

**Micromonospora ovatispora** sp. nov. isolated from mangrove soil

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An oval spore-forming actinomycete, designated 2701SIM06ᵀ, was isolated from mangrove soil in Sanya, Hainan province, China. The 16S rRNA gene sequence of strain 2701SIM06ᵀ showed highest similarity to *Micromonospora pattaloongensis* TJ2-2ᵀ (98.6 %), *Micromonospora polyrhachis* NEAU-ycm2ᵀ (98.6 %) and *Micromonospora sonneratiae* 274745ᵀ (98.5 %). Phylogenetic analysis based on both the 16S rRNA gene and the gyrB gene supported this relationship. The chemotaxonomic results confirmed the isolate as a member of the genus *Micromonospora*, but morphological, physiological and biochemical properties differentiated it from its closest relatives. Based on these observations, strain 2701SIM06ᵀ represents a novel species, for which the name *Micromonospora ovatispora* sp. nov. is proposed. The type strain is 2701SIM06ᵀ (=CCTCC AA 2012009ᵀ=DSM 45759ᵀ).

The genus *Micromonospora*, first described by Ørskov (1923), is the type genus of the family *Micromonosporaceae*. Members of this genus have distinct morphological characteristics in that they produce single spores on the substrate mycelium and lack aerial mycelium. In the last few years, several novel species have been isolated from diverse habitats, with more than 57 species described to date (www.bacterio.net).

During the course of studies into the discovery of new marine, drug-producing actinomycetes from mangroves in south China (Hong et al., 2009; Xu et al., 2014), strain 2701SIM06ᵀ was isolated from mangrove soil collected in Sanya, Hainan province. Soil samples were pretreated, diluted by an established procedure (Ren et al., 2013) and spread on selective agar (Li et al., 2013), then incubated at 28 °C for 4–12 weeks. A tiny colony of strain 2701SIM06ᵀ was noticed on the isolation plate after more than 70 days of incubation, then picked up, purified and maintained on ATCC 172 agar (www.atcc.org), and preserved as a suspension of mycelial fragments in glycerol (20 %, v/v) at −20 °C for study and at −80 °C for long-term preservation.

Morphological characteristics were assessed by scanning electron microscopy (TM3000; Hitachi) of 10-day-old cultures on ATCC 172 agar. Cultural characteristics of strain 2701SIM06ᵀ were determined on various agar media including ATCC 172 agar, M8 agar (Castiglione et al., 2008), ISP2–5 (Shirling & Gottlieb, 1966), nutrient agar (Waksman, 1967), potassium-dextrose agar (PDA) and Czapek’s agar (Waksman, 1967). The ISCC-NBS colour charts were used to determine colony colours (Kelly, 1964). Temperature range (4, 17, 20, 28, 30, 32, 34, 37, 40 and 45 °C), pH (4.5, 5, 5.5, 6, 7, 8, 8.5, 9 and 10, using phosphate buffer system at 0.25 mmol l⁻¹) and NaCl (0, 1, 2, 3, 5, 7 and 10 %, w/v) tolerance for growth were determined on ATCC 172 agar or in ATCC 172 broth for 14–21 days at 28 °C except for the temperature tests. Gelatin liquefaction and milk peptonization were determined as described by Arai (1975) and Williams & Cross (1971). Casein degradation was confirmed by the transparent zone produced on M8 agar. CM-cellulose hydrolysis was tested by Congo red (Teather & Wood, 1982) on ISP2 medium supplemented with 1 % CM-cellulose. Production of lipase was determined on ISP2 medium supplemented with 1 % Tween 20, 40, 60 or 80. Carbon source utilization was tested by using ISP9 medium (Shirling & Gottlieb, 1966) supplemented with 0.5 % (final concentration) of each carbon source. Production of melanoid pigments was examined using ISP6 and ISP7 media (Shirling & Gottlieb, 1966). Other physiological and biochemical characteristics were tested according to the procedures of Williams et al. (1983) and Kämpfer et al. (1991).

Freeze-dried cells used for chemotaxonomic studies were obtained from cultures grown in ATCC 172 broth on a rotary shaker (220 r.p.m.) at 28 °C for 4 days. Cell-wall
Strain 2701SIM06\textsuperscript{T} formed well-developed and branched mycelia using the HPLC method (Mesbah et al. 1999). The DNA G+C content was extracted using the procedures described by Pospiech & Neumann (1995). The DNA G+C content was determined by counting the identical and total bases. The alignment was manually verified and edited to mask the sequence’s length discrepancy, and then the sequence similarity was determined by counting the identical and total bases. The program MEGA 5 (Tamura et al., 2011) was used for the phylogenetic tree reconstruction by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms. The stability of the clad in the tree was determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Values for sequence similarity among the closest strains were calculated after obtaining pairwise alignments using CLUSTAL X (Thompson et al., 1997). PCR amplification and analysis of the gyrB gene were performed as described by Li et al. (2013).

Chromosomal DNA for determination of G+C content was extracted using the procedures described by Pospiech & Neumann (1995). The DNA G+C content was determined using the HPLC method (Mesbah et al., 1989).

Strain 2701SIM06\textsuperscript{T} formed well-developed and branched substrate hyphae, but no aerial mycelium was seen. Impressive oval-shaped, smooth, non-motile spores (0.5–0.6 × 0.9–1.0 μm) were observed borne on the tips of the substrate mycelium (Fig. 1). Good growth was observed on ATCC 172 agar with vivid orange yellow substrate hyphae, but no soluble pigment was observed on any of the media used (Table S1, available in the online Supplementary Material) except for orange diffusible pigments produced on ISP\textsubscript{9} medium supplemented with 0.5% of either fructose, maltose or sucrose during the carbon source utilization tests.

The cell wall of strain 2701SIM06\textsuperscript{T} contained meso-diaminopimelic acid and whole-cell sugars were glucose, mannose, xylose, ribose, galactose and arabinoise. The acyl type of the cell-wall polysaccharides was glycolyl. The characteristic polar lipids in the strain were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol (Fig. S1), corresponding to phospholipid type PII (Lechevalier et al., 1977), which is typical of the genus Micromonospora. The major menaquinones were MK-10(H\textsubscript{6}) (52.1 %) and MK-10(H\textsubscript{4}) (38.2 %). The major fatty acids were iso-C\textsubscript{16:0} (30.8 %), anteiso-C\textsubscript{17:0} (14.8 %) and iso-C\textsubscript{15:0} (11.7 %). These saturated and unsaturated iso-/anteiso-branched fatty acids are characteristic of the genus Micromonospora. Detailed fatty acid profiles of strain 2701SIM06\textsuperscript{T} and its closest phylogenetic relatives are given in Table S2.

The almost-complete 16S rRNA gene sequence of strain 2701SIM06\textsuperscript{T} (1478 nt) was obtained. The analysis results indicated the strain is a member of the genus Micromonospora. Strain 2701SIM06\textsuperscript{T} shared highest similarities with Micromonospora pattaloongensis TJ2-2\textsuperscript{T} (98.6 %; differences/total, 20/1477 nt) and Micromonospora polychromatic NEAU-ymc2\textsuperscript{T} (98.6 %; 20/1478 nt). In the phylogenetic tree (Fig. 2; the tree including all members of the genus is shown in Fig. S2), strain 2701SIM06\textsuperscript{T} was placed in a monophyletic clade comprising M. pattaloongensis TJ2-2\textsuperscript{T}, M. polychromatic NEAU-ymc2\textsuperscript{T}, Micromonospora sonneratiae 274745\textsuperscript{T} (98.5 %; 31/1453 nt) and Micromonospora pisi GUI 15\textsuperscript{T} (97.8 %; 31/1453 nt), which was supported by a bootstrap value of 90 % in the neighbour-joining tree and also confirmed by the maximum-parsimony and maximum-likelihood algorithms. Analysis of the gyrB gene supported the above phylogenetic relationship, with strain 2701SIM06\textsuperscript{T} sharing highest similarities with M. pisi GUI 15\textsuperscript{T} (89.3 %), followed by M. polychromatic NEAU-ymc2\textsuperscript{T} (87.7 %), M. sonneratiae 274745\textsuperscript{T} (86.7 %) and M. pattaloongensis TJ2-2\textsuperscript{T} (87.1 %). Furthermore, a stable clade consisting of these closest relatives was reproduced in the gyrB gene tree (Fig. S3). The low gene sequence similarities and divergence in all phylogenetic trees demonstrated...
that strain 2701SIM06\textsuperscript{T} is not a member of any known species of the genus \textit{Micromonospora}.

The G + C content of strain 2701SIM06\textsuperscript{T} was 70.4 %. As concluded by Meier-Kolthoff \textit{et al.} (2013), to determine whether DNA–DNA hybridization is needed in prokaryote species discrimination, a threshold range of 98.2–99.0 % 16S rRNA gene sequence similarity should be applied depending on the taxonomic group involved (Stackebrandt & Ebers, 2006). Therefore, DNA–DNA hybridization was not performed between strain 2701SIM06\textsuperscript{T} and its closest relatives based on their low 16S rRNA gene sequence similarity. Furthermore, detailed physiological, biochemical and chemotaxonomic characteristics listed in Table 1, along with the oval spore shape, and marked morphological characteristics, confirmed the distinction of 2701SIM06\textsuperscript{T} from its closest related species.

In summary, it is evident from the genotypic and phenotypic data presented above that strain 2701SIM06\textsuperscript{T} represents a novel species in the genus \textit{Micromonospora}, for which the name \textit{Micromonospora ovatispora} sp. nov. is proposed.

### Description of \textit{Micromonospora ovatispora} sp. nov.

\textit{Micromonospora ovatispora} (o.v.a.ti.spo'ra. L. adj. ovatus egg-shaped, ovate; Gr. n. spora seed and, in bacteriology, a spore; N.L. fem. n. \textit{ovatispora} having ovate spores).

Aerobic and Gram-stain-positive. Smooth, non-motile, oval spores (0.5–0.6 × 0.9–1.0 μm) are produced at the well-developed branched substrate hyphae. Aerial hyphae are absent. Well grown colonies are folded and raised, and vivid orange yellow. An orange diffusible pigment is produced on ISP9 medium supplemented with 0.5% of either fructose, maltose or sucrose. Growth occurs at 20–37 °C and at pH 6–9. The maximum NaCl concentration for growth is 3%. Positive for catalase production, gelatin liquefaction, and hydrolysis of aesculin, casein, CM-cellulose and Tweens 20, 40, 60 and 80. Negative in tests for melanin production, milk peptonization, nitrate reduction, starch hydrolysis and H\textsubscript{2}S production. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, sucrose and D-xylose and weakly utilizes arabinose, salicin and D-sorbitol as carbon source, but does not utilize α-ketoglutaric acid, α-lactose, melibiose, raffinose or succinic acid. The acyl type of the cell-wall polysaccharides is glycolyl. The cell wall contains meso-diaminopimelic acid and characteristic whole-cell sugars are mannose, xylose and arabinose. The polar lipid profile comprises phosphatidylethanolamine, phosphatidylserine, diphasatidylglycerol, phosphatidylglycerol, phosphatidylmannosides, and unknown phosphoglycolipid and unknown phospholipids. The major menaquinones are MK-10(H\textsubscript{4}) and MK-10(H\textsubscript{6})

Major fatty acids are iso-C\textsubscript{16}:0, anteiso-C\textsubscript{17}:0 and iso-C\textsubscript{15}:0.

The type strain, 2701SIM06\textsuperscript{T} (=CCTCC AA 201209\textsuperscript{T}=DSM 45759\textsuperscript{T}), was isolated from mangrove sediment soil collected in Sanya, Hainan province, China. The G + C content of the DNA of the type strain is 70.4 mol%.
Table 1. Differential characteristics between strain 2701SIM06<sup>T</sup> and its closest phylogenetic relatives

Strains: 1, 2701SIM06<sup>T</sup>; 2, M. pattaloongensis ICM 12833<sup>T</sup>; 3, M. polyrhachis NEAU-ycm2<sup>T</sup>; 4, M. sonneratiae 274745<sup>T</sup>; 5, M. pisi GUI 15<sup>T</sup>. +++, strongly positive; +, positive; −, negative; w, weakly positive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>4</th>
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<tr>
<td>Colony colour on ISP2</td>
<td>Vivid orange yellow</td>
<td>Pale orange yellow</td>
<td>Strong orange yellow</td>
<td>Moderate orange yellow</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>MK-10(H&lt;sub&gt;6&lt;/sub&gt;), MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;, MK-10(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MK-10(H&lt;sub&gt;6&lt;/sub&gt;)&lt;sup&gt;c&lt;/sup&gt;, MK-10(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;e&lt;/sup&gt;, MK-10(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>Major fatty acids</td>
<td>iso-C&lt;sub&gt;16 : 0&lt;/sub&gt; (30.8 %), iso-C&lt;sub&gt;15 : 0&lt;/sub&gt; (11.7 %)</td>
<td>iso-C&lt;sub&gt;16 : 0&lt;/sub&gt; (40.7 %), iso-C&lt;sub&gt;15 : 0&lt;/sub&gt; (15.5 %), anteiso-C&lt;sub&gt;17 : 0&lt;/sub&gt; (12.7 %)</td>
<td>iso-C&lt;sub&gt;15 : 0&lt;/sub&gt; (28.7 %), anteiso-C&lt;sub&gt;15 : 0&lt;/sub&gt; (20.7 %), iso-C&lt;sub&gt;17 : 0&lt;/sub&gt; (11.7 %)</td>
<td>iso-C&lt;sub&gt;15 : 0&lt;/sub&gt; (25.8 %), anteiso-C&lt;sub&gt;17 : 0&lt;/sub&gt; (20.3 %)</td>
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<tr>
<td>DNA G+C content (mol%)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>70.4</td>
<td>71.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.6&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Growth temperature range (℃)</td>
<td>20–37</td>
<td>20–40</td>
<td>16–37</td>
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<td>Utilization of:</td>
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<td>Fructose</td>
<td>+</td>
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<td>−</td>
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*Data are from: a, Thawai et al. (2008); b, Xiang et al. (2014); c, Li et al. (2013); d, García et al. (2010). (Continued)

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References


