Nocardia amamiensis sp. nov., isolated from a sugar-cane field in Japan

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An actinomycete, strain TT 00-78\textsuperscript{T}, was isolated from soil from a sugar-cane field on Amami Island in Japan, using an SDS/yeast extract pre-treatment method, and the taxonomy was studied using a polyphasic approach. The chemotaxonomic and morphological characterizations clearly demonstrated that the strain belongs to the genus *Nocardia*. 16S rRNA gene sequencing studies showed that the strain was closely related to the type strains of *Nocardia pneumoniae* (98.6%), *Nocardia araoensis* (98.1%), *Nocardia arthritidis* (97.9%) and *Nocardia beijingensis* (97.7%). However, the results of DNA–DNA hybridization and physiological and biochemical tests showed that strain TT 00-78\textsuperscript{T} could be differentiated from its closest phylogenetic relatives both genotypically and phenotypically. Therefore this strain represents a novel species of the genus *Nocardia*, for which the name *Nocardia amamiensis* sp. nov. is proposed. The type strain is TT 00-78\textsuperscript{T} (=NBRC 102102\textsuperscript{T} =DSM 45066\textsuperscript{T} =KCTC 19208\textsuperscript{T}).

The taxonomic position of strain TT 00-78\textsuperscript{T} by using a polyphasic approach.

The colonial properties of strain TT 00-78\textsuperscript{T} were recorded from colonies grown on a plate containing modified Bennett’s agar (Jones, 1949) and incubated for 14 days at 28 °C. Spore motility was examined in hanging drops by means of light microscopy. Gram staining was examined by using Hucker’s method (Gerhardt, 1981). Acid–alcohol-fastness was examined by using a modified version of the Ziehl–Neelsen method (Gordon, 1967) in which 0.5 % (v/v) sulfuric acid was used for decolorization. The hydrolysis of complex substrates and the utilization of carbon sources were examined by using well-established procedures (Gordon et al., 1974; Isik et al., 1999). The tests for aesculin and arbutin hydrolysis (Williams et al., 1983), nitrate reduction (Gordon & Mihm, 1962) and urea hydrolysis (Gordon et al., 1974) were performed using established procedures. Growth at 45 °C was recorded on GYE medium (Gordon & Mihm, 1962).

Diaminopimelic acid isomers and sugars in whole-cell hydrolysates were analysed on the basis of the methods established by Hasegawa et al. (1983) and Schaal (1985), respectively. Standard procedures were also used for the extraction and analysis of mycolic acids (Schaal, 1985), fatty acids (Tamura et al., 1994), isoprenoid quinones and polar lipids (Minnikin et al., 1984); comparisons were made with the appropriate controls. Chromosomal DNA from strain TT 00-78\textsuperscript{T} was isolated and purified by the method of Saito & Miura (1963) with a minor modification (Hatano et al., 2003). The DNA G+C content of the strain 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 TT 00-78\textsuperscript{T} was carried out according to the procedures described by Tamura & Hatano (2001) and directly sequenced using an ABI Prism BigDye Terminator cycle sequencing kit (PE Applied Biosystems) and an automatic DNA sequencer (model 3100 Genetic Analyzer; PE Applied Biosystems). The 16S rRNA gene sequence obtained in the present study was aligned with reference sequences for the genus Nocardia (available from EMBL/GenBank/DDBJ) by using the CLUSTAL\textsubscript{X} program (Thompson et al., 1997). Phylogenetic trees were constructed with MEGA, version 3.1 (Kumar et al., 2001) and CLUSTAL\textsubscript{X} (Thompson et al., 1997), using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution and maximum-parsimony methods (Takahashi & Nei, 2000). The topography of the resulting tree was evaluated by means of bootstrap analysis based on 1000 replicates (Felsenstein, 1985).

The 16S rRNA gene sequence derived from strain TT 00-78\textsuperscript{T} contained the signature nucleotides characteristic of the family Nocardiaceae (Stackebrandt et al., 1997). On the basis of the phylogenetic analysis, the strain falls within the radiation of the genus Nocardia (data not shown). The chemotaxonomic and morphological characteristics of strain TT 00-78\textsuperscript{T} were consistent with its assignment to the genus Nocardia (Goodfellow, 1998; Goodfellow et al., 1999). The whole-cell hydrolysate of the test strain contained meso-diaminopimelic acid, arabinine and galactose (wall chemotype IV sensu Lechevalier & Lechevalier, 1970). The major menaquinones were MK-8(H\textsubscript{4}) (30.3 %) and MK-8(H\textsubscript{2}) (16.8 %). The major polar lipids found were phosphatidylyethanolamine, phosphatidylinositol and diphosphatidylglycerol (phospholipid type PI sensu Lechevalier et al., 1977). In addition, the TLC analysis revealed that the strain contained mycolic acids with an \( R_f \) value (0.46) identical to that of the reference strain used as a control. The major cellular fatty acids were hexadecanoate (43 %), hexadecenoate (20 %), tuberculostearic acid (10-methyl octadecanoate; 17 %) and cis-9 octadecanoate (10 %). The formation of branched substrate hyphae, fragmenting into rod-shaped elements (Goodfellow & Lechevalier, 1989), and relatively short aerial hyphae with chains of arthrospores were observed by microscopy (Fig. 1).

The almost-complete 16S rRNA gene sequence (1476 nt) of strain TT 00-78\textsuperscript{T} was compared with sequences from recognized species of Nocardia. The phylogenetic tree obtained using the neighbour-joining method showed that the strain forms a monophyletic clade with Nocardia pneumoniae, Nocardia beijingensis, Nocardia arthritidis and Nocardia araoensis (Fig. 2). The 16S rRNA gene sequence similarities with phylogenetic neighbours were in the range 97.7–98.6 %. The closest phylogenetic species was N. pneumoniae, with 98.6 % sequence similarity to the type strain. The taxonomic integrity of strain TT 00-78\textsuperscript{T} was supported by the DNA relatedness data. DNA relatedness values of 6.9–20.3 % were obtained with respect to the type strains of related Nocardia species (N. pneumoniae, 20.3 %; N. beijingensis, 6.9 %; N. arthritidis, 20.3 %; N. araoensis, 18.3 %), the values being well below the 70 % cut-off point recommended for the assignment of bacterial strains to the same genomic species (Wayne et al., 1987). Strain TT 00-78\textsuperscript{T} was also distinguishable from its phylogenetic neighbours in comparisons of biochemical and phenotypic characteristics (Table 1).

On the basis of phenotypic and genotypic data, therefore, strain TT 00-78\textsuperscript{T} represents a novel species within the genus Nocardia, for which the name Nocardia amaniensis sp. nov. is proposed.

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**Fig. 1.** Scanning electron micrograph of hyphae of strain TT 00-78\textsuperscript{T} grown on modified Bennett’s agar at 28 °C for 14 days. Bar, 1 μm.

**Fig. 2.** Phylogenetic tree derived from 16S rRNA gene sequences, showing the relationships of strain TT 00-78\textsuperscript{T} with the closest Nocardia species. The tree was constructed using the neighbour-joining method and \( K_{\text{nuc}} \) values (Saitou & Nei, 1987). Asterisks indicate branches of the tree that were also recovered using the minimum-evolution and maximum-parsimony methods (Takahashi & Nei, 2000). Bar, 0.01 \( K_{\text{nuc}} \).
Nocardi a amamiensis sp. nov.

**Description of Nocardi a amamiensis sp. nov.**

Nocardi a amamiensis (a.ma.mi.en’sis. N.L. fem. adj. amami-ensis pertaining to Amami Island, from where the organism was first isolated).

Aerobic, Gram-positive, partially acid–alcohol-fast, non-motile actinomycete that forms moderately white aerial mycelium that fragments into rod-shaped elements. Diffusible pigments are not produced. Aesculin is hydrolysed and nitrate is reduced. Arbutin and urea are not hydrolysed. Mycelium that fragments into rod-shaped elements. Diffusible pigments are not produced. Aesculin is hydrolysed and nitrate is reduced. Arbutin and urea are not hydrolysed.

**Table 1. Phenotypic properties that serve to distinguish strain TT 00-78 from the type strains of related Nocardi a species**

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<th>Characteristic</th>
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<td>Arbutin hydrolysis</td>
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<td>Nitrile reduction</td>
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<td>Decomposition of:</td>
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<td>Testosterone</td>
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<td>Uric acid</td>
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<td>Xanthine</td>
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<td>Growth with sole carbon sources</td>
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<td>L-Arabinose</td>
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<td>meso-Erythritol</td>
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<td>D-Galactose</td>
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<td>D-Lactose</td>
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<td>l-Rhamnose</td>
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<tr>
<td>Growth at 45 °C</td>
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*Data from this study.

The type strain, TT 00-78 ( = NBRC 102102 = DSM 45066 = KCTC 19208 T), was isolated from a soil sample collected from a sugar-cane field on Amami Island in Japan.

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