Salinactinospora qingdaonensis gen. nov., sp. nov., a halophilic actinomycete isolated from a salt pond

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A novel halophilic, filamentous, actinomycete strain, designated CXB832T, was isolated from a salt pond in Qingdao, China. Optimal growth occurred at 37 °C, pH 7.0–8.0 and 9–12 % (w/v) NaCl. Strain CXB832T formed pale yellow to deep yellow branched substrate mycelium without fragmentation. Abundant white aerial mycelium differentiated into long chains of spores and the spores were rod-shaped with smooth surfaces. Strain CXB832T contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan, and glucose and xylose as the major whole-cell sugars. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipid and unidentified lipids. MK-10(H6), MK-9(H8), MK-10(H2) and MK-10(H4) were the predominant menaquinones. The major fatty acids were i-C16:0 (30.71 %), ai-C17:0 (13.31 %) and C16:0 (11.28 %). The G+C content of the DNA was 60.1 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. The highest sequence similarities were to Nocardiopsis arabia DSM 45083T (95.4 %) and Haloactinospora alba DSM 45015T (94.9 %). On the basis of phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain CXB832T represents a new genus and novel species in the family Nocardiopsaceae, for which the name Salinactinospora qingdaonensis gen. nov., sp. nov. is proposed. The type strain of the type species is CXB832T (=DSM 45442T=LMG 25567T).

The family Nocardiopsaceae was created, with Nocardiopsis as the type genus, by Rainey et al. (1996) based on data obtained using a polyphasic taxonomic approach. At present, the family Nocardiopsaceae contains seven genera, namely, Nocardiopsis (Meyer, 1976), Thermobifida (Zhang et al., 1998), Streptomonospora (Cui et al., 2001), Haloactinospora (Tang et al., 2008), Marinactinospora (Tian et al., 2009), Murinocardiospora (Kämpfer et al., 2010) and Spinactinospora (Chang et al., 2011). With more than 25 species and subspecies, the genus Nocardiopsis is the largest genus in the family Nocardiopsaceae. Most strains of the genus Nocardiopsis were isolated from hypersaline soils (M.-G. Li et al., 2003; W.-J. Li et al., 2004, 2006; Chen et al., 2008; Yang et al., 2008) and some strains were isolated from marine sediments (Sabry et al., 2004; Krokpenstedt & Evtushenko, 2006; Tian et al., 2009). The four species of the genus Thermobifida were isolated from composts or damp stored hay (Zhang et al., 1998), manure heaps (Kukolya et al., 2002) and a salt mine (Yang et al., 2008). The five species of the genus Streptomonospora were isolated from a salt lake (Cui et al., 2001; Cai et al., 2009) and soil (W.-J. Li et al., 2003; Cai et al., 2008). The genera Haloactinospora, Marinactinospora and Spinactinospora contain only one species and the type species of these genera were isolated from a salt lake (Tang et al., 2008) and marine sediments (Tian et al., 2009; Chang et al., 2011). Many strains of the family Nocardiopsis have been isolated from saline soils and are halophilic, some of which are strictly halophilic (Tang et al., 2008). A novel strain was isolated from a salt pond in Qingdao, China, designated CXB832T, and was found to be phylogenetically closely related to members of the genera Nocardiopsis and Haloactinospora. In the present study, the taxonomic position of strain CXB832T was determined by using a polyphasic taxonomic approach.

Strain CXB832T was isolated and maintained on starch–casein agar medium (pH 7.5) supplemented with 15 % (w/v) NaCl at 37 °C for 3 weeks. The composition of the starch–casein agar medium was (distilled water 1-L): 10 g starch, 0.3 g casein, 2 g KNO3, 0.05 g MgSO4, 7H2O, 2 g KH2PO4, 0.02 g CaCO3, 0.01 g FeSO4 and 15 g agar. After primary isolation and purification by the streak plate method, the isolates were preserved both on slants of International
**Streptomyces** Project medium 4 agar (ISP 4; Shirling & Gottlieb, 1966) supplemented with 10% (w/v) NaCl at 4 °C and in 20% (v/v) glycerol at −80 °C.

All the media were supplemented with 10% (w/v) NaCl for the observation of growth. Strain CXB832T 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). The novel strain did not produce diffusible pigments on any of the media tested. Micromorphology was observed by light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) using cells incubated for 7, 14, 21 and 28 days on ISP 2 agar supplemented with 10% (w/v) NaCl. The colours of the substrate and aerial mycelia were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). The aerial and substrate mycelia were well developed on most media tested, producing a pale yellow to deep yellow substrate mycelium that carried white aerial hyphae on ISP 4 agar supplemented with 10% (w/v) NaCl. The aerial mycelium formed long chains of spores at maturity that were rod-shaped with smooth surfaces; they were non-motile (Fig. 1a, b). Substrate mycelium was extensively branched with non-fragmenting hyphae (Fig. 1c).

Physiological characteristics, including temperature and pH ranges for growth and tolerance to NaCl, were tested using ISP 4 as the basal medium. Growth was tested at 4, 16, 20, 28, 37, 40, 43, 46, 49, 50, 51 and 52 °C on ISP 4 agar with 10% (w/v) NaCl. For tolerance experiments involving concentrations of NaCl between 0 and 25% (at intervals of 1%), ISP 4 was used as the basal medium. The pH range for growth was investigated between pH 4.0 and 11.0 at intervals of 1.0 pH unit using the buffer system described by Xu et al. (2005). Carbon-source utilization for growth was performed 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 peptonization and production of H2S and melanin were performed with 10% (w/v) NaCl as described by Gonzalez et al. (1978). Antibiotic susceptibility was examined according to Groth et al. (2004) using antibiotic discs on ISP 4 agar with 10% (w/v) NaCl. *Haloactinospora alba* DSM 45015T (the type species of the genus *Haloactinospora*) obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) was used as a reference strain and cultured under the same conditions. The detailed physiological properties of the novel strain are given in the genus and species description (see below) and are presented in Table 1.

Biomass for chemotaxonomic studies was obtained by centrifugation after cultivation in ISP 4 broth with 10% (w/v) NaCl for 7 days at 180 r.p.m. at 37 °C. For fatty acid analyses, cells were grown on tryptic soy agar (Difco) containing 10% (w/v) NaCl at 180 r.p.m. at 37 °C. For analysis of amino acids and sugars in the cell-wall hydrolysate, wet biomass was well suspended in isopropanol/water (1:1, v/v). For analysis of polar lipids and menaquinones, wet biomass was freeze-dried. Analysis of whole-cell sugars and amino acids in the cell-wall hydrolysate, polar lipids and menaquinones was carried out by the Identification Service of the DSMZ. Analysis of whole-cell sugars was performed according to the procedure described by Becker et al. (1965). Amino acids in the cell-wall hydrolysate were analysed by the method described by Schleifer & Kandler (1972). Menaquinones were extracted and analysed according to Collins et al. (1979) and Groth et al. (1996). Polar lipids extracted by the method of Minnikin et al. (1979) were identified by two-dimensional TLC as described by Collins & Jones (1980). The analysis of fatty acids was performed as described by Sasser (1990) and the results were compared with the database of fatty acids in the Microbial Identification System (MIDI) (TSBA6; version, 6.0B library). The whole-cell hydrolysates of strain CXB832T contained meso-diaminopimelic acid as the diagnostic cell-wall peptidoglycan and the whole-cell sugars were glucose, xylose with traces of ribose and arabinoose. Phospholipids (see Fig. S1 in IJSEM Online) comprised...
Table 1. Differentiating phenotypic and chemotaxonomic characteristics of strain CXB832<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>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Salt pond</td>
<td></td>
<td></td>
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<tr>
<td>Spore surface</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Sand dune soil</td>
<td>Marine sediment</td>
<td>Marine sediment</td>
<td>Salt mine</td>
<td>Indoor walls</td>
</tr>
<tr>
<td>Growth conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Smooth</td>
<td>No aerial mycelium</td>
</tr>
<tr>
<td>NaCl range (%, w/v)</td>
<td>1–23</td>
<td>9–21*</td>
<td>0–15</td>
<td>1–15</td>
<td>0–5</td>
<td>0–10</td>
<td>0–11</td>
<td>5–20</td>
</tr>
<tr>
<td>NaCl optimum (%, w/v)</td>
<td>9–12</td>
<td>15*</td>
<td>5</td>
<td>3–8</td>
<td>0–1</td>
<td>NT</td>
<td>NT</td>
<td>10</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>37</td>
<td>37*</td>
<td>28–30</td>
<td>37</td>
<td>28</td>
<td>45</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td>Maximum temperature (°C)</td>
<td>50</td>
<td>45*</td>
<td>40</td>
<td>44</td>
<td>55</td>
<td>50</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>Diagnostic sugars</td>
<td>Glu, Xyl</td>
<td>Gal, Rib</td>
<td>Gal, Glu</td>
<td>Rib, Glu</td>
<td>None</td>
<td>Gal, Xyl, Glu</td>
<td>None</td>
<td>Gal</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-10(H&lt;sub&gt;2&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;, H&lt;sub&gt;n&lt;/sub&gt;, H&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, 10-methyl-C&lt;sub&gt;18&lt;/sub&gt;:0 ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;18&lt;/sub&gt;:0</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;18&lt;/sub&gt;:0</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;18&lt;/sub&gt;:0</td>
<td>i-C&lt;sub&gt;16&lt;/sub&gt;:0, ai-C&lt;sub&gt;17&lt;/sub&gt;:0 C&lt;sub&gt;18&lt;/sub&gt;:0, 10-methyl-C&lt;sub&gt;18&lt;/sub&gt;:0, 10-methyl-C&lt;sub&gt;17&lt;/sub&gt;:0</td>
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*Data from this study for Haloactinospora alba. This strain was grown under the same culture conditions as strain CXB832<sup>T</sup>.
diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipid and unidentified lipids. The menaquinones consisted of MK-10(H₈) (32%), MK-9(H₈) (29%), MK-10(H₂) (20%), MK-10(H₉) (17%) with traces of MK-6(H₈) and MK-10(H₄). The fatty acid profile contained i-C₁₆ : 0 (30.71%), ai-C₁₇ : 0 (13.31%), C₁₆ : 0 (11.82%), C₁₈ : 0 (9.43%), i-C₁₈ : 0 (6.56%), 10-methyl-C₁₈ : 0 (6.08%), C₁₈ : 0 2-0H (2.73%), i-C₁₇ : 0 (2.63%), C₁₇ : 0 (2.63%), C₁₈ : 0 9c (2.52%), ai-C₁₅ : 0 (1.47%), C₁₄ : 0 (1.19%), C₁₇ : 0 iso 9c and/or 10-methyl-C₁₆ : 0 (1.14%) and i-C₁₄ : 0 (0.56%).

The G+C content of the chromosomal DNA was determined according to the methods described by Mesbah et al. (1989) using reverse-phase HPLC. The G+C content of strain CXB832T was 60.1 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. The 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) (the sequence alignment is available as supplementary material in IJSEM Online). The phylogenetic trees were constructed using the neighbour-joining and maximum-likelihood methods with Kimura 2-state parameter model analyses implemented in the MEGA version 5 program (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 CXB832T showed that its closest relatives were members of genera Nocardiopsis and Haloactinospora in the family Nocardiopsaceae, with the highest gene sequence similarity of 95.4% with Nocardiopsis arabia DSM 45083T and 94.9% with Haloactinospora alba DSM 45015T, respectively. In the neighbour-joining (Fig. 2) and maximum-likelihood trees (Fig. S2) based on the 16S rRNA gene sequences of representatives of all genera in family Nocardiopsaceae, strain CXB832T formed a distinct lineage, which showed the evolutionary divergence between strain CXB832T and the previously described genera in this family.

The phenotypic and chemotaxonomic characteristics of the novel strain could be readily distinguished from the closest

![Fig. 2. Phylogenetic dendrogram obtained by neighbour-joining analysis based on 16S rRNA gene sequences showing the position of strain CXB832T and its phylogenetic neighbours. Numbers at nodes are bootstrap values based on 1000 resamplings (only values >50% are indicated). Bar, 1% sequence divergence.](http://ijs.sgmjournals.org)
phylogenetic genera, *Nocardiopsis* and *Haloactinospora*, by the maximum NaCl concentration for growth, the diagnostic sugars, the predominant menaquinones and the diagnostic phospholipids. The maximum NaCl concentration for growth of strain CXB832<sup>T</sup> was 23% (w/v), but members of the genera *Nocardiopsis* and *Haloactinospora* are unable to grow at this concentration. The diagnostic sugars of strain CXB832<sup>T</sup> were glucose and xylose, which are different from those found for the other two genera. The predominant menaquinones for strain CXB832<sup>T</sup> were MK-10(H<sub>3</sub>,H<sub>6</sub>,H<sub>8</sub>) and MK-9(H<sub>8</sub>), but MK-10(H<sub>2</sub>) and MK-9(H<sub>6</sub>) are absent from members of the genera *Nocardiopsis* and *Haloactinospora*. The diagnostic phospholipids of the genera *Nocardiopsis* and *Haloactinospora* are phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM) and phosphatidylmethylthanolamine (PME), but none of these phospholipids were present in strain CXB832<sup>T</sup>. Other characteristics that differentiated strain CXB832<sup>T</sup> from members of the other seven genera of the family *Nocardiopsaceae* are shown in Table 1. Thus, based on the above phenotypic and genotypic results, strain CXB832<sup>T</sup> represents a novel genus for which the name *Salinactinospora* gen. nov. is proposed. The type species of the genus *Salinactinospora* qingdaonensis sp. nov. is described for the genus.

**Description of Salinactinospora gen. nov.**

*Salinactinospora* (Sa.lin.ac.ti.no’spo.ra. N.L. adj. salinus saline; Gr. n. actis actinus a ray; Gr. n. spora a seed; N.L. fem. n. *Salinactinospora* an actinomycete originating from a saline habitat).

Gram-positive-staining, aerobic, moderately halophilic, filamentous actinomycetes. The aerial mycelium forms long chains of spores. Spores are rod-shaped with smooth surfaces. Substrate mycelium is extensively branched with non-fragmenting hyphae. No diffusible pigments are produced. The whole-cell hydrolysates contain meso-diaminopimelic acid, glucose and xylose. The predominant menaquinones are MK-10(H<sub>3</sub>), MK-10(H<sub>6</sub>), MK-10(H<sub>8</sub>) and MK-9(H<sub>8</sub>). The phospholipids are diphosphatidylglycerol, phosphatidyglycerol, phospholipids, glycolipid and unidentified lipids. The major fatty acids are i-C<sub>16</sub>:0, ai-C<sub>17</sub>:0 and C<sub>16</sub>:0. The G+C content of the genomic DNA is 60.1 mol%.

**Description of Salinactinospora qingdaonensis sp. nov.**

*Salinactinospora qingdaonensis* (qing.da.o.nen’sis. N.L. fem. adj. *qingdaonensis* pertaining to Qingdao, a city in the coastal region of East China, from which the type strain was isolated).

Displays the following properties in addition to those described for the genus. Good growth on ISP 2, ISP 3, ISP 4 and ISP 5 agars, Czapek solution agar, nutrient agar and potato agar supplemented with 10% (w/v) NaCl. The colour of the aerial mycelium is white and the substrate mycelium is pale yellow to deep yellow on ISP 4 agars supplemented with 10% (w/v) NaCl. Growth occurs between 16 and 50 °C, but not at 4 or 51 °C. Grows at pH 6.0–9.0 and with 1–23% (w/v) NaCl, with optimum growth at pH 7.0–8.0 and 37 °C with 9–12% (w/v) NaCl. Utilizes x-cyclodextrin, dextrin, glycerogen, inulin, mannan, L-arabinose, D-arabitol, arbutin, celloheose, D-galactose, D-glucose, lactose, lactulose, maltose, maltotriose, D-mannose, melibiose, raffinose, L-rhamnose, sucrose, salicin, D-ribose, D-sorbitol, turanose, xylitol and inosine as sole carbon sources, but D-fructose, L-fucose, inositol, D-galacturonic acid, D-mannitol, melezitose, stachyose, D-xylose, D-tagatose and D-psicose are not utilized. Susceptible to (µg per disc): norfloxacin (10), vancomycin (30), amoxicillin (10), minocycline (30) and erythromycin (15). Resistant to (µg per disc): penicillin (10), oxacillin (1), ampicillin (10), carbenicillin (100), amikacin (30), gentamicin (10), kanamycin (30), neomycin (30), midecamycin (30), ofloxacin (5), norfloxacin (10), polymyxin B (300), furazolidone (300), cindamide (2), netilmicin (30), ciprofloxacin (5), tobramycin (10), trimethoprim (5), lincomycin (2) and streptomycin (10). Tests for hydrolysis of starch and cellulose, melanin production, H<sub>2</sub>S production and gelatin liquefaction are negative, whereas tests for Tweens 20, 40, 60 and 80, nitrate reduction, utilization of urea, milk coagulation and milk peptonization are positive. The fatty acid profile contained mainly i-C<sub>16</sub>:0, ai-C<sub>17</sub>:0 and C<sub>16</sub>:0.

The type strain, CXB832<sup>T</sup> (=DSM 45442<sup>T</sup>=LMG 25567<sup>T</sup>), was isolated from a salt pond in Qingdao, China. The DNA G+C content of the type strain is 60.1 mol%.

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**References**


