Nocardia jiangxiensis sp. nov. and Nocardia miyunensis sp. nov., isolated from acidic soils

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The taxonomic positions of two soil actinomycetes, strains 117T and 43401T, provisionally assigned to the genus Nocardia were determined in a polyphasic study. The organisms were found to have phenotypic properties typical of members of the genus Nocardia, and formed a distinct branch in the Nocardia 16S rRNA gene tree. It was evident from the phylogenetic data that the isolates were most closely, albeit loosely, associated with the type strains of Nocardia nova and Nocardia pseudobrasiliensis. However, all four of these organisms were readily distinguishable from one another using DNA–DNA relatedness and phenotypic data. It is evident from the genotypic and phenotypic data that the two isolates should be recognized as novel species of the genus Nocardia. It is proposed, therefore, that strains 117T (=CGMCC 4.1904T =JCM 12860T) and 43401T (=CGMCC 4.1905T =JCM 12861T) be classified in the genus Nocardia as the type strains of Nocardia miyunensis sp. nov. and Nocardia jiangxiensis sp. nov., respectively.

The genus Nocardia belongs to the family Nocardiaceae, a member of the suborder Corynebacterineae, proposed by Stackebrandt et al. (1997). At the time of writing, the genus encompasses 46 species with validly published names; the majority of these species have been described in the last 5 years by means of polyphasic taxonomic approaches (Maldonado et al., 2000; Yassin et al., 2001a, b; Kim et al., 2002; Zhang et al., 2003, 2004). Most of the novel species are associated with human and animal infections (Yassin et al., 2000a, b, 2003; Hamid et al., 2001; Kageyama et al., 2004a, b, c, d); nearly all of the remaining novel species are from soil collected from geographically diverse locations (Maldonado et al., 2000; Albuquerque de Barros et al., 2003; Li et al., 2004; Saintpierre-Bonaccio et al., 2004; Zhang et al., 2003, 2004).

Little is known about the functional roles of nocardiae in natural habitats, though it is clear that they form an integral part of the autochthonous soil microflora (Orchard, 1979, 1981). Nevertheless, there is evidence that nocardial species diversity in the soil ecosystem is greatly underestimated (Orchard & Goodfellow, 1980; Wang et al., 1999; Maldonado et al., 2000). It is important to establish the species richness of nocardiae in natural habitats for ecological reasons and for industrial purposes, as novel nocardiae are a source of commercially significant bioactive compounds (Kinoshita et al., 2001; Bringmann et al., 2005). The aim of the present study was to determine the taxonomic status of two Nocardia-like strains isolated from soil.

Strain 117T was isolated from a pine-forest soil sample (about pH 5.5) collected in Miyun County, Beijing (China), and strain 43401T was isolated from rhizosphere soil (about pH 3.5) of goose-grass (Eleusine indica) growing next to a copper mine in Wushan, Jiangxi Province, southern China. In each case, soil suspensions prepared using a dispersing and differentiation procedure (Wang et al., 2003) were inoculated onto plates of an acidified selective isolation medium (Huang et al., 2004), which were incubated for 3 weeks at 28°C. The strains were maintained on oatmeal agar (ISP medium 3; Küster, 1959), adjusted to an initial pH of 5-5 using a citric acid (0.1 M)/Na2HPO4 (0.2 M) buffer.

The colonial properties of the isolates were observed on modified Sauton’s agar (Mordarska et al., 1972) and acidified oatmeal agar (as above) plates that had been incubated for 7 days at 28°C. Micromorphological properties were recorded using samples taken from the Sauton’s agar plates and examined by using light microscopy (Axioskop 20; Zeiss) and scanning electron microscopy (FEI QUANTA instrument). Well-established procedures were used to...
determine a range of phenotypic properties (Goodfellow, 1971). In addition, acid production from carbohydrates was determined using media and methods described by Gordon et al. (1974), use of sole carbon sources for energy and growth was assessed according to the methods of Yassin et al. (1995) and biochemical and biodegradation tests were performed using procedures described by Gordon & Mihm (1957). Oatmeal agar adjusted to initial pH values of 3-5, 4-5, 5-5, 6-5, 7-5, 8-5, 9-5 and 10-5 using citric acid (0-1 M)/NaOH (0-2 M) and glycine (0-05 M)/Na2HPO4 (0-2 M) buffer systems was used to test the ability of strains to grow at different pHs. The chemotaxonomic properties of the isolates were determined by following standard chromatographic procedures as outlined by Zhang et al. (2003). Extraction of genomic DNA, PCR amplification of 16S rRNA and purification of the PCR products from strains 117T and 43401T were carried out by following the procedures described by Rainey et al. (1996); the purified PCR products were sequenced directly, according to the method of Lu et al. (2001). The 16S rRNA gene sequences of the strains were aligned manually with corresponding nucleotide sequences of the type strains of Nocardioid species, retrieved from the GenBank/EMBL/DDBJ databases, using CLUSTAL X 1.8 software (Thompson et al., 1997). Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices were generated as described by Kimura (1980). The resultant unrooted tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings, using the SEQBOOT and CONSENSE options from the PHYLIP package.

Chromosomal DNA was extracted and purified from the two isolates and from the type strains of Nocardia nova and Nocardia pseudobrasiliensis. DNA–DNA relatedness values between the tested strains were estimated using the fluorometric microplate method of Ezaki et al. (1989). The hybridization experiments were conducted at 50% formamide at 50°C. Fluorescence intensities were measured using a Fluostar Optima microplate reader (BMG LABTECH) at a wavelength of 360 nm for excitation and at 460 nm for emission. DNA–DNA relatedness values were calculated from quadruplicated hybridization experiments and expressed as means of the corresponding reciprocal values.

When the almost-complete 16S rRNA gene sequences of strains 117T (1405 nt) and 43401T (1409 nt) were examined, they were found to contain the signature nucleotide characteristics of the family Nocardiaceae (Stackebrandt et al., 1997) and of the genus Nocardia (Chun & Goodfellow, 1995). It was apparent from the phylogenetic analyses that the two strains fell within the 16S rRNA Nocardia gene tree (data not shown). The organisms were also shown to have phenotypic properties consistent with their classification in the genus Nocardia (Goodfellow et al., 1999), i.e. the strains are aerobic, Gram-positive, partially acid–alcohol-fast actinomycetes that form extensively branched substrate mycelia that fragment into irregular, rod-shaped, non-motile, rod-shaped elements (see Supplementary Fig. S1 in IJSEM Online). In addition, each strain produced whole-organism hydrolysates rich in meso-diaminopimelic acid, arabinose and galactose (wall chemotype IV sensu Lechevalier & Lechevalier, 1970) and contained major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol mannosides (phospholipid type II sensu Lechevalier et al., 1977), a predominance of hexahydrogenated menaquinones with eight isoprene units where the end two were cyclized, and fatty acid profiles consisting mainly of saturated, unsaturated and 10-methyl-branched components. They were also characterized by the presence of mycolic acids that co-migrated (Rf value around 0-47) with those from marker strains of Nocardia.

![Fig. 1. An unrooted neighbour-joining tree (Saitou & Nei, 1987), based on nearly-complete 16S rRNA gene sequences, showing the positions of strains 117T and 43401T amongst representatives of closely related Nocardia species. The symbols f, m and p denote branches that were also recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms, respectively. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given. Bar, 0-01 substitutions per nucleotide position.](image-url)
It is apparent from the abridged phylogenetic tree (Fig. 1) that the two strains form a phylogenetic branch that is supported by three out of the four tree-making algorithms and by a bootstrap value of 84% in the neighbour-joining analysis. The two organisms share a 16S rRNA gene sequence similarity of 98·5%, a value that corresponds to 15 nt differences at 1405 locations. Each organism is most closely, albeit loosely, related to the type strains of N. nova and N. pseudobrasiliensis. Strain 117T shares 16S rRNA gene sequence similarity of 98·1% with N. nova JCM 6044T and 98·2% with N. pseudobrasiliensis ATCC 51512T, values that are equivalent to 26 and 25 nt differences at 1405 positions; the corresponding values for strain 43401T are 98·7 and 97·9%, corresponding to 18 and 29 nt differences at 1395 positions. Much higher 16S rRNA gene sequence similarities than these have been found between the type strains of Nocardia species shown to have DNA–DNA relatedness values well below the 70% threshold recommended for the delineation of genomic species (Wayne et al., 1987). The type strains of Nocardia brevicatena and Nocardia paucivorans, for instance, were found to share a 16S rRNA gene sequence similarity of 99·6% and had a DNA relatedness value of 61·9% (Yassin et al., 2000a).

It is evident from the DNA–DNA relatedness data that strains 117T and 43401T represent two distinct genomic species. These strains were found to have a mean DNA–DNA hybridization value of 58% and corresponding relatedness values below 55% with N. nova JCM 6044T (54% with strain 117T and 46% with strain 43401T) and N. pseudobrasiliensis ATCC 51512T (42% with strain 117T and 39% with strain 43401T). The two strains can also be distinguished from one another and from phylogenetically close relatives, including the type strains of N. nova and N. pseudobrasiliensis, by using a combination of phenotypic properties (Table 1).

It is clear from the genotypic and phenotypic data that strains 117T and 43401T merit recognition as novel Nocardia species: the names proposed for these novel taxa are Nocardia miyunensis sp. nov. and Nocardia jiangxiensis sp. nov., respectively.

**Description of Nocardia jiangxiensis sp. nov.**

Nocardia jiangxiensis (jiang.xi.en'sis. N.L. fem. adj. jiang.xi.en'sis referring to Jiangxi Province, southern China, the source of the type strain).

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**Table 1. Phenotypic characteristics that distinguish strains 117T and 43401T from one another and from the type strains of phylogenetically close Nocardia species**

<table>
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<th>Characteristic</th>
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Aerobic, Gram-positive, catalase-positive, partially acid-alcohol-fast, non-motile actinomycete that forms an extensively branched substrate mycelium that fragments into rod-shaped elements. A pinkish white to orange substrate mycelium carrying white aerial hyphae is formed on oatmeal agar at an initial pH of 5-5. Diffusible pigments are not formed. Does not degrade starch, Tween 20 or 80 or xanthine. Acid is formed from D-arabinose, D-galactose, D-glucose, glycerol, myo-inositol, D-mannose and D-xylene, but not from D-lactose, D-maltose, D-mannitool, D-L-rhamnose or sodium citrate. D-Arabinose, D-cellobiose, meso-erythritol (weak), D-fructose, D-galactose, D-glucose, glycerol, myo-inositol (weak), D-lactose, D-maltose, D-melezitose, D-ribose, D-sorbitol, D-sucrose (weak), D-trehalose and D-xylene are used as sole carbon sources for energy and growth, but adonitol and sodium propionate are not. Growth occurs at an initial pH between 4-5 and 9-5, and between 17 and 37°C, but not at 10 or 45°C. Additional phenotypic properties are shown in Table 1. Chemotaxonomic properties are typical of the genus *Nocardia*. The major cellular fatty acids are C16:1ω7t (16·4%), C16:0 (27·2%), C18:1ω9c (7·7%), C18:0 (6·4%) and 10-methyl C18:0 (33·0%). The species description is based on a single strain, which therefore serves as the type strain.

The type strain, 117T (=CGMCC 4.1905T=JCM 12860T), was isolated from a pine-forest soil sample collected in Miyun County, Beijing, People’s Republic of China.

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**References**


