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

Tomohiko Tamura and Takeshi Sakane

Correspondence

Tomohiko Tamura
tamura-tomohiko@nite.go.jp

Biological Resource Center, National Institute of Technology and Evaluation,
2-5-8 Kazusakamatari, Kisarazu, Chiba, 292-0812, Japan

An actinomycete that developed sporangia containing four spores in a single row at the ends of short sporangiophores on branched aerial hyphae 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 00-51^T = NBRC 100141^T = DSM 44746^T).

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, having meso-diaminopimelic acid (meso-A₂pm) in the cell wall and galactose, arabinose, xylose, mannose and ribose in whole-cell hydrolysate. *Planotetraspora mira*, which was isolated from soil collected in China, was the only known species to belong to the genus *Planotetraspora*. Kudo (2001) reported that this organism contains madurose and rhamnose, but not arabinose or xylose in its whole-cell hydrolysate, has MK-9(H₄) as the major isoprenoid quinone and has phospholipid pattern PIV. This genus has been classified in the family *Streptosporangiaceae* by 16S rRNA gene sequence analysis (Stackebrandt *et al.*, 1997; Yokota, 1997).

During a study of the distribution of actinomycetes in the subtropical field, strain TT 00-51^T was isolated from soil of Amami Island, Japan. The isolate was judged to belong to the genus *Planotetraspora* by phylogenetic analysis and chemotaxonomic characteristics, and to represent a novel species of the genus *Planotetraspora* by DNA–DNA relatedness and physiological characteristics. Therefore, we propose the name *Planotetraspora silvatica* sp. nov. for strain TT 00-51^T.

Strain TT 00-51^T (=NBRC 100141^T = DSM 44746^T) was isolated from a sample of forest soil in Amami Island,

Kagoshima prefecture, Japan. This organism was isolated on humic acid/vitamin (HV) agar (Hayakawa & Nonomura, 1987) by using the yeast extract/SDS method (Hayakawa & Nonomura, 1989). Freeze-dried cells for chemotaxonomic analyses were grown in yeast extract/glucose broth [10 g yeast extract, 10 g D-glucose (1 distilled water)⁻¹, pH 7.0] on a rotary shaker at 28 °C. *P. mira* NBRC 15435^T was also analysed to compare whole-cell sugar patterns.

Cultural and physiological characteristics were determined as described previously (Gordon *et al.*, 1974; Seino *et al.*, 1985; Shirling & Gottlieb, 1966; Yokota *et al.*, 1993). Morphology was observed by scanning electron microscopy as described previously (Tamura *et al.*, 2000).

Strain TT 00-51^T developed sporangia at the ends of short sporangiophores on branched aerial mycelium. Each sporangium was seen to contain four spores. Globose bodies were not observed. Spores were oval to short rods (0.6–0.9 × 0.8–1.5 µm); motile spores were not observed. A micrograph is available as supplementary material in IJSEM Online.

The strain exhibited good growth on yeast extract/malt extract agar, glycerol/asparagine agar and tyrosine agar, and developed white colonies. In contrast, *P. mira* NBRC 15435^T developed yellow colonies on yeast extract/malt extract agar. The new strain developed white aerial mycelium on yeast extract/malt extract agar, inorganic salts/starch agar, glycerol/asparagine agar, tyrosine agar and HV agar.

The strain utilized mannose, lactose, galactose, methyl α-D-glucoside, maltose, rhamnose, melibiose and raffinose. Decomposition of hypoxanthine and L-tyrosine was positive, but resistance to 4% NaCl, hydrolysis of starch,

Abbreviation: A₂pm, diaminopimelic acid.

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

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

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^T 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 A₂pm, 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^T 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*-A₂pm, alanine and glutamic acid; this corresponds to murein type A1 γ , according to Schleifer & Kandler (1972). The predominant isoprenoid quinones of the new strain were MK-9(H₄), MK-9(H₂) and MK-9. Phosphatidylethanolamine and an unidentified phospholipid containing glucosamine were detected as diagnostic phospholipids, but phosphatidylglycerol and phosphatidylcholine were not detected. The major isoprenoid quinone and phospholipid pattern of strain TT 00-51^T 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_{18:0} and iso-C_{16:0} (> 14 %). The DNA G + C content of the isolate ranged from 70 to 71 mol%.

The microplate hybridization method developed by Ezaki *et al.* (1988, 1989) was applied with minor modifications to determine DNA–DNA relatedness (Tamura *et al.*, 1999).

The strain exhibited DNA–DNA relatedness levels of 38–42 % with *P. mira* NBRC 15435^T.

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^T, formed a line of descent that was distinct from other actinomycetes of this family (Fig. 1). The signature nucleotides of the 16S rRNA gene at positions 502–543 (A-U) and 1116–1184 (U-G) of *P. mira* NBRC 15435^T 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 *Acrocarpospora* (similarity values 95.8–97.1 %) and *Herbidospora* (similarity values 95.8–97.1 %).

Strain TT 00-51^T 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 *Planotetraspora*. The genus *Planotetraspora* 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^T formed a monophyletic cluster with *P. mira* NBRC 15435^T. Strain TT 00-51^T and *P. mira* are the phylogenetically closest neighbours of the genera *Herbidospora* (Kudo *et al.*, 1993) and *Acrocarpospora* (Tamura *et al.*, 2000). Members of the genus *Herbidospora* develop short chains of non-motile spores (10–30 spores per chain) that are borne on the tips of sporophores arising directly from the agar surface. Members of the genus *Acrocarpospora* develop a few club-shaped and many spherical sporangia that are borne on the tips of aerial

Fig. 1. Phylogenetic tree based on neighbour-joining (Saitou & Nei, 1987), derived from 16S rRNA gene sequences for members of the family *Streptosporangiaceae*. *Streptomyces ambofaciens* ATCC 23877^T (GenBank accession no. M27245) 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_{nuc} .

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 00-51^T 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 00-51^T = NBRC 100141^T = DSM 44746^T).

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-A₂pm. Wall chemotype is III, according to Lechevalier & Lechevalier (1970), and the peptidoglycan type is presumed to be A1γ, according to Schleifer & Kandler (1972). Madurose, 3-O-methylmannose, rhamnose, glucose and galactose are detected as whole-cell sugars. Major cellular fatty acid is 10-methylated C_{18:0}. 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 00-51^T from *P. mira* NBRC 15435^T

Species: 1, *P. silvatica* TT 00-51^T; 2, *P. mira* NBRC 15435^T.

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

*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 00-51^T (=NBRC 100141^T=DSM 44746^T). 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

- Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S.-L., Miura, H., Matsui, K. & Yabuuchi, E. (1988). Simple genetic method to identify viridans group streptococci by colorimetric dot hybridization and quantitative fluorometric hybridization in microdilution wells. *J Clin Microbiol* **26**, 1708–1713.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.
- Hayakawa, M. & Nonomura, H. (1989). A new method for the intensive isolation of actinomycetes from soil. *Actinomycetologica* **3**, 95–104.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Kudo, T. (2001). Family *Streptosporangiaceae*. In *Identification Manual of Actinomycetes*, pp. 259–276. Edited by S. Miyadoh, M. Hamada, K. Hotta, T. Kudo, A. Seino, K. Suzuki & A. Yokota. Tokyo, Japan: Business Center for Academic Societies.
- Kudo, T., Itoh, T., Miyadoh, S., Shomura, T. & Seino, A. (1993). *Herbidospora* gen. nov., a new genus of the family *Streptosporangiaceae* Goodfellow et al. 1990. *Int J Syst Bacteriol* **43**, 319–328.
- Lechevalier, M. P. & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P., De Bievre, C. & Lechevalier, H. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Rayner, R. W. (1970). *A Mycological Colour Chart*. Kew, UK: Commonwealth Mycological Institute and British Mycological Society.
- Runmao, H., Guizhen, W. & Junying, L. (1993). A new genus of actinomycetes, *Planotetraspora* gen. nov. *Int J Syst Bacteriol* **43**, 468–470.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Seino, A., Arai, M., Enokida, R., Okazaki, T. & Furuichi, A. (1985). *Identification Manual of Actinomycetes*. Tokyo, Japan: Society for Actinomycetes.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Tamura, T. & Hatano, K. (2001). Phylogenetic analysis of the genus *Actinoplanes* and transfer of *Actinoplanes minutisporangius* Ruan et al. 1986 and '*Actinoplanes aurantiacus*' to *Cryptosporangium minutisporangium* comb. nov. and *Cryptosporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* **51**, 2119–2125.
- Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E. & Yokota, A. (1994). A new genus of the order *Actinomycetales*, *Couchioplanes* gen. nov., with descriptions of *Couchioplanes caeruleus* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int J Syst Bacteriol* **44**, 193–203.
- Tamura, T., Hayakawa, M. & Hatano, K. (1999). *Sporichthya brevicatena* sp. nov. *Int J Syst Bacteriol* **49**, 1779–1784.
- Tamura, T., Suzuki, S. & Hatano, K. (2000). *Acrocarpospora* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Evol Microbiol* **50**, 1163–1171.
- Yokota, A. (1997). Phylogenetic relationship of actinomycetes. In *Atlas of Actinomycetes*, pp. 194–197. Edited by M. Hamada, K. Hotta, T. Kudo, S. Miyadoh, A. Seino, K. Suzuki & A. Yokota. Tokyo: Asakura Publishing Company.
- Yokota, A., Tamura, T., Hasegawa, T. & Huang, L. H. (1993). *Catenuloplanes japonicus* gen. nov., sp. nov., nom. rev., a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **43**, 805–812.