A novel psychrophilic, Gram-positive, yellow-pigmented and aerobic bacterium, strain 0543T, was isolated from the China No. 1 glacier. Strain 0543T was able to grow at 4–23 °C, with optimum growth at 18–19 °C. The major fatty acids were anteiso-C₁₅ : ₀ (58.36 %), iso-C₁₆ : ₀ (21.13 %), iso-C₁₄ : ₀ (10.25 %) and anteiso-C₁₇ : ₀ (7.16 %). The genomic DNA G+C content was 63.5 mol% and the major menaquinone was MK-10. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 0543T represented a lineage within the family Microbacteriaceae, with the highest similarity of 97.4 % with Salinibacterium amurskyense KMM 3673T. On the basis of polyphasic, chemotaxonomic, physiological and biochemical evidence from this study, the novel species Salinibacterium xinjiangense sp. nov. is proposed; the type strain is 0543T (=CGMCC 1.5381T = JCM 13926T).

The family Microbacteriaceae (Park et al., 1993; Stackebrandt et al., 1997) consists of numerous actinomycetes that are Gram-positive, irregularly shaped, non-spore-forming and have high genomic DNA G+C content. The genera of the family Microbacteriaceae are distinguished mainly by morphology, peptidoglycan diamino acids, respiratory menaquinone profiles and fatty acid compositions at the phenetic level (Komagata & Suzuki, 1987; Collins & Bradbury, 1992). Some members of the family Microbacteriaceae are cold-adapted micro-organisms and have been isolated from a wide range of habitats, including Antarctica, airborne dust and boreal groundwater (Suzuki et al., 1997; Kämpfer et al., 2000; Männistö et al., 2000; Sheridan et al., 2003; Reddy et al., 2003). The genus Salinibacterium was proposed by Han et al. (2003). It currently accommodates Gram-positive, aerobic, high G+C-content, non-motile, non-spore-forming, irregular rods and includes a single species, Salinibacterium amurskyense, which was isolated from seawater samples taken from the East Sea (Sea of Japan).

As 80% of the Earth’s biosphere is permanently below 5 °C, cold-adapted micro-organisms are widely distributed in nature (Margesin & Schinner, 1994). The China No. 1 glacier, which is located in Xinjiang Uygur Autonomous Region in north-western China, is a relatively simple and closed ecosystem, and four cold-adapted micro-organisms have been isolated from it in our lab (Zhu et al., 2003, Zhang et al., 2006, 2007). In this study, we report the isolation and identification of strain 0543T.

Strain 0543T was isolated from frozen soil collected from the China No. 1 glacier using previously described media and methods (Zhu et al., 2003). The strain was obtained in pure culture after three successive transfers to fresh agar medium and stored at −80 °C in 30 % (v/v) glycerol. Strain 0543T was routinely grown aerobically at 18 °C in PYG medium of the following composition (l⁻¹): 5 g Bacto peptone (Difco), 0.2 g yeast extract (Oxoid), 5 g glucose, 3 g beef extract (Oxoid), 0.5 g NaCl and 1.5 g MgSO₄·7H₂O (pH adjusted to 7.0). Rhodoglobus vestalii LV3T was kindly provided by Dr Vanya I. Miteva (Penn State University, University Park, PA, USA). Salinibacterium amurskyense JCM 12362T and Cryobacterium psychrophilum JCM 1463T were obtained from the Japan Collection of Microorganisms. These cultures were used as reference strains.

Cell morphology was examined under a light microscope (Olympus BX51) and transmission electron microscope (Hitachi H-600) (Supplementary Fig. S1, available in IJSEM Online). Colony morphology was observed on PYG medium after incubation at 18 °C for 3–4 days. Growth temperature was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined...
for cultures in PYG medium at various pH values adjusted with HCl or NaOH (1 M). General physiological tests were done according to Smibert & Krieg (1994) and Dong & Cai (2001). Acid production from carbohydrates was determined as described by Leifson (1963). API strips (API 20E, API 20NE, API ZYM; bioMérieux) were used to determine physiological and biochemical characteristics according to the manufacturer’s instructions.

DNA was extracted and purified as described by Sambrook et al. (1989). The gene encoding 16S rRNA was amplified by PCR with two universal primers (Zhang et al., 2006). The PCR product was sequenced by using an ABI Big Dye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). The 16S rRNA gene sequence of strain 0543T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. The phylogenetic tree was constructed using Kimura’s two-parameter and pairwise-deletion model analysis implemented in the program MEGA version 3.0 (Kumar et al., 2004). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 0543T represented a lineage within the family Microbacteriaceae, with the highest similarity of 97.4% with Salinibacterium amurskyense KMM 3673T (Fig. 1). This independent cluster was supported by a relatively high bootstrap value (99%) in the neighbour-joining tree. This topology was also supported by the maximum-parsimony and minimum-evolution algorithms at a confidence level of 99% (data not shown). In addition, it is noteworthy from the phylogenetic tree that the taxonomic position of Leifsonia aures and Leifsonia rubra was ambiguous, and further studies may be needed to resolve the confusion (Sheridan et al., 2003; An & Yokota, 2007).

Menaquinones were extracted and purified according to Komagata & Suzuki (1987) and were analysed by reversed-phase HPLC (Wu et al., 1989). The cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel F60; Merck) by TLC (Kates, 1986). Cell-wall peptidoglycan was prepared by the method of Komagata & Suzuki (1987). Amino acid compositions were determined using an automatic amino acid analyser (Sykam model S 433D) equipped with a separation column (LCA K06/Na). Cellular fatty acids were determined for a culture grown in PYG at 18°C for 4 days and were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990). The major isoprenoid quinone of strain 0543T was menaquinone, and the components were MK-10 (54.4%), MK-11 (28.9%), MK-9 (6.8%), MK-12 (5.4%) and MK-8 (4.3%). The polar lipids were diphosphatidylglycerol and phosphatidylglycerol. The cell-wall amino acid composition of strain 0543T was determined and contained glutamic acid, glycine, alanine, ornithine and lysine. The major cellular fatty acids were anteiso-C15:0 (58.36%), iso-C16:0 (21.13%), iso-C14:0 (10.25%) and anteiso-C17:0 (7.16%). Chemotaxonomic characteristics such as cell-wall peptidoglycan, the polar lipid profile and the fatty acid profile were in good agreement with those determined for Salinibacterium amurskyense KMM 3673T (Table 1). The fatty acid profiles of strain 0543T and its phylogenetic relatives are available in Supplementary Table S1.

The G+C content was determined by the thermal denaturation method with Escherichia coli K-12 as the reference, and DNA–DNA hybridization was done by the liquid renaturation method (De Ley et al., 1970) as modified by Huß et al. (1983); both experiments were carried out using a DU800 spectrophotometer (Beckman) with a thermal controller. The DNA G+C content of strain 0543T was 63.5 mol%. The

![Fig. 1. Phylogenetic dendrogram of strain 0543T and other members of the family Microbacteriaceae based on 16S rRNA gene sequence similarity. The tree was constructed using the neighbour-joining method. The sequence of Brevibacterium linens DSM 20425T served as an outgroup. Numbers at nodes represent percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. GenBank accession numbers are given in parentheses. Bar, 1% sequence divergence.](image-url)
DNA–DNA relatedness between strain 0543\(^{T}\) and \textit{Salinibacterium amurskyense} KMM 3673\(^{T}\) was 46.7\%, lower than the suggested cut-off value of 70\% for species differentiation.

On the basis of phylogenetic, chemotaxonomic and other taxonomic data from this study (Table 1), we found that strain 0543\(^{T}\) represents a novel species of the genus \textit{Salinibacterium}, for which the name \textit{Salinibacterium xinjiangense} sp. nov. is proposed.

**Description of \textit{Salinibacterium xinjiangense} sp. nov.**

\textit{Salinibacterium xinjiangense} (xin.jiang.en’se. N.L. neut. adj. xinjiangense pertaining to Xinjiang, where the type strain was isolated).

Cells are Gram-positive, psychophilic, irregular rods, non-spore-forming, non-flagellated and non-gliding, 1.4–2.3 µm long and 0.5–0.8 µm wide. Catalase-positive, oxidase-negative. Colonies are yellow, smooth, circular and convex with entire margins. Grows at 4–23\ C and pH 5.0–9.0, with optimum growth at 18–19\ C and approximately pH 6.0–8.0. Grows in the presence of 0–14\% (w/v) NaCl. Hydrolyses starch and Tweens 20, 60 and 80, but not gelatin or casein. Nitrate is reduced. Tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, alkaline phosphatase, acid phosphatase, indole production, \(\varepsilon\)-chymotrypsin, trypsin, urease, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\beta\)-glucosidase, \(\alpha\)-fucosidase and \(\alpha\)-mannosidase are negative. Tests for naphthol-AS-BI-phosphohydrolase, \(\alpha\)-galactosidase, \(\alpha\)-chymotrypsin, acid phosphatase, indole production, \(\varepsilon\)-chymotrypsin, trypsin, urease, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\beta\)-glucosidase, \(\alpha\)-fucosidase and \(\alpha\)-mannosidase are negative. Tests for naphthol-AS-BI-phosphohydrolase, \(\alpha\)-mannosidase, esterase (C4), esterase lipase (C8), cystine arylamidase, leucine arylamidase and valine arylamidase are positive. The following substrates are utilized as sole carbon sources: sucrose, glucose, cellobiose, \(\alpha\)-mannose, melibiose, maltose, galactose, arabinose and fructose. Acids are produced from glucose, fructose, \(\alpha\)-mannose and galactose.

The type strain is 0543\(^{T}\) (=CGMCC 1.5381\(^{T}\) =JCM 13926\(^{T}\)), isolated from the China No. 1 glacier (Xinjiang Uygur Autonomous Region).

**Acknowledgements**

We are grateful to Dr Vanya I. Miteva for the gift of the type strain of \textit{Rhodoglobus vestali}. This work was supported by the National Basic Research Program of China (2004CB719601).

**References**


**Table 1.** Comparison of strain 0543\(^{T}\) with phylogenetically related genera of the family \textit{Microbacteriaceae}

<table>
<thead>
<tr>
<th>Taxa:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Colony colour*</td>
<td>Y</td>
<td>Y</td>
<td>R</td>
<td>Y</td>
<td>Y, O</td>
<td>Y, WH</td>
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<tr>
<td>Growth temperature ((^{\circ})C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>4–23</td>
<td>4–37</td>
<td>–2 to 21</td>
<td>2–28</td>
<td>(\leq) 37</td>
<td>7–37</td>
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<td>NaCl range for growth (%)</td>
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<td>0–2.5</td>
<td>ND</td>
<td>0–6</td>
<td>0–5</td>
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<tr>
<td>Diamino acid(s)*</td>
<td>Orn/Lys</td>
<td>Orn/Lys</td>
<td>Orn</td>
<td>DAB</td>
<td>DAB/Orn</td>
<td>DAB</td>
</tr>
<tr>
<td>Major menaquinone(s)</td>
<td>10, 11</td>
<td>10, 11</td>
<td>11, 12</td>
<td>9, 10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Major fatty acids†</td>
<td>(\omega)-C15:0, (\omega)-C16:0, (\omega)-C17:0</td>
<td>(\omega)-C15:0, (\omega)-C16:0, (\omega)-C17:0</td>
<td>(\omega)-C15:0, (\omega)-C16:0, (\omega)-C17:0, (\omega)-C18:0</td>
<td>(\omega)-C15:0, (\omega)-C16:0, (\omega)-C17:0, (\omega)-C18:0</td>
<td>(\omega)-C15:0, (\omega)-C16:0, (\omega)-C17:0, (\omega)-C18:0</td>
<td>(\omega)-C15:0, (\omega)-C16:0, (\omega)-C17:0, (\omega)-C18:0</td>
</tr>
<tr>
<td>G + C content (mol%)</td>
<td>63.5</td>
<td>61</td>
<td>62</td>
<td>64–68</td>
<td>67</td>
<td>66–73</td>
</tr>
</tbody>
</table>

*O, Orange; r, red; WH, white; y, yellow.
†DAB, Diaminobutyric acid; Orn, ornithine; Lys, lysine.
‡\(\omega\), Anteiso-branched; i, iso-branched.

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