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EXPERIMENTAL ARTICLES

Halomonas mongoliensis sp. nov. and *Halomonas kenyensis* sp. nov., New Haloalkaliphilic Denitrifiers Capable of N_2O Reduction, Isolated from Soda Lakes

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Abstract—In the course of the search for N_2O-utilizing microorganisms, two novel strains of haloalkaliphilic denitrifying bacteria, Z-7009 and AIR-2, were isolated from soda lakes of Mongolia and Kenya. These microorganisms are true alkaliphiles and grow in the pH ranges of 8.0–10.5 and 7.5–10.6, respectively. They are facultative anaerobes with an oxidative type of metabolism, able to utilize a wide range of organic substrates and reduce nitrate, nitrous oxide, and, to a lesser extent, nitrite to gaseous nitrogen. They can oxidize sulfide in the presence of acetate as the carbon source and nitrous oxide (strain Z-7009) or nitrate (strain AIR-2) as the electron acceptor. The strains require Na^+ ions. They grow at 0.16–2.2 M Na^+ (Z-7009) and 0.04–2.2 M Na^+ (AIR-2) in the medium. The G+C contents of the DNA of strains Z-7009 and AIR-2 are 67.9 and 65.5 mol %, respectively. According to the results of 16S rRNA gene sequencing and DNA–DNA hybridization, as well as on the basis of physiological properties, the strains were classified as new species of the genus *Halomonas*: *Halomonas mongoliensis*, with the type strain Z-7009^T (=DSM 17332, =VKM B2353), and *Halomonas kenyensis*, with the type strain AIR-2^T (=DSM 17331, =VKM B2354).

Key words: haloalkaliphiles, *Halomonas*, soda lakes, denitrification, nitrous oxide, sulfide.

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In nature, denitrifying bacteria play an important role in the nitrogen cycle. Many microorganisms are able to utilize nitrate as an electron acceptor. As distinct from nitrate-reducing organisms which reduce nitrate to nitrite (nitrate respiration), true denitrifiers carry out complete reduction of nitrate to gaseous nitrogen in a series of successive stages of production and consumption of nitrogen oxides.

Denitrifying bacteria are extremely diverse, both in terms of their taxonomic positions and in their physiological properties. Various extremophilic microorganisms, including alkaliphilic ones from alkaline biotopes, participate in the process [1–8]. Several representatives of alkaliphilic denitrifying bacteria involved both in the complete reduction of nitrate to gaseous nitrogen and in the individual stages of this process have been described thus far. Phylogenetically, the heterotrophic alkaliphilic denitrifiers belong to the genus *Halomonas* [1–5], and the autotrophic alkaliphilic denitrifiers belong to the sulfur-oxidizing bacteria of the genus *Thioalkalivibrio* [6, 7]. Moreover, representatives of the *Alkalispirillum-Alkalilimnicola* group possess the capacity for denitrification as well [8]. Among them, only *T. denitrificans* [6], *Halomonas campisalis* Z-7398-2, described by us earlier [5], and strains Z-7008 and AGDZ [8] were isolated from soda lakes on media with nitrous oxide as a sole electron acceptor.

In the present work, novel species of alkaliphilic denitrifying bacteria, *Halomonas mongoliensis* sp. nov. and *Halomonas kenyensis* sp. nov., that are able to reduce nitrous oxide are described.

MATERIALS AND METHODS

Strains and their sources. Strain Z-7009 was isolated by T.N. Zhilina from a sediment sample collected by V.M. Gorlenko in Lake Dzun-Tukhem-Nur (northeastern Mongolia). At the moment of sampling, the pH value of the water was 9.1 and the total salt concentra-

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tion was 27 g/l [9]. Strain AIR-2 was isolated by D.Yu. Sorokin from a mixed sample which contained sediments collected by B.E. Jones from five Kenyan Lakes (Crater Lake, Lake Bogoria, Lake Elmenteita, Lake Nakuru, and Lake Magadi).

The type strains *Halomonas campisalis* $4A^T$ [2], *H. desiderata* $FB2^T$ [1], *H. ventosae* $Al12^T$ [10], and *H. campaniensis* $5AG^T$ [4], needed for comparison, were kindly provided to us by B.M. Peyton, B. Tindall, V. Béjar, and A. Gambacorta, respectively. The type strain *H. alimentaria* $YKJ\text{-}16^T$ [11] was obtained from the DSMZ collection.

Media and cultivation conditions. Enrichment culture of strain Z-7009 was obtained on a medium containing (g/l): Na_2CO_3, 59.0; $NaHCO_3$, 20.0; NaCl, 31.0; KH_2PO_4, 0.2; $MgCl_2 \times 6H_2O$, 0.05; yeast extract, 0.1; $Na_2S \times 9H_2O$, 1.0; and trace element solution, 1 ml (pH 10.0). In optimized medium, the concentrations of Na_2CO_3, $NaHCO_3$, and NaCl were 10.0, 15.0, and 28.0 g/l, respectively (pH 9.3); no changes were made in the other medium components; no sulfide was added. The optimized medium (pH 9.35) for strain AIR-2 contained (g/l) Na_2CO_3, 24.0 and $NaHCO_3$, 38.0; the other component concentrations were the same as for strain Z-7009. Sodium acetate (2 g/l) served as a carbon source and an electron donor, and sodium nitrate or N_2O as an electron acceptor. The effects of pH, salinity, and temperature on the cell growth, as well as the dependence of growth on Na^+, Cl^-, and HCO_3^- ions, were assessed as described previously [5]. In the latter case, 50 mM TABS buffer was used to stabilize the pH of the medium.

Phenotypic characteristics. To elucidate the substrates used for catabolism, they were added to a concentration of 2 g/l. In aerobic conditions, nitrate was replaced by ammonium chloride (0.5 g/l). The tested electron acceptors were added to the following concentrations (mM): $NaHCO_3$, 10; $NaNO_2$, 2; Na_2SO_4, 10; Na_2SO_3, 2 or 10; $Na_2S_2O_3$, 10; and nitrous oxide, 0.5 atm. The electron donors—sodium acetate, sodium formate, sodium sulfide, and hydrogen—were added to concentrations of 15 mM, 30 mM, 4mM, and 1.0 atm, respectively. The capacity of microorganisms for anaerobic fermentation was assessed in the absence of electron acceptors.

Analytical procedures. Growth rate was determined from the optical density of the cell suspension measured at 600 nm with a Spekol-10 spectrophotometer (Jena, Germany) directly in Hungate tubes. Nitrogen was determined chromatographically; nitrite and sulfide were determined colorimetrically, as previously described [5]. The fatty acid composition of microbial lipids was determined chromatographically according to the technique described in [12]. The cells (40–60 mg of wet biomass) were subjected to acid methanolysis at 80°C for one hour in order to release fatty acids from the cell membrane lipids, as well as from the cell wall lipopolysaccharide, in the form of methyl esters. They were extracted two times with 200 μl hexane and evaporated to 60 μl. The obtained extract was analyzed on a Microbial Identification System (Sherlock) chromatograph (MIDI Inc., Newark, United States). The separated fatty acids were identified using an Agilent Technologies AT-5971 SMART mass spectrometer.

Genotypic studies. DNA isolation and purification were performed as described earlier [13]. The G+C content of the DNA was determined from the thermal denaturation curves. The DNA homology levels were determined by the optical reassociation method [14]. Determination and analysis of the 16S rRNA gene nucleotide sequences were carried out as described earlier [13].

The 16S rRNA gene sequences of strains Z-7009 and AIR-2 have been deposited in the GenBank under the accession numbers AY 962236 and AY 962237, respectively.

RESULTS

Pure culture isolation. Strain Z-7009 was isolated under strictly anaerobic conditions with acetate as a substrate and N_2O as an electron acceptor. The pure culture was obtained by plating serial 10-fold dilutions of the enrichment culture onto solid medium (3% agar). The culture purity was confirmed by microscopic examination, as well as by homogeneity of the colonies.

Morphology. The cells of strain Z-7009 are straight or slightly curved short rods, 0.7–2 μm long and 0.5–1 μm in diameter (Fig. 1a). Stationary-phase cells can be up to 5 μm long. The cells are motile by means of a single subpolar flagellum only in young cultures (Fig. 1b) and are usually surrounded by a thick slimy capsule.

The cells of strain AIR-2, at the stage of exponential growth, are thin short rods 1.5–2.5 μm long and 0.5–0.7 μm in diameter (Fig. 2a). Under unfavorable conditions, they produce thin curved filaments. The cells are actively motile by means of long peritrichous flagella (Fig. 2b).

The organisms reproduce by binary division. Their cell wall structure is of the gram-negative type. On agarized medium, the strains form rounded cream-colored colonies, 1.0–2.0 mm in diameter, with smooth edges and a homogeneous structure. The colony surface is smooth, lustrous, and opaque. Both strains are catalase- and oxidase-positive; they do not form spores and are temperature-sensitive. After 10-min incubation at 70°C, no growth occurred.

Growth characteristics. Strain Z-7009 grows at 0.16–2.2 M Na^+ in the medium with a broad optimum at 0.7–1.7 M and may be assigned to moderate halophiles (Fig. 3a). The organism is an obligate alkaliphile, since it grows in a pH range of 8.0–10.5 with an optimum at 8.5–9.6 (Fig. 3b). At the optimum pH values and salinities, the culture grows within a wide temper-

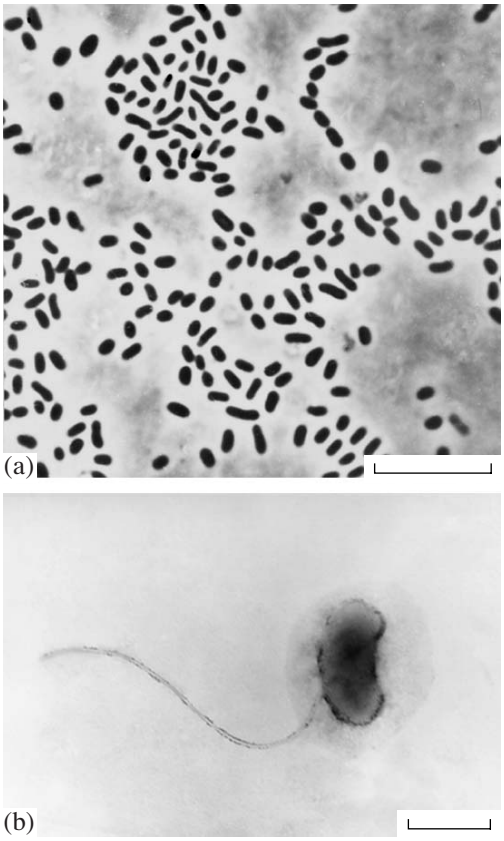

Fig. 1. Morphology of strain Z-7009: (a) cells under a light microscope (phase contrast; scale bar, 10 μm); (b) a cell with a flagellum and capsule under an electron microscope (negative staining with phosphotungstic acid; scale bar, 0.5 μm).

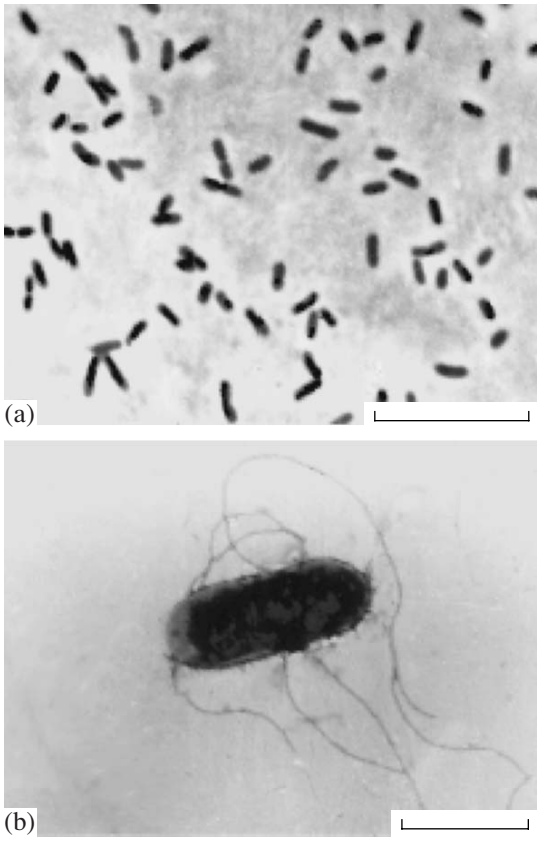

Fig. 2. Morphology of strain AIR-2: (a) cells under a light microscope (phase contrast; scale bar, 10 μm); (b) negatively stained cells with peritrichous flagella (scale bar, 1.0 μm).

ature range of 15–50°C with an optimum of 36–40°C (Fig. 3c). The growth rate reaches its peak at 40°C; however, under these conditions, the culture lyses rapidly, which does not occur at 36°C and lower temperatures.

Strain AIR-2 grows at 0.04–2.2 M Na^+ with an optimum at 0.5–1.2 M Na^+ in the medium (Fig. 3a). According to D. Kushner's classification, the organism can be considered a facultative halophile, since it can grow at a Na^+ concentration lower than 0.1 M [15]. It is a true alkaliphile and develops within the alkaline pH range (between 7.5 and 10.6), with an optimum of 9.5 (Fig. 3b). The strain grows in a temperature range from 10 to 55°C with an optimum at 36–40°C. (Fig. 3c). The strains obligately require Na^+ ions. Their growth is equally possible with NaCl + TABS buffer (pH 9.3) or in medium with sodium carbonates; thus, chloride and carbonate anions are interchangeable.

The novel microorganisms are facultative anaerobes, like the majority of the presently known denitrifying bacteria. They have the capacity for both aerobic and anaerobic oxidation of a wide spectrum of organic substrates of various types; however, they are not capable of fermentation. The spectra of substrates utilized by both strains are quite similar; however the organisms were found to be different in their reactions to sugars (see Tables 1 and 2 and species descriptions). Strain AIR-2 shows good growth on glucose, sucrose, maltose, xylose, fructose, and trehalose (and somewhat weaker growth on ribose and mannose) both under aerobic and anaerobic conditions. Strain Z-7009 utilizes pentoses (ribose, arabinose, xylose) and ketohexoses (fructose, sorbose) under aerobic conditions; ribose and xylose are preferential, whereas growth on other sugars was very weak and began after a prolonged lag phase. Under anaerobic conditions, the growth of this strain occurred only on ribose.

We tested nitrogen-containing compounds, such as nitrate, nitrite, and nitrous oxide, as electron acceptors in combination with various potential electron donors, such as acetate, formate, hydrogen, and sulfide. Acetate served as a carbon source in the hydrogen- and sulfide-containing media. It was found that, under anaerobic conditions, both strains reduced nitrate, nitrous oxide, and, to a lesser extent, nitrite to gaseous nitrogen, using acetate as a carbon source and electron donor. In the absence of acetate, growth, as well as acceptor reduc-

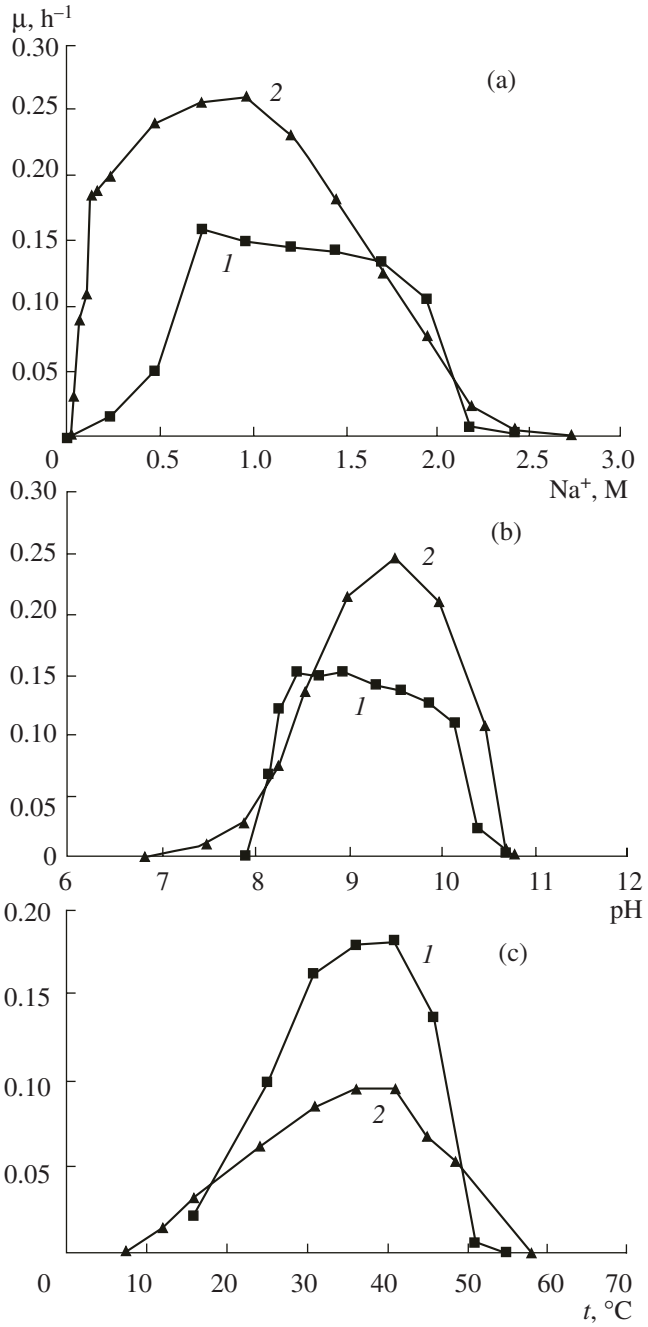

Fig. 3. Effect of (a) Na^+ concentration, (b) pH, and (c) temperature on the specific growth rates of (*1*) strain Z-7009 and (*2*) strain AIR-2.

tion, were not observed. The ability of microorganisms to utilize sulfide as an electron donor under heterotrophic conditions is of particular interest. Strain AIR-2 oxidized sulfide only with nitrate; strain Z-7009, only with N_2O. The strains differ with respect to their sensitivity to sulfide: the growth of Z-7009 was inhibited at the initial concentration of 9 mM, whereas strain AIR-2 grew at a concentration of 28 mM, which, possibly, allows it to survive in the zone of active sulfidogenesis. No other donor–acceptor pairs were utilized. The reduction of sulfur-containing acceptors, such as sulfate, sulfite, and thiosulfate, was not observed as well.

Analysis of the fatty acid composition. Table 3 shows the results of the analysis. The strains Z-7009 and AIR-2 are close with respect to their fatty acid profile, confirming their affiliation to one genus. It was found that 11,12-octadecenoic acid 18:1ω7c is their main fatty acid (67.97% in Z-7009 and 62.91% in AIR-2). The levels of hexadecanoic (16:0) and 9-hexadecenoic acids (16:1ω7) were relatively high; however, their proportions were different in these two organisms. In addition, there was also a difference in the contents of tetradecanoic acid (14:0), which were 0.47% and 3.39% in Z-7009 and AIR-2, respectively.

Phylogenetic analysis. According to the results of partial 16S rRNA gene sequencing (about 1370 nucleotides between *E. coli* positions 70 and 1448), strains Z-7009 and AIR-2 belong to the genus *Halomonas* of the class of *Gammaproteobacteria.* The level of similarity with the known species was 92.8–98.0%. The phylogenetic tree in Fig. 4 illustrates the taxonomic position of the strains within the genus *Halomonas.* The DNA–DNA homology between strains AIR-2 and Z-7009 (50%) conforms to an interspecific level of relatedness.

DISCUSSION

The genus *Halomonas* is a large group which contains more than 30 species of aerobic or facultatively anaerobic chemoorganotrophic halotolerant microorganisms with an oxidative type of metabolism. The facultatively anaerobic representatives of this genus can reduce nitrate; however, in most cases, this process is incomplete and terminates after the nitrite formation. Besides, only some *Halomonas* species may be considered true alkaliphiles, despite their ability to grow within the alkaline pH range. Hence, our isolates enhance the diversity of the few known obligately alkaliphilic nitrate reducers capable of complete reduction of nitrate to gaseous products [1, 3, 18].

The fact that the new halophilic *Halomonas* species utilize acetate indicates that they may fulfil an important ecological function in soda lakes. It is well known that acetate, along with formate, hydrogen, and carbon dioxide, is a main terminal product of organic matter fermentation by the anaerobic microbial community. In most habitats, acetate is utilized by aceticlastic methanogens and sulfate reducers in the anaerobic zone. However, these processes are blocked in hypersaline lakes [16], whereas aerobic respiration and denitrification (which yield more energy) may occur at high salt concentrations approximating saturation. Several strains of the *Alkalispirillum/Alkalilimnicola* group [8] and most *Halomonas* species [4, 10, 17] are distinguished by their capacity for anaerobic oxidation of acetate in hypersaline media. Taking into account the

Table 1. Differentiating characteristics of strain Z-7009 and phylogenetically close representatives of the genus *Halomonas*

Characteristic	Strain Z-7009	*H. ventosae* Al12^T	*H. alimentaria* YKJ-16^T
Cell size, μm	0.7–2.0 × 0.5–1.0	1.2–1.4 × 0.7–0.8	0.8–1.2 × 0.8–1.2
Motility	+	+	–
Flagellation type	monotrichous	N/D	–
pH (range/optimum)	8–10.5/8.5–9.6	6–10/ND	ND/6.5–7.5
NaCl (range/optimum)	0–12*/3–9*	3–15/8	1–24/1–13
Oxidized substrates:			
ethanol	+	–	–**
glycerol	–	+	–**
gluconate	–	+	ND
D-glucose	–	+	+**
sucrose	–	ND	+**
maltose	–	+	ND
lactose	–	+	–**
D-trehalose	–	+	–**
D-galactose	–	+	ND
L-arabinose	+	–	–**
L-sorbitol	–	+	–**
L-alanine	+	–	+**
Tween 80	+	–	–
$HS^- + N_2O$	+	ND	–**
SeO_3^{2-} reduction	–	+	ND
G+C, mol %	67.9	74.3***	63.0

Notes: Data for were taken from [10] and data for *H. alimentaria* are from [11].
* Against the background of the 0.16 M Na^+ of the carbonates.
** These data on substrate utilization were obtained by us.
*** 69 mol %, according to our data.

high degree of halophily of halomonads, it can be anticipated that this group is important in the provision of the acetate sink in the alkaliphilic microbial community.

The possible ecological role of haloalkaliphilic halomonads also consists in their involvement in the nitrogen cycle, including N_2O transformation. Thus, there is a potential for the natural utilization of this greenhouse gas in soda lakes.

An important trait of the new isolates is their capacity for heterotrophic sulfide oxidation. Strain AIR-2 oxidized sulfide only in combination with nitrate, as was reported earlier for several heterotrophs of the genus *Halomonas* [18]. On the contrary, the capacity of strain Z-7009 for sulfide oxidation in combination with N_2O is the first case of detection of such a capacity in obligately heterotrophic bacteria, including *Halomonas*. This trait was previously reported for the phylogenetically distant chemolithoautotroph *Thioalkalivibrio denitrificans* [6], as well as for the facultatively autotrophic strain Z-7008, closely related to *Alkalispirillum/Alkalilimnicola* [8]. In the latter case, the process was stimulated by acetate; however, the reaction occurred in the absence of acetate as well. It should be noted that marine denitrifiers are unable to oxidize sulfide, whereas this ability is frequent among microorganisms isolated from soda lakes. Oxidation of sulfide in combination with reduction of nitrogen compounds may point to the possible coupling of two important natural cycles, nitrogen and sulfur, in soda lakes.

The diversity of metabolic processes, as well as the ability to grow in a wide range of pH, salinity, and temperature and to use both aerobic and anaerobic respiration pathways, seem to enable the newly isolated organisms to easily adapt to various physicochemical conditions of the soda lakes of the equatorial and cryoarid

Table 2. Differentiating characteristics of strain AIR-2 and phylogenetically close representatives of the genus *Halomonas*

Characteristic	Strain AIR-2	*H. campisalis* 4A^T	*H. desiderata* FB2^T	*H. campaniensis* 5AG^T
Cell size, μm	1.5–2.5 × 0.5–0.7	3–5 × 1	1.0–2.6 × 0.4–0.6	2–2.2 × 0.3–0.6
Flagellation type	peritrichous	ND	peritrichous	ND
pH (range/optimum)	7.5–10.6/9.5	6–11/9.5	7–11/9.5	7–10/9.0
NaCl (range/optimum)	0–13*/3–7*	0.2–25/ND	0–18/ND	0–16/10
Oxidized substrates:				
propionate	+	+**	–***	ND
glycolate	+	–**	+**	ND
ethanol	+	+	–***	ND
formate	–	–**	+***	ND
betaine	–	–**	+**	ND
glycerol	–	+	+	+
ribose	+	–	+	ND
D-xylose	+	–	+	–
D-mannose	+	–	+	+
D-galactose	–	–	+	–
L-arabinose	–	–	+	–
N-acetyl-D-glucosamine	–	+	ND	ND
L-alanine	+	+**	–	ND
arginine	+	–**	+/–**	ND
Tween 80	+	ND	–	+
$HS^- + NO_3^-$	+	+**	+**	+**
G+C, mol %	65.5	66.0	66.0	63.7

Notes: Data for *H. campisalis* are from [2], data for *H. desiderata* from [1], and data for *H. campaniensis* from [4].
* Against the background of the 0.04 M Na^+ of the carbonates.
** These data on substrate utilization were obtained by us.
*** Data from [17].

zones. Utilization of a wide spectrum of various organic substrates allows them to use various pathways of organic matter utilization and survive the competition for the carbon source within the microbial community. This metabolic universality determines the importance of the representatives of the genus *Halomonas* in the trophic system of the community and explains their abundance in soda lakes.

According to their chemotaxonomic properties, the new isolates conform to their affiliation with the genus *Halomonas*. According to their fatty acid profile, they are close to the species H. *campisalis* [2], *H. desiderata* [1], and *H. alimentaria* [11], which are phylogenetically close. In general, the high level of unsaturated fatty acids, as well as the presence of 3-hydroxy-dodecanoic and C19 cyclopropanoic acids, are common features of the genus *Halomonas.* At the same time, the fatty acid profile cannot be a reliable differentiating feature of species within the genus, since the fatty acid composition may vary even within one species [20].

Strain Z-7009 is phylogenetically close to *H. alimentaria* (similarity level, 97.5%) and *H. ventosae* (97.8%). The levels of DNA–DNA hybridization with the type strains were 29 and 64%, respectively. The level of DNA–DNA hybridization with *H. ventosae* was high; however, it was insufficient to affiliate the strain to this species [19]. Table 1 shows the results of comparison of the three organisms. Strain Z-7009 is the first obligate haloalkaliphile of this cluster which does not grow at pH 7 and below. *H. alimentaria*, which has an optimal pH range of 6.5–7.5, is a typical representative of neutrophilic organisms. The pH range and optimum for *H. ventosae* A112^T growth are close to those of Z-7009. However, due to the ability of *H. ventosae* to grow at pH 6, we cannot consider it a true alkaliphile. The new isolate differs from *H. alimentaria* YKJ-16^T by its inability to grow on glucose and sucrose, as well

as by the capacity for growth on ethanol, arabinose, and Tween 80 and by the ability to oxidize sulfide in combination with N_2O. Strain Z-7009 differs from *H. ventosae* A112^T by the ability to utilize ethanol, arabinose, alanine, and Tween 80 as substrates and to oxidize sulfide, as well as by the inability to reduce selenite and to grow on glycerol, gluconate, glucose, maltose, lactose, or trehalose. On the basis of the existing genotypic and phenotypic differences, we propose that strain Z-7009 should be described as a novel species *Halomonas mongoliensis* sp. nov.

Strain AIR-2 fell into the group of alkaliphilic halomonads together with *H. campisalis* (similarity level 97.5%), *H. desiderata* (97.9%), and *H. campaniensis* (97.5%). The levels of DNA–DNA hybridization with the type strains of these species (36, 52, and 27%, respectively) indicate that strain AIR-2 is to be classified as a novel species. Table 2 shows the differentiating characteristics of the above-mentioned species. Strain AIR-2 differs from the closest (according to DNA–DNA homology) type strain *H. desiderata* FB2^T by the ability to grow on propionate, ethanol, alanine, and Tween 80, as well as by the inability to utilize formate, betaine, glycerol, galactose, and arabinose. AIR-2 differs from the type strain *H. campisalis* 4A^T by the cell size, the ability to utilize glycolate, ribose, xylose, mannose, and arginine as substrates, and by the inability to grow on glycerol or N-acetyl-D-glucosamine. On the basis of DNA–DNA hybridization data and the existing phenotypic differences, we propose that strain AIR-2 should be described as the type strain of the novel species *Halomonas kenyensis* sp. nov.

Description of *Halomonas mongoliensis* sp. nov. Mon.go.li`en.sis; N. L. fem. adj. Mongolian, of Mongolia, the region of isolation.

Rod-shaped, straight, or curved gram-negative cells measure 0.7–2 × 0.5–1 μm, are temperature sensitive and non-spore-forming. In young cultures, the cells are motile by means of a single subpolar flagellum.

True alkaliphile. Growth occurs in a pH range of 8.0–10.5, with an optimum at 8.5–9.6, and in a salinity range of 0.16–2.2 M Na^+, with an optimum at 0.7–1.7 M Na^+. Na^+ ions are obligately required; carbonate and chloride are not. Mesophile. The temperature range for growth is 15–50°C, with an optimum at 36–40°C.

Facultative anaerobe, reduces nitrate, nitrous oxide, and, to a lesser extent, nitrite. Catalase- and oxidase-positive. Incapable of fermentation. Chemoorganoheterotrophic. During anaerobic growth with nitrate, oxidizes acetate, propionate, butyrate, succinate, glycolate, fumarate, citrate, pyruvate, lactate, ethanol, ribose, peptone, yeast extract, Casamino acids, glutamate, aspartate, alanine, and proline. Under aerobic conditions, oxidizes, in addition, xylose and, to a lesser extent, arabinose, fructose, and sorbose. Hydrolyzes Tween 80. Does not utilize oxalate, formate, methanol, glycerol, trimethylamine, betaine, gluconate, glucose, sucrose, maltose, trehalose, mannose, lactose, galactose, fucose, rhamnose, sorbitol, dulcitol, N-acetyl-*D*-glucosamine, arginine, and histidine. Capable of heterotrophic oxidation of sulfide with nitrous oxide as an electron acceptor. Sensitive to high sulfide concentrations. 11,12-octadecenoic, hexadecanoic, and 9-hexadecenoic acids prevail in the cell membranes (67.97%, 12.13%, and 10.61%, respectively).

Table 3. Composition of fatty acids in the membranes of strains Z-7009 and AIR-2

Fatty acid	% of the total fatty acid content	
	Z-7009	AIR-2
10:0	2.20	2.80
12:1	0.22	
12:0	1.96	0.62
3h12	0.57	0.73
14:0	0.47	3.39
16:1ω7	10.61	4.79
16:0	12.13	20.70
18:1ω9c	0.36	0.41
18:1ω7c	67.97	62.91
18:0	1.06	1.53
11-Me-8:1	0.30	
19:0 cycω8	1.40	1.52
20:2ω6,9c	0.46	0.60
20:1ω9c	0.29	
Total	100	100

The G+C content of DNA is 67.9 mol %. The type strain is Z-7009^T (=DSM 17332, = VKM B2353), isolated from Lake Dzun-Tukhem-Nur (northeastern Mongolia).

Description of *Halomonas kenyensis* sp. nov. Ke.ny.en`sis; N. L. fem. adj. Kenyan, of Kenya, the region of isolation.

Rod-shaped, thin, short gram-negative cells measure 1.5–2.5 × 0.5–0.7 μm and are actively motile by means of peritrichous flagella. The cells are temperature sensitive and non-spore-forming.

True alkaliphile. Growth occurs in a pH range of 7.5–10.6, with an optimum at 9.5. Na^+ ions are obligately required; carbonate and chloride are not. Facultative halophile. Growth occurs in a salinity range of 0.04–2.2 M Na^+, with an optimum at 0.5–1.2 M Na. Mesophile. The temperature range for growth is 10–55°C, with an optimum at 36–40°C.

Facultative anaerobe, reduces nitrate, nitrous oxide, and, to a lesser extent, nitrite. Catalase- and oxidase-positive. Metabolism is of an oxidative type and chemoorganoheterotrophic. In the presence of oxygen

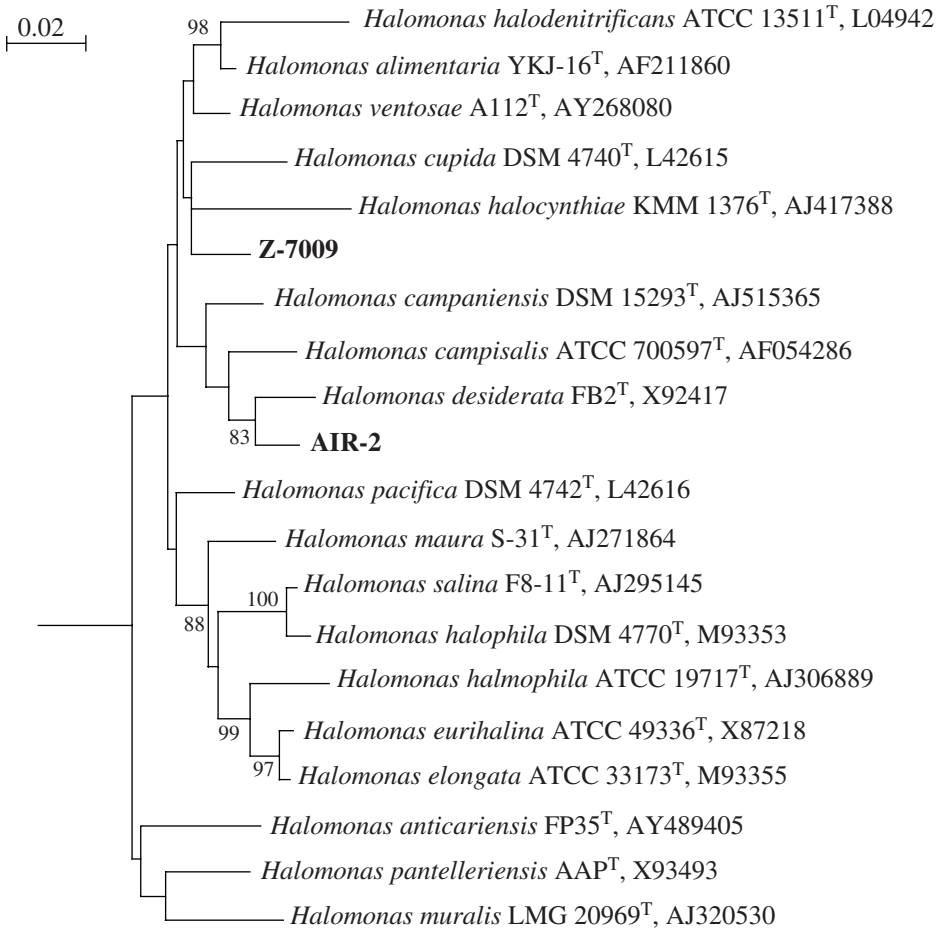

Fig. 4. Phylogenetic positions of strains Z-7009 and AIR-2 among members of the genus *Halomonas.* Bar corresponds to 2 nucleotide substitutions per 100 nucleotides.

or nitrate as an electron acceptor, the following substrates are oxidized: acetate, propionate, butyrate, succinate, glycolate, fumarate, citrate, pyruvate, lactate, ethanol, glucose, sucrose, maltose, xylose, fructose, trehalose, peptone, yeast extract, casamino acids, glutamate, aspartate, alanine, arginine, proline, histidine, and, to a lesser extent, ribose and mannose. Tween 80 is hydrolyzed. Oxalate, formate, methanol, glycerol, trimethylamine, betaine, galactose, arabinose, fucose, rhamnose, lactose, sorbitol, dulcitol, and N-acetyl-D-glucosamine are not oxidized. Sulfide can be oxidized heterotrophically with nitrate as an electron acceptor. Sulfide-tolerant. 11,12-octadecenoic and hexadecanoic acids prevail in the cell membranes (62.91% and 20.70%, respectively).

The G+C content of DNA is 65.5 mol %. The type strain is AIR-2^T (=DSM 17331; =VKM B2354), isolated from a mixed sample which contained sediments collected from five Kenyan Lakes (Crater Lake, Lake Bogoria, Lake Elmenteita, Lake Nakuru, and Lake Magadi).

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