Thermasporomyces composti gen. nov., sp. nov., a thermophilic actinomycete isolated from compost

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A thermophilic, Gram-positive bacterium that formed a branched vegetative mycelium was isolated from compost. The strain, designated I3^T, grew at temperatures between 35 and 62 °C, with optimum growth at 50–55 °C. No growth was observed below 29 °C or above 65 °C. The pH range for growth was 5.7–10.0, the pH for optimum growth was 7.0 and no growth was observed below pH 5.6 or above pH 10.8. The DNA G + C content of strain I3^T was 69.2 mol%. The major fatty acids found were C_{15:0} iso (14.2 %), C_{15:0} anteiso (12.1 %), C_{17:0} iso (16.3 %) and C_{17:0} anteiso (21.7 %). The major menaquinones were MK-9(H₄), MK-10(H₄) and MK-11(H₄). The cell wall contained glutamic acid, glycine, alanine and L-diaminopimelic acid in a molar ratio of 1.0 : 3.9 : 0.6 : 0.5. The polar lipids consisted of ninhydrin-positive phosphoglycolipids, phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. The cell-wall sugars were rhamnose and arabinose. 16S rRNA gene sequence analysis assigned this actinomycete to the family *Nocardioidaceae*, but its 16S rRNA gene sequence shared no more than 95.5 % similarity with those of other members of the family. The chemotaxonomic and phenotypic characteristics of strain I3^T differed in some respects from those of members of the genus *Actinopolymorpha*, the most closely related genus. Therefore, strain I3^T represents a novel species in a new genus of the family *Nocardioidaceae*, for which the name *Thermasporomyces composti* gen. nov., sp. nov. is proposed. The type strain of the type species is I3^T (=JCM 16421^T=DSM 22891^T).

The family *Nocardioidaceae* was described by Nesterenko *et al.* (1985). The name was validly published in 1990 (Nesterenko *et al.*, 1990) and the description of the genus has since been emended by Zhi *et al.* (2009). At the time of writing, the family *Nocardioidaceae* comprised six genera: *Nocardioides* (Prauser, 1976), *Aeromicrobium* (Miller *et al.*, 1991), *Kribbella* (Park *et al.*, 1999; Sohn *et al.*, 2003), *Marmoricola* (Urzì *et al.*, 2000), *Actinopolymorpha* (Wang *et al.*, 2001) and *Jiangella* (Song *et al.*, 2005). All recognized species in this family are mesophilic. In this study, a new thermophilic actinomycete belonging to the family *Nocardioidaceae* was isolated from compost. In order to determine the taxonomic and phylogenetic position of this organism, the morphological, physiological and biochemical characteristics of the novel strain were examined and

the chemotaxonomic markers and 16S rRNA gene sequence were analysed. The results indicated that the new strain represented a novel species of a new genus.

The sample was obtained from mature compost produced by a field-scale composter (Hazaka system; Hazaka Plant Kogyo Co., Ltd), which was used for the treatment of livestock excreta. The composter was an open strip furrow (100 m long × 3 m wide × 2 m deep) with an automatic scoop-type turner. The details of this system have been described previously (Yabe *et al.*, 2009). The sample was collected in a plastic bag (Ziploc), transported to the laboratory and stored at room temperature for 1 day.

The isolation medium was International Streptomyces Project (ISP) 3 agar (Shirling & Gottlieb, 1966). The medium was supplemented with 20 mg l⁻¹ trimethoprim, 10 mg l⁻¹ nalidixic acid and 20 mg l⁻¹ kanamycin. The sample (1 g wet weight) was serially diluted in saline solution and aliquots of the dilutions were spread on the

Abbreviation: ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Thermasporomyces composti* I3^T is AB535715.

plates. The plates were incubated at 50 °C for 7 days. One of the yellow and vegetative-mycelium-forming colonies was picked, grown and purified by plating three times on ISP 3. The colony was restreaked and a stock culture was prepared by inoculating agar slants from the second plate. The isolate was designated strain I3^T.

Cell morphology and colonial characteristics were determined after growth for 14 days at 50 °C on ISP 1, 2, 3, 4, 5, 6 agars and nutrient agar by phase-contrast microscopy and visual inspection, respectively. A suitable agar block containing a colony grown at 50 °C for 14 days on ISP 3 agar was used for scanning electron microscopy (JSM6700F; JEOL) observations. The full details of the method were as previously described by Yabe *et al.* (2010).

Strain I3^T grew well on ISP 3 agar and weakly on ISP 2, 4, 6 agars and nutrient agar. No growth occurred on ISP 1 or ISP 5 agars. Strain I3^T developed pale yellow colonies on ISP 3, pale yellow–orange colonies on ISP 2 and white colonies on other media. No aerial mycelia or diffusible pigments were observed on any of the media. Strain I3^T formed branched hyphae that fragmented into short chains or aggregates. The fragments were coccoid or short rods (Fig. 1). Spores were not observed.

In order to determine the effect of pH, temperature and NaCl on growth, strain I3^T was cultivated in ISP 3 agar. Temperature, pH and NaCl tolerance tests were carried out at 20–65 °C (pH 7.0), pH 5.6–10.8 (50 °C) and with NaCl 0–7 % (w/v) (50 °C, pH 7.0). The pH was adjusted to various values with HCl and NaOH at room temperature. Biochemical tests were carried out using API ZYM microbial identification strips (bioMérieux). Physiological characteristics, including reaction with milk, sugar fermentation analyses, utilization of organic acids, gelatin liquefaction, and hydrolysis or decomposition of starch, L-tyrosine, xanthine, carboxymethylcellulose, xylan and casein were evaluated as described by Gordon *et al.* (1974). Gram staining was carried out by the modified Hucker method (Smibert & Krieg, 1994). Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. The oxidase test was performed with cytochrome oxidase test paper (Nissui Seiyaku).

Fig. 1. Scanning electron micrograph of strain I3^T grown on ISP 3 agar for 14 days at 50 °C. Bar, 10 µm.

Strain I3^T stained Gram-positive and was catalase- and oxidase-positive. Growth occurred at temperatures between 35 and 62 °C, with an optimum temperature of 50–55 °C; there was no growth at 29 °C or 65 °C. Strain I3^T was able to grow over a pH range from 5.7 to 10.0, with an optimum at pH 7.0. No growth was observed at pH 5.6 or 10.8. Growth was observed with up to 5 % (w/v) NaCl. The results of the phenotypic examination of strain I3^T are given in the species description.

Cellular fatty acids of strain I3^T were prepared from cell mass grown in nutrient broth for 5 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). The amino acids in the cell-wall hydrolysate were identified by TLC (Harper & Davis, 1979) and HPLC as their phenylthio-carbamoyl derivatives with 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 equipped with a 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 concentration 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 (mol%) of the genomic DNA was determined by HPLC (Tamaoka & Komagata, 1984) using a COSMOSIL 5C 18-AR-II packed column (4.6 mm × 250 mm; Nacalai Tesque) with the DNA-GC kit (Yamasa Shouyu).

The DNA G+C content of strain I3^T was 69.2 mol%, as determined by HPLC analysis. The predominant mena-quinones were MK-9(H₄), MK-10(H₄) and MK-11(H₄). The major fatty acids were C_{15:0} iso (14.2 %), C_{15:0} anteiso (12.1 %), C_{17:0} iso (16.3 %) and C_{17:0} anteiso (21.7 %). The polar lipids consisted of ninhydrin-positive phosphoglycerolipids, phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. The peptidoglycan contained glutamic acid, glycine, alanine and LL-diaminopimelic acid in a molar ratio of 1.0:3.9:0.6:0.5. Cell-wall sugars were rhamnose and arabinose. Differential chemotaxonomic characteristics between strain I3^T and other genera belonging to the family *Nocardioideae* are summarized in Table 1.

A nearly complete 16S rRNA gene sequence of strain I3^T was amplified by PCR and the purified PCR product was sequenced as described previously by Yabe *et al.* (2010). The 16S rRNA gene sequence of strain I3^T was 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

Table 1. Differential characteristics of strain I3^T and related taxa of the family *Nocardioideaceae*

Taxa: 1, *Thermasporomyces composti* gen. nov., sp. nov.; 2, *Actinopolymorpha* (data from Wang *et al.*, 2001); 3, *Jiangella* (Song *et al.*, 2005); 4, *Kribbella* (Park *et al.*, 1999); 5, *Nocardioides* (Prauser, 1976; O'Donnell *et al.*, 1982; Collins *et al.*, 1989; Tamura & Yokota, 1994; Miller *et al.*, 1991; Park *et al.*, 1999); 6, *Aeromicrobium* (Miller *et al.*, 1991; Tamura & Yokota, 1994); 7, *Marmoricola* (Urzi *et al.*, 2000). PI, phosphatidylinositol; DPG, diphosphatidylglycerol; PIM, phosphatidylinositol mannosides; PG, phosphatidylglycerol; PC, phosphatidylcholine.

Characteristic	1	2	3	4	5	6	7
Major menaquinone	MK-9(H ₄), MK-10(H ₄), MK-11(H ₄)	MK-9(H ₆)	MK-9(H ₄)	MK-9(H ₄)	MK-8(H ₄)	MK-9(H ₄)	MK-8(H ₄)
Major fatty acids	C _{15:0} iso, C _{15:0} anteiso, C _{17:0} iso, C _{17:0} anteiso	C _{15:0} iso, C _{16:0} iso, C _{16:1} -iso-H	C _{15:0} anteiso, C _{16:0} iso	C _{15:0} anteiso, C _{16:0} iso	C _{16:0} iso	C _{16:0} , C _{16:0} 2-OH, C _{18:0} -10-Me, C _{18:1} <i>ω</i> 9 <i>c</i>	C _{16:0} , C _{18:1} (C _{16:0} iso)†
Phospholipids	PG, DPG	PIM, PI, PG, (DPG)*	PIM, PI, DPG	PC	PG	PG, DPG	PI, PG, DPG

**Actinopolymorpha cephalotaxi* lacks DPG.

†Major fatty acid of *Marmoricola bigeumensis* is C_{16:0} iso.

unidentified base positions were edited using BioEdit (Hall, 1999). Phylogenetic trees were 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,

Fig. 2. Neighbour-joining tree based on 1310 aligned positions of the 16S rRNA gene sequence showing the position of strain I3^T in relation to other members of the family *Nocardioideaceae*. The sequence of *Streptomyces sparsogenes* NRRL 2940^T was used as the outgroup. The numbers at the nodes are bootstrap percentages based on 1000 replicated datasets; only values $\geq 70\%$ are shown. Bar, 1% sequence dissimilarity.

1985). The evolutionary distances were computed using the Kimura two-parameter method (Kimura, 1980).

A phylogenetic tree was constructed based on 16S rRNA gene sequences (Fig. 2). All trees had similar topologies. The 16S rRNA gene sequence similarities between strain I3^T and its closest relatives were as follows: *Actinopolymorpha cephalotaxi* I06-2230^T (95.5%), *Actinopolymorpha rutila* YIM 45725^T (95.5%), *Actinopolymorpha alba* YIM 48868^T (95.5%) and *Actinopolymorpha singaporensis* IM 7744^T (95.3%). The 16S rRNA gene sequence similarities between strain I3^T and members of other genera were less than 93%.

The results of the phylogenetic analysis suggest that strain I3^T should be placed in the family *Nocardioideae*. However, strain I3^T had low 16S rRNA gene sequence similarities with members of the genus *Actinopolymorpha* (95.3–95.5%) and was separated from its closest neighbours (species of the genus *Actinopolymorpha*) by a long branch in the phylogenetic tree (Fig. 2). Furthermore, strain I3^T was thermophilic, whereas other species in the family *Nocardioideae*, including species of the genus *Actinopolymorpha*, are mesophilic. The chemotaxonomic properties of strain I3^T were very distinct from those of other species of the genus *Actinopolymorpha*, for example, strain I3^T had MK-11(H₄) as one of its major menaquinones and no phosphatidylinositol or phosphatidylinositol mannosides (Table 1). Therefore, strain I3^T represents a novel species in a new genus of the family *Nocardioideae*, for which the name *Thermasporomyces composti* gen. nov., sp. nov. is proposed.

Description of *Thermasporomyces* gen. nov.

Thermasporomyces [Ther.ma.spo.ro.my'ces. Gr. n. *therme* heat; Gr. prefix. *a* not; Gr. n. *spora* a seed, and in biology a spore; Gr. masc. n. *mukes* mushroom or other fungus; N.L. masc. n. *Thermasporomyces* the heat (-loving) non-spored fungus].

Gram-positive, aerobic, heterotrophic bacteria that produce branched vegetative mycelium. No spores are observed. Good growth occurs on ISP 3 medium. Growth occurs between 35 and 62 °C (optimum 50–55 °C), pH 5.7–10.0 (optimum pH 7.0) and in the presence of up to 5% (w/v) NaCl. The major fatty acids are C_{15:0} iso, C_{15:0} anteiso, C_{17:0} iso and C_{17:0} anteiso. The major menaquinones are MK-9(H₄) (41.2%), MK-10(H₄) (27.7%) and MK-11(H₄) (30.8%). The cell wall contains glutamic acid, glycine, alanine and LL-diaminopimelic acid in a molar ratio of 1.0:3.9:0.6:0.5. The polar lipids consist of ninhydrin-positive phosphoglycolipids, phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. Cell-wall sugars are rhamnose and arabinose. The type species is *Thermasporomyces composti*.

Description of *Thermasporomyces composti* sp. nov.

Thermasporomyces composti (com.pos'ti. N.L. n. *compositum* -i compost; N.L. gen. n. *composti* of compost).

Exhibits the following properties in addition to those given in the genus description. Catalase- and oxidase-positive. Positive for nitrate reduction, gelatin hydrolysis, milk coagulation and peptonization. Casein, carboxymethyl-cellulose and xylan are hydrolysed, while starch, xanthine and L-tyrosine are not hydrolysed. Utilizes raffinose, sorbitol, lactose, cellobiose and D-arabinose, but not sodium citrate or sodium succinate. Acid is produced from mannose and maltose, but not from L-rhamnose, sorbitol, inositol or D-galactose. Enzymic activities detected by API ZYM are alkaline phosphatase, C4 and C8 esterases, leucine arylamidase, α -chymotrypsin, naphthol phosphohydrolase, trypsin, α -galactosidase, α -glucosidase, lipase C14, valine arylamidase, N-acetyl- β -glucosaminidase and α -mannosidase. Activities not detected by API ZYM are β -galactosidase, β -glucosidase, β -glucuronidase and α -fucosidase.

The type strain is I3^T (=JCM 16421^T=DSM 22891^T). The DNA G+C content of the type strain is 69.2 mol%.

Acknowledgements

This work was partly supported by the Center for Integrated Nanotechnology Support at Tohoku University and also by 'Nanotechnology Network Project' of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of the Japanese Government. We thank Eiji Aoyagi (Institute for Materials Research, Tohoku University) for SEM technical guidance, and thank Ms Kim Jeeyoung for help with the G+C content and cell wall analysis. We acknowledge Professor Dr Jean Euzéby for support in the Latin etymology of the new genus names.

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