

Actinomycetospora chiangmaiensis gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae*

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A novel actinomycete strain, YIM 0006^T, was isolated from soil of a tropical rainforest in northern Thailand. The isolate displayed the following characteristics: aerial mycelium is absent, short spore chains are formed directly on the substrate mycelium, contains *meso*-diaminopimelic acid, arabinose and galactose (cell-wall chemotype IV), the diagnostic phospholipid is phosphatidylcholine, MK-9(H₄) is the predominant menaquinone and the G + C content of the genomic DNA is 69.0 mol%. Phylogenetic analysis and phenotypic characteristics showed that strain YIM 0006^T belongs to the family *Pseudonocardiaceae* but can be distinguished from representatives of all genera classified in the family. The novel genus and species *Actinomycetospora chiangmaiensis* gen. nov., sp. nov. are proposed, with strain YIM 0006^T (=CCTCC AA 205017^T =DSM 45062^T) as the type strain of *Actinomycetospora chiangmaiensis*.

The first description of the family *Pseudonocardiaceae* was given by Embley *et al.* (1988), and the description was emended by Stackebrandt *et al.* (1997) on the basis of 16S rRNA gene sequence analysis. The family currently consists of 14 genera with validly published names: *Actinoalloteichus* (Tamura *et al.*, 2000), *Actinopolyspora* (Gochnauer *et al.*, 1975), *Amycolatopsis* (Lechevalier *et al.*, 1986), *Crossiella* (Labeda, 2001), *Goodfellowia* (Labeda & Kroppenstedt, 2006), *Kibdelosporangium* (Shearer *et al.*, 1986), *Kutzneria* (Stackebrandt *et al.*, 1994), *Prauserella* (Kim & Goodfellow, 1999), *Pseudonocardia* (Henssen, 1957), *Saccharomonospora* (Nonomura & Ohara, 1971), *Saccharopolyspora* (Lacey & Goodfellow, 1975), *Streptoalloteichus* (Tomita *et al.*, 1987), *Thermobispora* (Wang *et al.*, 1996) and *Thermocrisum* (Korn-Wendisch *et al.*, 1995). Strain YIM 0006^T was isolated during an investigation of actinomycete diversity in soil from a tropical rainforest in Chiang Mai, in northern Thailand. Here, we report on the classification and characterization of strain YIM 0006^T and propose a novel genus and species of the family *Pseudonocardiaceae* to accommodate the strain.

Strain YIM 0006^T was isolated from a soil sample after 2 weeks incubation at 28 °C on starch-glycerol medium as described by Jiang *et al.* (2006). Cultural characteristics of

the strain were determined after growth at 28 °C for 2 weeks by methods used in the International Streptomyces Project (ISP; Shirling & Gottlieb, 1966) as well as by using Czapek's medium and nutrient agar (Dong & Cai, 2001). Colour determination was performed with colour chips from the ISCC-NBS Color Charts Standard Samples no. 2106 (Kelly, 1964). Morphological observations of spore chains and mycelia were made by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (Philips XL30 ESEM-TMP) after 20–50 days incubation. Gram staining (Hucker's modification; Society for American Bacteriologists, 1957) and Ziehl–Neelsen preparations (Gordon, 1967) were evaluated by light microscopy.

Growth of strain YIM 0006^T was poor on most media tested, although the strain grew well but slowly on yeast extract-malt extract agar (ISP 2). Soluble pigments were not produced under any conditions tested in this study. Aerial mycelium was not observed on any of the tested media. The vegetative mycelium fragmented into rod-shaped elements (Fig. 1a) and was pale to brilliant orange–yellow in colour. Short spore chains were formed directly on the vegetative mycelium (Fig. 1a, b). The strain displayed bud-like structures of the spore chains, as has also been described for members of the genus *Pseudonocardia* (Huang *et al.*, 2002). Spores were short and rod-shaped, 0.3–0.6 × 0.8–1.2 µm. The spore surface was smooth.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain YIM 0006^T is AM398646.

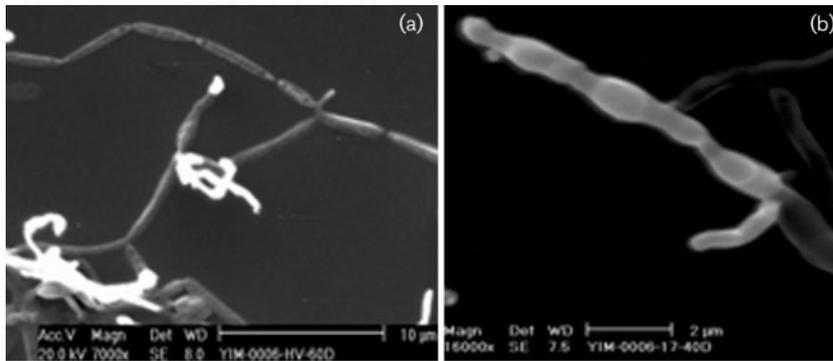


Fig. 1. (a) Scanning electron micrograph of fragments of vegetative mycelium and short spore chains of strain YIM 0006^T on HV medium (Hayakawa & Nonomura, 1987) after incubation for 60 days. Bar, 10 µm. (b) Scanning electron micrograph of short spore chains of strain YIM 0006^T on glycerol-asparagine medium (ISP 5) after incubation for 40 days. Bar, 2 µm.

All tests of physiological and biochemical characteristics of strain YIM 0006^T were performed at 28 °C and recorded after 7, 14, 20 and 30 days, except for the nitrate reduction test, which was recorded after 1, 3 and 5 days. Carbon- and nitrogen-source utilization as well as acid production from sugars under aerobic conditions were examined according to the method of Kämpfer *et al.* (1991). The isolate used a range of carbon sources (see species description). Galactose, arabinose, mannose, raffinose, inositol, mannitol and sodium citrate were not utilized. Tests of gelatin liquefaction, milk coagulation, milk peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, H₂S and melanin production were negative.

Cell material for the extraction of chromosomal DNA and chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in ISP 2 broth (Shirling & Gottlieb, 1966) supplemented with the vitamin mixture of HV medium (Hayakawa & Nonomura, 1987) as a shaking culture. Procedures for analysis of diagnostic cell-wall amino acids and sugars followed those described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Menaquinones were extracted according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). Chromosomal DNA for genomic DNA G + C content analysis was extracted as described by Marmur (1961). The DNA G + C content was determined by the HPLC method (Tamaoka & Komagata, 1984) with an Agilent 1100 LC system (IRIS Technologies).

The cell wall of strain YIM 0006^T contained meso-diaminopimelic acid as the diagnostic peptidoglycan diamino acid. Whole-cell hydrolysates contained arabinose and galactose as diagnostic sugars (cell-wall chemotype IV; Lechevalier & Lechevalier, 1970). Analysis of phospholipids revealed phosphatidylcholine, phosphatidylinositol and phosphatidylglycerol, indicating phospholipid type PIII (Lechevalier *et al.*, 1977). The predominant menaquinone was MK-9(H₄). The fatty acid profile consisted mainly of iso-branched saturated hexadecanoic acid. The predominant components, as proportions of the total fatty acid

composition, were iso-C_{14:0} (1.1%), iso-C_{15:0} (3.2%), iso-C_{16:1} H (2.6%), iso-C_{16:0} (29.8%), C_{16:1}ω7c/iso-C_{15:0} 2-OH (17.6%), C_{16:0} (10.8%), C_{16:0} 10-methyl (7.2%), iso-C_{17:0} (2.5%), anteiso-C_{17:0} (5.5%), C_{17:1}ω8c (4.4%), C_{17:0} (1.6%), C_{17:0} 10-methyl (1.3%), C_{18:1}ω9c (2.3%) and C_{18:0} (3.7%). The G + C content of genomic DNA of the strain was 69.0 mol%.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene of strain YIM 0006^T were carried out using procedures described by Xu *et al.* (2003). The 16S rRNA gene sequence (1456 nucleotides) was compared with corresponding sequences of the family *Pseudonocardiaceae* from the GenBank/EMBL/DDBJ database by using BLAST (Altschul *et al.*, 1997), BLAST 2 sequences (Tatusova & Madden, 1999) and FASTA (Pearson, 1990). The alignment was performed using CLUSTAL_X (Thompson *et al.*, 1997) and corrected manually. Phylogenetic analysis was conducted using MEGA version 3.1 (Kumar *et al.*, 2004) and the PhyML online web server (Guindon *et al.*, 2005). A distance matrix was generated according to Kimura's two-parameter model (Kimura, 1980, 1983) and a phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 resamplings. A maximum-likelihood tree was calculated using the GTR (general time-reversible) substitution model and bootstrap values from 500 resamplings.

A database search demonstrated that strain YIM 0006^T belongs to the family *Pseudonocardiaceae* (Stackebrandt *et al.*, 1997). Phylogenetic study was performed with 16S rRNA gene sequences of type strains of *Pseudonocardia* species with validly published names, as far as available, including [*Actinobispora*] *xinjiangensis*, [*Actinobispora*] *aurantiaca*, [*Actinobispora*] *alaniniphila* and [*Actinobispora*] *yunnanensis*, which were combined into the genus *Pseudonocardia* by Huang *et al.* (2002) as well as with sequences of representative type strains of the other 13 genera of the *Pseudonocardiaceae*. The closest relatives of strain YIM 0006^T were *Pseudonocardia halophobica* DSM 43089^T, with 95.24% sequence identity, *Pseudonocardia antarctica* DSM 44749^T (95.17%), *Pseudonocardia benzenivorans* DSM 44703^T and *Pseudonocardia alni* IMSNU

20049^T (both 95.10%). The sequence identity of YIM 0006^T to *Kibdelosporangium aridum* DSM 43828^T was 94.28%, and the identity to type strains belonging to other genera of the family *Pseudonocardiaceae* was below 94.20%.

According to the phylogenetic tree (Fig. 2), strain YIM 0006^T formed a distinct subclade between the genera *Pseudonocardia* and *Kibdelosporangium*. Although the 16S rRNA gene sequence similarity between strain YIM 0006^T and members of the genus *Pseudonocardia* fell into the range between the *Pseudonocardia* species (99.6–93.6%) given by Huang *et al.* (2002), the separate branching of the isolate is clearly supported by high bootstrap values of 96% (percentage of 1000 resamplings) and 97% (percentage of 500 resamplings) after calculation of the neighbour-joining tree (Fig. 2) and the maximum-likelihood tree (not shown), respectively.

Strain YIM 0006^T and representatives of the next most closely related genus *Pseudonocardia* have the same cell-wall chemotype (chemotype IV; meso-diaminopimelic acid, arabinose and galactose), fatty acid type and DNA G+C

content, while the menaquinone pattern clearly distinguishes the new isolate from members of the genus *Pseudonocardia* (Table 1). Several chemotaxonomic characteristics and the absence of sporangium-like structures clearly differentiate strain YIM 0006^T from representatives of the phylogenetically close genus *Kibdelosporangium* (Table 1).

On the basis of a combination of phylogenetic distinctness and differences in chemotaxonomic and morphological characteristics, we consider that strain YIM 0006^T represents a novel genus and species, for which the name *Actinomycetospira chiangmaiensis* gen. nov., sp. nov. is proposed.

Description of *Actinomycetospira* gen. nov.

Actinomycetospira (Ac.ti'no.my.ce.to.spo'ra. N.Gr. n. *actinomyces* -*etos* an actinomycete; Gr. fem. n. *spora* a seed and, in bacteriology, a spore; N.L. fem. n. *Actinomycetospira* referring to an actinomycete with spore chains).

Aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes. Substrate mycelium fragments into rod-shaped

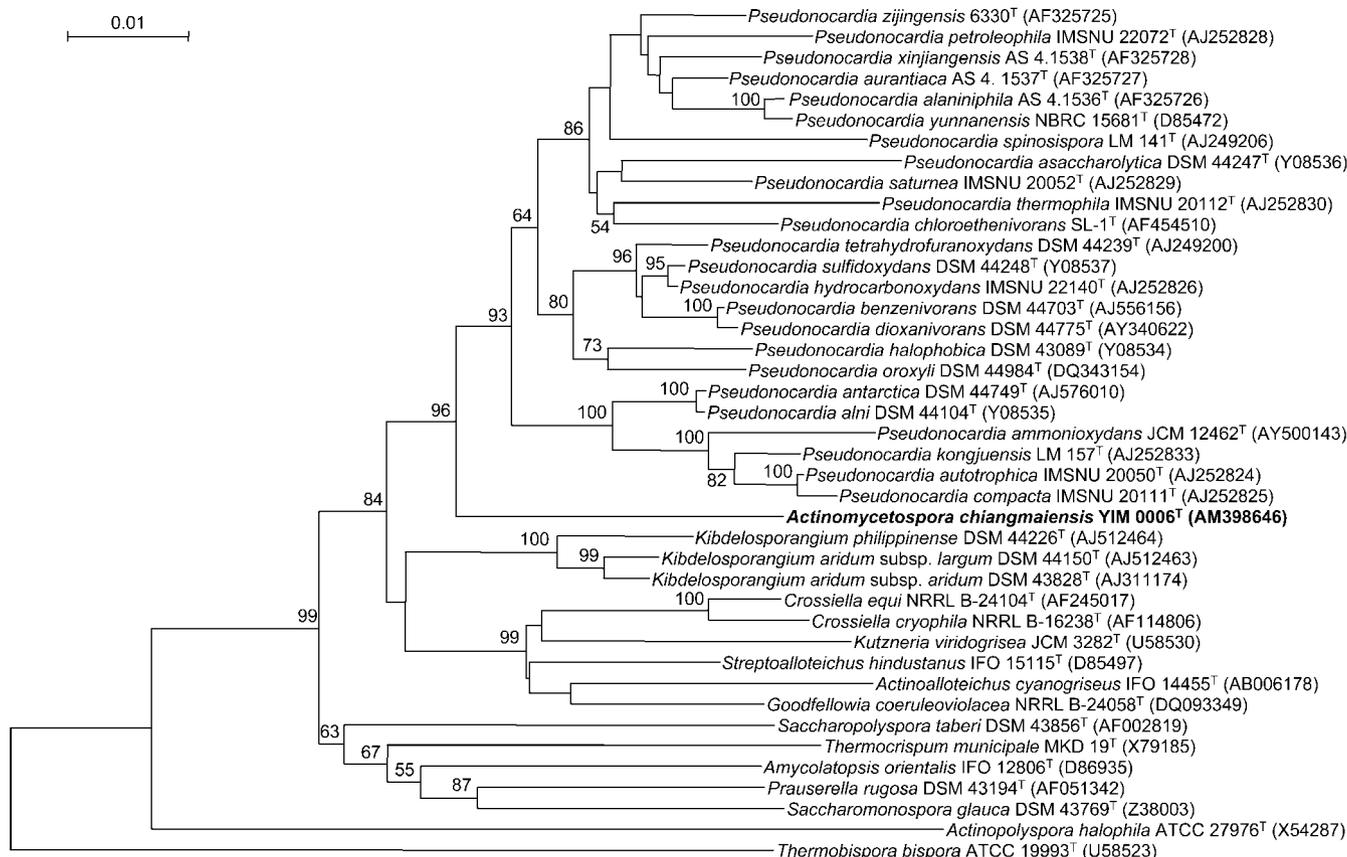


Fig. 2. Neighbour-joining tree derived from 16S rRNA gene sequences showing the relationship of YIM 0006^T and representative species of the 14 genera of the family *Pseudonocardiaceae*. Numbers at branch nodes are bootstrap percentages (1000 resamplings; only values over 50% are given). Bar, 1% sequence divergence.

Table 1. Morphological and chemotaxonomic characteristics of strain YIM 0006^T and related genera of the family Pseudonocardiaceae

Data for reference genera were derived from Shearer *et al.* (1986), Mertz & Yao (1988), Tomita *et al.* (1993), Hensen (1957), Huang *et al.* (2002), Reichert *et al.* (1998) and Kämpfer *et al.* (2006). Abbreviations: Ara, arabinose; Gal, galactose; Glc, glucose; Mad, madurose; Man, mannose; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylmethyl ethanolamine; PME, phosphatidylmethyl ethanolamine; GlcNu, N-acetylglucosamine-containing phospholipids; v, variable.

Genus	Some key morphological characteristics	Sugar pattern	Phospholipids*	Major menaquinone(s)	Fatty acid type†	G+C content (mol%)
<i>Kibdelosporangium</i>	Long chains of spores, sporangium-like structure of the aerial mycelium, fragmentation of the substrate mycelium	Ara, Gal, Mad (v), Glc (v), Rha (v)	PE, PI, PME, PG, DPG, PIM	MK-9(H ₂ , H ₄ , H ₆)	3c	66
<i>Pseudonocardia</i>	Long or short chains of spores, fragmentation of aerial and substrate mycelia (v), production of spores by budding or septation (v), swollen hyphal segments (v)	Ara, Gal	PE or PC, PME, PI, PG, DPG, GlcNu	MK-8(H ₄)	2b	68–79
<i>Actinomycetospira</i> gen. nov. (YIM 0006 ^T)	Short chains of spores, bud-like structures, fragmentation of substrate mycelium, absence of aerial mycelium	Ara, Gal	PC, PI, PG	MK-9(H ₄)	2b	69

*Data given in italics represent the diagnostic phospholipid according to Lechevalier *et al.* (1977).

†According to the classification of Kroppenstedt (1985).

elements. No aerial mycelium is produced on any medium tested. The cell wall contains *meso*-diaminopimelic acid. Whole-cell hydrolysates contain arabinose and galactose (cell-wall chemotype IV). Phosphatidylcholine is the diagnostic phospholipid, with phosphatidylinositol and phosphatidylglycerol. The predominant menaquinone is MK-9(H₄). The type species is *Actinomycetospira chiangmaiensis*.

Description of *Actinomycetospira chiangmaiensis* sp. nov.

Actinomycetospira chiangmaiensis (chiang.mai.en'sis. N.L. fem. adj. *chiangmaiensis* pertaining to Chiang Mai, a city in the north of Thailand in the vicinity of which the type strain was found).

In addition to the characteristics given in the genus description, this species has the following properties. Vegetative mycelium is pale to brilliant orange–yellow in colour. Short spore chains are formed directly from vegetative mycelium. Spores are short and rod-shaped. Spore surfaces are smooth. No soluble pigment is produced. Glucose, fructose, xylose, ribose, rhamnose, sucrose, lactose, sorbitol, glycerol, sodium acetate, asparagine, glycine, histidine and methionine are utilized as sole carbon sources. Acid is not produced from these carbon sources. Gelatin liquefaction, milk coagulation and peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, H₂S and melanin production are negative. The major cellular fatty acids are iso-C_{16:0} (29.8%), C_{16:1ω7c}/iso-C_{15:0} 2-OH (17.6%), C_{16:0} (10.8%) and C_{16:0} 10-methyl (7.2%). The G+C content of the DNA of the type strain is 69 mol%.

The type strain, YIM 0006^T (=CCTCC AA 205017^T =DSM 45062^T), was isolated from soil collected from a tropical rainforest located at Chiang Mai in the north of Thailand.

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