Thermus islandicus sp. nov., a mixotrophic sulfur-oxidizing bacterium isolated from the Torfajokull geothermal area

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Strains PRI 2268 and PRI 3838^T were isolated from two separate hot springs in the Torfajokull geothermal area of South Iceland. The cells were non-motile rods, approximately 0.3 µm in width and 1.5–2.5 µm in length. Electron microscopy revealed a Gram-negative cell-wall structure. The strains grew at 45–79 °C (optimum, 65 °C) and pH 5.5–10.5 (optimum, pH 6.0–7.0). 16S rRNA gene sequence analysis indicated that they formed a separate branch within the genus *Thermus* with '*Thermus kavarayensis*' KW11 as their closest cultured relative (96.5 % similarity). The gene sequence similarities of both new isolates to *Thermus aquaticus* YT-1^T and *Thermus igniterrae* RF-4^T were 96.1 % and 95.5 %, respectively. DNA–DNA relatedness between strain PRI 3838^T and '*T. kavarayensis*' was 46.1 %. The DNA G+C content of strain PRI 3838^T was 69.0 mol%. The predominant menaquinones, pigmentation, fatty acid profiles and phospholipid profiles of the novel strains were similar to those of other members of the genus *Thermus*. However, the new strains could be differentiated from the type strains of all other species of the genus *Thermus* by their lack of catalase activity and their utilization of only a few carbon sources. Furthermore, the novel strains exhibited mixotrophic growth with sulfur oxidation. On the basis of 16S rRNA gene sequence comparisons, DNA–DNA hybridization and physiological and biochemical characteristics, the new isolates represent a novel species. Since the species appears to be ubiquitous in Icelandic hot springs, the name *Thermus islandicus* sp. nov. is proposed. The type strain is PRI 3838^T (=DSM 21543^T=ATCC BAA-1677^T).

Bacteria of the genus *Thermus* have been prominent in the history of thermophile research since the discovery of *Thermus aquaticus* by Brock & Freeze in 1969. Since then, several additional *Thermus* species have been recognized. As a group, strains of the genus *Thermus* are both biochemically and metabolically quite homogeneous. Most strains are yellow-pigmented, non-sporulating rods that grow heterotrophically on various carbon sources (da Costa *et al.*, 2006). Optimal and maximal temperatures for

growth are usually about 65–70 °C and below 80 °C, respectively, and optimal pH for growth is around neutrality. Most *Thermus* strains have been described as obligate aerobes but some are known to use nitrate, ferric ion, elemental sulfur or arsenate as terminal electron acceptors (Kieft *et al.*, 1999; Gihring & Banfield, 2001; Skirnisdottir *et al.*, 2001). Mixotrophic growth with the oxidation of sulfur compounds has been reported as a common trait in members of the genus *Thermus* (Hreggvidsson *et al.*, 2006).

Species of the genus *Thermus* have been isolated from natural and artificial thermal environments around the world but exhibit different patterns of distribution. *Thermus aquaticus* and *Thermus filiformis* have so far only been encountered in the USA and New Zealand, respectively (Brock & Freeze, 1969; Hudson *et al.*, 1987). *Thermus Brockianus*, *Thermus oshimai* and *Thermus scotoductus*

Abbreviation: ICP-MS, inductively coupled plasma mass spectrometry.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences for strains PRI 2268 and PRI 3838^T are EU753248 and EU753247, respectively.

Scanning and transmission electron micrographs of cells of the novel strains and a graph showing the effect of temperature on the growth of strains PRI 2268 and PRI 3838^T are available as supplementary figures with the online version of this paper.

show global distributions (Kristjansson *et al.*, 1994; Williams *et al.*, 1995, 1996), while *Thermus igniterrae* and *Thermus antranikianii* have only been isolated from hot springs in Iceland (Chung *et al.*, 2000). *Thermus thermophilus* has been isolated from marine and coastal hot springs all over the world (Williams *et al.*, 1995). *T. thermophilus* was the only *Thermus* species isolated from Japanese hot springs until recently, when the discovery of '*Thermus kawarayensis*' was reported (Kurosawa *et al.*, 2005). During an environmental assessment of the Torfajokull geothermal area commissioned by the National Energy Authority of Iceland, we encountered a hot spring, HS-605, harbouring 16S rRNA gene sequences showing 96 % similarity to '*T. kawarayensis*' (unpublished results). Nearly identical sequences had previously been identified in two hot springs in south-western Iceland (Skirnisdottir *et al.*, 2000; Hjorleifsdottir *et al.*, 2001; Hobel *et al.*, 2004). The aim of this work was to isolate and describe this unknown species of the genus *Thermus*.

Samples were collected from hot spring HS-605, which is located 603 m above sea level in the Torfajokull geothermal area in southern Iceland. The temperature was 74 °C at the inlet of the opening and 60 °C at the borders. The pH was around 6.0 and the conductivity was 234 µS cm⁻¹. The concentrations of chloride, magnesium and sulfur were estimated as 0.6 mg l⁻¹, 2 mg l⁻¹ and 10 mg l⁻¹, respectively, using inductively coupled plasma mass spectrometry (ICP-MS). The concentration of hydrogen sulfide was determined to be 1.3 mg l⁻¹ using the methylene blue method (Eaton *et al.*, 2005). The hot spring was characterized by a conspicuous grey microbial mat that was at approximately 66–74 °C when sampled. Biomass was inoculated into R2A liquid medium (Becton Dickinson) and incubated for 24 h at 65 °C without shaking. Turbid cultures were streaked on agar plates containing R2A, which were then incubated at 65 °C for up to 7 days. Different colony types appeared and were picked at random with an emphasis on yellow-pigmented strains. Selected strains were purified by streaking on to the same medium. For phylogenetic characterization, DNA was isolated from cultured strains using Dynabeads DNA Direct (Invitrogen) and partial 16S rRNA gene sequences were determined as described below. Strain PRI 3838^T, which showed 99 % gene sequence similarity to the previously detected clone sequence SRI-248 (Skirnisdottir *et al.*, 2000), was chosen for phenotypic characterization.

During this work, we also examined yellow-pigmented strains that had been deposited uncharacterized in the Matis-Prokaria strain collection. This resulted in the identification of strain PRI 2268, which also exhibited 99 % sequence similarity to the clone sequence SRI-248. This strain was isolated from a hot spring at 65–70 °C and pH 5.5 in Hrafninnusker in the Torfajokull area. It was isolated on an R2A plate at 65 °C. The reference strains used in this study, '*T. kawarayensis*' (DSM 16200), *T. aquaticus* (DSM 625^T) and *T. igniterrae* (DSM 12459^T)

were obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen (DSMZ).

Isolates PRI 2268 and PRI 3838^T stained Gram-negative. They produced irregular, umbonate and undulate colonies of 1–1.5 mm after incubation at 65 °C on medium 166 (Hjorleifsdottir *et al.*, 2001) for 4 days. Both strains produced yellow pigments in contrast to the colourless '*T. kawarayensis*' and the absorbance spectra of acetone extracts showed a peak at 450 nm. Both novel strains exhibited oxidase activities but were catalase-negative and did not reduce nitrate, in contrast to '*T. kawarayensis*' (Table 1). The sensitivity of strains PRI 2268 and PRI 3838^T and the reference strains to antibiotics was examined. Plates containing medium 166 and standard (6 mm) antibiotic discs (Oxoid) were incubated at 65 °C. The inhibition zone was measured and the strains scored as sensitive to the antibiotic if the zone was >20 mm in diameter (Kristjansson *et al.*, 1994). All strains tested as sensitive to ampicillin (10 µg), gentamicin (10 µg), novobiocin (30 µg), kanamycin (30 µg), penicillin G (10 U) and streptomycin (10 µg). They were resistant to nalidixic acid (30 µg), polymyxin B (300 U) and rifampicin (2 µg). Cells of the novel strains were non-motile and a capsule was not

Table 1. Characteristics that distinguish the new *Thermus* isolates from their closest relatives

Strains: 1, PRI 2268; 2, PRI 3838^T; 3, '*T. kawarayensis*' KW11; 4, *T. aquaticus* DSM 625^T; 5, *T. igniterrae* DSM 12459^T. The values for the DNA G+C content of *T. aquaticus* and *T. igniterrae* were obtained from da Costa *et al.* (2001) and Chung *et al.* (2000), respectively. Other data are from this study. +, Positive; –, negative; w, weak growth.

Characteristic	1	2	3	4	5
Pigmentation	Yellow	Yellow	–	Yellow	Yellow
Catalase	–	–	–	+	+
Nitrate reduction	–	–	+	–	+
Thiosulfate oxidation	+	+	+	–	+
Growth at 75 °C	+	+	–	+	+
DNA G+C content (mol%)	ND	69.0	68.0	64	70.3
Utilization of:					
D-Arabinose	w	–	w	–	+
L-Arginine	–	–	–	+	+
D-Glucose	–	–	+	–	+
L-Glutamine	+	–	–	+	+
Glycerol	+	+	–	+	+
myo-Inositol	+	w	–	+	+
D-Mannose	–	–	w	+	+
L-Rhamnose	–	–	–	+	+
Ribitol	+	–	–	+	+
L-Serine	–	–	–	+	–
Starch	+	+	–	–	+
Sucrose	–	–	–	–	+
Trehalose	–	–	–	+	+

detected by crystal violet and copper sulfate or by India ink staining. Scanning electron micrographs of strain PRI 3838^T showed straight rod-shaped cells, approximately 0.3 µm in diameter and 1.5–2.5 µm in length (see Supplementary Fig. S1, available in IJSEM Online). Transmission electron micrographs showed a thick envelope and a typical Gram-negative cell-wall profile (see Supplementary Figs S2 and S3).

Strains PRI 2268 and PRI 3838^T and the reference strains were routinely grown at 65 °C in medium 166 (Hjorleifsdottir *et al.*, 2001). The new isolates were aerobic and did not grow anaerobically with nitrate. The pH ranges for growth were examined at 65 °C in medium 166 buffered with 30 mM acetate to pH 4.0–5.5, 30 mM MES to pH 6.0, 30 mM phosphate to pH 6.5–8.0, 30 mM Tricine to pH 9.0 and by 30 mM CAPS to pH 10.0–11.0. Strains PRI 2268 and PRI 3838^T grew optimally at pH 6.0–7.0 and pH 6.5–7.0, respectively. The maximum and minimum pH values for growth were pH 5.5 and pH 10.5 for both strains. Their growth temperature ranges were examined by measuring the turbidity (OD₆₀₀) of liquid cultures. Both strains PRI 2268 and PRI 3838^T grew optimally at about 65 °C and their maximum specific growth rates (µ) were determined as 0.37 h⁻¹ and 0.29 h⁻¹, respectively (see Supplementary Fig. S4, available in IJSEM Online). The minimum and maximum growth temperatures were about 44 °C and 79 °C, respectively, for both strains. Salt tolerance tests were performed on plates with medium 166 at 65 °C and in liquid cultures and neither novel strain grew in the presence of 1% (w/v) NaCl. Growth on 27 different single and complex carbon sources was tested on agar plates and compared with that of '*T. kavarayensis*' DSM 16200, *T. aquaticus* DSM 625^T and *T. igniterrae* DSM 12459^T. Modified minimal medium 162 (Degryse *et al.*, 1978) containing EGTA instead of Titriplex I was used. It also contained 0.05% NH₄Cl and 0.4% vitamin solution (Degryse *et al.*, 1978) and was prepared either with or without 0.2% yeast extract. Carbon sources were added in concentrations of 0.2% for L-amino acids and 0.4% for other substrates. Plates were scored as positive if growth was clearly visible after 7 days. A negative control without an added organic compound was also included. Strains PRI 2268 and PRI 3838^T assimilated very few carbon sources when compared with the reference strains. However, by lowering the growth temperature to 60 °C, several more carbon sources were utilized and both strains grew well on pyruvate, starch and glycerol, when supplemented with yeast extract. Strain PRI 2268 also grew well on L-glutamine, D-arabinose and ribitol (Table 1). Both novel strains showed some growth on cellobiose, lactose and myo-inositol. They also grew on minimal medium supplemented with yeast extract and thiosulfate (16 mM), both on plates and in liquid medium. Furthermore, when thiosulfate was added to medium 166, the maximum growth rate increased, but only at high aeration. This indicated mixotrophic growth and energy harnessing oxidation of thiosulfate. This was confirmed by

the production of sulfate from thiosulfate in the presence of yeast extract as determined by using BaCl₂ as described by Tabatabai (1974). Strains PRI 2268 and PRI 3838^T produced 4.3 and 4.5 mM sulfate from 16 mM thiosulfate after growth for 48 h at 65 °C with a concomitant decrease in pH from 7.7 to 5.3 and 5.0, respectively. The strains were also capable of sulfur oxidation in the presence of yeast extract, but less efficiently than with thiosulfate. Both produced 0.6 mM sulfate when incubated with sulfur (2 g l⁻¹) at 65 °C for 48 h. Similar results were obtained for both '*T. kavarayensis*' DSM 16200 and *T. igniterrae* DSM 12459^T (Table 1). Previously, *T. scotoductus* IT-7254 has been shown to oxidize thiosulfate and elemental sulfur (Skirnisdottir *et al.*, 2001). Furthermore, the growth of this strain in nutrient medium was enhanced by thiosulfate as shown here for strains PRI 2268 and PRI 3838^T.

Cellular fatty acids were extracted from bacteria grown in medium 166 at 65 °C according to the protocol of the MIDI system. Analysis by GC was controlled by the MIS software and the peaks were automatically integrated and identified by the Microbial Identification software package (Sasser, 1990). The predominant fatty acids of strain PRI 3838^T were iso-17:0 (26.2%), iso-15:0 (23.7%), anteiso-15:0 (20.3%) and anteiso-17:0 (15.2%). Other fatty acids detected were mainly 16:0 (5.5%), iso-16:0 (4.7%), iso-14:0 (1.2%) and 17:0 (1.1%). The ratio of fatty acids anteiso-15:0 and anteiso-17:0 for strain PRI 3838^T was relatively high compared with many other species of the genus *Thermus* (Chung *et al.*, 2000). The predominant fatty acids of strain PRI 3838^T were also detected in high ratios in the reference strain, '*T. kavarayensis*'. This strain contained mainly iso-15:0 (32.8%), iso-17:0 (25.5%), anteiso-15:0 (13.4%) and anteiso-17:0 (9.0%). Analyses of polar lipids and respiratory quinones were carried out by the DSMZ (B. J. Tindall). Menaquinones MK8 (96%) and MK7 (2%) were detected in strain PRI 3838^T. Two major phospholipids (PL1 and PN) and one major glycolipid (GL5) were identified along with a minor phospholipid (PL2) and several minor glycolipids (GL1–GL4 and GL6).

The species status of strains PRI 2268 and PRI 3838^T was demonstrated by a DNA–DNA hybridization value of 46.1% between strain PRI 3838^T and '*T. kavarayensis*' DSM 16200. DNA–DNA hybridization was performed by the DSMZ as described by De Ley *et al.* (1970) with the modifications of Huß *et al.* (1983). The G+C content of DNA was determined by the DSMZ according to Mesbah *et al.* (1989). DNA was purified as described by Cashion *et al.* (1977). The DNA G+C composition of strain PRI 3838^T was determined as 69.0 mol%. This was similar to the value for '*T. kavarayensis*', which was determined as 68.0 mol% and to that of *T. igniterrae* (Chung *et al.*, 2000). However, this value was higher than that obtained for other species of the genus *Thermus* (63–65 mol% G+C) (da Costa *et al.*, 2001).

The 16S rRNA genes of strains PRI 3838^T and PRI 2268 were amplified using Dynazyme (Finzymes) and primers

F9 (5'-GAGTTTGATCCTGGCTCAG-3') and R1544 (5'-AGAAAGGAGGTGATCCA-3') (Skirnisdottir *et al.*, 2000). Reactions were subjected to 5 min at 94 °C, 30 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1.5 min and finally to 7 min at 72 °C. Amplification products were purified using ExoSapIt (Amersham Biosciences) and their sequences determined using ABI 3730 DNA Analyzer and a BigDye Terminator Cycle Sequencing kit (Applied Biosystems). The primers F9 and R1544 were used for sequencing as well as R805 (5'-GACTACCCGGGTATC-TAATCC-3'), F338 (5'-ACICCTACGGGIGGCAGCAG-3'), R357 (5'-CTGCTGCCICCCGTAGG-3') and R1195 (5'-GACGTCITCCCCICCTTCCTC-3') (Skirnisdottir *et al.*, 2000). The sequences were assembled and analysed using Sequencher version 4.8 (GeneCodes). They were aligned with those of other species of the genus *Thermus* (1442 bp) by using CLUSTAL_X software (Thompson *et al.*, 1997). Evolutionary distance matrices were calculated using the Kimura two-parameter model (Kimura, 1980). A phylogenetic tree was constructed according to the neighbour-joining method of Saitou & Nei (1987). The tree was displayed with NJPLOT (Perrière & Gouy, 1996) and its topology was evaluated by a bootstrap analysis with 1000 trial replications. The 16S rRNA gene sequences of strain PRI 2268 and PRI 3838^T were found to be 99.7 % similar and 98.8 % similar to the previously identified sequence SRI-248 (Skirnisdottir *et al.*, 2000). The phylogenetic tree (Fig. 1) revealed that '*T. kawarayensis*' KW11 was the closest relative of the new isolates (96.5 % sequence similarity). Gene sequence similarity values of 96.1 % and 95.9 % were found to *T. aquaticus* YT-1^T and *T. igniterrae* RF-4^T, respectively.

On the basis of physiological differences between the novel strains and related organisms (Table 1) and on 16S rRNA

gene sequence similarity and DNA–DNA relatedness values of 96.5 % and 46.1 %, respectively, between strain PRI 3838^T and the closest relative '*T. kawarayensis*', a novel species of the genus *Thermus*, namely *Thermus islandicus* sp. nov., is proposed to accommodate strains PRI 2268 and PRI 3838^T (=DSM 21543^T=ATCC BAA-1677^T).

Description of *Thermus islandicus* sp. nov.

Thermus islandicus (is.lan'di.cus. N.L. masc. adj. *islandicus* from Iceland, pertaining to the location of its first isolation).

Cells are non-spore-forming, non-motile rods measuring 0.3 µm in width and 1.5–2.5 µm in length. Cells stain Gram-negative and contain a thick envelope and a typical Gram-negative cell-wall profile. Aerobic, oxidase-positive, catalase-negative and does not reduce nitrate. Colonies are light yellow, slightly irregular, umbonate, undulate and 1–1.5 mm after 4 days at 65 °C on medium 166. Grows between 45 and 79 °C (optimum, 65 °C) and from pH 5.5–10.5 (optimum, pH 6.0–7.0). Grows well in medium 166 and R2A. Pyruvate, glycerol and starch support growth of the type strain at 60 °C when supplemented with yeast extract. Weak growth is exhibited on cellobiose, lactose and inositol. Does not utilize arabinose, fructose, galactose, glucose, mannose, melibiose, raffinose, rhamnose, ribose, sucrose, trehalose, xylose, ribitol, mannitol, sorbitol, citrate, malate, L-arginine, L-glutamine, L-serine or Casamino acids. Capable of mixotrophic growth with sulfur-oxidation in medium 166 supplemented with thiosulfate and in minimal medium supplemented with thiosulfate and yeast extract. Susceptible to ampicillin, gentamicin, novobiocin, kanamycin, penicillin and streptomycin. Resistant to nalidixic acid, polymyxin B and rifampicin. The predominant menaquinone is MK8. The

Fig. 1. Neighbour-joining phylogenetic tree derived from analysis of 16S rRNA gene sequences (Kimura's two-parameter method) showing the position of strains PRI 2268 and PRI 3838^T among other members of the genus *Thermus*. GenBank accession numbers are given in parentheses. Bootstrap values (1000 replications) are shown as percentages at each node only if they are ≥ 50 %. Bar, 0.01 substitutions per nucleotide position.

major fatty acids are iso-17:0, iso 15:0, anteiso 15:0 and anteiso 17:0.

The type strain, PRI 3838^T (=DSM 21543^T=ATCC BAA-1677^T), was isolated from a hot spring in the Torfajokull geothermal area in Iceland. Strain PRI 2268 is a reference strain. The DNA G+C content of the type strain is 69 mol%.

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References

- Brock, T. D. & Freeze, H. (1969). *Thermus aquaticus* gen. n. and sp. n., a non-sporulating extreme thermophile. *J Bacteriol* **98**, 289–297.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chung, A. P., Rainey, F. A., Valente, M., Nobre, M. F. & da Costa, M. S. (2000). *Thermus igniterrae* sp. nov. and *Thermus antranikianii* sp. nov., two new species from Iceland. *Int J Syst Evol Microbiol* **50**, 209–217.
- da Costa, M. S., Nobre, M. F. & Rainey, F. A. (2001). Genus I. In *Bergey's Manual of Systematic Bacteriology*, pp. 404–414. Edited by G. Garrity, D. R. Boone & R. W. Castenholz. Vol. 1. New York: Springer.
- da Costa, M. S., Rainey, F. A. & Nobre, M. F. (2006). The genus *Thermus* and relatives. In *The Prokaryotes. A Handbook on the Biology of Bacteria*, 3rd edn, vol. 7, pp. 797–812. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Degryse, E., Glansdorff, N. & Piérard, A. (1978). A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch Microbiol* **117**, 189–196.
- Eaton, A. D., Clesceri, L. S., Rice, E. W. & Greenberg, A. E. (2005). *Standard Methods for the Examination of Water and Wastewater*. Washington DC: American Public Health Association.
- Gihring, T. M. & Banfield, J. F. (2001). Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. *FEMS Microbiol Lett* **204**, 335–340.
- Hjorleifsdottir, S., Skirnisdottir, S., Hreggvidsson, G. O., Holst, O. & Kristjansson, J. K. (2001). Species composition of cultivated and non-cultivated bacteria from short filaments in an Icelandic hot spring at 88 °C. *Microb Ecol* **42**, 117–125.
- Hobel, C. F. V., Marteinsson, V. T., Hauksdottir, S., Fridjonsson, O. H., Skirnisdottir, S., Hreggvidsson, G. O. & Kristjansson, J. K. (2004). Use of low nutrient enrichments to access novel amylase genes in silent diversity of thermophiles. *World J Microbiol Biotechnol* **20**, 801–809.
- Hreggvidsson, G. O., Skirnisdottir, S., Smit, B., Hjorleifsdottir, S., Marteinsson, V. T., Petursdottir, S. & Kristjansson, J. K. (2006). Polyphasic analysis of *Thermus* isolates from geothermal areas in Iceland. *Extremophiles* **10**, 563–575.
- Hudson, J. A., Morgan, H. W. & Daniel, R. M. (1987). *Thermus filiformis* sp. nov., a filamentous caldactive bacterium. *Int J Syst Bacteriol* **37**, 431–436.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kieft, T. L., Fredrickson, J. K., Onstott, T. C., Gorby, Y. A., Kostandarither, H. M., Bailey, T. J., Kennedy, D. W., Li, S. W., Plymale, A. E. & other authors (1999). Dissimilatory reduction of Fe(III) and other electron acceptors by a *Thermus* isolate. *Appl Environ Microbiol* **65**, 1214–1221.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kristjansson, J. K., Hjorleifsdottir, S., Marteinsson, V. & Alfredsson, G. A. (1994). *Thermus scotoductus*, sp. nov., a pigment-producing thermophilic bacterium from hot tap water in Iceland and including *Thermus* sp. X-1. *Syst Appl Microbiol* **17**, 44–50.
- Kurosawa, N., Itoh, Y. H. & Itoh, T. (2005). *Thermus kawarayensis* sp. nov., a new member of the genus *Thermus*, isolated from Japanese hot springs. *Extremophiles* **9**, 81–84.
- Mesbah, M., Premachandran, U. & Whitman, W. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Perrière, G. & Gouy, M. (1996). WWW-Query: An on-line retrieval system for biological sequence banks. *Biochimie* **78**, 364–369.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Skirnisdottir, S., Hreggvidsson, G. O., Hjorleifsdottir, S., Marteinsson, V. T., Petursdottir, S., Holst, O. & Kristjansson, J. K. (2000). Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats. *Appl Environ Microbiol* **66**, 2835–2841.
- Skirnisdottir, S., Hreggvidsson, G. O., Holst, O. & Kristjansson, J. K. (2001). Isolation and characterization of a mixotrophic sulfur-oxidizing *Thermus scotoductus*. *Extremophiles* **5**, 45–51.
- Tabatabai, M. A. (1974). A rapid method for determination of sulfate in water samples. *Environ Lett* **7**, 237–243.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X window interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Williams, R. A. D., Smith, K. E., Welch, S. G., Micallef, J. & Sharp, R. J. (1995). DNA relatedness of *Thermus* strains, description of *Thermus brockianus* sp. nov., and proposal to reestablish *Thermus thermophilus* (Oshima and Imahori). *Int J Syst Bacteriol* **45**, 495–499.
- Williams, R. A. D., Smith, K. E., Welch, S. G. & Micallef, J. (1996). *Thermus oshimai* sp. nov., isolated from hot springs in Portugal, Iceland, and the Azores and comment on the concept of a limited geographical distribution of *Thermus* species. *Int J Syst Bacteriol* **46**, 403–408.