Dactylosporangium maewongense sp. nov., isolated from soil

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Morphological and chemotaxonomic characterization of actinomycete strain MW2-25T, isolated from tropical forest soil in Nakhon Sawan Province, Thailand, clearly demonstrated that this strain belongs to the genus Dactylosporangium. Phylogenetic analysis using 16S rRNA gene sequences also indicated that this strain should be classified in the genus Dactylosporangium and showed that the closest relative was Dactylosporangium aurantiacum IFO 12592T (99.3% sequence similarity). DNA–DNA hybridization values and some physiological and biochemical properties indicated that this strain could be readily distinguished from its closest phylogenetic relatives. On the basis of these phenotypic and genotypic data, this strain represents a novel species, for which the name Dactylosporangium maewongense sp. nov. is proposed. The type strain is MW2-25T (=BCC 34832T =JCM 15933T).

The genus Dactylosporangium Thiemann et al. (1967) belongs to the family Micromonosporaceae in the order Actinomycetales (Stackebrandt et al., 1997). This genus is well established and based on morphological characteristics (i.e. formation of finger-shaped sporangia directly on substrate hyphae) and 16S rRNA gene sequence-based phylogenetic analyses (Koch et al., 1996). At the time of writing, the genus Dactylosporangium encompasses six species with validly published names: Dactylosporangium aurantiacum (Thiemann et al., 1967), Dactylosporangium fulvum (Shomura et al., 1986), Dactylosporangium matsu-zakiense (Shomura et al., 1980), Dactylosporangium roseum (Shomura et al., 1985), Dactylosporangium thailandense (Thiemann et al., 1967; Thiemann, 1970) and Dactylosporangium vinaceum (Shomura et al., 1983). Members of this genus can be distinguished from one another using morphological and biochemical properties and 16S rRNA gene sequence analysis.

During an investigation of new actinomycetes from tropical forest soil from Thailand, we isolated strain MW2-25T, which showed typical morphological and chemotaxonomic characteristics of the genus Dactylosporangium but was genotypically and phenotypically distinct from recognized species of the genus. Here, we describe the polyphasic characterization of the new strain and propose a novel species of the genus Dactylosporangium.

Strain MW2-25T was isolated from a soil sample collected from Maewong National Park 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 for 8 days. 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 hunic acid-vitamin (HV) agar, supplemented with (1−1) 25 mg nalidixic acid, 50 mg cycloheximide and 1 mg terbinafin. A pure culture was preserved by freezing at −80°C and freeze-drying.

The morphological properties of strain MW2-25T were observed after growth on modified soil extract agar, containing 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 CaC₂. 2H₂O, 0.1 g yeast extract, 0.1 g Casamino acids, 0.2 g glucose, 100 ml soil extract (Hamaki

Abbreviation: Apm, diaminopimelic acid.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MW2-25T is AB495209.

Supplementary tables showing the cellular fatty acid composition of strain MW2-25T and DNA–DNA relatedness values with the type strains of recognized species of the genus Dactylosporangium are available with the online version of this paper.

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et al., 2005), 18 g agar and 900 ml distilled water (pH 7.0), by light and scanning electron microscopy (JSM 5410 LV; JEOL). The sample for scanning electron microscopy was 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. The hydrolysis of various compounds was examined using the basal medium recommended by Gordon et al. (1974). The temperature, pH and NaCl tolerances were determined on yeast extract-malt extract agar (International Streptomyces Project, ISP 2 medium; Shirling & Gottlieb, 1966). Carbon source utilization was tested by using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1% of the carbon source and 0.05% Casamino acids. Gelatin liquefaction, milk peptonization, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Melanin and hydrogen sulfide production were examined on slants of tyrosine agar and peptone iron agar supplemented with 0.1% (w/v) yeast extract.

Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in ISP 2 broth on a rotary shaker at 30 °C for 4 days. Cell-wall peptidoglycan was prepared and hydrolyzed 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). The reducing sugars from whole-cell hydrolysates were analysed by GLC according to the instructions of the Microbial Identification System (MIDI) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The presence of mycolic acids was investigated according to the method of Minnikin et al. (1975). Isoprenoid quinones were extracted by the method of Collins et al. (1977) and were analysed by HPLC equipped with a Cosmosil 5C18 column (4.6 × 50 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 ISP 2 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) using an equimolar mixture of nucleotides (Yamasa Shoyu) as the quantitative standard. DNA–DNA hybridization was conducted in microdilution-well plates as reported by Ezaki et al. (1989). DNA–DNA relatedness was determined by using the colorimetric method (Verlander, 1992). PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described previously (Suriyachadkun et al., 2009). The 16S rRNA gene sequence was aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases by using CLUSTAL W version 1.81 (Thompson et al., 1994). The alignment was manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Felsenstein, 1985) methods in MEGA version 2.1 (Kumar et al., 2001). The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The values for sequence similarity among the closest strains were determined using the EzTaxon server (Chun et al., 2007).

It was apparent from both morphological and chemical properties that strain MW2-25T is a member of the genus Dactylosporangium (Thiemann et al., 1967; Shomura et al., 1983). Colonies of strain MW2-25T were compact and raised and produced well-developed branched substrate hyphae, but lacked aerial hyphae. Growth of the novel strain was good on yeast extract-malt extract agar (ISP 2), inorganic salts-starch agar (ISP 4) and glycerol-asparagine agar. The colour of the colonies was yellowish orange to strong yellowish orange. No soluble pigment was produced in these media. The novel strain formed finger-shaped sporangia on short sporangiophores that emerged directly from substrate hyphae (Fig. 1a). The sporangium surface

![Fig. 1. Scanning electron micrographs of sporangia (a) and globose bodies (b) of strain MW2-25T grown on humic acid-vitamin agar for 4 weeks at 28 °C. Bars, 1 μm.](http://ijs.sgmjournals.org)
was irregular rugose. Each sporangium contained a row of ovoid motile spores (0.9–1.1 μm). Globose bodies were observed on modified soil extract agar and HV agar (Fig. 1b). The cell-wall hydrolysates contained glutamic acid, glycine, alanine, meso-A2pm and 3-hydroxy-meso-A2pm, indicating that this strain has cell-wall chemotype II of Lechevalier & Lechevalier (1970). The acyl type of the cell-wall muramic acid was glycolyl. Glucose, xylose, rhamnose, ribose and trace amounts of arabinose were found as whole-cell sugars (whole-cell sugar pattern D of Lechevalier & Lechevalier, 1970). Characteristic phospholipids were diphosphatidylglycerol, phosphatidylglycerol, lysyl phosphatidylglycerol and phosphatidylethanolamine, but not phosphatidylcholine. This pattern corresponds to phospholipid type PII of Lechevalier et al. (1977). The fatty acid pattern of strain MW2-25T consists of iso-C16:0 (27.9 %), iso-C15:0 (25.9 %), anteiso-C15:0 (15.4 %), iso-C14:0 (7.9 %), anteiso-C17:0 (7.3 %), iso-C17:0 (2.3 %), C18:0 (2.2 %), C17:0 (2.2 %), cis-9-C18:1 (2.0 %), anteiso-C17:1 (1.1 %), cis-9-C17:1 (0.8 %) and 10-methyl-C17:0 (0.8 %) (see Supplementary Table S1, available in IJSEM Online). This pattern corresponds to fatty acid type 3b of Kroppenstedt (1985). Mycolic acids were absent. The predominant menaquinones were MK-9(H8) (43.7 %) and MK-9(H4) (37.4 %). The G+C content of the DNA was 73.2 mol%.

An almost-complete 16S rRNA gene sequence (1468 nt) was obtained for strain MW2-25T. This sequence exhibited a close relationship with members of the family Micro- monosporaceae and strain MW2-25T was placed within the clade of the genus Dactylosporangium. It is evident from Fig. 2 that strain MW2-25T formed a subclade with D. aurantiacum IFO 12592T with a high bootstrap value (99 % in the neighbour-joining tree). The relationship between these strains was supported by the results from the maximum-parsimony method. 16S rRNA gene sequence similarity values between strain MW2-25T and the type strains of all recognized species of the genus Dactylosporangium ranged from 97.6 % (D. fulvum IFO 14381T) to 99.3 % (D. aurantiacum IFO 12592T).

The characteristics shown in Table 1 clearly indicate that strain MW2-25T possesses some distinct phenotypic characteristics that separate it from its closest phylogenetic neighbour, D. aurantiacum NBRC 12592T. In particular, peptonization of milk, minimum pH tolerance and utilization of D-fructose, melibiose, D-ribose, lactose and L-arabinose are effective for the discrimination of strain MW2-25T from related organisms. Furthermore, low DNA–DNA relatedness values (9.6–31.7 %) were observed between strain MW2-25T and the type strains of the six recognized species of the genus Dactylosporangium (see Supplementary Table S2). The DNA–DNA relatedness

Fig. 2. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing the relationships between strain MW2-25T and members of the genera Dactylosporangium, Micro monospora and Actinoplanes. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. Asterisks indicate that the corresponding nodes were also found in the tree constructed using the maximum-parsimony method. Streptomyces ambofaciens ATCC 23877T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
value between strain MW2-25\(^T\) and \textit{D. aurantiacum} NBRC 12592\(^T\) was 31.7 ± 0.99 %.

It is evident from the phenotypic and genotypic data mentioned above that strain MW2-25\(^T\) can be distinguished from previously described species of the genus \textit{Dactylosporangium}. It is, therefore, proposed that this strain be classified as representing a novel species of the genus \textit{Dactylosporangium}, for which the name \textit{Dactylosporangium maewongense} sp. nov. is proposed.

\textbf{Description of \textit{Dactylosporangium maewongense} sp. nov.}

\textit{Dactylosporangium maewongense} (mae.wong.en’se. N.L. neut. adj. \textit{maewongense} pertaining to Maewong National Park, where the type strain was isolated).

Gram-positive, mesophilic actinomycete that forms finger-shaped sporangia on short sporangiophores emerging directly from the substrate hyphae. Colonies are yellowish orange on ISP 2. Each irregular rugose sporangium contains a row of ovoid motile spores. Globose bodies are observed on modified soil extract agar and HV agar. Aerial mycelium is absent. The spore surface appears smooth. Spores are motile. No soluble pigment is produced in test culture media. Nitrate is reduced to nitrite. Utilizes cellubiose, D-mannitol, raffinose, glycerol and salicin, weakly utilizes D-galactose, D-ribose, D-xylose and L-rhamnose, but does not utilize lactose, L-arabinose, D-fructose or melibiose. Peptonization of milk, hydrolysis of starch and liquefaction of gelatin are positive, but formation of melanin and production of H\(_2\)S are negative. Optimal temperature for growth is between 25 and 30 \(^\circ\)C; no growth occurs above 42 \(^\circ\)C. The maximum NaCl concentration for growth is 3 \%(w/v). Cell wall contains glutamic acid, glycine, alanine, \textit{meso-A}\(_{2}\)pm and 3-OH-\textit{meso-A}\(_{2}\)pm. The acyl type of the cell wall is the glycolyl type. The predominant menaquinone is MK-9(H\(_{8}\)). The characteristic whole-cell sugars are glucose, xylose, rhamnose, ribose and trace amounts of arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylglycerol, lysyl phosphatidylglycerol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids of the type strain are \textit{iso-C}\(_{16:0}\), \textit{iso-C}\(_{15:0}\) anteiso, \textit{iso-C}\(_{14:0}\) anteiso, \textit{iso-C}\(_{17:0}\) \textit{C}\(_{18:0}\), \textit{C}\(_{16:0}\) \textit{C}\(_{17:0}\), \textit{cis-9-C}\(_{18:1}\) anteiso, \textit{cis-9-C}\(_{17:1}\) and 10-methyl-\textit{C}\(_{17:0}\).

The type strain, MW2-25\(^T\) (=\text{BCC} \text{34832}\text{T}=\text{JCM} \text{15933}\text{T}), was isolated from a soil sample collected from Maewong National Park in Nakhon Sawan Province, Thailand. The DNA G+C content of the type strain is 73.2 mol%.
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