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TAXONOMIC DESCRIPTION

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Halomonas urmiana sp. nov., a moderately halophilic bacterium isolated from Urmia Lake in Iran

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Abstract

In the course of screening halophilic bacteria in Urmia Lake in Iran, which is being threatened by dryness, a novel Gram-negative, moderately halophilic, heterotrophic and short rod-shaped bacteria was isolated and characterized. The bacterium was isolated from a water specimen and designated as TBZ3^T. Colonies were found to be creamy yellow, with catalase- and oxidase-positive activities. The growth of strain TBZ3^T was observed to be at 10–45 °C (optimum, 30 °C), at pH 6.0–9.0 (optimum, pH 7.0) and in the presence of 0.5–20% (w/v) NaCl (optimum, 7.5 %). Strain TBZ3^T contained $C_{16:0}$, cyclo-$C_{19:0}\omega 8c$, summed feature 3 (comprising $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$) and summed feature 8 (comprising $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$) as major fatty acids and ubiquinone-9 as the only respiratory isoprenoid quinone. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, glycolipid, unidentified phospholipid and unidentified polar lipids were detected as the major polar lipids. Strain TBZ3^T was found to be most closely related to *Halomonas saccharevitans* AJ275^T, *Halomonas denitrificans* M29^T and *Halomonas sediminicola* CPS11^T with the 16S rRNA gene sequence similarities of 98.93, 98.15 and 97.60% respectively and in phylogenetic analysis strain TBZ3^T grouped with *Halomonas saccharevitans* AJ275^T contained within a large cluster within the genus *Halomonas*. Based on phenotypic, chemotaxonomic and molecular properties, strain TBZ3^T represents a novel species of the *Halomonas* genus, for which the name *Halomonas urmiana* sp. nov. is proposed. The type strain is TBZ3^T (=DSM 22871^T=LMG 25416^T).

The genus *Halomonas* belongs to the family *Halomonadaceae*, in class *Gammaproteobacteria,* typically includes marine and moderately halophilic microorganisms. The type species of this genus is described as Gram-negative, rod-shaped, facultative anaerobic, and halotolerant that are capable of growth in a wide range of salt concentrations [1]. At the time of writing, this genus comprised 102 validly published species (www.bacterio.net/index.html). Numerous *Halomonas* species are broadly distributed all over saline environments including saline soils [2, 3], salty foods [4], oceans and seas [5, 6], saline lakes [7–9], soda lakes [10], solar salterns [11–13] and deep-sea hydrothermal vents [14, 15] habitats regardless of their ecological location.

Urmia Lake, located in the north-west of Iran, is one of the largest hypersaline lakes in the world and in many features of morphology, chemistry, and sediments, is like the Great Salt Lake in the western USA. In spite of this similarity and its several values, little literature has been published on the lake's biota [16] and bacterial diversity [17]. The salinity of Urmia Lake has increased dramatically to more than 350 g l^{-1} during recent years, due to drought and increased demands for agricultural water in the lake's basin, which has greatly influenced almost all aspects of the lake [18–20].

Bacteria belonging to the *Halomonas* genus have applicable potentials in various fields of industry, ecology, and biotechnology [21–24]. The most promising application are the production of compatible solutes and extracellular compounds; for instance enzymes [25] and exopolysaccharides [26, 27]. Over the last decade, interest in *Halomonas* species has been focused on their ability to degrade

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbor-joining; Q-9, ubiquinone-9.
The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain TBZ3^T are EU305729 and VBUI00000000, respectively.
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One supplementary table and four supplementary figures are available wtith the online version of this article.

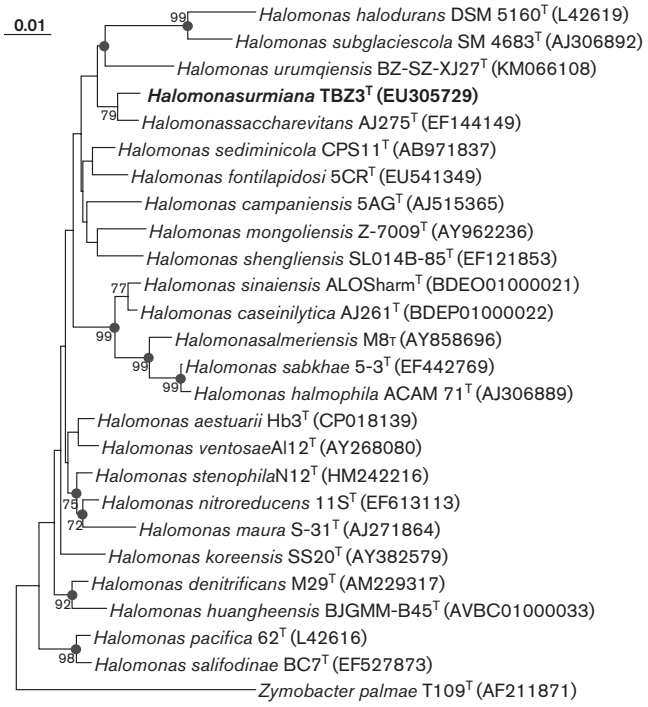

Fig. 1. A neighbour-joining tree showing the phylogenetic relationships between strain TBZ3^T and closely related taxa of genus *Halomonas*, based on 16S rRNA gene sequences. Filled circles (●) indicate that the same nodes were also recovered by the maximum-likelihood and maximum-parsimony algorithms. Bootstrap values are shown on nodes as percentages of 1000 replicates for values over 70%. *Zymobacter palmae* T109^T (AF211871) was used as an outgroup. The scale bar equals 0.01 changes per nucleotide position.

aromatic compounds such as benzoic acid, salicylic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, cinnamic acid, phenylacetic acid, *p*-aminosalicylic acid, para-amino acetanilide, and ferulic acid that eventually can be functional in environmental bioremediation processes [28–33]. The Fe^{3+} reduction ability of *Halomonas* is also reported [34]. Due to the importance of *Halomonas* bacteria, this study tried to screen Urmia Lake water for the presence of *Halomonas* strains. In this paper, a novel bacterium of the genus *Halomonas* was characterized taxonomically using a polyphasic approach, with the proposed name of *Halomonas urmiana* type strain TBZ3^T.

Strain TBZ3^T was isolated from water specimens collected from Islami (Shahi) island of Urmia Lake, Iran (37°48′18.1″N 45°23′14.4″E) at a depth of 0.5–1 metres (Fig. S1, available in the online version of this article). Strain TBZ3^T was initially cultured aerobically on Halomonas agar medium at 30 °C for 3–4 days. *Halomonas* agar medium [35] was composed of (per l): 80 g NaCl, 7.50 g casamino acids (with vitamins), 5 g protease peptone, 1 g yeast extract, 3 g Na_3-citrate, 20 g $MgSO_4.7H_2O$, 0.50 g K_2HPO_4, 0.05 g $Fe(NH_4)_2(SO_4)_2$ and 15 g agar, with pH adjusted to 7.5. Strain TBZ3^T was routinely cultured on marine agar [MA; Becton-Dickinson (BD)] and in marine broth [MB; Becton-Dickinson (BD)] at 30 °C for 2 days for further taxonomic study as described previously [7]. Strain TBZ3^T was preserved at −80 °C in MB supplemented with 15% (v/v) glycerol. For phylogenetic analysis, the 16S rRNA gene of strain TBZ3^T was amplified by PCR using 16F27 (5′-AGAGTTTGATCTGGCTCAG-3′) and 16R1488 (5′-TACCTTGTTAGGACTTCACC-3′) forward and reverse universal primers, respectively. Amplified product was sequenced at Macrogen (Korea) using the universal primers 16F27, 16R1488 and three other designed primers; H400 (5′-GGGTTGTAAAGCACTTTCAG-3′), H550 (5′-CCAGTAATTCCGATTAACGC-3′) and H900 (5′-ACTCAAATGAATTGACGGGG-3′) to obtain a complete 16S rRNA gene sequence (1485 nucleotides) [17]. The resulting 16S rRNA gene sequence of strain TBZ3^T was compared with those of all other reported type strains using the nucleotide similarity search program in the EzBioCloud server (http://www.ezbiocloud.net/identify) [36]. The 16S rRNA gene sequences of strain TBZ3^T and closely related type strains were aligned using the fast secondary-structure aware infernal aligner available in the Ribosomal Database Project [37]. Then, the phylogenetic trees were constructed with the Kimura two-parameter model, the nearest-neighbour-interchange heuristic search method and the partial deletion options with bootstrap values (1000 replications) for the construction of neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) trees in the MEGA7 software [38]. For the comparisons of phenotypic properties and fatty acid compositions, the three most closely related type strains based on 16S rRNA gene sequence similarity, *Halomonas sediminicola* (KACC 18262^T), *Halomonas saccharevitans* (JCM 14606^T) and *Halomonas denitrificans* (KCTC 12665^T) were selected and obtained from their corresponding collection centres to use them as reference strains.

The resulting 16S rRNA gene sequences comparison between strain TBZ3^T and other type strains showed that strain TBZ3^T was most closely related to *Halomonas saccharevitans* AJ275^T, *Halomonas denitrificans* M29^T and *Halomonas sediminicola* CPS11^T with 98.93, 98.15, and 97.60% sequence similarities, respectively. However, the phylogenetic analysis based on the 16S rRNA gene sequences using the NJ algorithm showed that strain TBZ3^T grouped with *Halomonas saccharevitans* AJ275^T contained within a large cluster within the genus *Halomonas* (Fig. 1). The phylogenetic trees built by the ML and MP algorithms also supported that strain TBZ3^T formed a phyletic lineage contained within a large cluster within the genus *Halomonas* of the family *Halomonadaceae* (Fig. S2).

For the whole genome sequencing of strain TBZ3^T, the genomic DNA of strain TBZ3^T was extracted using a Wizard Genomic DNA purification kit (Promega, USA) according to the procedure of the manufacturer and sequenced with 101 bp paired-end sequencing reads by an Illumina Hiseq 2500 instrument at Macrogen (Korea). The resulting sequencing reads were assembled using SPAdes [39]. The DNA G+C content of strain TBZ3^T was calculated using the EditSeq module of the Lasergene package (USA) from the draft whole genomic sequence. The SPAdes assembly of the genome sequencing data of strain TBZ3^T produced 122

contigs with an N50 of 122 kb and average genome coverage of 689×. The draft genome of strain TBZ3^T was 4028094 bp in size and contained 64 tRNA genes coding 20 amino acids. A total of 3804 genes were predicted and among these, 3624 protein-coding genes were identified. The draft genome sequence of strain TBZ3^T was deposited to GenBank with the accession number VBUI00000000. Based on the draft genome, the G+C content of strain TBZ3^T was calculated as 66.9 mol%. Average nucleotide identity (ANI) and digital DNA–DNA hybridization (DDH) values between strain TBZ3^T and *H. saccharevitans* AJ275^T (GenBank accession no., NZ_FPAQ00000000) and *H. denitrificans* M29^T (GenBank accession no., NZ_PYVX00000000) were calculated using a stand-alone software available in the EZGenome web server (www.ezbiocloud.net/sw/oat) [40] and the server-based Genome-to-Genome Distance Calculator version 2.1 (http://ggdc.dsmz.de/distcalc2.php) [41], respectively. The ANI and digital DDH values between strain TBZ3^T and the type strains of *H. saccharevitans* AJ275^T and *H. denitrificans* M29^T were 91.5 and 43% and 91.2 and 43.2%, respectively, which were low compared to the thresholds (ANI,~95%; *in silico* DDH, 70%) for prokaryotic species delineation [42]. In conclusion, the 16S rRNA based phylogenetic analysis and homology of genome with reference strains propose that strain TBZ3^T can represent a novel species of the genus *Halomonas*.

The growth of strain TBZ3^T was examined on several bacteriological agar media: marine agar (MA; BD), *Halomonas* agar, R2A agar, tryptic soy agar (TSA; BD), nutrient agar (NA; BD) and laboratory prepared Luria-Bertani (LB; MP Biomedicals) agar (supplemented with 7.5% optimum salt) for 2 days at 30 °C. The growth of strain TBZ3^T was evaluated at various temperatures (4, 15, 20, 25, 30, 37, 40 and 45 °C) and at different pH values (4.0–10.0 with 0.5 pH unit intervals) on MA and in MB at 30 °C, respectively for 2 days. MB with pH 4.0–5.5, pH 6.0–7.0 and pH 8.0–10.0 were prepared using sodium citrate buffer, Na_2HPO_4/NaH_2PO_4 and Tris-HCl buffers, respectively [43] and their pH values were adjusted again if necessary, after autoclaving for 15 min at 121 °C. The tolerance to NaCl of strain TBZ3^T was tested in NaCl-free-MB with different NaCl concentrations [0–10% (w/v) at 0.5% intervals], which were prepared in the laboratory according to the BD formula. The following biochemical physiological tests of strain TBZ3^T were conducted using cells grown on MA for 2 days at 30 °C. Cell morphology and flagella presence of strain TBZ3^T and *H. saccharevitans* JCM 14606^T were investigated using transmission electron microscopy (JEM-1010; JEOL) after negative staining and phase-contrast microscopy (Carl Zeiss Scope.A1, Germany) after flagellum staining using Ryu staining solution [44]. Gram-staining was investigated using the Gram-stain kit (bioMérieux) according to the procedure described by the manufacturer. Catalase and oxidase activities were tested by the production of oxygen bubbles in aqueous H_2O_2 [3% (v/v)] and by the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck) [45]. The anaerobic growth of strain TBZ3^T was assessed on MA and MA supplemented with various electron acceptors [disodium fumarate (10 mM), dimethyl sulfoxide (10 mM), sodium nitrate (10 mM) and sodium nitrite (5 mM)] at 30 °C for 21 days under an anaerobic condition formed by the GasPak Plus system (BBL). The following properties of strain TBZ3^T and three reference strains were examined in parallel under the same physiological conditions. Hydrolysis of tyrosine, casein, aesculin, starch, Tween 20 and Tween 80 was tested on MA, according to the methods described previously [45, 46]. H_2S generation was tested in HM broth supplemented with 0.01% (w/v) of L-cysteine. Additional biochemical features, enzymatic activities and acid production from carbohydrates were tested using the API 20NE, API ZYM and API 50CH system (bioMérieux), according to the instructions described by the manufacturers. Antibiotic sensitivity was tested by spreading a light suspension of bacteria in MB on the surface of MA and applying commercially available antibiotic sensitivity test discs (Mast group ltd, UK); ampicillin (10 µg), clotrimazole (25 µg), cefotaxime (30 µg), chloramphenicol (30 µg), rifampicin (5 µg), erythromycin (15 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), penicillin (10 IU), polymyxin B (300 µg), aztreonam (30 µg), kanamycin (30 µg), neomycin (30 µg), nystatin (100 IU), tobramycin 10 (30 µg) and streptomycin (30 µg) per disc. The plates were cultivated at 30 °C for 48 h.

Strain TBZ3^T grew well on MA and showed growth on *Halomonas* agar, R2A agar, LB agar, TSA and NA. Cells of strain TBZ3^T were found to be Gram-stain-negative, positive for catalase and oxidase activities, non-motile short rods with approximately 0.7–1.0 µm in width and 0.8–1.5 µm in length (Fig. S3). After 21 days of incubation, growth was observed under the anaerobic condition, which indicated strain TBZ3^T to be facultative aerobic. Strain TBZ3^T was found to be susceptible to ampicillin, clotrimazole, cefotaxime, chloramphenicol, rifampicin, erythromycin, nalidixic acid, nitrofurantoin, penicillin, polymyxin B, penicillin V100 rifampicin and aztreonam but not to kanamycin, neomycin, nystatin, tobramycin 10 and streptomycin. Strain TBZ3^T and all other reference strains hydrolysed Tween 20 and Tween 80 and did not hydrolyse tyrosine, starch, aesculin, and casein, while the *Halomonas sediminicola* KACC 18262^T was observed to be negative for the hydrolysis of Tween 20 and Tween 80. Strain TBZ3^T and all other reference strains were found to be positive for nitrate reduction except *Halomonas saccharevitans* JCM 14606^T, which was observed to be negative. Strain TBZ3^T and all other reference strains were negative for the following characteristics: H_2S production, indole production, fermentation of glucose, and activity of arginine dihydrolase, *β*-galactosidase, lipase (C14), cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase and assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and phenylacetic acid and production of acids from D-arabinose, L-arabinose, ribose, L-xylose, methyl-*β*-xyloside, D-mannose, L-sorbose, dulcitol, mannitol, *N*-acetyl-glucosamine, amygdaline, inulin, melezitose, xylitol, *β*-gentiobiose, D-lyxose, D-tagatose,

Table 1. Comparison of phenotypic characteristics of strain TBZ3^T and related taxa of the genus *Halomonas*

Strains: 1, strain TBZ3^T [this study]; 2, *Halomonas saccharevitans* JCM 14606^T [9]; 3, *Halomonas denitrificans* KCTC 12665^T [50]; 4, *Halomonas sediminicola* KACC 18262^T [13]. All strains are positive for the following characteristics: oxidase and catalase activities, activities* of urease, gelatinase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase, assimilation* of D-glucose, maltose, potassium gluconate, malic acid and trisodium citrate. All strains are negative for the following characteristics: H_2S production, hydrolysis* of starch, tyrosine, aesculin and casein, indole production*, fermentation of glucose*, and activities* of arginine dihydrolase, β-galactosidase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase and assimilation* of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and phenylacetic acid. Symbols: +, positive; –, negative

Characteristic	1*	2	3	4
Colony colour†	CY	LY	BY	CY
Cell size (width ×length; μm)	0.7–1.0×0.8–1.5	1.0–2.0×2.0–4.0	0.6–0.8×1.2–1.6	0.5–0.7×1.2–1.5
Cell shape	Short rod	Ovoid	Rod	Rod
Motility	–	+	+	+
Flagella	–	Lophotrichous*	Peritrichous	Lophotrichous
Growth at:				
Temperature (°C)	10–45	4–48	5–50	10–37
pH	6.0–9.0	6.0–10.0	7.0–10.0	5.0–9.0
NaCl (%, w/v)	0.5–20	0.5–15	2–20	1–20
Nitrate reduction	+	–	+	+
Hydrolysis* of				
Tween 80, Tween 20	+	+	+	–
Enzyme activity* of:				
valine arylamidase	+	+	+	–
acid phosphatase	–	+	+	–
α-Glucosidase	+	–	–	–
Assimilation (API 20NE)* of:				
capric acid	–	–	+	–
adipic acid	–	+	–	–
DNA G+C content (mol%)	66.9‡	67.0‡	68.0‡	64.3
Major polar lipids§	DPG, PG, PE, GL, PL	DPG, PG, PE, GL, PL*	PG, PE, GL, APL, PL*	PG, PE, APL, PL

*Data from this study.
†CY, Cream yellow; LY, light yellow; BY, brown yellow.
‡The DNA G+C content were calculated from their whole genome sequence.
§DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; GL, glycolipid; APL, aminophospholipid; PL, unidentified phospholipid.

L-fucose, gluconate and 2-keto-gluconate. Although some phenotypic properties of strain TBZ3^T including enzyme activity of urease and gelatinase, assimilation of D-glucose, maltose, potassium gluconate, malic acid and trisodium citrate and enzyme activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase were observed to be the same with those of reference strains. Many other taxonomic characteristics and phenotypic activities differentiated strain TBZ3^T from other reference strains were described in species description and in (Tables 1 and S1).

Isoprenoid quinones of strain TBZ3^T and *H. saccharevitans* JCM 14606^T were extracted and purified as described by Minnikin *et al.* [47] and examined with a high-performance liquid chromatography system (LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 Í 4.6 mm; Kromasil, Akzo Nobel), as described previously [48]. For the whole-cell fatty acids analysis, strain TBZ3^T and the three reference strains were cultured in MB at 30 °C and cells were harvested during the same growth phase (optical density=0.8 at 600 nm). The MIDI standard protocol was

followed for the saponification, methylation, and extraction of fatty acids from the harvested cells. The fatty acid methyl esters were investigated by gas chromatography (Hewlett Packard 6890) and identified using the RTSBA6 database of the Microbial Identification System (Sherlock ver. 6.0B). The polar lipids of strain TBZ3^T, *Halomonas saccharevitans* JCM 14606^T and *Halomonas denitrificans* KCTC 12665^T were analysed by two-dimensional thin-layer chromatography (TLC) using cells harvested during the exponential growth phase according to the method proposed by Minnikin *et al*. [49]. The polar lipids were detected by following spray reagents: 10% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer-Lester reagent (for phospholipids) and *α*-naphthol (for glycolipids).

Strain TBZ3^T and *Halomonas saccharevitans* JCM 14606^T contained ubiquinone-9 as the sole respiratory quinone, which was in accordance with those detected from other strains of the genus *Halomonas* [7, 50]. $C_{16:0}$, cyclo-$C_{19:0}$ ω*8c*, summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and summed feature 8 (comprising $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) were identified to be as the major cellular fatty acids (>10% of total fatty acids). The general fatty acids profile of strain TBZ3^T and other members of the genus *Halomonas* was found to be relatively similar with some differences in the respective proportions of some fatty acid components (Table 2). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, glycolipid and an unidentified phospholipid were detected as major polar lipids from strain TBZ3^T and *Halomonas saccharevitans* JCM 14606^T, while phosphatidylglycerol, phosphatidylethanolamine, glycolipid, an aminophospholipid and an unidentified phospholipid were identified as major polar lipids from *Halomonas denitrificans* KCTC 12665^T (Fig. S4). In conclusion, phylogenetic, chemotaxonomic and physiological features clearly support that strain TBZ3^T represents a novel species of the genus *Halomonas*, for which the name *Halomonas urmiana* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions of strain TBZ3^T and closely related taxa of the genus *Halomonas*

Taxa: 1, strain TBZ3^T; 2, *Halomonas saccharevitans* JCM 14606^T; 3, *Halomonas denitrificans* KCTC 12665^T; 4, *Halomonas sediminicola* KACC 18262^T. All data were from this study. Data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 0.5% in all strains are not shown. Major components (>5.0 %) are highlighted in bold. TR, trace amount (<0.5 %); –, not detected

Fatty acid	1	2	3	4
Saturated:				
$C_{10:0}$	2.6	2.7	4.3	3.5
$C_{12:0}$	0.7	1.0	1.1	TR
$C_{14:0}$	0.5	TR	TR	0.5
$C_{16:0}$	**23.8**	**22.2**	**19.5**	**18.4**
cyclo-$C_{17:0}$	3.4	1.0	3.2	3.3
$C_{18:0}$	TR	TR	TR	0.5
Unsaturated:				
cyclo-$C_{19:0}$ ω*8c*	**10.8**	**5.6**	**9.5**	**11.5**
11-methyl $C_{18:1}$ ω*7c*	TR	TR	TR	1.3
Hydroxy:				
$C_{10:0}$ 3-OH	TR	TR	0.6	0.5
$C_{12:0}$ 3-OH	**6.8**	**7.7**	**10.6**	**9.2**
Iso-$C_{15:0}$ 3-OH	–	–	TR	0.7
$C_{16:0}$ 3-OH	–	–	–	0.7
Summed feature*:				
3	**22.5**	**24.8**	**20.5**	**26.2**
7	TR	TR	0.5	1.5
8	**25.9**	**32.0**	**27.1**	**19.1**

*Summed features represent groups of two or three fatty acids that cannot be separated by gas-liquid chromatography with the MIDI system. Summed feature 3, $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; summed feature 7, $C_{19:1}$ *ω7c* and/or $C_{19:1}$ *ω6c*; summed feature 8, $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*.

DESCRIPTION OF *HALOMONAS URMIANA* SP. NOV.

Halomonas urmiana (ur.mi.a'na. N.L. fem. adj. *urmiana* pertaining to Urmia, a saline lake in Iran where the strain was isolated).

Cells are Gram-negative, short rods, facultative aerobic, moderately halophilic, heterotrophic with oxidase- and catalase-positive activities. Colonies are convex, smooth, translucent and creamy yellow after 48 h of incubation at 30 °C on MA medium. Growth occurs at 10–45 °C (optimum; 30 °C) and at pH 6.0 to 9.0 (optimum pH; 7.0) and in the presence of 0.5–20% (w/v) NaCl (optimum; 7.5%). No growth occurs in the absence of salt. H_2S production is negative. Tween 20 and Tween 80 are hydrolysed but tyrosine, starch, aesculin, and casein are not hydrolysed. Nitrates are reduced to nitrites. Indole production, glucose fermentation, the activity of arginine dihydrolase and *β*-galactosidase and assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, capric acid, adipic acid, and phenylacetic acid are negative. Positive for the activity of urease and gelatinase and assimilation of D-glucose, maltose, potassium gluconate, malic acid, and trisodium citrate. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, and *α*-glucosidase activities are positive and negative for lipase (C14), cystine arylamidase, trypsin, *α*-chymotrypsin, acid phosphatase, *α*-galactosidase, *β*- galactosidase, *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase. Acids are produced from L-arabinose, D-xylose, adonitol, D-glucose, inositol, sorbitol, aesculin, cellobiose, melibiose and turanose. Major polar lipids are

diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, glycolipid, and an unidentified phospholipid. The major cellular fatty acids are $C_{16:0}$, cyclo-$C_{19:0}$ $\omega 8c$, summed feature 3 (comprising $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$) and summed feature 8 (comprising $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$). The only respiratory quinone is ubiquinone-9.

The type strain is TBZ3^T (=DSM 22871^T=LMG 25416^T), isolated from a water sample of the Urmia Lake, Iran. The type strain has 66.9 mol% G+C content in the genomic DNA. The GenBank accession numbers of strain TBZ3^T for the 16S rRNA gene and genome sequences are EU305729 and VBUI00000000, respectively.

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Conflicts of interest
The authors declare no competing financial conflicts of interests.

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