Nocardioides mesophilus sp. nov., isolated from soil

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A short coccoid- to rod-shaped, motile, mesophilic actinobacterium, strain MSL-22^T, was isolated from soil on Bigeum Island, Korea. A polyphasic study was undertaken to establish the taxonomic position of this strain. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain MSL-22^T formed an evolutionary lineage within the radiation of the genus *Nocardioides*. In particular, it formed a monophyletic lineage with *Nocardioides jensenii* KCTC 9134^T with which it shared the highest sequence similarity of about 97.3%. However, DNA–DNA relatedness demonstrated that strain MSL-22^T was distinct from its closest phylogenetic neighbours. The cell-wall peptidoglycan of strain MSL-22^T contained LL-diaminopimelic acid. The predominant menaquinone was MK-8(H₄). Strain MSL-22^T had a cellular fatty acid profile containing straight-chain, branched, unsaturated and 10-methyl fatty acids, with iso-C_{16:0} as the major fatty acid. The DNA G + C content of the strain was 68.7 mol%. On the basis of phenotypic and phylogenetic evidence, the strain is separate from previously described members of the genus *Nocardioides* and represents a novel species in this genus, for which the name *Nocardioides mesophilus* sp. nov. is proposed. The type strain is MSL-22^T (=DSM 19432^T=KCTC 19310^T).

The genus *Nocardioides* was proposed by Prauser (1976) for nocardioform actinomycetes that form mycelia which fragment into irregular rod- or coccus-like elements. The organisms are Gram-positive, non-acid-fast, aerobic and mesophilic, and have a cell wall based on LL-diaminopimelic acid, the diagnostic diamino acid of cell-wall chemotype I. At the time of writing, the genus *Nocardioides* is composed of over 40 species with validly published names, including the recently described species *N. bigeumensis*, *N. agariphilus*, *N. koreensis*, *N. sediminis*, *N. halotolerans*, *N. tritolerans*, *N. islandensis*, *N. dilutus* (Dastager *et al.*, 2008a, b, c, d, e, 2009), *N. salarius* (Kim *et al.*, 2008), *N. hwasunensis* (Lee *et al.*, 2008), *N. hankookensis* (Yoon *et al.*, 2008), *N. fonticola* (Chou *et al.*, 2008), *N. dokdonensis* (Park *et al.*, 2008) and *N. daphniae* (Tóth *et al.*, 2008). The aim of the present study was to determine the taxonomic position of strain MSL-22^T using a polyphasic taxonomic approach.

Strain MSL-22^T was isolated after 7 days at 28 °C on 10 % R2A agar (Reasoner & Geldreich, 1985) from a soil sample

collected from Bigeum Island (Jeollanam-do Province, Korea) and prepared using the standard dilution technique. To investigate its morphological, physiological and biochemical characteristics, strain MSL-22^T was routinely cultivated at 28 °C on 50 % R2A agar and preserved in glycerol suspensions (20 %, v/v) at –80 °C. Gram-staining was performed using a Gram-stain kit (bioMérieux), according to the manufacturer's instructions. Cell morphology and motility were studied using phase-contrast microscopy and scanning electron microscopy (Philips SEM 515; JEOL).

The physiological characteristics of strain MSL-22^T were examined on 50 % R2A medium. Growth at various temperatures (4–50 °C) was measured on 50 % R2A agar. Growth at different pH values (pH 6.0–12.0) was determined by adjusting the pH with Na₂CO₃ prior to sterilization and incubating for 5 days. Growth at various NaCl concentrations from 0–20 % (w/v) was investigated on 50 % R2A agar at 28 °C for 5 days. Catalase and oxidase activities and hydrolysis of starch, casein, gelatin, hypoxanthine, Tween 80, L-tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described by Lányi (1987). Enzyme activity was determined by using the API ZYM system (bioMérieux). Utilization of various substrates as sole carbon

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MSL-22^T is EF466117.

A scanning electron micrograph of cells of strain MSL-22^T is available with the online version of this paper.

and energy sources was determined as described by Shirling & Gottlieb (1966).

Cell biomass for DNA extraction and analysis of cell components was harvested from 50 % R2A broth after incubation at 28 °C for 7 days. The G+C content of the genomic DNA of strain MSL-22^T was determined using HPLC with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM), according to the method of Tamaoka & Komagata (1984). The cell wall was purified and the amino acids of the peptidoglycan were analysed by TLC

(Lechevalier & Lechevalier, 1980). Analysis of the whole-cell sugar composition followed procedures described by Becker *et al.* (1965). Phospholipid analysis was carried out as described by Lechevalier *et al.* (1977). Menaquinones were determined using the procedures of Collins *et al.* (1977). For fatty acid methyl ester analysis, cell mass of strain MSL-22^T was harvested from 50 % R2A plates after incubation at 28 °C for 7 days. The fatty acids were extracted, methylated and analysed using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G+C content of

Table 1. Differential phenotypic properties of strain MSL-22^T and related strains of the genus *Nocardioides*

Strains: 1, MSL-22^T; 2, *N. jensenii* DSM 20641^T; 3, *N. dubius* KCTC 9992^T; 4, *N. alkalitolerans* KCTC 19037^T; 5, *N. dilutus* DSM 19318^T; 6, *N. daphniae* DSM 18664^T. Data from identical conditions were obtained from this study, Collins *et al.* (1994), Yoon *et al.* (1997, 2004), Dastager *et al.* (2008c) and Tóth *et al.* (2008). All strains were rods or cocci and positive for Gram-stain, casein, D-cellobiose, D-glucose, sucrose and D-xylose utilization and acid phosphatase, esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase. All strains were negative for L-arabinose, D-ribose, maltose, hypoxanthine and xanthine utilization and urease, α -galactosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. +, Positive; v, variable; w, weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Cell characteristics						
Size (µm)	0.3–0.8 × 0.9–1.4	0.6–0.8 × 3.0–7.0	0.8–1.0 × 1.5–2.5	0.8–1.0 × 1.5–2.0	0.4–0.8 × 1.9–4.0	0.8–1.0 × 1.2–2.2
Motility	+	–	+	–	+	–
Colony colour	Cream–white	Yellowish white	Yellowish white	Milky white	White–cream	Yellowish
Optimum for growth (°C)	28	ND	30	25–30	28	28–37
Reduction of nitrate	–	+	–	+	+	+
Hydrolysis of:						
Aesculin	–	–	+	–	–	+
Gelatin	–	+	w	v	–	+
Starch	+	–	+	–	–	+
Tween 80	–	+	–	+	+	+
L-Tyrosine	+	+	–	+	+	+
Carbon source utilization						
D-Fructose	–	–	+	–	–	+
D-Galactose	–	–	–	–	+	–
myo-Inositol	+	–	–	–	+	+
Lactose	–	–	–	–	+	+
D-Mannitol	+	–	+	–	+	–
D-Mannose	–	–	–	–	+	+
Melibiose	–	–	–	–	+	–
D-Raffinose	+	–	–	+	+	–
L-Rhamnose	–	+	–	–	+	–
API ZYM						
Alkaline phosphatase	+	w	w	+	–	w
Cystine arylamidase	–	+	–	–	–	–
Esterase (C4)	+	w	w	+	+	+
α -Glucosidase	–	+	+	–	–	–
β -Glucosidase	–	+	+	+	+	–
Leucine arylamidase	+	+	+	+	–	+
Lipase (C14)	–	w	–	–	+	w
Valine arylamidase	–	v	–	–	+	w
Major fatty acids (>5 %)	i-C _{16:0} , C _{16:1} , i-C _{14:0} , C _{16:0}	i-C _{16:0} , i-C _{16:1} H, C _{17:0} , C _{17:1} ω 6c	i-C _{16:0} , C _{17:0}	i-C _{16:0} , 10- methyl-C _{18:0} , C _{18:1} ω 9c	i-C _{16:0} , C _{18:1} ω 9c, C _{17:1} ω 8c	i-C _{16:0} , C _{18:1} ω 9c, 10-methyl-C _{17:0} , i-C _{17:1} ω 9c, i-C _{17:0}
DNA G+C content (mol%)	68.7	69.0	70.6	72.4–73.6	71.8	69.9

the genomic DNA of strain MSL-22^T was 68.7 mol%. The major respiratory lipoquinone of strain MSL-22^T was MK-8(H₄). The fatty acid profile of strain MSL-22^T showed a predominance of saturated fatty acids: iso-C_{16:0} (44.6 %), C_{16:1} (12.1 %), iso-C_{14:0} (11.0 %), C_{16:0} (6.5 %), C_{18:1ω9c} (3.1 %), 10-methyl-C_{16:0} (2.8 %), iso-C_{15:0} (2.3 %) and C_{15:0} (2.2 %). Similar fatty acid

profiles have been reported for type strains of species of the genus *Nocardioides*.

Strain MSL-22^T formed cream-white, glistening, translucent, slightly sticky, irregular and slightly raised colonies when grown on 50 % R2A agar at 28 °C for 3 days. Optimum growth was observed at 28 °C (range 20–37 °C)

Fig. 1. Neighbour-joining dendrogram based on 16S rRNA gene sequences indicating the phylogenetic position of strain MSL-22^T. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. *Terrabacter tumescens* KCTC 9133^T served as an outgroup. Bar, 1% sequence divergence.

and at pH 7.0–7.5. Cells of strain MSL-22^T were Gram-positive, motile, short irregular rods, 0.3–0.8 µm wide and 0.9–1.4 µm long (see Supplementary Fig. S1, available in IJSEM Online) with no flagella. Strain MSL-22^T was oxidase-negative and catalase-positive and did not reduce nitrate to nitrite. Growth was not observed after 5 days at 28 °C on 50 % R2A agar under anaerobic conditions.

The typical phenotypic characteristics of strain MSL-22^T are summarized and compared with those of the type strains of closely related taxa in Table 1 and additional characteristics are given in the species description. Strain MSL-22^T exhibited characteristics of the genus *Nocardioides* (iso-C_{16:0} as the major fatty acid and DNA G + C contents in the range of 67–74 mol%; Prauser, 1976, 1984; Yoon & Park, 2006).

Genomic DNA extraction and PCR amplification of the 16S rRNA gene of strain MSL-22^T were carried out using the procedure of Chun & Goodfellow (1995). The 16S rRNA gene sequence of strain MSL-22^T was compared with sequences available in GenBank by using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>) to determine the approximate phylogenetic affiliation and was aligned with close relatives by using CLUSTAL W (Thompson *et al.*, 1994). An almost-complete 16S rRNA gene sequence (1403 nt) of strain MSL-22^T was obtained and a neighbour-joining (Saitou & Nei, 1987) phylogenetic analysis showed that strain MSL-22^T was most closely affiliated to the genus *Nocardioides* (Fig. 1). Strain MSL-22^T exhibited the highest 16S rRNA gene sequence similarity (97.3 %) to *Nocardioides jensenii* KCTC 9134^T and formed a distinct monophyletic cluster with this type strain. Lower 16S rRNA gene sequence similarities were found with the type strains of other species of the genus *Nocardioides* (93.8–96.6 %) and the other taxa included in the phylogenetic analysis (<94.7 %). The topologies of the phylogenetic trees generated with the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms also showed that strain MSL-22^T belonged to the genus *Nocardioides* (data not shown). Because of the high pairwise 16S rRNA gene sequence similarity between strain MSL-22^T and *N. jensenii* KCTC 9134^T, DNA–DNA hybridization was performed using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) in duplicate under optimal hybridization conditions. DNA–DNA relatedness between strain MSL-22^T and *N. jensenii* DSM 20641^T was 34 ± 2.0 %, which is well below the 70 % threshold value recommended for the definition of bacterial species (Wayne *et al.*, 1987).

Thus, on the basis of physiological, biochemical and phylogenetic properties, strain MSL-22^T represents a novel species within the genus *Nocardioides*, for which the name *Nocardioides mesophilus* sp. nov. is proposed.

Description of *Nocardioides mesophilus* sp. nov.

Nocardioides mesophilus [me.so.phi'lus. Gr. adj. *mesos* middle; Gr. adj. *philos* loving; N.L. masc. adj. *mesophilus* middle (temperature)-loving, mesophilic].

Gram-positive, motile without flagella, aerobic, non-spore-forming, short irregular rods (0.3–0.8 × 0.9–1.4 µm). Colonies are cream-white, glistening, translucent, slightly sticky, irregular and slightly raised with entire margins after 72 h at 28 °C on 50 % R2A agar. Optimum growth occurs at 28 °C and pH 7.0–7.5. Catalase-positive and oxidase-negative. Casein, starch and tyrosine are hydrolysed, but aesculin, gelatin, Tween 80 and urea are not. Nitrate is not reduced to nitrite. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Negative for lipase (C14), cystine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. As sole carbon and energy sources, utilizes cellobiose, glucose, inositol, mannitol, raffinose, sucrose and xylose, but not aesculin, fructose, galactose, lactose, maltose, mannose, melibiose, rhamnose or ribose. Acid is not produced from any of the carbon sources tested. The main cell-wall amino acid is LL-diaminopimelic acid. The predominant menaquinone is MK-8(H₄). The cellular fatty acids are iso-C_{16:0}, C_{16:1}, iso-C_{14:0}, C_{16:0}, C_{18:1}ω9c, 10-methyl-C_{16:0}, iso-C_{15:0} and C_{15:0}.

The type strain, MSL-22^T (=DSM 19432^T=KCTC 19310^T), was isolated from a soil sample collected from Bigeum Island in South Korea. The DNA G + C content of the type strain is 68.7 mol%.

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