Nocardiopsis nikkonensis sp. nov., isolated from a compost sample

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An actinomycete strain, designated YU1183-22^T, was isolated from a compost sample collected in Nikko, Japan. The isolate formed white aerial mycelium with relatively long aerial hyphae showing chains of arthrospores. Strain YU1183-22^T grew with 0–10% (w/v) NaCl, at pH 6–11 and at 10–37 °C (optimum 30 °C). Strain YU1183-22^T contained *meso*-diaminopimelic acid and no diagnostic sugars. The predominant menaquinones were MK-10(H₁₀) and MK-10(H₈). The polar lipids were phosphatidylcholine and phosphatidylglycerol. The major cellular fatty acids were iso-C_{16:0}, anteiso-C_{17:0} and tuberculostearic acid. The G + C content of the DNA was 72.3 mol%. Chemotaxonomic and morphological characterization clearly demonstrated that strain YU1183-22^T belonged to the genus *Nocardiopsis*. Phylogenetic analysis using 16S rRNA gene sequences showed that the isolate was closely related to *Nocardiopsis salina* YIM 90010^T (98.0% 16S rRNA gene sequence similarity), *Nocardiopsis xinjiangensis* YIM 90004^T (97.9%) and *Nocardiopsis kunsanensis* HA-9^T (97.3%). However, DNA–DNA relatedness as well as physiological and biochemical analyses showed that strain YU1183-22^T could be differentiated from its closest phylogenetic relatives. It is proposed that this strain be classified as a representative of a novel species of the genus *Nocardiopsis*, with the name *Nocardiopsis nikkonensis* sp. nov. The type strain is YU1183-22^T (=NBRC 102170^T =KCTC 19666^T).

The genus *Nocardiopsis* is a member of the family *Nocardiopsaceae* (Meyer, 1976) and, at the time of writing, about 30 species have been described, including *Nocardiopsis arabia*, *N. valliformis*, *N. quinghaiensis* and *N. ganjiahuensis* (Hozzein & Goodfellow, 2008; Yang *et al.*, 2008; Chen *et al.*, 2008; Zhang *et al.*, 2008). Many members of the genus *Nocardiopsis* have been isolated from hypersaline or alkaline environments. However, others have been isolated from non-hypersaline environments, including the sputum of a kidney transplant patient, the rhizosphere of *Casuarina* sp. and the atmosphere of a composting facility (Yassin *et al.*, 1997; Evtushenko *et al.*, 2000; Kämpfer *et al.*, 2002). *Nocardiopsis* species are of value to industry as they are known to produce bioactive agents such as griseusin D, apoptolidin and methylpendolmycin (Li *et al.*, 2007; Kim *et al.*, 1997; Sun *et al.*, 1991). Hence, the discovery of additional species of this genus will

contribute both to an understanding of their ecological roles and to the provision of bioresources for industrial applications.

Strain YU1183-22^T was isolated from a matured compost sample collected in Nikko, Japan, by growth on HV agar (Hayakawa & Nonomura, 1987) containing (l⁻¹) 20 mg nalidixic acid, 50 mg cycloheximide and 100 g NaCl. After incubation at 28 °C for 14 days, colonies of strain YU1183-22^T showed a thin, flat morphology with sparse, white, aerial hyphae.

Morphological features of cells of strain YU1183-22^T grown on HV agar and oatmeal-YGG agar (Hayakawa *et al.*, 1982) at 28 °C for 14 days were analysed by light and scanning electron microscopy. Cultural characteristics were determined after 2 weeks at 28 °C by using the methods adopted in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). All media were supplemented with 3% (w/v) NaCl and the colours of both substrate and aerial mycelia were determined according to Japan Colour Research Institute (1954). The detailed results are shown in Table 1. Spore motility in hanging drops was examined by light microscopy. Gram staining was examined by Hucker's

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YU1183-22^T is AB491226.

A scanning electron micrograph of strain YU1183-22^T is available with the online version of this paper.

method (Gerhardt, 1981). Carbon source utilization was examined using well-established procedures (Isik *et al.*, 1999) with the addition of 3 % NaCl. Nitrate reduction was determined after 7 and 14 days of growth by the addition of 0.2 ml each of Griess-Ilosvay reagents I and II to stab cultures in sloppy nitrate medium supplemented with 0.2 % (w/v) KNO₃ and 0.6 % Bacto agar (Difco) (Gordon & Mihm, 1962). Lipolysis was assessed on Sierra's medium (Sierra, 1957) using Tween 80 as a substrate. Hydrogen sulfide formation was detected by insertion of sterile lead acetate filter paper strips into the necks of the culture tubes containing ISP 6 medium (Küster & Williams, 1964). Melanin production was determined after 1–4 days of growth on ISP 6 and ISP 7 (Shirling & Gottlieb, 1966). Conditions for growth were tested using modified CM + YE medium (JCM medium no. 275) as follows. Growth at 15, 20, 25, 30, 37, 42 and 45 °C and pH 5, 6, 7, 8, 9, 10 and 11 was measured after 7 and 14 days; growth at 10 °C was also assayed after 6 weeks. Growth with 0–20 % (w/v) NaCl was determined after 14 and 21 days (Williams *et al.*, 1983).

Diaminopimelic acid isomers and sugars in whole-cell hydrolysates were analysed based on the methods established by Hasegawa *et al.* (1983) and Schaal (1985), respectively. Standard procedures were used for extraction and analysis of fatty acids (Tamura *et al.*, 1994) and isoprenoid quinones and polar lipids (Minnikin *et al.*, 1984) and the results were compared with the appropriate controls. Chromosomal DNA from strain YU1183-22^T was isolated and purified by the method of Saito & Miura (1963) with a minor modification (Hatano *et al.*, 2003). The G + C content of DNA from strain YU1183-22^T was determined by HPLC, as described by Tamura *et al.* (1994). DNA–DNA hybridization was carried out as described by Kusunoki *et al.* (1991) using biotinylated DNA.

PCR amplification of the 16S rRNA gene from strain YU1183-22^T was carried out according to the procedures described by Tamura & Hatano (2001) and the amplification products were directly sequenced using an ABI Prism BigDye Terminator cycle sequencing kit (PE Applied Biosystems) and an automated DNA sequencer (model

3100 Genetic Analyzer; PE Applied Biosystems). The 16S rRNA gene sequence obtained was aligned with reference sequences of the genus *Nocardiopsis* available from public databases by using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed by using MEGA version 4 (Tamura *et al.*, 2007) and CLUSTAL X for the neighbour-joining method with K_{nuc} values (Saitou & Nei, 1987) and minimum-evolution and maximum-parsimony methods (Takahashi & Nei, 2000) and PHYLIM (Guindon *et al.*, 2005) for the maximum-likelihood method. The topologies of the trees were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings (neighbour-joining, maximum-parsimony and minimum-evolution) and 500 resamplings (maximum-likelihood). 16S rRNA gene sequence similarity was calculated using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007).

Chemotaxonomic and morphological characteristics of strain YU1183-22^T were consistent with its assignment to the genus *Nocardiopsis* (Li *et al.*, 2004; Hozzein & Goodfellow, 2008). A whole-cell hydrolysate of strain YU1183-22^T contained *meso*-diaminopimelic acid and displayed no diagnostic sugars (wall chemotype III *sensu* Lechevalier & Lechevalier, 1970). The major menaquinones were MK-10(H₈) (28.1 %) and MK-10(H₁₀) (24.1 %). The major polar lipids were phosphatidylcholine and phosphatidylglycerol (phospholipid type PIII *sensu* Lechevalier *et al.*, 1977). The major cellular fatty acids were iso-C_{16:0} (26.0 %), anteiso-C_{17:0} (16.4 %) and 10-methyl C_{18:0} (16.3 %; tuberculostearic acid). Strain YU1183-22^T formed branched substrate hyphae, which fragmented into rod-shaped elements, and relatively long aerial hyphae with chains of arthrospores (Supplementary Fig. S1, available in IJSEM Online).

The almost-complete 16S rRNA gene sequence (1488 nt) of strain YU1183-22^T was compared with sequences of members of the genus *Nocardiopsis*. The highest 16S rRNA gene sequence similarity was observed with *Nocardiopsis lucentensis* DSM 44048^T (98.2 %), *N. salina* YIM 90010^T (98.0 %), *N. dassonvillei* subsp. *albirubida* DSM 40465^T (X97882) (98.0 %), *N. xinjiangensis* YIM 90004^T (97.9 %), *N. alba* DSM 43377^T (97.8 %), *N. halotolerans* DSM 44410^T

Table 1. Cultural characteristics of strain YU1183-22^T

All media were adjusted to pH 7.0 and supplemented with 3 % (w/v) NaCl. The isolate formed white aerial mycelium on yeast extract/malt extract agar.

Medium	Growth	Substrate mycelium
Yeast extract-malt extract agar (ISP 2)	Good	Pale yellow
Oatmeal agar (ISP 3)	Poor	White
Inorganic salts-starch agar (ISP 4)	Poor	White
Glycerol-asparagine agar (ISP 5)	Moderate	White
Peptone-yeast extract-iron agar (ISP 6)	Moderate	Pale yellow
Tyrosine agar (ISP 7)	Moderate	White
Trypticase soy agar	Abundant	Pale yellow
Mueller–Hinton agar	Good	Pale yellow

(97.8 %), *N. synnemataformans* IMMIB D-1215^T (97.7 %), *N. dassonvillei* subsp. *dassonvillei* DSM 43111^T (97.7 %), *N. aegyptia* DSM 44442^T (97.5 %) and *N. kunsanensis* HA-9^T (97.3 %). Neighbour-joining phylogenetic analysis revealed that strain YU1183-22^T formed a monophyletic clade with *N. salina* YIM 90010^T, *N. kunsanensis* HA-9^T and *N. xinjiangensis* YIM 90004^T and this cluster was also recovered with the other algorithms tested (Fig. 1). *N. lucentensis* DSM 44048^T did not cluster with strain YU1183-22^T.

DNA–DNA relatedness between strain YU1183-22^T and *N. lucentensis* NBRC 15854^T and its closest phylogenetic neighbours was 45.7–52.9 % (*N. lucentensis*, 51.7 %; *N. salina* JCM 13364^T, 45.7 %; *N. kunsanensis* NBRC 100348^T, 52.9 %; and *N. xinjiangensis* NBRC 104162^T, 48.7 %). These values were well below the 70 % cut-off point recommended for the assignment of bacterial strains to the same genomic species (Wayne *et al.*, 1987).

Strain YU1183-22^T could also be distinguished from its phylogenetic neighbours by a range of biochemical and phenotypic characteristics (Table 2). The isolate could be differentiated from its closest phylogenetic neighbours and *N. lucentensis* NBRC 15854^T by its ability to lyse Tween 80 and from its closest phylogenetic neighbours by its ability to grow without NaCl. *N. salina*, *N. kunsanensis* and *N. xinjiangensis* have been reported as being halophilic (Li *et al.*, 2004; Chun *et al.*, 2000; Li *et al.*, 2003).

Phenotypic, genotypic and chemotaxonomic characteristics show that strain YU1183-22^T represents a novel species

within the genus *Nocardiopsis*, for which the name *Nocardiopsis nikkonensis* sp. nov. is proposed.

Description of *Nocardiopsis nikkonensis* sp. nov.

Nocardiopsis nikkonensis (nik.ko.nen'sis. N.L. fem. adj. *nikkonensis* pertaining to Nikko City, Tochigi, Japan, where the type strain was isolated).

Aerobic, Gram-positive, non-motile actinomycete that produces a white aerial mycelium. Substrate mycelium is pale yellow. Diffusile pigments are not produced. Melanoid pigments are not formed on ISP 6 or ISP 7. Short-branched substrate mycelium fragments into non-motile elements. Aerial mycelium shows a typical zig-zag formation prior to sporulation. Grows with 0–20 % (w/v) NaCl, at pH 6–11 and at 25 and 30 °C, but not at 42 °C. H₂S is produced, starch is hydrolysed and Tween 80 is lysed. Nitrate is not reduced. Catalase-positive but oxidase-negative. As sole carbon sources, utilizes L-arabinose, D-fructose, D-galactose, D-glucose, L-rhamnose, sucrose and D-xylose, but not cellobiose, lactose, maltose, D-mannose, melibiose, raffinose, D-ribose, sodium acetate, glycerol, *myo*-inositol or D-mannitol. Contains *meso*-diaminopimelic acid but no diagnostic sugars. The predominant menaquinones are MK-10(H₁₀) and MK-10(H₈). The polar lipids are phosphatidylcholine and phosphatidylglycerol. The major cellular fatty acids are iso-C_{16:0}, anteiso-C_{17:0} and tuberculostearic acid. The DNA G + C content of the type strain is 72.3 mol%.

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of strain YU1183-22^T with the type strains of related species of the genus *Nocardiopsis*. Filled circles indicate that the corresponding nodes are also recovered in trees generated with the minimum-evolution, maximum-parsimony and maximum-likelihood methods. Bootstrap values (>50 %) based on 1000 resamplings are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Table 2. Phenotypic characteristics that distinguish strain YU1183-22^T from the type strains of the most closely related species of the genus *Nocardiopsis*

Strains: 1, *Nocardiopsis nikkonensis* sp. nov. YU1183-22^T; 2, *N. salina* JCM 13364^T; 3, *N. kunsanensis* NBRC 100348^T; 4, *N. xinjiangensis* NBRC 104162^T; 5, *N. lucentensis* NBRC 15854^T; +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5
Growth at/with:					
10 °C	w	–	–	w	w
37 °C	w	w	w	–	+
pH 11	+	w	+	+	w
0 % (w/v) NaCl	+	–	–	–	+
20 % (w/v) NaCl	w	w	w	+	–
Starch hydrolysis	+	–	+	+	+
Nitrate reduction	–	+	–	–	+
Tween 80 lipolysis	+	–	–	–	–
H ₂ S production	+	+	+	–	+
Sole carbon sources*					
D-Galactose	+	–	+	–	–
D-Glucose	+	–	+	–	+
Raffinose	–	+	–	–	–
D-Ribose	–	+	–	+	–
Sucrose	+	+	+	–	+
Sodium acetate	–	+	+	–	–

*Data for columns 2–4 were taken from Li *et al.* (2004).

The type strain is YU1183-22^T (=NBRC 102170^T =KCTC 19666^T), isolated from compost.

Acknowledgements

We are grateful to Miss Haruna Ashizawa for her excellent technical assistance.

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