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## Short Communication

# *Micromonospora narathiwatensis* sp. nov., from Thai peat swamp forest soils

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The genus *Micromonospora* is well established according to its morphological and chemotaxonomic properties as well as 16S rRNA gene-based phylogenetic analyses (Kawamoto, 1989; Kroppenstedt, 1985; Lechevalier and Lechevalier, 1970; Lechevalier et al., 1977; Stackebrandt et al., 1997). Reclassification of *Micromonospora* species was carried out by Kasai et al. (2000) on the basis of *gyrB* sequence analyses and DNA-DNA hybridization experiments, including the recently described species, *Micromonospora eburnea*, *M. aurantinigra*, *M. siamensis*, *M. endolithica*, *M. mirobrigensis*, *M. citrea*, *M. echinaurantiaca*, *M. echinofusca*, *M. fulviviridis*, *M. inyonensis*, *M. peucetia*, *M. sagamiensis*, *M. viridifaciens*, *M. coriariae*, *M. chokoriensis*, and *M. coxensis* (Ara and Kudo, 2007; Hirsch et al., 2004; Kroppenstedt et al., 2005; Thawai et al., 2004, 2005a, b; Trujillo et al., 2005, 2006). During investigation of new actinomycetes from peat swamp forest soils in the southern area of Thailand, we isolated six actinomycete strains, showing typical morphological characteristics of the genus *Micromonospora* and they were phenotypically and genotypically distinguished from all validly described *Micromonospora* species. In this paper, we report taxonomic characterization and classification of these isolates and propose a new species *Micromonospora narathiwatensis* sp. nov. for the strains.

Strains BTG4-1$^T$, BTG1-1 and BTG1-4 were isolated from peat swamp forest soils collected in Narathiwat whereas strains KM1-9 and KM1-6 were from Pattaloong, and LK2-12 was from Yala Province, Thailand as described by Thawai et al. (2004, 2005b). The colonies of these strains were transferred onto yeast extract-malt extract agar (International *Streptomyces* Project medium no. 2, ISP medium 2). Morphological properties of these strains grown on ISP medium 2 agar were observed by light and scanning electron microscopy (model JSM-5410 LV; JEOL, Ltd., Tokyo, Japan) as described by Itoh et al., 1989. The phenotypic properties were examined by using several standard methods and the 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 and NaCl tolerance were determined on ISP medium 2. Carbon source utilization was tested by using ISP medium 9 (Shirling and Gottlieb, 1966) sup-

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plemented with a final concentration of 1% of the tested carbon sources and 0.05% casamino acids. 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). Melanin and hydrogensulfide production were examined on slants of tyrosine agar (ISP medium 7) and peptone-yeast extract iron agar (ISP medium 6) supplemented with 0.1% (w/v) yeast extract, respectively.

Cell wall peptidoglycan was prepared and hydrolyzed by the methods of Kawamoto et al. (1981), and the amino acid composition was analyzed with an automatic amino acid analyzer. The isomers of diaminopimelic acid ($A_2$pm) in cell walls were determined by the method of Staneck and Roberts (1974). The acyl group of muramic 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 in cells 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 5$C_{18}$ 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).

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). DNA-DNA hybridization was carried out at 55°C for 2 h and measured fluorometrically using the microplate reader as described by Ezaki et al. (1989). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene 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 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 (Saitou and Nei, 1987) and maximum-parsimony method (Kluge and Farris, 1969) in the MEGA program version 2.1. Confidence values of branches of the phylogenetic tree were determined using the bootstrap analyses (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. The DDBJ accession numbers for the 16S rRNA gene sequences of strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1 are AB193559, AB193560, AB193561, AB193562, AB193563, and AB193564, respectively.

Six strains exhibited a range of phenotypic and chemotaxonomic properties that were consistent with their classification in the genus *Micromonospora* (Kawamoto, 1989). These strains produced well-developed and branched substrate hyphae on ISP medium 2, but no aerial hyphae. Spores were borne singly on the substrate hyphae having a diameter of 0.4–0.5 μm. The spores were rough and nodular on the surface and non-motile. The color of the substrate mycelium was yellowish white to grayish yellow. Pale yellow soluble pigment is produced in ISP medium 2. These organisms contained glutamic acid, glycine, alanine and *meso*-diaminopimelic acid in the cell wall, indicating that these strains have wall chemotype 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; xylose and arabinose as diagnostic whole-cell sugar in whole-organism hydrolysates (pattern D of Lechevalier and Lechevalier (1970); diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides and phosphatidylethanolamine as major polar lipids, but not phosphatidylcholine (phospholipid type II of Lechevalier et al., 1977); iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$, $C_{17:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$ as major cellular fatty acids (fatty acid type 3b of Kroppenstedt, 1985). Mycolic acids were absent. The predominant menaquinones were MK-10($H_8$), MK-10($H_6$), and MK-10($H_4$). The DNA base compositions of strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1 were 71.6, 71.4, 72.0, 71.8, 71.6 and 71.5 mol%, respectively.

Comparison of the almost complete 16S rRNA gene sequences obtained for strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1 (1,509, 1,494, 1,509, 1,508, 1,508, and 1,507 nucleotides, respec-

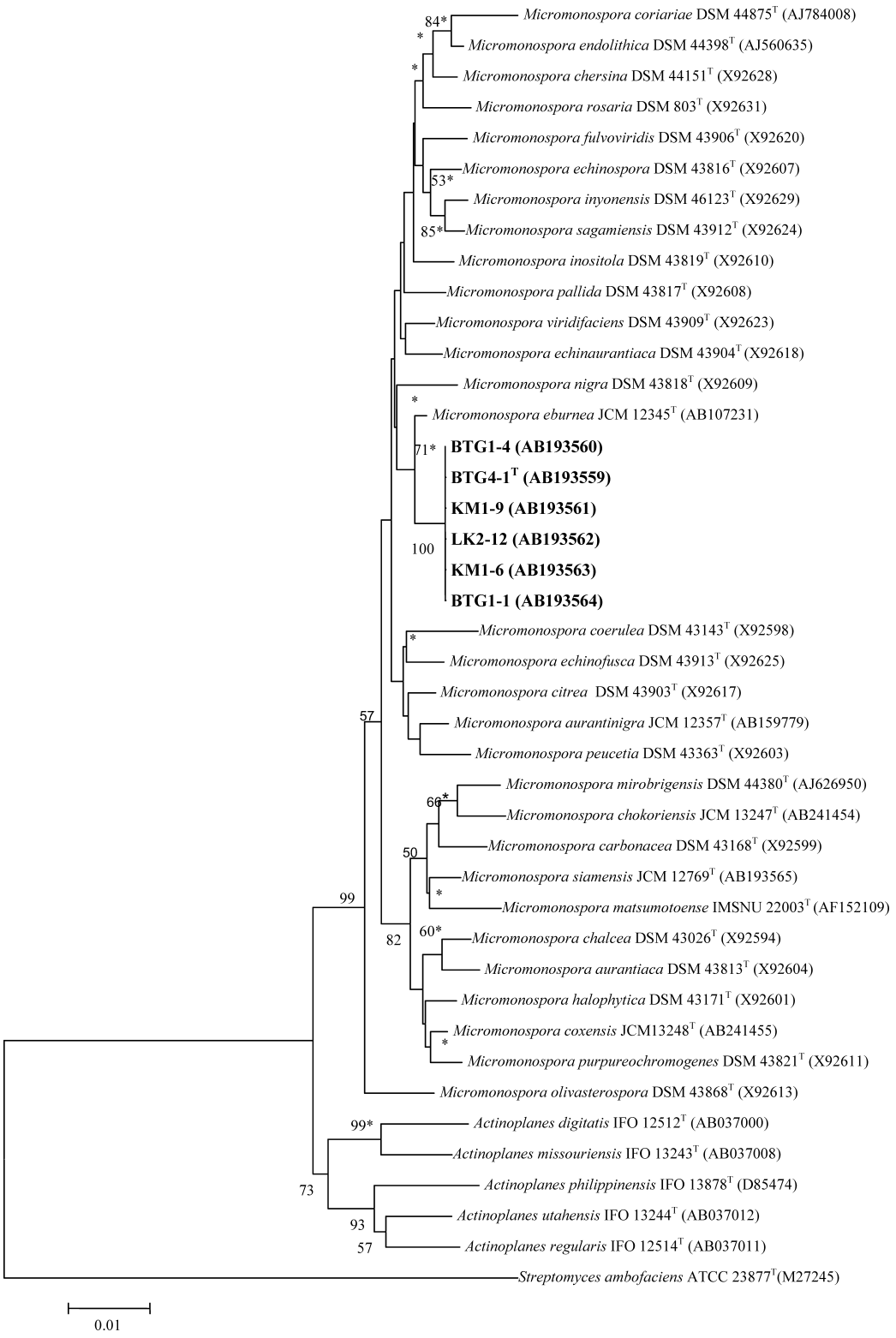


Fig. 1. Neighbor-joining tree (Saitou and Nei, 1987) based on almost-complete 16S rRNA gene sequences showing relationships among strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, BTG1-1, *Micromonospora* species, and related taxa of the family *Micromonosporaceae*.

Asterisks indicate branches of the tree that were also found using the maximum-parsimony (Kluge and Farris, 1969) method. The numbers on the branches indicate the percentage bootstrap values of 1,000 replicates and only values $>$50% are indicated. Bar, 0.01 substitutions per nucleotide position.

tively) with corresponding sequences of all of the type strains of validly described *Micromonospora* species, representative sequences of the genus *Actinoplanes* and the 16S rRNA gene sequence of *Streptomyces ambofaciens* (as an outgroup) indicated that these six strains, BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1, had identical 16S rRNA gene sequences and formed a clade closely associated with the *Micromonospora eburnea* JCM 12345$^T$, which was supported by bootstrap value of 71%. In addition, the tree constructed by using the maximum-parsimony method showed the same topography as the neighbor-joining method, which the branches of the tree were indicated as asterisks (Fig. 1). The 16S rRNA gene sequence similarities between these six strains and all validly described *Micromonospora* species ranged from 99.5% (*M. eburnea*), and 98.8% (*M. nigra*) to 98.0% (*M. olivasterospora*), which corresponds to the range 13 to 31 nucleotide differences, respectively. Strain BTG4-1$^T$ showed different color of colonies on ISP medium 2, ISP medium 4, ISP medium 6, and the soluble pigment on ISP medium 3 from *M. eburnea* JCM 12345$^T$ as shown in Table 1. They were distinguished from the phylogenetically closest *M. eburnea* JCM 12345$^T$ and *M. nigra* JCM 8973$^T$ in growth at 45°C and the utilization of L-arabinose, D-fructose, D-mannitol, L-rhamnose, and D-ribose as shown in Table 2. The levels of DNA-DNA relatedness among strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1 ranged from 88–103% while the levels of DNA-DNA relatedness between these six strains and the closest *Micromonospora* species, *M. eburnea* JCM 12345$^T$ and *M. nigra* JCM 8973$^T$ ranged from 26.0–48.3%. The amounts of cellular fatty acids of iso-$C_{15:0}$ and iso-$C_{16:0}$ of BTG4-1$^T$ and BTG1-1 were different from *M. eburnea* JCM 12345$^T$ as shown in Table 3. These phenotypic and genotypic data showed that strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1 belong to the same species and merit classification as a new species of the genus *Micromonospora*. Therefore, we propose *Micromonospora narathiwatensis* sp. nov. for the strains BTG4-1$^T$, BTG1-4, KM1-9, LK2-12, KM1-6, and BTG1-1.

Table 1. Cultural characteristics of the strain BTG4-1$^T$ and the closely related *Micromonospora* species.

| Medium | 1 | | | 2[a] | | |
|---|---|---|---|---|---|---|
| | Growth | Color of colony | Soluble pigment | Growth | Color of colony | Soluble pigment |
| Yeast extract-malt extract agar (ISP medium 2) | Abundant | Black | Pale yellow (5.5Y 9/1.5) | Good | Greyish black | Pale yellow (5.5Y 9/1.5) |
| Oatmeal agar (ISP medium 3) | Abundant | Dull orange (5YR 7/4) | — | Abundant | Dull orange (5YR 7/4) | Pale yellow (5.5Y 9/1.5) |
| Inorganic salts-starch agar (ISP medium 4) | Poor | Yellowish white (2.5Y 9/0.5) | — | Poor | Colorless | — |
| Glycerol-asparagine agar (ISP medium 5) | Poor | Yellowish white (2.5Y 9/0.5) | — | Poor | Yellowish white (2.5Y 9/0.5) | — |
| Peptone-yeast extract iron agar (ISP medium 6) | Abundant | Light yellowish brown (9YR 6.5/5) | — | Good | Dark yellowish brown (9YR 3/3) | — |
| Tyrosine agar (ISP medium 7) | Poor | Yellowish white (2.5Y 9/0.5) | — | Poor | Yellowish white (2.5Y 9/0.5) | — |
| Glucose-asparagine agar | Poor | Yellowish white (2.5Y 9/0.5) | — | Poor | Yellowish white (2.5Y 9/0.5) | — |
| Czapek's agar | Poor | Yellowish white (2.5Y 9/0.5) | — | Poor | Yellowish white (2.5Y 9/0.5) | — |
| Nutrient agar | Good | Dark yellowish brown (9YR 3/3) | Pale yellow (5.5Y 9/1.5) | Moderate | Dark yellowish brown (9YR 3/3) | Pale yellow (5.5Y 9/1.5) |

Abbreviations: 1, BTG4-1$^T$; 2, *M. eburnea* JCM 12345$^T$.

[a] Data from Thawai et al. (2005b).

Table 2. Differential characteristics of strains and the closely related *Micromonospora* species.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7[a] | 8[a] |
|---|---|---|---|---|---|---|---|---|
| Growth at 45°C | − | − | − | − | − | − | + | − |
| Utilization of : | | | | | | | | |
| L-Arabinose | w | w | w | w | w | w | − | w |
| D-Fructose | − | − | − | − | − | − | − | w |
| D-Mannitol | + | + | w | w | w | w | w | − |
| L-Rhamnose | − | − | − | − | − | − | + | − |
| D-Ribose | + | + | + | w | + | + | − | − |

Abbreviations: 1, BTG4-1$^T$; 2, BTG1-4; 3, KM1-9; 4, LK2-12; 5, KM1-6; 6, BTG1-1; 7, *M. eburnea* JCM 12345$^T$; 8, *M. nigra* JCM 8973$^T$. +, Positive; −, negative; w, weakly positive.

[a] Data from Thawai et al. (2005b).

Table 3. Cellular fatty acid compositions of BTG4-1$^T$, BTG1-1, and JCM 12345$^T$.

| Fatty acid[a] | BTG4-1$^T$ | BTG1-1 | JCM 12345$^T$ |
|---|---|---|---|
| Saturated | | | |
| $C_{15:0}$[b] | 2 | 3.5 | 1.6 |
| $C_{16:0}$ | 1.4 | 1.5 | 1.1 |
| $C_{17:0}$ | 8.3 | 9.5 | 7.9 |
| $C_{18:0}$ | 1.7 | 1.1 | 2.5 |
| $C_{19:0}$ | 0.5 | | 1.4 |
| Unsaturated | | | |
| 2OH-$C_{16:1}$ | 0.9 | | |
| $C_{17:1}$ (ω8c) | 3.3 | 4.2 | 5.2 |
| $C_{18:1}$ (ω9c) | 0.6 | 0.6 | 1.9 |
| Branched | | | |
| i-$C_{14:0}$ | | 0.7 | 0.8 |
| i-$C_{15:0}$ | 39.6 | 37.7 | 24.1 |
| a-$C_{15:0}$ | 6.4 | 10.3 | 8.5 |
| i-$C_{16:0}$ | 9.6 | 8.4 | 17.9 |
| i-$C_{17:0}$ | 10.8 | 7.8 | 7.7 |
| a-$C_{17:0}$ | 7.2 | 8.2 | 10.1 |
| i-$C_{17:1}$ω9c | 2.5 | 2.6 | 1.6 |
| i-$C_{18:0}$ | | | 0.7 |
| 10-Methylated | | | |
| $C_{17:0}$ | 2.9 | 1.1 | 4.2 |
| $C_{18:0}$ | 0.5 | | 0.9 |

[a] Values are percentages of total cellular fatty acids. Trace amounts (less than 0.5%) are not shown.

[b] Number of carbon atoms : number of double bonds.

Abbreviations: i, iso; a, anteiso.

Description of *Micromonospora narathiwatensis* sp. nov.

*Micromonospora narathiwatensis* (na.ra.thi.wat.en'sis. N.L.fem.adj.*narathiwatensis* referring to the province where the soil samples were collected).

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are yellowish white to grayish yellow and turn to black after sporulation in ISP medium 2. Single spores are formed on substrate hyphae. Aerial mycelium is absent. The spore surface appears rough and nodular. Spores are non-motile. Pale yellow soluble pigment is produced in ISP medium 2. Nitrate is reduced to nitrite. Utilizes cellobiose, D-glucose, glycerol, D-melibiose, lactose, D-raffinose, D-ribose, salicin, and D-xylose; weakly utilizes L-arabinose, D-galactose, and D-mannitol, but not D-fructose and L-rhamnose. Peptonization of milk, hydrolysis of starch and gelatin liquefaction are positive, but formation of melanin and $H_2S$ production are negative. Optimal temperature for growth is between 25 and 30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 4%. Cell wall contains glutamic acid, glycine, alanine and *meso*-$A_2$pm. The acyl type of the cell wall is the glycolyl type. The predominant menaquinones are MK-10($H_8$), MK-10($H_6$), and MK-10($H_4$). The characteristic whole-cell sugars are xylose and arabinose. The phospholipids profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major fatty acid pattern of the type strain consists of iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$, $C_{17:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$. The G+C content of the DNA is 71.4–72.0 mol%. Habitat is soil. The type strain is BTG4-1$^T$ (=JCM 12394$^T$=PCU 265$^T$=TISTR 1570$^T$).

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