Nocardia takedensis sp. nov., isolated from moat sediment and scumming activated sludge

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Chemotaxonomic and morphological characterization of two actinomycete strains, MS1-3^T and AS4-2, respectively isolated from moat sediment and scumming activated sludge, was carried out. This characterization clearly demonstrated that strains MS1-3^T and AS4-2 belong to the genus *Nocardia*. 16S rRNA gene sequencing studies showed that these isolates are most closely related to *Nocardia beijingensis* (98.1–98.3 % similarity), *Nocardia brasiliensis* (97.9–98.0 %) and *Nocardia tenerifensis* (97.8–97.9 %). However, the results of DNA–DNA hybridizations and physiological and biochemical tests showed that strains MS1-3^T and AS4-2 could be differentiated from their closest phylogenetic relatives both genotypically and phenotypically. It is proposed that the two isolates be classified as representatives of a novel species of *Nocardia*, *Nocardia takedensis* sp. nov. The type strain is MS1-3^T (=NBRC 100417^T=DSM 44801^T); AS4-2 (=NBRC 100418=DSM 44802) is a reference strain.

The genus *Nocardia* Trevisan 1889 belongs to the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997) and currently encompasses about 40 species of mycolic-acid-containing actinomycetes, including the recently described species *Nocardia puris*, *Nocardia inohanensis*, *Nocardia yamanashiensis*, *Nocardia tenerifensis* and *Nocardia neocaledoniensis* (Yassin *et al.*, 2003; Kageyama *et al.*, 2004; Kämpfer *et al.*, 2004; Saintpierre-Bonaccio *et al.*, 2004).

The *Nocardia* selective isolation method utilizing sucrose density-gradient centrifugation was developed previously (Yamamura *et al.*, 2003a). Using this technique, two *Nocardia* strains, MS1-3^T and AS4-2, were isolated. MS1-3^T was from a sediment sample taken from the moat surrounding Takeda Shrine in Yamanashi Prefecture, Japan, and AS4-2 was from a scumming activated sludge obtained from a

sewage treatment plant in Saitama Prefecture, Japan (Yamamura *et al.*, 2003b, 2004). These isolates could be differentiated from all known species of *Nocardia* in terms of their 16S rRNA gene restriction fragment length polymorphism (RFLP) patterns (Yamamura *et al.*, 2003b). The aim of present study was to determine the taxonomic position of isolates MS1-3^T and AS4-2 using a polyphasic approach.

Colony morphology and micromorphological properties of strains MS1-3^T and AS4-2 were observed by light and scanning electron microscopy according to a previously described procedure (Hayakawa *et al.*, 1996). Physiological and biochemical properties were recorded according to well-established procedures (Gordon *et al.*, 1974; Isik *et al.*, 1999). Diaminopimelic acid (A₂pm) 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 also used for extraction and analysis of mycolic acids (Schaal, 1985), fatty acids (Tamura *et al.*, 1994) and isoprenoid quinones and polar lipids (Minnikin *et al.*, 1984), and compared to the appropriate controls. Genomic DNA was prepared as described by Torres *et al.* (1996). The G + C contents of DNA from the isolates were 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.

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Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Nocardia takedensis* MS1-3^T and AS4-2 are AB158277 and AB158278.

SEMs of cells of strain MS1-3^T and an extended phylogenetic tree derived from 16S rRNA gene sequences are available as supplementary material in IJSEM Online.

16S rRNA genes were amplified by PCR and sequenced following procedures described previously (Yamamura *et al.*, 2003a). The 16S rRNA gene sequences obtained in the present study were manually aligned with sequences of available from EMBL/GenBank/DDBJ. A phylogenetic tree was inferred using neighbour-joining tree-making algorithms (Saitou & Nei, 1987). The program CLUSTAL W (Thompson *et al.*, 1994) was used to calculate evolutionary distances and similarity values. Topography of the constructed tree was evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985).

Chemotaxonomic and morphological characteristics of isolates MS1-3^T and AS4-2 were consistent with their assignment to the genus *Nocardia* (Goodfellow, 1998; Goodfellow *et al.*, 1999). Whole-cell hydrolysates of the test strains were rich in *meso*-A₂pm, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The predominant menaquinone component was MK-8(H₄, ω-cycl.). They also contained phosphatidylethanolamine (phospholipid type PII *sensu* Lechevalier *et al.*, 1977). In addition, TLC revealed that the strains contained methyl mycolates, which are equivalent in mobility to nocardomycolic acid. C_{16:0}, *cis*-9 C_{18:1} and 10-methyl C_{16:0} were present as major cellular fatty acids. Microscopic observation confirmed the formation of branched substrate hyphae, fragmenting into rod-shaped elements (Goodfellow & Lechevalier, 1989), and relatively short aerial hyphae with chains of arthrospores (see Supplementary Fig. A in IJSEM Online).

Almost complete 16S rRNA gene sequences (1345 nt) of isolates MS1-3^T and AS4-2 were compared with sequences of recognized species of *Nocardia*. The 16S rRNA gene sequences of the test strains and those representative of the genus *Nocardia* had 95.3–98.3% similarity. Highest sequence similarities were shown with *Nocardia beijingensis* (98.1–98.3%), *Nocardia brasiliensis* (97.9–98.0%) and *N. tenerifensis* (97.8–97.9%). The 16S rRNA gene-based tree, constructed using the neighbour-joining method, showed that the test strains formed a monophyletic clade that is loosely associated with *N. brasiliensis*, *N. tenerifensis* and *Nocardia transvalensis* (Fig. 1). The taxonomic integrity of the test strains was supported by DNA relatedness data. Strain MS1-3^T showed DNA relatedness values of 8.1–23.1% to *N. beijingensis*, *N. brasiliensis* and *N. tenerifensis*, results that were well below the 70% cut-off point recommended for assignment of bacterial strains to the same genomic species (Wayne *et al.*, 1987). On the other hand, the 16S rRNA gene sequence similarity between MS1-3^T and AS4-2 was 99.8% and the DNA relatedness value was 91.0%. The phenotypic properties of these two strains also clearly distinguished them from representatives of *N. beijingensis*, *N. brasiliensis*, *N. tenerifensis* and *N. transvalensis* (Table 1).

Based on 16S rRNA gene sequence data, DNA–DNA hybridization studies and biochemical properties, as well as on RFLP patterns (Yamamura *et al.*, 2003b), it is proposed that strains MS1-3^T and AS4-2 represent a novel species in the

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of strains MS1-3^T and AS4-2 to the nearest *Nocardia* species. The tree was constructed using the neighbour-joining method and K_{nuc} values (Saitou & Nei, 1987). Only values above 50% significance are indicated. A tree based on available 16S rRNA gene sequences of all *Nocardia* species is available as Supplementary Fig. B in IJSEM Online.

Table 1. Phenotypic properties that differentiate strains MS1-3^T and AS4-2 from representatives of phylogenetically related *Nocardia* species

Strains: 1, MS1-3^T and AS4-2; 2, *N. beijingensis* IFO (now NBRC) 16342^T; 3, *N. brasiliensis* ATCC 19296^T; 4, *N. tenerifensis* DSM 44704^T; 5, *N. transvalensis* DSM 43405^T. ND, Not determined; +, positive; –, negative; w, weak reaction. Data for reference strains were taken from Goodfellow & Lechevalier (1989), Wang *et al.* (2001), Yassin *et al.* (2003) and Kämpfer *et al.* (2004).

Character	1	2	3	4	5
Aesculin hydrolysis	+	+	+	+	–
Decomposition of:					
Casein	–	–	+	+	–
Elastin	–	–	+	ND	–
Hypoxanthine	–	–	+	+	–
Tyrosine	–	–	+	+	–
Uric acid	+	–	–	ND	ND
Xanthine	–	+	–	–	–
Growth on sole carbon sources:					
Adonitol	–	–	–	–	+
L-Arabinose	–	+	–	ND	–
Cellobiose	–	+	–	ND	–
D-Fructose	–	+	+	–	ND
<i>meso</i> -Erythritol	–	+	–	ND	+
<i>meso</i> -Inositol	–	–	+	+	–
D-Mannitol	–	+	+	+	–
D-Mannose	+	+	–	ND	+
Raffinose	–	+	–	ND	–
L-Rhamnose	–	+	–	–	+
D-Sorbitol	–	+	–	–	–
D-Trehalose	–	+	+	+	+
D-Xylose	–	+	–	ND	–
Isoamyl alcohol	–	–	w	ND	+
Citrate	–	+	+	+	+
<i>p</i> -Hydroxybenzoic acid	–	+	–	+	–
Growth at 45 °C	–	–	+	–	–

genus *Nocardia*. The name *Nocardia takedensis* sp. nov. is proposed for these two strains.

Description of *Nocardia takedensis* sp. nov.

Nocardia takedensis (ta.ke.den'sis. N.L. fem. adj. *takedensis* pertaining to the Takeda Shrine, from where the organism was first isolated).

Aerobic, Gram-positive, non-motile actinomycete. Forms an orange substrate and white aerial mycelium, which fragments into rod-shaped elements. Diffusible pigments are not produced. Aesculin and urea are hydrolysed and nitrate is reduced. Does not degrade adenine, casein, elastin, hypoxanthine, tyrosine or xanthine, but does degrade uric acid. Grows well at 30 °C, but does not grow at 45 °C. Assimilates 2,3-butanediol, D-galactose, D-glucose, D-mannose, sucrose (all at 1 %, w/v) and acetate (at 0.1 %, w/v) as sole carbon sources, but not adonitol, L-arabinose, cellobiose, D-fructose, *meso*-erythritol, *meso*-inositol, isoamyl alcohol, D-mannitol, D-melezitose, D-melibiose, 1,2-propanediol, raffinose, L-rhamnose, D-sorbitol, D-trehalose, D-xylose (at 1 %, w/v), adipic acid, citrate, D-gluconate, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, pimelic acid or sebacic acid (at 0.1 %, w/v). The major cellular fatty acids are C_{16:0} (45.0 %), *cis*-9 C_{18:1} (20.4 %) and 10-methyl C_{18:0} (10.4 %). The G + C content of the DNA is 68.6 mol%.

The type strain is MS1-3^T (=NBRC 100417^T=DSM 44801^T), isolated from a sediment sample taken from the moat surrounding Takeda Shrine in Yamanashi Prefecture, Japan.

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