NOTE

Nocardia veterana sp. nov., isolated from human bronchial lavage

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A nocardioform bacterium was isolated from the bronchoscopic lavage of a 78-year-old man with a past history of tuberculous pleurisy, who presented with bilateral upper lobe lesions at Austin and Repatriation Medical Centre, Heidelberg, Australia. The strain was aerobic, Gram-positive, produced beige substrate mycelium and scant white aerial mycelium. It showed chemotaxonomic markers which were consistent with the classification of *Nocardia*: i.e. *meso*-diaminopimelic acid, *N*-glycolylmuramic acid, arabinose and galactose as diagnostic sugars; phospholipids phosphatidylinositol mannosides, phosphatidylinositol, phosphatidylethanolamine and diphosphatidylglycerol; a menaquinone with a cyclic isoprene side chain, MK-8(H_{4cycl}); a fatty acid pattern composed of unbranched saturated and monounsaturated fatty acids with a considerable amount of tuberculostearic acid; and mycolic acids composed of 54–62 carbon atoms with three principal mycolic acids which were mono- and polyunsaturated, showing a chain length C_{56}, C_{58} and C_{60} and accounting for over 70% of the entire pattern. The 16S rDNA sequence showed the highest similarity to the type strain of *Nocardia vaccinii*; the DNA–DNA similarity of the two strains was 31%. These data, together with distinct physiological traits and molecular biological analyses, as well as chemotaxonomic results, led to the conclusion that the novel isolate represents a new species within the genus *Nocardia* for which the name *Nocardia veterana* sp. nov. is proposed. The type strain is M157222^T (DSM 44445^T = NRRL B-24136^T).

Keywords: *Nocardia veterana* sp. nov., polyphasic taxonomy

As some nocardiae cause a variety of suppurative diseases in humans and animals, notably actinomycete mycetoma and nocardiosis (Boiron *et al*., 1993; Schaal & Lee, 1992; Tsukamura, 1982), it is important to differentiate the nocardiae to species level. *Nocardia brasiliensis* and *Nocardia transvalensis* are the main causal agents of actinomycete mycetoma (McNeil & Brown, 1994; McNeil *et al*., 1992), and the predominant agents of nocardiosis are *Nocardia asteroides*, *Nocardia farcinica* and *Nocardia nova* (Schaal & Lee, 1992; Wallace *et al*., 1991). *Nocardia seriolae* (Kudo *et al*., 1988) and *Nocardia salmonicida* (Isik *et al*., 1999) are fish pathogens, whereas *Nocardia crassostreae* is the causal agent of nocardiosis in Pacific oysters (Friedman *et al*., 1998). *Nocardia paucivorans* has been isolated from patients with chronic lung diseases (Yassin *et al*., 2000), but here the clinical relevance has not been clarified. The aim of this study was to clarify the taxonomic position of a novel *Nocardia* isolate, M157222^T (= DSM 44445^T), by morphological, physiological, chemotaxonomical and molecular biological methods.

Strain M157222^T was isolated from the bronchoscopic lavage of a 78-year-old man with a past history of tuberculous pleurisy, who presented with bilateral upper lobe lesions. The strain was thought not to be clinically significant and was deposited as *Nocardia veterana* DSM 44445^T at the Deutsche Sammlung von

The EMBL accession number for the 16S rDNA sequence of strain DSM 44445^T is AF278572.

Table 1. Comparison of the physiological properties of *N. veterana* and its phylogenetic neighbour, *N. vaccinii*

Assimilation of auxanographic substrates was detected photometrically by means of reduction of the redox dye MTT. +, A_{540} (test) − A_{540} (control) > 0·129; −, A_{540} (test) − A_{540} (control) < 0·129. No assimilation was detected in *N. veterana* and *N. vaccinii* with glucarate, gluconate, D-galactose, D-sucrose, D-turanose, D-arabitol, caprate, succinate 4-aminobutyrate, 4-aspartate, L-leucine, L-serine, L-valine, acetamide, tyramine, benzoate, pimelate, 3-hydroxybenzoate, 4-hydroxybenzoate, quinate, pNp-fl-D-xyloside and 2-deoxythymidine-5-pNp-phosphate; L-proline was used by both strains.

Compounds assimilated	*N. veterana*	*N. vaccinii*
N-Acetyl-D-glucosamine	−	+
D-Glucosaminic acid	+	−
L-Rhamnose	+	−
D-Ribose	−	+
i-Inositol	+	−
Citrate	−	+
2-Hydroxyvalerate	−	+
2-Oxoglutarate	+	−
L-Alanine	+	−
Putrescine	+	−
Phenylacetic acid	+	−
pNp-phosphorylcholine	+	−

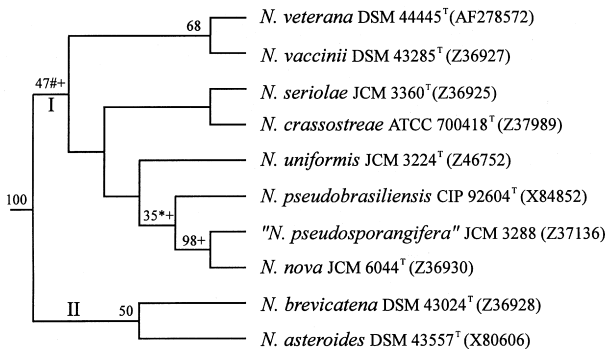

Fig. 1. Consensus phylogenetic tree derived from aligned 16S rRNA sequences, showing the position of *Nocardia veterana* DSM 44445^T among the phylogenetically nearest neighbours. Bootstrap values were available for all six phylogenetic analysis methods listed in Methods except for '*' where bootstrap values were not available because of unresolved branches using PAUP, '+' because of a different branch order using DNAML and '#' because of unresolved branches using PAUP and a different branch order using NEIGHBOR.

Mikroorganismen und Zellkulturen. For the determination of the colour, morphological studies and biochemical tests (Kämpfer *et al.*, 1990, modified by Kirchhof *et al.*, 1992 and Klatte *et al.*, 1994), *N. veterana* was grown on GYM agar medium (DSMZ no. 65) at 28 °C. For biochemical tests, *N. veterana* was harvested from agar after 3 d. Morphological studies and the examination of the colour were carried out after 1, 3 and 7 d. Strain DSM 44445^T showed the typical macroscopic and microscopic picture of a *Nocardia* species (Goodfellow, 1992; Goodfellow & Lechevalier, 1989) with beige substrate mycelium and a scant dirty white aerial mycelium. The reverse side of the mycelium was yellowish. In contrast to many other nocardiae, the highly branched substrate and aerial mycelium was quite stable and did not fragment during ageing. The results of the physiological tests obtained from microtitre plates revealed that M157222^T was unable to utilize most of the 31 carbon sources by means of an MTT reduction test (Table 1). The utilization pattern separates *N. veterana* from the closely related *Nocardia vaccinii* DSM 43286^T.

Determination of the 16S rDNA sequence (Rainey *et al.*, 1996) and phylogenetic analyses (Felsenstein, 1993) followed described methods. The oligonucleotide sequences used for PCR and sequencing are listed in Gürtler *et al.* (1991) except for R514 (CGTGCCAGCAGCCGCGGTAA) and R907R (CCGTCAATTCCTTTGAGTTT). The 16S rDNA sequence from DSM 44445^T was aligned with 16S rDNA sequences from representatives of the actinomycete sublines available from the public databases using CLUSTAL X (Thompson *et al.*, 1997). Pairwise evolutionary distances were computed using DNADIST in the PHYLIP suite of programs (Felsenstein, 1993). Phylogenetic and bootstrap analyses of the 16S rDNA sequence alignment were done using methods for parsimony (Swofford, 1999), distance [FITSCH, KITSCH and NEIGHBOR (neighbour and UPGMA methods); Felsenstein, 1993] and maximum-likelihood (DNAML; Felsenstein, 1993). The bootstrap values (from four, five or six methods) for 100 trees were averaged. Determination of DNA–DNA similarity was performed by the spectrophotometric reassociation method as described by Kleespies *et al.* (1996). The 16S rDNA sequence of strain DSM 44445^T has been deposited in the EMBL database under the accession number AF278572; those of reference organisms are indicated in Fig. 1. The partial 16S rDNA sequence (1328 nt) of strain DSM 44445^T was compared to the same region of the 16S rDNA sequence from 13 species of *Nocardia* and two species of *Rhodococcus*, only some of which, namely the nearest neighbours of strain DSM 44445^T, are shown in Fig. 1. Two clusters (I and II) were found in 100 % (bootstrap value) of the 100 trees analysed for each of the six phylogenetic analysis methods (Fig. 1). Strain DSM 44445^T was found in cluster I and was most closely related to *N. vaccinii* with a similarity value of 98·6 %. Within cluster I the next most closely related strains were *N. nova* (98·1 %) and '*Nocardia pseudosporangifera*' (98·0 %) and the other four cluster I strains had similarity values ranging from 96·5 to 97·2 %. In general there is good agreement with the branching pattern obtained in the present study to that obtained in previous studies (Yassin *et al.*, 2000), e.g. *Nocardia brevicatena*,

Nocardia carnea and *Nocardia flavorosea* are closely related with high bootstrap values. One exception is that in the present study the similarity value between *Nocardia uniformis* and *Nocardia otitidiscaviarum* was found to be low (95%) while in the study by Yassin *et al*. (2000) they were found to be closely related (not shown). The 16S rDNA data indicated that strain DSM 44445^T belongs to the genus *Nocardia* but is different from previously described species. The level of DNA–DNA relatedness between strain DSM 44445^T and the type strain of *N. vaccinii*, DSM 43285^T, was only 31%.

For the analysis of fatty acids (Miller, 1982) and mycolic acid composition *N. veterana* was grown on TSB agar [3%, w/v, Trypticase soy broth (BBL); 1·5%, w/v, Bacto Agar (Difco)] for 4 d at 28 °C. For fatty acid methyl ester analysis, standard Microbial Identification System (MIS) conditions were used (Kämpfer & Kroppenstedt, 1996). For the analyses of cell wall amino acids and sugars (Stanek & Roberts, 1974), acyl-type of murein (Uchida & Aida, 1977), peptidoglycan and quinone analysis (Minnikin *et al*., 1984; Kroppenstedt, 1982, 1985) and polar lipids (Minnikin *et al*., 1977), cells were grown in Trypticase soy broth (BBL) for 4 d at 28 °C on a rotary shaker, harvested by centrifugation and washed twice with distilled water. The chemotaxonomic properties of DSM 44445^T were also consistent with their classification into the genus *Nocardia* (Goodfellow, 1992; Goodfellow & Lechevalier, 1989). Whole-cell hydrolysates of DSM 44445^T contained *meso*-diaminopimelic acid as the only diamino acid of the peptidoglycan, and glucose and arabinose as major cell wall sugars. As expected for *Nocardia* and related taxa, the glycolated cell wall sugars were *N*-glycolylmuramic acid and *N*-glycolylglucosamine. In the lipid extraction the *Nocardia* diagnostic quinone, MK-8(II, III $H_{4cycl.}$), was found, a menaquinone with a tetrahydrogenated isoprenoid chain of eight isoprene units of which the terminal two isoprene moieties are cycled. The polar lipid pattern was composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and some unspecified glycolipids. This pattern matched quite well with those of *Nocardia* spp. reported by Minnikin *et al*. (1977).

The fatty acid pattern was composed of straight-chain saturated and unsaturated fatty acids and a 10-methyl branched fatty acid: i.e. tetradecanoic acid ($C_{14:0}$), 2·0%; pentadecanoic acid ($C_{15:0}$), 1·2%; palmitic acid ($C_{16:0}$), 37·4%; palmitoleic acid (*cis*-9 $C_{16:1}$), 13·8%; hexadecenoic acid (*cis*-7 $C_{16:1}$), 0·4%; heptadecanoic acid ($C_{17:0}$), 1·1%; heptadecenoic acid ($C_{17:1}$), 0·7%; stearic acid ($C_{18:0}$), 1·0%; oleic acid (*cis*-9 $C_{18:1}$) 25·8%; tuberculostearic acid (10-methyl branched $C_{18:0}$), 15·3%; nonadecenoic acid ($C_{19:1}$), 0·8%; and a small amount (0·5%) of an unknown fatty acid. DSM 44445^T synthesized a homologous series of mycolic acids ranging from 54 to 64 carbon atoms with C_{56}, C_{58} and C_{60} being the three principal mycolic acids accounting for more than 70% of the whole mycolic acids.

Based on the phenotypic and genotypic data we can conclude that the strain, which was isolated from a bronchoscope of a patient suffering from a respiratory disease, merits species status in the genus *Nocardia*. We therefore propose the name *Nocardia veterana* for the isolate M157222^T (= DSM 44445^T = NRRL B-24136^T)

Description of *Nocardia veterana* sp. nov.

Nocardia veterana [ve.te.ra′na. L. fem. adj. *veteranus* old in service (as soldiers), referring to the veteran's hospital where the organism was isolated].

Aerobic, Gram-positive, non-motile actinomycete which forms beige substrate and white aerial mycelium. Reverse side of the colonies is yellowish. DSM 44445^T is able to utilize L-rhamnose, D-glucosaminic acid, 2-oxoglutarate, L-alanine, i-inositol, putrescine, L-proline and phenylacetate. No reduction of MTT is found with acetamide, D-arabitol, L-aspartate, benzoate, 4-aminobutyrate, 3-hydroxybenzoate, 4-hydroxybenzoate, caprate, citrate, D-galactose, gluconate, *N*-acetylglucosamine, 2-oxoglutarate, L-leucine, pimelate, quinate, D-ribose, L-serine, succinate, sucrose, D-turanose, tyramine, 2-hydroxyvalerate or L-valine. *p*-Nitrophenyl (pNp) phosphorylcholine could be hydrolysed by DSM 44445^T but none of the other chromogenic substrates, i.e. pNp-fl-D-xyloside and 2-desoxythymidine-5-pNp-phosphate, were hydrolysed. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose (cell wall chemotype IV *sensu* Lechevalier & Lechevalier). Sugars of the peptidoglycan are glycolated. Predominant menaquinone is MK-8($H_{4cycl.}$). Polar lipids are composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. Fatty acid pattern is composed of $C_{14:0}$ (2·0%), $C_{15:0}$ (1·2%), $C_{16:0}$ (37·4%), *cis*-9 $C_{16:1}$ (13·8%), *cis*-7 $C_{16:1}$ (0·4%), $C_{17:0}$ (1·1%), $C_{17:1}$ (0·7%), $C_{18:0}$ (1·0%), $C_{18:1}$ (25·8%), 10-methyl $C_{18:0}$ (15·3%) $C_{19:1}$ (0·8%) and unknown (0·5%). The principal mycolic acids have a chain length of 56, 58 and 60 carbon atoms. DSM 44445^T was thought not to be clinically significant and was isolated in a hospital at Heidelberg, Australia, from a man with a past history of tuberculous pleurisy who presented with bilateral upper lobe lesions. Type strain is *Nocardia veterana* M157222^T (= DSM 44445^T = NRRL B-24136^T).

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