**Prauserella shujinwangii** sp. nov., from a desert environment

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A Gram-positive, spore-forming, rod-shaped actinomycete, designated XJ46T, was isolated from Xinjiang Uyghur Autonomous Region, China and subjected to a polyphasic taxonomic analysis. Morphological and chemotaxonomic characteristics of XJ46T were identified as being similar to those of members of the genus *Prauserella*. The phylogenetic tree based on 16S rRNA gene sequences showed that XJ46T shared the highest similarity (95.9%) with *Prauserella marina* MS498T. Based on its phenotypic characteristics, chemotaxonomic analysis and 16S rRNA gene sequence analysis, strain XJ46T is proposed to represent a novel species of the genus *Prauserella*, named *Prauserella shujinwangii* sp. nov. The type strain is XJ46T (=CGMCC 4.7125T = JCM 19736T).

Rare actinomycetes are usually regarded as the actinomy- cete strains with a lower isolation frequency than the members of the genus *Streptomyces* isolated by conventional methods (Fiedler et al., 2008; Kurtböke, 2012). The identification of rare actinomycetes of previously under- represented genera from unexplored environments is considered to be an effective avenue for discovery of novel antibiotics (Tiwari & Gupta, 2012). The study of organisms from unique, untapped ecological niches, such as deep-sea, salt lakes and deserts, provides an efficient approach for identification of novel microbes which have the significant potential for producing unique active compounds. The most significant examples include the isolation of strains representing members of the genera *Salinispora* and *Verru- cosispora* from marine sediments, producing salinospor- amides and abyssomicins, respectively (Bister et al., 2004; Buck, 1982; Fenical et al., 2009; Fiedler et al., 2008; Goodfellow & Fiedler, 2010; Keller et al., 2007; Maldonado et al., 2005; Riedlinger et al., 2004; Wang et al., 2013; Williams et al., 2005). Our laboratory has been devoted to systematics-guided bioprospecting for discovery of anti- infective natural products from actinomycetes, especially the rare ones from sea sediments, salt lakes and deserts (Bian et al., 2009; Dai et al., 2010; Mao et al., 2011; Wang et al., 2010). Recently, the investigation of samples from deserts led us to isolate a novel actinomycete strain belonging to the genus *Prauserella*.

The genus *Prauserella* (Kim & Goodfellow, 1999) belongs to the family *Pseudonocardiaceae*. This genus is described as containing aerobic, Gram-positive, non-acid-fast actinomycetes that form a well-developed substrate mycelium with irregular rod-shaped elements and aerial mycelium with spores in chains. Whole cells of the members of this genus contain meso-diaminopimelic acid in the peptidoglycan and major amounts of galactose and arabinose. The predominant menaquinone is MK-9 (H4) and the DNA G+C contents are between 65.8 and 69.9 mol%. The polar lipid profiles have diphosphatidylglycerol and phosphati- dylethanolamine as diagnostic components and the fatty- acid profiles are rich in branched chain and saturated components (Kim & Goodfellow, 1999; Li et al., 2003). To date, this genus comprises nine known species, namely

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†These authors contributed equally to this work.

**Abbreviations:** ME, minimum-evolution; ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain XJ46T is KJ125073.

A supplementary table and four supplementary figures are available with the online version of this paper.
**Prauserella rugosa** (Kim & Goodfellow, 1999), **Prauserella halophila**, **Prauserella alba** (Li et al., 2003), **Prauserella salsuginis**, **Prauserella flava**, **Prauserella aidingensis**, **Prauserella sediminis** (Li et al., 2009), **Prauserella muralis** (Schäfer et al., 2010) and **Prauserella marina** (Wang et al., 2010).

Strain XJ46<sup>T</sup> was originally isolated from a desert sample collected from Xinjiang, China (GPS coordinates for the sampling site are 40° 36.804' N 79° 41.260' E) after two weeks of incubation at 28 °C on SCN agar (Küster & Williams, 1964) and maintained on International Streptomyces Project medium 2 (ISP2; Shirling & Gottlieb, 1966) agar at 4 °C and as suspensions of mycelia fragments in glycerol (20%, v/v) at −80 °C. Biomass for molecular and chemical studies was obtained by cultivation in shake flasks using ISP2 (pH 7.2) at 28 °C, with shaking at 200 r.p.m. for 8 days. XJ46<sup>T</sup> and the reference strains *P. marina* MS498<sup>T</sup> and *P. muralis* 05-Be-005<sup>T</sup> were subjected to polyphasic taxonomic procedures.

Genomic DNA extraction, PCR amplification and 16S rRNA gene sequencing of XJ46<sup>T</sup> were carried out according to the procedures described by Kim et al. (1998). The phylogenetic trees were reconstructed using three methods, neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-likelihood (ML) (Fitch, 1971) and maximum-evolution (ME) (Rzhetsky & Nei, 1992), using the software package MEGA version 5.0 (Tamura et al., 2011). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein, 1985). Genomic DNA for the determination of the G + C content was prepared according to a method previously reported (Marmur, 1961) and was determined by the thermal denaturation (T<sub>m</sub>) method (Mandel & Marmur, 1968) with *Escherichia coli* K-12 (CGMCC 1.748) as the reference strain using a LAMBDA 35 UV/Vis spectrophotometer (PerkinElmer) fitted with a thermal controller.

The highest degree of 16S rRNA gene sequence similarity to strain XJ46<sup>T</sup> (1493 nt) was found for *P. marina* (95.9%). The phylogenetic tree shown in Fig. 1 indicated that XJ46<sup>T</sup> represented a member of the genus *Prauserella*. Phylogenetic analysis based on 16S rRNA sequence analysis revealed that XJ46<sup>T</sup> fell into a subclade between the genera *Prauserella* and *Saccharomonospora* with a bootstrap value above 85% and was associated with *P. marina* and *P. muralis* (Fig. 1). ML (Fig. S1, available in the online Supplementary Material) and ME (Fig. S2) trees were similar to the NJ tree. The DNA G + C content of XJ46<sup>T</sup> was 67.5%. Considering the morphology and chemotaxonomic characteristics of XJ46<sup>T</sup> were similar to those of members of the genus *Prauserella*, we concluded that strain XJ46<sup>T</sup> should be assigned to the genus *Prauserella*.

Chemosystematic studies were carried out to compare the chemical profile of XJ46<sup>T</sup> and that of *P. marina* MS498<sup>T</sup> (= DSM 45268<sup>T</sup>) and *P. muralis* 05-Be-005<sup>T</sup> (= DSM 45305<sup>T</sup>). Cell-wall peptidoglycans and whole-cell sugars were analysed according to the procedures developed by Hasegawa et al. (1983). Polar lipids were extracted and examined by two-dimensional TLC and identified using procedures described by Minnikin et al. (1984). Menaquinones were isolated according to the protocol of Minnikin et al. (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid was identified as described by Sasser (1990) using the Microbial Identification System (MIDI) and the TSB6 database. Strain XJ46<sup>T</sup> contained *meso*-diaminopimelate acid as the diagnostic diamino acid. The whole-cell sugars of XJ46<sup>T</sup> mainly consisted of arabinose, glucose and galactose. The polar lipids profile of XJ46<sup>T</sup> consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylinositol and other unknown phospholipids (Fig. S3). The major polar lipids of XJ46<sup>T</sup> were diphosphatidylglycerol and phosphatidylethanolamine, consistent with its assignment to the genus *Prauserella*. There were three polar lipids (PL1, PL3 and PL4) found only in XJ46<sup>T</sup>. The predominant menaquinone of XJ46<sup>T</sup> was MK-9 (H<sub>4</sub>). XJ46<sup>T</sup> was found to contain iso-C<sub>16:0</sub>, C<sub>17:1</sub>ω6c and C<sub>16:0</sub> as the major fatty acid components, which was similar to *P. marina* and *P. muralis*. The fatty acid profile of XJ46<sup>T</sup> was very similar to that of *P. marina* with almost the same components (except for C<sub>14:0</sub> and C<sub>17:0</sub>) (Table 1). The chemotaxonomic data for XJ46<sup>T</sup> was consistent with the classification of XJ46<sup>T</sup> as representing a member of the genus *Prauserella* (Kim & Goodfellow, 1999; Lechevalier et al., 1986; Li et al., 2003).

Cell morphology, Gram staining and morphological characteristics were observed by light microscopy (BH 2; Olympus) and spore morphology was observed by scanning electron microscopy (Quanta 200; FEI). Gram staining of XJ46<sup>T</sup> was carried out using the standard Gram reaction and was confirmed by using the non-staining method (Buck, 1982). For XJ46<sup>T</sup>, aerial mycelium was white on most media and substrate mycelium was light yellow to yellowish white. For *P. marina* MS498<sup>T</sup>, the colour of substrate mycelium ranged from soft yellow (GT agar and Czapek’s agar; GT agar contains 20 g soluble starch, 0.5 g l-asparagine, 1 g KNO<sub>3</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>·H<sub>2</sub>O, 0.5 g NaCl, 0.5 g MgSO<sub>4</sub>, 7H<sub>2</sub>O, 1 g CaCO<sub>3</sub> and 20 g agar per litre) to pale pink (ISP3) to moderate reddish brown (ISP2), while substrate mycelium of *P. muralis* 05-Be-005<sup>T</sup> was yellowish white to strong reddish orange. The novel isolate can be distinguished from two reference strains by a battery of cultural characteristics (Table S1). Spore chains were borne on aerial mycelium and non-motile (Fig. S4).

With regard to physiological and biochemical properties of XJ46<sup>T</sup>, growth at different pH values (pH 4.0–10.0, at intervals of 1.0 pH units), temperatures (4, 10, 16, 28, 37, 45, 55 and 65 °C) and NaCl concentrations (0–20%, w/v; at intervals of 1%) was observed on ISP2. The pH tolerance experiment was investigated using the following buffer systems: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH<sub>2</sub>PO<sub>4</sub>/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO<sub>3</sub>/0.1 M Na<sub>2</sub>CO<sub>3</sub>. Carbon/nitrogen utilization and physiological tests were carried out according to the methods described by Williams et al.
XJ46T could be distinguished from the reference strains by a series of morphological and physiological properties (Table 2). Growth of XJ46T occurred at temperatures of 16–45 °C, pH 6.0–9.0 and in the presence of 0–7% (w/v) NaCl. Oxidase was positive, but negative in *P. marina* MS498T and weakly positive in *P. muralis* 05-Be-005T. D-Cellobiose, lactose, D-fructose, D-glucose and D-xylose could be utilized as carbon sources, but D-mannitol, D-sorbitol, sucrose, L-arabinose, D-galactose, L-rhamnose, trehalose, D-ribose, maltose and raffinose could not be utilized. L-Lysine, L-methionine, L-arginine, L-glycine, L-serine, L-threonine, L-asparagine and L-glutamic acid could be utilized as nitrogen sources, but L-tryptophan could not be utilized as a nitrogen source.

XJ46T is related to *P. marina* MS498T and *P. muralis* 05-Be-005T according to the 16S rRNA gene sequence analysis and has the highest degree of 16S rRNA gene sequence similarity with *P. marina*. It shows sufficient physiological and cultural differences from other species of the genus *Prauserella*. Compared with species of the genus *Saccharomonospora*, strain XJ46T also presents differences in chemotaxonomic properties, such as having diphosphatidylglycerol and phosphatidylethanolamine as major polar lipids and MK-9 (H4) as the predominant menaquinone. Thus, based on the polyphasic taxonomic characteristics, strain XJ46T represents a novel species of the genus *Prauserella*, for which the name *Prauserella shujinwangii* sp. nov. is proposed.

**Description of *Prauserella shujinwangii* sp. nov.**

*Prauserella shujinwangii* (shu.jin.wang’i.ii. N.L. gen. masc. *n. shujinwangii* of Shujin Wang, named in honour of the famous Chinese microbiologist Professor Shujin Wang).

**Table 1.** Fatty acid content of strain XJ46T, *P. marina* MS498T and *P. muralis* 05-Be-005T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>–</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>C15:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>14.9</td>
<td>21.8</td>
<td>13.8</td>
</tr>
<tr>
<td>C17:0</td>
<td>3.1</td>
<td>–</td>
<td>2.4</td>
</tr>
<tr>
<td>C17:0 10-methyl</td>
<td>–</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>1.8</td>
<td>5.3</td>
<td>1.9</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>23.5</td>
<td>24.9</td>
<td>26.8</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>3.1</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>antteiso-C17:0</td>
<td>5.6</td>
<td>3.8</td>
<td>3.1</td>
</tr>
<tr>
<td>C15:1o6c</td>
<td>–</td>
<td>–</td>
<td>0.8</td>
</tr>
<tr>
<td>iso-C16:1H</td>
<td>5.6</td>
<td>4.9</td>
<td>5.5</td>
</tr>
<tr>
<td>C17:1o8c</td>
<td>3.3</td>
<td>2.1</td>
<td>3.4</td>
</tr>
<tr>
<td>C17:1o6c</td>
<td>15.4</td>
<td>9.2</td>
<td>13.0</td>
</tr>
<tr>
<td>C18:1o9c</td>
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<td>6.7</td>
<td>9.8</td>
</tr>
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<td>Summed feature 5*</td>
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<td>3.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Summed feature 8*</td>
<td>–</td>
<td>–</td>
<td>0.7</td>
</tr>
<tr>
<td>Summed feature 9*</td>
<td>3.9</td>
<td>10.0</td>
<td>11.4</td>
</tr>
</tbody>
</table>

*Data for all the strains are based on the Microbial Identification System. Summed feature 3 consists of C16:1ω7cC16:1ω6c, summed feature 5 consists of C18:2ω6cC18:1ω9c, summed feature 8 consists of C18:1ω7c and summed feature 9 consists of iso-C17:1ω9c.

**Fig. 1.** Neighbour-joining phylogenetic tree for XJ46T reconstructed using MEGA5. Numbers at nodes indicate levels of bootstrap support (percentages) based on the 16S rRNA gene sequences for XJ46T and the most closely related species; only values >50% are given. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and minimum-evolution trees. Bar, 0.01 nucleotide substitutions per site. *Mycobacterium tuberculosis* was used as the outgroup.
Aerobic, Gram-staining-positive, non-motile. Aerial mycelium is white on GT agar and white–yellow on ISP2. Spores are borne on aerial mycelium. Spores appear to be rod-shaped and have smooth surfaces. Optimum growth occurs on ISP2 medium in the absence of NaCl at 28 °C, pH 7.0. Growth occurs at temperatures of 16–45 °C, pH 6.0–9.0 and in the presence of 0–7% (w/v) NaCl. All strains utilized d-xylose as the sole carbon source and l-arginine, l-glycine, l-serine, l-threonine, l-asparagine and l-glutamic acid as nitrogen sources. All strains had L-asparagine and L-glutamic acid as nitrogen sources. Oxidase, catalase and esterase (Tween 20, 40 and 80 hydrolysis) tests are positive; hydrogen sulfide production, starch hydrolysis, urease activity and gelatin liquefaction tests are negative. Cell walls contain meso-diaminopimelic acid as the main peptidoglycan component. Glucose, arabinose and galactose are present in whole-cell hydrolysates. The polar lipids profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylinositol and other unknown phospholipids. The predominant menaquinone is MK-9 (H4), and MK-8 (H4), MK-9 (H6) and MK-10 (H4) are also present. The major components of cellular fatty acids are iso-C15:0, C17:0 3-0c and C16:0.

The type strain, XJ46T (=CGMCC 4.7125T =JCM 19736T), was isolated from samples collected in Xinjiang, China. The DNA G+C content of the type strain is 67.5 mol%.

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**References**


