Nocardia kroppenstedtii sp. nov., an actinomycete isolated from a lung transplant patient with a pulmonary infection

Amanda L. Jones,1,2,3 Andrew. J. Fisher,4 Rahul Mahida,4 Kate Gould,2 John D. Perry,2 Margaret M. Hannan,5 Eoin P. Judge,5 Ros Brown,1 Kimberley Boagey3 and Michael Goodfellow1

A novel actinomycete, strain N1286T, isolated from a lung transplant patient with a pulmonary infection, was provisionally assigned to the genus Nocardia. The strain had chemotaxonomic and morphological properties typical of members of the genus Nocardia and formed a distinct phylectic line in the Nocardia 16S rRNA gene tree. Isolate N1286T was most closely related to Nocardia farcinica DSM 43665T (99.8 % gene sequence similarity) but could be distinguished from the latter by the low level of DNA–DNA relatedness. These strains were also distinguishable on the basis of a broad range of phenotypic properties. It is concluded that strain N1286T represents a novel species of the genus Nocardia for which the name Nocardia kroppenstedtii sp. nov. is proposed. The type strain is N1286T (=DSM 45810T=NCTC 13617T).

Improvements in the classification of the genus Nocardia due to the application of polyphasic taxonomy provide a sound framework for the recognition of additional species (Goodfellow & Maldonado, 2012). At the time of writing, the genus encompasses 85 species with validly published names (http://www.bacterio.net/n/nocardia.html), including the recently described Nocardia grenadensis (Kämpfer et al., 2012), Nocardia rhamnosiphila (Everest et al., 2011), Nocardia goodfellowii and Nocardia thracensis (Sazak et al., 2012). Nocardiae form a clade within the evolutionary radiation occupied by mycolic acid-containing actinomycetes; microorganisms belonging to genera assigned to the order ‘Corynebacteriales’ (Goodfellow & Jones, 2012). Most recently described species of the genus Nocardia are associated with human infections (Brown-Elliott et al., 2006; Goodfellow & Maldonado, 2012), as exemplified by Nocardia mikamii (Jannat-Khah et al., 2010) and Nocardia niwae (Moser et al., 2011). Here we describe the results of phenotypic and phylogenetic analyses of another strain isolated from clinical material and suggest that it represents a novel species of the genus Nocardia.

Strain N1286T was isolated from bronchial lavage cultured on chocolate agar incubated at 37 °C in 5 % CO2 for 2 days. Organisms were maintained on glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) at room temperature and as 20 % (v/v) glycerol suspensions at 2 °C, as were Nocardia asteroides DSM 43757T and Nocardia farcinica DSM 43665T. Biomass of all strains analysed for chemotaxonomic and molecular systematic studies, was grown in shake flasks of GYE broth for 5 days at 28 °C, checked for purity and harvested by centrifugation. Cells for chemosystematic analyses were washed twice in distilled water and freeze-dried; those for molecular systematic studies were washed in NaCl/EDTA buffer (0.1 M EDTA, 0.1 M NaCl, pH 8.0) and stored at −20 °C until required.

The phylogenetic position of isolate N1286T was determined by 16S rRNA gene sequence analysis. Chromosomal DNA was isolated, PCR fragments amplified and direct sequencing of the purified products carried out (Kim et al., 1998). The almost-complete 16S rRNA gene sequence (1544 nt) was aligned manually against corresponding sequences of genera of the order ‘Corynebacteriales’, retrieved from DDBJ/EMBL/GenBank databases, using

Abbreviation: A2pm, diaminopimelic acid.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N1286T is DQ157924.

Two supplementary figures are available with the online version of this paper.
the pairwise alignment option and 16S rRNA gene secondary structural information held in the MEGA5 program (Tamura et al., 2011). Phylogenetic trees were inferred using the maximum-parsimony (Kluge & Farris, 1969), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms using MEGA5 software. The Jukes & Cantor (1969) model was used to generate an evolutionary distance matrix for the neighbour-joining algorithm. Topologies of the resultant unrooted trees were evaluated by bootstrap analysis of the neighbour-joining method (Felsenstein, 1985) based upon 1000 replicates using MEGA5 software.

It can be seen from Figs. 1 and S1 (available in IJSEM Online) that strain N1286T formed a distinct subclade in the Nocardia 16S rRNA gene tree together with N. farcinica DSM 43665T, an association supported by all of the tree-making algorithms and by a 99 % bootstrap value in the neighbour-joining analysis. The strains shared a 16S rRNA gene sequence similarity of 99.8 %, a value that corresponded to 3 nt differences at 1544 locations. The two strains were associated with Nocardia higoensis DSM 44732T and Nocardia shimofusensis DSM 44733T, as shown in Fig. 1; strain N1286T shared 16S rRNA gene sequence similarities of 98.9 % with N. higoensis DSM 44732T and N. shimofusensis DSM 44733T, a value equivalent to 17 nt differences.

Strain N1286T was examined for key chemotaxonomic markers considered to be characteristic of strains of species of the genus Nocardia using N. asteroides DSM 43757T as a reference strain. Standard procedures were used to determine the diagnostic isomers of diaminopimelic acid (A2pm; Staneck & Roberts, 1974), cellular fatty acids (Sutcliffe, 2000), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida et al., 1999), polar lipids (Minnikin et al., 1975), and whole-organism sugars (Hasegawa et al., 1983). The novel isolate contained arabinose, galactose and meso-A2pm in whole-organism hydrolysates (wall chemotype IV sensu; Lechevalier & Lechevalier, 1970); N-glycyl muramic acid; hexahydrogenated menaquinone with eight isoprene units, where the two end units were cyclized [MK-8(H4), ω cyclo], as the sole isoprenologue; major proportions of straight-chain saturated, unsaturated and tuberculostearic acids (fatty acid type 1b sensu; Kroppenstedt, 1985); diphosphatidylglycerol, phosphatidyldithanolamine, phosphatidylglycerol and phosphatidylinositollamnosides as major polar lipids (Fig. S2); and mycolic acids that co-migrated with those from the type strain of N. asteroides. This chemotaxonomic profile is consistent with the classification of isolate N1286T in the genus Nocardia (Goodfellow & Maldonado, 2012).

DNA–DNA relatedness values (ΔTm) were determined, in triplicate, between isolate N1286T and N. farcinica DSM 43665T using the fluorimetric method described by Gonzalez & Saiz-Jimenez (2005); the optimum temperatures for reassociation (Tor) were calculated using the equation Tor = 0.51 (%GC) + 47. The melting temperatures (Tm) at which 50 % of the initial double-stranded DNA molecules denatured into single-stranded DNA for isolate N1286T g DNA and isolate N1286T/N. farcinica DSM 43665T hybrid DNA preparations were compared and the differences (ΔTm) calculated. The %GC was 80.2 %, the mean ΔTm between isolate N1286T g DNA and isolate N1286T/N. farcinica DSM 43665T hybrid DNA was 9.6 ± 1.2 °C, which represents a DNA–DNA relatedness value of 44 ± 4 % (Gonzalez & Saiz-Jimenez, 2005).

A range of phenotypic properties of isolate N1286T and N. farcinica DSM 43665T were examined using well-established media known to be of value in nocardial systematics (Andrews, 2001; Goodfellow, 1971; Goodfellow & Maldonado, 2012; Isik et al., 1999). A number of differential characteristics separated the two strains; isolate N1286T, unlike N. farcinica DSM 43665T, grew at 37 °C, did not produce aerial mycelium, degraded starch, hydrolysed aesculin and arbutin; grew on meso-inositol and methyl α-D-glucopyranoside as the sole carbon source (1 %, w/v) and was not inhibited by bacitracin (10 units). N. farcinica DSM 43665T, unlike the isolate, degraded DNA and RNA, reduced nitrate, grew on dulcitol and i-erythritol (both 1 %, w/v), on sodium benzoate, oxalic acid and pimelic acid (all 0.1 %, w/v) as sole carbon sources, and in the presence of fusidic acid (10 µg).

It can be concluded that isolate N1286T forms a distinct phyletic line in the Nocardia 16S rRNA gene tree and can

---

**Fig. 1.** A section of the neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain N1286T relative to its nearest neighbours. Asterisks indicate branches of the tree that were also found with the maximum-likelihood and maximum-parsimony tree-making algorithms. Numbers at nodes indicate the levels of bootstrap support based on neighbour-joining analysis of 1000 resampled datasets; only values >50 % are shown. Bar, 0.005 substitutions per nucleotide position.
be readily distinguished from *N. farcinica* DSM 43665T, its nearest phylogenetic neighbour, by a combination of phenotypic features. Consequently, it is suggested that isolate N1286T represents a novel species of the genus *Nocardi*a, for which the name *Nocardi*a *kroppenstedtii* sp. nov. is proposed.

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

*Nocardi*a *kroppenstedtii* (krop.pen.sted’t.i. N.L. masc. gen. n. kroppenstedtii of Kroppenstedt, to honour Reiner Kroppenstedt, a German microbiologist, for his many contributions to actinobacterial systematics).

Aerobic, Gram-stain-positive, non-motile, non-spore-forming, partially acid–alcohol-fast, catalase-positive, actinomycete, which forms a mycelium that fragments into rods and cocci. Irregular, wrinkled, matt, pale orange–yellow-pigmented colonies are formed on modified Bennett’s agar after 5 days growth at 30°C. Growth occurs at pH 6.0–10.0, between 25°C and 37°C and optimally at 28°C. Uric acid is not degraded. D-Arabinol, arbutin, D-fucose, glycerol and D-ribose (all 1% w/v), 1% (v/v) n-propanol and 0.1% γ-hydroxybutyric acid, sodium fumarate, sodium-DL-malate and sodium suberate (all w/v) are used as sole carbon sources. Growth occurs in the presence of filter paper discs soaked in bacitracin (10 U), cephalaxin (30 μg), clindamycin hydrochloride (2 μg), colis-tin (25 μg), cotrimoxazole (25 μg), erythromycin (5 μg), nalidixic acid (30 μg), novobiocin (5 μg), penicillin (1 μg) and tetracycline hydrochloride (10 μg), but not in the presence of discs soaked in ciprofloxacin (1 μg) and fusidic acid (10 μg). Additional phenotypic properties are cited in the text. The major cellular fatty acid components are hexadecanoic (C\textsubscript{16:0}), monosaturated hexadecanoic (C\textsubscript{16:1}), octadecanoic (C\textsubscript{18:0}), monosaturated octadecanoic (C\textsubscript{18:1}), tuberculostearic acid (TSA\textsubscript{18}) and eicosanoic (C\textsubscript{20:0}), also typical of *Nocardi*a. The type strain, N1286\textsuperscript{T} (=DSM 45810\textsuperscript{T}=NCTC 13617\textsuperscript{T}), was isolated from a lung transplant patient with a pulmonary infection. The species description is based on a single strain and hence serves as a description of the type strain.

**Acknowledgements**

The authors are indebted to Professor Iain Sutcliffe and Karen Walker (University of Northumbria, UK) for help with fatty acid analysis and amplification of the 16S rRNA gene of strain N1286\textsuperscript{T}, respectively.

**References**


