

## *Nocardioides deserti* sp. nov., an actinobacterium isolated from desert soil

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A rod- or coccus-shaped, non-spore-forming actinobacterium, designated strain SC8A-24<sup>T</sup>, was isolated from a soil sample collected from the rhizosphere of *Alhagi sparsifolia* on the southern edge of the Taklimakan desert, Xinjiang, China, and examined by a polyphasic approach to clarify its taxonomic position. This actinobacterium was Gram-staining-positive and aerobic. Substrate and aerial mycelia were not observed, and no diffusible pigments were observed on the media tested. Strain SC8A-24<sup>T</sup> grew optimally without NaCl at 28–30 °C and pH 7.0–8.0. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain SC8A-24<sup>T</sup> belonged to the genus *Nocardioides* and shared the highest 16S rRNA gene sequence similarity with *Nocardioides salarius* CL-Z59<sup>T</sup> (96.51%), *N. pyridinolyticus* OS4<sup>T</sup> (96.43%) and *N. ginsengagri* BX5-10<sup>T</sup> (96.37%). The DNA G+C content of strain SC8A-24<sup>T</sup> was 71 mol%. The cell-wall peptidoglycan contained LL-2,6-diaminopimelic acid, and MK-8(H<sub>4</sub>) was the predominant menaquinone. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, an unidentified glycolipid and an unidentified phospholipid. The major fatty acids were C<sub>17:1</sub>ω8c, 10-methyl C<sub>17:0</sub> and C<sub>18:1</sub>ω9c. On the basis of phylogenetic analysis and phenotypic and chemotaxonomic characteristics, strain SC8A-24<sup>T</sup> represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides deserti* sp. nov. is proposed. The type strain is SC8A-24<sup>T</sup> (=DSM 26045<sup>T</sup>=CGMCC 4.7183<sup>T</sup>).

The genus *Nocardioides*, with *Nocardioides albus* as the type strain, was proposed by Prauser (1976). At the time of preparing this paper, the genus contained 73 species with validly published names (<http://www.bacterio.net/nocardioides.html>; Euzéby, 1997). Some of the 73 species were isolated from saline–alkaline environments; for instance, four type strains were isolated from alkaline soils (Yoon *et al.*, 2005a, b, c, 2006), nine type strains were isolated from marine sediments (Yi & Chun, 2004a, b; Lee *et al.*, 2007; Lee, 2007; Park *et al.*, 2008; Dastager *et al.*, 2009; Kim *et al.*, 2009; Fan *et al.*, 2014; Zhang *et al.*, 2014), one species was isolated from a salt lake (Lawson *et al.*, 2000) and three species were isolated from seawater (Choi *et al.*, 2007; Cho *et al.*, 2013a, b).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SC8A-24<sup>T</sup> is KM816582.

Two supplementary figures and a supplementary table are available with the online Supplementary Material.

During a study on cultivable actinobacterial diversity of desert soil (Dong *et al.*, 2013), strain SC8A-24<sup>T</sup> was isolated from a saline–alkaline soil sample (37° 02' 38" N 80° 15' 01" E) in the rhizosphere of *Alhagi sparsifolia* collected from the southern edge of the Taklimakan desert, Xinjiang, China. Based on phylogenetic analysis, strain SC8A-24<sup>T</sup> showed high levels of 16S rRNA gene sequence similarity to members of the genus *Nocardioides*. Our polyphasic taxonomic study showed that strain SC8A-24<sup>T</sup> was distinguished from previously described species of the genus *Nocardioides* and represents a novel species. In this paper, the taxonomic position of this strain is reported.

Strain SC8A-24<sup>T</sup> was isolated by using the dilution plating technique on modified starch casein agar (containing per 1 distilled water: 10.0 g glucose, 0.3 g casein, 2.0 g KNO<sub>3</sub>, 0.05 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.0 g K<sub>2</sub>HPO<sub>4</sub>, 1.0 g CaCl<sub>2</sub>, 0.01 g FeSO<sub>4</sub>, 36.0 g NaCl, 12.0 g KCl, 2.0 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 20.0 g agar, pH 8.0) after 8 weeks of incubation at 28 °C.

Colonies were transferred and streaked onto ISP 2 agar (Shirling & Gottlieb, 1966) until pure strains were obtained. The strain was cultivated, maintained on ISP 2 agar slants at 4 °C and stored as an aqueous glycerol suspension (20 %, v/v) at -80 °C.

Cultural, physiological and biochemical characteristics of strain SC8A-24<sup>T</sup> were tested with the following three reference strains tested under the same conditions: *Nocardiooides ginsengagri* BX5-10<sup>T</sup> and *Nocardiooides plantarum* NCIMB 12834<sup>T</sup> from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and *Nocardiooides fonticola* NAA-13<sup>T</sup> from the Bioresources Collection and Research Center (Taiwan). Cultural characteristics were determined by observing growth of the strain at 28 °C for 3–4 weeks on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 7 agars (Shirling & Gottlieb, 1966), nutrient agar (Waksman, 1961), R2A agar (Difco), tryptic soy agar (TSA; Bacto) and Bennett's agar (Gordon & Smith, 1955); the ISCC-NBS colour charts (Kelly, 1964) were used to assess colony colours. Morphological characteristics were observed after incubation at 28 °C for 9 days on ISP 2 agar by light microscopy (model BH2; Olympus) and recorded by scanning electron microscopy (Quanta 200; FEI) using gold-coated, dehydrated specimens. The Gram-staining test was performed as described by Magee *et al.* (1975). Growth under anaerobic conditions was determined after incubation in the BBL GasPak Anaerobic System (Difco) at 28 °C for 14 days. The temperature range for growth was determined by incubation of the strain on R2A agar at 4, 10, 20, 25, 28, 30, 37, 42 and 50 °C for 14 days. The pH range for growth was measured in R2A broth at pH 4.0–13.0 (at intervals of 1 pH units) for 4 weeks. For the pH experiments, the buffers used were as described by Xu *et al.* (2005). Salt tolerance was tested in R2A broth without added NaCl and supplemented with 1, 3, 5, 7 and 10 % (w/v) NaCl for 14 days. Catalase activity was determined by bubble production in 3 % (v/v) H<sub>2</sub>O<sub>2</sub>. Oxidase activity was assessed by using 1 % (w/v) tetramethyl *p*-phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of starch, cellulose, gelatin and Tweens 20, 40 and 80, production of H<sub>2</sub>S and melanin, milk coagulation and peptonization were examined as described by Gonzalez *et al.* (1978). Acid production from carbon sources was tested using the API 50CH system (bioMérieux) according to the manufacturer's instructions. Oxidation of carbon sources and sensitivity to antimicrobial compounds were tested using Biolog GEN III MicroPlates (Biolog). Other biochemical characteristics and enzyme activities were tested by using the API 20NE, API Coryne and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Strain SC8A-24<sup>T</sup> was Gram-staining-positive, non-spore-forming and aerobic. Colonies of strain SC8A-24<sup>T</sup> on ISP 2 agar were circular, smooth and entire and brilliant yellow. Substrate and aerial mycelia were not observed, and no diffusible pigments were produced on the media tested. Cells were rod- or coccus-shaped. Strain SC8A-24<sup>T</sup> grew well on TSA, ISP 2 agar, ISP 3 agar, R2A agar and nutrient agar. Poor growth occurred on Bennett's agar, ISP 4 and

ISP 5 agars. No growth occurred on ISP 7 agar. The temperature range for growth of strain SC8A-24<sup>T</sup> was 10–42 °C, the pH range was pH 5.0–12.0, and the range of NaCl concentration was 0–7 %. No growth occurred at 4 or 50 °C, pH 4.0 or 13.0 or in the presence of 10 % NaCl. The best growth occurred at pH 7.0–8.0, 28–30 °C and without NaCl. The detailed physiological and biochemical characteristics of strain SC8A-24<sup>T</sup> are given in Table 1 and the species description.

For molecular systematic study and chemotaxonomic analyses of menaquinones and polar lipids, strain SC8A-24<sup>T</sup> and the reference strains were grown in ISP 2 broth for 7 days at 28 °C with shaking at 180 r.p.m., except strain *N. ginsengagri* BX5-10<sup>T</sup>, which was cultivated in R2A broth under the same conditions, since *N. ginsengagri* BX5-10<sup>T</sup> did not grow in ISP 2 broth. The isomers of diaminopimelic acid in whole-cell hydrolysates of strain SC8A-24<sup>T</sup> were identified by TLC as described by Schleifer & Kandler (1972). The polar lipids were extracted and analysed by two-dimensional TLC on a silica gel 60 F<sub>254</sub> plates (Merck) as described by Minnikin *et al.* (1984); the solvent systems of the first and second dimensions were chloroform/methanol/water (64:27:5, by vol.) and chloroform/methanol/acetic acid/water (80:18:12:5, by vol.), respectively. Menaquinones were isolated and purified according to the method of Collins *et al.* (1977), and then analysed and confirmed by HPLC (Wu *et al.*, 1989) and by using a single quadrupole mass spectrometer LCMS-2020 (Shimadzu), respectively. The parameters for separation and molecular ion peak identification of menaquinones were as follows: a UFLC system was used, equipped with an SPD-M20A photodiode array detector and an atmospheric pressure chemical ionization (APCI) interface, a reversed-phase column (Shim-pack XR-ODS, 3.0 mm i.d. × 75 mm; Shimadzu) with methanol/isopropanol (60:40, v/v) as the mobile phase at a flow rate of 0.3 ml min<sup>-1</sup>. The APCI interface in positive ionization mode was used for MS analysis with the following operating settings: nebulizer gas flow rate, 4 l min<sup>-1</sup>; drying gas flow rate, 15 l min<sup>-1</sup>; APCI interface temperature, 350 °C; DL temperature, 250 °C; heat block temperature, 200 °C; APCI interface voltage, 4.5 kV; detector voltage, 1.20 kV. Data acquisition and processing were accomplished using Shimadzu LCMS solution software. For the analysis of whole-cell fatty acids, cell mass of strain SC8A-24<sup>T</sup> and the reference strains was harvested from R2A agar at 28 °C, when the bacterial cultures reached the late-exponential stage of growth according to the four-quadrant steak method (Sasser, 1990). The whole-cell fatty acids were saponified, methylated and extracted according to the standard protocol described by Sasser (1990), and analysed by using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C single quadrupole mass spectrometer equipped with Nist08 Library software database. A capillary column HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Agilent Technologies) was used for separation of fatty acid methyl esters. The initial temperature of 90 °C was maintained for 1 min, raised to 180 °C at the rate of 10 °C min<sup>-1</sup>, raised to

**Table 1.** Differential characteristics of strain SC8A-24<sup>T</sup> and type strains of related species of genus *Nocardioides*

Strains: 1, SC8A-24<sup>T</sup>; 2, *N. ginsengagri* BX5-10<sup>T</sup>; 3, *N. fonticola* NAA-13<sup>T</sup>; 4, *N. plantarum* NCIMB 12834<sup>T</sup>. Data were obtained in this study unless indicated otherwise. All strains were Gram-staining-positive, positive for hydrolysis of Tweens 20 and 40 and positive for catalase, but negative for hydrolysis of cellulose, urease activity and production of H<sub>2</sub>S and indole. In API 20NE kits, all strains were negative for assimilation of adipate and phenylacetate. In API ZYM kits, all strains were positive for acid phosphatase, esterase lipase,  $\alpha$ -glucosidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -chymotrypsin,  $\alpha$ -fucosidase,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase, lipase,  $\alpha$ -mannosidase. All four strains have MK-8(H<sub>4</sub>) as the major menaquinone. +, Positive; -, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4
Cell morphology	Cocci, rods	Short rods <sup>a*</sup>	Rods <sup>b</sup>	Short rods, cocci <sup>c</sup>
Cell size ( $\mu$ m)	0.3–0.6 $\times$ 0.3–1.1	0.4–0.7 $\times$ 1.5–2.8 <sup>a</sup>	0.8 $\times$ 2.0–9.0 <sup>b</sup>	0.5–0.7 $\times$ 1.0–1.6 <sup>c</sup>
Colony colour on ISP 2	Brilliant yellow	ND	Vivid yellow	White
Growth pH range	5.0–12.0	6.0–9.0	5.0–9.0	5.0–9.0
Growth temperature ( $^{\circ}$ C)				
Range	10–42	10–37	20–37	4–30
Optimum	30	30	30	25
NaCl concentration for growth (% w/v)				
Range	0–7	0–1	0–1	ND
Optimum	0	0	1	ND
Oxidase	–	– (+ <sup>a</sup> )	–	–
Milk peptonization and coagulation	–	–	+	–
Nitrate reduction	–	–	+ (– <sup>b</sup> )	–
Hydrolysis of:				
Starch	+	–	+	+ (– <sup>c</sup> )
Tween 80	+	– <sup>a</sup>	+	+
Gelatin	–	–	+	–
Carbon source utilization				
D-Glucose	–	–	+	– <sup>a</sup>
L-Arabinose	–	– (+ <sup>a</sup> )	– (+ <sup>b</sup> )	– <sup>a</sup>
D-Mannose	–	–	– (+ <sup>b</sup> )	– <sup>a</sup>
D-Mannitol	–	–	+	– <sup>a</sup>
N-Acetyl-D-glucosamine	–	–	–	+ <sup>a</sup>
Maltose	–	–	+ (– <sup>b</sup> )	+ <sup>a</sup>
Potassium gluconate	–	–	–	+ <sup>a</sup>
Captate	–	–	–	+ <sup>a</sup>
Malic acid	–	–	–	+ <sup>a</sup>
Trisodium citrate	–	–	–	– <sup>a</sup>
Enzyme activities (API ZYM)				
Alkaline phosphatase	+	–	+	+
Esterase	+	+ (– <sup>a</sup> )	+	+
Valine arylamidase	–	+	–	+
Cystine arylamidase	+	–	–	+
Trypsin	+	–	–	+
$\beta$ -Galactosidase	–	–	+	– (+ <sup>a</sup> )
$\beta$ -Glucosidase	w	–	+	+
Major fatty acids (>10%) <sup>†</sup>	C <sub>17:1</sub> $\omega$ 8c, 10-Me C <sub>17:0</sub> , C <sub>18:1</sub> $\omega$ 9c	C <sub>18:1</sub> $\omega$ 9c, C <sub>16:0</sub> , 10-Me C <sub>18:0</sub> , C <sub>17:1</sub> $\omega$ 8c	i-C <sub>16:0</sub> , C <sub>17:0</sub>	i-C <sub>16:0</sub> , 10-Me C <sub>17:0</sub>
Polar lipids <sup>‡</sup>	DPG, PG, PL, GL, PME, PE, L	DPG, PG, PL, AL	DPG, PG, PL	DPG, PG, PL
DNA G + C content (mol%)	71.0	70.3 <sup>a</sup>	71.8 <sup>b</sup>	69.0 <sup>c</sup>

\*Data from: a, Lee *et al.* (2012); b, Chou *et al.* (2008); c, Collins *et al.* (1994).

<sup>†</sup>i, Iso-branched; Me, methyl.

<sup>‡</sup>DPG, Disphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmethylethanolamine; AL, unidentified aminolipid(s); GL, unidentified glycolipid(s); PL, unidentified phospholipid(s); L, unidentified lipid(s).

210 °C at the rate of 2 °C min<sup>-1</sup> and finally increased to 270 °C at the rate of 20 °C min<sup>-1</sup> and held for 2 min. Helium was used as the carrier gas with a flow rate of 1.0 ml min<sup>-1</sup>. Injection (2 µl) was made in splitless mode at an injector temperature of 270 °C. Mass spectra were obtained using electron impact (EI; 70 eV). To determine the G + C content, genomic DNA of strain SC8A-24<sup>T</sup> was prepared according to the method described by Marmur (1961) and the G + C content was determined by reversed-phase HPLC as described by Mesbah *et al.* (1989).

The whole-cell hydrolysate of strain SC8A-24<sup>T</sup> contained LL-2,6-diaminopimelic acid and the predominant menaquinone was MK-8(H<sub>4</sub>). The polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, an unidentified glycolipid, phosphatidylethanolamine, phosphatidylmethylethanolamine, five unidentified phospholipids and other lipids. The polar lipid profiles are shown in Fig. S1, available in the online Supplementary Material. The whole-cell fatty acid profile contained large amounts of C<sub>17:1</sub>ω8c (12.63%), 10-methyl C<sub>17:0</sub> (11.58%) and C<sub>18:1</sub>ω9c (10.28%) and small amounts of C<sub>16:0</sub> (9.57%), iso-C<sub>17:0</sub> (9.39%), iso-C<sub>16:0</sub> (8.94%), C<sub>17:0</sub> (6.04%), C<sub>17:1</sub>ω6c (4.93%), iso-C<sub>15:0</sub> (4.86%), 10-methyl C<sub>18:0</sub> (4.10%), C<sub>16:1</sub>ω9c (3.43%), anteiso-C<sub>17:0</sub> (3.42%), C<sub>18:0</sub> (3.31%), C<sub>15:0</sub> (2.89%), C<sub>16:1</sub>ω7c (1.70%), 10-methyl C<sub>16:0</sub> (1.54%), C<sub>17:0</sub> cyclo (<1%) and anteiso-C<sub>15:0</sub> (<1%). The cellular fatty acid profiles of strain SC8A-24<sup>T</sup> and the reference strains are given in Table S1. The DNA G + C content of strain SC8A-24<sup>T</sup> was 71 mol%. The major menaquinones, fatty acids and polar lipids of the three reference strains were similar to those reported previously (Collins *et al.*, 1994; Chou *et al.*, 2008; Lee *et al.*, 2012); differences in the proportions of fatty acids and slight differences in the types of polar lipids may be due to the different experimental conditions used.

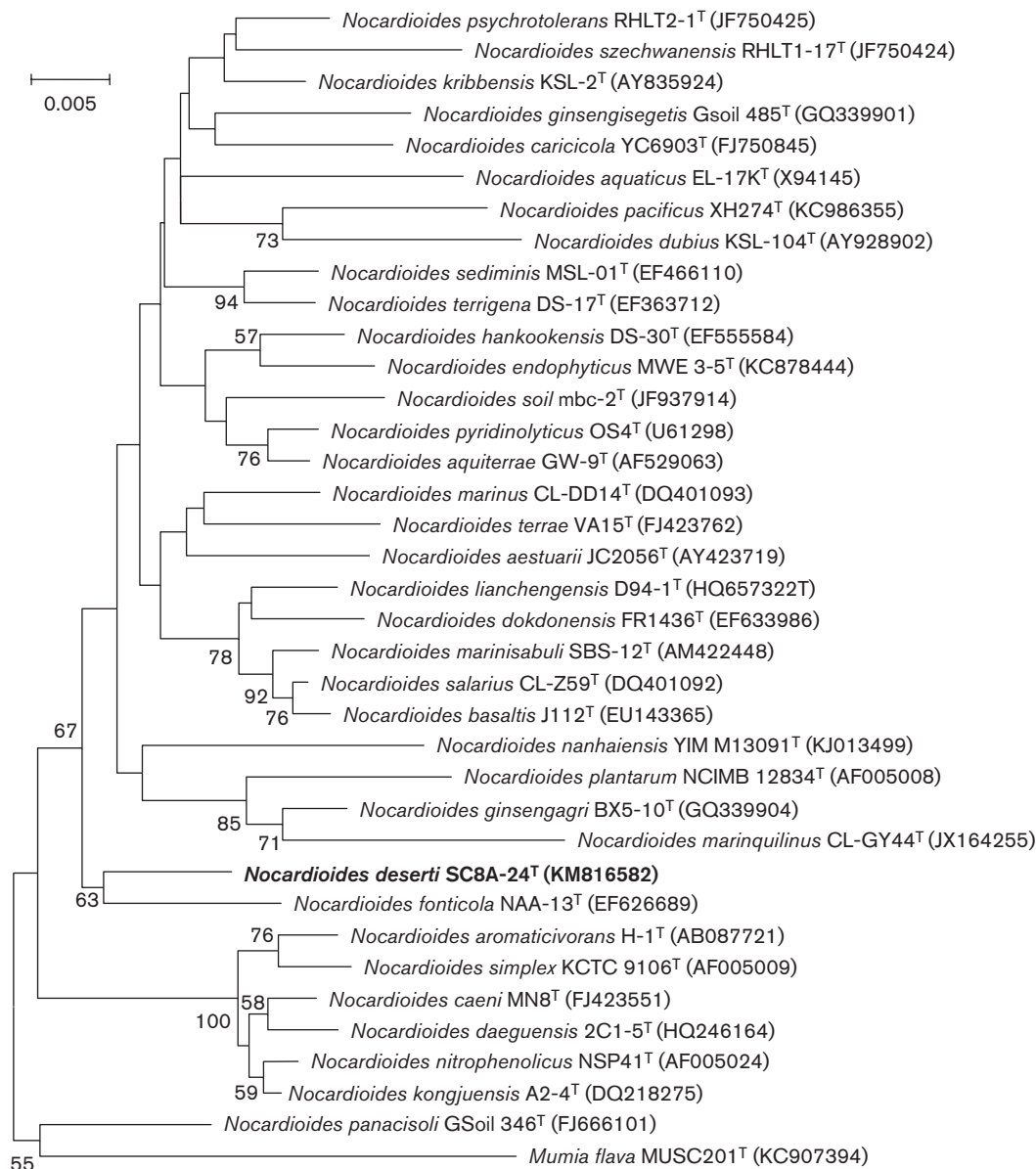
Extraction of genomic DNA from strain SC8A-24<sup>T</sup> and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). The purified PCR products were cloned using the pEASY-T1 Cloning kit (TransGen Biotech) according to the manufacturer's instructions, and sequenced by an ABI PRISM 3730XL DNA Analyser. 16S rRNA gene sequence similarity between strain SC8A-24<sup>T</sup> and type strains of related species was obtained from the EzTaxon server (<http://eztaxon-e.ezbiocloud.net/>; Chun *et al.*, 2007). Multiple alignments were made using CLUSTAL\_X (Thompson *et al.*, 1997). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods with MEGA version 5.0 (Tamura *et al.*, 2011). The topologies of the phylogenetic trees were evaluated by using the bootstrap method of Felsenstein (1985) with 1000 repeats.

The almost full-length 16S rRNA gene sequence (1482 bp) of strain SC8A-24<sup>T</sup> was obtained; BLAST searches showed that strain SC8A-24<sup>T</sup> exhibited the highest levels of 16S

rRNA gene sequence similarity with *Nocardioides salarius* CL-Z59<sup>T</sup> (96.51%), *N. pyridinolyticus* OS4<sup>T</sup> (96.43%) and *N. ginsengagri* BX5-10<sup>T</sup> (96.37%). Levels of similarity to other related strains in the genus *Nocardioides* were less than 96.37%. Phylogenetic trees based on 16S rRNA gene sequences generated by using all three tree-making methods showed that strain SC8A-24<sup>T</sup> formed a distinct cluster with *N. fonticola* NNA-13<sup>T</sup> (Figs 1 and S2) within the genus *Nocardioides*, which indicated that strain SC8A-24<sup>T</sup> was affiliated phylogenetically to the genus *Nocardioides*. However, the relatively low levels of sequence similarity to recognized species of the genus *Nocardioides* indicated that strain SC8A-24<sup>T</sup> represents a novel species.

Important chemotaxonomic characteristics of strain SC8A-24<sup>T</sup>, such as the presence of LL-2,6-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan and MK-8(H<sub>4</sub>) as the predominant menaquinone, were consistent with those of members of the genus *Nocardioides* (Evtushenko *et al.*, 2012). The DNA G + C content of strain SC8A-24<sup>T</sup> was 71 mol%, which falls within the range reported for members of the genus *Nocardioides* (68.7–74.9 mol%; Fan *et al.*, 2014). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SC8A-24<sup>T</sup> should be assigned to the genus *Nocardioides*, but also could be clearly distinguished from all other known species of the genus. In the polar lipid profiles, strain SC8A-24<sup>T</sup> apparently contained more components than the reference strains. Though strain SC8A-24<sup>T</sup> shared the polar lipids diphosphatidylglycerol and phosphatidylglycerol with the reference strains, only strain SC8A-24<sup>T</sup> contained phosphatidylmethylethanolamine, phosphatidylethanolamine and an unidentified glycolipid. In addition, differences in unidentified lipids between SC8A-24<sup>T</sup> and the reference strains were also significant. Strain SC8A-24<sup>T</sup> contained unidentified lipids L1, L2 and L4, which were absent from the reference strains (Fig. S1). In general, the cellular fatty acids of members of the genus *Nocardioides* are complex mixtures of saturated and monounsaturated, straight-chain and iso-, anteiso- and 10-methyl-branched components, including 10-methyl octadecanoic acid, among which iso-C<sub>16:0</sub> usually predominates (Evtushenko *et al.*, 2012). Although strain SC8A-24<sup>T</sup> contained iso-C<sub>16:0</sub>, the proportion (8.94%) of iso-C<sub>16:0</sub> was significantly lower than that of the reference strains *N. fonticola* NAA-13<sup>T</sup> (47.15%) and *N. plantarum* NCIMB 12834<sup>T</sup> (34.93%); meanwhile, the dominant fatty acids (>10%) of strain SC8A-24<sup>T</sup> were C<sub>17:1</sub>ω8c (12.63%), 10-methyl C<sub>17:0</sub> (11.58%) and C<sub>18:1</sub>ω9c (10.28%), which differentiated it from the reference strain *N. ginsengagri* BX5-10<sup>T</sup> [C<sub>18:1</sub>ω9c (30.67%), C<sub>16:0</sub> (19.56%), 10-methyl C<sub>18:0</sub> (11.20%), C<sub>17:1</sub>ω8c (10.54%)] (Table S1). Other characteristics that differentiated strain SC8A-24<sup>T</sup> from closely related members of the genus *Nocardioides* are shown in Table 1.

In conclusion, based on the phylogenetic analysis and phenotypic and chemotaxonomic characterization, strain SC8A-24<sup>T</sup> represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides deserti* sp. nov. is proposed.



**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences of strain SC8A-24<sup>T</sup> and related strains in the genus *Nocardiooides*. Numbers at nodes are percentage bootstrap values (based on 1000 replicates; only values >50% are shown). Bar, 5 substitutions per 1000 nucleotide positions.

### Description of *Nocardiooides deserti* sp. nov.

*Nocardiooides deserti* (de.ser' ti. L. gen. n. *deserti* of a desert).

Cells are Gram-staining-positive, aerobic and rod- or coccus-shaped (measuring 0.3–0.6 × 0.3–1.1 μm). Substrate and aerial mycelia are not observed, and no diffusible pigments are produced on any tested media. Colonies on ISP 2 agar are circular, smooth and entire and brilliant yellow. Grows well on ISP 2 agar, ISP 3 agar, TSA, R2A agar and nutrient agar; poor growth occurs on ISP 4 agar, ISP 5 agar and Bennett's agar, and no growth occurs on ISP 7 agar. Growth occurs at 10–42 °C (optimum, 28–30 °C) and pH 5.0–12.0 (optimum,

pH 7.0–8.0) and with NaCl concentrations of 0–7% (w/v) (optimum growth in the absence of added NaCl). No growth at 10 or 50 °C or at pH 4.0 or 13.0. Cells are positive for catalase activity and hydrolysis of starch and Tweens 20, 40 and 80. Hydrolysis of cellulose, nitrate reduction, urease production, milk peptonization and coagulation and oxidase activity are negative. Positive for acid and alkaline phosphatases, cystine arylamidase, esterase (C4), esterase lipase (C8), α-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and trypsin. Weakly positive for β-glucosidase. Negative for *N*-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase,

lipase (C14),  $\alpha$ -mannosidase and valine arylamidase. Produces acid from aesculin, D-fructose, D-galactose, D-glucose, L-rhamnose and D-xylose, but not from N-acetylglucosamine, D-adonitol, amygdalin, D- and L-arabinose, D- and L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, glycogen, glycol, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, maltose, mannitol, D-mannose, melezitose, melibiose, methyl  $\alpha$ -D-glucopyranoside, methyl  $\alpha$ -D-mannopyranoside, methyl  $\beta$ -D-xylopyranoside, raffinose, D-ribose, sucrose, salicin, sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose, L-xylose and xylitol. In the Biolog system, positive for oxidation of acetoacetic acid, N-acetylneuraminic acid, L-arginine, cellobiose, dextrin, D-galactose, gelatin, gentiobiose,  $\alpha$ -D-glucose, L-glutamic acid, L-histidine, *p*-hydroxyphenylacetic, L-lactic acid, D-lactic acid methyl ester, D-malic acid, maltose, L-pyroglytamic acid, L-rhamnose, stachyose, sucrose, trehalose, turanose and Tween 40. Negative for oxidation of acetic acid, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl- $\beta$ -D-mannosamine, D-alanine,  $\gamma$ -aminobutyric acid, D-arabitol, D-aspartic acid, L-aspartic acid, bromosuccinic acid, citric acid, formic acid, D-fructose, D-fructose 6-phosphate, D- and L-fucose, galacturonic acid, D-galactonic acid lactone, D-gluconic acid, D-glucose 6-phosphate, glucuronamide, D-glucuronic acid, glycerol, glycyl L-proline,  $\alpha$ -hydroxybutyric acid,  $\beta$ -hydroxy-DL-butyric acid, inosine,  $\alpha$ -ketobutyric acid,  $\alpha$ -ketoglutaric acid, lactose, L-malic acid, D-mannitol, D-mannose, melibiose, 3-methyl D-glucose, methyl  $\beta$ -D-glucoside, methyl pyruvate, mucic acid, *myo*-inositol, pectin, propionic acid, quinic acid, raffinose, D-saccharic acid, salicin, D-serine, L-serine and D-sorbitol. Sensitive to aztreonam, fusidic acid, guanidine hydrochloride, lincomycin, lithium chloride, minocycline, nalidixic acid, niaproof 4, rifamycin SV, sodium bromate, sodium butyrate, tetrazolium blue, tetrazolium violet, troleandomycin, vancomycin, pH 5 and 6 and 8% NaCl; tolerates potassium tellurite, 1% sodium lactate and 1 and 4% NaCl. The cell-wall peptidoglycan contains LL-2,6-diaminopimelic acid as the diagnostic diamino acid. The predominant menaquinone is MK-8(H<sub>4</sub>). The polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, an unidentified glycolipid, phosphatidylethanolamine, phosphatidylmethylethanolamine, five unidentified phospholipids and other lipids. The major fatty acids are C<sub>17:1</sub>ω8c, 10-methyl C<sub>17:0</sub> and C<sub>18:1</sub>ω9c.

The type strain, SC8A-24<sup>T</sup> (=DSM 26045<sup>T</sup>=CGMCC 4.7183<sup>T</sup>), was isolated from a rhizosphere soil sample of *Alhagis parsifolia* collected from the Taklimakan desert, Xinjiang province, north-west China. The G + C content of the genomic DNA of the type strain is 71 mol%.

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