Pseudonocardia babensis sp. nov., isolated from plant litter

Yayoi Sakiyama,1 Nguyen K. N. Thao,2 Hoang V. Vinh,2 Nguyen M. Giang,2 Shinji Miyadoh,1 Duong V. Hop2 and Katsuhiko Ando1

1NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Kazusakamata 2-5-8, Kisarazu, Chiba, 292-0818, Japan
2Institute of Microbiology and Biotechnology (IMBT), Vietnam National University, Hanoi (VNUH), Japan

A novel actinomycete, designated strain VN05A0561T, 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 VN05A0561T 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 quione, phosphatidylglycerol as the diagnostic phospholipid and iso-C16:0 as the major cellular fatty acid. Strain VN05A0561T 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 VN05A0561T represents a novel species of the genus Pseudonocardia, for which the name Pseudonocardia babensis sp. nov. is proposed. The type strain is VN05A0561T (=VTCC-A-1757T=NBRC 105793T).

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 VN05A0561T was isolated after incubation for more than 10 days at room temperature.

Strain VN05A0561T 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...
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\textsuperscript{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\textsuperscript{T} aerial mycelia developed on ISP 2 and YS medium. A moderate yellow colour appeared on ISP 2, 6 and 7. White mycelia were observed on ISP 2 and YS medium. Raised and wrinkled colonies with moderate yellow colour appeared on ISP 2, 6 and 7.

Fig. 1. Scanning electron micrograph of cells of strain VN05A0561\textsuperscript{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\textsuperscript{T} was extracted and amplified by using the procedures described by Sakiyama \textit{et al.} (2009). The 16S rRNA gene sequence of strain VN05A0561\textsuperscript{T} was aligned with the \textit{CLUSTAL_X} program by using the corresponding sequences of the type strains of all recognized species of the genus \textit{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\textsuperscript{T} was determined by using the method described by Mesbah \textit{et al.} (1989).

The 16S rRNA gene sequence of strain VN05A0561\textsuperscript{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 \textit{Pseudonocardia}. Strain VN05A0561\textsuperscript{T} was most closely related to the type strains of \textit{Pseudonocardia xinjiangensis} (96.9% 16S rRNA gene sequence similarity), \textit{Pseudonocardia chloroethenivorans}, \textit{Pseudonocardia hydrocarbonoxydans}, \textit{Pseudonocardia alaminiphila}, \textit{Pseudonocardia aurantica} (all 96.6%) and \textit{Pseudonocardia asccharolytica} (96.4%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain VN05A0561\textsuperscript{T} formed a cluster with the type strains of \textit{P. asccharolytica} and \textit{Pseudonocardia acaciae} (Fig. 2), but did not form any definite clusters with other closely related \textit{Pseudonocardia} strains. 16S rRNA gene sequence analysis thus suggested that strain VN05A0561\textsuperscript{T} represents a novel species of the genus \textit{Pseudonocardia} (Stackebrandt & Goebel, 1994; Stackebrandt & Ebers, 2006). The DNA G+C content of strain VN05A0561\textsuperscript{T} was 73 mol%.

The physiological and biochemical characteristics of strain VN05A0561\textsuperscript{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 described by Sakiyama \textit{et al.} (2009).

Strain VN05A0561\textsuperscript{T} could be differentiated from \textit{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\textsuperscript{T} represents a novel species of the genus \textit{Pseudonocardia}, for which the name \textit{Pseudonocardia babensis} sp. nov. is proposed.

**Description of \textit{Pseudonocardia babensis} sp. nov.**

\textit{Pseudonocardia babensis} (ba. ben’sis. N.L. fem. adj. babensis referring to Ba Be National Park, Vietnam, from where the type strain was isolated).
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 fragments. 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, 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, 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, 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, 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, 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, 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, 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, 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.
The type strain, VN05A0561\(^T\) (=VTCC-A-1757\(^T\)=NBRC 105793\(^T\)), was isolated from plant litter collected in Vietnam.

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