</td>
<td>G (A)</td>
<td></td>
</tr>
<tr>
<td>1393</td>
<td>T</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific for Acidilobus group and Desulfurococcales</td>
<td>784:798</td>
<td>C:G</td>
<td>C:G</td>
<td>G (A):C (T)</td>
</tr>
<tr>
<td>1364</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

*According to the E. coli numbering system.

Fig. 2. 16S rDNA sequence-based phylogenetic tree, showing the position of Caldisphaera lagunensis IC-154T (1066 base positions). Numbers at nodes indicate bootstrap values of 1000 trials (values >80% are shown). GenBank accession numbers are given in parentheses. Bar, 0.02% evolutionary distance.
Thermosphaera (Huber et al., 1998), favour weakly acidic to neutral pH for growth (optimal pH, 5.5–7.5). Considering the outlying position of the Acidilobus group on the phylogenetic tree (Fig. 2) and the fact that only a few signature sequences are shared with other species of the order Desulfurococcales (Table 2), inclusion of the Acidilobus group in the order Desulfurococcales may be controversial. At the moment, however, sequence analysis of 16S rDNA from more related strains, or of molecules other than 16S rDNA, is required to settle the taxonomic status of the Acidilobus group at order or family level.

Strains IC-154T and IC-163 can be differentiated from Acidilobus aceticus, and even from strain NC12, by the following characteristics. Strains IC-154T and IC-163 are extreme thermophiles that grow optimally around 75 °C and not at 82 °C, whereas Acidilobus aceticus and NC12 are hyperthermophilic and grow optimally at 85 and 92 °C, respectively. Moreover, Acidilobus aceticus has a genomic DNA G+C content of 53.8 mol%, requires yeast extract when grown with starch as the carbon source and does not produce H2 during growth. On the phylogenetic tree, strains IC-154T and IC-163 occupy an independent position and show at least 7.7% sequence dissimilarity from any other crenarchaeote strains. Therefore, these two strains represent a novel genus in the Acidilobus group. Except for a few phenotypic differences between strains IC-154T and IC-163 (e.g. presence or absence of pili and spectrum of electron acceptors), the two strains are similar to each other and their 16S rDNA sequences are identical, suggesting that they belong to a single species. Thus, we propose the name Caldisphaera lagunensis gen. nov., sp. nov. to accommodate the four strains IC-154T, IC-158, IC-163 and IC-165. The type strain of the novel species is IC-154T (=JCM 11604T=MCC-UPLB 1331T=ANMR 0165T).

Our attempt to isolate thermophilic organisms from an acidic hot spring in the Philippines resulted in the detection of five different crenarchaeote species, as determined by analysis of their partial 16S rDNA sequences. Rod-shaped crenarchaeotes, Caldibirga maquililingensis and Thermoproteus sp., were isolated in an atmosphere of N2 (pH 5.0 and 85 °C, as shown previously (Itoh et al., 1999)). Otherwise, cocoid crenarchaeotes, Metallosphaera and Sulfolobus–Sulfurisphaera-related strains and Caldisphaera lagunensis were obtained, as shown in this study. The growth conditions for isolation of Metallosphaera strains (air, pH 5.0, 70 °C) are consistent with the optimal growth conditions of known Metallosphaera species, except for pH (range, 1.0–4.5; Huber et al., 1989; Fuchs et al., 1995). The Sulfolobus–Sulfurisphaera isolates prevailed in enrichment cultures grown under various conditions, as shown in Table 1. Indeed, representative strains (IC-146, IC-147, IC-155, IC-156 and IC-157) of the Sulfolobus–Sulfurisphaera isolates grew aerobically and anaerobically in a H2/CO2 (4:1, v/v) gas atmosphere (data not shown). Sulfolobus yangningensis is phylogenetically most closely related to our Sulfolobus–Sulfurisphaera isolates; nevertheless, Sulfolobus yangningensis, as well as Sulfolobus tokodaii, are described as obligate aerobes (Jan et al., 1999; Suzuki et al., 2002), whereas Sulfurisphaera ohwakuenensis (Kuroswa et al., 1998) is able to grow anaerobically by utilizing H2 as an electron donor and sulfur as an electron acceptor (which is the same as our isolates). The taxonomic delineation of the genera Sulfolobus and Sulfurisphaera should be re-evaluated in the near future.

Caldisphaera lagunensis was able to grow by dissimilatory fermentative sulfur reduction in an atmosphere of N2. Therefore, employment of N2 as the gas phase in enrichment cultures may be advantageous for selective isolation of strains related to the genus Caldisphaera from sample sites where Caldisphaera cohabits with anaerobically growing Sulfolobales species, such as Sulfurisphaera ohwakuenensis. The isolation of two genera (Caldibirga and Caldisphaera) from a single hot spring site suggests that further undescribed archaean genera may be isolated from unexplored geothermal habitats.

Description of Caldisphaera gen. nov.

Caldisphaera (Cal.di.spha’ra. L. adj. caldus hot; L. fem. n. sphaera sphere; N.L. fem. n. Caldisphaera a hot spherical cell).

Cells are mostly regular cocci, 0.8–1.1 μm in diameter, and occur singly or in pairs. Pili may be present. Nonmotile. Extremely high temperature (70–78 °C) and acidic conditions (pH 3.5–4.5) are preferred for growth. Grows anaerobically. Resistant to chloramphenicol, kanamycin, oleandomycin, streptomycin and vancomycin. Sensitive to erythromycin, novobiocin and rifampicin. Possesses cyclic and acyclic tetraether core lipids. DNA G+C content of the type species is 31 mol%. The 16S rDNA is of a crenarchaeote in sequence signature analysis. Phylogenetically, the genus represents an independent lineage related to the order Desulfurococcales. Inhabits terrestrial hot springs. The type species is Caldisphaera lagunensis.

Description of Caldisphaera lagunensis sp. nov.

Caldisphaera lagunensis (la.gu.nen’sis. N.L. fem. adj. lagunensis pertaining to Laguna, the province in the Philippines where the type strain was isolated).

Grows anaerobically and tolerates low levels of oxygen (up to 2%). Heterotrophic. Growth occurs at 45–80 °C and pH 2.3–5.4. Under optimal growth conditions, doubling time is 5 h. Growth occurs at low salinity (≤1.5% NaCl). Chemo-organotrophic; utilizes starch, glycogen, gelatin, beef extract, yeast extract and peptone as carbon and energy sources. Forms acetate, propionate, isobutyrate, butyrate, isovalerate, H2 and CO2 as metabolic products. Growth is stimulated by the presence of sulfur. Hydrogen sulfide is formed. DNA G+C content is 31 mol%.

The type strain is IC-154T (=JCM 11604T=MCC-UPLB 1331T=ANMR 0165T).
1331^T = ANMR 0165^T). Reference strains are IC-158 (= ANMR 0169), IC-163 (= JCM 11605 = MCC-UPLB 1332 = ANMR 0174) and IC-165 (= ANMR 0176). Isolated from a hot spring on Mt Maquiling, Laguna, the Philippines.

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