Spinactinospora alkalitolerans gen. nov., sp. nov., an actinomycete isolated from marine sediment

Xianbo Chang, Wenzheng Liu and Xiao-Hua Zhang

A novel marine actinomycete, designated CXB654T, was isolated from marine sediment collected at a depth of 17.5 m near the Yellow Sea Cold Water Mass, China. Optimal growth occurred at 37.0 °C, at pH 7.0–8.0 and in 3–8 % (w/v) NaCl. Strain CXB654T formed branched substrate mycelium without fragmentation. Abundant aerial mycelium differentiated into long or short chains of spores and spores were elliptical and cylindrical with spiny surfaces. Strain CXB654T contained meso-diaminopimelic acid as the diagnostic diamino acid, and ribose and glucose as the major whole-cell components. Phospholipids were diphytanylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. MK-10(H8), MK-10(H6) and MK-9(H6) were the predominant menaquinones. The major fatty acids were i-C16:0 (24.46 %), ai-C17:0 (20.66 %) and C18:0 (20.14 %). The DNA G+C content was 71.1 mol%. Comparative analysis of 16S rRNA gene sequences showed that the novel strain was most closely related to genera within the family Nocardiopsaceae, but formed a separate lineage. Highest sequence similarities were to Murinocardiopsis flavida DSM 45312T (96.6 %), Thermobifida halotolerans YIM 90462T (96.5 %) and Marinactinospora thermotolerans DSM 45154T (96.1 %). On the basis of phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain CXB654T was considered to represent a novel species in a new genus in the family Nocardiopsaceae, and the name Spinactinospora alkalitolerans gen. nov., sp. nov. is proposed; the type strain is CXB654T (=DSM 45414T =LMG 25485T).

Actinomycetes are Gram-positive bacteria with filamentous growth and high DNA G+C content (60–78 mol%) that are widely distributed in various ecological habitats. The family Nocardiopsaceae was created, with Nocardiosis as the type genus, by Rainey and co-workers in 1996 based on data from polyphasic studies (Rainey et al., 1996). At present, the family Nocardiopsaceae contains six genera, namely, Nocardiosis (Meyer, 1976), Thermobifida (Zhang et al., 1998), Streptomomonospora (Cui et al., 2001), Haloactinospora (Tang et al., 2008), Marinactinospora (Tian et al., 2009) and Murinocardiopsis (Kämpfer et al., 2010). Most strains of the genus Nocardiosis have been isolated from hypersaline soils (Li et al., 2003, 2004, 2006; Chen et al., 2008; Yang et al., 2008b), but some strains have been isolated from marine sediment (Sabry et al., 2004; Kroppenstedt &Evtushenko, 2006; Tian et al., 2009); most Nocardiosis strains are strictly halophilic. All four Thermobifida species were isolated from manure heaps, composts, damp stored hay or salt mines and have outstanding cellulose decomposition abilities (Yang et al., 2008a). Members of the two genera Streptomomonospora and Haloactinospora are strictly halophilic. Marinactinospora and Murinocardiopsis both contain only a single species, isolated from marine sediment and indoor walls, respectively. Members of this family can form long or short spore chains or sporangia with abundant spores, which increase the survival rate of these organisms in many extreme environments. A novel strain belonging to this family, designated CXB654T, was isolated recently from marine sediment and found to be phylogenetically closely related to members of the genera Murinocardiopsis, Thermobifida and Marinactinospora. In the present study, the taxonomic position of strain CXB654T was determined by a polyphasic taxonomic approach.

Samples were collected from sediment near the Yellow Sea Cold Water Mass (32°00.398’N 122°09.722’E), China, in July 2008. The surface layer of the sediment, about 40 cm in depth, was collected by a grab bucket and samples from the top 5–10 cm layer were obtained aseptically and stored in sterile 50 ml conical tubes at 4 °C. Samples were...
transported to the laboratory and processed for cultivation experiments. A 2 g wet sample was suspended in 18 ml sterile seawater and 0.1 ml aliquots of the suspension were spread on sodium propionate–aspartic acid agar plates (0.1 g aspartic acid, 2.0 g peptone, 4.0 g sodium propionate, 0.05 g K₂HPO₄, 3H₂O, 0.1 g MgSO₄.7H₂O, 0.01 g FeSO₄.7H₂O, 20.0 g agar, 1.0 l 2- to 3-week old seawater, pH 7.2–7.4). After incubation at 28 °C for 3 weeks, colonies were isolated, purified and maintained on International Streptomyces Project (ISP) medium 2 agar (Shirling & Gottlieb, 1966) modified with 2- to 3-week-old seawater instead of distilled water. Purified strains were suspended in sterile 0.9 % (w/v) saline supplemented with 15 % (v/v) glycerol and stored at −80 °C.

All media were supplemented with seawater for observation of growth. Strain CXB654ᵀ grew well on ISP 2, ISP 3, ISP 4 and ISP 5 agars (Shirling & Gottlieb, 1966), Czapek solution agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961). During incubation at 28 °C for 7 days, white aerial mycelia developed well on ISP 2 agar, nutrient agar (Difco) and potato agar, and yellow–white substrate mycelia grew well on all media. By comparing the cultures with colour chips from the ISCC-NBS colour charts (Kelly, 1964), it was evident that the novel strain did not produce diffusible pigments on any of the media tested. Micromorphology was observed by light microscopy (model BH2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) using cells incubated for 7, 14, 21 and 28 days on modified ISP 2 agar. After 14 days, the white aerial mycelia differentiated into short or long spore chains. Spore surfaces were spiny (Fig. 1).

Physiological characteristics, including temperature and pH ranges for growth and NaCl tolerance, were tested using ISP 2 as the basal medium. Growth was tested at 4, 16, 20, 28, 37, 40, 42, 44 and 45 °C on ISP 2 agar modified with seawater instead of distilled water. For tolerance experiments involving NaCl concentrations of 0–16 % (at intervals of 1 %), ISP 2 was used as the basal medium. The pH range for growth was investigated between pH 4.0 and 11.0 at intervals of 0.5 units using the buffer system described by Xu et al. (2005). Carbon source utilization for growth was carried out as described by Shirling & Gottlieb (1966). Tests for hydrolysis of cellulose, gelatin, starch, and Tweens 20, 40, 60 and 80, nitrate reduction, utilization of urea, milk coagulation and peptonisation, and production of H₂S and melanin were performed in 3 % (w/v) NaCl as described by González et al. (1978). Antibiotic susceptibility was examined as described by Groth et al. (2004) using antibiotic discs on modified ISP 2 agar. Thermobifida alba DSM 43795ᵀ (type species of the genus Thermobifida) and Marinactinospora thermotolerans DSM 45154ᵀ (the type species of the genus Marinactinospora) obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) GmbH, Braunschweig, Germany, were used as reference strains under the same culture conditions. Detailed physiological properties of the isolate are given in the genus and species descriptions (see below) and Table 1.

Biomass for chemotaxonomic studies was obtained by centrifugation after cultivation in modified ISP 2 broth at 28 °C for 5 days. For analysis of sugars, amino acids and peptides in the cell-wall hydrolysate, wet biomass was thoroughly suspended in isopropanol/water (1:1, v/v). For analysis of menaquinones, phospholipids and fatty acids, wet biomass was freeze-dried. Analysis of whole-cell sugars, amino acids and peptides in the cell-wall hydrolysate and menaquinones, phospholipids and fatty acids were carried out by the Identification Service of the DSMZ and B. J. Tindall, (DSMZ). Whole-cell hydrolysates of strain CXB654ᵀ contained meso-diaminopimelic acid and whole-cell sugars were ribose, glucose and traces of galactose and fucose. The menaquinones consisted of MK-10(H₄) (44 %), MK-10(H₈) (20 %), MK-9(H₈) (14 %), MK-10(H₈) (5 %) and MK-10(H₂) (3 %), in addition to some long-chain minor components, which could not be identified. Phospholipids (see Supplementary Fig. S1 available in IJSEM Online) comprised diphostatidylglycerol, phosphatidylcholine, phosphatidlyglycerol, phosphatidylinositol and unknown phospholipids. The fatty acid profile contained mainly...

Fig. 1. Scanning electron micrographs showing the long or short spore chains and spores of Spinactinospora alkalitolerans gen. nov., sp. nov. CXB654ᵀ after incubation on modified ISP 2 for 14 days at 28 °C. Bars: (a) 10 μm; (b) 10 μm; (c) 1 μm.
Table 1. Differential phenotypic and chemotaxonomic characteristics of strain CXB654<sup>T</sup> and 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>
</tr>
</thead>
<tbody>
<tr>
<td>Number of species</td>
<td>1 marine</td>
<td>1 indoor walls</td>
<td>4 terrestrial</td>
<td>1 marine</td>
<td>38 terrestrial or marine</td>
<td>1 salt lake</td>
<td>5 terrestrial or salt lake</td>
</tr>
<tr>
<td>Isolation source</td>
<td>Marine</td>
<td>Indoor walls</td>
<td>Terrestrial</td>
<td>Marine</td>
<td>Terrestrial or marine</td>
<td>Salt lake</td>
<td>Terrestrial or salt lake</td>
</tr>
<tr>
<td>Spore surface</td>
<td>Spiny</td>
<td>No aerial mycelium</td>
<td>Smooth</td>
<td>Wrinkled</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Wrinkled</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6.0–10.5</td>
<td>5–9</td>
<td>7–9</td>
<td>6–9</td>
<td>6–14</td>
<td>6–9</td>
<td>5–9</td>
</tr>
<tr>
<td>NaCl concentration range</td>
<td>1–15</td>
<td>0–11</td>
<td>0–5</td>
<td>0–5</td>
<td>0–20</td>
<td>0–20</td>
<td>9–21</td>
</tr>
<tr>
<td>for growth (%) (w/v)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range for optimum growth</td>
<td>37</td>
<td>28</td>
<td>40–45</td>
<td>28</td>
<td>25–40</td>
<td>37</td>
<td>28–37</td>
</tr>
<tr>
<td>Maximum</td>
<td>44</td>
<td>34</td>
<td>55</td>
<td>55</td>
<td>45</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Diagnostic sugars*</td>
<td>Rib, Glu</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Gal, Rib</td>
<td>Gal</td>
<td>Gal</td>
</tr>
<tr>
<td>Predominant menaquinoines</td>
<td>MK-10(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>MK-11(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Diagnostic phospholipids†</td>
<td>PG, PC, DPG, PL</td>
<td>DPG, PME, PC, PL</td>
<td>DPG, PC, PG, PL</td>
<td>DPG, PC, PG, PL</td>
<td>PC, PME, PC, PL</td>
<td>PC, PME, PC, PL</td>
<td>PC, PME, PC, PL</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, i-C&lt;sub&gt;17:0&lt;/sub&gt;, C&lt;sub&gt;18:1&lt;/sub&gt; &amp; 10-methyl-C&lt;sub&gt;18:1&lt;/sub&gt; (10.24 %), ai-C&lt;sub&gt;17:0&lt;/sub&gt; (20.66 %) and C&lt;sub&gt;18:0&lt;/sub&gt; (20.14 %); minor components were ai-C&lt;sub&gt;15:0&lt;/sub&gt; (7.12 %), 10-methyl-C&lt;sub&gt;18:0&lt;/sub&gt; (6.29 %), i-C&lt;sub&gt;18:1&lt;/sub&gt; (5.21 %), i-C&lt;sub&gt;17:0&lt;/sub&gt; (2.89 %), C&lt;sub&gt;16:0&lt;/sub&gt; (2.21 %), i-C&lt;sub&gt;14:0&lt;/sub&gt; (2.13 %), C&lt;sub&gt;17:0&lt;/sub&gt; (1.58 %), 10-methyl-C&lt;sub&gt;17:0&lt;/sub&gt; (1.49 %), i-C&lt;sub&gt;15:0&lt;/sub&gt; (1.18 %), C&lt;sub&gt;18:10c&lt;/sub&gt; (1.09 %), ai-C&lt;sub&gt;19:0&lt;/sub&gt; (1.03 %), C&lt;sub&gt;19:0&lt;/sub&gt; (0.46 %), i-C&lt;sub&gt;19:0&lt;/sub&gt; (0.45 %), C&lt;sub&gt;20:0&lt;/sub&gt; (0.44 %), ai-C&lt;sub&gt;13:0&lt;/sub&gt; (0.17 %) and C&lt;sub&gt;14:0&lt;/sub&gt; (0.17 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rib, Ribose; Glu, glucose; Gal, galactose.
†DPG, Diphosphatidylglycerol; GL, glycolipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PIM, phosphatidylinositol mannoside; PME, phosphatidymethylethanolamine; PL, unknown phospholipids; GL, glycolipid.

The chromosomal DNA G + C content was determined according to the methods described by Mesbah et al. (1989) using reverse-phase HPLC. The DNA G + C content of strain CXB654<sup>T</sup> was 71.1 mol%.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were conducted as described by Li et al. (2007). The 16S rRNA gene sequence was aligned and compared with available sequences in the GenBank/EMBL/ DDBJ database using BLAST searches. Identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007). Sequences were aligned using CLUSTAL X 1.8 (Thompson et al., 1997) and edited manually using BioEdit Sequence Alignment Editor version 5.0.9 (Hall, 1999). Phylogenetic trees were constructed using the neighbour-joining and maximum-likelihood methods with Kimura 2-parameter model analyses implemented in the program MEGA version 4 (Tamura et al., 2007). In each case, bootstrap values were calculated based on 1000 replicates.

BLAST results for the 16S rRNA gene sequence of strain CXB654<sup>T</sup> showed that its closest relatives were members of genera *Murinocardiopsis*, *Thermobifida* and *Marinactinospora* in the family Nocardiopsaceae, with the highest gene sequence similarity of 96.6% with *Murinocardiopsis flavidus* DSM 45312<sup>T</sup>; similarities were 96.5 and 96.1% with *Thermobifida halotolerans* YIM 90462<sup>T</sup> and *Marinactinospora thermotolerans* DSM 45154<sup>T</sup>, respectively. The 16S rRNA gene-based neighbour-joining (Fig. 2) and maximum-likelihood trees (Supplementary Fig. S2 available in IJSEM Online) showed the phylogenetic relationship between strain CXB654<sup>T</sup> and members of closely related genera and revealed that strain CXB654<sup>T</sup> formed a distinct lineage among members of the family Nocardiopsaceae.
Strain CXB654<sup>T</sup> could be differentiated from members of the phylogenetically most closely related genera *Murinocardiopsis*, *Thermobifida* and *Marinactinospora* as follows. (1) The surfaces of spores of strain CXB654<sup>T</sup> were spiny, unlike those of all other genera in the family *Nocardiopsaceae* apart from the genus *Murinocardiopsis*, which has no aerial mycelium. (2) Strain CXB654<sup>T</sup> required NaCl for growth, enabling it to be distinguished from members of the genera *Murinocardiopsis*, *Thermobifida* and *Marinactinospora*. (3) The diagnostic sugars of strain CXB654<sup>T</sup> were ribose and glucose, but closely related genera had no diagnostic sugar. (4) The predominant menaquinones for strain CXB654<sup>T</sup> were MK-10(H<sub>8</sub>), MK-10(H<sub>6</sub>) and MK-9(H<sub>8</sub>), which differed from those of the genera *Murinocardiopsis* [MK-10(H<sub>4</sub>) and MK-11(H<sub>4</sub>)], *Thermobifida* [MK-10(H<sub>6</sub>) and MK-10(H<sub>8</sub>)] and *Marinactinospora* [MK-11(H<sub>8</sub>) and MK-11(H<sub>10</sub>)] in that strain CXB654<sup>T</sup> possessed MK-9(H<sub>8</sub>), but the other genera did not. (5) Diagnostic phospholipids of the genera *Murinocardiopsis*, *Thermobifida* and *Marinactinospora* are glycolipid, phosphatidylmethylethanolamine and phosphatidylinositol mannoside, respectively, which are not found in strain CXB654<sup>T</sup>. Other characteristics that differentiate strain CXB654<sup>T</sup> from members of the other genera of the family *Nocardiopsaceae* are shown in Table 1. Based on data from this polyphasic study, strain CXB654<sup>T</sup> represents a novel species in a new genus for which the name *Spinactinospora alkalitolerans* gen. nov., sp. nov. is proposed.

**Description of Spinactinospora gen. nov.**

*Spinactinospora* (Spin.ac.ti.no’sp.o.ra. L. n. *spina* spine; Gr. n. *actinos* a ray; Gr. n. *spora* a seed; N.L. fem. n. *Spinactinospora* an actinomyctete with spiny spores).

Gram-positive, aerobic, moderately alkalitolerant, filamentous actinomycete. Aerial mycelium forms short or long chains of spores; spore surfaces are spiny. Substrate mycelium is branched with non-fragmenting hyphae. No diffusible pigments are produced. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, ribose and glucose. Predominant menaquinones are MK-10(H<sub>9</sub>), MK-10(H<sub>8</sub>) and MK-9(H<sub>9</sub>). The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and unknown phosphoglycolipids. Major fatty acids are i-C<sub>16:0</sub>, ai-C<sub>17:0</sub> and C<sub>18:0</sub>. The type species is *Spinactinospora alkalitolerans*.
Description of *Spinactinospora alkalitolerans* sp. nov.

*Spinactinospora alkalitolerans* (al.ka.li.to’le.rans. N.L. n. alkali alkal; L. part. adj. tolerans, tolerating; N.L. part. adj. alkalitolerans alkali-tolerating).

Displays the following properties in addition to those described for the genus. Growth is good on ISP 2, ISP 3, ISP 4 and ISP 5 agars, Czapek solution agar, nutrient agar. The colour of the aerial mycelium is white to light yellow or deep yellow. Spores are elliptical and cylindrical with spiny surfaces. No diffusible pigments are produced. Growth occurs between 16 and 44°C, but not at 4 or 45°C. Grows at pH 6.0–10.5 and in 1–15% (w/v) NaCl, with optimum growth at pH 7.0–8.0, at 37°C and in 3–8% (w/v) NaCl. Utilizes dextrin, glycogen and starch and cellulose, melanin production, H2S production, ofloxacin (5), profloracin (5), polymyxin B (300), sulfamethoxazole (75), sulfamethazine (300), sulfa-acetyl-D-glucosamine, D-arabitol, cello- and 80, nitrate reduction, utilization of urea, catalase and oxidase are positive. The fatty acid profile contains i-C16 : 0, C16 : 0, i-C14 : 0, C17 : 0, 10-methyl-C16 : 0, i-C18 : 0, C18 : 0, C18 : 1, C16 : 0, C17 : 0, 10-methyl-C17 : 0, i-C15 : 0, Cl, C18 : 1, i-C19 : 0, C19 : 0, i-C18 : 0, 20 : 0, C18 : 0, 16 : 0 and C14 : 0.

The type strain is CXB654T (=DSM 45414T =LMG 25485T), isolated from sediment at a depth of about 17.5 m near the Yellow Sea Cold Water Mass, China. The DNA G+C content of the type strain is 71.1 mol%.

Acknowledgements

This work was supported by a grant from the National High Technology Research and Development Program of China (863 Program, no. 2007AA092434) and a grant from the National Natural Science Foundation of China (no. 40876067).

References


http://ijs.sgmjournals.org


