Halolamina salifodinae sp. nov. and *Halolamina salina* sp. nov., two extremely halophilic archaea isolated from a salt mine

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Two strictly aerobic, extremely halophilic archaea, strains WSY15-H1^T and WSY15-H3^T, were isolated from a salt mine in Wensu county, Xinjiang province, China. Cells of the two strains were Gram-stain-negative, non-motile and pleomorphic. Colonies were pink- and red-pigmented, respectively. Strain WSY15-H1^T grew at 20–45 °C (optimum 37–42 °C), 1.6–5.4 M NaCl (optimum 3.4–3.9 M), 0–2.0 M $MgCl_2$ (optimum 0.1–0.5 M) and pH 6.0–9.0 (optimum 7.0), whereas strain WSY15-H3^T grew at 20–50 °C (optimum 37 °C), 1.9–5.4 M NaCl (optimum 3.4 M), 0.02–2.5 M $MgCl_2$ (optimum 0.5-1.0 M) and pH 6.0–7.5 (optimum 6.5). The minimal NaCl concentrations to prevent cell lysis were 9 % (w/v) for strain WSY15-H1^T and 8 % (w/v) for strain WSY15-H3^T. The major polar lipids of the two isolates were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulfate, as well as nine glycolipids for strain WSY15-H1^T and seven glycolipids for strain WSY15-H3^T; two of these glycolipids (GL1 and GL3) were chromatographically identical to bis-sulfated diglycosyl diether (S_2-DGD-1) and sulfated diglycosyl diether (S-DGD-1), respectively. The genomic DNA G+C contents of strains WSY15-H1^T and WSY15-H3^T were 65.4 and 66.2 mol%. On the basis of 16S rRNA gene sequence analysis, strains WSY15-H1^T and WSY15-H3^T shared 97.0 % similarity with each other and showed respectively 98.4 and 97.6 % sequence similarity to *Halolamina pelagica* TBN21^T, which was the only type strain that had higher than 91 % sequence similarity with the two isolates. Analysis of phylogenetic relationships and DNA–DNA relatedness indicated that strains WSY15-H1^T and WSY15-H3^T represent two novel lineages with closest affinity to *H. pelagica* TBN21^T. Based on phenotypic, chemotaxonomic and genotypic characteristics, two novel species of the genus *Halolamina* are proposed, *Halolamina salifodinae* sp. nov. (type strain WSY15-H1^T=JCM 18548^T=GCMCC 1.12371^T) and *Halolamina salina* sp. nov. (type strain WSY15-H3^T=JCM 18549^T=GCMCC 1.12285^T).

The genus *Halolamina* (Cui *et al.*, 2011) was proposed based on low 16S rRNA gene sequence similarity (<90 %) with all other extremely halophilic archaea and a distinctive polar lipid composition. At the time of writing, this genus comprised a single species, *Halolamina pelagica*, the type strain of which (TBN21^T) was isolated from the Taibei marine solar saltern in China. During our surveys on halophilic archaeal diversity in a salt mine in Wensu county, Akesu area of Xinjiang province, China, two extremely halophilic archaea, strains WSY15-H1^T and WSY15-H3^T, were obtained. Although the three strains described above were all screened from dried salt crystals, *H. pelagica* TBN21^T was isolated from brine sampled from Taibei marine solar saltern (Cui *et al.*, 2011) while strains WSY15-H1^T and WSY15-H3^T were isolated from a salt mine. In this paper, we propose that strains WSY15-H1^T and WSY15-H3^T represent two novel species of the genus *Halolamina*.

Abbreviations: PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerol phosphate methyl ester; PGS, phosphatidylglycerol sulfate; S-DGD-1, sulfated diglycosyl diether; S_2-DGD-1, bis-sulfated diglycosyl diether.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains WSY15-H1^T and WSY15-H3^T are JX014295 and JX192605.

Six supplementary figures are available with the online version of this paper.

About 3 g salt sample was suspended in 30 ml NOM-3 medium containing 100 μg streptomycin ml^{-1} and 100 μg ampicillin ml^{-1} to enrich halophilic archaea at 37 °C for 2 weeks. The enrichment culture was diluted and spread onto NOM-3 plates which were incubated at 37 °C. The NOM-3 medium contained (per litre distilled water) 5.4 g KCl, 0.3 g K_2HPO_4, 0.25 g $CaCl_2$, 0.25 g NH_4Cl, 26.8 g $MgSO_4 . 7H_2O$, 23.0 g $MgCl_2 . 6H_2O$, 184.0 g NaCl, 1.0 g yeast extract, 0.25 g fish peptone, 0.25 g sodium formate, 0.25 g sodium acetate, 0.25 g sodium lactate and 0.25 g sodium pyruvate (pH 7.0). The medium was solidified with 2.0 % agar. After 3 weeks of incubation, colonies were picked up and purified by repeated restreaking. The isolates were routinely cultured on NOM-3 medium and maintained at −80 °C with 25 % (v/v) glycerol.

Phenotypic tests were performed according to the proposed minimal standards for description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). *H. pelagica* TBN21^T was selected as a reference strain. Cell morphology and motility were determined by using optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) (Huo *et al.*, 2010). Gram staining was performed according to Dussault (1955). To determine the salt dependence, the strains were cultured in NOM-3 medium with different NaCl concentrations (0.9–5.4 M, at intervals of 0.5 M) or $MgCl_2 . 6H_2O$ concentrations (0, 0.1, 0.2, 0.5, 0.8, 1, 1.5, 1.8, 2, 2.5 M). The pH range for growth was determined at pH 4.0–10.0 (at intervals of 0.5 pH units) with the following buffers: ammonium acetate (pH 4.0–5.0), MES (pH 5.5–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.0) at a concentration of 30 mM. The temperature range for growth was determined by incubation at 10, 15, 20, 28, 32, 37, 40, 42, 45, 48, 50 and 55 °C. Minimal salt concentrations to prevent cell lysis were tested by suspending washed cells in a set of sterile saline solutions containing 0–15 % (w/v) NaCl and the stability of the cells was detected by light microscopic examination. All tests were performed in triplicate and medium without inoculation was used as a control.

All the following biochemical and physiological tests were carried out on strains WSY15-H1^T, WSY15-H3^T and *H. pelagica* TBN21^T, and were performed in triplicate in NOM-3 medium. Oxidase and catalase activity, and nitrate and nitrite reduction were tested according to Dong & Cai (2001). Indole, methyl red, Voges–Proskauer and Simmons citrate tests, H_2S production, and hydrolysis of starch, aesculin, casein and gelatin were tested as described by Zhu (2011). Esterase activity was detected as outlined by Gutiérrez & González (1972). Production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, phosphatase and β-galactosidase were assessed as described by Oren *et al.* (1997). Anaerobic growth was tested in the presence of sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM), L-arginine or DMSO (5 g l^{-1}). Oxygen was removed as described by Grishchenkov *et al.* (2000). The same medium lacking L-cysteine and resazurin was used as aerobic control. To investigate the assimilation of single carbon sources, fish peptone, sodium formate, sodium acetate, sodium lactate and sodium pyruvate were omitted from NOM-3 medium, and yeast extract was added at a concentration of 0.05 g l^{-1}. Organic acids were autoclaved for 20 min at 121 °C. Sugars, alcohols and amino acids were sterilized by UV light. The concentration of each added carbon source was 0.5 % (w/v). Acid production from carbohydrates was determined according to Gerhardt *et al.* (1994). Susceptibility to antibiotics was determined on NOM-3 agar plates using antibiotic discs (μg per disc, unless indicated): ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), cefotaxime (30), cefoxitin (30), cephalothin (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), nitrofurantoin (300), norfloxacin (10), novobiocin (30), nystatin (100), penicillin G (10 IU), rifampicin (5), rocephin (30), streptomycin (10), tetracycline (30) and vancomycin (30).

Polar lipids were extracted using a chloroform/methanol system. They were separated by TLC using silica gel 60 F_{254} aluminium-backed thin-layer plates (Merck) (Kates, 1986). One-dimensional chromatography was performed by using a chloroform/glacial acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.) solvent system. In addition, two-dimensional chromatography was performed by using chloroform/methanol/water (65 : 25 : 4, by vol.) in the first dimension and chloroform/glacial acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.) in the second dimension. The TLC plates were sprayed with sulfuric acid/ethanol (1 : 1, v/v) and heated at 120 °C for 10 min to detect phospholipids and glycolipids.

Genomic DNA was extracted and purified using the method described by Ng *et al.* (1995). The 16S rRNA gene was amplified by PCR using universal archaeal primers: forward, 5′-ATTCCGGTTGATCCTGCCGGA-3′ (positions 1–20 in the *Halobacterium salinarum* numbering and 6–26 in the *Escherichia coli* numbering); and reverse, 5′-AGGAGGTGATCCAGCCGCAG-3′ (*Hbt. salinarum* 1454–1473; *E. coli* 1540–1521). PCR products were cloned into vector pMD18-T (TaKaRa) and then sequenced. Sequence similarity was analysed with the EzTaxon-e server (Kim *et al.*, 2012) and the BLAST program. The *rpoB*' gene was obtained by using the method described by Enache *et al.* (2007). The PCR primers were 444F (5′-TCCCGTACCCNGARCAYAAY-3′) and 1743R (5′-TTGAAYGCGTAGSWSATCTC-3′). Multiple sequence alignment was performed with CLUSTAL W1.8 (Thompson *et al.*, 1994). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods with the MEGA 5 program package (Tamura *et al.*, 2011). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method. The DNA G+C content was determined by reversed-phase HPLC as

described by Mesbah & Whitman (1989). DNA–DNA hybridizations were performed using the thermal denaturation and renaturation method (De Ley *et al.*, 1970) as modified by Huss *et al.* (1983), using a Beckman DU 800 spectrophotometer. The experiments were carried out in quadruplicate.

Cells of the two isolates were Gram-stain-negative, aerobic, non-motile and pleomorphic (Fig. S1, available in IJSEM Online). Colonies of strain WSY15-H1^T were pink while those of strain WSY15-H3^T were red. The minimal NaCl concentrations to prevent cell lysis were 9 % (w/v) for strain WSY15-H1^T and 8 % (w/v) for strain WSY15-H3^T. Mg^{2+} was required for growth for strain WSY15-H3^T but not for strain WSY15-H1^T. Catalase activity, oxidase activity, phosphatase activity, Voges–Proskauer test and hydrolysis of aesculin were positive for the two isolates. Nitrate reduction, indole, methyl red and Simmons citrate tests, hydrolysis of starch, casein, gelatin, Tweens 20, 40, 60 and 80, production of H_2S, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and β-galactosidase were negative for the two isolates. Acid was produced from D-glucose and D-ribose by strain WSY15-H1^T while no acid was produced by strain WSY15-H3^T. More detailed characteristics of the two strains are given in the species descriptions.

The polar lipids of the two strains consisted of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and phosphatidylglycerol sulfate (PGS), as well as nine glycolipids (GL1, GL2, GL3, GL4, GL5, GL6, GL7, GL8, GL9) for strain WSY15-H1^T and seven glycolipids (GL1, GL2, GL3, GL6, GL7, GL8, GL9) for strain WSY15-H3^T; two of these glycolipids (GL1 and GL3) were chromatographically identical to bis-sulfated diglycosyl diether (S_2-DGD-1) and sulfated diglycosyl diether (S-DGD-1), respectively (Figs S5 and S6).

Seventeen complete 16S rRNA gene sequences of strain WSY15-H1^T and 15 complete 16S rRNA gene sequences of strain WSY15-H3^T were obtained. No heterogeneity was found in the 16S rRNA genes of strains WSY15-H1^T and WSY15-H3^T. Based on the alignment of 16S rRNA gene sequences, strains WSY15-H1^T and WSY15-H3^T were affiliated with the family *Halobacteriaceae*. Strains WSY15-H1^T and WSY15-H3^T shared 97.0 % 16S rRNA gene sequence similarity with each other. The two strains showed 98.4 and 97.6 % sequence similarity to *H. pelagica* TBN21^T, respectively, and showed low sequence similarity (<91 %) to other species. Phylogenetic trees revealed that strains WSY15-H1^T and WSY15-H3^T clustered with *H. pelagica* TBN21^T and *H. pelagica* TBN49 (a second reference strain of *H. pelagica*) at high bootstrap values, which indicated that these four strains form a distinct lineage of the genus *Halolamina* (Fig. 1, Figs S2 and S3). Based on the alignment of *rpoB*' gene sequences, strains WSY15-H1^T and WSY15-H3^T shared 93.6 % sequence similarity with each other. The two strains showed 94.6 and 94.8 % *rpoB*' gene sequence similarity to *H. pelagica* TBN21^T, respectively, and showed low sequence similarity (<88 %) to other species. These levels of gene sequence similarity indicate that strains WSY15-H1^T, WSY15-H3^T and *H. pelagica* TBN21^T are members of the same genus (Minegishi *et al.*, 2010). The neighbour-joining phylogenetic tree based on *rpoB*' gene sequences also supported strains WSY15-H1^T and WSY15-H3^T as representing two novel lineages with closest affinity to *H. pelagica* TBN21^T (Fig. S4). The genomic G+C contents of strains WSY15-H1^T and WSY15-H3^T were 65.4 and 66.2 mol%. The level of DNA–DNA relatedness between strains WSY15-H1^T and WSY15-H3^T was 40.4 %. Moreover, the DNA–DNA relatedness values between the two new isolates and *H. pelagica* TBN21^T were 41.9 and 44.6 %, respectively, lower than the accepted threshold value (70 %) to separate species (Stackebrandt & Goebel, 1994).

Strains WSY15-H1^T and WSY15-H3^T differed in several characteristics. Strain WSY15-H3^T grew more slowly than strain WSY15-H1^T. The upper temperature for growth was 50 °C for strain WSY15-H3^T and 45 °C for strain WSY15-H1^T. Mg^{2+} was required for growth of strain WSY15-H3^T but not for strain WSY15-H1^T. The pH, NaCl and $MgCl_2$ concentration ranges for growth and the pigments were different between the two isolates. In contrast to strain WSY15-H3^T, strain WSY15-H1^T utilized L-alanine, DL-lactate, L-malate and L-ornithine as sole carbon sources for growth but not D-galactose, produced acid from D-glucose and D-ribose, and was sensitive to bacitracin and nitrofurantoin. Strain WSY15-H1^T contained glycolipids GL4 and GL5 whereas strain WSY15-H3^T did not (Fig. S4). The 16S rRNA gene sequences of strains WSY15-H1^T and WSY15-H3^T were only 97.0 % similar. The *rpoB*' gene sequences of strains WSY15-H1^T and WSY15-H3^T shared only 93.6 % similarity with each other, and the neighbour-joining phylogenetic tree based on *rpoB*' gene sequences also revealed that strains WSY15-H1^T and WSY15-H3^T represented two novel lineages. DNA–DNA hybridization between strains WSY15-H1^T and WSY15-H3^T showed a low relatedness value of 40.4 %. We therefore suggest that strains WSY15-H1^T and WSY15-H3^T represent two novel species of the genus *Halolamina*.

Strains WSY15-H1^T and WSY15-H3^T were phylogenetically closest to *H. pelagica* TBN21^T, and they shared high 16S rRNA gene sequence similarity (98.4 and 97.6 %, respectively) with *H. pelagica* TBN21^T. Differences among the three strains were that *H. pelagica* TBN21^T could grow only up to 0.7 M $MgCl_2$ while the two novel strains could grow up to 2 M $MgCl_2$ and the optimum $MgCl_2$ concentration for growth was also different among them. In addition, the two new strains were negative for acid production from D-mannose, starch hydrolysis and H_2S production from sodium thiosulfate, and strain WSY15-H1^T was positive for acid production from D-ribose. *H. pelagica* TBN21^T also lacked GL4 and GL5 according to analysis of polar lipids. DNA G+C contents were different among the three strains. Strains WSY15-H1^T and WSY15-H3^T shared low DNA–DNA relatedness values with *H.*

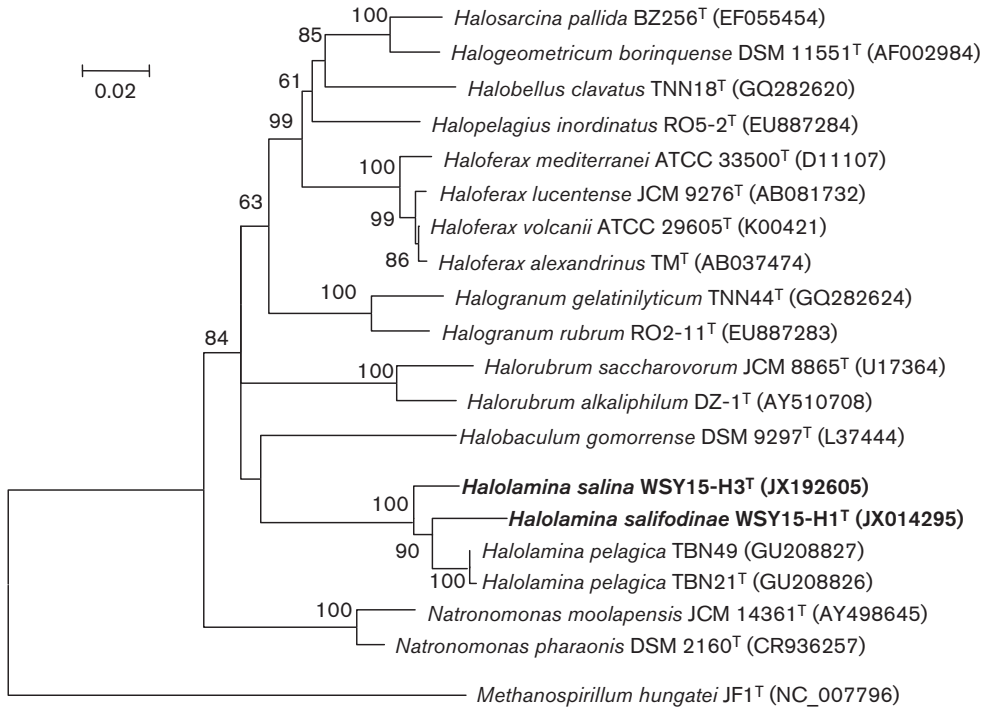

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences, showing the relationships of the novel isolates and related taxa. Bootstrap values based on 1000 replicates are shown for branches with more than 60 % support. Bar, 0.02 substitutions per nucleotide position.

pelagica TBN21^T (41.9 and 44.6 %, respectively). Differences in the physiological and biochemical characterization among the three strains are detailed in Table 1.

Based on the phenotypic, chemotaxonomic and genotypic characteristics described above, strains WSY15-H1^T and WSY15-H3^T are identified as representing two novel species of the genus *Halolamina*, for which the names *Halolamina salifodinae* sp. nov. and *Halolamina salina* sp. nov. are proposed, respectively.

Description of *Halolamina salifodinae* sp. nov.

Halolamina salifodinae (sa.li.fo.di′na.e. L. gen. n. *salifodinae* of a saltpit, salt mine).

Cells are non-motile, pleomorphic and Gram-stain-negative. Colonies are pink-pigmented. Extremely halophilic. Growth occurs at 20–45 °C (optimum, 37–42 °C), 1.6–5.4 M NaCl (optimum, 3.4–3.9 M), 0–2.0 M $MgCl_2$ (optimum, 0.1–0.5 M) and pH 6.0–9.0 (optimum, 7.0–7.5). The minimal NaCl concentration to prevent cell lysis is 9 % (w/v). Anaerobic growth does not occur with sodium thiosulfate, sodium sulfite, sodium sulfate, sodium nitrite, sodium nitrate, L-arginine or DMSO. Catalase- and oxidase-positive. Production of indole and methyl red, Voges–Proskauer and Simmons citrate tests are negative. Does not hydrolyse starch, casein, gelatin, or Tweens 20, 40, 60 or 80. Does not produce arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease or β-galactosidase. Nitrate is not reduced to nitrite. H_2S is not produced from sodium thiosulfate. Tests for hydrolysis of aesculin and phosphatase activities are positive. Able to use the following substrates as sole carbon sources: acetate, L-alanine, L-arginine, D-glucose, L-glutamate, DL-lactate, L-malate, L-ornithine, pyruvate, D-ribose, starch and sucrose. No growth occurs on L-aspartate, citrate, D-fructose, fumarate, D-galactose, glycerol, glycine, lactose, L-lysine, maltose, D-mannitol, D-mannose, D-sorbitol, L-sorbose, succinate or D-xylose. Acid is produced from D-glucose and D-ribose. Sensitive to the following antibiotics (μg per disc, unless indicated): bacitracin (0.04 IU), ciprofloxacin (5), norfloxacin (10), novobiocin (30), rifampicin (5), nitrofurantoin (300) and nystatin (100). Resistant to ampicillin (10), carbenicillin (100), cefotaxime (30), cefoxitin (30), cephalothin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), penicillin G (10 IU), rocephin (30), streptomycin (10), tetracycline (30) and vancomycin (30). The polar lipids are PG, PGP-Me, PGS and nine glycolipids; two of these glycolipids (GL1 and GL3) are chromatographically identical to S_2-DGD-1 and S-DGD-1, respectively.

The type strain, WSY15-H1^T (=JCM 18548^T=GCMCC 1.12371^T), was isolated from a salt mine taken from Wensu county, Xinjiang province, China. The DNA G+C content of the type strain is 65.4 mol% (HPLC).

Table 1. Differential characteristics between strains WSY15-H1^T, WSY15-H3^T and *Halolamina pelagica* TBN21^T

Strains: 1, WSY15-H1^T; 2, WSY15-H3^T; 3, *H. pelagica* TBN21^T. Data were taken from this study. All strains were positive for catalase and oxidase activity, Voges–Proskauer test; all strains were negative for hydrolysis of gelatin, casein and Tween 80, indole, methyl red test, nitrate reduction and anaerobic growth. The following substrates could be used by all strains as single carbon sources: acetate, L-arginine, D-glucose, L-glutamate, pyruvate, D-ribose and starch. No growth occurred on L-aspartate, citrate, D-fructose, fumarate, glycerol, glycine, lactose, L-lysine, maltose, D-mannitol, D-sorbitol, L-sorbose, succinate or D-xylose. All strains were sensitive to novobiocin and rifampicin, but resistant to ampicillin, carbenicillin, cefotaxime, cefoxitin, cephalothin, chloramphenicol, erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, penicillin G, rocephin, streptomycin, tetracycline and vancomycin. −, Negative; +, positive; R, resistant; S, susceptible.

Characteristic	1	2	3
Pigmentation	Pink	Red	Red
Temperature range (°C)	20–45	20–50	25–50
Temperature optimum (°C)	37–42	37	37
pH range	6.0–9.0	6.0–7.5	5.5–9.5
pH optimum	7.0	7.0	7.0–7.5
NaCl range (M)	1.6–5.4	1.9–5.4	1.4–5.1
NaCl optimum (M)	3.4–3.9	3.4	3.4–3.9
$MgCl_2$ range (M)	0–2	0–2.5	0–0.7
$MgCl_2$ optimum (M)	0.1–0.5	0.02–2.5	0.01–0.05
Minimal NaCl concentration to prevent cell lysis (%)	9	8	8
H_2S production	−	−	+
Hydrolysis of starch	−	−	+
Carbon source used for growth:			
L-Alanine	+	−	+
D-Galactose	−	+	+
DL-Lactate	+	−	−
L-Malate	+	−	−
D-Mannose	−	−	+
L-Ornithine	+	−	+
D-Ribose	+	+	−
Sucrose	+	+	−
Acid production from:			
D-Galactose	−	−	+
D-Glucose	+	−	+
D-Mannose	−	−	+
D-Ribose	+	−	−
Norfloxacin (10 μg)	S	S	R
Ciprofloxacin (5 μg)	S	S	R
Nystatin (100 μg)	S	S	R
Bacitracin (0.04 IU)	S	R	S
Nitrofurantoin (300 μg)	S	R	S
Polar lipids	PG, PGP-Me PGS, GL1, GL2, GL3, GL4, GL5, GL6, GL7, GL8, GL9	PG, PGP-Me PGS, GL1, GL2, GL3, GL6, GL7, GL8, GL9	PG, PGP-Me PGS, GL1, GL2, GL3, GL6, GL7, GL8, GL9
DNA G+C content (mol%)*	65.4	66.2	66.9

*The DNA G+C contents of strains WSY15-H1^T, WSY15-H3^T and *H. pelagica* TBN21^T were determined by HPLC under the same conditions.

Description of *Halolamina salina* sp. nov.

Halolamina salina (sa.li'na. L. fem. adj. *salina* of or belonging to salt).

Cells are non-motile, pleomorphic and Gram-stain-negative. Colonies are red-pigmented. Extremely halophilic. Growth occurs at 20–50 °C (optimum, 37–42 °C), 1.9–5.4 M NaCl (optimum, 3.4 M), 0–2.5 M $MgCl_2$ (optimum 0.5–1.0 M) and pH 6.0-7.5 (optimum, 6.5). The minimal NaCl concentration to prevent cell lysis is 8 % (w/v). Anaerobic growth does not occur with sodium thiosulfate, sodium sulfite, sodium sulfate, sodium nitrite, sodium nitrate, L-arginine or DMSO. Catalase- and oxidase-positive. Production of indole and methyl red, Voges–Proskauer and Simmons citrate tests are negative. Does not hydrolyse starch, casein, gelatin, or Tweens 20, 40, 60 or 80. Does not produce arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease or β-galactosidase.

Nitrate is not reduced to nitrite. H_2S is not produced from sodium thiosulfate. Tests for hydrolysis of aesculin and phosphatase activities are positive. Able to use the following substrates as sole carbon sources: acetate, L-arginine, D-galactose, D-glucose, L-glutamate, pyruvate, D-ribose, starch and sucrose. No growth occurs on L-alanine, L-aspartate, citrate, D-fructose, fumarate, glycerol, glycine, DL-lactate, lactose, L-lysine, L-malate, maltose, D-mannitol, D-mannose, L-ornithine, D-sorbitol, L-sorbose, succinate or D-xylose. No acid is produced. Sensitive to the following antibiotics (μg per disc, unless indicated): ciprofloxacin (5), norfloxacin (10), novobiocin (30), rifampicin (5) and nystatin (100). Resistant to ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), cefotaxime (30), cefoxitin (30), cephalothin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), nitrofurantoin (300), penicillin G (10 IU), rocephin (30), streptomycin (10), tetracycline (30) and vancomycin (30). The polar lipids are PG, PGP-Me, PGS and seven glycolipids; two of these glycolipids (GL1 and GL3) are chromatographically identical to S_2-DGD-1 and S-DGD-1, respectively.

The type strain, WSY15-H3^T (=JCM 18549^T=GCMCC 1.12285^T), was isolated from a salt mine taken from Wensu county, Xinjiang province, China. The DNA G+C content of the type strain is 66.2 mol% (HPLC).

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