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

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A novel Gram-positive, aerobic, short-rod-shaped bacterium, designated strain J112T, 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 J112T shared the highest 16S rRNA gene sequence similarities with Nocardioides marinisabuli SBS-12T (99.2 %), Nocardioides terrigena DS-17T (97.3 %), Nocardioides kribbensis KCTC 19038T (97.1 %) and type strains of other Nocardioides species with validly published names (>97 %). The DNA–DNA hybridization values between strain J112T 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 J112T represents a novel species of the genus Nocardioides, for which the name Nocardioides basaltis sp. nov. is proposed. The type strain is J112T (=KCTC 19365T=JCM 14945T).

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 J112T, from black beach sand and performed a polyphasic taxonomic study. On the basis of the results of this study, strain J112T is proposed as a novel species of the genus Nocardioides.

Strain J112T 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 Premix (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 J112T had the greatest levels of similarity with respect to the following strains with validly published names: N. marinisabuli SBS-12T (99.2 %), Nocardioides terrigena DS-17T (97.3 %), Nocardioides kribbensis KCTC 19038T (97.1 %), N. aquaticus DSM 11439T (96.8 %), Nocardioides aquiterrae GW-9T (96.8 %), Nocardioides dubius KCTC 9992T (96.8 %) and Nocardioides pyridinolyticus KCTC 0074BP (96.7 %). Related sequences of
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 J112T fell within the radiation of the genus *Nocardioides*, forming a clade with *N. marinisabuli* SBS-12T (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 J111T and type strains of the most closely related species, *N. marinisabuli* DSM 18965T, *N. terrigena* DS-17T and *N. kribbensis* KCTC 19038T, were 15.8 ± 1.5, 7.0 ± 1.7 and 28.7 ± 2.7 %, respectively. These low relatedness values confirmed that strain J112T 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) H2O2 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 J112T 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 J112T 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 J112T was similar to those of *N. albus* (67 mol%), *Nocardioides luteus* (68 mol%) and *N. aquatilis* (69 mol%). Cellular fatty acids were characterized for strain J112T and *N. marinisabuli* DSM 18965T, 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 J112T contained the following cellular fatty acids (>1 %): iso-C16:0 (70.3 %), C17:1ω8c (4.3 %), iso-C16:0 3-H (3.7 %), and iso-C14:0 3-OH (28.2 %).
Table 1. Physiological and biochemical characteristics of strain J112T and related species of the genus *Nocardioides*


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</table>

*Different results were reported by Yi & Chun (2004a).
(3.5%), 10-methyl C17:0 (3.2%), C18:1ω9c (2.8%), iso-C18:0 (2.7%), C18:1ω7c (1.7%), summed feature 3 (1.7%); comprising iso-C15:0 2-0H and/or C16:1ω7c and C16:0 (1.3%). The following fatty acids are present in trace amounts (<1%); iso-C12:0, iso-C14:0, iso-C15:0, C15:0, C16:0 N alcohol, 10-methyl C16:0, iso-C17:0, anteiso-C17:0, C17:0ω6c, C17:0, iso-C18:1 H, C18:0 and C17:0 3-0H. The large proportion of iso-C16:0 and the presence of 10methyl C17:0 found in strain J112T are typical of members of the genus Nocardioides. N. marinisabuli DSM 8965T contains the following fatty acids: iso-C16:0 (35.9%), C18:1ω9c (15.4%), iso-C17:0 (12.3%), C17:1ω8c (5.6%), anteiso-C17:0 (4.1%), iso-C18:0 (3.8%), summed feature 3 (2.7%); comprising iso-C15:0 2-0H and/or C16:1ω7c, iso-C15:0 (2.6%), 10-methyl C17:0 (2.0%), C17:0 (1.5%), C18:0 (1.5%) and C16:0 (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 J112T differed from that of N. marinisabuli DSM 8965T in that it contained a greater proportion of iso-C16:0 and smaller proportions of iso-C17:0, C18:1ω9c and anteisoC17:0. The dimaminopilic acid of the peptidoglycan was analysed by using TLC (Staneck & Roberts, 1974). LL-Diaminopilic acid was detected as the diamin acid in the cell-wall peptidoglycan of strain J112T. 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(H4) was found to be the predominant menaquinone in strain J112T.

The phylogenetic data, chemotaxonomic properties and physiological characteristics determined for strain J112T 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 J112T does not belong to any Nocardioides species with a validly published name. Therefore, strain J112T 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(H4) is the predominant menaquinone. Contains the following cellular fatty acids (>1%): iso-C16:0, C17:0ω8c, iso-C16:0 H, iso-C14:0, 10-methyl C17:0, C18:1ω9c, iso-C18:0, C18:1ω7c, summed feature 3 (comprising iso-C15:0 2-0H and/or C16:1ω7c) and C16:0. The following fatty acids are present in trace amounts: iso-C12:0, C14:0, iso-C15:0, C15:0ω6c, C15:0, C16:0 N alcohol, 10-methyl C16:0, iso-C17:0, anteiso-C17:0, C17:0ω6c, C17:0, iso-C18:1 H, C18:0 and C17:0 3-0H. 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-arabinol, 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, malose, D-mannose, melibiase, methyl a-D-glucopyranoside, methyl a-D-mannopyranoside, methyl b-D-xyllose, raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbate, D-tagatose, D-xylose, L-xylose and xylitol. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), b-glucosidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase; weakly positive for cystine arylamidase and trypsin; and negative for N-acetyl-b-glucosaminidase, acid phosphatase, a-chymotrypsin, a-fucosidase, a-galactosidase, b-galactosidase, b-glucosidase, b-glucuronidase, lipase (C14), a-mannosidase and valine arylamidase. Other physiological characteristics of strain J112T are given in Table 1. The DNA G+C content of the type strain is 68 mol%.

The type strain, J112T (=KCTC 19365T=JCM 14945T), was isolated from black sand collected from Soesoggak, Jeju Island, Korea.

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