**Allosalinactinospora lopnorensis** gen. nov., sp. nov.,
a new member of the family *Nocardiopsaceae*
isolated from soil

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A novel actinomycete, designated strain CA15-2T, was isolated from a soil sample collected from the rhizosphere of tamarisk in the Lop Nor region, Xinjiang, China, and was characterized by using a polyphasic taxonomic approach. Optimal growth occurred at 37 °C and pH 7.5–8.0 and with 5% (w/v) NaCl. Strain CA15-2T formed white to pale-yellow branched substrate mycelium without fragmentation and sparse aerial mycelium with wavelike curves. Whole-cell hydrolysates of the isolate contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell wall but no diagnostic sugars. The polar lipids comprised diphosphatidyglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylethanolamine, one unidentified glycolipid, one unidentified phospholipid and other unidentified lipids. MK-9(H₈), MK-10(H₆) and MK-10(H₈) were the predominant menaquinones. The major fatty acids were iso-C₁₆ : 0 and C₁₆ : 0. The G+C content of the genomic DNA was 69.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CA15-2T formed a distinct subclade in the family *Nocardiopsaceae*, with less than 95% 16S rRNA gene sequence similarity to all known members of the family *Nocardiopsaceae*. On the basis of the evidence from our polyphasic study, a novel genus, *Allosalinactinospora* gen. nov., is proposed, with the type species *Allosalinactinospora lopnorensis* gen. nov., sp. nov. The type strain of *Allosalinactinospora lopnorensis* is strain CA15-2T (≡DSM 45697T=CGMCC 4.7074T).

The family *Nocardiopsaceae*, with *Nocardiopsis* as the type genus, was proposed by Rainey et al. (1996) based on polyphasic analysis. At the time of preparing this manuscript, the family *Nocardiopsaceae* contained eight genera: *Nocardiopsis* (Meyer, 1976), *Thermobifida* (Zhang et al., 1998), *Streptomonospora* (Cui et al., 2001), *Haloactinospora* (Tang et al., 2008), *Marinactinospora* (Tian et al., 2009), *Murinocardiopsis* (Kämper et al., 2010), *Spinactinospora* (Chang et al., 2011) and *Salinactinospora* (Chang et al., 2012). As the largest genus in the family *Nocardiopsaceae*, *Nocardiopsis* contained 37 species and two subspecies (http://www.bacterio.net/nocardiopsis.html; Euzéby, 1997), more than half of which were isolated from saline environments such as salterns (Chun et al., 2000), saline or hypersaline soils (Al-Zarban et al., 2002; Li et al., 2003a, 2004, 2006; Hozzein et al., 2004; Zhang et al., 2008; Yang et al., 2008a; Chen et al., 2008, 2010; Hamedi et al., 2010, 2011) or marine samples (Sabry et al., 2004; Kroppenstedt & Evtushenko, 2006; Tian et al., 2009; Chen et al., 2009; Fang et al., 2011; Li et al., 2012). In addition, one of four species in the genus *Thermobifida* was isolated from a salt mine (Yang et al., 2008b) and eight of nine species in the genus *Streptomonospora* were isolated from a salt lake or hypersaline soils (Cui et al., 2001; Li et al., 2003b; Cai et al., 2008, 2009; Meklat et al., 2014) or marine samples (Zhang et al., 2013). The genera *Haloactinospora*, *Marinactinospora*, *Spinactinospora* and *Salinactinospora* each contain only one species, and the type species of these genera were isolated from a salt lake (Tang et al., 2008) or marine sediments (Tian et al., 2009; Chang et al., 2011, 2012).

During a study of the diversity of cultivable rhizosphere actinomycetes from psammophytes in Xinjiang, China, strain CA15-2T was isolated from a saline soil sample from the rhizosphere of tamarisk collected in the Lop Nor region (40.186° N 90.455° E), a dried-up salt lake located between the Taklamakan and Kumtag deserts in the south-eastern...
portion of Xinjiang Uygur Autonomous Region, China (Tuo et al., 2012). Based on phylogenetic analysis, strain CA15-2\textsuperscript{T} could be readily distinguished from previously described genera of the family Nocardiopsaceae and represents a new genus. In this paper, the taxonomic description of this strain is reported.

Strain CA15-2\textsuperscript{T} was isolated and purified by the dilution plating method on HV agar (Hayakawa & Nonomura, 1987) after incubated at 28 °C for 8 weeks. The purified strain was maintained on slants of tryptic soy agar (TSA; Difco) containing 5 % (w/v) NaCl at 4 °C and in 20 % (v/v) glycerol at −80 °C. All culture media were supplemented with 5 % (w/v) NaCl for observation of growth at 37 °C for 3–4 weeks. Strain CA15-2\textsuperscript{T} grew well on TSA (Difco) and R2A agar (Difco), and poor growth occurred on ISP 2 and ISP 4 agars (Shirling & Gottlieb, 1966), Czapek’s agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961). No growth occurred on ISP 5 agar (Shirling & Gottlieb, 1966). The isolate did not produce diffusible pigments on any of the media tested. Morphological characteristics were observed by light microscopy (model BH2; Olympus) using the coverslip technique described by Zhou et al. (1998) and then recorded by scanning electron microscopy (Quanta 200; FEI) using gold-coated dehydrated specimens of 3-month cultures from TSA supplemented with 5 % NaCl at 37 °C. Strain CA15-2\textsuperscript{T} formed white to pale-yellow branched substrate mycelium without fragmentation, and sparse aerial mycelium was observed as wavelike curves with no fragmentation (Fig. 1).

Physiological characteristics, including temperature and pH ranges and NaCl tolerance, were tested using TSA or tryptic soy broth (TSB; Difco) as the basal medium. Growth was tested at 0, 4, 10, 15, 20, 25, 28, 32, 37, 42, 45 and 50 °C on TSA supplemented with 5 % (w/v) NaCl. For NaCl tolerance experiments at 37 °C, the concentration of NaCl added to TSB was 0, 1, 3, 5, 8, 10 or 15 % (w/v). The pH range for growth was tested between pH 5.0 and 11.0 at intervals of 0.5 pH units in TSB using the buffer system described by Xu et al. (2005). Strain CA15-2\textsuperscript{T} was able to grow at 20–42 °C and grew well at 28–37 °C; no growth occurred at 15 or 45 °C. The optimum growth temperature was 37 °C. Growth was observed at pH 6.0–9.0 and optimum growth occurred at pH 7.5–8.0. Strain CA15-2\textsuperscript{T} could grow with 0–10 % (w/v) NaCl, but no growth occurred at 15 % (w/v) NaCl; the optimum concentration of NaCl for growth was 5 % (w/v). Carbon utilization and acid production from carbohydrates were tested using Biolog GENIII MicroPlates and the API 50CH (bioMérieux) system, respectively, according to the manufacturers’ protocols. Sole nitrogen sources were determined using the following basal liquid medium (1 L): 1.0 g D-glucose, 0.05 g MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O, 0.05 g NaCl, 0.001 g FeSO\textsubscript{4} \cdot 7H\textsubscript{2}O and 0.01 g K\textsubscript{2}HPO\textsubscript{4}. All of the physiological tests above were observed consistently for 1 month. Qualitative enzyme tests were carried out using API ZYM (bioMérieux) and oxidase was detected using API oxidase reagent (bioMérieux) as described by the manufacturer’s instructions. Catalase was determined by production of bubbles after a drop of 3 % H\textsubscript{2}O\textsubscript{2} was added. Other physiological and biochemical properties of strain CA15-2\textsuperscript{T} were examined according to the methods of Williams et al. (1983) and Kämpfer et al. (1991). The detailed physiological and biochemical characteristics of strain CA15-2\textsuperscript{T} are given in the species description.

Biomass for molecular systematic and chemotaxonomic studies was obtained from cultures grown in TSB supplemented with 5 % (w/v) NaCl at pH 7.5 for 14 days at 37 °C and with shaking at 180 r.p.m. The diagnostic isomer of diaminopimelic acid in whole-cell hydrolysates (4M HCl, 100 °C, 15h) of strain CA15-2\textsuperscript{T} was identified by TLC on cellulose plates using the solvent system of Schleifer & Kandler (1972). The diagnostic sugar in whole-cell hydrolysates was identified by TLC as described by Staneck & Roberts (1974). For analysis of menaquinones, lipids and fatty acids, Salinactinospora qingdaonensis CXB832\textsuperscript{T}, the closest phylogenetic neighbour of strain CA15-2\textsuperscript{T}, was used as a reference strain and cultured under the same conditions as strain CA15-2\textsuperscript{T}.
Polar lipids were extracted and analysed by two-dimensional TLC on silica gel 60 F254 plates (Merck) as described by Minnikin et al. (1984). The solvent systems of the first and second dimensions were respectively chloroform/methanol/water (64:27:5, by vol.) and chloroform/methanol/acetic acid/water (80:18:12:5, by vol.). Menaquinones were extracted according to the method of Collins et al. (1977) and analysed by HPLC (Groth et al., 1997), and then confirmed by using a single quadrupole mass spectrometer LCMS-2020 (Shimadzu). The parameters for separation and molecular ion peak identification of menaquinones were as follows. A UFLC system was equipped with an SPD-M20A photodiode array detector, an atmospheric pressure chemical ionization (APCI) interface and a reversed-phase column (Shim-pack XR-ODS, 3.0 mm i.d. × 75 mm; Shimadzu). The mobile phase was methanol/isopropanol (60:40, v/v) at a flow rate of 0.3 ml min⁻¹. The APCI interface in positive ionization mode was used for MS analysis with the following operating settings: nebulizer gas flow rate, 4.0 l min⁻¹; drying gas flow rate, 15.0 l min⁻¹; APCI interface temperature, 350 °C; DL temperature, 250 °C; heat block temperature, 200 °C; APCI interface voltage, 4.5 kV; detector voltage, 1.20 kV. Data acquisition and processing were accomplished using Shimadzu LCMS solution software. For analysis of fatty acids, strain CA15-2T and the reference strain were cultured on TSA supplemented with 5 % (w/v) NaCl at 37 °C for 2 weeks. Whole-cell fatty acids were prepared according to the standard protocol of Sasser (1990), and analysed using MIDI Sherlock version 6.0 and the ACTIN1 database.

The whole-cell hydrolysate of strain CA15-2T contained meso-diaminopimelic acid but no characteristic sugars. The major polar lipids were diphsophatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylglyceroethanolamine, phosphatidylethanolamine, one unidentified glycolipid, one unidentified phospholipid and other lipids. The polar lipid profiles of strain CA15-2T and the reference strain are shown in Fig. S1 (available in the online Supplementary Material). The predominant menaquinones were MK-9(H4) (35.48 %), MK-10(H6) (29.82 %) and MK-10(H6) (14.45 %); minor amounts of MK-9(H4) (2.84 %), MK-9(H6) (7.64 %), MK-9(H10) (0.51 %), MK-10(H6) (0.57 %), MK-10(H4) (5.78 %), MK-10(H10) (2.12 %) and MK-9(H2) (0.8 %) were also present (Fig. S2). The fatty acid profile contained iso-C16 : 0 (35.48 %), C16 : 0 (29.82 %), anteiso-C17 : 0 (8.44 %), C18 : 0 (5.19 %), C18:1ω7c (5.19 %), iso-C18:0 (4.17 %), 10-methyl C18 : 0 (3.73 %), C18:1ω9c (1.93 %), iso-C17 : 0 (1.58 %) and anteiso-C15 : 0 (1.41 %) (Table S1). The major components of the menaquinone, polar lipid and fatty acid profiles of Salinactinospora qingdaoensis CXBX382T were similar to those reported previously (Chang et al., 2012). The slight differences in the proportions of menaquinones and fatty acids and types of polar lipids may be due to the different experimental conditions used.

To determine the DNA G+C content, genomic DNA was prepared according to the method described by Marmur (1961). The DNA G+C content of strain CA15-2T was 69.6 mol% as determined by the reversed-phase HPLC method (Mesbah et al., 1989).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene from strain CA15-2T were performed as described by Li et al. (2007). The PCR products were cloned by the pEASY-T1 Cloning kit (Transgen Biotechnology) and sequenced by an ABI PRISM 3730XL DNA Analyzer. Similarity values of the 16S rRNA gene sequence were calculated by using the EzTaxon-e server (http://eztaxon-e. ezbiocloud.net/; Chun et al., 2007). Multiple alignments with sequences of closely related taxa in the family Nocardiopsaceae were done by using CLUSTAL_X (Thompson et al., 1997). A neighbour-joining tree based on 16S rRNA gene sequences was reconstructed using the method of Saitou & Nei (1987) from Knu values (Kimura, 1980) and MEGA version 5.0 (Tamura et al., 2011) (Fig. 2); a maximum-likelihood tree was reconstructed using the method of Felsenstein (1981) with MEGA version 5.0 (Tamura et al., 2011) (Fig. S3). Bootstrap resampling analysis using the method of Felsenstein (1985) was employed to evaluate the topology of the phylogenetic trees with 1000 replicates.

BLAST searches showed that strain CA15-2T had the highest 16S rRNA gene sequence similarities (<95 %) to members of the family Nocardiopsaceae. In the neighbour-joining and maximum-likelihood trees based on 16S rRNA gene sequences, strain CA15-2T fell in the family Nocardiopsaceae and formed a distinct clade with the genera Salinactinospora and Haloactinospora, which supported the suggestion that strain CA15-2T represents a new genus of the family Nocardiopsaceae (Figs 2 and S3).

Strain CA15-2T was similar to members of the family Nocardiopsaceae in that it contained iso-C16 : 0 as the major fatty acid and meso-diaminopimelic acid as the diagnostc amino acid. The DNA G+C content of strain CA15-2T also fell within the range of 64–76 mol% reported as the DNA G+C content range of the family Nocardiopsaceae. BLAST sequence analysis of 16S rRNA gene sequences and the phylogenetic tree also indicated that strain CA15-2T was assigned to the family Nocardiopsaceae. However, strain CA15-2T had low 16S rRNA gene sequence similarity (<95 %) to members of the family Nocardiopsaceae and formed a distinct clade among members of the family in the phylogenetic tree. The phenotypic and chemotaxonomic characteristics of strain CA15-2T could be readily distinguished from those of the phylogenetically closest genera Salinactinospora, Haloactinospora and Nocardiopsis. In the family Nocardiopsaceae, almost all genera have aerial mycelium with spore chains, except the genus Murinocardiosis, members of which do not form aerial mycelium. Strain CA15-2T formed aerial mycelium with wavelike curves, but no fragmentation or spore chains were observed. No diagnostic sugars were detected from the whole-cell hydrolysate of strain CA15-2T, but diagnostic sugars were detected from the closely related genera Salinactinospora (glucose, xylose) and Haloactinospora (ribose, galactose).
The predominant menaquinones of strain CA15-2T were MK-9(H8), MK-10(H8) and MK-10(H6), but MK-9(H8) was absent from the genera Haloactinospora and Nocardiopsis. Although MK-9(H8) and MK-10(H8) were detected from the genus Salinactinospora, the content was significantly different from that of strain CA15-2T (Table 1). Strain CA15-2T shared the presence of diphosphatidylglycerol with the genera Haloactinospora and Salinactinospora with the genera Salinactinospora, Haloactinospora and Nocardiopsis, but some significant differences also existed between strain CA15-2T and the genus Salinactinospora, e.g. strain CA15-2T contained phosphatidylmethylethanolamine and phosphatidylethanolamine, which were not detected in Salinactinospora qingdaonensis CXB832T. On the other hand, Salinactinospora qingdaonensis CXB832T contained unidentified glycolipids (GL2, GL4) that were not detected in strain CA15-2T. Other differences in polar lipids are apparent in Fig. S1. The major fatty acids of strain CA15-2T were iso-C16:0 (54.14 %) and C16:0 (14.02 %), which differed significantly from Salinactinospora qingdaonensis CXB832T [anteiso-C17:0 (35.79 %), iso-C16:0 (30.86 %) and C16:0 (13.67 %)] (Table S1). Other characteristics that differentiated strain CA15-2T from members of the other genera of the family Nocardiopsaceae are shown in Table 1.

Based on the data from our polyphasic study, it is clear that strain CA15-2T is different from the eight genera in the family Nocardiopsaceae, and that it represents a novel genus, for which the name Allosalinactinospora gen. nov. is proposed. The type species of the genus is Allosalinactinospora lopnorensis sp. nov.

**Description of Allosalinactinospora gen. nov.**

Allosalinactinospora (Al.lo.sa.lin.ac.ti’no.sp’ra. Gr. adj. allos another, the other, different; N.L. fem. n. Salinactinospora a bacterial genus name; N.L. fem. n. Allosalinactinospora the other Salinactinospora, referring to the fact that the genus is related phylogenetically to Salinactinospora).

Aerobic, Gram-positive-staining, non-motile actinomycetes. The aerial mycelium is wave-shaped with no fragmentation or spore chains. Substrate mycelium is branched with non-fragmented hyphae. Catalase-positive and oxidase-negative. Whole-cell hydrolysates contain meso-diaminopimelic acid as the diamino acid and no characteristic sugars. The predominant menaquinones are MK-9(H8), MK-10(H8) and MK-10(H6). The major fatty acids (>10 %) are iso-C16:0 and C16:0. The G+C content of the genomic DNA of the type strain of the type species is 69.6 mol%. The type species is Allosalinactinospora lopnorensis.

**Description of Allosalinactinospora lopnorensis sp. nov.**

Allosalinactinospora lopnorensis (lop.no.ren’sis. N.L. fem. adj. lopnorensis pertaining to the Lop Nor region of Xinjiang, north-west China, the source of the type strain). Displays the following properties in addition to those given in the genus description. Colonies grow well on TSA and R2A agar and poorly on ISP 2 agar, ISP 4 agar, Czapek’s agar, nutrient agar and potato agar; no growth occurs on ISP 5 agar. Growth occurs at pH 6.0–9.0 and 20–42 °C.
**Table 1.** Differential characteristics of strain CA15-2T^T and related genera of the family *Nocardiopsaceae*

Taxa: 1, strain CA15-2T^T; 2, *Salinactinospora* (unless indicated, data from Chang et al., 2012); 3, *Nocardiopsis* (Kroppenstedt &Evtushenko, 2006; Hozzein & Trujillo, 2012); 4, *Thermobifida* (Yang et al., 2008b); 5, *Marinactinospora* (Tian et al., 2009); 6, *Halocactinospora* (Tang et al., 2008); 7, *Streptomonospora* (Cai et al., 2008); 8, *Spinactinospora* (Chang et al., 2011); 9, *Marinocardiopsis* (Kämpfer et al., 2010). Cell walls of all taxa contain meso-diaminopimelic acid. ND, No data available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial mycelium</td>
<td>Wave shaped, non-fragmented</td>
<td>Forms long spore chains</td>
<td>Differentiates into straight to flexuous spore chains</td>
<td>Forms dichotomously branched sporophores</td>
<td>Forms long spore chains</td>
<td>Forms long spore chains</td>
<td>Forms short spore chains</td>
<td>Forms long or short spore chains</td>
<td>No aerial mycelium</td>
</tr>
<tr>
<td>Substrate mycelium</td>
<td>Branched, non-fragmented</td>
<td>Branched, non-fragmented</td>
<td>Branched, fragmented</td>
<td>Branched, non-fragmented</td>
<td>Branched, non-fragmented</td>
<td>Branched, non-fragmented</td>
<td>Branched, non-fragmented</td>
<td>Non-fragmented</td>
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<tr>
<td>Diagnostic sugar(s)</td>
<td>None</td>
<td>Glu, Xyl</td>
<td>None</td>
<td>Gal, Xyl, Glu</td>
<td>Glu</td>
<td></td>
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<tr>
<td>Predominant menaquinones</td>
<td>9(H₈) (35.48%), 10(H₈) (29.82%), 10(H₆) (14.45%)</td>
<td>9(H₈) (53.49%), 9(H₈) (22.29%), 10(H₈) (10.77%)</td>
<td>10(H₄, H₆, H₈)</td>
<td>10(H₄, H₆, H₈)</td>
<td>11(H₄, H₆, H₈)</td>
<td>10(H₄, H₆, H₈)</td>
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<td>10(H₄, H₆, H₈)</td>
<td>10(H₄, H₆, H₈)</td>
</tr>
<tr>
<td>Diagnostic phospholipids†</td>
<td>DPG, PG, PC, PE, PME, PL, GL</td>
<td>DPG, PG, PC, PE, PME, PL, PE</td>
<td>PME, PE</td>
<td>PC, PME, PG, DPG</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>69.6</td>
<td>60.1</td>
<td>64–76</td>
<td>66–72</td>
<td>72</td>
<td>68</td>
<td>72–75</td>
<td>71.1</td>
<td>ND</td>
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</tbody>
</table>

*Data from this study for *Salinactinospora qingdaonensis* CXB832^T.*
†DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidymethyllethanolamine; GL, unknown glycolipid(s); PL, unknown phospholipid(s).
‡ai, anteiso; i, iso; Me, methyl.


