Nocardia exalbida sp. nov., isolated from Japanese patients with nocardiosis

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Two bacterial strains isolated from different hospitals in Japan were subjected to a polyphasic analysis. Strains IFM 0803^T and IFM 10383 were found to have morphological, biochemical, physiological and chemotaxonomic properties consistent with their classification in the genus *Nocardia*. Strains IFM 0803^T and IFM 10383 clustered with the type strain of *Nocardia xishanensis*, showing 16S rRNA gene sequence similarities of 98.6–98.9% with this species. The novel strains could be distinguished from *N. xishanensis* by a range of phenotypic properties. Based on their phenotypic and phylogenetic characteristics, the two isolates are proposed as members of a novel species of the genus *Nocardia*, *Nocardia exalbida* sp. nov., with the type strain IFM 0803^T (=NBRC 100660^T=JCM 12667^T=DSM 44883^T).

Members of the genus *Nocardia* have been implicated as agents of infectious diseases in both immunocompetent and immunocompromised patients, but immunocompromised patients are particularly at risk (Conville *et al.*, 2004). The primary treatment of nocardial infections in immunocompromised patients is by antibiotic therapy (Conville *et al.*, 2004). The initial selection of antibiotic therapy should take account of the species involved as nocardial strains are known to show species-specific drug-susceptibility patterns (Mikami & Yazawa, 1989). The most prevalent species is considered to be *Nocardia farcinica* (Hitti & Wolff, 2005). However, it has been repeatedly reported that *N. farcinica* is resistant to most of the antibiotics that are typically used for the treatment of nocardial infections (Hitti & Wolff, 2005; Kageyama *et al.*, 2004c). Recently, the whole genome of

N. farcinica was sequenced and the study revealed that this organism has more sophisticated drug-resistance mechanisms than previously thought (Ishikawa *et al.*, 2004). These data further suggest that nocardial species determination is necessary in order to choose the most appropriate therapeutic drug. Two bacterial strains, IFM 0803^T and IFM 10383, isolated from clinical specimens in Japan, were initially identified in our laboratory as *Nocardia asteroides* from morphological and biochemical characteristics. However, 16S rRNA gene sequencing and DNA–DNA hybridization of the two novel strains showed that they belong to a distinct species. This study was performed to determine the taxonomic position of the two novel strains using a polyphasic taxonomic approach.

Strains IFM 0803^T and IFM 10383 were isolated from a bronchoalveolar lavage of a 43-year-old immunocompromised patient with lung abscess at Chiba University Hospital, Chiba, Japan, and from a 60-year-old immunocompromised patient diagnosed with pemphigus vulgaris at St Luke's International Hospital, Tokyo, Japan, respectively.

All nocardial strains used in this study were cultured on Muller Hinton II (MH II; Difco) agar slants with 1% glucose

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Nocardia exalbida* strains IFM 0803^T and IFM 10383 are AB187522 and AB187521, respectively.

An extended phylogenetic tree based on 16S rRNA gene sequences, the TLC mycolic acid patterns of strain IFM 0803^T and a comparison of the physiological properties of 53 recognized *Nocardia* species are available as supplementary material in IJSEM Online.

and 1% glycerol at 37 °C for 1 week. Colony morphology and micromorphological properties of strains IFM 0803^T and IFM 10383 were observed by light and scanning electron microscopy according to previously described procedures (Kageyama *et al.*, 2004b). Physiological and biochemical properties were examined using published procedures (Gordon *et al.*, 1974; Poonwan *et al.*, 1995). Arylsulfatase activity was determined using the method of Kubica & Beam (1961). The minimum inhibitory concentration (MIC) values for seven drugs, arbekacin, ampicillin, clarithromycin, cefetamet pivoxil, cefotiam, erythromycin and a mixture of sulfamethoxazole–trimethoprim (152:8) were determined using the method described by Mikami & Yazawa (1989). Drug susceptibility tests were performed with TRIDISK (Eiken Chemical Co.) using the method described by Kageyama *et al.* (2004b). Strains were stained by a modified method (Chapin & Murray, 1999), using 0.5% sulfuric acid for determination of acid–alcohol-fastness.

The 16S rRNA gene was amplified using PCR employing six prokaryotic 16S rRNA universal primers and sequenced following procedures described previously (Kageyama *et al.*, 2004a). Species related to the novel isolates were identified by performing a nucleotide sequence database search using BLAST programs (nucleotide–nucleotide BLAST: <http://www.ncbi.nlm.nih.gov/blast>) from GenBank. Sequences of related species were also retrieved from GenBank. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972) and phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). Tree topology was evaluated using a bootstrap analysis (based on 1000 resamplings) of sequence data using CLUSTAL W software (Thompson *et al.*, 1994). Sequence similarity values were determined through visual comparison and manual calculation. Genomic DNA was prepared as described previously (Kageyama *et al.*, 2004b, c). The DNA G + C composition was estimated by HPLC (Tamaoka & Komagata, 1984). Levels of DNA–DNA relatedness were determined using the method of Ezaki *et al.* (1989) using photobiotin and microplates.

Whole-cell hydrolysates were analysed for diaminopimelic acid (A₂pm) isomers using TLC (Staneck & Roberts,

1974). Whole-cell sugars were prepared according to published protocols (Lechevalier & Lechevalier, 1980) and analysed using the TLC method (Miyadoh, 2001). Mycolic acids were prepared according to Minnikin *et al.* (1980). Menaquinones were analysed as described by Chun & Goodfellow (1995). Fatty acid methyl esters were prepared and analysed as described previously using the standard Microbial Identification System (MIDI) for automated GC analysis (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Comparison of the almost-complete 16S rRNA gene sequences of strains IFM 0803^T and IFM 10383 with those of representatives of genera classified in the suborder *Corynebacterineae* showed that they contained all the signature nucleotides that are expected for members of the family *Nocardiaceae* (Stackebrandt *et al.*, 1997) and the genus *Nocardia* (Chun & Goodfellow, 1995). Highest sequence similarities were found with *Nocardia beijingensis*, *Nocardia arthritidis*, *Nocardia abscessus*, *Nocardia brevicatena*, *Nocardia paucivorans* and *Nocardia xishanensis*, with sequence similarities of 98.3, 98.9, 98.6, 98.4, 98.3 and 98.9%, respectively. Gene sequence similarity between strains IFM 0803^T and IFM 10383 was 99.7%. A tree constructed using the neighbour-joining method depicting the phylogenetic placement of strains IFM 0803^T and IFM 10383 within a subset of the genus *Nocardia* is shown in Fig. 1. This tree shows that the two novel strains form a monophyletic clade that is loosely associated with *N. xishanensis* JCM 12160^T. Sequence similarity values between strains IFM 0803^T and IFM 10383 and *N. xishanensis* were 98.6% (1367/1389) and 98.9% (1396/1412).

DNA–DNA relatedness between the two novel strains and *N. xishanensis* was 57–59%. This value is below the 70% cut-off point for species classification, as recommended by Wayne *et al.* (1987).

Chemotaxonomic and morphological characteristics of strains IFM 0803^T and IFM 10383 were consistent with their assignment to the genus *Nocardia* (Goodfellow, 1998; Goodfellow *et al.*, 1999). Both contain galactose and arabinose as characteristic whole-cell sugars and meso-A₂pm

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences. The tree was created using the neighbour-joining method and K_{nuc} values. Numbers at branch points indicate bootstrap values. Bar, 1 inferred substitution per 100 nucleotides. An extended tree is available as Supplementary Fig. S2 in IJSEM Online.

as the diagnostic cell-wall diamino acid (chemotype IV *sensu* Lechevalier & Lechevalier, 1980). The major menaquinone was MK-8(H4 ω -cycl). The mycolic acid major chain length ranged from C48 to C56 for strains IFM 0803^T and IFM 10383. TLC separation of whole-cell *trans* esterification extracts of strains IFM 0803^T and IFM 10383 revealed two or three spots (see Supplementary Fig. S1 in IJSEM Online). The lower spot co-migrated with nocardia-mycolic acids. The second spot, an unknown lipid, migrated to a position between nocardia-mycolic acid and non-hydroxylated fatty acids and the R_F value of the higher spot (third spot) correlated with non-hydroxylated fatty acids. The higher spot of strain IFM 0803^T co-migrated with extracts from *N. xishanensis* JCM 12160^T (Zhang *et al.*, 2004). Our TLC analysis revealed the presence of the higher spot of mycolic acid in a few species of *Nocardia*, such as *N. abscessus* DSM 44432^T, *N. beijingensis* JCM 10666^T and *Nocardia asiatica* IFM 0245^T (Poonwan *et al.*, 2005). Analyses of fatty acids of strains IFM 0803^T and IFM 10383 by GLC revealed the diagnostic pattern of members of the genus *Nocardia* and related taxa: straight-chain saturated and unsaturated fatty acids, together with a diagnostic amount of tuberculostearic acid.

Phenotypic characteristics of the two novel strains were also examined and compared with those of recognized species of the genus *Nocardia*. The novel strains were able to decompose casein, but *N. xishanensis* JCM 12160^T was not. *N. xishanensis* utilizes adipic acid, but IFM 0803^T and IFM 10383 do not. Citrate is used by the novel strains, but not

by *N. xishanensis* JCM 12160^T. Based on these phenotypic characteristics, strains IFM 0803^T and IFM 10383 can be distinguished from *N. xishanensis* JCM 12160^T. The two novel strains could also be distinguished from *N. xishanensis* JCM 12160^T on the basis of drug susceptibility tests. The differential features of the phenotypes are summarized in Table 1. A comparison of phenotypic characteristics between the two novel strains and additional species of *Nocardia* is available as Supplementary Table S1 in IJSEM Online. Both phenotypic and genotypic data show that the two novel isolates represent a novel species within the genus *Nocardia*, for which the name *Nocardia exalbida* sp. nov. is proposed.

Description of *Nocardia exalbida* sp. nov.

Nocardia exalbida (ex'al.bi.da. L. fem. adj. *exalbida* whitish or white, referring to the colour of the aerial mycelium).

Aerobic, Gram-positive, partially acid-fast, non-motile actinomycetes that form an extensively branched substrate mycelium that fragments into rod-shaped elements (0.4–0.8 × 0.8–1.7 μ m). Orange–tan to tan colonies with abundant white to off-white aerial mycelium. No soluble pigment is produced. Colonies are 1.0–2.5 mm in diameter after 7 days at 30 °C on MH II medium with 0.2 % glucose. Casein is decomposed, but adenine, arbutin, elastin, hypoxanthine, tyrosine and xanthine are not decomposed. Urea is decomposed. Glucose is utilized, but arabinose, erythritol, galactose, inositol, maltose, mannose, rhamnose and sorbitol are not. Citrate and testosterone are utilized,

Table 1. Phenotypic properties of strains IFM 0803^T, IFM 10383, *N. xishanensis* JCM 12160^T and *N. abscessus* JCM 6043

+, Positive; –, negative; w, weakly positive; ND, not determined. All strains are positive for the decomposition of aesculin and urea. All strains are negative for decomposition of adenine, elastin, hypoxanthine, tyrosine and xanthine.

Characteristic	IFM 0803 ^T	IFM 10383	JCM 12160 ^T	JCM 6043
Growth at 45 °C	–	w	–	–
Acid from rhamnose (Gordon test)	–	–	–	+
Decomposition of:				
Arbutin	–	+	+	+
Casein	+	w	–	–
Utilization of:				
Adipic acid	–	–	+	–
Citrate	+	+	–	+
Susceptibility to:*				
Arbekacin	<2	<2	4	ND
Ampicillin	16	>16	4	ND
Clarithromycin	1	2	<0.006	ND
Cefetamet pivoxil	0.5	1	0.12	ND
Cefotiam	8	4	2	ND
Erythromycin	2	4	<0.006	ND
Sulfamethoxazole–trimethoprim (152:8)	2.4	2.4	9.5	ND

*MIC values at μ g ml^{–1}.

but adipic acid, acetamide and gluconate are not. No growth at 45 °C. The G + C content of the DNA is 68 mol%. Susceptible to imipenem and tobramycin (growth inhibition zone around a paper disc with 2.5 µg drug per disc), moderately susceptible to kanamycin (10 µg disc), but not susceptible to 5-fluorouracil (30 µg disc).

The type strain, IFM 0803^T (=NBRC 100660^T=DSM 44883^T=JCM 12667^T), was isolated from the bronchoalveolar lavage of a patient with lung nocardiosis in Japan.

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