EXPERIMENTAL ARTICLES

Halonatronum saccharophilum **gen. nov. sp. nov.: A New Haloalkaliphilic Bacterium of the Order** *Haloanaerobiales* **from Lake Magadi**

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Abstract—A new alkaliphilic and moderately halophilic chemoorganotrophic anaerobic bacterium (strain Z-7986), which is spore-forming, rod-shaped, and has a gram-negative cell wall pattern, was isolated from the coastal lagoon mud of the highly mineralized Lake Magadi (Kenya). The organism is an obligatorily carbonate- and sodium chloride-dependent motile peritrichously flagellated rod that grows within a 3–17% NaCl concentration range (with an optimum at 7–12% NaCl) and within a pH range of 7.7–10.3 (with an optimum at pH values of 8–8.5). It is a moderate thermophile with a broad temperature optimum at $36-55^{\circ}$ C; maximum growth temperature is 60°C. The bacterium catabolizes glucose, fructose, sucrose, maltose, starch, glycogen, *N*-acetyl-D-glucosamine, and, to a slight degree, peptone and yeast extract. Its anabolism requires yeast extract or casamino acids. Glucose fermentation yields formate, acetate, ethanol, H_2 , and CO_2 . The bacterium is sulfide-tolerant and capable of the nonspecific reduction of S^0 to H₂S. The G+C content of the DNA is 34.4 mol %. The analysis of the 16S rRNA sequence revealed that strain Z-7986 belongs to the order *Haloanaerobiales* and represents a new genus in the family *Halobacteroidaceae.* We suggest the name *Halonatronum saccharophilum* gen. nov. sp. nov. The type strain of this species is $Z-7986$ ^T (= DSM13868, = Uniqem*211).

Key words: alkaliphiles, halophiles, anaerobes, saccharolytic bacteria, the order *Haloanaerobiales.*

The order *Haloanaerobiales* [1] includes gram-negative organotrophic bacteria whose spore-forming representatives form the family *Halobacteroidaceae.* Haloanaerobes are strictly dependent on high ambient concentrations of Na+ ions in the form of sodium chloride (for halophiles) and/or sodium carbonate (for alkaliphiles). Most of the haloanaerobes described to date are halophilic bacteria that ferment carbohydrates and develop within the neutral range of pH values. Bodies of water of marine origin are their typical habitats in which they represent the group of primary anaerobes in the trophic system of the anaerobic halophilic community [2].

Alkaliphilic haloanaerobes growing at pH 10 and inhabiting intracontinental soda-containing bodies of water are represented by only one species of the genus *Natroniella* in the order *Haloanaerobiales* [3]. This homoacetic bacterium that was isolated from Lake Magadi (Kenya) does not use carbohydrates and is analogous to *Acetohalobium* with respect to its metabolism [4].

The research on alkaliphilic saccharolytic anaerobes forming part of the anaerobic community of soda lakes has been initiated relatively recently [5]. Several works dealt with the phylogenetic diversity of these organisms [6, 7]. It was established that they are widespread inhabitants of soda lakes and include representatives of different phylogenetic lineages. New alkaliphilic spirochetes from Lake Magadi and from lakes of Central Asia were isolated and characterized [8]. Several new saccharolytic clostridia that have not yet acquired a taxonomic status were isolated from the Elementeite, Bogoria, and Magadi lakes in Africa [6]. The continuation of our studies involved the isolation of new saccharolytic obligate and facultative anaerobic bacteria from Lake Magadi and soda lakes of the Transbaikal Region to determine the functional and phylogenetic diversity of the alkaliphilic anaerobic community [9]. It was established that two of the strains isolated were phylogenetically related to bacilli, two other strains were related to clostridia, and strain Z-7986 was related to haloanaerobes [7].

This work deals with the alkaliphilic and moderately halophilic strain Z-7986 that was isolated from the continental soda Lake Magadi. It is a new alkaliphilic representative of the order *Haloanaerobiales.* It belongs to a separate lineage in the family *Halobacteroidaceae.* The strain is described herein as a new genus and a new species termed *Halonatronum saccharophilum* gen. nov., sp. nov.

^{*}Uniqem (Institute of Microbiology, RAS) (URL: http://inmi.da.ru).

Fig. 1. Morphology of strain Z-7986. (a) Vegetative cells of a young culture and (b) cells of an old culture under a phase-contrast microscope; note the round spheroplasts and the cells with forming spores. (c) Cells with peritrichous flagellation under an electron microscope.

Fig. 2. Ultrathin sections of (a) a vegetative and (b) a sporulating cell.

MATERIALS AND METHODS

Source. Strain Z-7986 was isolated from mud sampled by G.A. Zavarzin in the coastal lagoon of Lake Magadi (Kenya) in September 1998. The water in the lagoon was saturated with soda and was characterized by a pH value of 10.2 and a mineralization degree of 260 g/l. Its temperature was 39°C.

Cultivation conditions. Initial enrichment and isolation of strain Z-7986 was carried out using a modification of the medium described earlier [3]. The

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Fig. 3. Dependence of the specific growth rate on (a) salinity, (b) pH, and (c) temperature values.

medium composition was as follows (g/l): KH_2PO_4 , 0.2; MgCl₂, 0.1; NH₄Cl, 0.5; KCl, 0.2; NaCl, 50.0; $Na₂CO₃$, 68.0; NaHCO₃, 38.0; Na₂S · 9H₂O, 0.7; yeast extract, 0.2; sucrose, 5.0; trace element solution [10], 1 ml/l; vitamin solution [11], 10 ml/l; and resazurin, 0.001 (pH 9.75). Catabolizable substrates were identified as follows. The substrates were added at a concentration of 3 g/l against a background of 0.2 g/l of yeast extract. To avoid the caramelization of carbohydrates in the alkaline medium during sterilization, their sterile aqueous solutions were added to the medium immediately before inoculation. Medium preparation and cultivation were carried out under strictly anaerobic conditions in an N_2 atmosphere.

Physiological properties. Electron acceptors were added as concentrated solutions with a microsyringe to sterile media. Their final concentrations are as follows: $Na₂S₂O₄$, 1mM; $Na₂SO₃$, 2 mM; $Na₂SO₃$, NaNO₂, NaNO₃, Na₂S₂O₃ · 5H₂O, and Na₂SO₄, 10 mM; $Na_2S_2O_3 \cdot 5H_2O$, 20 mM; and S^0 , 2% (w/v). The utilization of sulfur-containing acceptors was determined from hydrogen sulfide evolution with sodium thioglycollate used as a reductant. Nitrite reduction was determined using the Griess reagent. The $N₂$ -fixation capacity was tested in a medium lacking nitrogen sources other than N_2 . In order to investigate the pH dependence of the tested activities, the pH value was adjusted by titration of the medium with 10% HCl or 10% NaOH, and sodium carbonate was replaced by sodium bicarbonate at a concentration decreased by a factor of 10. The sodium carbonate dependence of the tested processes was investigated using a system in which an equimolar amount of NaCl was substituted for sodium carbonate; the pH was adjusted to 9.0 using 50 mM serine buffer. The NaCl requirement was determined by replacing NaCl with equimolar amounts of sodium bicarbonate and carbonate; all other chlorides were replaced with sulfates. The temperature dependence was investigated within the 6–65°C range at optimum pH values and sodium chloride concentrations.

Analytic methods. Bacterial growth was estimated by measuring the optical density in Hungate tubes with a Specol-10 spectrophotometer (Jena) at 600 nm. Glucose was determined using the phenol reaction [12]. Hydrogen and nitrogen were determined with an LKhM-80 gas chromatograph equipped with a katharometer; volatile fatty acids were determined using a model 3700 gas chromatograph with a flame ionization detector. Formate was measured colorimetrically [13]. Dissolved hydrogen sulfide was determined colorimetrically based on the methylene blue formation reaction [14].

Morphology. Cell morphology was investigated under a phase-contrast light microscope (ZETOPAN, Austria). Photographs were taken using agarose slide film [15]. Sections were prepared as described earlier [3]. Sections and cells (stained with 1% phosphotungstic acid to detect flagella) were examined under a JEM-100C electron microscope (Japan).

Determination of the nucleotide sequence of the 16S rRNA gene. Amplification and sequencing of the fragments of the 16S rRNA gene of the tested strain was performed as described earlier [7].

Analysis of the nucleotide sequence of the 16S rRNA gene. The nucleotide sequence of the 16S rRNA gene of strain Z-7986 was manually aligned with the corresponding sequences of the known species of the order *Haloanaerobiales* using the sequence editor BIOEDIT. A rooted phylogenetic tree of the tested strain was constructed with the help of several algorithms contained in the program packages TREECON [16] and PHYLIP [17]. *Bacillus subtilis* was used as an outgroup.

The 16S rDNA sequence of strain Z-7986^T is deposited with GenBank (accession number AY014858).

RESULTS

Isolation. An enrichment culture was obtained in an anaerobically prepared liquid medium with sucrose as the main energy source at 37°C. Growth was detected one day later and long flexible motile rods dominated the culture. Part of the rods contained plectridial spores. The organism displays the *Halobacteroidaceae*-typical morphological pattern characterized by flexible cells that tend to curl up and form spheroplasts at the end of the growth period. As early as in the first stage of our study, this suggested that the organism might be a haloanaerobe. A pure culture was obtained using a series of subcultures in a medium with sucrose based on the limiting dilution method. The culture purity was confirmed by the fact that only one type of colony formed on an agarized medium. The colonies were greenish in color and were elevated above the medium surface. They were 0.5–1 mm in diameter with indistinctly shaped smooth edges. The culture obtained from one of these colonies, strain Z-7986, was used in subsequent studies.

Morphology and ultrastructure. The morphology of the cells of strain Z-7986 markedly depends on the growth stage. The cells are actively motile and slender during the exponential growth phase, which lasts an average of 17 h. Their average diameter and length are 0.4–0.6 and 3.5–3.75 µm, respectively, if the cells divide evenly. The organism reproduces by binary division with a constriction, but the division is not always even, and the culture may contain short $(1.8-2 \mu m)$ and long $(10 \mu m)$ and longer) cells (Fig. 1a). Cells that initiate sporulation or contain mature spores are significantly thicker than vegetative cells (Figs. 1a, 1b). At the late exponential growth stage which lasts 3 h, the cells are curved in a horseshoe-like fashion or curled up to form a ring; some of the cells lyse, yielding spheroplasts. Mature spores form at this growth stage. They are spherical in shape (1.25 µm in diameter) and located at one of the cell's poles (Fig. 1b).

Using an electron microscope, we detected thin flagella that are peritrichously arranged along the whole length of the cell (Fig. 1c). Ultrathin sections revealed cell walls with outer membranes, which is typical of gram-negative bacteria (Fig. 2).

Growth parameters. Strain Z-7986 grows within a wide salinity range. Its growth occurred at 3 to 17% NaCl (w/v) with an optimum at 7–10% NaCl and an upper limit at 18% NaCl (Fig. 3a). Cells are lysed at NaCl concentrations below 3%. The strain obligatorily requires NaCl, and its growth did not occur with NaCl replaced by an equimolar amount of Na_2CO_3 + $NaHCO₃$.

Strain Z-7986 develops in the alkaline pH range with a pH optimum of $8-\overline{8}$.5 and pH limits of $7.7-10.2$. No growth occurred at pH values of 7.0 and 10.5 (Fig. 3b). The organism obligatorily requires $Na_2CO_3 + NaHCO_3$, and it did not grow if carbonates were replaced by an

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Substrates	Maximum optical density, $\lambda = 600$ nm; 18 h
D-Glucose	0.290
D-Fructose	0.200
Sucrose	0.280
D-Maltose	0.370
Starch	0.280
Glycogen	0.380
N -Acetyl-D-glucosamine	0.085
Peptone	0.025
Yeast extract	0.050

Note: Strain Z-7986^T does not use the following substrates: D-ribose, L-arabinose, D-xylose, D-mannose, D-galactose, sorbose, D-fucose, D-lactose, trehalose, D-cellobiose, melibiose, glycerol, L-sorbitol, mannitol, dulcitol, L-inositol, erythritol, xylan, microcrystalline cellulose, pectin, gum arabic, carboxymethylcellulose, agar-agar, gelatin, formate, acetate, propionate, butyrate, glycolate, lactate, pyruvate, malonate, succinate, methanol, ethanol, trimethylamine, choline chloride, betaine, and casamino acids.

equimolar amount of NaCl while maintaining the medium pH by 50 mM serine buffer (pK_a = 9.2).

At optimum pH values and NaCl concentrations, the temperature optimum for the growth of strain Z-7986 corresponds to a broad range of values (36–55°C). Growth is possible within the range of 18 to 60°C (Fig. 3c). No growth occurred at 6 or 65°C.

Strain Z-7986 is an obligate anaerobe. It does not grow aerobically or in a medium without reductants. With thioglycollate as the reductant, its growth is somewhat worse than with sodium sulfide. Cysteine can be used as the reductant and as the sulfur and nitrogen source. The strain is incapable of $N₂$ fixation or of dissimilatory reduction of inorganic nitrogen compounds $(NO₃⁻, and NO₂⁻)$ or sulfur compounds $(SO₄²⁻, SO₃⁻,$ and $S_2O_3^{2-}$). However, it can reduce S^0 , forming up to 16.5 mM of H_2S . The strain exhibited a relatively high tolerance to sulfide, but an initial hydrogen sulfide concentration of 28 mM completely suppressed its growth. In a medium with glucose, growth was neither inhibited nor stimulated in the presence of the tested acceptors and sodium dithionite.

Strain Z-7986 utilizes glucose, fructose, sucrose, maltose, starch, glycogen, *N*-acetyl-D-glucosamine, and, to a slight degree, peptone and yeast extract as the energy sources (Table 1). Growth was lacking with hexoses (other than those listed above), pentoses, disaccharides, sugar alcohols, cellulose, mono- and dicarboxy-

Fig. 4. Unrooted phylogenetic tree of haloanaerobes (based on the comparative analysis of the sequences of the 16S rRNA gene) that indicates the position of strain Z-7986 in the system of the order *Haloanaerobiales.* Bar, 5 nucleotide substitutions per 100 nucleotides. Numbers refer to the statistical significance of each branching order based on the data of bootstrap analysis (values over 85 are considered significant).

lic acids, monoatomic alcohols, amines, casamino acids, and insoluble polysaccharides. The strain failed to liquefy gelatin or agar (see note in Table 1). Its growth with glucose required yeast extract, which could be replaced by casamino acids. The yield was proportional to the casamino acid concentration within the 250–1000 mg/l concentration range. The amino acids methionine and serine are the growth factors required; 10 mg/l of each are sufficient for optimal growth.

Glucose fermentation yields acetate, formate, ethanol, $H₂$, and $CO₂$. If the strain uses 24.4 mmol of glucose, it produces 25.8 mmol of acetate, 12.7 mmol of ethanol, 52.6 mmol of formate, and 1.7 mmol of hydrogen. The fermentation is, therefore, characterized by a 97 or 95.5% balance (based on carbon and hydrogen, respectively). Doubling time under optimal growth conditions is 2.5 h.

DNA analysis. The G+C content of the DNA of strain Z-7986 was 34.4 mol %, based on its melting point [7].

Phylogenetic analysis. We obtained an almost complete 16S rDNA sequence of strain Z-7986. It consists of 1457 nucleotides, corresponding to positions 45−1470 in the *E. coli* numbering. A preliminary comparative analysis of the sequence obtained was carried out using the BLAST programs and GenBank data. We confirmed our earlier data on the phylogenetic relationship between strain Z-7986 and haloanaerobes [7]. The position of this strain on the phylogenetic tree, including all known representatives of the order *Haloanaerobiales*, is as follows. The tested strain belongs to a subdivision of the family *Halobacteroidaceae*, forming a separate lineage in a cluster that also includes other spore-forming haloanaerobes, such as species of the genus *Orenia* and the alkaliphilic homoacetogenic bacteria *Natroniella* (Fig. 4). The degrees of similarity of the 16S rDNA of strain Z-7986 and its closest relatives in the family *Halobacteroidaceae* to the species of the genus *Orenia* and to *Natroniella acetigena* were 91.4−92.8% and 90.3%, respectively. The analysis of the secondary structure of the 16S rRNA of strain

Table 2

Note: ND, no data; "+", used as energy source; "-", not used as growth substrate; "+/-", used as growth substrate to a slight degree.

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Z-7986 revealed a *Halobacteroidaceae*-specific helix at positions 60–100 in the *E. coli* numbering [1], a shortened helix (characteristic of the genera *Orenia, Natroniella, Halobacteroides*, and *Haloanaerobacter*) at positions 100–200 [18], and an extended helix (characteristic of the genera *Orenia, Halobacteroides*, and *Haloanaerobacter*) at positions 1435–1466 [18].

DISCUSSION

The strain isolated by us is a gram-negative sporeforming moderately halophilic obligatorily anaerobic chemoorganotrophic bacterium with a low G+C content of the DNA. Based on these properties, it is related to haloanaerobes of the family *Halobacteroidaceae.*

Phylogenetic data obtained by analyzing the primary and secondary structures of the sequence of the 16S rRNA gene indicated that the strain really belongs to this family. The fact that strain Z-7986 should be placed in the order *Haloanaerobiales* is of considerable interest, because most representatives of this order are thalassophiles adapted to bodies of water that contain evaporating seawater. A peculiarity of strain Z-7986 is its alkaliphilic nature. It was the first saccharolytic haloanaerobe that was isolated from a continental soda lake and is able to develop at a pH value of 10. Another alkaliphilic haloanaerobe described earlier, *Natroniella* [3], does not use carbohydrates and exhibits a homoacetogenic type of metabolism.

The organism isolated in the present work is a zymogenic copiotroph, which is a rapidly growing fermenter that uses a limited set of substrates, predominantly soluble polysaccharides composed of polyglucose and their degradation products. These compounds are the substrates of catabolism. The organism also requires amino acids or yeast extract to accomplish the processes of anabolism.

Like *Natroniella*, the new organism depends both on

 Cl^- and HCO_3^- ions in a highly mineralized medium (with a mineralization degree of 0.5 to 2.9 M). Therefore, the organism conforms to the definition of a haloalkaliphile, because its growth depends both on high sodium chloride concentrations and on alkalinity. It should be considered moderately alkaliphilic, since the pH optimum for its growth is 8–8.5, and it can grow at the near-neutral pH value of 7.7. The organism can grow at 60°C and is characterized by a broad range of optimum temperatures (36–55°C). It can be regarded as thermotolerant or moderately thermophilic. Thermotolerance is quite compatible with its lifestyle in the equatorial Lake Magadi, whose temperature may considerably increase when directly heated by the sun.

While growing with elemental sulfur, strain Z-7986 proved capable of nonspecific sulfur reduction, which is peculiar to some haloanaerobes [2, 18, 19]. Based on its high sulfide tolerance and the incapacity to utilize sulfur catabolically, the organism is likely to be adapted to the sulfidogenesis zone. The growth of the strain was not completely inhibited by sodium sulfite and dithionite, in contrast to the species of the genera *Halobacteroides* and *Haloanaerobium* [2, 10].

The capacity to fix N_2 was demonstrated in *Orenia salinaria*, one of the species of the order *Haloanaerobiales* [20]. Strain Z-7986 does not grow with N_2 in the absence of other nitrogen sources and it is not capable of nitrogen fixation.

Strain Z-7986 possesses heat-resistant spores. In contrast to many other haloanaerobic and thermoalkaliphilic species, it sporulates when cultivated in a laboratory and this process does not require special conditions. A significant part of the late stationary phase cells convert into spores.

The organism is adapted to function at the beginning of the anaerobic saccharolytic trophic chain that degrades water-soluble polysaccharides in the trophic system of an anaerobic community. Its ecological and physiological features conform to the physical and chemical conditions of highly mineralized soda lakes.

Despite the indubitable phylogenetic relationship between strain Z-7986 and the representatives of the family *Halobacteroidaceae*, the analysis of the 16S rDNA sequences did not give us grounds for classifying this organism into any existing taxon of this family. Although strain Z-7986 forms one cluster with the species of the genera *Orenia* and *Natroniella*, the divergence level of the 16S rDNA sequences between Z-7986 and these closest relatives is relatively high (at least 8%). This prevents us from placing the organism in one of these genera. Therefore, strain Z-7986 is a representative of a new genus in the family *Halobacteroidaceae*.

Strain Z-7986 differs from the marine saccharolytic species of the genus *Orenia* in that it is alkaliphilic, by the set of sugars it utilizes, and in the metabolic products of glucose fermentation. The capacity to utilize sugars and differences in the type of metabolism distinguish it from the obligate alkaliphile *Natroniella* that was also isolated from Lake Magadi (Table 2). Based on the phenotypic and genotypic peculiarities of the new organism, we suggest classifying it into a new genus and a new species, *Halonatronum saccharophilum.* Its diagnosis follows.

Description of *Halonatronum* **gen. nov.** Hal.o.nat.ron.um. Hal.o, from Gr. n. *hals, halos,* salt; Nat.ron, from Gr. n. *natron*, soda, the mineral Na_2CO_3 . 10H₂O. An organism growing with sodium chloride and carbonate.

Gram-negative spore-forming motile rods with peritrichous flagellation. An obligate anaerobe that ferments carbohydrates including soluble polysaccharides. A moderate haloalkaliphile with a low G+C content of DNA, belonging to the spore-forming branch of the order *Haloanaerobiales* and the family *Halobacteroidaceae.* The genus is monotypic, with *Halonatronum saccharophilum* as the type species.

Description of *Halonatronum saccharophilum* **sp. nov.** Sac.cha.ro.philum. From Gr. n. *sacchar*, sugar and Gr. adj. *philus*, liking. A sugar-liking organism.

Slender flexible rods, $0.4-0.6 \times 3.5-10 \,\mu m$ in young cultures; long thickened degenerate cells, spheroplasts, and spores are characteristic of aging cultures. Spores are round, terminal, thermostable, of the plectridial type. The cell wall is characterized by a gram-negative structural pattern. Cells are motile by means of peritrichous flagella.

A moderate haloalkaliphile obligatorily requiring sodium chloride and carbonate and growing within the NaCl concentration range of 3–17% with an optimum at $7-12\%$ and at pH 7.7–10.3 with a pH optimum of 8.0–8.5. A moderate thermophile growing at 18–60°C with an optimum within the broad temperature range of 36–55°C. The doubling time is 2.5 h under optimum conditions.

An obligate anaerobe with the fermentative type of metabolism. Ferments glucose, fructose, sucrose, maltose, starch, glycogen, *N*-acetyl-D-glucosamine, and, to a slight degree, peptone and yeast extract. A chemoorganotroph whose metabolism requires yeast extract or amino acids. Formate, ethanol, acetate, H_2 , and CO_2 form as glucose fermentation products. Uses sulfur as electron acceptor in a process that yields no energy. Sulfide-tolerant.

The G+C content of DNA is 34.3 mol %.

Isolated from the bottom deposits of the coastal lagoon of Lake Magadi (Kenya). Z -7986^T (=DSM13868, =Uniqem 211) is the type strain of the species.

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REFERENCES

- 1. Rainey, F.A., Zhilina, T.N., Boulygina, E.S., Stackebrandt, E., Tourova, T.P., and Zavarzin, G.A., The Taxonomic Status of the Fermentative Halophilic Anaerobic Bacteria: Description of *Haloanaerobiales* ord. nov., *Halobacteroidaceae* fam. nov., *Orenia* gen. nov., and Further Taxonomic Rearrangements at the Genus and Species Level, *Anaerobe*, 1995, vol. 1, pp. 185–199.
- 2. Zhilina, T.N. and Zavarzin, G.A., Anaerobic Degradative Bacteria in the Halophilic Cyanobacterial Community, *Zh. Obshch. Biol.*, 1991, vol. 52, pp. 302–318.
- 3. Zhilina, T.N., Zavarzin, G.A., Detkova, E.N., and Rainey, F.A., *Natroniella acetigena* gen. nov., sp. nov.,

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an Extremely Haloalkaliphilic, Homoacetic Bacterium: A New Member of *Haloanaerobiales, Curr. Microbiol.*, 1996, vol. 32, pp. 320–326.

- 4. Zhilina, T.N. and Zavarzin, G.A., A New Extremely Halophilic Homoacetic Bacterium *Acetohalobium arabaticum* gen. nov., sp. nov., *Dokl. Akad. Nauk SSSR*, 1990, vol. 311, pp. 745–747.
- 5. Zhilina, T.N. and Zavarzin, G.A., Alkaliphilic Anaerobic Community at pH 10, *Curr. Microbiol.*, 1994, vol. 29, pp. 109–112.
- 6. Jones, B.E., Grant, W.D., Duckworth, A.W., and Owenson, G.G., Microbial Diversity of Soda Lake, *Extremophiles*, 1998, vol. 2, pp. 191–200.
- 7. Tourova, T.P., Garnova, E.S., and Zhilina, T.N., Phylogenetic Diversity of Alkaliphilic Anaerobic Saccharolytic Bacteria Isolated from Soda Lakes, *Mikrobiologiya,* 1999, vol. 68, pp. 701–709.
- 8. Zhilina, T.N., Zavarzin, G.A., Rainey, F.A., Kevbrin, V.V., Kostrikina, N.A., and Lysenko, A.M., *Spirochaeta alkalica* sp. nov., *Spirochaeta africana* sp. nov., and *Spirochaeta asiatica* sp. nov., Alkaliphilic Anaerobes from the Continental Soda Lakes in Central Asia and East African Rift, *Int. Syst. Bacteriol*, 1996, vol. 46, pp. 305–312.
- 9. Zavarzin, G.A., Zhilina, T.N., and Kevbrin, V.V., The Alkaliphilic Microbial Community and Its Functional Diversity, *Mikrobiologiya*, 1999, vol. 68, pp. 579–599.
- 10. Kevbrin, V.V. and Zavarzin, G.A., The Effect of Sulfur Compounds on the Growth of the Halophilic Homoacetic Bacterium *Acetohalobium arabaticum, Mikrobiologiya*, 1992, vol. 61, pp. 812–817.
- 11. Wolin, E.A., Wolin, M.J., and Wolfe, R.S., Formation of Methane by Bacterial Extracts, *J. Biol. Chem.,* 1963, vol. 238, pp. 2882–2886.
- 12. Hanson, R. and Phillips, J., Chemical Composition of the Bacterial Cell, in *Manual of Methods for General Bacteriology*, Gerhardt, P. *et al.*, Eds., Washington: Am. Soc. Microbiol., 1981.
- 13. Lang, E. und Lang, N., Spezifische Farbreaktion zum direkten Nachweis der Ameisensäure, *Fres. Z. Anal. Chem.*, 1972, vol. 260, no. 1, pp. 8–10.
- 14. Trüper, H.G. and Schlegel, H.G., Sulfur Metabolism in *Thiorhodaceae*: Quantitative Measurements on Growing Cells of *Chromatium okenii, Antonie van Leeuwenhoek*, 1964, vol. 30, pp. 225–238.
- 15. Pfennig, N. and Wagener, S., An Improved Method of Preparing Wet Mounts for Photomicrographs of Microorganisms, *J. Microbiol. Methods*, 1986, vol. 4, pp. 303– 306.
- 16. Van de Peer, Y. and De Wachter, R., TREECON for Windows: A Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Appl. Biosci.*, 1994, vol. 10, pp. 569–570.
- 17. Felsenstein, J., PHYLIP Manual Version 3.3., *University Herbarium*, Univ. of California, Berkeley, 1990.

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- 18. Zhilina, T.N., Tourova, T.P., Kuznetsov, B.B., Kostrikina, N.A., and Lysenko, A.M., *Orenia sivashensis* sp. nov., a New Moderately Halophilic Anaerobic Bacterium from Lake Sivash Lagoons, *Mikrobiologiya,* 1999, vol. 68, pp. 517–527.
- 19. Ravot, G., Magot, M., Olliver, B., Patel, B.K.C., Ageron, E., Grimont, P.A.D., Thomas, P., and Garcia, J.L., *Haloanaerobium congolense* sp. nov., an Anaerobic, Thiosulfate and Sulfur-reducing Bacterium from an African Oil Field, *FEMS Microbiol. Lett.*, 1997, vol. 147, pp. 81–88.
- 20. Moune, S., Eatock, C., Matheron, R., Willison, J.C., Hirschler, A., Herbert, R., and Caumette, P., *Orenia salinaria* sp. nov., a Fermentative Bacterium Isolated from Anaerobic Sediments of Mediterranean Salterns, *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 721–729.
- 21. Oren, A., Pohla, H., and Stackebrandt, E., Transfer of *Clostridium lortetii* to a New Genus, *Sporohalobacter* gen. nov. as *Sporohalobacter lortetii* comb. nov. and Description of *Sporohalobacter marismortui* sp. nov., *Syst. Appl. Microbiol.*, 1987, vol. 9, pp. 239–246.