Halobacterium jilantaiense sp. nov., a halophilic archaeon isolated from a saline lake in Inner Mongolia, China

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A novel halophilic archaeon, NG4^T, was isolated from Jilantai salt lake in Inner Mongolia, China. The taxonomy of strain NG4^T was studied by polyphasic methods. Strain NG4^T grew at pH 5.5–8.5 and at a temperature of 22–55 °C. It was chemo-organotrophic, aerobic and required concentrations of 2.7–5.2 M NaCl and 0.05–0.3 M Mg²⁺ for growth. Cells were Gram-negative, slender rods. Colonies on agar plates containing 25 % (w/v) total salts were red, elevated and round. According to 16S rRNA gene sequence similarity, strain NG4^T was phylogenetically related to *Halobacterium salinarum* DSM 3754^T (98.2 %) and *Halobacterium noricense* A1^T (97.3 %). The DNA G + C content was 64.2 mol%. DNA–DNA relatedness values with *Hbt. salinarum* DSM 3754^T and *Hbt. noricense* A1^T were 47 and 35 %, respectively. The polar lipids of strain NG4^T consisted of phosphatidylglycerol, methylated phosphatidylglycerol phosphate, phosphatidylglycerol sulfate, triglycosyl diether, sulfated triglycosyl diether and sulfated tetraglycosyl diether. It was concluded that strain NG4^T represents a novel species of the genus *Halobacterium*, for which the name *Halobacterium jilantaiense* sp. nov. is proposed. The type strain is NG4^T (=CGMCC 1.5337^T=JCM 13558^T).

Members of the genus *Halobacterium* (Grant, 2001) are obligately aerobic and heterotrophic and live in highly saline environments such as salt lakes and salterns. Several former members of the genus *Halobacterium* have been excluded from the genus, based on distant relationships as determined by phylogenetic analysis (McGenity & Grant, 1995). Currently, the genus *Halobacterium* contains two recognized species: *Halobacterium salinarum* (Ventosa & Oren, 1996; Grant, 2001) and *Halobacterium noricense* (Gruber *et al.*, 2004).

Strain NG4^T was isolated from the salt lake of Jilantai (105° 42' E 39° 45' N) in Inner Mongolia, China, by enrichment and subsequent plating on agar medium containing the following ingredients (g l⁻¹): Casamino acids, 7.5; yeast extract, 10.0; MgSO₄·7H₂O, 20.0; trisodium citrate, 3.0; KCl, 2.0; FeSO₄·7H₂O, 0.05; NaCl, 200; agar, 15 (pH adjusted to 7.4 with 1 M NaOH). The strain grew at a temperature range of 22–55 °C (optimum 40 °C) and at a pH range of 5.5–8.5 (optimum 7.0–7.5). Routine cultivation was conducted at 40 °C and pH 7.4. Strain NG4^T required

2.7–5.2 M NaCl (optimum 3.1–3.5 M) and 0.05–0.3 M MgSO₄ (optimum 0.1–0.2 M) for growth.

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 by growth on agar medium after incubation at 40 °C for 7 days. Anaerobic growth was tested in the presence of 5 g potassium nitrate l⁻¹, L-arginine and DMSO in filled, stoppered tubes. Tests for catalase and oxidase activity and hydrolysis of starch and of Tweens 20, 40, 60 and 80 were performed as described previously (Gonzalez *et al.*, 1978). Nitrate reduction, H₂S 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 by thin layer chromatography on silica gel plates (10 × 10 cm) and were analysed according to the method of Xin *et al.* (2001).

DNA base composition was determined by thermal denaturation (*T*_m) (Marmur & Doty, 1962). The 16S rRNA gene was amplified by PCR using the halophilic archaea-specific primers 5'-TTCCGGTTGATCTGCC-3'

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NG4^T is DQ256409.

A two-dimensional thin-layer chromatograph of polar lipids extracted from strain NG4^T is available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain NG4^T, other members of the genus *Halobacterium* and members of other selected genera of the family *Halobacteriaceae*. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 70% bootstrap support. Bar, 0.02 expected changes per site.

and 5'-AAGGAGGTGATCCAGCC-3'. The PCR product was sequenced using an ABI BigDye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI 3730; Applied Biosystems). DNA–DNA hybridization of strain NG4^T to the type strains of *Hbt. salinarum* and *Hbt. noricense* was performed using the method of De Ley *et al.* (1970), as modified by Huß *et al.* (1983). A phylogenetic tree (Fig. 1) was constructed by the neighbour-joining method with Kimura's two-parameter calculation model in MEGA version 3.1 (Kumar *et al.*, 2004).

Results from BLAST searches based on 16S rRNA gene sequence similarities indicated that strain NG4^T was related phylogenetically to *Hbt. salinarum* DSM 3754^T (98.2%) and *Hbt. noricense* A1^T (97.3%). Strain NG4^T contained C₂₀C₂₀ derivatives of phosphatidylglycerol (PG), methylated phosphatidylglycerol phosphate (PGP-Me),

phosphatidylglycerol sulfate (PGS), triglycosyl diether (S-TGD-1), sulfated triglycosyl diether (S-TeGD) and sulfated tetraglycosyl diether (TGD-1), similar to *Hbt. salinarum* (see Supplementary Fig. S1 available in IJSEM Online). The lipid composition, physiological properties (see species description below) and DNA characteristics indicated that NG4^T was a member of the genus *Halobacterium*. The DNA G + C content was 64.2 mol%. DNA–DNA relatedness values between strain NG4^T and *Hbt. salinarum* DSM 3754^T and *Hbt. noricense* A1^T were 47 and 35%, respectively. Moreover, strain NG4^T was distinguishable from *Hbt. salinarum* DSM 3754^T and *Hbt. noricense* A1^T in phenotypic and physiological properties (Table 1).

Based on these results, it was concluded that strain NG4^T represents a novel species of the genus *Halobacterium*, for which the name *Halobacterium jilantaiense* sp. nov is proposed.

Table 1. Differentiation of strain NG4^T from other members of the genus *Halobacterium*

Data for *Hbt. salinarum* and *Hbt. noricense* were obtained from Grant (2001) and Gruber *et al.* (2004), respectively. ND, No data available.

Characteristic	<i>Hbt. salinarum</i>	<i>Hbt. noricense</i>	NG4 ^T
Cell morphology	Small rods	Irregular rods	Slender rods
Pigmentation	Red	Light red	Red
Optimum [NaCl] for growth (M)	3.5–4.5	2.5–3.0	3.1–3.5
Optimum [MgCl ₂] for growth (M)	0.05–0.1	0.6–0.9	0.1–0.2
Temperature range for growth (°C)	20–55	28–50	22–55
Optimum growth temperature (°C)	50	45	40
pH range for growth	5.5–8.0	5.2–7.0	5.5–8.5
Oxidase	+	–	+
Urease	–	ND	+
Esterase (C ₄)	–	+	–
Nitrate reduced	+	–	–
Gelatin liquefaction	+	–	+
Anaerobic growth with DMSO	+	–	+
DNA G + C content (mol%)	67.1–71.2	54.5	64.2

Description of *Halobacterium jilantaiense* sp. nov.

Halobacterium jilantaiense (ji.lan.tai.en'se. N.L. neut. adj. *jilantaiense* pertaining to Jilantai salt lake, where the type strain was isolated).

Cells are slender rods ($0.5\text{--}1.0 \times 1.0\text{--}3.0\text{ }\mu\text{m}$) and Gram-negative. Colonies on agar plates containing 25% (w/v) total salts are red, elevated and round. Growth is chemo-organotrophic and aerobic; it occurs at $2.7\text{--}5.2\text{ M NaCl}$ and $0.05\text{--}0.3\text{ M Mg}^{2+}$ at pH $5.5\text{--}8.5$ and at $22\text{--}55\text{ }^{\circ}\text{C}$. Optimal NaCl concentration, Mg^{2+} concentration, pH and temperature for growth are $3.1\text{--}3.5\text{ M}$, $0.1\text{--}0.2\text{ M}$, pH $7.0\text{--}7.5$ and $40\text{ }^{\circ}\text{C}$, respectively. Catalase- and oxidase-positive. Grows anaerobically in the presence of L-arginine, nitrate and DMSO. Nitrate reduction to nitrite is observed and H_2S is produced from cysteine. Indole formation is positive. Tween 80 and starch are not hydrolysed. Casein and gelatin are hydrolysed. Amino acids are required for growth. Starch is not hydrolysed. Glucose, galactose, sucrose, maltose, fructose, sorbose, xylose, mannose, lactose, D-ribose, mannitol and sorbitol are not utilized in 7–10 days. Glycerol is utilized without acid production. Susceptible to anisomycin and novobiocin. Resistant to penicillin, ampicillin, rifampicin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, bacitracin and erythromycin. Cells contain $\text{C}_{20}\text{C}_{20}$ derivatives of PG, PGP-Me, PGS, S-TGD-1, S-TeGD and TGD-1. The DNA G+C content is 64.2 mol%.

The type strain, NG4^T (=CGMCC 1.5337^T=JCM 13558^T), was isolated from Jilantai salt lake in Inner Mongolia, China.

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References

- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Dussault, H. P. (1955). An improved technique for staining halophilic bacteria. *J Bacteriol* **70**, 484–485.
- Gonzalez, C., Gutierrez, C. & Ramirez, C. (1978). *Halobacterium vallismortis* sp. nov. An amylolytic and carbohydrate-metabolizing extremely halophilic bacterium. *Can J Microbiol* **24**, 710–715.
- Grant, W. D. (2001). Genus I. *Halobacterium* Elazari-Volcani 1957, 207^{AL} emend. Larsen and Grant 1989, 2222. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 301–305. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Gruber, C., Legat, A., Pfaffenhuemer, M., Radax, C., Weidler, G. W., Busse, H.-J. & Stan-Lotter, H. (2004). *Halobacterium noricense* sp. nov., an archaeal isolate from a bore core of an alpine Permian salt deposit, classification of *Halobacterium* sp. NRC-1 as a strain of *Hbt. salinarum* and emended description of *Hbt. salinarum*. *Extremophiles* **8**, 431–439.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- McGenity, T. J. & Grant, W. D. (1995). Transfer of *Halobacterium saccharovorum*, *Halobacterium sodomense*, *Halobacterium trapanicum* NRC 34021 and *Halobacterium lacusprofundi* to the genus *Halorubrum* gen. nov., as *Halorubrum saccharovorum* comb. nov., *Halorubrum sodomense* comb. nov., *Halorubrum trapanicum* comb. nov., and *Halorubrum lacusprofundi* comb. nov. *Syst Appl Microbiol* **18**, 237–243.
- Oren, A., Ventosa, A. & Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* **47**, 233–238.
- Ventosa, A. & Oren, A. (1996). *Halobacterium salinarum* nom. corrig., a name to replace *Halobacterium salinarium* (Elazari-Volcani) and to include *Halobacterium halobium* and *Halobacterium cutirubrum*. *Int J Syst Bacteriol* **46**, 347.
- Xin, H., Itoh, T., Zhou, P., Suzuki, K. & Nakase, T. (2001). *Natronobacterium nitratireducens* sp. nov., a haloalkaliphilic archaeon isolated from a soda lake in China. *Int J Syst Evol Microbiol* **51**, 1825–1829.