Nocardioides basaltis sp. nov., isolated from black beach sand

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A novel Gram-positive, aerobic, short-rod-shaped bacterium, designated strain J112^T, was isolated from black sand collected from Soesoggak, Jeju Island, Korea. The strain was found to be oxidase-negative and catalase-positive. Cells grew at 10–37 °C, at pH 5.5–8.0 and with 1–10 % NaCl. Growth occurred on marine agar but not on R2A or trypticase soy agar. A phylogenetic analysis based on 16S rRNA gene sequences showed that the strain belongs to the radiation of the genus *Nocardioides*. Strain J112^T shared the highest 16S rRNA gene sequence similarities with *Nocardioides marinisabuli* SBS-12^T (99.2 %), *Nocardioides terrigena* DS-17^T (97.3 %), *Nocardioides kribbensis* KCTC 19038^T (97.1 %) and type strains of other *Nocardioides* species with validly published names (<97 %). The DNA–DNA hybridization values between strain J112^T and the three most closely related strains were low enough to justify the assignment of this strain to a novel species. On the basis of these phenotypic, phylogenetic and chemotaxonomic data, strain J112^T represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides basaltis* sp. nov. is proposed. The type strain is J112^T (=KCTC 19365^T=JCM 14945^T).

The genus *Nocardioides* was originally described by Prauser (1976), who designated *Nocardioides albus* as the type species of the genus. Currently, the genus *Nocardioides* comprises more than 27 species with validly published names. Some have been isolated from saline environments: *Nocardioides aestuarii* (Yi & Chun, 2004a) and *Nocardioides ganghwensis* (Yi & Chun, 2004b) were isolated from tidal flat sediments; *Nocardioides aquaticus* (Lawson *et al.*, 2000) was from a hypersaline Antarctic lake; *Nocardioides marinus* (Choi *et al.*, 2007) was from seawater; and *Nocardioides furvisabuli* (Lee, 2007) and *Nocardioides marinisabuli* (Lee *et al.*, 2007) were from beach sand. We isolated a *Nocardioides*-like bacterium, designated strain J112^T, from black beach sand and performed a polyphasic taxonomic study. On the basis of the results of this study, strain J112^T is proposed as a novel species of the genus *Nocardioides*.

Strain J112^T was isolated on marine agar 2216 (MA; Difco) by means of the standard dilution-plating method, using a sample of black sand collected from Soesoggak, Jeju Island, Korea. Colonies were creamy, smooth, circular and convex and measured 0.5–1.5 mm in diameter after incubation for 3 days at 30 °C on MA. Cells grew on MA, but did not grow on R2A (BBL) or trypticase soy agar (TSA; BBL).

Genomic DNA was extracted using a commercial kit (G-spin; iNtRON Biotechnology). The 16S rRNA gene was PCR-amplified from chromosomal DNA using PCR Pre-Mix (Solgent). A BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) was used according to the manufacturer's instructions to sequence the PCR product purified with a PCR purification kit (Cosmo Genetech). Automated DNA analysis (PRISM 3730XL DNA analyser; Applied Biosystems) was used to analyse the resulting reaction mixtures. 16S rRNA gene sequence analysis was conducted as described previously (Roh *et al.*, 2008). Comparison with related sequences showed that strain J112^T had the greatest levels of similarity with respect to the following strains with validly published names: *N. marinisabuli* SBS-12^T (99.2 %), *Nocardioides terrigena* DS-17^T (97.3 %), *Nocardioides kribbensis* KCTC 19038^T (97.1 %), *N. aquaticus* DSM 11439^T (96.8 %), *Nocardioides aquiterrae* GW-9^T (96.8 %), *Nocardioides dubius* KCTC 9992^T (96.8 %) and *Nocardioides pyridinolyticus* KCTC 0074BP^T (96.7 %). Related sequences of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain J112^T is EU143365.

members of the genus *Nocardioides* were collected from NCBI GenBank and phylogenetic trees were constructed as described previously (Kim *et al.*, 2006). In phylogenetic trees based on the neighbour-joining and maximum-likelihood methods, strain J112^T fell within the radiation of the genus *Nocardioides*, forming a clade with *N. marinisabuli* SBS-12^T (Fig. 1). DNA–DNA hybridization was performed using photobiotin-labelled DNA probes and microwell plates, as described previously (Ezaki *et al.*, 1989). The DNA–DNA hybridization values for strain J112^T and type strains of the most closely related species, *N. marinisabuli* DSM 18965^T, *N. terrigena* DS-17^T and *N. kribbensis* KCTC 19038^T, were 15.8±1.5, 7.0±1.7 and 28.7±2.7 %, respectively. These low relatedness values confirmed that strain J112^T should not be assigned to any recognized species of the genus *Nocardioides*.

The Gram reaction was determined using 3 % KOH (Buck, 1982). A phase-contrast microscope (Nikon) was used to investigate morphology and motility in cells grown on MA for 3 days at 30 °C. Catalase and oxidase activities were determined using bubble production with 3 % (v/v) H_2O_2 and by assessing any colour change with 1 % (w/v) tetramethyl-*p*-phenylenediamine, respectively. Physiological and biochemical characteristics were determined using API 20NE, API ZYM and API 50 CH galleries, according to the instructions of the manufacturer (bioMérieux). AUX medium (bioMérieux) containing 1.5 % (w/v) NaCl was used for the API 50 CH test. Growth temperature (4, 10, 15, 25, 30, 37, 41 and 45 °C) and pH ranges (pH 4.0–13.0) were tested using MA and marine broth (Difco), respectively. Salt ranges were determined using marine broth containing 0–30 % (w/v) NaCl. Strain J112^T was shown to be a Gram-positive, non-motile, short-rod-shaped bacterium. The strain was found to be oxidase-negative and catalase-positive. Cells grew at 10–37 °C, at pH 5.5–8.0 and with 1–10 % NaCl. Physiological and biochemical characteristics of strain J112^T and representative type strains of members of the genus *Nocardioides* are presented in Table 1.

Thermal denaturing methods involving fluorescent dyes were used (Gonzalez & Saiz-Jimenez, 2002) to determine the DNA G+C content. The DNA G+C content (68 mol%) of strain J112^T was similar to those of *N. albus* (67 mol%), *Nocardioides luteus* (68 mol%) and *N. aquaticus* (69 mol%). Cellular fatty acids were characterized for strain J112^T and *N. marinisabuli* DSM 18965^T, using cells grown on MA for 3 days at 30 °C. The cellular fatty acids were extracted and analysed using gas chromatography, according to the protocol of the Sherlock Microbial Identification System (Sasser, 1990). Strain J112^T contained the following cellular fatty acids (>1 %): iso-$C_{16:0}$ (70.3 %), $C_{17:1}\omega 8c$ (4.3 %), iso-$C_{16:0}$ H (3.7 %), iso-$C_{14:0}$

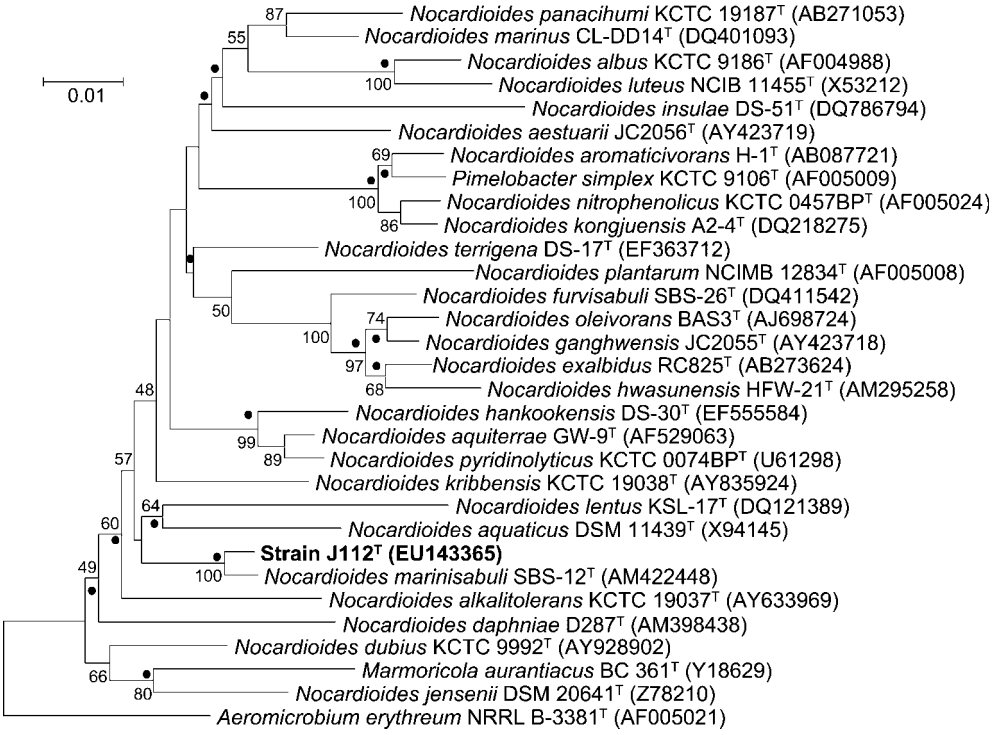

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain J112^T and *Nocardioides* species with validly published names. Filled circles indicate branches that were also found in the maximum-likelihood tree. Numbers at nodes indicate bootstrap percentages (based on 1000 resamplings) >45 %. GenBank accession numbers are shown in parentheses. Bar, 0.01 nucleotide substitutions per site.

Table 1. Physiological and biochemical characteristics of strain J112^T and related species of the genus *Nocardioides*

Strains: 1, strain J112^T; 2, *N. marinisabuli* SBS-12^T; 3, *N. terrigena* DS-17^T; 4, *N. kribbensis* KCTC 19038^T; 5, *N. marinus* CL-DD14^T; 6, *N. aestuarii* JC2056^T; 7, *N. ganghwensis* JC2055^T; 8, *N. aquaticus* EL-17K^T; 9, *N. aquiterrae* GW-9^T; 10, *N. pyridinolyticus* KCTC 0074BP^T; 11, *N. albus* JCM 3185^T; 12, *N. luteus* JCM 3358^T. For taxa 2–12, data are from previous work (Choi *et al.*, 2007; Lawson *et al.*, 2000; Lee *et al.*, 2007; Prauser, 1976, 1984; Yi & Chun, 2004a, b; Yoon *et al.*, 1997, 2004, 2005, 2007). +, Positive; −, negative; W, weakly positive; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell morphology	Short rods	Rods	Rods, cocci	Short rods, cocci	Rods, cocci	Rods	Rods	Cocci, short rods	Rods, cocci	Rods, cocci	Hyphae	Hyphae
Motility	−	−	−	−	−	−	−	−	+	+	−	−
Temperature (optimum) (°C)	10–37 (25–30)	4–40	4–35 (30)	4–35 (30)	10–40 (25–30)	20–35 (30)	10–40 (30)	(6–26)	15–42 (30)	20–40 (35)	(28)	(28)
NaCl range (optimum) (%)	1–10 (1–2)	0–3 (0.5–1.0)	0–3 (0.5–1.0)	0–3	0.5–8 (1–3)	0–8 (0–2)	0–8 (0–1)	ND	ND	ND	ND	ND
pH range (optimum)	5.5–8.0 (6–7)	(7.1–8.1)	(8)	6–11 (9)	6–9 (7–8)	6–10 (7)	6–10 (7)	ND	(6–7)	5–9 (8)	ND	ND
Oxidase	−	−	+	+	−	−	−	−	+	−	−	−
Nitrate reduction	−	−	+	+	−	−	+	+	+	+	−	−
Gelatinase	+	−	+	+	−	+	+	+	+	+	+	+
Decomposition of:												
Starch	−	W	+	−	−	−	+	W*	−*	+	+	+
Tween 80	−	−	W	+	+	+	+	+	+	−	+	+
Tyrosine	−	−	−	+	−	−	+	+	−	+	+	+
Hypoxanthine	−	−	−	−	−	−	−	−	−	−	+	+
Xanthine	−	−	−	−	−	−	W	−	−*	+	+	−*
API ZYM test results												
Acid phosphatase	−	−	−	+	W	W	W	+	+	+	−	−
Alkaline phosphatase	+	+	+	+	+	W	+	V	−	+	+	V
α-Chymotrypsin	−	−	−	+	−	+	−	−	−	−	−	−
Cystine arylamidase	W	−	−	−	−	−	W	V	W	−	−	−
α-Galactosidase	−	−	−	−	−	−	+	−	−	−	−	−
Esterase (C4)	+	−	+	+	W	+	V	W	−	−	+	+
α-Mannosidase	−	−	−	−	−	−	−	−	−	−	−	+
Naphthol-AS-BI-phosphohydrolase	+	−	+	+	−	W	−	−	+	+	W	V
Trypsin	W	−	−	−	+	+	−	W	+	+	+	+
Valine arylamidase	−	−	−	−	W	+	+	W	−	W	−	−
Carbon utilization												
N-Acetyl-D-glucosamine	−	−	−	ND	−	−	+	−	−	−	W	+
L-Arabinose	−	−	ND	−	−	−	+	−	−	−	+	+
Cellobiose	+	−	+	+	ND	+	+	−	+	+	+	+
Citrate	−	+	−	ND	−	+	+	−	W	−	+	+
D-Fructose	−	+	+	−	+	+	+	+	+	+	+	−*

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
D-Galactose	−	+	+	+	+	+	+	−*	+	+	+	−
Glycerol	−	−	−	ND	−	W	+	+	−	−	W	−
myo-Inositol	−	−	−	−	−	−	−	W*	−	+	−	−
Lactose	−	−	+	−	−	W	+	−	+*	+*	−	−
D-Mannitol	+	−	+	+	+	+	+	+	+*	−	+	+
D-Mannose	−	−	−	−	−	−	+	−	+*	+*	+	+
Raffinose	−	−	−	+	−	W	+	−	−	−	V	−
L-Rhamnose	−	−	+	+	+	−	−	+*	W*	+	+	−
D-Ribose	−	−	−	+	−	−	−	−	W*	+	−	−
Salicin	+	−	−	ND	ND	−	+	−	−	−	W	−
Sucrose	+	−	+	+	+	+	+	+	+	+	+*	+*
D-Xylose	−	+	+	−	+	+	+	W*	+	+	+	+
DNA G+C content (mol%)	68	73.1	71.5	73–74	73	70	72	69	73	73	67	68
Isolation source	Beach sand	Beach sand	Soil	Alkaline soil	Seawater	Tidal flat	Tidal flat	Saline lake (Antarctica)	Groundwater	Oil shale	Soil	Soil

*Different results were reported by Yi & Chun (2004a).

(3.5 %), 10-methyl $C_{17:0}$ (3.2 %), $C_{18:1}\omega9c$ (2.8 %), iso-$C_{18:0}$ (2.7 %), $C_{18:1}\omega7c$ (1.7 %), summed feature 3 (1.7 %; comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) and $C_{16:0}$ (1.3 %). The following fatty acids are present in trace amounts (<1 %): iso-$C_{12:0}$, $C_{14:0}$, iso-$C_{15:0}$, $C_{15:1}\omega6c$, $C_{15:0}$, $C_{16:0}$ N alcohol, 10-methyl $C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$, $C_{17:1}\omega6c$, $C_{17:0}$, iso-$C_{18:1}$ H, $C_{18:0}$ and $C_{17:0}$ 3-OH. The large proportion of iso-$C_{16:0}$ and the presence of 10-methyl $C_{17:0}$ found in strain J112^T are typical of members of the genus *Nocardioides*. *N. marinisabuli* DSM 18965^T contains the following fatty acids: iso-$C_{16:0}$ (35.9 %), $C_{18:1}\omega9c$ (15.4 %), iso-$C_{17:0}$ (12.3 %), $C_{17:1}\omega8c$ (5.6 %), anteiso-$C_{17:0}$ (4.1 %), iso-$C_{18:0}$ (3.8 %), summed feature 3 (2.7 %; comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{15:0}$ (2.6 %), 10-methyl $C_{17:0}$ (2.0 %), $C_{17:0}$ (1.5 %), $C_{18:0}$ (1.5 %) and $C_{16:0}$ (1.3 %). This composition is similar to that obtained for cells grown on TSA for 3 days at 30 °C (Lee *et al.*, 2007). The fatty acid profile of strain J112^T differed from that of *N. marinisabuli* DSM 18965^T in that it contained a greater proportion of iso-$C_{16:0}$ and smaller proportions of iso-$C_{17:0}$, $C_{18:1}\omega9c$ and anteiso-$C_{17:0}$. The diaminopimelic acid of the peptidoglycan was analysed by using TLC (Staneck & Roberts, 1974). LL-Diaminopimelic acid was detected as the diamino acid in the cell-wall peptidoglycan of strain J112^T. The menaquinone composition was determined as described previously (Hiraishi *et al.*, 1996): briefly, quinones were extracted sequentially with chloroform/methanol (2 : 1, v/v) and n-hexane/water (1 : 1). The extracted quinones were separated by using Sep-Pak Vac silica cartridges (Waters) and analysed by HPLC. MK-8(H_4) was found to be the predominant menaquinone in strain J112^T.

The phylogenetic data, chemotaxonomic properties and physiological characteristics determined for strain J112^T were in accordance with those for the genus *Nocardioides*. On the basis of its 16S rRNA gene sequence, DNA–DNA hybridization values, fatty acid profile and differential phenotypic characteristics, strain J112^T does not belong to any *Nocardioides* species with a validly published name. Therefore, strain J112^T represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides basaltis* sp. nov. is proposed.

Description of *Nocardioides basaltis* sp. nov.

Nocardioides basaltis (ba.sal′tis. L. masc. gen. n. *basaltis* of basalt, pertaining to the source of isolation).

Cells are Gram-positive, aerobic, non-motile, short rods (0.7–1.0 μm wide and 1.2–2.0 μm long). After 3 days growth on MA, colonies are creamy, smooth, circular and convex and measure 0.5–1.5 mm in diameter. Cells are oxidase-negative and catalase-positive. Cells grow on MA, but not on R2A or TSA. Cells grow at 10–37 °C, at pH 5.5–8.0 and with 1–10 % NaCl. MK-8(H_4) is the predominant menaquinone. Contains the following cellular fatty acids (>1 %): iso-$C_{16:0}$, $C_{17:1}\omega8c$, iso-$C_{16:0}$ H, iso-$C_{14:0}$, 10-methyl $C_{17:0}$, $C_{18:1}\omega9c$, iso-$C_{18:0}$, $C_{18:1}\omega7c$, summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) and $C_{16:0}$. The following fatty acids are present in trace amounts: iso-$C_{12:0}$, $C_{14:0}$, iso-$C_{15:0}$, $C_{15:1}\omega6c$, $C_{15:0}$, $C_{16:0}$ N alcohol, 10-methyl $C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$, $C_{17:1}\omega6c$, $C_{17:0}$, iso-$C_{18:1}$ H, $C_{18:0}$ and $C_{17:0}$ 3-OH. Negative for indole production, glucose acidification and for the presence of arginine dihydrolase and urease. Positive for assimilation of D-arabitol, cellobiose, gluconate, D-glucose, D-mannitol, melezitose, salicin, sucrose, trehalose and turanose. Negative for assimilation of *N*-acetyl-D-glucosamine, D-adonitol, starch, amygdalin, D-arabinose, L-arabinose, L-arabitol, arbutin, dulcitol, erythritol, aesculin, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, D-lactose, D-lyxose, maltose, D-mannose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylose, raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, D-tagatose, D-xylose, L-xylose and xylitol. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase; weakly positive for cystine arylamidase and trypsin; and negative for *N*-acetyl-β-glucosaminidase, acid phosphatase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase and valine arylamidase. Other physiological characteristics of strain J112^T are given in Table 1. The DNA G+C content of the type strain is 68 mol%.

The type strain, J112^T (=KCTC 19365^T=JCM 14945^T), was isolated from black sand collected from Soesoggak, Jeju Island, Korea.

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