Nocardioides koreensis sp. nov., Nocardioides bigeumensis sp. nov. and Nocardioides agariphilus sp. nov., isolated from soil from Bigeum Island, Korea

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Three Gram-positive, coccoid to rod-shaped actinobacteria, designated strains MSL-09T, MSL-19T and MSL-28T, were isolated from a soil sample collected from Bigeum Island in Korea, and were subjected to a polyphasic taxonomic analysis. All three isolates grew optimally at pH 7.5–9.0 and 28–30°C. 16S rRNA gene analysis revealed that all three strains belonged to the genus Nocardioides, with strains MSL-09T, MSL-19T and MSL-28T respectively showing the highest sequence similarity to Nocardioides aquaticus DSM 11439T (96.25%), Nocardioides aquiterrae GW-9T (95.75%) and Nocardioides terrigena DS-17T (95.61%).

Chemotaxonomically, they contain LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan, MK-8(H4) as the predominant menaquinone and diphosphatidylglycerol, phosphatidylglycerol and some unknown lipids as the polar lipids found in the cell wall. iso-C16:0 is a major fatty acid. The G+C content of the DNA was respectively 69.9, 69.3 and 69.4 mol% for strains MSL-09T, MSL-19T and MSL-28T. Based on morphological, physiological, biochemical and chemotaxonomic characters presented in this study, the three strains represent novel species of the genus Nocardioides. The names Nocardioides koreensis sp. nov. (type strain MSL-09T = KCTC 19272T = DSM 19266T), Nocardioides bigeumensis sp. nov. (type strain MSL-19T = KCTC 19290T = DSM 19320T) and Nocardioides agariphilus sp. nov. (type strain MSL-28T = KCTC 19276T = DSM 19323T) are proposed.

The genus Nocardioides was proposed by Prauser (1976) for Gram-positive, non-acid-fast, aerobic and mesophilic nocardioform actinomycetes that develop a mycelium that fragments into irregular rod- to coccus-like elements. Originally, the genus contained two species, Nocardioides albus and Nocardioides luteus (Prauser, 1984), and, at the time of writing, the genus harbours 23 recognized species. In this study, isolates MSL-09T, MSL-19T and MSL-28T were the subject of a taxonomic investigation.

Strain MSL-09T, MSL-19T and MSL-28T were isolated from a soil sample collected from Bigeum Island in Korea using R2A (1:10-diluted) medium (Difco). The isolates were routinely maintained on R2A (1:2-diluted) medium at 28°C and maintained as glycerol suspensions (20%, w/v) at −70°C. Morphological, physiological, cultural and biochemical properties were examined as described by Yoon & Park (2006). Morphological properties were examined by light microscopy (model Nikon HFX-DX) and electron microscopy (JEOL apparatus Philips SEM 515). Growth in the presence of NaCl was investigated in 2-fold-diluted R2A at various NaCl concentrations (0.5–7.0%, w/v, at intervals of 0.5%). The pH range for growth was determined by adjusting the pH at intervals of 0.5 pH units from pH 4.5 to 12.0. The pH was adjusted prior to sterilization by the addition of HCl or Na2CO3. Metabolic properties were determined using API ZYM test kits (bioMérieux) according to the manufacturer’s instructions. Physiological and biochemical characteristics were examined at 28°C and properties were recorded after 7–10 days except for the nitrate reduction test, which was recorded after 2–3 days. Other tests, including cell morphology, motility, acid production and assimilation of carbon sources, were performed as described by Kämpfer (1991). Cell biomass for DNA extraction and for analyses of cell-wall and isoprenoid quinones was obtained by cultivation at 28°C in 2-fold-diluted R2A broth (pH 7.5). Chemotaxonomic and molecular systematic studies were performed as described by Yoon et al. (2005a, b, c). 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 of the strains was harvested from 2-fold-diluted R2A broth (pH 7.5) after incubation for 10 days at 28 °C.

Morphological, cultural, physiological and biochemical characteristics of strains MSL-09T, MSL-19T and MSL-28T are given in the species descriptions and presented in Table 1. Cells are coccoid to short rods, arranged singly or in groups (Supplementary Fig. S1, available in IJSEM Online). Extraction of chromosomal DNA, amplification and purification of the 16S rRNA gene by PCR and direct sequencing of the purified PCR product were performed as described previously (Lee et al., 2000; Lee, 2006). Almost-complete sequences of the 16S rRNA genes of strains MSL-09T (1398 nt), MSL-19T (1398 nt) and MSL-28T (1426 nt) were compared with those of representatives of the family Nocardioidaceae using the CLUSTAL_X program (Thompson et al., 1997). Comparative 16S rRNA gene sequence analysis showed that strains MSL-09T, MSL-19T and MSL-28T were affiliated phylogenetically to the genus Nocardioides (Fig. 1). In phylogenetic trees based on the neighbour-joining, maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms, strain MSL-09T clustered with Nocardioides

<table>
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<th>Characteristic</th>
<th>1</th>
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<td>W</td>
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<td>DNA G+C content (mol%)</td>
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<td>69.3</td>
<td>69.4</td>
<td>74.8*</td>
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<td>Alkaline soil*</td>
<td>Saline lake*</td>
<td>Soil*</td>
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</table>

*Data for the type strain.
aquaticus DSM 11439T, MSL-19T formed a separate lineage
with Nocardioides aestuarii JC2056T and MSL-28T formed a
cluster with Nocardioides lentus KSL-17T (Fig. 1). The
highest similarity values of the 16S rRNA gene sequences of
strains MSL-09T, MSL-19T and MSL-28T with those of
recognized Nocardioides species were 96.25 % (MSL-09T,
with Nocardioides aquaticus DSM 11439T), 95.75 % (MSL-
19T, with Nocardioides aquiterrae GW-9T) and 95.61 %
(MSL-28T, with Nocardioides terrigena DS-17T).

Phylogenetic analyses based on 16S rRNA gene sequences
and chemotaxonomic data revealed that strains MSL-09T,
MSL-19T and MSL-28T can be assigned to the genus
Nocardioides (Urzà et al., 2000; Wang et al., 2001) as
representatives of three novel species. This conclusion is
supported by a number of phenotypic differences between
the isolates and their phylogenetic neighbours (Table 1),
as well as chemotaxonomic differences and fatty acid
compositions. On the basis of the combination of
physiological and chemotaxonomic properties and their
phylogenetic distinctness, strains MSL-09T, MSL-19T and
MSL-28T represent novel members of the genus
Nocardioides, for which the names Nocardioides koreensis
sp. nov., Nocardioides bigeumensis sp. nov. and
Nocardioides agariphilus sp. nov. are proposed.

Description of Nocardioides koreensis sp. nov.

Nocardioides koreensis (ko.re.en’sis. N.L. masc. adj. koreen-
sis pertaining to Korea).

Cells are aerobic, non-spore-forming, Gram-positive,
irregular short rods (0.2–0.7 × 0.8–3.2 μm). Colonies are
circular, smooth, flat, cream to whitish in colour and 0.9–
1.4 mm in diameter after 4–5 days incubation on R2A
medium at 30°C. No formation of aerial or substrate
mycelium. Growth occurs at 27–37°C but not at or below
25°C or above 37°C. Optimum growth at pH 7.0–8.0 and
30°C. Growth is observed in the presence 0–5 % NaCl (w/v).
Cellulose is not hydrolysed and tests for H2S
production, nitrate reduction, DNase and citrate
hydrolysis are negative. Positive for starch hydrolysis.
Cell-wall peptidoglycan contains LL-DAP as the diagnostic
amino acid. The predominant menaquinone is MK-8(H4)
and phospholipids are diphosphatidylglycerol, phosphatidyl-
glycerol and some unidentified glycolipids were detected. The
fatty acid profiles comprised large amounts of branched,
unsaturated, straight-chain and 10-methyl fatty acids, as
detailed in the species descriptions. The chemotaxonomic
characteristics were consistent with the affiliation of the
strains to the genus Nocardioides (Yoon et al., 1997, 2004,
2005a, b, c; Yoon & Park, 2006). The DNA G+C content of
the type strain is 69.9 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences
and chemotaxonomic data revealed that strains MSL-09T,
MSL-19T and MSL-28T can be assigned to the genus
Nocardioides (Urzà et al., 2000; Wang et al., 2001) as
representatives of three novel species. This conclusion is
supported by a number of phenotypic differences between
the isolates and their phylogenetic neighbours (Table 1),
as well as chemotaxonomic differences and fatty acid
compositions. On the basis of the combination of
physiological and chemotaxonomic properties and their
phylogenetic distinctness, strains MSL-09T, MSL-19T and
MSL-28T represent novel members of the genus
Nocardioides, for which the names Nocardioides koreensis
sp. nov., Nocardioides bigeumensis sp. nov. and
Nocardioides agariphilus sp. nov. are proposed.

Description of Nocardioides koreensis sp. nov.

Nocardioides koreensis (ko.re.en’sis. N.L. masc. adj. koreen-
sis pertaining to Korea).

Cells are aerobic, non-spore-forming, Gram-positive,
irregular short rods (0.2–0.7 × 0.8–3.2 μm). Colonies are
circular, smooth, flat, cream to whitish in colour and 0.9–
1.4 mm in diameter after 4–5 days incubation on R2A
medium at 30°C. No formation of aerial or substrate
mycelium. Growth occurs at 27–37°C but not at or below
25°C or above 37°C. Optimum growth at pH 7.0–8.0 and
30°C. Growth is observed in the presence 0–5 % NaCl (w/v).
Cellulose is not hydrolysed and tests for H2S
production, nitrate reduction, DNase and citrate
hydrolysis are negative. Positive for starch hydrolysis.
Cell-wall peptidoglycan contains LL-DAP as the diagnostic
amino acid. The predominant menaquinone is MK-8(H4)
and phospholipids are diphosphatidylglycerol, phosphatidyl-
glycerol and some unknown phospholipids. Major fatty
acids are i-C16:0 (62.87 %), C18:1ω9c (5.88 %), i-C14:0
(3.96 %), 10-methyl C17:0 (3.42 %), C16:0 (3.30 %), i-C16:1
(3.13 %), ai-C17:0 (1.73 %), C18:0 (1.57 %), C17:1ω8c
(1.33 %), C17:0 (1.20 %) and i-C15:0 (1.12 %). The DNA
G+C content of the type strain is 69.9 mol%.

Fig. 1. Phylogenetic dendrogram obtained by
neighbour-joining analysis based on 16S
rRNA gene sequences, showing the position
of strains MSL-9T, MSL-19T and MSL-28T in
the genus Nocardioides. Asterisks indicate
branches that were also recovered using
maximum-likelihood and maximum-parsimony.
Terrabacter tumescens DSM 20308T served
as the outgroup. Numbers on branch nodes
are bootstrap values expressed as percentages
(1000 resamplings). Bar, 0.01 substitutions
per nucleotide position.
The type strain, MSL-09T (=KCTC 19272T =DSM 19266T), was isolated from a soil sample collected from Bigeum Island, Republic of Korea.

**Description of Nocardioides bigeumensis sp. nov.**

_Nocardioides bigeumensis_ (bi.ge.um.en’sis. N.L. masc. adj. _bigeumensis_ pertaining to Bigeum Island, Korea, the source of the soil sample from which the type strain was isolated).

Cells are aerobic, non-spore-forming, Gram-positive cocci to short rods (0.3–0.8 × 0.8–4.0 μm). Cells show coccus-to-rod-like appearance from early exponential phase to stationary phase. Colonies are irregular, smooth, flat, cream in colour and 1.0–2.5 mm in diameter after 4–5 days incubation on R2A medium at 28 °C. No aerial or substrate mycelium is observed. Growth occurs at 20–35 °C, with optimum growth at 28 °C. Optimum pH for growth is pH 7.5–9.0. No growth is observed in the presence of 1% NaCl or more. Cellulose, casein, starch and urea are not hydrolysed and tests for H2S production, nitrate reduction, DNase and citrate hydrolysis are negative. Cell-wall peptidoglycan contains LL-DAP as the diagnostic diamino acid. MK-8(H4) is the predominant menaquinone detected and diphosphatidylglycerol and phosphatidylglycerol are the phospholipids found in the cell wall. Major fatty acids are i-C16 : 0 (38.3%), i-C15 : 0 (13.1%), i-C14 : 0 (9.0%), C18 : 1ω9c (6.25%), 10-methyl C16 : 0 (4.8%), C16 : 0 (4.40%), C17 : 0ω8c (3.9%), C17 : 0 (3.0%), 10-methyl C17 : 0 (1.9%), ai-C15 : 0 (1.6%), ai-C17 : 0 (1.2%), C15 : 0 (1.1%) and i-C16 : 1 (1.1%). The DNA G+C content of the type strain is 69.4 mol%. Other phenotypic properties are given in Table 1.

The type strain, MSL-28T (=KCTC 19276T =DSM 19323T), was isolated from a soil sample collected from Bigeum Island, Republic of Korea.

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**References**


