Thermogemmatispora onikobensis gen. nov.,
sp. nov. and *Thermogemmatispora foliorum* sp. nov.,
isolated from fallen leaves on geothermal soils, and
description of *Thermogemmatisporaceae* fam. nov.
and *Thermogemmatisporales* ord. nov. within the
class *Ktedonobacteria*

Shuhei Yabe,¹ Yoshifumi Aiba,² Yasuteru Sakai,¹ Masaru Hazaka¹
and Akira Yokota²

Correspondence

Shuhei Yabe

kennan-6@kennan-e.co.jp

¹Hazaka Plant Research Center, Kennan Eisei Kogyo Co., Ltd, 44 Aza-Inariyama, Oaza-Ashitate, Murata-cho, Shibata-gun, Miyagi 989-1311, Japan

²Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi Bunkyo-ku, Tokyo 113-0032, Japan

Two thermophilic, Gram-stain-positive, sporulating bacterial strains, which formed branched vegetative and aerial mycelia, were isolated from fallen leaves sampled from geothermal soils and designated ONI-1^T and ONI-5^T. Strain ONI-1^T grew at 50–74 °C, with optimum growth at 60–65 °C, and strain ONI-5^T grew at 45–74 °C, with optimum growth at 60–65 °C. The pH range for growth of the strains was pH 4.6–8.0, with optimum growth at pH 7.0. The DNA G+C contents of strains ONI-1^T and ONI-5^T were 60.2 and 58.1 mol%, respectively. The major fatty acid was iso-C_{17:0} and the major menaquinone was MK-9(H₂). The cell walls of the strains contained glutamic acid, serine, glycine, histidine, alanine and ornithine. The polar lipids consisted of phosphatidylinositol, phosphatidylglycerol and a glycolipid. The cell-wall sugar was rhamnose. Detailed phylogenetic analysis based on 16S rRNA gene sequences indicated that the strains belong to the class *Ktedonobacteria* and that strains ONI-1^T and ONI-5^T are most closely related to *Thermosporothrix hazakensis* SK20-1^T (85.3 and 84.5 % sequence similarity, respectively). 16S rRNA gene sequence similarity between the two strains was 96.6 %. Based on the phenotypic features and phylogenetic position, we propose that strains ONI-1^T and ONI-5^T constitute a novel genus containing two novel species, for which we propose the names *Thermogemmatispora onikobensis* gen. nov., sp. nov. (the type species; type strain ONI-1^T = JCM 16817^T = KCTC 19768^T) and *Thermogemmatispora foliorum* sp. nov. (type strain ONI-5^T = JCM 16818^T = KCTC 19767^T), within the new family *Thermogemmatisporaceae* fam. nov. and order *Thermogemmatisporales* ord. nov.

Currently, the phylum *Chloroflexi* is divided phylogenetically into the classes *Chloroflexi* (Garrrity & Holt, 2001), *Caldilineae* (Yamada *et al.*, 2006), *Thermomicrobia* (Hugenholtz & Stackebrandt, 2004), *Anaerolineae* (Yamada *et al.*, 2006) and ‘*Dehalococcoidetes*’ (Hugenholtz & Stackebrandt, 2004) and a class-level clone cluster of uncultured bacteria (Morris *et al.*, 2004). The class *Ktedonobacteria*, which contains two cultured species with the validly published names, *Ktedonobacter racemifer* (Cavaletti *et al.*, 2006) and

Thermosporothrix hazakensis, was recently affiliated with the phylum *Chloroflexi* by Yabe *et al.* (2010a). *Ktedonobacter* and *Thermosporothrix* form branched substrate and aerial mycelia with spores and the colony morphology of the organisms is very similar to that of aerial mycelium-forming actinomycetes. Sporulation of *Thermosporothrix hazakensis* SK20-1^T has been observed by scanning and transmission electron microscopy of cells grown at different stages of incubation by Yabe *et al.* (2010b); multiple exospores in each mother cell were formed by budding on the branched aerial mycelium. Spores originated from a lateral position in the mycelium. Although branched aerial mycelium is a characteristic feature of actinomycetes, multiple budding sporulation has not been described previously for prokaryotes (Yabe *et al.*, 2010b).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ONI-1^T and ONI-5^T are AB547912 and AB547913, respectively.

A supplementary table is available with the online version of this paper.

We isolated two novel thermophilic bacteria from fallen leaves on geothermal soils that form branched aerial mycelium and belong to the phylum *Chloroflexi*. To determine the taxonomic and phylogenetic position of these organisms, we examined their morphological, physiological, biochemical and chemotaxonomic characteristics and 16S rRNA gene sequences. The results indicated that these strains should be placed in two novel species of a new genus belonging to a new family and order of the class *Ktedonobacteria*.

We sampled fallen leaves deposited on steaming soil (60–90 °C at 10–15 cm depth) at Jigokudani in Onikobe Hot Springs, a geothermal area in Miyagi Prefecture, Japan. The samples were collected in a plastic bag (Ziploc), transported to the laboratory and stored at room temperature for 2 days.

We used a modified FS1V medium (MFS) for isolation, in which FS1V medium was supplemented with 100 mg kanamycin l⁻¹ and 1% (w/v) microcrystalline cellulose (Stott *et al.*, 2008). The isolation medium was adjusted to pH 7.0 with NaOH at room temperature. Fallen leaves were spread on the plates and incubated at 65 °C for 3 weeks. Two colonies that formed aerial mycelium were selected and serially diluted in saline solution and the dilutions were then plated. Each single colony was purified by plating three times. Colonies were restreaked and each stock culture was prepared by inoculating agar slants from the second plate. The isolates were designated strains ONI-1^T and ONI-5^T.

Colony characteristics were determined after growth for 14 days at 65 °C on FS1V medium and International Streptomyces Project (ISP) medium 1 and 3 agar (Shirling & Gottlieb, 1966) by visual inspection. Morphology of cells grown at 60 °C for 14 days on FS1V medium was examined using a phase-contrast microscope. Suitable agar blocks including colonies of ONI-1^T and ONI-5^T grown at 60 °C for 5 or 14 days on FS1V medium were subjected to scanning electron microscopy (SU8000; Hitachi). For transmission electron microscopy, suitable agar blocks including colonies of ONI-1^T and ONI-5^T grown at 60 °C for 10 days on FS1V plates were used. These were cut using an ultramicrotome into 80–90 nm slices, triple-stained with tannic acid, uranyl acetate and lead citrate and observed and photographed using a JEOL JEM1200EX electron microscope at an acceleration voltage of 80 kV. Scanning and transmission electron microscopy methodologies were as described previously (Yabe *et al.*, 2010a).

Strains ONI-1^T and ONI-5^T grew well on FS1V medium and ISP1 agar and grew weakly on ISP3 agar. Strain ONI-5^T developed orange colonies on ISP1 agar, whereas colonies of strain ONI-1^T were white. Aerial mycelium of these strains was observed on FS1V medium, but the strains did not form aerial mycelium on ISP1 or ISP3 agar.

Phase-contrast microscopy showed that strains ONI-1^T and ONI-5^T formed vegetative and aerial mycelia in FS1V

medium and that both were irregularly branched and had morphology similar to that of common actinomycetes. Spores exhibited refractivity under the phase-contrast microscope. They were formed by budding on the aerial mycelium (Fig. 1a, c; arrows); mature spores were 1.0–2.0 µm long and 0.5–1.5 µm in diameter (Fig. 1b, d), covered with a double membrane and surrounded by thick cell walls (Fig. 1e, g). As shown in Fig. 1(f, h), the strains formed two or more spores in a mother cell. These morphological features are similar to those of *Thermosporothrix hazakensis* SK20-1^T (Yabe *et al.*, 2010b). The spores were oval in shape and had rough surfaces (Fig. 1a–d).

To determine the effects of pH, temperature and NaCl concentration on growth, strains ONI-1^T and ONI-5^T were cultivated in ISP1 medium. Temperature, pH and NaCl concentration tests were performed at 40–80 °C (pH 7.0), pH 3.0–8.7 (60 °C) and 0–3% (w/v) NaCl (60 °C, pH 7.0). The pH was adjusted with HCl and NaOH at room temperature. Anaerobic growth was observed in test tubes by adding 0.1% (w/v) NaNO₃, Na₂SO₃, NaNO₂ or Na₂S₂O₃ to ISP1 medium where air was replaced with N₂. Assimilation of various sole carbon sources (0.5%) (L-arabinose, D-xylose, D-glucose, D-fructose, sucrose, L-rhamnose, raffinose, inositol and D-mannitol) was assessed using FS1V medium containing agar in place of gellan gum as a solidifying agent (FS1V agar) and determined to be positive or negative by the method reported by Shirling & Gottlieb (1966). Hydrolysis of microcrystalline cellulose (Avicel), CM-cellulose, xylan, chitin, starch, gelatin and casein was evaluated using ISP1 agar supplemented with each substrate at a concentration of 0.5% (w/v). The presence of a clear zone around the colony indicated that hydrolysis had occurred. Catalase activity was detected by bubble production in a 3% hydrogen peroxide solution. Gram staining was performed using the modified Hucker method (Smibert & Krieg, 1994).

Strains ONI-1^T and ONI-5^T stained Gram-positive. Strain ONI-1^T grew at 50–74 °C and strain ONI-5^T grew at 45–74 °C; both strains showed optimum growth at 60–65 °C. No growth of either strain was observed below 45 or above 78 °C. The strains were able to grow at pH 4.6–8.0 with an optimum pH of 7.0; no growth was observed at pH 4.0 or below or at pH 8.7 or above. The strains did not require NaCl for growth. Strain ONI-1^T grew in the presence of 1% NaCl, whereas strain ONI-5^T did not. No growth of strain ONI-1^T was observed at 2% NaCl. Both strains grew only under aerobic conditions. Strain ONI-1^T utilized D-xylose, D-glucose, sucrose, L-rhamnose and D-mannitol as sole carbon sources and hydrolysed gelatin, Avicel, CM-cellulose, xylan and chitin but not starch or casein. Strain ONI-5^T utilized D-mannitol as a sole carbon source and hydrolysed Avicel, CM-cellulose, xylan and chitin but not gelatin, starch or casein. Both strains grew well in FS1V medium but not on FS1V agar.

Cellular fatty acids from strains ONI-1^T and ONI-5^T were prepared from cells grown on ISP1 medium for 7 days at

Fig. 1. Photomicrographs of cells of strains ONI-1^T and ONI-5^T. (a–d) Scanning electron micrographs of strains ONI-1^T (a, b) and ONI-5^T (c, d). Bars, 10 µm (a), 2 µm (b), 5 µm (c) and 3 µm (d). (e–h) Transmission electron micrographs of ultrathin-sectioned cells of strains ONI-1^T (e, f) and ONI-5^T (g, h). Bars, 500 nm (e, g) and 100 nm (f, h).

60 °C and then separated and identified using a commercial microbial identification system (MIDI Inc.). An unidentified fatty acid methyl ester peak was analysed further using a GC-mass spectrometer (GC-17A/GCMS-QP5050). Cell walls were prepared using 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 phenylthiocarbamoyl derivatives using 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 GC equipped with an Rtx 2330 (0.32 mm × 30 m) column. Phospholipids were extracted and identified by two-dimensional TLC and then sprayed with appropriate detection reagents based on the method reported by Tindall (1990a, b). Genomic DNA of the strains 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 a mixture of RNase A and RNase T₁ (50 µl ml⁻¹; Wako). The G+C content of genomic DNA was determined by HPLC (Tamaoka & Komagata, 1984) using a Cosmosil 5C₁₈-AR-II packed column (4.6 × 250 mm; Nacalai Tesque) with a DNA-GC kit (Yamasa Corp.).

The G+C contents of strains ONI-1^T and ONI-5^T were 60.2 and 58.1 mol%, respectively, as determined by HPLC analysis (Table 1). The predominant menaquinone was MK-9(H₂) and the major fatty acids were iso-C_{17:0} (44.1 % for strain ONI-1^T and 44.8 % for ONI-5^T), iso-C_{19:0} (19.3 and 27.3 %) and an unknown fatty acid [equivalent chain-length (ECL) of 19.032; 26.6 and 14.6 %]. Electron-impact MS of the unknown fatty acid ECL 19.032 was characterized by the presence of *m/z* 326 (molecular ion peak, M), 297 (M–29), 283 (M–43), 199, 143 [CH₃CO(CH)₆], 87 [CH₃OC(OH)-CH-CH₂] and 74 [CH₃OC(OH)=CH₂], which suggests that the unknown fatty acid corresponds to C_{20:0} methyl ester. The compositions of cellular fatty acids are shown in Supplementary Table S1, available in IJSEM Online. The polar lipids of the strains consisted of phosphatidylinositol, phosphatidylglycerol and a glycolipid (Table 1). The peptidoglycan of the strains contained glutamic acid, serine, glycine, histidine, alanine and ornithine in molar ratios of 1.0:0.8:2.5:2.2:2.2:1.2 in strain ONI-1^T and 1.0:1.2:2.6:1.8:2.5:1.0 in strain ONI-5^T. It is rare to observe histidine in a cell-wall peptidoglycan. The only cell-wall sugar in these strains was rhamnose. Differential chemotaxonomic and phenotypic characteristics of strains ONI-1^T and ONI-5^T, *T. hazakensis* SK20-1^T and *K. racemifer* SOSP1-21^T are summarized in Table 1.

Almost-complete 16S rRNA gene sequences from these strains were amplified by PCR and the purified PCR products were sequenced as described previously (Yabe *et al.*, 2010a). The 16S rRNA gene sequences of strains ONI-1^T and ONI-5^T were compared with sequences obtained 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). Phylogenetic trees were constructed using the maximum-likelihood method (Felsenstein, 1981) in PhyML (Guindon & Gascuel, 2003) and the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in 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).

In the class *Ktedonobacteria*, two cultured species with validly published names are currently known, *Ktedonobacter racemifer* and *Thermosporothrix hazakensis*. The closest strain to strains ONI-1^T and ONI-5^T was *T. hazakensis* SK20-1^T, with 85.3 and 84.5 % 16S rRNA gene sequence similarity, respectively. 16S rRNA gene sequence similarities of strains ONI-1^T and ONI-5^T to *K. racemifer* SOSP1-21^T were respectively 83.1 and 83.0 %. Although these similarities are quite low, the morphology of strains ONI-1^T and ONI-5^T, which exhibit aerial and vegetative mycelia as well as spores, is similar to that of *K. racemifer* SOSP1-21^T and *T. hazakensis* SK20-1^T. As shown in Fig. 2, the bootstrap support for the node of the class *Ktedonobacteria* is 100 % according to the maximum-likelihood, neighbour-joining and maximum-parsimony methods, suggesting that strains ONI-1^T and ONI-5^T belong to the class *Ktedonobacteria*. Strains ONI-1^T and ONI-5^T are also present in the 'GS cluster' described previously by Yabe *et al.* (2010a); the cluster was formed at a high bootstrap value of 100 %. The cluster *Ktedonobacteria* is subdivided into two major clusters (*Ktedonobacterales* and GS). Furthermore, *K. racemifer* SOSP1-21^T and *T. hazakensis* SK20-1^T are divided at the family level in the *Ktedonobacterales* cluster. Therefore, the *Ktedonobacterales* and GS clusters, including strains ONI-1^T and ONI-5^T, should be divided at the order level.

Strains ONI-1^T and ONI-5^T can be differentiated from other species of the *Ktedonobacteria* by the following chemotaxonomic characteristics. Strains ONI-1^T and ONI-5^T have C_{20:0} (ECL 19.032) as a cellular fatty acid and do not have diphosphatidylglycerol as a polar lipid. The peptidoglycans of strains ONI-1^T and ONI-5^T contain histidine.

Operational taxonomic units in the GS cluster in addition to strains ONI-1^T and ONI-5^T consist of four cultivated strains (*Chloroflexi* bacteria P359, P352, T81 and T104) isolated

Table 1. Characteristics of strains ONI-1^T and ONI-5^T and related type strains

Data for reference strains were taken from Yabe *et al.* (2010a) (*T. hazakensis* SK20-1^T) and Cavaletti *et al.* (2006) (*K. racemifer* SOSP1-21^T). All four strains stain Gram-positive and have MK-9(H₂) as the major quinone. ND, No data available.

Characteristic	Strain ONI-1 ^T	Strain ONI-5 ^T	<i>T. hazakensis</i> SK20-1 ^T	<i>K. racemifer</i> SOSP1-21 ^T
Colony colour on ISP1 agar	Orange	White	White	ND
DNA G + C content (mol%)	60.2	58.1	54.0	53.9
Gelatin liquefaction	+	–	–	+
Growth in the presence of:				
1 % NaCl	+	–	+	+
2 % NaCl	–	–	–	+
Temperature for growth (°C)				
Range	50–74	45–74	31–58	17–40
Optimum	60–65	60–65	50	28–33
pH for growth				
Range	4.6–8.0	4.6–8.0	5.4–8.7	4.2–7.2
Optimum	7.0	7.0	7.0	6.0
Hydrolysis of:				
Starch	–	–	–	+
Avicel	+	+	+	–
Xylan	+	+	+	–
Chitin	+	+	+	–
Utilization of:				
L-Arabinose	–	–	–	ND
D-Xylose	+	–	+	ND
D-Glucose	+	–	+	ND
D-Fructose	–	–	–	ND
Sucrose	+	–	+	ND
L-Rhamnose	+	–	–	ND
Raffinose	–	–	+	ND
Inositol	–	–	+	ND
D-Mannitol	+	+	+	ND
Major cellular fatty acid(s)	iso-C _{17:0}	iso-C _{17:0}	iso-C _{17:0}	C _{16:1} 2-OH, iso-C _{17:0}
Cell-wall amino acids*	Glu, Ser, Gly, His, Ala, Orn	Glu, Ser, Gly, His, Ala, Orn	Glu, Ser, Gly, Ala, Orn	Glu, Ser, Gly, Ala, Orn
Cell-wall sugar(s)†	Rha	Rha	Rha, Man	Ara, Gal
Polar lipids‡	PI, PG	PI, PG	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.

from steam-affected geothermal soils in New Zealand (Stott *et al.*, 2008), but these strains have not been described formally. Sequence similarities of 16S rRNA genes among strains ONI-1^T and ONI-5^T and the four *Chloroflexi* bacteria were 96.4–98.1 %. Therefore, all strains in the GS cluster may be affiliated with the same genus. However, the level of 16S rRNA gene sequence similarity between strains ONI-1^T and ONI-5^T (96.6 %) indicates that these are distinct species, since 16S rRNA gene sequence divergence of more than 3 % is generally regarded as indicative of different species (Stackebrandt & Goebel, 1994).

There were also phenotypic differences between strains ONI-1^T and ONI-5^T. Strain ONI-1^T grew at 1 % NaCl, but strain ONI-5^T did not. Strain ONI-1^T was able to utilize glucose, sucrose and rhamnose, while strain ONI-5^T did

not grow when these sugars were the sole carbon source. Gelatin was hydrolysed by strain ONI-1^T but not by strain ONI-5^T. Strain ONI-5^T developed orange colonies on ISP1 agar, whereas those of strain ONI-1^T were white.

Overall, the low levels of 16S rRNA gene sequence similarity, together with differences in chemotaxonomic, physiological and biochemical properties, indicate that strains ONI-1^T and ONI-5^T represent novel species of a new genus, and the names *Thermogemmatispora onikobensis* gen. nov., sp. nov. and *Thermogemmatispora foliorum* sp. nov., respectively, are proposed. We also propose the names *Thermogemmatisporaceae* fam. nov. and *Thermogemmatisporales* ord. nov. based on the distinct phylogenetic position of *Thermogemmatispora* within the class *Ktedonobacteria*.

Fig. 2. Neighbour-joining tree based on 1172 aligned positions of the 16S rRNA gene sequence. Numbers at nodes are bootstrap percentages from the neighbour-joining method based on 1000 replicated datasets; only values greater than 70% are shown. Bar, 2% sequence dissimilarity. The outgroup consisted of sequences from *Bacillus subtilis* Lactipan (GenBank accession no. AJ277905), *Streptomyces arenae* ISP 5293^T (AJ399485), *Thermus thermophilus* HB8^T (X07998), *Thermotoga maritima* MSB-8^T (M21774) and *Aquifex pyrophilus* Kol5a^T (M83548). DDBJ/EMBL/GenBank accession numbers are shown in parentheses. Closed circles indicate nodes that were supported by bootstrap values greater than 70% with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Classes: A, Ktedonobacteriales; B, Anaerolineae; C, Caldilineae; D, 'Dehalococcoides'; E, clone cluster; F, Chloroflexi; G, Thermomicrobia.

Although strains ONI-1^T and ONI-5^T differ from *K. racemifer* and *T. hazakensis* at the order level, these species have similar morphological features, i.e. formation of branched substrate and aerial mycelia and spores. Therefore, the species belonging to the class Ktedonobacteria may have common morphological features.

Description of *Thermogemmatisspora* gen. nov.

Thermogemmatisspora (Ther'mo.gem.ma'ti.spo'ra. Gr. adj. *thermos* hot; L. adj. *gemmatus* provided with buds; Gr. n. *spora* a seed and, in biology, a spore; N.L. fem. n. *Thermogemmatisspora* thermophilic budding-spored organism).

Filamentous, Gram-stain-positive, spore-forming bacteria. Thermophilic aerobic heterotrophs with glutamic acid,

serine, glycine, histidine, alanine and ornithine as peptidoglycan amino acids and rhamnose as a cell-wall sugar. iso-C_{17:0}, iso-C_{19:0} and C_{20:0} are the major fatty acids and MK-9(H₂) is the major menaquinone. The polar lipids consist of phosphatidylinositol, phosphatidylglycerol and a glycolipid. The type species is *Thermogemmatisspora onikobensis*.

Description of *Thermogemmatisspora onikobensis* sp. nov.

Thermogemmatisspora onikobensis (o.ni.ko.ben'sis. N.L. fem. adj. *onikobensis* pertaining to Onikobe, named for Onikobe hot springs, the source of the geothermal soils from which the type strain was isolated).

Displays the following properties in addition to those in the genus description. Produces branched vegetative and

aerial mycelia. Spores are borne in a cluster and are non-motile, oval in shape (1.0–2.0 µm long and 0.5–1.5 µm wide) with rough surfaces. Grows at 50–74 °C (optimum 60–65 °C) and pH 4.6–8.0 (optimum pH 7.0). Does not require NaCl; growth is inhibited completely by 2 % NaCl. Avicel, CM-cellulose, xylan, chitin and gelatin are hydrolysed, whereas starch and casein are not. Catalase-positive. D-Xylose, D-glucose, sucrose, L-rhamnose and D-mannitol are utilized as sole carbon sources. The G+C content of genomic DNA of the type strain is 60.2 mol%.

The type strain is ONI-1^T (=JCM 16817^T =KCTC 19768^T), isolated from fallen leaves on geothermal soils at Onikobe hot springs, Miyagi, Japan.

Description of *Thermogemmatispora foliorum* sp. nov.

Thermogemmatispora foliorum (fo.li.o'rum. L. pl. gen. n. *foliorum* of leaves, referring to the isolation of the type strain from fallen leaves).

Displays the following properties in addition to those in the genus description. Produces branched vegetative and aerial mycelia. Spores are formed in a cluster and are non-motile, oval in shape (1.0–2.0 µm long and 0.5–1.5 µm wide) with rough surfaces. Grows at 45–74 °C (optimum 60–65 °C) and pH 4.6–8.0 (optimum pH 7.0). Does not require NaCl; growth is inhibited completely by 1 % NaCl. Avicel, CM-cellulose, xylan and chitin are hydrolysed, whereas gelatin, starch and casein are not. Catalase-positive. D-Mannitol is utilized as a sole carbon source. The G+C content of genomic DNA of the type strain is 58.1 mol%.

The type strain is ONI-5^T (=JCM 16818^T =KCTC 19767^T), isolated from fallen leaves on geothermal soils at Onikobe hot springs, Miyagi, Japan.

Description of *Thermogemmatisporaceae* fam. nov.

Thermogemmatisporaceae (Ther'mo.gem.ma'ti.spo.ra'ce.ae. N.L. fem. n. *Thermogemmatispora* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Thermogemmatisporaceae* the *Thermogemmatispora* family).

The description is the same as for the genus *Thermogemmatispora*. The type genus is *Thermogemmatispora*.

Description of *Thermogemmatisporales* ord. nov.

Thermogemmatisporales (Ther'mo.gem.ma'ti.spo.ra'les. N.L. fem. n. *Thermogemmatispora* type genus of the order; -ales ending to denote an order; N.L. fem. pl. n. *Thermogemmatisporales* the order of the genus *Thermogemmatispora*).

The description is the same as for the genus *Thermogemmatispora*. The order contains the family *Thermogemmatisporaceae*. The type genus is *Thermogemmatispora*.

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