Nocardioides insulae sp. nov., isolated from soil

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A Gram-positive, rod-shaped or coccoid bacterial strain, DS-51^T, was isolated from a soil in Dokdo, Korea, and its taxonomic position was investigated by using a polyphasic approach. Strain DS-51^T grew optimally at pH 8.0 and 30 °C without NaCl. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain DS-51^T forms a distinct line of descent within the radiation enclosed by the genus *Nocardioides*. The chemotaxonomic properties of strain DS-51^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} was the major fatty acid. The DNA G+C content was 71.1 mol%. The 16S rRNA gene sequence of strain DS-51^T had similarity levels of 92.5–95.1 % with the sequences of the type strains of *Nocardioides* species. Strain DS-51^T could be distinguished from other *Nocardioides* species by differences in some phenotypic characteristics. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain DS-51^T represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides insulae* sp. nov. is proposed. The type strain is DS-51^T (=KCTC 19180^T=DSM 17944^T).

The genus *Nocardioides* was proposed by Prauser (1976) and, at the time of writing, comprises 18 recognized species, including the recently described species *Nocardioides lentus* (Yoon *et al.*, 2006a) and *Nocardioides kongjuensis* (Yoon *et al.*, 2006b). Here, we report on the taxonomic characterization of a *Nocardioides*-like strain, DS-51^T, which was isolated from a soil collected in Dokdo, an island of Korea.

Strain DS-51^T was isolated by using the standard dilution plating technique at 30 °C on 10 × diluted nutrient agar (NA; Difco). To investigate its morphological, physiological and biochemical characteristics, strain DS-51^T was routinely cultivated at 30 °C on NA and in nutrient broth (NB; Difco). The morphological, physiological, cultural and biochemical properties were examined as described by Yoon *et al.* (2005a). Growth at various NaCl concentrations (0–8 %, w/v, using increments of 1.0 %) was investigated in trypticase soy broth prepared according to the formula of Difco medium except that no NaCl was used. The pH range for growth was determined in NB that was adjusted to various pH values (pH 4.0–10.5, using increments of 0.5 pH units) by the addition of HCl and Na₂CO₃. The susceptibility of strain DS-51^T to various antibiotics was tested on NA plates by using antibiotic discs, as follows: 100 U polymyxin B, 50 µg streptomycin, 20 U penicillin G, 100 µg chloramphenicol, 10 µg ampicillin, 30 µg cephalothin, 30 µg gentamicin, 5 µg novobiocin, 30 µg tetracycline, 30 µg

kanamycin, 15 µg lincomycin, 15 µg oleandomycin, 30 µg neomycin and 100 µg carbenicillin. Other physiological and biochemical properties were tested by using the API 20E and API ZYM systems (bioMérieux). Cell biomass for DNA extraction and for analyses of cell walls and isoprenoid quinones was obtained by cultivation in NB at 30 °C for 3 days. For fatty acid methyl ester analysis, cell mass of strain DS-51^T was harvested from NA plates after incubation at 30 °C for 7 days. Chemotaxonomic and molecular systematic 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 method described by Komagata & Suzuki (1987).

Morphological, cultural, physiological and biochemical characteristics of strain DS-51^T are given in the species description (see below) or are shown in Table 1. Strain DS-51^T was found to be susceptible to polymyxin B, streptomycin, chloramphenicol, cephalothin, gentamicin, tetracycline, carbenicillin, kanamycin, lincomycin, neomycin and oleandomycin, but not to ampicillin, novobiocin or penicillin G. The almost-complete 16S rRNA gene sequence of strain DS-51^T, comprising 1484 nt (approx. 96 % of the *Escherichia coli* 16S rRNA gene sequence), was determined in this study. Comparative 16S rRNA gene sequence analyses showed that strain DS-51^T is most closely affiliated to the genus *Nocardioides* (Fig. 1). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DS-51^T formed a distinct phylogenetic lineage with the radiation of the cluster comprising *Nocardioides* species (Fig. 1). Strain DS-51^T exhibited 16S rRNA gene sequence

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-51^T is DQ786794.

Table 1. Differential phenotypic characteristics of strain DS-51^T and other *Nocardioide*s species

Taxa: 1, strain DS-51^T; 2, *N. albus*; 3, *N. luteus*; 4, *N. simplex*; 5, *N. plantarum*; 6, *N. pyridinolyticus*; 7, *N. nitrophenolicus*; 8, *N. aquaticus*; 9, *N. aquiterrae*; 10, *N. ganghwensis*; 11, *N. aestuarii*; 12, *N. alkalitolerans*; 13, *N. kribbensis*; 14, *N. oleivorans*; 15, *N. aromaticivorans*; 16, *N. lentus*; 17, *N. kongjuensis*. Data are from Collins *et al.* (1989, 1994), Lawson *et al.* (2000), Prauser (1976, 1984, 1989), Suzuki & Komagata (1983), Yoon *et al.* (1997, 1999, 2004, 2005a, b, 2006a, b), Yi & Chun, (2004a, b), Schippers *et al.* (2005) and Kubota *et al.* (2005). +, Positive reaction; –, negative reaction; ND, not determined; w, weakly positive reaction; v, variable reaction. Data in parentheses are for the type strain. All species are positive for the Gram stain, for catalase and for esterase lipase (C8) (not determined for *N. oleivorans*). All species are negative for β -glucuronidase, *N*-acetyl- β -glucosaminidase and α -fucosidase activities (not determined for *N. oleivorans*).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Cell morphology	Rods, cocci	Hyphae	Hyphae	Rods, cocci	Short rods, cocci	Rods, cocci	Rods, cocci	Cocci, short rods	Rods, cocci	Rods	Rods	Rods, cocci	Short rods, cocci	Irregular rods	Rods	Rods, cocci	Rods, cocci
Cell size (μm)	0.6–1.0 $\times$ 1.3–6.0	0.5–1.0*	0.5–1.0*	1.0–1.2 $\times$ 1.5–6.0	ND	0.5–0.6 $\times$ 1.2–1.6	0.5–0.8 $\times$ 1.0–3.0	0.9–1.0 $\times$ 0.9–1.4	0.8–1.0 $\times$ 1.7–2.0	0.4–0.5 $\times$ 0.9–4.5	0.3–0.4 $\times$ 0.9–2.1	0.8–1.0 $\times$ 1.5–2.0	0.8–1.0 $\times$ 1.5–2.0	0.3 $\times$ ~1.1	0.5–0.7 $\times$ 1.0–2.0	0.4–0.7 $\times$ 1.0–4.5	0.4–0.7 $\times$ 0.8–3.0
Motility	–	–	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–
Colony colour†	Ivory	Whitish to faintly brownish	Yellow to orange–yellow or cream	Yellowish white	ND	Cream	Yellowish white	Dull orange	Cream	Ivory	Ivory	Milky white	Cream	Orange	Milky white	Yellow	Yellowish white
Optimum temperature (°C)	30	28	28	26–37	25	35	30	16–26	30	30	30	25–30	30	30	30	28	30–37
Nitrate reduction	+	(–)	–	(–)	–	+	–	+	+	+	–	+	+	ND	–	+	–
Hydrolysis of:																	
Aesculin	–	(w)	+	(+)	w	+	+	–	+	w	w	–	+	ND	+	–	–
Casein	+	(+)	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	+
Gelatin	+	(+)	+	+	+	+	+	+	+	+	+	v(+)	+	ND	ND	+	+
Hypoxanthine	–	(+)	+	(–)	–	–	–	–	–	–	–	–	–	ND	+	–	–
Starch	–	(+)	+	(w)	–	+	+	w‡	–	+	–	–	–	ND	–	–	–
Tween 80	+	(+)	+	(+)	+	–	+	+	+	+	+	+	+	ND	–	+	+
Tyrosine	–	(+)	+	(+)	–	+	+	+	–	+	–	+	+	ND	–	v(+)	–
Urea	–	(–)	–	–	–	–	+	–	–	–	–	–	–	ND	+	–	–
Xanthine	–	(+)	–‡	(–)	–	+	–	–	–	w	–	–	–	ND	–	–	–
Utilization as sole carbon and energy sources:																	
L-Arabinose	–	+	+	–	–	–	–	–	–	+	–	+	–	–	+ or w	w	–
D-Cellobiose	+	(+)	+	(–)	+	+	–	–	+	+	+	+	+	+	+	+	–
D-Fructose	–	+	+	(–)	+	+	+	+	+	+	+	–	–	+	+	–	–
D-Galactose	+	(+)	–	(–)	–	+	–	–‡	+	+	+	–	+	+	–	+	–
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Maltose	w	ND	ND	ND	+	+	–	w	+	ND	ND	v(–)	+	+	+ or w	+	–
D-Mannose	w	(+)	+	(–)	–	+	w‡	–	+	+	–	–	–	+	w	–	w
Sucrose	–	v(+‡)	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
D-Trehalose	+	ND	ND	ND	+	+	+	w	+	ND	ND	+	+	+	ND	+	+
D-Xylose	w	+	+	–	+	+	+	w‡	+	+	+	–	–	ND	+	v(+)	–
API ZYM tests:																	
Acid phosphatase	+	(–)	–	(w)	w	+	+	+	+	w	w	+	+	ND	–	+	+
Alkaline phosphatase	+	(+)	v	(+)	–	+	+	v	–	+	w	+	+	ND	–	+	+
α -Chymotrypsin	–	(–)	–	(–)	–	–	–	–	–	–	+	–	+	ND	–	–	–
Cystine arylamidase	–	(–)	–	(w)	+	–	w	v	w	w	–	–	–	ND	–	–	–
Esterase (C4)	+	(+)	+	(–)	+	–	–	w	–	v	+	+	+	ND	–	+	–
α -Galactosidase	–	(–)	–	(–)	–	–	–	–	–	+	–	–	–	ND	–	–	–
β -Galactosidase	–	(v)	v	(–)	–	–	–	–	–	+	+	–	w	ND	–	–	–
α -Glucosidase	–	(+)	v	(+)	+	+	+	+	+	+	+	+	+	ND	+	–	+
β -Glucosidase	–	(w)	–	(w)	+	–	w	–	w	–	–	–	–	ND	–	+	–
Leucine arylamidase	–	(+)	+	(+)	+	+	+	+	+	+	+	–	+	ND	+	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Lipase (C14)	—	(—)	—	(—)	W	—	—	—	—	—	—	+	—	ND	—	—	—
α -Mannosidase	—	(—)	+	(—)	—	—	—	—	—	—	—	—	—	ND	—	—	—
Naphthol-AS-BI-phosphohydrolase	—	(w)	v	(—)	+	+	+	—	+	—	w	w	+	ND	—	+	w
Trypsin	—	(+)	+	(+)	—	+	+	w	+	—	+	—	—	ND	—	—	—
Valine arylamidase	—	(—)	—	(w)	w	w	+	w	—	+	+	—	—	ND	—	—	—
DNA G+C content (mol%)	71	67	68	72–74	69	73	71	69	73	72	70	72–74	73–74	ND	72.0–72.4	74.6–74.8	72.1
Isolation source	Soil	Soil	Soil	Soil	Herbage	Oil shale column	Industrial wastewater	Saline lake, Antarctica	Groundwater	Tidal flat	Tidal flat	Alkaline soil	Alkaline soil	Crude oil	River water, sediment, soil	Alkaline soil	Soil

*Cell width is shown.

†Differences may be caused by different cultivation conditions.

‡Data from Prauser (1976, 1984), Collins *et al.* (1994), Yoon *et al.* (1997, 1999, 2004) and Lawson *et al.* (2000); a different result was reported by Yi & Chun (2004a).

milarity values of 92.5–95.1 % with respect to the type strains of other *Nocardioide*s species and showed values of less than 92.1 % sequence similarity with other species used in the phylogenetic analysis.

The chemotaxonomic properties supported the result of the monothetic phylogenetic classification suggesting that strain DS-51^T could be 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 present in strain DS-51^T was LL-2,6-diaminopimelic acid, which is characteristic of wall chemotype I *sensu* Lechevalier & Lechevalier (1970). The predominant isoprenoid quinone detected in strain DS-51^T was a tetrahydrogenated menaquinone with eight isoprene units [MK-8(H₄)]. The cellular fatty acid profile (i.e. fatty acids representing >1 % of the total) comprised the following: branched fatty acids iso-C_{16:0} (49.7 %), ante-iso-C_{17:0} (4.9 %), iso-C_{17:0} (2.6 %), iso-C_{18:0} (2.3 %), iso-C_{15:0} (1.6 %) and iso-C_{16:1} (2.3 %); unsaturated fatty acids C_{18:1} ω 9c (7.4 %), C_{17:1} ω 6c (5.4 %) and C_{17:1} ω 8c (1.1 %); straight-chain fatty acids C_{18:0} (2.3 %), C_{17:0} (1.9 %) and C_{16:0} (1.7 %); 10-methyl fatty acids C_{18:0} (8.3 %), C_{17:0} (2.9 %) and C_{16:0} (1.8 %), and summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH) (1.6 %). This fatty acid profile is similar to those of *Nocardioide*s species, although there are differences in the proportions of some fatty acids, perhaps because of differences in the extraction and cultivation conditions (Yoon *et al.*, 1997, 1999, 2004, 2005a, b; Lawson *et al.*, 2000; Yi & Chun, 2004a, b; Kubota *et al.*, 2005). The DNA G + C content of strain DS-51^T was 71.1 mol%. The differential phenotypic properties of DS-51^T, together with its phylogenetic distinctiveness, are sufficient to categorize the strain as a novel species of the genus *Nocardioide*s (Stackebrandt & Goebel, 1994; Table 1), for which the name *Nocardioide*s *insulae* sp. nov. is proposed.

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

*Nocardioide*s *insulae* (in.su'lae. L. fem. gen. n. *insulae* of an island, referring to the source of isolation of the type strain).

Cells are aerobic, non-spore-forming, Gram-positive rods or cocci (0.6–1.0 $\times$ 1.0–6.0 μ m) in the exponential phase of growth. Cells show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Oxidase-positive. Colonies are circular, smooth, glistening, slightly convex, ivory in colour and 1.0–1.5 mm in diameter after 7 days incubation on NA at 30 °C. Neither substrate nor aerial mycelium is formed. Growth occurs at 10 and 34 °C, but not at 4 or 35 °C. The optimal pH for growth is 8.0; growth occurs at pH 6.5, but not at pH 6.0. Growth occurs in the presence of 0–3 % (w/v) NaCl, but is optimum in the absence of NaCl. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are absent. H₂S and indole are not produced. Tweens 20, 40 and 60 are hydrolysed. Acetate, L-malate, pyruvate and L-glutamate are utilized as sole carbon and energy sources and salicin is weakly utilized, but citrate, succinate,

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain DS-51^T and some related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at the branch points. Bar, 0.01 substitutions per nucleotide position.

benzoate and formate are not utilized. 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 acid (> 10 % of total fatty acids) is iso-C_{16:0}. The DNA G+C content is 71.1 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-51^T (=KCTC 19180^T=DSM 17944^T), was isolated from soil.

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