**Nocardia uniformis nom. rev.**

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**A soil isolate representing the putatively novel species 'Nocardia uniformis' was found to have morphological, staining and chemotaxonomic properties consistent with its classification in the genus Nocardia. An almost complete sequence of the 16S rDNA of the strain was determined following cloning and sequencing of the amplified gene. The sequence was aligned with those available for nocardiae and phylogenetic trees were inferred using four tree-making algorithms. The organism was consistently associated with the type strain of *Nocardia otitidiscaviarum* albeit with a relatively low bootstrap value recorded for neighbour-joining analysis. The strain was also readily separated from representatives of all validly described *Nocardia* species using a set of phenotypic properties. The genotypic and phenotypic data indicate that the strain should be assigned to the genus *Nocardia* as a new species. The name proposed for the new species is *Nocardia uniformis*. The type strain is JCM 3224T.**

**Keywords:** *Nocardia uniformis* nom. rev., polyphasic taxonomy, 16S rDNA sequencing

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**INTRODUCTION**

The genus *Nocardia* is well-defined for the first time in its long and tortuous taxonomic history mainly due to the application of chemotaxonomic and molecular systematic methods (Lechevalier, 1976; Goodfellow, 1997). The revised genus encompasses fourteen validly described species which form a monophyletic clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes, the mycolata (Chun & Goodfellow, 1995; Friedman et al., 1998; Isik, 1998). Despite improvements in nocardial systematics, the taxonomic position of a number of strains putatively assigned to the genus needs to be resolved.

The name 'Nocardia uniformis' was proposed by Marton & Szabó (1959) for actinomycetes isolated from the B1 horizon of a solonchak-solonetz soil in eastern Hungary. The organism, which was described on the basis of morphological and nutritional properties, was subsequently found to have chemical features consistent with its classification in the genus *Nocardia* (Mordarska et al., 1972; Yano et al., 1990). Strains of this actinomycete were found to be most closely related to *Nocardia otitidiscaviarum* in an extensive numerical phenetic survey of the genus *Nocardia* (Goodfellow, 1971) though the putative type strain was later found to form a distinct single-membered cluster (Yano et al., 1990). 'Nocardia uniformis' was described as a species incertae sedis in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (McClung, 1974), but was neither mentioned in the corresponding ninth edition (Goodfellow & Lechevalier, 1989) nor cited in the Approved Lists of Bacterial Names (Skerman et al., 1980).

The aim of the present investigation was to determine the taxonomic relationships of strain JCM 3224T using a combination of genotypic and phenotypic properties. It was clear from the resultant data that the organism merits recognition as a new species of *Nocardia*, namely *Nocardia uniformis* nom. rev.

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**METHODS**

**Bacterial strains and cultivation conditions.** Strain JCM 3224T was grown in shake flasks containing modified Sauton's broth (Mordarska et al., 1972) for 7 d at 30 °C; biomass was harvested by centrifugation and washed twice with distilled water. The strain was maintained as glycerol suspensions (20 %, v/v) at −20 °C.

**Phenotypic characterization.** The test strain was examined for a broad range of phenotypic properties as described in an earlier investigation (Isik et al., 1999). Additional enzymic tests were carried out using an API ZYM kit (bioMérieux) following the instructions of the manufacturer. The inoculated kit was incubated at 37 °C for 4 h.
16S rDNA sequencing. Isolation of chromosomal DNA and PCR, cloning and sequencing of the resultant 16S rDNA preparation of strain JCM 3224T was carried out using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer, as described previously (Chun & Goodfellow, 1995). The 16S rDNA sequence of the test strain was aligned manually against sequences of representative mycolata strains retrieved from the GenBank and EMBL databases. Evolutionary trees were inferred using the Fitch & Margoliash (1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated after Jukes & Cantor (1969). The unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. The phylogenetic analyses were carried out using the PHYLIP package (Felsenstein, 1993).

Chemotaxonomy. The diagnostic isomer of diaminopimelic acid and predominant whole-organism sugars of the test strain were detected using established procedures (Lechevalier & Lechevalier, 1980). Menaquinones were extracted from freeze-dried biomass (50 mg) and analysed as described previously (Chun & Goodfellow, 1995).

RESULTS AND DISCUSSION

An almost complete 16S rDNA sequence (1471 nt) was obtained for strain JCM 3224T. Comparison of this sequence with corresponding nucleotide sequences of representative mycolata clearly indicated that the organism belongs to the genus Nocardia (data not shown). The test strain contained meso-diaminopimelic acid and major amounts of arabinose and galactose (wall chemotype IV sensu Lechevalier & Lechevalier, 1970) and predominant amounts of hexahydrogenated menaquinone with eight isoprene units where the end two are cyclized. These results confirm and extend those of earlier studies which indicated that strain JCM 3224T has chemical and morphological properties characteristic of nocardiae (Marton & Szabo, 1959; Goodfellow, 1971; Mordarska et al., 1972; Yano et al., 1990).

The phylogenetic trees show that strain JCM 3224T is most closely associated with N. otitidiscaviarum albeit with a relatively low bootstrap value (48%) in the analysis based on the neighbour-joining method (Fig. 1). The 16S rDNA sequence similarity between strain JCM 3224T and N. otitidiscaviarum NCTC 19349T is 98.1%, a value which corresponds to 22 nt differences out of 1351 nt positions. Similarity values around this level have been reported between several validly described Nocardia species, for instance, between N. otitidiscaviarum and Nocardia seriolae (98.0%) and Nocardia farcinica and Nocardia nova (98.4%) (Chun & Goodfellow, 1995); these taxa can readily be distinguished using standard phenotypic tests (Goodfellow, 1971; Isik, 1998). Strain JCM 3224T can be distinguished from representatives of all validly described species of Nocardia, including N. otitidiscaviarum using a set of phenotypic properties (Table 1). The genotypic and phenotypic data clearly show that
The description is based on data derived from earlier studies (Marton & Szabó, 1959; Goodfellow, 1971). Aerobic, Gram-positive, catalase-positive, acid–alcohol-fast, non-motile actinomycete which forms an extensively branched substrate mycelium which fragments in situ into rod-shaped to coccoid elements (0.7–1.1 μm). A yellowish-orange substrate mycelium which is resistant to lysozyme. 2-Naphthyl caprylate, 2-naphthyl phosphate (pH 8.5), 2-cystyl-2-naphthylamide, L-leucyl-2-naphthylamide, 2-glutaryl-phenylalanine-2-naphthylamide, naphthol AS-BI phosphate, 2-naphthyl α-D-glucopyranoside and 6-bromo-2-naphthyl-β-D-glucopyranoside are cleaved but 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), L-valyl-naphthylamide, N-benzyol-DL-arginine-2-naphthylamide, 2-naphthyl α-L-fucopyranoside, 2-naphthyl β-D-galactopyranoside, 1-naphthyl N-acetyl-β-D-glucosaminide and naphthol AS-BI β-D-glucuronide are not. Acid is formed from arbutin, D(−)fructose, D(+)-glucose, glyceral and meso-inositol but not from adonitol, amygdalin, D(+)- or L(−)-arabinose, D(+)-cellobiose, dulcitol, ethanol, D(+)galactose, glycosgen, inulin, L(+)lactose, D(+)melizitose, D(+)raffinose, α-L-rhamnose, salicin, D(+)sorbitol, D(+)sucrose or D(+)xyllose. D(+)Glucose, meso-inositol, D(+)mannitol, D(+)mannose, paraffin, sebacic acid, sodium acetate, sodium n-butylate, sodium fumarate, sodium hydro-

strain JCM 3224\textsuperscript{T} merits recognition as a distinct species in the genus \textit{Nocardia}. It is therefore proposed that the organism be classified in the genus \textit{Nocardia} as \textit{Nocardia uniformis} nom. rev.

### Description of \textit{Nocardia uniformis} nom. rev.

\textit{Nocardia uniformis} (u.ni.for'mis, L. masc. adj. uniformis having only one form, uniform).

The description is based on data derived from earlier studies (Marton & Szabó, 1959; Goodfellow, 1971). Aerobic, Gram-positive, catalase-positive, acid–alcohol-fast, non-motile actinomycete which forms an extensively branched substrate mycelium which fragments in situ into rod-shaped to coccoid elements (0.7–1.1 μm x 1–4 μm). A yellowish-orange substrate mycelium which is resistant to lysozyme. 2-Naphthyl caprylate, 2-naphthyl phosphate (pH 8.5), 2-cystyl-2-naphthylamide, L-leucyl-2-naphthylamide, 2-glutaryl-phenylalanine-2-naphthylamide, naphthol AS-BI phosphate, 2-naphthyl α-D-glucopyranoside and 6-bromo-2-naphthyl-β-D-glucopyranoside are cleaved but 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), L-valyl-naphthylamide, N-benzyol-DL-arginine-2-naphthylamide, 2-naphthyl α-L-fucopyranoside, 2-naphthyl β-D-galactopyranoside, 1-naphthyl N-acetyl-β-D-glucosaminide and naphthol AS-BI β-D-glucuronide are not. Acid is formed from arbutin, D(−)fructose, D(+)-glucose, glyceral and meso-inositol but not from adonitol, amygdalin, D(+)- or L(−)-arabinose, D(+)-cellobiose, dulcitol, ethanol, D(+)galactose, glycosgen, inulin, L(+)lactose, D(+)melizitose, D(+)raffinose, α-L-rhamnose, salicin, D(+)sorbitol, D(+)sucrose or D(+)xyllose. D(+)Glucose, meso-inositol, D(+)mannitol, D(+)mannose, paraffin, sebacic acid, sodium acetate, sodium n-butylate, sodium fumarate, sodium hydro-

### Table 1. Phenotypic characters which distinguish \textit{Nocardia uniformis} strain JCM 3224\textsuperscript{T} from other nocardiae

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Symbols: +, >90% of strains positive; −, 90% of strains negative; ND, not determined; T, type strain.
gen malate, sodium propionate, sodium pyruvate, sodium succinate and testosterone are used as sole sources of carbon for energy and growth but not D(-)fucose, D(+)-arabitol, arbutin, D(+)-cellobiose, dulcitol, D(-)fucose, D(+)-galactose, glycogen, inulin, L(+)-lactose, D(-)-maltose, D(+)-mannitol, α-D-melibiose, D(+)-melezitose, D(+)-raffinose, α-L-rhamnose, D(-)-ribose, salicin, D(+)-sorbitol, D(+)-sucrose, D(+)-trehalose, D(+)-xylulose, xylitol, D(-)-alanine, betaine-HCl, benzamide, m- or p-hydroxybenzoic acid, o-hydroxybenzaldehyde, D(-)-norleucine, D-mandelic acid, pimelic acid, L-proline, protocatechuic acid, sodium acetate, sodium adipate, sodium benzoate, sodium azelate, sodium citrate, sodium gluconate, sodium hippurate, sodium lactate, sodium malonate, sodium n-octoate, sodium oleate, sodium oxalate, sodium tartrate, sodium valerate, L-threonine, L-tryptophan or L-tyrosine. The organism grows from 14 to 40 °C, from pH 6.0 to 10 and in the presence of crystal violet (0.001 w/v), potassium tellurite (0.01 w/v) or thallous acetate (0.01 w/v) but not at 10 °C, pH 5.0 or in the presence of crystal violet (0.001 w/v), potassium tellurite (0.01 w/v), sodium azide (0.0002 w/v), sodium chloride (10%, w/v), potassium tellurite (0.01%, w/v), sodium chloride (10%, w/v), tetrazolium (0.1%, w/v) or thallous acetate (0.01%, w/v). The organism was isolated from a degraded solonetz soil collected from the Hortobagy steppe in eastern Hungary. The species description is based on a limited carbon utilization pattern.

ACKNOWLEDGEMENTS

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