Nocardia concava sp. nov., isolated from Japanese patients

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Two actinomycete strains, IFM 0354^T and IFM 0576, isolated from Japanese patients, were found to have morphological, biochemical and chemotaxonomic properties consistent with their classification in the genus *Nocardia*. The strains resembled *Nocardia otitidiscaviarum* and *Nocardia uniformis* in their phenotypic properties. Comparative 16S rRNA gene sequence analysis showed that the strains are closely related to *Nocardia seriolae*. DNA–DNA relatedness values and phenotypic differences from *N. seriolae* indicated that the strains belong to a novel species of *Nocardia*, for which the name *Nocardia concava* sp. nov. is proposed. The type strain is IFM 0354^T (=NBRC 100430^T=JCM 12351^T=DSM 44804^T).

The genus *Nocardia* has been well defined on the basis of chemotaxonomic, molecular genetic and numerical phenetic methods (Goodfellow *et al.*, 1999). Members of the genus are common and widely distributed in terrestrial habitats (Gordon *et al.*, 1974; Goodfellow, 1998; Wang *et al.*, 2001; Albuquerque de Barros *et al.*, 2003; Zhang *et al.*, 2003). Many *Nocardia* species are associated with human and animal infections that are difficult to diagnose because they do not present specific clinical manifestations and histological data are lacking (Gordon *et al.*, 1974; Boiron *et al.*, 1992; Goodfellow, 1998; Yassin *et al.*, 2000, 2003). The two bacterial strains studied in this work (IFM 0354^T and IFM 0576) were isolated from Japanese patients with cutaneous nocardiosis. The strains were most similar to *Nocardia otitidiscaviarum* and *Nocardia uniformis* in their biochemical characteristics.

Strains IFM 0354^T and IFM 0576 were isolated from a 76-year-old female patient in 1991 and from a 69-year-old male patient in 1995, respectively. The strains were cultured for morphological observations and chemotaxonomic and molecular analyses at 30 °C as described previously (Kageyama *et al.*, 2004a, b). The reference strain *Nocardia seriolae* JCM 3360^T (Kudo *et al.*, 1988) was grown on the same medium at 25 °C. For fatty acid and mycolic acid study, strain IFM 0354^T and type strains of *N. seriolae*,

N. otitidiscaviarum and *N. uniformis* were grown on trypticase soy broth agar (DSMZ medium 535) at 28 °C for 7 days.

Biochemical, physiological and chemotaxonomic characteristics, except for mycolic acid patterns, were examined as reported by Kageyama *et al.* (2004a, b), using the methods described by Gordon *et al.* (1974), Stanek & Roberts (1974), Lechevalier & Lechevalier (1980), Miyadoh (2001), Sasser (1990), Kämpfer & Kroppenstedt (1996) and Chun & Goodfellow (1995). Mycolic acid trimethylsilyl ethers were prepared and analysed following the methods of Klatte *et al.* (1994). Extraction of genomic DNA, DNA–DNA hybridization experiments and determination and analysis of 16S rRNA gene sequences were performed as reported by Kageyama *et al.* (2004a, b).

Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the trees was evaluated by a bootstrap analysis of the sequence data using CLUSTAL W software (Thompson *et al.*, 1994). Sequence similarity values were determined by visual comparison and manual calculation.

The nearly complete 16S rRNA gene sequences were determined for strains IFM 0354^T (1472 bp) and IFM 0576 (1447 bp). Phylogenetic analysis demonstrated that the strains belonged to the family *Nocardiaceae* of the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997) and formed a monophyletic clade associated with *N. seriolae* (Fig. 1; an extended tree showing a larger selection of reference sequences is available as Supplementary Fig. S1 in IJSEM Online). 16S rRNA gene sequence similarity between IFM 0354^T and IFM 0576 was 99.5%; similarity between

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Nocardia concava* IFM 0354^T and IFM 0576 are AB126880 and AB126881, respectively.

An extended phylogenetic tree and tables detailing the fatty acid profiles, mycolic acid patterns and phenotypic properties of IFM 0354^T, IFM 0576 and other *Nocardia* species are available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequence analysis. The tree was created using the neighbour-joining method and K_{nuc} values. Numbers on the tree indicate bootstrap values for the branch points. An extended version of this tree including a wider selection of reference strains is available as Supplementary Fig. S1 in IJSEM Online.

N. seriolae and the above strains was nearly 98.1 %. Lower levels of sequence similarity (<98 %) were observed with other neighbouring species, including *N. otitidiscaviarum* and *N. uniformis*. Genomic delineation of the isolated strains from *N. seriolae* was supported by DNA–DNA relatedness data. The two isolates shared 97 % DNA–DNA relatedness, whereas relatedness values of IFM 0354^T and IFM 0576 to *N. seriolae* were only 16 and 12 %, respectively. These values are well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987).

The chemotaxonomic characters of the two isolated strains were consistent with their assignment to the genus *Nocardia* (Goodfellow, 1998; Goodfellow *et al.*, 1999). The strains contained galactose and arabinose as characteristic whole-cell sugars in addition to *meso*-diaminopimelic acid as the diagnostic cell-wall diamino acid. The major menaquinone was MK-8(H_{4ω-cycl}). The fatty acid profile of IFM 0354^T included straight-chain saturated and unsaturated fatty acids plus a significant amount of tuberculostearic acid (10-methyl octadecanoic acid) (see Supplementary Table S1 available in IJSEM Online). The strain also contained a homologous series of mycolic acids ranging from 52 to 62 carbon atoms, with C56 and C54 being the principal chain lengths (Supplementary Table S2). In contrast to IFM 0354^T, *N. seriolae* synthesized mycolic acids in the range from 46 to 58 carbon atoms, with the principal mycolic acid being of chain length C52 (Kudo *et al.*, 1988), whereas *N. otitidiscaviarum* and *N. uniformis* had mainly quantitative differences in the proportions of mycolic acids present.

Strains IFM 354^T and IFM 0576 were also examined for a set of biochemical and physiological characteristics in comparison with *N. seriolae* and *Nocardia asteroides*. The two strains could be readily distinguished from these species by a combination of physiological and biochemical characteristics, including decomposition of adenine, hypoxanthine, urea and xanthine (Table 1). They also differed phenotypically from other *Nocardia* species (see Supplementary Table S3 in IJSEM Online).

Table 1. Phenotypic properties of the isolated strains, *N. asteroides* and *N. seriolae*

Strains: 1, IFM 0354^T; 2, IFM 0576; 3, *N. asteroides* ATCC 19247^T; 4, *N. seriolae* JCM 3360^T. Data were taken from Kinoshita *et al.* (2001), Kudo *et al.* (1988), Yassin *et al.* (2000) and this study. +, Positive; –, negative; ND, no data.

Characteristic	1	2	3	4
Decomposition of:				
Adenine	+	+	–	–
Hypoxanthine	+	+	–	–
Tyrosine	+	+	–	–
Urea	+	+	+	–
Xanthine	+	+	–	–
Utilization of:				
Arabinose	+	+	–	–
Galactose	+	+	–	–
Inositol	+	+	–	–
Mannose	+	+	ND	ND
Growth at 37 °C	+	+	+	–
Susceptibility tests: ^a				
Imipenem ^a	–	–	3+	ND
Tobramycin ^a	2+	2+	3+	ND
5-Fluorouracil ^b	+	+	–	ND
Kanamycin ^c	3+	3+	1+	ND

^aScored as follows. *a*: 3+, highly susceptible, growth inhibition at 2.5 µg per disc; 2+, moderately susceptible, growth inhibition at 5.0 µg per disc; –, not susceptible, no growth inhibition at 10.0 µg per disc. *b*: +, susceptible, growth inhibition at 30.0 µg per disc; –, not susceptible, no growth inhibition at 30.0 µg per disc. *c*: 3+, highly susceptible, growth inhibition at 5.0 µg per disc; 1+, slightly susceptible, growth inhibition at 30.0 µg per disc.

On the basis of the genotypic and phenotypic evidence described above, it was concluded that strains IFM 354^T and IFM 0576 represented a novel species within the genus *Nocardia*. We therefore propose the name *Nocardia concava* sp. nov.

Description of *Nocardia concava* sp. nov.

Nocardia concava (con.ca'va. L. fem. adj. *concava* hollow, concave, referring to the colony morphology on agar plates).

Aerobic Gram-positive, acid–alcohol-fast, non-motile actinomycete, which forms greyish-orange to faint-orange substrate mycelium that fragments into irregular rod-shaped elements. Aerial mycelium is absent to scanty. Grows at 37 °C, but not at 45 °C. Colonies are 1.0–3.5 mm in size after growth for 7 days at 30 °C on MH II agar medium with 0.2 % glucose. Erythritol, maltose, mannose, rhamnose and sorbitol are not utilized. Arabinose, galactose, glucose, inositol and citrate are utilized. Casein and tyrosine are not decomposed. Adenine, hypoxanthine, urea and xanthine are decomposed. The DNA G + C content is 67–68 mol%.

The type strain is IFM 0354^T (=NBRC 100430^T=JCM 12351^T=DSM 44804^T). A second strain is IFM 0576. These strains are isolates from Japanese patients.

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