Nocardioides aestuarii sp. nov., isolated from tidal flat sediment

Hana Yi and Jongsik Chun

Correspondence
Jongsik Chun
jchun@snu.ac.kr

School of Biological Sciences, Seoul National University, 56-1 Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

A strictly aerobic, non-motile, rod-shaped actinomycete, designated strain JC2056^T, was isolated from a sediment sample of getbol, the tidal flat of Korea. Results of 16S rRNA gene sequence analysis indicated that the isolate belonged to the genus *Nocardioides*, with the highest similarity being to *Nocardioides luteus* KCTC 9575^T (95.7%). The major menaquinone was MK-8(H₄), and predominant cellular fatty acids were iso-16:0 and iso-16:1 H. The DNA G+C content was 70 mol%. Based on the morphological, physiological, biochemical and chemotaxonomical data presented in this study, strain JC2056^T should be classified as a novel species, for which the name *Nocardioides aestuarii* sp. nov. is proposed; the type strain is JC2056^T (=IMSNU 14029^T=KCTC 9921^T=JCM 12125^T).

Recently, a novel *Nocardioides* species was isolated from getbol (Korean tidal flat) of Ganghwa Island in Korea, and described as *Nocardioides ganghwensis* (Yi & Chun, 2004). An additional *Nocardioides* strain, JC2056^T, was also isolated from getbol sediment near the site (37°36'22.3"N, 126°22'59.4"E). In this study, the newly isolated strain was the subject of a taxonomic investigation to elucidate the identity; the name *Nocardioides aestuarii* sp. nov. is proposed for this isolate.

Strain JC2056^T was isolated using MR2A [R2A (Difco) supplemented with artificial sea salts (Sigma)] and was routinely cultured on marine agar 2216 (MA; Difco) at 30 °C and maintained as a glycerol suspension (20%, w/v) at –80 °C.

Primers, PCR condition and sequencing method for the 16S rRNA gene were as described previously (Chun & Goodfellow, 1995). Phylogenetic analyses were performed following the same methods and procedure described previously (Yi & Chun, 2004) using the jPHYDIT (available at <http://chunlab.snu.ac.kr/jphydit>) and PAUP 4.0 (Swofford, 1998). Initial BLAST searching against GenBank using the 16S rRNA gene sequence of strain JC2056^T (1380 bp) indicated that the test strain belonged to the genus *Nocardioides*. The newly determined sequence was then aligned manually against representatives of the family *Nocardioideaceae* using secondary-structure information of bacterial 16S rRNA. Domains used to construct the phylogenetic trees were the regions available for all sequences

(positions 38–1431; *Escherichia coli* numbering system), excluding positions showing ambiguous alignment (positions 76–95, 199–219, 455–478, 1025–1037 and 1135–1140). On the basis of 16S rRNA gene sequence similarity, the closest cultured bacterial relatives were *Nocardioides* species (93.6–95.7%), *Marmoricola aurantiacus* DSM 12652^T (93.1%) and *Aeromicrobium* species (92.7–93.0%). The highest sequence similarity was observed with *Nocardioides luteus* KCTC 9575^T (95.7%), which is below the recommended cut-off value of 97% for bacterial species definition (Stackebrandt & Goebel, 1994). This relationship between our isolate and other members of the family *Nocardioideaceae* was also evident in the phylogenetic tree (Fig. 1). Although the branching position varied depending on the tree-making algorithm, the phyletic line of strain JC2056^T was clearly placed within the *Nocardioides* clade based on all four tree-inferring methods used in this study. Our isolate was recovered as a sister group to the subclade containing *Nocardioides albus* KCTC 9186^T and *N. luteus* KCTC 9575^T in the neighbour-joining and Fitch–Margoliash trees with low bootstrap values (35–37%), or formed a clade with *Nocardioides plantarum* NCIMB 12834^T in the maximum-likelihood tree with less than 50% bootstrap support. The most-parsimonious tree showed that our isolate branched out between *N. plantarum* NCIMB 12834^T and the subclade containing *N. albus* KCTC 9186^T and *N. luteus* KCTC 9575^T with less than 50% bootstrap support.

All the phenotypical characters were investigated as described previously (Yi & Chun, 2004). Morphological, physiological and biochemical tests were performed using MA as the basal medium at 30 °C. The results of biochemical, physiological and morphological tests are given in Table 1 and the species description.

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The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA gene sequence of strain JC2056^T is AY423719.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationship between strain JC2056^T and members of the genus *Nocardioide*. Percentage bootstrap values of >50% (1000 resamplings) are given at the nodes, and solid circles indicate that the corresponding nodes (groupings) are also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. *Nocardia asteroides* ATCC 19247^T (GenBank/EMBL/DBJ accession no. Z36934) was used as the outgroup (not shown). Bar, 0.01 nt substitution per position.

Chemotaxonomical characteristics of strain JC2056^T were determined using the cells grown at 30 °C for 3 days on MA or in MB (marine broth 2216; Difco). Menaquinone and DNA G+C content (mol%) were determined by HPLC as described previously (Yi & Chun, 2004). Analysis of fatty acid methyl esters was performed by GLC according to the instruction of the Microbial Identification System (MIDI). Cell-wall diamino acid was determined as described by Stanek & Roberts (1974). Most chemotaxonomical characters of strain JC2056^T were typical of the genus *Nocardioide* (Collins *et al.*, 1994; Lawson *et al.*, 2000; O'Donnell *et al.*, 1982; Prauser, 1976, 1986; Suzuki & Komagata, 1983; Yoon *et al.*, 1997, 1999, 2003); MK-8(H₄) as a major menaquinone, LL-DAP as a cell-wall diamino-pimelic acid, 70 mol% as the DNA G+C content. The cellular fatty acid composition of our isolate also resembled that of other *Nocardioide* species, but was differentiated by high proportions of iso-16:0 (52.0%) and iso-16:1 H (14.5%).

On the basis of 16S rRNA gene phylogeny and chemotaxonomy, it is clear that our isolate belongs to the genus *Nocardioide*. Low 16S rRNA gene sequence similarity values to *Nocardioide* species (93.6–95.7%) indicate that strain JC2056^T represents a novel genomic species in the genus. Moreover, several morphological and physiological characteristics can be weighted to differentiate our isolate from other *Nocardioide* species (Table 1). Based on the polyphasic evidence presented in this study, it is fair to conclude that strain JC2056^T merits novel species status in the genus *Nocardioide*. The name *Nocardioide aestuarii* sp. nov. is proposed for strain JC2056^T.

Description of *Nocardioide aestuarii* sp. nov.

Nocardioide aestuarii (aes.tu'ar.i.i. N.L. gen. n. *aestuarii* of the tidal flat).

Gram-positive and strictly aerobic bacterium. Oxidase-negative, catalase-positive and not acid-fast. Spores are not formed. Cells are non-motile rods of approximately 0.9–2.1 × 0.3–0.4 μm. Colonies on MA are ivory, circular, convex, glistening, opaque and butyaceous and have an entire margin. Colonies are approximately 0.5–1 mm in diameter after 3 days on MA at 30 °C; they reach the maximum diameter of 1–2 mm after 5 days. Substrate or aerial mycelium is not observed. Grows at 20–35 °C, pH 6–10 and 0–8% (w/v) NaCl; optimal growth at 30 °C, pH 7 and 0–2% (w/v) NaCl. Major menaquinone is MK-8(H₄). Cell-wall diamino acid is LL-DAP. Major fatty acids are iso-16:0 (52.0%) and iso-16:1 H (14.5%). In addition, 17:1ω8c (7.0%), 10Me-17:0 (3.1%), 18:1ω9c (2.7%), iso-14:0 (1.8%), anteiso-17:0 (1.8%), 10:0 (1.4%), 10Me-16:0 (1.3%), anteiso-15:0 (1.3%), iso-15:0 (1.2%) and a mixture of iso-15:0 2-OH and 16:1ω7c (1.3%; MIDI system cannot differentiate these two fatty acids) are present. Trace amount of tuberculostearic acid (0.3%) also exists. DNA G+C content is 70 mol%. Results of API 20NE, API ZYM kits, decomposition of high-molecular-weight substrates and carbon source utilization are given in Table 1.

The type strain is JC2056^T (IMSNU 14029^T=KCTC 9921^T=JCM 12125^T). Isolated from sediment of getbol (tidal flat) in Ganghwa Island, Korea (37°36'22.3"N, 126°22'59.4"E).

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Table 1. Characteristics that differentiate strain JC2056^T from other *Nocardioides* strains

Strains: 1, Strain JC2056^T; 2, *N. albus* IMSNU 20105^T; 3, *N. aquaticus* IMSNU 13113^T; 4, *N. aquiterrae* KCCM 41647^T; 5, *N. ganghwensis* IMSNU 14028^T; 6, *N. jensenii* IMSNU 22036^T; 7, *N. luteus* IMSNU 22020^T; 8, *N. nitrophenolicus* KCTC 0457BP^T; 9, *N. plantarum* IMSNU 22067^T; 10, *N. pyridinolyticus* KCTC 0074BP^T; 11, *N. simplex* IMSNU 21329^T. +, Positive reaction; –, negative reaction; w, weakly positive; v, variable. All *Nocardioides* strains are positive for Gram reaction, catalase, gelatin hydrolysis, esterase lipase (C8) and leucine arylamidase; negative for acid-fastness, arginine dihydrolase, acid production from glucose (API 20NE kit), indole production from tryptophan, decomposition of adenine, alginate and cellulose, β -glucuronidase, *N*-acetyl- β -glucosaminidase and α -fucosidase. Acetate was utilized by all strains; D-sorbitol, inulin, 2-propanol, L-ascorbate, polyethylene glycol, salicylate and thiamin were not. Data from Yi & Chun (2004) except for *Nocardioides aestuarii* strain JC2056^T.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Motility	–	–	–	+	–	–	–	+	–	+	+
Mycelium and spore formation*	–	+	–	–	–	–	+	–	–	–	–
Oxidase	–	–	–	+	–	–	–	–	–	–	+
β -Galactosidase	+	+	–	+	+	–	+	–	–	+	+
Nitrate reduction	–	–	+	+	+	+	–	–	–	+	–
Urease	–	–	–	–	–	+	–	–	–	–	–
Decomposition of:											
Casein	+	+	w	+	+	+	+	+	–	+	+
DNA	+	w	+	+	+	+	+	+	–	+	+
Aesculin	w	w	–	+	w	–	+	w	w	+	+
Hypoxanthine	–	+	–	–	–	+	+	–	–	–	–
Starch	–	+	–	+	+	–	+	–	–	+	w
Tween 80	+	+	+	+	+	+	+	–	+	–	+
Tyrosine	–	+	+	–	+	+	+	+	–	+	+
Xanthine	–	+	–	+	w	+	+	–	–	+	–
API ZYM											
Alkaline phosphatase	w	+	v	–	+	w	v	+	–	+	+
Esterase (C4)	+	+	w	–	v	w	+	–	+	–	–
Lipase (C14)	–	–	–	–	–	–	–	–	w	–	–
Valine arylamidase	+	–	w	–	+	v	–	+	w	w	w
Cystine arylamidase	–	–	v	w	w	–	–	w	+	–	w
Trypsin	+	+	w	+	–	w	+	+	–	+	+
α -Chymotrypsin	+	–	–	–	–	–	–	–	–	–	–
Acid phosphatase	w	–	+	+	w	v	–	+	w	+	w
Naphthol-AS-BI-phosphohydrolase	w	w	–	+	–	v	v	+	+	+	–
α -Galactosidase	–	–	–	–	+	–	–	–	–	–	–
β -Galactosidase	+	v	–	–	+	–	v	–	–	–	–
α -Glucosidase	+	+	+	+	+	v	v	+	+	+	+
β -Glucosidase	–	w	–	w	–	–	–	w	+	–	w
α -Mannosidase	–	–	–	–	–	–	+	–	–	–	–
Carbon sources utilized:											
Acetamide	–	–	–	–	–	–	–	–	–	–	+
Benzoate	–	+	–	–	–	+	+	–	–	–	–
Citrate	+	+	–	w	+	+	+	+	+	–	+
D-Cellobiose	+	+	–	+	+	–	+	–	+	+	–
D-Fructose	+	+	+	+	+	–	–	+	+	+	–
D-Galactose	+	+	+	+	+	–	–	–	–	+	–
D-Glucose	+	+	+	+	+	+	+	+	–	+	+
D-Mannitol	+	+	+	+	+	–	+	–	–	–	–
D-Mannose	–	+	–	–	+	–	+	–	–	–	–
D-Raffinose	w	–	–	–	+	–	–	–	–	–	–
D-Ribose	–	–	–	–	–	–	–	+	–	+	–
D-Salicin	–	w	–	–	+	–	–	–	+	–	–
D-Xylose	+	+	+	+	+	–	+	+	+	+	–
Glycerol	w	w	+	–	+	w	–	–	+	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Inositol	—	—	—	—	—	—	—	—	—	+	—
Lactose	W	—	—	—	+	—	—	—	—	—	—
L-Arabinose	—	+	—	—	+	—	+	—	—	—	—
L-Arginine	—	—	—	—	—	—	—	+	+	—	+
L-Asparagine	—	W	—	+	+	+	—	—	—	+	+
L-Lysine	W	—	—	+	—	W	—	—	—	—	—
L-Ornithine	—	W	—	—	+	—	—	—	+	—	+
L-Rhamnose	—	+	W	—	—	+	—	+	+	+	—
N-Acetylglucosamine	—	W	—	—	+	—	+	—	—	—	—
Succinate	+	+	+	+	+	—	+	+	+	+	+
Sucrose	+	—	+	+	+	W	—	+	+	+	+
Tartrate	—	—	—	W	—	W	—	—	—	—	—

*On agar media tested in this study.

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