Nocardia niwae sp. nov., isolated from human pulmonary sources

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Members of the genus Nocardia are responsible for cutaneous, pulmonary and disseminated human infections. From 2003 to 2008, four nocardioform strains (W8027, W8681, W9071 and W9241T) were isolated from patients in the state of Florida, USA. Ribosomal gene sequencing analysis suggested that a novel species of the genus Nocardia had been isolated. These strains were subjected to a taxonomic analysis using a polyphasic approach. Phenotypic analyses included morphological examination, biochemical profiling and antimicrobial susceptibility testing. Molecular studies included 16S rRNA and DNA gyrase B subunit (gyrB) gene sequence analyses and DNA–DNA hybridization. Phylogenetic neighbours were determined through 16S rRNA and gyrB gene sequence analyses. Phenotypic characteristics that differentiated the novel isolates from phylogenetically related species were growth at 45 °C, and three of the four novel strains utilized L-rhamnose. The antimicrobial profiles could not reliably distinguish the novel species from related nocardiae. Analysis showed that the 16S rRNA gene sequences of the four novel isolates were identical. The BLAST analysis of the near full-length 16S rRNA gene showed 99.2% sequence similarity to Nocardia araoensis DSM 44729T, Nocardia arthritidis DSM 44731T and Nocardia beijingensis JCM 10666T, 98.7% to Nocardia amaniensis DSM 45066T, 98.2% to Nocardia pneumoniae JCM 12119T and 97.8% to Nocardia takedensis JCM 13313T. Analysis of partial gyrB gene sequences showed that the novel isolates had 95.4% similarity to N. arthritidis DSM 44731T, 95.3% to Nocardia gamkensis DSM 44956T, 94.4% to N. pneumoniae JCM 12119T, 93.8% to Nocardia asiatica DSM 44668T, 93.5% to N. amaniensis DSM 45066T, 93.4% to N. beijingensis JCM 10666T and 93.2% to N. araoensis DSM 44729T. The DNA–DNA relatedness values between the four novel strains were 86–89%; the relatedness value for strain W9241T compared with N. beijingensis JCM 10666T was 47% and 46% with N. araoensis DSM 44729T, 44% with N. arthritidis DSM 44731T, 32% with N. amaniensis DSM 45066T and 20% with N. asiatica DSM 44668T. The results of the taxonomic analysis suggested that the new isolates represent a novel species of the genus Nocardia for which the name Nocardia niwae sp. nov. is proposed. The type strain is W9241T (=DSM 45340T=CCUG 57756T).

Species of the genus Nocardia are ubiquitous environmental pathogens that cause a variety of different disease manifestations in humans and animals. Members of the genus Nocardia have been identified as the source of non-specific pulmonary, extrapulmonary, cutaneous and catheter-related infections in humans (Brown-Elliott et al., 2006). Since members of the genus Nocardia do not present definitive clinical manifestations, nocardial infections associated with humans and animals are difficult to diagnose (Gordon et al., 1974; Boiron et al., 1992; Goodfellow, 1998) and Nocardia species are challenging to identify phenotypically (Conville & Witebsky, 2007). However, during the last ten years, with the advent of molecular techniques, the genus Nocardia has undergone
many taxonomic changes (Roth et al., 2003). Current methods of classification of species of the genus Nocardia investigate not only morphological, biochemical and chemotaxonomic characteristics, but also include phylogenetic analyses and DNA–DNA hybridization (Stackebrandt et al., 2002).

In this paper, the morphological, biochemical and chemotaxonomic characteristics along with analyses of the 16S rRNA and gyrB gene sequences and DNA–DNA hybridization are reported for four isolated strains of the genus Nocardia. As part of a taxonomic analysis based on a polyphasic approach, these four strains were compared by 16S rRNA gene sequence analysis with neighbouring reference strains of the genus Nocardia in order to characterize a novel species.

From 2003 to 2008, four clinical strains were sent to the Special Bacteriology Reference Laboratory (SBRL) at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA. The four strains examined (W8027, W8681, W9071 and W9241\textsuperscript{T}) were isolated from clinical pulmonary sources and were initially identified as a putative novel species of the genus Nocardia through 16S rRNA gene sequence analysis. Strains W8681 and W9071 were isolated from bronchial washings of a 37-year-old female and a 92-year-old female, respectively. The remaining two strains, W8027 and W9241\textsuperscript{T}, were isolated from a pleural mass aspirate of a 72-year-old female and a lung biopsy of a 60-year-old male, respectively. All four strains originated from individuals residing in the state of Florida, USA.

The morphological features of these Nocardia strains were studied from aerobic growth on heart infusion agar with rabbit blood (BBL) and Middlebrook 7H11 agar slants (Remel) for 7 days at 35 °C (Brown-Elliott et al., 2006). The organisms were aerobic, Gram-positive and weakly acid–fast by modified Kinyoun stain (Berd, 1973). They formed colonies with white aerial and substrate hyphae on rabbit blood media and orange–coral-coloured hyphae on Middlebrook 7H11 media after 7 days at 35 °C; the reverse side was also orange–coral-coloured on Middlebrook agar. The phenotypic characteristics of the strain designated as the type strain, W9241\textsuperscript{T}, are given in the species description and were determined using methods described previously by Berd (1973).

Tests for the utilization of 22 carbohydrates, production of arylsulfatase, decomposition of adenine, ascin, casein, hypoxanthine, tyrosine and xanthine, utilization of acetamide, citrate and urea, nitrate reduction, growth in lysozyme and growth at 25, 35 and 45 °C were conducted according to previously described methods (Berd, 1973; Conville & Witebsky, 2007). The phenotypic characteristics that could be used to differentiate strains W8027, W8681, W9071 and W9241\textsuperscript{T} and the type strains of phylogenetically related species of the genus Nocardia are presented in Table 1.

The minimum inhibitory concentrations (MIC) for ten antimicrobial agents: amikacin, amoxicillin/clavulinate, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline, tigecycline and trimethoprim/sulfamethoxazole were determined using panels from PML Microbiologicals, Inc. following guidelines and breakpoints established for members of the genus Nocardia (NCCLS, 2003). The results of the antimicrobial susceptibility testing indicated that the four clinical strains were susceptible to amikacin, ceftriaxone, clarithromycin, linezolid, minocycline and trimethoprim/sulfamethoxazole. All strains showed resistance to amoxicillin/clavulanate and varied in their resistance to ciprofloxacin. The results for the four novel strains and the type strains of related species are presented in Supplementary Table S1 (available in IJSEM Online).

Table 1. Differential phenotypic characteristics of strains W8027, W8681, W9071, W9241\textsuperscript{T} and some phylogenetically related type strains

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Analyses of diaminopimelic acid isomers were performed according to the methods described by Rhuland et al. (1984), a small-scale integrated procedure was used to extract and purify quinones and polar lipids. A 200 µl aliquot of 2-propanol was used to dissolve the preparations and 1–10 µl aliquots, without further purification, were separated

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through HPLC and analysed as described by Kroppenstedt (1982, 1985). Extracted polar lipids were examined through two-dimensional TLC and identified according to Minnikin et al. (1977). The Microbial Identification System (MIDI Inc.) for GC analyses (Sasser, 1990) was utilized for the analysis of fatty acid methyl esters and mycolic acid trimethylsilyl esters prepared as described by Klatte et al. (1994). The properties of the novel strains were chemotaxonomically consistent with those expected for members of the genus Nocardia (Kämpfer et al., 2004). The fatty acid patterns of the novel strains are presented in Supplementary Table S2 (see IJSEM Online). Whole cell hydrolysates of strain W9241T contained meso-diaminopimelic acid as the diagnostic amino acid of the cell-wall peptidoglycan. The major cell-wall arabinos were xylos, galactose, glucose and ribose; mannose, xylose and rhamnose were not present. MK-8 (H₄cyc) was the major quinone for this strain. The polar lipids present were phosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol and diphosphatidylglycerol. These corresponded with lipids reported previously for members of the genus Nocardia by Minnikin et al. (1977). The fatty acid patterns of the four novel strains were of straight chain saturated and unsaturated fatty acids plus tuberculostearic acid. Mono- and di-unsaturated mycolic acids ranged from saturated and unsaturated fatty acids plus tuberculostearic acid.

Almost complete 16S rRNA gene sequences (1441 bp) were determined for the four novel strains and amplification of the 16S rRNA gene was performed as described by Morey et al. (2006) except for the modification of primers FL1 (5’-CCGAATTCCGTCGACA) and RL1 (5’-CCGGGATCC-GAAGCT). BLAST software (nucleotide–nucleotide BLAST: https://www.ncbi.nlm.nih.gov/BLAST) was used to identify related sequences in GenBank. Bootstrap analysis with 1000 replicates was performed using MEGA4 software to assess the support for each node of the phylogenetic tree (Tamura et al., 2007). The 16S rRNA gene sequences for the four novel strains were identical. The phylogenetically related neighbours of the novel strains (Fig. 1) were Nocardia araoensis DSM 44729T (99.2% similarity), N. arthritidis DSM 44731T (99.2%) and N. beijingensis JCM 10666T (99.2%), N. amamiensis DSM 45066T (98.7%), N. pneumoviae JCM 12119T (98.2%) and N. takedensis JCM 13313T (97.8%).

DNA extraction, amplification of a ~1245-bp gyrB gene fragment, primers and sequencing followed methods previously described by Shen et al. (2006). The MEGA4 program was used for phylogenetic assessment (Tamura et al., 2007). The gyrB gene sequences for the four novel strains were identical. The closest phylogenetic neighbours were revealed (Fig. 2) as N. arthritidis DSM 44731T (95.4% similarity), N. gamsensis DSM 44956T (95.3%), N. pneumoviae JCM 12119T (94.4%), N. asiatica DSM 44668T (93.8%), N. amamiensis DSM 45066T (93.5%), N. beijingensis JCM 10666T (93.4%) and N. araoensis DSM 44729T (93.2%).

DNA was extracted from the four novel strains and from five closely related phylogenetic neighbours as described by Loefelholz & Scholl (1989) and DNA–DNA hybridization studies were performed in the Special Bacteriology Reference Laboratory at CDC following the protocol by Brenner et al. (1982). DNA–DNA hybridization studies were performed between DNA samples from strains W8027, W8681, W9071 and W9241T as well as between the DNA of strain W9241T and five of the most closely phylogenetically related members of the genus Nocardia.

![Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain W9241T and a few closely related Nocardia species. The tree was constructed using the neighbour-joining method and was based on a comparison of ~1441 nt. The tree was rooted using Mycobacterium tuberculosis ATCC 27294T as the outgroup (not shown). Bootstrap percentages were expressed as percentages of 1000 replicates and only values >50% are presented. Bar, 0.005 substitutions per nucleotide position. The full tree from which this area is taken is available as Supplementary Fig. S1 in IJSEM Online.](https://www.microbiologyresearch.org/ijsem/article-pdf/61/6/440/7023764/ijsem6161.pdf)
The mean DNA–DNA relatedness values between strains W8027, W8681, W9071 and W9241T ranged from 86 to 89%. The levels of DNA–DNA relatedness between strain W9241T and N. beijingensis DSM 10666T, N. araoensis DSM 44729T, N. arthritidis DSM 44731T, N. amaniensis DSM 45066T and N. asiatica DSM 44668T were 47%, 46%, 44%, 32% and 20%, respectively. These values were below the 70% cut-off recommended by Wayne et al. (1987) for species delineation.

As a result of the polyphasic approach applied to our taxonomic investigation, we found that the four strains isolated from pulmonary sources represented a novel species of the genus Nocardia. Phenotypic analysis showed that the new strains showed differential characteristics of growth at 45 °C and L-rhamnose utilization (except for strain W9071) from related species of the genus Nocardia. The results of the phylogenetic analysis of 16S rRNA and gyrB gene sequences and DNA–DNA hybridization demonstrated that these strains represented a distinct entity for which we propose the name Nocardia niwae sp. nov.

Description of Nocardia niwae sp. nov.

Nocardia niwae (ni’wa.e. N.L. gen. n. niwae of Niwa, named in honour of Dr Hidekazu Niwa, our Japanese colleague; a veterinarian and microbiologist).

Aerobic, Gram-positive, weakly acid-fast, non-motile actinomycete, forming orange–coral-coloured aerial and substrate hyphae on Middlebrook 7H11 media. Utilizes and produces acid from D-glucose, glycerol, L-rhamnose, succrose and trehalose, but does not utilize adonitol, L-arabinose, cellobiose, dulcitol, L-erythritol, D-fructose, D-galactose, myo-inositol, lactose, maltose, D-mannitol, mannose, melibiose, raffinose, salicin, D-sorbitol or D-xyllose. Utilizes citrate as sole carbon source, but not acetamide as a carbon and nitrogen source. Grows in the presence of lysozyme, reduces nitrate, but has no arylsulfatase activity. Hydrolyses urea, but does not hydrolyse adenine, aesculin, casein, hypoxanthine, tyrosine or xanthine. Grows at 25, 35 and 45 °C.

The type strain, W9241T (=DSM 45340T =CCUG 57756T), was isolated from the lung tissue of a 60-year-old male in the state of Florida, USA. The G+C value of the DNA for the type strain is 68.5 mol%.

Fig. 2. Phylogenetic tree, based on gyrB gene sequences, showing the relationships of strain W9241T and a few closely related Nocardia species. For construction details, see legend of Fig. 1. Bar, 0.01 substitutions per nucleotide position. The full tree from which this area is taken is available as Supplementary Fig. S2 in ISEM Online.

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References


