Planotetraspora thailandica sp. nov., isolated from soil in Thailand

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A Gram-positive-staining, filamentous bacterial strain that developed cylindrical sporangia containing four oval- to rod-shaped spores at the ends of short sporangiophores on branched aerial mycelium was isolated from tropical rainforest soil near a hot spring. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid, glutamic acid and alanine as cell-wall amino acids; the whole-cell hydrolysate contained rhamnose, madurose, glucose, galactose and 3-O-methylmannose as whole-cell sugars. The predominant menaquinone was MK-9(H₄). Mycolic acids were not detected. The diagnostic phospholipid was phosphatidylethanolamine. The predominant cellular fatty acids were iso-C_{16:0} and 10-methylated C_{17:0}. The G + C content of the DNA was 71.1 mol%. The phenotypic and chemotaxonomic analyses showed that the isolate had characteristics typical of members of the genus *Planotetraspora*. Furthermore, 16S rRNA gene sequence analysis also indicated that this strain belongs to the genus *Planotetraspora*, but as a putative novel species. Following phenotypic, chemotaxonomic and genotypic studies, the isolate is proposed to be a representative of a novel species, to be named *Planotetraspora thailandica* sp. nov. The type strain is BCC 21825^T (=NBRC 104271^T). An emended description of the genus *Planotetraspora* is also presented.

The genus *Planotetraspora* was first proposed by Runmao *et al.* (1993) and classified in the family *Streptosporangiaceae*. At the time of writing, it includes two species: *Planotetraspora mira* (Runmao *et al.*, 1993) as the type species and *Planotetraspora silvatica* (Tamura & Sakane, 2004). Cells are Gram-positive-staining, non-acid-fast and aerobic. They develop cylindrical sporangia containing four non-motile spores at the ends of short sporangiophores on the aerial mycelium. Substrate mycelium is non-fragmented. Vegetative mycelia are white to pale yellow and good growth is observed between 25 and 30 °C. These strains

Abbreviation: *meso*-A₂pm, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BCC 21825^T is AB370244.

Supplementary tables showing cultural and physiological characteristics of type strains of *Planotetraspora* species are available with the online version of this paper.

have *meso*-diaminopimelic acid (*meso*-A₂pm), glutamic acid and alanine in the cell wall. They contain madurose and rhamnose as whole-cell sugars. Mycolic acids are not detected. MK-9(H₄) is the major isoprenoid quinone and the phospholipid pattern is type PIV. The DNA G + C content is 70–71 mol%. The genus *Planotetraspora* is related closely to the genera *Microbispora* (Nonomura & Ohara, 1957), *Microtetraspora* (Thiemann *et al.*, 1968), *Herbidospora* (Kudo *et al.*, 1993) and *Acrocarpospora* (Tamura *et al.*, 2000). Members of these genera exhibit high 16S rRNA gene sequence similarity to the genus *Planotetraspora*, but the genus can be distinguished from the other four genera on the basis of morphological and chemotaxonomic criteria.

During a project on the diversity of actinomycetes in Thailand, strain BCC 21825^T was isolated from forest soil in Krabi Province. The isolate was identified as a novel

member of the genus *Planotetraspora* based on morphological and chemotaxonomic studies, phylogenetic analysis and DNA–DNA relatedness studies. Here, we report the taxonomic characterization and classification of strain BCC 21825^T and propose that the strain is a representative of a novel species, *Planotetraspora thailandica* sp. nov.

Strain BCC 21825^T was isolated from tropical lowland rainforest soil collected near a hot spring in Krabi Province, Thailand. The sample was taken from the organic layer on the surface of the soil (pH 7) and kept at 4 °C. The sample was dried at room temperature for 8 days and then treated with 1.5 % phenol in distilled water as recommended by Hayakawa *et al.* (1991). The organism was isolated on humic acid–salts vitamin agar (HV) supplemented with (l⁻¹): 25 mg nalidixic acid, 50 mg cycloheximide and 1 mg terbinafin. The pure culture was preserved by freezing at –80 °C in glycerol (15 %, v/v) and freeze-drying.

For determination of the morphological characteristics, growth on modified soil extract agar [0.5 g CaSO₄·2H₂O, 0.25 g Ca(NO₃)₂·4H₂O, 0.05 g MgSO₄·7H₂O, 0.03 g K₂SO₄, 0.02 g KH₂PO₄, 0.1 g NaHCO₃, 0.02 g CaCl₂·2H₂O, 0.1 g yeast extract, 0.1 g Casamino acids, 0.2 g glucose, 100 ml soil extract, 18 g agar, 900 ml distilled water, pH 7.0], yeast extract–starch agar (2 g yeast extract, 10 g starch, 18 g agar, 1 l distilled water, pH 7.0) and inorganic salts–starch agar (ISP4 medium; Shirling & Gottlieb, 1966) for 7–14 days at 28 °C was observed with a light microscope and a scanning electron microscope (model JSM-5410 LV; JEOL). The sample on modified soil extract agar for scanning electron microscopy was prepared as described previously (Itoh *et al.*, 1989).

Strain BCC 21825^T had characteristics typical of the genus *Planotetraspora*. It developed cylindrical sporangia (0.8–1.0 × 2.0–3.0 µm) containing four oval- to short rod-shaped spores (0.4–0.6 × 0.8–1.0 µm) at the ends of short sporangioophores on the aerial mycelium (Fig. 1). Globose bodies

Fig. 1. Scanning electron micrograph of cells of strain BCC 21825^T grown on modified soil extract agar at 28 °C for 21 days. Bar, 1 µm.

were not observed. The spores were not motile, as determined with a light microscope using cells grown on HV agar for 10 days at 28 °C and then incubated at 28 °C for 30–60 min in 0.1 M potassium phosphate buffer (pH 7).

Phenotypic characteristics were examined by using several standard methods. Cultural characteristics were tested by using 14-day-old cultures grown at 28 °C on various agar media (Shirling & Gottlieb, 1966). The NBS-ISCO colour system was used for determining colour designations and names (Kelly, 1964). Acid production from carbon sources was examined by using the medium recommended by Gordon *et al.* (1974). Temperature, pH and NaCl tolerances were determined on ISP2 medium. Carbon-source utilization was tested by using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the carbon sources. Gelatin liquefaction, peptonization of milk, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Arai (1975) and Williams & Cross (1971). The results are indicated in detail in the species description, Supplementary Tables S1 and S2 (available in IJSEM Online) and Table 1.

Freeze-dried cells for chemotaxonomic analyses were obtained from a culture grown in glucose–yeast extract broth (Tamura *et al.*, 1994) on a rotary shaker at 30 °C for 7 days. Cell-wall peptidoglycan was prepared and hydrolysed according to the methods of Kawamoto *et al.* (1981) and the amino acid composition was determined by HPLC. The isomer of diaminopimelic acid in the cell wall was determined by using the method of Stanek & Roberts (1974). The acyl group of the muramic acid in the peptidoglycan was determined by using the method of Uchida & Aida (1984). The reducing sugars from whole-cell hydrolysates were analysed by HPLC using a Sugar-Pak column (Waters) according to Mikami & Ishida (1983). The phospholipids in the cells were extracted and analysed by using the method of Minnikin *et al.* (1984). Fatty acid methyl ester analysis was performed by GLC according to the instructions of the Microbial Identification System (MIDI) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones were extracted according to the method of Collins *et al.* (1977) and were analysed by HPLC [Cosmosil 5C₁₈ column (4.6 × 150 mm); Nacalai Tesque] with a mixture of methanol and 2-propanol (2 : 1, v/v) as elution solvent.

The chemotaxonomic characteristics of strain BCC 21825^T were similar to those of members of the genus *Planotetraspora*. Cell-wall amino acids of the novel isolate contained *meso*-A₂pm, alanine and glutamic acid. These amino acids correspond to murein type A1γ as described by Schleifer & Kandler (1972). This strain contained rhamnose, madurose, glucose, galactose and 3-*O*-methylmannose as whole-cell sugars, but not arabinose or xylose [whole-cell sugar pattern B of Lechevalier & Lechevalier (1970)]. Phosphatidylethanolamine was detected but phosphatidylglycerol, phosphatidylcholine and glucosamine-containing phospholipids were not detected. This

Table 1. Characteristics differentiating *Planotetraspora thailandica* sp. nov., *P. mira* and *P. silvatica*

Species: 1, *P. thailandica* BCC 21825^T; 2, *P. mira* NBRC 15435^T; 3, *P. silvatica* NBRC 100141^T. +, Positive; ±, weakly positive; –, negative. All strains were positive or weakly positive for production of acid from D-glucose and D-xylose and utilization of L-rhamnose, melibiose, glycerol, D-lactose, D-galactose and cellobiose. All strains were negative for starch hydrolysis and production of acid from raffinose and maltose.

Characteristic	1	2	3
Colour of colonies on ISP 2			
Aerial mycelium or spore	Pale yellow	Pale yellow	White
Substrate mycelium	Pale yellow	Pale yellow	White to colourless
Colour of colonies on ISP 3			
Aerial mycelium or spore	Colourless	Pale yellow	Yellowish white
Substrate mycelium	Colourless	Pale yellow	Yellowish white
NaCl tolerance (%)	≤ 1	≤ 1	≤ 3
Acid production from:			
D-Galactose	–	+	+
D-Lactose	–	+	–
D-Mannitol	+	+	–
D-Mannose	+	+	–
Melibiose	+	–	–
L-Rhamnose	+	+	–
Utilization of:			
D-Fructose	+	–	–
D-Glucose	±	+	–
D-Mannitol	+	+	–
Raffinose	+	–	+
D-Xylose	+	+	–
Salicin	+	–	–
Skimmed milk peptonization	–	–	+
Skimmed milk coagulation	–	+	–

pattern corresponds to phospholipid type PIV of Lechevalier *et al.* (1977). The cellular fatty acid compositions of strain BCC 21825^T and the two recognized *Planotetraspora* species are shown in Table 2. Tamura & Sakane (2004) proposed that the major cellular fatty acids of *Planotetraspora* species were iso-C_{16:0} and 10-methylated C_{18:0}, which corresponds to fatty acid pattern 3d of Kroppenstedt (1985). In this study, the three *Planotetraspora* species mentioned above were analysed by GLC according to the instructions of MIDI (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). It showed that major fatty acids of *Planotetraspora* species were iso-C_{16:0}, 10-methylated C_{17:0}, iso C_{15:0}, C_{15:0}, 10-methylated C_{16:0}, C_{16:0}, anteiso-C_{17:0}, C_{17:0}, anteiso-C_{15:0}, iso-C_{14:0} and C_{17:1}, corresponding to fatty acid pattern 3c of Kroppenstedt (1985), but not 10-methylated C_{18:0}. Therefore, we propose that all members of the family *Streptosporangiaceae* express fatty acid pattern 3c of Kroppenstedt (1985). Mycolic acids were absent. The menaquinones found in the isolates were MK-9(H₄) (61.45%), MK-9(H₈) (18.63%), MK-9(H₂) (13.16%), MK-9(H₀) (5.06%) and MK-9(H₆) (1.7%).

Genomic DNA was prepared according to the method of Saito & Miura (1963) using a slight modification. The 16S rRNA gene sequence was amplified by PCR as described

previously (Katsura *et al.*, 2001; Kawasaki *et al.*, 1993; Yamada *et al.*, 2000). Two primers, 20F (5'-GAGTTTGATCCTGGCTCAG-3', positions 9–27) and 1500R (5'-GTTACCTTGTTACGACTT-3', positions 1509–1492), were used for preliminary phylogenetic analysis. Positions in the 16S rRNA gene fragments were based on the *Escherichia coli* numbering system (GenBank accession no. V00348; Brosius *et al.*, 1981). Amplified PCR products were sequenced directly with an ABI PRISM BigDye Terminator cycle sequencing ready-reaction kit on an ABI PRISM model 310 Genetic Analyzer (both from Applied Biosystems). The following primers were used for sequencing: 20F, 520F, 920F, 520R, 920R and 1500R (Yukphan *et al.*, 2004). Multiple alignments of the sequences were carried out with the program BioEdit Sequence Alignment Editor (version 7.0.0). Distance matrices for the aligned sequences were calculated by using the two-parameter method of Kimura (1980). The neighbour-joining method (Saitou & Nei, 1987) was used to construct a phylogenetic tree. Comparison of the sequence data obtained was made on 1376 bases and the robustness for individual branches was estimated by bootstrapping with 1000 replications (Felsenstein, 1985). The novel strain BCC 21825^T exhibited highest 16S rRNA gene sequence similarity of 98.0 and 98.1% to *P. mira* NBRC 15435^T and *P. silvatica* TT 00-51^T, respectively. A

Table 2. Cellular fatty acid compositions of the three *Planotetraspora* species

Species: 1, *P. thailandica* BCC 21825^T; 2, *P. mira* NBRC 15435^T; 3, *P. silvatica* NBRC 100141^T. Values are percentages of total fatty acids. —, Not detected.

Fatty acid	1	2	3
Saturated fatty acids			
C _{13:0}	0.53	0.44	0.39
C _{14:0}	1.59	2.26	1.08
C _{15:0}	7.26	9.45	9.94
C _{16:0}	5.02	7.30	5.57
C _{17:0}	4.00	8.72	7.24
C _{18:0}	0.68	0.94	0.71
Unsaturated fatty acids			
C _{15:1}	0.30	—	—
C _{16:1}	2.00	1.83	2.43
C _{17:1}	3.03	4.61	6.32
C _{18:1}	0.35	0.62	0.77
Branched fatty acids			
i-C _{14:0}	3.20	1.64	1.36
i-C _{15:0}	7.82	15.46	15.18
ai-C _{15:0}	3.96	7.72	11.80
i-C _{16:0}	32.65	15.38	10.82
i-C _{16:1}	0.68	—	—
i-C _{17:0}	0.95	1.44	1.44
ai-C _{17:0}	4.87	4.96	9.16
i-C _{18:0}	0.39	—	—
10-Methylated fatty acids			
C _{16:0}	6.22	3.72	2.79
C _{17:0}	13.71	12.40	12.22
C _{18:0}	0.80	1.12	0.81

neighbour-joining tree showing the phylogenetic relationship between strain BCC 21825^T and members of the family *Streptosporangiaceae* revealed that this strain should be classified within the genus *Planotetraspora* and that it was clearly separated from its closest relatives (Fig. 2).

The G+C content of the DNA was determined by the method of Tamaoka & Komagata (1984) after treatment with P1 nuclease and alkaline phosphatase and with HPLC using a Shimadzu LC-6AD apparatus equipped with a Cosmosil 5C₁₈-AR column (4.6 × 150 mm; Nacalai Tesque). DNA–DNA hybridization was carried out by using the photobiotin-labelling method with microdilution wells as described by Ezaki *et al.* (1989). DNA–DNA relatedness (%) was determined by using the colorimetric method (Verlander, 1992). The G+C content of the genomic DNA from strain BCC 21825^T was 71.1 mol%. The strain exhibited DNA–DNA relatedness levels of 25.44 ± 2.51 % with *P. mira* NBRC 15435^T and 26.48 ± 1.47 % with *P. silvatica* NBRC 100141^T (mean values from triplicate measurements). The results of 16S rRNA gene sequence analysis and DNA–DNA relatedness were sufficient to categorize strain BCC 21825^T as distinct from previously described *Planotetraspora* species.

These phenotypic (Table 1) and genotypic data indicated that strain BCC 21825^T merits classification as a member of a novel species of the genus *Planotetraspora*, for which we propose the name *Planotetraspora thailandica* sp. nov.

Emended description of the genus *Planotetraspora* Runmao *et al.* 1993 emend. Tamura and Sakane 2004

The description of the genus is as emended by Tamura & Sakane (2004), with the exception that the fatty acid profile is 3c and contains iso-C_{16:0}, 10-methylated C_{17:0}, iso-C_{15:0}, C_{15:0}, 10-methylated C_{16:0}, C_{16:0}, anteiso-C_{17:0}, C_{17:0}, anteiso-C_{15:0} and C_{17:1}.

Description of *Planotetraspora thailandica* sp. nov.

Planotetraspora thailandica (thai.lan'di.ca. N.L. fem. adj. *thailandica* of Thailand, where the type strain was isolated).

Fig. 2. Phylogenetic relationships based on neighbour-joining analysis (Saitou & Nei, 1987) of *Planotetraspora thailandica* BCC 21825^T, derived from 16S rRNA gene sequences for members of the family *Streptosporangiaceae*. *Actinomadura madurae* DSM 43067^T was used as an outgroup. Numerals at nodes indicate bootstrap percentages derived from 1000 replications (only values >50 % are indicated). Bar, 0.005 substitutions per nucleotide position.

Gram-positive-staining, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are pale yellow on ISP medium no. 2. Cylindrical sporangia containing four oval- to rod-shaped spores are formed at the ends of short sporangiospores on the aerial mycelium. Spores are non-motile. No soluble pigment is detected in ISP media 2, 3, 4 and 5. Nitrate is not reduced. Utilizes cellobiose, D-fructose, D-galactose, D-lactose, D-mannitol, D-melibiose, D-raffinose, L-rhamnose, D-xylose, glycerol and salicin, but not L-arabinose or D-ribose. Acid is produced from D-glucose, D-mannitol, D-mannose, melibiose, L-rhamnose and D-xylose, but not D-galactose, D-lactose, maltose or D-raffinose. Peptonization of milk, hydrolysis of starch and gelatin liquefaction are negative. Optimal temperature for growth is 25–30 °C. No growth occurs above 37 °C. The maximum NaCl concentration for growth is 1 %. Cell wall contains glutamic acid, alanine and meso-A₂pm. These amino acids correspond to murein type A1γ. The predominant menaquinones are MK-9(H₄), MK-9(H₈) and MK-9(H₂). The characteristic whole-cell sugars are rhamnose, madurose, glucose, galactose and 3-O-methylmannose. The diagnostic phospholipid is phosphatidylethanolamine; phosphatidylglycerol, phosphatidylcholine and glucosamine-containing phospholipids are not detected. The major fatty acids of the type strain are iso-C_{16:0}, 10-methylated C_{17:0}, iso-C_{15:0}, C_{15:0}, 10-methylated C_{16:0}, C_{16:0}, anteiso-C_{17:0}, C_{17:0}, anteiso-C_{15:0} and C_{17:1}. The G+C content of the DNA is 71.1 mol%.

The type strain is BCC 21825^T (=NBRC 104271^T), isolated from soil in Thailand.

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