Halorubrum pallidum sp. nov., an extremely halophilic archaeon isolated from a subterranean rock salt

Shaoxing Chen,1,2 Hong-Can Liu,3 Jian Zhou1 and Hua Xiang1

1State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China
2University Key Laboratory of Crop High Quality and High Effective Cultivation and Safety Control in Yunnan Province, Honghe University, Mengzi 66110, Yunnan, PR China
3China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

An extremely halophilic archaeon, strain PJ61T, was isolated from a subterranean rock salt of Yuanyongjing Salt Mine, Yunnan, China. Colonies were pale, smooth, convex, and round (1.0–2.0 mm in diameter) on nutrient agar plates. Cells of strain PJ61T were spherical or oval, stained Gram-negative, and were non-motile. Optimal growth was observed with 3.4 M NaCl and at 38 °C in aerobic conditions. Mg2+ was required for growth. Phylogenetic analysis based on 16S rRNA gene sequence similarities showed that strain PJ61T belonged to the genus Halorubrum and was closely related to Halorubrum laminariae R60T (98.3 % 16S rRNA gene sequence similarity), Halorubrum salinum GX71T (98.2 %) and other species of the genus Halorubrum (<98 %). Sequence similarities of ropB' gene and ef-2 gene between strain PJ61T and the species of the genus Halorubrum also showed that strain PJ61T was closely related to strain Halorubrum salinum GX71T (93.4 % for ropB' and 94.8 % for ef-2). The DNA–DNA relatedness between strains PJ61T and Halorubrum laminariae R60T was 33±0.5 %, while it was 37±0.4 % for Halorubrum salinum GX71T. The DNA G+C content of strain PJ61T was 65.1 mol%. The major polar lipids of strain PJ61T consisted of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl glucosyl diether. The phenotypic, chemotaxonomic and phylogenetic properties suggest that strain PJ61T represents a novel species of the genus Halorubrum, for which the name Halorubrum pallidum sp. nov. is proposed. The type strain is PJ61T (=CGMCC 1.15212T =JCM 30955T).

Hypersaline environments are distributed globally exhibiting a wide range of ecosystem types, including salt lakes, soda lakes, solar salterns, and ancient salt deposits (Oren, 2002). Extensive studies of hypersaline environments in various geographical locations have led to the isolation and characterization of the microbial diversity found in these environments (Ventosa et al., 2015). Based on the culture-dependent or culture-independent approaches, numerous 16S rRNA gene sequences of isolates or clones were assigned to the species of genus Halorubrum which was proposed by McGenity & Grant (1995) (Burns et al., 2004; Boutilier et al., 2011; Tazi et al., 2014).

Most of these studies were carried out on aquatic habitats, however, the halohaloclastic communities in subterranean rock salt remains mostly unknown. Two years ago, Xiao et al. (2013) uncovered the prokaryotic diversity in Yunnan salt mines (China) by using culture-independent approach of the 16S rRNA gene sequences. The halohaloclastic strain, PJ61T, with light-red color on nutrient agar plates, was isolated from a subterranean rock salt, Yuanyongjing Salt Mine (25° 16′ 49″ N 101° 54′ 02″ E), Yunnan province, China. Based on the polyphasic taxonomic standards for the description of new taxa in the order Halobacterales (Oren et al., 1997) and the new proposal for division of the class Halobacteria into three orders (Halobacterales,
Haloferales, and Natrualiales) (Gupta et al., 2015), strain PJ61T is considered to represent a novel species of the rapidly growing genus Halorubrum in family Haloferales.

The subterranean rock salt sample constituted of sandy soil and salt crystal was dissolved in sterilized 5% (w/v) NaCl solution for screening of haloorachael strains. The mixture was filtrated with filter paper (Whatman 3MM). The pH of the filtrate was determined to be neutral (pH 7.01). The soluble elements accounted for 17.8% (w/w) of the total weight of the sample (Fig. S1, available in the online Supplementary Material). The sample was rich in Ca2+ and Mg2+ (~95 g kg⁻¹), whereas a trace amount of SO2− was detected by precipitation method. Simultaneously, filtrates were spread onto solid AS-168 medium containing (g l⁻¹): NaCl 200, MgSO4.7H2O 20, yeast extract (Difco) 5, casamino acids (Difco) 5, sodium citrate 3, KCl 2, monosodium glutamate 1.8, FeSO4 0.036, and MnCl2.4H2O 0.0036 (Li et al., 2003) for the isolation of haloorachae. The petri dishes were sealed with paraffins, and cultured at 37°C in a plastic bag for two or three weeks. A light red colony (1–2 mm in diameter), strain PJ61T, was isolated for detailed identification (Fig. S2).

Pure cultures of strain PJ61T was obtained by repeated streaking on solid AS-168 plates. The 16S rRNA gene was amplified with the primer pair 18F (5′-ATTCCGGTT-GATCCGCC-3′) and 1518R (5′-AGGAGGTGATC-CAGCCGC-3′) (Cui et al., 2009). The PCR products were inserted into the pMD-18T vector (TaKaRa) for sequencing. The 16S rRNA gene sequence (1471 nt) was taken as the query to search the public database via the BLAST searching tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzBioCloud (Kim et al., 2012). The analysis of 16S rRNA gene sequence similarity showed that strain PJ61T was closely related to species of the genus Halorubrum, Halorubrum laminariae R60T (98.3%), Halorubrum salinarum GX71T (98.2%) and other species of the genus Halorubrum (16S rRNA gene sequence similarity <98%).

Cell morphology was observed using phase contrast microscopy (OLYMPUS BX51 equipped with OLYMPUS DP72) and scanning electron microscopy (HITACHI SU8010) (Fig. S2). Gram-staining was performed referring to the method described by Dussault (1955). Most experiments including the optimal growth factors of pH, NaCl concentration, Mg2+ requirement, growth temperature and others unless indicated, were performed in the AS-168 medium (Li et al., 2003). According to requirements, the components or pH of the medium were modified. The range of salinity was set from 0.85 M to 1.2 M with intervals of 0.1 M, while the range of pH was set from 5.0–9.0 with intervals of 0.5 units. The pH was adjusted using different types of buffers: MES for pH 5.0–6.0, PIPES for pH 6.5–7.0, HEPES for pH 7.5–8.0, Tricine for pH 8.5 and CHES for pH 9.0; all at 50 mM. The temperature range for growth was set to 4, 15, 20, 25, 30, 35, 38, 40, 42, 45, 50 and 55°C in a medium of pH 7.2 with 3.4 M NaCl. The range of Mg2+ concentration (0, 0.005, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5, 0.7, 1.0 M) for growth was tested using MgCl2.6H2O instead of MgSO4.7H2O to avoid the impacts of SO42−. Cells of strain PJ61T were non-motile, spherical or oval shaped (0.5–0.8×1.0–1.2 µm) and stained Gram-negative. Cell lysis occurs in distilled water. Colonies on solid medium after incubation at 38°C for 2 weeks are pale, smooth, convex, round and ~1.0 mm in diameter (Fig. S2). Strain PJ61T grew at temperatures in the range 25–45°C (optimally at 38°C), with 2.6–5.1 M NaCl (optimally at 3.4 M), and at pH 7.5–8.5 (optimally at pH 8.0). Mg2+ was required for growth (optimally at 0.1 M).

Phenotypic characterization was carried out in accordance with the recommended minimal standards for the description of novel taxa in the order Halobacterales (Oren et al., 1997). Anaerobic growth was tested in the presence of 5% (w/v) nitrate and 3% (w/v) L-arginine, and 5% (w/v) DMSO in filled, stoppered tubes. The tests for catalase and oxidase activities and the hydrolysis of starch, gelatin, casein and Tweens 20, 40, 60 and 80 were carried out as described by Gonzalez et al. (1978). H2S formation was detected using a filter-paper strip impregnated with lead acetate (Cui et al., 2007). Indole production from tryptophan and the utilization of sugars and organic acids were assessed as described by Oren et al. (1997). Reduction of nitrate and nitrite were detected by using the sulfanilic acid and α-naphthylamine reagent (Smibert & Krieg, 1994). To assess the utilization of sole carbon and energy sources, the minimal medium containing (g l⁻¹): NaCl 200, yeast extract (Difco) 0.1, NH4Cl 0.5, KH2PO4 0.05, MgSO4.7H2O 20, KCl 2, FeCl2.4H2O 0.036, and MnCl2.4H2O 0.00036, was supplemented with 1% (w/v) of the following substrates: acetate, L-alanine, L-arginine, L-aspartate, citrate, D-fructose, fumarate, D-galactose, D-glucose, L-glutamate, glycerol, glycine, DL-lactate, lactose, L-lysine, L-malate, maltose, D-mannitol, D-mannose, L-ornithine, pyruvate, D-ribose, D-sorbitol, L-sorbose, starch, succinate, sucrose and D-xylene (Oren et al., 1997). All phenotypic characteristics tested above of strain PJ61T are described in the species description, while the differential features between strain PJ61T and other closely related species are summarized in Table 1.

Antimicrobial compounds sensitivity tests were performed by spreading the culture suspension on solid AS-168 medium plates. Antibiotics tested included (µg per disc, unless otherwise indicated): ampicillin (10), bacitracin (0.04 IU), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), kanamycin (10), neomycin (30), norfloxacin (10), novobiocin (30), penicillin G (10 IU), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30). Strain PJ61T was sensitive to novobiocin and neomycin, but resistant to vancomycin, norfloxacin, ampicillin, bacitracin, penicillin G, ciprofloxacin, tetracycline, erythromycin, kanamycin, chloramphenicol, rifampin and streptomycin, which differs from closely related species of genus Halorubrum.

Polar lipids were extracted using the chloroform/methanol system and detected using one- and two-dimensional thin-
layer chromatography (TLC) on silica gel 60 F254 aluminium-backed thin-layer plates (Merck) (Minnikin et al., 1984; Kamekura, 1993). The first dimensional solvent was chloroform/methanol/water (65:25:4, by vol.), and the second dimensional solvent was chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Glycolipids were detected as dark purple spots by spraying with 0.5% (w/v) α-naphthol in methanol/water (1:1, v/v) and then with sulfuric acid/ethanol (1:1, v/v), followed by heating at 120°C for 10 min to detect the total polar lipids. The presence of phospholipids and glycolipids on the plates was confirmed by comparing with the polar lipid profile of Halorubrum saccharovorum CGMCC 1.2147T, the type species of genus Halorubrum. The major polar lipids of strain PJ61T were phosphatidylglycerol phosphate methyl ester (PG-PME), phosphatidylglycerol sulfate (PGS), phosphatidylglycerol (PG) and sulfated diglycosyl dietherglycolipids (S-DGD-3) (Fig. S3), which were similar to the closely related strains Hrr. salinum GX71T and Hrr. laminariae R60T.

Table 1. Differential characteristics between strain PJ61T and type strains of closely related species of the genus Halorubrum

<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>Shape</td>
<td>Spherical or oval</td>
<td>Rod</td>
<td>Pleomorphic</td>
<td>Rod</td>
<td>Pleomorphic</td>
<td>Rod</td>
<td>Rod</td>
<td>Rods and pleomorphic</td>
</tr>
<tr>
<td>Size (µm)</td>
<td>0.5–1.2</td>
<td>0.6–1.2</td>
<td>0.8–1.0×1.5</td>
<td>2–3×0.9</td>
<td>0.5–0.6×0.9</td>
<td>0.9–1.1</td>
<td>1.2×1.8</td>
<td>1.3×2.5</td>
</tr>
<tr>
<td>NaCl for growth (M)</td>
<td>Optimum</td>
<td>38</td>
<td>30</td>
<td>30</td>
<td>37</td>
<td>35–40</td>
<td>45–48</td>
<td>40</td>
</tr>
<tr>
<td>pH for growth</td>
<td>Range</td>
<td>7.5–8.5</td>
<td>6.0–9.5</td>
<td>5.5–9.5</td>
<td>6.0–9.0</td>
<td>7.0–9.0</td>
<td>7.0–9.0</td>
<td>7.0–9.0</td>
</tr>
<tr>
<td>Reduction of nitrate to nitrite</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+*</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tween 80 hydrolysis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>H2S formation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+*</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Antimicrobial susceptibility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacitracin (0.04 IU)</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin (15 µg)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Rifampin (5 µg)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>ND</td>
<td>S</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>65.1</td>
<td>63.0</td>
<td>67.0</td>
<td>69.4</td>
<td>64.6</td>
<td>65.9</td>
<td>71.2</td>
<td>64.2</td>
</tr>
</tbody>
</table>

*R Determined in this study.
Halorubrum sodomense ATCC 33755 \(^T\) (D13379)
Halorubrum californiense SF3-213 \(^T\) (EF139654)
Halorubrum chaoviator HALO-G \(^T\) (AM048786)
Halorubrum ezzemouliense 5.1 \(^T\) (DQ118426)
Halorubrum coriense Ch2 \(^T\) (L00922)
Halorubrum litoreum Fa-1 \(^T\) (EF028067)
Halorubrum distributum JCM 9100 \(^T\) (D63572)
Halorubrum xinjiangense BD-1 \(^T\) (AY510707)
Halorubrum trapanicum NRC 34021 \(^T\) (X82168)
Halorubrum ejinorense EJ-32 \(^T\) (AM491830)
Halorubrum tebenquichense JCM12290 \(^T\) (EF468473)
Halorubrum terrestre VKM B-1739 \(^T\) (AB090169)
Halorubrum arcis AJ201 \(^T\) (DQ355793)
Halorubrum pallidum PJ61 \(^T\) (KJ644242)
Halorubrum salinum GX71 \(^T\) (HM063951)
Halorubrum laminariae R60 \(^T\) (KF680551)
Halorubrum saccharovorum JCM 8865 \(^T\) (U17364)
Halorubrum persicum C49 \(^T\) (HG421000)
Halorubrum halophilum BB \(^T\) (EF077637)
Halorubrum lipolyticum 9-3 \(^T\) (DQ355814)
Halorubrum kocuri BG-1 \(^T\) (AM900832)
Halorubrum aidingense 31-hong \(^T\) (DQ355813)
Halorubrum yunnanense Q85 \(^T\) (KJ644187)
Halorubrum rutilum YJ-18-S1 \(^T\) (KC918819)
Halorubrum orientale EJ-52 \(^T\) (AM235786)
Halorubrum aquaticum EN-2 \(^T\) (AM268115)
Halorubrum rubrum YC87 \(^T\) (JQ237124)
Halorubrum cibi B31 \(^T\) (EF077639)
Halorubrum vacuolatum JCM 9060 \(^T\) (D87972)
Halorubrum alkalophilum DZ-1 \(^T\) (AY510708)
Halorubrum luteum CGSA15 \(^T\) (DQ987877)
Halorubrum gandharaense MK13 \(^T\) (AB563178)
Halopenitus persicus DC30 \(^T\) (JF979130)
Haloferax lucentense JCM 9276 \(^T\) (AB081732)

Fig. 1. Maximum-likelihood phylogenetic tree reconstructions based on 16S rRNA gene (a) and rpoB \(^T\) gene (b) sequences, showing the relationships between strain PJ61 \(^T\) and related members of the genus Halorubrum in the family Haloferacaceae. Halopenitus persicus DC30 \(^T\) and Haloferax lucentense JCM 9276 \(^T\) were taken as outgroups for the 16S rRNA gene tree, while Halobacterium jilantaiense JCM 13558 \(^T\) and Halobacterium salinarum JCM 8978 \(^T\) were used as outgroups for the rpoB \(^T\) gene tree. Bootstrap values (%) are based on 1000 replicates and are shown for branches with >50% bootstrap support. Bar, 0.01 expected substitutions per nucleotide position.
Primer pair HrpoB2 1420F (5'-TGTGGGCTNGTGAA-GAACTT-3') and HrpoA 153R (5'-GGGTCCATCAGCCC-CATGTC-3') were used to amplify the rpoB (RNA polymerase beta subunit) gene (Minegishi et al., 2010), while EF-2f (5'-ATGGGYMGACGHAAGAA-3') and EF-2r (5'-GCBGGRCCRCGGTGGAT-3') were used to amplify the ef-2 (elongation factor 2) gene (Han & Cui, 2015). The complete rpoB gene (1830 nt) and partial ef-2 gene (1802 nt) of strain PJ61T were sequenced via the same procedure as used for the 16S rRNA gene. Sequence similarity analysis of the rpoB gene showed that strain PJ61T was closely related to Hrr. salinum GX71T (93 %), Hrr. laminariae R60T (92 %) and Halorubrum aidingense JCM 13560T (91 %). In addition, the sequence similarity analysis based on the ef-2 gene showed that strain PJ61T was closely related to Hrr. salinum GX71T (94 %), Halorubrum aquaticum CGMCC 1.6377T (94 %), Halorubrum rubrum YC87T (93 %) and Hrr. laminariae R60T (93 %). The sequence similarity between strain PJ61T and other species of the genus Halorubrum is lower.
The related sequences including outgroups and species with validly published names were retrieved from the NCBI database. Multiple sequence alignments were performed using the Clustal W program implemented in the BioEdit software (Hall, 1999). Phylogenetic trees were reconstructed using maximum-likelihood and neighbour-joining algorithms in the MEGA 5 software (Tamura et al., 2011). The maximum-likelihood phylogenetic trees of the 16S rRNA gene and rpoB gene sequences revealed that strain PJ61T tightly clustered with Hrr. laminariae R60T and Hrr. salinum GX71T and formed an independent branch in the genus Halorubrum (Fig. 1). This independent lineage was also presented on the neighbour-joining phylogenetic trees of the 16S rRNA gene and rpoB gene (Fig. S4). The topology of the maximum-likelihood phylogenetic tree based on the 16S rRNA gene and rpoB gene sequences is similar, but slightly differs from that of the ef-2 gene phylogenetic tree (Fig. S5).

The DNA G+C content was determined using the thermal denaturation method (Marmur & Doty, 1962) with a Beckman-Coulter DU800 spectrophotometer. DNA–DNA hybridizations were performed on a Perkin-Elmer Lambda 35 spectrophotometer equipped with a high-performance temperature controller (PTP-6 Peltier system; PerkinElmer) according to the thermal denaturation and renaturation method (De Ley et al., 1970). The DNA G+C content of strain PJ61T was determined to be 65.1 mol%. Strain PJ61T showed low DNA–DNA relatedness with Hrr. salinum GX71T (37±0.4 %), and Hrr. laminariae R60T (33±0.5 %), far below the accepted threshold value (70 %) for separating two different species (Stackebrandt & Goebel, 1994).

In brief, the sequence similarity and phylogenetic analysis based on multiple gene sequences supported the placement of strain PJ61T in the genus Halorubrum. The differential characteristics listed in Table 1 can easily distinguish strain PJ61T from the other closely related species. Thus, based on this taxonomic study using a polyphasic approach, strain PJ61T is considered to represent a novel species of the genus Halorubrum, for which the name Halorubrum pallidum sp. nov. is proposed.

**Description of Halorubrum pallidum sp. nov.**

Halorubrum pallidum (pal’li.dum. L. neut. adj. pallidum pale).

Cells are non-motile, spherical or oval-shaped (0.5–0.8 × 1.0–1.2 μm) and stain Gram-negative. Colonies are achenomatos or pale, smooth, convex, round, and 1.0–2.0 mm in diameter when it cultured on the AS-158 agar plate at 37 °C. Cell lysis occurs in distilled water. Growth occurs in the presence of 2.6–5.1 M NaCl (optimum 3.4 M), at 25–45 °C (optimum 38 °C) and pH 7.5–8.5 (optimum pH 8.0). Mg2⁺ is required for growth (optimum 0.1 M). Oxidase- and catalase-positive. Anaerobic growth does not occur in the presence of nitrate, L-arginine or DMSO. H₂S is produced from cysteine, but indole is not produced from tryptophan. Nitrate and nitrite cannot be reduced, and no gas formation occurs. Starch, gelatin, casein and Tween 20, 40, 60 and 80 are not hydrolysed. The following substrates are utilized for growth as sole source of carbon and energy: D-glucose, D-mannose, D-galactose, D-fructose, D-xylene, maltose, glycerol, malate, L-glutamate, acetate, pyruvate, lactate, succinate and fumarate. The following compounds are not utilized as sole source of carbon and energy: D-sorbose, D-ribose, sucrose, lactose, starch, D-mannitol, D-sorbitol, citrate, glycine, L-alanine, L-arginine, L-aspartate, L-lysine, and L-ornithine. D-Glucose, D-mannose, D-galactose, D-fructose, D-sorbose and D-xylene can be utilized as sole carbon source, and acids were produced. The major polar lipids are phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated diglycosyl dietherglycolipids.

The type strain, PJ61T (=CGMCC 1.15212T =JCM 30955T), was isolated from a subterranean rock salt of Yunnanying Salt Mine, Yunnan, China. The DNA G+C content of the type strain is 65.1 mol% (Tm method).

**Acknowledgements**

We thank Professor Zhu L. Yang from the Kunming Institute of Botany, Chinese Academy of Sciences, for the help in sample collection and strain isolation, and thank Professor Heng-Lin Cui from Jiangsu University for providing some type strains. Dr Sumit Kumar from the Institute of Microbiology, Chinese Academy of Sciences is thanked for the comments to improve the manuscript. This work was supported by grants from the National Natural Science Foundation of China (31330001 and 31460003).

**References**


http://ijs.microbiologyresearch.org


