**Nocardia abscessus sp. nov.**

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**INTRODUCTION**

Numerous taxonomic studies have revealed the heterogeneity of the species *Nocardia asteroides* by using a variety of parameters, including numerical taxonomy (Gordon & Mihm, 1957; Kurup & Schmitt, 1973; Tsukamura, 1977, 1982; Schaal & Reuterberg, 1978; Orchard & Goodfellow, 1980), antigen-induced delayed hypersensitivity (Magnusson & Mariat, 1968), DNA homology (Franklin & McClung, 1976; Mordarski et al., 1978) and antimicrobial susceptibility (Wallace et al., 1988). Goodfellow & Lechevalier (1986) recognized three subgroups of *N. asteroides*. Besides *N. asteroides sensu stricto*, these are *Nocardia farcinica* and *Nocardia nova*. *N. farcinica* was validly described by Trevisan (1889) and had served as the type species of the genus from 1954 to 1985 because the strain originally isolated from a case of bovine farcy by Nocard in 1888 appeared to be still extant as the type strain of the species. However, strains ATCC 3318⁷ and NCTC 4524, which were both thought to represent Nocard’s original isolate and thus to be identical, were found to be very different, even belonging to two different genera (*Nocardia* and *Mycobacterium*). Because of these inconsistencies the type species of the genus was changed to *N. asteroides* Eppinger & Blanchard 1896 in 1985, but the species *N. farcinica* was retained with ATCC 3318⁷ as type strain, representing a particularly homogeneous subgroup of the so-called *N. asteroides* complex (Tsukamura, 1977; Schaal & Reuterberg, 1978).

The species *N. nova* was proposed by Tsukamura in 1982 for another subgroup delineated within the *N. asteroides* complex. However, the primary description of this species was rather vague so that it was hardly possible to recognize members of this species.

Tests and characteristics suitable for identification of *N. farcinica* and *N. nova* under routine conditions were published by Wallace et al. (1990, 1991). However, they did not really solve the diagnostic dilemma because the tests recommended for the identification of *N. farcinica* by Schaal and co-workers (Goodfellow & Schaal, 1979; Schaal, 1984, 1992) were sufficient and were even more reliable than those of Wallace et al. (1990), but the identification of *N. nova* remained dubious despite the additional characters devised by Wallace et al. (1991).

Applying chemotaxonomic methods for identification of clinical isolates, four bacterial strains, namely IMMIB D-1592⁵, IMMIB D-1599, IMMIB V-12 and HIK N-63 (= *N. asteroides* ATCC 23824), were found to have chemotaxonomic characteristics which suggested their inclusion in the genus *Nocardia*. Tra-
ditional physiological markers primarily suggested their inclusion in the so-called *N. asteroides* complex, which is stressed by the fact that one of the strains (HIK N-63 = ATCC 23824) had formerly been identified as *N. asteroides*. However, further physiological and phylogenetic investigations indicated that they belong to a new independent species which is different from previously described species of the genus *Nocardia*. In this paper we describe the morphological, chemotaxonomic, physiological and phylogenetic characteristics of this new species for which the name *N. abscessus* sp. nov. is proposed.

**METHODS**

**Bacterial strains.** Strain IMMIB D-1592<sup>+</sup> was isolated from a joint abscess of a 56-year-old male patient with a complete endoprosthes in one of his knees. Strain IMMIB D-1599 had been obtained from a severely extended abscess of the fibula of a 61-year-old immunosuppressed patient. Strain IMMIB V-12 was recovered from the drainage of the leg of a 34-year-old patient. Strain HIK N-63 (= IMMIB N-63) was received from M. Tsukamura who suggested it to be the neotype of *N. asteroides* (Tsukamura, 1969). This isolate was also deposited in the American Type Culture Collection (ATCC), Manassas, VA, USA, under the number ATCC 23824 by the same author. The other strains, *N. asteroides* ATCC 19247<sup>+</sup> and *N. nova* ATCC 33726<sup>+</sup> were obtained from the ATCC. *Nocardia brasiliensis* DSM 43758<sup>+</sup>, *N. farcinica* DSM 43665<sup>+</sup> and *Nocardia otitidiscaviarum* DSM 43242<sup>+</sup> were provided by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). *Nocardia paucivorans* DSM 44386<sup>+</sup> was described previously (Yassin et al., 2000).

**Morphology and pigmentation.** Strains IMMIB D-1592<sup>+</sup>, IMMIB D-1599, IMMIB V-12 and HIK N-63 were grown on Brain Heart Infusion (BHI; Difco) agar and were examined for pigmentation, production of aerial hyphae and other morphological characteristics. Cultures were grown for 4 weeks and observed weekly. Air-dried smears from BHI agar cultures were stained by the Gram and Ziehl–Neelsen methods to determine the Gram reaction and acid-fastness, respectively.

**Physiological characteristics.** To determine decomposition of adenine, guanine, hypoxanthine, xanthine, tyrosine, elastin, keratin and testosterone, the method of Gordon (1967) was used. Aesculin decomposition was tested by the method of Gordon (1966) and a gelatin hydrolysis test was performed by the method of Gordon & Mihm (1957). The urea decomposition test was carried out using urea agar base (catalogue no. CM 53; Oxoid) after 2–2.5% urea had been added. To determine the utilization of a substrate as carbon and nitrogen source, we used media described previously (Yassin et al., 1995).

**Cell chemistry.** The strains studied were cultivated at 37 °C in shake flasks containing BHI broth for 1 week. After checking for purity at maximum growth, the organisms were killed with formaldehyde (1%, v/v), harvested by centrifugation, washed with distilled water and freeze-dried. Analysis of whole-cell hydrolysates for amino acids was performed by the method of Becker et al. (1964) and analysis of whole-cell sugars was performed by the method of Lechevalier (1968) using 20 × 20 cm pieces of Merck 5574 cellulose F aluminium sheets. Lipids were extracted using acid methanolysis and mycolic acids were detected with one-dimensional TLC; pyrolysis GC of the mycolate was performed as described previously (Yassin et al., 1993a); mycolic acid composition was determined using electron-impact MS according to Collins et al. (1982). Non-hydroxylated fatty acids were purified with preparative TLC and then separated, identified and quantified by GC as described by Yassin (1988). Menaquinones were extracted and purified by the method of Collins et al. (1977) and were identified by using a Finnigan Mat 212 mass spectrometer. Phospholipids were extracted, purified and identified as described previously (Yassin et al., 1993b).

**DNA isolation and characterization.** DNA was isolated by a modification of the phenol method (Saito & Miura, 1963) as described previously (Yassin et al., 2000). The isolated DNA was then purified by chromatography on hydroxyapatite using the method of Cashion et al. (1977). G+C contents were determined by HPLC (Mesbah et al., 1989) using λ phage DNA as reference.

**DNA–DNA hybridization studies.** Levels of DNA–DNA hybridizations between the DNAs from strains IMMIB D-1592<sup>+</sup>, IMMIB D-1599, IMMIB V-12, *N. paucivorans* DSM 44386<sup>+</sup> and *N. asteroides* ATCC 19247<sup>+</sup> were determined spectrophotometrically from renaturation rates (De Ley et al., 1970; Huss et al., 1983; Jahne, 1992).

**16S rRNA gene sequence determination.** Genomic DNA extraction, PCR-mediated amplification of 16S rDNA and purification of PCR products were carried out using procedures described previously (Rainey et al., 1996). Purified PCR products were sequenced using the Tag DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) as described by the manufacturer. The Applied Biosystems 310 DNA Genetic Analyzer was used for the electrophoresis of the ethanol-precipitated sequence reaction products.

**Phylogenetic analyses of 16S rRNA gene sequence data.** The ae2 editor (Maidak et al., 1999) was used to align the 16S rRNA gene sequences of strains IMMIB D-1592<sup>+</sup>, IMMIB D-1599, IMMIB V-12 and HIK N-63 against the 16S rRNA gene sequences of the validly described species of the genus *Nocardia* available from the public databases. The strain designations and nucleotide sequence accession numbers of the analysed sequences are as follows: *N. asteroides* ATCC 19247<sup>+</sup>, X84850; *N. brasiliensis* DSM 43758<sup>+</sup>, X80608; *Nocardia brevicatena* ATCC 15333<sup>+</sup>, X80600; *Nocardia carnea* DSM 43397<sup>+</sup>, X80607; *Nocardia crassa* ATCC 700418<sup>+</sup>, Z37989; *N. farcinica* ATCC 3318<sup>+</sup>, X80595; *Nocardia flavosea* JCM 3332<sup>+</sup>, Z46754; *N. nova* ATCC 33726<sup>+</sup>, X80593; *N. otitidiscaviarum* ATCC 14629<sup>+</sup>, X80599; *N. paucivorans* DSM 44386<sup>+</sup>, AF 179865; *Nocardia pseudo-brasiliensis* ATCC 51512<sup>+</sup>, X84857; *Nocardia salmonicida* JCM 4826<sup>+</sup>, Z46750; *Nocardia seriolutea* ATCC 43993<sup>+</sup>, X80592; *Nocardia transvalensis* DSM 43405<sup>+</sup>, X80609; *Nocardia uniformis* JCM 3224<sup>+</sup>, Z46752; *Nocardia vaccinii* DSM 43285<sup>+</sup>, Z36927; *Nocardia species* HIK N-63 (= ATCC 23824), X84851. The alignment used in the phylogenetic analyses comprised 1381 nt positions between positions 38 and 1449 (*Escherichia coli* numbering; Brosius et al., 1978). The programs of the PHYLIP package, including DNADIST and NEIGHBOUR, were used for the phylogenetic analyses (Felsenstein, 1993). The tree topology was reanalysed using 1000 bootstrapped data sets and the programs SEQBOOT, DNADIST and CONSENSE of the PHYLIP package (Felsenstein, 1993).
RESULTS

Micromorphology

The hyphae of strains IMMIB D-1592<sup>T</sup>, IMMIB D-1599, IMMIB V-12 and HIK N-63 were Gram-positive and slightly acid/alcohol-fast. The vegetative hyphae were well-developed with irregular branches penetrating the agar and bearing white aerial hyphae. At a late stage of growth the filaments fragment into rod-shaped elements characteristic of nocardiae.

Physiological characteristics

The physiological properties of strains IMMIB D-1592<sup>T</sup>, IMMIB D-1599, IMMIB V-12 and HIK N-63 are shown in Table 1. They were able to utilize acetate, citrate glucose, maltose, hypoxanthine, keratin, tyrosine and xanthine. All of adenine, aesculin, casein, elastin, gelatin, guanine, testosterone and urea but were not able to hydrolyse are shown in Table 1. They were able to hydrolyse other validly described Nocardia

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<th>HIK N-63</th>
<th>N. asteroides ATCC 19247&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. nova ATCC 33720&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. brasiliensis DSM 43758&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. farcinica DSM 43665&lt;sup&gt;T&lt;/sup&gt;</th>
<th>N. otitidiscaviarum DSM 43242&lt;sup&gt;T&lt;/sup&gt;</th>
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Utilization as sole sources of carbon and energy:

- Acetamide
- Acetate
- Citrate
- Gluconate
- l-Arabinose
- Galactose
- Malate
- Maltose
- Rhamnose
- Succinate
- Trehalose
- Xylose
- myo-Inositol
- Mannitol
- Isoamyl alcohol
- 2,3-Butanediol
- 1,2-Propanediol
- m-Hydroxybenzoate
- p-Hydroxybenzoate

Utilization as sole sources of carbon and nitrogen:

- Acetamide
- L-Alanine
- Gelatin
- Proline
- Serine

Table 1. Differential physiological characteristics of strains IMMIB D-1592<sup>T</sup>, IMMIB D-1599, IMMIB V-12, HIK N-63 and other validly described Nocardia species
IMMIB D-1599, a C16:0 acid represents the main pyrolysis product (58-3-60-8%) of the mycolic acids isolated from strains IMMIB V-12 and HIK N-63. Mass spectral analysis of the pure mycolate reveals the presence of mycolic acids with 46-56 carbon atoms with 0-4 double bonds. GC analyses of the non-hydroxylated fatty acid methyl esters revealed the presence of tetradecanoate (0-91±0-28% of total fatty acids), pentadecanoate (0-31±0-23%), hexadecanoate (3-36±0-76%), hexadecanoate (42-32±0-52%), 10-methylhexadecanoate (0-81±0-11%), heptadecanoate (0-38±0-23%), octadecanoate (7-41±0-57%), octadecanoate (8-93±0-06%), tuberculostearic acid (10-methylloctadecanoate; 28-15±2-8%), eicosanoate (0-87±0-14%), eicosanoate (0-28±0-0%), docosanoate (0-33±0-05%), docosanoate (3-38±1-37%), tetradecanoate (0-91±0-36%) and tetradecanoate (1-67±0-65%) as cellular fatty acids.

Polar lipid analysis showed that strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 contain phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol mannoside and diphosphatidylglycerol as characteristic phospholipids (i.e. they have phospholipid type PII sensu Lechevalier et al., 1977).

The composition of the respiratory quinones of strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 was examined. On reverse phase TLC using RP-18, the lipid extracts from the first two strains showed only one band while the extracts from strains IMMIB V-12 and HIK N-63 produced two separate bands. MS analysis of the main component from the four strains showed a strong peak at m/z 720 attributable to M² in the high mass region as well as peaks of considerable intensity at m/z 582 and 594. These correspond to a hexahydrogenated menaquione with eight isoprene units in which the terminal two isoprene moieties are cyclized (Howarth et al., 1986). The second band isolated from strains IMMIB V-12 and HIK N-63 displayed, in the high mass region, a strong peak at m/z 736 attributable to M², with a second intense peak at m/z 720 (corresponding to loss of oxygen from M²), while in the low mass region it showed a peak of medium intensity at m/z 241 (indicative of an additional oxygen in the naphthoquinone ring system). This mass spectrum of the second band corresponds to a hexahydrogenated epoxymenaquinone with eight isoprene units in which the last two isoprene moieties are cyclized (Collins et al., 1987).

**Phylogenetic analysis**

The almost complete 16S rRNA gene sequence of strains IMMIB D-1592T, IMMIB D-1599 and IMMIB V-12, comprising 1457 nt (95% of the *E. coli* sequence; Brosius et al., 1978), were determined in this study. A partial sequence of strain HIK N-63 (= ATCC 23824) comprising 1236 nt was also determined and found to be identical to the sequence of the same strain available from the public databases under the accession number X84851. Therefore, the database sequence was used in all analyses. The unrooted phylogenetic tree shown in Fig. 1 was constructed from evolutionary distances by the neighbour-joining method. A total of 1381 nt present in all strains between positions 38 and 1449 (*E. coli* numbering) was used for these analyses and compared with the

![Unrooted phylogenetic tree showing the position of strains IMMIB D-1592T (= DSM 44432T), IMMIB D-1599, V-12 and N-63 within the radiation of species of the genus Nocardioid. Bootstrap values, expressed as a percentage of 1000 replications, are shown at the branching points. The scale bar indicates 2 inferred nt substitutions per 100 nt.](image-url)
sequences of the type strains of the 16 validly described Nocardia species. The 16S rRNA gene-sequence-based phylogenetic tree shown in Fig. 1 indicates the position of strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 within the radiation of species of the genus Nocardia. The 16S rDNA sequences of these strains contain all of the 16S rDNA signature nucleotides designated for the family Nocardiaceae (Stackebrandt et al., 1997). 16S rRNA gene sequence similarity values between strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 and the other Nocardia species included in the tree (Fig. 1) show these four strains to be identical at the 16S rRNA gene sequence level. The 16S rDNA gene sequence similarity values of the sequences of strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 to other Nocardia species are in the range 96–98.9% (data not shown). These values and the results shown in the phylogenetic tree (Fig. 1) indicate that strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 are members of the genus Nocardia and show a high degree of relatedness to the type strain of N. asteroides, sharing 98.9% 16S rDNA gene sequence similarity. The bootstrap values for the internal branches are for the most part not significant, the majority being less than 95% (Fig. 1).

### DNA base composition

The results of triplicate determinations of the G+C content of the DNA of strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 were 67.9±0.2, 69.8±0.6, 67.5±0.2 and 68.1±0.5 mol%, respectively.

### DNA–DNA hybridization

The results of DNA–DNA hybridization experiments are shown in Table 2. The levels of relatedness (binding rates) between the DNAs of strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 ranged from 72.0 to 100%. These results clearly show that these isolates belong to the same genospecies. The level of binding between their DNAs and the DNA of N. asteroides ATCC 19247T (23.7–56.8%) as well as the DNA of N. paucivorans DSM 44386T (32–60%) clearly shows that they differ from both species.

### DISCUSSION

The 16S rDNA sequence comparison clearly shows that strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 are members of the family Nocardiaceae (Stackebrandt et al., 1997), since the determined sequences for these strains contain all of the signature nucleotides designated for this lineage. The high 16S rDNA gene sequence similarity values to other previously described members of the genus Nocardia (Stackebrandt et al., 1997) support the addition of strains IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 to this genus. Although it has been demonstrated previously that high levels (>99%) of 16S rDNA gene sequence similarity between strains belonging to the same genus do not indicate membership of the same species (Yassin et al., 1995, 1996), in this case it is clear from the results of DNA–DNA hybridization studies that the four strains are members of the same species, a new species which is distinct from those currently belonging to the genus Nocardia. The 16S rDNA gene sequence analyses and the results of DNA–DNA hybridization studies demonstrate the distinctness of the new isolates from the type strain of N. asteroides. Although the new strains and the type strain of N. asteroides share 98.9% 16S rDNA gene sequence similarity they have low reassociation values at the DNA level (23.7–56.8%), indicating their novel species status. The low bootstrap value (24%) for the branching point of the new isolates and the type strain of N. asteroides demonstrates that this relationship has no significance and that these strains could cluster with other lineages within this phylogenetic group. This also supports the proposal to assign them to a new species.

The physiological and chemotaxonomic characteristics of isolates IMMIB D-1592T, IMMIB D-1599, IMMIB V-12 and HIK N-63 were compared with those of the validly described species of the genus Nocardia abscessus sp. nov.
Nocardia. Chemotaxonomically, the test strains contained chemical markers which supported their assignment to the genus. These chemotaxonomic similarities are supported by the high levels of 16S rRNA gene sequence similarity observed between isolates IMMIB D-1592<sup>T</sup>, IMMIB D-1599, IMMIB V-12, HIK N-63 and the 16 validly described species of the genus.

In contrast to the chemotaxonomic similarities of the four isolates and the validly described species of Nocardia, the results of our physiological tests (Table 1) revealed both clear similarities and clear differences between the four strains and representatives of validly described Nocardia species. The physiological similarities between the test strains are supported by the high levels of reassociation between their DNAs (Table 2). Their physiological differences to N. asteroides ATCC 19247<sup>T</sup> and N. paucivorans DSM 44386<sup>T</sup> are supported by the low level of reassociation of their DNAs with the DNAs of the latter two species (Table 2).

Based on the phenotypic and the genotypic data presented here, strains IMMIB D-1592<sup>T</sup>, IMMIB D-1599, IMMIB V-12 and HIK N-63 can be assigned to a new species, Nocardia abscessus sp. nov.

**Nocardia abscessus sp. nov.**

*Nocardia abscessus* (abs.ces’sus. L. gen. masc. n. abscessus of or derived from an abscess, referring to the characteristic clinical conditions from which the organisms were isolated).

The filamentous cells formed in early growth stages are Gram-positive, slightly acid/alcohol-fast and fragment, in a later stage of growth, into rod-shaped elements. Mature colonies measure 0.5–1.0 mm in diameter. The substrate mycelium is well developed and shows an orange colour. Aerial hyphae are abundant and white. Type of metabolism and chemotaxonomic properties are of the genus Nocardia. D-Glucose, maltose, rhamnose (some strains are not able to utilize this sugar), sucrose, trehalose, galactose, lactose, D-melezitose, raffinose, xylose, adonitol, meso-erythritol, myo-inositol, D-mannitol, D-sorbitol, isoamyl alcohol, 2,3-butanediol, 1,2-propanediol, m-hydroxybenzoate, p-hydroxybenzoate, adipate, benzoate, glutonate and lactate are not utilized. Acetamide, L-alanine, gelatin, proline and serine are not utilized as simultaneous nitrogen and carbon sources. Testosterone and urea are hydrolysed but adenine, asesculin, casein, elastin, gelatin, guanine, hypoxanthine, keratin, tyrosine and xanthine are not. The G+C content is 67.9±0.2 mol% for the type strain and ranges from 67.5 to 69.8 mol% for the species. The type strain of *N. abscessus* is strain IMMIB D-1592<sup>T</sup> (= DSM 44432<sup>T</sup>), isolated from a joint abscess of a 56-year-old man with a complete endoprosthesis of one of his knees.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


