Nocardioides hankookensis sp. nov., isolated from soil

Jung-Hoon Yoon, So-Jung Kang, Mi-Hwa Lee and Tae-Kwang Oh

Correspondence

Jung-Hoon Yoon

jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejeon, Republic of Korea

A Gram-positive, non-motile, rod-shaped bacterial strain, DS-30^T, was isolated from soil from Dokdo, Korea, and its taxonomic position was investigated by means of a polyphasic study. Strain DS-30^T grew optimally at pH 6.0–7.0 and 25 °C in the presence of 0–0.5 % (w/v) NaCl. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-30^T fell within the genus *Nocardioides*, forming a coherent cluster with the type strains of *Nocardioides aquiterrae* and *Nocardioides pyridinolyticus*, with which it exhibited 16S rRNA gene sequence similarity values of 98.1 and 98.3 %, respectively. Strain DS-30^T exhibited 16S rRNA gene sequence similarity values below 96.8 % with respect to the type strains of the other *Nocardioides* species. The chemotaxonomic properties of strain DS-30^T were consistent with those of the genus *Nocardioides*: the cell-wall peptidoglycan type was based on LL-diaminopimelic acid, the predominant menaquinone was MK-8(H₄) and the major fatty acids were iso-C_{16:0}, C_{17:1ω8c} and C_{18:1ω9c}. The DNA G + C content was 71.3 mol%. Strain DS-30^T exhibited DNA–DNA relatedness levels of 19 and 22 % with respect to the type strains of *N. aquiterrae* and *N. pyridinolyticus*, respectively, and could be differentiated from these species with reference to a number of phenotypic characteristics. On the basis of the data obtained, strain DS-30^T represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides hankookensis* sp. nov. is proposed. The type strain is DS-30^T (=KCTC 19246^T =CCUG 54522^T).

The genus *Nocardioides* was created by Prauser (1976) with a single species, *Nocardioides albus*. Recently, the number of *Nocardioides* species has increased considerably through the continual description of novel species. Many of the novel *Nocardioides* species described recently have been isolated from various regions of Korea (Yoon *et al.*, 2005a, b, c, 2006a, b, 2007; Lee, 2007; Choi *et al.*, 2007). In this study, we describe a *Nocardioides*-like bacterial strain, DS-30^T, isolated from soil from Dokdo (37° 14' 12" N 131° 52' 07" E), an island of Korea.

Strain DS-30^T was isolated by means of the standard dilution plating technique at 25 °C with 10-fold-diluted nutrient agar (Difco). *Nocardioides pyridinolyticus* OS4^T and *Nocardioides aquiterrae* GW-9^T (Yoon *et al.*, 1997, 2004) were used as reference strains for DNA–DNA hybridization. The morphological, physiological and biochemical characteristics of strain DS-30^T were investigated using routine cultivation on R2A agar (Difco) or trypticase soy agar (Difco) at 25 °C. Morphological, physiological, cultural and biochemical properties were examined as described by Yoon *et al.* (2005a). Growth at various NaCl concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 %, w/v) was

investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. The pH range for growth was determined in trypticase soy broth (Difco) adjusted to various pH values (pH 4.5–10.5, in increments of 0.5 pH units) by the addition of HCl and Na₂CO₃. Susceptibility to antibiotics was tested by placing antibiotic-impregnated discs on R2A agar plates seeded with suspensions of the test strain. Substrate utilization, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux); the utilization of various substrates was determined by inoculating an API 50 CH strip with cells suspended in AUX medium (bioMérieux). Morphological, cultural, physiological and biochemical characteristics of strain DS-30^T are given in the species description or in Table 1. Cell biomass for DNA extraction and for the analysis of cell walls and isoprenoid quinones was obtained from cultivation for 3 days at 25 °C in trypticase soy broth. For fatty acid methyl ester analysis, cell mass of strain DS-30^T was harvested from R2A agar plates after incubation for 7 days at 25 °C. Molecular systematic and chemotaxonomic studies were performed as described by Yoon *et al.* (2005a). The isomer type of the diamino acid in the cell-wall peptidoglycan was analysed using TLC according to

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain DS-30^T is EF555584.

the method described by Komagata & Suzuki (1987). DNA–DNA hybridization was performed fluorometrically using the method of Ezaki *et al.* (1989) with photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values.

The almost-complete 16S rRNA gene sequence of strain DS-30^T determined in this study comprised 1447 nt (approx. 96 % of the *Escherichia coli* 16S rRNA sequence). Comparative 16S rRNA gene sequence analysis showed that strain DS-30^T is most closely related phylogenetically to members of the genus *Nocardioides* (Fig. 1). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DS-30^T joined the cluster comprising *N. aquiterrae* and *N. pyridinolyticus* at a bootstrap resampling value of 100 %. Strain DS-30^T exhibited 16S rRNA gene sequence similarity values of 98.1 and 98.3 % with respect to the type strains of *N. aquiterrae* and *N. pyridinolyticus*, respectively, and showed values of 94.2–96.8 % with respect to the type strains of the other *Nocardioides* species. The sequence similarity values with respect to other species used in the phylogenetic analysis were below 94.0 %.

Chemotaxonomic properties confirm the phylogenetic results, namely that strain DS-30^T is affiliated to the genus *Nocardioides* (Tamura & Yokota, 1994; Yoon *et al.*, 1997; Lawson *et al.*, 2000; Urzi *et al.*, 2000; Wang *et al.*, 2001). Strain DS-30^T contained LL-2,6-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan.

MK-8(H₄) was the predominant isoprenoid quinone. The fatty acid profile was composed of the following (each constituting >0.5 % of total fatty acids): branched fatty acids iso-C_{16:0} (42.7 %), anteiso-C_{17:0} (3.5 %), iso-C_{17:0} (2.8 %), iso-C_{15:0} (2.3 %), iso-C_{18:0} (1.9 %) and iso-C_{14:0} (1.4 %); unsaturated fatty acids C_{17:1ω8c} (14.8 %), C_{18:1ω9c} (13.1 %) and C_{18:1ω7c} (0.6 %); straight-chain fatty acids C_{17:0} (2.4 %), C_{16:0} (1.4 %), C_{15:0} (0.8 %) and C_{18:0} (0.6 %); 10-methyl fatty acids C_{17:0} (5.5 %), C_{18:0} (2.3 %) and C_{16:0} (0.5 %); and summed features 6 (C_{19:1ω9c} and/or C_{19:1ω11c}; 1.9 %) and 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH; 0.7 %). This fatty acid profile was similar to those of *Nocardioides* species, although there were differences in the proportions of some fatty acids, probably because of differences in cultivation and extraction conditions (Yoon *et al.*, 1999, 2005a, 2006a, 2007; Kubota *et al.*, 2005; Choi *et al.*, 2007). The DNA G+C content of strain DS-30^T was 71.3 mol %.

Strain DS-30^T exhibited DNA–DNA relatedness levels of 19 and 22 % with respect to *N. aquiterrae* GW-9^T and *N. pyridinolyticus* OS4^T, respectively, the type strains of the two most closely phylogenetically related *Nocardioides* species. Strain DS-30^T was distinguishable from *N. aquiterrae* and *N. pyridinolyticus* through differences in several phenotypic characteristics (Table 1). On the basis of the phylogenetic distinctiveness of strain DS-30^T, the DNA–DNA relatedness data and the differential phenotypic properties, this strain represents a novel species of the genus *Nocardioides* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), for which the name *Nocardioides hankookensis* sp. nov. is proposed.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain DS-30^T and some related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of strain DS-30^T and two phylogenetically related *Nocardioides* species

Strains: 1, strain DS-30^T; 2, *N. pyridinolyticus* OS4^T; 3, *N. aquiterrae* GW-9^T. Data are from Yoon *et al.* (1997, 2004), Yi & Chun (2004) and this study. All strains give positive results for the Gram stain, catalase, hydrolysis of aesculin, casein and gelatin, utilization of D-xylose, glucose, fructose, cellobiose, maltose, sucrose, trehalose, starch and turanose and for esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All strains are negative for hydrolysis of hypoxanthine and urea, utilization of glycerol, erythritol, D-arabinose, L-xylose, adonitol, methyl β -D-xyloside, mannose, sorbose, dulcitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, salicin, inulin, raffinose, glycogen, xylitol, D-lyxose, D-tagatose, D- and L-fucose, L-arabitol, 2-ketogluconate, 5-ketogluconate, caprate, citrate and phenylacetate and for lipase (C14), α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Cell morphology	Rods	Rods, cocci	Rods, cocci
Cell size (μm)	0.4–0.8 $\times$ 1.5–10.0	0.5–0.6 $\times$ 1.2–1.6	0.8–1.0 $\times$ 1.7–2.0
Motility	–	+	+
Colony colour*	White	Cream	Cream
Oxidase	+	–	+
Optimal temperature ($^{\circ}\text{C}$)	25	35	30
Nitrate reduction	–	+	+
Hydrolysis of:			
Starch	+	+	–
Tween 80	w	–	+
Xanthine	–	+	–
Tyrosine	–	+	–
Utilization of sole carbon and energy sources†			
L-Arabinose	+	–	–
Ribose	–	+	–
Galactose	+	–	–
L-Rhamnose	+	+	–
Inositol	–	+	–
D-Mannitol	+	–	+
Aesculin	+	–	+
Lactose	–	–	+
Melibiose	+	–	–
Melezitose	–	+	–
Gentiobiose	w	–	+
D-Arabitol	–	+	–
Gluconate	w	–	+
Adipate	+	+	–
Malate	+	–	+
Enzyme activities (API ZYM)			
Alkaline phosphatase	+	+	–
Esterase (C4)	+	–	–
Valine arylamidase	–	w	–
Cystine arylamidase	–	–	w
Trypsin	–	+	+
α -Glucosidase	–	+	+
β -Glucosidase	–	–	w
DNA G + C content (mol%)	71.3	73	73
Isolation source	Soil	Oil-shale column	Groundwater

*Differences may be caused by different cultivation conditions.

†Data for *N. pyridinolyticus* OS4^T and *N. aquiterrae* GW-9^T are from this study.

Description of *Nocardioides hankookensis* sp. nov.

Nocardioides hankookensis (han.koo.ken'sis. N.L. masc. adj. *hankookensis* of Hankook, the Korean name for South Korea, from where the type strain was isolated).

Cells are aerobic, non-spore-forming, non-motile, curved or straight rods ($0.4\text{--}0.8 \times 1.5\text{--}10.0\ \mu\text{m}$) in the exponential phase of growth. Cells do not show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Gram-positive, but Gram-variable in old cultures. Colonies on R2A agar are circular to slightly irregular with entire margins, convex, smooth, glistening, white in colour and $0.7\text{--}1.0\ \text{mm}$ in diameter after 7 days incubation on R2A agar at $25\ ^\circ\text{C}$. Neither substrate nor aerial mycelium is formed. Growth occurs at 10 and $34\ ^\circ\text{C}$, but not at 4 or $35\ ^\circ\text{C}$. Optimal pH for growth is $6.0\text{--}7.0$. Growth occurs at pH 5.5 and 8.0 , but not at pH 5.0 or 8.5 . Growth occurs in the presence of $0\text{--}2\%$ (w/v) NaCl, with an optimum at $0\text{--}0.5\%$ (w/v) NaCl. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H_2S and indole are not produced. Tweens 20, 40 and 60 are hydrolysed. Susceptible to the following antibiotics (μg per disc, unless indicated otherwise): carbenicillin (100), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), oleandomycin (15), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Resistant to the following antibiotics (μg per disc, unless indicated otherwise): ampicillin (10), cephalothin (30), novobiocin (5) and penicillin G (20 U). The diagnostic diamino acid in the cell-wall peptidoglycan is LL-2,6-diaminopimelic acid. The predominant menaquinone is MK-8(H_4). The major fatty acids ($>10\%$ of total fatty acids) are iso- $\text{C}_{16:0}$, $\text{C}_{17:1}\omega 8\text{c}$ and $\text{C}_{18:1}\omega 9\text{c}$. The DNA G+C content of the type strain is $71.3\ \text{mol}\%$ (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-30^T (=KCTC 19246^T =CCUG 54522^T), was isolated from soil from Dokdo, an island of Korea.

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

References

- Choi, D. H., Kim, H. M., Noh, J.-H. & Cho, B. C. (2007). *Nocardioides marinus* sp. nov. *Int J Syst Evol Microbiol* **57**, 775–779.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

- Kubota, M., Kawahara, K., Sekiya, K., Uchida, T., Hattori, Y., Futamata, H. & Hiraishi, A. (2005). *Nocardioides aromaticivorans* sp. nov., a dibenzofuran-degrading bacterium isolated from dioxin-polluted environments. *Syst Appl Microbiol* **28**, 165–174.
- Lawson, P. A., Collins, M. D., Schumann, P., Tindall, B. J., Hirsch, P. & Labrenz, M. (2000). New LL-diaminopimelic acid-containing actinomycetes from hypersaline, heliothermal and meromictic Antarctic Ekho Lake: *Nocardioides aquaticus* sp. nov. and *Friedmanniella lacustris* sp. nov. *Syst Appl Microbiol* **23**, 219–229.
- Lee, S. D. (2007). *Nocardioides furvisabuli* sp. nov., isolated from black sand. *Int J Syst Evol Microbiol* **57**, 35–39.
- Prauser, H. (1976). *Nocardioides*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **26**, 58–65.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamura, T. & Yokota, A. (1994). Transfer of *Nocardioides fastidiosa* Collins and Stackebrandt 1989 to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. *Int J Syst Bacteriol* **44**, 608–611.
- Urzi, C., Salamone, P., Schumann, P. & Stackebrandt, E. (2000). *Marmoricola aurantiacus* gen. nov., sp. nov., a coccoid member of the family *Nocardioidaceae* isolated from a marble statue. *Int J Syst Evol Microbiol* **50**, 529–536.
- Wang, Y. M., Zhang, Z. S., Xu, X. L., Ruan, J. S. & Wang, Y. (2001). *Actinopolymorpha singaporensis* gen. nov., sp. nov., a novel actinomycete from the tropical rainforest of Singapore. *Int J Syst Evol Microbiol* **51**, 467–473.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yi, H. & Chun, J. (2004). *Nocardioides ganghwensis* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **54**, 1295–1299.
- Yoon, J.-H., Rhee, S.-K., Lee, J.-S., Park, Y.-H. & Lee, S. T. (1997). *Nocardioides pyridinoliticus* sp. nov., a pyridine-degrading bacterium isolated from the oxic zone of an oil shale column. *Int J Syst Bacteriol* **47**, 933–938.
- Yoon, J.-H., Cho, Y.-G., Lee, S. T., Suzuki, K., Nakase, T. & Park, Y.-H. (1999). *Nocardioides nitrophenolicus* sp. nov., a *p*-nitrophenol-degrading bacterium. *Int J Syst Bacteriol* **49**, 675–680.
- Yoon, J.-H., Kim, I.-G., Kang, K. H., Oh, T.-K. & Park, Y.-H. (2004). *Nocardioides aquiterrae* sp. nov., isolated from groundwater in Korea. *Int J Syst Evol Microbiol* **54**, 71–75.
- Yoon, J.-H., Kim, I.-G., Lee, M.-H., Lee, C.-H. & Oh, T.-K. (2005a). *Nocardioides alkalitolerans* sp. nov., isolated from an alkaline serpentine soil in Korea. *Int J Syst Evol Microbiol* **55**, 809–814.
- Yoon, J.-H., Kim, I.-G., Lee, M.-H. & Oh, T.-K. (2005b). *Nocardioides kribbensis* sp. nov., isolated from an alkaline soil. *Int J Syst Evol Microbiol* **55**, 1611–1614.
- Yoon, J.-H., Lee, C.-H. & Oh, T.-K. (2005c). *Nocardioides dubius* sp. nov., isolated from an alkaline soil. *Int J Syst Evol Microbiol* **55**, 2209–2212.
- Yoon, J.-H., Lee, C.-H. & Oh, T.-K. (2006a). *Nocardioides lentus* sp. nov., isolated from an alkaline soil. *Int J Syst Evol Microbiol* **56**, 271–275.
- Yoon, J.-H., Lee, J.-K., Jung, S.-Y., Kim, J.-A., Kim, H.-K. & Oh, T.-K. (2006b). *Nocardioides kongjuensis* sp. nov., an *N*-acylhomoserine lactone-degrading bacterium. *Int J Syst Evol Microbiol* **56**, 1783–1787.
- Yoon, J.-H., Kang, S.-J., Lee, C.-H. & Oh, T.-K. (2007). *Nocardioides insulae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 136–140.