

## A new member of the family *Micromonosporaceae*, *Planosporangium flavigriseum* gen. nov., sp. nov.

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A novel actinomycete, designated strain YIM 46034<sup>T</sup>, was isolated from an evergreen broadleaved forest at Menghai, in southern Yunnan Province, China. Phenotypic characterization and 16S rRNA gene sequence analysis indicated that the strain belonged to the family *Micromonosporaceae*. Strain YIM 46034<sup>T</sup> showed more than 3% 16S rRNA gene sequence divergence from recognized species of genera in the family *Micromonosporaceae*. Characteristic features of strain YIM 46034<sup>T</sup> were the production of two types of spores, namely motile spores, which were formed in sporangia produced on substrate mycelia, and single globose spores, which were observed on short sporophores of the substrate mycelia. The cell wall contained meso-diaminopimelic acid, glycine, arabinose and xylose, which are characteristic components of cell-wall chemotype II of actinomycetes. Phosphatidylethanolamine was the major phospholipid (phospholipid type II). Based on morphological, chemotaxonomic, phenotypic and genetic characteristics, strain YIM 46034<sup>T</sup> is considered to represent a novel species of a new genus in the family *Micromonosporaceae*, for which the name *Planosporangium flavigriseum* gen. nov., sp. nov. is proposed. The type strain of *Planosporangium flavigriseum* is YIM 46034<sup>T</sup> (=CCTCC AA 205013<sup>T</sup> =DSM 44991<sup>T</sup>).

The family *Micromonosporaceae* was proposed by Krasil'nikov (1938), and the description of the family has subsequently been emended by Koch *et al.* (1996) and Stackebrandt *et al.* (1997) on the basis of chemotaxonomic data and 16S rRNA gene sequence analysis. These groups of authors included the following genera within the family: *Micromonospora* (Ørskov, 1923), *Actinoplanes* (Couch, 1950), *Catellatospora* (Asano & Kawamoto, 1986), *Catenuloplanes* (Yokota *et al.*, 1993), *Couchioplanes* (Tamura *et al.*, 1994), *Dactylosporangium* (Thiemann *et al.*, 1967) and *Pilimelia* (Kane, 1966). The genera *Actinocatenispora* (Thawai *et al.*, 2006), *Asanoa* (Lee & Hah, 2002), *Luedemanella* (Ara & Kudo, 2007a, b), *Longispora* (Matsumoto *et al.*, 2003), *Polymorphospora* (Tamura *et al.*, 2006), *Salinispora* (Maldonado *et al.*, 2005), *Spirilliplanes* (Tamura *et al.*, 1997), *Verrucosispora* (Rheims *et al.*, 1998) and *Virgisporangium* (Tamura *et al.*, 2001)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 46034<sup>T</sup> is AM232832.

Scanning electron micrographs of cells of strain YIM 46034<sup>T</sup> grown on GYM agar are available as supplementary material with the online version of this paper.

have since been described as additional members of the family.

During the course of a study on actinomycete diversity, a new isolate was obtained from an evergreen broadleaved forest soil sample from Yunnan Province, China, and this is shown here to represent a novel species of a new genus within the family *Micromonosporaceae*.

Strain YIM 46034<sup>T</sup> was isolated from soil collected in Menghai, Yunnan Province, China, on fucose-proline medium [per litre distilled water: 5 g fucose, 1 g proline, 1 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g NaCl, 2 g CaCl<sub>2</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>, 1 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 20 g agar, pH 7.2; nalidixic acid (20 mg l<sup>-1</sup>) and nystatin (100 mg l<sup>-1</sup>) were added as inhibitors of bacteria and fungi, respectively] incubated at 28 °C.

The phenotypic properties of strain YIM 46034<sup>T</sup> were examined by using various standard procedures (Williams *et al.*, 1983). Cultural characteristics were determined following growth on potato-dextrose agar (PDA; Difco), Czapek's agar (30 g sucrose, 2 g NaNO<sub>3</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g KCl, 0.01 g FeSO<sub>4</sub>, 20 g agar, pH 7.2–7.4), nutrient agar (10 g peptone, 5 g beef extract,

5 g NaCl, 20 g agar, pH 7.2–7.4), GYM agar (4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g CaCO<sub>3</sub>, 12 g agar, pH 7.2) and International *Streptomyces* Project media ISP2, ISP3, ISP4 and ISP5 after 25 days at 28 °C according to the methods of Shirling & Gottlieb (1966). Morphology of spores, sporangia, flagella and mycelia was observed after incubation at 28 °C for 15–30 days by light microscopy (BH-2; Olympus), scanning electron microscopy [JSM 5600LV (JEOL) and XL30 ESEM-TMP (Philips)] and transmission electron microscopy (H 800; Hitachi). Negative staining was used to demonstrate flagellation of the spores. Colours and hues were determined according to Kelly (1964). The Gram reaction was performed according to Gregersen (1978) by using KOH for cell lysis. Acid-fastness was determined by using carbol–fuchsin solution for cell staining, acid alcohol treatment and counter-staining with methylene blue (Ziehl–Neelsen method).

Biomass for molecular systematic and most of the chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in shaken cultures with yeast extract–malt extract broth (ISP2) supplemented with the vitamin mixture of HV medium (Hayakawa & Nonomura, 1987). Cell walls were purified and amino acids in the peptidoglycan were analysed by TLC (Lechevalier & Lechevalier, 1980). Whole-cell sugar composition was analysed according to the methods of Becker *et al.* (1965) and Lechevalier & Lechevalier (1980). Phospholipid analysis was carried out as described by Lechevalier *et al.* (1981). Menaquinones were determined by using the procedures of Collins *et al.* (1977). Biomass for quantitative fatty acid analysis was prepared by scraping colonies from TSA plates [3% (w/v) trypticase soy broth (BBL), 1.5% (w/v) Bacto agar (Difco)] that had been incubated for 7 days at 28 °C. Fatty acids were extracted, methylated and analysed by using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

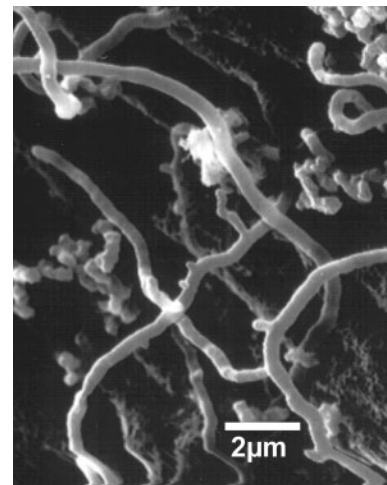
Chromosomal DNA of strain YIM 46034<sup>T</sup> was extracted as described by Marmur (1961). The DNA G + C content was determined by thermal denaturation (Mandel & Marmur, 1968).

Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene of strain YIM 46034<sup>T</sup> were carried out according to the procedures described by Xu *et al.* (2003). The 16S rRNA gene sequence of strain YIM 46034<sup>T</sup> (1470 nt) was compared with sequences in the DDBJ, EMBL and GenBank databases by using BLAST searches (Altschul *et al.*, 1997). For initial taxonomic classification of the sequence, the classifier program of the Ribosomal Database Project II (<http://rdp.cme.msu.edu/index.jsp>) was used. For phylogenetic analysis, sequences of representative species of different families belonging to the *Actinobacteria* and, in a second step, of all 16 recognized genera in the family *Micromonosporaceae* were used. Neighbour-joining trees (Saitou & Nei, 1987) were calculated by using distances corrected according to the Kimura two-parameter model (Kimura, 1980, 1983) with

the software package MEGA version 3.1 (Kumar *et al.*, 2001) after multiple alignment of the data by CLUSTAL\_X (Thompson *et al.*, 1997). For construction of the maximum-likelihood tree, the online version of PhyML (Guindon *et al.*, 2005) was used. The topology of the trees was evaluated by performing a bootstrap analysis (Felsenstein, 1985) of 1000 resamplings. *Nocardiosis alba* DSM 43377<sup>T</sup> was used as outgroup.

To examine the secondary structures of nine variable areas of the 16S rRNA gene (V1–V9) we used the procedures described by Bouthinon & Soldano (1999) and Akutsu (2000). The sequences were cut by using the program CLUSTAL\_X and the secondary structures were evaluated and viewed via the programs RNA structure 3.7 (De Rijk & De Wachter, 1997) and RnaViz 2.0 (De Rijk *et al.*, 2003).

Cells of strain YIM 46034<sup>T</sup> were aerobic, Gram-positive and non-acid-fast. No growth was observed on Czapek's agar, inorganic salts–starch agar (ISP4) or nutrient agar. Cultures grew very slowly, but developed well, within 25 days on yeast extract–malt extract agar (ISP2), oatmeal agar (ISP3), glycerol–asparagine agar (ISP5), PDA and GYM agar. No soluble pigments were produced on all media tested. The substrate mycelium branched extensively, and the colour of colonies on different media was pale grey (ISP5), orange yellow/light yellow (ISP2/PDA), orange (GYM agar) and pale grey–olive (ISP3). No aerial mycelium was observed on these media, except on GYM agar. Colonies on GYM agar were tough and wrinkled, with a smooth surface. Older cultures (9 weeks) partially developed areas with aerial mycelium at the edge of the colony. A scanning electron micrograph of the substrate mycelium with filament diameters of 0.6–0.7 µm is shown in Fig. 1. Strain YIM 46034<sup>T</sup> formed two types of spores. Motile spores were formed in finger-like, short, narrow



**Fig. 1.** Scanning electron micrograph of substrate mycelium of a 9-week-old agar culture of strain YIM 46034<sup>T</sup> grown on GYM agar. Bar, 2 µm.

**Table 1.** Characteristics of genera in the family *Micromonosporaceae*

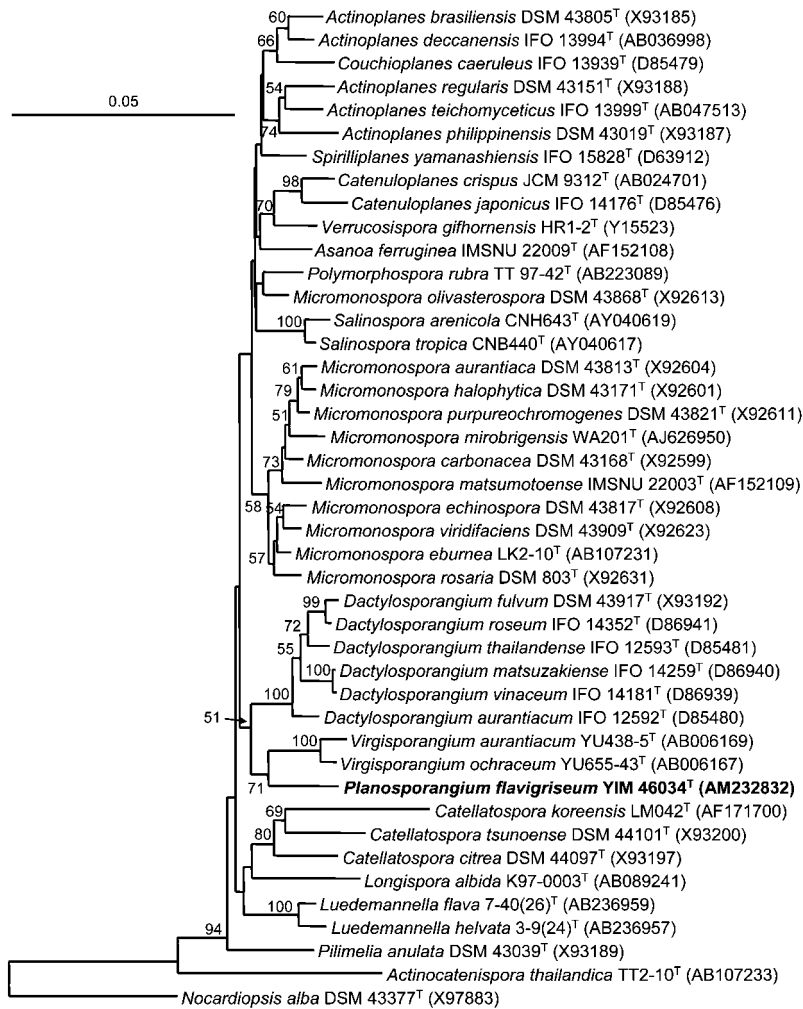
Taxa: 1, *Actinocatenispora*; 2, *Actinoplanes*; 3, *Asanoa*; 4, *Catellatospora*; 5, *Catenuloplanes*; 6, *Couchioplanes*; 7, *Dactylosporangium*; 8, *Longispora*; 9, *Luedemannella*; 10, *Micromonospora*; 11, *Pilimelia*; 12, *Polymorphospora*; 13, *Salinispora*; 14, *Spirilliplanes*; 15, *Verrucosipora*; 16, *Virgisporangium*; 17, strain YIM 46034<sup>T</sup>. +, Present; –, absent; ND, no data available; *m*-DAP, meso-diaminopimelic acid; Data taken from Thawai *et al.* (2006), Couch (1950), Lee & Hah (2002), Asano & Kawamoto (1986), Ara & Kudo (2006, 2007a), Yokota *et al.* (1993), Kudo *et al.* (1999), Tamura *et al.* (1994, 1997, 2001, 2006), Thiemann *et al.* (1967), Matsumoto *et al.* (2003), Ørskov (1923), Kane (1966), Maldonado *et al.* (2005) and Rheims *et al.* (1998).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Spore motility	–	+	–	–	+	+	+/–	–	–	–	+	–	–	+	–	+	+/–
Sporangia/ spore vesicles	–	+	–	–	–	–	+	–	+	–	+	–	–	–	–	+	+
Diamino acid	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	L-Lys	L-Lys	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP
Characteristic whole-cell sugar(s)*	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Man, Rha, Rib, Xyl or only Xyl	Man, Rib, Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Man, Rha, Rib, Xyl	Ara, Gal, Man, Rha, Rib, Xyl	Ara, Xyl	Ara, Xyl	Ara, Gal, Xyl	Gal, Man, Xyl	Man, Xyl	Ara, Gal, Man, Rha, Xyl	Ara, Xyl
Fatty acid type†	3b	2c	2d	3b	2c	2c	2d	2d	2d	3b	2b	2a	3a	2d	2b	2d	3b
Major mena- quinone(s)	9(H <sub>4,6</sub> )	9(H <sub>4</sub> ), 10(H <sub>4</sub> )	10(H <sub>6,8</sub> )	9(H <sub>4,6</sub> ) or 10(H <sub>4</sub> )	10(H <sub>4</sub> ), 11(H <sub>4</sub> )	9(H <sub>4</sub> )	9(H <sub>4,6,8</sub> )	10(H <sub>4,6</sub> )	9(H <sub>4,6</sub> )	9(H <sub>4,6</sub> ), 10(H <sub>4,6</sub> )	9(H <sub>2,4</sub> )	9(H <sub>4,6</sub> ), 10(H <sub>4,6</sub> )	9(H <sub>4</sub> )	10(H <sub>4</sub> )	9(H <sub>4</sub> )	10(H <sub>4</sub> , H <sub>6</sub> ,H <sub>8</sub> )	9(H <sub>4</sub> ), 10(H <sub>4</sub> )
Phospholipid type‡	II	II	II	II	III	II	II	II	II	II	II	II	II	II	II	II	II
DNA G + C content (mol%)	72	72–73	71–72	70–72	70–73	70–72	72–73	70	71	71–73	ND	71	70–73	69	70	71	71.4

\*Ara, Arabinose; Gal, galactose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

†According to the classification of Kroppenstedt (1985).

‡According to the classification of Lechevalier *et al.* (1977).



**Fig. 2.** Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences showing the relationship between strain YIM 46034<sup>T</sup> and the type strains of species of related genera in the family *Micromonosporaceae*. Numbers on branch nodes are bootstrap percentages (1000 replications; only values >50 % are given). Bar, 5 % sequence divergence.

**Table 2.** Signature nucleotides of the 16S rRNA gene sequence of members of the family *Micromonosporaceae*

Taxa: 1, *Micromonosporaceae* (Stackebrandt *et al.*, 1997); 2, *Actinoplanes*; 3, *Asanoa*; 4, *Catellatospora*; 5, *Catenuloplanes*; 6, *Couchioplanes*; 7, *Dactylosporangium*; 8, *Longispora*; 9, *Luedemannella*; 10, *Micromonospora*; 11, *Pilimelia*; 12, *Salinospora*; 13, *Spirilliplanes*; 14, *Verrucosipora*; 15, *Virgisporangium*; 16, *Polymorphospora*; 17, *Actinocatenispora*; 18, strain YIM 46034<sup>T</sup>. R, Purine; Y, pyrimidine.

<i>E. coli</i> position(s)	1, 3, 4, 9, 11, 16	2, 10, 13	5, 6	7, 18	8	12	14	15	17
66:103	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C
127:234	A-T	A-T	A-T	A-T	A-T	A-T	A-T	A-T	A-T
153:168	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G
502:543	G-C	G-C	G-C	G-C	A-T	G-C	G-C	A-T	T-G
589:650	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G
747	A	A	A	A	A	A	A	A	G
811	T	T	T	T	T	T	T	T	T
840:846	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G
952:1229	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G
1116:1184	C-G	C-G	C-G	T-G	C-G	T-G	C-G	T-G	T-G
1133:1141	G-C	G-G	R-Y	G-C	G-G	G-G	A-T	G-C	G-C

mentary Fig. S1a in IJSEM Online) produced directly on substrate mycelia. Sporangium envelopes became visible by light microscopy after spore liberation following rupture of the sporangia. Light microscopy also revealed that each sporangium contained a single row of three or more straight or slightly curved rod-shaped spores (1.0–1.5 × 0.4–0.5 µm). Cells of similar dimensions were detected by scanning electron microscopy (Supplementary Fig. S1b). Spore motility was demonstrated by light microscopy observation, and transmission electron microscopy revealed that the spores possessed a single polar flagellum. In contrast, non-motile globose spores with a smooth surface and diameters varying between 0.5 and 1.5 µm were also detected at the tip of short sporophores of the substrate mycelium (Supplementary Fig. S1c).

Colony and cell morphology of strain YIM 46034<sup>T</sup> were quite similar to those of *Dactylosporangium* species as described by Vobis (1992), especially with regard to the two types of spores produced. Non-motile spores of *Dactylosporangium* species were larger (1.7–2.8 µm in diameter) than those of strain YIM 46034<sup>T</sup>. A further difference from *Dactylosporangium* is apparent in the flagellation of the spores. Whereas spores of strain YIM 46034<sup>T</sup> possessed only a single flagellum, those of *Dactylosporangium* species are reported to possess a polar or subpolar tuft of flagella (Vobis, 1987).

The physiological, chemotaxonomic and genomic characteristics of strain YIM 46034<sup>T</sup> are given in Table 1 and also in the genus and species descriptions below. The major menaquinones were MK-9(H<sub>4</sub>) (52 %) and MK-10(H<sub>4</sub>) (48 %). The fatty acid profile comprised iso-C<sub>15:0</sub> (7.3 %), anteiso-C<sub>15:0</sub> (3.3 %), C<sub>15:0</sub> (1.2 %), iso-C<sub>16:1</sub> (1.9 %), iso-C<sub>16:0</sub> (17.5 %), C<sub>16:0</sub> (2.1 %), iso-C<sub>17:1</sub>ω9c (4.2 %), anteiso-C<sub>17:1</sub>ω9c (1.9 %), iso-C<sub>17:0</sub> (5.9 %), anteiso-C<sub>17:0</sub> (19.1 %), C<sub>17:1</sub>ω8c (14.5 %), C<sub>17:0</sub> (5.0 %), C<sub>17:0</sub> 10-methyl (1.6 %), C<sub>18:1</sub>ω9c (7.0 %), C<sub>18:0</sub> (1.7 %) and C<sub>19:1</sub>ω11c/C<sub>19:1</sub>ω9c (1.9 %).

Strain YIM 46034<sup>T</sup> showed 16S rRNA gene sequence similarities of ≤97 % to recognized representatives of genera belonging to the family *Micromonosporaceae*. Highest levels of similarity were to *Micromonospora aurantiaca* ATCC 27029<sup>T</sup> (97.0 %), *Micromonospora halophytica* DSM 43171<sup>T</sup> (97.0 %), *Micromonospora purpureochromogenes* DSM 43821<sup>T</sup> (96.9 %), *Micromonospora carbonacea* DSM 43168<sup>T</sup> (96.7 %), *Virgisporangium ochraceum* YU655-43<sup>T</sup> (96.2 %), *Virgisporangium aurantiacum* YU438-5<sup>T</sup> (95.9 %) and *Dactylosporangium aurantiacum* IFO 12592<sup>T</sup> (95.6 %). Although strain YIM 46034<sup>T</sup> showed highest levels of 16S rRNA gene sequence similarity to members of the genus *Micromonospora*, phylogenetic analysis revealed that strain YIM 46034<sup>T</sup> formed a distinct lineage within the family *Micromonosporaceae*. It did not cluster within the genus *Micromonospora* but was related to the genus *Virgisporangium* (Fig. 2). The maximum-likelihood tree showed a similar topology (data not shown).

Signature nucleotides of the 16S rRNA gene sequence of strain YIM 46034<sup>T</sup> were shared in all but one position (1116:1184 according to the *Escherichia coli* numbering scheme) with species of the family *Micromonosporaceae* as described by Stackebrandt *et al.* (1997) (Table 2). Other recognized species of the family *Micromonosporaceae* also differ in this position with regard to signature nucleotides (e.g. *Dactylosporangium*, *Virgisporangium*, *Salinispora* and *Actinocatenospora*; Table 2). Within the *Micromonosporaceae* (signature nucleotides according to Stackebrandt *et al.*, 1997), strain YIM 46034<sup>T</sup> differs from the genera *Micromonospora* (positions 1116:1184 and 1133:1141) as well as from *Virgisporangium* (position 502:543), but clusters with the genus *Dactylosporangium* (Table 2). However, signature nucleotides of strain YIM 46034<sup>T</sup> at positions based on Koch *et al.* (1996) were different from those of the genus *Virgisporangium* in one position (141:222) and from those of *Dactylosporangium* in several positions (141:222,

**Table 3.** Signature nucleotides of the 16S rRNA gene sequence of strain YIM 46034<sup>T</sup> and its most closely related genera modified according to Koch *et al.* (1996)

Numbers of 16S rRNA gene sequences taken for the determination of signature nucleotides are indicated. S, G or C.

<i>E. coli</i> position(s)	<i>Dactylosporangium</i> (n=18)	<i>Virgisporangium</i> (n=4)	Strain YIM 46034 <sup>T</sup>
141:222	G–C	G–C	A–T
129:232	T–G	T–G	T–G
415:428	A–G	C–G	C–G
441	G	G	G
442:491	A–G	A–G	A–G
560	T	T	T
600:638	A–T	G–T	G–T
601:637	G–C	A–T	A–T
614:626	G–T	G–C	G–C
653	T	A	A
998	T	G	G
1002:1038	S–S	C–G	G–C

415:428, 600:638, 601:637, 614:626, 653, 998), clearly differentiating the novel strain from both of these genera (Table 3).

The secondary structure of the variable regions of the 16S rRNA gene sequence of strain YIM 46034<sup>T</sup> V1 (positions 61–106), V2 (136–227; Fig. 3), V3 (437–497), V4 (588–651), V5 (821–879), V6 (997–1044), V7 (1118–1155), V8 (1241–1296) and V9 (1435–1466) closely matched those of strains and type strains of species of more distantly related genera. The secondary structure of the V2 region of YIM

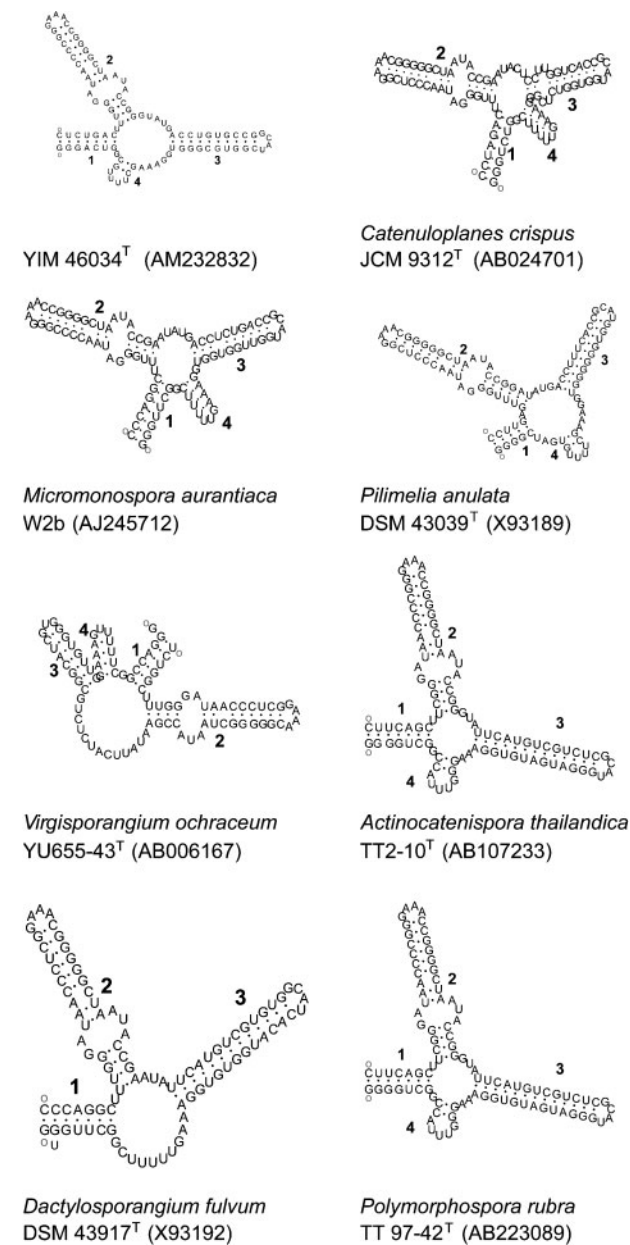
46034<sup>T</sup>, for example, is different from that of *M. aurantiaca* ATCC 27029<sup>T</sup>, *Virgisporangium ochraceum* CIP 107213<sup>T</sup>, *Dactylosporangium fulvum* DSM 43917<sup>T</sup>, *Actinocatenispora thailandica* JCM 12343<sup>T</sup>, *Catenuloplanes crispus* JCM 9312<sup>T</sup>, *Pilimelia anulata* DSM 43039<sup>T</sup> and *Polymorphospora rubra* DSM 44947<sup>T</sup> (Fig. 3). Different members of a single genus can display identical V2 secondary structures, as found in, for example, the genus *Micromonospora* (e.g. *M. halophytica* DSM 43171<sup>T</sup>, *M. carbonacea* DSM 43168<sup>T</sup>, *M. purpureochromogenes* DSM 43821<sup>T</sup>; data not shown). Thus, the different V2 secondary structure of strain YIM 46034<sup>T</sup> compared with related and recognized species further supports the placement of this novel strain within a new genus.

Characteristic properties that differentiate strain YIM 46034<sup>T</sup> from related species are given in Table 1. Diagnostic features of YIM 46034<sup>T</sup> are the production of motile spores enclosed within sporangia. This clearly differentiates strain YIM 46034<sup>T</sup> from species of the genus *Micromonospora*. Whole-cell sugar, fatty acid and menaquinone patterns of strain YIM 46034<sup>T</sup> were different from those of recognized *Virgisporangium* species. The menaquinone and fatty acid patterns of strain YIM 46034<sup>T</sup> were different from those of members of the genus *Dactylosporangium*. In addition, signature nucleotides (as described by Stackebrandt *et al.*, 1997) of strain YIM 46034<sup>T</sup> were not consistent with any of the described genera of the family *Micromonosporaceae* except *Dactylosporangium* (Table 2). However, the signature nucleotides as defined by Koch *et al.* (1996) clearly distinguished the new isolate from members of the genus *Dactylosporangium* (Table 3). Furthermore, strain YIM 46034<sup>T</sup> was not affiliated with any other recognized genus of the family *Micromonosporaceae*. Therefore, we suggest that strain YIM 46034<sup>T</sup> represents a novel species of a new genus, for which the name *Planosporangium flavigriseum* gen. nov., sp. nov. is proposed.

### Description of *Planosporangium* gen. nov.

*Planosporangium* (Pla.no.spo.ran'gi.um. Gr. n. *planes* a wanderer; N.L. neut. n. *sporangium* sporangium, spore case; N.L. neut. n. *Planosporangium* wandering sporangium, referring to the production of sporangia with motile spores).

Aerobic, Gram-positive and non-acid-fast. Short and narrow sporangia. Each sporangium contains a single row of three or more straight or slightly curved rod-shaped and motile spores with a single flagellum. A second spore type is represented by globose spores on the tip of short sporophores. The cell wall contains *meso*-diaminopimelic acid and glycine as diagnostic amino acids. Whole-cell hydrolysates contain arabinose and xylose as characteristic sugars (cell-wall chemotype II). Phospholipids are of type II (phosphatidylethanolamine as major component). Major menaquinones are MK-9(H<sub>4</sub>) and MK-10(H<sub>4</sub>). Major fatty acids are anteiso- and iso-branched. The type species is *Planosporangium flavigriseum*.



**Fig. 3.** Secondary structure of the V2 variable region of the 16S rRNA gene sequence of strain YIM 46034<sup>T</sup> and strains of related species.

## Description of *Planosporangium flavigriseum* sp. nov.

*Planosporangium flavigriseum* (fla.vi.gri'se.um. L. adj. *flavus* yellow; L. neut. adj. *griseum* grey; N.L. neut. adj. *flavigriseum* yellowish grey, referring to the colour of substrate mycelium of the type strain).

Displays the following properties in addition to those given in the genus description. No growth on Czapek's agar, inorganic salts-starch agar (ISP4) or nutrient agar. Slow but good growth after 25 days on PDA. The substrate mycelium is extensively branched with pale grey, orange-yellow, orange or pale grey-olive colour, depending on media and culture conditions. Diffusible pigments are not formed. The surface of spores is smooth. D-Glucose, D-galactose, D-mannose, D-arabinose, D-xylose, D-ribose, D-rhamnose, sucrose, maltose, melibiose, cellobiose, raffinose, mannitol, sorbitol and galactitol are utilized, but no acid is produced from these carbon sources. Fructose, lactose, inositol, erythritol, sodium acetate, ammonium acetate, sodium citrate, urea, L-glycine, L-histidine and L-methionine are not utilized. Negative for gelatin liquefaction, milk coagulation and peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, production of H<sub>2</sub>S and melanin. Major fatty acids are anteiso-C<sub>17:0</sub>, iso-C<sub>16:0</sub> and C<sub>17:1</sub>ω8; lesser components (<10% of total) are iso-C<sub>15:0</sub>, C<sub>18:1</sub>ω9, iso-C<sub>17:0</sub> and C<sub>17:0</sub>; minor components (<5% of total) are anteiso-C<sub>15:0</sub>, C<sub>15:0</sub>, iso-C<sub>16:1</sub>, C<sub>16:0</sub>, iso-C<sub>17:1</sub>ω9, anteiso-C<sub>17:1</sub>ω9, iso-C<sub>17:0</sub>, C<sub>17:0</sub> 10-methyl, C<sub>18:0</sub> and C<sub>19:1</sub>ωllc/C<sub>19:1</sub>ω9c. The G+C content of the genomic DNA of the type strain is 71.4 mol%.

The type strain, YIM 46034<sup>T</sup> (=CCTCC AA 205013<sup>T</sup> =DSM 44991<sup>T</sup>), was isolated from an evergreen broad-leaved forest soil at Menghai, southern Yunnan Province, China.

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