Nocardioides sediminis sp. nov., isolated from a sediment sample

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A strictly aerobic, motile, short-rod-shaped, Gram-positive-staining actinomycete strain, designated MSL-01^T, was isolated from a sediment sample from Bigeum Island of Korea. 16S rRNA gene sequence analysis revealed that the isolate MSL-01^T belonged to the genus *Nocardioides*, with the highest sequence similarity to *Nocardioides terrigena* DS-17^T (98.54 %), but the DNA–DNA relatedness to this type strain was 34 %. The physiological properties of strain MSL-01^T differ from those of *Nocardioides terrigena* DS-17^T and other species of *Nocardioides*. The diamino acid in the cell-wall peptidoglycan of strain MSL-01^T is LL-diaminopimelic acid, the major menaquinone is MK-8(H₄) and iso-C_{16:0} is the major fatty acid component. The name *Nocardioides sediminis* sp. nov. is proposed for the novel species, with the type strain MSL-01^T (=DSM 19263^T =KCTC 19271^T).

The genus *Nocardioides* was proposed by Prauser (1976) for Gram-positive-staining, non-acid-fast, aerobic and mesophilic nocardioform actinomycetes that develop a mycelium that fragments into irregular rod-coccus-like elements. Originally, the genus contained the two species *Nocardioides albus* and *Nocardioides luteus* (Prauser, 1984). The number of species belonging to the genus *Nocardioides* has increased considerably, revealing the morphological heterogeneity of the genus. Presently, the genus includes 28 recognized species, including the recently described species *Nocardioides terrigena* (Yoon *et al.*, 2007), *Nocardioides marinisabuli* (Lee *et al.*, 2007), *Nocardioides daphniae* (Tóth *et al.*, 2008) and *Nocardioides hankookensis* (Yoon *et al.*, 2008). In this study, we report the taxonomic characterization of a *Nocardioides*-like strain, MSL-01^T, which was isolated from a sediment sample collected on Bigeum Island, Jeollanam-do Province, Korea.

Strain MSL-01^T was isolated from a sediment sample taken during May 2006 at a depth of 60–70 m. The top 10 cm of the sediment surface was obtained aseptically for sampling and then processed for cultivation experiments by using standard dilution techniques at 28 °C on tenfold-diluted R2A agar (Reasoner & Geldreich, 1985; Difco). To investigate the morphological, physiological and biochemical characteristics of strain MSL-01^T, the strain was routinely cultivated at 28 °C on twofold-diluted R2A agar and the strain was stored as glycerol suspensions (20 %, v/v) at –70 °C. Gram staining was performed using a bioMérieux Gram-stain kit according to the manufac-

turer's instructions. Cell morphology and motility were studied using phase-contrast and scanning electron microscopy (JEOL apparatus Philips SEM 515). The physiological characteristics of strain MSL-01^T were examined by growing the isolate on diluted R2A medium. Growth at various temperatures (4–50 °C) was measured on twofold-diluted R2A agar. Growth at different pH levels (pH 6.0–12.0) was determined after adjusting the pH of the medium by the addition of Na₂CO₃ prior to sterilization. Growth at various NaCl concentrations (0–20 % w/v) was investigated on twofold-diluted R2A agar. Catalase and oxidase activities and hydrolysis of starch, casein, gelatin, hypoxanthine, Tween 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Oxidase activity was tested by assessing the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by assessing the production of bubbles from a 3 % (v/v) aqueous H₂O₂ solution. Hydrolysis of aesculin and nitrate reduction were studied as described previously by Lányi (1987). Enzyme activities were determined by using the API ZYM system (bioMérieux); detailed information is given in the species description. Utilization of various substrates as sole sources of carbon and energy was determined as described by Shirling & Gottlieb (1966).

Cell walls were purified and peptidoglycan amino acids were analysed by TLC as described by 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.* (1981). Menaquinones were determined using the procedures of Collins *et al.* (1977).

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain MSL-01^T is EF466110.

Cell biomass for DNA extraction and for analysis of cell walls and isoprenoid quinones was obtained by cultivation in R2A broth at 28 °C for 7 days. For fatty acid methyl ester analysis, cell mass of strain MSL-01^T was harvested from R2A plates after incubation at 28 °C for 7 days. The fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Analyses of isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The G+C content of the genomic DNA of strain MSL-01^T was determined using an HPLC apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM), according to the method of Tamaoka & Komagata (1984).

Strain MSL-01^T formed cream-white, glistening, translucent, slightly sticky, irregular and slightly raised colonies when grown on R2A agar at 28 °C for 2 days. Growth was observed at 20–37 °C; optimum growth occurred at 28 °C and pH 7.0–7.5. Cells of the isolate were motile, short, irregular rods (0.3–0.8 mm wide and 0.9–1.4 µm long) without any flagella (Fig. 1). Cells of strain MSL-01^T strained Gram-positive and were oxidase-negative and catalase-positive and reduced nitrate to nitrite. Growth was not observed under anaerobic conditions after 5 days at 28 °C on R2A agar. The major respiratory lipoquinone of strain MSL-01^T was MK-8(H₄). The fatty acid profile of the strain showed a predominance of fatty acids iso-C_{16:0} (48.3 %), C_{17:1ω8c} (17.1 %), iso-C_{17:0} (7.0 %), 10-methyl C_{17:0} (4.8 %) and C_{18:1ω9c} (4.3 %). Similar fatty acid profiles have been reported for type strains of the genus *Nocardioides*. The G+C content of the genomic DNA of strain MSL-01^T was 71.5 mol%.

In Table 1, the phenotypic characteristics of strain MSL-01^T are summarized and compared with those of the type strains of closely related species. Members of the genus *Nocardioides* possess iso-C_{16:0} as the major fatty acid and

have DNA G+C contents in the range 67–74 mol% (Prauser, 1976, 1984; Yoon & Park, 2006). Strain MSL-01^T possesses phenotypic characteristics typical of the genus *Nocardioides*.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene of strain MSL-01^T were carried out using the procedure of Chun & Goodfellow (1995). An almost-complete 16S rRNA gene sequence (1437 nt) was obtained from strain MSL-01^T. The sequence of strain MSL-01^T was compared with 16S rRNA gene sequences available in GenBank by using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine the approximate phylogenetic affiliation and the sequence was then aligned with those of its close relatives by using the CLUSTAL W software (Thompson *et al.*, 1994). Phylogenetic trees were generated based on the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MSL-01^T formed a distinct, monophyletic cluster with *N. terrigena* DS-17^T (Fig. 2). The topologies of trees built using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that the isolate belongs to the genus *Nocardioides* (not shown). Comparative 16S rRNA gene sequence analyses showed that the isolate was most closely related to *N. terrigena* DS-17^T, having a similarity of 98.54 %. Because of the high pairwise similarity between strain MSL-01^T and *N. terrigena* DS-17^T, the DNA–DNA relatedness of these two strains was determined. The value obtained was 34 %, which is well below the threshold value of 70 % recommended for the definition of bacterial species (Wayne *et al.*, 1987). Sequence similarities to other members included in the phylogenetic analysis ranged from 97.38 to 94.87 %. Thus, on the basis of the physiological, biochemical and phylogenetic properties of strain MSL-01^T, this isolate represents a novel species within the genus *Nocardioides*, for which the name *Nocardioides sediminis* sp. nov. is proposed.

Description of *Nocardioides sediminis* sp. nov.

Nocardioides sediminis (se.di.mi'nis. L. n. *sedimen* a sediment; L. gen. n. *sediminis* of a sediment, referring to the source from which the type strain was isolated).

Gram-positive-staining, motile without any flagellum, aerobic and non-spore-forming. Cells appear as short, irregular rods, approximately 0.3–0.8 × 0.9–1.4 µm after 72 h incubation at 28 °C. Colonies are cream-whitish, glistening, translucent, slightly sticky, irregular and slightly raised with entire margins. Optimum growth at 28 °C and pH 7.0–7.5. Positive for catalase test and negative for oxidase test. Casein, gelatin, starch and Tween 80 are hydrolysed but aesculin, hypoxanthine and xanthine are not decomposed. Nitrate is reduced to nitrite and urea hydrolysis is negative. Positive for

Fig. 1. Scanning electron micrograph of cells of strain MSL-01^T grown on R2A agar for 5 days at 30 °C. Bar, 1.0 µm.

Table 1. Differential phenotypic characteristics of strain MSL-01^T and the type strains of phylogenetically related *Nocardioides* species

Strains: 1, MSL-01^T; 2, *N. terrigena* DS-17^T; 3, *N. aquiterrae* GW-9^T; 4, *N. pyridinoliticus* OS4^T. Data were taken from Yoon *et al.* (1997, 2004, 2007) and this study. +, Positive; –, negative; w, weakly positive. All strains are positive for Gram staining, catalase, nitrate reduction, hydrolysis of casein and gelatin, utilization of D-xylose, glucose, fructose, cellobiose, maltose, sucrose, trehalose and turanose and activities of esterase lipase (C8) and leucine arylamidase. All are negative for hydrolysis of hypoxanthine and urea, utilization of glycerol, erythritol, D-arabinose, L-xylose, adonitol, mannose, sorbose, dulcitol, sorbitol, salicin, inulin, raffinose, glycogen, xylitol, D-fucose, citrate and phenylacetate and activities of lipase (C14), valine arylamidase, α -chymotrypsin, α -galactosidase, β -glucosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Characteristic	1	2	3	4
Cell morphology	Short rods	Rods, cocci	Rods, cocci	Rods, cocci
Cell size (μm)	0.3–0.8 $\times$ 0.9–1.4	0.4–0.7 $\times$ 0.7–2.0	0.8–1.0 $\times$ 1.7–2.0	0.5–0.6 $\times$ 1.2–1.6
Motility	+	+	–	–
Colony colour	Cream	Ivory	Cream	Cream
Oxidase	–	+	+	–
Optimal temperature ($^{\circ}\text{C}$)	28	30	30	35
Hydrolysis of:				
Aesculin	–	–	+	+
Starch	+	w	–	+
Tween 80	+	w	+	–
Xanthine	–	–	–	+
Tyrosine	+	–	–	+
Carbon-source utilization				
Aesculin	–	–	+	–
Adipate	–	+	–	+
Arbutin	–	–	+	–
D-Arabitol	–	–	–	+
Galactose	+	+	–	–
Gentiobiose	–	+	+	–
Gluconate	–	+	+	–
Inositol	+	–	–	+
Lactose	+	+	–	+
D-Lyxose	–	w	–	–
Malate	–	+	+	–
Melezitose	–	–	–	+
Melibiose	+	+	–	–
Mannitol	+	+	+	–
Ribose	–	–	–	+
L-Rhamnose	+	+	–	+
API ZYM tests				
Alkaline phosphatase	+	+	–	+
Esterase (C4)	+	+	–	–
Cystine arylamidase	–	–	–	w
Trypsin	–	–	w	+
Acid phosphatase	–	–	+	+
β -Galactosidase	–	+	+	–
DNA G + C content (mol%)	71.5	71.5	73	73
Source of isolation	Sediment	Soil	Oil-shale column	Groundwater

alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase. Negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Uses cellobiose, fruct-

ose, galactose, glucose, inositol, lactose, melibiose, mannitol, mannose, maltose, rhamnose, sucrose, trehalose, turanose and xylose as sole carbon and energy sources, but not arabinose, aesculin, adonitol, adipate, D-arabitol, arbutin, dulcitol, erythritol, D-fucose, gentiobiose, gluconate, glycerol, glycogen, inulin, D-lyxose, mannose, malate, melezitose, raffinose, ribose, salicin,

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain MSL-01^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

sorbose, sorbitol, xylitol or L-xylose. 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 major cellular fatty acids are iso-C_{16:0}, C_{17:1}ω8c, iso-C_{17:0}, 10-methyl C_{17:0} and C_{18:1}ω9c. The DNA G+C content of the type strain is 71.5 mol%.

The type strain, MSL-01^T (=DSM 19263^T =KCTC 19271^T), was isolated from a sediment sample collected from Bigeum Island in South Korea.

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