**Micromonospora zhanjiangensis** sp. nov., isolated from mangrove forest soil

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A novel actinomycete, designated strain 2902at01\(^T\) was isolated from soil collected at a mangrove forest in Zhanjiang, Guangdong province, China. The strain was identified using a polyphasic classification method. The 16S rRNA gene sequence of strain 2902at01\(^T\) showed the highest similarity to *Micromonospora equina* Y22\(^T\) (98.3 %) and *Micromonospora pattaloongensis* TJ2-2\(^T\) (98.1 %). Phylogenetic analysis based on the gyrB gene sequence also clearly showed that the strain was different from any previously discovered species of the genus *Micromonospora*. The characteristic whole-cell sugars were ribose and xylose. The cell-wall hydrolysates contained alanine, asparagine, glycine and *meso*-diaminopimelic acid. MK-10(H\(_6\)) and MK-10(H\(_8\)) were the major menaquinones of the novel strain. The predominant fatty acids were iso-C\(_{15}:0\), anteiso-C\(_{15}:0\) and iso-C\(_{16}:0\). The characteristic polar lipids of strain 2902at01\(^T\) were phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and diphosphatidylglycerol. The DNA G + C content was 70.2 mol%. DNA–DNA hybridization data combined with other physiological and biochemical features could distinguish strain 2902at01\(^T\) from the reference strains *M. equina* Y22\(^T\) and *M. pattaloongensis* TJ2-2\(^T\).

On the basis of these phenotypic and genotypic data, strain 2902at01\(^T\) represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora zhanjiangensis* sp. nov. is proposed. The type strain is 2902at01\(^T\) (=CCTCC AA2014018\(^T\)=DSM 45902\(^T\)).

The genus *Micromonospora* first described by Ørskov (1923) is the type genus of the family *Micromonosporaceae*. Members of this genus typically exhibited single, non-motile spores directly on the substrate mycelium, which did not fragment, and was absent of aerial mycelium (Kawamoto, 1989). At the time of writing, the genus *Micromonospora* comprised 59 species with validly published names isolated from diverse habitats. This genus is well established in terms of morphological and chemotaxonomic properties (Lechevalier & Lechevalier, 1980; Lechevalier et al., 1977; Kroppenstedt, 1985) as well as 16S rRNA gene sequence (Stackebrandt et al., 1997).

Mangroves are unique woody plant communities of intertidal coasts in tropical and subtropical coastal regions (Costanza et al., 1997). Since 2002 to 2013, thousands of actinomycetes have been isolated from different mangrove habitats including mangrove soil, rhizosphere soil and plant endogenous environments in China by the Hong group, and these actinomycetes could be categorized into 25 genera, 11 families and 8 suborders (Hong, 2013). There was evidence that mangrove soil contained high populations of *Micromonospora* (Hong et al., 2009), and several novel species were reported, including *Micromonospora rifamycinica* (Huang et al., 2008), *Micromonospora pattaloongensis* (Thawai et al., 2011), *Micromonospora rhizosphaerae* (Wang et al., 2011), *Micromonospora haikouensis* (Xie et al., 2012), *Micromonospora maritima* (Songsumanus et al., 2013) and *Micromonospora sonneratiae* (Li et al., 2013).

In the course of exploring *Micromonosporaceae* from mangrove forests, strain 2902at01\(^T\) was isolated from a soil sample collected in Zhanjiang mangrove, Guangdong province, China.

A mangrove soil sample was pretreated by an established procedure (Xie et al., 2011), and was then spread over the surface of 1/10 ATCC 172 agar, supplemented with (µg ml\(^{-1}\)) nalidixic acid (10), novobiocin (10), nystatin (50) and K\(_2\)Cr\(_2\)O\(_7\) (20) and incubated at 28 °C for 4–6 weeks until the outgrowth of strong orange red colonies were discerned. Isolates were purified and maintained on International *Streptomyces* Project (ISP) 2 agar (Shirling & Gottlieb, 1966), and preserved as a suspension of mycelia fragments in glycerol (20 %, v/v) at −40 °C for study and at −80 °C for long-term preservation.

**Abbreviations:** DAP, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and gyrB sequences of strain 2902at01\(^T\) are KJ742700 and KJ742701, respectively.

Six supplementary figures and two supplementary tables are available with the online Supplementary Material.
The morphological characteristics of strain 2902at01T were assessed by scanning electron microscopy (TM 3000; Hitachi) using 7-day-old cultures on ISP 2 agar. Cultural characteristics of strain 2902at01T were determined on various agar media including ATCC 172 agar, Czapek’s agar, ISP 1–7 agar, MB agar, and potato-glucose agar as previously used (Li et al., 2013a), and MB agar (Castiglione et al., 2008) and nutrient agar (Waksman, 1967). The ISCC-NBS colour chart was used to determine the names and designations of colony colours (Kelly, 1964). Temperature tolerance (4, 10, 20, 28, 37, 40, 45 and 50 °C) was determined on ISP 2 agar for 14–21 days. The range of pH (pH 4–11 at intervals 0.5 units, using 0.25 mmol l⁻¹ phosphate buffer systems) and NaCl (0, 1, 2, 3, 5, 7, 10, 15 and 25 %, w/v) for growth were determined with the same cultural condition above at 28 °C. Gelatin liquefaction, milk peptonization, nitrate reduction and starch hydrolysis were determined as described by Arai (1975) and Williams & Cross (1971). Carboxymethyl-cellulose (CM-cellulose) hydrolysis was tested using Congo red as the developing agent (Teather & Wood, 1982) on ISP 2 medium supplemented with 1 % CM-cellulose. Carbon source utilization was tested using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with 1 % (final concentration) of each carbon source. The utilization of amino acids as nitrogen sources was tested as described by Williams et al. (1983). Production of melanoid pigments was examined using ISP 6 and ISP 7 media (Shirling & Gottlieb, 1966). Other physiological and biochemical characteristics including hydrolysis of aesculin and production of catalase and H₂S were tested by the procedures of Williams et al. (1983) and Kämper et al. (1991).

Freeze-dried cells were used for the chemotaxonomic studies. The strain was first cultured in ISP 2 broth at 28 °C for 3 days on a rotary shaker (220 r.p.m.). Cell-wall amino acids and whole-cell sugars were analysed according to the procedure of Lechevalier & Lechevalier (1980). Analysis of phospholipids was carried out using TLC according to Minnikin et al. (1984). Fatty acids were extracted from fresh cells obtained with the same cultural conditions above using the method of Sasser (1990) and the composition was determined by GC-MS performed on a GC instrument (Li et al., 2013a). Menaquinones were extracted from freeze-dried biomass, purified according to Minnikin et al. (1984) and finally analysed by HPLC with an ODS-BP C18 column (4.6x250 mm); the elution solvent was methanol and 2-propanol (3:2, v/v) based on the method of Li et al. (2013a).

DNA for PCR was obtained by Fast Pre-24 instrument (MP Biomedicals). PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Nakajima et al. (1999). The 16S rRNA gene sequence of strain 2902at01T was subjected to multiple alignments with selected sequences obtained from the GenBank database using CLUSTAL X software. The alignment was manually verified and adjusted prior to the sequence similarity determination and reconstruction of the phylogenetic trees. The phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), and maximum-parsimony (Kluge & Farris, 1969) methods with MEGA 5 software (Tamura et al., 2011). The stability of the clades of the phylogenetic tree was determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. PCR amplification of the gyrB gene was carried out using primers GYF1 and GYRB3 (Garca et al., 2010) and the PCR program for 16S rRNA gene. Sequencing and phylogenetic analysis was performed as described above.

Genomic DNA was extracted as described by Pospiech & Neumann (1995). The DNA G+C content of strain 2902at01T was measured using the HPLC method (Mesbah et al., 1989). DNA–DNA hybridization between strain 2902at01T and the closest related species was determined on nylon membranes using the method described by Wang et al. (2011), with labelled denatured DNA (250 ng) of test strain and immobilized denatured DNA (500 ng) of all strains. Reciprocal hybridizations were performed where the denatured DNA was swapped between the three strains. Each test was repeated twice.

The morphological, cultural and chemotaxonomic properties of strain 2902at01T were consistent with its classification in the genus Micromonospora. The strain formed well-developed and branched substrate hyphae on ISP 2 medium, but did not form aerial mycelium on any of the media tested. Single or cluster spores were spherical and non-motile borne from the substrate mycelium at a diameter of approximately 0.5–0.7 μm (Fig. S1, available in the online Supplementary Material). Good growth was observed on ATCC 172 agar, ISP 2 agar medium, MB agar and NA medium. Melanin was not produced on ISP 6 or ISP 7 agar, and no soluble pigment was produced on any of the tested media (Table S1).

The cell wall of strain 2902at01T contained alanine, asparagine, glycine, meso-diaminopimelic acid (DAP), DD-DAP and a small amount of LL-DAP (Fig. S2). The whole-cell sugars were ribose, xylose and a small amount of galactose (Fig. S3); arabinose was not detected though it can be found in most of the members of the genus Micromonospora. Similar results have been reported for other species of the genus (Trujillo et al., 2005; Carro et al., 2012). The polar lipids of strain 2902at01T were phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, diphosphatidylglycerol, an unknown aminolipid, an unknown phosphoglycolipid and unknown phospholipids, corresponding to phospholipid type PII (Fig. S4). The predominant menaquinones were MK-10(H₄) and MK-10(H₈). The major fatty acids of strain 2902at01T were iso-C₁₅:0 anteiso-C₁₅:0 and iso-C₁₆:0 (Table S2). The DNA G+C content was 70.2 mol%.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence (1480 nt) of strain 2902at01T indicated that the strain should be assigned to the genus Micromonospora with the closest related species being Micromonospora equina Y22T (98.3 % 16S rRNA gene sequence similarity) and M. pattaloongensis TJ2-2T (98.1 %). Phylogenetic trees presented in Fig. 1 and Fig. S5 showed strain
2902at01^T was located in the root of the branch comprised of *M. equina* Y22^T and *M. pattaloongensis* TJ2-2^T with bootstrap values lower than 50%; i.e. 18% and 20% of the branches connected with *M. equina* Y22^T and *M. pattaloongensis* TJ2-2^T, respectively, which indicated that the location of the novel strain was rather unstable. In addition, a partial *gyrB* gene sequence (1147 nt) was obtained, and *gyrB* gene phylogeny clearly showed that strain 2902at01^T should be assigned to the genus *Micromonospora*, but was different from any established species of the genus *Micromonospora* (Fig. S6). Further study showed that the DNA–DNA hybridization values with *M. equina* Y22^T and *M. pattaloongensis* TJ2-2^T were 26.7–27.7% and 34.8–36.0%, respectively, and these results supported the assumed relationship between strain 2902at01^T and the reference strains.

In summary, the morphological, biochemical and chemotaxonomic features and phylogenetic data suggested that strain 2902at01^T was a member of the genus *Micromonospora* when compared with the reference strains *M. equina* Y22^T and *M. pattaloongensis* TJ2-2^T, but could be distinguished from previously described species (Table 1). It is proposed that strain 2902at01^T represents a novel species of the genus *Micromonospora* with the name *Micromonospora zhanjiangensis* sp. nov.

**Description of Micromonospora zhanjiangensis** sp. nov.

*Micromonospora zhanjiangensis* (zhan.jiang.en’sis. N.L. fem. adj. zhanjiangensis pertaining to Zhanjiang city in
Guangdong, province of China, from where the type strain was isolated.

Aerobic, Gram-stain-positive actinomycete that forms well-developed and branched substrate hyphae. Aerial hyphae are absent. Smooth, non-motile single spores are produced at the tip of substrate hyphae. Colonies are folded and raised. The colonies are strong orange red. No soluble pigment is produced on any of the tested media. Growth occurs at 10–40°C and pH 5–10 (optimum pH 7). The maximum NaCl concentration for growth is 2%. Positive for catalase production, hydrolysis of aesculin, gelatin liquefaction, milk peptonization and starch hydrolysis. Negative result in tests for CM-cellulose hydrolysis, H2S production and melanin production. Nitrate is reduced to nitrite. Grows well on ISP 9 agar medium to which the following sole carbon sources are added: cellobiose, D-galactose, inositol, a-lactose, maltose, D-mannitol, D-mannose, melibiose, D-ribose, D-salicin and D-xylose. Can weakly utilize L-arabinose, D-fructose, trehalose, raffinose and L-rhamnose as sole carbon sources, but not adonitol, D-maltose, melezitose or succinic acid. Utilizes the following sole nitrogen sources: L-alanine, L-asparagine, L-cysteine, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-proline, L-serine, L-threonine, L-tryptophan and L-valine. The characteristic whole-cell sugars contain ribose and xylose. The characteristic cell-wall hydrolysates include alanine, asparagine, glycine, meso-DAP, glycine, alanine, glutamic acid.

**Table 1. Differential characteristics of strain 2902at01T and the reference strains**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour on ISP 2 medium</td>
<td>Red orange yellow</td>
<td>Deep orange</td>
<td>Yellowish-white</td>
</tr>
<tr>
<td>Major menaquinones (%)</td>
<td>MK-10(H4) (57.4), MK-10(H6) (21.6)</td>
<td>MK-10(H4) (30.0), MK-9(H4) (22.0), MK-10(H4) (16.0)</td>
<td>MK-10(H4) (56.1), MK-10(H6) (22.8)</td>
</tr>
<tr>
<td>Major polar lipids†</td>
<td>DPG, PIM, PI, PE</td>
<td>PE, PI, PIM</td>
<td>DPG, PIM, PI, PE</td>
</tr>
<tr>
<td>Major fatty acids (%)</td>
<td>iso-C15:0 (30.5) anteiso-C15:0 (10.2) iso-C16:0 (14.2)</td>
<td>iso-C15:0 (29.0) anteiso-C15:0 (9.6) iso-C16:0 (7.5)</td>
<td>iso-C15:0 (24.4) anteiso-C15:0 (10.0) iso-C16:0 (38.9)</td>
</tr>
<tr>
<td>Whole-cell sugars</td>
<td>Xylose, ribose, galactose</td>
<td>Xylose, ribose, arabinose, glucose</td>
<td>Xylose, arabinose</td>
</tr>
<tr>
<td>Major cell-wall amino acid</td>
<td>meso-DAP, glycine, alanine asparagine</td>
<td>meso-DAP, glycine</td>
<td>meso-DAP, glycine, alanine, glutamic acid</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>70.2</td>
<td>ND</td>
<td>71.5†</td>
</tr>
<tr>
<td>CM-cellulose decomposition</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Milk peptonization</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 40°C</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Utilization of sole carbon source</td>
<td>Succinic acid</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>Melibiose</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>D-Mannitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Inositol</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>Raffinose</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of sole nitrogen source</td>
<td>L-Proline</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>L-Serine</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>L-Methionine</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td>L-Valine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>L-Cysteine</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Data from: a, Everest & Myers (2013); b, Thawai et al. (2008).
†PE, Phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; DPG, diphasphatidylglycerol.
The type strain, 2902a01T (≡CCTCC AA2014018T = DSM 45902T), was isolated from mangrove soil in Zhanjiang, Guangdong province, China. The DNA G+C content of the type strain is 70.2 mol%.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (no. 31170467) and the EU FP7 project PharmaSea (312184).

References


