**Planotetraspora silvatica** sp. nov. and emended description of the genus *Planotetraspora*

Tomohiko Tamura and Takeshi Sakane

The genus *Planotetraspora* was proposed by Runmao *et al.* (1993) to accommodate an actinomycete that was characterized by forming long, cylindrical sporangia containing four spores in a single row at the ends of short sporangiophores on aerial hyphae. This organism was isolated from subtropical forest soil. The isolate contained menaquinone MK-9(H4), glutamic acid, alanine and meso-diaminopimelic acid as cell-wall amino acids and madurose in the whole-cell hydrolysate. The 16S rRNA gene sequence indicated that the isolate formed a monophyletic cluster with *Planotetraspora mira*. On the basis of morphological and chemotaxonomic characteristics, phylogenetic analysis and DNA–DNA relatedness data, a novel species of the genus *Planotetraspora* is proposed, *Planotetraspora silvatica* sp. nov. (type strain, TT 00-51T = NBRC 100141T = DSM 44746T).

**Abbreviation:** A2pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Planotetraspora silvatica* TT 00-51T is AB112082.

A scanning electron micrograph of strain TT 00-51T is available as supplementary material in IJSEM Online.

The strain utilized mannose, lactose, galactose, methyl z-D-glucoside, maltose, rhamnose, melibiose and raffinose. Decomposition of hypoxanthine and L-tyrosine was positive, but resistance to 4% NaCl, hydrolysis of starch,
utilization of lactate, malate, succinate, citrate and oxalate and decomposition of urea, adenine, cellulose and calcium malate were negative. The strain showed good growth at 25–30 °C. In contrast, *P. mira* NBRC 15435<sup>T</sup> did not utilize maltose or galactose or decompose L-tyrosine.

Analyses of whole-cell sugar pattern, cell-wall amino acids, menaquinones, cellular fatty acids, isomers of A<sub>2pm</sub>, acyl type of peptidoglycan, mycolic acid and DNA G+C content were performed as described previously (Tamura et al., 1994).

The new strain contained glucose, madurose, galactose, rhamnose and 3-O-methylmannose as whole-cell sugars. In this study, consistent with the results of Kudo (2001), *P. mira* NBRC 15435<sup>T</sup> was found to contain madurose and rhamnose, but not xylose or arabinose. The amino acids in the cell wall of the new strain were meso-A<sub>2pm</sub>, alanine and glutamic acid; this corresponds to murein type A<sub>1/2</sub>, according to Schleifer & Kandler (1972). The predominant isoprenoid quinones of the new strain were MK-9(H<sub>4</sub>), MK-9(H<sub>2</sub>) and MK-9. Phosphatidylethanolamine and an unidentified phospholipid containing glucosamine were detected as diagnostic phospholipids, but phosphatidyglycerol and phosphatidylcholine were not detected. The major isoprenoid quinone and phospholipid pattern of strain TT 00-51<sup>T</sup> were consistent with those of *P. mira* (Kudo, 2001). Mycolic acids were not detected. The glycan moiety of the murein contained acetyl residues. The cellular fatty acids consisted of iso-branched, anteiso-branched, saturated, unsaturated and 10-methylated fatty acids, corresponding to fatty acid pattern 3d of Kroppenstedt (1985). The strain contained diagnostic amounts of 10-methylated C<sub>18:0</sub> and iso-C<sub>16:0</sub>(>14%). The DNA G+C content of the isolate ranged from 70 to 71 mol%.

The microplate hybridization method developed by Ezaki et al. (1999) was applied with minor modifications to determine DNA–DNA relatedness (Tamura et al., 1994).

The strain exhibited DNA–DNA relatedness levels of 38–42 % with *P. mira* NBRC 15435<sup>T</sup>.

PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Tamura & Hatano, 2001) with a model ABI PRISM 3100 genetic analyser (Applied Biosystems) according to the manufacturer’s protocol. Phylogenetic analysis of 16S rRNA gene sequences was performed as described previously (Tamura & Hatano, 2001). This phylogenetic analysis revealed that the isolate fell within the cluster of the family *Streptosporangiaceae* and, with *P. mira* NBRC 15435<sup>T</sup>, formed a line of descent that was distinct from other actinomycetes of this family (Fig. 1).

Strain TT 00-51<sup>T</sup> developed cylindrical sporangia at the ends of short sporangiophores on aerial hyphae, with each sporangium containing four spores in a single row. These morphological characteristics are consistent with those of the genus *Planetetraspora*. The genus *Planetetraspora* resembles the genus *Microtetraspora* in this characteristic (Kudo, 2001), but the two genera can be distinguished by their 16S rRNA gene sequences. Strain TT 00-51<sup>T</sup> formed a monophyletic cluster with *P. mira* NBRC 15435<sup>T</sup> and the isolate were different from those of other members of the family *Streptosporangiaceae* (positions 502–543 (A-U) and 1116–1184 (U-G) of *P. mira* NBRC 15435<sup>T</sup> and the isolate were different from those of other members of the family *Streptosporangiaceae* (positions 502–543 (G-C), 1116–1184 (C-G)). The closest neighbours are members of the genera *Acrocarpaspora* (similarity values 95.8–97.1%) and *Herbidospora* (similarity values 95.8–97.1%).

\[ \begin{align*}
\text{Fig. 1.} & \quad \text{Phylogenetic tree based on neighbour-joining (Saitou & Nei, 1987), derived from 16S rRNA gene sequences for members of the family *Streptosporangiaceae*. Streptomycyes ambofaciens ATCC 23877<sup>T</sup> (GenBank accession no. MT7245) was used as the root. Numbers on branches are confidence limits (expressed as percentages) estimated from a bootstrap analysis with 1000 replicates (only percentages above 50% are indicated). Bar, 0.01 K_{\text{nuc}}.}
\end{align*} \]
hyphae. Members of the genus *Planotetraspora* can be distinguished from these two genera on the basis of morphological criteria, although the three genera show high 16S rRNA gene sequence similarity.

On the basis of morphological, chemotaxonomic and phylogenetic criteria, strain TT 00-51T is considered to belong to the genus *Planotetraspora* and to represent a distinct species, based on DNA–DNA relatedness and physiological characteristics (Table 1). We propose that the isolate should be classified as the type strain of a novel species, *Planotetraspora silvatica* sp. nov. (type strain, TT 00-51T = NBRC 100141T = DSM 44746T).

**Emended description of the genus *Planotetraspora* Runmao et al. 1993**

The description is based on data taken from earlier studies (Runmao et al., 1993; Kudo, 2001) and our own studies. Cells are Gram-positive, non-acid-fast and aerobic, with branching hyphae. Non-fragmentary substrate mycelia are present. Long, cylindrical sporangia are formed at the ends of short sporangiophores on aerial hyphae, with each sporangium containing four spores in a single row. Spores are short cylindrical, short rod or oval in shape (0·4–1·4 × 0·8–1·5 μm) and may exhibit motility. Good growth occurs between 25 and 30 °C. In general, vegetative mycelia are pale yellow to white. Cell walls contain glutamic acid, alanine and meso-Δ2pm. Wall chemotype is III, according to Lechevalier & Lechevalier (1970), and the peptidoglycan type is presumed to be A1γ, according to Schleifer & Kandler (1972). Maltose, 3-O-methylmannose, rhamnose, glucose and galactose are detected as whole-cell sugars. Major cellular fatty acid is 10-methylated C₁₈ : 0. Major menaquinone is MK-9(H₄). Phosphatidylethanolamine is present as the diagnostic phospholipid [phospholipid pattern type PIV, according to Lechevalier et al. (1977)]. Acyl type of cell-wall polysaccharides is acetyl. Mycolic acid is not detected. DNA G+C content is 71 mol%. Habitat is soil. The type species is *Planotetraspora mira*.

**Description of *Planotetraspora silvatica* sp. nov.**

*Planotetraspora silvatica* (sil.va’ti.ca. L. fem. adj. *silvatica* of the forest).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Brownish, soluble pigment is produced on tyrosine agar (ISP medium 7). Starch is not hydrolysed. Hydrolysis of gelatin is negative or weakly positive. Calcium malate is not decomposed.

**Table 1. Diagnostic characteristics that differentiate *P. silvatica* TT 00-51T from *P. mira* NBRC 15435T.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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</thead>
<tbody>
<tr>
<td><strong>Cultural characteristics</strong></td>
<td></td>
<td></td>
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<tr>
<td>ISP medium no. 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>Good, white to colourless</td>
<td>Good, pale yellow (45)*</td>
</tr>
<tr>
<td>Aerial mycelium or spore</td>
<td>Good, white</td>
<td>Moderate, white</td>
</tr>
<tr>
<td>Reverse colour</td>
<td>White to colourless</td>
<td>Pale yellow (45)</td>
</tr>
<tr>
<td>ISP medium no. 4:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>Good, white to colourless</td>
<td>Poor, colourless</td>
</tr>
<tr>
<td><strong>Physiological characteristics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Acid from:</td>
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<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+ +</td>
<td>±</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+ +</td>
<td>±</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Decomposition of xanthine</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*The code of colours corresponds to the mycological colour chart of Rayner (1970).*
Coagulation and peptonization of milk are positive. Optimum temperature for growth is 25–30 °C. Does not grow at 37 °C. Does not grow on 4% NaCl. Glucose, mannose, lactose, galactose, methyl α-D-glucoside, maltose, rhamnose and melibiose are utilized, but dulcitol, erythritol, adonitol and arabinose are not. As major cellular fatty acids, 10-methylated C_{18:0} and iso-C_{16:0} are present. DNA G+C content is 71 mol%.

The type strain is TT 00-51T (＝NBRC 100141T＝DSM 44746T). Habitat is soil.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research (C) (2) no. 11660326 from the Japan Society for the Promotion of Science. The authors are grateful to Drs A. Nakagiri, Y. Nakagawa, I. Okane and K. Ueda-Nishimura for kind help.

References


