Planosporangium mesophilum sp. nov., isolated from rhizosphere soil of Bletilla striata

Yan-Ru Cao,1,2 Qian Wang,2 Rong-Xian Jin,2 Yi Jiang,2 Hang-Xian Lai,1 Wen-Xiang He,1 Li-Hua Xu2 and Cheng-Lin Jiang2

A Gram-stain-positive, non-motile actinomycete, designated strain YIM 48875T, was isolated from rhizosphere soil of Bletilla striata and its taxonomic position was established by using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequence data showed that strain YIM 48875T belonged to the genus Planosporangium, supported by a bootstrap value of 100%. Cells of strain YIM 48875T showed two kinds of sporangia, which also supported its classification in the genus Planosporangium. Strain YIM 48875T grew optimally at 28°C, at pH 6.0–8.0 and in the presence of 2% (w/v) NaCl. The level of 16S rRNA gene sequence similarity between strain YIM 48875T and Planosporangium flavigriseum YIM 46034T was 98.6%. Strain YIM 48875T exhibited a quinone system with menaquinones MK-9(H₄), MK-9(H₆) and MK-9(H₈) as the predominant compounds, a polar lipid profile comprising diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannoside and the major fatty acids iso-C₁₅ : 0 and iso-C₁₆ : 0; these data were markedly different from those for P. flavigriseum YIM 46034T. The level of DNA–DNA relatedness between strain YIM 48875T and P. flavigriseum YIM 46034T was 45.5%. It is apparent from the genotypic and phenotypic data that strain YIM 48875T represents a novel species of the genus Planosporangium, for which the name Planosporangium mesophilum sp. nov. is proposed. The type strain is YIM 48875T (=CCTCC AA 209049T =KCTC 19779T).

The genus Planosporangium, belonging to the family Micromonosporaceae, was proposed by Wiese et al. (2008) to accommodate Gram-positive actinomycetes that had motile or non-motile spores. At the time of writing, the genus comprises one recognized species, Planosporangium flavigriseum (Wiese et al., 2008). In the present polyphasic taxonomic study, a novel actinomycete, designated strain YIM 48875T, isolated from a rhizosphere soil is shown to represent a novel species of the genus Planosporangium.

During investigations into the selective isolation of members of the genus Actinoplanes, strain YIM 48875T was recovered from a Bletilla striata rhizosphere soil collected from Xishuangbanna, Yunnan Province, southwest China. One gram of the soil sample (dried for 7 days at room temperature and then for 1 h at 100°C) was first suspended in 9 ml sterilized distilled water in a small plate, a capillary tube (Palleroni, 1980) full of pollen (Hayakawa et al., 1991) solution was placed into the plate, and this was then incubated at 28°C for 1 h. The solution in the capillary tube was extracted, diluted and spread onto improved medium for the isolation of Actinoplanes, which contained (per litre distilled water): 60 g oatmeal, 2.5 g yeast, 1 g K₂HPO₄, 0.5 g KCl, 0.5 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O and 15 g agar (pH 7.5). Nystatin (100 mg l⁻¹) and nalidixic acid (25 mg l⁻¹) were sterilized separately before being added to the medium. Incubation for isolation was performed at 28°C for 30 days. The strain thus isolated was maintained on medium YIM 38 (Jiang et al., 2007) at 28°C and showed a light orange-coloured substrate mycelium on this medium, with white aerial hyphae. Biomass for chemotaxonomic and molecular systematic studies was derived from a 14-day-old (6-day-old for fatty acid analysis) Bacto trypticase soy broth shake culture incubated at 28°C, harvested by centrifugation and washed twice with distilled water.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 48875T is GU126491. A supplementary figure is available with the online version of this paper.
48875T was aligned with available corresponding sequences. The resulting 16S rRNA gene sequence of strain YIM 48875T was 71.6 mol% similar with *P. flavigriseum* similarity calculations indicated that the closest relative of strain YIM 46034T (98.6 %) and the tree evolutionary distance matrices (distance options according to Kimura's two-parameter model) were performed according to the method described by Farris et al. (1969) and neighbour-joining (Saitou & Nei, 1987). DNA–DNA hybridization experiments between strain YIM 48875T and *P. flavigriseum* YIM 46034T were performed according to the method described by Ezaki et al. (1989) and He et al. (2005). Genomic DNA for analysis of G+C content was extracted as described by Marmur (1961). The G+C content of the DNA was determined by HPLC (Mesbah et al., 1989).

In the neighbour-joining phylogenetic tree, strain YIM 48875T clustered with *P. flavigriseum* YIM 46034T (Fig. 1). This demonstrated that the novel strain belongs to the genus *Planosporangium*, supported by a bootstrap value of 100 %, although strain YIM 48875T formed a distinct phyletic line in this genus. This relationship was also supported in the tree generated with the maximum-parsimony method. Although strain YIM 48875T shared 98.6 % 16S rRNA gene sequence similarity with *P. flavigriseum* YIM 46034T, the level of DNA–DNA relatedness between the two strains was only 45.5 %, which is below the 70 % cut-off point recommended for the delineation of genomic species (Wayne et al., 1977) were analysed by HPLC (Groth et al., 1997), fatty acids by using the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) and polar lipids were detected as described by Minnikin et al. (1979) and Collins & Jones (1980). Strain YIM 48875T was characterized by a cell wall containing meso-diaminopimelic acid and whole-cell hydrolysates containing mannose, ribose, glucose and galactose. The major menaquinones were MK-9(H6), MK-9(H8) and MK-9(H10) and the polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine and

Gram staining was confirmed by using the standard Gram reaction (Gram, 1884). Cell morphology was examined by light microscopy (BH-2; Olympus) and scanning electron microscopy (ISM 5600LV; JEOL) of 9-week cultures grown on ISP 2 medium. Cell motility was confirmed by the presence of turbidity throughout tubes containing semi-solid medium (Leifson, 1960). Cultural characteristics were observed on Czapek's agar (Waksman, 1967), potato dextrose agar (PDA) and ISP media 1–5 (Shirling & Gottlieb, 1966) at 28 °C. Colony colours were recorded with reference to Kelly (1964). The pH and temperature ranges for growth were established as described by Xu et al. (2005), and tolerance of NaCl was tested on ISP 2 medium. Other physiological tests, including the determination of enzyme activities, utilization of sole carbon sources and decomposition of test substrates, were carried out as described by Gordon et al. (1974). Formation of characteristic sporangia was observed (Fig. 2) and spore motility was not found. Both pestle-like and globose spores (Fig. 2) were observed clearly. Strain YIM 48875T formed a white aerial mycelium after 3 weeks on all the test media except nutrient agar and PDA. The substrate mycelium of strain YIM 48875T was white on Czapek’s agar and light orange to light salmon pink on other test media. Good growth occurred on ISP 2 and ISP 3, and weak growth on Czapek's agar, nutrient agar and PDA. No diffusible pigment was produced on any of the test media. Other physiological characteristics of strain YIM 48875T are given in Table 1 and in the species description.

Standard procedures were used to extract and analyse the cell-wall amino acids and sugars in whole-cell hydrolysates (Hasegawa et al., 1983). Menaquinones (Collins et al., 1977) were analysed by HPLC (Groth et al., 1997), fatty acids by using the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) and polar lipids were detected as described by Minnikin et al. (1979) and Collins & Jones (1980). Strain YIM 48875T was characterized by a cell wall containing meso-diaminopimelic acid and whole-cell hydrolysates containing mannose, ribose, glucose and galactose. The major menaquinones were MK-9(H6), MK-9(H8) and MK-9(H10) and the polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine and

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**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain YIM 48875T and the type strains of related members of the *Micromonosporaceae*. Asterisks denote branches that were also recovered by using the maximum-parsimony method. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >50 % are given. Bar, 0.5 % sequence divergence.

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phosphatidylinositol mannoside (see Supplementary Fig. S1, available in IJSEM Online). The fatty acid profile was dominated by iso-C15:0 (27.56 % of the total) and iso-C16:0 (20.47 %); anteiso-C15:0 (6.50 %), anteiso-C17:0 (8.98 %), summed feature 9 (iso-C17:1ω9c and/or 10-methyl C16:0 8.98 %) and C17:1ω8c (5.20 %) were found as minor components.

The morphological and chemical characteristics of strain YIM 48875T together with data from phylogenetic analysis indicated that it is a member of the genus Planosporangium. However, it is evident that strain YIM 48875T can be distinguished from the sole recognized species of the genus, P. flavigriseum, by using a combination of phenotypic properties. In contrast to P. flavigriseum YIM 46034T, strain YIM 48875T was able to utilize inositol, lactose, D-fructose and sodium acetate as sole carbon sources, but not galactitol, sorbitol, L-rhamnose, cellobiose, arabinose or sucrose. Strain YIM 48875T can be differentiated from P. flavigriseum YIM 46034T based on the presence of phosphatidylinositol mannoside in the polar lipid profile. The profiles of major menaquinones [MK-9(H4), MK-9(H6) and MK-9(H8)] and fatty acids (iso-C15:0 and iso-C16:0) were highly significant in distinguishing strain YIM 48875T from P. flavigriseum YIM 46034T. The level of DNA–DNA relatedness between the two strains (45.5 %) confirmed this separate species status. It can be concluded from the genotypic and phenotypic data that strain YIM 48875T represents a novel species of the genus Planosporangium, for which the name Planosporangium mesophilum sp. nov. is proposed.

**Description of Planosporangium mesophilum sp. nov.**

Planosporangium mesophilum [me.so’phi.lum. Gr. adj. mesos middle; Gr. adj. philos loving; N.L. neut. adj. mesophilum middle (temperature)-loving, mesophilic].

Gram-stain-positive, non-motile actinomycete that produces pestle-like and globose spores. Forms a light orange to light salmon-pink vegetative mycelium. Aerial mycelium is white after 3 weeks and no diffusible pigment is produced. Grows at pH 6–8 and 28–30 °C. Tolerates 2 % NaCl in the culture medium. Catalase-positive and oxidase-negative. Degrades Tweens 20, 40 and 60. Negative for urease activity, H2S production, nitrate reduction, milk coagulation and peptonization and hydrolysis of gelatin, cellulose, starch and Tween 80. Utilizes inositol, lactose, D-fructose, D-galactose, raffinose, glucose, sodium acetate, D-mannose, maltose, D-xylose, D-ribose, calcium DL-malate, methanol and sodium oxalate as sole carbon sources, but not dulcitol, mannitol, sorbitol, L-rhamnose, cellobiose, sucrose, glycerol or arabinose. Utilizes DL-methionine and L-leucine as sole nitrogen sources, but not urea or L-histidine. The peptidoglycan contains meso-diaminopimelic acid. Diagnostic sugars are mannose, ribose, glucose and galactose. MK-9(H4), MK-9(H6) and MK-9(H8) are the predominant menaquinones. Polar lipids comprise diphasphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannoside. Major

### Table 1. Phenotypic properties that serve to distinguish strain YIM 48875T from P. flavigriseum YIM 46034T

Data for P. flavigriseum YIM 46034T were obtained in this study in parallel experiments. W, Weakly positive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>YIM 48875T</th>
<th>P. flavigriseum YIM 46034T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore motility</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
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<td>+</td>
</tr>
<tr>
<td>D-Fructose</td>
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<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
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<td>+</td>
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<tr>
<td>Raffinose</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>+</td>
</tr>
<tr>
<td>Sodium acetate</td>
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<td>–</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Polar lipid(s)*</td>
<td>DPG, PE, PIM</td>
<td>PE</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>9(H4), 9(H6), 9(H8) 9(H4), 10(H4)</td>
<td></td>
</tr>
<tr>
<td>Major fatty acids (&gt;10 %)†</td>
<td>i-C15:0, i-C16:0 ai-C17:0, i-C18:0 C17:1ω8c</td>
<td></td>
</tr>
</tbody>
</table>

*DPG, Diphasphatidylglycerol; PE, phosphatidylethanolamine; PIM, phosphatidylinositol mannoside.
†ai, Anteiso-branched; i, iso-branched.
fatty acids (>10%) are iso-C₁₅:₀ and iso-C₁₆:₀; smaller amounts (>5%) of anteiso-C₁₅:₀; anteiso-C₁₇:₀; summed feature 9 (iso-C₁₇:₁₀₇c and/or 10-methyl C₁₆:₀) and C₁₇:₁₀₈c are found. The DNA G+C content of the type strain is 71.6 mol%.

The type strain, YIM 48875T (= CCTCC AA 209049T = KCTC 19777T), was isolated from a rhizosphere soil of Bletilla striata in Xishuangbanna, Yunnan Province, China.

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References


