Nocardia pigrifrangens sp. nov., a novel actinomycete isolated from a contaminated agar plate

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A polyphasic study was undertaken to establish the taxonomic position of an actinomycete strain isolated from a contaminated agar plate. The strain, designated 7031T, had morphological and chemotaxonomic properties typical of the genus Nocardia. An almost-complete 16S rRNA gene sequence determined for the strain was aligned with available sequences for nocardiae, and phylogenetic trees were inferred using three tree-generating algorithms. Strain 7031T clustered with the type strains of Nocardia carnea and Nocardia flavorosea, showing low 16S rRNA gene sequence similarities to these species (97·2 and 97·5 %, respectively). The strain was also distinguished from the closest species by a range of phenotypic properties. It is proposed that the strain be recognized as a novel species of Nocardia, Nocardia pigrifrangens sp. nov., the type strain of which is 7031T (=AS 4.1808T = JCM 11884T).

The genus Nocardia is well defined as a result of the application of complementary genotypic and phenotypic methods (Goodfellow et al., 1999; Yassin et al., 2003). The improved classification provides an invaluable framework for the delineation of additional species. The genus encompasses 36 recognized species at the time of writing, 20 of which have been described in the last 5 years. Members of most of the latter have been isolated either from clinical material (Yassin et al., 2000a, b, 2001a, b, 2003; Gürtler et al., 2001; Hamid et al., 2001; Kageyama et al., 2004a, b) or from environmental samples, notably soil (Maldonado et al., 2000; Kinoshita et al., 2001; Wang et al., 2001; Kämpfer et al., 2004; Saintpierre-Bonaccio et al., 2004; Albuquerque de Barros et al., 2003). Despite this rapid expansion in the number of described species, it is evident that additional nocardial diversity has still to be formally described (Orchard & Goodfellow, 1980; Wang et al., 1999; Maldonado et al., 2000; Roth et al., 2003).

In the course of ongoing studies on unusual actinomycetes, a strain isolated from a contaminated Bennett’s agar plate (Zhang et al., 2003) was considered to belong to the genus Nocardia based on morphological criteria. The aim of the present study was to determine the taxonomic position of this isolate using a polyphasic taxonomic approach.

The colonial properties of strain 7031T were recorded from a modified Bennett’s agar plate (Zhang et al., 2003) that had been incubated for 7 days at 28 °C. It was also examined for a broad range of phenotypic properties using standard procedures (Goodfellow, 1971). The biochemical and degradative tests were carried out after Gordon & Mihm (1957) and the carbon utilization tests according to Yassin et al. (1995). Biomass for most of the chemotaxonomic tests was prepared in Sauton’s broth as described by Zhang et al. (2003). Established TLC procedures were used to determine the diagnostic isomers of diaminopimelic acid (Lechevalier & Lechevalier, 1980) and menaquinone (Collins et al., 1987), polar lipid (Yassin et al., 1993) and whole-organism sugar patterns (Lechevalier & Lechevalier, 1980). Mycolic acids were detected using the acid methanolysis procedure (Minnikin et al., 1975). Biomass for the quantitative fatty acid analysis was prepared from trypticase soy broth agar plates after Zhang et al. (2003). The non-hydroxylated fatty acids were extracted, purified, methylated, identified and quantified by GC using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kropfenstedt, 1996).

Chromosomal DNA from strain 7031T was isolated and purified following the procedure described by Yassin et al. (2000a). 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. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR products were carried out following the procedures of Rainey et al. (1996). Purified PCR products were sequenced directly using a Taq Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems) and universal primers as described by 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.

An almost-complete 16S rRNA gene sequence of strain 7031T (1419 nt) was aligned manually with corresponding nucleotide sequences of representatives of recognized Nocardia species, available from public databases, using the PHYDIT program (Chun, 1995). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms. The robustness of the resultant trees was evaluated by a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset using the SEQBOOT and CONSENSE options from the PHYLIP package.

Strain 7031T exhibited a range of phenotypic properties typical of members of the genus Nocardia (Goodfellow et al., 1999). The organism is an aerobic, Gram-positive, slightly acid–alcohol-fast actinomycete, which forms an extensively branched orange substrate mycelium that fragments into rod-shaped, non-motile elements and supports abundant white aerial hyphae on modified Bennett's agar. Whole-organism hydrolysates of the organism are rich in meso-diaminopimelic acid, arabinoze and galactose (wall chemotype IV sensu Lechevalier & Lechevalier, 1970) and diphosphatidyglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol (phospholipid lipid type II sensu Lechevalier et al., 1977). The isolate contains hexahydrogenated menaquinones with eight isoprene units, in which the end two are cyclized; this menaquinone is restricted to members of the genera Nocardia and Skermania (Chun et al., 1997; Goodfellow et al., 1999). The fatty acid profile mainly consists of straight-chain saturated, unsaturated and 10-methyl branched components. The G+C content of the DNA is 68-7 mol%.

The unrooted phylogenetic tree (Fig. 1) shows that strain 7031T and the type strains of Nocardia carnea and Nocardia flavosea form a clade that is loosely associated with a taxon that encompasses the type strains of Nocardia brevicatenae and Nocardia paucivorans. The taxonomic integrity of the two cladcs is supported by analyses based on all three tree-making algorithms and by a high bootstrap value with the neighbour-joining method. It is known that the type strains of N. carnea and N. flavosea share a high 16S rRNA gene sequence similarity (99.2% or 12 nt differences out of 1472 positions) but belong to genomic species with a mean DNA–DNA relatedness value of 5% (Chun et al., 1998). It is, therefore, evident that the low 16S rRNA gene sequence values found between strain 7031T and the type strains of N. carnea and N. flavosea, namely 97.2% and 97.5%, respectively, indicate that the test strain forms a distinct centre of taxonomic variation in the N. carnea clade. It is clear from Table 1 that strain 7031T can be distinguished readily from the type strains of N. carnea and N. flavosea using a combination of phenotypic properties. These organisms can also be distinguished by a number of 16S rRNA signatures (results are available as supplementary data in IJSEM Online).

![Fig. 1. Unrooted neighbour-joining tree (Saitou & Nei, 1987), based on almost-complete 16S rRNA gene sequences, showing the position of strain 7031T within the genus Nocardia. Asterisks indicate the branches of the tree that were also recovered using both the least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at the nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given.](image-url)
The combined genotypic and phenotypic data indicate that strain 7031T merits recognition as a novel species in the genus Nocardia; the name proposed for this new taxon is Nocardia pigrifrangens sp. nov.

**Description of Nocardia pigrifrangens sp. nov.**

Nocardia pigrifrangens (L. adj. piger slow; L. part. adj. frangens from L. v. frango to break up small; N.L. part. adj. pigrifrangens slow to break up; frango referring to the fact that the substrate mycelium remains stable for up to 14 days before undergoing fragmentation).

Aerobic, Gram-positive, catalase-positive, slightly acid-alcohol-fast, non-motile actinomycete that produces an orange substrate mycelium that fragments in situ into irregular rod-shaped elements and which bears sparse white to pinkish aerial hyphae on modified Bennett’s, modified Sauton’s and oatmeal agars. Colonies are convex with to pinkish aerial hyphae on modified Bennett’s, modified Sauton’s and oatmeal agars. Colonies are convex with to pinkish aerial hyphae on modified Bennett’s, modified Sauton’s and oatmeal agars.

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<th>Character</th>
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<td>+</td>
<td>−</td>
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<tr>
<td>Arbutin hydrolysis</td>
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<td>Nitrate reduction</td>
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<td>(−)-D-Myo-Inositol (1-0)</td>
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<td>Growth at 45 °C</td>
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L-Alanine, L-aspartate, L-glutamate, monoethanolamine, L-proline, uric acid and L-valine are used as sole carbon and nitrogen sources, but not acetamide, gelatin, L-leucine, L-phenylalanine or urea (all at 0-1%, w/v). Additional phenotypic properties are shown in Table 1. The major cellular fatty acids are C16:0 (8.9%), C16:1ω7c (18.0%), C18:1ω9c (17.6%), C18:0 (30.5%), 10-methyl-C18:0 (21%) and C21:1ω9c (4.1%). The G+C content of the DNA is 68.7 mol%.

The type strain, 7031T (= AS 4.1808T = JCM 11884T), was isolated from a contaminated agar plate of a clinically significant strain in Shanghai, People’s Republic of China.

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**References**


