Nocardia goodfellowii sp. nov. and *Nocardia thraciensis* sp. nov., isolated from soil

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The taxonomic position of two soil actinomycetes, strains A2012^T and A2019^T, isolated from Turkish soils, was determined using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that the strains belonged to the family *Nocardiaceae*. Strains A2012^T and A2019^T were most closely related to *Nocardia caishijiensis* DSM 44831^T (98.9%) and *Nocardia mexicana* CIP 108295^T (98.6%), respectively; similarity to other type strains of the genus *Nocardia* ranged from 96.9 to 97.9%. However, DNA–DNA relatedness and phenotypic data demonstrated that strains A2012^T and A2019^T could be clearly distinguished from members of the most closely related *Nocardia* species. It is evident from the genotypic and phenotypic data that the two isolates represent two novel species of the genus *Nocardia*. It is proposed, therefore, that strains A2012^T and A2019^T be classified in the genus *Nocardia* as representatives of *Nocardia goodfellowii* sp. nov. (type strain A2012^T=DSM 45516^T=NRRL B-24833^T=KCTC 19986^T) and *Nocardia thraciensis* sp. nov. (type strain A2019^T=DSM 45517^T=NRRL B-24834^T=KCTC 19985^T), respectively.

The genus *Nocardia* belongs to the family *Nocardiaceae*, a member of the suborder *Corynebacterineae*, proposed by Stackebrandt *et al.* (1997), and members of the genus form extensively branched, substrate hyphae that fragment into rod-shaped, non-motile elements; aerial hyphae are usually formed but are sometimes only visible microscopically (Goodfellow & Lechevalier, 1989; Gordon & Mihm, 1957, 1962). *Nocardiae* are also characterized by a number of chemical markers, including the presence of *meso*-diaminopimelic acid in the whole peptidoglycan, arabinose and galactose as the characteristic sugars, mycolic acids with 40–60 carbon atoms and genomic DNA G+C contents of 64 to 72 mol%. The application of chemotaxonomic and molecular systematic methods has promoted a fundamental reassessment of the genus *Nocardia* (Trevisan, 1889; Lechevalier, 1976; Goodfellow, 1998). Much of the emphasis in nocardial systematics has focussed on the causal agents of actinomycetoma and nocardiosis (Goodfellow, 1992, 1998; McNeil & Brown, 1994), although it is evident that nocardiae are common in natural habitats, notably soil (Orchard *et al.*, 1977; Orchard, 1979, 1981; Wang *et al.*, 1999; Maldonado *et al.*, 2000). It is important to establish the species richness of *Nocardia* in natural habitats for ecological reasons and for industrial purposes, as novel members of the genus may be a source of

commercially significant bioactive compounds such as nocardicin, tubelactomisin A and brasilicardin A (Aoki *et al.*, 1976; Komaki *et al.*, 1999; Igarashi *et al.*, 2000). During the course of a screening programme designed to isolate novel actinomycetes from soil, two *Nocardia*-like strains, designated A2012^T and A2019^T were isolated and tentatively assigned to the genus *Nocardia* (Saintpierre, 2001). The aim of the present study was to establish the taxonomic position of the organisms; results indicate that the strains represent two novel species.

Strains A2012^T and A2019^T were isolated from a suspension of an arid soil that was used to inoculate Gauze medium no. 2 (Gauze *et al.*, 1957) supplemented with cycloheximide (50 µg ml⁻¹), nystatin (50 µg ml⁻¹), nalidixic acid (10 µg ml⁻¹) and novobiocin (10 µg ml⁻¹) and incubated at 28 °C for 14 days. Soil samples were collected in the village of Sinankoy, in Edirne, Turkey. The isolates were purified and maintained on modified Bennett's agar (MBA; Jones, 1949) and preserved as a suspension of mycelial fragments in glycerol (20%, w/v) at -20 °C.

The morphological and physiological characteristics of strains A2012^T, A2019^T, *Nocardia caishijiensis* DSM 44831^T, *Nocardia alba* DSM 44684^T, *Nocardia mexicana* DSM 44952^T, *Nocardia terpenica* DSM 44935^T, *Nocardia takedensis* DSM 44801^T and the type species of the genus *Nocardia*, *Nocardia asteroides* ATCC 19247^T were studied after incubation for 14 days at 28 °C on various media described by Shirling & Gottlieb (1966): yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salt-starch agar (ISP 4),

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *Nocardia goodfellowii* A2012^T and *Nocardia thraciensis* A2019^T are HQ157183 and HQ157184, respectively.

Five supplementary figures and three supplementary tables are available with the online version of this paper.

glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), MBA (Jones, 1949) and nutrient agar (NA; Difco). Growth was tested at various temperatures (4, 10, 20, 25, 28, 30, 37 and 45 °C), pH values (4, 5, 6, 7, 8, 9, 10 and 11) and NaCl concentrations (1–5 %, w/v) using ISP 2 as the basal medium. Gram staining was examined by using Hucker's method (Gerhardt, 1981). Acid–alcohol-fastness was examined by using Ziehl–Neelsen method (Gordon, 1967). Colony morphology and micro-morphological properties of isolates A2012^T and A2019^T were determined by examination of gold-coated dehydrated specimens of 14 day cultures from ISP 2 medium (Fig. S1, available in IJSEM Online) using a Cambridge Stereoscan 240 instrument. The test strains were examined for a range of phenotypic properties using well-established procedures (Gordon *et al.*, 1974; Williams *et al.*, 1983; Isik *et al.*, 1999).

Preparation of genomic DNA and PCR amplification of the 16S rRNA gene were carried out following Chun & Goodfellow (1995). Nearly complete 16S rRNA gene sequences were determined for strains A2012^T (1469 bp) and A2019^T (1482 bp) by using an ABI PRISM 3730XL automatic sequencer. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). CLUSTAL W version 1.8 (Thompson *et al.*, 1994) was used to align the sequences of strains A2012^T and A2019^T with those of related taxa retrieved from public databases. Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making algorithms from the program MEGA version 3 (Kumar *et al.*, 2004), and the maximum-likelihood method (Felsenstein, 1981) from the PHYLIP suite of programs (Felsenstein, 1993). The evolutionary distance model of Jukes & Cantor (1969) was used to generate evolutionary distance matrices for the neighbour-joining algorithm. Topologies of the resultant trees were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the CONSENSE and SEQBOOT options from the PHYLIP package.

DNA–DNA relatedness values between strain A2012^T and related type species of *N. caishijiensis* DSM 44831^T, and between strain A2019^T and *N. mexicana* DSM 44952^T were performed by the Identification Service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) [incorporating the modifications described by Huß *et al.* (1983)] using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in glucose-yeast extract-malt extract (GYM) broth on a rotary shaker at 160 r.p.m. at 28 °C for 14 days, checked for purity, harvested by centrifugation and stored at room temperature until required. The isomers of diaminopimelic acid in the cell wall and whole cell-wall sugars were determined by the method of Stanek & Roberts (1974). Isoprenoid quinones were extracted by the method of Collins *et al.* (1977) and were analysed by HPLC (Wu *et al.*, 1989). Mycolic acids were prepared according to the methods of Minnikin *et al.* (1980). Fatty acids were determined from cells grown for 5 days in trypticase soy broth (TSB) at 28 °C with shaking at 150 r.p.m. After 5 days of incubation, 5 ml starter culture was inoculated into 50 ml TSB. The inoculated flask was incubated as before for 5 days. After harvesting by cellulose membrane filtration (0.45 µm), wet cells (200 mg) were placed in an extraction tube. Cellular fatty acids were extracted, derivatized to their fatty acid methyl esters and separated by the Microbial Identification System (MIDI; Microbial ID Inc.), utilizing an Agilent Technologies 6890N GC with a G2614A autosampler and a 6783 injector (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Fatty acid methyl ester peaks were analysed using software version TSBA 5.0.

The DNA G+C contents of strains A2012^T and A2019^T were determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

Strains A2012^T and A2019^T, isolated from arid soil, grew well on various inorganic or organic media; vegetative and aerial mycelium grew well on the media tested. Table 1 shows the degree of growth and colour of both aerial and vegetative hyphae of isolates and members of the most closely related species.

The chemotaxonomic and morphological characteristics of the two isolates were consistent with their assignment to the genus *Nocardia* (Goodfellow, 1998; Goodfellow *et al.*, 1999). Both strains contained galactose and arabinose as characteristic whole sugars plus glucose, mannose and ribose, in addition to *meso*-diaminopimelic acid as the dominant cell-wall diamino acid (chemotype IV *sensu* Lechevalier & Lechevalier, 1980). The predominant menaquinone of strains A2012^T and A2019^T was MK-8(H₄ω-cycl) (60 and 80 %, respectively), although substantial amounts of MK-9 (25 and 7.0 %, respectively), an unknown component (8.0 %) and traces of other menaquinones were present (Figs S2 and S3, available in IJSEM Online). The mycolic acid chain-length ranged from C₄₈ to C₅₈ for strain A2012^T and from C₅₄ to C₆₂ for strain A2019^T (Table S1, available in IJSEM Online). The fatty acid patterns of strains A2012^T and A2019^T were composed of straight-chain saturated and unsaturated fatty acids plus tuberculostearic acid (10-methyl octadecanoic acid) (Table S2, available in IJSEM Online) although there are qualitative and quantitative differences between the strains.

Table 1. Growth and cultural characteristics of strains A2012^T and A2019^T and their closest neighboursStrains: 1, A2012^T; 2, *N. caishijiensis* DSM 44831^T; 3, A2019^T; 4, *N. mexicana* DSM 44952^T. + + +, Abundant; + +, moderate; +, poor.

Medium	Growth and cultural characteristics	1	2	3	4
ISP 2	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	Pale orange yellow	Light brown	Light orange yellow	None
	Reverse colour	Dark yellow brown	Dark brown	Strong orange yellow	Vivid orange yellow
ISP 3	Soluble pigment	None	Dark orange yellow	None	None
	Growth	+ + +	+ +	+ + +	No growth
	Aerial mycelium	White	Pale orange yellow	White	
ISP 4	Reverse colour	Pale orange yellow	Pale orange yellow	Pale yellow	
	Soluble pigment	None	None	None	
	Growth	+ + +	+ +	+ + +	No growth
ISP 5	Aerial mycelium	White	Pale orange yellow	White	
	Reverse colour	White	Pale orange yellow	Pale yellow	
	Soluble pigment	None	None	None	
ISP 6	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	Light orange	Light yellowish brown	White	Pale orange yellow
	Reverse colour	Moderate brown	Dark greyish brown	Light orange	Light orange
ISP 7	Soluble pigment	Pale orange yellow	Dark orange yellow	None	Light orange yellow
	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	Light orange	Light orange	White	None
GYM	Reverse colour	Vivid orange yellow	Strong orange	Vivid orange	Dark orange yellow
	Soluble pigment	None	None	Strong orange yellow	Brilliant orange yellow
	Growth	+ + +	+ + +	+ + +	+ + +
Czapek's agar	Aerial mycelium	Light brown	Light yellowish brown	Light greyish brown	Yellowish grey
	Reverse colour	Dark greyish reddish brown	Dark greyish brown	Dark brown	Strong yellowish brown
	Soluble pigment	Light orange yellow	Brownish orange	Strong brown	Dark orange yellow
NA	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	Pale orange yellow	Light brown	Light orange yellow	None
	Reverse colour	Dark orange yellow	Dark brown	Strong orange yellow	Vivid orange yellow
TSA	Soluble pigment	None	Dark orange yellow	None	None
	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	White	White	None
MBA	Reverse colour	White	Dark greyish olive	White	
	Soluble pigment	None	Light greyish olive	None	
	Growth	+ +	+ + +	+ + +	+ + +
	Aerial mycelium	White	Light orange	None	None
	Reverse colour	Light orange yellow	Strong orange	Light orange yellow	Light orange yellow
	Soluble pigment	None	Moderate orange	None	None
	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	Light orange	Brownish orange	White	None
	Reverse colour	Strong orange	Deep orange	Strong yellowish brown	Brilliant orange yellow
	Soluble pigment	None	Strong orange	Dark orange yellow	None
	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	Light greyish olive	Greyish yellowish brown	Light orange yellow	White
	Reverse colour	Dark yellowish brown	Dark greyish brown	Deep brown	Pale yellow
	Soluble pigment	None	Dark yellow	Light orange	Pale orange yellow

The genomic DNA G + C contents of strains A2012^T and A2019^T were 70.6 and 69.5 mol%, respectively.

Comparison of the almost-complete 16S rRNA gene sequences of isolates A2012^T and A2019^T with corresponding sequences from representatives of genera in the suborder *Corynebacterineae*, with the exception of position

600–638 (U : G) for strain A2019^T, 843 (U) for strains A2012^T and A2019^T and 1008–1021 (A : U) for strain A2012^T according to the *Escherichia coli* numbering system, revealed the presence of signature nucleotides (Table S3, available in IJSEM Online) that are characteristic for members of the family *Nocardaceae* (Stackebrandt *et al.*, 1997) and the genus *Nocardia* (Chun & Goodfellow, 1995).

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing the positions of strains A2012^T and A2019^T among their nearest neighbours. Numbers at the nodes indicate the levels of bootstrap support (%); only values above 50 % are cited. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site. The root position of the neighbour-joining tree was obtained using *Rhodococcus corynebacterioides* DSM 20151^T as the outgroup.

16S rRNA gene sequence similarities between strains A2012^T and the closely related type strains *N. caishijiensis* DSM 44831^T, *N. alba* YIM 30243^T, *Nocardia anaemiae* IFM 0323^T, *Nocardia pseudovaccinii* DSM 43406^T, *N. asteroides* ATCC 19247^T, *Nocardia nova* JCM 6044^T, *Nocardia ignorata* DSM 44496^T, *Nocardia thailandica* IFM 10145^T and *Nocardia cyriacigeorgica* DSM 44484^T were 98.6 % (20 nt differences at 1426), 97.9 % (30 nt differences at 1442), 97.9 % (31 nt differences at 1469), 97.8 % (31 nt differences at 1455), 97.8 % (32 nt differences at 1455), 97.8 % (32 nt differences at 1455), 97.5 % (36 nt differences at 1460), 97.5 % (36 nt differences at 1430) and 97.5 % (37 nt differences at 1457), respectively.

Strain A2019^T was most closely related to *N. mexicana* CIP 108295^T; the two strains shared 98.94 % 16S rRNA gene sequence similarity, which corresponds to 14 nt differences at 1329 locations. Relatively high similarity values were shown with the type strains of *Nocardia artemisiae* YIM 65623^T (97.4 %; 39 nt differences at 1468), *N. terpenica* IFM 0706^T (97.3 %; 40 nt differences at 1468), *N. takedensis* MS1-3^T (97.2 %; 40 nt differences at 1448), *Nocardia lijiangensis* YIM 33378^T (97.2 %; 41 nt differences at 1468) and *Nocardia endophytica* KLBMP 1256^T (97.2 %; 41 nt differences at 1459). It is also apparent from Fig. 1 that strains A2012^T and A2019^T are part of two different subclades and shared 95.8 % 16S rRNA gene sequence similarity, which corresponds to 58 nt differences at 1460 locations. It is clear from the phylogenetic analysis that strains A2012^T and A2019^T formed two distinct phyletic

lines, with *N. caishijiensis* DSM 44831^T and *N. mexicana* DSM 44952^T, respectively, in the *Nocardia* 16S rRNA gene tree (Fig. 1; Figs S4 and S5, available in IJSEM Online). This was supported by all four tree-making algorithms and by a 100 % bootstrap value.

The taxonomic integrity of the test strains was supported by DNA relatedness data. Strain A2012^T showed a DNA relatedness value of 15.4 % to *N. caishijiensis* DSM 44831^T, whereas strain A2019^T showed a DNA relatedness value 27.2 % to *N. mexicana* DSM 44952^T. On the other hand, the DNA relatedness value between A2012^T and A2019^T was 22.6 %. The phenotypic properties of these two strains also clearly distinguished them from each other and from the most closely related members of the genus *Nocardia* (Table 2).

It is evident from the genotypic and phenotypic data that isolates A2012^T and A2019^T represent two novel species within the genus *Nocardia*. It is, therefore, proposed that these organisms be classified in this taxon as *Nocardia goodfellowii* sp. nov. and *Nocardia thraciensis* sp. nov.

Description of *Nocardia goodfellowii* sp. nov.

Nocardia goodfellowii (good.fel.low'i.i. N.L. gen. masc. n. *goodfellowii* of Goodfellow, named in honour of Michael Goodfellow for his contributions to *Nocardia* systematics).

Aerobic, Gram-positive, weakly acid-alcohol-fast and non-motile actinomycete that forms white or yellow to brown

Table 2. Phenotypic properties that differentiate strains A2012^T and A2019^T and representatives of the phylogenetically most closely related species

Strains: 1, A2012^T; 2, *N. caishijiensis* DSM 44831^T; 3, *N. alba* DSM 44684^T; 4, *N. anaemiae* DSM 44821^T [data taken from Kageyama *et al.* (2005)]; 5, *N. asteroides* ATCC 19247^T; 6, A2019^T; 7, *N. mexicana* DSM 44952^T; 8, *N. terpenica* DSM 44935^T; 9, *N. takedensis* DSM 44801^T. Data from this study except where specified. ND, Not determined; +, positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9
Biochemical tests									
Aesculin	+	–	ND	ND	+	+	+	+	+
Arbutin	+	–	ND	ND	+	+	+	+	ND
Allantoin	–	–	ND	ND	+	+	–	–	ND
Nitrate	+	+	+	ND	+	–	+	–	ND
Degradation of:									
Adenine (0.5 %)	–	–	–	–	–	–	+	+	–
DNA (0.5 %)	–	–	–	ND	–	–	–	+	–
Gelatine (0.5 %)	+	+	+	ND	–	+	–	+	–
Hypoxanthine (0.4 %)	+	–	–	+	–	+	+	–	–
Starch (1 %)	–	–	–	ND	–	–	–	–	+
Tween 20 (1 %)	–	–	–	ND	+	+	+	–	+
Uric acid	+	+	+	+	–	+	+	+	–
Growth on sole carbon sources (1.0 %, w/v):									
Methyl α -D-glucoside	–	–	–	ND	–	+	+	–	+
Adonitol	+	–	–	–	–	–	+	+	+
Arabinose	–	–	–	+	–	–	+	+	–
Dextrin	–	+	–	ND	–	+	+	+	–
Galactose	+	–	–	+	–	+	+	–	–
Inositol	–	–	–	–	–	–	+	+	–
Mannitol	+	+	–	ND	+	+	+	+	+
Melezitose	–	–	–	ND	–	+	+	+	+
Rhamnose	–	–	+	–	–	+	+	–	+
Salicin	–	–	–	ND	–	+	+	+	–
Sorbitol	+	–	–	+	–	+	+	+	–
Starch	–	+	–	ND	–	+	+	–	+
Sucrose	–	+	–	ND	+	+	+	–	+
Xylitol	–	–	–	ND	–	–	+	+	+
Xylose	–	–	–	ND	–	+	–	–	+
Growth on sole nitrogen sources (0.1 %, w/v):									
α -Isoleucine	+	–	–	ND	+	+	+	–	+
Glycine	–	–	–	ND	–	+	+	+	+
L-Alanine	+	–	+	ND	–	+	+	+	+
L-Histidine	+	–	–	ND	–	+	+	+	+
Growth at/in:									
10 °C	+	+	ND	ND	ND	–	–	–	ND
pH 10.0	+	+	ND	ND	+	–	+	–	ND
NaCl (4.0 %)	+	+	ND	ND	ND	–	+	–	ND

aerial mycelium, depending on the culture medium, that fragments into rod-shaped elements. Produces orange–yellow diffusible pigments on ISP 5 and ISP 7 media. Grows at pH 5.0–10.0 and 10–37 °C, but not at pH 4.0 or 11.0, or at temperatures of 4 or 45 °C. Optimal growth is at 28 °C and pH 7.0. Growth is observed in 0–4 % (w/v) NaCl. Aesculin and arbutin are hydrolysed and nitrate is reduced but not allantoin. Degrades gelatin, hypoxanthine and uric acid but not adenine, casein, chitin, DNA, guanine, RNA, Tweens 20 and 80, xanthine and xylan. Assimilates adonitol, (–)-D-fructose, (+)-D-galactose,

(+)-D-glucose, (+)-D-mannose, (+)-D-mannitol, maltose, propionic acid, (–)-D-sorbitol and succinic acid but not methyl α -D-glucoside, (–)-D-arabinose, (–)-D-cellobiose, (–)-D-salicin, (+)-D-melezitose, (+)-D-melibiose, dextran, dextrin, inulin, (–)-L-sorbose, (+)-L-arabinose, (+)-L-rhamnose, lactose, L-glutamate, *myo*-inositol, raffinose, sucrose, xylitol or xylose as sole carbon sources. Utilizes α -isoleucine, L-alanine, L-arginine, L-cysteine, L-histidine, L-proline, L-serine, L-tyrosine and L-valine but not D-phenylalanine, glycine, L-hydroxyproline, L-methionine or L-threonine as sole nitrogen sources. Predominant menaquinone

is MK-8(H₄ω-cycl) (60 %), although substantial amounts of MK-9 (25 %) and other components are also present. The major fatty acids are hexadecanoic acid, oleic acid and tuberculostearic acid [10-methyl branched C_{18:0}]. Mycolic acids with 48–56 carbons are present.

The type strain, A2012^T (=DSM 45516^T=NRRL B-24833^T=KCTC 19986^T) was isolated from arid soil from Sinankoy, Lalapasa, Edirne, Turkey. The genomic DNA G+C content of the type strain is 70.6 mol%.

Description of *Nocardia thraciensis* sp. nov.

Nocardia thraciensis (thra.ci.en'sis. N.L. fem. adj. *thraciensis* from Thrace, the European region of Turkey, the source of the organism).

Aerobic, Gram-positive, partially acid-alcohol-fast, non-motile actinomycete that forms white aerial mycelium on ISP 3, ISP 4, ISP 5, ISP 6, Czapek's and TSA media, yellow to brown substrate mycelium depending on the culture medium that fragments into rod-shaped elements, and orange-yellow to strong brown soluble pigments produced on ISP 6, ISP 7, TSA and MBA media. Growth occurs at pH 6.0–9.0 and 20–37 °C but not at pH 4.0, 5.0 or 10.0, or at temperatures of 4, 10 and 45 °C. Optimal growth occurs at pH 7.0 and 28 °C. Growth is observed in 0–4 % (w/v) NaCl. Aesculin, allantoin, arbutin and urea are hydrolysed but nitrate is not reduced. Degrades gelatin, hypoxanthine, Tween 20 and uric acid but not adenine, casein, chitin, DNA, guanine, RNA, Tween 80, xanthine or xylan. Utilizes methyl α-D-glucoside, (–)-D-fructose, (–)-D-salicin, (+)-D-galactose, (+)-D-glucose, (+)-D-mannose, (+)-D-mannitol, dextrin, maltose, propionic acid, (–)-D-sorbitol, succinic acid, (+)-L-arabinose, (+)-L-rhamnose, (+)-D-melezitose, sucrose and xylose but not adonitol, (–)-D-arabinose, (–)-D-cellobiose, (+)-D-melibiose, dextran, inulin, (–)-L-sorbose, lactose, L-glutamate, *myo*-inositol, raffinose or xylitol as sole carbon sources. Utilizes α-isoleucine, glycine, L-alanine, L-arginine, L-cysteine, L-histidine, L-proline, L-serine, L-tyrosine and L-valine but not D-phenylalanine, L-hydroxyproline, L-methionine or L-threonine as sole nitrogen sources. Predominant menaquinone is MK-8(H₄ω-cycl) (80 %), although substantial amounts of MK-9 (7 %), an unknown component (8 %) and other menaquinones are also present. The major fatty acids are hexadecanoic acid, oleic acid and tuberculostearic acid [10-methyl branched C_{18:0}]. Mycolic acids with 54–62 carbons are present.

The type strain, A2019^T (=DSM 45517^T=NRRL B-24834^T=KCTC 19985^T), was isolated from arid soil in Sinankoy, Lalapasa, Edirne, Turkey. The genomic DNA G+C content of the type strain is 69.5 mol%.

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References

- Aoki, H., Sakai, H., Kohsaka, M., Konomi, T., Hosoda, J., Kubochi, Y., Iguchi, E. & Imanaka, H. (1976). Nocardicin A, a new monocyclic beta-lactam antibiotic. I. Discovery, isolation and characterization. *J Antibiot (Tokyo)* **29**, 492–500.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gauze, G. F., Preobrazhenskaya, T. P., Kudrina, E. S., Blinov, N. O., Ryabova, I. D. & Sveshnikova, M. A. (1957). *Problems of Classification of Actinomycete-Antagonists*. Moscow: Government Publishing House of Medical Literature, Mediz.
- Gerhardt, P. (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2005). A simple fluorimetric method for the estimation of DNA-DNA relatedness between closely related microorganisms by thermal denaturation temperatures. *Extremophiles* **9**, 75–79.
- Goodfellow, M. (1992). The family Nocardiaceae. In *The Prokaryotes*, 2nd edn, pp. 1188–1213. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Goodfellow, M. (1998). *Nocardia* and related genera. In *Topley & Wilson's Microbiology & Microbial Infections*, 9th edn, vol. 2, pp. 463–489. Edited by A. Balows & B. I. Duerden. London: Edward Arnold.
- Goodfellow, M. & Lechevalier, M. P. (1989). Genus *Nocardia*. Trevisan 1889, 9^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1458–1471. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Goodfellow, M., Isik, K. & Yates, E. (1999). Actinomycete systematics: an unfinished synthesis. *Nova Acta Leopold* **312**, 47–82.
- Gordon, R. E. (1967). The taxonomy of soil bacteria. In *The Ecology of Soil Bacteria*, pp. 293–321. Edited by T. R. G. Gray & D. Parkinson. Liverpool: Liverpool University Press.
- Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J Bacteriol* **73**, 15–27.
- Gordon, R. E. & Mihm, J. M. (1962). The type species of the genus *Nocardia*. *J Gen Microbiol* **27**, 1–10.

- Gordon, R. E., Barnett, D. A., Handerman, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridisation from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Igarashi, M., Hayashi, C., Homma, Y., Hattori, S., Kinoshita, N., Hamada, M. & Takeuchi, T. (2000). Tubelactomicin A, a novel 16-membered lactone antibiotic, from *Nocardia* sp. I. Taxonomy, production, isolation and biological properties. *J Antibiot (Tokyo)* **53**, 1096–1101.
- Isik, K., Chun, J., Hah, Y. C. & Goodfellow, M. (1999). *Nocardia salmonicida* nom. rev., a fish pathogen. *Int J Syst Bacteriol* **49**, 833–837.
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kageyama, A., Yazawa, K., Nishimura, K. & Mikami, Y. (2005). *Nocardia anaemiae* sp. nov. isolated from an immunocompromised patient and the first isolation report of *Nocardia vinacea* from humans. *Nippon Ishinkin Gakkai Zasshi* **46**, 21–26.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Komaki, H., Nemoto, A., Tanaka, Y., Takagi, H., Yazawa, K., Mikami, Y., Shigemori, H., Kobayashi, J., Ando, A. & Nagata, Y. (1999). Brasilicardin A, a new terpenoid antibiotic from pathogenic *Nocardia brasiliensis*: fermentation, isolation and biological activity. *J Antibiot (Tokyo)* **52**, 13–19.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lechevalier, M. P. (1976). The taxonomy of the genus *Nocardia*: some light at the end of the tunnel? In *The Biology of the Nocardiae*, pp. 1–33. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy* (Special Publication no. 6), pp. 227–291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.
- Maldonado, L., Hookey, J. V., Ward, A. C. & Goodfellow, M. (2000). The *Nocardia salmonicida* clade, including descriptions of *Nocardia cummidelensis* sp. nov., *Nocardia fluminea* sp. nov. and *Nocardia soli* sp. nov. *Antonie van Leeuwenhoek* **78**, 367–377.
- McNeil, M. M. & Brown, J. M. (1994). The medically important aerobic actinomycetes: epidemiology and microbiology. *Clin Microbiol Rev* **7**, 357–417.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.
- Orchard, V. A. (1979). Effect of sewage sludge additions on *Nocardia* in soil. *Soil Biol Biochem* **11**, 217–220.
- Orchard, V. A. (1981). The ecology of *Nocardia* and related taxa. *Zentralbl Bakteriell* **11** (Suppl.), 167–180.
- Orchard, V. A., Goodfellow, M. & Williams, S. T. (1977). Selective isolation and occurrence of *Nocardiae* in soil. *Soil Biol Biochem* **9**, 233–238.
- Saintpierre, D. (2001). *Identification de souches originales d'actinomycètes isolées de sols ultramafiques de Nouvelle-Calédonie. Caractérisation chimique de quelques antibiotiques produits*. PhD thesis, Institut National Polytechnique de Toulouse, France (in French).
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL_W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Trevisan, V. (1889). *I Generi e le Specie delle Batteriacee*. Milan: Zanaboni & Gabuzzi.
- Wang, Y., Zhang, Z. S., Ruan, J. S., Wang, Y. M. & Ali, S. M. (1999). Investigation of actinomycete diversity in the tropical rainforests of Singapore. *J Ind Microbiol Biotechnol* **23**, 178–187.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology (English translation of Mikrobiologia)* **16**, 176–178.