Thermosporothrix hazakensis gen. nov., sp. nov.,
isolated from compost, description of
Thermosporotrichaceae fam. nov. within the class
Ktedonobacteria Cavaletti *et al.* 2007 and
emended description of the class *Ktedonobacteria*

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We isolated from compost an aerobic, thermophilic, Gram-stain-positive, spore-forming bacterium that formed branched vegetative and aerial mycelia. This strain, designated SK20-1^T, grew at 31–58 °C, with optimum growth at 50 °C, while no growth was observed below 28 or above 60 °C. The pH range for growth was 5.4–8.7, with optimum growth at pH 7.0, while no growth was observed below pH 5.0 or above pH 9.1. Strain SK20-1^T was able to hydrolyse polysaccharides such as cellulose, xylan and chitin. The DNA G + C content was 54.0 mol%. The major fatty acid was iso-C_{17:0} and the major menaquinone was MK-9(H₂). The cell wall contained glutamic acid, serine, alanine and ornithine in a molar ratio of 1.00 : 1.07 : 2.64 : 0.83. The polar lipids consisted of phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. Cell-wall sugars were rhamnose and mannose. Detailed phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SK20-1^T belongs to the class *Ktedonobacteria*, and that the strain is most closely related to *Ktedonobacter racemifer* SOSP1-21^T (88.5%). On the basis of its phenotypic features and phylogenetic position, we propose that SK20-1^T represents a novel genus and species, *Thermosporothrix hazakensis* gen. nov., sp. nov., within the new family *Thermosporotrichaceae* fam. nov. The type strain of *Thermosporothrix hazakensis* is strain SK20-1^T (=JCM 16142^T =ATCC BAA-1881^T). In addition, we propose an emended description of the class *Ktedonobacteria* to classify the class in the phylum *Chloroflexi*.

The phylum *Chloroflexi* is currently divided phylogenetically into at least five classes, *Chloroflexi* (Garrity & Holt, 2001), *Caldilineae* (Yamada *et al.*, 2006), *Thermomicrobia* (Hugenholtz & Stackebrandt, 2004), *Anaerolineae* (Yamada *et al.*, 2006) and ‘*Dehalococcoidetes*’ (Hugenholtz & Stackebrandt, 2004). It also contains a clone cluster of uncultured bacteria (Morris *et al.*, 2004). The class

Ktedonobacteria, which contains one cultured species with a validly published name, *Ktedonobacter racemifer*, has not yet been assigned to a phylum. However, the highest levels of binary similarity of 16S rRNA gene sequences were with representatives of the phylum *Chloroflexi* (Cavaletti *et al.*, 2006). The association between *K. racemifer* SOSP1-21^T and species in the phylum *Chloroflexi* was not supported by high bootstrap values in the phylogenetic tree, and there are several differences in morphology and chemotaxonomy between the two. Therefore, Cavaletti *et al.* (2006) reported that *K. racemifer* SOSP1-21^T may belong to a new phylum. However, they did not propose a new phylum, since the phylum *Chloroflexi* is highly heterogeneous with respect to phylogenetic, morphological and physiological characteristics.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain SK20-1^T and *Ktedonobacter racemifer* DSM 44963^T are AB500145 and AB510917.

A photograph of a colony of strain SK20-1^T and a global 16S rRNA gene sequence-based neighbour-joining tree are available as supplementary material with the online version of this paper.

In this study, we report the cultivation and isolation of a novel thermophilic and branched mycelium-forming bacterium from compost belonging to the class *Ktedonobacteria*. We formally propose to amend the description of the class *Ktedonobacteria* as suggested by Cavaletti *et al.* (2006), by placing it in the phylum *Chloroflexi*.

The sample was obtained from ripe compost produced by a field-scale composter (Hazaka system; Hazaka Plant Kogyo Co., Ltd), which had been used for the treatment of livestock excreta. The composter is an open strip furrow (100 m long by 3 m wide by 2 m deep) with an automatic scoop-type turner. The details of this system were described previously (Yabe *et al.*, 2009). The sample was collected in a plastic bag (Ziploc), transported to the laboratory and stored at room temperature until use.

The isolation medium contained (per litre distilled water) 1 g yeast extract, 2 g tryptone, 1 g NaCl, 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 g compost powder (described below) and 20 g agar. The medium was supplemented with 20 mg trimethoprim, 10 mg nalidixic acid and 20 mg kanamycin 1^{-1} . The compost powder was prepared by grinding the compost to a fine powder in a mortar after drying at 60 °C overnight. The pH of the isolation plates was adjusted to 7.0 with NaOH at room temperature. The sample (1 g wet weight) was serially diluted in saline solution, and aliquots of the dilutions were plated on the plates. The plates were incubated at 50 °C for 7 days. One of the colonies that formed aerial mycelium was picked, grown and purified by plating three times. The colony was restreaked, and a stock culture was prepared by inoculating agar slants from the second plate. The isolate was designated strain SK20-1^T.

Morphology of cells grown at 50 °C for 7 days on ISP3 agar (Shirling & Gottlieb, 1966) was examined with a phase-contrast microscope. For scanning electron microscopy (JSM6700F; JEOL Inc.), a suitable agar block including a colony grown at 50 °C for 14 days on ISP3 agar was cut out with a scalpel, mounted on a glass slide, fixed for 24 h in a sealed bin containing 2 % osmium tetroxide and freeze-dried for 4 h. The dried sample was mounted on a specimen support with carbon tape, covered with gold film by sputter coating under a vacuum and observed in high-vacuum mode at 3 kV. For transmission electron microscopy (TEM), a suitable agar block including a colony grown at 50 °C for 5 days on ISP3 agar was fixed overnight by steeping with 2 % glutaraldehyde buffered with 0.1 M sodium cacodylate. After washing with the same buffer, the block was post-fixed for 90 min with 1 % permanganic acid potassium salt and washed with distilled water. TEM preparations were carried out at 4 °C. The block was then dehydrated in an acetone series at room temperature and embedded in Spurr resin at 60 °C for 24 h. It was cut by ultramicrotome into 80–90 nm slices, triple-stained with tannic acid, uranyl acetate and lead citrate and observed and photographed on a JEOL JEM1200EX at an acceleration voltage of 80 kV.

Colonies of SK20-1^T were 3–5 cm in diameter after incubation for 7 days at 50 °C on ISP3 agar (see Supplementary Fig. S1, available in IJSEM Online). Strain SK20-1^T formed vegetative and thick aerial mycelia on solid medium; both were irregularly branched and similar in morphology to actinomycetes (Fig. 1a, b). Spores, 1.0–2.0 µm long and 0.7–1.0 µm wide, were borne in a cluster and had a fig-like shape (Fig. 1c). The spores exhibited refractivity under a phase-contrast microscope (Fig. 1b). As shown in Fig. 1(d), the spore cells were covered with a double membrane and surrounded by thick cell walls, with the cells being clearly separated from the mycelium by membranes and walls.

To test for heat resistance of the spores, colonies of strain SK20-1^T were incubated at 50 °C for 7 days on ISP3 agar, suspended in 0.5 % NaCl (1 ml) and heated at 60, 70 or 80 °C in a water bath for 10 or 20 min. After heat treatment, 0.1 ml of the treated sample was added to 100 ml ISP1 medium and incubated at 50 °C for 2 weeks. After heating at 60 or 70 °C for 20 min, spores grew after 2 days; those heated at 80 °C for 10 min grew after 5 days, while those heated at 80 °C for 20 min showed no growth. Thus, spores of strain SK20-1^T are sensitive to heat.

The dipicolinic acid content of spores of strain SK20-1^T was determined by the method of Janssen *et al.* (1958). *Bacillus cereus* NBRC 3836 and *Thermobifida fusca* NBRC 14071^T were similarly tested and used as positive and negative controls, respectively. Spores of strain SK20-1^T and *Thermobifida fusca* NBRC 14071^T were raked with a sterilized toothpick from well-sporulated colonies, incubated at 50 °C for 7 days on ISP3 agar and suspended in 0.5 % NaCl (1 ml). Preparation of spore suspensions of *Bacillus cereus* NBRC 3836 was performed according to the method of Bhothipaksa & Busta (1978). The wet weight of each spore sample was about 50 mg. Dipicolinic acid was detected in spores of *Bacillus cereus* NBRC 3836 but not in those of strain SK20-1^T or *Thermobifida fusca* NBRC 14071^T.

In order to determine the effect of pH, temperature and NaCl on growth, strain SK20-1^T was cultivated in ISP3 medium. Temperature, pH and NaCl concentration tests were carried out at 20–60 °C (pH 7.0), pH 4.5–10.5 (50 °C) and 0–3 % NaCl (50 °C, pH 7.0). The pH was adjusted with HCl or NaOH at room temperature. Anaerobic growth was observed in test tubes by adding 0.1 % (w/v) NaNO_3 , Na_2SO_3 , NaNO_2 or $\text{Na}_2\text{S}_2\text{O}_3$ to ISP1 medium (Shirling & Gottlieb, 1966). Air was replaced with N_2 . Assimilation of various sole carbon sources (at 1 %; L-arabinose, D-xylose, D-glucose, D-fructose, sucrose, L-rhamnose, raffinose, inositol and mannitol) was assessed using Pridham–Gottlieb agar (Shirling & Gottlieb, 1966) and determined to be positive or negative by the method of Shirling & Gottlieb (1966). Hydrolysis of cellulose, xylan, chitin, starch, gelatin and casein was evaluated on isolation plates supplemented with each substrate at a concentration of 1 % (w/v). The presence of a clear zone

Fig. 1. Photomicrographs of cells of strain SK20-1^T. (a, c) Scanning electron micrographs of strain SK20-1^T. Bars, 10 µm (a) and 1 µm (c). (b) Phase-contrast photomicrograph of cells of strain SK20-1^T grown on ISP3 agar at 50 °C for 7 days. Bar, 10 µm. (d) Transmission electron micrograph of an ultrathin-sectioned spore of strain SK20-1^T. Bar, 200 nm.

around the colony indicated that there was hydrolysis. Catalase activity was determined by bubble production in a 3% hydrogen peroxide solution. Gram staining was carried out with the modified Hucker method (Smibert & Krieg, 1994).

Differential phenotypic characteristics between strain SK20-1^T and *K. racemifer* SOSP1-21^T are summarized in Table 1. Strain SK20-1^T is Gram-stain-positive. It grows at 31–58 °C, with optimum growth at 50 °C; there was no growth below 28 or above 60 °C. Strain SK20-1^T is able to grow at pH 5.4–8.7, with optimum growth at pH 7.0; no growth was observed below pH 5.0 or above pH 9.1. It does not require NaCl for growth, but growth is inhibited by NaCl at concentrations higher than 2%. It grows only under aerobic conditions. Strain SK20-1^T utilizes D-xylose, D-glucose, sucrose, L-rhamnose, inositol and mannitol as sole carbon sources and hydrolyses cellulose, xylan, chitin and casein but not starch. It is catalase-positive and does not produce H₂S.

Cellular fatty acids of strain SK20-1^T were prepared from cell mass grown on ISP1 medium for 4 days at 50 °C and

then separated and identified with the Microbial Identification Systems (MIDI Inc.). Cell walls were prepared by the methods described by Schleifer & Kandler (1972) and amino acids in the cell-wall hydrolysate were identified by TLC (Harper & Davis, 1979) and HPLC, as their phenylthiocarbamoyl derivatives, with an HPLC apparatus (LC-10AD; Shimadzu) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries) as described by Yokota *et al.* (1993). Cell-wall sugars were determined as alditol acetate derivatives by GLC using a Shimadzu GC-17A apparatus equipped with an Rtx 2330 (0.32 mm × 30 m) column. Phospholipids were extracted and identified using two-dimensional TLC, followed by spraying with appropriate detection reagents, according to the method of Tindall (1990a, b). Genomic DNA of the isolate was prepared using a method modified from Marmur (1961), in which achromopeptidase and lysozyme (final concentrations 0.5 and 0.75 mg ml⁻¹, respectively) were used for cell lysis. RNA was digested with RNase mix solution (50 µl ml⁻¹) (Wako). The G+C content of the genomic DNA was determined by HPLC (Tamaoka & Komagata, 1984) using a COSMOSIL 5C₁₈-AR-II packed

Table 1. Differential characteristics of strain SK20-1^T and *Ktedonobacter racemifer* SOSP1-21^T

Data for *K. racemifer* SOSP1-21^T were taken from Cavaletti *et al.* (2006). Both strains stain Gram-positive, have MK-9(H₂) as the major quinone and are positive for catalase, growth with 1% NaCl and hydrolysis of casein. Both strains are negative for growth with 3% NaCl. ND, No data available.

Characteristic	Strain SK20-1 ^T	<i>K. racemifer</i> SOSP1-21 ^T
Spore size (μm)	1.0–2.0 × 0.7–1.0	1.6–1.8
DNA G + C content (mol%)	54.0	53.9
Gelatin liquefaction	–	+
Production of H ₂ S	–	+
Growth in the presence of 2% NaCl	–	ND
Temperature for growth (°C)		
Range	31–58	17–40
Optimum	50	28–33
pH for growth		
Range	5.4–8.7	4.2–7.2
Optimum	7.0	6.0
Hydrolysis of:		
Starch	–	+
Cellulose	+	–
Xylan	+	–
Chitin	+	–
Utilization of:		
L-Arabinose	–	ND
D-Xylose, D-glucose, D-fructose, sucrose, L-rhamnose, raffinose, inositol, mannitol	+	ND
Major cellular fatty acid(s)	iso-C _{17:0}	C _{16:1} 2-OH, iso-C _{17:0}
Cell-wall amino acids*	Glu, Ser, Ala, Orn	Glu, Gly, Ala, Ser, Orn
Cell-wall sugars*	Rha, Man	Ara, Gal
Polar lipids*	PI, PIM, PG, DPG	PI, PG, DPG

*Orn, Ornithine; Ara, arabinose; Gal, galactose; Man, mannose; Rha, rhamnose; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides.

column (4.6 mm × 250 mm) (Nacalai Tesque) with a DNA-GC kit (Yamasa Shouyu).

The G + C content of strain SK20-1^T was 54.0 mol%, as determined by HPLC analysis (Table 1). The predominant menaquinone (85%) was MK-9(H₂); MK-9(H₄) was present as a minor component (5%). The major fatty acid was iso-C_{17:0} (52.8%). The composition of cellular fatty acids is shown in Table 2. The polar lipids consisted of phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid (Table 1). The peptidoglycan contained

Table 2. Cellular fatty acid compositions of strain SK20-1^T and *K. racemifer* SOSP1-21^T

Data for *K. racemifer* SOSP1-21^T were taken from Cavaletti *et al.* (2006). Values are percentages of total fatty acids; fatty acids present at less than 1% in both strains are not shown. ND, Not detected/not reported.

Fatty acid	Strain SK20-1 ^T	<i>K. racemifer</i> SOSP1-21 ^T
iso-C _{15:0}	0.64	2.37
C _{16:0}	10.03	6.66
iso-C _{16:0}	1.14	11.54
10-Methyl C _{16:0}	1.26	7.79
C _{16:1} 2-OH	9.39	29.65
C _{17:0}	1.76	ND
iso-C _{17:0}	52.79	25.00
anteiso-C _{17:0}	10.30	9.61
C _{18:0}	7.28	2.01
C _{18:1} ω9c	0.71	1.21

glutamic acid, serine, alanine and ornithine in a molar ratio of 1.00:1.07:2.64:0.83. Cell-wall sugars of strain SK20-1^T were rhamnose and mannose. Differential chemotaxonomic characteristics of strain SK20-1^T and *K. racemifer* SOSP1-21^T are summarized in Table 1.

The 16S rRNA gene of *K. racemifer* DSM 44963^T was sequenced because the sequence reported previously for strain SOSP1-21^T was short (GenBank accession no. AM180156; 1386 bp). The 16S rRNA genes of strain SK20-1^T and *K. racemifer* DSM 44963^T were amplified with primers F27 (5'-AGAGTTTGTATCATGGCTCGA-3'; positions 8–27 of the *Escherichia coli* 16S rRNA gene) and R1492 (5'-GGCTACCTTGTTACGACTT-3'; 1510–1492). PCR thermal cycling was carried out in a TaKaRa PCR Thermal Cycler MP-3000 (TaKaRa Bio) using the following parameters: 94 °C for 2 min followed by 30 cycles at 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min with a final extension cycle of 72 °C for 2 min. Purified PCR products were sequenced as described by Lin *et al.* (2004).

To construct a global phylogenetic tree, the 16S rRNA gene sequence of strain SK20-1^T was automatically aligned using the ARB program (<http://www.arb-home.de/>) to a set of 7006 pre-aligned 16S rRNA gene sequences of type strains in the *Bacteria* available from the All-Species Living Tree Project (LTP) dataset based on SILVA release_s95 (ARB format, 17 October 2008; Yarza *et al.*, 2008). After manual refinement of the alignment, the sequence of strain SK20-1^T was added to the reference tree provided with the LTP_s95 database using quick parsimony. Reference tree construction had been performed by using the maximum-likelihood algorithm RaxML (Stamatakis, 2006) in LTP (Yarza *et al.*, 2008). Representatives of several phyla or close relatives of strain SK20-1^T were chosen, and detailed phylogenetic trees were reconstructed by the following methods (Figs 2 and 3). The 16S rRNA gene sequence of strain SK20-1^T was compared with sequences obtained

Fig. 2. Neighbour-joining tree based on 1191 aligned positions of the 16S rRNA gene showing the position of strain SK20-1^T and *K. racemifer* DSM 44963^T among members of other phyla. The sequences of *Aquifex pyrophilus* Kol5a^T (GenBank accession no. M83548) and *Thermotoga maritima* MSB8^T (accession no. M21774) were used as an outgroup (not shown). Numbers at nodes are bootstrap percentages based on 1000 replicated datasets; only values greater than 50 % are shown. Bar, 2 % sequence dissimilarity. DDBJ/EMBL/GenBank accession numbers for reference strains are shown in parentheses.

from GenBank. Multiple alignments of the sequences were performed using CLUSTAL W (version 1.83) (Thompson *et al.*, 1994), and gaps and unidentified base positions were edited using BioEdit (Hall, 1999). A phylogenetic tree was constructed with the maximum-likelihood method (Felsenstein, 1981) using PhyML (Guindon & Gascuel, 2003), the neighbour-joining method (Saitou & Nei, 1987) and the maximum-parsimony method (Fitch, 1971) using MEGA version 4.1 (Tamura *et al.*, 2007), with bootstrap values based on 100, 1000 and 1000 replications, respectively (Felsenstein, 1985). Evolutionary distances were computed using Kimura's two-parameter method (Kimura, 1980).

The closest relative of strain SK20-1^T with a validly published name is *K. racemifer* DSM 44963^T, with 88.5 % sequence similarity. All other cultured species of the phylum *Chloroflexi* with validly published names are related more distantly, showing 16S rRNA gene sequence similarities of less than 81 % (*Caldilinea aerophila*, 81 %; *Sphaerobacter thermophilus*, 81 %; *Anaerolinea thermolimosa*, 79 %). The uncultured bacterial clone B424, detected from Hawaiian volcanic deposits and belonging to the same cluster as strain SK20-1^T, has 16S rRNA gene sequence similarity of 87 % with strain SK20-1^T. Hawaiian volcanic bacterial clones are also present in clusters 1 and 3 (see Fig. 3). The sequence similarities of strain SK20-1^T with clusters 1, 2, 3 and GS were 85–88, 87–88, 85–87 and 84–85 %, respectively. Phylogenetic trees based on 16S rRNA gene sequences (Fig. 2 and Supplementary Fig. S2) indicate that strain

SK20-1^T and *K. racemifer* DSM 44963^T belong to the phylum *Chloroflexi*, because the *Chloroflexi* cluster, including strain SK20-1^T and *K. racemifer* DSM 44963^T, was formed at a high bootstrap value of 89 %; the global phylogenetic tree (Supplementary Fig. S2) includes 7007 type strain sequences from the *Bacteria*, indicating that strain SK20-1^T and *K. racemifer* SOSP1-21^T were placed in the cluster *Chloroflexi*. As shown in Fig. 3, the bootstrap value for the node of the class *Ktedonobacteria* is 100 % according to the maximum-likelihood, neighbour-joining and maximum-parsimony methods, suggesting that strain SK20-1^T belongs to the class *Ktedonobacteria*. The cluster *Ktedonobacteria* is subdivided into two major clusters (*Ktedonobacterales* and GS); therefore, the class *Ktedonobacteria* has two major orders, and strain SK20-1^T belongs to the order *Ktedonobacterales*. The GS cluster consists of four cultivated strains isolated from geothermal soils in New Zealand (Stott *et al.*, 2008), but no names have been validly published for these strains. These strains also form spores. The cluster *Ktedonobacterales* is subdivided into four subclusters (*Thermosporotrichaceae* fam. nov. and clusters 1–3); therefore, the order *Ktedonobacterales* has at least four lineages at the family level. Furthermore, strain SK20-1^T shows only 87–88 % 16S rRNA gene sequence similarity with members of cluster 2, including *K. racemifer* DSM 44963^T. These facts suggest that strain SK20-1^T and *K. racemifer* DSM 44963^T should be separated at the family level.

In addition, physiological and chemotaxonomic characteristics clearly differentiate strain SK20-1^T from the most

Fig. 3. Neighbour-joining tree based on 1180 aligned positions of the 16S rRNA gene showing the position of strain SK20-1^T and *K. racemifer* DSM 44963^T among members of the phylum *Chloroflexi*. The sequences of *Aquifex pyrophilus* Kol5a^T and *Thermotoga maritima* MSB8^T were used as an outgroup (not shown). Numbers at nodes are bootstrap percentages based on 1000 replicated datasets; only values greater than 60 % are shown. Bar, 2 % sequence dissimilarity. Closed circles indicate nodes that were recovered using neighbour-joining method (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Classes are indicated as follows: A, *Ktedonobacteria*; B, *Anaerolineae*; C, *Caldilineae*; D, '*Dehalococcoides*'; E, clone cluster; F, *Chloroflexi*; G, *Thermomicrobia*.

closely related species, *K. racemifer*. Strain SK20-1^T is thermophilic, whereas *K. racemifer* SOSP1-21^T is mesophilic. Strain SK20-1^T hydrolyses cellulose, xylan and chitin, whereas *K. racemifer* SOSP1-21^T does not. The major fatty acid of *K. racemifer* SOSP1-21^T is C_{16:1} 2-OH (29.65 %), while that of strain SK20-1^T is iso-C_{17:0} (52.79 %). The maximum colony diameter of strain SK20-1^T is 5 cm after incubation for 7 days under optimum conditions, while that of *K. racemifer* SOSP1-21^T is 3–4 mm after incubation for 3–4 weeks under optimum conditions. The features that strain SK20-1^T and *K. racemifer* have in common are being Gram-stain-positive and forming branched mycelium, which are not characteristic of the phylum *Chloroflexi*, but rather are similar to actinomycetes. The results of morphological, physiological and chemotaxonomic investigations, together with the phylogenetic analysis, reveal that strain SK20-1^T is clearly distinguished from *K. racemifer* at the family level. Therefore, we put forward the name *Thermosporotrichaceae* fam. nov. in the class *Ktedonobacteria*. We also propose the

name *Thermosporothrix hazakensis* gen. nov., sp. nov. to accommodate strain SK20-1^T. In addition, we propose the classification of the class *Ktedonobacteria* in the phylum *Chloroflexi*.

Description of *Thermosporothrix* gen. nov.

Thermosporothrix (Ther'mo.spo'ro.thrix. Gr. n. *therme* heat; Gr. n. *spora* a seed and, in biology, a spore; Gr. fem. n. *thrix* hair; N.L. fem. n. *Thermosporothrix* thermophilic spore-forming hair).

Filamentous, spore-forming bacteria. Gram-stain-positive. Thermophilic, aerobic heterotrophs. Contain glutamic acid, serine, alanine and ornithine as peptidoglycan amino acids. Cell-wall sugars are rhamnose and mannose. iso-C_{17:0} is the major fatty acid and MK-9(H₂) is the major menaquinone. The G + C content of the genomic DNA of the type strain of the type species is 54.0 mol%. The type species is *Thermosporothrix hazakensis*.

Description of *Thermosporothrix hazakensis* sp. nov.

Thermosporothrix hazakensis (ha.za.ken'sis. N.L. fem. adj. *hazakensis* pertaining to Hazaka, referring to the isolation of the type strain at Hazaka Plant Research Center, Japan).

Displays the following properties in addition to those described for the genus. Produces branched vegetative and aerial mycelium. Spores are borne in a cluster. The non-motile spores are fig-like in shape and are 1.0–2.0 µm long and 0.7–1.0 µm wide. Growth occurs at 31–58 °C (optimum 50 °C) and pH 5.4–8.7 (optimum pH 7.0). Spore cells are covered with a membrane and surrounded by thick cell walls. Does not require NaCl; growth is inhibited completely by 3 % NaCl. Casein, cellulose, xylan and chitin are hydrolysed, while starch and gelatin are not. Catalase-positive. D-Xylose, D-glucose, sucrose, L-rhamnose, inositol and mannitol are utilized as sole carbon sources.

The type strain is SK20-1^T (=JCM 16142^T =ATCC BAA-1881^T), isolated from compost.

Description of *Thermosporotrichaceae* fam. nov.

Thermosporotrichaceae (Ther'mo.spo'ro.tri.cha'ce.ae. N.L. fem. n. *Thermosporothrix* -trichos type genus of the family; L. suff. -aceae ending to denote a family; N.L. fem. pl. n. *Thermosporotrichaceae* the family of the genus *Thermosporothrix*).

The description is the same as for the genus *Thermosporothrix*. The family is a member of the order *Ktedonobacterales*. The type genus is *Thermosporothrix*.

Emended description of the class *Ktedonobacteria* Cavaletti et al. 2007

On the basis of comparative 16S rRNA gene sequence analysis, the class *Ktedonobacteria* belongs to the phylum *Chloroflexi*. The class contains the order *Ktedonobacterales*.

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