**Micromonospora wenchangensis** sp. nov., isolated from mangrove soil

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An actinomycete, strain 2602GPT1-05T, was isolated from a composite mangrove soil sample collected from Wenchang, Hainan province, China. Strain 2602GPT1-05T showed closest 16S rRNA gene sequence similarity to *Micromonospora haikouensis* 232617T (99.05%), and phylogenetically clustered with *Micromonospora haikouensis* 232617T, *Micromonospora matsumotoense* IMSNU 22003T (98.7%) and *Micromonospora rifamycinica* AM105T (98.6%) based on the 16S rRNA and *gyrB* gene sequence phylogenetic analysis. The strain harboured meso-DAP and glycine as major cell-wall amino acids, and MK-10(H6) and MK-9(H6) as predominant menaquinones. The characteristic whole-cell sugars were xylose, arabinose, glucose and galactose. The polar lipid profile comprised phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, unknown phospholipid and an unknown phosphoglycolipid. The major cellular fatty acids were C18:1ω9c, iso-C15:0 10-methyl C18:0 (tuberculostearic acid), C16:0, C18:0 and iso-C16:0. The DNA G+C content was 71.7 mol%. Furthermore, some physiological and biochemical properties and low DNA–DNA relatedness values enabled the strain to be differentiated from members of closely related species. On the basis of these phenotypic, genotypic and chemotaxonomic data, strain 2602GPT1-05T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora wenchangensis* sp. nov. is proposed. The type strain is 2602GPT1-05T (=CCTCC AA 2012002T =DSM 45709T).

**Abbreviation:** TBSA, tuberculostearic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of *Micromonospora wenchangensis* 2602GPT1-05T are J0768361 and J0768362, respectively.

Two supplementary figures and two supplementary tables are available with the online version of this paper.

*Micromonospora* proposed by Ørskov (1923) is the type genus of the family *Micromonosporaceae* and has gradually been recognized as an important source of secondary metabolites (Be´rdy, 2005). Members of this genus typically exhibit single, non-motile spores on the substrate mycelium and lack aerial mycelium. A number of novel species of the genus *Micromonospora* have been described in the last decade, with a total species number of 49 at the time of writing, including recently described *Micromonospora kuihong31* (Supong et al., 2012), *Micromonospora zamorensis* (Everest & Meyers, 2012), *Micromonospora maritima* (Songsumanus et al., 2013), *Micromonospora rifamycinica* (Huang et al., 2008), *Micromonospora pattalondaensis* (Thawai et al., 2008), *Micromonospora rhizosphaerae* (Wang et al., 2011), *Micromonospora haikouensis* (Xie et al., 2012) and *Micromonospora maritima* (Songsumanus et al., 2013). During an exploration of *Micromonospora* resources in mangrove soil in 2009, hundreds of strains of the genus *Micromonospora* were isolated. Strain 2602GPT1-05T was isolated from a composite soil sample collected from Wenchang mangrove (19° 37.647’ N, 110° 50.083’ E). The soil sample was air-dried at room temperature for 1 week, then sieved to exclude large particles and triturated using a sterile pestle. A 1 g sample of soil was added to 10 ml Ringer’s solution (NaCl 9 g, KCl 0.12 g, CaCl2 0.24 g, NaHCO3 0.2 g, MnSO4 0.1 g and KH2PO4 0.1 g) and 1 ml of 10% peptone solution (W, 2 g, L-arginine, 0.5 g, NaCl, 2 g, KCl, 0.1 g, glucose, 2 g) in a 100 ml Erlenmeyer flask. The flask was inoculated with 1 ml of the soil sample, then incubated on a rotary shaker for 24 h at 28 °C. The method was repeated three times with a fresh 100 ml Erlenmeyer flask containing 10 ml Ringer’s solution and incubated at 28 °C. The flask was then transferred to a 100 ml Erlenmeyer flask containing 10 ml of 0.3 M NaCl solution and 1 ml of 10% peptone solution, incubated at 28 °C for 24 h and then centrifuged at 3000 g for 10 min. The supernatant was acidified to pH 2.0 with 5 N HCl for 5 min, then centrifuged for 10 min at 3000 g. The supernatant was filtered through a 0.45 μm filter, a 10 μl filter and a 0.22 μm filter. The final solution was subjected to bioassay screening using the TLC method.
double-distilled H₂O 1000 ml), wet heated at 55 °C for 20 min, then diluted to 10⁻³. Aliquots of 100 µl of the resultant solutions were inoculated on plates of glucose–peptone–tryptone agar (GPT), supplemented with 50 mg nystatin l⁻¹, 50 mg cycloheximide l⁻¹, 25 mcg novobiocin l⁻¹ and 20 mg nalidixic acid l⁻¹. After 21 days of aerobic incubation at 28 °C, the isolate, which formed a strong orange colony, was transferred and purified on yeast extract–malt extract agar (ISP 2) and maintained as working culture on ISP 2 medium.

The morphology of strain 2602GPT1-05T was assessed as described by Shirling & Gottlieb (1966) using 21 day-old cultures on ISP 2. Spore-surface ornamentation and sporophore morphology were observed by using light microscopy (Zeiss Axioskop) and scanning electron microscopy (TM3000, Hitachi). Cultural characteristics of strain 2602GPT1-05T and the reference strains were determined using 14 day cultures at 28 °C on various agar media, including ISP 2, oatmeal agar (ISP 3), inorganic salts–starch agar (ISP 4), glycerol–asparagine agar (ISP 5), Peptone-yeast extract iron agar (ISP 6), tyroline agar (ISP 7), GYM agar (Ochi, 1987), ATCC172 agar (http://www. atcc.org), M8 agar (Castiglione et al., 2008), Czapek's agar (Waksman, 1967), nutrient agar and potato-glucose agar (PDA). The Inter-Society Color Council–National Bureau of Standards (ISCC–NBS) colour charts were used to determine the designations of colony colours (Kelly, 1964).

Physiological and biochemical characteristics of strain 2602GPT1-05T and its phylogenetically closest neighbours were examined according to the following methods: The range of growth temperatures (4, 10, 16, 28, 37, 40, 45, 50 and 55 °C), pH (4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11) and NaCl tolerance [0–7 % (w/v), at intervals of 1 %] for growth were determined on ISP 2 for 14–21 days at 28 °C except for temperature tests. KH₂PO₄/HC1, KH₂PO₄/K₂HPO₄ and K₂HPO₄/NaOH buffer systems were used to maintain the pH values of the media. Gelatin liquefaction, nitrate reduction and starch hydrolysis were determined through cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Hydrogen sulfide production and melanoid pigment production was determined on Tresner and ISP 6 agar (Shirling & Gottlieb, 1966). Aesculin utilization, CMC degradation and catalase activity were determined according to the method of Trujillo et al. (2005, 2006). Carbon source utilization was tested on basal medium (Shirling & Gottlieb, 1966) and each sterile carbon source was added to the medium aseptically to give a concentration of approximately 1 %.

For molecular systematic and chemotaxonomic analyses, the strain was cultivated in ISP 2 broth for 4 days at 28 °C on rotary shakers at 220 r.p.m. to prepare biomass. Biomass was harvested, washed in distilled water and freeze-dried. Cell-wall amino acids were analysed following the procedure of Lechevalier & Lechevalier (1980). Sugars in whole-cell hydrolysates were concentrated, dissolved in 0.2 ml sterile distilled water and subsequently analysed by TLC on microcrystalline cellulose plates (Wang, 1986). The N-acyl group of muramic acid in the peptidoglycan was determined by the method of Uchida & Aida (1984). Menaquinones and phospholipids were extracted and purified according to the protocol of Minnikin et al. (1984). Menaquinones were analysed by HPLC with an ODS-BP C₁₈ column (4.6 × 250 mm) and phospholipids were separated and identified on two-dimensional TLC (silica gel 60; 10 × 10 cm). TLC plates were developed with anisaldehyde and molybdenum blue reagents. Cellular fatty acids for strain 2602GPT1-05T and related strains were prepared from cells grown on ISP 2 broth at 28 °C under shaking conditions until good growth was obtained (Sasser, 1990) and the composition was analysed by GLC using the standard Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were identified by using the Microbial Identification software package (Sherlock Version 6.0; MIDI database: ACTIN6).

Genomic DNA was extracted as described by Pospiech & Neumann (1995). The DNA G+C content of strain 2602GPT1-05T was determined by the HPLC method (Mesbah et al., 1989). The level of DNA–DNA relatedness between strain 2602GPT1-05T and the related strains were measured on nylon membranes using the method described by Wang et al. (2011). Both standard experiments (DNA of 2602GPT1-05T was labelled, DNA of 2602GPT1-05T and related strains were immobilized) and reciprocal experiments (DNA of related strains were labelled, DNA of related strains and 2602GPT1-05T were immobilized) were determined in duplicate.

PCR amplification and sequencing of the 16S rRNA and gyrB genes were carried out as described by García et al. (2010). The 16S rRNA gene sequence of strain 2602GPT1-05T was multiply aligned with those deposited in the GenBank database and sequence similarities were calculated with the EzTaxon-e server (Kim et al., 2012). The 16S rRNA gene sequence of the strain was aligned with corresponding sequences of 49 species of the genus Micromonospora with validly published names retrieved from GenBank using the program CLUSTAL_X (Thompson et al., 1997) with Catellatospora citrea DSM 44097T (GenBank accession no. X93197) as an outgroup. Alignment was manually verified and adjusted prior to the construction of a phylogenetic tree for alignment inconsistencies, MEGA version 4.0 (Tamura et al., 2007) was used for phylogenetic tree construction using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Phylogenetic distances were calculated with Kimura's two-parameter model (Kimura, 1980) and the stability of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. As it has been revealed that gyrB gene sequence analysis is superior for inferring intrageneric relationships (Kasai et al., 2000), which was proved to be a useful marker for the delineation of species of the genus Micromonospora by recent reports (García et al., 2010; Wang et al., 2011; Zhang et al., 2012; Carro et al.,
Fig. 1. Neighbour-joining phylogenetic tree (Saitou & Nei, 1987), based on almost-complete 16S rRNA gene sequences (>1400 nt), showing the relationships between strain 2602GPT1-05T and species of the genus Micromonospora. Catellatospora citrea DSM 44097T was used as an outgroup. Asterisks indicate branches of the tree that were also found using the maximum-parsimony method (Fitch, 1971). Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values >50% are indicated. Bar, 0.005 substitutions per nucleotide position.
Phylogenetic analysis of gyrB gene was performed as described by García et al. (2010).

An almost complete 16S rRNA gene sequence (1476 bp) for strain 2602GPT1-05T was obtained. A preliminary comparison of the sequence with those in GenBank indicated that strain 2602GPT1-05T belonged to the genus Micromonospora. Pairwise sequence similarities calculated on the EzTaxon-e server (Kim et al., 2012) between the new isolate and species of the genus Micromonospora indicated strain 2602GPT1-05T has a similarity of 99.05% with Micromonospora haikouensis 232617T and 99.01% with Micromonospora siamensis TT2-4T. The phylogenetic position of the isolate in relation to other species of the genus Micromonospora based on the neighbour-joining method is shown in Fig. 1. The closest relatives of strain 2602GPT1-05T were Micromonospora haikouensis 232617T, Micromonospora matsumotoense IMSNU 22003T and Micromonospora rifamycinica AM105T, which shared 99.05%, 98.7% and 98.6% gene sequence similarity with the isolate respectively. A bootstrap value of 77% supported the clade in which the strain and Micromonospora rifamycinica AM105T were recovered. A partial sequence (1161 bp) of gyrB for strain 2602GPT1-05T was obtained and the phylogenetic analysis of the gyrB sequence using neighbour-joining method supported placing strain 2602GPT1-05T in the genus Micromonospora, also near to Micromonospora matsumotoense IMSNU 22003T and Micromonospora rifamycinica AM105T by a 99% bootstrap value (Fig. S1, available in IJSEM Online). Strains clustered with the novel isolate in 16S rRNA and the gyrB gene sequence trees were somewhat different, but both topologies were supported by using the maximum-parsimony (Fitch, 1971) tree-making algorithm as shown in Fig. 1 and Fig. S1. Strain 2602GPT1-05T did not cluster close to M. haikouensis 232617T, which shared the highest 16S rRNA gene sequence similarity, but shared the highest DNA–DNA hybridization value.

The DNA G+C contents of strain 2602GPT1-05T, Micromonospora rifamycinica AM105T, Micromonospora matsumotoense IMSNU 22003T, Micromonospora haikouensis 232617T and Micromonospora siamensis TT2-4T were 71.7%, 71% (Huang et al., 2008), 71% (Lee et al., 1999), 71.5% (Xie et al., 2012) and 73% (Thawai et al., 2005), respectively. The DNA–DNA relatedness values between strain 2602GPT1-05T and the phylogenetically closest species Micromonospora rifamycinica AM105T, Micromonospora matsumotoense IMSNU 22003T, Micromonospora haikouensis 232617T and Micromonospora siamensis TT2-4T were 17.22 ± 1.1% (20.65 ± 0.65%), 19.11 ± 0.54% (23.06 ± 0.37%), 59.6 ± 0.80% (59.8 ± 0.21%) and 23.91 ± 0.88% (27.19 ± 0.56%), respectively, the values in parentheses are from reciprocal experiments, all of which are below the 70% threshold value proposed by Wayne et al. (1987), the key marker for the identification of a novel species. These results demonstrate that strain 2602GPT1-05T represents a separate genomic species of the genus Micromonospora.

The morphological properties of strain 2602GPT1-05T are consistent with its classification as a member of the genus Micromonospora (Kawamoto, 1989). Strain 2602GPT1-05T produced well-developed and branched substrate hyphae on ISP 2 medium, approximately 0.4 μm in diameter, but no aerial hyphae. Spores were borne singly from the substrate mycelium with a diameter of approximate 0.5 μm, the spore surface appeared warty (Fig. 2). Strain 2602GPT1-05T grew well on ISP 2 and ATCC172, moderate growth was observed on ISP 3, ISP 4, GYM agar, M8 and PDA, poor growth on Czapek’s agar, nutrient agar, ISP 5, ISP 6 and ISP 7. Raised colonies become black with increased age on most media. The colour of the substrate hyphae was yellowish white to strong yellowish brown. No soluble pigment was produced on any of the 12 tested media (Table S1).

The cell-wall amino acids of strain 2602GPT1-05T contained meso-diaminopimelic acid, glycine and alanine. The whole-cell sugars were arabinose, xylose, glucose, galactose, mannose, ribose and rhamnose. The acyl type of the cell-wall muramic acid was glycolyl. The characteristic polar lipids were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol mannosides (PIMs), unknown phospholipid (PL) and unknown phosphoglycolipid (PGL) (Fig. S2), corresponding to phospholipid type II of Lechevalier et al. (1977). The major menaquinones of strain 2602GPT1-05T contained MK-10(H4) (61.1%), MK-9(H4) (17.1%) and MK-9(H2) (8.9%). The major cellular fatty acids were C18:1ω9c (18.54%), iso-C15:0 (12.48%), 10methyl C18:0 [tuberculostearic acid (TBSA)] (10.27%), C16:0 (9.00%), C18:0 (7.95%) and iso-C16:0 (6.66%) (Table S2). Strain 2602GPT1-05T differs from related strains in some physiological and biochemical characteristics. In particular, it is positive for nitrate reduction, negative for gelatin.

![Image](https://example.com/image.png)
liquefaction, the growth temperature, pH range, NaCl tolerance and utilization of (+)-D-galactose, α-Lactose, melibiose, (+)-L-rhamnose and (+)-L-arabinose effectively discriminated strain 2602GPT1-05T from the phylogenetically related type strains. Detailed physiological and biochemical properties of strain 2602GPT1-05T are given in Table 1 and the species description.

The characteristics shown in Table 1 clearly indicate that strain 2602GPT1-05T possesses some distinct phenotypic and chemotaxonomic profiles that distinguish it from its closest phylogenetic relatives, *Micromonospora matsumotoense* IMSNU 22003T, *Micromonospora rifamycinica* AM105T, *Micromonospora haikouensis* 232617T and *Micromonospora siamensis* TT2-4T. Furthermore, a low level of DNA–DNA relatedness was observed between strain 2602GPT1-05T and its closest phylogenetic relatives. It is evident from the genotypic and phenotypic data presented above that strain 2602GPT1-05T is distinguishable from previously described species of the genus *Micromonospora*. Therefore, strain 2602GPT1-05T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora wenchangensis* sp. nov. is proposed.

**Description of *Micromonospora wenchangensis* sp. nov.**

*Micromonospora wenchangensis* (wen.chang.en’sis. N.L. fem. adj. wenchangensis pertaining to Wenchang, Hainan province, China, from where the strain was isolated).

Aerobic, Gram-positive, mesophilic actinomycete that forms well-developed and branched substrate hyphae. Colonies are

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**Table 1. Differential characteristics of strain 2602GPT1-05T and its closest relatives**

<table>
<thead>
<tr>
<th>Strains: 1, 2602GPT1-05T; 2, <em>M. rifamycinica</em> AM105T; 3, <em>M. matsumotoense</em> IMSNU 22003T; 4, <em>M. siamensis</em> TT2-4T; 5, <em>M. haikouensis</em> 232617T. Symbols: ++, Strongly positive; +, positive; −, negative.</th>
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<tr>
<td><strong>Symbols:</strong></td>
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<td>++, Strongly positive; +, positive; −, negative.</td>
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<td><strong>Growth at 45 °C</strong></td>
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<tr>
<td>Growth at pH range</td>
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<td>NaCl tolerance (%)</td>
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<td>Colony colour on ISP 2</td>
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<td>Colony colour on ISP 3</td>
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<tr>
<td>Major whole-organism sugars</td>
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<td>Major fatty acid (%)</td>
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<td>Major menaquinones (%)</td>
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<td>DNA G+C content (mol%)</td>
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<td>Nitrate reduction</td>
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<td>Gelatin liquefaction</td>
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<tr>
<td>Utilization of:</td>
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<td>(+)-D-Galactose</td>
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<td>α-Lactose</td>
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<td>Melibiose</td>
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<tr>
<td>(+)-L-Rhamnose</td>
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<td>L-Arabinose</td>
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*Data from Huang et al. (2008). †Data from Lee et al. (1999). §Data from Thawai et al. (2005). $Data from Xie et al. (2012).
yellowish white to strong yellowish brown and raised colonies become black with increased age on most media. Single spores are formed on substrate hyphae, but aerial hyphae are absent. No soluble pigment is produced on agar plates. Utilizes (+)-D-cellulobiose, (-)-D-fructose, (+)-D-galactose, (+)-D-glucose, z-lactose, maltose, melibiose, sucrose and (+)-D-xylene and weakly utilizes (+)-D-mannose, but does not utilize (+)-L-arabinose, (-)-D-mannitol and (+)-L-rhamnose as sole carbon sources. Nitrate is reduced to nitrite. Positive in tests for starch hydrolysis, aesculin degradation and catalase production.

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References


