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Pseudonocardia kongjuensis sp. nov., isolated from a gold mine cave

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The taxonomic position of an isolate that was recovered from a gold mine cave near Kongju, Republic of Korea, was determined by 16S rDNA sequence studies and chemotaxonomic characterization. Comparative studies of 16S rDNA sequences indicated that this organism was phylogenetically related to members of the genus *Pseudonocardia*, branching outside a cluster encompassing *Pseudonocardia autotrophica* and *Pseudonocardia compacta*. The affiliation to the genus was also supported by the cell chemistry, which was represented by a type IV cell wall, MK-8(H_4) as the major menaquinone, a phospholipid type PIII pattern (phosphatidylcholine as a diagnostic phospholipid) and a DNA G+C content of 71 mol%. The fatty acid profile contained saturated, unsaturated and 10-methyl branched fatty acids, but tuberculostearic acid and hydroxy fatty acids were not present. The isolate differed from its phylogenetic neighbours in the presence of phosphatidylethanolamine, dodecanoate, 16-methylheptadecenoate and 16-methylheptadecanoate and the absence of phosphatidylinositol mannoside and phosphatidylmethylethanolamine. The unique combination of physiological properties, the cellular fatty acid profile and DNA–DNA hybridization data indicates that this organism is readily differentiated from the type strains of all of the validly published species of the genus *Pseudonocardia*. The name *Pseudonocardia kongjuensis* sp. nov. is proposed for the type strain, LM 157^T (= IMSNU 50583^T = KCTC 9990^T = DSM 44525^T).

Keywords: *Pseudonocardia kongjuensis* sp. nov., soil bacteria, 16S rDNA sequence studies, chemotaxonomy

INTRODUCTION

The genus *Pseudonocardia*, proposed originally by Henssen (1957), was recently emended by Reichert *et al.* (1998) with the proposal of two new dimethyl disulfide-degrading species and currently contains 12 validly described species: *Pseudonocardia alni*, *Pseudonocardia asaccharolytica*, *Pseudonocardia autotrophica*, *Pseudonocardia compacta*, *Pseudonocardia halophobica*, *Pseudonocardia hydrocarbonoxydans*, *Pseudonocardia nitrificans*, *Pseudonocardia petroleophila*, *Pseudonocardia saturnea*, *Pseudonocardia spinosa*, *Pseudonocardia sulfidoxydans* and *Pseudonocardia thermophila*. Phylogenetically, the genus forms a distinct lineage within the radiation of the family *Pseudonocardiaceae* (McVeigh *et al.*, 1994; Warwick *et al.*, 1994; Reichert *et al.*, 1998) and has a following characteristics: vegetative and aerial mycelium with spore chains produced by acropetal budding or fragmentation, type IV cell wall, major menaquinone is MK-8(H_4), DNA G+C content of 68–79 mol%, no mycolic acids and phospholipid type II or III pattern, according to the species. The inter- and intrageneric relationships of the genera *Pseudonocardia* and *Actinobispora* (Jiang *et al.*, 1991) based on 16S rDNA sequences were investigated in our recent study (Lee *et al.*, 2000b). In this paper, we describe the characterization and classification of a soil isolate, LM 157^T, for which a new species is proposed, *Pseudonocardia kongjuensis* sp. nov.

The EMBL accession number for the 16S rDNA sequence of strain LM 157^T is AJ252833.

METHODS

Micro-organisms and culture conditions. Strain LM 157^T was isolated from soil collected at a gold mine cave in Kongju, Korea, by the dilution plating method on tap water agar (Lee, 1996). To obtain cells, the organism were grown on trypticase soy broth (BBL) with shaking at 30 °C for 3 d. For comparison, the following *Pseudonocardia* type strains were used: *P. alni* IFO 14491^T (= IMSNU 20049^T), *P. autotrophica* IFO 12743^T (= IMSNU 20050^T) and *P. compacta* IFO 14343^T (= IMSNU 20111^T).

For morphological observation, strain LM 157^T was grown on yeast extract/malt extract agar (ISP 2 medium) for 1–2 weeks at 28 °C. Morphological, physiological and chemotaxonomic characteristics were determined as described previously (Lee *et al.*, 2000a).

Phylogenetic analyses. The 16S rDNA sequences of strain LM 157^T and reference organisms were aligned using the CLUSTAL W program (Thompson *et al.*, 1994) and a phylogenetic tree was constructed by the neighbour-joining method (Saito & Nei, 1987) using the distance coefficient of Jukes & Cantor (1969). Parsimony analysis was performed with the PAUP program for the Macintosh (Swofford, 1998). DNA base composition and the level of DNA–DNA relatedness between isolate LM 157^T and reference organisms were determined by methods described previously (Lee *et al.*, 2000c).

RESULTS AND DISCUSSION

The almost complete 16S rDNA sequence of strain LM 157^T was compared with those of representatives of the family *Pseudonocardiaceae* and subsequently with those of species of the genera *Pseudonocardia* and *Actinobispora*. A total of 1441 unambiguous nucleotides present in all strains between positions 29 and 1152 (*Escherichia coli* numbering; Brosius *et al.*, 1978) were used in construction of the final tree. Phylogenetic analysis using distance-matrix and maximum-parsimony methods resulted in trees showing similar topologies. A phylogenetic tree (Fig. 1) indicated that strain LM 157^T formed a branch between the *P. autotrophica–P. compacta* cluster and the *P. alni–Pseudonocardia* sp. IMSNU 22071 cluster. This relationship was supported by a high bootstrap value (99 %). Strain LM 157^T showed levels of 16S rDNA sequence similarity ranging from 94·0 to 98·8 % to members of the genera *Pseudonocardia* and *Actinobispora*. Of the *Pseudonocardia* species, *P. autotrophica*

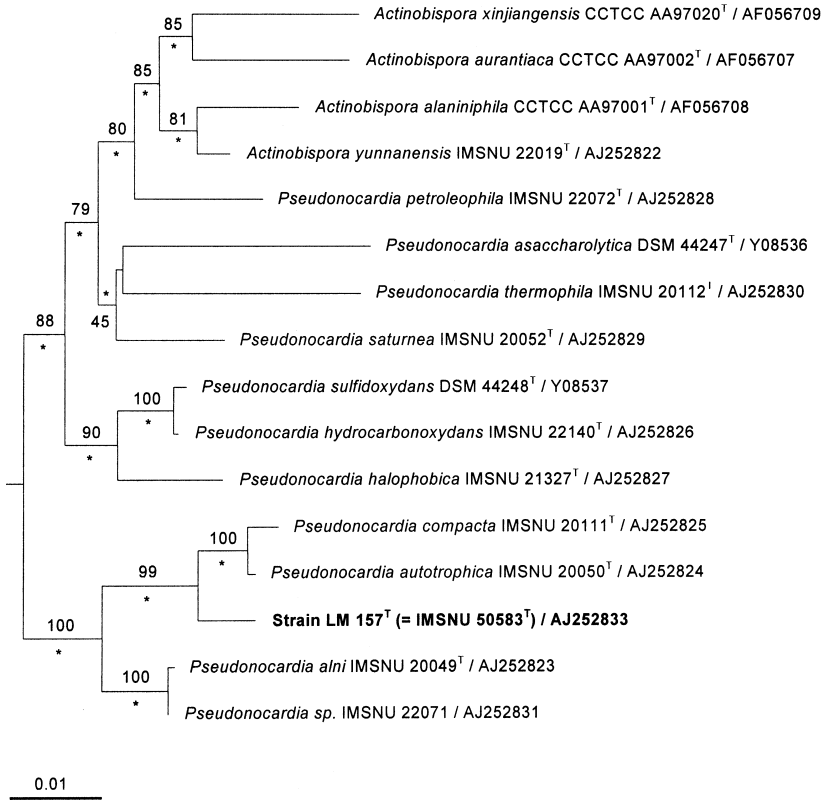

Fig. 1. Phylogenetic tree showing the position of strain LM 157^T within the radiation of the genera *Pseudonocardia* and *Actinobispora*. The tree was constructed by the neighbour-joining method (Saito & Nei, 1987) based on a comparison of 1441 unambiguous nucleotide positions. *Saccharothrix violacea* (Lee *et al.*, 2000c) was used as an outgroup (not shown). Asterisks indicate branching nodes that were also recovered in the parsimony analysis. Numbers at the branching points are percentages of occurrence in 1000 bootstrapped trees (only values greater than 40 % are indicated). Bar, 1 substitution per 100 nucleotides.

Table 1. Phospholipid and fatty acid compositions of strain LM 157^T and related taxa of the genus *Pseudonocardia*

All taxa shown contained diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. Abbreviations: PE, phosphatidylethanolamine; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethylethanolamine; PL, unknown phospholipid(s); i, iso; ai, anteiso; 10Me, 10-methyl. Percentages of total fatty acid content are given; fatty acids present at less than 0·5 % in all taxa are not shown. ND, Not detected.

Component	Strain LM 157^T	*P. compacta* IMSNU 20111^T	*P. autotrophica* IMSNU 20050^T	*P. alni* IMSNU 20049^T
Phospholipids				
PE	+	+	−	+
PIM	−	+	+	−
PME	−	+	+	+
PL	−	+	+	−
Fatty acids				
$C_{12:0}$	0·6	ND	ND	ND
i-$C_{14:0}$	ND	1·9	ND	ND
i-$C_{15:1}$	ND	ND	ND	0·6
i-$C_{15:0}$	2·4	3·6	4·3	11·2
$C_{15:1}$	ND	5·0	ND	ND
$C_{15:0}$	ND	10·0	ND	ND
i-$C_{16:1}$	6·7	4·2	10·0	18·2
i-$C_{16:0}$	31·4	34·5	33·5	28·0
$C_{16:1}$	7·4	3·8	4·4	4·9
$C_{16:0}$	6·0	3·0	2·0	1·0
i-$C_{17:1}$	4·4	ND	5·5	9·7
10Me-$C_{16:0}$	3·6	1·1	6·1	4·2
i-$C_{17:0}$	6·2	1·0	7·4	6·3
$C_{17:1}$	13·8	19·9	10·0	12·1
$C_{17:0}$	0·7	4·8	ND	ND
i-$C_{18:1}$	1·7	ND	0·6	ND
10Me-$C_{17:0}$	3·8	3·8	10·0	1·6
i-$C_{18:0}$	0·6	ND	ND	ND
$C_{18:0}$	9·6	2·6	3·3	1·8

(98·8 %) and *P. compacta* (98·5 %) revealed the highest sequence similarity to strain LM 157^T.

16S rDNA sequence studies indicated that isolate LM 157^T is related to members of the genera *Pseudonocardia* and *Actinobispora* (Fig. 1). We recently examined the inter- and intrageneric relationships of the genera *Pseudonocardia* and *Actinobispora* based on 16S rDNA sequence studies (Lee *et al*., 2000b). In our previous study, all of the validly named species of genus *Pseudonocardia* formed a coherent cluster with members of the genus *Actinobispora*, within the radiation of the family *Pseudonocardiaceae*. The type strains of both genera were intermixed and separated well from members of the other genera of the family *Pseudonocardiaceae*. This relationship was supported by a high bootstrap value of 100 % and the presence of 16S rDNA signature nucleotides unique for both genera (Lee *et al*., 2000b). Our recent study showed that *Actinobispora yunnanensis* IMSNU 22019^T possessed MK-8(H_4) as a major menaquinone (Lee *et al*., 2000b), which was not consistent with the results of Jiang *et al*. (1991) and Xu *et al*. (1999). Thus, phylogenetic evidence and chemical data, with the exception of phospholipid composition, suggest the unification of the two genera.

The affiliation of strain LM 157^T to the genus *Pseudonocardia* was confirmed by chemotaxonomic data. Strain LM 157^T has chemotaxonomic properties consistent with those of the genus *Pseudonocardia* (Reichert *et al*., 1998) as follows: the presence of *meso*-diaminopimelic acid, arabinose and galactose (type IV cell wall; Lechevalier & Lechevalier, 1970), the acetyl type of murein, no mycolic acid, MK-8(H_4) as the major menaquinone and phospholipid type PIII pattern (phosphatidylcholine as a diagnostic phospholipid).

Strain LM 157^T is differentiated from related *Pseudonocardia* species by the presence and/or the absence of phosphatidylinositol mannoside, phosphatidylethanolamine, phosphatidylmethylethanolamine and an unknown phospholipid(s) (Table 1). Since the results of fatty acid analyses can differ to some extent according to the laboratory and the methods used, the fatty acid profiles of the isolate and the type strains of related taxa were determined

Table 2. Characteristics that differentiate strain LM 157^T from reference *Pseudonocardia* strains

All taxa were negative for production of acid from D-xylitol.

Characteristic	Strain LM 157^T	*P. compacta* IMSNU 20111^T	*P. autotrophica* IMSNU 20050^T	*P. alni* IMSNU 20049^T
Acid produced from:				
L-Arabinose	−	−	+	+
D-Cellobiose	+	+	+	−
D-Galactose	+	−	+	+
Maltose	+	−	+	+
D-Mannose	+	−	+	−
D-Melezitose	+	−	+	+
Methyl α-D-glucoside	−	−	+	−
Salicin	−	−	−	+
Sucrose	+	−	+	+
D-Trehalose	+	−	+	+
D-Xylose	+	−	+	+
Adonitol	+	−	+	+
meso-Erythritol	−	−	−	+
meso-Inositol	+	−	+	−
D-Mannitol	+	+	+	−
1,2-Propanediol	−	−	+	+
D-Sorbitol	−	−	+	+
H_2S production	+	−	+	+
Urease activity	+	−	+	+
Decomposition of:				
Hypoxanthine	+	−	−	+
Tyrosine	+	−	−	+
Xanthine	−	−	−	+
Hydrolysis of:				
Casein	+	−	−	−
Gelatin	−	−	−	+
Starch	−	−	−	+
Growth at/on:				
4 °C	+	+	−	−
37 °C	+	−	+	+
7% NaCl	+	−	+	+

under standardized conditions (Table 2). All test strains had saturated, unsaturated and 10-methyl branched fatty acids but did not possess tuberculostearic acid or hydroxy fatty acids. However, strain LM 157^T differed from the *Pseudonocardia* reference strains in the amounts of hexadecanoic ($C_{16:0}$), hexadecenoic ($C_{16:1}$) and octadecanoic ($C_{18:0}$) acids and by the presence of small amounts of dodecanoic ($C_{12:0}$), 16-methylheptadecanoic (i-$C_{18:0}$) and 16-methylheptadecenoic (i-$C_{18:1}$) acids.

The physiology of the new isolate was compared with that of reference strains for a total of 47 characters. Most of the physiological properties of the reference strains were confirmed in this study, in agreement with those described previously (Reichert *et al*., 1998). However, *P. alni* IMSNU 20049^T did not produce acid from methyl α-D-glucoside or D-mannitol and *P. autotrophica* IMSNU 20050^T did not produce acid from *meso*-erythritol, in disagreement with previous results (Reichert *et al*., 1998). All of the reference strains produced acid from D-glucose, D-fructose and glycerol, whereas isolate LM 157^T showed a weak positive reaction in acid production from D-fructose and glycerol. None of the test strains produced acid from D-lactose, melibiose, methyl α-D-mannoside, D-raffinose, L-rhamnose, L-sorbose, 2,3-butanediol or dulcitol. Isolate LM 157^T has a morphology typical of the genus *Pseudonocardia*, in that aerial mycelium is fragmented into non-motile, rod-shaped spores (Fig. 2). The spore surface was smooth. No specific structures, such as sporangia, synnemata or sclerotia, were observed. Isolate LM 157^T grew well on all of the media used. Substrate mycelium was well-developed and yellowish-brown. No distinctive pigment was produced.

Strain LM 157^T differs physiologically from *P. autotrophica* IMSNU 20050^T in acid production from L-arabinose, methyl α-D-glucoside, 1,2-propanediol and D-sorbitol, in the decomposition of hypoxanthine and tyrosine and in the hydrolysis of casein (Table 2). The

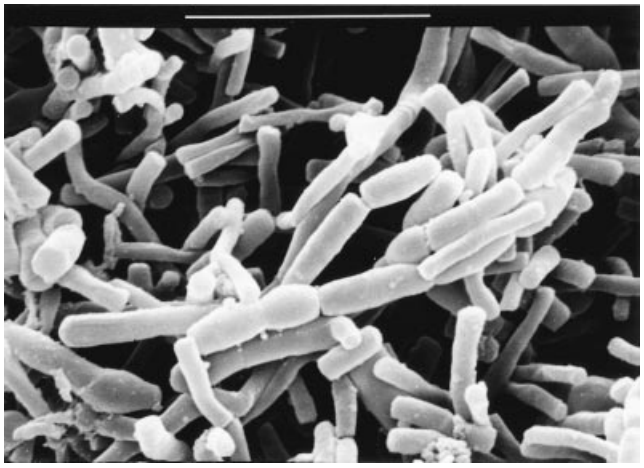

Fig. 2. Scanning electron micrograph of strain LM 157^T grown on yeast extract/malt extract agar (ISP medium 2). Bar, 5 μm.

isolate is readily distinguished from *P. compacta* IMSNU 2011^T by most of the physiological characters tested (Table 2). DNA–DNA hybridization studies showed that the isolate exhibited low levels of DNA relatedness to its phylogenetic neighbours *P. autotrophica* (32%) and *P. compacta* (14%). The DNA G+C content of isolate LM 157^T was 71 mol%.

Despite high phylogenetic similarity, *P. compacta* IMSNU 20111^T can be readily differentiated from *P. autotrophica* IMSNU 20050^T by its fatty acid profile, the presence of phosphatidylethanolamine and its physiological properties (Tables 1 and 2). *Pseudonocardia* sp. IMSNU 22071 (= DSM 44061), which had previously been described as '*Streptomyces nitrificans*' (Schatz *et al*., 1954), shows the same result as *P. alni* IMSNU 20049^T for phospholipid composition and all of the physiological tests except for acid production from D-mannose, D-trehalose and D-xylitol. These facts, together with the high level of 16S rDNA sequence similarity (99·9%), indicate that *Pseudonocardia* sp. IMSNU 22071 (= DSM 44061) is a strain of *P. alni*.

The unique combination of phenotypic and genotypic properties support the conclusion that isolate LM 157^T merits species status in the genus *Pseudonocardia*, and the name *Pseudonocardia kongjuensis* sp. nov. is proposed.

Description of *Pseudonocardia kongjuensis* sp. nov.

Pseudonocardia kongjuensis (kong.ju.en′sis. N. L. adj. *kongjuensis* of Kongju, Republic of Korea).

Aerobic, Gram-positive, non-acid-alcohol-fast. Forms a white aerial mycelium that fragments into rod-shaped spores. The spore surface is smooth. Substrate mycelium is abundant and yellowish-brown. Growth occurs between 4 and 37 °C. Catalase-positive. Urease-positive. H_2S is produced. Acid is produced from D-cellobiose, D-galactose, D-glucose, maltose, D-mannose, D-melezitose, sucrose, D-trehalose, D-xylose, adonitol, *meso*-inositol and D-mannitol. No acid production from L-arabinose, D-lactose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, D-raffinose, L-rhamnose, salicin, L-sorbose, 2,3-butanediol, dulcitol, *meso*-erythritol, 1,2-propanediol, D-sorbitol or D-xylitol. Weak acid production from D-fructose and glycerol. Casein is hydrolysed. Gelatin and starch are not hydrolysed. Hypoxanthine and tyrosine are decomposed. Xanthine is not decomposed. Growth occurs on 7% NaCl. Type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose in the cell wall). The acyl type of muramic acid is acetyl. The major menaquinone is MK-8(H_4). The phospholipid profile contains phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol (phospholipid type PIII pattern). The fatty acid profile contains saturated, unsaturated and 10-methyl branched fatty acids but tuberculostearic acid and hydroxy fatty acids are not present. Small amounts of dodecanoate, 16-methylheptadecenoate and 16-methylheptadecanoate are also present. The G+C content of the DNA is 71 mol%.

Isolated from a gold mine cave in Kongju, Republic of Korea. The type strain is strain LM 157^T (= IMSNU 50583^T = KCTC 9990^T = DSM 44525^T).

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