Thermus composti sp. nov., isolated from oyster mushroom compost

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A Gram-stain-negative, rod-shaped bacterium (strain K-39^T) was isolated from the thermophilic phase of the composting process for oyster mushroom substrate preparation. The strain grew at 40–80 °C (optimum, 65–75 °C), at pH 5–9 (optimum, pH 7), in media containing up to 1.5 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain K-39^T formed a distinct lineage within the genus *Thermus*. Its closest cultivated relative was *Thermus islandicus* PRI 3838^T (96.8 % similarity). The DNA G+C content of strain K-39^T was 71.3 mol%. The new strain could be differentiated from the related taxa by not being able to hydrolyse starch. The predominant fatty acids of strain K-39^T were iso-$C_{17:0}$ and anteiso-$C_{17:0}$. Strain K-39^T contained a lower amount of the fatty acid iso-$C_{15:0}$ as compared to related species of the genus *Thermus*. The predominant respiratory quinone of the new isolate was menaquinone MK-8. On the basis of a taxonomic study using a polyphasic approach, strain K-39^T is considered to represent a novel species of the genus *Thermus*, for which the name *Thermus composti* sp. nov. is proposed. The type strain is K-39^T (=DSM 21686^T=NCAIM B 02340^T).

The genus *Thermus* was established by Brock & Freeze (1969) with the description of *Thermus aquaticus*. Nine further species have been described (Bjornsdottir *et al.*, 2009; Zhang *et al.*, 2010), originating from natural and artificial thermal environments such as hydrothermal areas, hot water taps, self-heating compost piles and rock surfaces (da Costa *et al.*, 2006). In a recent article, Vajna *et al.* (2010) investigated the microbiota during oyster mushroom (*Pleurotus ostreatus*) substrate preparation. The preparation had three main stages: (1) wheat straw supplemented with alfalfa was chopped, moistened and kept in heaps for composting for 7 days with daily mixing, during which the inside temperature rose to 70–75 °C; (2) the partially composted substrate was packed into tunnels for pasteurization (65 °C for 18 h); (3) it was conditioned (48 °C for 48 h), and left to cool to approximately 25 °C. During that study several strains were isolated from the thermophilic phase (70–75 °C). One strain gave low pairwise similarity values of partial 16S rRNA gene sequences to recognized members of the genus *Thermus*. This paper describes the detailed taxonomic characterization of strain, K-39^T, which represents a novel species of the genus *Thermus*.

Strain K-39^T was isolated with the use of the standard dilution plating technique on oat flake medium (DSMZ Medium 189) incubated at 60 °C. The reference strains used in this study for side by side analyses, *Thermus arciformis* TH92^T (=JCM 15153^T) and *Thermus islandicus* PRI 3838^T (=DSM 21543^T) were obtained from the Japan Collection of Microorganisms (JCM) and the Deutsche Sammlung von Micro-organismen und Zellkulturen (DSMZ), respectively. Strain K-39^T was maintained on R2A medium (DSMZ Medium 830); subcultivation for detailed taxonomical analyses was performed at 65 °C. Colony morphology was tested on R2A agar by direct observation of single colonies. Cell morphology and motility were studied with native preparations and by Gram staining according to Claus (1992). Growth at different temperatures in water baths [40, 50, 60, 65, 70, 75, 80, 82 and 85 °C, modified *Thermus* 162 medium (DSMZ Medium 878), 1 week], NaCl tolerance [0.5, 1, 1.5, 2 % (w/v), 65 °C, R2A broth, 1 week] and pH tolerance (from pH 3–11 by steps of 1, R2A, 1 week) were determined. *Thermus* 162 medium was modified according to Bjornsdottir *et al.* (2009) as follows: it contained EGTA instead of Titriplex I, 0.05 % NH_4Cl and 0.4 % vitamin solution (10 mg of *p*-aminobenzoic acid, nicotinic acid, calcium pantothenate, thiamine, pyridoxine, biotin and cyano-cobalamine

Abbreviations: GL, glycolipid; PL, phospholipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K-39^T is EU701067.

Three supplementary figures are available with the online version of this paper.

in 1 l distilled water). Single-carbon-source assimilation tests were performed in the modified *Thermus* 162 medium without yeast extract and tryptone, adding 2 g l^{-1} filter-sterilized (0.22 μm; Millipore) carbon sources. Growth was examined by measuring the turbidity of cultures incubated at 65 °C for 7 days in 10 ml screw-capped tubes containing 5 ml medium as recommended by Santos *et al.* (1989). Hydrolysis of different substrates was checked on *Thermus* agar (ATCC Medium 697) supplemented with casein (1 %), starch (10 %), Tween 20 (10 %), 40 (10 %), 60 (10 %) and 80 (10 %). Oxidase activity was studied by the method of Tarrand & Gröschel (1982) and by the Microbiology Bactident Oxidase (Merck). Catalase production was demonstrated by the method of Cowan & Steel (1974). Reduction of NO_3^- was studied according to Smibert & Krieg (1994). Other enzyme activities and hydrolysis capacities were tested on API ZYM and API 20 NE (bioMérieux) in accordance with the manufacturer's instructions. They were read after 4 h and 24 h incubation at 65 °C, respectively.

Isoprenoid quinones were extracted according to the method of Collins *et al.* (1977), and the profile was analysed by HPLC (HP 9001) (Groth *et al.*, 1997) after the cells were cultivated in liquid Rich medium (Yamada & Komagata, 1972). For fatty acid, polar lipid and peptidoglycan analyses, the cells were grown in the modified *Thermus* 162 medium for 72 h at 65 °C. Cellular fatty acids were analysed by using the Microbial Identification System (MIDI, Sherlock version 6.1; method TSBA40; gas chromatograph model 6890N, Agilent Technologies) following the method of Stead *et al.* (1992). Polar lipids were determined according to the method described by Minnikin *et al.* (1979) and identified by two-dimensional TLC. Peptidoglycan of strain K-39^T was isolated and its structure analysed according to Rusznyák *et al.* (2011).

The DNA base composition was determined from bacterial cells disrupted by using a French press. After purification of the DNA on hydroxyapatite according to the procedure of Cashion *et al.* (1977), the DNA was degraded to nucleosides using P1 nuclease and bovine intestinal mucosa alkaline phosphatase as described by Mesbah *et al.* (1989). The nucleosides were separated by reversed-phase HPLC (HP 9001) with the methods described by Tamaoka & Komagata (1984). The G+C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine.

The 16S rRNA gene was analysed using the primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′; Lane, 1991), 534R (5′-ATTACCGCGGCTGCTGG-3′; Muyzer *et al.*, 1993), 907R (5′-CCGTCAATTCMTTTGAGTTT-3′; Casamayor *et al.*, 2000), 1055F (5′-ATGGCTGTCGTCAGCT-3′; Ferris *et al.*, 1996) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′; Polz & Cavanaugh, 1998) as described previously by Sipos *et al.* (2007). Sequence similarity analysis was performed via the EzTaxon service (Chun *et al.*, 2007). Alignment of this sequence with *Thermus* type strains was carried out with SILVA aligner (Pruesse *et al.*, 2007). The alignment was corrected manually according to secondary structure, and phylogenetic trees were prepared with the MEGA5 software (Tamura *et al.*, 2011).

On the basis of almost complete 16S rRNA gene sequence, K-39^T gave low pairwise similarity values (91.8–96.8 %) with the recognized members of the genus *Thermus*. Closest relatives of strain K-39^T were *T. islandicus* PRI 3838^T (96.8 %) and *T. arciformis* TH92^T (96.6 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain K-39^T can be distinguished from species of the genus *Thermus* (Fig. 1; Figs S1 and S2, available in IJSEM Online).

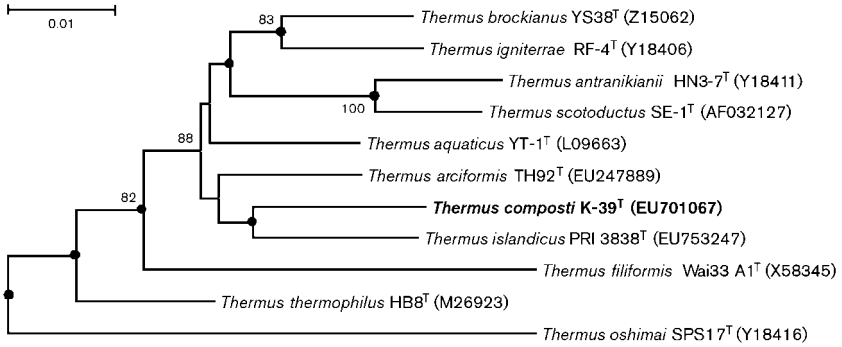

Fig. 1. Phylogenetic position of strain K-39^T and type strains within the genus *Thermus*. The phylogenetic tree is based on the SILVA alignment of almost complete 16S rRNA gene sequences with manual correction according to secondary structure. All positions containing gaps and missing data were eliminated. There were a total of 1401 positions in the final dataset. The phylogenetic tree was reconstructed with the neighbour-joining method (distance options according to the Kimura two-parameter model) with bootstrapping (based on 1000 resampling) using the software package MEGA5 (Tamura *et al.*, 2011). Only bootstrap values >70 % are shown. GenBank accession numbers are given in parentheses. Sequence of *T. oshimai* was used as outgroup. Filled circles indicate branches that were also recovered in the maximum-parsimony and maximum-likelihood tree. Bar, 0.01 substitutions per nucleotide position.

The results of the physiological and biochemical examination of strain K-39^T and the above-mentioned related type strains are summarized in Table 1. It shows that strain K-39^T had three unique phenotypic characteristics, which can differentiate the new isolate from related type strains of members of the genus *Thermus*: K-39^T was not able to hydrolyse starch and was not able to utilize glycerol and lactose as sole carbon source.

The predominant respiratory quinone of the new isolate was menaquinone MK-8, which is common to the genus *Thermus* (da Costa *et al.*, 2006); menaquinone MK-6 and menaquinone MK-7 were found as minor fractions. The fatty acid composition of strain K-39^T and related type strains is shown in Table 2. Its predominant fatty acids were iso-$C_{17:0}$ and anteiso-$C_{17:0}$. Strain K-39^T contained more fatty acid anteiso-$C_{17:0}$ but less fatty acid iso-$C_{15:0}$ compared with the related strains of the genus *Thermus*. The predominant polar lipids of strain K-39^T were one major phospholipid (PL1) and one major glycolipid (GL2) (Fig. S3), which is common to the genus *Thermus* (da Costa *et al.*, 2006) and further confirmed by our results for *T. islandicus* and *T. arciformis* (Fig. S3). Strain K-39^T showed the peptidoglycan type A3β L-Orn–Gly–Gly, also published for other members of the genus *Thermus* (Quintela *et al.*, 1995; da Costa *et al.*, 2006). The DNA G+C content of strain K-39^T was 71.3 mol%, which is the highest in the genus (Brock & Freeze, 1969; Oshima & Imahori, 1974; Hudson *et al.*, 1987; Kristjánsson *et al.*, 1994; Williams *et al.*, 1995, 1996; Chung *et al.*, 2000; Bjornsdottir *et al.*, 2009; Zhang *et al.*, 2010).

On the basis of the low similarity values of 16S rRNA gene sequence of strain K-39^T to *T. islandicus* and *T. arciformis*, and the differences in fatty acid profiles and in phenotypic characteristics, strain K-39^T is considered to represent a novel species within the genus *Thermus*, for which the name *Thermus composti* sp. nov. is proposed.

Table 1. Characteristics that distinguish strain K-39^T from the type strains of related species of the genus *Thermus*

Strains: 1, K-39^T; 2, *T. islandicus* PRI 3838^T (=DSM 21543^T); 3, *T. arciformis* TH92^T (=JCM 15153^T). +, Positive; w, weakly positive; −, negative. Data are from the present study unless otherwise indicated.

Characteristic	1	2	3
Pigmentation	White	Yellow	Yellow
Growth in 1 % NaCl*	+	−	+
Maximum growth temperature (°C)*	80	79	78
Production of:			
α-Galactosidase	−	−	+
β-Galactosidase	−	−	+
Catalase	+	−	+
Degradation of β-Glucopyranoside	−	−	+
Hydrolysis of starch	−	+	w
Reduction of nitrate	−	−	+
Utilization of:†			
Casamino acids	+	−	+
Cellobiose	w	w	+
D-Fructose	+	−	+
D-Galactose	−	−	+
D-Glucose	+	−	+
Melibiose	w	−	+
Trehalose	+	−	+
Glycerol	−	+	w
Lactose	−	w	+
L-Arginine	w	−	w
L-Glutamine	w	−	+
L-Serine	−	−	+
Malate	w	−	w
myo-Inositol	−	w	−
DNA G+C content (mol%)*	71.3	69	68.3

*Data for growth in NaCl, maximum growth temperature and DNA base composition for *T. islandicus* and *T. arciformis* were taken from Bjornsdottir *et al.* (2009) and Zhang *et al.* (2010).
†Carbon-source utilization data for *T. islandicus* were taken from Bjornsdottir *et al.* (2009).

Description of *Thermus composti* sp. nov.

Thermus composti (com.pos′ti. N.L. gen. n. *composti* of/from compost).

Cells are Gram-stain-negative, non-motile rods (2–8 μm in length and 0.4–0.8 μm in width). Aerobic, oxidase- and catalase-positive, and does not reduce nitrate. Colonies on R2A medium after 2 days incubation at 65 °C are small (0.05–0.1 mm in diameter), white, bright and round. Growth occurs at 40–80 °C (optimum, 65–75 °C), at pH 5–9 (optimum, pH 7) and with 0.5–1.5 % (w/v) NaCl. Grows well on modified *Thermus* medium 162 and

Table 2. Fatty acid composition of strain K-39^T and the type strains of related species of the genus *Thermus*

Strains: 1, K-39^T; 2, *T. islandicus* PRI 3838^T (=DSM 21543^T); 3, *T. arciformis* TH92^T (=JCM 15153^T). −, Not detected (<0.5 %). Values are percentages of total fatty acids. Fatty acids present at <0.5 % in all tested strains are not shown. Data are from the present study.

Fatty acid	1	2	3
iso-$C_{13:0}$	−	0.7	0.6
iso-$C_{14:0}$	−	1.3	1.3
iso-$C_{15:0}$	7.8	23.4	28.7
anteiso-$C_{15:0}$	2.25	20.3	8.7
$C_{15:0}$	−	0.6	0.7
iso-$C_{16:0}$	4.1	4.5	11.1
$C_{16:0}$	10.2	8.3	6.9
iso-$C_{17:0}$	49.0	24.1	31.2
anteiso-$C_{17:0}$	22.4	14.6	8.5
$C_{17:0}$	0.6	−	−
iso-$C_{18:0}$	1.0	−	0.6
anteiso-$C_{19:0}$	0.7	−	−
$C_{18:0}$	0.7	0.7	−

R2A. Degrades α-glucopyranoside and aesculin, but not β-glucopyranoside. Negative in tests for α- and β-galactosidase. Hydrolyses gelatin and Tweens 20, 40, 60 and 80, but not casein and starch. Utilizes acetate, Casamino acids, D-fructose, D-glucose, trehalose, maltose and pyruvate. Does not utilize acetamide, citrate, D-galactose, raffinose, D-sorbitol, D-xylose, erythritol, formate, glycerol, lactose, L-arabinose, L-rhamnose, L-serine, L-sorbose, *myo*-inositol or propionate. Weak growth is exhibited on cellobiose, melibiose, L-arginine, L-asparagine, L-glutamate, L-glutamine, malate and succinate. The predominant polar lipids are a major phospholipid (PL1) and one major glycolipid (GL2). It contains peptidoglycan type A3β L-Orn–Gly–Gly.

The type strain, K-39^T (=DSM 21686^T=NCAIM B 02340^T), was isolated from the thermophilic phase of oyster mushroom substrate preparation. The DNA G+C content of strain K-39^T is 71.3 mol%.

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