**Nocardia jinanensis** sp. nov., an amicoumacin B-producing actinomycete

Wei Sun, Yue-Qin Zhang, Ying Huang, Yu-Qin Zhang, Zhao-Yong Yang and Zhi-Heng Liu

A novel actinomycete, strain 04-5195\(^\text{T}\), that produces amicoumacin B, which targets bone morphogenetic protein-2, was isolated from a soil sample collected in Jinan, Shandong Province, China. Strain 04-5195\(^\text{T}\) had morphological, biochemical, physiological and chemotaxonomic properties that were consistent with its classification in the genus *Nocardia* and it formed a phyletic line in the *Nocardia* 16S rRNA gene tree. It was evident from the phylogenetic data that strain 04-5195\(^\text{T}\) was most closely associated with *Nocardia speluncae* N2-11\(^\text{T}\). However, the two organisms were distinguishable from one another using DNA–DNA relatedness and phenotypic data. The isolate was readily differentiated from other related *Nocardia* strains by a set of phenotypic properties and by its phylogenetic position. Therefore, it is proposed that the isolate represents a novel species in the genus *Nocardia*, *Nocardia jinanensis* sp. nov.; the type strain is 04-5195\(^\text{T}\) \((\text{=CGMCC 4.3508}\text{T} =\text{DSM 45048})\).

The genus *Nocardia* was proposed by Trevisan (1889) with *Nocardia farcinica* as the original type species (the type species is now *Nocardia asteroides*). The genus belongs to the mycolic-acid-containing group of actinomycetes, members of which form extensively branched mycelia and substrate hyphae that fragment into rod-shaped, non-motile elements (Goodfellow & Lechevalier, 1989). The application of chemotaxonomic, numerical phenetic and molecular systematic methods has led to improved classification of members of the genus *Nocardia* (Goodfellow, 1998; Goodfellow *et al*., 1999). At the time of writing, the genus contained 71 species with validly published names. Many *Nocardia* species have been shown to be agents of human disease, such as *N. asteroides*, *N. farcinica* and *Nocardia nova* (Schaal & Lee, 1992; Wallace *et al*., 1991), although it has also been shown that some species produce secondary metabolites of potential industrial value (Isik *et al*., 1999; Kinoshita *et al*., 2001), e.g. *Nocardia uniformis*, which can produce nocardicin. In the course of screening micro-organisms for new anti-osteoporosis agents targeting bone morphogenetic protein-2 (BMP-2), an active compound (5195A) with the potential to increase expression of the BMP-2 gene was found in fermentation broth of a nocardioform actinomycete, strain 04-5195\(^\text{T}\), that had been isolated from soil. The compound 5195A was identified as amicoumacin B (Yang *et al*., 2007), which has been reported previously to be produced by *Bacillus pumilus* (Itoh *et al*., 1982). A polyphasic taxonomic investigation based on genotypic and phenotypic characteristics revealed that isolate 04-5195\(^\text{T}\) represents a novel species of the genus *Nocardia*.

Strain 04-5195\(^\text{T}\) was isolated on a modified Sauton’s agar plate (Mordarska *et al*., 1972) that had been incubated at 28 °C for 2 weeks following inoculation with a suspension of a soil sample collected from Jinan, Shandong Province, China. The isolate was maintained on yeast extract-malt extract agar (ISP 2; Shirling & Gottlieb, 1966) slopes at 4 °C and as glycerol suspensions (20%, v/v) at −20 °C. All cultures were incubated at 28 °C unless otherwise indicated. Biomass for chemotaxonomic and molecular genetic studies was prepared as described previously (Sun *et al*., 2007).

The colonial properties of the isolate were observed on ISP 2 agar plates that had been incubated for 8 days. Morphological properties were detected following growth on ISP 2 plates and examined by using light microscopy (Axiostkop 20; Zeiss) and scanning electron microscopy (Quanta; FEI). Well-established methods were used to determine a range of phenotypic properties (Goodfellow,
Acid production from carbohydrates was determined using media and methods described by Gordon et al. (1974) and the utilization of sole carbon sources was investigated according to Gordon & Mihm (1957). pH, temperature and NaCl tolerances were determined on ISP 2 agar plates incubated for up to 14 days. Established TLC procedures were used to determine diagnostic dianaminopimelic acid isomers (Hasegawa et al., 1983), whole-cell sugar composition (Lechevalier & Lechevalier, 1980) and polar lipids (Minnikin et al., 1984). Menaquinones were extracted and estimated using the methods of Collins (1985). The acid methanalysis procedure was used for extraction and analysis of mycolic acids (Minnikin et al., 1975). Fatty acids were extracted, methylated and estimated by GC using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Chromosomal DNA was extracted from biomass of strain 04-5195T grown in modified Sauton’s broth for 5 days at 28°C. DNA–DNA relatedness between strain 04-5195T and related type strains Nocardia speluncae N2-11T, N. carnea DSM 43397T, N. flavosea JCM 3332T, N. testacea JCM 12235T and N. sienata IFM 10088T were 99.0 % (14 nt differences at 1397 sites), 98.2 % (25/1404), 97.8 % (31/1406), 97.7 % (33/1406) and 97.8 % (31/1402), respectively. It is clear from the phylogenetic analysis that strain 04-5195T forms a distinct phylogenetic line with N. speluncae N2-11T in the Nocardia 16S rRNA gene tree (Fig. 1; see also Supplementary Fig. S2). This line was supported by all four tree-making algorithms and by a 100 % bootstrap value. Moreover, the four tree-making algorithms and the high bootstrap value supported the position of the strain 04-5195T/ N. speluncae N2-11T phylogenetic line in the same clade as the other four related type strains above. The shortest phylogenetic distance (0.008) was observed between strain 04-5195T and N. speluncae N2-11T. DNA–DNA hybridization was conducted between strain 04-5195T and N. speluncae DSM 45078T; DNA–DNA relatedness values between these two strains were 22.5 ± 2.1 %, which is well below the 70 % cut-off point generally recognized for genomic species (Wayne et al., 1987). A number of phenotypic properties (Table 1) also separated strain 04-5195T from the type strains of the most closely related species.

The genotypic and phenotypic data indicate that strain 04-5195T merits recognition as a representative of a novel species of Nocardia. It is therefore proposed that the isolate be classified in the genus Nocardia as the type strain of Nocardia jinanensis sp. nov.

**Description of Nocardia jinanensis sp. nov.**

*Nocardia jinanensis* (ji.nan.en’sis. N.L. fem. adj. jinanensis pertaining to Jinan, the capital city of Shandong Province, China, soil of which was the source of the type strain).

Aerobic, Gram-positive, catalase-positive, partially acid–alcohol-fast, non-motile actinomycete that forms branched substrate mycelium that fragments in situ into irregular rod-shaped elements. White to yellow substrate hyphae that bear sparse to abundant, white to yellowish aerial hyphae are formed on a number of agar media. Diffusible pigments are not produced. Aesculin is hydrolysed. Nitrate is not reduced to nitrite. Amylase and gelatinase are not produced. No
antibiosis is observed against strains of *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa* or *Staphylococcus epidermidis*. Grows in the presence of 3% NaCl, but not in 5–10% NaCl. Acid is formed from D-fructose, D-galactose, mannitol, melezitose, raffinose, D-ribose and salicin, but not from D-arabinose, inositol, inulin, D-mannose, L-rhamnose or D-xylene. Dextrin, D-galactose, D-glucose, D-mannose and trehalose are utilized (all at 1%, w/v), but L-cysteine (0.1%, w/v), D-ribose (1%, w/v), sodium citrate (0.1%, w/v) and D-xylene are not. Negative for decomposition of elastin (0.3%, w/v) and Tween 60. Growth occurs at an initial pH of 5.5–10.5 and between 15 and 37°C, but not at pH 3.5, pH 4.5 or 45°C. Sensitive to filter-paper discs soaked in tobramycin (10 mg l⁻¹) and kanamycin (30 mg l⁻¹). Additional phenotypic properties are shown in Table 1. The predominant cellular fatty acids are C₁₈:₁ω₉c, C₁₆:₀ and 10-methyl C₁₈:₀. The species description is based on a single strain, which therefore serves as the type strain. The type strain is 04-5195T (＝CGMCC 4.3508T＝DSM 45048T), isolated from a soil sample collected from Jinan city, Shandong Province, northern China. The DNA G+C content of the type strain is 65.0 mol%.

Acknowledgements

This research was supported by the National Facilities and Information Infrastructure for Science and Technology (grant no. 2005DKA21203) and the Natural Science Foundation of China (grant no. 30770002).

Table 1. Phenotypic properties that distinguish strain 04-5195T from type strains of related *Nocardia* species

<table>
<thead>
<tr>
<th>Property</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Decomposition of 0.5% (w/v) uric acid</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>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Methyl x-D-glucopyranoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Mannitol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melezitose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x-L-Rhamnose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth on sole carbon and nitrogen sources:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Valine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All at 1.0% (w/v) apart from sodium acetate (0.1%, w/v).*

**Fig. 1.** Neighbour-joining tree (Saitou & Nei, 1987) derived from aligned 16S rRNA gene sequences showing the position of strain 04-5195T among its nearest phylogenetic neighbours. Branches were also recovered using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods are indicated by the letters f, p and m, respectively; asterisks indicate branches that were recovered using all three methods. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are indicated. Bar, 0.01 substitutions per nucleotide position. An extended tree is available as Supplementary Fig. S2.
References


