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Micromonospora siamensis sp. nov., isolated from Thai peat swamp forest

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Morphological and chemotaxonomic characterization of actinomycete strain TT2-4^T isolated from peat swamp forest soil in Pattaloong Province, Thailand, clearly demonstrated that this strain belongs to the genus *Micromonospora*. 16S rDNA sequence analysis for the strain supported the assignment of the strain to the genus *Micromonospora* and the similarity value of sequences between this strain and the closely related species, *Micromonospora mirobrigensis* was 99.1%, and *M. carbonacea* and *M. matsumotoense* were 98.8%. The DNA-DNA hybridization result and some physiological and biochemical properties indicated that strain TT2-4^T was distinguished from the phylogenetically closest relatives. Based on these genotypic and phenotypic data, strain TT2-4^T merits a new species in the genus *Micromonospora* and the name *Micromonospora siamensis* sp. nov. is proposed for the strain. The type strain is strain TT2-4^T (=JCM 12769^T =PCU 266^T =TISTR 1554^T).

Key Words—actinomycetes; *Micromonospora*; *Micromonosporaceae*; peat swamp forest

Introduction

The genus *Micromonospora* Ørskov 1923 belongs to the family *Micromonosporaceae* (Stackebrandt et al., 1997) and currently encompasses 18 validly described species (the type strain NTCT 4582^T of *Micromonospora gallica* is not available any longer, Kawamoto, 1989), including the recently described species *M. eburnea*, *M. aurantinigra*, *M. endolithica* and *M. miro-*

brigensis (Hirsch et al., 2004; Thawai et al., 2004, 2005; Trujillo et al., 2005). This genus is well established according to its morphological and chemotaxonomic properties (Kroppenstedt, 1985; Lechevalier and Lechevalier, 1970; Lechevalier et al., 1977) as well as through 16S rDNA-based phylogenetic analysis (Stackebrandt et al., 1997).

During investigation of new actinomycetes from peat swamp forest soil in the southern area of Thailand, we isolated strain TT2-4^T showing typical morphological characteristics of the genus *Micromonospora* and it was genotypically and phenotypically distinguished from all validly described *Micromonospora* species. In this paper, we report taxonomic characterization and classification of this isolate and propose a new species *Micromonospora siamensis* sp. nov. for the strain.

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Abbreviations: JCM, Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama, Japan; PCU, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand; TISTR, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand.

Materials and Methods

Isolation. Strain TT2-4^T was isolated from soil samples collected from peat swamp forest in Pat-taloong Province, Thailand. A sample was taken from surface of soil, half a gram of soil was suspended in 4.5 ml sterile distilled water, and the suspension was treated by the wet-heat technique. A 100 µl diluted (10^{-3}) soil sample was spread onto starch-casein nitrate agar (pH 7.2) supplemented with novobiocin and mycostatin as described by Thawai et al., 2005. After a 21-day aerobic incubation at 30°C, the colony of this strain was transferred onto yeast extract-malt extract agar (ISP medium no. 2).

Morphology. Strain TT2-4^T was grown on ISP medium no. 2 for 14 days at 30°C and observed by light and scanning electron microscopy (model JSM-5410 LV; JEOL, Ltd., Tokyo, Japan) as described by Itoh et al., 1989.

Cultural, physiological, and biochemical characteristics. The phenotypic properties were examined by using several standard methods; cultural characteristics were tested by using 14-day cultures grown at 30°C on various agar media. The *Jacal Color Card L2200* (Japan Color Research Institute) was used for determining color designations and names. Decomposition of various compounds was examined by using the basal medium recommended by Gordon et al. (1974). The temperature for growth and NaCl tolerance were determined on ISP medium no. 2. Carbon source utilization was tested by using ISP medium no. 9 (Shirling and Gottlieb, 1966) supplemented with a final concentration of 1% of the tested carbon sources and 0.05% casamino acid. Gelatin liquefaction, peptonization of milk, nitrite production, cellulose decomposition and starch hydrolysis were determined by cultivation on various media described by Arai (1975), and Williams and Cross (1971). Melanoid pigment and H₂S production were examined on slants of tyrosine agar and peptone iron agar supplemented with 0.1% (w/v) yeast extract, respectively.

Chemotaxonomy. Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in yeast extract-malt extract broth (containing 0.4% glucose, 0.4% yeast extract and 1% malt extract; pH 7.3) on a rotary shaker at 30°C. Cell wall peptidoglycan was prepared and hydrolyzed by the method of Kawamoto et al. (1981), and the amino acid composition was analyzed with an automatic amino

acid analyzer. The isomers of diaminopimelic acid (A₂pm) in cell walls were determined by the method of Stanek and Roberts (1974). The acyl group of mu-ramic acid in peptidoglycan was determined by the method of Uchida and Aida (1984). The reducing sugars from whole-cell hydrolysates were analyzed by the HPLC method of Mikami and Ishida (1983). Phospholipids were extracted and analyzed by 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) (Kämpfer and Kroppenstedt, 1996; Sasser, 1990). Isoprenoid quinones were extracted by the method of Collins et al. (1977) and were analyzed by HPLC equipped with a Cosmosil 5C₁₈ column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan). The elution solvent was a mixture of methanol and 2-propanol (2:1, v/v).

Preparation of DNA, DNA base composition and DNA-DNA hybridization. Chromosomal DNA was isolated from cells grown in yeast extract-malt extract broth according to the method of Tamaoka (1994). The G+C content of the DNA was determined using the HPLC method of Tamaoka and Komagata (1984). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu, Choshi, Japan) was used as the quantitative standard. DNA-DNA relatedness was measured fluorometrically using the microplate hybridization method devised by Ezaki et al. (1989). Hybridization was carried out at 55°C for 2 h.

16S rDNA sequence analysis. Genomic DNA extraction, PCR-mediated amplification of 16S rDNA, and sequencing of the PCR products were carried out as described previously (Nakajima et al., 1999). The sequence was multiply aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases by using the CLUSTAL W version 1.81. The alignment was manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987) in the MEGA program version 2.1. The confidence values of branches of the phylogenetic tree were determined using the bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings. The values for sequence similarity among the closest strains were calculated manually after pairwise alignments obtained using the CLUSTAL X program (Thompson et al., 1997). Gaps and ambiguous nucleotides were eliminated from the calculations.

Nucleotide sequence accession number. The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain TT2-4^T is AB193565.

Results and Discussion

Strain TT2-4^T exhibited a range of phenotypic and chemotaxonomic properties that is consistent with their classification in the genus *Micromonospora* (Kawamoto, 1989). This strain produced well-developed and branched substrate hyphae on ISP medium no. 2, but no aerial hyphae. Spores were borne singly on the substrate hyphae having a diameter of 0.3–0.5 µm. The spores were smooth on the surface and non-motile. The color of the substrate mycelium was vivid orange. Pale yellow soluble pigment was produced in ISP medium no. 2 (Table 1). The cell wall peptidoglycan contained glutamic acid, glycine, alanine and *meso*-diaminopimelic acid, indicating that these strains have cell wall type II of Lechevalier and Lechevalier (1970) and peptidoglycan type A1γ of Schleifer and Kandler (1972). The acyl type of cell wall muramic acid was glycolyl type. This strain contained xylose and arabinose as diagnostic whole-cell sugar in whole-organism hydrolysates (whole-cell sugar pattern D of Lechevalier and Lechevalier, 1970). This strain contained phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol-mannosides but not phosphatidylcholine (phospholipid type II of Lechevalier et al., 1977). Major cellular fatty acids were iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{17:0} and C_{17:0} as major components (fatty acid type 3b of Kroppenstedt, 1985). Mycolic acids were absent. The predominant menaquinones were MK-10(H₄) and MK-10(H₆). The DNA base composition was 73.0%.

Comparison of the almost complete 16S rDNA sequence of strain TT2-4^T (1,444 nucleotides) with corresponding sequences of the type strains of validly described *Micromonospora* species indicated that this strain was closely related to *M. mirobriensis* DSM 44380^T (Trujillo et al., 2005), which was supported by a high bootstrap value (Fig. 1). The 16S rDNA similarities between strain TT2-4^T and the type strains of validly described *Micromonospora* species ranged from 97.8% (*M. olivasterospora*) to 99.1% (*M. mirobriensis*), which corresponds to 36 to 17 nucleotide differences. Characteristics shown in Table 2 clearly indicate that strain TT2-4^T possesses a distinct phenotypic profile that separates it from the phylogenetically closest species, *M. carbonacea* DSM 43168^T and *M. matsumotoense* IMSNU 22003^T (Lee et al., 1999). In particular, the maximum NaCl concentration for growth, nitrate reduction, and utilization of D-ribose, L-rhamnose, D-melibiose, glycerol, salicin, and L-arabinose were effective for discrimination between strain TT2-4^T and these related species. In addition, TT2-4^T was differentiated from the recent new species, *M. mirobriensis* DSM 44380^T by the production of diffusible pigment, the maximum NaCl (5%) tolerance, urease, the utilization of L-rhamnose (Trujillo et al., 2005), and the DNA base composition (Table 3). Furthermore, the levels of DNA-DNA relatedness between strain TT2-4^T and type strains of validly described *Micromonospora* species ranged from 24.0–50.0% (Table 3).

It is evident from the genotypic and phenotypic data that strain TT2-4^T is distinguished from previously described species of the genus *Micromonospora*. It is, therefore, proposed that this strain be classified as a new species of the genus *Micromonospora* and the name *Micromonospora siamensis* is proposed for the

Table 1. Cultural characteristics of strain TT2-4^T.

Medium	Growth	Color of colony	Soluble pigment
Yeast extract-malt extract agar (ISP medium no. 2)	Abundant	Vivid orange	Pale yellow
Oatmeal agar (ISP medium no. 3)	Abundant	Vivid orange	—
Inorganic salts-starch agar (ISP medium no. 4)	Abundant	Dark brown	—
Glycerol-asparagine agar (ISP medium no. 5)	Poor	Light yellowish brown	—
Peptone-yeast extract iron agar (ISP medium no. 6)	Abundant	Deep orange	—
Tyrosine agar (ISP medium no. 7)	Moderate	Yellowish brown	—
Glucose-asparagine agar	Moderate	Light yellowish brown	—
Czapek's agar	Abundant	Vivid orange	—
Nutrient agar	Growth	Vivid orange	—

Fig. 1. Neighbor-joining tree (Saitou and Nei, 1987) based on almost-complete 16S rRNA gene sequences showing relationships among strain TT2-4^T, the 18 validly described *Micromonospora* species and representatives of the genus *Actinoplanes*.

Streptomyces ambifaciens is used as an outgroup. The numbers on the branches indicate the percentage bootstrap values of 1,000 replicates; only values >50% are indicated. Bar, 0.01 substitutions per nucleotide position.

Table 2. Differential physiological and biochemical characteristics of strain TT2-4^T and phylogenetically closest relatives.

Characteristic ^a	Strain TT2-4 ^T	<i>M. carbonacea</i> JCM 3139 ^T	<i>M. matsumotoense</i> JCM 9104 ^T
Max. NaCl tolerance (%)	5	3	3
Nitrate reduction	—	+	+
Utilization ^b of :			
D-Ribose	—	—	w
L-Rhamnose	+	—	—
D-Melibiose	+	+	—
Glycerol	—	+	—
Salicin	+	+	—
L-Arabinose	+	—	+

^a All strains were positive for gelatin liquefaction, starch hydrolysis, peptonization of milk, utilization of D-glucose, D-raffinose, lactose, D-galactose, cellobiose, D-fructose and D-xylose, and growth at 40°C. All strains were negative for decomposition of adenine, hypoxanthine and xanthine, and utilization of D-mannitol.

^b Growth: +, positive; —, negative; w, weakly positive.

Table 3. DNA base composition and DNA-DNA relatedness between strain TT2-4^T and type strains of validly described *Micromonospora* species.

Strain	G+C content (mol%)	Percentage DNA complementarity with labeled DNA from TT2-4 ^T
TT2-4 ^T	73.0	100
<i>M. mirobrigensis</i> JCM 13240 ^T	68.6 ^a	24
<i>M. chersina</i> JCM 9459 ^T	72.9	50
<i>M. coerulea</i> JCM 3175 ^T	71.7	38
<i>M. purpureochromogenes</i> JCM 3156 ^T	73.0	37
<i>M. echinospora</i> JCM 3073 ^T	71.7	33
<i>M. carbonacea</i> JCM 3139 ^T	73.3	30
<i>M. chalcea</i> JCM 3031 ^T	71.9	43
<i>M. inositola</i> JCM 6239 ^T	71.4	34
<i>M. nigra</i> JCM 8973 ^T	71.7	41
<i>M. halophytica</i> JCM 3125 ^T	72.5	47
<i>M. aurantiaca</i> JCM 10878 ^T	71.6	40
<i>M. matsumotoense</i> JCM 9104 ^T	71.0	26
<i>M. pallida</i> JCM 3133 ^T	71.1	31

^a Trujillo et al. (2005)

strain.

Description of *Micromonospora siamensis* sp. nov.

Micromonospora siamensis (si.am.en'sis. M.L. fem. adj. *siamensis* pertaining to Thailand, the place of the soil from which the type strain was isolated).

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are vivid orange and turn to brownish black after sporulation in ISP medium no. 2. Spores are singly formed on substrate hyphae. Aerial mycelium is absent. The spore surface appears smooth. Spores are non-motile. Pale yellow soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, D-melibiose, D-raffinose, salicin, lactose, D-galactose, L-arabinose, cellobiose, D-xylose, L-rhamnose; weakly utilizes D-fructose but not D-mannitol, D-ribose, or glycerol. Peptonization of milk, hydrolysis of starch and gelatin liquefaction are positive, but urease, nitrate reduction, formation of melanoid pigment and H₂S production are negative. Optimal temperature for growth is 25–30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 5%. Cell wall contains glutamic acid, glycine, alanine and meso-A₂pm. The acyl type of the cell wall is the glycolyl type. The predominant menaquinones are MK-10(H₄) and MK-10(H₆). The characteristic whole-cell

sugars are xylose and arabinose. Contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositolmannosides and phosphatidylinositol but not phosphatidylcholine. The major cellular fatty acids are iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{17:0} and C_{17:0}. The G+C content of the DNA is 73.0 mol%. Habitat is soil. The type strain is strain TT2-4^T (=JCM 12769^T =PCU 266^T =TISTR 1554^T).

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