NOTE

***Nocardia cyriacigeorgici* sp. nov.**A. F. Yassin,¹ F. A. Rainey² and U. Steiner³¹ Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, 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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Chemotaxonomic and 16S rRNA gene sequence analyses of an isolate from the bronchial secretions of a patient with chronic bronchitis demonstrated clearly that it belongs to the genus *Nocardia*. The 16S rRNA gene sequence data, as well as the biochemical characteristics of the isolate, indicated that it belongs to a new species that differs from previously described members of the genus *Nocardia*. The name *Nocardia cyriacigeorgici* sp. nov. is proposed for this isolate, and is represented by strain IMMIB D-1627^T (= DSM 44484^T).

Keywords: *Nocardia cyriacigeorgici*, *Nocardia asteroides*, chemotaxonomy, DNA–DNA hybridization, 16S rRNA gene sequence

The application of chemotaxonomic and molecular-systematic methods promoted a radical reappraisal of the genus *Nocardia* (Trevisan, 1889; Goodfellow, 1997; Lechevalier, 1976). The improved classification provides a sound framework for the recognition of additional species. The genus now encompasses 17 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). Interest in the taxonomy of the nocardiae has risen as some nocardiae cause a variety of suppurative diseases in human and animals, notably actinomycete mycetoma and nocardiosis (Boiron *et al.*, 1992; Lerner, 1996; Schaal & Lee, 1992). During the course of identification of clinical isolates under routine diagnostic conditions, strain IMMIB D-1627^T was found to have chemotaxonomic characteristics that suggested its inclusion in the genus *Nocardia*. This isolate was readily distinguished from other *Nocardia* species by biochemical tests. Based on phylogenetic and phenotypic evidence, it is proposed that strain IMMIB D-1627^T be classified as *Nocardia cyriacigeorgici* sp. nov.

Strain IMMIB D-1627^T was isolated from the bronchial secretion of a patient with chronic bronchitis. The strain 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 de-

scribed 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 by 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); the composition of the mycolic acids was determined by electron-impact mass spectrometry according to Collins *et al.* (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). The G+C content was 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 the purification of PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced by 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 the electrophoresis of the sequence reaction products. The ae2 editor (Maidak *et al.*, 1999) was used to align the

The GenBank accession number for the 16S rDNA sequence of strain DSM 44484^T is AF282889.

Fig. 1. Unrooted phylogenetic tree showing the position of strain IMMIB D-1627^T (= DSM 44484^T) within the radiation of species of the genus *Nocardia*. Bar, 2.0 inferred nucleotide substitutions per 100 nucleotides.

16S rRNA gene sequence of strain IMMIB D-1627^T against 16S rRNA gene sequences of the 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 analysis (Felsenstein, 1993). The tree topology was reanalysed using 1000 bootstrapped datasets and the programs SEQBOOT, DNADIST and CONSENSE of the PHYLIP package (Felsenstein, 1993).

The almost complete 16S rRNA gene sequence of strain IMMIB D-1627^T, comprising 1467 nucleotides, was determined in this study. The unrooted phylogenetic tree (shown in Fig. 1) was constructed from evolutionary distances by the neighbour-joining method. A total of 1377 nucleotides present in all strains between positions 38 and 1449 (*Escherichia coli* numbering) were used for these analyses and compared with the sequences of the type strains of the 17 validly described *Nocardia* species. The 16S rDNA sequence comparison showed clearly that strain IMMIB D-1627^T is a member of the family *Nocardiaceae* (Stackebrandt *et al.*, 1997), since the determined sequence 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* (96.6–

98.8%) support the addition of strain IMMIB D-1627^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 D-1627^T shares 98.8% gene sequence similarity but only 45% reassociation with *Nocardia abscessus*.

Chemotaxonomically, strain IMMIB D-1627^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 D-1627^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 the non-hydroxylated fatty acids. Pyrolysis gas chromatography of the pure mycolate from strain IMMIB D-1627^T released fatty acid methyl esters of C14:0 (10.04% of total cleavage products), C16:0 (63.09%), C18:1 (8.7%) and C18:0 (18.16%)

Table 1. Physiological characteristics of strain IMMIB D-1627^T and other significant validly described *Nocardia* species

Taxa are identified as: 1, IMMIB D-1627^T; 2, *N. abscessus* DSM 44432^T; 3, *N. abscessus* IMMIB V-12; 4, *N. paucivorans* DSM 44386^T; 5, *N. asteroides* ATCC 19247^T; 6, *Nocardia nova* ATCC 33726^T; 7, *Nocardia brasiliensis* DSM 43758^T; 8, *Nocardia farcinica* DSM 43665^T; 9, *Nocardia otitidiscaviarum* DSM 43242^T; 10, *Nocardia vaccinii* DSM 43285^T. Characteristics are scored as: +, positive; w, utilized weakly after 3 weeks of incubation; –, negative.

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

as pyrolysis cleavage products. Mass spectral analysis of the pure mycolate revealed the presence of mycolic acids with 46–54 carbon atoms with 0–3 double bonds. Gas chromatographic analyses of the non-hydroxylated fatty acid methyl esters revealed the presence of tetradecanoate (0.90 % of total fatty acids), pentadecanoate (0.24 %), *cis*-hexadecanoate (1.64 %), *trans*-hexadecanoate (32.17 %), hexadecanoate (0.35 %), heptadecanoate (0.82 %), octadecanoate (2.80 %), octadecanoate (11.76 %), tuberculostearic acid (10-methyl octadecanoate, 35.85 %), eicosenoate (0.55 %), eicosanoate (8.97 %), docosenoate (0.41 %) and docosanoate (3.54 %) as the major cellular fatty acid methyl esters. Polar lipid analysis showed that strain IMMIB D-1627^T contains phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol

mannoside and diphosphatidylglycerol as the characteristic phospholipids (i.e. phospholipid type PII *sensu* Lechevalier *et al.*, 1977). Mass spectral analysis of the respiratory quinones showed that strain IMMIB D-1627^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 corresponds to 2,3-epoxy-MK-8(H₆). The result of triplicate determinations of the G + C content of the DNA of strain IMMIB D-1627^T was 68.1 ± 0.1 mol %. These chemotaxonomic similarities to the 17 validly described species of the genus *Nocardia* are supported by the high levels of 16S rRNA gene sequence similarity observed between isolate IMMIB D-1627^T and members of the genus *Nocardia*.

Strain IMMIB D-1627^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 vegetative hyphae are well-developed with irregular branches penetrating the agar and bearing white aerial hyphae. At a late stage of growth, the hyphae fragment into rod-shaped elements characteristic of nocardiae.

The novelty of strain IMMIB D-1627^T is supported by its unique physiological properties (Table 1). The physiological differences from the newly described species *N. abscessus* and *Nocardia paucivorans* are underpinned by the low reassociation rates (45.0 and 32.0%, respectively) between their DNAs. Strain IMMIB D-1627^T is similar to the type species, *Nocardia asteroides*, with regard to hydrolysis of complex substrates. However, strain IMMIB D-1627^T can be differentiated from *N. asteroides* on the basis of carbon assimilation tests as well as on the basis of utilization of substrates as simultaneous carbon and nitrogen sources. These differences are also underlined by the low binding rate (61.1%) between the DNA of strain IMMIB D-1627^T and that of *N. asteroides*. Differences from some of the validly described species of the genus *Nocardia* examined in this study can be taken from Table 1. On the basis of the results of the polyphasic taxonomic study reported here, we consider that strain IMMIB D-1627^T merits classification as a new species of the genus *Nocardia*, for which the name *Nocardia cyriacigeorgici* sp. nov. is proposed.

Description of *Nocardia cyriacigeorgici* sp. nov.

Nocardia cyriacigeorgici (cy.ri.a.ci.ge.or'gi.ci. Gr. neut. n. *kyriako'n* house of the lord, church; M.L. neut. n. *cyriacum*, *cyriaci* church; Gr. adj. *georgikos* related to agriculture, related to St George; M.L. adj. *georgicus* of St George; M.L. adj. *cyriacigeorgici* of St George's church, referring to the origin of the name of the German town Gelsenkirchen where the type strain was isolated).

Hyphae are Gram-positive and slightly acid-alcohol-fast. The 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. The organism contains *meso*-diaminopimelic acid, galactose and arabinose in whole-cell hydrolysates (i.e. cell wall chemotype IV). It has mycolic acids with 46–54 carbon atoms and 0–3 double bonds (C_{46:0}, C_{46:3}, C_{48:0}, C_{48:3}, C_{50:0}, C_{50:1}, C_{50:3}, C_{52:0}, C_{52:1} and C_{54:1}) that are cleaved on pyrolysis to release fatty acids with C_{14:0}, C_{16:0}, C_{18:1} and C_{18:0} carbon atoms with C_{16:0} as the major cleavage product; thus *Nocardia cyriacigeorgici* has a cellular fatty acid profile that comprises 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. It hydrolyses aesculin, testosterone and urea but not adenine, casein, elastin, gelatin, guanine, hypoxanthine, keratin, tyrosine or xanthine. Assimilates acetate, gluconate, sucrose and paraffin as carbon sources but not adipate, benzoate, citrate, lactate, arabinose, cellobiose, galactose, glucose, lactose, maltose, melezitose, raffinose, rhamnose, trehalose, xylose, adonitol, *meso*-erythritol, *myo*-inositol, mannitol, sorbitol, isoamyl alcohol, 2,3-butanediol, 1,2-propanediol, *m*-hydroxybenzoate or *p*-hydroxybenzoate. Utilizes acetamide, but not alanine, gelatin, proline or serine, as simultaneous carbon and nitrogen sources. The G+C content of the DNA is 68.1 mol%. The type strain is IMMIB D-1627^T (= DSM 44484^T), which was isolated from the bronchial secretion of a patient suffering from chronic bronchitis.

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