NOTE

***Nocardia ignorata* sp. nov.**A. F. Yassin,¹ F. A. Rainey² and U. Steiner³¹ Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, D-53127 Bonn, Germany² Department of Biological Sciences, 508 Life Sciences Building, Louisiana State University, Baton Rouge, LA 70803, USA³ DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen, D-38124 Braunschweig, Germany

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An isolate that was received during a mycobacterial quality control test and which was thought to be a *Mycobacterium* species was subjected to a polyphasic taxonomic study after mycolic acid analysis showed that it possessed mycolates characteristic of the nocardiae. Further chemotaxonomic and 16S rRNA gene sequence analyses of this isolate demonstrated that it belongs to the genus *Nocardia*. 16S rRNA gene sequence data, DNA–DNA hybridization studies and the biochemical characteristics of the isolate indicate that it belongs to a novel species that differs from previously described members of the genus *Nocardia*. The name *Nocardia ignorata* sp. nov. is proposed for this isolate with the type strain IMMIB R-1434^T (= DSM 44496^T = NRRL B-24141^T).

Keywords: *Nocardia salmonicida*, mycobacterial quality control tests, chemotaxonomy, DNA–DNA hybridization, 16S rRNA gene sequence

Data quality is a central requirement of scientific research and external monitoring is essential in multi-centric clinical trials. Performance of quality control in the clinical microbiological laboratory includes a wide variety of specimens that are issued. These include specimens for general bacteriology, including isolation, sensitivity testing and serology; mycobacterial bacteriology; virus isolation; general viral serology; rubella serology, hepatitis B antigen detection; electron microscopy; mycology; parasitology; and public health specimens including milk and water. During a mycobacterial quality control test, the Microbiological Quality Control Laboratory (MQCL) dispatched, among other specimens, one that was evaluated at the MQCL as a species of *Mycobacterium*. However, after subjecting this specimen, labelled by us as strain IMMIB R-1434^T, to chemotaxonomic as well as molecular taxonomic methods in our laboratory, it became clear that it belongs to the genus *Nocardia*. This case raises questions about the authenticity of some of the test specimens that are used in quality control assurance programmes. It also underlines the requirement of the MQCL for reference strains that should be identified directly by reliable taxonomic methods at the MQCL before being dispatched to participants.

Chemotaxonomic and molecular systematic data promoted a radical reappraisal of the genus *Nocardia* (Goodfellow, 1997; Goodfellow *et al.*, 1999; Lechevalier, 1976). The improved classification provides a sound framework for the recognition of additional species. The genus now encompasses more than 27 validly described species that form a distinct phylogenetic line within the evolutionary radiation occupied by the mycolic acid-containing actinomycetes (Chun & Goodfellow, 1995; Rainey *et al.*, 1995; Ruimy *et al.*, 1994). On the basis of phylogenetic and phenotypic evidence, it is proposed that strain IMMIB R-1434^T be classified as *Nocardia ignorata* sp. nov.

Strain IMMIB R-1434^T was cultured on brain–heart infusion agar to determine its morphological properties. The strain was characterized biochemically by using tests to determine hydrolysis of complex substrates, as described previously (Gordon, 1966, 1967; Gordon & Mihm, 1957), as well as tests to determine carbon source utilization according to Yassin *et al.* (1995). The isomeric form of diaminopimelic acid was determined by the methods of Becker *et al.* (1964) and whole-cell sugars were determined by the method of Lechevalier (1968). Lipids were extracted using acid methanolysis and mycolic acids were detected with TLC as described by Minnikin *et al.* (1980); pyrolysis gas chromatography of the mycolate was performed according to Yassin *et al.* (1993a) and the composition of the mycolic acids was determined using electron impact-mass spectrometry according to Collins *et al.*

Abbreviation: MQCL, Microbiological Quality Control Laboratory.

The GenBank accession number for the 16S rDNA sequence of strain IMMIB R-1434^T (= DSM 44496^T = NRRL B-24141^T) is AJ303008.

Fig. 1. Unrooted phylogenetic tree showing the position of strain IMMIB R-1434^T (= DSM 44496^T) within the radiation of species of the genus *Nocardia*. Bar, 2.0 inferred nucleotide substitutions per 100 nucleotides.

(1982). Non-hydroxylated fatty acids were purified, identified and quantified by gas chromatography as described by Yassin (1988). Menaquinones were extracted, purified and identified according to Collins *et al.* (1977). Phospholipids were extracted, purified and identified as described previously (Yassin *et al.*, 1993c).

DNA was isolated and purified as described previously (Yassin *et al.*, 2000). G + C contents were determined by HPLC (Mesbah *et al.*, 1989). DNA–DNA hybridization studies were carried out by using the thermal renaturation method (Yassin *et al.*, 1993b). Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) as described in the manufacturer's protocol. An Applied Biosystems 310 DNA Genetic Analyzer was used for electrophoresis of the sequence reaction products. The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rRNA gene sequence of strain IMMIB R-1434^T against 16S rRNA gene sequences of validly described species of the genus *Nocardia* available from public databases. The programs of the PHYLIP package, including DNADIST and NEIGHBOR, were used for phylogenetic analyses (Felsenstein, 1993). The tree topology was reanalysed using 1000 bootstrapped datasets with the programs SEQBOOT, DNADIST and CONSENSE of the PHYLIP package (Felsenstein, 1993).

Strain IMMIB R-1434^T has morphological properties consistent with its assignment to the genus *Nocardia*. It is an aerobic organism that forms hyphae that are Gram-positive and slightly acid–alcohol-fast. The orange-coloured vegetative hyphae were well-developed with irregular branches penetrating the agar and bearing white aerial hyphae. At a late stage of growth, the hyphae fragmented into rod-shaped elements characteristic of nocardiae.

Chemotaxonomically, strain IMMIB R-1434^T contained chemical markers that support its assignment to the genus *Nocardia*. The cell wall contains meso-diaminopimelic acid as well as arabinose and galactose (i.e. wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). One-dimensional TLC of whole-cell acid methanolysates of strain IMMIB R-1434^T revealed the presence of two lipid spots on the chromatogram. The lower one corresponds to mycolic acids, as identified by its *R_f* value (0.47), and the higher spot corresponds to non-hydroxylated fatty acids. Pyrolysis gas chromatography of the purified mycolic acid methyl esters from strain IMMIB R-1434^T released fatty acid methyl esters of C_{16:0} (67.14 % of total cleavage products), C_{17:0} (3.75 %), C_{18:1} (3.82 %) and C_{18:0} (25.30 %) as pyrolysis cleavage products. Mass spectrum analysis of the purified mycolate revealed the presence of mycolic acids with 46–57 carbon atoms and 0–3 double bonds. Gas chromatographic analyses of the non-hydroxylated fatty acid methyl esters revealed the presence of tetradecanoate (0.51 % of total fatty acids), pentadecanoate (0.26 %), *cis*-

Table 1. Physiological characteristics of strain IMMIB R-1434^T and other validly described *Nocardia* species

Taxa are identified as: 1, strain IMMIB R-1434^T; 2, *Nocardia salmonicida* JCM 4826^T; 3, *Nocardia cyriacigeorgica* DSM 44484^T; 4, *Nocardia abscessus* DSM 44432^T; 5, *Nocardia paucivorans* DSM 44386^T; 6, *Nocardia asteroides* ATCC 19247^T; 7, *Nocardia nova* ATCC 33726^T; 8, *Nocardia brasiliensis* DSM 43758^T; 9, *Nocardia farcinica* DSM 43665^T; 10, *Nocardia otitidiscaviarum* DSM 43242^T; 11, *Nocardia vaccinii* DSM 43285^T; 12, *Nocardia transvalensis* NCTC 2392^T. d, Differs between strains (some positive and others negative); w, utilized weakly after 3 weeks of incubation.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Hydrolysis of:												
Aesculin	+	+	+	–	–	+	–	+	+	+	–	–
Casein	–	–	–	–	–	–	–	+	–	–	–	–
Elastin	–	–	–	–	–	–	–	+	–	–	–	–
Gelatin	+	+	–	–	–	–	–	–	–	–	–	+
Hypoxanthine	–	–	–	–	–	–	–	+	–	+	–	–
Testosterone	+	+	+	+	–	+	–	–	+	+	–	+
Tyrosine	–	–	–	–	–	–	–	+	–	–	–	–
Xanthine	–	–	–	–	–	–	–	–	–	+	–	–
Utilization as sole sources of carbon and energy of:												
Acetate	+	+	+	+	+	+	+	+	+	+	–	+
Citrate	–	+	–	+	–	–	–	+	–	–	+	+
Gluconate	+	+	+	–	–	+	–	–	–	w	+	+
L-Arabinose	–	–	–	–	–	–	–	–	–	–	+	–
Cellobiose	–	+	–	–	–	–	–	–	–	–	–	–
Galactose	–	+	–	–	–	–	+	+	–	–	+	+
Glucose	+	+	–	+	–	+	+	+	+	+	+	+
Maltose	+	–	–	+	–	–	–	+	+	–	–	+
Raffinose	–	+	–	–	–	–	–	–	–	–	–	–
L-Rhamnose	–	–	–	d	–	–	–	–	+	–	+	–
Sucrose	w	–	+	+	–	+	–	w	+	–	–	+
Trehalose	+	+	–	+	+	–	–	+	+	w	+	+
Xylose	–	–	–	–	–	–	–	–	–	–	+	–
meso-Erythritol	–	–	–	–	–	–	–	–	–	–	–	+
myo-Inositol	–	–	–	–	–	–	–	+	–	+	–	–
Mannitol	+	+	–	–	–	–	–	w	–	+	+	+
Isoamyl alcohol	–	+	–	–	–	–	+	w	+	–	–	+
2,3-Butanediol	–	–	–	–	–	–	–	–	+	–	–	–
1,2-Propanediol	–	–	–	–	–	–	–	–	+	–	–	–
m-Hydroxybenzoate	–	–	–	–	–	–	–	–	+	–	–	w
p-Hydroxybenzoate	–	–	–	–	–	–	–	–	–	–	w	–
Utilization as sole sources of carbon and nitrogen of:												
Acetamide	–	–	+	–	–	–	–	–	+	–	–	–
L-Alanine	–	–	–	–	–	–	–	+	–	+	–	–
Gelatin	w	–	–	–	–	–	–	+	–	–	–	–
Proline	+	+	–	–	–	+	–	+	–	–	–	+
Serine	–	+	–	–	–	–	–	w	–	w	–	+

hexadecenoate (3.17%), hexadecanoate (20.84%), heptadecanoate (1.40%), octadecenoate (6.27%), octadecanoate (38.33%), tuberculostearic acid (10-methyl octadecanoate, 15.94%), nonadecanoate (0.54%), eicosenoate (2.00%), eicosanoate (7.75%), heneicosanoate (0.39), docosenoate (1.05%) and docosanoate (2.52%) as the major cellular fatty acid methyl esters. Polar lipid analysis showed that strain IMMIB R-1434^T contains phosphatidylethanolamine,

phosphatidylinositol, phosphatidylinositol mannoside and diphosphatidylglycerol as characteristic phospholipids (i.e. phospholipid type PII *sensu* Lechevalier *et al.*, 1977). Mass spectrum analysis of the respiratory quinones showed that strain IMMIB R-1434^T possesses hexahydrogenated menaquinones with eight isoprene units in which the two terminal isoprene moieties are cyclized. The main component corresponds to MK-8(H₆) and the minor component corre-

sponds to 2,3-epoxy-MK-8(H₆). The mean of triplicate determinations of the G+C content of the DNA of strain IMMIB R-1434^T was 68.08 mol%. These chemotaxonomic similarities to the validly described species of the genus *Nocardia* are supported by the high levels of 16S rRNA gene sequence similarity observed between isolate IMMIB R-1434^T and members of the genus *Nocardia*.

The almost complete 16S rRNA gene sequence of strain IMMIB R-1434^T, comprising 1466 nucleotides, was determined in this study. The unrooted phylogenetic tree (Fig. 1) was constructed from evolutionary distances by the neighbour-joining method. A total of 1400 nucleotides present in all strains between positions 38 and 1449 (*Escherichia coli* positions) were used for these analyses and compared with the sequences of the type strains of validly described *Nocardia* species. The 16S rDNA sequence comparison showed clearly that strain IMMIB R-1434^T is a member of the family *Nocardiaceae* (Stackebrandt *et al.*, 1997), since the sequence determined contains all of the signature nucleotides designated for this lineage. The high values for 16S rRNA gene sequence similarity to other previously described members of the genus *Nocardia* (95.4–98.6%) support the addition of strain IMMIB R-1434^T to this genus. It has been demonstrated previously that high levels of 16S rRNA gene sequence similarity between strains belonging to the same genus do not necessarily indicate membership of the same species (Yassin *et al.*, 1995, 1996). Here again, it is clear that high 16S rRNA gene sequence similarities can be found between strains that have low DNA–DNA reassociation values, since strain IMMIB R-1434^T shares 98.6% gene sequence similarity but only 19.8% reassociation with *Nocardia salmonicida*.

The novelty of strain IMMIB R-1434^T is supported by its unique physiological properties (Table 1). The physiological differences from *Nocardia salmonicida* are underpinned by the low reassociation rate (19.8%) between their DNAs. Differences from some of the validly described species of the genus *Nocardia* examined in this study can be taken from Table 1. The chemical (and morphological) data indicate clearly that the organism belongs to the genus *Nocardia*; 16S rDNA sequencing, DNA–DNA pairing and the biochemical results indicate that it forms a novel species when compared with the type strains of validly described species of *Nocardia*. Thus, on the basis of the results of the reported polyphasic taxonomic study, we consider that strain IMMIB R-1434^T merits classification as a novel species of the genus *Nocardia*, for which the name *Nocardia ignorata* sp. nov. is proposed.

Description of *Nocardia ignorata* sp. nov.

Nocardia ignorata (ig.no.ra'ta. L. fem. part. adj. *ignorata* unrecognized, unknown, pertaining to the fact that the type strain was mistakenly used as a member of the genus *Mycobacterium* in a mycobacterial quality control test).

Hyphae are Gram-positive and slightly acid-alcohol-fast. The orange-coloured vegetative hyphae are well-developed with irregular branches penetrating the agar and bear white aerial hyphae. At a late stage of growth, the hyphae fragment into rod-shaped elements. Grows at temperatures between 22 and 45 °C. Contains *meso*-diaminopimelic acid, galactose and arabinose in whole-cell hydrolysates (i.e. cell wall chemotype IV). Has mycolic acids with 46–54 carbon atoms and 0–3 double bonds (C_{46:2}, C_{48:0}, C_{48:2}, C_{49:0}, C_{49:1}, C_{50:0}, C_{50:1}, C_{50:3}, C_{51:0}, C_{51:1}, C_{52:0}, C_{52:1}, C_{53:1}, C_{54:0}, C_{54:1}, C_{55:1}, C_{56:1} and C_{57:1}) that are cleaved on pyrolysis to release fatty acids of C_{14:0}, C_{16:0}, C_{18:1} and C_{18:0}, with C_{16:0} as the major cleavage product. The cellular fatty acid profile is composed of saturated, unsaturated and 10-methyl branched fatty acids. Possesses type PII phospholipids with significant amounts of phosphatidylethanolamine. The menaquinone system consists of partially saturated menaquinones with eight isoprene units in which the two terminal isoprene moieties are cyclized; MK-8(H₆) is the major menaquinone and minor amounts of 2,3-epoxy-MK-8(H₆) are also present. Hydrolyses aesculin, gelatin, testosterone and urea but not adenine, casein, elastin, guanine, hypoxanthine, keratin, tyrosine or xanthine. Assimilates acetate, gluconate, glucose, maltose, mannitol, sucrose, trehalose and paraffin as carbon sources but not adipate, isoamyl alcohol, benzoate, citrate, lactate, arabinose, cellobiose, galactose, lactose, melzitose, raffinose, rhamnose, xylose, adonitol, *meso*-erythritol, *myo*-inositol, sorbitol, 2,3-butanediol, 1,2-propanediol, *m*-hydroxybenzoate or *p*-hydroxybenzoate. Utilizes gelatin and proline but not acetamide, alanine or serine as simultaneous carbon and nitrogen sources. The G+C content of the DNA is 68.08 mol%.

The type strain is strain IMMIB R-1434^T (= DSM 44496^T = NRRL B-24141^T).

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