**Nocardiopsis rhizosphaerae** sp. nov., isolated from rhizosphere soil of *Halocnermum strobilaceum* (Pall.) Bieb

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An alkalitolerant actinomycete strain, designated EGI 80674ᵀ, was isolated from a rhizosphere soil of *Halocnermum strobilaceum* (Pall.) Bieb in Xinjiang, north-west China and subjected to a taxonomic characterization using a polyphasic approach. Strain EGI 80674ᵀ formed white aerial hyphae with long spore chains. Whole-cell hydrolysates of the isolate contained meso-diaminopimelic acid as the diagnostic diamino acid with no diagnostic sugars. The major fatty acids identified were iso-C₁₆:₀, anteiso-C₁₇:₀ and 10-methyl-C₁₈:₀ TBSA. The predominant menaquinones detected were MK-10(H₈) and MK-10(H₆). The G+C content of the genomic DNA of strain EGI 80674ᵀ was 70.9 mol%. Strain EGI 80674ᵀ showed the highest 16S rRNA gene sequence similarity (97.24 %) to *Nocardiopsis nikkonensis* NBRC 102170ᵀ. The DNA–DNA relatedness value of strain EGI 80674ᵀ and *N. nikkonensis* NBRC 102170ᵀ was 18.4 ±1.3 %. Phenotypical, chemotaxonomic and phylogenetic characteristics and DNA–DNA hybridization data suggest that strain EGI 80674ᵀ represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis rhizosphaerae* sp. nov. is proposed. The type strain is EGI 80674ᵀ (=CGMCC 4.7228ᵀ =KCTC 39673ᵀ).

**Abbreviation:** meso-DAP, meso-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EGI 80674ᵀ is KU992916.

Four supplementary figures and three supplementary tables are available with the online Supplementary Material.

The genus *Nocardiopsis* was firstly proposed by Meyer (1976), and later affiliated with the family of *Nocardiopsaceae* (Rainey et al., 1996). At time of writing, the genus comprises 42 species with validly published names and two subspecies (http://www.bacterio.net/nocardiopsis.html), except that the species *Nocardiopsis arabia* was recently reclassified as *Streptomonospora arabia* (Zhang et al., 2013).

Recently, two new members of the genus *Nocardiopsis* were reported, *Nocardiopsis mwathae* which was isolated from haloalkaline lake Elmenteita (Akhwale et al., 2016), and *Nocardiopsis ansamitocini* that was isolated from a saline–alkali soil sample (Zhang et al., 2016). Species of the genus *Nocardiopsis* harbour distinct features from other genera, such as variably hydrogenated menaquinones with ten isoprene units as major menaquinones phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol as main polar lipids and no diagnostic cell-wall amino acids (Hozein & Trujillo, 2012). Members of the genus *Nocardiopsis* are widely distributed in nature, especially in hypersaline or alkaline soils (Hozein & Trujillo, 2012), and have great potential in biotechnology (Bennur et al., 2015).
Alkaliphilic or alkalitolerant species of the genus *Nocardiosis* have great potential to produce bioactive pharmaceuticals (Horikoshi, 1999; Ding et al., 2010, 2012; Zhang et al., 2016). During a study of the diversity of culturable actinobacteria, strain EGI 80674 was isolated from rhizosphere soil of *Halocnemum strobilaceum* (Pall.) Bieb in Xinjiang, north-west China. In this research, a polyphasic approach was used to study the taxonomy of strain EGI 80674, the results suggest that EGI 80674 is a novel member of the genus *Nocardiosis*.

A sample of rhizosphere soil of *Halocnemum strobilaceum* (Pall.) Bieb [pH 8.8; total salts, 10.3%, (w/w)] was collected from Karamay, Xinjiang, north-west China. The sample was diluted to the concentrations of 10^{-3} and 10^{-4} (w/v), 0.1 ml of each were spread on yeast extract-malt extract agar (ISP 2) (Shirling & Gottlieb, 1966) modified with final pH adjusted to 10.0 with autoclaved 10M NaOH, and incubated at 30 °C for 4 weeks. Strain EGI 80674 was picked, and then maintained on ISP 2 medium modified with addition of 2.0 % NaCl (w/v) and adjusting pH to 9.0. The strain was preserved as glycerol suspensions (20%, w/v) at −80 °C. Biomass for chemical and molecular studies was obtained by cultivation at 30 °C for 7 days in shake flasks (about 150 r.p.m.) using ISP 2 broth modified as described above.

Cultural characteristics were determined after incubation for 4 weeks at 30 °C according to the methods described by Shirling & Gottlieb (1966) except that all media were supplemented with 2.0 % NaCl (w/v) and adjusted to pH 9.0 with 10M NaOH. The colours of aerial and substrate mycelia, and soluble pigments were determined with the ISCC-NBS colour charts (Kelly, 1964). Strain EGI 80674 grew well on modified ISP 2 and potato-dextrose-agar (PDA), moderately on modified oatmeal agar (ISP 3), glycero-l-asparagine agar (ISPS), Czapek’s agar and nutrient agar, and weakly on modified inorganic starch agar (ISP 4) and Gauze No. 1 agar. No soluble pigment was observed on the media tested (Table S1, available in the online Supplementary Material). The colour of aerial mycelium was white, while the substrate mycelium colours were variable depending on the medium used (Table S1). Morphological characteristics of strain EGI 80674 were observed by light microscopy (BH-2; Olympus) and scanning electron microscopy (Quanta 200; FEI) after incubation at 30 °C for 2 or 4 weeks on ISP 2 agar (Shirling & Gottlieb, 1966) modified with addition of 2.0 % NaCl (w/v) and adjusting pH to 9.0 with 10M NaOH. The substrate mycelium was non-fragmented (Fig. S1a). The abundant well-developed aerial hyphae formed long rod-like spore chains, and the surface of the spore was smooth (Fig. S1b).

Growth at different temperatures (5–60 °C, at intervals of 5 °C) was examined by growing the strain on tryptic soy agar (TSA) modified with addition of 2.0 % NaCl (w/v) and adjusting pH to 9.0 with NaOH. Growth at various NaCl concentrations (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25 and 30%, w/v) was tested. The pH growth range was tested between pH 4.0 and 12.0, at intervals of 1.0 pH unit, in tryptic soy broth (TSB) modified by addition of 2.0 % NaCl (w/v) with the buffer system described by Xu et al. (2005). Carbon-source utilization tests were performed according to the methods of Shirling & Gottlieb (1966). Nitrogen-source utilization tests were analysed as described by Williams et al. (1983). Physiological and biochemical characteristics were examined as described previously (Goodfellow, 1971; Williams et al., 1983). All media used above were modified with addition of 2.0 % NaCl (w/v) and adjusting pH to 9.0 except those mentioned specifically. Strain EGI 80674 could grow at 20–40 °C, pH 6.0–10.0 and 0–20 % (w/v) NaCl. The optimal growth was determined to occur at 30 °C, pH 8.0–9.0 with 2–4 % NaCl (w/v) addition. Other physiological characteristics of strain EGI 80674 are given in Table 1 and the species description.

Amino acids in whole-cell hydrolysates were analysed by TLC as described by Stanek & Roberts (1974). Cell-wall sugars were detected according to the method used by Tang et al. (2009). Polar lipids were extracted and identified by two-dimensional TLC following the method of Minnikin et al. (1984). Menaquinones were extracted and prepared as described previously (Collins et al., 1977). The purified menaquinones were dissolved in methanol and separated by atmospheric pressure photo-ionization-LC-MS. The chromatographic system consisted of an AB SCIEX API 4000+TM LC/MS/MS system and a column oven (ABI). The chromatography and ionization conditions were set as described by Tang et al. (2008). For fatty acid analysis, strain EGI 80674 was cultured at 30 °C for 3 days on TSB medium adjusted to pH 9.0 with NaOH. Cellular fatty acids analysis was performed as described by Sasser (1990) according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System. For determination of G+C content, the genomic DNA of strain EGI 80674 was prepared according to Marmur (1961). The G+C content of the DNA was determined by the HPLC method (Mesbah et al., 1989).

Whole-cell hydrolysates of strain EGI 80674 contained meso-diaminopimelic acid (DAP) as diagnostic diamino acids, and mannose as predominant sugar while no diagnostic sugars were detected. The predominant menaquinones detected were MK-10(H8) (39.3 %) and MK-10(H6) (33.8 %), while minor components were MK-9(H8) (8.7 %), MK-10(H4) (7.2 %), MK-9(H6) (6.1 %), MK-9(H4) (2.5 %) and MK-10(H4) (2.3 %). The polar lipids determined were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, six unknown phospholipids, one phosphatidylinositollamnoside and one unknown polar lipid (Fig. S2). The major fatty acids identified were iso-C16:0 (35.74 %), 10-methyl-C18:0 (TBSA) (22.03 %) and anteiso-C17:0 (12.25 %); other minor fatty acids are listed in Table S2. The genomic DNA G+C content of strain EGI 80674 was 70.9 mol%.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were carried out using procedures described by Li et al. (2007). Multiple alignments with sequences of type strains of species of the genus *Nocardiosis*, and calculations of levels of sequence similarity were carried out using the
Table 1. Characteristics that distinguish strain EGI 80674T from its closest phylogenetic relative Nocardiopsis nikkonensis NBRC 102170T.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Spore chain</td>
<td>Straight</td>
<td>Zig-zag*</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH range</td>
<td>6–10</td>
<td>6–11</td>
</tr>
<tr>
<td>NaCl range (w/v %)</td>
<td>0–20</td>
<td>0–22</td>
</tr>
<tr>
<td>Optimal NaCl (w/v %)</td>
<td>2–4</td>
<td>0</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>20–40</td>
<td>15–40</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Carbon sources utilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Rhamnose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Raffinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Ribose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Xylitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrogen sources utilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Arginine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Glutamine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Lysine</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Methionine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Phenylalanine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Tryptophan</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major menaquinones (MK) (&gt;30%)</td>
<td>10(H4) (39.1 %), 10(H2) (33.7 %)</td>
<td>10(H4) (37.7 %), 9(H2) (30.7 %)</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10 %)</td>
<td>iso-C16:0, anteiso-C17:0</td>
<td>iso-C16:0, anteiso-C17:0</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>DPG, PG, PC, PLs, PIM, UL</td>
<td>DPG, PG, PC, PLs, PIM, Uls, GL</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>70.9</td>
<td>72.3*</td>
</tr>
</tbody>
</table>

*Data from Yamamura et al. (2010).
represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis rhizosphaerae* sp. nov. is proposed.

**Description of Nocardiopsis rhizosphaerae** sp. nov.

*Nocardiopsis rhizosphaerae* (rhizo.sphae’ræ. N.L. gen. n. *rhizosphaerae* of the rhizosphere).

Gram-reaction-positive, aerobic actinomycete. Substrate mycelia are non-fragmented. The colour of the substrate mycelia vary from white to yellow-white, which is dependent on the culture medium. White aerial mycelium is well-developed and forms rod-like spore chains. Grows well on ISP 2 and PDA, moderately on ISP 3, ISP 5, Czapek’s agar and nutrient agar, and weakly on ISP 4 and ISP 6. Whole-cell hydrolysates contain meso-DAP as a diagnostic diamino acid, and no diagnostic sugars. The predominant menaquinones detected are MK-10 (H1) and MK-10(H4). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, six unknown phospholipids, one phosphatidylinositolmannoside and one unknown polar lipid. The major fatty acids are iso-C16:0, anteiso-C17:0 and 10-methyl-C18:0.

The type strain is EGI 80674T (=CGMCC 4.7228=KCTC 39673T) isolated from rhizosphere soil of *Halocnemum strobilaceum* (Pall.) Bib in Xinjiang, north-west China. The G+C content of the genomic DNA of the type strain is 70.9 mol%.

**Acknowledgements**

The authors are grateful to Dr Tomohiko Tamura (NBRC, Japan) for kindly providing the reference type strain. This research was supported by National Natural Science Foundation of China (NSFC) (nos U1403101 and 31400009) and the West Light Foundation of the Chinese Academy of Sciences (RCPY201203). W.-J.L. was also supported by the ‘Hundred Talents Program’ of the
References


