Dactylosporangium siamense sp. nov., isolated from soil

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A novel actinomycete strain, designated MW4-36ᵀ, was isolated from tropical forest soil in Nakhon Sawan Province, Thailand. Morphological and chemotaxonomic characteristics of this strain clearly demonstrated that it belongs to the genus Dactylosporangium. The strain formed finger-shaped sporangia on short sporangiophores that emerged directly from substrate hyphae. The cell-wall peptidoglycan contained glutamic acid, glycine, alanine and meso-diaminopimelic acid including 3-hydroxy-meso-diaminopimelic acid; arabinose, glucose, rhamnose, ribose and xylose were found as whole-cell sugars. The diagnostic phospholipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol; no phosphatidylcholine was found. The predominant menaquinones were MK-9(H₈) and MK-9(H₆). Mycolic acids were not detected. The predominant cellular fatty acids were iso-C₁₅ :₀, iso-C₁₆ :₀, anteiso-C₁₅ :₀and anteiso-C₁₇ :₀. The G + C content of the genomic DNA was 72.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequences also indicated that the strain should be classified in the genus Dactylosporangium and showed that the closest relative was Dactylosporangium maewongense JCM 15933ᵀ (99.4 % similarity). These taxonomic data revealed that strain MW4-36ᵀ could be readily distinguished from its phylogenetically closest relative. On the basis of these phenotypic and genotypic data, strain MW4-36ᵀ is considered to represent a novel species, for which the name Dactylosporangium siamense sp. nov. is proposed. The type strain is MW4-36ᵀ (=BCC 34901ᵀ =NBRC 106093ᵀ).

The genus Dactylosporangium Thiemann et al. (1967) belongs to the family Micromonosporaceae in the order Actinomycetales (Zhi et al., 2009). Members of the genus Dactylosporangium typically form both finger-shaped sporangia and globose bodies directly on substrate hyphae. At the time of writing, the genus Dactylosporangium comprised 12 species with validly published names, Dactylosporangium aurantiacum (Thiemann et al., 1967), D. thailandense (Thiemann et al., 1967; Thiemann, 1970), D. matsuakiense (Shomura et al., 1980), D. vinaceum (Shomura et al., 1983), D. roseum (Shomura et al., 1985), D. fulvum (Shomura et al., 1986), D. darangshiense (Seo & Lee, 2010), D. maewongense (Chiaraphongphon et al., 2010), D. luridum, D. luteum and D. salmoneum (Kim et al., 2010) and D. tropicum (Thawai et al., 2011).

During an investigation of novel actinomycetes from tropical forest in Thailand, we successfully isolated strain MW4-36ᵀ, which showed morphological and chemotaxonomic characteristics typical of the genus Dactylosporangium, but was genotypically and phenotypically distinct from all recognized Dactylosporangium species. Here, we describe the polyphasic characterization of this strain and suggest that it represents a novel species of the genus Dactylosporangium.

Strain MW4-36ᵀ was isolated from a soil sample collected from tropical forest in Nakhon Sawan Province, Thailand. The sample was taken from the soil surface and kept at 4 °C. The sample was air-dried at room temperature. The dried soil (1 g) was treated with 1.5 % (v/v) phenol in...
distilled water (9 ml) and then serially diluted in sterile distilled water as recommended by Hayakawa et al. (1991). This organism was isolated on humic acid-salts vitamin agar (HV) supplemented with (per litre) 25 mg nalidixic acid, 50 mg cycloheximide and 1 mg terbinafin and the pure culture was preserved by freezing at −80 °C and freeze-drying.

Morphological properties of the strain grown on HV agar were observed by light and scanning electron microscopy (model JSM-5410 LV; JEOL). The samples for scanning electron microscopy were prepared as described by Itoh et al. (1989).

Phenotypic characteristics were examined by using several standard methods. Cultural characteristics were tested using 14 day cultures grown at 30°C on various agar media. The Jacal Colour Card L2200 (Japan Colour Research Institute) was used for determining colour designations. Spore motility was examined by observing cells suspended in phosphate buffer (1 mM; pH 7.0) under a light microscope. Hydrolysis of various compounds was examined using the basal medium recommended by Gordon et al. (1974). Temperature tolerances were determined on yeast extract-malt extract agar [(International Streptomyces Project (ISP) 2 medium; Shirling & Gottlieb (1966)] for 14 days. pH and NaCl tests were determined on ISP 2 medium at 30°C for 14 days. Carbon source utilization was tested by using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1% of the carbon sources and 0.05% Casamino acids. 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). Results are given in detail in the species description and Table 1.

Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in ISP 2 broth on a rotary shaker at 30°C for 7 days. Cell-wall peptidoglycan was prepared and hydrolysed by the methods of Kawamoto et al. (1981), and the amino acid composition was analysed by TLC (Lechevalier & Lechevalier, 1980). The isomer of diaminopimelic acid (A2pm) in the cell wall was determined by the method of Staneck & Roberts (1974). The acyl group of muramic acid in the peptidoglycan was determined by the method of Uchida & Aida (1984). Reducing sugars from whole-cell hydrolysates were analysed by the cellulose TLC method of Komagata & Suzuki (1987). Phospholipids in cells were extracted and analysed according to Minnikin et al. (1984). Fatty acid methyl ester analysis was performed by GLC according to the instructions of the Microbial Identification System (MIDI) Sherlock version 4.5 (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) with the TSBA40 MIDI database. The presence of mycolic acids was investigated by using the method of Minnikin et al. (1975). Isoprenoid quinones were extracted according to Collins et al. (1977) and were analysed by HPLC using a Cosmosil 5C18 column (4.6 by 150 mm; Nacalai Tesque). The elution solvent was a mixture of methanol and 2-propanol (2:1, v/v).

Chromosomal 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 determined using the HPLC method of Tamaoka & Komagata (1984).

Table 1. Differential characteristics between strain MW4-36T and its phylogenetically closest Dactylosporangium relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Formation of globose bodies on HV agar</td>
<td>None</td>
<td>Moderate</td>
<td>Abundant</td>
<td>Abundant</td>
<td>Abundant</td>
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<tr>
<td>Colour of colonies on ISP 2</td>
<td>Orange yellow</td>
<td>Yellowish orange</td>
<td>Deep yellowish orange</td>
<td>Orange</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Reduction of nitrate</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Coagulation of milk</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Peptonization of milk</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Growth at 40°C</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at pH 5</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Utilization of:</td>
<td></td>
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<tr>
<td>D-Fructose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>D-Galactose</td>
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<td>w</td>
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<td>w</td>
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<td>w</td>
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<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>−</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

Strains: 1, MW4-36T; 2, D. maewongense MW2-25T; 3, D. aurantiacum NBRC 12592T; 4, D. lutum BK51T; 5, D. luridum BK63T. All data were determined in this study. +, Positive; −, negative; w, weakly positive.
An equimolar mixture of nucleotides for analysis of the DNA base composition (Yamasa Shoyu) was used as the quantitative standard. DNA–DNA hybridization was conducted in micro-dilution-well plates, as reported by Ezaki et al. (1989). DNA–DNA relatedness was determined by using the colorimetric method (Verlander, 1992). The 16S rRNA gene was amplified as described by Suriyachadkun et al. (2009). The PCR products were sequenced (Macrogen) using universal primers 27F (5′-GTTTGATCCTGGCTCAG-3′), 350F (5′-TACGGAGGCA-GCG-3′), 780F (5′-GATTAGATACCCGTGTA-3′), 1100F (5′-GCAACGGGCGAAACC-3′), 350R (5′-CTTGGCGCTC- CCGTAG-3′), 780R (5′-CTACCGGGTGATCTAATCG-3′) and 1492R (5′-GTGATCCCGCTGCTGCCTC-3′) (Lane, 1991). The 16S rDNA gene sequence was multiply aligned with selected sequences obtained from the GenBank/EMBL/ DDBJ databases by using the CLUSTAL W program version 1.81 (Thompson et al., 1994). The alignment was manually verified and adjusted prior to construction of a phylogenetic tree. The phylogenetic tree was reconstructed by using the neighbour-joining method (Saitou & Nei, 1987), with genetic distances computed by using Kimura’s two-parameter model (Kimura, 1980). DNA–DNA relatedness among strains MW4-36T and the phylogenetically closest relatives, Dactylosporangium and the 16S rRNA gene sequence similarities between strain MW4-36T and the type strains of all recognized species of the genus Dactylosporangium, representative sequences of the genera Actinoplanes and Micromonaspora and the 16S rRNA gene sequence of Streptomyces ambofaciens NRBC 12836T (as an outgroup) indicated that strain MW4-36T formed a distinct phylectic line related to recognized Dactylosporangium species in the neighbour-joining tree (Fig. 2). 16S rRNA gene sequence similarities between strain MW4-36T and the type strains of its phylogenetically closest relatives were 99.4% (D. maewongense), 99.3% (D. luteum), 99.2% (D. aurantiacum) and 98.8% (D. luidum). Strain MW4-36T showed different colour of the soluble pigment on ISP 2 medium from D. maewongense MW2-25T and D. luidum BK51T (Table 1). It was distinguished from the phylogenetically closest Dactylosporangium relatives in growth at pH 5, reduction of nitrate to nitrite, coagulation and peptonization of milk, gelatin liquefaction, formation of globose bodies on HV agar, and utilization of D-fructose, D-galactose, D-mannitol, raffinose, glycerol, lactose, L-arabinose and L-rhamnose (Table 1).

Comparison of the almost-complete 16S rRNA gene sequence obtained for strain MW4-36T (1433 nt) with corresponding sequences of the type strains of all recognized species of the genus Dactylosporangium, representative sequences of the genera Actinoplanes and Micromonaspora and the 16S rRNA gene sequence of Streptomyces ambofaciens NRBC 12836T (as an outgroup) indicated that strain MW4-36T formed a distinct phylectic line related to recognized Dactylosporangium species in the neighbour-joining tree (Fig. 2). 16S rRNA gene sequence similarities between strain MW4-36T and the type strains of its phylogenetically closest relatives were 99.4% (D. maewongense), 99.3% (D. luteum), 99.2% (D. aurantiacum) and 98.8% (D. luidum). Strain MW4-36T showed different colour of the soluble pigment on ISP 2 medium from D. maewongense MW2-25T and D. luidum BK51T (Table 1). It was distinguished from the phylogenetically closest Dactylosporangium relatives in growth at pH 5, reduction of nitrate to nitrite, coagulation and peptonization of milk, gelatin liquefaction, formation of globose bodies on HV agar, and utilization of D-fructose, D-galactose, D-mannitol, raffinose, glycerol, lactose, L-arabinose and L-rhamnose (Table 1).

Levels of DNA–DNA relatedness among strains MW4-36T and the phylogenetically closest Dactylosporangium relatives, D. maewongense MW2-25T, D. aurantiacum NBRC 12592T, D. luteum BK51T and D. luidum BK63T, ranged from 22.6 ± 0.71 to 40.1 ± 0.18% (mean ± SD of five determinations; Table S2). Strain MW4-36T produced iso-C15:0, in contrast to D. maewongense (Table S1). These phenotypic and genotypic data showed that strain MW4-36T merits classification as

**Fig. 1.** Scanning electron micrograph of sporangia and sporangiospore of strain MW4-36T grown on HV agar for 4 weeks at 28 °C. Bar, 10 μm.
representing a novel species of the genus *Dactylosporangium*, for which the name *Dactylosporangium siamense* sp. nov. is proposed.

**Description of Dactylosporangium siamense** sp. nov.

*Dactylosporangium siamense* (si.am’en.se. N.L. neut. adj. siamense pertaining to Siam, the old name for Thailand, the country of origin of the soil from which the type strain was isolated).

Gram-stain-positive, mesophilic actinomycete that forms finger-shaped sporangia on short sporangiophores which emerge directly from the substrate hyphae. Colonies are orange yellow on ISP 2. Each irregular rugose sporangium contains a row of ovoid motile spores. Globose bodies are not observed on humic acid–vitamin agar. Aerial mycelium is absent. The spore surface appears smooth. Spores are motile. Greenish yellow soluble pigment is produced in ISP 2 medium. Nitrate is not reduced to nitrite. Utilizes cellobiose, D-fructose, D-galactose, D-mannitol, melibiose, raffinose, D-xylene, glycerol, lactose, L-arabinose, L-rhamnose and salicin but not D-ribose. Coagulation of milk and hydrolysis of starch are positive, but gelatin liquefaction and peptonization of milk are negative. Optimal temperature for growth is 28–30 °C. No growth occurs above 40 °C. The maximum NaCl concentration for growth is 3 %. Cell wall contains glutamic acid, glycine, alanine, meso-A2pm and 3-OH-meso-A2pm. The acyl type of the cell wall is glycolyl. The predominant menaquinones are MK-9(H8) and MK-9(H6). The characteristic whole-cell sugars are arabinose, glucose, rhamnose, ribose and xylose. The phospholipid profile contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphoglycolipid, ninhydrin-positive lipid, three unknown phospholipids and four unknown lipids but not phosphatidylcholine. The fatty acid profile of the type strain consists of iso-C15 : 0, iso-C16 : 0, anteiso-C15 : 0, anteiso-C17 : 0, iso-C17 : 0, C18 : 0, C16 : 0, C17 : 0, iso-C14 : 0, iso-C16 : 1, cis-9-C18 : 1, anteiso-C17 : 1, cis-9-C18 : 1, 10-methyl C17 : 0 (Table S1).

**Fig. 2.** Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing relationships between strain MW4-36T, the type strains of recognized *Dactylosporangium* species and members of the genera *Micromonospora* and *Actinoplanes*. *Streptomyces ambofaciens* NBRC 12836T was used as an outgroup. Asterisks (*) indicate branches of the tree that were also found using the maximum-parsimony and maximum-likelihood methods; hashes (#) indicate clades that were found using the maximum-likelihood method. Numbers at branch nodes indicate the percentage bootstrap values of 1000 replicates; only values ≥50% are indicated. Bar, 0.01 substitutions per nucleotide position.
The type strain is MW4-36T (=BCC 34901T=NBRC 106093T), which was isolated from a soil sample collected from tropical forest in Nakhon Sawan Province, Thailand. The DNA G+C content of the type strain is 72.9 mol%.

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