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

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A Gram-stain-positive, non-motile actinomycete, designated strain YIM 48875^T, 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 48875^T belonged to the genus *Planosporangium*, supported by a bootstrap value of 100 %. Cells of strain YIM 48875^T showed two kinds of sporangia, which also supported its classification in the genus *Planosporangium*. Strain YIM 48875^T 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 48875^T and *Planosporangium flavigriseum* YIM 46034^T was 98.6 %. Strain YIM 48875^T exhibited a quinone system with menaquinones MK-9(H_4), MK-9(H_6) and MK-9(H_8) as the predominant compounds, a polar lipid profile comprising diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannoside and the major fatty acids iso-$C_{15:0}$ and iso-$C_{16:0}$; these data were markedly different from those for *P. flavigriseum* YIM 46034^T. The level of DNA–DNA relatedness between strain YIM 48875^T and *P. flavigriseum* YIM 46034^T was 45.5 %. It is apparent from the genotypic and phenotypic data that strain YIM 48875^T represents a novel species of the genus *Planosporangium*, for which the name *Planosporangium mesophilum* sp. nov. is proposed. The type strain is YIM 48875^T (=CCTCC AA 209049^T =KCTC 19779^T).

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 48875^T, 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 48875^T 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 K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$ 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 48875^T is GU126491.

A supplementary figure is available with the online version of this paper.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out as described previously (Li *et al.*, 2007). The resulting 16S rRNA gene sequence of strain YIM 48875^T was aligned with available corresponding sequences of type strains of members of the *Micromonosporaceae* retrieved from the DDBJ/EMBL/GenBank databases by using the EzTaxon server (http://www.eztaxon.org/) (Chun *et al.*, 2007). Multiple alignments of the data were performed by using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic analysis was performed by using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic trees were inferred by using the maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms. Evolutionary distance matrices (distance options according to Kimura's two-parameter model) were generated as described by Kimura (1980) and the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. 16S rRNA gene sequence similarity calculations indicated that the closest relative of strain YIM 48875^T was *P. flavigriseum* YIM 46034^T (98.6 %). DNA–DNA hybridization experiments between strain YIM 48875^T and *P. flavigriseum* YIM 46034^T 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 48875^T clustered with *P. flavigriseum* YIM 46034^T (Fig. 1). This demonstrated that the novel strain belongs to the genus *Planosporangium*, supported by a bootstrap value of 100 %, although strain YIM 48875^T 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 48875^T shared 98.6 % 16S rRNA gene sequence similarity with *P. flavigriseum* YIM 46034^T, 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.*, 1987). The genomic G+C content of the DNA of strain YIM 48875^T was 71.6 mol%.

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 (JSM 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 48875^T formed a white aerial mycelium after 3 weeks on all the test media except nutrient agar and PDA. The substrate mycelium of strain YIM 48875^T 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 48875^T 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 48875^T 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(H_4), MK-9(H_6) and MK-9(H_8) and the polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine and

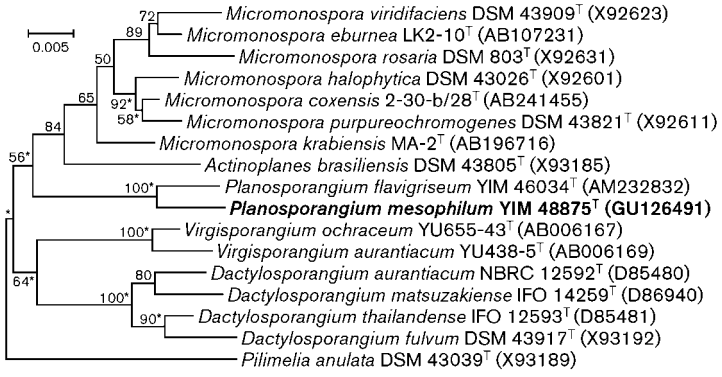

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain YIM 48875^T and the type strains of related members of the *Micromonosporaceae*. Asterisks denote branches that were also recovered by using the maximum-parsimony method. Numbers at 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.

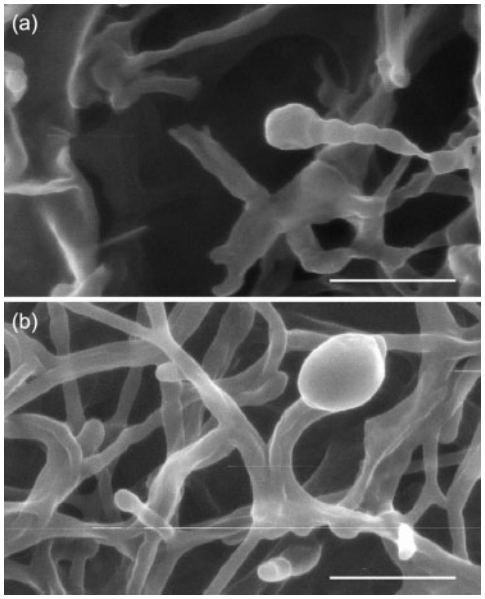

Fig. 2. Scanning electron micrographs of cells of strain YIM 48875^T grown on ISP 2 medium for 9 weeks at 28 °C. (a) Pestle-like spore; (b) globose spore. Bars, 2 μm.

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

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

Characteristic	YIM 48875^T	*P. flavigriseum* YIM 46034^T
Spore motility	−	+
Utilization of:		
Galactitol	−	+
Inositol	W	−
Sorbitol	−	+
Lactose	+	−
L-Rhamnose	−	+
Cellobiose	−	+
D-Fructose	+	−
D-Galactose	W	+
Raffinose	W	+
Arabinose	−	+
Glucose	W	+
Sucrose	−	+
Sodium acetate	+	−
DL-Methionine	W	−
Polar lipid(s)*	DPG, PE, PIM	PE
Predominant menaquinones	9(H_4), 9(H_6), 9(H_8)	9(H_4), 10(H_4)
Major fatty acids (>10 %)†	i-$C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$, i-$C_{16:0}$, $C_{17:1}\omega8c$

*DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PIM, phosphatidylinositol mannoside.
†ai, Anteiso-branched; i, iso-branched.

phosphatidylinositol mannoside (see Supplementary Fig. S1, available in IJSEM Online). The fatty acid profile was dominated by iso-$C_{15:0}$ (27.56 % of the total) and iso-$C_{16:0}$ (20.47 %); anteiso-$C_{15:0}$ (6.50 %), anteiso-$C_{17:0}$ (8.98 %), summed feature 9 (iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$, 8.98 %) and $C_{17:1}\omega8c$ (5.20 %) were found as minor components.

The morphological and chemical characteristics of strain YIM 48875^T together with data from phylogenetic analysis indicated that it is a member of the genus *Planosporangium*. However, it is evident that strain YIM 48875^T 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 46034^T, strain YIM 48875^T 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 48875^T can be differentiated from *P. flavigriseum* YIM 46034^T based on the presence of phosphatidylinositol mannoside in the polar lipid profile. The profiles of major menaquinones [MK-9(H_4), MK-9(H_6) and MK-9(H_8)] and fatty acids (iso-$C_{15:0}$ and iso-$C_{16:0}$) were highly significant in distinguishing strain YIM 48875^T from *P. flavigriseum* YIM 46034^T. 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 48875^T 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, H_2S 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(H_4), MK-9(H_6) and MK-9(H_8) are the predominant menaquinones. Polar lipids comprise diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannoside. Major

fatty acids (>10 %) are iso-$C_{15:0}$ and iso-$C_{16:0}$; smaller amounts (>5 %) of anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, summed feature 9 (iso-$C_{17:1}\omega 9c$ and/or 10-methyl $C_{16:0}$) and $C_{17:1}\omega 8c$ are found. The DNA G+C content of the type strain is 71.6 mol%.

The type strain, YIM 48875^T (=CCTCC AA 209049^T =KCTC 19779^T), was isolated from a rhizosphere soil of *Bletilla striata* in Xishuangbanna, Yunnan Province, China.

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