Halomonas xinjiangensis sp. nov., a halotolerant bacterium isolated from a salt lake

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A novel bacterium, TRM 0175^T, belonging to the genus *Halomonas*, was isolated from a soil sample taken from a salt lake in Xinjiang Province, north-west China. The isolate was Gram-negative, aerobic, rod-shaped and motile by means of peritrichous flagella. It was catalase-positive and oxidase-negative. Growth occurred at NaCl concentrations of 0–20% (optimum at 10–13%), at 15–50 °C (optimum at 37 °C) and at pH 6.0–9.0 (optimum at pH 7.0). Metabolism was respiratory with oxygen as terminal electron acceptor. Acid was produced from D-ribose, D- and L-arabinose, D-xylose, D-galactose, D-mannose, L-rhamnose, cellobiose, maltose, trehalose and D- and L-fucose and was produced weakly from aesculin. The predominant ubiquinone was Q-9. The major fatty acids were C_{18:1}ω7c and C_{19:0} cyclo ω8c. The G+C content of the genomic DNA was 60.0 mol%. The affiliation of strain TRM 0175^T with the genus *Halomonas* was confirmed by 16S rRNA gene sequence comparisons. The most closely related species was *Halomonas anticariensis*; 16S rRNA gene sequence similarity between *H. anticariensis* FP35^T and strain TRM 0175^T was 95.3%. Phenotypically, some characteristics of TRM 0175^T differed from those of *H. anticariensis*. On the basis of data from this polyphasic study, strain TRM 0175^T represents a novel species of the genus *Halomonas*, for which the name *Halomonas xinjiangensis* sp. nov. is proposed; the type strain is TRM 0175^T (=CCTCC AB 208329^T =KCTC 22608^T).

The family *Halomonadaceae* currently includes four genera of halophilic bacteria, *Halomonas*, *Chromohalobacter*, *Cobetia* and *Modicisolibacter*, and three genera of non-halophilic bacteria, *Carnimonas*, *Halotalea* and *Zymobacter* (Franzmann *et al.*, 1988; Ventosa *et al.*, 1989; Okamoto *et al.*, 1993; Dobson & Franzmann, 1996; Garriga *et al.*, 1998; Arahal *et al.*, 2002, 2007; Ntougias *et al.*, 2007; Ben Ali Gam *et al.*, 2007). The genus *Halomonas* was proposed by Vreeland *et al.* (1980), with *Halomonas elongata* as the type species. At the time of writing, the genus *Halomonas* contained the greatest number of species (54 species with validly published names; <http://www.bacterio.cict.fr/h/halomonas.html>) of all genera in the family *Halomonadaceae*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TRM 0175^T is EU822512.

Phylogenetic trees based on maximum-parsimony and maximum-likelihood analysis of the 16S rRNA gene sequences and a transmission electron micrograph of cells of strain TRM 0175^T are available as supplementary material with the online version of this paper.

Strain TRM 0175^T, which belongs to the genus *Halomonas*, was isolated from a soil sample from Lop Nur salt lake (40° 23' N 90° 18' E; 778 m altitude) in Xinjiang Province, north-west China. The high salinity (30.5%), low nutrient levels, dry climate and high UV intensity make it an extreme environment. A soil sample (approx. 100 g) was collected from Lop Nur salt lake in autumn. The temperature and pH of the soil sample, measured with portable meters, were 16.2 °C and pH 7.4, respectively. The sample was isolated on trypticase soy agar (TSA) containing 15% (w/v) NaCl by using a tenfold dilution series method. The organism was grown and maintained at 37 °C on TSA containing 10% (w/v) NaCl.

Cell morphology and motility were examined by optical microscopy (Olympus BX40) and transmission electron microscopy (Philips CM200). For NaCl tolerance experiments, growth was studied at 37 °C in TSA containing 0, 0.5, 1, 3, 5, 8, 10, 13, 15, 18, 20, 25 and 30% (w/v) NaCl. Growth was investigated on TSA containing 10% (w/v) NaCl at 5–55 °C (at intervals of 5 °C) and at pH 4–12 (at

1 pH unit intervals) 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_2PO_4 /0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO_3 /0.1 M Na_2CO_3 . Biochemical tests (on TSA containing 10% NaCl) and nutritional tests [in medium containing (1^{-1} distilled water): 100 g NaCl, 2 g KCl, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g KNO_3 , 1 g $(\text{NH}_4)_2\text{HPO}_4$ and 0.5 g KH_2PO_4] were carried out according to Mata *et al.* (2002). In addition, antimicrobial susceptibility tests were performed on TSA using antimicrobial compounds (BBL) as outlined by Mata *et al.* (2002). Detailed results are given in the species description.

Genomic DNA of strain TRM 0175^T was extracted from cells grown on TSA containing 10% NaCl for 3 days at 37 °C and used as a template for subsequent PCR amplification. Reaction conditions and amplification protocols used were as described by Hezayen *et al.* (2002). The almost-complete 16S rRNA gene sequence of strain TRM 0175^T (1420 nt) was obtained and compared with available 16S rRNA gene sequences in GenBank using BLAST searches (Altschul *et al.*, 1990). The 16S rRNA gene sequence of strain TRM 0175^T was aligned with those of available strains of *Halomonas* species obtained from GenBank. Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA3 program package (Kumar *et al.*, 2004) and by the maximum-likelihood method (Felsenstein, 1981) with the PHYLIP 3.6 program. Phylogenetic tree topologies were evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Results indicated that strain TRM 0175^T clustered within the genus *Halomonas* and exhibited the closest phylogenetic affinity (Fig. 1) and highest sequence similarity to *Halomonas anticariensis* FP35^T (95.3%). 16S rRNA gene sequence similarity values between strain TRM 0175^T and strains of other *Halomonas* species were below 95%. Phylogenetic trees based on maximum-parsimony and maximum-likelihood analysis of 16S rRNA gene sequences are shown in Supplementary Fig. S1 (available in IJSEM Online). The DNA G + C content of strain TRM 0175^T was determined by using the HPLC method (Mesbah *et al.*, 1989). The DNA G + C content of strain TRM 0175^T (60.0 mol%) was distinct from that of *H. anticariensis* FP35^T, the most closely related type strain (61.4 mol%; Martínez-Cánovas *et al.*, 2004).

Menaquinones were extracted using the method of Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI). The major ubiquinone of strain TRM 0175^T was Q-9 (93%); a small amount of Q-8 (7%) was also present. The major fatty acids (greater than 1%) of strain TRM 0175^T were $\text{C}_{18:1}\omega 7\text{c}$ (39.6%), $\text{C}_{19:0}$ cyclo $\omega 8\text{c}$ (24.0%), $\text{C}_{16:0}$ (12.3%), $\text{C}_{16:1}\omega 7\text{c}/\text{C}_{16:1}\omega 6\text{c}$ (7.4%), $\text{C}_{12:0}$ (4.8%), $\text{C}_{17:0}$ cyclo (3.2%), $\text{C}_{12:0}$ 3-OH (2.9%), $\text{C}_{18:0}$ (2.1%), $\text{C}_{10:0}$ (2.0%) and $\text{C}_{17:0}$ (1.8%). Differential characteristics and fatty acid compositions of

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of the novel isolate and related taxa. Bootstrap values are based on 1000 replicates; only values >50% are shown. Bar, 0.02 substitutions per nucleotide position.

strain TRM 0175^T and *H. anticariensis* FP35^T are shown in Tables 1 and 2.

Combined morphological, phenotypic and genotypic data indicate that the isolate represents a novel species in the genus *Halomonas*, for which the name *Halomonas xinjiangensis* sp. nov. is proposed.

Description of *Halomonas xinjiangensis* sp. nov.

Halomonas xinjiangensis (xin.ji.ang.en'sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, a region of China, from where the type strain was isolated).

Cells are Gram-negative, aerobic, motile by means of peritrichous flagella and rod-shaped, approximately $1.2\text{--}2.0 \times 0.6\text{--}1.0$ μm in size (Supplementary Fig. S2). Colonies on TSA medium are 1–2 mm in diameter, smooth, circular and brown–orange after 5 days. Growth occurs at 15–50 °C and pH 6.0–9.0, with optimum growth at 37 °C and pH 7.0. Grows in 0–20% (w/v) NaCl, with optimum growth at 10–13% (w/v) NaCl. Metabolism is respiratory with oxygen as terminal electron acceptor. Does not grow anaerobically in the presence of nitrate, nitrite or fumarate. Oxidase-negative and catalase-positive. Phosphatase is produced, but not exopolysaccharide or poly- β -hydroxyalkanoate. Nitrate is reduced to nitrite. Selenite is reduced. Aesculin and Tweens 20 and 80 are hydrolysed, but casein, DNA, gelatin, starch and urea are not. H_2S is formed from L-cysteine. Positive for gluconate oxidation. Negative for lysine decarboxylase, ornithine decarboxylase, phenylalanine deamination, indole production, the methyl

Table 1. Differential characteristics of strain TRM 0175^T and its closest relative *H. anticariensis* FP35^T

Data for *H. anticariensis* FP35^T were taken from Martínez-Cánovas *et al.* (2004).

Characteristic	TRM 0175 ^T	<i>H. anticariensis</i> FP35 ^T
Cell size (µm)	1.20–2.00 × 0.60–1.00	3.00–3.50 × 0.75–1.00
Exopolysaccharide production	–	+
Oxidase	–	+
Nitrate reduction	+	–
H ₂ S production	+	–
Temperature range (°C)	15–50	20–45
NaCl range (% w/v)	0–20	0.5–15
NaCl optimum (% w/v)	10–13	7.5
Hydrolysis of:		
Aesculin	+	–
Tween 80	+	–
Urea	–	+
Sole source of carbon and energy		
L-Arabinose	–	+
<i>myo</i> -Inositol	–	+
D-Mannitol	–	+
D-Galactose	+	–
Sole source of carbon, nitrogen and energy		
L-Alanine	–	+
L-Histidine	–	+
Acid production from:		
L-Arabinose	+	–
D-Ribose	+	–
D-Xylose	+	–
D-Galactose	+	–
L-Rhamnose	+	–
Cellobiose	+	–
Maltose	+	–
Trehalose	+	–
D-Fucose	+	–
Susceptible to:		
Tobramycin	–	+
Trimethoprim/sulfamethoxazole	–	+
Lipoquinone(s)	Q-9, Q-8*	Q-9
DNA G + C content (mol%)	60.0	61.4

*93 % Q-9 and 7 % Q-8.

red and Voges–Proskauer tests, *o*-nitrophenyl β-D-galactopyranosidase and fermentation of glucose. Utilizes L-rhamnose, D-mannose, D-fructose, D-galactose, trehalose, aesculin, xanthine, L-asparagine, L-serine and hypoxanthine as sole sources of carbon, nitrogen (where appropriate) and energy, but not D-mannitol, maltose, D-sorbitol, cellobiose, D-ribose, D-xylose, *myo*-inositol, lactose, raffinose, D-salicin, arabinose, xylitol, dulcitol, L-alanine, L-histidine, L-lysine or L-arginine. Acid is produced from D-ribose, D-

Table 2. Fatty acid contents of strain TRM 0175^T and *H. anticariensis* FP35^T

Data for *H. anticariensis* FP35^T were taken from Martínez-Cánovas *et al.* (2004). Values are percentages of total fatty acids; fatty acids that account for less than 1.0 % total fatty acids in both strains are not shown. NA, Not applicable/not reported.

Fatty acid	TRM 0175 ^T	<i>H. anticariensis</i> FP35 ^T
10:0	2.0	2.7
12:0	4.8	3.4
12:0 3-OH	2.9	5.2
16:0	12.3	25.5
16:1ω7c/16:1ω6c	7.4	NA
16:1ω7c/15:0 iso 2-OH	NA	12.5
17:0 cyclo	3.2	NA
17:0	1.8	NA
18:0	2.1	NA
18:1ω7c	39.6	47.6
19:0 cyclo ω8c	24.0	1.1

and L-arabinose, D-xylose, D-galactose, D-mannose, L-rhamnose, cellobiose, maltose, trehalose, D- and L-fucose. Acid is produced weakly from aesculin, but not from erythritol, D-adonitol, glycerol, L-xylose, D-fructose, L-sorbose, methyl β-D-xyloside, dulcitol, *myo*-inositol, D-mannitol, D-glucose, D-sorbitol, D-lactose, arbutin, salicin, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, xylitol, turanose, D-lyxose, D-tagatose or D- or L-arabitol. Susceptible to streptomycin (10 µg), amoxicillin (25 µg), chloramphenicol (30 µg), cefoxitin (30 µg), kanamycin (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), ampicillin (10 µg), sulfamide (250 µg), cefotaxime (30 µg), erythromycin (15 µg), polymyxin B (300 IU), rifampicin (30 µg) and carbenicillin (100 µg), but not to tobramycin (10 µg) or trimethoprim/sulfamethoxazole (1.25/23.75 µg). The predominant ubiquinone is Q-9. The major fatty acids (greater than 5 %) are C_{18:1}ω7c, C_{19:0} cyclo ω8c, C_{16:0} and C_{16:1}ω7c/C_{16:1}ω6c.

The type strain is TRM 0175^T (=CCTCC AB 208329^T =KCTC 22608^T), isolated from a hypersaline soil sample from Xinjiang Province, north-west China. The DNA G + C content of the type strain is 60.0 mol%.

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