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

Yi Jiang,1,2 Jutta Wiese,1 Shu-Kun Tang,2 Li-Hua Xu,2 Johannes F. Imhoff1 and Cheng-Lin Jiang2

1Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR, Düsternbrooker Weg 20, D-24105 Kiel, Germany
2Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, China

A novel actinomycete strain, YIM 0006T, 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 0006T 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 0006T (=CCTCC AA 205017T =DSM 45062T) 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), Streptothalliteichus (Tomita et al., 1987), Thermobispora (Wang et al., 1996) and Thermocrispum (Korn-Wendisch et al., 1995). Strain YIM 0006T 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 0006T and propose a novel genus and species of the family Pseudonocardiaceae to accommodate the strain.

Strain YIM 0006T 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 0006T 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 cell wall of strain YIM 0006T contained system (IRIS Technologies). (Tamaoka & Komagata, 1984) with an Agilent 1100 LC DNA analysis was extracted as described by Marmur (1961). The acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, 1982). Cellular fatty acids 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 0006T 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-C14:0 (1.1 %), iso-C15:0 (3.2 %), iso-C16:1 H (2.6 %), iso-C16:0 (29.8 %), C16:1ω7c/iso-C15:0 2-OH (17.6 %), C16:0 (10.8 %), C16:0 10-methyl (7.2 %), iso-C17:0 (2.5 %), anteiso-C17:0 (5.5 %), C17:1ω8c (4.4 %), C17:0 (1.6 %), C17:0 10-methyl (1.3 %), C18:1ω9c (2.3 %) and C18:0 (3.7 %). The G+C content of genomic DNA of the strain was 69.0 mol%.

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).

All tests of physiological and biochemical characteristics of strain YIM 0006T 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, mannotol and sodium citrate were not utilized. Tests of gelatin liquefaction, milk coagulation, milk peptonization, starch hydrolysis, nitrate reduction, growth on cellulose, H2S and melamin production were negative.

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

**Fig. 1.** (a) Scanning electron micrograph of fragments of vegetative mycelium and short spore chains of strain YIM 0006T 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 0006T on glycerol-asparagine medium (ISP 5) after incubation for 40 days. Bar, 2 μm.
20049\textsuperscript{T} (both 95.10 %). The sequence identity of YIM 0006\textsuperscript{T} to Kibdelosporangium aridum DSM 43828\textsuperscript{T} was 94.28 %, and the identity to type strains belonging to other genera of the family Pseudonocardiaeae was below 94.20 %.

According to the phylogenetic tree (Fig. 2), strain YIM 0006\textsuperscript{T} formed a distinct subclade between the genera Pseudonocardia and Kibdelosporangium. Although the 16S rRNA gene sequence similarity between strain YIM 0006\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{T} represents a novel genus and species, for which the name Actinomycetospora chiangmaiensis gen. nov., sp. nov. is proposed.

**Description of Actinomycetospora gen. nov.**

Actinomycetospora (Ac.ti.no.my.ces -otos an actinomyces; Gr. fem. n. spora a seed and, in bacteriology, a spore; N.L. fem. n. Actinomycetospora referring to an actinomyces with spore chains).

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

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

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

<table>
<thead>
<tr>
<th>Genus</th>
<th>Some key morphological characteristics</th>
<th>Phospholipids*</th>
<th>Major menaquinone(s)</th>
<th>G+C content (mol%)</th>
<th>Fatty acid G+C content (mol%)</th>
<th>Type menaquinone(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kibdelosporangium</td>
<td>Long chains of spores, sporangium-like structure of the aerial mycelium, fragmentation of the substrate mycelium</td>
<td>Ara, Gal, Mad, V</td>
<td>PE, PI, PG, DPG, MK-9(H2,H4,H6)</td>
<td>4</td>
<td>66</td>
<td>MK-9(H2,H4,H6)</td>
</tr>
<tr>
<td>Pseudonocardia</td>
<td>Long or short chains of spores, fragmentation of the substrate mycelium</td>
<td>Ara, Gal, Mad, V</td>
<td>PE, PI, PG, DPG, MK-9(H2,H4,H6)</td>
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</tr>
<tr>
<td>Actinomycetospora</td>
<td>Long or short chains of spores, fragmentation of the substrate mycelium</td>
<td>Ara, Gal, Mad, V</td>
<td>PE, PI, PG, DPG, MK-9(H2,H4,H6)</td>
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<td>66</td>
<td>MK-9(H2,H4,H6)</td>
</tr>
</tbody>
</table>

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

Description of Actinomycetospora chiangmaensis sp. nov.

Actinomycetospora chiangmaensis (chiang.mai.en’sis. N.L. fem. adj. chiangmaensis pertaining to Chiang Mai, a city in the north of Thailand, in the vicinities 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 starch hydrolysis, nitrate reduction, growth on cellulose, H2S and melanin production are negative. The major cellular fatty acids are iso-C16:0 (29.8%), C16:1ω7c/iso-C15:0 2-OH (17.6%), C16:0 (10.8%) and C16:0 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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References


