Pseudonocardia babensis sp. nov., isolated from plant litter

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A novel actinomycete, designated strain VN05A0561^T, was isolated from plant litter collected at Ba Be National Park, Vietnam. The substrate mycelia and spore chains fragmented in a manner similar to nocardioform actinomycetes; the spores had smooth surfaces and were rod-shaped. Strain VN05A0561^T had the following chemotaxonomic characteristics: meso-diaminopimelic acid in the cell-wall peptidoglycan, arabinose and galactose as characteristic sugars, MK-8(H₄) as the major isoprenoid quinone, phosphatidylcholine as the diagnostic phospholipid and iso-C_{16:0} as the major cellular fatty acid. Strain VN05A0561^T shared low levels of 16S rRNA gene sequence similarity (<97%) with the type strains of recognized species of the genus *Pseudonocardia* and could be differentiated from its closest phylogenetic relatives based on phenotypic characteristics. These results suggested that strain VN05A0561^T represents a novel species of the genus *Pseudonocardia*, for which the name *Pseudonocardia babensis* sp. nov. is proposed. The type strain is VN05A0561^T (=VTCC-A-1757^T=NBRC 105793^T).

The genus *Pseudonocardia* was established as a nocardioform actinomycete by Henssen (1957), and its description has been emended based on both chemotaxonomic and morphological variations (Reichert *et al.*, 1998; Huang *et al.*, 2002; Park *et al.*, 2008). On the basis of the lack of mycolic acids and the presence of cell-wall type IV, the genera *Amycolata* (Lechevalier *et al.*, 1986) and *Pseudoamycolata* (Akimov *et al.*, 1989) were also proposed as nocardioform actinomycetes. However, the latter two genera were found to have chemotaxonomic properties similar to those of the genus *Pseudonocardia* (Kothe *et al.*, 1989; Takeuchi *et al.*, 1992), and were incorporated in the genus *Pseudonocardia* on the basis of 16S rRNA gene sequence analysis (Warwick *et al.*, 1994; McVeigh *et al.*, 1994). Subsequently, members of the genus *Actinobispora* (Jiang *et al.*, 1991) were also transferred to the genus *Pseudonocardia* based on identification with the use of specific PCR primers, and re-analysis of 16S rRNA gene sequences and menaquinones of the type species (Huang *et al.*, 2002).

At the time of writing, the genus *Pseudonocardia* comprises 30 recognized species. Strains of this genus have been isolated from various environmental samples, such as active sludge soil, including those polluted by industrial chemicals

(Lee *et al.*, 2004; Kämpfer & Kroppenstedt, 2004; Mahendra & Alvarez-Cohen, 2005; Liu *et al.*, 2006; Kämpfer *et al.*, 2006; Park *et al.*, 2008) and plant samples, including stems, leaves, root nodules, tree-bark compost and a traditional Chinese medicinal plant (Evtushenko *et al.*, 1989; Reichert *et al.*, 1998; Gu *et al.*, 2006; Chen *et al.*, 2009).

In the present study, a *Pseudonocardia*-like strain isolated from a plant litter sample collected during the course of a study on the diversity of actinomycetes inhabiting Vietnam (Sakiyama *et al.*, 2009) was studied by using a polyphasic taxonomic approach.

Plant litter samples were collected at the Ba Be National Park, Bac Kan, in the mountainous area of northern Vietnam. Samples were dried at room temperature for 5–7 days and subsequently used for isolation. Rehydration and centrifugation methods (Hayakawa *et al.*, 2000) were employed for isolation by using humic acid-vitamin agar (Hayakawa & Nonomura, 1987) containing nalidixic acid (20 mg l⁻¹) and kabicidine (0.75 mg l⁻¹). Strain VN05A0561^T was isolated after incubation for more than 10 days at room temperature.

Strain VN05A0561^T was cultured on yeast extract-soluble starch medium (YS medium; per litre distilled water: 2 g yeast extract, 10 g soluble starch and 15 g agar; pH 7.3) at 28 °C for 10–14 days. Pale yellow colonies with white aerial

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain VN05A0561^T is AB514449.

mycelia appeared on the YS medium. The substrate mycelia of the strain fragmented as per those of a nocardioform actinomycete. The spore chains sometimes formed zig-zag-shaped and intercalary swellings. The spores were rod-shaped (width, 0.3–0.5 µm; length, 1.0–2.0 µm) with a smooth surface (Fig. 1). Spores were non-motile. The cultural characteristics of strain VN05A0561^T were observed on ISP media 2–7 (Shirling & Gottlieb, 1966) and YS medium at 28 °C for 3 weeks. Moderate growth was observed on ISP 2–7 and YS medium. Raised and wrinkled colonies with moderate yellow colour appeared on ISP 2, 6 and 7. White aerial mycelia developed on ISP 2 and YS medium.

For chemotaxonomic analysis, biomass of strain VN05A0561^T was obtained by centrifugation and lyophilization after incubation in yeast extract-glucose broth (per litre distilled water: 10 g yeast extract and 10 g glucose; pH 7.3) for 10 days at 28 °C. Diaminopimelic acids and whole-cell sugars were analysed according to the method described by Stanek & Roberts (1974), isoprenoid quinones and phospholipids according to Minnikin *et al.* (1984), and cellular fatty acids according to Tamura *et al.* (1994) and by using the MIDI Sherlock Microbial Identification system (Microbial ID). Strain VN05A0561^T had meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, and arabinose and galactose as characteristic sugars. The cell wall was of type IV (Lechevalier & Lechevalier, 1970). The major isoprenoid quinone was MK-8(H₄). The cellular fatty acid profile of strain VN05A0561^T contained iso-C_{15:0} (7.9%), iso-C_{16:1} H (10.5%), iso-C_{16:0} (54.4%) and anteiso-C_{17:0} (14.1%) as major components, with minor amounts of iso-C_{14:0} (3.4%), anteiso-C_{15:0} (1.3%), C_{15:1} B (1.7%), C_{16:0} 9? methyl (1.8%), iso-C_{17:0} (1.3%) and anteiso-C_{17:0} (3.7%). The phospholipid was of type PIII, and the polar lipid profile consisted of phosphatidyl-*N*-methyl-ethanolamine, phosphatidylcholine, diphosphatidylglycerol, phosphatidylinositol and three unknown glycolipids. The chemotaxonomic properties of strain VN05A0561^T were consistent with the characteristics described for the genus *Pseudonocardia*.

Fig. 1. Scanning electron micrograph of cells of strain VN05A0561^T grown on ISP 2 medium for 14 days at 28 °C. Bar, 5 µm.

For 16S rRNA gene sequence analysis, the DNA of strain VN05A0561^T was extracted and amplified by using the procedures described by Sakiyama *et al.* (2009). The 16S rRNA gene sequence of strain VN05A0561^T was aligned via the CLUSTAL_X program (Thompson *et al.*, 1997) by using the corresponding sequences of the type strains of all recognized species of the genus *Pseudonocardia* available in the DDBJ/EMBL/GenBank databases. Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods. The topology of the phylogenetic trees was evaluated by bootstrap analysis of 1000 replicates (Felsenstein, 1985). The DNA G + C content of strain VN05A0561^T was determined by using the method described by Mesbah *et al.* (1989).

The 16S rRNA gene sequence of strain VN05A0561^T determined herein was 1454 nt long and showed 93.9–96.9% nucleotide sequence similarity with those of the type strains of all recognized species of the genus *Pseudonocardia*. Strain VN05A0561^T was most closely related to the type strains of *Pseudonocardia xinjiangensis* (96.9% 16S rRNA gene sequence similarity), *Pseudonocardia chloroethenivorans*, *Pseudonocardia hydrocarbonoxydans*, *Pseudonocardia alaniniphila*, *Pseudonocardia aurantiaca* (all 96.6%) and *Pseudonocardia asaccharolytica* (96.4%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain VN05A0561^T formed a cluster with the type strains of *P. asaccharolytica* and *Pseudonocardia acaciae* (Fig. 2), but did not form any definite clusters with other closely related *Pseudonocardia* strains. 16S rRNA gene sequence analysis thus suggested that strain VN05A0561^T represents a novel species of the genus *Pseudonocardia* (Stackebrandt & Goebel, 1994; Stackebrandt & Ebers, 2006). The DNA G + C content of strain VN05A0561^T was 73 mol%.

The physiological and biochemical characteristics of strain VN05A0561^T were examined after incubation at 28 °C for 3 weeks. YS medium containing 0, 1, 2, 3, 4 and 5% NaCl (w/v) was used for tests of NaCl tolerance. Urease production, degradation of casein and other compounds, utilization of citrate, other organic acids and carbohydrates, and acid production from carbohydrates were examined according to the methods of Sakiyama *et al.* (2009).

Strain VN05A0561^T could be differentiated from *P. xinjiangensis* based on NaCl tolerance, urease production, hypoxanthine decomposition and acid production from D-sorbitol and adonitol (Table 1).

On the basis of data from the present taxonomic study, we suggest that strain VN05A0561^T represents a novel species of the genus *Pseudonocardia*, for which the name *Pseudonocardia babensis* sp. nov. is proposed.

Description of *Pseudonocardia babensis* sp. nov.

Pseudonocardia babensis (ba.ben'sis. N.L. fem. adj. *babensis* referring to Ba Be National Park, Vietnam, from where the type strain was isolated).

Fig. 2. Neighbour-joining phylogenetic tree reconstructed based on 16S rRNA gene sequences showing the position of strain VN05A0561^T among members of the genus *Pseudonocardia*. Numbers on branches are confidence limits estimated by bootstrap analysis of 1000 replicates (only values above 500 are presented). Closed circles indicate branches that were also recovered with both the maximum-parsimony and the maximum-likelihood method; open circles indicate branches that were also recovered with the maximum-likelihood method. Bar, 0.005 sequence divergence (K_{nuc}).

Aerobic, Gram-positive, non-motile actinomycete. Colonies on YS medium are pale yellow and on ISP media 2, 6 and 7 are moderate yellow. Substrate mycelium frag-

Table 1. Differential physiological and biochemical characteristics between strain VN05A0561^T and closely related species of the genus *Pseudonocardia*

Taxa: 1, strain VN05A0561^T; 2, *P. xinjiangensis* CCTCC AA97020^T; 3, *P. chloroethenivorans* SL – 1^T; 4, *P. hydrocarbonoxydans* DSM 43281^T; 5, *P. alaniniphila* CCTCC AA97001^T; 6, *P. aurantiaca* CCTCC AA97002^T; 7, *P. asaccharolytica*; 8, *P. acaciae* GMKU 095^T. Data for reference strains were taken from Lee *et al.* (2004) and Duangmal *et al.* (2009). +, Positive; –, negative; w, weakly positive; ND, no data.

Characteristic	1	2	3	4	5	6	7	8
Acid production from:								
D-Sorbitol	+	–	–	–	–	–	–	–
Adonitol	–	+	–	–	–	–	–	+
Decomposition of:								
Urea	–	+	–	–	–	+	–	+
Hypoxanthine	–	+	+	–	+	+	–	ND
Growth on 3 % (w/v) NaCl	+	–	w	–	–	–	–	+

ments. White aerial mycelia and rod-shaped spores (width, 0.3–0.5 µm; length, 1.0–2.0 µm) with a smooth surface form on ISP 2 and YS medium. Grows at 10–45 °C and in the presence of 0–3 % (w/v) NaCl. Decomposes aesculin, casein and testosterone, but not adenine, hypoxanthine, xanthine, L-tyrosine or urea. Utilizes carbohydrates such as L-arabinose, D-arabitol, cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, melezitose, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, starch, sucrose, trehalose, xylitol and D-xylose, but not adonitol. Utilizes organic acids such as fumarate, malate and succinate, but not benzoate, mucate, oxalate, or L-tartrate. Produces acid from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, melezitose, D-sorbitol, sucrose and D-xylose, but not from adonitol or i-erythritol. The cell-wall diamino acid is *meso*-diaminopimelic acid. Whole-cell sugars are arabinose, mannose, glucose and galactose. The major menaquinone is MK-8(H₄). Major fatty acids are iso-C_{16:0}, iso-C_{16:1} and anteiso-C_{17:0}. Phospholipids are phosphatidyl-*N*-methylethanolamine, phosphatidylcholine, diphosphatidylglycerol, phosphatidylinositol and three unknown glycolipids. The DNA G + C content of the type strain is 73 mol%.

The type strain, VN05A0561^T (=VTCC-A-1757^T=NBRC 105793^T), was isolated from plant litter collected in Vietnam.

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