**Thermotoga petrophila** sp. nov. and
**Thermotoga naphthophila** sp. nov., two hyperthermophilic bacteria from the Kubiki oil reservoir in Niigata, Japan

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Two hyperthermophilic bacteria, strains RKU-1T and RKU-10T, which grew optimally at 80 °C, were isolated from the production fluid of the Kubiki oil reservoir in Niigata, Japan. They were strictly anaerobic, rod-shaped fermentative heterotrophs. Based on the presence of an outer sheath-like structure (toga) and 16S rDNA sequences, they were shown to belong to the genus *Thermotoga*. Cells of strain RKU-1T were 2–7 µm by 0.7–1.0 µm, with flagella. They grew at 47–88 °C on yeast extract, peptone, glucose, fructose, ribose, arabinose, sucrose, lactose, maltose, starch and cellulose as sole carbon sources. Cells of strain RKU-10T were 2–7 µm by 0.8–1.2 µm, with flagella. They grew at 48–86 °C on yeast extract, peptone, glucose, galactose, fructose, mannitol, ribose, arabinose, sucrose, lactose, maltose and starch as sole carbon sources. While strains RKU-1T and RKU-10T reduced elemental sulfur to hydrogen sulfide, their final cell yields and specific growth rates decreased in the presence of elemental sulfur. Thiosulfate also inhibited growth of strain RKU-1T but not strain RKU-10T. The G+C contents of the DNA from strains RKU-1T and RKU-10T were 46.8 and 46.1 mol%.

Phenotypic characteristics and 16S rDNA sequences of the isolates were similar to those of *Thermotoga maritima* and *Thermotoga neapolitana*, both being hyperthermophilic bacteria isolated from hydrothermal fields. However, the isolates differed from these species in their minimum growth temperatures, utilization of some sugars, sensitivity to rifampicin and the effects of elemental sulfur and thiosulfate on growth. The low levels (less than 31%) of DNA reassociation between any two of these hyperthermophilic *Thermotoga* strains indicated that the isolates were novel species. Analysis of the *gyrB* gene sequences supported the view that the isolates were genotypically different from these reference species. The isolates were named *Thermotoga petrophila* sp. nov., with type strain RKU-1T (= DSM 13995T = JCM 10881T), and *Thermotoga naphthophila* sp. nov., with type strain RKU-10T (= DSM 13996T = JCM 10882T).

**Keywords:** *Thermotoga petrophila*, *Thermotoga naphthophila*, hyperthermophilic bacteria, oil reservoir

**INTRODUCTION**

A large number of hyperthermophiles with optimum growth temperatures higher than 80 °C have been isolated from various hot environments (Stetter, 1996). While most of them are members of the domain...
Archaea, some hyperthermophiles belong to the genera Aquifex and Thermotoga in the domain Bacteria (Blöchl et al., 1995). According to 16S rRNA gene sequencing, they represent the deepest phylogenetic branches within the domain Bacteria (Winker & Woese, 1991). The members of the genus Thermotoga, in the order Thermotogales, include Thermotoga maritima (Huber et al., 1986), Thermotoga neapolitana (Belkin et al., 1986; Jannasch et al., 1988), Thermotoga thermarum (Windberger et al., 1989), Thermotoga elfii (Ravot et al., 1995a), Thermotoga subterranea (Jeanthon et al., 1995) and Thermotoga hypogea (Fardeau et al., 1997). T. maritima and T. neapolitana grow optimally at 80 °C and are hyperthermophilic species. In the last decade, hyperthermophilic archaea, including T. elfii, T. subterranea and T. hypogea, have been isolated from the production fluids of oil reservoirs. Thermotoga-like bacteria with togas, growing at 65–85 °C, have also been reported from oil reservoirs in Alaska and California (Orphan et al., 2000; Stetter et al., 1993). We describe two novel hyperthermophilic Thermotoga species, Thermotoga petrophila sp. nov. and Thermotoga naphthophila sp. nov., isolated from the Kubiki oil reservoir.

METHODS

Reference micro-organisms. T. maritima DSM 3109®, T. neapolitana DSM 4359® and T. thermarum DSM 5069® were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), Braunschweig, Germany.

Collection of the sample. The production fluid was taken from the no. 3 storage tank of the Kubiki oil reservoir in Niigata, Japan. The geological structure and physical properties of the Kubiki oil reservoir have been described previously (Takahata et al., 2000). The production fluid was collected in sterile glass bottles, which were then sealed with sterile silicon stoppers and plastic screw caps. They were chilled in a cooler box with ice and transported to the laboratory.

Isolation and cultivation. Anaerobic, heterotrophic hyperthermophiles were grown on YE medium (Takahata et al., 2000) containing 0.2% (w/v) yeast extract in artificial sea water (ASW; Jannasch et al., 1995). One litre of ASW contained 20 g NaCl, 3 g MgCl₂·6H₂O, 6 g MgSO₄·7H₂O, 1 g (NH₄)₂SO₄, 0.3 g CaCl₂·2H₂O, 0.2 g KH₂PO₄, 0.5 g KCl, 0.05 g NaBr, 0.025 g H₃BO₃, 0.02 g SrCl₂·6H₂O, 0.01 g ferric ammonium citrate, 2.25 g bis-tris propane, 10 ml of a trace mineral solution (Wolin et al., 1963), 10 ml of a vitamin solution (Bazyliński et al., 1989) and 0.6 mg resazurin as a redox indicator. The pH was adjusted to 7.0 with 6 M HCl after supplementing the medium with 0.2% yeast extract at room temperature. Aliquots (9 ml) of the liquid medium were dispensed into 30 ml Hungate tubes (Sanshin Kogyo) and autoclaved at 121 °C for 20 min. The tubes were flushed with oxygen-free nitrogen through a heated, H₂-reduced, copper furnace (Sanshin Kogyo). The tubes were then sealed tightly with sterile butyl-rubber stoppers. Sodium sulfide, which had been sterilized by filtration (disk capsule of 0.2 μm pore size; Fuji), was added to a final concentration of approximately 400 μM. After autoclaving, flushing with nitrogen and adding sodium sulfide, the final pH in the medium was between 6.9 and 7.1 at room temperature. One millilitre of the formation water in the production fluid was inoculated into 9 ml of YE medium in a Hungate tube and incubated at 85 °C for 1–7 d. Hyperthermophiles were purified by the Gelrite plating method as described previously (Takahata et al., 2000).

Electron microscopy. Cell morphology and flagellation were observed with a transmission electron microscope (H-7000; Hitachi) as described previously (Takahata et al., 2000). The cells were fixed with 25% glutaraldehyde and post-fixed with 1% osmium tetroxide. Ultrathin sections of the cells were embedded in Epon 812 resin were cut with a Reichert-Nissei Ultrakut-N ultramicrotome and doubly stained with uranyl acetate and lead citrate (Reynolds, 1963). After fixation with 2.5% glutaraldehyde, whole mounted cells were negatively stained with 4% (w/v) uranyl acetate (Kurr et al., 1991).

Determination of cell numbers. Cell growth was monitored by the acridine orange direct-counting method (Hobbie et al., 1977) under an epifluorescence microscope (model BX-60; Olympus).

Growth characteristics. The effect of temperature on microbial growth was examined in YE medium with a mineral oil bath (model OH-16; Taitec). The effects of pH and NaCl on growth were examined in a YE-based medium incubated at 80 °C. The pH dependency of growth was determined with various buffer systems, as described previously (Hoaki et al., 1994). The pH of the medium was checked and readjusted at room temperature after autoclaving, flushing with nitrogen and adding sodium sulfide. The effects of various gaseous phases on microbial growth were examined by using ASW for autotrophic growth or YE medium for heterotrophic growth. The gaseous phase in a Hungate tube was filled with CO₂, H₂, air or a mixture of H₂ and CO₂ (4:1). To determine the nutritional requirements of the isolates, their growth was examined in 10 ml ASW medium (pH 7.0) without shaking at 80 °C with one of the following carbon sources at a final concentration of 0.1% (w/v): yeast extract, peptone, casein, albumin, Casamino acids, a mixture of 20 amino acids, glucose, galactose, fructose, mannitol, ribose, arabinose, xylose, sucrose, lactose, maltose, starch, acetate, lactate, formate, propionate, maleic and citric acids, methanol and ethanol. Growth was also examined in the presence of 1% (v/v) cellulose, chitin, kerosene, light oil, A-heavy oil (Nippon Mitsubishi Oil) or crude oil in 30 ml ASW medium (pH 7.0) with shaking. Crude oil was obtained from the Kubiki oil reservoir. To confirm growth on any of the substrates, the isolate was subcultured twice in the same medium.

Metabolic products. Metabolic products [H₂, CO₂ and low-molecular-mass (C₅-C₁₀) organic acids] were analysed by HPLC and GC as described previously (Takahata et al., 2000). d-Glucose was analysed with a glucose analysis kit (no. 716251; Boehringer Mannheim).

Effect of electron acceptors. The effects of sulfate (20 mM), thiosulfate (20 mM) and elemental sulfur (2%) as electron acceptors on growth of the isolates were examined in YE-based medium in which MgSO₄·7H₂O and (NH₄)₂SO₄ had been replaced with MgCl₂·6H₂O and NH₄Cl. Hydrogen sulfide production was analysed with a gas analysis kit (Gastec).
Sensitivity to antibiotics. The sensitivity of the isolates to rifampicin, streptomycin, vancomycin and chloramphenicol was examined at a concentration of 100 µg ml⁻¹ in MY medium at 70 °C for 7 d.

Isolation of DNA. Genomic DNA was extracted from each isolate by using a procedure described elsewhere (Ausubel et al., 1987) with a slight modification. RNA was digested with 20 µg DNase-free RNase ml⁻¹ at 37 °C for 1 h after extracting with chloroform/isoamyl alcohol.

DNA base composition. The nucleotide composition was determined by HPLC with a Develosil ODS-HG-5 column (4.6 × 250 mm) and a UV detector (model UV 8010; Tosoh) at 270 nm after digesting the DNA with nuclease P1 (Zillig et al., 1980).

16S rDNA sequence analysis. PCR was used to amplify 16S rDNA from the genomic DNA of each isolate using primers B0R (Escherichia coli positions 8–27; 5'-AGAGTTTGATCTTGGCTCAG-3') and B9 (1491–1512; 5'-TACGGGTCA-CCTTGTACCACTT-3') (Takahata et al., 2000). A total of 35 cycles of amplification was performed with template DNA denaturation at 94 °C for 1 min, primer annealing at 58 °C for 1 min and primer extension at 72 °C for 2 min. After purifying the PCR products with a QIAEX II gel extraction kit (Qiagen), the 16S rDNA sequence was determined in this study. Outgroup: Thermosiphon marilensis DSM 12029T (Z70248).

RESULTS AND DISCUSSION

Isolation and 16S rDNA sequence analysis

Two hyperthermophilic bacterial strains, RKU-1T and RKU-10T, were isolated from a deep subterranean oil reservoir in Niigata, Japan. They were obligately anaerobic heterotrophic rods with a ‘toga’, a sheath-like structure with ballooning at both ends, which is characteristic of members of the order Thermotogales. Phylogenetic analysis using 16S rDNA sequences showed that the novel isolates belong to the genus Thermotoga and they were most closely related to the hyperthermophilic species T. maritima and T. neapolitana (Fig. 1), which were isolated from marine hydrothermal fields (Belkin et al., 1986; Huber et al., 1986). Three previously described Thermotoga species, T. subterranea, T. elfii and T. hypogea, have also been isolated from oil reservoirs (Fardeau et al., 1997; Jeanthon et al., 1995; Ravot et al., 1995a). However, the phylogenetic tree of 16S rDNA sequences revealed that these three species formed a clade distinct from the hyperthermophilic Thermotoga species (Fig. 1).

Morphological and phenotypic properties

Cells of strain RKU-1T were 2–7 µm long and 0.7–1.0 µm wide. Cells of strain RKU-10T were 2–7 µm long and 0.8–1.2 µm wide. In the stationary growth phase, the cell shape became spherical, with a diameter of 1–2 µm. While T. maritima has a single subpolar flagellum (Huber et al., 1986) and T. neapolitana lacks flagella (Belkin et al., 1986), strains RKU-1T and RKU-10T possessed several subpolar and lateral flagella (Fig. 2a, b). Similar flagellation has been
shown for cells of *T. elfii* and *T. hypogea*. Electron microscopy of thin sections of the isolates showed a cell wall approximately 50 nm thick (Fig. 2c, d).

The temperature ranges for growth were 47–88 °C with an optimum at 80 °C for strain RKU-1T and 48–86 °C with an optimum at 80 °C for RKU-10T (Fig. 3a). No growth was apparent at 46 or 89 °C (RKU-1T) and at 47 or 87 °C (RKU-10T). At 80 °C, the respective doubling times of strains RKU-1T and RKU-10T were 54 and 59 min. While *T. maritima* and *T. neapolitana* also grow optimally at 80 °C, they are not able to grow at temperatures below 55 °C (Belkin et al., 1986; Huber et al., 1986). The optimum temperatures of other *Thermotoga* species isolated from oil reservoirs have been reported to be 66–70 °C (Fardeau et al., 1997; Jeanthon et al., 1995; Ravot et al., 1995a).

No growth was apparent at concentrations of 0 or 6% (RKU-1T) and at 0 or 6.5% (RKU-10T). In contrast, thermophilic *Thermotoga* species from oil reservoirs required lower levels of salinity (NaCl concentration range for growth 0–2.8%) (Fardeau et al., 1997; Jeanthon et al., 1995; Ravot et al., 1995a).

No growth was apparent at pH 5 or 9 (RKU-1T) and at pH 5-2 or 9-5 (RKU-10T). The pH ranges for growth were 5-2–9-0 (RKU-1T) and 5-4–9-0 (RKU-10T), with the optimum at 7-0 for both (Fig. 3b). No growth was apparent at pH 5-0 or 9-5 (RKU-1T) and at pH 5-2 or 9-5 (RKU-10T). These ranges are about the same as those of *T. maritima* and *T. neapolitana* (Belkin et al., 1986; Huber et al., 1986). The NaCl concentration ranges for growth were 0-1–5.5% (RKU-1T) and 0-1–6.0% (RKU-10T), with the optimum at 1-0% for both (Fig. 3c). No growth was apparent at concentrations of 0 or 6% (RKU-1T) and at 0 or 6.5% (RKU-10T). In contrast, thermophilic *Thermotoga* species from oil reservoirs required lower levels of salinity (NaCl concentration range for growth 0–2.8%) (Fardeau et al., 1997; Jeanthon et al., 1995; Ravot et al., 1995a).

Carbon dioxide did not affect heterotrophic growth of the isolates in YE medium. They could grow in YE medium with a hydrogen atmosphere, but their final cell yields were only about 10% of those with nitrogen. They grew on yeast extract, peptone and various sugars. Growth of the novel isolates, *T. maritima* and *T. neapolitana* on various sugars is shown in Table 1. All of the strains could grow on all the sugars tested except mannitol or xylose as the sole carbon and energy source, although the final cell densities after incubation for 48 h at 80 °C were different (Table 1). Strain RKU-1T grew weakly on cellulose, but strain RKU-10T did not. Neither strain RKU-1T nor RKU-10T grew on proteins (casein or albumin), amino acids (Casamino acids or a mixture of 20 amino acids), organic acids (acetate, lactate, formate, propionate, maleic or citric acid), alcohols (methanol or ethanol),
Two novel *Thermotoga* species

![Graphs showing effects of temperature, pH, and NaCl concentration on specific growth rates of strains RKU-1T and RKU-10T.](image)

**Table 1. Growth of hyperthermophilic *Thermotoga* strains on various sugars**

The initial cell density was adjusted to approximately $10^4$ cells ml$^{-1}$. Growth was evaluated from the final cell density after incubation for 48 h at 80 °C, and is scored as: $+++$, $>10^9$ cells ml$^{-1}$; $++$, $10^7$–$10^8$ cells ml$^{-1}$; $+$, $10^5$–$10^7$ cells ml$^{-1}$; $-$, no growth. All four taxa were scored as $+$ for growth on arabinose.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>RKU-1$^T$</th>
<th>RKU-10$^T$</th>
<th><em>T. maritima</em></th>
<th><em>T. neapolitana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>$+$</td>
<td>$+$</td>
<td>$+++$</td>
<td>$+$</td>
</tr>
<tr>
<td>Galactose</td>
<td>$++$</td>
<td>$++$</td>
<td>$+++$</td>
<td>$+++$</td>
</tr>
<tr>
<td>Fructose</td>
<td>$++$</td>
<td>$++$</td>
<td>$+$</td>
<td>$+$</td>
</tr>
<tr>
<td>Mannitol</td>
<td>$-$</td>
<td>$+$</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>Ribose</td>
<td>$++$</td>
<td>$++$</td>
<td>$+$</td>
<td>$+++$</td>
</tr>
<tr>
<td>Xylose</td>
<td>$-$</td>
<td>$-$</td>
<td>$+++$</td>
<td>$+$</td>
</tr>
<tr>
<td>Sucrose</td>
<td>$++$</td>
<td>$++$</td>
<td>$+++$</td>
<td>$+++$</td>
</tr>
<tr>
<td>Lactose</td>
<td>$++$</td>
<td>$++$</td>
<td>$+$</td>
<td>$+++$</td>
</tr>
<tr>
<td>Maltose</td>
<td>$++$</td>
<td>$++$</td>
<td>$+++$</td>
<td>$+++$</td>
</tr>
<tr>
<td>Starch</td>
<td>$++$</td>
<td>$++$</td>
<td>$+++$</td>
<td>$+++$</td>
</tr>
</tbody>
</table>

**Table 2. Fermentation of glucose by strains RKU-1$^T$ and RKU-10$^T$**

Glucose consumed was calculated by subtracting the glucose concentration after cultivation from that before cultivation. Metabolites produced were measured after incubation for 100 h in ASW containing 0.1% glucose at 80 °C.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cell density (cells ml$^{-1}$)</th>
<th>Glucose consumed (mM)</th>
<th>Production of (mM):</th>
<th>Total carbon recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td>Lactate</td>
</tr>
<tr>
<td>RKU-1$^T$</td>
<td>$5.2 \times 10^4$</td>
<td>$7.3 \times 10^6$</td>
<td>0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>RKU-10$^T$</td>
<td>$6.3 \times 10^4$</td>
<td>$1.2 \times 10^7$</td>
<td>1.36</td>
<td>1.05</td>
</tr>
</tbody>
</table>
chitin or hydrocarbons (kerosene, light oil, A-heavy oil or crude oil) as the sole carbon and energy source.

Lactate, acetate, CO₂ and H₂ were produced by strains RKU-1 and RKU-10 during growth on glucose. The concentration of glucose consumed and those of the final fermentation products after cultivation at 80 °C for 100 h are shown in Table 2. The ratio of acetate/lactate in strain RKU-10 was higher than those in strain RKU-1 and T. maritima (0:40; Huber et al., 1986).

Sulfur utilization was reported to be one of the discriminatory characteristics between hyperthermophilic Thermotoga species from hydrothermal fields and thermophilic Thermotoga species from oil reservoirs (Fardeau et al., 1997). While elemental sulfur has been reported to enhance the final cell yields and specific growth rates of T. maritima and T. neapolitana by removing growth-inhibitory H₂ (Ravot et al., 1995b), it inhibited the growth of T. elfii and T. subterranea (Jeanthon et al., 1995; Ravot et al., 1995a). Strains RKU-1* (data not shown) and RKU-10 (Fig. 4a) did not reduce sulfate to hydrogen sulfide but did reduce sulfur and thiosulfate. The final yields of H₂S production from elemental sulfur by these two strains were almost the same (about 400 μg ml⁻¹; Fig. 4a). While RKU-1* (data not shown) and RKU-10 (Fig. 4b) reduced elemental sulfur to H₂S, their final cell yields and specific growth rates were reduced. Growth of six Thermotoga reference strains was stimulated by thiosulfate reduction (Ravot et al., 1996). Strain RKU-1 also reduced thiosulfate to H₂S (120 μg ml⁻¹; data not shown), but its final cell yield and specific growth rate were reduced. The final yield of H₂S from thiosulfate by strain RKU-10 was much less than that of strain RKU-1 (approx. 10 μg ml⁻¹; Fig. 4a). The final cell yield and specific growth rate were not affected significantly by the presence of thiosulfate (Fig. 4b). These results indicate that the utilization of sulfur or thiosulfate by these isolates from Japanese oil reservoirs was quite different from that of Thermotoga reference species.

Strains RKU-1 and RKU-10 did not grow in YE medium containing 100 μg rifampicin, streptomycin, vancomycin or chloramphenicol ml⁻¹ at 70 °C within 7 d. In contrast, T. maritima and T. neapolitana were not sensitive to rifampicin at the same concentration (Belkin et al., 1986; Huber et al., 1986).

These results indicated that the morphological and phenotypic properties of strains RKU-1* and RKU-10, such as type of flagellum, minimum growth temperature, sugar utilization, effects of sulfur and thiosulfate on growth and rifampicin sensitivity, were clearly different from those of T. maritima and T. neapolitana. They also differed from other Thermotoga species isolated from oil reservoirs in their optimum growth temperature, range of NaCl concentrations for growth and effects of sulfur and thiosulfate on growth. However, the morphological and phenotypic properties of these two isolates were quite similar, except in mannitol and cellulose utilization and thiosulfate reduction.

Genotypic analysis

The DNA G+C contents of strains RKU-1* and RKU-10 were 46.6 and 46.1 mol%. These G+C contents are similar to that of T. maritima (46 mol%). DNA reassociation values between any two of the hyperthermophilic Thermotoga strains were 110–308% (Table 3). According to the criteria of Wayne et al. (1987), these results indicate that each of the new isolates should be classified as a distinct species.

The 16S rDNA sequence evolves so slowly that it is not suitable for distinguishing closely related species. The gyrB gene, encoding the subunit B protein of DNA gyrase (topoisomerase type II), has been proposed to be more suitable to distinguish closely related species (Yamamoto et al., 1999). Amplified gyrB gene fragments from strains RKU-1* and RKU-10, T. maritima and T. neapolitana were sequenced directly and their partial gyrB sequences (358 bp) were determined. The similarity (77.8–94.7%; Table 3) of gyrB sequences between any two of the four hyper-
thermophilic *Thermotoga* strains was much less than that in their 16S rDNA sequences (99.1–99.6 %). These results support the genotypic difference among them.

On the basis of their phenotypic characteristics and phylogenetic relationships, the two isolates from a Japanese oil reservoir are thought to be novel species. They are designated *Thermotoga petrophila* sp. nov., with the type strain RKU-1<sup>T</sup>, and *Thermotoga naphthophila* sp. nov., with the type strain RKU-10<sup>T</sup>.

**Description of Thermotoga petrophila** sp. nov.

*Thermotoga petrophila* (pe.тро'phi.la. Gr. n. petros rock; Gr. adj. philos loving; N.L. fem. adj. petrophilia rock-loving).

Cells are rods, 2–7 µm long by 0.7–1 µm wide. Cells possess an outer sheath-like structure (toga) and several subpolar and lateral flagella. Heterotrophic and obligate anaerobe of the domain *Bacteria*. The temperature for growth is 48–86 °C, with the optimum at 80 °C. The pH range for growth is 5.4–9.0, with the optimum at 7.0. The NaCl concentration range for growth is 0.1–6.0 %, with the optimum at 1.0 %. The doubling time at the optimum temperature is 59 min. Requires yeast extract, peptone, glucose, galactose, fructose, mannitol, ribose, arabinose, sucrose, lactose, maltose or starch as the sole carbon and energy source. The end products from glucose fermentation are acetate, lactate, H<sub>2</sub>S, their growth rate and cellular yield decrease in the presence of elemental sulfur. While the species reduces thiosulfate weakly to H<sub>2</sub>S, thiosulfate shows no effect on the growth rate or cellular yield. Sensitive to 100 µg rifampicin, streptomycin, vancomycin or chloramphenicol ml<sup>–1</sup>. The DNA G+C content is 46.1 mol %. Isolated from the formation water of production fluid in the Kubiki oil reservoir.

The type strain is RKU-1<sup>T</sup> (= DSM 13995<sup>T</sup> = JCM 10882<sup>T</sup>).

**Description of Thermotoga naphthophila** sp. nov.

*Thermotoga naphthophila* (nap.tho’phi.la. Gr. n. naphtha bitumen; Gr. adj. philos loving; N.L. fem. adj. naphthophila bitumen-loving).

Cells are rods, 2–7 µm long by 0.8–1.2 µm wide. Cells possess an outer sheath-like structure (toga) and several subpolar and lateral flagella. Heterotrophic and obligate anaerobe of the domain *Bacteria*. While the cells reduce elemental sulfur to H<sub>2</sub>S, their growth rate and cellular yield decrease in the presence of elemental sulfur. While the species reduces thiosulfate weakly to H<sub>2</sub>S, thiosulfate shows no effect on the growth rate or cellular yield. Sensitive to 100 µg rifampicin, streptomycin, vancomycin or chloramphenicol ml<sup>–1</sup>. The DNA G+C content is 46.6 mol %. Isolated from the formation water of production fluid in the Kubiki oil reservoir.

The type strain is RKU-10<sup>T</sup> (= DSM 13995<sup>T</sup> = JCM 10882<sup>T</sup>).

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**REFERENCES**


Stetter, K. O., Huber, R., Bloch, E., Kurr, M., Eden, R. D., Fielder,
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