Pseudonocardia ailaonensis sp. nov., isolated from soil in China

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A Gram-positive, aerobic actinomycete, designated strain YIM 45505^T, was isolated from a soil sample collected from Yunnan province, south-west China. Cells exhibited cream–white aerial mycelium and orange–yellow to yellow–brown substrate mycelium. The substrate mycelium fragmented into rod-shaped elements, and the aerial mycelium formed long chains of spores. 16S rRNA gene sequence analysis revealed that strain YIM 45505^T was related most closely to *Pseudonocardia oroxyli* DSM 44984^T (98.5 % similarity) and *Pseudonocardia halophobica* IMSNU 21327^T (97.5 %). The G+C content of the DNA of strain YIM 45505^T was 74.1 mol%. Combined with results of DNA–DNA hybridization and phenotypic tests, these data revealed that strain YIM 45505^T represents a novel species of the genus *Pseudonocardia*, for which the name *Pseudonocardia ailaonensis* sp. nov. is proposed. The type strain is YIM 45505^T (=KCTC 19315^T =DSM 44979^T).

The genus *Pseudonocardia* was erected by Henssen (1957) to accommodate nocardioform actinomycetes that have a type IV cell wall and lack mycolic acids. Its description was later emended by Warwick *et al.* (1994), McVeigh *et al.* (1994), Reichert *et al.* (1998) and Huang *et al.* (2002). At the time of writing, the genus *Pseudonocardia* comprises 26 recognized species, the majority of which have been described based on data from polyphasic approaches (Lee *et al.*, 2002; Kämpfer & Kroppenstedt, 2004; Gu *et al.*, 2006).

During the course of our study on isolation methods of rare actinomycetes, a *Pseudonocardia*-like strain was isolated from a soil sample. The aim of the present study was to characterize the taxonomic position of this strain, designated YIM 45505^T. Genotypic and phenotypic data showed that the new isolate should be recognized as representing a novel species of the genus *Pseudonocardia*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 45505^T is DQ344632.

A scanning electron micrograph of cells of strain YIM 45505^T, an extended neighbour-joining tree based on 16S rRNA gene sequences and a table giving the fatty acid contents of strain YIM 45505^T and related species are available as supplementary material with the online version of this paper.

Strain YIM 45505^T was isolated from a soil sample collected from Ailao Mountain in Yunnan province, south-west China. The medium used for isolation was modified starch-casein agar [0.3 g mannose, 2 g KNO_3, 0.3 g casein, 0.05 g $MgSO_4.7H_2O$, 2 g NaCl, 2 g K_2HPO_4, 0.02 g $CaCO_3$, 10 mg $FeSO_4.7H_2O$ and 15 g agar in 1000 ml tap water (pH 7.2–7.4)], which was incubated at 28 °C for 2 weeks. Biomass for molecular systematic and chemotaxonomic characterization studies was obtained by cultivation in yeast extract-malt extract broth (ISP 2; Shirling & Gottlieb, 1966) (28 °C, 1 week, with shaking at 150 r.p.m.). Cultural characteristics of the new isolate were determined after 2–3 weeks at 28 °C in accordance with methods recommended by the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966). Morphological features of spores and mycelia were observed by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (JSM 5600LV; JEOL). Growth was tested at 0, 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C on ISP 2. The ability of the strain to grow at different pH values (pH 5.0–10.0, at intervals of 0.5 pH units) and NaCl concentrations (0–10 %, w/v, at intervals of 0.5 %) was examined by using ISP 2 as the basal medium. Media and procedures used for determination of physiological features and carbon source utilization were those

described by Shirling & Gottlieb (1966) and Smibert & Krieg (1981). Acid production from carbohydrates was assessed by using the media and methods described by Gordon *et al.* (1974). Colony colour was determined in accordance with Kelly (1964).

Amino acid and sugar analysis of whole-cell hydrolysates followed standard procedures (Hasegawa *et al.*, 1983). Phospholipids were extracted, examined by two-dimensional TLC and identified by using recognized procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were isolated by using the method of Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). Analysis of the whole-cell fatty acid pattern followed the instructions of the MIDI System (Microbial ID) (Kroppenstedt, 1985) by using exponential phase cultures.

Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were performed according to Li *et al.* (2007). Multiple alignment with sequences of the most closely related actinobacteria and calculations of levels of sequence similarity were carried out by using CLUSTAL_X (Thompson *et al.*, 1997). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) trees were constructed by using MEGA version 3.1 (Kumar *et al.*, 2004). Pairwise distances for the neighbour-joining algorithm were calculated with Kimura's two-parameter model (Kimura, 1980) and close-neighbour-interchange (search level=2, random additions=100) was applied in maximum-parsimony analysis. The topology of the trees was evaluated by performing a bootstrap analysis (Felsenstein, 1985) with 1000 resamplings.

The DNA G+C content of strain YIM 45505^T was determined by using the HPLC method (Mesbah *et al.*, 1989). DNA–DNA hybridization between strain YIM 45505^T and its closest phylogenetic neighbours was carried out by using the method described by He *et al.* (2005).

Strain YIM 45505^T developed well on most media tested, including ISP 2, ISP 5 (glycerol-asparagine agar), potato agar and Czapek's agar (Waksman, 1967). The cells produced cream–white aerial mycelium on all the media. The colour of the substrate mycelium was orange–yellow on Czapek's agar, yellow–brown on ISP 5 and deep orange–yellow on ISP 2 and potato agar media. The substrate mycelium fragmented into rod-shaped elements, and the aerial mycelium formed long chains of spores. The spores were smooth and non-motile (see Supplementary Fig. S1 in IJSEM Online). No diffusible pigments were produced on any of the media. The optimal pH and temperature for growth of strain YIM 45505^T were about pH 7.0 and 28 °C. Detailed physiological and biochemical properties of strain YIM 45505^T are provided in the species description. A comparison of the physiological properties of strain YIM 45505^T, *Pseudonocardia oroxyli* DSM 44984^T and *Pseudonocardia halophobica* IMSNU 21327^T is given in Table 1.

Whole-organism hydrolysates of strain YIM 45505^T were rich in *meso*-diaminopimelic acid, arabinose, ribose,

Table 1. Comparison of the physiological characteristics of strain YIM 45505^T and the type strains of the two most closely related *Pseudonocardia* species

Strains: 1, YIM 45505^T; 2, *P. oroxyli* DSM 44984^T; 3, *P. halophobica* IMSNU 21327^T. +, Positive; −, negative; (+), weakly positive. Data were obtained in this study. All strains are positive for acid production from D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, sucrose and trehalose, but negative for acid production from cellobiose, methyl α-D-glucoside, D-lactose, D-lactulose, raffinose and salicin. All strains are positive for assimilation of L-arabinose, citrate, dulcitol, D-fructose, D-glucose, glycerol, glycogen, D-mannose, L-methionine, L-phenylalanine, L-proline, raffinose, trehalose and L-valine, but negative for assimilation of L-leucinamide.

Characteristic	1	2	3
Acid production from:			
L-Arabinose	−	−	+
Dulcitol	(+)	−	+
Erythritol	−	−	+
Inositol	−	−	+
Melezitose	−	+	+
D-Sorbitol	−	−	+
D-Sorbose	−	−	+
D-Xylose	+	−	(+)
Assimilation of sole carbon sources			
Acetate	−	+	+
Cellobiose*	+	(+)	(+)
D-Galactose*	−	(+)	−
Inositol	+	+	−
D-Lactose*	+	(+)	(+)
D-Mannitol	+	+	−
Melezitose	(+)	+	−
Oxalate	(+)	(+)	−
D-Rhamnose*	+	(+)	(+)
D-Ribose*	+	+	(+)
Salicin	(+)	(+)	−
D-Sorbitol	+	+	−
Sucrose	+	+	−
D-Xylose*	−	+	(+)
Assimilation of sole nitrogen sources			
L-Arginine	+	−	+
L-Cysteine	+	−	(+)
L-Ornithine	+	+	(+)
L-Threonine	+	−	+
L-Tyrosine*	+	−	+
Decomposition of:			
Adenine	−	+	(+)
Hypoxanthine	+	+	(+)
L-Tyrosine	+	+	(+)
Xanthine	(+)	+	+
Growth in the presence of:			
5 % NaCl	+	+	(+)
7 % NaCl	−	(+)	(+)

*Tests on *P. oroxyli* and *P. halophobica* were also carried out by Gu *et al.* (2006) and gave congruent results.

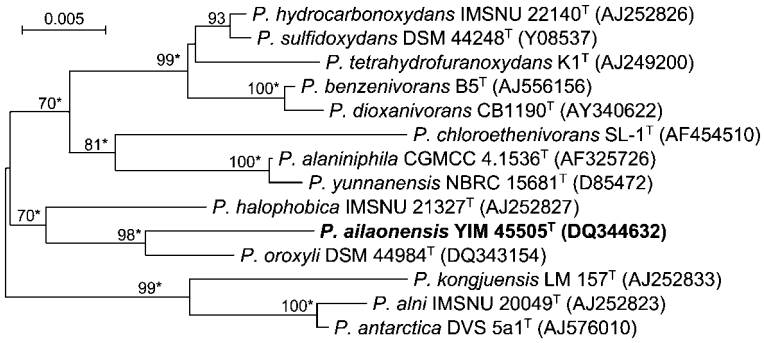

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships between strain YIM 45505^T and related representatives of *Pseudonocardia* species. Asterisks indicate branches that were conserved in both the neighbour-joining and maximum-parsimony trees. Bootstrap values at branch points are expressed as percentages from 1000 replications (only values greater than 50 % are indicated). Bar, 0.5 % sequence divergence. An extended version of this tree is available as Supplementary Fig. S1.

galactose and glucose. The predominant menaquinone was MK-8(H_4). Cells of strain YIM 45505^T had a type PIII phospholipid pattern (Lechevalier *et al.*, 1977). Major phospholipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The whole-cell fatty acid profile of strain YIM 45505^T mainly consisted of iso-$C_{16:0}$ (35.5 %), iso-$C_{16:0}$ 2-OH (10.8 %) and $C_{16:0}$ 10-methyl (9.0 %). A comparison of the fatty acid profiles of strain YIM 45505^T, *P. oroxyli* DSM 44984^T and *P. halophobica* IMSNU 21327^T is given in Supplementary Table S1.

The almost-complete sequence of the 16S rRNA gene of strain YIM 45505^T (1467 bp) was used for phylogenetic analysis. A neighbour-joining tree (Supplementary Fig. S2) based on available 16S rRNA gene sequences of the type strains of recognized *Pseudonocardia* species confirmed the placement of strain YIM 45505^T in the genus *Pseudonocardia*. Strain YIM 45505^T exhibited 98.5 and 97.5 % 16S rRNA gene sequence similarity to its closest neighbours, *P. oroxyli* DSM 44984^T and *P. halophobica* IMSNU 21327^T, respectively, and these formed a distinct subclade separate from other representatives of the genus *Pseudonocardia* (Fig. 1 and Supplementary Fig. S2). Levels of 16S rRNA gene sequence similarity between strain YIM 45505^T and the type strains of other recognized species of the genus *Pseudonocardia* were below 97 %. Strain YIM 45505^T showed relatively low levels of DNA–DNA relatedness with *P. oroxyli* DSM 44984^T (44.3 $\pm$ 5.4 %) and *P. halophobica* IMSNU 21327^T (33.8 $\pm$ 2.9 %), which are much lower than the recommended threshold value of 70 % for the delineation of genomic species (Stackebrandt & Goebel, 1994). This clearly indicated that strain YIM 45505^T represented a genomic species separate from *P. oroxyli* and *P. halophobica*.

Therefore, based on its phenotypic and genotypic properties, strain YIM 45505^T is considered to represent a novel species of the genus *Pseudonocardia*, for which the name *Pseudonocardia ailaonensis* sp. nov. is proposed.

Description of *Pseudonocardia ailaonensis* sp. nov.

Pseudonocardia ailaonensis (ai.lao.nen′sis. N.L. fem. adj. *ailaonensis* pertaining to Ailao Mountain, Yunnan province, China, the source of the soil sample from which the type strain was isolated).

Cells are Gram-positive and aerobic. Forms cream–white aerial mycelium and orange–yellow to yellow–brown substrate mycelium on ISP 2, ISP 5, potato agar and Czapek's agar. Both substrate and aerial mycelia fragment into rod-shaped elements. No pigment is produced. The temperature range for growth is 15–37 °C, with optimal growth at 28 °C. The pH range for growth is 6.0–8.0. The NaCl concentration range for growth is 0–5 % (w/v). Positive for urease, catalase, melanin production (ISP 6 medium; peptone-yeast extract-iron agar), nitrate reduction, milk coagulation and milk peptonization, but negative for gelatin liquefaction, hydrolysis of cellulose and starch and production of H_2S. The cell wall contains *meso*-diaminopimelic acid. The whole-cell sugar pattern consists of arabinose, ribose, glucose and galactose. MK-8(H_4) is the predominant menaquinone. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol (type PIII phospholipid). The major cellular fatty acid is iso-$C_{16:0}$ (35.5 %). The G+C content of the genomic DNA of the type strain is 74.1 mol%. Physiological properties, including acid production, carbon source utilization, biodegradation and growth in the presence of sodium chloride, are given in Table 1.

The type strain, YIM 45505^T (=KCTC 19315^T =DSM 44979^T), was isolated from a soil in Yunnan province, south-west China.

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