Planobispora siamensis sp. nov., isolated from soil

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A novel actinomycete strain, A-T 4600T, which developed cylindrical sporangia containing a longitudinal pair of motile spores forming singly or in bundles on short ramifications of the aerial mycelium, was isolated from soil collected from an evergreen forest in Thailand. The cell-wall peptidoglycan contained meso-diaminopimelic acid. The whole-cell sugars contained ribose, madurose, mannose and glucose. The predominant menaquinones were MK-9(H2). Mycolic acids were not detected. The diagnostic phospholipids were phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannoside, diphasatidylglycerol and aminophosphoglycolipid. The predominant cellular fatty acids were unsaturated C17 : 1, C18 : 1, saturated C16 : 0, and C17 : 0. The G+C content of the DNA was 70.8 mol%. Phenotypic and chemotaxonomic analyses showed that the isolate had the typical characteristics of members of the genus Planobispora. Furthermore, 16S rRNA gene sequence analysis also indicated that this strain belonged to the genus Planobispora but as a putative novel species. DNA–DNA relatedness values that differentiate the isolate from previously described members of the genus Planobispora were significantly below 70%. Following an evaluation of phenotypic, chemotaxonomic and genotypic studies, it is proposed that the isolate represents a novel species, Planobispora siamensis sp. nov.; the type strain is A-T 4600T (=BCC 39469T =NBRC 107568T).

The genus Planobispora was first proposed by Thiemann & Beretta (1968) and classified in the family Streptosporangiaceae by Goodfellow et al. (1990). It includes two recognized species: Planobispora longispora (Thiemann & Beretta, 1968) as the type species and Planobispora rosea (Thiemann, 1970). Cells are Gram-positive, non-acid-fast and aerobic. They develop cylindrical to clavate spore vesicles that contain a longitudinal pair of spores forming singly or in bundles on short ramifications of the aerial mycelium. Spores are motile by peritrichous flagella (Thiemann & Beretta, 1968). This genus has N-acetylated muramic acid and meso-diaminopimelic acid (A2pm) in the cell wall. It has madurose as whole-cell diagnostic sugar. Mycolic acids are not detected. The predominant menaquinones are MK-9(H4), MK-9(H2) and MK-9(H). The phospholipid pattern is type PIV. The DNA G+C content is 70–71 mol% (Ara & Kudo, 2007; Vobis, 1989). The genus Planobispora is closely related to the genus Planomonospora (Thiemann et al., 1967). Members of the two genera exhibit a high 16S rRNA gene sequence similarity, but can be distinguished from each other on the basis of morphological criteria, i.e. members of the genus Planomonospora develop single spores in cylindrical spore vesicles.

During a study on the diversity of actinomycetes in Thailand, strain A-T 4600T was isolated from soil in Kanchanaburi Province. The isolate was identified as a new member of the genus Planobispora based on morphological and chemotaxonomic studies, phylogenetic analysis and DNA–DNA relatedness studies. Here, we report the taxonomic characterization and classification of strain A-T 4600T and propose a novel species as Planobispora siamensis sp. nov. to accommodate the strain.
Strain A-T 4600T was isolated from soil collected in Kanchanaburi Province (14° 41’ N 98° 24’ E), Thailand. The sample was taken from the organic layer on the soil surface, and was kept at 4 °C. The sample was dried at room temperature for 10 days and then dry-heated at 100 °C for 1 h. The dried sample was treated using the flooding method of Suzuki (2001) with slight modifications. The organism was isolated on humic acid-salts vitamin agar (Nonomura & Hayakawa, 1988) supplemented with 25 mg nalidixic acid l⁻¹, 50 mg cycloheximide l⁻¹ and 1 mg tetrabutylphosphine l⁻¹. The pure culture was preserved at −80 °C in glycerol (10 %, v/v) and in liquid-drying.

Morphological characteristics of the strain A-T 4600T grown on modified soil extract agar, yeast extract-starch agar (Suriyachadkun et al., 2009) and inorganic salts-starch agar (ISP 4 medium; Shirling & Gottlieb, 1966) at 28 °C, 14–21 days, was observed with a light microscope and a scanning electron microscope (model JSM-5410 LV; JEOL). Samples on modified soil extract agar for scanning electron microscopy were prepared as described previously (Itoh et al., 1989).

Phenotypic characteristics of the novel strain were examined using several standard methods. Cultural characteristics were tested using 14–21 day culture grown at 28 °C on various agar media (Shirling & Gottlieb, 1966). The ISCC–NBS Color Charts standard sample no. 2106 was used to determine colour designations (Kelly, 1964). The temperature, pH and NaCl tolerance for growth were determined on ISP 2 medium at 28 °C for 14 days. Utilization of carbohydrates as sole carbon sources was tested using ISP 9 medium as a basal medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % carbon sources. The hydrolysis of various compounds was examined using the basal medium recommended by Gordon et al. (1974). Tests using the commercial API ZYM (bioMérieux) system were performed according to the manufacturer’s instruction. Gelatin liquefaction, peptonezation of milk, nitrate reduction and starch hydrolysis were determined by cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Catalase and oxidase activities were determined using the method of Greenwood & Pickett (1979). The results are indicated in the species description and Table 1.

Freeze-dried cells used 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. The A₂pm isomer in the cell wall was determined by the method of Staneck & Roberts (1974). The acyl group of muramic acid in peptidoglycan was determined by the method of Uchida & Aida (1984). Reducing sugars from whole-cell hydrolysates were analysed using cellulose TLC according to the method of Komagata & Suzuki (1987). Cellular phospholipids were extracted and analysed using the method of Minnikin et al. (1984). Fatty acid methyl ester analysis was performed by GLC according to the instructions of ACTINO method of the Microbial Identification System, MIDI, Sherlock version 4.0B (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The presence of mycolic acids was investigated using the method of Minnikin et al. (1975). Isoprenoid quinones were extracted according to the method of Collins et al. (1977) and 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.

Chromosomal DNA was isolated from cells grown in yeast extract-glucose broth according to a slightly modified

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<td>Colour of substrate mycelium on ISP 2</td>
<td>Vivid red</td>
<td>Vivid reddish orange</td>
<td>Strong orange</td>
<td>No growth</td>
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<td>DNA G+C content (mol%)</td>
<td>70.8</td>
<td>71.2</td>
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<td>Major menaquinones</td>
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<td>MK-9(H₄), MK-9(H₂)</td>
<td>MK-9(H₂), MK-9(H₄)</td>
<td>MK-9(H₂), MK-9(H₄), MK-9</td>
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<td>NaCl tolerance (%)</td>
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<td>D-Galactose</td>
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method of Saito & Miura (1963). The 16S rRNA gene was amplified by PCR as described previously (Yamada et al., 2000; Katsura et al., 2001; Kawasaki et al., 1993). Two primers, 20F and 1500R (Suriyachadkun et al., 2009), were used for preliminary phylogenetic analysis. An amplified 16S rRNA gene was sequenced by Macrogen Inc. using universal primers. Multiple alignments of the sequences were carried out with Clustal W (Thompson et al., 1997) and the software BioEdit Sequence Alignment Editor (version 7.0.0). Sequence similarity values among strains of all recognized members of the genus Planobispora were first determined using the EzTaxon server (Kim et al., 2012). 16S rRNA gene sequence similarities among closely related species were calculated manually after pairwise alignments obtained using the Clustal W (Thompson et al., 1997). Phylogenetic trees were constructed by neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1981) methods and maximum-likelihood (Felsenstein, 1981) methods by using MEGA 5. The robustness for the trees was tested by 1000 replications (Felsenstein, 1985). Bootstrap values below 50 % are not shown. The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) following treatment with P1 nuclease and alkaline phosphatase and analysis with HPLC using Shimadzu LC-6AD apparatus equipped with a Cosmosil 5C18-AR column (4.6 × 150 mm; Nacalai Tesque). DNA–DNA hybridization was carried out using the photobiotin-labelling method with microdilution wells as described by Ezaki et al. (1989). DNA–DNA relatedness (%) was determined using the colorimetric method (Verlander, 1992).

Strain A-T 4600T had characteristics typical of members of the genus Planobispora. It developed cylindrical sporangia (1.0 μm wide, 5.0 μm long) that contained a longitudinal pair of spores forming singly or in bundles on short ramifications of the aerial mycelium (Fig. 1). Spores were straight rod-shaped (1.0 μm wide, 2.0–3.0 μm long). The spores were motile as observed with a light microscope after incubating sporangia in 0.1 M potassium phosphate buffer (pH 7) at 28 °C for 30 to 60 min. Strain A-T 4600T grew well on ISP 2, moderately well on ISP 3, ISP 6 and nutrient agar, and weakly on ISP 4, ISP 5, ISP 7. Colonies of strain A-T 4600T on ISP 2 were vivid red.

Chemotaxonomic characteristics of strain A-T 4600T were similar to those of members of the genus Planobispora. Cell-wall amino acids of the novel strain contained meso-Apm. The acyl type of the cell wall muramic acid was N-acetylated muramic acid. The strain contained ribose, madurose, mannone and glucose as whole cell sugars, but not arabinose, galactose or xylose (whole-cell sugar pattern B of Lechevalier & Lechevalier, 1970). Phosphatidylethanolamine, phosphatidyl-methylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannoside, diphosphatidylglycerol and aminophosphoglycolipid were detected, but not phosphatidylinositol (Fig. S1, available in IJSEM Online). The major cellular fatty acid composition of strain A-T 4600T was unsaturated C17:1 (24.82 %), C18:1 (11.57 %), saturated C16:0 (16.56 %) and C17:0 (13.56 %), and small amounts of saturated C15:0 (6.14 %), anteiso-C15:0 (4.78 %), anteiso-C17:0 (4.27 %) and unsaturated C16:1 (4.22 %). Cellular fatty acid compositions of strain A-T 4600T compared with the two recognized Planobispora species and Planomonospora venezuelensis are shown in Table 2. The table shows that major fatty acid compositions of strain A-T 4600T were similar to those of the type strain of Planobispora venezuelensis.
acids of the genus *Planobispora* were unsaturated C_{17:1}, C_{18:1}, saturated C_{16:0} and C_{17:0}, but the major fatty acids of *Planomonospora venezuelensis* were unsaturated C_{17:1}, saturated C_{13:0} and C_{17:0} (Table 2). Mycolic acids were absent. Menaquinones found in strain A-T 4600^T were MK-9(H_2) (77.86%), MK-9 (11.10%), MK-9(H_4) (10.49%) and MK-9(H_6) (0.55%).

Almost complete 16S rRNA gene sequence (1480 nt) was obtained for strain A-T 4600^T. These sequences exhibited a close relationship with members of the family *Streptosporangiaceae* and the strain was placed within the clade of the genus *Planobispora*. Strain A-T 4600^T exhibited the highest 16S RNA gene sequence similarity of 97.4% to *Planobispora longispora* IFO 13918^T, 97.5% to *Planobispora rosea* JCM 3166^T and 97.46% to *Planomonospora venezuelensis* JCM 3167^T. The phylogenetic trees constructed by the neighbour-joining (Fig. 2), maximum-parsimony (Fig. S2A) and maximum-likelihood (Fig. S2B) methods showed a phylogenetic relationship between strain A-T 4600^T and the members of the family *Streptosporangiaceae*. They revealed that strain A-T 4600^T should be classified in the genus *Planobispora* and that it could be clearly separated from its closest relatives. The G+C content of genomic DNA from strain A-T 4600^T was 70.8 mol%. The strain exhibited DNA–DNA relatedness levels of 22.28–36.49% with *Planobispora longispora* IFO 13918^T, 32.68–35.33% with *Planobispora rosea* JCM 3166^T and 33.30–33.60% with *Planomonospora venezuelensis* JCM 3167^T. Results of 16S rRNA gene sequence analysis and DNA–DNA relatedness were sufficient to categorize strain A-T 4600^T as distinct from previously described *Planobispora* species.

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Planobispora siamensis sp. nov.

**Description of Planobispora siamensis sp. nov.**

Planobispora siamensis. (sia.men’sis. N.L. fem. adj. siamensis pertaining to Siam, the old name of Thailand, where the type strain was isolated).

Colonies are vivid red on ISP medium no. 2. Cylindrical sporangia containing a longitudinal pair of motile spores form singly or in bundles on short ramifications of the aerial mycelium. No soluble pigment is detected in ISP medium no. 2, 3, 4 or 5. Substrates utilized as single carbon sources include L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, melibiose, raffinose, L-rhamnose, D-xylene and salicin, but not lactose, D-ribose or glycerol. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and catalase. Nitrate reduction, hydrolysis of starch and gelatin liquefaction are positive, but peptonization and coagulation of milk are negative. The maximum NaCl concentration for growth is 1%.

Optimal temperature for growth is between 25 and 30 °C. Cell wall contains glutamic acid, alanine and meso-A2pm. The acyl type of the cell wall muramic acid is N-acetylated muramic acid. The predominant menaquinones are MK-9(H2) (77.86 %) and small amounts of MK-9(H4) and MK-9(H6). The characteristic whole-cell sugars are ribose, madurose, mannose and glucose. The diagnostic phospholipids are phosphatidylethanolamine, phosphatidyglycerol, phosphatidylmethylethanolamine, diphosphatidyglycerol, phosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylglycerol and aminophosphoglycolipid. The major fatty acids are unsaturated C17:1, C18:1, saturated C16:0 and C17:0. The type strain is strain A-T 4600T (=BCC 39469T=NBRC 107568T), isolated from soil. The DNA G+C content of the type strain is 70.8 mol%.

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**References**


