**Thermoproteus thermophilus** sp. nov., a hyperthermophilic crenarchaeon isolated from solfataric soil

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A hyperthermophilic crenarchaeon, designated strain CBA1502T, was isolated from volcanic soil in the Mayon volcano in the Philippines. The 16S rRNA gene sequence of strain CBA1502T was most closely related to that of *Thermoproteus uzoniensis* DSM 5263T (99.2 % similarity) and *Thermoproteus tenax* Kra1T (99.0 %). The organism grew at 75–90 °C and pH 4.0–6.0 and in the presence of 0–0.5 % (w/v) NaCl, with optimal growth at 85 °C and pH 5.0. Strain CBA1502T utilized D-arabinose, beef extract, Casamino acids, formate, fumarate, peptone, pyruvate, trimethylamine and yeast extract as energy substrates, and D-arabinose, formate, pyruvate and yeast extract as carbon sources. Fumarate, sulfate, sulfur and thiosulfate functioned as electron acceptors, but not ferric chloride, nitrate, malate or oxidized glutathione. DNA–DNA hybridization studies showed that there was less than 46.1 % relatedness between strain CBA1502T and other members of the genus *Thermoproteus*. The DNA G+C content of strain CBA1502T was 62.0 mol%. We conclude that, according to its phylogenetic, phenotypic and genotypic characteristics, strain CBA1502T represents a novel species of the genus *Thermoproteus*, and propose the name *Thermoproteus thermophilus* sp. nov., with the type strain CBA1502T (=ATCC BAA-2416T=JCM 17229T).

The genus *Thermoproteus* accommodates hyperthermophilic archaeons, growing optimally above 80 °C and belonging to the family *Thermoproteaceae* in the order *Thermoproteales* of the phylum *Crenarchaeota*. Micro-organisms belonging to the order *Thermoproteales* have been isolated from hot springs and hydrothermal systems and grow chemolithotrophically (Zillig et al., 1981; Burggraf et al., 1997). The genus *Thermoproteus* proposed by Zillig et al. (1981) had included three species with validly published names: *Thermoproteus tenax* (Zillig et al., 1981), *Thermoproteus neutrophilus* (Stetter & Zillig, 1989) and *Thermoproteus uzoniensis* (Bonch-Osmolovskaya et al., 1990) (http://www.bacterio.net/thermoproteus.html). However, based on phylogenetic analysis, *T. neutrophilus* has been recently reclassified to the genus *Pyrobaculum* as *Pyrobaculum neutrophilum* (Chan et al., 2013). In the present study, we determined the phylogenetic, phenotypic and genotypic characteristics of a hyperthermophilic isolate (designated strain CBA1502T) from solfataric soil, and propose it to be representative of a novel species of the genus *Thermoproteus*.

A soil sample from a solfataric thermal field was collected, as previously described (Yim et al., 2015). A 5 g sample of this was enriched at 80 °C for one month using Japan Collection of Microorganisms (JCM) medium number 236 (M236), containing components as follows: 2.94 g trisodium citrate dihydrate, 0.5 g yeast extract (BD), 10.0 ml trace vitamins, 1.0 mg resazurin, 0.5 g Na2S.9H2O and 10.0 g sulfur in 1 litre salt base solution, prepared anaerobically according to the JCM culture medium guidelines. Serial dilutions were made, with pure growth culture representing the highest dilution. This procedure was repeated twice, yielding the same isolate both times. The purity of the isolated strain, which was designated CBA1502T, was validated by light microscopy (BA210; Motic), sequencing of its PCR-amplified 16S rRNA gene, and phylogenetic analysis. *T. uzoniensis* DSM 5263T and *T. tenax* DSM 2078T were obtained from the Deutsche Sammlung von...
Mikroorganismen und Zellkulturen (DSMZ) and used as reference strains for analysis by DNA–DNA hybridization. The genomic DNA extraction, PCR-amplification and sequencing of the 16S rRNA gene, and phylogenetic analysis were carried out as previously described (Yim et al., 2015). A nearly full-length (1435 bp) 16S rRNA gene sequence of strain CBA1502T was obtained. The 16S rRNA gene sequence of strain CBA1502T had 99.2, 99.1, 99.0 and <97.0 % similarity to the sequences of *T. uzoniensis* DSM 5263T (982 bp; data from this study), *T. uzoniensis* 768-20, *T. tenax* Kra 1T and other members of the family Thermoproteaceae, respectively. Phylogenetic trees, based on 16S rRNA gene sequences, indicated that strain CBA1502T falls within the cluster of species of the genus *Thermoproteus* of the family Thermoproteaceae, with high bootstrap values (99, 98 and 97 % in neighbour-joining, maximum-parsimony and maximum-likelihood trees, respectively) (Fig. 1).

Cells of strain CBA1502T were observed using electron microscopy (Tecnai G2 Spirit; FEI), as described previously (Lee et al., 2013). They were rod-shaped (mainly 0.3–0.5 μm wide × 2.9–7.7 μm long) and motile with peritrichous flagellation. Cells of *T. uzoniensis* and *T. tenax* were sometimes branching or had spherical protrusions on the ends or at branching points and had no flagella (Zillig et al., 1981; Bonch-Osmolovskaya et al., 1990), but cells of strain CBA1502T were straight rods and had flagella. The optimum growth conditions for strain CBA1502T were determined by analysing growth between 65 and 95 °C at intervals of 5 °C, and at pH values of between pH 3.0 and 8.0 at intervals of 1.0 pH unit using M236 medium. The pH was adjusted using buffers as follows: 1 M acetic acid and sodium acetate, pH 3.0; 10 mM MES, pH 4.0–6.0, and 10 mM TAPS, pH 7.0 and 8.0. NaCl tolerance was tested using M236 medium with NaCl added (0–3 %, w/v, at intervals of 0.5 %). Growth was estimated from cell counting using a haemocytometer. The isolate grew at 75–90 °C and pH 4.0–6.0 (w/v) and in the presence of 0–0.5 % (w/v) NaCl, with optimal growth at 85 °C and pH 5.0. The strain grew with a doubling time of 14.6 h (under optimal growth conditions in M236 medium without citrate); at the stationary phase, the culture contained approximately 7.0 × 10⁶ cells ml⁻¹.

Fig. 1. Phylogenetic tree derived from the 16S rRNA gene sequences of strain CBA1502T and closely related species. Branch nodes represented by filled circles indicate generic branches present in phylogenetic trees that were generated by the neighbour-joining as well as the maximum-parsimony and maximum-likelihood algorithms. Numbers at nodes indicate bootstrap percentages, calculated by the neighbour-joining, maximum-parsimony and maximum-likelihood methods, respectively. Bootstrap analyses were performed using 1000 replicates and values greater than 70 % are shown at branching points. Bar, 0.01 changes per nucleotide position.
The following tests were carried out on cells growing in cultures transferred three times under optimal growth conditions using M236 medium (without citrate) with a pH buffer (10 mM MES). For testing the utilization of different energy substrates, potential energy sources (0.5 %, w/v) were added individually to medium with a small amount of yeast extract also present (0.1 g l⁻¹). The differences to control (with yeast extract only) cell yields were also confirmed by hydrogen sulfide formation, as described by Cui et al. (2007). D-Arabinose, beef extract, Casamino acids, formate, fumarate, peptone, pyruvate and trimethylamine were utilized as energy substrates, but not acetate, butyrate, citrate, D-fructose, D-galactose, D-glucose, lactose, L-malate, D-mannose, methanol, methylamine, starch, succinate, sucrose or D-xylene. For testing the utilization of different carbon sources, potential carbon sources (0.5 %, w/v) were added individually to medium with yeast extract removed. D-Arabinose, formate and pyruvate were utilized as carbon sources, but not acetate, beef extract, butyrate, Casamino acids, citrate, D-fructose, fumarate, D-galactose, D-glucose, lactose, L-malate, D-mannose, methanol, methylamine, peptone, starch, succinate, sucrose, trimethylamine or D-xylene. For testing possible electron acceptors, strain CBA1502T was cultured in medium, which had ferric chloride (10 mM) rather than sulfur present, or fumarate (20 mM), malate (20 mM), nitrate (40 mM), oxidized glutathione (2.5 mM), sulfate (20 mM) or thiosulfate (20 mM). When sulfur-containing compounds were used as electron acceptors, results were also confirmed by hydrogen sulfide formation. Fumarate, sulfate and thiosulfate were utilized as electron acceptors, but not ferric chloride, malate, nitrate or oxidized glutathione. Strain CBA1502T showed no growth in medium with only yeast extract present in the absence of other energy substrates, carbon sources and electron acceptors. Strain CBA1502T showed weak growth in a low-oxygen atmosphere [5.0 % (v/v) air in N₂], but no growth in 5.5 % (v/v) air in N₂. The strain showed no growth under the chemolithothrophic conditions of a H₂/CO₂ (4 : 1, v/v) gas mixture in medium without yeast extract. To test antibiotic sensitivity, strain CBA1502T was inoculated in medium with the following present (all at 100 µg ml⁻¹): erythromycin, novobiocin, rifampicin, ampicillin, chloramphenicol, kanamycin, streptomycin and vancomycin. The strain was susceptible to erythromycin, novobiocin, chloramphenicol, kanamycin, streptomycin and vancomycin, but resistant to rifampicin and ampicillin. The differential characteristics of strain CBA1502T and its close relatives in the genus Thermoproteus are shown in Table 1. Strain CBA1502T had lower maximum and optimal growth temperatures than those of both T. uzoniensis and T. tenax (Bonch-Osmolovskaya et al., 1990; Garrity and Holt, 2001). Strain CBA1502T also differed from T. uzoniensis and T. tenax due to its motility, utilization of formate and reduction of sulfate.

We used photobiotinylated DNA probes, as described by Ezaki et al. (1989), and found that the homology between the genomic DNA of CBA1502T and that of T. uzoniensis DSM 5263T and T. tenax DSM 2078T was 46.1 and 24.9 %, respectively. Lower than 70 % homology indicates that an isolate represents a distinct genomospecies (Wayne et al., 1987; Stackebrandt & Goebel, 1994). The DNA G+C content of strain CBA1502T was determined to be 62.0 mol% from a fluorimetric method (González & Saiz-Jimenez, 2002) using SYBR Green I and a real-time PCR thermocycler. This value is higher than the range of 55.5–56.5 mol% previously reported for members of the genus Thermoproteus (Zillig et al., 1981; Bonch-Osmolovskaya et al., 1990).

In summary, the physiological, biochemical and genomic characteristics of strain CBA1502T differ from those of other members of the genus Thermoproteus. Thus, strain CBA1502T represents a novel species of the genus Thermoproteus in the family Thermoproteaceae and we propose the name Thermoproteus thermophilus sp. nov.

### Description of Thermoproteus thermophilus sp. nov.

Thermoproteus thermophilus (ther.mo’phi.lus. Gr. n. therm heat; Gr. adj. philos friendly, loving; N.L. masc. adj. thermophilus heat-loving, referring to its growth temperature).

Cells are anaerobic, hyperthermophilic, acidophilic, obligately chemo-organotrophic, rod-shaped (0.3–0.5 µm wide × 2.9–7.7 µm long) and motile with peritrichous flagellation. Growth occurs at 75–90 °C (optimum, 85 °C), pH 4.0–6.0 (optimum, pH 5.0) and in medium containing 0–0.5 % (w/v) NaCl. Under optimal growth conditions the doubling

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**Table 1. Characteristics that differentiate between strain CBA1502T and type strains of its closest relatives in the genus Thermoproteus**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Motility</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>75–90</td>
<td>74–102</td>
<td>&lt;96</td>
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<tr>
<td>Optimum temperature</td>
<td>85</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>pH range for growth</td>
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<td>2.5–6.0</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>5.0</td>
<td>5.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Carbon source</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Formate</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Glucose</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Electron acceptor</td>
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<tr>
<td>Malate</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Sulfate</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Thiosulfate</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol %)</td>
<td>62.0</td>
<td>56.5</td>
<td>55.5</td>
</tr>
</tbody>
</table>

*Data for T. tenax DSM 2078T from this study.*
time is 14.6 h. D-Arabinose, beef extract, Casamino acids, formate, fumarate, peptone, pyruvate, trimethylamine and yeast extract, but not acetate, butyrate, citrate, D-fructose, D-galactose, D-glucose, lactose, L-malate, D-mannose, methanol, methylene, starch, succinate, sucrose or D-xylene are utilized as energy substrates. D-Arabinose, formate, pyruvate and yeast extract, but not acetate, beef extract, butyrate, Casamino acids, citrate, D-fructose, fumarate, D-galactose, D-glucose, lactose, L-malate, D-mannose, methanol, methylene, peptone, starch, succinate, sucrose, trimethylamine or D-xylene are utilized as carbon sources. Fumarate, sulfate, sulfur and thiosulfate are utilized as electron acceptors, but not ferric chloride, malate, nitrate or oxidized glutathione. Cells can tolerate a low oxygen level [5.0 % (v/v) air in N₂].

The type strain is CBA1502ᵀ (=ATCC BAA-2416ᵀ=JCM 17229ᵀ), isolated from solfataric soil from the Mayon volcano on the island of Luzon in the Republic of the Philippines. The DNA G+C content of the type strain is 62.0 mol%.

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References


