INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Zhou *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:2981–2987

DOI 10.1099/ijsem.0.003856

MICROBIOLOGY SOCIETY

OPEN MICROBIOLOGY

Nocardia colli sp. nov., a new pathogen isolated from a patient with primary cutaneous nocardiosis

Tao Zhou[1]†, Xiao-Yun Wang[2,*],†, Dan-Qi Deng[2]†, Li-Hua Xu[3], Xiao-Lan Li[2], Yun Guo[2], Wen-Hua Li[2], Hong Xie[2], Pei-Lian Zhang[2] and Xiao-Hong Zhou[2]

Abstract

A novel nocardioform strain, CICC 11023^T, was isolated from a tissue biopsy of neck lesions of a patient with primary cutaneous nocardiosis and characterized to establish its taxonomic position. The morphological, biochemical, physiological and chemotaxonomic properties of strain CICC 11023^T were consistent with classification in the genus *Nocardia*. Whole-cell hydrolysates were rich in meso-diaminopimelic acid, galactose, arabinose and fructose. Mycolic acids were present. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and two unidentified lipids, and the predominant menaquinone was cyclo MK-8 (H4, ω-cyclo). The main fatty acids (>5%) were $C_{18:0}$ 10-methyl (TBSA), $C_{16:0}$, summed feature 4 ($C_{16:1}$ trans 9/$C_{15:0}$ iso 2OH), $C_{15:0}$ and $C_{17:0}$ 10-methyl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that the isolate is most closely related (>98% similarity) to the type strains *Nocardia ninae* OFN 02.72^T, *Nocardia iowensis* UI 122540^T and *Nocardia alba* YIM 30243^T, and phylogenetic analysis of *gyrB* gene sequences showed similarity (89.1–92.2%) to *Nocardia vulneris* NBRC 108936^T, *Nocardia brasiliensis* IFM 0236^T and *Nocardia exalbida* IFM 0803^T. DNA–DNA hybridization results for strain CICC 11023^T compared to *Nocardia* type strains ranged from 20.4 to 35.4%. The genome of strain CICC 11023^T was 8.78 Mbp with a G+C content of 67.4 mol% overall. The average nucleotide identity (ANI) values between strain CICC 11023^T and *N. alba* YIM 30243^T were low (OrthoANIu=77.47%), and the ANI values between strain CICC 11023^T and *N. vulneris* NBRC 108936 ^{T} were low (OrthoANIu=83.75 %). Consequently, strain CICC 11023^T represents a novel *Nocardia* species on the basis of this polyphasic study, for which the name *Nocardia colli* sp. nov. is proposed. The type strain is CICC 11023^T (=KCTC 39837^T).

The genus *Nocardia*, belonging to the suborder Corynebacterineae [1], was established by Trevisan in 1889 [2] and consists of Gram-stain-positive, variably acid-fast, strictly aerobic bacteria that form filamentous, branched cells that fragment into pleomorphic, rod-shaped or coccoid elements [3]. Since Pijper and Pullinger identified *Nocardia transvalensis* as the pathogenic micro-organism associated with a case of mycetoma in a South African patient in 1927 [4], more and more cases of clinical *Nocardia* infection have been reported worldwide every year. This increased prevalence is partly due to advances in phylogenetic analyses based on 16S rRNA and partial *gyrB* gene sequences, allowing for the more rapid identification of nocardial isolates compared to standard phenotypic techniques [5, 6]. More than 40 species within the genus *Nocardia* have been reported as clinically relevant, and many of these show resistance to several classes of antimicrobials [7]. *Nocardia* species are widely distributed in the environment and cause a variety of suppurative and granulomatous infections of humans and animals, including cutaneous, subcutaneous, lymphocutaneous, pulmonary, cerebral or disseminated nocardiosis. Treatment of nocardiosis often requires long-term therapy with a combination of drugs [8]. In the present study, a novel strain of *Nocardia* was isolated from a patient with primary cutaneous nocardiosis and the

Author affiliations: [1]Department of Clinical Laboratory Medicine, Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, PR China; [2]Department of Dermatology, Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, PR China; [3]The Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education and Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China.
***Correspondence:** Xiao-Yun Wang, sunwindwang@ymail.com
Keywords: *Nocardia colli* sp. nov.; pathogen; cutaneous nocardiosis.
Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; TSB, trypticase soy broth.
The nucleotide sequence of the 16S rRNA gene of strain CICC 11023^T that we determined has been submitted to GenBank under the accession number KJ659849. The *gyrB* gene sequence of strain CICC 11023^T determined in this study has been deposited in GenBank under the accession number MH580561. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession VXLC00000000. The version described in this paper is version VXLC01000000.
†These authors contributed equally to this work
Two supplementary tables are available with the online version of this article.

phenotypic, morphological, chemotaxonomic and molecular characteristics of strain CICC 11023^T are presented.

A 36-year-old woman, who is a farmer by occupation, presented to the Department of Dermatology of the Second Affiliated Hospital of Kunming Medical University (Kunming, Yunnan Province, PR China) with a 10 year history of gradually enlarging and infiltrating painless papulo-nodular lesions of the neck and chest [9]. Two strains were isolated from an aerobic culture of the biopsied skin tissue specimens at 25 °C in Sabouraud agar medium after 1 week. One of the strains showed 99.8% 16S rRNA gene sequence similarity to *Staphylococcus epidermidis* ATCC 14990^T. *S. epidermidis* is part of the normal human flora, typically the skin flora, and is less commonly found in the mucosal flora [10]. The other strain, KY2-1, was deposited in the China National Research Institute of Food and Fermentation Industries, China Centre of Industrial Culture Collection (CICC) as strain CICC 11023^T and the Korean Collection for Type Cultures (KCTC), Biological Resource Centre (BRC), Korea Research Institute of Bioscience and Biotechnology as strain KCTC 39837^T.

Characteristic chemotaxonomic properties of the genus *Nocardia* are based on mycolic acids and fatty acid compositions [11]. To identify the whole-cell fatty acid composition, strain CICC 11023^T and reference *Nocardia* type strains were grown in trypticase soy broth (TSB) with shaking at 150 r.p.m. for 7 days at 28 °C. Extraction and analysis of the cellular fatty acids were based on the standard protocol of the Sherlock Microbial Identification (MIDI) System, version 6.0 [12], and peaks were identified using the peak-naming table TSBA6 compiled by the China General Microbiological Culture Collection Centre (CGMCC) [13]. Analysis of the acyl cell wall was performed according to a glycolate test by diethyl ether extraction as previously reported [14] . The whole-cell sugars and diaminopimelic acids were determined by thin-layer chromatography using previously described methods [15, 16]. Menaquinones were extracted and purified through the method described by Collins *et al.* [17] and analysed by high-performance liquid chromatography [18] . Phospholipids were extracted by two-dimensional thin-liquid chromatography [19] and identified by following a previously reported procedure [20]. Analysis of mycolic acids was carried out using a previously described method [19].

In general, a >5% fatty acid content is considered to present a 'major fatty acid' [21]. Analyses of the fatty acids by gas–liquid chromatography revealed that the main fatty acids (>5%) of strain CICC 11023^T were $C_{18:0}$ 10-methyl (TBSA, 30.36%), $C_{16:0}$ (20.52%), summed feature 4 ($C_{16:1}$ trans 9/$C_{15:0}$ iso 2OH; 14.33%), $C_{15:0}$ (13.01%) and $C_{17:0}$ 10-methyl (5.41%). The fatty acid patterns of the novel strain and the reference strains are presented in Table 1. Comparisons of the fatty acid profiles showed that all seven tested strains contained $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{18:0}$ 10-methyl (TBSA) and $C_{18:1}\omega9c$; however, strain CICC 11023^T exhibited relatively large amounts of $C_{17:0}$ and $C_{18:0}$ 10-methyl (TBSA), and small amounts of $C_{16:0}$ and $C_{18:1}\omega9c$. Thus, compared to the six reference strains, strain CICC 11023^T showed a distinct major fatty acid pattern. The whole-cell hydrolysates were rich in meso-diaminopimelic acid, galactose, arabinose and fructose. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and two unidentified lipids, and the predominant menaquinone was cyclo MK-8(H4, ω-cyclo) (86.5%). The strain also contained mycolic acid, which is characteristic of the genera *Nocardia* and *Rhodococcus* [22]. The chemotaxonomic features of strain CICC 11023^T were consistent with those of members of the genus *Nocardia* [6].

Pigmentation, production of aerial hyphae, and morphological characteristics were observed under a light microscope (Olympus CX41) and a scanning fiber-optic electron microscope (FEI Quanta). Strain CICC 11023^T was grown

Table 1. Main fatty acid compositions (>5%) of strain CICC 11023^T and the type strains of related *Nocardia* species

Strains: 1, CICC 11023^T; 2, *Nocardia ninae* OFN 02.72^T; 3, *Nocardia iowensis* UI 122540^T; 4, *Nocardia alba* YIM 30243^T; 5, *Nocardia vulneris* NBRC 108936^T; 6, *Nocardia brasiliensis* IFM 0236^T; 7, *Nocardia exalbida* IFM 0803^T. All data are from this study. Values are percentages (%) of total fatty acids. –, Not detected.

Fatty acid	1	2	3	4	5	6	7
$C_{15:0}$	13.0	–	–	–	–	<5	–
$C_{16:0}$	20.5	35.8	31.4	24.7	32.9	40.0	25.9
$C_{17:0}$ 10-methyl	5.4	–	<5	<5	–	–	–
$C_{18:0}$	<5	<5	<5	5.0	<5	7.9	5.7
$C_{18:0}$ 10-methyl (TBSA)	30.3	8.1	15.7	11.7	6.2	12.9	8.2
$C_{18:1}\omega9c$	<5	24.0	11.4	19.5	18.0	24.0	17.1
Summed feature 3*	–	13.4	30.8	18.3	16.0	–	12.8
Summed feature 4*	14.3	–	–	–	–	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC using the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 4 contained $C_{16:1}$ trans 9 and/or $C_{15:0}$ iso 2OH.

separately on Gause 1, ISP 2, ISP 3, ISP 4 and ISP 5 at 30 °C for 5 days, and then examined for colour determination using colour chips from the ISCC–NBS colour charts (standard sample no. 2106). Growth at 21, 28, 37 and 45 °C was measured on ISP 2 for 5 days. The pH range for growth using the buffer system described in [23] (pH 4–10 at intervals of 0.5 pH units) and the requirement for NaCl (1, 4, 7 and 10%) were determined in ISP 2 broth. Phenotypic characteristics such as Gram-staining, catalase and oxidase activity, and hydrolysis of casein, Tweens 20 and 80, egg yolk and starch were examined using the methods described by Smibert and Krieg [24]. Utilization of various substrates as sole carbon sources was tested at the CICC using the GN2 MicroPlate Gram-negative identification test panel (Biolog), and the result was determined after incubation at 30 °C for 24 h. Physiological and biochemical properties were further determined with API 20NE, API 20E and API ZYM strips (bioMérieux). Tests were generally performed according to the manufacturer's instructions. The API 20NE tests were read after 24–48 h at 28 °C, the API 20E tests were read after 18–24 h at 36 °C, and the API ZYM tests were read after 4 h of incubation at 37 °C [13].

Morphological characteristics of strain CICC 11023^T presented typical properties of the genus *Nocardia*. Strain CICC 11023^T was aerobic, Gram-stain-positive, non-motile, with modified acid alcohol-positive actinomycetes forming extensively branched grey-white substrate mycelium and aerial mycelium with fragments that appeared as short coli-like bodies under scanning electronic microscopy (0.5×0.7–0.9 µm in diameter). When grown on Gause 1, ISP 3 and ISP 5 media at 30 °C for 5 days, the surface of colonies appeared as a velvet powder, with a grey aerial mycelium and substrate mycelium, and a grey spore heap. When grown on ISP 2 at 30 °C for 5 days, the colonies had a corrugated surface, with a white aerial mycelium, light brown substrate mycelium and a white spore heap. Culture inserts on ISP 2 at 30 °C for 5 days showed formation of short spore chains, a spore chain flex and mycelium breaking into a rod-like curved body after 8 days. Growth was weak on ISP 4 at 30 °C for 5 days. No soluble pigments were found on any medium. Strain CICC 11023^T grew at 21, 28 and 37 °C, but not at 45 °C. Positive reactions were observed for milk peptonization, catalase, aesculin, urease and cellulose; negative reactions were observed for milk coagulation, nitrate reduction, gelatin liquefaction, tyrosinase, hydrolysis of starch, casein, xanthine, hypoxanthine and production of H_2S. Strain CICC 11023^T utilized L-arabinose, rhamnose, D-fructose, salicin, D-xylose, inositol, lactose, melibiose, D-glucose, raffinose, sucrose, D-mannitol, maltose, trehalose and arabinose as sole carbon sources. The main differential characteristics between strain CICC 11023^T and closely related *Nocardia* species are presented in Table 2.

Antibiotic sensitivity analysis of strain CICC 11023^T was performed using Etest (bioMérieux) to determine the minimal inhibitory concentration values for some antibiotics according to the manufacturer instructions. Sulfonamides have been the mainstay of antimicrobial therapy for human nocardiosis [25]. Thus, the patient was treated with oral Co-SMZ (containing 0.4 g sulfamethoxazole and 0.08 g trimethoprim; two tablets/time, three times/day, twice the first dose) for 8 weeks and achieved very good improvement with this treatment: the nocardiosis resolved 6 months after the administration of Co-SMZ [9]. No recurrence of the infection was observed for approximately 3 years. Although the majority of these infections can be treated with sulfonamides, there are *in vitro* differences noted in the antimicrobial susceptibility among different cases of *Nocardia*. The drug susceptibility testing showed that strain CICC 11023^T was susceptible to levofloxacin, ofloxacin, tobramycin and clindamycin, and was resistant to fosfomycin, imipenem, vancomycin and erythromycin.

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene from strain CICC 11023^T were performed as described previously [26] . The program CLUSTAL X was used to conduct multiple alignments with sequences of the most closely related *Actinobacteria* strains and for calculations of sequence similarity [27]. Phylogenetic trees were reconstructed using the neighbour-joining [28], maximum-parsimony [29] and maximum-likelihood [30] algorithms in MEGA version 4.0 [31]. The stability of the clades in the trees was appraised using a bootstrap value with 1000 replications [32] . The 16S rRNA gene sequence (1506 bp) of strain CICC 11023^T was determined. Phylogenetic analysis showed that strain CICC 11023^T was most closely related to members of the genus *Nocardia*, and sequence similarity calculations obtained by pairwise comparisons indicated that the closest relatives of strain CICC 11023^T were *Nocardia ninae* OFN 02.72^T (98.4%), *Nocardia iowensis* UI 122540^T (98.3%) and *Nocardia alba* YIM 30243^T (98.1%; Fig. 1). The *gyrB* gene sequence for strain CICC 11023^T (1094 bp) was also determined and analysed according to the methods reported by Takeda *et al.* [5]. The closest phylogenetic neighbours were *Nocardia vulneris* NBRC 108936^T (92.2%), *Nocardia brasiliensis* IFM 0236^T (91.8%) and *Nocardia exalbida* IFM 0803^T (89.1%; Fig. 2). The *Nocardia* type species were clustered based on *gyrB* sequence similarity values of 93.5% and above [5]. Therefore, *N. ninae* OFN 02.72^T, *N. iowensis* UI 122540^T, *N. alba* YIM 30243^T, *N. vulneris* NBRC 108936^T, *N. brasiliensis* IFM 0236^T and *N. exalbida* IFM 0803^T as reference strains were used for phenotypic comparisons and DNA–DNA hybridization (DDH) tests.

The G+C content was determined using the method of Mesbah *et al.* [33] and was found to be 65.6 mol%. DDH experiments were carried out at the CGMCC using dot-blot hybridization and a simple fluorimetric method based on thermal denaturation temperatures [34] to evaluate the DNA–DNA relatedness between strain CICC 11023^T and its most closely related species: *N. ninae* OFN 02.72^T (35.4%), *N. iowensis* UI 122540^T (20.4%), *N. alba* YIM 30243^T (25%), *N. vulneris* NBRC 108936^T (21.6%), *N. brasiliensis* IFM 0236^T (22.2%) and *N. exalbida* IFM 0803^T (23.3%). In accordance with the recommended threshold value of 70% DNA–DNA relatedness for species delineation [35], strain CICC 11023^T represents a species distinct from *N. ninae* OFN 02.72^T, *N. iowensis* UI 122540^T, *N. alba* YIM 30243^T, *N. vulneris* NBRC 108936^T, *N. brasiliensis* IFM 0236^T and *N. exalbida* IFM 0803^T.

Table 2. Differential phenotypic characteristics between strain CICC 11023^T and closely related *Nocardia* species

Strains: 1, CICC 11023^T; 2, *Nocardia ninae* OFN 02.72^T; 3, *Nocardia iowensis* UI 122540^T; 4, *Nocardia alba* YIM 30243^T; 5, *Nocardia vulneris* NBRC 108936^T; 6, *Nocardia brasiliensis* IFM 0236^T; 7, *Nocardia exalbida* IFM 0803^T. All data were obtained in this study unless indicated otherwise. +, positive; –, negative; w, weak.

Characteristic	1	2	3	4	5	6	7
Growth at 37 °C	+	+	+	–	+	+	+
Growth at 45 °C	–	–	+	–	–	–	–
Milk coagulation	–	–	–	–	–	–	–
Milk peptonization	+	–	–	–	–	–	–
Carbon utilization:							
Glucose	+	+	+	+	+	+	+
Mannitol	+	–	–	+	+	w	+
Inositol	+	–	–	+	–	–	–
Arabinose	+	+	–	–	–	–	–
Maltose	+	w	+	+	–	+	+
Galactose	+	+	–	–	+	+	w
Raffinose	+	–	–	–	–	–	–
Rhamnose	w	–	–	+	–	–	–
Sorbitol	w	–	–	–	–	–	–
Decomposition:							
Adenine	+	+	–	–	–	–	–
Casein	–	–	–	–	+	–	–
Tyrosine	–	–	+	–	+	+	+
Xanthine	–	–	+	–	–	–	+
Hypoxanthine	–	+	+	–	+	+	+
Uric acid	+	+	+	–	+	–	–
Aesculin	+	–	–	+	–	–	–
Polar lipids*	DPG, PE, uPL, uL1, uL2	DPG, PE, PI, PIM	DPG, PE, PI, PIM	DPG, PE, PI, PIM, GL	DPG, PE, PI, PIM	DPG, PE, PI, PIM	DPG, PE, PI, PIM
DNA G+C content (mol%)	65.6	67.6	70.5	72	68.4	69.6	68

*DPG, diphosphatidylglycerol; GL, glycolipid; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; uL, unidentifiedlipid; uPLunidentifiedphospholipid.

The extracted genomic DNA of strain CICC 11023^T was sequenced by combining Illumina HiSeq at the CGMCC. An Illumina library with an insert size of about 400 bp was prepared from 500 ng of DNA using the TruSeq DNA Sample Prep Kit according to the manufacturer's instructions. Genes were predicted within the completed genomic sequence using Glimmer software 3.02 [36]. tRNA genes were predicted using tRNAscan-SE 1.3.1 [37], and rRNA genes were identified using RNAmmer 1.2 [38]. The protein sequence of the predicted gene was BLASTP-aligned with the Nr, Swiss-prot, string and GO databases, respectively (BLAST 2.2.28+), thereby obtaining annotation information for the predicted gene. Konstantinidis and Tiedje [39] proposed that the 70% DDH standard seen as a pragmatic cut-off value for the delineation of species corresponds to 94% average nucleotide identity (ANI) value in the definition of prokaryotic species. The orthologous ANI algorithm used the USEARCH program [40]. The final genome of strain CICC 11023^T comprised 48 scaffolds with a total size of 8.78 Mb and a G+C content of 67.4 mol% overall, 68.05 mol% for the gene regions and 62.84 mol% for the intergenetic regions. The assembled contigs were annotated with the NCBI Prokaryotic Genome Annotation Pipeline pipeline [41], yielding a total of 9563 coding genes. General features of the genome of strain CICC

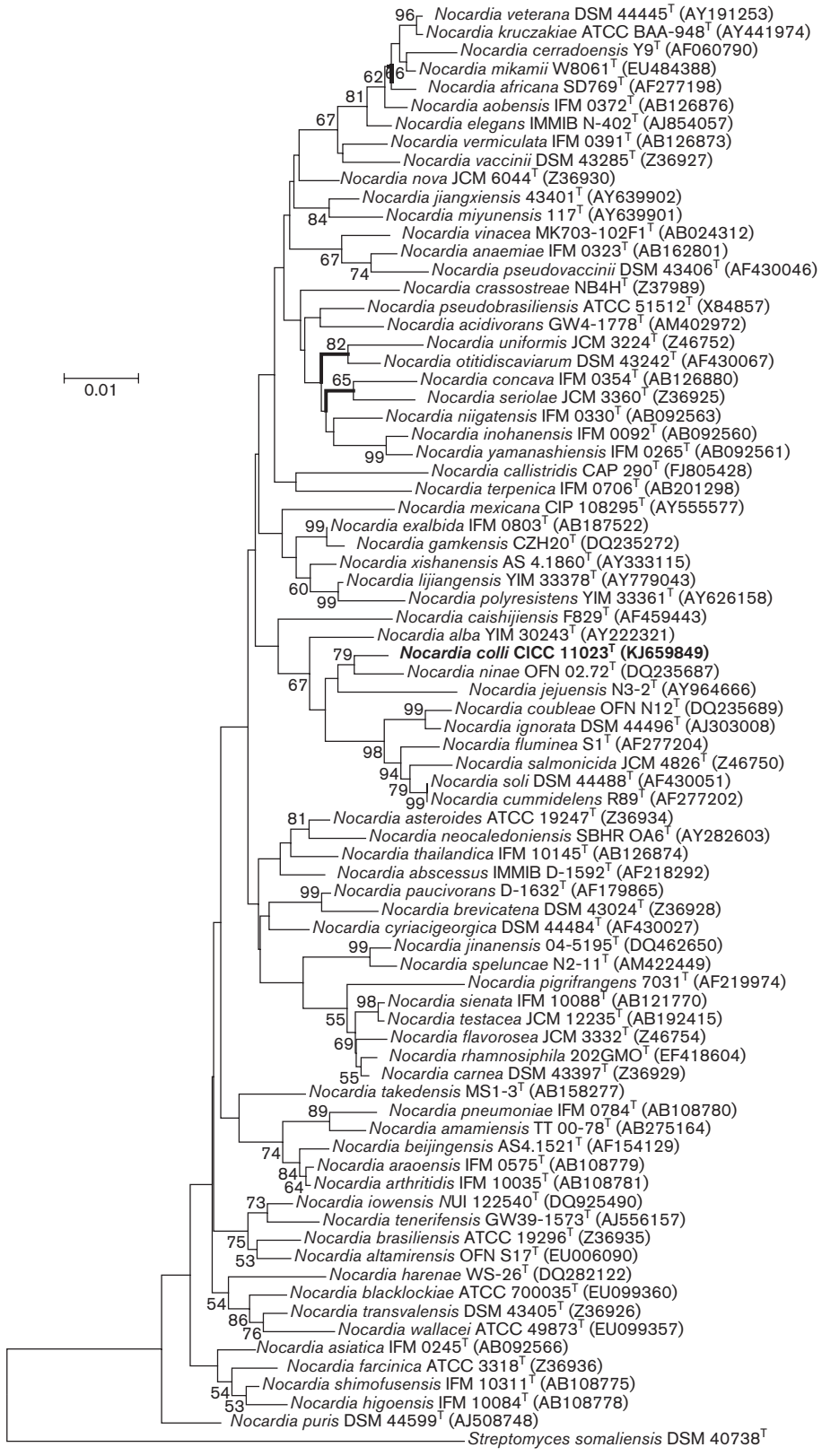

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing relationships between Nocardia *colli* CICC 11023^T and closely related type strains of the genus *Nocardia*. *Streptomyces somaliensis* DSM 40738^T was used as outgroup. Bootstrap values were expressed as percentages of 1000 replications. The branching is supported by the results from the three algorithms used. Bar, 0.01 substitutions per nucleotide position. GenBank accession numbers are given in parentheses.

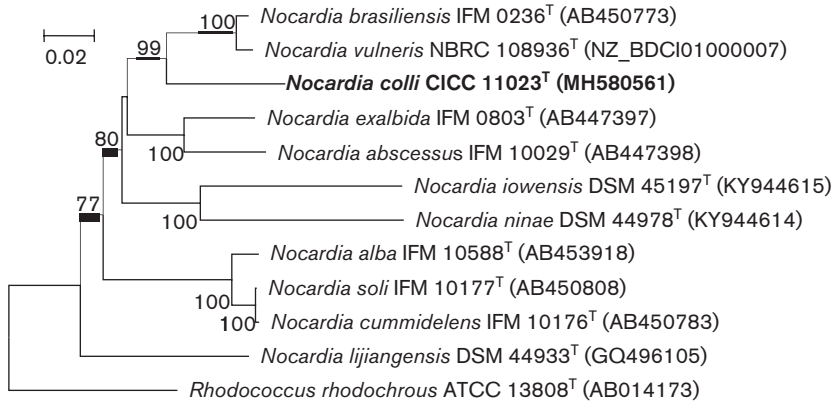

Fig. 2. Phylogenetic trees derived from the *gyrB* gene sequences showing relationships between Nocardia *colli* CICC 11023^T and closely related type strains of the genus *Nocardia*. The trees were created using the neighbour-joining method. *Rhodococcus rhodochrous* ATCC 13808^T was used as outgroup. Bootstrap values were expressed as percentages of 1000 replications. Bar, 0.02 substitutions per nucleotide position. GenBank accession numbers are given in parentheses. The numbers on the tree represent bootstrap values for the branch points. Bootstrap values greater than 50 % significance are indicated.

11023^T are shown in Table S3. The ANI values of strain CICC 11023^T were calculated between *N. alba* YIM 30243^T and *N. vulneris* NBRC 108936^T, respectively. The OrthoANIu value between CICC 11023^T and *N. alba* YIM 30243^T was 77.47%, the DDH value was 25%, and the OrthoANIu value between CICC 11023^T and *N. vulneris* NBRC 108936^T was 83.75%), the DDH value was 21.6%.

In conclusion, the morphological and chemotaxonomical characteristics and the results of phylogenetic analyses support that strain CICC 11023^T had characteristics typical of a member of the genus *Nocardia*. The differential characteristics shown in Table 2 indicate that strain CICC 11023^T has several different phenotypic properties that allow discrimination from the closest related species of the genus *Nocardia*, including utilization of raffinose, sorbitol, milk peptonization, and decomposition of aesculin. In addition, the cellular fatty acid analysis clearly suggested that CICC 11023^T contained relatively large amounts of $C_{17:0}$ and $C_{18:0}$ 10-methyl (TBSA), and small amounts of $C_{16:0}$ and $C_{18:1}\omega9c$. The unique 16S rRNA and *gyrB* gene sequences and low level of DDH support that strain CICC 11023^T represents a new species of the genus *Nocardia* with low ANI values (<94%). The name *Nocardia colli* sp. nov. is proposed.

DESCRIPTION OF *NOCARDIA COLLI* SP. NOV.

Nocardia colli (col'li. L. neut. gen. n. *colli* of the neck).

Strain CICC 11023^T is an aerobic, Gram stain-positive, non-motile, modified acid alcohol-positive actinomycetes bacterium, which forms an extensively branched grey-white substrate mycelium and aerial mycelium with fragments forming short coli-like bodies under scanning electronic microscopy (0.5×0.7–0.9 µm in diameter). The growth temperature range of strain CICC 11023^T is 21–37 °C with an optimum growth temperature of 28 °C. The salt tolerance is in the range of 1–4% and the optimum growth salinity is 1%. The strain shows positive reactions for milk peptonization, catalase, aesculin, urease and cellulose; negative reactions for milk coagulation, nitrate reduction, gelatin liquefaction, tyrosinase, hydrolysis of starch, casein, xanthine, hypoxanthine and production of H_2S. Strain CICC 11023^T can utilize L-arabinose, rhamnose, D-fructose, salicin, D-xylose, inositol, lactose, melibiose, D-glucose, raffinose, sucrose, D-mannitol, maltose, trehalose and arabinose as sole carbon sources. No soluble pigments are produced. The main fatty acids (>5%) are $C_{18:0}$ 10-methyl (TBSA), $C_{16:0}$, summed feature 4 ($C_{16:1}$ trans 9/$C_{15:0}$ iso 2OH), $C_{15:0}$ and $C_{17:0}$ 10-methyl. The main menaquinone is cyclo MK-8(H_4, ω-cyclo). The phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and two unidentified lipids. The type strain is susceptible to levofloxacin, ofloxacin, tobramycin and clindamycin; resistant to fosfomycin, imipenem, vancomycin and erythromycin. The organism is a pathogen of cutaneous infection in normal immunocompetent patients. The DNA G+C content of the type strain is 65.6 mol%.

The type strain, CICC 11023^T (=KCTC 39837^T), was isolated from aerobic culture of a biopsied skin tissue specimen from a 36-year-old female patient with primary cutaneous nocardiosis in Yunnan Province, south-west China.

Funding information
This work was supported by the Special Coordination Funds of Science and Technology Department of Yunnan Province and Kunming Medical University (2010CD174).

Acknowledgements
We are grateful to Professor Lihua Xu (Yunnan Institute of Microbiology, Yunnan University, China) for technical assistance.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new Hierarchic classification system, actinobacteria classis nov. *Int J Syst Bacteriol* 1997;47:479–491.
2. Trevisan VB.1889. I Generi E Le specie delle Batteriacee. L. Zanaboni E Gabuzzi. http://scholar.google.com/scholar?cluster=12213764842208350008&hl=en&oi=scholarr
3. Beaman BL, Beaman L. *Nocardia* species: host-parasite relationships. *Clin Microbiol Rev* 1994;7:213–264.
4. Conville PS, Brown JM, Steigerwalt AG, Brown-Elliott BA, Witebsky FG. *Nocardia wallacei* sp. nov. and *Nocardia blacklockiae* sp. nov., Human Pathogens and Members of the "Nocardia transvalensis Complex". *J Clin Microbiol* 2008;46:1178–1184.
5. Takeda K, Kang Y, Yazawa K, Gonoi T, Mikami Y. Phylogenetic studies of *Nocardia* species based on *gyrB* gene analyses. *J Med Microbiol* 2010;59:165–171.
6. Tindall BJ, Busse H-J, Ludwig W, Rosselló-Móra R, Kämpfer P. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;60:249–266.
7. Conville PS, Nocardia WFG, Rhodococcus G. *Actinomadura, Streptomyces*, and other aerobic *Actinomycetes*. *In: Manual of Clinical Microbiology*, 10th ed. American Society of Microbiology;; 2011.
8. Welsh O, Vera-Cabrera L, Salinas-Carmona MC. Current treatment for Nocardia infections. *Expert Opin Pharmacother* 2013;14:2387–2398.
9. Wang X, Zhou T, Deng D, Guo Y. A case of cutaneous nocardiosis with involvement of the trachea, anterior mediastinum and sternum. *Case Rep Dermatol* 2010;2:177–182.
10. Fey PD, Olson ME. Current concepts in biofilm formation of *Staphylococcus epidermidis*. *Future Microbiol* 2010;5:917–933.
11. Li WJ, Jiang Y, Kroppenstedt RM, Xu LH, Jiang C-L. *Nocardia alba* sp.nov., a novel actinomycete strain isolated from soil in China. *Syst Appl Microbiol* 2004;27:308–312.
12. Athalye M, Noble WC, Minnikin DE. Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. *J Appl Bacteriol* 1985;58:507–512.
13. Kong BH, Li YH, Liu M, Liu Y, Li CL *et al*. *Massilia namucuonensis* sp. nov., isolated from a soil sample. *Int J Syst Evol Microbiol* 2013;63:352–357.
14. Uchida K, Kudo T, Suzuki K-ichiro, Nakase T. A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* 1999;45:49–56.
15. Lechevalier MP, Lechevalier H. Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20:435–443.
16. Becker B, Lechevalier MP, Gordon RE, Lechevalier HA. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl Microbiol* 1964;12:421–423.
17. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
18. Groth I, Schumann P, Rainey FA, Martin K, Schuetze B *et al*. *Demetria terragena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* 1997;47:1129–1133.
19. Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M. Thin-Layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* 1980;188:221–233.
20. Collins MD, Jones D. Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* 1980;48:459–470.
21. Wang Y, Jiang Y. Chemotaxonomy of Actinobacteria. In: Dhanasekaran D (editor). *Actinobacteria - Basics and Biotechnological Applications*. InTech; 2016.
22. Kageyama A, Torikoe K, Iwamoto M, Masuyama J-I, Shibuya Y *et al*. *Nocardia arthritidis* sp. nov., a new pathogen isolated from a patient with rheumatoid arthritis in Japan. *J Clin Microbiol* 2004;42:2366–2371.
23. Nie G-X, Ming H, Li S, Zhou E-M, Cheng J *et al*. *Amycolatopsis dongchuanensis* sp. nov., an actinobacterium isolated from soil. *Int J Syst Evol Microbiol* 2012;62:2650–2656.
24. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA and Krieg NR (editors). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society of Microbiology; 1994.
25. Hornef MW, Gandorfer A, Heesemann J, Roggenkamp A. Humoral response in a patient with cutaneous nocardiosis. *Dermatology* 2000;200:78–80.
26. Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R *et al*. *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* 2007;57:1424–1428.
27. Thompson J, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–4882.
28. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
29. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Biol* 1971;20:406–416.
30. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 2003;52:696–704.
31. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007;24:1596–1599.
32. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
33. Mesbah M, Premachandran U, Whitman WB. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159–167.
34. Gillis M, Ley JD, Cleene MD. The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur J Biochem* 1970;12:143–153.
35. Wayne LG. International Committee on systematic bacteriology announcement of the report of the ad hoc Committee on reconciliation of approaches to bacterial Systematics. *J Appl Bacteriol* 1988;64:283–284.
36. Delcher AL. *Glimmer Release Notes Version 3.02*; 2006.
37. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997;25:955–964.
38. Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007;35:3100–3108.
39. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 2005;102:2567–2572.
40. Edgar RC. Search and clustering orders of magnitude faster than blast. *Bioinformatics* 2010;26:2460–2461.
41. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP *et al*. Ncbi prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44:6614–6624.