Dactylosporangium maewongense sp. nov., isolated from soil

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Morphological and chemotaxonomic characterization of actinomycete strain MW2-25^T, 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 12592^T (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 MW2-25^T (=BCC 34832^T=JCM 15933^T).

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 matsuzakiense* (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.

Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MW2-25^T is AB495209.

Supplementary tables showing the cellular fatty acid composition of strain MW2-25^T 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.

During an investigation of new actinomycetes from tropical forest soil from Thailand, we isolated strain MW2-25^T, 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 MW2-25^T 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 humic 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 MW2-25^T 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 CaCl₂·2H₂O, 0.1 g yeast extract, 0.1 g Casamino acids, 0.2 g glucose, 100 ml soil extract (Hamaki

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 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 Stanek & 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 the cellulose TLC method of Komagata & Suzuki (1987). Phospholipids in cells 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 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 deter-

mined 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 (Kluge & Farris, 1969) 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 MW2-25^T is a member of the genus *Dactylosporangium* (Thiemann *et al.*, 1967; Shomura *et al.*, 1983). Colonies of strain MW2-25^T 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 MW2-25^T grown on humic acid-vitamin agar for 4 weeks at 28 °C. Bars, 1 µm.

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 MW2-25^T consists of iso-C_{16:0} (27.9 %), iso-C_{15:0} (25.9 %), anteiso-C_{15:0} (15.4 %), iso-C_{14:0} (7.9 %), anteiso-C_{17:0} (7.3 %), iso-C_{17:0} (2.3 %), C_{18:0} (2.3 %), C_{16:0} (2.2 %), C_{17:0} (2.2 %), *cis*-9-C_{18:1} (2.0 %), anteiso-C_{17:1} (1.1 %), *cis*-9-C_{17:1} (0.8 %) and 10-methyl-C_{17:0} (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(H₆) (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 MW2-25^T. This sequence exhibited a close relationship with members of the family *Micromonosporaceae* and strain MW2-25^T was placed within the clade of the genus *Dactylosporangium*. It is evident from Fig. 2 that strain MW2-25^T formed a subclade with *D. aurantiacum* IFO 12592^T 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 MW2-25^T and the type strains of all recognized species of the genus *Dactylosporangium* ranged from 97.6 % (*D. fulvum* IFO 14381^T) to 99.3 % (*D. aurantiacum* IFO 12592^T).

The characteristics shown in Table 1 clearly indicate that strain MW2-25^T possesses some distinct phenotypic characteristics that separate it from its closest phylogenetic neighbour, *D. aurantiacum* NBRC 12592^T. 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 MW2-25^T from related organisms. Furthermore, low DNA–DNA relatedness values (9.6–31.7 %) were observed between strain MW2-25^T 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 MW2-25^T and members of the genera *Dactylosporangium*, *Micromonospora* 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 23877^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of strain MW2-25^T and the type strains of recognized species of the genus *Dactylosporangium*

Strains: 1, MW2-25^T; 2, *D. aurantiacum* NBRC 12592^T; 3, *D. thailandense* NBRC 12593^T; 4, *D. vinaceum* NBRC 14181^T; 5, *D. matsuzakiense* NBRC 14259^T; 6, *D. roseum* NBRC 14352^T; 7, *D. fulvum* NBRC 14381^T. All data were taken from this study. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6	7
Formation of globose bodies on HV agar	Moderate	Abundant	Abundant	Poor	Poor	Poor	Abundant
Colour of colonies on ISP 2	Yellowish orange	Deep yellowish orange	Deep orange	Vivid red	Orange	Vivid yellowish pink	Orange
Colour of soluble pigment on ISP 2	None	None	Yellowish brown	Deep red	Pale yellow	None	Yellowish brown
Liquefaction of gelatin	+	+	–	+	–	–	–
Reduction of nitrate	+	+	–	–	–	+	+
Peptonization of milk	+	–	–	+	–	–	–
Hydrolysis of starch	+	+	+	+	–	–	+
Growth at 42 °C	w	w	–	–	–	w	w
Maximum NaCl tolerance (%)	3	3	1.5	3	1.5	2	3
Minimum pH tolerance	5	6	6	6	6	6.5	6.5
Utilization of:							
L-Arabinose	–	+	w	w	w	+	w
D-Fructose	–	+	+	+	w	+	w
D-Galactose	w	+	+	+	–	w	w
Glycerol	+	w	–	w	+	w	–
Lactose	–	+	w	w	–	w	w
D-Mannitol	+	w	w	+	+	–	+
Melibiose	–	+	–	w	w	w	w
Raffinose	+	w	+	w	+	–	–
L-Rhamnose	w	+	w	+	w	–	–
D-Ribose	w	–	–	w	–	w	w

value between strain MW2-25^T and *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 *Dactylosporangium*. It is, therefore, proposed that this strain be classified as representing a novel species of the genus *Dactylosporangium*, for which the name *Dactylosporangium maewongense* sp. nov. is proposed.

Description of *Dactylosporangium maewongense* sp. nov.

Dactylosporangium maewongense (mae.wong.en'se. N.L. neut. adj. *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

cellobiose, 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 H₂S are negative. Optimal temperature for growth is between 25 and 30 °C; no growth occurs above 42 °C. The maximum NaCl concentration for growth is 3 % (w/v). Cell wall contains glutamic acid, glycine, alanine, *meso*-A₂pm and 3-OH-*meso*-A₂pm. The acyl type of the cell wall is the glycolyl type. The predominant menaquinone is MK-9(H₈). 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 iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{14:0}, anteiso-C_{17:0}, iso-C_{17:0}, C_{18:0}, C_{16:0}, C_{17:0}, *cis*-9-C_{18:1}, anteiso-C_{17:1}, *cis*-9-C_{17:1} and 10-methyl-C_{17:0}.

The type strain, MW2-25^T (=BCC 34832^T=JCM 15933^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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