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Halorubrum xinjiangense sp. nov., a novel halophile isolated from saline lakes in China

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A novel halophilic archaeon, strain BD-1^T, was isolated from Xiao-Er-Kule Lake in Xinjiang, China. The taxonomy of strain BD-1^T was studied by polyphasic methods. According to 16S rRNA gene sequence similarity, strain BD-1^T was phylogenetically related to *Halorubrum trapanicum* (98·4 %), *Halorubrum sodomense* (98·0 %), *Halorubrum distributum* (97·8 %) and *Halorubrum coriense* (97·3 %). Strain BD-1^T is able to grow at 10 °C and further differs physiologically from the above species in the assimilation of sugars. The G + C content of DNA is 68·0 % (T_m). The DNA–DNA relatedness values to *Hrr. trapanicum* and *Hrr. distributum* are 47 and 24 %, respectively. It is concluded that strain BD-1^T represents a novel species of the genus *Halorubrum*, for which the name *Halorubrum xinjiangense* sp. nov. is proposed. The type strain is BD-1^T (=AS 1.3527^T=JCM 12388^T).

Members of the genus *Halorubrum* (McGenity & Grant, 1995) are widely distributed in hypersaline environments such as salt and/or soda lakes and solar salterns (Ochsenreiter *et al.*, 2002, as cited therein). Being fast-growing species and active in the conversion of sugars and amino acids, *Halorubrum* species are considered to play important roles in the carbon and nitrogen cycles of hypersaline environments. Currently, the genus *Halorubrum* contains nine species with validly published names: *Halorubrum sodomense* (Oren, 1983), *Halorubrum lacusprofundi* (Franzmann *et al.*, 1988), *Halorubrum saccharovorum* (Tomlinson & Hochstein, 1976), *Halorubrum trapanicum*, *Halorubrum coriense* (Kamekura & Dyall-Smith, 1995), *Halorubrum distributum*, *Halorubrum vacuolatum* (Kamekura *et al.*, 1997; Grant & Larsen, 1989), *Halorubrum tebenquichense* (Lizama *et al.*, 2002) and *Halorubrum tibetense* (Fan *et al.*, 2004).

Strain BD-1^T was isolated from sludge samples of Xiao-Er-Kule saline lake in Xinjiang, China, by enrichment and subsequent plating of the enriched cultures on agar medium containing the following ingredients (l^{-1}): Casamino acids, 1 g; yeast extract, 1 g; trisodium citrate, 3 g; NaCl, 200 g; KCl, 2 g; $MgSO_4.7H_2O$, 5 g; $MnSO_4.7H_2O$, 0·2 mg; $FeSO_4.7H_2O$, 0·05 g; glucose, 10 g (pH adjusted to 7·0 with 1 M NaOH solution). This strain grew over a temperature range of 10–54 °C (optimum 40 °C) as determined by using a temperature gradient incubator and over a pH range of 6·0–10·0 (optimum 7·0–7·5) as determined with various pH buffers. Routine cultivation was conducted at 40 °C and pH 7·5. The requirements for NaCl and magnesium for growth were determined in media with 0·9–5·2 M NaCl or 0–0·5 M $MgSO_4$.

Phenotypic tests were performed according to the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). Cell motility and morphology were examined by phase-contrast and transmission electron microscopy of exponentially growing liquid cultures. Gram staining was carried out as described by Dussault (1955). Colony morphology was observed on optimal growth agar medium after incubation at 40 °C for 4 days. Anaerobic growth was tested in the presence of 5 g potassium nitrate, L-arginine or DMSO l^{-1} in filled, stoppered tubes. Tests for catalase and oxidase activities and hydrolysis of starch and Tween 80 were performed as described previously (Gonzalez *et al.*, 1978). Nitrate reduction, H_2S formation, indole formation and the utilization of sugars, alcohols, amino acids and organic acids were examined as described by Oren *et al.* (1997). Phospholipids and glycolipids were separated on silica gel plates (10 × 10 cm) by TLC and were analysed according to Kamekura *et al.* (1997) and Xin *et al.* (2001).

DNA base composition was determined by thermal denaturation (T_m). The 16S rRNA gene sequence was amplified with archaeon-specific primers under conditions described previously (Zhang *et al.*, 2003). 16S rRNA gene sequence alignments were performed by using CLUSTAL W version 1.3b (Van de Peer & De Wachter, 1994). A phylogenetic tree (Fig. 1) was constructed by the neighbour-joining method with Kimura's two-parameter calculation model in TREECON W version 1.3b. DNA–DNA hybridizations of strain BD-1^T to the type strains of *Hrr. trapanicum* and *Hrr.*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BD-1^T is AY510707.

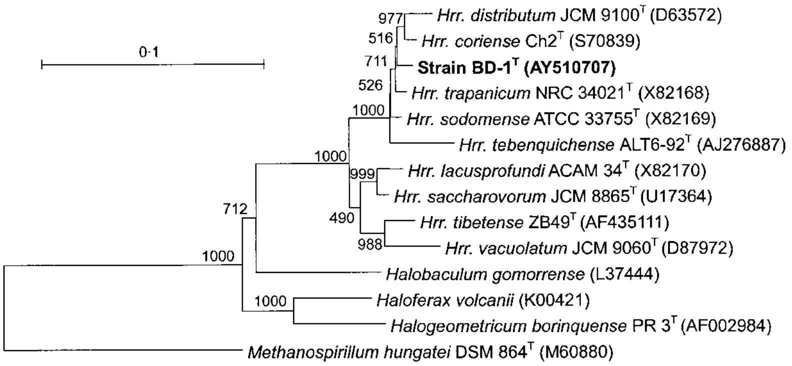

Fig. 1. Phylogenetic tree showing the position of strain BD-1^{T} among the species of genus *Halorubrum* and other genera of extremely halophilic archaea. The 16S rRNA gene sequence of *Methanospirillum hungatei* DSM 864^{T} was used as outgroup. Numbers at branch points indicate the level of bootstrap support, based on 1000 resamplings.

distributum were performed by thermal denaturation and the renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983).

Strain BD-1^{T} contained $C_{20}C_{20}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl-glucosyl glycerol diether. The lipid composition, physiological properties and characteristics of the DNA molecule indicated that strain BD-1^{T} was a member of the genus *Halorubrum*. 16S rRNA gene sequence similarity searches indicated that strain BD-1^{T} was phylogenetically related to *Hrr. trapanicum* (98·4 %), *Hrr. sodomense* (98·0 %), *Hrr. distributum* (97·8 %) and *Hrr. coriense* (97·3 %). However, they differed in phenotypic and physiological properties. Strain BD-1^{T} grew at 10 °C, while neither *Hrr. trapanicum* nor *Hrr. sodomense* grew at this temperature. Strain BD-1^{T} assimilated glucose, sucrose, fructose and maltose, but not lactose, galactose or glycerol. Detailed phenotypic and physiological properties of this strain are given in the species description, and differentiating characteristics of this strain BD-1^{T} from other *Halorubrum* species are listed in Table 1.

Members of the genus *Halorubrum* gathered into three main clusters based on 16S rRNA gene sequence similarities, with 100 % bootstrap support (Grant & Larsen, 1989). Cluster 1 contains *Hrr. saccharovorum* and *Hrr. lacusprofundi* (with similarity of 98·3 %). Cluster 2 contains *Hrr. sodomense*, *Hrr. trapanicum*, *Hrr. coriense* and *Hrr. distributum* (with similarity >97·7 %). Cluster 3 is phylogenetically distant

Table 1. Differentiation of *Halorubrum xinjiangense* sp. nov. from other *Halorubrum* species

Species: 1, *Hrr. xinjiangense* sp. nov.; 2, *Hrr. saccharovorum*; 3, *Hrr. coriense*; 4, *Hrr. distributum*; 5, *Hrr. lacusprofundi*; 6, *Hrr. sodomense*; 7, *Hrr. trapanicum*; 8, *Hrr. vacuolatum*; 9, *Hrr. tebenquichense*. ND, Not described; PGS, phosphatidylglycerol sulfate; S-DGD, sulfated mannosyl-glucosyl glycerol diether.

Property	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Motility	+	+	+	+	ND	+	−	−	−
Gas vacuole	−	−	−	−	−	−	−	+	−
Growth at 2 M NaCl	+	+	−	+	+	+	−	−	+
Mg^{2+} required	−	+	+	ND	+	+	ND	−	−
Growth at 10 °C	+	−	−	−	+	−	−	−	−
Growth at pH 10·5	−	−	−	−	−	−	−	+	−
Starch hydrolysis	−	−	ND	−	−	+	−	ND	−
Indole production	−	−	−	−	−	−	−	ND	−
Sugar assimilation:									
Glucose	+	+	+	−	+	+	+	+	+
Sucrose	+	+	+	−	ND	+	+	+	−
Lactose	−	+	+	ND	+	+	−	ND	−
Fructose	+	+	ND	−	−	+	+	−	+
Galactose	−	+	ND	−	+	−	+	+	+
Maltose	+	+	ND	−	−	+	+	−	−
Glycerol assimilation	−	+	+	+	−	+	+	−	+
Amino acids required	−	+	−	ND	+	+	+	+	−
Presence of PGS	+	+	+	+	+	+	+	−	−
Presence of S-DGD	+	+	+	+	+	+	+	−	+
G+C content (mol%)	68·0	71·2	ND	63·6	65·3	67·4	64·3	62·7	63·2

from the above two clusters and contains *Hrr. vacuolatum*. The recent discovery of *Hrr. tebenquichense* (Lizama *et al.*, 2002), *Hrr. tibetense* (Fan *et al.*, 2004) and strain BD-1^T (*Hrr. xinjiangense* sp. nov.) significantly reduced the bootstrap support (49 %) of clusters 1 and 3 (Fig. 1), while cluster 2 is still supported by 100 % bootstrap. Strain BD-1^T clearly belongs to cluster 2, and the closest relative was *Hrr. trapanicum* (98·4 %). The DNA–DNA relatedness of strain BD-1^T to *Hrr. trapanicum* and *Hrr. distributum* was 47 and 24 %, respectively.

Description of *Halorubrum xinjiangense* sp. nov.

Halorubrum xinjiangense (xin.ji.ang.en′se. N.L. neut. adj. *xinjiangense* pertaining to Xinjiang, where the strain was isolated).

Cells are short rods (0·8–1·2 × 1·8–2·6 μm) and Gram-negative. Colonies on agar plates containing 25 % (w/v) total salts are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at 2·0–5·2 M NaCl, pH 6–10 and 10–54 °C, with optimal growth at 3·1–3·4 M NaCl, pH 7·0–7·5 and 40 °C. Magnesium is not required for growth. Catalase and oxidase are positive. Anaerobic growth with nitrate, arginine and DMSO does not occur and nitrate reduction to nitrite or dinitrogen is not observed. H_2S is produced from cysteine, but indole formation is negative. Tween 80 and starch are not hydrolysed. Amino acids are not required for growth. Fructose, glucose, sucrose and maltose are utilized with production of acids. No growth or acid production is observed on mannose, lactose, raffinose, rhamnose, galactose, D-ribose, glycerol, mannitol or sorbitol. Organic acids are not utilized. Cells contain $C_{20}C_{20}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl-glucosyl glycerol diether. The G + C content of DNA is 68·0 % (T_m).

The type strain is BD-1^T (= AS 1.3527^T = JCM 12388^T), isolated from Xiao-Er-Kule Lake in Xinjiang, China.

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