Nocardioides dokdonensis sp. nov., an actinomycete isolated from sand sediment

Seong Chan Park,¹ Keun Sik Baik,¹ Mi Sun Kim,¹ Jong sik Chun² and Chi Nam Seong¹

¹Department of Biology, College of Natural Sciences, Sunchon National University, Suncheon 540-742, Republic of Korea
²School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea

A strictly aerobic, non-motile, short rod-shaped actinomycete, designated strain FR1436T, was isolated from sand sediment from a beach on Dokdo Island, Republic of Korea. The major menaquinone was MK-8(H₄), the predominant cellular fatty acid was iso-C₁₆:₀ (40.4 %) and the G+C content of the DNA was 69.1 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain FR1436T formed a lineage within the genus Nocardioides and was closely related to Nocardioides marinisabuli SBS-12T (97.8 % sequence similarity) and Nocardioides salarius CL-Z59T (97.4 %). Phenotypic characteristics and DNA–DNA relatedness data served to distinguish strain FR1436T from N. marinisabuli. On the basis of the evidence presented in this study, strain FR1436T represents a novel species of the genus Nocardioides, for which the name Nocardioides dokdonensis sp. nov. is proposed. The type strain is FR1436T (=KCTC 19309T =JCM 14815T).

The genus Nocardioides is characterized chemotaxonomically by the combination of L-l-diaminopimelic acid in the peptidoglycan and MK-8(H₄) as the major menaquinone (O’Donnell et al., 1982; Prauser, 1976). Since the original description by Prauser (1976), the number of Nocardioides species described has increased rapidly because of improved classification resulting from the polyphasic approach. At the time of writing, there are more than 20 Nocardioides species with validly published names. Species of the genus have been isolated from various environments including soil, herbage, groundwater, an oil-shale column, black sand and beach sand, crude oil, a saline lake, a tidal flat, seawater and a water flea (Yoon et al., 2005, 2006a, b, 2007, 2008; Lee, 2007; Lee et al., 2007, 2008; Tóth et al., 2008). Of these, Nocardioides ganghwensis (Yi & Chun, 2004a), Nocardioides aestuarii (Yi & Chun, 2004b) and Nocardioides marinus (Choi et al., 2007) were isolated recently from tidal flat sediments.

Recently, during the course of a study on the cultivable aerobic bacterial community in sand sediment, a Gram-positive bacterium that showed high levels of 16S rRNA gene sequence similarity to members of the genus Nocardioides was isolated from Dokdo Island, Korea (37°05’ N 131°13’ E). The sediment isolate, designated strain FR1436T, was the subject of this study.

Strain FR1436T was isolated from a sediment sample during February 2005, using the standard dilution plating technique. Isolation was achieved using MR2A [R2A (Difco) supplemented with 3.5 % artificial sea salts (Sigma)]. The isolate was routinely cultured on tryptic soy agar (TSA; Difco) at 25 °C and maintained as a glycerol suspension (20 %, w/v) at −80 °C.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resultant sequence of strain FR1436T (1385 nt) was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available in all sequences (positions 39–1447; Escherichia coli numbering system) by using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated as described by Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at http://plaza.snu.ac.kr/~jchun/jphydit/) and PAUP 4.0 (Swofford, 1998), as described previously (Chun et al., 2000).

Preliminary sequence comparisons with 16S rRNA gene sequences held in GenBank indicated that strain FR1436T
was closely related to the genus *Nocardioides*. The newly
determined sequence was then aligned manually against
representatives of the genus *Nocardioides*. Strain FR1436T
showed the highest 16S rRNA gene sequence similarity
with respect to *Nocardioides marinisabuli* SBS-12T (97.8 %)
and *Nocardioides salarius* CL-Z59T (97.4 %); the sequence
similarities with respect to all other species of the genus
*Nocardioides* were below 96.6 %. Also, strain FR1436T
occupied a distinct position in the neighbour-joining tree,
clustering only with *N. marinisabuli* and *N. salarius* (Fig.
1). The tree based on the Fitch–Margoliash method showed
essentially similar topology (data not shown).

In view of the results of the 16S rRNA gene sequence
analysis and the subsequent phylogenetic analysis, DNA–
DNA hybridization between FR1436T and the type strain of
*N. marinisabuli* was the only test needed to define the
strain as a novel species, because the sequence similarities (<97 %)
between strain FR1436T and all *Nocardioides* species other than *N. marinisabuli* and *N. salarius* were below the level indicative of relatedness at the species level (Stackebrandt & Goebel, 1994). DNA–DNA hybridization was performed using a membrane filter technique according to the method described by Baik et al. (2006). The DNA–DNA relatedness between strain FR1436T and *N. marinisabuli* KCCM 42681T was 23.1 ± 5.6 %. It is clear from the 16S rRNA gene sequence and DNA–DNA

hybridization data that strain FR1436T represents a novel
species in the genus *Nocardioides* (Wayne et al., 1987).

Bacterial growth was tested on nutrient agar (Difco), TSA,
R2A agar, glucose-yeast extract agar (Gordon & Mihm, 1962) and marine agar 2216 (Difco). Formation of mycelium and spores and production of pigment and melanin were examined for 3 weeks on several culture media, namely ISP 3, ISP 4 (Difco), ISP 5 and ISP 6 (Williams et al., 1983). The following features were determined as reported previously (Park et al., 2008): Gram stain, motility, oxidase and catalase activities, temperature, pH and NaCl tolerance for growth, acid production from sugars and hydrolysis of casein, L-tyrosine and starch. Acid-fastness was determined by means of Ziehl–Neelsen staining (Hendrickson & Krenz, 1991). Degradation of urea (0.15 %, w/v) and allantoin (0.15 %, w/v) was tested using the medium of Gordon et al. (1974) containing 1 % KH₂PO₄, 0.95 % Na₂HPO₄, 0.1 % yeast extract and 0.04 % phenol red. Degradation of arbutin (0.1 %, w/v) was tested using the medium of Williams et al. (1983) containing 0.3 % yeast extract, 0.05 % ferric ammonium citrate and 1.5 % agar. Hydrolysis of Tween 20 (1 %, w/v) and Tween 80 (1 %, w/v) was tested using TSA as the basal medium (Barrow & Feltham, 1993). Hydrolysis of hippurate (1 %, w/v) was tested using hippurate agar (Williams et al., 1983). DNase test agar

---

**Fig. 1.** Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing relationships between strain FR1436T and members of the genus *Nocardioides*. Numbers at nodes are bootstrap percentages (based on 1000 resampled datasets). The sequence of *Nocardia asteroides* ATCC 19247T was used as an outgroup. Bar, 0.01 nucleotide substitutions per position.
(Difco) was used to assay DNase activity. Decomposition of elastin (0.3 %, w/v), guanine (0.3 %, w/v), xanthine (0.4 %, w/v) and xylan (0.4 %, w/v) was checked using modified Bennett’s agar as described by Gordon et al. (1974). Utilization of compounds as sole carbon sources was examined on Stevenson medium (Stevenson, 1967) suitably supplemented with each carbon source, and growth was recorded after 10 days incubation at 30 °C. Utilization of compounds as sole nitrogen sources was examined on Tsukamura’s medium (Tsukamura, 1975) suitably supplemented with each nitrogen source, and growth was recorded after 10 days incubation at 30 °C. Other biochemical features and enzyme activities were determined using API 20NE, API 50 CH and API ZYM kits (all from bioMérieux). Growth in the presence of various chemicals was determined on appropriately supplemented TSA after 10 days incubation at 30 °C. Antibiotic resistance was determined using the disc diffusion method with

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Oxidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utilization of cellobiose (API 50 CH)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of maltose (API 20NE)</td>
<td>−</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>Utilization of carbon sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Lactose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Raffinose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>w</td>
<td>w</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>v*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>a-Chymotrypsin</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>−</td>
<td>v*</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>−</td>
<td>−</td>
<td>w</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>w*</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>w</td>
<td>w*</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69.1</td>
<td>69</td>
<td>73–74 †</td>
<td>74.6–74.8 †</td>
<td>73.1</td>
<td>73</td>
</tr>
</tbody>
</table>

*Data for strain N. aquaticus DSM 11439T from Yoon et al. (2006a).
†Ranges of values for three strains, including the type strain, in each case.

Table 1. Characteristics that serve to differentiate strain FR1436T from closely related species

Strains: 1, FR1436T; 2, N. aquaticus DSM 11439T; 3, N. kribbensis KCTC 19039T; 4, N. lentes KCTC 19039T; 5, N. marinisabuli KCCM 42681T; 6, N. salarius CL-Z59T. Data for reference strains are from Lawson et al. (2000), Lee et al. (2007), Kim et al. (2008) and Yoon et al. (2005, 2006a). All of the strains are positive for Gram stain, catalase, esterase lipase (C8)* and leucine arylamidase* and negative for xanthine hydrolysis and production of lipase (C14)*, α-galactosidase*, β-glucuronidase*, N-acetyl-β-glucosaminidase*, α-mannosidase* and α-fucosidase*. Mannitol and trehalose were utilized by all of the strains. +, Positive; −, negative; v, variable; W, weakly positive; ND, no data available.

DNA G+C content (mol%) 69.1 69 73–74† 74.6–74.8† 73.1 73

http://ijs.sgmjournals.org
commercial antibiotic-impregnated discs (BBL Becton Dickinson). The results were interpreted according to the guidelines set forth by the NCCLS (2003). Antibiosis against micro-organisms was checked using an overlay technique (Williams et al., 1983) on TSA and growth inhibition of target micro-organisms was measured after 24 h incubation at 30 °C.

Cells of strain FR1436T grown on TSA for 2 days at 25 °C were prepared and analysed for fatty acids by GLC according to the instructions of the Microbial Identification System (MIDI, 1999). The dianimopinic acid isomer in the cell wall was determined as described by Seong et al. (1999). Isoprenoid quinones were isolated according to the method of Minnikin et al. (1984) and were analysed using HPLC as described by Collins (1985). The G + C content of the DNA was determined using the thermal denaturation method of Marmur & Doty (1962).

The fatty acid profile of strain FR1436T is shown in Supplementary Table S1 (available in IJSEM Online). The predominant fatty acid was iso-C₁₆:0 (40.4 %); 10-methyl C₁₇:0 (1.4 %), which is characteristic of the genus Nocardioides, was present. Considerable amounts of C₁₈:1ω9c (11.2 %), C₁₆:0 (7.7 %) and C₁₈:0 (7.2 %) were detected. The major menaquinone was MK-8(H₄), as is the case for all Nocardioides species. The DNA G + C content of strain FR1436T was 69.1 mol%, which is similar for the type strain of Nocardioides aquaticus and lower than those of the type strains of Nocardioides kribbensis, Nocardioides lentus and N. marinisabui.

A number of phenotypic characteristics clearly distinguish strain FR1436T from other phylogenetically related Nocardioides species (Table 1). Therefore, strain FR1436T represents a novel species within the genus Nocardioides, for which the name Nocardioides dokdonensis sp. nov. is proposed.

**Description of Nocardioides dokdonensis sp. nov.**

Nocardioides dokdonensis (dok.do.nen’s.is. N.L. masc. adj. dokdonensis pertaining to Dokdo, the Korean island from where the type strain was isolated).

Gram-positive and strictly aerobic bacterium. Oxidase-negative, catalase-positive and not acid-fast. Spores are not formed. Cells are non-motile short rods (0.6–0.9 × 1.2–1.8 μm) in the exponential phase of growth. Substrate and aerial mycelia are not observed. Colonies on TSA are cream, circular, convex, entire, glistening and opaque. Colonies are approximately 1.0–2.0 mm in diameter after 3 days (on TSA at 25 °C) and reach a maximum diameter of 3 mm after 7 days. Growth occurs in 0–7 % (w/v) NaCl (optimum, 0–3 %), at pH 5–10 (optimum, pH 7) and at 4–30 °C (optimum, 25 °C). Diffusible pigment and melanin are not produced. Acid is not produced from 0.5 % (w/v) glucose or maltose. Does not produce H₂S or indole. Arginine dihydrolase is absent. Allantoin, guanine, Tween 20 and xylan are hydrolysed, but arbutin, DNA, elastin and hippurate are not hydrolysed. The following substrates are utilized as sole carbon and energy sources: adonitol, glycerol, melezitose, melibiose, ribose, sodium acetate, sodium citrate, sodium propionate and sodium pyruvate. Does not utilize N-acetylglucosamine, adipate, caprate, dextran, gentiobiose, myo-inositol, DL-malate, phenyl acetate, salicin or xylitol. The following substrates are utilized as sole nitrogen sources: l-cysteine, l-hydroxyproline, l-phenylalanine, l-threonine, l-valine and potassium nitrate. Does not utilize DL-9-amino-n-butyric acid or l-histidine. Cells are sensitive to the following antibiotics (μg per disc, unless otherwise indicated): amikacin (30), ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), penicillin (10 U), polymyxin B (300 U), streptomycin (10), tetracycline (30) and vancomycin (30). The major menaquinone is MK-8(H₄). The cell-wall diamino acid is LL-dianimopinic acid. The major fatty acids are iso-C₁₆:0, C₁₈:1ω9c, C₁₆:0 and C₁₈:1ω9c. The fatty acid 10-methyl C₁₇:0 is present, but tuberculostearic acid is absent. The DNA G + C content of the type strain is 69.1 mol%. Other physiological and biochemical characteristics are given in Table 1 and Supplementary Tables S1 and S2.

The type strain, FR1436T (=KCTC 19309T =JCM 14815T), was isolated from sand sediment from a beach on Dokdo Island, Republic of Korea.

**Acknowledgements**

This work was supported by the BK21 programme (Ministry of Education and Human Resources Development), Republic of Korea.

**References**


