Nocardiopsis akesuensis sp. nov., an actinomycete isolated from a salt water beach

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The taxonomic position of a novel actinomycete, strain TRM 46250T, isolated from the sediment of a salt water beach at Baicheng, Xinjiang, China, was determined by a polyphasic approach. Strain TRM 46250T grew optimally in the presence of 2% (w/v) NaCl and an optimum temperature range for growth of 28–37 °C. The whole-cell sugars of strain TRM 46250T were ribose, xylose, mannose and galactose. The diagnostic amino acid was meso-diaminopimelic acid. The polar lipids were phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and six unidentified phospholipids. The predominant menaquinones were MK-10, MK-10(H4) and MK-10(H6). The major fatty acids were 10-methyl C18:0, iso-C16:0, C16:0, iso-G C16:1 and C18:1ω9c. Based on morphological and chemotaxonomic characteristics the isolate was determined to belong to the genus Nocardiopsis. The phylogenetic tree based on its nearly complete 16S rRNA gene sequence (1493 nt) with those of representative strains showed that the strain consistently falls into a distinct phyletic line together with Nocardiopsis gilva YIM 90087T (97.68% similarity) and a subclade consisting of Nocardiopsis composta KS9T (97.52%), Nocardiopsis rosea YIM 90094T (97.44%) and Nocardiopsis rhodophaea YIM 90096T (97.16%). However, DNA–DNA hybridization studies between strain TRM 46250T and N. gilva YIM 90087T showed only 36.94% relatedness. On the basis of these data, strain TRM 46250T should be designated as a representative of a novel species of the genus Nocardiopsis, for which the name Nocardiopsis akesuensis sp. nov. is proposed. The type strain is TRM 46250T (=CCTCC AA 2015027=KCTC 39725T).

The genus Nocardiopsis was first described by Meyer (1976) on the basis of chemotaxonomic and morphological characteristics. Species of the genus Nocardiopsis have been found in an Antarctic glacier, in marine sediments, in the actinorhizal plant rhizosphere, in the gastrointestinal tract of animals, as endophytes of yam bean, in salterns and in clinical material (Hamedi et al., 2011). At the time of writing, the genus Nocardioides comprised 49 species with validly published names (http://www.bacterio.net/nocardiopsis.html). In this paper we describe the results of a taxonomic study using a polyphasic approach on strain TRM 46250T and propose that this strain represents a novel species of the genus Nocardiopsis.

Strain TRM 46250T was isolated from a salt water beach of the Gobi desert, in Baicheng, Xinjiang province, China (41°55’ N 81°23’ E), at an altitude of 1385 m. The strain was isolated using ISP (International Streptomyces Project) medium 4 with 5% (w/v) NaCl after 7 days of aerobic incubation at 37 °C. The composition of the ISP medium 4 was (per litre distilled water): 10.0 g Difco soluble starch, 1.0 g K2HPO4, 1.0 g MgSO4.7H2O, 1.0 g NaCl, 2.0 g (NH4)2SO4, 2.0 g CaCO3, 1.0 ml trace salts solution and 20 g agar. The organism was grown and maintained on ISP medium 4 (Shirling & Gottlieb, 1966) at 4 °C and as a glycerol suspension (20%, v/v) at −20 °C. Biomass for chemical and molecular studies was obtained by cultivation in ISP medium 5 on a shaker at 180 r.p.m. and 28 °C for 7 days. Cultural characteristics were determined after incubation according to the methods given for the ISP 1–7 media. Cultural characteristics were recorded on seven standard media after incubation at 28 °C for 7 days.

The organism showed poor growth on ISP medium 3, ISP medium 4, ISP medium 7, Gauze’s Medium No. 1 and Czapek’s agar medium, moderate growth on ISP medium 1, ISP medium 2 and ISP medium 6, and relatively good growth
on ISP medium 5 and nutrient agar medium. There was no soluble pigment produced on any of the media. The morphological characteristics of strain TRM 46250 T were determined using the coverslip technique of Kawato & Shinobu (1959), and observed by light microscopy (Axioskop 20; Zeiss) and scanning electron microscopy (Quanta; FEI) after incubation on ISP medium 5 at 28 °C for 7 days. The colour of aerial hyphae and substrate mycelium was bright white on ISP medium 5. The aerial mycelium was sparse, flexuous and smooth-surfaced with irregular branches, and sporangia were formed (Fig. 1).

For NaCl tolerance tests, growth was studied at 28 °C on ISP medium 5. Growth was investigated on ISP medium 5 containing 0–10 % (w/v) NaCl at 4–55 °C and at pH 4–12 (at 1 pH unit intervals). Media and procedures used for determination of physiological features and carbon source utilization were those described by Williams et al. (1989). Enzyme activity and acid production from carbohydrates were determined by using the API ZYM and API 50CH systems (bio-Mérieux) according to the manufacturer’s instructions. Strain TRM 46250 T could grow at 25–50 °C, at pH 6.0–11.0 and with 0–10 % (w/v) NaCl; optimum growth occurred at pH 7.0–9.0, at 28 °C and with 2 % (w/v) NaCl. The phenotypic characteristics of strain TRM 46250 T differed from those of other species of the genus Nocardiopsis (Table 1). The detailed physiological and biochemical characteristics of strain TRM 46250 T are given in the species description.

Diaminopimelic acid isomers and sugar analysis of whole-cell hydrolysates were performed according to the procedures described by Stanec & Roberts (1974) and Hasegawa et al. (1983). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted using the method of Collins et al. (1984) and analysed by HPLC (Groth et al., 1997). Cellular fatty acid composition was determined using the Microbial Identification System (MIDI Sherlock version 6.0). DNA G+C content was determined by HPLC as described by Tamaoa & Komagata (1984). The cell wall of strain TRM 46250 T contained meso-diaminopimelic acid. Whole-cell hydrolysates contained ribose, xylose, mannose and galactose. The menaquinones were MK-10 (16.1 %), MK-10(H 4) (26.4 %) and MK-10(H 6) (30.8 %). The polar lipids were phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphostatidyglycerol, phosphatidymethyl ethanolamine and six unidentified phospholipids (Fig. S1, available in the online Supplementary Material). Major cellular fatty acids were 10-methyl C 18:0 (36.9 %), iso-C 16:0 (15.5 %), C 16:0 (7.2 %), iso-G C 16:1 (5.6 %) and C 18:1ω9c (5.5 %); fatty acids present in smaller amounts (between 1 and 5 %) were anteiso-C 17:0 (4.9 %), C 18:0 (2.7 %), 10-methyl C 17:0 (2.0 %), anteiso-C 15:0 (1.8 %), iso-C 18:0 (1.8 %), anteiso-C 17:1ω9c (1.5 %) and iso-C 17:0 (1.0 %). The G+C content of the DNA was 71.05 mol%.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene from strain TRM 46250 T were performed using an established method (Chun & Goodfellow, 1995). Multiple alignments with sequences from closely related members of the genus Nocardiopsis and calculations of sequence similarity were carried out using the EzTaxon-e server (Kim et al., 2012). Phylogenetic analyses were performed using three tree-making algorithms: neighbour-joining, maximum-parsimony and maximum-likelihood, using MEGA version 5 (Tamura et al., 2011). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The phylogenetic analysis based on 16S rRNA gene sequences revealed that strain TRM 46250 T falls within the radius of the genus Nocardiopsis and has the highest sequence similarity to Nocardiopsis gilva YIM 90087 T (GenBank accession no. AY619712; 97.68 %), N. composta K9 T (AF360734; 97.52 %), N. rosea YIM 90094 T (AY619713; 97.44 %) and N. rhodophaeae YIM 90096 T (AY619714; 97.16 %). All other Nocardiopsis species showed sequence similarity of lower than 97 % to strain TRM 46250 T. In the phylogenetic tree based on the neighbour-joining algorithm, strain TRM 46250 T clustered clearly with N. gilva YIM 90087 T, N. rosea YIM 90094 T and N. rhodophaeae YIM 90096 T (Fig. 2). This relationship was supported by other tree-making methods used in this study. According to Stackebrandt & Ebers (2006), when 16S rRNA gene sequence similarity values are above 98.7–99 % a novel isolate should be tested for its genomic uniqueness, a revision from the previously accepted value of 97 % similarity (Stackebrandt et al., 1994). Strain TRM 46250 T was subjected to a DNA–DNA relatedness test against N. gilva YIM 90087 T. The DNA–DNA hybridization experiment was carried out in microplate wells, as described by Ezaki et al. (1989). The strain was used to generate the labelled probes for DNA–DNA hybridization. The level of DNA–DNA relatedness between strain TRM 46250 T and N. gilva YIM 90087 T was 36.94 %, which indicates that the strain represents a distinct species. All of the above data confirmed

Fig. 1. Scanning electron micrograph of aerial mycelium of strain TRM 46250 T grown on ISP medium 5 at 28 °C for 7 days. Bar, 20 µm.
that strain TRM 46250\textsuperscript{T} should be assigned to the genus *Nocardiopsis*.

Strain TRM 46250\textsuperscript{T} was different from members of other species of the genus *Nocardiopsis* in some morphological and physiological properties (Table 1): scanning electron microscopic observations of strain TRM 46250\textsuperscript{T} showed the aerial mycelium to be sparse, with irregular branches, sporangia were formed and there were no spore chains on the aerial mycelium, which differentiated it from *N. gilva* YIM 90087\textsuperscript{T}, the nearest neighbouring species, and other phylogenetically closely related species. Moreover, strain TRM 46250\textsuperscript{T} exhibited some chemotaxonomic differences from *N. gilva* YIM 90087\textsuperscript{T}, *N. composta* KS9\textsuperscript{T}, *N. rosea* YIM 90094\textsuperscript{T} and *N. rhodophaea* YIM 90096\textsuperscript{T}; strain TRM 46250\textsuperscript{T} contained ribose, xylose, mannose and galactose in whole-cell hydrolysates; MK-10(H\textsubscript{8}), MK-10(H\textsubscript{6}) and MK-10 were the predominant menaquinones; and the polar lipids were phosphatidylglycerol, phosphatidylylcholine, phosphatidylethanolamine, phosphatidyglycerol, diphasphatidylglycerol, phosphatidylcholine and six unidentified phospholipids. By contrast, the phylogenetically closely related species did not contain ribose as a whole-cell sugar, and the diagnostic polar lipids and predominant cellular fatty acids also differed compared with strain TRM 46250\textsuperscript{T}.

On the basis of DNA–DNA hybridization data and a combination of phylogenetic distinctness and differences in chemotaxonomic, biochemical, physiological and morphological characteristics, strain TRM 46250\textsuperscript{T} represents a novel species in the genus *Nocardiopsis*, for which the name *Nocardiopsis akesuensis* sp. nov. is proposed.

### Description of *Nocardiopsis akesuensis* sp. nov.

*Nocardiopsis akesuensis* (a.ke.su.en’sis. N.L. fem. adj. akesuensis of or belonging to Akesu city, north-west China, from where the type strain was isolated).

Aerobic, Gram-stain-positive actinomycete, with sparse aerial mycelium with flexuous branches. Grows poorly on ISP medium 3, ISP medium 4, ISP medium 7, Gauze’s Medium No.1 and Czapek’s agar medium, with moderate growth on ISP medium 1, ISP medium 2 and ISP medium 6, but relatively good growth on ISP medium 5 and nutrient agar medium. Growth occurs at 25–50 °C, at pH 6.0–11.0 and with 0–10 % (w/v) NaCl; optimum growth is at 28 °C, at pH 7.0–9.0 and with 2 % (w/v) NaCl. L-Fucose, glucose and L-rhamnose can be used as sole carbon sources for growth, while most other carbon sources, such as arabinose, cellobiose, chitosan, fructose, D-galactose, inositol, lactose, maltose, melezitose, raffinose, ribose, D-saligenin, L-sorbose, sucrose, xylan, xylitol and D-xylose, cannot. Positive for oxidase reaction and catalase production, but negative for milk

### Table 1. Morphological, physiological and biochemical characteristics of strain TRM 46250\textsuperscript{T} and phylogenetically related species of the genus *Nocardiopsis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Aerial mycelium</td>
<td>Long</td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
<td>Short</td>
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<tr>
<td>Spore chains</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Spore shape</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth, irregular</td>
<td>Smooth</td>
<td>Smooth</td>
</tr>
<tr>
<td>Optimum temperature (°C)</td>
<td>28</td>
<td>28–30</td>
<td>15–45</td>
<td>37–40</td>
<td>37–40</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>8.0</td>
<td>7.2</td>
<td>6.5–9.5</td>
<td>7.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Utilization as sole carbon source</td>
<td>10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, 10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, C\textsubscript{18:1ω9c}</td>
<td>10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, 10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, C\textsubscript{18:1ω9c}</td>
<td>10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, 10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, C\textsubscript{18:1ω9c}</td>
<td>10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, 10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, C\textsubscript{18:1ω9c}</td>
<td>10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, 10-methyl C\textsubscript{18:0}, iso-G C\textsubscript{16:1}, C\textsubscript{18:1ω9c}</td>
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<tr>
<td>Ribose</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Sucrose</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Rhamnose</td>
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<tr>
<td>Fructose</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Inositol</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<td>Maltose</td>
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<td>–</td>
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<td>+</td>
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<tr>
<td>D-Xylose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>D-Galactose</td>
<td>–</td>
<td>+</td>
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<td>Gelatin liquefaction</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Urease</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</table>

Strains: 1, TRM 46250\textsuperscript{T}; 2, *Nocardiopsis gilva* YIM 90087\textsuperscript{T} (data from Li et al., 2006); 3, *Nocardiopsis composta* KS9\textsuperscript{T} (Kämpfer et al., 2002); 4, *Nocardiopsis rosea* YIM 90094\textsuperscript{T} (Li et al., 2006); 5, *Nocardiopsis rhodophaea* YIM 90096\textsuperscript{T} (Li et al., 2006). +, Positive; −, negative.
peptonization, urease, gelatin liquefaction, nitrate reduction, starch hydrolysis, melanin production and H₂S production. Tweens 20, 40, 60 and 80 are hydrolysed, but cellulose is not. The cell wall contains meso-diaminopimelic acid. The whole-cell sugar pattern consists mainly of ribose, xylose, mannose and galactose. The predominant menaquinone is MK-10, MK-10(H₄) and MK-10(H₈). The polar lipids are phosphatidylinositol, phosphatidylcholine, phospha
dyl ethanolamine, phosphatidylglycerol, diphosphatidylycerol, phosphatidyl ethanolamine and six unidentified phospholipids. Major fatty acids are 10-methyl C₁₈:0, iso-C₁₆:0, C₁₆:0, iso-G C₁₆:1 and C₁₈:1ω9c.

The type strain is TRM 46250T (=CCTCC AA 2015027T =KCTC 39725T), isolated from sediments collected from a salt water beach located at Akesu city in the north-west of China. The G + C content of the genomic DNA of the type strain is 71.05 mol%.

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References


