Nocardioides terrigena sp. nov., isolated from soil

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A Gram-positive, rod- or coccoid-shaped bacterial strain, DS-17^T, was isolated from a soil in Dokdo, Korea, and its taxonomic position was investigated by using a polyphasic approach. Strain DS-17^T grew optimally at around pH 8.0 and 30 °C in the presence of 0.5–1.0% (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain DS-17^T belonged to the genus *Nocardioides*. The chemotaxonomic properties of strain DS-17^T were consistent with those of the genus *Nocardioides*: the cell-wall peptidoglycan type was based on LL-2,6-diaminopimelic acid, MK-8(H₄) was the predominant menaquinone and iso-C_{16:0}, C_{17:1}ω8c and C_{17:0} were the major fatty acids. The DNA G + C content was 71.5 mol%. Strain DS-17^T exhibited 16S rRNA gene sequence similarity values of 94.5–96.9% to the type strains of recognized *Nocardioides* species. Strain DS-17^T could be distinguished from recognized *Nocardioides* species by differences in phenotypic characteristics. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain DS-17^T is considered to represent a novel species of the genus *Nocardioides*, for which the name *Nocardioides terrigena* sp. nov. is proposed. The type strain is DS-17^T (=KCTC 19217^T=JCM 14582^T).

The genus *Nocardioides* was proposed by Prauser (1976). At the time of writing, it comprised 23 recognized species, including the recently described species *Nocardioides lentus* (Yoon *et al.*, 2006a), *N. kongjuensis* (Yoon *et al.*, 2006b), *N. furvisabuli* (Lee, 2007), *N. insulae* (Yoon *et al.*, 2007), *N. marinus* (Choi *et al.*, 2007) and *N. panacihumi* (An *et al.*, 2007). Here we report the taxonomic characterization of a *Nocardioides*-like strain, DS-17^T, which was isolated from soil in Dokdo, Korea.

A soil sample collected from Dokdo (37° 14' 12" N 131° 52' 07" E) was used as a source for the isolation of bacterial strains. Strain DS-17^T was isolated by using the standard dilution plating technique at 25 °C on 10 × diluted nutrient agar (Difco). The morphological, physiological and biochemical characteristics of strain DS-17^T were investigated via routine cultivation on R2A 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 liquid R2A medium (R2A broth) prepared according to the formula of the Difco medium except that agar was not added. The pH range for growth was determined in R2A broth that was adjusted to various pH values (pH 4.0–10.5 at intervals of 0.5 pH units) by the addition of HCl and

Na₂CO₃. The susceptibility to various antibiotics was tested on R2A agar plates by using antibiotic discs as follows: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg), carbenicillin (100 µg). Utilization of various substrates, 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); utilization of various substrates was determined by inoculating the API 50 CH strip with cells suspended in AUX medium (bioMérieux). Morphological, cultural, physiological and biochemical characteristics of strain DS-17^T are given in the species description (see below) or are detailed in Table 1 and Supplementary Table S1 (available in IJSEM Online).

Cell biomass for DNA extraction and for the analyses of cell-wall and isoprenoid quinones was obtained by cultivation for 3 days at 30 °C in R2A broth. For fatty acid methyl ester analysis, cell mass of strain DS-17^T was harvested from R2A agar plates after incubation for 7 days at 30 °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 by using TLC according to the method described by Komagata & Suzuki (1987).

The almost complete 16S rRNA gene sequence of strain DS-17^T determined in this study comprised 1470 nt (approximately 96% of the *Escherichia coli* 16S rRNA gene

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-17^T is EF363712.

A table detailing the differential phenotypic characteristics of strain DS-17^T and related *Nocardioides* species is available as supplementary material with the online version of this paper.

Table 1. Differential phenotypic characteristics of strain DS-17^T and two phylogenetically related *Nocardioides* species

Taxa: 1, strain DS-17^T; 2, *N. pyridinoliticus*; 3, *N. aquiterrae*. Data taken from Yoon *et al.* (1997, 2004) and this study. +, Positive reaction; –, negative reaction; w, weakly positive reaction. All are positive for Gram-stain, catalase, nitrate reduction, hydrolysis of casein and gelatin, utilization of D-xylose, glucose, fructose, cellobiose, maltose, sucrose, trehalose, starch and D-turanose, esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α -glucosidase. All 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-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate, 5-ketogluconate, caprate, citrate and phenylacetate, lipase (C14), α -chymotrypsin, α -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Characteristic	1	2	3
Cell morphology	Rods, cocci	Rods, cocci	Rods, cocci
Cell size (μm)	0.4–0.7 $\times$ 0.7–2.0	0.5–0.6 $\times$ 1.2–1.6	0.8–1.0 $\times$ 1.7–2.0
Motility	–	+	+
Colony colour*	Ivory	Cream	Cream
Oxidase	+	–	+
Optimal temperature ($^{\circ}\text{C}$)	30	35	30
Hydrolysis of:			
Aesculin	–	+	+
Starch	w	+	–
Tween 80	w	–	+
Xanthine	–	+	–
Tyrosine	–	+	–
Utilization as sole carbon and energy sources of:†			
Ribose	–	+	–
Galactose	+	–	–
L-Rhamnose	+	+	–
Inositol	–	+	–
Mannitol	+	–	+
Arbutin	–	–	+
Aesculin	–	–	+
Lactose	+	+	–
Melibiose	+	–	–
D-Melezitose	–	+	–
Gentiobiose	+	–	+
D-Lyxose	w	–	–
D-Arabitol	–	+	–
Gluconate	+	–	+
Adipate	+	+	–
Malate	+	–	+
API ZYM tests			
Alkaline phosphatase	+	+	–
Esterase (C4)	+	–	–
Valine arylamidase	–	w	–
Cystine arylamidase	–	–	w
Trypsin	–	+	+
Acid phosphatase	–	+	+
β -Galactosidase	+	–	–
β -Glucosidase	–	–	w
DNA G + C content (mol%)	71.5	73	73
Isolation source	Soil	Oil shale column	Groundwater

*Differences may be caused by different cultivation conditions.

†Data for *N. pyridinoliticus* and *N. aquiterrae* are from this study.

sequence). Comparative 16S rRNA gene sequence analyses showed that strain DS-17^T was affiliated most closely to the genus *Nocardioide*s (Fig. 1). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DS-17^T fell within the radiation of the cluster comprising *Nocardioide*s species (Fig. 1). Strain DS-17^T exhibited highest 16S rRNA gene sequence similarity values (96.9%) to the type strains of *Nocardioide*s *aquiterrae*, *Nocardioide*s *pyridinolyticus* and *Nocardioide*s *kribbensis*. Strain DS-17^T exhibited 16S rRNA gene sequence similarity values of 94.5–96.7% to the type strains of other *Nocardioide*s species and of less than 94.7% to other species used in the phylogenetic analysis.

Chemotaxonomic properties confirmed the phylogenetic affiliation of strain DS-17^T as a member of the genus *Nocardioide*s (Tamura & Yokota, 1994; Yoon *et al.*, 1997; Lawson *et al.*, 2000; Urzì *et al.*, 2000; Wang *et al.*, 2001). The diagnostic diamino acid found in strain DS-17^T was LL-2,6-diaminopimelic acid, which is characteristic of wall chemotype I *sensu* Lechevalier & Lechevalier (1970). Strain DS-17^T contained menaquinone-8(H₄) as the predominant isoprenoid quinone. The fatty acid profile of strain DS-17^T included the following (each constituting >0.5% of the total fatty acids): branched fatty acids iso-C_{16:0} (35.5%), iso-C_{17:0} (2.3%), iso-C_{15:0} (1.6%), iso-C_{16:1} (1.5%), iso-C_{14:0} (1.3%), iso-C_{18:0} (1.3%) and anteiso-C_{17:0} (1.2%); unsaturated fatty acids C_{17:1}ω8*c* (30.2%), C_{18:1}ω9*c* (2.7%), C_{17:1}ω6*c* (1.1%) and C_{15:1}ω6*c* (0.8%); straight-chain fatty acids C_{17:0} (11.5%), C_{15:0} (2.6%) and C_{16:0} (1.7%); 10-methyl fatty acid C_{17:0} (1.8%); summed feature 3 (C_{16:1}ω7*c* and/or iso-C_{15:0} 2-OH, 1.6%); and

hydroxy fatty acid C_{17:0} 3-OH (1.1%). Tuberculostearic acid was not detected. This fatty acid profile was similar to those of recognized *Nocardioide*s species, although there were differences in the proportions of some components, particularly iso-C_{16:0}, C_{17:1}ω8*c* and C_{17:0}, perhaps because of differences in cultivation and extraction conditions (Yoon *et al.*, 1997, 1999, 2004, 2005a, b, 2006a, b, 2007; Yi & Chun, 2004; Kubota *et al.*, 2005). The DNA G+C content of strain DS-17^T was 71.5 mol%. Strain DS-17^T differed from recognized *Nocardioide*s species in several phenotypic characteristics (Table 1 and Supplementary Table S1 in IJSEM Online). The phylogenetic distinctiveness, together with differential phenotypic properties, were sufficient to allocate strain DS-17^T to a species that is separate from all recognized *Nocardioide*s species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-17^T is considered to represent a novel species of the genus *Nocardioide*s, for which the name *Nocardioide*s *terrigena* sp. nov. is proposed.

Description of *Nocardioide*s *terrigena* sp. nov.

*Nocardioide*s *terrigena* (ter.ri.ge'na. L. masc. or fem. n. *terrigena* child of the earth, referring to the isolation of the type strain from soil).

Cells are aerobic, non-spore-forming, rods or cocci (0.4–0.7 µm × 0.7–2.0 µm) in the exponential phase of growth. Cells show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Gram-positive but Gram-variable in old cultures. Colonies are circular, slightly convex, smooth, glistening, ivory in colour and 1.0–1.2 mm in diameter after 7 days incubation on R2A

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DS-17^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood or maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

agar at 30 °C. Neither substrate nor aerial mycelia are formed. Growth occurs at 4 and 35 °C, but not at 36 °C. Optimal pH for growth is around 8.0. Growth occurs in the presence of 0–3 % (w/v) NaCl with optimum growth in the presence of 0.5–1.0 % (w/v) NaCl. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H₂S and indole are not produced. Tweens 20, 40 and 60 are hydrolysed. Adipate, L-malate, starch, gentiobiose, D-turanose and gluconate are utilized as sole carbon and energy sources and D-lyxose is weakly utilized, but caprate, citrate, phenylacetate, glycerol, erythritol, D-arabinose, L-xylose, methyl β -D-xyloside, sorbose, dulcitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, inulin, glycogen, xylitol, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate and 5-ketogluconate are not. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, kanamycin, lincomycin, neomycin, novobiocin, oleandomycin, penicillin G and streptomycin and weakly susceptible to tetracycline, but not to gentamicin or polymyxin B. The cell-wall peptidoglycan contains LL-2,6-diaminopimelic acid as the diagnostic diamino acid. The predominant menaquinone is MK-8(H₄). The major fatty acids (>10 % of the total fatty acids) are iso-C_{16:0}, C_{17:1} ω 8c and C_{17:0}. The DNA G+C content is 71.5 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1 and Supplementary Table S1 (available in IJSEM Online).

The type strain, DS-17^T (=KCTC 19217^T=JCM 14582^T), was isolated from soil.

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References

- An, D.-S., Im, W.-T., Lee, S.-T. & Yoon, M.-H. (2007). *Nocardioides panacihumi* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **57**, 2143–2146.
- 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.
- 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.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: VEB Gustav Fischer.
- 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.
- 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 pyridinolyticus* 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.-I., 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 serpentinite 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. (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.