Halomonas salifodinae sp. nov., a halophilic bacterium isolated from a salt mine in China

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A Gram-negative, aerobic, motile, halophilic bacterium, designated strain BC7^T, was isolated from a salt mine in north-western China and was subjected to a polyphasic taxonomic characterization. The isolate was able to grow in the presence of 0.5–20 % (w/v) NaCl and at pH 6.0–9.0 and 4–48 °C; optimum growth was observed with 3 % (w/v) NaCl and at pH 7.0 and 30 °C. Cells were long rods, 0.8–1.2 μm wide and 4.0–6.0 μm long. The major fatty acids were $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:0}\omega7c$. The DNA G+C content was 65.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BC7^T belonged to the genus *Halomonas* and showed highest sequence similarity to the type strain of *Halomonas pacifica* (99.2 %). Levels of DNA–DNA relatedness between strain BC7^T and *H. pacifica* CGMCC 1.2314^T and *Halomonas taeanensis* DSM 16463^T were 51 and 38 %, respectively. On the basis of phenotypic and genotypic data, strain BC7^T is considered to represent a novel species of the genus *Halomonas*, for which the name *Halomonas salifodinae* sp. nov. is proposed. The type strain is BC7^T (=CGMCC 1.6774^T =JCM 14803^T).

At the time of writing, the genus *Halomonas*, belonging to the family *Halomonadaceae* within the class *Gammaproteobacteria*, comprises 49 species of Gram-negative, rod-shaped, halophilic bacteria (Vreeland *et al.*, 1980; Franzmann *et al.*, 1988; Euzéby, 2008). Members of the genus *Halomonas* have been isolated from various natural habitats, such as salt lakes, solar salterns, saline sand or soil, subterranean saline wells, mineral pools, marine (including deep-sea) environments, animals and seafood, as well as artificial sewage installations and mural paintings (Ventosa *et al.*, 1998; Arahal *et al.*, 2002, 2007; Arahal & Ventosa 2006; Xu *et al.*, 2007). Here we present the results of a polyphasic study describing a novel *Halomonas* strain isolated from a salt mine in north-western China.

Strain BC7^T was isolated from a saline soil sample collected from a salt mine (41° 35′ 24″ N 81° 40′ 48″ E) at Baicheng, Xinjiang Province, China. A soil sample of approximately 100 mg was incubated for 24 h in halophilic medium (HM) containing 10 % NaCl (w/v). HM medium contained (per litre distilled water): NaCl as indicated, 2.0 g KCl, 1.0 g $MgSO_4$, 0.36 g $CaCl_2.2H_2O$, 0.23 g NaBr, 0.06 g $NaHCO_3$, trace $FeCl_3$, 10.0 g yeast extract (Difco), 5.0 g peptone (Difco) and 1.0 g glucose (pH 7.5) (Ventosa *et al.*, 1982). The liquid was plated on HM agar plates with 4 % NaCl (w/v), by using a tenfold dilution series method. After 3 days incubation at 30 °C, a number of brown–yellow colonies had developed, and one representative colony was picked and purified by repeated restreaking.

Phenotypic tests were performed according to the proposed minimal standards for describing new taxa in the family *Halomonadaceae* (Arahal *et al.*, 2007). Unless stated otherwise, the growth media contained 4 % NaCl (w/v). Cell morphology and motility were examined by scanning electron (Cambridge S260), transmission electron (Philips TECNAI 10) and optical (Olympus BX40) microscopy with cells from exponentially growing cultures. The optimal conditions for growth were determined in HM medium with different salt concentrations (0, 0.5, 1, 3, 5, 7.5, 10, 15, 20, 25 and 30 %, w/v). The pH range for growth was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BC7^T is EF527873.

Micrographs of cells of strain BC7^T, an extended neighbour-joining phylogenetic tree based on 16S rRNA gene sequences and detailed fatty acid compositions of strain BC7^T and *H. pacifica* CGMCC 1.2314^T are available as supplementary material with the online version of this paper.

determined in HM medium by adding MES (pH 5.0–6.0, 25 mM), PIPES (pH 6.5–7.0, 25 mM), Tricine (pH 7.5–9.0, 25 mM) and Na_2CO_3/$NaHCO_3$ (pH 9.5–10.0). The temperature range for growth was determined by incubating at 4, 15, 20, 25, 30, 37, 45 and 48 °C. Biochemical and nutritional tests were performed in HM medium according to Xu *et al.* (2007) as described previously (Mata *et al.*, 2002). Antimicrobial susceptibility tests were performed in liquid HM medium containing antimicrobial agents at 50 μg ml^{-1} (Xu *et al.*, 2007).

Fatty acid methyl esters were prepared from lipids extracted from cells grown on HM plates for 24 h at 30 °C and analysed by using GC/MS (Kuykendall *et al.*, 1988).

Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with methyl chloride/methanol (2 : 1) and analysed by reversed-phase HPLC. The genomic DNA G+C content was determined by thermal denaturation (T_m) (Marmur & Doty, 1962) with *Escherichia coli* K-12 DNA as calibration standard.

The 16S rRNA gene of strain BC7^T was amplified and analysed as described by Xu *et al.* (2007). The sequence was compared with those of closely related reference organisms from the EzTaxon service (Chun *et al.*, 2007). Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed according to the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods within the MEGA 3 program package (Kumar *et al.*, 2004). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method.

The morphological, physiological and biochemical characteristics of strain BC7^T are given in the species description below or are detailed in Table 1. Cells of strain BC7^T were long rods, 0.8–1.2 μm wide and 4.0–6.0 μm long (see Supplementary Fig. S1, available in IJSEM Online). Cells were motile by means of a single flagellum. Strain BC7^T was oxidase- and catalase-positive. It was able to hydrolyse tyrosine, starch was degraded only weakly and hydrolysis of aesculin, casein, DNA, gelatin and Tweens 20 and 80 was not observed. The major fatty acids of strain BC7^T were $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:0}\omega7c$. These fatty acids were also predominant components of *Halomonas pacifica* CGMCC 1.2314^T (Supplementary Table S1). However, $C_{18:0}$ was detected in strain BC7^T but not in *H. pacifica* CGMCC 1.2314^T.

The almost-complete 16S rRNA gene sequence (1428 nt) of strain BC7^T was obtained. Strain BC7^T showed highest 16S rRNA gene sequence similarity to the type strains of *H. pacifica* (99.2 %), *Halomonas ventosae* (97.6 %), *Halomonas salina* (97.4 %) and *Halomonas denitrificans* (97.1 %), and less than 97.0 % sequence similarity with respect to the type strains of other recognized *Halomonas* species. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain BC7^T formed a coherent cluster with *H. pacifica* with a high bootstrap resampling value (97 % by the neighbour-joining method) (Fig. 1). A similar topology was found in an extended phylogenetic tree with 16S rRNA gene sequences of all recognized species of the genus *Halomonas* (Supplementary Fig. S2).

DNA–DNA hybridization experiments were performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983), by using a Beckman DU 800 spectrophotometer. The hybridization temperature used was 77 °C, and the experiments were carried out in triplicate. Levels of DNA–DNA relatedness between strain BC7^T and *H. pacifica* CGMCC 1.2314^T and *Halomonas taeanensis* DSM 16463^T (96.6 % 16S rRNA gene sequence similarity) were 50.8 ± 3.1 % (mean ± SD) and 37.9 ± 6.9 %, respectively. Strain BC7^T could be differentiated from recognized *Halomonas* species based on several phenotypic properties (Table 1) such as colony pigmentation, cell morphology and size, salt or temperature range for growth, formation of H_2S, reduction of nitrate, hydrolysis of various substrates, utilization of hydrocarbons, production of acids from sugars and DNA G+C content.

Therefore, based on 16S rRNA gene sequence analysis, DNA–DNA hybridization data and differential phenotypic properties, we suggest that strain BC7^T represents a novel species of the genus *Halomonas*, for which the name *Halomonas salifodinae* sp. nov. is proposed.

Description of *Halomonas salifodinae* sp. nov.

Halomonas salifodinae (sa.li.fo.di′nae. L. n. *sal*, *salis* salt; L. n. *fodina* mine; N.L. gen. n. *salifodinae* of a salt mine).

Cells are Gram-negative, motile, long rods, 0.8–1.2 × 4.0–6.0 μm. Colonies on complex agar medium are 1–2 mm in diameter, smooth, circular, elevated and brown–yellow after 2 days growth. No growth in the absence of salt. Grows in the presence of total salt concentrations of 0.5–20 % (w/v), with optimum growth at 3 % (w/v). Grows at pH 6.0–9.0 and 4–48 °C (optimum growth at pH 7.0 and 30 °C). Does not grow anaerobically in the presence of nitrate, nitrite or fumarate. Oxidase- and catalase-positive. Grows on MacConkey agar but not on cetrimide agar. Does not produce exopolysaccharide or poly-β-hydroxyalkanoate. Starch and tyrosine are hydrolysed, but aesculin, casein, DNA, gelatin and Tweens 20 and 80 are not. Positive for methyl red test, oxidation of gluconate, reduction of selenite and urease. Negative for indole production, lecithinase, lysine decarboxylase, *o*-nitrophenyl β-D-galactopyranosidase, ornithine decarboxylase, phenylalanine deaminase and Voges–Proskauer test. H_2S is formed from L-cysteine. No nitrate or nitrite reduction. The following substrates are utilized for growth: acetate, adonitol, L-alanine, L-arginine, L-aspartate, citrate, D-fructose, fumarate, gluconate, glucose, L-glutamate, glycerol, glycine, L-histidine, *myo*-inositol, isoleucine, lactate, malate, maltose, mannitol, L-ornithine, propionate, pyr-

Table 1. Differential characteristics between strain BC7^T and related species of the genus *Halomonas*

Taxa: 1, strain BC7^T; 2, *H. pacifica* CGMCC 1.2314^T; 3, *H. taeanensis* DSM 16463^T (phenotypic tests for *H. pacifica* CGMCC 1.2314^T and *H. taeanensis* DSM 16463^T were performed in our laboratory in parallel with the tests for strain BC7^T); 4, *H. gomseomensis* (data from Kim *et al.*, 2007); 5, *H. denitrificans* (Kim *et al.*, 2007); 6, *H. saccharevitans* (Xu *et al.*, 2007); 7, *H. koreensis* (Lim *et al.*, 2004; Xu *et al.*, 2007); 8, *H. elongata* (Mata *et al.*, 2002); 9, *H. meridiana* (James *et al.*, 1990; Mata *et al.*, 2002). +, Positive; −, negative; ND, no data available. All taxa are Gram-negative, motile and oxidase-positive.

Characteristic	1	2	3	4	5	6	7	8	9
Colony pigmentation	Brown–yellow	Cream	Cream	Cream–beige	Brown–yellow	Cream	Cream	White	White
Morphology	Long rods	Rods[a*]	Rods[b]	Rods	Short rods	Rods	Short rods	Rods	Long rods
Cell size (μm)	0.8–1.2 × 4.0–6.0	ND	0.6–1.0 × 2.0–3.0[b]	0.6–0.8 × 1.8–2.4	0.6–0.8 × 1.2–1.6	0.5–1.0 × 3.0–5.0	0.8–1.0 × 1.8–2.2	ND	0.8–1.2 × 2.0–4.0
NaCl concentration for growth (%, w/v)									
Range	0.5–20	0–20[a]	1–25[b]	1–20	2–20	0–15	1–20	0–20	0.5–20
Optimum	3	0.5–3[a]	10–12[b]	8–12	8–10	1.0–5	1–12	3–8	7.5–10
Growth temperature range (°C)	4–48	4–45[a]	10–45[b]	5–45	5–50	4–48	10–47	4–45	15–37
Growth pH range	6–9	5–10[a]	7–10[b]	6–10	7–10	6–10	5.5–10	5–10	5–10
Oxidase	+	+	+	−	+	−	+	−	+
H_2S formation	+	−	+	−	−	ND	ND	ND	−
Nitrate reduction	−	−	+	−	+	ND	+	ND	−
Hydrolysis of:									
Aesculin	−	−	−	+	−	−	−	−	−
Casein	−	−	−	−	−	+	−	−	−
DNA	−	+	−	+	−	−	ND	−	−
Starch	+	−	−	−	−	−	−	−	+
Tween 20	−	−	+	ND	ND	+	ND	−	+
Tween 80	−	−	−	−	−	−	−	−	+
Tyrosine	+	−	+	ND	ND	−	+	−	−
Urea	+	+	+	−	−	+	+	ND	+
Acid production from:									
Adonitol	+	−	−	−	−	ND	−	−	+
Arabinose	−	−	+	+	−	+	−	+	+
D-Fructose	+	−	+	+	+	+	−	−	+
Glucose	+	+	+	+	−	+	+	+	+
myo-Inositol	+	−	+	+	−	+	ND	−	+
Lactose	−	−	+	−	−	ND	−	+	+
Maltose	+	−	−	+	−	+	−	+	+
Mannitol	+	+	+	−	−	+	−	+	+
Sorbitol	+	+	+	−	−	+	ND	+	+
Sucrose	+	−	+	+	−	+	−	+	+
Trehalose	+	−[a]	+	+	−	+	−	+	+
DNA G+C content (mol%)	65.5	67–68[c]	65[b]	62.0–63.6	53.8–55.2	57.6	70	60.5[c]	58.2–59.9

*Data from: *a*, Mata *et al.* (2002); *b*, Lee *et al.* (2005); *c*, Arahal *et al.* (2002).

uvate, ribose, L-serine, sorbitol, succinate, sucrose, trehalose and L-valine. The following compounds are not utilized as sole carbon and energy sources: L-arabinose, cellobiose, ethanol, formate, D-galactose, lactose, malonate, D-mannose, raffinose, rhamnose, sorbose and xylose. Acid is produced from adonitol, D-fructose, glucose, *myo*-inositol, maltose, mannitol, sorbitol, sucrose and trehalose, but not from L-arabinose, D-galactose, lactose, D-mannose, rhamnose or sorbose. Susceptible to ampicillin, carbenicillin, cefotaxime, chloramphenicol, nalidixic acid, neomycin, nitrofurantoin, penicillin, polymyxin B, rifampicin and streptomycin, but not to bacitracin, erythromycin, kanamycin, novobiocin, nystatin or tetracycline. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:0}\omega7c$. The predominant isoprenoid quinone is ubiquinone-9. The DNA G+C content of the type strain is 65.5±0.2 mol% (T_m).

The type strain, BC7^T (=CGMCC 1.6774^T =JCM 14803^T), was isolated from a salt mine at Baicheng in Xinjiang Province, China.

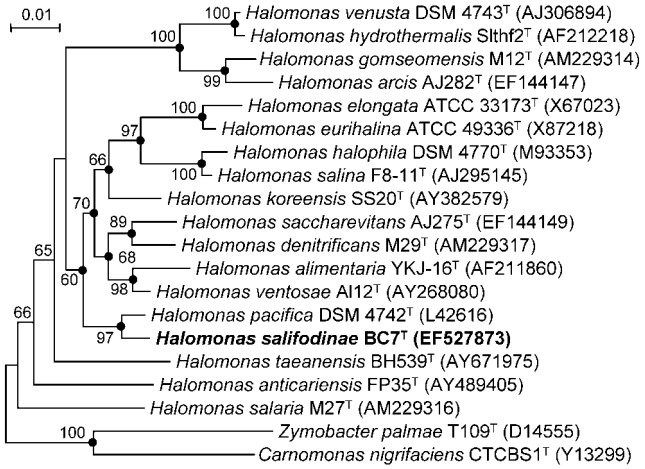

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain BC7^T and related taxa. Bootstrap values are based on 1000 replicates; only values >60 % are shown. Dots indicate branches of the tree that were also retrieved by using the maximum-parsimony method. Bar, 0.01 substitutions per nucleotide position. An extended neighbour-joining tree based on sequences of all recognized species of the genus *Halomonas* is shown in Supplementary Fig. S2.

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