Asanoa siamensis sp. nov., isolated from soil from a temperate peat swamp forest

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A novel actinomycete strain, PS7-2T, which produced spore chains borne on the tips of short sporophores, was isolated from soil collected from a temperate peat swamp forest in Phu-Sang National Park, Phayao province, Thailand. The isolate contained glutamic acid, glycine, alanine, 3-hydroxy-diaminopimelic acid and meso-diaminopimelic acid in the cell-wall peptidoglycan. The whole-cell sugars were glucose, mannose, rhamnose and xylose, and the major phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylethanolamine. The predominant menaquinones were MK-10(H8) and MK-9(H8) and the predominant cellular fatty acids were iso-C15:0, anteiso-C15:0 and anteiso-C17:0. The G+C content of the genomic DNA was 72.3 mol%. On the basis of 16S rRNA gene sequence analysis, strain PS7-2T clustered with members of the genus Asanoa and appeared most closely related to the type strains of Asanoa hainanensis (99.5 % sequence similarity), Asanoa iromotensis (99.0 %), Asanoa ishikariensis (98.9 %) and Asanoa ferruginea (98.5 %). DNA–DNA hybridizations and some physiological and biochemical properties indicated that strain PS7-2T could be readily distinguished from its closest phylogenetic relatives. Based on the phenotypic and genotypic evidence and DNA–DNA relatedness values, strain PS7-2T represents a novel species in the genus Asanoa, for which the name Asanoa siamensis sp. nov. is proposed; the type strain is PS7-2T (=BCC 41921T =NBRC 107932T).

The genus Asanoa, which was proposed by Lee & Hah (2002), belongs to the family Micromonosporaceae (Zhi et al., 2009). At the time of writing, this genus comprised four species: Asanoa ferruginea (basonym Catellatospora ferruginea; Asano & Kawamoto, 1986), Asanoa ishikariensis (formerly ‘Catellatospora ishikariense’; Lee & Hah, 2002), Asanoa iromotensis (Tamura & Sakane, 2005) and Asanoa hainanensis (Xu et al., 2011). Representatives of the genus Asanoa are widely distributed in nature and have been isolated from diverse sources, including soil samples from both mangrove forest (Tamura & Sakane, 2005; Xu et al., 2011) and woodland (Asano & Kawamoto, 1986). Members of the genus Asanoa have meso-diaminopimelic acid and 3-hydroxy-diaminopimelic acid in their cell walls, xylose as a characteristic sugar in their whole-cell hydrolysates, and fatty acid profiles characterized by major amounts of anteiso-C15:0, anteiso-C17:0, iso-C15:0, C17:0 and iso-C16:0. The major diagnostic phospholipid is phosphatidylethanolamine and the predominant menaquinones are MK-10(H6), MK-10(H8), MK-9(H6) and MK-9(H8).

During an investigation of novel actinomycetes from temperate peat swamp forest soil in northern Thailand, we isolated a novel strain, PS7-2T, that showed morphological and chemotaxonomic characteristics typical of established members of the genus Asanoa. The taxonomic...
position of strain PS7-2T was investigated by following a polyphasic approach.

Strain PS7-2T was isolated from a soil sample collected in a peat swamp forest in the Phu-Sang National Park in Phayao province, Thailand. The soil sample was air-dried at room temperature for 14 days. A portion of the dried soil sample (1 g) was mixed with 9 ml 0.05 % (w/v) SDS in distilled water, incubated at 30°C for 30 min, and then serially diluted with sterile distilled water, as recommended by Thawai et al. (2005). The novel strain was isolated on humic acid-salts vitamin (HV) agar (Hayakawa & Nonomura, 1987) supplemented with nalidixic acid (25 mg l⁻¹) and terbinafin (50 mg l⁻¹). A pure culture was preserved by freezing at −80°C and freeze-drying.

The morphological characteristics of strain PS7-2T grown on 1/10 strength yeast extract-malt extract agar [International Streptomyces Project (ISP) medium 2 (Shirling & Gottlieb, 1966)] were observed under a light microscope and in a scanning electron microscope (JSM-5410 LV, JEOL). The samples for scanning electron microscopy were prepared as described by Thawai et al. (2005).

The phenotypic characteristics were examined by using several standard methods. Cultural characteristics were tested using cultures grown at 30°C for 14 days on various agar media. ISCC-NBS colour charts were used for determining colony colour (Kelly, 1964). The hydrolysis of various compounds and acid production from carbon sources were examined using the basal medium recommended by Gordon et al. (1974). Temperature tolerances were determined on ISP 2 for 14 days. The pH range for growth and salt tolerance were also determined on ISP 2, with incubation at 30°C for 14 days. Gelatin liquefaction, peptonization and coagulation of milk, nitrate reduction and starch hydrolysis were determined by cultivation on various media, as described by Arai (1975) and Williams & Cross (1971). The results are indicated in detail in the species description and Table 1.

Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in yeast extract-glucose broth

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**Table 1. Differential characteristics of strain PS7-2T and type strains of established members of the genus Asanoa**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of 3-OH-diaminopimelic acid*</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Whole-cell sugars*†</td>
<td>Glu, Rha, Rib, Man, Xyl</td>
<td>Glu, Rib, Man, Xyl</td>
<td>Glu, Gal, Man, Xyl</td>
<td>Glu, Gal, Rha, Rib, Man, Xyl, Ara</td>
<td>Glu, Gal, Rha, Rib, Man, Xyl, Ara</td>
</tr>
<tr>
<td>Phospholipids*‡</td>
<td>DPG, PG, PE, PI, three PL, GL, four lipids containing glucosamine</td>
<td>PE, PI, containing glucosamine, other PL</td>
<td>PE, PL containing glucosamine, other PL</td>
<td>DPG, PG, PE, PI, PIM, PL</td>
<td>DPG, PG, PE, PI, PIM, PL</td>
</tr>
<tr>
<td>DNA G+C content (mol%)*</td>
<td>72.3</td>
<td>70.3</td>
<td>69.0</td>
<td>71.5</td>
<td>71.1</td>
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<tr>
<td>Nitrate reduction</td>
<td>w</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Gelatin liquefaction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Coagulation of milk</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Maximum pH for growth</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Maximum NaCl concentration</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
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<td>Acidity production from:</td>
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<tr>
<td>Raffinose</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbon utilization:</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td>w</td>
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<td>+</td>
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<tr>
<td>Glycerol</td>
<td>w</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Data shown for the established members of the genus *Asanoa* were taken from Asano & Kawamoto (1986), Lee & Hah (2002), Tamura & Sakane (2005) and Xu et al. (2011).
†Glu, Glucose; Gal, galactose; Rha, rhamnose; Rib, ribose; Man, mannose; Xyl, xylose; Ara, arabinose.
‡DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unidentified phospholipid(s); GL, unidentified glycolipid.
on a rotary shaker at 30 °C for 5 days. Cell-wall peptidoglycan was prepared and hydrolysed by the methods of Kawamoto et al. (1981) and amino acid composition of the hydrolysates was analysed by TLC (Lechevalier & Lechevalier, 1980). The acyl group of muramic acid in the peptidoglycan was determined by the method of Uchida & Aida (1984). The reducing sugars from whole-cell hydrolysates were analysed by the cellulose TLC method of Komagata & Suzuki (1987). Phospholipids in cells were extracted and analysed by the method of Minnikin et al. (1984). Fatty acid methyl ester analysis was performed by GLC according to the standard protocol of version 6.0 of the Sherlock Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kropfenstedt, 1996), with the RTSBA6 database (MIDI) used for reference. The presence of mycolic acids was investigated by using the method of Minnikin et al. (1975). Isoprenoid quinones were extracted by the method of Collins et al. (1977) and then analysed by reverse-phase liquid chromatography-electrospray mass spectrometry with UV detection. The solvent system used in the chromatography was 2-propanol/methanol (1:2, v/v) at a flow rate of 0.6 ml min⁻¹.

Genomic DNA was isolated from cells grown in yeast extract-glucose broth according to the method of Tamaoka (1994). The G+C content of the DNA was then determined using the HPLC method of Tamaoka & Komagata (1984), with an equimolar mixture of nucleotides for analysis of DNA base composition (Yamas Shoyu) used as the quantitative standard. DNA–DNA hybridizations were performed in microdilution-well plates, as reported by Ezaki et al. (1989). DNA–DNA relatedness values (%) were determined by the colorimetric method described by Verlander (1992). The 16S rRNA gene was amplified as described by Suriyachadkun et al. (2009). The PCR products were sequenced (Macrogen, Seoul, Korea) using universal primers (Lane, 1991). The 16S rRNA gene sequence was then multiply aligned, with selected 16S rRNA gene sequences obtained from GenBank/EMBL/DDBJ by using version 1.81 of the CLUSTAL W program (Thompson et al., 1994). The alignment was verified manually and adjusted prior to the construction of phylogenetic trees using both the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) algorithms, within version 4.0.2 of the MEGA program (Tamura et al., 2007). The topology of each tree was evaluated by bootstrap analysis (Felsenstein, 1985), with 1000 resamplings. Close relatives of the novel strain were determined using the EzTaxon-e database (Kim et al., 2012). 16S rRNA gene sequence similarities between the novel strain and its close relatives were calculated manually after the corresponding pairwise alignments were made within the CLUSTAL_X program (Thompson et al., 1997).

Strain PS7-2ᵀ grew well on ISP 2, moderately well on ISP 3, ISP 6 and nutrient agar, and weakly on ISP 4, ISP 5 and ISP 7. On ISP 2, the colonies of strain PS7-2ᵀ were vivid orange–yellow in colour and formed well-developed and branched substrate hyphae but no detectable aerial hyphae. A strong reddish-orange, soluble pigment was only observed in cultures on ISP 2. Strain PS7-2ᵀ formed spore chains, each with >10 non-motile spores, on the tips of short sporophores that arose directly from the agar surface (Fig. S1, available in IJSEM Online).

Chemotaxonomic characteristics of strain PS7-2ᵀ were similar to those of established members of the genus Asanoa. The cell wall of strain PS7-2ᵀ contained meso-diaminopimelic acid, 3-hydroxy-diaminopimelic acid, glutamic acid, glycine and alanine. No 3-hydroxy-diaminopimelic acid was detected in the cell wall of the novel strain’s phylogenetically closest relative, A. hainanensis (Xu et al., 2011). The reducing sugars in whole-cell hydrolysates of the novel strain were glucose, mannose, rhamnose, ribose and xylose. Rhamnose was reported to be a component of the cell-wall sugars of A. ferruginea and A. ishikariensis (Lee & Hah, 2002) but was not detected in cell-wall hydrolysates of A. iriomotensis (Tamura & Sakane, 2005) or A. hainanensis (Xu et al., 2011). The predominant menaquinones of strain PS7-2ᵀ were identified as MK-10(H₄) (59.8 %), MK-9(H₄) (24.7 %), MK-10(H₆) (9.1 %) and MK-9(H₆) (3.2 %). The cellular fatty acid profile comprised iso-C₁₅:0 anteiso-C₁₅:0 anteiso-C₁₇:0 C₁₇:0 iso-C₁₆:0 iso-C₁₇:0 C₁₆:0 iso-C₁₄:0 iso-C₁₅:0 G C₁₈:0 and C₁₇:0(9)c (Table S1). Mycolic acids were absent. The phospholipid profile comprised diposphatidylglycerol, phosphatidyglycerol, phosphatidylinositol, phosphatidyethanolamine, three unidentified phospholipids, an unidentified glycolipid and four unidentified lipids (Fig. S2). A similarly complex pattern of polar lipids was found in A. ferruginea and A. ishikariensis (Lee & Hah, 2002). The unidentified phospholipids, diposphatidylglycerol, phosphatidyglycerol and phosphatidylinositol found in the novel strain were not detected in A. iriomotensis (Tamura & Sakane, 2005) or A. hainanensis (Xu et al., 2011). The novel strain’s cell-wall peptidoglycan was of the glycolyl acyl type. The genomic DNA G+C content was 72.3 mol%.

The almost-complete 16S rRNA gene sequence of strain PS7-2ᵀ (1440 nt) was determined and found to be similar to the 16S rRNA gene sequences of members of the family Micromonosporaceae. It is evident from Fig. 1 that strain PS7-2ᵀ formed a 16S rRNA gene subclade with A. hainanensis 210121ᵀ that was supported by a high bootstrap value (99 % in the neighbour-joining tree). The relationship between these two strains was supported by results from both the neighbour-joining and maximum-parsimony methods. 16S rRNA gene sequence similarity values between the novel isolate and type strains of all species of the genus Asanoa ranged from 98.5 % (A. ferruginea) to 99.5 % (A. hainanensis). Based on the morphological, chemotaxonomic and phylogenetic data, the novel isolate should be classified as a member of the genus Asanoa.

The characteristics shown in Table 1 clearly indicate that strain PS7-2ᵀ possesses some distinct chemotypic and phenotypic characteristics that separate it from A.
**Asanoa siamensis sp. nov.**

Asanoa siamensis (siam.en’ sis. N.L. fem. adj. siamensis of or belonging to Thailand, the origin of the soil from which the type strain was isolated).

Gram-staining-positive, mesophilic actinomycete that forms chains, each of more than 10 non-motile spores, on the tips of short sporophores that, in culture, arise directly from the agar surface. Aerial hyphae are not observed. A strong reddish-orange, soluble pigment is produced on ISP 2. Nitrate is weakly reduced to nitrite. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, melibiose, sucrose, D-xylene, lactose, L-arabinose and L-rhamnose as sole carbon sources, and weakly utilizes maltose, raffinose and glycerol. Produces acid from cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, melibiose, sucrose, D-xylene, lactose, L-arabinose and L-rhamnose as sole carbon sources, and weakly utilizes maltose, raffinose and glycerol. Produces acid from cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, melibiose, raffinose, D-ribose, D-salicin, sucrose, D-xylene, glycerol, lactose, L-arabinose and L-rhamnose. Positive for the peptonization of milk and hydrolysis of starch but negative for the liquefaction of gelatin and coagulation of milk. The optimal temperature for growth lies between 25 and 30 °C; no growth occurs above 37 °C. The maximum NaCl concentration for growth is 2% (w/v). Grows at pH 6–12. The cell-wall peptidoglycan contains meso-diaminopimelic acid, 3-hydroxy-diaminopimelic acid, glutamic acid, glycine and alanine. The predominant menaquinones are MK-10(H8) and MK-9(H8), with minor amounts of MK-10(H6) and MK-9(H6). Diaminopimelic acid in its cell wall, rhamnose in whole cell hydrolysate and the ability to liquefy gelatin, coagulate milk and produce acid from raffinose, sucrose, glycerol and lactose (Table 1). These two strains also differ in their phospholipid profiles, salt tolerance and pH range (Table 1). Furthermore, low DNA–DNA relatedness values (19.2 ± 1.3 % to 32.1 ± 1.3 %) were recorded between strain PS7-2T and type strains of the four species currently in the genus Asanoa (Table S2), which were well below the 70% cut-off point recommended for the assignment of a pair of bacterial strains to the same genomic species (Wayne et al., 1987).

Based on the phenotypic, chemotaxonomic, genotypic and phylogenetic evidence and the results of the DNA–DNA hybridizations, strain PS7-2T represents a novel species within the genus Asanoa, for which the name Asanoa siamensis sp. nov. is proposed.

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the relationships between strain PS7-2T and established members of the genera Asanoa, Micromonospora, Dactylosporangium, Catellatospora, Pilimelia, Planosporangium and Actinoplanes. Streptomyces ambolliciens NBRC 12836T was used as an outgroup. Asterisks indicate branches that were also recovered using the maximum-parsimony algorithm. Bootstrap values (≥50%) based on 1000 resamplings are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.
profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, three unidentified phospholipids, an unidentified glycolipid and four other unidentified lipids but no detectable phosphatidycholine. The cellular fatty acid profile is dominated by iso-C_{15:0}, anteiso-C_{15:0}, anteiso-C_{17:0} and C_{17:0}ω9c with smaller amounts of iso-C_{16:0}ω7c, iso-C_{17:0}ω4c, iso-C_{14:0}ω5c, G, C_{18:0}ω3c and C_{17:1}ω8c.

The type strain, PS7-2^T (=BCC 41921^T=NBRC 107932^T), was isolated from soil collected in a temperate peat swamp forest in the Phu-Sang National Park, Phayao province, Thailand. The genomic DNA G+C content of the type strain is 72.3 mol%.

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**References**


