Nocardioides lentus sp. nov., isolated from an alkaline soil

Jung-Hoon Yoon, Choong-Hwan Lee and Tae-Kwang Oh

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

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr

Gram-positive, rod- or coccoid-shaped bacterial strains KSL-17^T, KSL-18 and KSL-19 were isolated from an alkaline soil in Korea. They were subjected to analysis using polyphasic taxonomy. Strains KSL-17^T, KSL-18 and KSL-19 grew optimally at pH 8·0 and 28 °C and in the presence of 0·5 % (w/v) NaCl. They were characterized chemotaxonomically as having LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan, MK-8(H_4) as the predominant menaquinone and iso-$C_{16:0}$ as the major fatty acid. Their DNA G+C contents were in the range 74·6–74·8 mol%. Strains KSL-17^T, KSL-18 and KSL-19 were identical in terms of their 16S rRNA gene sequences and exhibited a mean level of DNA–DNA relatedness of 85–90 %. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains KSL-17^T, KSL-18 and KSL-19 formed a distinct phylogenetic lineage within the genus *Nocardioides*. The levels of 16S rRNA gene sequence similarity between the three strains and the type strains of *Nocardioides* species were in the range 92·6–95·2 %. On the basis of phenotypic, genetic and phylogenetic data, strains KSL-17^T (=KCTC 19039^T=DSM 16315^T), KSL-18 and KSL-19 should be classified as members of a novel species of the genus *Nocardioides*, for which the name *Nocardioides lentus* sp. nov. is proposed.

The genus *Nocardioides* was proposed by Prauser (1976), and, at the time of writing, the genus comprises 15 recognized species, including the recently described species *Nocardioides alkalitolerans* (Yoon *et al.*, 2005a), *Nocardioides kribbensis* (Yoon *et al.*, 2005b), *Nocardioides oleivorans* (Schippers *et al.*, 2005) and *Nocardioides dubius* (Yoon *et al.*, 2005c). In this study, we report the taxonomic characterization of three *Nocardioides*-like strains, KSL-17^T, KSL-18 and KSL-19, which were isolated from an alkaline soil (approximately pH 9·0–10·0) collected in Kwangchun, Korea.

Strains KSL-17^T, KSL-18 and KSL-19 were isolated by using the standard dilution plating technique at 30 °C on 10× diluted nutrient agar (Difco) with the pH adjusted to 9·0 using Na_2CO_3. To investigate their morphological, physiological and biochemical characteristics, strains KSL-17^T, KSL-18 and KSL-19 were routinely cultivated at 28 °C on 2× diluted nutrient agar with the pH adjusted to 8·0. Morphological, physiological, cultural and biochemical properties were examined as described by Yoon *et al.* (2005a). Growth in the absence of NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. Growth at various NaCl concentrations (0·5 %, w/v, and 1·0–7·0 %, w/v, at intervals of 1·0 %) was investigated in trypticase soy broth (Difco). The pH range for growth was determined in 2× diluted nutrient broth (Difco) that was adjusted to various pH values (initial pH 4·5–10·5 at intervals of 0·5 pH units). The pH was adjusted, prior to sterilization, to various levels by the addition of HCl or Na_2CO_3. Cell biomass for DNA extraction and for the analyses of cell-wall and isoprenoid quinones was obtained by cultivation at 28 °C in 2× diluted nutrient broth (pH 8·0). 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). For fatty acid methyl ester analysis, cell mass from the three strains was harvested from 2× diluted nutrient agar (pH 8·0) after incubation for 10 days at 28 °C.

The morphological, cultural, physiological and biochemical characteristics of strains KSL-17^T, KSL-18 and KSL-19 are given in the species description (see later) or are shown in Table 1. The 16S rRNA gene sequences of strains KSL-17^T, KSL-18 and KSL-19 were identical and each comprised 1491 nt, representing approximately 96 % of the *Escherichia*

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KSL-17^T, KSL-18 and KSL-19 are DQ121389, DQ121390 and DQ121391, respectively.

A table giving the cellular fatty acid content of strains KSL-17^T, KSL-18 and KSL-19 is available as supplementary material in IJSEM Online.

Table 1. Differential phenotypic characteristics of *Nocardioides lentus* sp. nov. and related *Nocardioides* species

Species: 1, *Nocardioides lentus* sp. nov.; 2, *Nocardioides albus*; 3, *Nocardioides luteus*; 4, *Nocardioides simplex*; 5, *Nocardioides plantarum*; 6, *Nocardioides pyridinolyticus*; 7, *Nocardioides nitrophenolicus*; 8, *Nocardioides aquaticus*; 9, *Nocardioides aquiterrae*; 10, *Nocardioides ganghwensis*; 11, *Nocardioides aestuarii*; 12, *Nocardioides alkalitolerans*; 13, *Nocardioides kribbensis*; 14, *Nocardioides oleivorans*. 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), Yi & Chun (2004a, b) and Schippers *et al.* (2005). +, Positive reaction; −, negative reaction; ND, not determined; W, weakly positive reaction; V, variable reaction. Data for the type strain are shown in parentheses. All species are positive for Gram stain, catalase and esterase lipase (C8) (not determined for *N. oleivorans*). All species are negative for *β*-glucuronidase, *N*-acetyl-*β*-glucosaminidase and *α*-fucosidase (not determined for *N. oleivorans*).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
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
Cell size (μm)	0·4–0·7 × 1·0–4·5	0·5–1·0	0·5–1·0	1·0–1·2 × 1·5–6·0	ND	0·5–0·6 × 1·2–1·6	0·5–0·8 × 1·0–3·0	0·9–1·0 × 0·9–1·4	0·8–1·0 × 1·7–2·0	0·4–0·5 × 0·9–4·5	0·3–0·4 × 0·9–2·1	0·8–1·0 × 1·5–2·0	0·8–1·0 × 1·5–2·0	0·3 × ∼1·1
Motility	−	−	−	+	−	+	+	−	+	−	−	−	−	−
Colony colour*	Yellow	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
Optimal temp. (°C)	28	28	28	26–37	25	35	30	16–26	30	30	30	25–30	30	30
Nitrate reduction	+	(−)	−	(−)	−	+	−	+	+	+	−	+	+	ND
Hydrolysis of:														
Aesculin	−	(W)	+	(+)	W	+	+	−	+	W	W	−	+	ND
Casein	+	(+)	+	+	+†	+	+	+	+	+	+	+	+	ND
Starch	−	(+)	+	(W)	−	+	+	W†	−	+	−	−	−	ND
Gelatin	+	(+)	+	+	+	+	+	+	+	+	+	V (+)	+	ND
Tween 80	+	(+)	+	(+)	+	−	+	+	+	+	+	+	+	ND
Urea	−	(−)	−	−	−	−	+	−	−	−	−	−	−	ND
Hypoxanthine	−	(+)	+	(−)	−	−	−	−	−	−	−	−	−	ND
Xanthine	−	(+)	−†	(−)	−	+	−	−	−	W	−	−	−	ND
Tyrosine	V (+)	(+)	+	(+)	−	+	+	+	−	+	−	+	+	ND
Utilization of substrates as sole carbon and energy sources														
L-Arabinose	W	+	+	−	−	−	−	−	−	+	−	+	−	−
D-Cellobiose	+	(+)	+	(−)	+	+	−	−	+	+	+	+	+	+
D-Fructose	−	+	+†	(−)	+	+	+	+	+	+	+	−	−	+
D-Galactose	+	(+)	−	(−)	−	+	−	−†	+	+	+	−	+	+
D-Glucose	+	+	+	+	+†	+	+	+	+	+	+	−	+	+
myo-Inositol	−	−	−	(−)	−	+	−	W†	−	−	−	−	−	ND
Lactose	V (+)	(−)	−	(−)	−	+†	−	−	+	+	W	−	−	ND
Maltose	+	ND	ND	ND	+	+	−	W	+	ND	ND	V (−)	+	+
D-Mannitol	+	+	+	−	−	+†	−	+	+	+	+	−	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D-Mannose	−	(+)	+	(−)	−	+†	W†	−	+	+	−	−	−	+
Melezitose	+	ND	ND	ND	+	ND	−	−	−	ND	ND	+	−	ND
D-Melibiose	−	ND	ND	ND	ND	−	−	W	−	ND	ND	−	−	+
D-Raffinose	+	V (−)	−	−	−	−	−	−	−	+	W	+	+	ND
L-Rhamnose	+	+	+†	−	+	+	+	+†	W†	−	−	−	+	+
D-Ribose	−	(−)	−	(−)	−	+	+	−	W†	−	−	−	+	−
Sucrose	+	V (+†)	+†	+	+	+	+	+	+	+	+	−	+	+
D-Trehalose	+	ND	ND	ND	+	+	+	W	+	ND	ND	+	+	+
D-Xylose	V (+)	+	+	−	+	+	+	W†	+	+	+	−	−	ND
API ZYM														
Alkaline phosphatase	+	(+)	V	(+)	−	+	+	V	−	+	W	+	+	ND
Esterase (C4)	+	(+)	+	(−)	+	−	−	W	−	V	+	+	+	ND
Lipase (C14)	−	(−)	−	(−)	W	−	−	−	−	−	−	+	−	ND
Leucine arylamidase	+	(+)	+	(+)	+	+	+	+	+	+	+	−	+	ND
Valine arylamidase	−	(−)	−	(W)	W	W	+	W	−	+	+	−	−	ND
Cystine arylamidase	−	(−)	−	(W)	+	−	W	V	W	W	−	−	−	ND
Trypsin	−	(+)	+	(+)	−	+	+	W	+	−	+	−	−	ND
α-Chymotrypsin	−	(−)	−	(−)	−	−	−	−	−	−	+	−	+	ND
Acid phosphatase	+	(−)	−	(W)	W	+	+	+	+	W	w	+	+	ND
Naphthol-AS-BI-phosphohydrolase	+	(W)	V	(−)	+	+	+	−	+	−	w	W	+	ND
α-Galactosidase	−	(−)	−	(−)	−	−	−	−	−	+	−	−	−	ND
β-Galactosidase	−	(V)	V	(−)	−	−	−	−	−	+	+	−	W	ND
α-Glucosidase	−	(+)	V	(+)	+	+	+	+	+	+	+	+	+	ND
β-Glucosidase	+	(W)	−	(W)	+	−	W	−	W	−	−	−	−	ND
α-Mannosidase	−	(−)	+	(−)	−	−	−	−	−	−	−	−	−	ND
DNA G+C content (mol%)	74·6–74·8	67	68	72–74	69	73	71	69	73	72	70	72–74	73–74	ND
Isolation source	Alkaline soil	Soil	Soil	Soil	Herbage	Oil-shale column	Industrial wastewater	Saline lake, Antarctica	Ground-water	Tidal flat	Tidal flat	Alkaline soil	Alkaline soil	Crude oil

*Differences may be caused by different cultivation conditions.

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

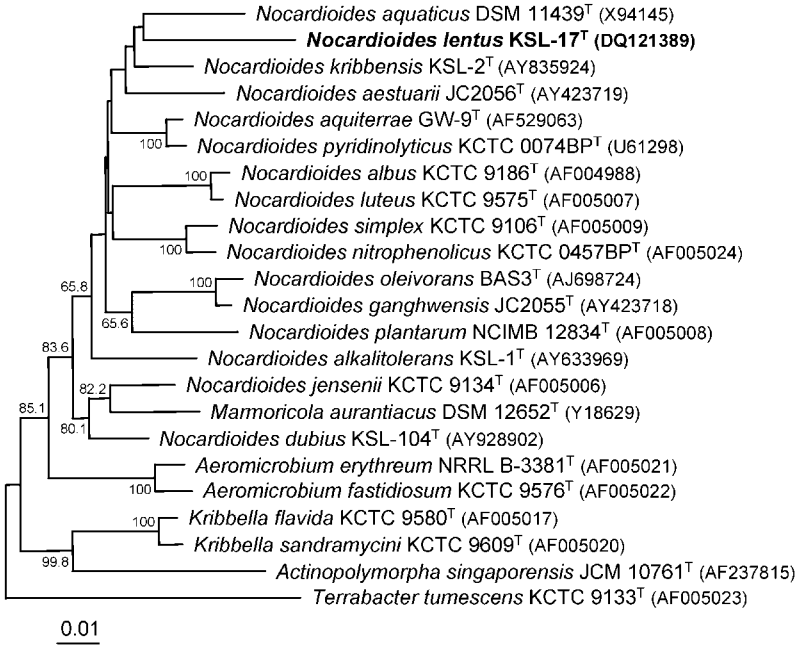

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain KSL-17^T and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0·01 substitutions per nucleotide position.

coli 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analyses showed that the three strains were phylogenetically affiliated to the genus *Nocardioides* (Fig. 1). In the phylogenetic trees based on the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms, strains KSL-17^T, KSL-18 and KSL-19 formed a distinct phylogenetic lineage within the radiation of the cluster comprising *Nocardioides* species (Fig. 1). Similarity values between the 16S rRNA gene sequences of the three strains and those of recognized *Nocardioides* species ranged from 92·6 % (*Nocardioides albus* KCTC 9186^T) to 95·2 % (*N. kribbensis* KSL-2^T). Values for sequence similarity to other species included in the phylogenetic analysis were below 93·3 %.

Strains KSL-17^T, KSL-18 and KSL-19 contained LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan, the predominant menaquinone was MK-8(H_4) and the fatty acid profiles comprised mainly iso-$C_{16:0}$ (60·9–80·9 %), with smaller amounts of $C_{17:1}\omega 8c$ (0·5–6·6 %), 10-methyl $C_{17:0}$ (1·5–5·8 %), iso-$C_{14:0}$ (2·3–6·5 %) and so on (see the supplementary table in IJSEM Online). The chemotaxonomic characteristics were consistent with the affiliation of the strains to the genus *Nocardioides* (Yoon *et al.*, 1997, 2004, 2005a, b; Lawson *et al.*, 2000; Urzì *et al.*, 2000; Yi & Chun, 2004a, b; Schippers *et al.*, 2005). The DNA G+C contents of strains KSL-17^T, KSL-18 and KSL-19 were 74·8, 74·7 and 74·6 mol%, respectively.

Phylogenetic analyses based on 16S rRNA gene sequences and chemotaxonomic data revealed that the three strains can be assigned to the genus *Nocardioides* (Miller *et al.*, 1991; Tamura & Yokota, 1994; Park *et al.*, 1999; Urzì *et al.*, 2000; Wang *et al.*, 2001). The mean DNA–DNA relatedness levels between strains KSL-17^T, KSL-18 and KSL-19 were 85–90 %, indicating that the three strains represent the same genomic species (Wayne *et al.*, 1987). They also shared almost identical phenotypic properties. The levels of 16S rRNA gene sequence similarity are low enough to exclude the possibility of assigning the three strains to a previously described *Nocardioides* species (Stackebrandt & Goebel, 1994). The three strains were distinguishable from the other *Nocardioides* species on the basis of some phenotypic properties, as shown in Table 1. Therefore, on the basis of the data presented, strains KSL-17^T, KSL-18 and KSL-19 should be classified in the genus *Nocardioides* as members of a novel species, for which the name *Nocardioides lentus* sp. nov. is proposed.

Description of *Nocardioides lentus* sp. nov.

Nocardioides lentus (len′tus. L. masc. adj. *lentus* slow, delayed, referring to slow growth).

Cells are aerobic, non-endospore-forming rods (0·4–0·7 × 1·0–4·5 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, convex, smooth, glistening, yellow in colour and 0·5–1·0 mm in diameter after 10 days incubation on 2× diluted nutrient agar at 28 °C. Neither substrate mycelium nor aerial mycelium is formed. Growth occurs at 4 and 34 °C, but not at 35 °C. The optimal pH for growth is 8·0; growth occurs at pH 6·5 and 9·5, but not at pH 6·0 or 10·0. Growth occurs in the presence of 0–5 % (w/v) NaCl, with an optimum at 0·5 % (w/v) NaCl. Weakly positive for oxidase activity. Tweens 20, 40 and 60 are hydrolysed. D-Sorbitol is utilized, but adonitol is not. The cell-wall peptidoglycan contains LL-2,6-diaminopimelic acid as the diagnostic diamino acid.

The predominant menaquinone is MK-8(H_4). The major fatty acid is iso-$C_{16:0}$; 10-methyl fatty acids are present. The DNA G+C content is 74·6–74·8 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, KSL-17^T (=KCTC 19039^T=DSM 16315^T), was isolated from an alkaline soil in Kwangchun, Korea. Reference strains are KSL-18 and KSL-19.

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