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

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The GenBank accession number for the 16S rRNA gene sequence of strain N1286ᵀ is DQ157924.
Abstract

An actinomycete, strain N1286\textsuperscript{T}, isolated from a lung transplant patient with a pulmonary infection, was provisionally assigned to the genus \textit{Nocardia}. The strain had chemotaxonomic and morphological properties typical of members of the genus \textit{Nocardia} and formed a distinct phyletic line in the \textit{Nocardia} 16S rRNA gene tree. It was most closely related to \textit{Nocardia farcinica} DSM 43665\textsuperscript{T} (99.8\% gene similarity) but was distinguished from the latter by a low level of DNA:DNA relatedness. These strains were also distinguished by a broad range of phenotypic properties. On the basis of these data, it is proposed that isolate N1286\textsuperscript{T} (=DSM 45810\textsuperscript{T} = NCTC 13617\textsuperscript{T}) should be classified as the type strain of a new \textit{Nocardia} species for which the name \textit{Nocardia kroppenstedii} is proposed.

Improvements in the classification of the genus \textit{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 validly published species (http://www.bacterio.net/n/nocardia.html), including the recently described \textit{Nocardia grenadensis} (Kämpfer \textit{et al.}, 2012), \textit{Nocardia rhamnosiphila} (Everest \textit{et al.}, 2011), \textit{Nocardia goodfellowii} and \textit{Nocardia thraciensis} (Sazak \textit{et al.}, 2012). \textit{Nocardiae} form a clade within the evolutionary radiation occupied by mycolic acid-containing actinomycetes, that is, microorganisms belonging to genera assigned to the order \textit{Corynebacteriales} (Goodfellow & Jones, 2012). Most recently described \textit{Nocardia} species are associated with human infections (Brown-Elliott \textit{et al.}, 2006; Goodfellow & Maldonado, 2012), as exemplified by \textit{Nocardia mikamii} (Jannat-Khah \textit{et al.}, 2010) and \textit{Nocardia niwae} (Moser \textit{et al.}, 2011). Here we describe the results of phenotypic and phylogenetic
analyses of another strain isolated from clinical material and show that it represents a new *Nocardia* species.

Strain N1286<sup>T</sup> was isolated from bronchial lavage cultured on chocolate agar incubated at 37°C in 5% CO<sub>2</sub> for 2 days. The organism was maintained on glucose-

yeast extract agar (GYEA; Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at -20°C, as were *Nocardia asteroides* DSM 43757<sup>T</sup> and *Nocardia farcinica* DSM 43665<sup>T</sup>. Biomass of all strains analysed, for the 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 the chemosystematic analyses were washed twice in distilled water and freeze-dried; those for the molecular systematic work were washed in NaCl/EDTA buffer (0.1M EDTA, 0.1M NaCl, pH 8.0) and stored at -20°C until required.

The phylogenetic position of isolate N1286<sup>T</sup> was determined by 16S rRNA gene sequence analysis. Chromosomal DNA was isolated, PCR fragments amplified and direct sequencing of the purified products carried out after Kim *et al.*, (1998). The almost complete 16S rRNA gene sequence (1544 nucleotides [nt]) was aligned manually against corresponding sequences of genera classified in the order *Corynebacteriales*, retrieved from the DDBJ/EMBL/GenBank databases, using the pairwise alignment option and 16S rRNA 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 from the MEGA5 software. The Jukes and 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 MEGA 5 software.

It can be seen from Figures 1 and S2, that strain N1286\(^T\) formed a distinct subclade in 
the 16S rRNA \textit{Nocardia} gene tree together with the type strain of \textit{N. farcinica}, 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 
similarity of 99.8%, a value that corresponded to 3 nt differences at 1544 locations. 
The two strains were associated with the type strains of \textit{Nocardia higoensis} and 
\textit{Nocardia shimofuensis}, as shown in Figure 1; strain N1286\(^T\) shared 16S rRNA 
similarities of 98.9% with the \textit{N. higoensis} and \textit{N. shimofuensis} strains, a value 
equivalent to 17 nt differences.

Strain N1268\(^T\) was examined for key chemotaxonomic markers considered to be 
characteristic of \textit{Nocardia} strains using \textit{N. asteroides} DSM 43757\(^T\) as control.

Standard procedures were used to determine the diagnostic isomers of diaminopimelic 
acid (A\(_2\)pm; Staneck & Roberts, 1974), cellular fatty acids (Sutcliffe, 2000), 
isoprenoid quinones (Collins, 1994), muramic acid type (Uchida \textit{et al.}, 1999), mycolic 
acids (Minnikin \textit{et al.}, 1975), polar lipids (Minnikin \textit{et al.}, 1984) and whole-organism 
sugars (Hasegawa \textit{et al.}, 1983). The organism contained meso-A\(_2\)pm, arabinose and 
galactose, in whole-organism hydrolysates (wall chemotype IV sensu, Lechevalier & 
Lechevalier, 1970); N-glycolyl muramic acid; hexahydrogenated menaquinone with 
eight isoprene units where the two end units were cyclized (MK- 8 [H\(_4\)], \(\omega\) cyclo) as 
the sole isoprenologue; major proportions of straight chain saturated, unsaturated and
tuberculostearic acids (fatty acid type 1b sensu, Kroppenstedt, 1985),
diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol
phosphatidylinositolmannosides as major polar lipids (Fig S1.); 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 N1268<sup>T</sup> in the
genus *Nocardia* (Goodfellow & Maldonado, 2012).

DNA:DNA relatedness values (Δ<sub>T</sub><sub>m</sub>) were determined, in triplicate, between isolate
N1286<sup>T</sup> and *N. farcinica* DSM 43665<sup>T</sup> using the fluorimetric method described by
Gonzalez and Sait-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 N1286<sup>T</sup> g DNA and isolate N1286<sup>T</sup> / *N. farcinica*
hybrid DNA preparations were compared and the differences (Δ<sub>T</sub><sub>m</sub>) calculated. The
%GC was 80.2%, the mean Δ<sub>T</sub><sub>m</sub> between isolate N1286<sup>T</sup> g DNA and isolate N1286<sup>T</sup> / *N. farcinica*
hybrid DNA was 9.6 ± 1.2°C, a value which represents a DNA:DNA
relatedness value of 44 ± 4% (Gonzalez & Saiz-Jimenez, 2005).

Isolate N1286<sup>T</sup> and the type strain of *N. farcinica*, were examined for a range of
phenotypic properties 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 N1286<sup>T</sup>, unlike the *N. farcinica* strain, 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 a sole carbon source (1% w/v) and was not
inhibited by bacitracin (10 units). Similarly, *N. farcinica* DSM 43665\(^T\), unlike the isolate, degraded DNA, and RNA; reduced nitrate, and grew on dulcitol and *i*-erythritol (1% w/v) and on sodium benzoate, oxalic acid and pimelic acid (0.1% w/v) as sole carbon sources and in the presence of fusidic acid (10 µg).

It can be concluded that isolate N1286\(^T\) forms a distinct phyletic line in the *Nocardia* 16S rRNA gene tree and can be distinguished readily from *N. farcinica* DSM 43665\(^T\), its nearest phylogenetic neighbour, using a combination of phenotypic features. Consequently, it is proposed that isolate N1286\(^T\) should be recognised as a new species, *Nocardia kroppenstedtii*.

**Description of Nocardia kroppenstedtii sp. nov.**

*Nocardia kroppenstedtii* (krop. pen. sted'ti.i. N.L. n. kroppenstedtii, of Kroppenstedt to honour Reiner Kroppenstedt, a German microbiologist, for his many contributions to actinobacterial systematics).

Aerobic, Gram-positive, nonmotile, nonsporeforming, 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, from 25°C to 37°C and optimally ~ 28°C. Uric acid is not degraded. D-arabitol, arbutin, D-fucose, glycerol and D-ribose (1%, w/v), *n*-propanol (1%, v/v) and *γ*-hydroxybutyric acid, sodium fumarate, sodium-DL-malate and sodium suberate (0.1%, w/v) are used as sole carbon sources. Growth occurs in the presence of filter paper discs soaked in bacitracin (10 units), cephalixin (30 µg), clindamycin
hydrochloride (2 μg), colistin (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 (C16:0; 30.8 %),
monosaturated hexadecanoic (C16:1; 18.6 %), octadecanoic (C18:0; 7.2 %),
monosaturated octadecanoic (C18:1; 6.4 %), tuberculostearic acid (TSA18; 30.2 %)
and eicosanoic (C 20:0; 5.2 %). Additional chemotaxonomic properties are also
typical of nocardiae.

The type strain, N1286T (=DSM 45810 = NCTC 13617T), 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

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Northumbria) for help with the fatty acid analysis and amplification of the 16S rRNA
gene of strain N1286T, respectively.

References

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Chichester: John Wiley & Sons.


Fig. 1. A section of the neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain N1286<sup>T</sup> 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; L and M indicate branches found using the maximum-likelihood and maximum-parsimony methods, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 re-sampled datasets; only values above 50% are given. The scale bar indicates 0.005 substitutions per nucleotide position. <sup>T</sup>, type strain.
Supplementary figures

Fig. S1: Polar lipid composition of strain N1286\textsuperscript{T}. The polar lipids were identified as follows: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannosides.

Fig. S2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain N1286\textsuperscript{T}. Asterisks indicate branches of the tree that were also found with the maximum-likelihood and maximum-parsimony tree-making algorithms; L and M indicate branches found using the maximum-likelihood and maximum-parsimony methods, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50\% are given. The scale bar indicates 10 substitutions per nucleotide position. \textsuperscript{T}, type strain.
Nocardia braakenschiolae DSM 45157T (HO157184)

Nocardia actinopharyngis DSM 44099T (AY426382)

Nocardia pseudobrasiliensis DSM 44298T (AF430002)

Nocardia cruziana DSM 44698T (AF430049)

Nocardia otitidisaviarum DSM 43242T (AF430067)

Nocardia uniformis DSM 43431T (AF430844)

Nocardia concava DSM 44884T (AB126880)

Nocardia seriolae DSM 44129T (AF430039)

Nocardia nitens DSM 44671T (DQ659910)

Nocardia inohakensis DSM 44687T (AB092360)

Nocardia yamashitensis DSM 44658T (AB092561)

Nocardia anaurae DSM 44821T (D89723)

Nocardia pseudovinacea DSM 44306T (AF430046)

Nocardia vinacea DSM 44368T (AB024312)

Nocardia catisii DSM 44315T (Z46751)

Nocardia goodfellowi DSM 45516T (HO157183)

Nocardia jejunis DSM 13281T (AY964666)

Nocardia alba DSM 44684T (AY222321)

Nocardia nivea DSM 44978T (DQ235687)

Nocardia corynebacterium DSM 44717T (AY282603)

Nocardia breviscuta DSM 43024T (AF430040)

Nocardia panzai DSM 43863T (DQ235857)

Nocardia gyipseigera DSM 44484T (AF430027)

Nocardia jinnanensis DSM 44933T (AY770043)

Nocardia polyresistens DSM 13509T (AY626338)

Nocardia farcinica DSM 43665T (Z36956)

Isolate N1286T (DQ157924)

Nocardia bigeminata DSM 44732T (AB108778)

Nocardia shimojimensis DSM 44733T (AB108775)

Nocardia asiatica DSM 44688T (AB092566)

Nocardia puris DSM 44599T (AJ305074)

Nocardia abscessus DSM 44423T (AF128192)

Nocardia shahlandica DSM 44880T (AB126874)

Nocardia asteroides DSM 43757T (AF430019)

Nocardia necrotriangulenta DSM 44717T (AY282603)

Nocardia breviscuta DSM 43024T (AF430040)

Nocardia panzai DSM 43863T (DQ235857)

Nocardia gyipseigera DSM 44484T (AF430027)

Nocardia jinnanensis DSM 44933T (AY770043)

Nocardia pigrafrangens DSM 11884T (AF219974)

Nocardia flavoreora DSM 44480T (Z46754)

Nocardia rhamnosiphila DSM 45147T (EF148604)

Nocardia stenara DSM 44786T (AB121770)

Nocardia testace DSM 44765T (AF192415)

Nocardia transvalensis DSM 43405T (AF430047)

Nocardia wallae DSM 45136T (EU099357)

Nocardia blacklockiae DSM 45135T (EU099360)

Nocardia harennae DSM 45485T (AB282122)

Nocardia takedeni DSM 44801T (AB158277)

Nocardia albanensis DSM 44997T (AJ306090)

Nocardia brasiliensis DSM 43758T (AF430038)

Nocardia vernieri DSM 44971T (DQ295480)

Nocardia ammoniensis DSM 45066T (AF219974)

Nocardia pseumoniaca DSM 44370T (AB108780)

Nocardia nivea DSM 45340T (F765056)

Nocardia amkaicantorenalis DSM 45539T (GU855442)

Nocardia arborescens DSM 44731T (AB108781)

Nocardia beijingensis DSM 44636T (AF430438)

Tomitella hifomata DSM 45403T (AF362837)

Sanguinoparum robustum JCM 13587T (AY068918)

Sanginiparasiticus nigrolineatus DSM 44881T (AB243070)

Wittisella muralis DSM 44341T (Y17384)

Millino brevis DSM 44465T (AJ354742)

Gordonia bronchialis DSM 43247T (X792845)

Derminia parfaonis DSM 43988T (Z453453)

Erythrococcus rhodosporous DSM 43243T (X792888)

Myco tuberculosis ATCC 27294T (X52917)

Amycolaciaceae subflavus DSM 45089T (EF654479)

Nasoyella altamorensis DSM 45258T (FJ794845)

Dietzia maris DSM 43672T (X79290)

Trachonella pumiceiflora DSM 20162T (Z46751)

Corynebacterium diptheriae DSM 44123T (X84248)

Tesorilla oitidis DSM 8821T (X73976)