Micromonospora humi sp. nov., isolated from peat swamp forest soil

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A novel actinomycete, strain P0402^T, was isolated from peat swamp forest soil collected in Thailand. Its taxonomic position was determined by using a polyphasic taxonomic approach. The chemotaxonomic characteristics of this strain matched those of the genus *Micromonospora*, i.e. the presence of *meso*-diaminopimelic acid and *N*-glycolyl muramic acid in the peptidoglycan, whole-cell sugar pattern D, phospholipid type II, and cellular fatty acid type 3b. Phylogenetic analysis based on 16S rRNA gene sequences revealed a close relationship between strain P0402^T and *Micromonospora coxensis* JCM 13248^T (99.0 % similarity), *Micromonospora eburnea* JCM 12345^T (99.0 %), *Micromonospora marina* JCM 12870^T (98.9 %), *Micromonospora halophytica* JCM 3125^T (98.7 %), *Micromonospora chalcea* JCM 3031^T (98.7 %), *Micromonospora purpureochromogenes* JCM 3156^T (98.6 %) and *Micromonospora aurantiaca* JCM 10878^T (98.5 %). It could be clearly distinguished from these type strains based on low levels of DNA–DNA relatedness and phenotypic differences. On the basis of the data presented, strain P0402^T is suggested to represent a novel species of the genus *Micromonospora*, for which the name *Micromonospora humi* sp. nov. is proposed. The type strain is P0402^T (=JCM 15292^T =PCU 315^T =TISTR 1883^T).

Micromonospora is a genus of the family *Micromonosporaceae* that was described by Ørskov (1923). Strains representing members of the genus *Micromonospora* have distinct morphological characteristics in that they produce single spores on the substrate mycelium and lack aerial mycelium. The rate at which novel species of the genus *Micromonospora* have been discovered has increased (Kawamoto, 1989; Kasai *et al.*, 2000; Hirsch *et al.*, 2004; Kroppenstedt *et al.*, 2005; Trujillo *et al.*, 2005, 2006, 2007; Thawai *et al.*, 2004, 2005a, b, 2007; Ara & Kudo, 2007; Jongrungruangchok *et al.*, 2008a, b; Huang *et al.*, 2008; Garcia *et al.*, 2010; Tanasupawat *et al.*, 2010; Kirby & Meyers, 2010; Wang *et al.*, 2011). At the time of writing, there are 44 species of the genus *Micromonospora* with validly published names (Euzéby, 2010), many of which were isolated from Thailand, including *Micromonospora auratinigra*, *M. eburnea*, *M. siamensis*, *M. narathiwatensis*, *M. chaiyaphumensis*, *M. krabiensis* and *M. marina* (Thawai *et al.*, 2004, 2005a, b, 2007; Jongrungruangchok *et al.*, 2008a, b; Tanasupawat *et al.*, 2010). In this study, we describe a novel strain of the genus *Micromonospora* isolated during an investigation of the biodiversity of actinomycetes in peat swamp forest soil in Thailand.

Strain P0402^T was isolated at Phu Sang National Park, Phayao province, in the northern part of Thailand by using wet heat at 70 °C for 15 min and the standard dilution technique on starch casein nitrate agar (Tanasupawat *et al.*, 2010) supplemented with nystatin (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). Plates were incubated at 30 °C for 14 days and a single colony was then transferred to and purified on yeast extract-malt extract agar [International *Streptomyces* Project (ISP) medium no. 2] as described by

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P0402^T is GU459068.

Three supplementary tables are available with the online version of this paper.

Shirling & Gottlieb (1966). The pure isolate was maintained on ISP 2 slants at 4–10 °C. Strain P0402^T grown on ISP 2 for 14 days was observed by light microscopy. Cell morphology was observed by scanning electron microscopy (Itoh *et al.*, 1989). The Hucker–Conn method was used for Gram staining (Hucker & Conn, 1923). Phenotypic properties were examined by using standard methods (Arai, 1975; Williams & Cross, 1971; Gordon *et al.*, 1974). For determination of cultural characteristics, the strain was grown at 30 °C for 14 days on various agar media (Shirling & Gottlieb, 1966; Asano & Kawamoto, 1986) after which colony colours were determined with reference to Jacobson *et al.* (1958). Temperature, pH and NaCl concentration ranges for the growth of strain P0402^T were tested on ISP 2 at 30 °C for 14 days. Carbon utilization medium (ISP 9) supplemented with 1 % sole carbon source was used to determine the carbon utilization profile of the strain. Production of melanin and H_2S was examined on tyrosine agar (ISP 7) and peptone iron agar (ISP 6).

For chemotaxonomic investigations, freeze-dried cells were collected from 4-day-old cultures grown in ISP 2 broth on a rotary shaker at 30 °C. The cell-wall peptidoglycan was prepared by the method of Kawamoto *et al.* (1981). The isomer of diaminopimelic acid was determined by the TLC method of Staneck & Roberts (1974). The *N*-acyl group of the muramic acid in the peptidoglycan was analysed spectrophotometrically by using the method of Uchida & Aida (1984). Isoprenoid quinones were extracted by the method of Collins *et al.* (1977) and were then analysed by HPLC with the chromatograph equipped with a Cosmosil 5C18 column (4.6 × 150 mm; Nacalai Tesque). Whole-cell sugars were analysed according to Mikami & Ishida (1983). Methyl esters of cellular fatty acids were prepared from cells grown on ISP 2, Nonomura's yeast-starch agar (NYS, JCM medium no. 61; per litre: 15 g soluble starch, 4 g yeast extract, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 . 7H_2O$ and 15 g agar, adjusted to pH 7.4) and sucrose Bennett's agar [SB, JCM medium no. 104; per litre: 1 g yeast extract, 1 g beef extract, 2 g N-Z amine (type A), 10 g sucrose and 15 g agar, adjusted to pH 7.3] and were identified by GLC according to the Microbial Identification System (MIDI) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) as described previously (Kudo *et al.*, 1993). Phospholipids were extracted and analysed by the two-dimensional TLC method of Minnikin *et al.* (1984).

Chromosomal DNA was extracted from cells grown in ISP 2 broth supplemented with 0.1 % glycine (Tamaoka, 1994; Yamada & Komagata, 1970). The G+C content of the DNA was determined by HPLC (Tamaoka & Komagata, 1984). Levels of DNA–DNA relatedness were determined according to Ezaki *et al.* (1989). The 16S rRNA gene was amplified by using primers 27F and 1492R as described by Nakajima *et al.* (1999). The 16S rRNA gene sequence was multiply aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases by using the CLUSTAL_X program, version 1.83 (Thompson *et al.*, 1997). The aligned sequences were manually edited before reconstructing the phylogenetic tree by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods in the MEGA 4 software (Tamura *et al.*, 2007). Confidence levels for the branches of the phylogenetic tree were examined by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. Gaps and ambiguous nucleotides were deleted manually before analysing sequence similarity values by CLUSTAL_X (Thompson *et al.*, 1997).

Strain P0402^T had morphological, cultural and chemotaxonomic properties consistent with its classification in the genus *Micromonospora*. The strain formed well-developed and branched substrate hyphae. No aerial mycelium was produced. Spores at maturity were spherical to oval, smooth and non-motile (Fig. 1). The phenotypic characteristics of strain P0402^T are presented in the species description below and in Table 1 and Supplementary Table S1 (available in IJSEM Online). The strain contained *meso*-diaminopimelic acid in the cell wall. The acyl type of the cell wall in the peptidoglycan was determined to be the glycolyl type. Whole-cell sugars detected were glucose, xylose, mannose, ribose, galactose, arabinose and rhamnose [pattern D of Lechevalier & Lechevalier (1970)]. The polar lipid profile comprised diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and phosphatidylethanolamine [pattern type PII of Lechevalier *et al.* (1977)]. Predominant fatty acids of strain P0402^T were iso-$C_{15:0}$ (12.72 %), iso-$C_{16:0}$ (22.61 %), anteiso-$C_{17:0}$ (7.47 %), $C_{17:0}$ (13.56 %), $C_{17:1}\omega 8c$ (12.51 %) and anteiso-$C_{15:0}$ (10.61 %) (Supplementary Table S2). This pattern corresponded to fatty acid type 3b of Kroppenstedt (1985). The predominant menaquinones were MK-10(H_4) (64.5 %) and MK-10(H_6) (35.6 %). The DNA G+C content of strain P0402^T was 73.0 mol%.

Analysis of the almost-complete 16S rRNA gene sequence (1478 nt) of strain P0402^T indicated that it was placed in a monophyletic clade with *Micromonospora coxensis* JCM 13248^T (99.0 % similarity), *M. eburnea* JCM 12345^T (99.0 %), *M. marina* JCM 12870^T (98.9 %), *Micromonospora halophy-*

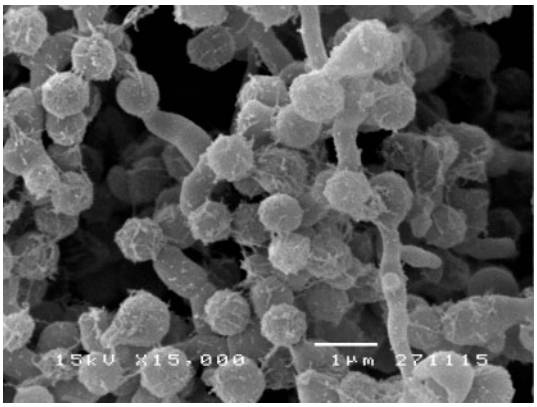

Fig. 1. Scanning electron micrograph of cells of strain P0402^T grown on ISP 2 agar medium at 30 °C for 14 days. Bar, 1 μm.

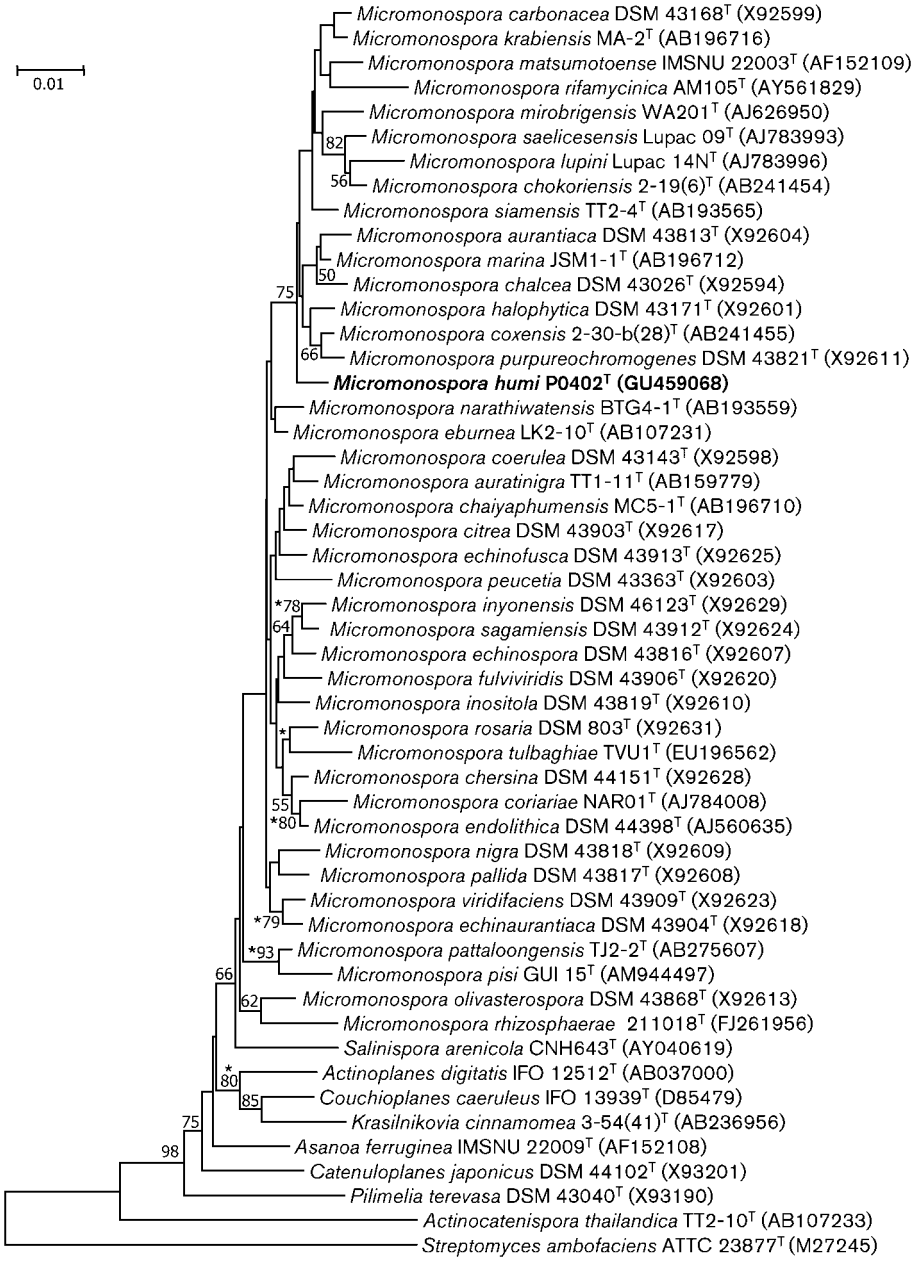

Fig. 2. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing relationships among strain P0402^T, the type strains of recognized species of the genus *Micromonospora* and representatives of the family *Micromonosporaceae*. *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. Asterisks indicate branches of the tree that were also found by using the maximum-parsimony method. The numbers on the branches indicate the percentage bootstrap values based on 1000 replicates; only values $\geqslant$50 % are indicated. Bar, 0.01 substitutions per nucleotide position.

tica JCM 3125^T (98.7 %), *Micromonospora chalcea* JCM 3031^T (98.7 %), *Micromonospora purpureochromogenes* JCM 3156^T (98.6 %) and *Micromonospora aurantiaca* JCM 10878^T (98.5 %), based on the results from both the neighbour-joining and the maximum-parsimony methods (Fig. 2). Comparison with the descriptions of these previously characterized species of the genus *Micromonospora* showed that strain P0402^T could be distinguished from them based on a combination of biochemical and physiological properties, in particular decomposition of L-tyrosine, utilization of D-fructose, glycerol, melibiose, raffinose and D-ribose, growth at pH 5, and maximum NaCl tolerance (5 %) (Table 1).

Levels of DNA–DNA relatedness between strain P0402^T and the type strains of the above species were $\leqslant$15.19 % (Supplementary Table S3). These values were obtained

Table 1. Differential characteristics between strain P0402^T and the type strains of related species of the genus *Micromonospora*

Strains: 1, P0402^T; 2, *M. coxensis* JCM 13248^T; 3, *M. eburnea* JCM 12345^T (data from Thawai *et al.*, 2005a); 4, *M. marina* JCM 12870^T (Tanasupawat *et al.*, 2010); 5, *M. halophytica* JCM 3125^T; 6, *M. chalcea* JCM 3031^T; 7, *M. purpureochromogenes* JCM 3156^T; 8, *M. aurantiaca* JCM 10878^T. W, Weakly positive.

Characteristic	1	2	3	4	5	6	7	8
Decomposition of tyrosine	−	−	−	+	−	−	−	+
Growth at pH 5	+	W	+	+	−	−	−	+
Maximum NaCl tolerance (%)	5	3	4	7	4	5	1.5	4
Utilization of:								
L-Arabinose	+	+	−	+	+	W	−	W
D-Fructose	−	+	−	+	+	W	+	+
Glycerol	−	+	+	W	W	−	−	−
Lactose	+	+	+	−	+	+	W	−
Melibiose	−	+	+	+	+	+	+	+
Raffinose	−	+	+	+	+	+	+	−
D-Ribose	−	−	−	+	−	−	−	−

from the means of three determinations and are below the threshold value of 70 % for distinguishing genomic species (Wayne *et al.*, 1987). It is evident from the genotypic and phenotypic data presented that strain P0402^T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora humi* sp. nov. is proposed.

Description of *Micromonospora humi* sp. nov.

Micromonospora humi sp. nov. (hu′mi. L. gen. n. *humi* of earth, soil, the source from which the type strain was isolated).

Aerobic, Gram-stain-positive, mesophilic actinomycete that forms a well-developed and extensively branched substrate mycelium. No aerial mycelium is produced. The colour of the vegetative mycelium on ISP 2 is sepia brown, turning to brownish black upon sporulation. Spores are spherical to oval, smooth and non-motile. Positive for starch hydrolysis, gelatin liquefaction and milk peptonization. Negative for nitrate reduction and L-tyrosine decomposition. Utilizes L-arabinose, D-galactose, D-glucose and lactose as sole carbon sources for energy, but not D-fructose, glycerol, raffinose, D-mannitol, L-rhamnose, inositol, melibiose, cellobiose, D-ribose or salicin. Grows at 20–30 °C, at pH 5–8 and in the presence of up to 5 % NaCl. Grows optimally at 30 °C, at pH 7.3–8 and in the presence of <4 % NaCl. The cell-wall peptidoglycan contains glutamic acid, alanine and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The predominant menaquinones are MK-10(H_4) and MK-10(H_6). Major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:0}$, $C_{17:1}\omega8c$ and anteiso-$C_{15:0}$. The DNA G+C content of the type strain is 73.0 mol%.

The type strain, P0402^T (=JCM 15292^T =PCU 315^T = TISTR 1883^T), was isolated from peat swamp forest soil in Thailand.

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