Planifilum fimeticola gen. nov., sp. nov. and *Planifilum fulgidum* sp. nov., novel members of the family 'Thermoactinomycetaceae' isolated from compost

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Four thermophilic, Gram-positive strains, designated H0165^T, 500275^T, C0170 and 700375, were isolated from a composting process in Japan. The isolates grew aerobically at about 65 °C on a solid medium with formation of substrate mycelia; spores were produced singly along the mycelia. These morphological characters resembled those of some type strains of species belonging to the family 'Thermoactinomycetaceae', except that aerial mycelia were not formed. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the closest related species to the isolates were members of the family 'Thermoactinomycetaceae', but that the isolates formed an independent phylogenetic lineage. Some chemotaxonomic characters of the isolates, such as DNA G + C contents of 58.7–60.3 mol%, MK-7 as the major menaquinone and cellular fatty acid profiles, differed from those of members of the family 'Thermoactinomycetaceae'. DNA–DNA hybridization showed that the isolates could be divided into two genomic groups, strain H0165^T and the other three strains. These results indicated that the four isolates should be classified into two species of a novel genus in the family 'Thermoactinomycetaceae', for which the names *Planifilum fimeticola* gen. nov., sp. nov. (type strain H0165^T = ATCC BAA-969^T = JCM 12507^T) and *Planifilum fulgidum* sp. nov. (type strain 500275^T = ATCC BAA-970^T = JCM 12508^T) are proposed.

The family 'Thermoactinomycetaceae' was initially described to accommodate the genus *Thermoactinomyces* (Lacey & Cross, 1989). However, based on 16S rRNA gene sequence analysis, as well as chemotaxonomic and physiological characterization of the genus, Yoon & Park (2000) suggested that *Thermoactinomyces* was a heterogeneous group containing more than one genus. Based on further chemotaxonomic characterization, it was proposed that this genus should be

divided into four genera, *Thermoactinomyces sensu stricto*, *Laceyella*, *Thermoflavimicrobium* and *Seinonella* (Yoon *et al.*, 2005). The members of this family are aerobic, Gram-positive, thermophilic bacteria, except the mesophilic genus *Seinonella*, and show filamentous growth like actinomycetes. Members of the four genera have been isolated from a variety of natural sources, such as mushroom compost (Song *et al.*, 2001), moulding hay, cereal grains, soil, river water, dairy products and marine sediments, and also from humidifiers of air-conditioning systems (Lacey & Cross, 1989).

In our previous study to characterize the microbial flora in a hyperthermal composting process in Japan (Hatayama *et al.*, 2005), we isolated four thermophilic bacteria showing filamentous growth at approximately 65 °C. From phylogenetic analyses based on 16S rRNA gene sequences as well as chemotaxonomic, physiological and phenotypic characterizations of these bacteria, we propose that they constitute

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Planifilum fimeticola* H0165^T and *Planifilum fulgidum* 500275^T, C0170 and 700375 are AB088364, AB088362, AB088363 and AB088361, respectively.

Tables detailing levels of DNA–DNA relatedness among the novel strains and cellular fatty acid profiles of the four strains plus representative members of the family 'Thermoactinomycetaceae' are available as supplementary material in IJSEM Online.

Fig. 1. Phase-contrast microscope images of spore-forming cells of strain H0165^T (a) and strain 500275^T (b) grown on LB agar. Bars, 10 μm. Arrows indicate spores.

a new genus in the family 'Thermoactinomycetaceae', consisting of two species.

Strains H0165^T, 500275^T, C0170 and 700375 were isolated from samples of the hyperthermal composting process (Hatayama *et al.*, 2005) by cultivation on Luria–Bertani (LB) agar plates at 65 °C. Growth of strain H0165^T was observed at 50–65 °C with an optimum at 55–63 °C, and the other three strains grew at 50–67 °C with optima at 60–65 °C in liquid LB medium. Morphological characteristics of these strains resembled those of the genus *Thermoactinomyces sensu stricto* in formation of substrate mycelia with single spores along the mycelia (Fig. 1). However, formation of aerial mycelia was not observed in any of the isolates grown on LB agar, CYC agar (Lacey & Cross, 1989), SY agar (Yoon

et al., 2005) or Bacto nutrient agar (Difco); colonies were lustrous, cream-yellow with radial wrinkles. This is at variance with the description that species in the family 'Thermoactinomycetaceae' form aerial mycelia on plates of at least one of these agars (Lacey & Cross, 1989).

To determine the phylogenetic position of the four strains, 16S rRNA gene sequence analysis was undertaken. Extraction of DNA, PCR amplification and sequencing and phylogenetic analyses of the 16S rRNA gene sequences were performed as previously described (Hatayama *et al.*, 2005). From searches using BLASTN (Altschul *et al.*, 1997) and FASTA (Pearson, 2000), the four isolates showed closest 16S rRNA gene sequence similarities to the unidentified low-G + C, Gram-positive bacterium HTA1417 (94.2–94.8 % similarity), which was isolated from the deepest sea mud of the Mariana Trench (Takami *et al.*, 1997). The closest recognized species to the isolates were those of the genus *Thermoactinomyces sensu stricto* (91.2–91.6 % 16S rRNA gene sequence similarity), *Laceyella* (88.8–89.4 %), *Thermoflavimicrobium* (89.3–89.7 %) and *Seinonella* (87.8–88.1 %). To visualize the phylogenetic relationship between the new isolates and these four genera, two phylogenetic trees were constructed using the maximum-likelihood (data not shown) and neighbour-joining (Fig. 2) methods. The two methods produced essentially the same phylogenetic trees, and indicated that the four isolates formed an independent cluster close to that of the genera *Thermoactinomyces sensu stricto*, *Laceyella*, *Thermoflavimicrobium* and *Seinonella* with a high bootstrap value (Fig. 2). This indicated that the isolates should be classified within a novel genus of the family 'Thermoactinomycetaceae'. Moreover, the analyses suggested that the isolates could be divided into two genomic groups, H0165^T and the other three strains (97.1–97.6 % sequence similarity between the two groups). This split was confirmed by the results of DNA–DNA hybridization experiments, determined with the method of Ezaki *et al.* (1989) (see Supplementary Table S1 available in IJSEM Online).

The DNA G + C contents of the isolates were determined using the method of Tamaoka & Komagata (1984). The values obtained were over 10 mol% higher than those of the other genera of the family 'Thermoactinomycetaceae' (Table 1).

Fig. 2. Phylogenetic tree based on the 16S rRNA gene sequences of the four new isolates, strains of the family 'Thermoactinomycetaceae' and type strains of *Bacillus subtilis* and *Geobacillus stearothermophilus*. The tree was constructed using the neighbour-joining method. Bootstrap values were calculated from 1000 repeats and those greater than 50 % are shown at branch points. Numbers in parentheses are GenBank accession numbers. Bar, 0.01 substitutions per nucleotide position.

The chemotaxonomic characteristics of strains H0165^T and 500275^T were determined using cells cultured in SY medium at the exponential phase of growth. Compositions of amino acids and sugars in the cell-wall peptidoglycan were determined by the method of Kawamoto *et al.* (1981) using cellulose HPTLC plates (Merck). Profiles of menaquinones and cellular fatty acids, conducted by the National Collections of Industrial and Marine Bacteria (NCIMB), Japan, Co. Ltd, were determined by using the methods of Tamaoka (1986) and Nishijima *et al.* (1997), and Yoon *et al.* (2005), respectively. The cell-wall peptidoglycan of the isolates contained *meso*-diaminopimelic acid, alanine and glutamic acid but no diagnostic sugars, indicating that the wall chemotype was of type III (Lechevalier & Lechevalier, 1970). This result was consistent with those of the members of the family 'Thermoactinomycetaceae'. However, the isolates contained almost only one menaquinone type, MK-7 (>98.1 % peak area ratio), which distinguished them from the genera *Thermoactinomyces sensu stricto*, *Laceyella* and *Seinonella* (Table 1). Moreover, the cellular fatty acid profiles of the isolates differed from those of the other four genera (Table 1 and Supplementary Table S2 in IJSEM Online). In particular, the new isolates contained longer branched cellular fatty acids, such as iso-C_{17:0} and anteiso-C_{17:0}, as major components compared with the type species of the four genera. In addition, differences in the major components of the cellular fatty acids were seen between strains H0165^T and 500275^T.

Utilization of sugars was tested using the methods of Shirling & Gottlieb (1966) and Fortina *et al.* (2001). No reproducible results were obtained because of the weak growth of the strains (data not shown). Degradation of substrates was tested using LB agar with 1 % casein, 1 % starch, 1 % avicel, 1 % carboxymethyl cellulose, 1 % xylan, 0.5 % hypoxanthine or 0.5 % xanthine, and using CYC agar with 0.5 % L-tyrosine. All the isolates degraded casein and starch but not other substrates, except that strain H0165^T was able to degrade L-tyrosine (Table 1).

Strains H0165^T, 500275^T, C0170 and 700375 were clearly distinguished from members of the related genera *Thermoactinomyces sensu stricto*, *Laceyella*, *Thermoflavimicrobium* and *Seinonella* based on the absence of aerial mycelia, growth temperatures, DNA G+C contents and compositions of menaquinones and cellular fatty acids. Phylogenetic analyses based on the 16S rRNA gene sequences showed that the isolates constituted an independent cluster close to that of these four genera. The results indicated that the isolates did not belong to *Thermoactinomyces sensu stricto*, *Laceyella*, *Thermoflavimicrobium* or *Seinonella*. Moreover, strain H0165^T could be clearly discriminated from the other three isolates based on 16S rRNA gene sequence similarities, levels of DNA–DNA relatedness, the cellular fatty acid profile and the ability to degrade L-tyrosine. On the basis of these results, the four isolates described herein have been assigned to two species of a novel genus in the family 'Thermoactinomycetaceae', for which the names *Planifilum*

Table 1. Differential phenotypic characteristics of the new isolates and the four genera of the family 'Thermoactinomycetaceae'

Taxa: 1, H0165^T; 2, 500275^T; 3, *Thermoactinomyces*; 4, *Laceyella*; 5, *Thermoflavimicrobium*; 6, *Seinonella*. +, Positive reaction; –, negative reaction; ND, not detected; NO, not observed. Data for reference taxa were taken from Lacey & Cross (1989) and Yoon *et al.* (2005).

Characteristic	1	2	3	4	5	6
Aerial mycelium	NO*	NO*	White	White	Yellow	White
Optimal growth temperature (°C)	55–63	60–65	50–55	48–55	55	35
Degradation of:						
Casein	+	+	+	+	+	–
Starch	+	+	–	+	–	–
Hypoxanthine	–	–	–	–	+	–
Xanthine	–	–	–	–	+	–
L-Tyrosine	+	–	–	+ / –	–	–
Predominant menaquinone	MK-7	MK-7	MK-7	MK-9	MK-7	MK-7
Other menaquinone(s) of > 10 % peak area ratio	ND†	ND‡	MK-8 or MK-9	MK-7 or MK-8 or MK-10	ND	MK-8, MK-9, MK-10
Major fatty acids	iso-C _{16:0} , iso-C _{17:0} , anteiso-C _{17:0}	iso-C _{17:0} , anteiso-C _{17:0} , iso-C _{15:0}	iso-C _{15:0} , iso-C _{17:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{16:0}	iso-C _{14:0} , anteiso-C _{15:0} , iso-C _{16:0}
DNA G+C content (mol%)	60.3	60.0§	48	48–49	43	40

*Aerial mycelia were not observed on LB, CYC, SY or Bacto nutrient agar plates.

†MK-8 detected at trace levels (1.2 % peak area ratio).

‡MK-8 detected at trace levels (1.9 % peak area ratio).

§DNA G+C contents of C0170 and 700375 were 58.7 and 60.0 mol%, respectively.

fimeticola gen. nov., sp. nov. (H0165^T) and *Planifilum fulgidum* sp. nov. (500275^T, C0170 and 700375) are proposed.

Description of *Planifilum* gen. nov.

Planifilum (Pla.ni.fi'lum. L. adj. *planus* flat; L. neut. n. *filum* a thread; N.L. neut. n. *Planifilum* a flat thread).

Cells are Gram-positive, aerobic and thermophilic. Substrate mycelia are formed on LB agar, CYC agar, SY agar and Bacto nutrient agar, but aerial mycelia are not formed. Endospores are produced singly along mycelia. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid, alanine and glutamic acid but no diagnostic sugars. The major menaquinone is MK-7. Major fatty acids are iso-C_{17:0}, anteiso-C_{17:0} and iso-C_{15:0} or C_{16:0}. DNA G + C content is 58.7–60.3 mol%. The type species is *Planifilum fimeticola*.

Description of *Planifilum fimeticola* sp. nov.

Planifilum fimeticola (fi.me.ti.co'la. L. n. *fimetum* a dung-hill and, by extension, compost; L. masc. suffix *-cola* inhabitant; N.L. masc. n. *fimeticola* inhabitant of compost, referring to the habitat of the type strain).

Colonies are lustrous, cream-yellow with radial wrinkles. Growth occurs at 50–65 °C, optimally at 55–63 °C. Casein, starch and L-tyrosine are degraded. The major cellular fatty acids are iso-C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}. DNA G + C content of the type strain is 60.3 mol%.

The type strain, H0165^T (=ATCC BAA-969^T=JCM 12507^T), was isolated from a hyperthermal composting process in Okinawa prefecture, Japan.

Description of *Planifilum fulgidum* sp. nov.

Planifilum fulgidum (ful'gi.dum. L. neut. adj. *fulgidum* lustrous, referring to the colony character).

Colonies are lustrous, cream-yellow with radial wrinkles. Growth occurs at 50–67 °C, optimally at 60–65 °C. Casein and starch are degraded. The major cellular fatty acids are iso-C_{17:0}, anteiso-C_{17:0} and iso-C_{15:0}. The DNA G + C content is 58.7–60.0 mol%.

The type strain, 500275^T (=ATCC BAA-970^T=JCM 12508^T), was isolated from a hyperthermal composting process in Okinawa prefecture, Japan. Two additional strains, C0170 and 700375, are included in this species.

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