Nocardia caishijiensis sp. nov., a novel soil actinomycete

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A soil isolate, strain F829T, which had provisionally been assigned to the genus Nocardia, was subjected to a polyphasic taxonomic study. An almost complete 16S rDNA sequence was determined for this strain; the sequence was aligned with available sequences for nocardiae and phylogenetic trees were inferred using three tree-making algorithms. The organism showed a combination of phenotypic properties typical of nocardiae and formed a distinct phyletic line within the evolutionary radiation of species of the genus Nocardia, being most closely related to Nocardia asteroides ATCC 19247T. Strain F829T was readily distinguished from representatives of species of Nocardia with validly published names on the basis of phenotypic data, notably from the type strain of N. asteroides. It is proposed that the organism be recognized as a novel species of Nocardia, Nocardia caishijiensis sp. nov. The type strain is F829T (= AS 4.1728T = JCM 11508T).

The application of chemotaxonomic, numerical phenetic and molecular systematic methods has led to an improved description of the genus Nocardia (Goodfellow et al., 1999). The genus belongs to the mycolic-acid-containing group of actinomycetes, i.e. the suborder Corynebacterineae Stackebrandt et al. 1997, which encompasses the genera Corynebacterium, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus, Skermania, Tsukamurella and Williamsia, and the genus Turicella, which lacks mycolic acids (Goodfellow et al., 1998, 1999). Members of these taxa form a distinct phyletic line in the 16S rDNA tree and can be distinguished from one another using a combination of biochemical, chemical and morphological features (Goodfellow et al., 1999). The revised genus Nocardia encompasses 27 species with validly published names at the time of writing; the taxonomic integrity of most of the latter is underpinned by a plethora of genotypic and phenotypic data (Goodfellow et al., 1999; Maldonado et al., 2000; Gürtler et al., 2001; Hamid et al., 2001; Wang et al., 2001; Yassin et al., 2001).

Much of the emphasis in nocardial systematics has focussed on the causal agents of actinomycetoma and nocardiosis (Goodfellow, 1992, 1998; McNeil & Brown, 1994), though it is evident that nocardiae are common in natural habitats, notably soil (Orchard et al., 1977; Orchard, 1979, 1981; Maldonado et al., 2000). It is also becoming increasingly clear that nocardial species diversity is underestimated in both clinical and non-clinical settings (Gürtler et al., 2001; Hamid et al., 2001; Wang et al., 2001; Yassin et al., 2001; Albuquerque de Barros et al., 2003). It is important to unravel the species richness of nocardiae, especially to determine the roles that members of particular species play in the flow of nutrients and energy in natural habitats.

The aim of the present study was to determine the taxonomic position of a Nocardia-like strain, isolated from soil, using a polyphasic approach. The resultant data show that strain F829T should be recognized as a novel species of Nocardia. The name Nocardia caishijiensis sp. nov. is proposed for this organism.

Strain F829T was isolated on a Bennett’s agar plate [1 % (w/v) D-glucose, 0.1 % (w/v) yeast extract, 0–1 % (w/v) beef extract, 0.2 % (w/v) casein enzymic hydrolysate (Sigma), 1.5 % (v/v) agar] that had been incubated at 28 °C for 7 days following inoculation with a suspension of a soil sample collected from Caishiji in Anhui Province, China. The isolate and the marker cultures used in the DNA–DNA relatedness and phenotypic characterization studies were maintained on modified Sauton’s agar slants (Mordarska et al., 1972) at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C.

The colonial properties of isolate F829T were recorded from modified Sauton’s and Bennett’s agar plates that had been incubated for up to 7 days at 28 °C. The micromorphological properties of the isolate were recorded using samples taken from the modified Sauton’s agar plate by light and

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The GenBank accession number for the 16S rDNA sequence of strain F829T (= AS 4.1728T) is AF459443.
scanning electron microscopy; in the latter case gold-coated dehydrated preparations from a 7 day culture were examined using a Hitachi S-570 scanning electron microscope. Gram (Hucker’s modification; Society for American Bacteriologists, 1957) and Ziehl–Neelsen (Gordon, 1967) preparations were also observed by light microscopy.

The test strain was examined for a range of phenotypic properties using standard procedures (Goodfellow, 1971; Williams et al., 1983). In addition, acid production from carbohydrates was carried out using media and methods described by Gordon et al. (1974) and the utilization of sole carbon and sole carbon/nitrogen sources was investigated after Gordon & Mihm (1957) and Tsukamura (1966). Resistance to lysozyme was determined by the method of Gordon et al. (1974). Tolerance of pH, temperature and sodium chloride regimes were determined on modified Sauton’s agar plates incubated for up to 14 days. Resistance to antibiotics was examined using chloramphenicol (30 μg), erythromycin (15 μg), gentamicin sulfate (10 μg), midecamycin (15 μg), minocycline hydrochloride (30 μg), penicillin G (10 U), rifampicin (5 μg), streptomycin sulfate (10 μg), tobramycin sulfate (10 μg) and vancomycin (30 μg) disks (Goodfellow & Orchard, 1974) with glucose-yeast extract agar (Gordon & Mihm, 1962) as the basal medium; the results were recorded following incubation at 28 °C for up to 14 days.

Biomass for most of the chemotaxonomic studies was prepared following growth of the isolate and marker strains in shake flasks of modified Sauton’s broth for 5 days at 28 °C; after checking for purity, the biomass was harvested by centrifugation, washed twice in distilled water and freeze-dried. Established TLC procedures were used to determine the diagnostic isomers of dianimonopellic acid (Lechevalier & Lechevalier, 1980), whole-organism sugars (Lechevalier & Lechevalier, 1980) and polar lipids (Minnikin et al., 1984). The acid methanolysis procedure was used to detect mycolic acids (Minnikin et al., 1975). The predominant isoprenoid quinones were extracted and purified by the method of Collins et al. (1977, 1987); purified menaquinones were determined by reversed-phase HPLC (Wu et al., 1989). Biomass for the quantitative fatty acid analysis was prepared by scraping growth from TS agar plates [trypticase soy broth (BBL), 3 % (w/v); Bacto agar (Difco), 1·5 % (w/v)] that had been incubated for 4 days at 28 °C. The fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Chromosomal DNA was extracted from biomass of strain F829T grown in modified Sauton’s broth for 3 days at 28 °C and purified following the methods of Saito & Miura (1963) and Whipple et al. (1987). The G + C content of the DNA was determined using the thermal denaturation method (Marmur & Doty, 1962) with Escherichia coli AS 1.365 as control. DNA–DNA relatedness values between strain F829T and Nocardia asteroides ATCC 12947T were determined spectrophotometrically from renaturation rates using established procedures (De Ley et al., 1970; Huß et al., 1983) and software (Jahnke, 1992).

Genomic DNA extraction, PCR amplification of 16S rDNA and purification of the PCR product from strain F829T were carried out using procedures described by Rainey et al. (1996). The purified PCR product was sequenced directly using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) and universal primers as described previously (Lu et al., 2001). Sequence gel electrophoresis was carried out and nucleotide sequences were obtained automatically using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer.

The 16S rDNA sequence of strain F829T was aligned manually with corresponding nucleotide sequences of representatives of the suborder Corynebacterineae, including the type strains of Nocardiopsis species, retrieved from the DDBJ/EMBL/GenBank databases using the program CLUSTAL X 1.8 (Thompson et al., 1997). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were generated according to the method of Kimura (1980). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP suite of programs.

When the almost complete 16S rDNA sequence (1426 nt) obtained for strain F829T was compared with corresponding sequences from representatives of genera in the suborder Corynebacterineae, it was found to contain signature nucleotides that are characteristic for members of the family Nocardiaceae (Stackebrandt et al., 1997) and the genus Nocardia (Chun & Goodfellow, 1995). The high 16S rDNA gene sequence similarities found between the tested strain and representatives of the genus Nocardia (94·8–97·6 %) also support its assignment to this taxon.

Strain F829T showed a range of phenotypic properties typical of members of the genus Nocardia (Goodfellow, 1998; Goodfellow et al., 1999). The organism is an aerobic, Gram-positive, slightly acid–alcohol-fast actinomycete which forms an extensively branched substrate mycelium that fragments into non-motile, rod-shaped elements on modified Sauton’s agar. Whole-organism hydrolysates of the organism were rich in meso-diaminopimelic acid, arabinose and galactose (wall chromotype IV sensu Lechevalier & Lechevalier, 1970) and diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl inositol mannosides (phospholipid type II sensu Lechevalier et al., 1977). The isolate also contained predominately hexahydrogenated menaquinones with eight isoprene units, the end two being cyclized; this menaquinone is restricted to members of the genera Nocardia and Skermania (Chun et al., 1997; Goodfellow et al., 1999).
One-dimensional TLC of whole-organism acid methanolysates revealed the presence of two lipid spots, the lower one corresponded to mycolic acids, as identified by its relative front value (0-47), and the higher one to non-hydroxylated fatty acids. The fatty acid profile contained mainly straight chain saturated, unsaturated and 10-methyl-branched fatty acids. The predominant components, as a proportion of the total fatty acid composition, were: C15:0, 12.2%; C16:0, 21.8%; C17:0, 5.9%; C18:0, 31.7%; cis9-C16:1, 10.7%; cis9-C17:1, 1.3%; cis9-C18:1, 16.1%; cis11,14-C20:2, 2.4%; and 10-methyl-C18:0, 7.8%. In addition, the DNA of strain F829T was rich in guanine and cytosine (G+C content 69.4 mol%).

The position of strain F829T in the unrooted 16S rDNA tree based on three tree-making algorithms is shown in Fig. 1. Strain F829T is most closely related to the type strain of N. asteroides. The two strains share 97.6% 16S rDNA sequence similarity, which corresponds to 34 nt differences over 1400 positions. However, it is evident from the 16S rDNA tree that the isolate and the type strain of N. asteroides are in different subclades; these organisms also show a relatively low level of DNA–DNA relatedness, 32%, a value well below the 70% cut-off point recommended for assignment of bacterial strains to the same genomic species (Wayne et al., 1987). Strain F829T can be distinguished from the type strains of all validly described species of Nocardia, including N. asteroides ATCC 19247T, using a combination of phenotypic properties (Table 1).

The genotypic and phenotypic data show that strain F829T merits recognition as a novel species in the genus Nocardia. It is, therefore, proposed that the organism be classified in this taxon as Nocardia caishijiensis sp. nov.

**Description of Nocardia caishijiensis sp. nov.**

Nocardia caishijiensis (cai.shi.ji.en’sis. N.L. adj. caishijiensis referring to Caishiji, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, slightly acid–alcohol-fast, non-motile actinomycete that forms an extensively branched substrate mycelium that fragments *in situ* into rod-shaped elements. An orange to brown substrate mycelium carries sparse to abundant, white to pinkish aerial hyphae on modified Sauton's agar. A brown substrate mycelium bears white to greyish aerial hyphae on Bennett's agar. Colony elevation is convex to irregular and colony margins are filamentous. Diffusible pigments are not formed. The organism is catalase-positive and reduces nitrate, but is oxidase-negative. Aesculin and urea are hydrolysed, but not arbutin. Tween 20 and 80 are degraded, but not adonine, casein, elastin, guanine, hypoxanthine, starch, Tween 60, tyrosine or xanthine. Acid is formed from adenine, casein, elastin, guanine, hypoxanthine, starch, Tween 60, tyrosine or xanthine. Acid is formed from D-fructose, D-galactose, D-glucose, glycerol, D-mannose, D-ribose, D-trehalose and D-xylene, but not from arbutin, D-cellulobiose, myo-inositol, inulin, D-maltose, D-melezitose, D-melibiose, D-raffinose, z-L-rhamnose, starch, D-sucrose or D-turanose. Arbutin, D-cellulobiose, D-fructose, D-galactose, D-glucose, glycerol, inulin, D-maltose, D-mannose, D-melezitose, D-melibiose, methyl z-D-glucoside, D-raffinose, z-L-rhamnose, D-ribose, starch (weak), D-sucrose, D-trehalose, D-turanose, D-xylene, acetate, fumarate (weak), lactic acid, propionate, pyruvate and succinate (weak) are utilized as sole carbon and energy sources, but not adonitol, L-arabinose, arbutin, dulcitol, meso-erythritol, ethanol, D-fucose, glycerogen, myo-inositol, lactose, D-mannitol, paraffin, salicin, D-sorbitol, xylitol, adipic acid, benzoate, citrate, formate, hippurate, malate, malonate, sebacic acid, oxalate or tartrate. L-Alanine, L-aspartate, L-glutamate and D-serine are used as sole carbon and nitrogen sources, but not acetamide, L-asparagine, gelatin, L-leucine, phenylalanine or L-valine. Grows between 17 and 37 °C, from pH 5 to 10 and in the presence of sodium chloride at 5%, but not at 6 or 7% (w/v). Resistant to lysozyme, gentamicin sulfate, penicillin G and streptomycin sulfate, but sensitive to
Table 1. Phenotypic characteristics that distinguish strain F829<sup>T</sup> from the type strains of *Nocardia* species

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chloramphenicol, erythromycin, midecamycin, minocycline hydrochloride, rifampicin, tobramycin sulfate and vancomycin. The major cellular fatty acids are C16:0 (21.8%), C16:1 (37.7%), cis9-C16:1 (10.7%), cis9-C18:1 (16.1%) and 10-methyl-C18:0 (7.8%). The G+C content of the DNA is 69.4 mol%.

The type strain, F829T (=AS 4.1728T = JCM 11508T), was isolated from a soil sample collected in Caishiji, Anhui Province, China.

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References


