Lipingzhangella halophila gen. nov., sp. nov., a new member of the family Nocardiopsaceae

Yong-Guang Zhang,1 Xin-Hua Lu,2 Yan-Bo Ding,2 Su-Juan Wang,3 Xing-Kui Zhou,3 Hong-Fei Wang,1,4 Jian-Wei Guo,1,5 Yong-Hong Liu,1 Yan-Qing Duan3 and Wen-Jun Li1,6

1Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürümqi 830011, PR China
2New Drug Research & Development Center, North China Pharmaceutical Group Corporation, National Microbial Medicine Engineering & Research Center, Shijiazhuang 050015, PR China
3China Tobacco Yunnan Industrial Co, Ltd, Kunming 650231, PR China
4College of Life Science, Liaoning Normal University, Dalian 116029, PR China
5Key Laboratory of Higher Quality and Efficient Cultivation and Security Control of Crops for Yunnan Province, Honghe University, Mengzi 661100, PR China
6State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China

An alkaliphilic and halophilic actinomycete strain, designated EGI 80537T, was isolated from a saline-alkali soil sample of Xinjiang, north-west China and subjected to a taxonomic characterization using a polyphasic approach. Strain EGI 80537T formed reticulate long aerial hyphae. Whole-cell hydrolysates of the isolate contained meso-diaminopimelic acid as the cell-wall diamino acid and mannose as the diagnostic sugar. 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 80537T was 67.6 mol%. Strain EGI 80537T showed the highest 16S rRNA gene sequence similarity to Allosalinactinospora lopnorensis CA15-2T (96.7 %). Phylogenetic analysis showed that strain EGI 80537T clustered with the members of the family Nocardiopsaceae. Based on phenotypic, chemotaxonomic and phylogenetic characteristics, strain EGI 80537T represents a novel species of a new genus in the family Nocardiopsaceae, for which the name Lipingzhangella halophila gen. nov., sp. nov. is proposed. The type strain of the type species is EGI 80537T (=CGMCC 4.7224= DSM 102030T).

The family Nocardiopsaceae was proposed by Rainey et al. (1996) based on phylogenetic analysis. At the time of writing, this family contains ten genera: Nocardiopsis (Meyer, 1976), Thermobifida (Zhang et al., 1998), Streptomonospora (Cui et al., 2001), Haloactinospora (Tang et al., 2008), Marinactinospora (Tian et al., 2009), Marinocardiopsis (Kämpfer et al., 2010), Spinactinospora (Chang et al., 2011), Salinactinospora (Chang et al., 2012), Allosalinactinospora (Guo et al., 2015) and Actinorugispora (Liu et al., 2015). Most members of the family Nocardiopsaceae were isolated from saline and hypersaline environments (Guo et al., 2015). During a special research programme on biodiversity of actinobacterial resource in extreme environments in Xinjiang, a novel alkaliphilic and halophilic strain, designated EGI 80537T, was isolated from a desert soil collected from western Gurbantünggüt Desert, Xinjiang, north-west China. In this research, strain EGI 80537T was subjected to a taxonomic analysis using a polyphasic approach, and the results suggest that strain EGI 80537T represents a novel species of a new genus in the family Nocardiopsaceae.

Strain EGI 80537T was isolated and purified by the dilution plating method on marine agar 2216 modified with addition of 2 % NaCl (w/v) and adjusting pH to 10.0 with autoclaved
10 M NaOH; plates were incubated at 30 °C for 4 weeks. The strain was maintained on slants of marine agar 2216 modified as above, and preserved as 20 % glycerol (v/v)/5 % NaCl (w/v) suspension at −80 °C. Biomass for chemical and molecular studies was obtained by cultivation at 30 °C for 21 days in shake flasks (about 150 r.p.m.) containing tryptic soy broth (TSB) supplemented with 5 % NaCl (w/v) and pH adjusted to 10.0.

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 modified with addition of 5 % NaCl (w/v) and adjusting pH to 10.0. The colours of aerial and substrate mycelia, and soluble pigments were determined with the ISCC–NBS colour charts (Kelly, 1964). Strain EGI 80537T grew well on modified Gauze No. 1 agar, moderately on modified oatmeal agar (ISP3), glycerol-asparagine agar (ISP 5) and nutrient agar, and weakly on modified yeast extract-malt extract agar (ISP 2), inorganic starch agar (ISP4), Czapek’s agar and potato-dextrose-agar. No soluble pigment was observed on the media tested. The detailed colours of substrate mycelium and aerial hyphae varied depending on the medium used (Table S1, available in the online Supplementary Material).

Morphological characteristics of strain EGI 80537T were observed by light microscopy (BH-2; Olympus) and scanning electron microscopy (Quanta 200; FEI) after incubation at 30 °C for 14–28 days on marine agar 2216 modified with addition of 3 % NaCl (w/v) and adjusting pH to 10.0 with 10 M NaOH. The strain formed non-fragmented substrate mycelium (Fig. 1a), which was in yellow–white or light yellow–brown. The isolate formed non-fragmented aerial hyphae, which were typical cobweb-like (Fig. 1b), and some were fasciculate (Fig. 1c, d). No spore was observed. The detailed cultural characteristics are given in Table S1.

Growth at different temperatures (5–60 °C, at intervals of 5 °C) was tested on tryptic soy agar (TSA) modified with addition of 5 % NaCl (w/v) and adjusting pH to 10.0 with NaOH. Tolerance to various NaCl concentrations (0–20 % at intervals of 2 %, 25 and 30 %, w/v) was examined by growing the strain on TSA modified with adjusting pH to 10.0 with NaOH. The pH growth range was tested between 4.0 and 12.0, at intervals of 1.0 pH unit, on TSA medium supplemented with 5 % NaCl (w/v) by using the buffer system described by Xu et al. (2005). Carbon-source utilization tests were performed according to the methods of Shirling & Gottlieb (1966) except that the basic medium was modified with addition of 5 % NaCl (w/v) and adjusting pH to 10.0. Nitrogen-source utilization tests were analysed as described by Williams et al. (1983) except that the basic medium was modified with addition of 5 % NaCl (w/v) and adjusting pH to 10.0. Catalase activity was determined by addition of 3 % H₂O₂ (v/v), and gas production was identified as a positive reaction. Physiological and biochemical characteristics were examined as described previously (Goodfellow, 1971; Williams et al., 1983) except that the culture media were modified as described above. Strain EGI 80537T could grow at 15–40 °C, at pH 7.0–11.0 and with 4–18 % (w/v) NaCl. Optimal growth was determined to occur at 30 °C, pH 9.0–10.0 and with 5–7 % (w/v) NaCl. Other physiological characteristics of strain EGI 80537T are given in Table 1 and the species description below.

Amino acids in whole-cell hydrolysates were analysed by TLC as described by Staneck & 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). Menaquinoles were extracted and prepared as described previously (Collins et al., 1977). The purified menaquinoles 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 80537T was cultured at 30 °C for 14 days on TSB medium modified with addition of 5 % NaCl (w/v) and adjusting pH to 10.0 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. The prepared fatty acid methyl esters were separated with the Sherlock Microbial Identification System (MIS) (MIDI) and analysed with the Microbial Identification software package (Sherlock version 6.1). During the procedure, an Agilent Technologies 7890A...
Table 1. Differential characteristics of strain EGI 80537<sup>T</sup> and related genera of the family Nocardiopsaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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<tbody>
<tr>
<td>Growth</td>
<td></td>
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<tr>
<td>NaCl range (%, w/v)</td>
<td>18–21</td>
<td>0–10</td>
<td>1–23</td>
<td>9–21</td>
<td>0–20</td>
<td>5–25</td>
<td>0–5</td>
<td>0–5</td>
<td>0–1</td>
<td>1–15</td>
<td>0–5.5</td>
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<tr>
<td>pH range</td>
<td>7–11</td>
<td>6–9</td>
<td>6–9</td>
<td>6–9</td>
<td>6–4</td>
<td>5–9</td>
<td>6–9</td>
<td>7–9</td>
<td>No aerial mycelium</td>
<td>6–9</td>
<td>6.0–10.5</td>
</tr>
<tr>
<td>Aerial mycelium</td>
<td>Reticulate mycelia, non-fragmented</td>
<td>Wave shaped, non-fragmented*</td>
<td>Long spore chains</td>
<td>Long spore chains</td>
<td>Straight to flexuous spore chains</td>
<td>Short spore chains</td>
<td>Long spore chains</td>
<td>Dichotomously branched sporophores</td>
<td>No aerial mycelium</td>
<td>Long or short spore chains</td>
<td>Wrinkled spora</td>
</tr>
<tr>
<td>Substrate mycelium</td>
<td>Non-fragmented</td>
<td>Non-fragmented*</td>
<td>Non-fragmented</td>
<td>Spore chains with pseudosporangia at the end</td>
<td>Fragmented</td>
<td>Non-fragmented</td>
<td>Non-fragmented</td>
<td>Non-fragmented</td>
<td>Non-fragmented</td>
<td>Non-fragmented</td>
<td>Non-fragmented</td>
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<tr>
<td>Diagnostic sugars</td>
<td>Man</td>
<td>None</td>
<td>Xyl</td>
<td>Gal, Rib</td>
<td>None</td>
<td>Gal</td>
<td>None</td>
<td>Gal</td>
<td>None</td>
<td>Rib</td>
<td>Gal</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>10(H&lt;sub&gt;5&lt;/sub&gt;, H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>9(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, 11(H&lt;sub&gt;2&lt;/sub&gt;, H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;12&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;10&lt;/sub&gt;)</td>
<td>11(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>10(H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;6&lt;/sub&gt;, H&lt;sub&gt;8&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Major polar lipids</td>
<td>PG, DPG, PC, PE, PL, GL</td>
<td>PG, DPG, PC, PE, PME, PL, GL</td>
<td>DPG, PG, PC, PL, UL</td>
<td>DPG, PG, PC, GL</td>
<td>PC, PME, DPG</td>
<td>PC, PME, DPG</td>
<td>PC, PME, DPG</td>
<td>PC, PME, DPG</td>
<td>PC, PME, DPG</td>
<td>PC, PME, DPG</td>
<td>PC, PME, DPG</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, 10-Me C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, 10-Me C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
</tr>
<tr>
<td>DNA G+C content (mol %)</td>
<td>67.6</td>
<td>60.1</td>
<td>68</td>
<td>64–76</td>
<td>69–67</td>
<td>72</td>
<td>66–72</td>
<td>ND</td>
<td>71.1</td>
<td>63.1</td>
<td></td>
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</tbody>
</table>

*Data from Guo et al. (2015).
GC system (gas chromatographic column, 0.2 mm × 25 m, ultra 25% Phenyl Methyl Silox; Agilent) and MIDI database (TSBA6) were used. For determination of G+C content, the genomic DNA of strain EGI 80537T was prepared according to Marrmur (1961). The G+C content of the DNA was determined by the HPLC method (Mesbah et al., 1989).

Whole-cell hydrolysates of strain EGI 80537T contained meso-diaminopimelic acid (DAP) as the cell-wall diamino acid and mannose as the diagnostic sugar as well as minor amounts of galactose. The predominant menaquinones detected were MK-10(H6) (51.3%) and MK-10(H4) (29.6%), while the minor components were MK-10(H6) (6.8%), MK-11(H6) (3.7%), MK-9(H6) (3.2%), MK-10(H10) (2.9%) and MK-10(H2) (2.5%). The major fatty acids identified were iso-C16:0, anteiso-C17:0, 10-methyl-C18:0 (TBSA) and other minor fatty acids (Table S2). The genomic DNA G+C content of strain EGI 80537T was 67.6 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 the type strains of members of the family Nocardiopsaceae, and calculations of levels of sequence similarity were carried out using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012) on the basis of 16S rRNA gene sequence data. Phylogenetic analysis was performed using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods by using the software MEGA 6 (Tamura et al., 2013). The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

An almost complete 16S rRNA gene sequence (1528 bp) was determined for strain EGI 80537T. Blast searches indicated that the isolate showed higher 16S rRNA gene sequence similarities to Allosalinactinospora lopnioresis CA15-2T (96.7%), Salinactinospora qingdaonensis CBX832T (94.3%) and Halocactinospora alba YIM 90648T (94.1%), and lower 16S rRNA gene sequence similarities to other members of the family Nocardiopsaceae (below 94.0%). Phylogenetic analysis based on the 16S rRNA gene sequences showed that the strain fell within the clade of the family Nocardiopsaceae. In the phylogenetic tree based on the neighbour-joining algorithm, strain EGI 80537T formed a distinct clade with A. lopnioresis CA15-2T (Fig. 2). The

![Phylogenetic tree](image-url)

**Fig. 2.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain EGI 80537T. Bootstrap values (expressed as percentages of 1000 replications) > 50% are shown at the branch nodes. Asterisks indicate that the clades were conserved when maximum-parsimony and maximum-likelihood methods were used to reconstruct the phylogenetic trees. Bar, 0.005 sequence divergence.
distinction was supported by the other two tree-making methods used in the study (Fig. 2).

As described above, strain EGI 80537\textsuperscript{T} exhibited typical characteristics of the family Nocardiopsaceae (Table 1): mycelium differentiation, meso-DAP as diagnostic diamino acid, and predominant menaquinones (MK-10 with varying degrees of hydrogenation), saturated fatty acids, and the DNA G+C content 67.6 mol\% within the range of the family (64–76 mol\%) (Goodfellow & Trujillo, 2012), as well as the closest phylogenetic relationship, which suggest that novel strain belongs to the family Nocardiopsaceae. Although strain EGI 80537\textsuperscript{T} forms a clade with A. loporensis CA15-2\textsuperscript{7}, the isolate differs significantly from its closest phylogenetic neighbour by reticulate aerial mycelia, alkali-philic physiology, mannose as diagnostic sugar and 10-methyl-C\textsubscript{18:0} (TBSA) as one of the major fatty acids (Tables 1 and S1). These distinct features suggest that strain EGI 80537\textsuperscript{T} does not belong to the genus Allosalinactinospora as well as other genera of the family Nocardiopsaceae. Based on the characteristics described above, strain EGI 80537\textsuperscript{T} represents a novel member of the family Nocardiopsaceae, for which the name Lipingzhangella halophila gen. nov., sp. nov. is proposed.

Description of Lipingzhangella gen. nov.

Lipingzhangella (li.ping.zhang.eI’la. N.L. fem. dim. n. Lipingzhangella named after Li-Ping Zhang (born 1955), a Chinese microbiologist, in recognition for her work on actinobacterial resources and their utilization).

Cells are non-motile, Gram-stain-positive actinomycetes that form well-developed substrate mycelium. The aerial mycelium is reticulate with no fragmentation and spore chains. Some aerial mycelia are fasciculate. Whole-cell hydrolysates contain meso-DAP and mannose as diagnostic markers. The predominant menaquinones are MK-10(H\textsubscript{8}) and MK-10(H\textsubscript{4}). The polar lipids are phosphatidylglycerol, phosphatidylycholine, diphosphatidylglycerol, phosphatidyl-ethanolamine, unknown phospholipids and unknown glycolipids. The major fatty acids are iso-C\textsubscript{16:0} anteiso-C\textsubscript{17:0} and 10-methyl-C\textsubscript{18:0} (TBSA).

The type species is Lipingzhangella halophila. The DNA G+C content of the genomic DNA of the type strain of the type species is about 67.6 mol\%.

Description of Lipingzhangella halophila sp. nov.

Lipingzhangella halophila (ha.lo’phi.ila. Gr. n. hals halos salt; Gr. adj. philos loving; N.L. fem. adj. halophila salt-loving, referring to the ability of the type strain to grow at high NaCl concentrations).

Displays the following properties in addition to those given in the genus description. Aerial mycelium forms when cultured on ISP 2, ISP 3, ISP 5, Czapek’s agar, PDA and Gauze No. 1 modified with addition of 5 % NaCl (w/v) and adjusting pH to 10.0 with 10 M NaOH, while not on ISP 4 and nutrient agar modified as described above. Diffusible pigment is not observed on all media tested. Growth occurs at pH 7.0–11.0, with 4–18.0 % (w/v) NaCl, and at 15–40°C; optimal growth occurs at pH 9.0–10.0, with 5.0–7.0 % (w/v) NaCl and at 30°C. Cellobiose, dulcitol, D-glucose, glycerol, myo-inositol, D-mannitol, melibiose, raffinose, sucrose, trehalose, sodium acetate and sodium pyruvate can be used as sole carbon source, but not D-arabinose, L-arabinose, D-galactose, D-mannose, L-rhamnose, D-ribose, D-xylitol and D-xylene. L-Alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, L-histidine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-valine and xanthine can be utilized as sole nitrogen source, but not ad- nine, L-cysteine, glycine and L-tryptophan. Positive for catalase activity, but negative for oxidase activity, gelatin liquefaction, coagulation and peptonization of skimmed milk, nitrate reduction, H\textsubscript{2}S production, hydrolysis of casein, cellulose, and Tweens 20, 40, 60 and 80. Major cellular fatty acids are iso-C\textsubscript{16:0} anteiso-C\textsubscript{17:0} and 10-methyl-C\textsubscript{18:0} (TBSA). Small proportion of 10-methyl-C\textsubscript{17:0} and C\textsubscript{18:0} are also found.

The type strain is EGI 80537\textsuperscript{T} (=CGMCC 4.7224\textsuperscript{T}=DSM 102030\textsuperscript{T}) isolated from a desert soil collected from western Gurbantunggut Desert, Xinjiang, north-west China.

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References


