Nocardia uniformis nom. rev.

Kamil Isik,1 Jongsik Chun,2 Yung Chi 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

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.

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

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.

The GenBank accession number for the 16S rDNA sequence of strain JCM 3224T is Z46752.
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 ATCC 14629T 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
Table 1. Phenotypic characters which distinguish Nocardia uniformis strain JCM 3224T from other nocardiae

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical tests:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Casein</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elastin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Testosterone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uric acid</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Xanthine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth on sole carbon sources:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D(+) Mannitol (1 %, w/v)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α-L(−) Rhamnose (1 %, w/v)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D(+) Sorbitol (1 %, w/v)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sodium citrate (0-1 %, w/v)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 45 °C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

strain JCM 3224T 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.formis. 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-mobile actinomycete which forms an extensively branched substrate mycelium which fragments in situ into rod-shaped to cocoid elements (0.7–1.1 x 1.4–4.0 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 hydrolysed, 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, glyceral and meso-inositol but not from adonitol, amygdalin, D(+) or L(−)arabinose, D(+)-cellobiose, dulcitol, ethanol, D(+)galactose, glycosgen, inulin, L(+)lactose, D(+)melitzitose, D(+)raffinose, α-L-rhamnose, salicin, D(+)sorbitol, D(+)sucrose or D(+)xylose. D(+)Glcucose, 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(+)-arabitol, arbutin, D(+)-cellobiose, dulcitol, D(-)-fucose, D(+)-galactose, glycogen, inulin, L(+)-lactose, D(+)-maltose, D(+)-mannitol, D(+)-melibiose, D(+)-melezitose, D(+)-raffinose, D(-)-rhamnose, D(-)-ribose, salicin, D(+)-sorbitol, D(+)-sucrose, D(+)-trehalose, D(+)-turanose, D(+)-xylitol, D(+)-xylene, betaine-HCl, benzamide, m- or p-hydroxybenzoic acid, o-hydroxybenzaldehyde, d- or L-proline, protocatechuic acid, sodium acetate, sodium adipate, sodium benzoate, sodium azelate, sodium citrate, sodium oxalate, sodium tartrate, sodium valerate, L-threonine, 1,3-propanediol, pyruvic acid, 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 g/v) and sodium chloride (10 g/v) but not at 10 °C, pH 5.0 or in the presence of crystal violet (0.001 w/v), potassium tellurite (0.01 g/v), sodium azide (0.0002 g/v) or thallous acetate (0.01 g/v, 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 3224T (= CBS 224.60T = DSM 43136T = IF0 13702T = NCIB 9633T). 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 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 Mayis, Samsun and the Government of Turkey.

**REFERENCES**


