Pseudonocardia oroxyli sp. nov., a novel actinomycete isolated from surface-sterilized *Oroxylum indicum* root

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A high-G + C-content, Gram-positive bacterium, strain D10^T, was isolated from the root of *Oroxylum indicum*, a Chinese medicinal plant. Based on 16S rRNA gene sequence analysis, strain D10^T was a member of the genus *Pseudonocardia* and was most closely related, albeit loosely, to *Pseudonocardia halophobica*. Morphological and chemotaxonomic characteristics support the affiliation of strain D10^T to the genus *Pseudonocardia*. Results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain D10^T from related *Pseudonocardia* species. Strain D10^T (= CGMCC 4.3143^T = DSM 44984^T) therefore represents a novel species, for which the name *Pseudonocardia oroxyli* sp. nov. is proposed.

Pseudonocardia, initially proposed by Henssen (1957), is the type genus of the family *Pseudonocardiaceae* (Embley *et al.*, 1988; Warwick *et al.*, 1994), which belongs to the suborder *Pseudonocardineae* (Stackebrandt *et al.*, 1997). The genus currently comprises 26 species, the majority of which have been described by polyphasic taxonomic approaches (Warwick *et al.*, 1994; Reichert *et al.*, 1998; Huang *et al.*, 2002). Most of these micro-organisms were isolated from soil samples, although they have also been isolated from plant samples, such as root nodules and tree-bark compost (Evtushenko *et al.*, 1989; Reichert *et al.*, 1998). In this paper, a novel *Pseudonocardia* species is proposed for a strain that was isolated from the surface-sterilized root of a higher plant.

Strain D10^T was isolated from the root elongation zone of *Oroxylum indicum*, a traditional Chinese medicinal plant, which was collected in the rainforest around Liusha River, southwest of Jinghong City, Yunnan Province, China. After being surface-sterilized by the procedure established by Coombs & Franco (2003), root samples were sliced into pieces with a sterile blade followed by plating on BL-2 agar plates (glucose, 5 g; soluble starch, 5 g; acid hydrolysates of casein, 2 g; yeast extract, 1 g; NaCl, 5 g; CaCO₃, 5 g; agar, 15 g; distilled water, 1 l; supplemented with 100 µg

penicillin ml⁻¹), which were incubated at 27 °C for 2–4 weeks. The organism, seen around the root sample under a light microscope, was transferred onto fresh yeast extract/malt extract (ISP medium 2) agar (Shirling & Gottlieb, 1966) and well maintained.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene from strain D10^T were performed using an established method (Chun & Goodfellow, 1995). The PCR product was purified and sequenced directly using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and universal primers 27F and 1492R. Sequence gel electrophoresis was carried out and the nucleotide sequences were obtained automatically using an Applied Biosystems DNA sequencer (model 3730XL) and software provided by the manufacturer. Preliminary phylogenetic analysis based on the results of the search program BLAST showed that strain D10^T is closely related to members of the genus *Pseudonocardia*. Alignment of 16S rRNA gene sequences and construction of neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) trees were carried out using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar *et al.*, 2004). Pairwise distances for the neighbour-joining algorithm were calculated with the Kimura two-parameter model (Kimura, 1980) and close-neighbour-interchange (search level = 2, random additions = 100) was applied in maximum-parsimony analysis. Bootstrap values were based on 1000 replicates. A neighbour-joining tree (see Supplementary Fig. S1 in IJSEM Online) containing available 16S rRNA gene sequences of type strains of *Pseudonocardia* species

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain D10^T is DQ343154.

A neighbour-joining tree based on 16S rRNA gene sequences of type strains of *Pseudonocardia* species and SEMs of strain D10^T grown on ISP medium 2 agar are available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic analyses based on 16S rRNA gene sequences (accession nos are given in parentheses), displaying relationships between strain D10^T and related representatives of *Pseudonocardia* species. Neighbour-joining and maximum-parsimony trees were both performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar *et al.*, 2004). Bootstrap values based on 1000 replicates are listed as percentages; those inferred from the neighbour-joining method are above the branches and those from the maximum-parsimony algorithm are below. Bar, 0.01 substitutions per nucleotide position.

confirmed placement of strain D10^T in the genus *Pseudonocardia*. It is evident from Fig. 1 that strain D10^T is closely related to *Pseudonocardia halophobica* IMSNU 21327^T (16S rRNA gene sequence similarity of 97.8%), *Pseudonocardia hydrocarbonoxydans* IMSNU 22140^T (97.5%), *Pseudonocardia sulfidoxydans* DSM 44248^T (97.5%), *Pseudonocardia benzenivorans* B5^T (97.3%) and *Pseudonocardia dioxanivorans* CB1190^T (97.2%). The 16S rRNA gene sequence similarities between strain D10^T and other *Pseudonocardia* species are below 97%. The relationship between strain D10^T and its closest neighbour, *P. halophobica*, is supported by both neighbour-joining and maximum-parsimony algorithms with bootstrap values of 82 and 69%, respectively (Fig. 1).

Morphological characteristics of isolate D10^T were examined by light (Axioskop 20; Zeiss) and scanning electron (FEI QUANTA) microscopy of 14-day-old cultures grown on ISP medium 2 agar. The organism showed morphology that was typical of the genus *Pseudonocardia* in that both substrate and aerial mycelium fragmented into rod-shaped, smooth elements and swollen segments were formed at the tip of mycelium (see Supplementary Fig. S2 in IJSEM Online). The physiological characteristics of strain D10^T and its closest neighbours were tested together using well-established procedures, i.e. utilization of sole carbon sources for energy and growth was assessed as described by Yassin *et al.* (1995), acid production from carbohydrates was determined using media and methods described by Gordon *et al.* (1974), and biochemical and biodegradation tests were performed according to the method of Gordon & Mihm (1957). The results of these tests enabled strain D10^T to be distinguished easily from related *Pseudonocardia* species (Table 1).

Standard methods were used for extraction and analysis of the isomers of diaminopimelic acid (Hasegawa *et al.*, 1983), whole-cell sugars (Lechevalier & Lechevalier, 1980), menaquinones (Collins, 1985; Wu *et al.*, 1989), polar lipids (Lechevalier *et al.*, 1977; Minnikin *et al.*, 1984) and fatty acids (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Mycolic acids were detected by the acid methanolysis procedure (Minnikin *et al.*, 1975). Strain D10^T produced

whole-organism hydrolysates rich in *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970) and contained MK-8 (H₄) as its predominant menaquinone. Major polar lipids were phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, two phospholipids of unknown structure containing glucosamine and two glycolipids. The fatty acid profile of strain D10^T was composed of C_{16:0} iso (45.1%), C_{16:0} 10-methyl (11.1%), C_{17:1}ω6c (7.4%), C_{16:1} iso H (7.1%), C_{18:1}ω9c (5.1%), C_{15:0} iso (4.4%), C_{16:1}ω7c (4.3%), C_{14:0} iso (3.5%) and C_{16:0} (3.0%), but it lacked mycolic acids. The G + C content of genomic DNA of strain D10^T (70.6 mol%) was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* K-12 as a control. All data supported affiliation of strain D10^T to the genus *Pseudonocardia* (Reichert *et al.*, 1998; Huang *et al.*, 2002).

Genomic hybridization experiments between strain D10^T and its closest phylogenetic neighbour, *P. halophobica* IMSNU 21327^T, were carried out using the method described by He *et al.* (2005), with the result that they shared low DNA–DNA relatedness of 35%. Moreover, strain D10^T could be easily differentiated from the type strain of *Pseudonocardia spinosa*, whose 16S rRNA gene sequence is not available, in that the latter produces spiny spores, grows extremely slowly and lacks septa in hyphae (Henssen & Schäfer, 1971).

The combined genotypic and phenotypic data show that strain D10^T should be classified as a representative of a novel species within the genus *Pseudonocardia*. The name proposed for this novel species is *Pseudonocardia oroxyli* sp. nov.

Description of *Pseudonocardia oroxyli* sp. nov.

Pseudonocardia oroxyli (o.ro.xy'li. N.L. gen. n. *oroxyli* of the plant genus *Oroxylum*).

Aerobic, Gram-positive actinomycete that forms cinnamon-buff substrate mycelium and pale pinkish cinnamon aerial mycelium. Both types of mycelium fragment into

Table 1. Physiological characteristics of the type strains of some *Pseudonocardia* species

Strains: 1, D10^T; 2, *P. halophobica* IMSNU 21327^T; 3, *P. sulfidoxydans* DSM 44248^T; 4, *P. hydrocarbonoxydans* IMSNU 22140^T; 5, *P. benzenivorans* B5^T; 6, *P. dioxanivorans* CB1190^T. +, Positive; –, negative; (+), weakly positive. All strains are positive for acid production from D-galactose and D-glucose, but negative for acid production from D-cellobiose, methyl α -D-glucoside, D-lactose and D-raffinose. All strains are positive for assimilation of D-fructose, D-glucose, glycerol, malate, D-mannose, succinate and D-trehalose, but negative for assimilation of nicotinamide and D-sorbose. Assimilation of D-glucose, glycerol, D-mannose and D-trehalose by *P. sulfidoxydans*, *P. hydrocarbonoxydans* and *P. dioxanivorans* was tested by Mahendra & Alvarez-Cohen (2005) using a different method; congruent results were obtained.

Characteristic	1	2	3	4	5	6
Acid produced from:						
D-Fructose, glycerol	+	+	+	–	+	+
D-Mannitol, D-melezitose, maltose	+	+	–	–	–	–
D-Sorbitol, dulcitol	–	+	–	–	–	–
D-Mannose	+	+	+	–	+	–
D-Trehalose	+	+	+	–	–	+
D-Xylose	–	(+)	+	+	+	(+)
Erythritol	–	+	+	–	–	–
Inositol	–	+	–	+	–	–
L-Arabinose	–	+	+	+	–	–
D-Lactulose	–	–	–	+	(+)	–
D-Sorbose	–	+	–	–	–	–
Salicin	–	–	–	+	–	–
Sucrose	+	+	+	+	–	–
Assimilation of sole carbon sources:						
Acetate	+	+	–	+	+	+
Citrate	+	+	(+)	–	+	+
D-Inulin	+	+	(+)	–	+	–
D-Cellobiose	+	(+)	(+)	+	–	+
D-Galactose, L-methionine, L-valine	+	+	+	–	–	+
D-Raffinose	+	+	+	–	+	–
D-Glutamic acid	(+)	+	–	+	–	+
D-Lactulose	+	+	–	–	+	–
D-Lactose*, L-alanine*	+	+	+	+	+	–
D-Maltose*, L-leucine	+	–	+	+	+	+
D-Mannitol	+	–	–	(+)	+	+
D-Melezitose	+	–	(+)	+	(+)	+
D-Rhamnose	+	(+)	+	–	–	+
D-Ribose, L-arabinose*	+	–	–	–	–	–
D-Sorbitol	+	–	(+)	–	+	–
D-Xylose	+	–	+	–	+	+
Dulcitol	+	+	+	–	(+)	(+)
Glycogen	+	+	(+)	–	–	–
Inositol	+	–	(+)	–	–	–
L-Arginine	–	+	–	–	–	+
L-Cysteine	–	(+)	–	–	–	–
L-Leucinamide	–	–	–	+	–	+
L-Ornithine	+	(+)	–	–	–	+
L-Phenylalanine	+	+	(+)	+	+	–
L-Proline	+	+	–	+	–	+
L-Tyrosine	–	(+)	+	+	–	–
Malonate	–	(+)	+	–	(+)	(+)
Methyl α -D-glucoside*	+	(+)	+	–	(+)	+
Oxalate, salicin	(+)	–	(+)	–	(+)	–
Sucrose	+	–	(+)	+	+	–

Table 1. cont.

Characteristic	1	2	3	4	5	6
Decomposition of:						
Adenine	+	(+)	—	—	—	—
Hypoxanthine	+	(+)	+	—	+	+
L-Tyrosine	+	(+)	+	—	—	—
Xanthine	+	+	+	—	—	+
Growth at/on:						
3 % NaCl*	+	+	+	—	+	+
5 % NaCl*	+	(+)	—	—	—	(+)
7 % NaCl	(+)	(+)	—	—	—	—

*Tests on *P. sulfidoxydans*, *P. hydrocarbonoxydans* and *P. dioxanivorans* were also carried out by Mahendra & Alvarez-Cohen (2005) using a different method and gave congruent results.

rod-shaped smooth elements. Swellings are produced at the tip of hyphae. No pigment is produced. Good growth occurs after 3 days incubation on ISP medium 2 agar at 25–30 °C. Decomposition of urea and production of H₂S are negative. Main polar lipids are phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, two phospholipids of unknown structure containing glucosamine and two glycolipids. The predominant fatty acid is C_{16:0} iso (45.1 %). Physiological properties, including acid production, carbon source utilization, biodegradation and growth in the presence of sodium chloride, are indicated in Table 1.

The type strain is D10^T (=CGMCC 4.3143^T=DSM 44984^T), isolated from a surface-sterilized root of *Oroxylum indicum* collected in the rainforest around Liusha River, southwest of Jinghong City, Yunnan Province, China. The G+C content of genomic DNA of strain D10^T is 70.6 mol%.

Acknowledgements

This work was supported by the Knowledge Innovation Project of the Chinese Academy of Sciences and by a grant from Hisun Pharmaceutical Company. The authors are grateful to Professor R. M. Kroppenstedt (DSMZ) for providing some type strains of *Pseudonocardia*, and to Professors Cheng-Lin Jiang and Li-Hua Xu (Yunnan University) for their help in plant sample collection.

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