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# *Thermus igniterrae* sp. nov. and *Thermus antranikianii* sp. nov., two new species from Iceland

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**Several yellow-pigmented isolates, with optimum growth temperatures of about 65–70 °C, were recovered from hot springs in Iceland. Phylogenetic analysis of the 16S rDNA and DNA–DNA reassociation values showed that these organisms represented two new species of the genus *Thermus*. Strains RF-4$^T$ and HN1-8 had maximum temperatures for growth below 80 °C, while strains HN3-7$^T$ and HN2-7, unlike all other strains of the species of the genus *Thermus* except those belonging to *Thermus thermophilus*, grew at 80 °C. The new isolates from Iceland could not be distinguished easily from each other or from other strains of the species of the genus *Thermus* by biochemical characteristics; however, strains RF-4$^T$ and HN1-8 assimilated ribitol, a characteristic which was not detected in any of the other strains examined. Moreover, the species represented by strains RF-4$^T$ and HN1-8 and the species represented by strains HN3-7$^T$ and HN2-7 could be distinguished clearly from the other species of *Thermus* by their fatty acid composition. Strains RF-4$^T$ and HN1-8 have the highest combined levels of iso-15:0 and iso-17:0 and the lowest levels of iso-16:0 of any of the strains of the species of *Thermus*, while strains HN3-7$^T$ and HN2-7 are characterized by a very low iso-15:0/iso-17:0 ratio. On the basis of the phylogenetic analysis, DNA–DNA reassociation values, physiological and biochemical characteristics and fatty acid composition, the name *Thermus igniterrae* sp. nov. is proposed for the species represented by strains RF-4$^T$ and HN1-8 and the name *Thermus antranikianii* sp. nov. is proposed for the species represented by strains HN3-7$^T$ and HN2-7.**

**Keywords:** *Thermus igniterrae*, *Thermus antranikianii*, *Bacteria*, thermophiles

## INTRODUCTION

Strains of the species of the genus *Thermus* have been isolated from numerous hydrothermal areas throughout the world with water temperatures higher than about 55 °C and pH ranging from neutral to alkaline (Kristjansson & Alfredsson, 1983; Williams & da Costa, 1992). Many strains also originate from artificial thermal environments (Brock & Boylen, 1969; Degryse *et al*., 1978; Kristjansson *et al*., 1994) and some strains have also been isolated from abyssal geothermal sites (Marteinsson *et al*., 1995). Six species of the genus *Thermus* have been validly described. These are *Thermus aquaticus* (Brock & Freeze, 1969), *Thermus thermophilus* (Oshima & Imahori, 1974; Manaia *et al*., 1994; Williams *et al*., 1995), *Thermus filiformis* (Hudson *et al*., 1987), *Thermus scotoductus* (Kristjansson *et al*., 1994), *Thermus brockianus* (Williams *et al*., 1995) and *Thermus oshimai* (Williams *et al*., 1996).

Elucidation of the taxonomy of the genus *Thermus* has been hindered for several reasons: the number of strains used to describe some species is small and is sometimes limited to one isolate and the number of phenotypic characteristics used to describe the species is, in many cases, also small. Moreover, the large phenotypic diversity within closely related isolates, which presumably belong to the same species, inevitably leads to the description of species without

The EMBL accession numbers for the *Thermus* sp. 16S rDNA sequences determined in this study are Y18406–Y18416.

representative phenotypes or characteristics that can be used to distinguish the species from one another. For example, *T. filiformis* was described on the basis of one naturally filamentous strain that contains large relative proportions of anteiso fatty acids (Hudson *et al.*, 1987; Ferraz *et al.*, 1994). However, other strains from New Zealand assigned to this species on the basis of DNA–DNA reassociation values are not filamentous and contain large proportions of iso fatty acids (Georganta *et al.*, 1993; Ferraz *et al.*, 1994). The species *T. aquaticus* and *T. brockianus*, originally recovered from Yellowstone National Park, can be distinguished easily from each other (Brock & Freeze, 1969; Munster *et al.*, 1986; Williams *et al.*, 1995). However, when biochemical and physiological characteristics of these organisms are compared with those of other species and with strains of *T. brockianus* from other geothermal areas, the distinctiveness of these species breaks down, even though there is no doubt that these two species constitute distinct genospecies. On the other hand, two species of the genus *Thermus* have characteristics that distinguish them from other species of the genus. *T. aquaticus* can be distinguished from all other species of the genus *Thermus* by its fatty acid composition since, at present, it is the only species that contains moderate levels of 3-hydroxy iso fatty acids. However, all known isolates originate from Yellowstone National Park and may be very closely related (Nobre *et al.*, 1996). Strains of *T. thermophilus* can also be distinguished from all other species of the genus *Thermus* by their ability to grow at temperatures above 80 °C and in medium containing 2–3% NaCl (Manaia & da Costa, 1991; Manaia *et al.*, 1994).

In this paper, we report the description of two new species of the genus *Thermus* isolated from hot springs in Iceland. At the phenotypic level, the two new species can only be distinguished clearly from other species on the basis of their fatty acid composition and by the ability of the strains of one of the new species to grow at 80 °C. However, at the genetic level, DNA–DNA reassociation values and phylogenetic analyses of the 16S rRNA gene sequences show that the new isolates belong to two species that have not been described previously. On the basis of the results presented in this study, we propose the names *Thermus igniterrae* sp. nov. for strains RF-4$^T$ and HN1-8 and *Thermus antranikianii* sp. nov. for strains HN3-7$^T$ and HN2-7.

## METHODS

**Isolation and bacterial strains.** Strains RF-4$^T$, HN1-8, GE-2, HN3-7$^T$, HN2-7, HN2-3, HN3-10 and HE-5 were isolated from hot springs within several geothermal areas in eastern and south-eastern Iceland. Water samples were transported without temperature control and filtered through membrane filters (Gelman type GN-6; pore size 0·45 μm, diameter 47 mm), which were placed on the surface of *Thermus* agar plates (Williams & da Costa, 1992). These preparations were wrapped in plastic bags and incubated at 70 °C for up to 7 d. Cultures were purified by subculturing and were preserved at −80 °C in *Thermus* medium containing 15% glycerol. Several strains of the species of the genus *Thermus* were used for comparative purposes and their sources are listed in Table 1. *T. scotoductus* strain X-1 (ATCC 27978) occurs as two colony variants that differ in their polar lipid composition (Tenreiro *et al.*, 1995; Wait *et al.*, 1997). Colony type t1 has the usual polar lipid composition of strains of the genus *Thermus* and was used here for comparative purposes.

**Morphological, biochemical and tolerance characteristics.** Cell morphology and motility were examined by phase-contrast microscopy during the exponential growth phase in *Thermus* medium. Cell dimensions were determined with an ocular micrometer calibrated with a stage micrometer. Unless otherwise stated, all biochemical and tolerance tests were performed as described previously (Santos *et al.*, 1989; Manaia & da Costa, 1991) in *Thermus* liquid medium or on *Thermus* agar, incubated at 70 °C for up 5 d. Catalase activity was determined by the formation of bubbles with a 3% hydrogen peroxide solution and oxidase activity was determined by the oxidation of 1% aqueous tetramethyl *p*-phenylenediamine on filter paper at room temperature. The growth temperature ranges of strains RF-4$^T$ and HN3-7$^T$ and the type strains of *T. brockianus* and *T. scotoductus* were examined by measuring the turbidity ($OD_{610}$) of cultures incubated in 300 ml metal-capped Erlenmeyer flasks containing 100 ml *Thermus* medium in a reciprocal water-bath shaker. Growth of all strains was also assessed in 20 ml metal-capped tubes containing 5 ml *Thermus* medium placed in water-bath incubators at 78, 80 and 82 °C for 5 d. The pH range for growth was examined at 70 °C in the same medium by using 30 mM MES for pH values between 5·0 and 6·5, 30 mM Tris for pH values between 7·0 and 8·5 and 30 mM CAPSO for pH values between 9·0 and 10·5; the pH of each buffer was adjusted with HCl or NaOH. The pH values were determined at room temperature. Control media containing each buffer adjusted to pH 8·2 were used to assess possible inhibitory effects of the buffering agents.

Single-carbon-source assimilation tests were performed in a minimal medium composed of *Thermus* basal salts with yeast extract (0·1 g $l^{-1}$) and without nitrate, to which filter-sterilized ammonium sulphate (0·5 g $l^{-1}$) and the carbon sources (2·0 g $l^{-1}$) were added. Growth was examined by measuring the turbidity of cultures incubated at 70 °C in 20 ml screw-capped tubes containing 10 ml medium after 5 d growth. Positive and negative control cultures were grown in *Thermus* medium and in minimal medium without a carbon source, respectively.

**Polar lipid and fatty acid composition.** The cultures used for polar lipid analysis were grown in 1 l Erlenmeyer flasks containing 200 ml *Thermus* medium at 70 °C in a reciprocal water-bath shaker until the exponential phase of growth. Harvesting of the cultures and the extraction of lipids was performed as described previously (Prado *et al.*, 1988; Donato *et al.*, 1990). The individual polar lipids were separated by one-dimensional TLC on silica gel G plates (Merck; 0·25 mm thick) with a solvent system consisting of chloroform:methanol:acetic acid:water (80:12:15:4 by vol.).

Cultures for fatty acid analysis were grown on *Thermus* medium plates in sealed plastic bags submerged in a water bath at 70 °C for 24 h. Fatty acid methyl esters were obtained from fresh wet biomass by saponification, methylation and extraction as described previously (Kuykendall *et al.*, 1988). The fatty acid methyl esters were separated by using a Hewlett Packard model 5890 GC equipped with a flame-

***Table 1.*** *Thermus* strains used in this study

| Strain | Source | Site of isolation | Reference |
|---|---|---|---|
| *T. aquaticus* | | | |
| YT-1$^T$ (= ATCC 25104$^T$) | ATCC | Yellowstone NP, USA | Brock & Freeze (1969) |
| YS031 | R. Sharp | Yellowstone NP, USA | Munster *et al.* (1986) |
| *T. thermophilus* | | | |
| HB8$^T$ (= ATCC 27634$^T$) | ATCC | Mine, Japan | Oshima & Imahori (1974) |
| B (= NCIMB 11247) | R. A. D. Williams | Hruni, Iceland | Pask-Hughes & Williams (1977) |
| *T. filiformis* | | | |
| Wai33.A1$^T$ (= ATCC 43280$^T$) | ATCC | Waimangu, New Zealand | Hudson *et al.* (1987) |
| *T. scotoductus* | | | |
| ITI-252$^T$ (= DSM 8553$^T$) | J. K. Kristjansson | Selfoss, Iceland | Kristjansson *et al.* (1994) |
| X-1 (= ATCC 27978) | ATCC | Bloomington, USA | Ramaley & Hixson (1970) |
| *T. brockianus* | | | |
| YS038$^T$ (= NCIMB 12676$^T$) | NCIMB | Yellowstone NP, USA | Williams *et al.* (1995) |
| D13-1 | R. Sharp | Kamchatka, Russia | Unpublished |
| *T. oshimai* | | | |
| SPS-17$^T$ (= NCIMB 13400$^T$) | Our isolate | S. Pedro do Sul, Portugal | Williams *et al.* (1996) |
| JK-91 | R. A. D. Williams | Hveragerdi, Iceland | Williams (1989) |

NP, National Park.

ionization detector fitted with a 5% phenylmethyl silicone capillary column (0·2 mm × 25 m; Hewlett Packard). The carrier gas was high-purity $H_2$, the column head pressure was 60 kPa, the septum purge was 5 ml min$^{-1}$, the column split ratio was 55:1 and the injection port temperature was 300 °C. The temperature of the oven was programmed from 170 to 270 °C at a rate of 5 °C min$^{-1}$. Identification and quantification of the fatty acid methyl esters and numerical analysis of the fatty acid profiles were performed by using the standard MIS Library Generation Software (Microbial ID Inc.).

**Determination of mean base composition of DNA and DNA–DNA reassociation studies.** The DNA used to determine DNA base compositions was isolated as described by Cashion *et al.* (1977). The G+C content of the overall genome was determined by HPLC as described by Mesbah *et al.* (1989). DNA for DNA–DNA reassociation studies was extracted and purified by the procedure of Marmur (1961). The degree of DNA reassociation was determined spectrophotometrically from the initial renaturation rates, according to De Ley *et al.* (1970). The renaturation rates were measured in 1 × SSC (0·15 M NaCl, 0·015 M trisodium citrate, pH 7·0) by using a Uvikon 940 spectrophotometer (Kontron) equipped with a thermostat-controlled cuvette chamber. The optimal renaturation temperature used in each case was calculated from the G+C content (De Ley *et al.*, 1970). Each hybridization experiment was executed at least twice.

**16S rRNA gene sequence determination and phylogenetic analyses.** The extraction of genomic DNA, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described previously (Rainey *et al.*, 1996). Sequence reaction products were purified by ethanol precipitation and electrophoresed with a model 373A or a model 310 Genetic Analyzer (Applied Biosystems). The 16S rRNA sequences obtained in this study were aligned against previously determined *Meiothermus*/*Thermus* sequences available from the public databases by using the ae2 editor (Maidak *et al.*, 1994). The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were generated by using various treeing algorithms contained in the PHYLIP package (De Soete, 1983; Felsenstein, 1993).

**Nucleotide sequence accession numbers.** The 16S rRNA gene sequences determined in this study are deposited with EMBL under the following accession numbers: Y18406 (strain RF-4$^T$), Y18407 (HN1-8), Y18408 (GE-2), Y18411 (HN3-7$^T$), Y18415 (HN2-7), Y18414 (HN2-3), Y18413 (HN3-10), Y18412 (HE-5), Y18409 (*T. brockianus* YS038$^T$), Y18410 (*T. scotoductus* ITI-252$^T$) and Y18416 (*T. oshimai* SPS-17$^T$). The accession numbers and strain designations of the reference 16S rRNA gene sequences used in the phylogenetic analyses are as follows: *T. aquaticus* YT-1$^T$ (L09663), *T. thermophilus* HB8$^T$ (X07998), *T. filiformis* Wai33.A1$^T$ (L09667), strain ZF1.A2 (L09662) and *Meiothermus ruber* DSM 1279$^T$ (L09672).

## RESULTS

### Isolation of strains, morphological and biochemical characteristics

Strains with designations beginning with HN were isolated from the hot spring and run-offs at Hruni, Iceland, where the water temperature varied between 80 and 88 °C and the pH was 9·1. Strain HE-5 was isolated from the hot spring at Hellur, Iceland, with a water temperature of 86 °C and a pH of 9·0. Strain RF-4$^T$ was isolated from a hot spring with a water temperature of 73 °C and a pH of 8·9 at Reykyaflot, Iceland, and strain GE-2 was isolated from a hot spring within the Geysir geothermal area, Iceland, with a water temperature of 90 °C and pH of 9·0.

All strains formed yellow-pigmented colonies, like most other strains of the genus *Thermus*, and stained

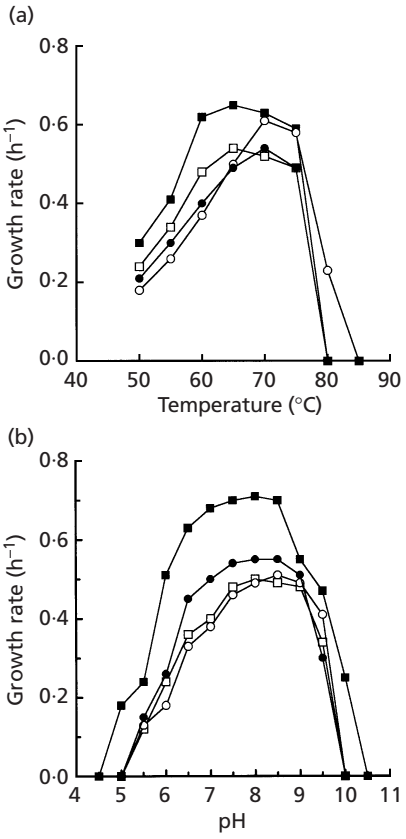


***Fig. 1.*** Effects of temperature (a) and pH (b) on the growth of *T. scotoductus* ITI-252$^T$ (●), HN3-7$^T$ (○), *T. brockianus* YS038$^T$ (■) and RF-4$^T$ (□).

Gram-negative. Filaments and short rods were seen by phase-contrast microscopy. The strains were not motile and did not produce spores. The optimum growth temperature was about 65 °C for strain RF-4$^T$ and about 70 °C for strain HN3-7$^T$ (Fig. 1a). Growth did not occur at or below 45 °C. The maximum growth temperature of strains HN3-7$^T$ and HN2-7 was about 80 °C, while strains RF-4$^T$ and HN1-8 did not grow at this temperature. Of the type and reference strains examined, only the two strains of *T. thermophilus* grew at 80 and at 82 °C. Strains RF-4$^T$ and HN3-7$^T$ had a pH range of growth from about 5·0 or 5·5 to 9·5 or 10·0, with an optimum pH for growth between 7·5 and 8·5 (Fig. 1b).

The new isolates had many biochemical characteristics in common (Table 2). Nevertheless, some characteristics distinguished the two groups of strains. For example, strains RF-4$^T$ and HN1-8 hydrolysed elastin but were α-galactosidase-negative, while strains HN3-7$^T$ and HN2-7 did not hydrolyse elastin but produced α-galactosidase. Some differences in the assimilation of single carbon sources were found among the isolates, reference strains and type strains of the species of the genus *Thermus*. Strains RF-4$^T$ and HN1-8 assimilated fewer single carbon sources than all other strains examined. Moreover, only these isolates, of all the organisms examined, assimilated ribitol. The carbon-source utilization of strains HN3-7$^T$ and HN2-7 closely resembled that of the other *Thermus* strains, the only difference being the utilization of malate as a single carbon source by these isolates.

### Polar lipids and fatty acid composition

The polar lipids of the new isolates had the canonical pattern, on TLC, found in all *Thermus* species. One major phospholipid (PL-2) and one major glycolipid (GL-1), along with a minor glycolipid (GL-2) and phospholipid (PL-1), dominated the polar lipid fraction of these isolates (results not shown).

Branched-chain iso and anteiso fatty acids were the predominant aliphatic components of the polar lipids of all organisms examined in this study (Table 3). The fatty acid composition of strains RF-4$^T$, HN1-8 and GE-2 was characterized by very large relative proportions of iso-15:0, reaching about 50% of the total, and iso-17:0, which reached levels of about 31%, and by the extremely low levels of iso-16:0, which did not exceed 1% of the total acyl chains. None of the other strains examined in this or a previous study (Nobre *et al*., 1996) had such high combined levels of iso-15:0 and iso-17:0 or such low levels of iso-16:0. Strains HN3-7$^T$, HN2-7, HN2-3 and HE-5 could be distinguished from all other strains of the species of the genus *Thermus* by very high levels of iso-17:0 (40–50%) and relatively low levels of iso-15:0 (10–15%). The type strain of *T. filiformis* and *T. thermophilus* strain B also had low levels of iso-15:0 (4–6%), but had very high levels of anteiso-branched fatty acids, which can easily distinguish them from all other *Thermus* species. It was noteworthy that the fatty acid composition of strain D13-1 had the highest relative proportion of iso-15:0 of all strains of the genus *Thermus* examined so far (Nobre *et al*., 1996).

### 16S rRNA gene sequence comparison

Almost-complete 16S rRNA gene sequences of between 1462 and 1475 nucleotides were determined for the eight new isolates and the type strains of the species *T. brockianus*, *T. scotoductus* and *T. oshimai*. Phylogenetic analyses based on a dataset consisting of 1414 unambiguous nucleotides between positions 33 and 1516 (*Escherichia coli* positions; Brosius *et al*., 1978) showed the new isolates to fall into two distinct clusters within the radiation of the genus *Thermus* (Fig. 2). One cluster contained strains RF-4$^T$, HN1-8 and GE-2 while strains HN3-7$^T$, HE-5, HN3-10, HN2-3 and HN2-7 formed the second cluster (Fig. 2). The 16S rRNA gene sequence similarity values showed that, within each strain cluster, the 16S rRNA gene sequences were highly similar (99·9%) or identical. However, only 95·9–96·2% 16S rRNA gene sequence similarity was seen between the strains of the two clusters. Strains RF-4$^T$, HN1-8 and GE-2 had identical

***Table 2.*** Biochemical characteristics that distinguish strains and species of the genus *Thermus*

Tests are scored as negative (−), positive (+) or weak (w). All organisms were catalase- and cytochrome oxidase-positive; grew in medium containing 1% NaCl and 0·1% tellurite and degraded *p*-nitrophenyl α-glucopyranoside, palmitate, laurate, hippurate, hide-powder azure and Tweens 20, 40 and 60. D-Mannose, maltose, pyruvate, L-glutamine, L-glutamate and L-proline were utilized by all strains. None of the strains hydrolysed xylan, grew in medium containing 5 or 6% NaCl or utilized D-mannitol, D-sorbitol, xylitol, erythritol, L-rhamnose, L-arabinose or L-fucose.

| Characteristic | *T. aquaticus* | | *T. thermophilus* | | *T. filiformis* Wai33.A1$^T$ | *T. scotoductus* | | *T. brockianus* | | *T. oshimai* | | *T. igniterrae* | | *T. antranikianii* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | YT-1$^T$ | YS031 | HB8$^T$ | B | | ITI-252$^T$ | X1-t1 | YS038$^T$ | D13-1 | SPS-17$^T$ | JK-91 | RF-4$^T$ | HN1-8 | HN3-7$^T$ | HN2-7 |
| Pigmentation | Yellow | Yellow | Yellow | Yellow | Yellow | White | White | Yellow | Yellow | Yellow | Yellow | Yellow | Yellow | Yellow | Yellow |
| Presence of: | | | | | | | | | | | | | | | |
| α-Galactosidase | − | − | + | + | + | + | + | + | + | + | − | − | − | + | + |
| β-Galactosidase | − | − | + | + | + | − | + | + | + | + | + | + | + | + | + |
| Degradation of *p*-nitrophenyl substrates: | | | | | | | | | | | | | | | |
| β-Glucopyranoside | + | − | + | + | + | − | + | + | + | + | + | + | + | + | + |
| Degradation of: | | | | | | | | | | | | | | | |
| Arbutin | w | w | + | + | + | w | + | + | + | + | + | + | + | + | + |
| Aesculin | − | − | + | + | + | w | + | + | + | + | + | + | + | + | w |
| Hydrolysis of: | | | | | | | | | | | | | | | |
| Elastin | + | + | − | − | − | − | + | − | − | + | + | + | + | − | − |
| Starch | + | + | + | + | + | + | + | − | − | + | + | + | + | + | + |
| Fibrin | + | + | − | + | − | + | + | + | + | + | + | + | + | − | + |
| Gelatin | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + |
| Casein | + | + | + | + | + | + | + | − | − | + | + | + | + | + | + |
| Tween 80 | − | + | + | w | − | − | − | + | + | + | w | + | − | + | + |
| Reduction of nitrate | − | − | − | + | − | + | + | + | + | + | − | + | − | + | + |
| Growth at/in: | | | | | | | | | | | | | | | |
| 80 °C | − | − | + | + | − | − | − | − | − | − | − | − | − | + | + |
| 82 °C | − | − | + | + | − | − | − | − | − | − | − | − | − | − | − |
| 2% NaCl | + | − | + | + | − | − | − | − | − | w | + | − | − | − | − |
| 3% NaCl | w | − | + | + | − | − | − | − | − | − | − | − | − | − | − |
| 4% NaCl | − | − | + | + | − | − | − | − | − | − | − | − | − | − | − |
| Utilization of: | | | | | | | | | | | | | | | |
| D-Glucose | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + |
| D-Fructose | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + |
| D-Galactose | − | + | − | + | + | + | − | − | − | + | − | − | − | + | + |
| L-Sorbose | − | − | − | − | − | − | + | − | − | − | w | − | − | − | − |
| D-Xylose | − | − | + | − | − | − | − | + | + | − | − | − | − | − | − |
| D-Ribose | − | − | − | − | − | − | + | − | + | − | − | − | − | − | + |
| D-Cellobiose | w | + | + | + | + | − | − | + | + | − | + | − | − | + | + |
| D-Melibiose | − | − | − | − | + | − | − | + | + | + | − | − | − | − | − |
| D-Trehalose | + | w | + | + | + | + | − | + | + | + | + | + | − | − | − |
| Sucrose | + | − | + | − | + | + | − | + | + | + | + | + | − | − | − |
| Lactose | − | w | − | + | + | − | − | + | + | + | − | − | − | + | − |
| D-Raffinose | − | − | − | − | + | − | − | + | + | + | − | − | − | − | − |
| Glycerol | − | − | + | + | − | + | + | − | − | − | − | w | − | − | + |
| Ribitol | − | − | − | − | − | − | − | − | − | − | − | + | + | − | − |
| *myo*-Inositol | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − |
| Citrate | − | − | + | + | − | + | + | + | + | − | + | − | − | − | w |
| Malate | − | − | − | − | − | w | − | − | − | − | − | − | − | + | + |
| L-Asparagine | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + |
| L-Serine | + | + | + | + | − | + | + | + | + | − | + | − | − | + | + |
| L-Arginine | + | + | − | + | − | − | + | + | + | − | + | − | − | − | + |

16S rRNA gene sequences and shared 99·9% sequence similarity with the previously isolated strain ZF1.A2 (Hudson *et al*., 1989), the 16S rRNA gene sequence of which has been described previously (Saul *et al*., 1993), and showed 97·3% similarity to the species *T. brockianus* and *T. aquaticus*. Four of the isolates of the second cluster, strains HE-5, HN3-10, HN2-3 and HN2-7, had identical 16S rRNA gene sequences and shared 99·9% sequence similarity with strain HN3-7$^T$. These strains showed highest 16S rRNA gene sequence similarity, 98·0–98·2%, to the sequence of *T. scotoductus*. The 16S rRNA gene sequence similarity values between the new isolates and the other validly described *Thermus* species, namely *T. thermophilus*, *T. filiformis* and *T. oshimai*, were in the range 92·3–95·4%. *Meiothermus ruber*, which was used as the outgroup in the phylogenetic analyses (Fig. 2), showed less than 86·9% 16S rRNA gene sequence similarity to any of the *Thermus* strains included.

### Mean base composition of DNA and DNA–DNA reassociation studies

The G+C content of the DNA of strains RF-4$^T$ and HN3-7$^T$ was 70·3 and 65·4 mol%, respectively. The distinct species status of strains RF-4$^T$ and HN1-8 on the one hand and strains HN3-7$^T$, HN2-7 and HN2-3 on the other was demonstrated by values for DNA–DNA reassociation with the type strains and representative strains of each species of the genus *Thermus*

***Table 3.*** Fatty acid composition of *Thermus* strains after growth at 70 °C

Percentages of total fatty acid content are shown. Values for fatty acids present at less than 0·5% in all strains are not shown. i, Iso; a, anteiso.

| Strain | 13 : 0i | 14 : 0i | 14 : 0 | 15 : 0i | 15 : 0a | 15 : 0 | 16 : 0i | 16 : 0 | ECL 16·09* | 15 : 0i 3-OH | 17 : 0i | 17 : 0a | 17 : 0 | 16 : 0i 3-OH | 16 : 0 3-OH | 18 : 0i | 17 : 0i 3-OH | 17 : 0a 3-OH | 19 : 0a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. aquaticus* | | | | | | | | | | | | | | | | | | | |
| YT-1$^T$ | – | 1·0 | 1·4 | 19·3 | 2·1 | – | 13·4 | 16·2 | – | 3·2 | 24·9 | 2·6 | – | 2·6 | 2·3 | 0·6 | 7·6 | 0·8 | – |
| YS031 | – | – | – | 22·0 | 1·6 | – | 9·8 | 7·9 | – | 3·1 | 38·7 | 2·9 | – | 1·6 | – | – | 10·0 | 0·6 | – |
| *T. thermophilus* | | | | | | | | | | | | | | | | | | | |
| HB8$^T$ | – | 0·7 | – | 32·4 | 4·3 | – | 5·3 | 10·0 | 0·7 | – | 41·4 | 5·1 | – | – | – | – | – | – | – |
| B | – | 0·8 | – | 5·5 | 8·2 | – | 13·9 | 6·6 | 1·9 | – | 25·0 | 31·1 | – | – | – | 3·4 | – | – | 1·6 |
| *T. filiformis* | | | | | | | | | | | | | | | | | | | |
| Wai33.A1$^T$ | – | 0·9 | – | 4·0 | 17·9 | – | 8·7 | 4·1 | 4·0 | – | 6·3 | 35·5 | – | 0·9 | – | 1·0 | 2·3 | 8·6 | 1·0 |
| *T. scotoductus* | | | | | | | | | | | | | | | | | | | |
| ITI-252$^T$ | – | – | – | 17·9 | 13·8 | 1·0 | 1·6 | 8·6 | 2·4 | – | 30·3 | 22·1 | 1·3 | – | – | – | – | – | 0·6 |
| X1-t1 | – | 1·7 | – | 19·5 | 4·5 | 0·9 | 23·7 | 8·7 | – | – | 30·0 | 6·7 | – | – | – | 2·3 | – | – | – |
| *T. brockianus* | | | | | | | | | | | | | | | | | | | |
| YS038$^T$ | 0·7 | 1·3 | 0·7 | 31·8 | 2·5 | 0·8 | 11·0 | 12·3 | – | – | 35·2 | 2·8 | – | – | – | 0·6 | – | – | – |
| D13-1 | 0·6 | 1·1 | 1·0 | 58·6 | 4·8 | 1·1 | 6·7 | 9·5 | 1·1 | – | 12·3 | 3·1 | – | – | – | – | – | – | – |
| *T. oshimai* | | | | | | | | | | | | | | | | | | | |
| SPS-17$^T$ | 0·7 | – | – | 36·2 | 2·9 | 3·1 | 2·5 | 8·8 | – | – | 38·3 | 3·2 | 2·1 | – | – | – | – | – | – |
| JK-91 | 0·9 | – | – | 40·2 | 2·9 | 1·6 | 2·8 | 10·5 | – | – | 35·6 | 3·1 | 0·8 | – | – | – | – | – | – |
| *T. igniterrae* | | | | | | | | | | | | | | | | | | | |
| RF-4$^T$ | 1·1 | – | – | 50·7 | 2·9 | 1·3 | 1·0 | 9·0 | – | – | 31·1 | 1·9 | – | – | – | – | – | – | – |
| HN1-8 | 1·9 | – | – | 49·4 | 2·5 | 1·7 | 0·9 | 8·9 | – | – | 31·9 | 1·5 | 1·0 | – | – | – | – | – | – |
| GE-2 | 1·0 | – | – | 49·5 | 2·7 | 2·2 | 1·4 | 8·9 | – | – | 30·2 | 1·8 | 0·8 | – | – | – | – | – | – |
| *T. antranikianii* | | | | | | | | | | | | | | | | | | | |
| HN3-7$^T$ | – | – | – | 10·8 | 1·7 | 1·9 | 9·6 | 11·9 | – | – | 51·0 | 6·2 | 3·1 | – | – | 1·4 | – | – | – |
| HN2-7 | – | 0·7 | – | 13·8 | 2·5 | 0·8 | 18·9 | 10·0 | 0·6 | – | 43·1 | 6·0 | 0·6 | – | – | 1·4 | – | – | – |
| HN2-3 | 0·6 | 0·7 | – | 14·9 | 2·9 | 0·8 | 17·6 | 11·0 | 0·8 | – | 40·4 | 7·1 | – | – | – | 1·3 | – | – | – |
| HE-5 | – | – | – | 12·7 | 1·8 | 2·7 | 8·8 | 12·2 | 0·8 | – | 49·0 | 5·6 | 4·4 | – | – | 1·1 | – | – | – |

*Unknown fatty acid or alcohol with an equivalent chain-length (ECL) of 16·090.

(Table 4). The DNA–DNA reassociation values of strain RF-4$^T$ were low with all strains except strain HN1-8, while strains HN3-7$^T$, HN2-7 and HN2-3 had the highest DNA–DNA reassociation values with each other, in the range 86–91%. Strain D13-1 from the Kamchatka Peninsula was also shown to have a very high DNA–DNA reassociation value (83%) with the type strain of *T. brockianus* from Yellowstone National Park.

## DISCUSSION

Biochemical and physiological characteristics are not generally useful for distinguishing species of the genus *Thermus* from each other. In fact, most species cannot be distinguished from each other on the basis of these characteristics and their description has been based, almost entirely, on DNA–DNA reassociation values (Williams *et al*., 1995, 1996).

Fatty acid analysis has been used successfully in the description and identification of many species of bacteria, but it is too difficult to use in distinguishing the species of this genus. The strains assigned to the species *T. thermophilus*, for example, have variable fatty acid compositions even though some strains originate from hot springs in the same general area; strains HB8, HB27, AT-62 and GK-24 from Japan are notorious in this respect (Nobre *et al*., 1996). It should also be noted that strain D13-1 from the Kamchatka Peninsula belongs to *T. brockianus* because of the high DNA–DNA reassociation value with the type strain and the very similar biochemical and physiological characteristics. However, the fatty acid composition of strain D13-1 is radically different from all isolates of this species from Yellowstone National Park. The large diversity of phenotypic characteristics within each species is difficult to explain. However, extensive DNA–DNA reassociation studies and 16S rRNA gene

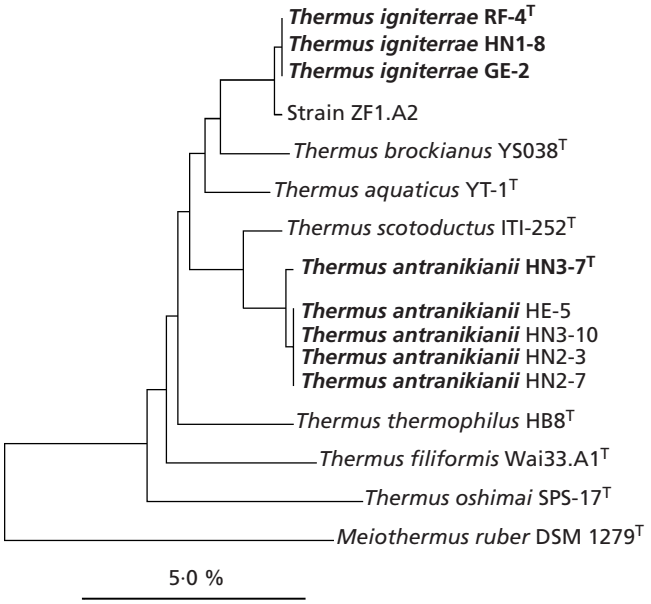


***Fig. 2.*** Phylogenetic dendrogram based on 16S rRNA gene sequence comparisons. The dendrogram was reconstructed from evolutionary distances by using the neighbour-joining method. The scale bar represents five inferred nucleotide changes per 100 nucleotides.

sequence analyses indicate that the species of the genus *Thermus* are composed of closely related strains that are, nevertheless, phenotypically variable (Saul *et al*., 1993; A. P. Chung, M. F. Nobre, M. S. da Costa & F. A. Rainey, unpublished results).

The description of species of the genus *Thermus* should include isolates from several geographical areas, but this has not generally been the case because isolates of new species are sometimes limited to one geographical area; *T. aquaticus* and *T. filiformis*, for example, have only been isolated from Yellowstone National Park and from New Zealand, respectively. The same is true for the isolates described in this study, which we propose represent two new species of the genus *Thermus*. Physiological, biochemical and tolerance characteristics do not distinguish the two groups of Icelandic strains clearly from the other species of the genus *Thermus*, although the species represented by strains HN3-7$^T$ and HN2-7 grows at 80 °C. This is a very rare characteristic among species of the genus *Thermus*, only being present in strains of *T. thermophilus*, which can also grow at 82 °C. However, the fatty acid composition of isolates representing these two new species of the genus *Thermus* is, at present, fairly homogeneous and distinguishes these two groups of strains from each other and from all the other strains of this genus (Nobre *et al*., 1996). Strains RF-4$^T$, HN1-8 and GE-2 have the highest combined levels of iso-15:0 and iso-17:0 and the lowest levels of iso-16:0 of any strain of the species of the genus *Thermus* examined so far. On the other hand, strains HN3-7$^T$, HN2-7, HN2-3 and HE-5 can be distinguished from all other strains of the species of the genus *Thermus* by the very low iso-15:0/iso-17:0 ratio.

All the isolates of the two new species of *Thermus* originated from Iceland, but were recovered from different hot springs, indicating that the organisms are widespread and may not represent a single clone. Phylogenetic analyses show that these isolates represent two distinct lineages within the *Thermus* species group. Strains RF-4$^T$, HN1-8 and GE-2 are most closely related to *T. brockianus* and *T. aquaticus*, while strains HN3-7$^T$, HN2-7, HN2-3, HN3-10 and HE-5 are most closely related to *T. scotoductus*. Although these new isolates show more than 97% 16S rRNA gene sequence similarity to previously described *Thermus* species, the results of DNA–DNA reassociation studies show that the isolates represent two new species of the genus *Thermus*. On the basis of these

***Table 4.*** DNA–DNA reassociation values between *Thermus* strains

Values are means of at least two determinations. –, Not determined.

| Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *T. aquaticus* YT-1$^T$ | 100 | | | | | | | | | | | | |
| 2. *T. thermophilus* HB8$^T$ | 35 | 100 | | | | | | | | | | | |
| 3. *T. filiformis* Wai33.A1$^T$ | – | – | 100 | | | | | | | | | | |
| 4. *T. scotoductus* ITI-252$^T$ | 19 | 30 | 26 | 100 | | | | | | | | | |
| 5. *T. scotoductus* X1-t1 | 55 | – | – | 79 | 100 | | | | | | | | |
| 6. *T. brockianus* YS038$^T$ | – | – | – | – | – | 100 | | | | | | | |
| 7. *T. brockianus* D13-1 | – | – | – | – | 41 | 83 | 100 | | | | | | |
| 8. *T. oshimai* SPS-17$^T$ | 47 | 34 | – | 27 | – | – | 36 | 100 | | | | | |
| 9. *T. igniterrae* RF-4$^T$ | 38 | 57 | 32 | 34 | 59 | 34 | 40 | 39 | 100 | | | | |
| 10. *T. igniterrae* HN1-8 | – | – | – | – | – | 20 | 32 | – | 83 | 100 | | | |
| 11. *T. antranikianii* HN3-7$^T$ | 35 | 24 | 28 | 56 | 58 | 41 | 32 | 39 | 40 | – | 100 | | |
| 12. *T. antranikianii* HN2-7 | – | – | – | 55 | 54 | – | – | – | – | – | 91 | 100 | |
| 13. *T. antranikianii* HN2-3 | – | – | – | 41 | 50 | – | – | – | – | – | 86 | 94 | 100 |

findings, we propose that strains RF-4$^T$ and HN1-8 represent a new species of the genus *Thermus*, for which we propose the name *Thermus igniterrae* sp. nov., and that strains HN3-7$^T$ and HN2-7 represent another novel species of this genus, for which we propose the name *Thermus antranikianii* sp. nov.

### Description of *Thermus igniterrae* sp. nov.

*Thermus igniterrae* (ig.ni.ter′rae. L. n. *ignis* fire; L. fem. n. *terra* land/earth; M. L. gen. fem. n. *igniterrae* of the land of fire, referring to Iceland).

*T. igniterrae* strains form rod-shaped cells of variable length that are 0·5–0·8 μm wide. Filaments are also present. Gram stain is negative. The cells are non-motile and spores are not formed. Colonies on *Thermus* medium are yellow-pigmented and 1–2 mm in diameter after 72 h growth. Growth occurs above 45 °C and below 80 °C; the optimum growth temperature for strain RF-4$^T$ is about 65 °C. The optimum pH is between 7·5 and 8·5; growth does not occur at pH 5·0 or pH 10·0. The major fatty acids are 15:0 iso and 17:0 iso; 3-OH fatty acids are not present. All strains are oxidase-positive and catalase-positive. Strain RF-4$^T$ reduces nitrate to nitrite, while strain HN1-8 does not; α-galactosidase is negative and β-galactosidase is positive. Elastin, starch, fibrin, casein, gelatin and hide-powder azure are hydrolysed. Arbutin and aesculin are degraded. Strains RF-4$^T$ and HN1-8 utilize D-glucose, D-fructose, D-mannose, maltose, ribitol, pyruvate, L-glutamate, L-glutamine and L-proline. Strains RF-4$^T$ and HN1-8 do not utilize D-galactose, D-melibiose, D-cellobiose, D-raffinose, D-xylose, D-ribose, lactose, D-mannitol, D-sorbitol, xylitol, erythritol, glycerol, malate, citrate, *myo*-inositol, L-sorbose, L-fucose, L-arabinose, L-rhamnose, L-serine or L-arginine.

The DNA of strain RF-4$^T$ has a G+C content of 70·3 mol%. This bacterium was isolated from hot springs at Reykyaflot in Iceland. Strain RF-4$^T$ has been deposited in the DSMZ as strain DSM 12459$^T$. Strain HN1-8 (DSM 12460) is an additional strain of this species.

### Description of *Thermus antranikianii* sp. nov.

*Thermus antranikianii* (an.tra.ni.ki.a′ni.i. M.L. adj. *antranikianii* in honour of Garabed Antranikian, for his contribution to our knowledge of thermophilic and hyperthermophilic organisms).

*T. antranikianii* strains form rod-shaped cells of variable length that are 0·5–0·8 μm wide. Filaments are also present. Gram stain is negative. The cells are non-motile and spores are not formed. Colonies on *Thermus* medium are yellow-pigmented and 1–2 mm in diameter after 72 h growth. Growth occurs above 50 °C and at 80 °C; the optimum growth temperature for strain HN3-7$^T$ is about 70 °C. The optimum pH is between 7·5 and 8·5; growth does not occur at pH 4·5 or pH 10·5. The major fatty acids are 17:0 iso, 16:0 iso and 15:0 iso; 3-OH fatty acids are not present. All strains are oxidase-positive and catalase-positive. Nitrate is reduced to nitrite. α- and β-galactosidase-positive. Starch, casein, hide-powder azure and Tween 80 are hydrolysed. Degradation of arbutin and aesculin is positive. Strains HN3-7$^T$ and HN2-7 do not hydrolyse elastin. Strains HN3-7$^T$ and HN2-7 utilize D-glucose, D-fructose, D-mannose, D-galactose, D-cellobiose, maltose, pyruvate, malate, L-glutamate, L-asparagine, L-glutamine, L-serine and L-proline. Strains HN3-7$^T$ and HN2-7 do not utilize D-xylose, D-melibiose, D-trehalose, D-raffinose, sucrose, D-mannitol, D-sorbitol, ribitol, xylitol, erythritol, *myo*-inositol, L-sorbose, L-arabinose, L-rhamnose or L-fucose.

The DNA of strain HN3-7$^T$ has a G+C content of 65·4 mol%. This bacterium was isolated from hot springs at Hruni in Iceland. Strain HN3-7$^T$ has been deposited in the DSMZ as strain DSM 12462$^T$. Strain HN2-7 (DSM 12461) is an additional strain of this species.

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