Nocardia uniformis nom. rev.

Kamil Isik,¹ Jongsik Chun,² Yung Chil Hah² and Michael Goodfellow¹

Author for correspondence: Michael Goodfellow. Tel: +44 191 222 7706. Fax: +44 191 222 5228.
e-mail: m.goodfellow@ncl.ac.uk

¹ Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

² Department of Microbiology, College of Natural Sciences and Research Center for Molecular Biology, Seoul National University, Seoul 151-742, Republic of Korea

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 3224^T.

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

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 3224^T 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.

METHODS

Bacterial strains and cultivation conditions. Strain JCM 3224^T 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.

The GenBank accession number for the 16S rDNA sequence of strain JCM 3224^T is Z46752.

Fig. 1. Unrooted neighbour-joining tree based on 1407 unambiguously aligned nucleotide positions. Asterisks indicate the branches that were also recovered using the Fitch-Margoliash, maximum-likelihood and maximum-parsimony methods. The numbers at the nodes indicate the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled data sets. Scale bar, 0.01 nucleotide substitutions per position.

16S rDNA sequencing. Isolation of chromosomal DNA and PCR, cloning and sequencing of the resultant 16S rDNA preparation of strain JCM 3224^T 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 corresponding 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 3224^T. 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 3224^T has chemical and morphological properties characteristic of nocardiae (Marton & Szabó, 1959; Goodfellow, 1971; Mordarska *et al.*, 1972; Yano *et al.*, 1990).

The phylogenetic trees show that strain JCM 3224^T 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 3224^T and *N. otitidiscaviarum* NCTC 19349^T 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 3224^T 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

Table 1. Phenotypic characters which distinguish *Nocardia uniformis* strain JCM 3224^T from other nocardiae

Strains: 1, *Nocardia uniformis* JCM 3224^T; 2, *Nocardia asteroides* N 317^T; 3, *Nocardia brasiliensis* (five strains); 4, *Nocardia brevicatena* DSM 43024^T; 5, *Nocardia carnea* DSM 43397^T; 6, *Nocardia crassostreae* (seven strains); 7, *Nocardia farcinica* (12 strains); 8, *Nocardia flavorosea* JCM 3332^T; 9, *Nocardia nova* (three strains); 10, *Nocardia otitidiscaviarum* (nine strains); 11, *Nocardia pseudobrasiliensis* (four strains); 12, *Nocardia salmonicida* JCM 4826^T; 13, *Nocardia seriolae* (four strains); 14, *Nocardia transvalensis* (four strains); 15, *Nocardia vaccinii* DSM 43285^T. Symbols: +, >90% of strains positive; –, 90% of strains negative; ND, not determined; T, type strain. Data were taken from the present study and from Chun *et al.* (1998), Friedman *et al.* (1998), Isik (1998) and Isik *et al.* (1999).

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Biochemical tests:															
Aesculin hydrolysis	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Nitrate reductase	+	+	+	–	+	ND	+	–	+	+	–	+	+	+	+
Urea hydrolysis	+	+	+	–	+	–	+	–	+	+	+	+	–	+	+
Decomposition of:															
Adenine	–	–	–	–	–	ND	–	–	–	–	+	–	–	–	–
Casein	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–
Elastin	+	–	+	–	–	ND	–	–	–	–	+	–	–	+	–
Hypoxanthine	+	–	+	–	–	–	–	–	–	+	+	–	–	+	–
Testosterone	+	+	+	+	+	ND	+	+	+	–	–	+	–	+	–
Tyrosine	+	–	+	–	–	–	–	–	–	–	+	+	–	–	–
Uric acid	+	–	–	ND	–	ND	–	–	–	+	+	–	–	+	–
Xanthine	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Growth on sole carbon sources:															
D(+) Mannitol (1%, w/v)	–	+	+	–	+	ND	–	–	+	+	+	+	–	+	+
α-L(–) Rhamnose (1%, w/v)	–	–	–	+	–	ND	+	–	–	–	–	–	–	+	+
D(+) Sorbitol (1%, w/v)	–	+	–	–	+	ND	–	–	+	–	+	+	–	+	–
Sodium citrate (0.1%, w/v)	–	+	+	–	–	–	–	–	–	–	+	+	+	–	–
Growth at 45 °C	–	–	+	–	–	–	+	+	–	+	–	–	–	–	–

strain JCM 3224^T merits recognition as a distinct species in the genus *Nocardia*. It is therefore proposed that the organism be classified in the genus *Nocardia* as *Nocardia uniformis* nom. rev.

Description of *Nocardia uniformis* nom. rev.

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 × 1.1 × 4.0 µm). A yellowish-orange substrate mycelium carries whitish, sparse to abundant, aerial hyphae. Colony elevation is convex to irregular and colony margins are filamentous. Diffusible pigments are not formed. DNA, elastin, guanine, hypoxanthine, RNA, starch, testosterone, Tweens 20 and 80, L-tyrosine, uric acid and xanthine are degraded, but not adenine, casein, cellulose, chitin, gelatin, keratin or xylan. Aesculin, allantoin, arbutin and urea are hydro-

lysed, nitrate is reduced but activity is not shown against hippurate. The organism is oxidase-negative, does not produce *m*- or *p*-nitrophenoloxidases and 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-benzoyl-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, glycerol and *meso*-inositol but not from adonitol, amygdalin, D(+) or L(–)arabinose, D(+)cellobiose, dulcitol, ethanol, D(+)galactose, glycogen, inulin, L(+)lactose, D(+)melezitose, D(+)raffinose, α-L-rhamnose, salicin, D(+)sorbitol, D(+)sucrose or D(+)xylose. D(+)Glucose, *meso*-inositol, D(+)mannitol, D(+)mannose, paraffin, sebacic acid, sodium acetate, sodium n-butyrate, sodium fumarate, sodium hydro-

gen malate, sodium propionate, sodium pyruvate, sodium succinate and testosterone are used as sole sources of carbon for energy and growth but not adonitol, amygdalin, D(+) or L(-)arabinose, 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(+)turanose, D(+)xylose, xylitol, D- α -alanine, betaine-HCl, benzamide, *m*- or *p*-hydroxybenzoic acid, *o*-hydroxybenzaldehyde, DL-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-octate, 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 (0.0001 %, w/v), phenyl ethanol (0.1 %, v/v), potassium tellurite (0.01 %, w/v) and sodium azide (0.0001 %, w/v) but not at 10 °C, pH 5.0 or in the presence of crystal violet (0.001 %, w/v), sodium azide (0.0002 %, 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 solonchak-solonetz soil collected from the Hortobagy steppe in eastern Hungary. The species description is based on a single strain and hence serves as the type strain description. The type strain is JCM 3224^T (= CBS 224.60^T = DSM 43136^T = IFO 13702^T = NCIB 963^T). Marton & Szabó (1959) considered the most characteristic features of *Nocardia uniformis* to be its uniform appearance on a variety of media and its limited carbon utilization pattern.

ACKNOWLEDGEMENTS

Part of this work was supported through the UK-Korea Actinomycete Research Programme (BBSRC grant R185/05688/01), the British Council Academic Link Scheme (Seoul, Korea) and by a Korean Science and Engineering Council (KOSEF) grant to the Research Center for Molecular Microbiology. K.I. was supported by a scholarship from the University of Ondokuz Mayıs, Samsun and the Government of Turkey.

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