Extremophiles (2006) 10:563–575
DOI 10.1007/s00792-006-0530-3

ORIGINAL PAPER

Gudmundur O. Hreggvidsson · Sigurlaug Skirnisdottir
Bart Smit · Sigridur Hjorleifsdottir
Viggo Th. Marteinsson · Solveig Petursdottir
Jakob K. Kristjansson

Polyphasic analysis of *Thermus* isolates from geothermal areas in Iceland

Received: 30 November 2005 / Accepted: 12 April 2006 / Published online: 24 June 2006

Abstract Genetic relationships and diversity of 101 *Thermus* isolates from different geothermal regions in Iceland were investigated by using multilocus enzyme electrophoresis (MLEE) and small subunit ribosomal rRNA (SSU rRNA) sequence analysis. Ten polymorphic enzymes were used and seven distinct and genetically highly divergent lineages of *Thermus* were observed. Six of seven lineages could be assigned to species whose names have been validated. The most diverse lineage was *Thermus scotoductus*. In contrast to the other lineages, this lineage was divided into very distinct genetic sub-lineages that may represent subspecies with different habitat preferences. The least diverse lineage was *Thermus brockianus*. Phenotypic and physiological analysis was carried out on a subset of the isolates. No relationship was found between growth on specific single carbon source to the grouping obtained by the isoenzyme analysis. The response to various salts was distinguishing in a few cases. No relationship was found between temperature at the isolation site and the different lineages, but pH indicated a relation to specific lineages.

Keywords *Thermus* · Polyphasic taxonomic · Iceland · Hot springs · Multilocus enzyme electrophoresis

Communicated by G. Antranikian

G. O. Hreggvidsson (✉) · S. Skirnisdottir · B. Smit · S. Hjorleifsdottir · V. Th. Marteinsson · S. Petursdottir · J. K. Kristjansson
Prokaria Ltd., Gylfaflot 5, IS-112 Reykjavik, Iceland
Tel.: +354-5707900
Fax: +354-5707901
e-mail: gudmundo@prokaria.com

G. O. Hreggvidsson
Institute of Biology, University of Iceland,
IS-108 Reykjavik,
Iceland

Introduction

The first *Thermus* species, *Thermus aquaticus*, was isolated from hot springs in Yellowstone National Park in the late 1960s (Brock and Freeze 1969). Since then, seven species of the genus have been described whose names have been validated. They are *Thermus thermophilus* (Oshima and Imahori 1974), *Thermus filiformis* (Hudson et al. 1987), *Thermus scotoductus* (Kristjansson et al. 1994), *Thermus brockianus* (Williams et al. 1995), *Thermus oshimai* (Williams et al. 1996), *Thermus igniterra* and *Thermus antranikianii* (Chung et al. 2000). *Thermus* spp. are relatively easy to isolate and are found in both natural and man-made thermal systems worldwide. All *Thermus* species except *T. aquaticus* and *T. filiformis* have been found in Iceland.

Thermophilic microorganisms encounter the unique situation that they can only grow in relatively few small spots on the planet. Furthermore, their habitats are comparatively rare. Therefore, they can be considered as islands in the ecological sense with large dispersal barriers between them. According to the currently known global distribution of terrestrial *Thermus* species, there appears to be a distinct difference between widely separated geothermal regions in species composition, with a mixture of unique endemic lineages and more cosmopolitan species. Certain regions appear also to be more diverse than others. Thus, *T. aquaticus* has only been found in Yellowstone National Park in the USA. Another distinct species, *T. filiformis* has only been isolated or detected in New Zealand and is so far the only terrestrial species found there. One species, *T. thermophilus* seems to be confined to marine or coastal hot springs and has a worldwide distribution (Kristjansson et al. 2000; Williams and Sharp 1995).

Many of the methods of bacterial systematics, such as restriction fragment length polymorphisms (RFLP) and multilocus enzyme electrophoresis (MLEE) are both fast and reliable, and a large number of samples or isolates can be processed at the same time (Vandamme et al. 1996). Therefore, it is both practical and

feasible as never before to measure various population parameters to describe and compare microbial communities. MLEE is an electrophoretic technique used to map the distribution of different alleles of a number of enzymes and to calulate diversity indices of populations. Genetic relationships are calculated from similarity in genotype on the basis of percentage of shared alleles. MLEE is an inexpensive technique and a great number of isolates can be processed at the same time. It is comparable with DNA:DNA hybridization in delineating species but as a taxonomic tool, it is more appropriate for hierarchical classification as it is very sensitive at and below the species level. In contrast to DNA:DNA hybridization, MLEE is particularly suitable for analyzing spatial and temporal dynamics of microbial populations and has proved its value in epidemiological studies (Goodfellow and O'Donnell 1993; Selander et al. 1986; Vandamme et al. 1996; Whittam 1995; Whittam et al. 1983).

Ideally, a taxonomic study of bacteria should be polyphasic, based on phylogenetic, phenotypic, biochemical and physiologic analysis on a large number of isolates from different regions in order to define species boundaries. Species boundaries should also ideally circumscribe ecologically distinct populations whether they exhibit a large or a narrow range of phenotypic, biochemical or genetic properties (Chung et al. 2000; Goodfellow et al. 1993; Vandamme et al. 1996). We have used the MLEE method and SSU rRNA sequence analysis in hand with typical phenotypic and physiological studies to examine genetic diversity and species boundaries of *Thermus* species isolated in Iceland. The aim of this study is to contribute to the growing taxonomic body of the genus and help in establishing basis for the study of the ecological factors and evolutionary mechanisms that direct the evolution and divergence of *Thermus* species.

Materials and methods

Strains

Strains were isolated at different temperatures and with some variations in medium composition and purified by repeated streaking onto medium 160 and 166 agar plates (Hjorleifsdottir et al. 2001). Medium 160 is the same as the Degryse medium 162 with only 1/10 of the phosphate buffer strength. It contains (per liter) 2.5 g of yeast extract, 2.5 g of tryptone, 1.0 g of nitrilotriacetic acid, 0.4 g of $CaSO_4$ $2H_2O$, 2.0 g of $MgSO_4 \cdot 7H_2O$, 1.5 ml of 0.2 M $Na_2HPO_4 \cdot 12H_2O$; 1.0 ml of 0.2 M KH_2PO_4, 0.5 ml of 0.01 M Fe(III) citrate $\cdot 5H_2O$ and 5.0 ml of trace element solution, pH 7.5. Medium 166 contains per liter geothermal tap water, 0.3 g of K_2HPO_4, 1 g of yeast extract, 1 g of peptone, 1 g of tryptone, 0.5 g of glucose, 0.5 g of starch, 0.6 g of pyruvic acid, 0.3 g of proline and 0.18 g of Na_2CO_3, 0.5 ml of 0.01 M Fe(III) citrate $\cdot 5H_2O$ and 5.0 ml of trace element solution, pH 7.5. The trace solution contains (per liter): $MnSO_4 \cdot H_2O$, 0.22 g; $ZnSO_4 \cdot 7H_2O$, 0.05 g; H_3BO_3, 0.05 g; $CuSO_4 \cdot 5H_2O$, 0.0025 g; $Na_2MoO_4 \cdot 2H_2O$, 0.0025 g and $CoCl_2 \cdot 6H_2O$, 0.0046 g.

The study also included reference strains deposited in culture collections, which were isolated from different parts of the world. *T. aquaticus* strain YT-1 (type strain, DSM 625) isolated in Yellowstone National Park, USA, *T. filiformis* strain Wai33 A.1 (type strain, DSM 4687, ATCC 43280) isolated in New Zealand, *T.thermophilus* strain HB8 (type strain, DSM 579, ATCC 27634) isolated in Japan, *T. thermophilus* strain AT-62 (DSM 674, ATCC 33923) isolated in Japan, *T. brockianus* strain YS38 (type strain NCIMB 12676) isolated from hot springs in Yellowstone National Park, USA, *Thermus* sp. strain X-1 (ATCC 27978) isolated from man-made geothermal systems in USA and *T. scotoductus* strain SE-1 (type strain ATCC 51532) isolated from hot tap water in Iceland.

Sampling sites

Snaefellsnes, Oxarfjordur and Reykjanes are coastal geothermal sites and the hot water systems sampled were saline. The sampling sites in Oxarfjordur were runoff streams from boreholes on the coast in Snaefellsnes, and they were typical coastal hot springs. In Reykjanes, the sampling sites were ponds formed by runoffs from boreholes. Salinity values at these three sites were respectively, 0.8, 0.2–0.4 and 2.2–5.8%. The hot water running into the ponds in Reykjanes has a salinity of approximately 3% and temperature between 70 and 80°C. The geothermal diversity was poor and therefore, only one to two hot water systems were sampled in each area (Petursdottir et al. 2000).

Hveravellir, Hrafntinnusker and Hagongur are in the sparsely vegetated highlands (> 500 m above sea level). Hrafntinnusker is a high-temperature geothermal field with steam holes and solfatara fields. These regions are characterized by emissions of steam and volcanic gases. The gas is composed primarily of N_2 and CO_2, but H_2S and H_2 can be up to 10% each of the total gas fraction. Because of the weak acids, CO_2 (pK = 6.3) and H_2S (pK = 7.2), the pH of the subsurface steam is near neutrality. On the surface the H_2S is oxidized chemically and biologically, first to sulfur and then to sulfuric acid. This lowers the pH, causing corrosion of the surrounding rocks and formation of the typical acidic mud of solfatara fields. The pH often stabilizes around 2–2.5, where sulfuric acid (pK_2 = 1.92) is the effective buffering agent. Because of the high temperature, there is little water that comes to the surface and the hot springs are mostly steam holes or fumaroles. These areas are usually unstable, and the individual openings often disappear or move to another location within the geothermal field.

Where groundwater levels are high, the water percolates into the hot areas, warms up, and returns to the surface containing dissolved minerals, such as silica and

some dissolved gases, mainly CO_2. Usually there is little H_2S in such fluids. Also here the subsurface pH is near neutrality, but there is usually much more water and little sulfide so its surface oxidation has no effect on the pH. On the surface, however, the CO_2 escapes and the silica precipitates, both resulting in increased pH. The pH is often stabilized at 9–10, where silicate (pK_n = 9.7) and carbonate (pK_2 = 10.25) start to act as effective buffering agents (Kristjansson and Hreggvidsson 1995).

The Hagongur region is also in a high temperature region characterized by solfatara fields and steam holes. The two hot springs sampled were untypical for the area having high water turnover rate and relatively high pH for the area, 7.1 and 7.6. Hveravellir is also situated in a high-temperature geothermal field. However, the area has an unusually large number of alkaline hot springs with a high water turnover rate besides having more typical solfatara fields, steam holes and acidic hot springs with little water.

Hveragerdi is a high-temperature geothermal field situated in the lowlands and has abundant vegetation. The groundwater level is high and therefore, neutral and alkaline hot springs with high water turnover are common. The Geysir area is in between the highland and lowland and has characteristics of both. It has a number of highly alkaline water bubbling hot springs and acidic solfatara fields.

Preparation of samples for MLEE analysis

After purification, isolates were grown overnight at 65°C on medium 160 agar plates and harvested by scraping of the plates. The cells were suspended in TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) giving about 1 g in 5 ml, and then disrupted in a French Press at 700 psi. The crude extract was centrifuged at 20000*g* for 30 min at 4°C and the supernatant collected and kept at –80°C until use. Before use, the samples were spun again and the clear supernatant was collected. The samples were run on 7.5% (w/v) polyacrylamide gels and after running, the gels were assayed for alkaline phosphatase (AP), aspartate aminotransferase (AAT), non-specific esterase (EST), glucose-6-phosphate isomerase (GPI), hexokinase (HK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), nucleoside phosphorylase (NP), superoxide dismutase (SOD) and an unspecific dehydrogenase (UDH). Demonstration of the enzyme stainings has been described elsewhere except 0.2 M Tris buffer (pH 8.0) that was used in our study (Manchenko 1994; Petursdottir et al. 2000). Distinctive electromorphs of each enzyme, numbered in order of decreasing anodal mobility were equated with alleles at the corresponding structural gene locus. An absence of enzyme activity was attributed to a null allele. Distinctive combinations of alleles over the ten enzyme loci (multilocus genotypes) were designated as electrophoretic types (ETs).

Statistical analysis

Genetic diversity and relationships among the 101 *Thermus* strains characterized by MLEE was analyzed by two computer softwares, ETDIV and ETCLUS, kindly provided by the author, Dr. T.S. Whittam (Department of Biology, Pennsylvania State University, University Park, PA, USA). Genetic diversity at an enzyme locus among either ETs or isolates was calculated from the allele frequencies among ETs or isolates as $h = (1 - \sum x_i^2)(n/n - 1)$, where x_i is the frequency of the ith allele and n is the number of ETs or isolates. Mean genetic diversity (H) is the arithmetic average of h values over all loci. H_S, the within group diversity was calculated as the mean of the diversity values obtained for the separate sampling sites (subpopulations). Total genetic diversity, H_T, is the diversity value calculated for the population as a whole. For a subdivided population the total diversity H_T will be greater than the diversity within subpopulations. Nei and Chesser (1983) coefficient of genetic differentiation G_{ST}, was then calculated as $(H_T - H_S)/H_T$ using the ETDIV software. This coefficient indicates how big proportion of the overall variation is due to differences between subsamples. A dendrogram based on the average linkage algorithm of all the isolates with the reference strains included was made. Distance was measured as the proportion of mismatched loci between pairs of ETs. Genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred (mismatches) and clustering of ETs was performed from a matrix of genetic distances by the average linkage method (UPGMA) (Caugant et al. 1987; Petursdottir et al. 2000).

Phenotypic characterization

A phenotypic and physiological analysis was carried out on a subset of the isolates from the MLEE analysis representing different clones of the same lineages and from different geographic regions. Growth was examined at temperatures 50, 65 and 78°C on agar plates with medium 160. Growth was analyzed at pH 5.0, 6.0, 8.0, 8.7 and 9.5 on agar plates with medium 160, but the pH was adjusted with HCl and NaOH. Salt and ion tolerance was tested on medium 160 agar plates supplied with 0.5, 1 and 2% NaCl, 50 mM of $MgSO_4$, 50 mM of $CaSO_4$, 2 mM of $CuSO_4$, 50 mM of Na_2SO_4, 50 mM of Na_2SO_3 and 50 mM $Na_2S_2O_3$. Growth in the presence of the chelating agent EDTA was analyzed on medium 160 agar plates supplemented with 2 and 5 mM EDTA. Utilization of single carbon sources was tested on minimal medium agar plates containing 0.2–0.4% of the organic compound as described before (Kristjansson et al. 1994; Petursdottir and Kristjansson 1996). Growth was examined in the presence of 35 carbon sources. Carbon sources, salts and other growth conditions are listed in Table 5. Growth was examined after 1, 3 and 5 days of incubation. Nitrate reduction was tested with 2- and 5-day-old cultures as described before with the

modification of using microtiter plates (Smibert and Krieg 1994).

Thiosulfate oxidation

A few representative strains were selected for the thiosulfate oxidation test on the basis of the clustering obtained by the MLEE analysis. The production of sulfate from 16 mM of thiosulfate was tested in liquid minimal medium supplied with 0.2% acetate, pyruvate or proline after 2 and 4 days incubation at 65°C (Skirnisdottir et al. 2001). The concentration of sulfate was determined by using $BaCl_2$ and turbidity (Tabatabai 1974).

Phylogenetic analysis

Strains for the phylogenetic studies were selected on the basis of the UPGMA clustering obtained by the MLEE analysis. The phylogenetic position of a few representatives of each lineage was determined by using the SSU rRNA gene as the phylogenetic marker by partial sequencing. The following strains were analyzed: 165, 220, 346, 2101, 2103, 2121, 2123, 2126, 2127, 2133, 2135, 2789, 2791 and 6230. DNA was isolated with a Dynabeads DNA Direct Kit (Dynal) according to the manufacturer. PCR amplifications of the SSU rRNA has been described elsewhere (Skirnisdottir et al. 2001). The SSU rRNA genes from strains were partially sequenced with an ABI 377 DNA sequencer by using BigDye Terminator Cycle Sequencing Ready Reaction kit according to the manufacturer (PE Applied Biosystems). Primer R805 (5'-GACTACCCGGGTATCTAATCC'-3; 805-785) was used for the sequencing. After BLAST searches, the sequences were manually aligned with other sequences within the *Thermus* group obtained from the Ribosomal Database Project (Maidak et al. 1999). Evolutionary distances were computed from pairwise similarities by using the correction of Jukes and Cantor (1969). ClustalX (http://bips.u-strasbg.fr/fr/Documentation/ClustalX/) was used to construct the phylogenetic tree by the neighbor-joining algorithm.

The GenBank accession numbers of the SSU rRNA sequences of the organisms used in this analysis are as follows: *T. scotoductus* IT252 (SE-1) (AF032127), *Thermus* sp. NMX2 A.1 (L09661), *Thermus* sp. IT-7254 (AF257219), *T. antranikianii* HE5 (Y18412), *Thermus* sp. ac-1 (L37520), *T. igniterrae* RF-4T (Y18406), unidentified *Thermus* sp. SRI248 (AF255591), *T. aquaticus* YT-1 (L09663), unidentified *Thermus* OPB31 (AF027020), *Thermus* sp. ac-7 (L37522), unidentified *Thermus* OPB32 (AF027021), unidentified *Thermus* OPB19 (AF027019), *T. thermophilus* HB-8 (X07998), *T. filiformis* WAI 33 A.1 (X58345), *Thermus* sp. YS38 (Z15062), *T. oshimai* SPS-17 (Y18416), *Thermus* YSPID A.1 (L10070), *Thermus* ZHGIB A.4 (L10071), *T. thermophilus* AT-62 (L09660), *Thermus* sp. Tok8 A.1 (L09666), *Thermus* sp. Tok20 A.1 (L09665), *Thermus* sp. W28 A.1 (L10068), *Thermus* sp. T351 (L09671) *Thermus rehai* RH99-02 (AF331969), *Thermus* sp. RH-1514 (AF521188), *Thermus yunnanensis* RH1003 (AY861385) and *Thermus kawarayensis* KW11 (AB071811).

DNA:DNA reassociation analysis was performed between *T. brockianus* YS38 (type strain, NCIMB 12676) and strain 2789. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (1977). DNA:DNA reassociation analysis was carried out as described by de Ley et al. (1970) with modification described by Huss et al. (1983) and Escara and Hutton (1980).

Results

Origin and isolation of strains

The 101 *Thermus* strains used in this study cover a wide geographic range and sample sites different in physicochemical and ecological characteristics. The sites could be broadly assigned to eight widely separated geothermal areas in Iceland (Fig. 1). The hot springs varied in pH, temperature, salt concentration, sulfide concentration, altitude and water turnover rate. A total of 45 hot springs and three man-made hot water geothermal systems were sampled. Three hot springs were in the pH range 4.5–5.9, nine hot springs in the pH range 6.0–6.9, twelve hot springs in the pH range 7.0–7.9, nine hot springs in the pH range 8.0–8.9 and twelve hot springs in the pH range 9.0–9.9. Most of the strains were isolated from alkaline hot springs, but a number of isolates were also obtained from hot water springs with pH lower than 7 (Table 1).

Genetic relationships of ETs

Based on the MLEE analysis of the 101 strains, seven distinct and genetically highly divergent lineages of *Thermus* were observed (Fig. 2). This grouping was consistent with the SSU rRNA sequencing results (Fig. 3). All except one of the population lineages (represented by isolates 2139 and 2789) could be affiliated to known species. The known species lineages were *T. brockianus, T. thermophilus, T. igniterrae*, *T. scotoductus, T. antranikiaii* and *T. oshimai.*

The *T. thermophilus* lineage consisted of marine isolates. These strains were isolated from only two locations, both of them coastal and saline (Oxarfjordur and Reykjanes). Isolates from each region were clustered together (Fig. 2).

The most commonly isolated species was *T. scotoductus* with 29 isolates. This lineage, in contrast to the other lineages was further divided into six distinct genetic sublineages, designated as A–F for convenience. These sublineages may represent subspecies with different habitat preferences as clustering of regional isolates could be observed. Strains belonging to sublineage A

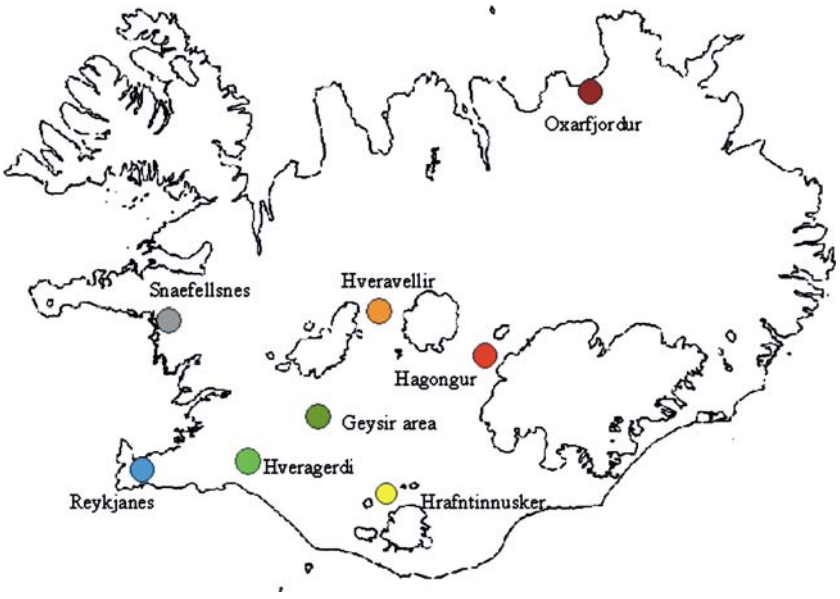

Fig. 1 Location of sample sites in Iceland for 101 *Thermus* strains used in this study

were mainly isolated from hot springs in Hagongur and Hveravellir, whereas strains in the very distinctive sub-lineage F were mainly isolated in Hrafntinnusker. Colorless strains of *T. scotoductus*, including the type strain (SE-1) from Iceland and *Thermus* sp. X1 from USA, clustered together in subgroup C. Subgroups B, D and E are only represented by few strains. In contrast to the *T. scotoductus* lineage, no inter-regional clustering was observed for isolates in the *T. igniterrae* and *T. brockianus* lineages. Only one *T. antranikianii* isolate was obtained.

One of the lineages consisted of strains that were highly divergent from the other lineages. According to the SSU rRNA sequencing results, bacteria belonging to this lineage were most closely related to *T. brockianus*, with 98.3% sequence similarity. However, as seen from the MLEE results it was genetically distant from *T. brockianus* with different alleles at all loci. Results of DNA:DNA reassociation between *Thermus* 2789 from this lineage and *T. brockianus* YS38 (type strain) gave a value of 72.9%.

Genetic analysis of isolates from different regions

Thermus brockianus

The *T. brockianus* group consisted of 21 isolates from three of the most varied inland geothermal areas, that is, Hveragerdi, Hveravellir and the Geysir areas. Thirteen ETs could be distinguished (Fig. 2). Six of the ten enzymes were polymorphic and the number of alleles ranged from 1 to 4 with an average of 2.1. SOD, AP, GPI and the unspecific dehydrogenase were invariant in the population (Table 2). Little genetic diversity was found within this population, H_T = 0.254 (Table 3). Some sharing of ETs was observed between the different areas. One ET was shared between the Geysir area and Hveravellir, one ET was represented by four isolates from the Geysir area and one from Hveragerdi. Two ETs were shared between isolates from Hveravellir and Hveragerdi (Fig. 2). The G_{ST} value was noticeably low for the whole cluster, 0.016. This indicates that the different geographic regions harbor the same genetic population.

Thermus thermophilus

This population consisted of 15 isolates from only two coastal locations, eight from Reykjanes and seven from Oxarfjordur. No ET was shared between the populations of these two areas, but 14 ETs could be distinguished. Six of the ten enzymes were polymorphic and the number of alleles ranged from 1 to 6 with an average of 2.8. SOD, HK, AP and IDH were invariant in the population (Table 2). The overall diversity was moderately high, H_T = 0.438 (Table 3), but significantly lower within the regions, 0.329 in Reykjanes and 0.340 in Oxarfjordur. The G_{ST} value showed that the inter-regional component of diversity was high between these two areas, 0.242, which strongly indicates that the separate populations are genetically different and are evolving independently. This divergence of the *T. thermophilus* population was also clearly observed in the UPGMA clustering of the isolates (Fig. 2).

Thermus igniterrae

The population of *T. igniterrae* consisted of 22 isolates found in all the sampling areas except the three coastal sites. Nineteen ETs could be distinguished (Fig. 2). Eight of the ten enzymes were polymorphic and the

Table 1 Characteristics of the geothermal sampling sites and isolated *Thermus* species

Sampling area	Hot spring	Temperature (°C)	PH	Description of hot springs	Number of species (number of strains)
Hveravellir	HL-2	84	6.8	Bubbling pool with no microbial mat	*T. broc* (2103), *T. scoto* (2101, 2100)
	HL-7	80–95	7.9	Very small pool, no microbial mat	*T. scoto* (2102)
	HL-9	60–70	5.5	Mud pit, green microbial mat at the edges	*T. scoto* (2127)
	HL-11	90	8.5	Pool with grey microbial mat	*T. scoto* (2108), *T. ignit* (2105), *T. broc* (2106)
	HL-13	70–80	9.3	Pool with green microbial mat	*T. ignit* (2104, 2107)
	HL-14	70–80	9.8	Pool with grey microbial mat, grey sediment	*T. ignit* (2110, 2114)
	HL-15	70–80	7.1	Bubbling pool, green microbial mat at the edges	*T. broc* (2111), *T. scoto* (2109)
	HL-16	70–80	7.5	Pool with gray microbial mat, grey precipitations	*T. broc* (2115)
Hagongur	KK-6	73	7.1	Hot spring in a river bank, disappeared during flood	*T. scoto* (6168, 6113, 6230, 6226, 6208, 6219, 6204. 6183)
	VS-1	75	7.5	Grey microbial mat	*T. ignit* (6213, 6298, 6224) *T. scoto* (6177, 6116)
Geysir area	Geysir	62–90	9.0–9.2	Bubbling pool	*T. broc* (2153, 319, 360) *T. scoto* (346)
	Lau-6, 7	70–72	9.5	White microbial mat	*T. oshim* (2131), *T. ignit* (2130)
	Lau-9, 11	65	9.1	Hot creek	*T. ignit* (2132, 2133, 2134)
	FL-19	90	9.2	Bubbling pool	*T. broc* (338, 339)
	FL-18	97	9.2	Bubbling pool	*T. broc* (340)
	FL-15	92	9.2	Bubbling pool	*T. broc* (334)
Hveragerdi	Badstofuhver	70–85	8.2–8.9	Orange and white microbial mat	*T. broc* (2788), *T.* sp. (2789), *T. oshim* (51) *T. ignit* (2790, 2791, 2792, 2793)
	HG-8	90	8.1	Bubbling pool	*T.* sp. (2795)
	HG-10	65	9.2	Water pool, no microbial mat	*T. broc* (2797) *T. scoto* (2811) *T.* sp. (2798)
	AC-3	65–75	8.6	Black microbial mat, gas effluent	*T. scoto* (52)
	HV-11	62	8.3	Green microbial mat	*T. broc* (79)
	HV-11	65	9.8	Green microbial mat	*T. broc* (2128, 2129)
	HV-11	88	9.7	Bubbling pool, black sediment	*T. ignit* (2136, 2443)
	HV-16	65–75	7.0	Grey microbial mat	*T. scoto* (53)
	GD-13	84	9.7	Steam hole	*T. ignit* (2137)
	HV-1	77	8.9	Green and brown microbial mat	*T. oshim* (77)
	HV-19	61	7.7	Pool with yellow and black microbial mat	*T. oshim* (80)
	Jackoshver	72–80	8.0	Pool with no microbial mat	*T. broc* (140)
	Thiobacillushver	61	6.8	Pool with grey microbial mat	*T. oshim* (2135)
	JK-1	90–99	8.0	Bubbling pool	*T. broc* (129)
	RD-1	78	7.0	Iron-rich sediment	*T. scoto* (2154)
	Spring 1	65–80	7.0	Grey microbial mat	*T. ignit* (165)
	Selfoss	75–85	7.5	Water pipeline	*T. scoto* (154)
	Lactosahver	78	6.8	Pool with grey and black microbial mat	*T. broc* (206, 210, 211) *T. oshim* (220)
Snaefellsnes	AK-7	70	6.2	Green microbial mat	*T.* sp. (2138, 2139)
Hrafntinnusker	HS-5	76	6.6	Bubbling pool, grey microbial mat at the edges	*T. ignit* (2117)
	HS-6	74–80	6.2	Bubbling pool, grey microbial mat	*T. scoto* (2118)
	HS-14	72	5.9	Small brown pool with brown microbial mat	*T. scoto* (2631)
	HS-15	70	6.1	White, green, orange microbial mat	*T. scoto* (2119)
	HS-16	88	4.5	Water pool, grey microbial mat	*T. scoto* (2126), *T.* sp. (2123)
	HS-17	90	8.5	Gray, green, orange microbial mat	*T. antr* (2120)
	HS-18	68	6.8	Grey microbial mat	*T. scoto* (2122, 2125)
	HS-20	75–86	7.6	Grey microbial mat	*T.* sp. (2124)
	HS-21	84–92	7.6	Grey microbial mat	*T. ignit* (2144)
	HS-22	95	7.6	Grey microbial mat	*T. scoto* (2121)
Reykjanes	Salt factory	55	6.8	Hot stream, silica precipitations	*T. therm* (781, 791)
	Blue Lagoon	45–75	7.5	Hot water pipeline	*T. therm* (797, 761, 759, 871, 847, 872)
Oxarfjordur	OX	75–82	6.1	Hot water from a drill hole	*T. therm* (1285, 1318, 1373, 1251, 1262 1270, 1340)

Abbreviations: *T. broc*, *Thermus brockianus*; *T. therm*, *Thermus thermophilus*; *T. ignit*, *Thermus igniterra*; *T.* sp., *Thermus* species; *T. antr*, *Thermus antranikianii*; *T. scoto*, *Thermus scotoductus*; *T. oshim, Thermus oshimai*

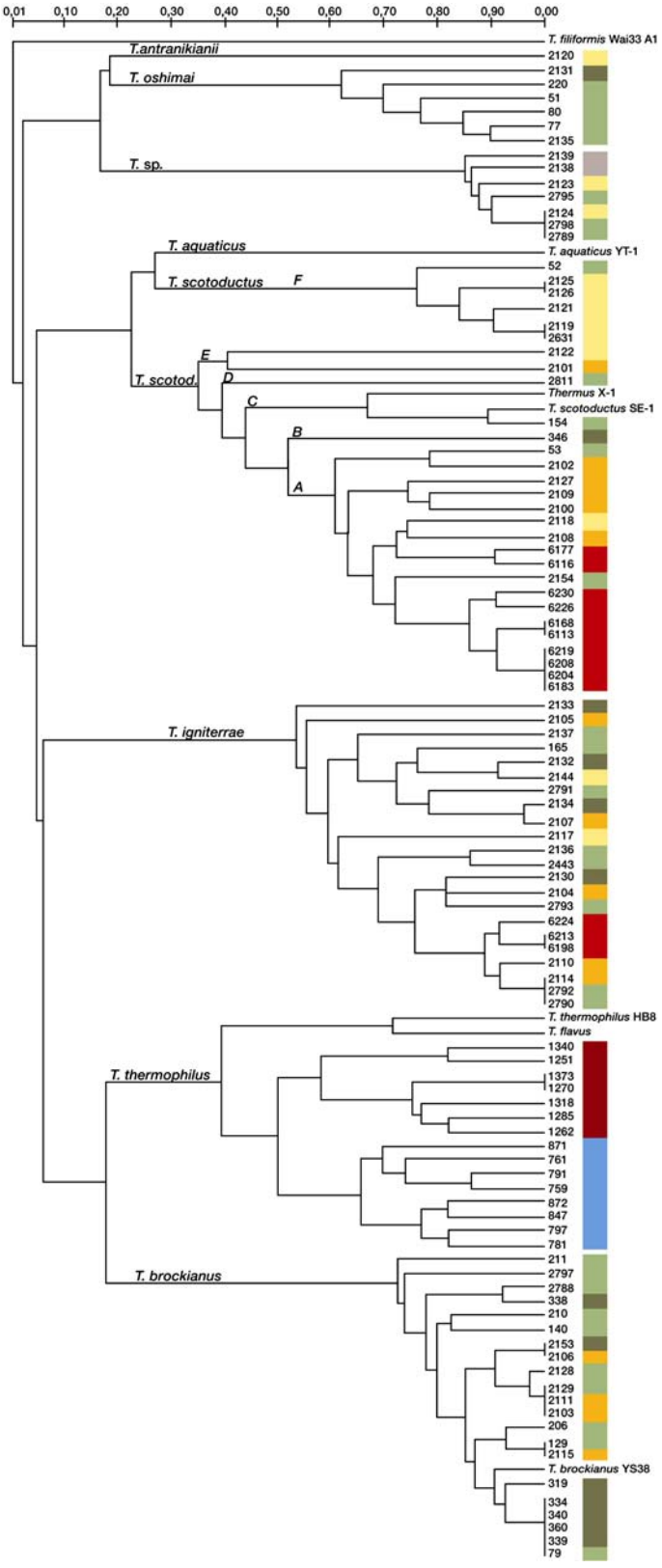

Fig. 2 Genetic relationship between 101 *Thermus* strains isolated from hot springs in Iceland. The dendrogram was generated with average linkage algorithm of clustering from matrix of genetic distances based on ten enzyme loci. The sample sites are defined according to the colors in Fig. 1

number of alleles ranged from 1 to 6, with an average of 2.9. SOD and IDH were invariant in the population (Table 2). The overall population was genetically relatively tight, as reflected by genetic diversity index, $H_T = 0.376$ (Table 3). There was a little sharing of ETs within the lineage from the five geographical areas. One ET was shared between Hveragerdi and Hveravellir. The G_{ST} value was relatively high for the whole population,

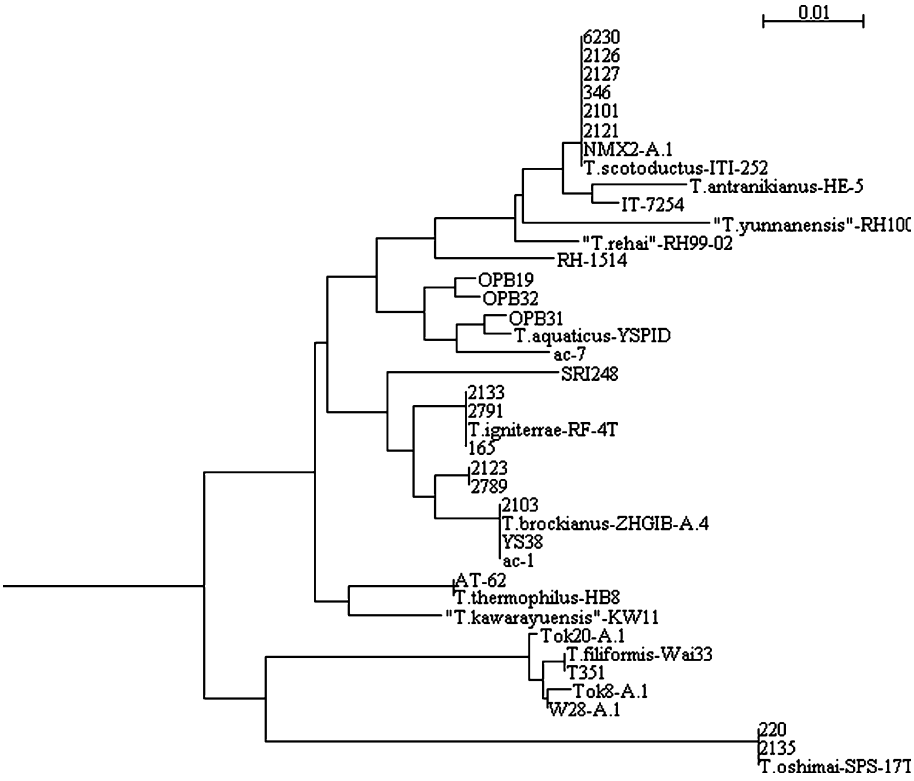

Fig. 3 Phylogenetic positon of 14 subset *Thermus* strains used in this study among representatives of all major *Thermus* lineages

0.179, indicating inter-regional genetic differentiation. However, as isolates from all of the locations were relatively few, this high value should be cautiously interpreted.

Thermus scotoductus

This population consisted of 29 isolates, which were found in all the sampling sites except the three coastal sites. The genetic diversity was highest for this *Thermus* population, H_T = 0.583, including *T. scotoductus* SE-1 (Table 3). All ten enzymes were polymorphic (Table 2), but the number of alleles for each enzyme ranged from 4 to 10, with an average of 5.4. Twenty-three distinct ETs could be distinguished and there was no sharing of ETs between the different geographic regions. The Nei's coefficient of genetic differentiation for the whole population was very high, G_{ST} = 0.362, indicating inter-regional genetic differences in this population. However, this high G_{ST} value may be misleading as it may be due to the very distinct genetic lineages observed within the population. These lineages may be ecologically distinct subpopulations, which have different habitat preferences and are evolving independently. The lineages B–F consisted of very few isolates. With larger sample size, these lineages might be found in their preferred habitats in other geographical regions as well, and this would consequently lower the overall G_{ST} value.

Table 2 Allele frequencies within lineages for different loci

Locus/no. of alleles	Lineage[a]					
	Thermus brockianus	*Thermus thermophilus*	*Thermus igniterrae*	*Thermus scotoductus*	*Thermus* sp.	*Thermus oshimai*
Alkaline phosphatase	1	1	5	4	2	2
Aspartate aminotransferase	4	4	6	4	2	2
Esterase	3	6	2	5	1	3
Glucose-6-phosphate isomerase	1	4	4	6	1	3
Hexokinase	2	1	2	4	2	1
Isocitrate dehydrogenase	2	1	1	4	1	1
Malate dehydrogenase	3	2	2	4	2	1
Nucleoside phosphorylase	3	4	3	8	1	3
Superoxide dismutase	1	1	1	5	1	2
Unspecific dehydrogenase	1	4	3	10	1	1
Mean	2.1	2.8	2.9	5.4	1.4	1.9

[a]Only one *Thermus antranikianii* strain was found in this analysis, therefore it is not included in the table

Table 3 Genetic diversity within lineages

Lineage[a]	No. of strains	No. of ETs	Polymorphic loci	Mean no. of alleles	ETs H_T	Isolates H_T
Thermus brockianus	21	13	0.6	2.1	0.254	0.193
Thermus thermophilus	15	14	0.6	2.8	0.438	0.433
Thermus igniterrae	22	19	0.8	2.9	0.376	0.358
Thermus scotoductus[b]	30	24	1	5.4	0.583	0.55
*Thermus*sp.	7	5	0.4	1.4	0.16	0.047
Thermus oshimai	6	6	0.6	1.9	0.293	0.293

[a]Only one *Thermus antranikianii* strain was found in this analysis, therefore it is not included in the table[b]*T. scotoductus* SE-1 includedH_T total genetic diversity; *ET* electrophoretic types

Thermus oshimai, Thermus sp. and Thermus antranikianii

The low genetic diversity observed for the new *Thermus* sp. and *T. oshimai* lineages is based on the low isolate numbers within them (Table 3). Therefore, these values are not significant. Only one *T. antranikianii* strain was found.

Environmental variables

The origin of the *Thermus* strains selected for this study was investigated in relation to two different physical parameters of the sample sites, pH and temperature. No clear relationship was found between temperature at the isolation site and the different lineages. Place of origin of three species *T. igniterrae*, *T. brockianus* and *T. scotoductus* showed some relation to pH (Tables 1 and 4). The *T. igniterrae* strains examined in this study originated from 12 different hot springs. Sixty-seven percent of them were above pH 8 and 50% were in the pH range 9–9.9 (Table 4). This tendency can also be estimated on the basis of sampling effort. The majority of strains belonging to this lineage were isolated from hot springs with pH above 8.1 (up to pH 9.8). Twenty-one of the sampled hot springs had pH at and above 8.0 and 16. *T. igniterrae* strains were isolated from these hot springs. On the other hand, 21 hot springs were in the pH range 6–7.9 and only six *T. igniterrae* strains were isolated from these hot springs. Therefore, per sampling effort, *T. igniterrae* was found three times more common in the highest pH hot springs than in the hot springs with pH range 6–7.9.

The *T. brockianus* strains originated from 15 different hot springs. Seventy-eight percent were above pH 8 and 40% were in the pH range 9–9.9. None of the hot springs had pH lower than 6 and only two hot springs were below pH 7. This tendency is also clear on the basis of sampling effort where *T. brockianus* (a total of 21 strains) was found almost three times more common in the hot springs with the highest pH (pH 8–9.9) than in the hot springs with pH range 6–7.9.

T. scotoductus was isolated from 18 hot springs and one man-made system. Approximately 78% of these had pH below 8.0 and 17% had pH below pH 6.0. Also, per sampling effort, *T. scotoductus* (total of 29 strains) was found more than six times more common in hot springs in the pH range 4.5–7.9 (25 strains) than in hot springs of pH range 8–9.9 (a total of four strains) (Table 4). This is in clear contrast with what was observed with *T. igniterrae* and *T. brockianus*.

Information on conductivity was not available for the sampling sites, but the coastal sites were saline. *T. thermophilus* was only found in the coastal sites and the only other species found in a coastal hot spring was the unaffiliated *Thermus* lineage represented by isolates 2789 and 2139.

T. oshimai was found in two separate geothermal areas. However, all these hot springs had a striking common characteristic, that is, a bacterial filamentous biomass that is typical of high-sulfide hot springs.

Table 4 Percentage of total hot springs in which each species was found, at different pH ranges. Number of isolates isolated at each pH range is also shown

Species	Total number of hot springs in which found	Percentage of total hot springs in which each species was found at different pH ranges (number of isolates isolated at each pH range)				
		pH 4.5–5.9	pH 6.0–6.9	pH 7.0–7.9	pH 8.0–8.9	pH 9.0–9.9
Thermus brockianus	15	0	13 (4)	13 (2)	34 (5)	40 (10)
Thermus igniterrae	12	0	8 (1)	25 (5)	17 (5)	50 (11)
Thermus scotoductus	19	17 (3)	22 (6)	39 (16)	11 (2)	11 (2)
Thermus oshimai	6	0	33 (2)	17 (1)	33 (2)	17 (1)
Thermus sp	6	16.7 (1)	16.7 (2)	16.7 (1)	33 (2)	16.7 (1)
Thermus antranikianii	1	0	0	0	100 (1)	0

Physiologic and phenotypic characterization

The numerical taxonomy results did not reflect the genetic clustering. No correlation was found between species lineages and growth on specific single carbon source or nitrate reduction. However, *T. igniterrae* as a group could be distinguished from other lineages, as usually only about half of the strains grew on any particular single carbon source (Table 5).

The salt and ion tolerance patterns varied between the different lineages (Table 5). For example, the isolates from coastal habitats belonging to *T. thermophilus* had the highest salt tolerance of all. All of them tolerated 1% NaCl and the majority tolerated 2% NaCl (67%). Two isolates of the new *Thermus* sp. (lineage 5) grew at 2% NaCl, but these isolates were also from a saline coastal hot spring. *T. igniterrae* was on the other hand noticeably more sensitive to salt concentration than the other

Table 5 Percentage of positive phenotypic tests for the strain lineages derived from the multilocus enzyme electrophoresis analysis

Phenotypic test[a]	Lineage (no. analyzed)					
	Thermus brockianus (10)	*Thermus thermophilus* (5)	*Thermus igniterrae* (9)	*Thermus scotoductus* (12)	*Thermus* sp. (5)	*Thermus oshimai* (4)
Acetate	0	0	11	0	0	0
Arabinose	10	0	0	7	40	0
Arginine	100	60	44	93	100	75
Casein	0	0	0	7	0	0
Citrate	20	0	33	14	0	50
Formic acid	100	80	44	71	100	75
Galactose	50	100	33	100	100	100
Glutamine	30	100	33	29	80	50
Glucose	63	60	44	79	80	75
Glycerol	100	100	56	93	100	100
Histidine	0	17	0	0	20	0
Lactose	10	0	0	0	0	0
Leucine	0	0	11	0	20	0
Malate	20	0	33	8	40	25
Maltose	10	0	0	0	40	0
Minimal	0	0	0	0	0	0
Ornithine	22	20	33	15	40	33
Proline	100	60	33	62	100	100
Pyruvate	56	50	38	14	80	75
Raffinose	0	0	0	7	0	0
Rhamnose	10	0	0	0	0	25
Sorbitol	88	100	67	75	100	67
Succinate	100	60	33	62	100	100
Sucrose	0	0	0	7	0	0
Starch	56	50	38	15	80	75
Tartrate	0	0	0	0	40	0
Threonine	22	20	33	15	40	33
Valine	10	0	0	0	0	25
Nitrate reduction	43	33	95	18	43	50
Growth at 50°C	100	56	76	79	86	100
Growth at 65°C	95	89	100	100	100	100
Growth at 78°C	62	67	14	21	86	33
0.5% NaCl	100	100	5	100	100	100
1.0% NaCl	43	100	0	21	100	0
2.0% NaCl	5	67	0	0	29	0
2 mm EDTA	100	100	86	86	100	100
50 mm Mg^{2+}	100	100	52	96	71	67
50 mm Ca^{2+}	67	78	33	82	71	67
2 mm Cu^{2+}	67	57	29	96	71	67
50 mm SO_4^{2-}	76	67	19	93	86	100
50 mm SO_3^{2-}	100	100	0	93	86	100
50 mm $SO_2O_3^{2-}$	100	100	52	100	86	100
pH 5	29	11	10	25	86	0
pH 6	95	100	100	100	100	100
pH 8	100	100	100	97	100	100
pH 8.7	100	100	29	96	100	100
pH 9.5	62	78	5	14	100	17
Thosulfate oxidation[b]	100	60	80	78	0	100

[a]No growth was observed on aspargine, aspartate, fructose, glutamine, alpha-ketoglutarate, lysine, serine, xylose and minimal media[b]Fewer strains were examined for thiosulfate oxidation, *T. brockianus* (5), *T. thermophilus* (5), *T. igniterrae* (5), *T. scotoductus* (9), *T.* sp. (5) and *T. oshimai* (4)*EDTA* Ethylenediaminetetra-acetic acid

species. Only 5% of the isolates grew at NaCl concentration as low as 0.5%. Similarly, only 19% of the *T. igniterrae* strains grew on Na_2SO_4, none grew on Na_2SO_3, and 29 and 33% grew on and 2 mM of $CuSO_4$ and 50 mM of $CaSO_4$, respectively. The great majority of strains belonging to all the other *Thermus* species grew under these conditions (Table 5).

Over 90% of the strains of all the *Thermus* species grew well at pH 8.7, except strains in the *T. igniterrae* lineage, where only 29% of strains grew. This was unexpected as they were isolated from the hot springs with the highest pH. Of the terrestrial species, *T. brockianus* was the other species most often isolated from hot springs with high alkalinity, and 62% of the strains from this lineage grew at pH 9.5. *Thermus* species appear generally to be adapted to slightly alkaline conditions; however, *T. scotoductus* was most often isolated from hot springs in the lower pH range. Twenty-five percent of the strains in of this lineage grew at pH 5.0, which is in the higher range compared with other species. Notably, only 14% of the strains grew at pH 9.5, which agrees well with the pH of the hot springs origin of most isolates. The unaffiliated *Thermus* lineage grew very well at both extremes, pH 5.0 and 9.5 (86 and 100%, respectively), and was also isolated from hot springs in this range.

Ethylenediaminetetra-acetic acid (EDTA) is a scavenger of divalent metal ions but bacteria need these ions, that is, as coenzymes in enzyme complexes and in the cell wall. If these ions are not present, the enzymes does not work and no growth occurs (Madigan et al. 1997). Most strains tolerated 2 mM of EDTA, but 5 mM was too much for all of them.

Thiosulfate oxidation

We tested four to nine strains in each lineage and in some of the lineages (*T. thermophilus, T. igniterrae* and *T. scotoductus*), a part of the strains could oxidize thiosulfate (Table 5). However, all of the *T. brockianus* and *T. oshimai* strains were able to do this. Furthermore, none of the strains in the new *Thermus* sp. lineage were thiosulfate-oxidative.

Discussion

Based on MLEE analysis of 101 Icelandic *Thermus* strains, seven distinct and genetically highly divergent populations of *Thermus* were observed. This grouping (Fig. 2) was consistent with the SSU rRNA sequencing results (Fig. 3). As expected, the two trees had different tree topologies as the MLEE method can only be used for demarcation of lineages, but not for phylogenetic relationship as the SSU rRNA sequencing method.

Six of the lineages could be assigned to validly described *Thermus* species that have previously been found in Iceland: *T. brockianus*, *T. thermophilus*, *T. oshimai*, *T. scotoductus, T. antranikianii* and *T. igniterrae*. The two species, *T. aquaticus* and *T. filiformis* seem to have no close relatives in Iceland. One lineage of seven isolates differed in all loci from other groups and may represent a new *Thermus* species. According to SSU rRNA sequencing, the isolates 2123 and 2789 that belong to this new lineage are approximately midway between *T. brockianus* (strain YS38) and *T. igniterrae* (strain RF-4) giving 98.3 and 97.8% sequence similarity, respectively. However, results of DNA:DNA reassociation between *Thermus* 2789 and *T. brockianus* strain YS38 (type strain) gave a borderline value of 72.9% (Amann et al. 1995; Goodfellow and O'Donnell 1993; Stackebrandt and Goebel 1994).

Some *Thermus* strains are able to oxidize thiosulfate and grow mixotropically (Skirnisdottir et al. 2001). Our results here show that this is a relatively common trait within the genus *Thermus*.

This study illustrates that the MLEE method is very effective in delimiting *Thermus* species. It can also be used for a rapid identification of species. For example, the SOD and the IDH were different between the different *Thermus* species, but almost invariable within the species.

An apparent geographic component was observed in the distribution within the *T. thermophilus* lineage. Two genetically distinct populations were found in the two widely separated locations sampled. Similar results have been obtained with another species, *Rhodothermus marinus*, living in coastal hot springs (Petursdottir et al. 2000).

Geographically distinct populations could not be discerned within other *Thermus* species identified in this study. Genetic diversity was generally high, but isolates of each species were too few and scattered between too many geographic areas. Of the lineages with the greatest number of isolates, genetic diversity was far greatest in the *T. scotoductus* lineage, moderate in *T. igniterrae* and *T. thermophilus* lineages and very low in the *T. brockianus* lineage. The *T. scotoductus* lineage appeared also to be divided into very distinct sublineages that may represent different ecotypes of the species. The opposite was true for the *T. brockianus* lineage. Genetic diversity in this lineage was unusually low for a natural species, despite the fact that the isolates were from widely separate regions and is indicatieve of pandemic distribution. It is noteworthy that the type species isolated in Yellowstone National Park in USA had a very similar allelic pattern to the Icelandic isolates.

Number of studies have shown that phenotypical properties are not very useful in defining taxonomic groups at the species level of *Thermus*. Furthermore, physiological properties, such as temperature and pH growth optimum and maximum appear to be similar from species to species (Chung et al. 2000; Williams et al. 1995). Our study corroborates previous findings that phenotypic characteristics, especially carbon source utilization patterns, do not discriminate between different *Thermus* species as no correlation was found between

growth on specific single carbon source to the clustering obtained by the MLEE analysis. Thus, no metabolic phenotype could be attributed to the genetic lineages.

Balkwill et al. (2004) have noted that *T. scotoductus* may be phenotypically more variable than other *Thermus* species as observed in lipid composition, pigmentation, carbon utilization and not the least in utilization of diverse inorganic compounds, such as iron and sulfur compounds. This could explain the considerable subdivision of these lineages by MLEE in this study where the different lineages may represent quite different ecotypes.

Water activity may be important for habitat preferences of *Thermus* species, either ability or inability to tolerate salts. Thus, the capacity of *T. thermophilus* growing at higher concentrations of sodium chloride may be sufficient to explain its habitat preference. In contrast, the majority of *T. igniterrae* strains appear to be sensitive to ionic strength of various salts. Noteworthy is its sensitivity to sulfur compounds (Table 5).

T. igniterrae shows discontinuous but very unexpected distribution. It has so far, only been found in Iceland and northeast Australia. In Australia it was detected and isolated as the dominant *Thermus* species at the higher temperatures in nonvolcanically heated hot runoff from a borehole (New Lorne Bore) in the Great Artesian Basin (GBA) aquifer in Blackall, central Queensland (Spanevello and Patel 2004). The water from this borehole is very pure and has a sodium bicarbonate chloride hydrochemistry typically found in bores in this region. The total ionic strength is about 430 mg/L of which CO_3^-–HCO_3^- is 60%. It has low concentration of Na^+, 3.7 mg/L and bivalent cations, 2 mg/L of Ca^{2+}, 0.1 mg/L of Mg^{2+} and 0.01 mg/L of Mn^{2+}. No sulfide is reported and a low sulfate concentration (4.3 mg/L) observed. The pH is relatively high, 8.5, and temperature at the source is 88°C (Spanevello 2001). The physiochemical characteristic of this *T. igniterrae* habitat in Australia agrees well with our findings, which indicate that the species prefers high alkaline and high temperature hot springs generally low in mineral salt content and is sensitive to NaCl, bivalent cations and sulfur compounds in the culture media (Tables 1, 4 and 5).

Two important niche parameters that may explain growth in any particular habitat are pH and temperature. In this study specific temperature did not relate the isolation sites of any of the species lineages. Regular temperature fluctuations in hot springs appear to be common. Up to 10°C difference have been observed between the lowest and the highest temperature within a 24–60-h period (Marteinssson, unpublished). Also, due to temperature gradients in hot springs, isolates may originate from preferred neighboring temperature zones that may have higher or lower temperature. The effect of temperature on the distribution of *Thermus* species may, therefore be masked.

Most hot springs in Iceland are alkaline and high alkalinity hot springs with pH up to at least 9.0 are relatively common in Iceland. Higly acidic hot springs with high water turnover rate are, scarce and only single or very few isolates are usually obtained from these habitats. These isolates are interesting as representatives of strains tolerating or growing at or close to the extreme low pH limit of growth for a particular species. In this study, pH of the sample site indicates a relationship to lineage formation. *T. igniterrae* and *T. brockianus* were generally isolated from hot springs with pH > 8.0, but *T. scotoductus* from hot springs around neutral pH.

In conclusion, our results indicate that some of the genospecies of *Thermus* may be ecologically distinct populations distinguished by adaptation to different physicochemical conditions. The results of this study also suggest that an ecological approach will perhaps reveal more about the factors that distinguish between different *Thermus* species or populations than phenotypic analyses in the laboratory. Such an approach should be based on a greater number of isolates randomly selected from each of a selected few characteristic hot springs of different types. The isolates should also be selected over a period of time to see how stable the populations are and environmental conditions carefully monitored for the same reason. In such a study, the MLEE methodology would provide fast and relatively inexpensive means for species identification besides giving valuable genotypic data for population structure studies.

References

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbial Rev 59:143–169

Balkwill DL, Kieft TL, Tsukuda T, Kostandarithes HM, Onstott TC, Macnaughton S, Bownas J, Fredrickson JK (2004) Identification of iron-reducing *Thermus* strains as *Thermus scotoductus*. Extremophiles 8:37–44

Brock TD, Freeze H (1969) *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. J Bacteriol 98:289–297

Cashion P, Holder-Franklin MA, McCully J, Franklin M (1977) A rapid method for the base ratio determination of bacterial DNA. Anal Biochem 81:461–466

Caugant DA, Mocca LF, Frasch CE, Froholm LO, Zollinger WD, Selander RK (1987) Genetic structure of *Neisseria meningitidis* populations in relation to serogroup, serotype, and outer membrane protein pattern. J Bacteriol 169:2781–2792

Chung AP, Rainey FA, Valente M, Nobre MF, da Costa MS (2000) *Thermus igniterrae* sp. nov. and *Thermus antranikianii* sp. nov., two new species from Iceland. Int J Syst Evol Microbiol 50:209–217

Escara JF, Hutton JR (1980) Thermal stability and renaturation of DNA in dimethylsulphoxide solutions: acceleration of the renaturation rate. Biopolymers 19:1315–1327

Goodfellow M, O'Donnell AG (1993) Roots of bacterial systematics. In: Goodfellow M, O'Donnell AG (eds) Handbook of new bacterial systematics. Academic, London, pp 3–54

Hjorleifsdottir S, Skirnisdottir S, Hreggvidsson GO, Holst O, Kristjansson JK (2001) Species composition of cultivated and non-cultivated bacteria from short filaments in an Icelandic hot spring at 88°C. Microbial Ecol 42:117–125

Hudson JA, Morgan HW, Daniel RM (1987) *Thermus filiformis* sp. nov., a filamentous caldoactive bacterium. Int J Syst Bacteriol 37:431–436

Huss VAR, Festl H, Schleifer KH (1983) Studies on the spectrometric determination of DNA hybridization from renaturation rates. J Syst Appl Microbiol 4:184–192

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Muntu HN (ed) Mammalian protein metabolism, 3rd edn. Academic Press, New York, pp 21–132

Kristjansson JK, Hreggvidsson GO (1995) Ecology and habitats of extremophiles. World J Microbiol Biotechnol 11:17–25

Kristjansson JK, Hreggvidsson GO, Grant WD (2000) Taxonomy of extremophiles. In: Priest FG, Goodfellow M (eds) Applied microbial systematics. Kluwer Academic, Dordrecht, pp 229–289

Kristjansson JK, Hjorleifsdottir S, Marteinsson VT, Alfredsson GA (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

de Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Madigan MT, Parker J, Brock TD, Martinko JM (1997) Brock biology of microorganisms, 8th edn. Prentice-Hall, Upper Saddle River

Maidak BL, Cole JR, Parker Jr CT, Garrity GM, Larsen N, Li B, Lilburn TG, McCaughey MJ, Olsen GJ, Overbeek R, Pramanik S, Schmidt TM, Tiedje JM, Woese CR (1999) A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res 27:171–173

Manchenko GP (1994) Handbook of detection of enzymes on electrophoretic gels. CRC Press, Boca Raton

Nei M, Chesser RK (1983) Estimation of fixation indices and gene diversities. Ann Hum Genet 47:253–259

Oshima T, Imahori K (1974) Description of *Thermus thermophilus* (Yoshida and Oshima) comb. nov., a nonsporulating thermophilic bacterium from a Japanese thermal spa. Int J Syst Bacteriol 24:102–112

Petursdottir SK, Kristjansson JK (1996) The relationship between physical and chemical conditions and low microbial diversity in the Blue Lagoon geothermal lake in Iceland. FEMS Microbiol Ecol 19:39–45

Petursdottir SK, Hreggvidsson GO, da Costa MS, Kristjansson JK (2000) Genetic diversity analysis of *Rhodothermus* reflects geographical origin of the isolates. Extremophiles 4:267–274

Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS (1986) Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 51:873–884

Skirnisdottir S, Hreggvidsson GO, Holst O, Kristjansson JK (2001) Isolation and characterization of a mixotrophic sulfur-oxidizing *Thermus scotoductus*. Extremophiles 5:45–51

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654

Spanevello MD (2001) The phylogeny of prokaryotes associated with Australia's Great Basin. PhD thesis. Griffith University

Spanevello MD, Patel BKC (2004) The phylogenetic diversity of *Thermus* and *Meiothermus* from microbial mats of an Australian subsurface aquifer runoff channel. FEMS Microbiol Ecol 50:63–73

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Tabatabai MA (1974) A rapid method for determination of sulfate in water samples. Environ Lett 7:237–243

Vandamme P, Pot B, Gillis M, de Vos P, Kersters K, Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60:407–438

Whittam TS (1995) Genetic population structure and pathogenicity in enteric bacteria. In: Baumberg S, Young JPW, Wellington EMH, Saunders JR (eds) Population genetics of bacteria. Society for General Microbiology, Symposium 52. Cambridge University Press, Cambridge, pp 217 – 245

Whittam TS, Ochman H, Selander RK (1983) Multilocus genetic structure in natural populations of *Escherichia coli*. Proc Natl Acad Sci USA 80:1751–1755

Williams R, Sharp R (1995) The taxonomy and identification of *Thermus*. In: Sharp R, Williams R (eds) *Thermus* species. Plenum, New York, pp 1–42

Williams RAD, Smith KE, Welch SG, Micallef J, Sharp RJ (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 RAD, Smith KE, Welch SG, 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