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EXPERIMENTAL ARTICLES

Elemental Composition of Extremely Alkaliphilic Anaerobic Bacteria

A. V. Pitryuk, M. A. Pusheva[1], and V. V. Sorokin

Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

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Abstract—The contents of several chemical elements were assessed in the haloalkaliphilic acetogenic bacterium *Natroniella acetigena* and the alkaliphilic sulfate-reducing bacterium *Desulfonatronum lacustre* using X-ray microanalysis, stereoscanning microscopy, and mass spectrometry. The organisms were found to differ significantly in their relative contents of S, K, P, and Cl. The P/S ratio in cells of the alkaliphilic bacteria incubated in mineral media at different pH was pH-dependent. With a pH increase from 9 to 10, potassium extrusion from cells was observed, suggesting that secondary K^+/H^+ antiport activity accounts for the homeostasis of cytosolic pH. Deenergization of bacterial cells in the presence of inhibitors and ionophores results in specific changes in the P/S ratio, which may be considered an indicator of the cell energetic state. In *Natroniella acetigena*, the content of intracellular Cl was directly proportional to the NaCl concentration in the medium. Some metals were shown to be necessary for the *N. acetigena* viability; the requirement for Ni and Co was absolute. Although little demand for Mg was characteristic of the bacteria studied, their growth was stimulated by an increase in Mg concentration, and the cell resistance to lysis was enhanced.

Key words: alkaliphiles, anaerobes, homoacetogens, sulfate-reducers, X-ray microanalysis, elemental composition of cells, bioenergetics.

The elemental composition of alkaliphilic anaerobes isolated from soda lakes varying in degree of mineralization is of great interest because of their adaptation to extreme environmental conditions. Various mechanisms for ion transport across their cytoplasmic membrane are used by these bacteria to generate ionic gradients necessary for maintaining the optimal intracellular ion concentration. Alkaliphilic bacteria develop under conditions of low proton and high sodium concentrations and are thus to be different from neutrophilic bacteria in energy metabolism.

Epicontinental soda lakes, inhabited by alkaliphilic microbial communities of great functional diversity, are prominent among the alkaline ecosystems [1]. In particular, in the alkaline, highly mineralized Lake Magadi (Kenya), organotrophic acetogenesis is driven by a homoacetogenic bacterium *Natroniella acetigena*, showing optimal growth at pH 9.7 and 1.6% NaCl. In the low-mineralization soda Lake Khadyn (Tuva), the hydrogen sink in the microbial community is dependent on the hydrogenotrophic sulfate-reducer *Desulfonatronum lacustre*, displaying optimal growth at pH 9.5 in the absence of NaCl [2].

The high alkalinity of soda lakes results in the deficiency of most metals, primarily, bivalent cations (magnesium and calcium), against the background of a high content of sodium ions (the dominant cations), carbonate, bicarbonate, and chloride (the dominant anions) [1]. Under these conditions, the accessibility for bacteria of microelements and magnesium is of interest. In addition, the organisms inhabiting Lake Magadi develop at high concentration of chlorides and sodium carbonate and, hence, like halophiles, they have to cope with the problem of osmotic stress.

In this work, we aimed to determine the elemental composition of cells and the effect produced on it by ionophores and pH and the role of Mg^{2+}, Mn^{2+}, Ni^{2+}, and Co^{2+} in extremely alkaliphilic anaerobic bacteria.

MATERIALS AND METHODS

Bacteria and cultivation conditions. The type strains of *Natroniella acetigena* Z-7937^T (=DSM 9952) and *Desulfonatronum lacustre* Z-7951^T (=DSM 10312) from the Laboratory of Microbial Communities (Institute of Microbiology, Russian Academy of Sciences) were used in this study.

N. acetigena was cultivated under strict anaerobic conditions at 37°C on a medium with pH 9.7 containing ethanol (0.5%) and the following components (g/l): KH_2PO_4, 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; NH_4Cl, 1.0; KCl, 0.2; NaCl, 15.7; Na_2CO_3, 68.3; $NaHCO_3$, 38.3; Whitman's solution of microelements, 1 ml; 0.04% resazurin, 2 ml; Wolin's solution of vitamins, 2 ml; yeast extract, 0.2; $Na_2S \cdot 9H_2O$, 1.0 [3]. The total Na concentration in the medium was 2 M, and the NaCl content was 1.57%.

[1]Corresponding author. E-mail: pusheva@inmi.da.ru

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D. lacustre was cultivated under strict anaerobic conditions at 37°C on mineral medium, pH 9.5, containing the following components (g/l): KH K_2HPO_4, 0.2; KCl, 0.2; $MgCl_2 \cdot 6H_2O$, 0,1; NH_4Cl, 1,0; Na_2SO_4, 3.0; Na_2CO_3, 2.76; $NaHCO_3$, 10; yeast extract, 1.0; Whitman's solution of microelements, 1 ml; Wolin's solution of vitamins, 2 ml; 0.04% resazurin, 2 ml; $Na_2S \cdot 9H_2O$, 0.5; formate (5%) served as the substrate [4]. The total Na concentration in the medium was 0.2 M.

The content of Whitman's microelements in the media was the following (μg/l): $MnCl_2 \cdot 4H_2O$, 720; $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$, 400; $FeSO_4 \cdot 7H_2O$, 200; $CoCl_2 \cdot 7H_2O$, 200; $ZnSO_4 \cdot 7H_2O$, 200; $NiCl_2 \cdot 6H_2O$, 100; $CuSO_4 \cdot 5H_2O$, 20; $KAl(SO_4) \cdot 12H_2O$, 20; H_3BO_3, 200; $Na_2MoO_4 \cdot 2H_2O$, 20; EDTA, 1000. Analytic-grade reagents (Russia) were used for preparation of the media.

Cultures were grown in 100-ml hermetically sealed serum flasks under a nitrogen atmosphere.

Analysis of cell elemental composition. At the end of the exponential growth phase, the cultures were sampled (1 ml) to precipitate cells by centrifugation at 15 000 g for 5 min. After resuspending in an inhibitor-containing medium or in a medium with definite pH, the cells were incubated at 37°C for 10 min. To obtain the required pH, the medium was acidified with 0.1 N HCl or made alkaline with 0.1 N NaOH. Cell suspensions were air-dried on a copper grid covered with a Formvar film. X-ray microanalysis of the samples was performed using a JEM-100CX2 electron microscope (Jeol, Japan) equipped with an EM-ASID4D scanning device, and a LINK-860 X-ray analyzer with an E5423 detector (Link-System, United Kingdom). After the examination of 30 cells, integral spectra were obtained and processed using standard Link-System software [5].

To quantify the content of microelements in cell lysates, exponential phase cells of *N. acetigena* were precipitated by centrifugation at 4000 g for 30 min and washed three times with a mineral medium lacking Mg and microelements. The cells were disrupted by osmotic shock imposed by dilution with deionized water (an Elix-3 deionizing device, Millipore, Austria). Analysis was performed on a VR Plasma QUAD PQ2 Turbo Plus mass spectrometer (United Kingdom) (discharge power, 1.3 kW; transport gas (argon) flow rate, 0.89 l/min; plasma-forming gas (argon) consumption, 12 l/min). An aqueous solution of indium (25 μg/l) served as the internal standard.

The content of intracellular Cl^- was determined using ionic chromatography. The cells were precipitated and treated as described above, except that the mineral solution used for cell washing contained no chlorides. The Cl^- ions were detected on an IC 5000 Biotronic chromatograph (Germany) (equipped with a thermal conductivity detector and an ion-exclusive system of columns) using the Ekokhrome software package (Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences).

The intracellular osmotic volume was calculated based on geometric cell size and the ratio of protein content to cell number [6]. Protein was determined by the Lowry method.

When the effects of Mg, Mn, Ni, and Co were studied, several culture transfers were performed on media lacking the relevant element. Theoretical calculations of the concentrations of soluble and insoluble forms of magnesium in the medium were performed using Gibbs software (Geological Faculty, Moscow State University). Magnesium was determined in the deficient medium by the trilonometric method using the eriochrome black dye [7].

Inhibitors of metabolism, monensin, 3,5-di-tert-butyl-4-hydroxybenzylidenyl malononitrile (SF-6847), valinomycin (Serva, Germany), and rhodamine G (Reakhim, Russia) were added to the incubation medium as ethanol solutions. Corresponding amounts of ethanol (less than 0.1%) were added to the control solutions. Amiloride was added as an aqueous solution.

RESULTS AND DISCUSSION

X-ray microanalysis of intact cells revealed some differences in the elemental composition of the cultures studied (Figs. 1a and 1b). In *D. lacustre* cells, S was the dominant element (50% of the total pool of the elements determined, as compared to 20% in *N. acetigena*), which is, probably, a result of sulfate accumulation in the cells of this sulfate-reducing bacterium. The relative content of K in *D. lacustre* and *N. acetigena* comprised 20 and 10%, respectively. The relative content of P was twice as high in *N. acetigena* as in *D. lacustre*. It should be noted that in the cells of the halophilic alkaliphilic *N. acetigena*, larger amounts of chlorine were present (most probably, in the chloride form) than in the cells of the alkaliphilic bacterium *D. lacustre*, which has no growth requirement for NaCl (Figs. 1a and 1b). A small amount of Ca was detectable in cells of both cultures. Although Ca salts were not added to the media, they are present as contaminations in the chemicals and can be leached from the glass flasks used for cultivation.

The P/S ratio in the bacterial cells incubated for 10 min at different pH values was found to be clearly dependent on this parameter of the incubation medium (Fig. 2). *N. acetigena* grows in a pH range from 8.1 to 10.7, and the maximum P/S ratio was determined at pH 9.5 (Fig. 2, curve *1*). *D. lacustre* grows in a pH range from 8 to 10, and the maximum P/S ratio in the cells was observed at pH 8–8.5 (Fig. 2, curve *2*).

Analysis of the K/Ca ratio as dependent on the pH of the incubation medium in the alkaliphilic *D. lacustre* and haloalkaliphilic *N. acetigena* showed that the content of K in *D. lacustre* cells decreased 3.5-fold with the increase in the pH of the incubation medium from 7 to 10.5 (Fig. 3, curve *2*), whereas in *N. acetigena* cells, the maximum K/Ca ratio was recorded at pH 9 (Fig. 3,

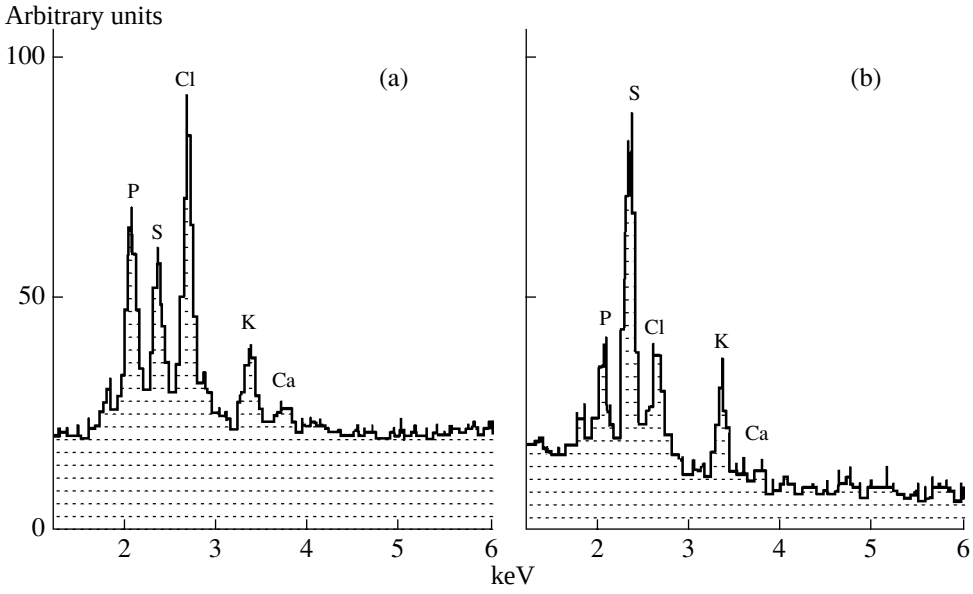

Fig. 1. X-ray spectra of elements dominating the cells of (a) *N. acetigena* and (b) *D. lacustre.*

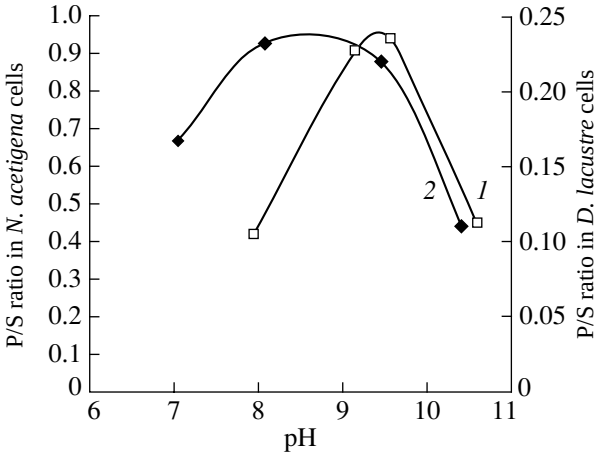

Fig. 2. P/S ratio in (*1*) *N. acetigena* and (*2*) *D. lacustre* cells as dependent on medium pH.

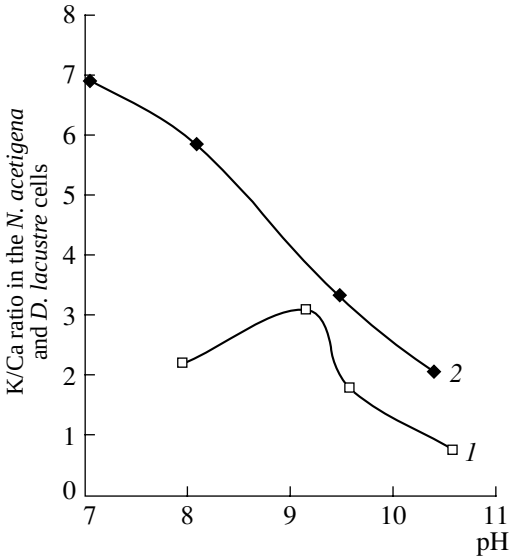

Fig. 3. K/Ca ratio in (*1*) *N. acetigena* and (*2*) *D. lacustre* cells as dependent on medium pH.

curve *1*). Potassium is known to play an important role in maintaining cellular osmotic pressure; it is involved in nonspecific activation of many enzymes, in bacterial energy metabolism (as the coupling ion), and in the regulation of intracellular pH [8]. In the alkaliphilic methanogen *Methanolobus taylorii* the intracellular potassium concentration decreased two- to threefold with an increase in the extracellular pH [9]. Potassium extrusion from alkaliphilic cells is probably necessary to make their cytosol more acidic than the external medium, and secondary K^+/H^+ antiport activity is likely to account for the K^+ extrusion. The organisms studied were almost indistinguishable in potassium content (Figs. 1a and 1b), which suggests that the haloalkaliphilic bacterium *N. acetigena* does not accumulate potassium in large amounts to use it as an osmoprotecting agent.

Calcium is known to stabilize bacterial membranes. In a number of alkaliphilic cyanobacteria, it is involved in energy metabolism [10]. At alkaline pH and high carbonate concentration, calcium precipitates from the solution and, therefore, it is not included in the media used for cultivation of alkaliphilic bacteria. The cells of alkaliphilic bacteria react to changes in the medium pH by changes in their K/Ca ratio. The cells of *N. acetigena* suspended in media with a pH lower than 8 were

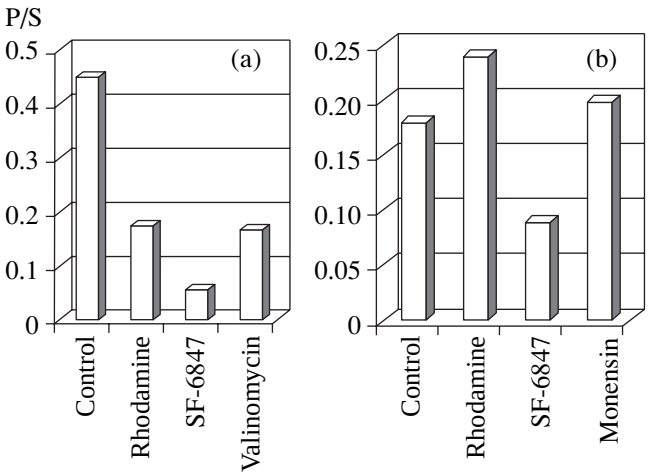

Fig. 4. Effect of inhibitors and ionophores on the P/S ratio in (a) *N. acetigena* and (b) *D. lacustre* cells.

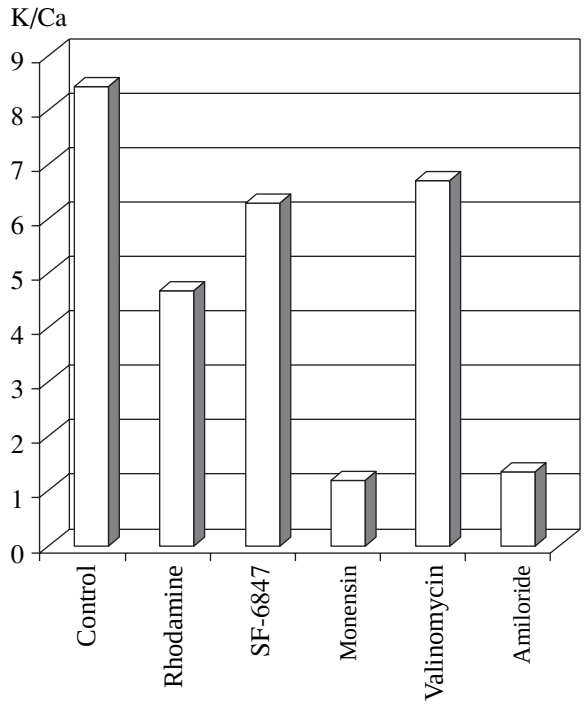

Fig. 5. Effect of inhibitors and ionophores on the K/Ca ratio in *D. lacustre* cells.

rapidly disrupted, whereas the cells of *D. lacustre* remained intact at the medium pH lower than 7 (Fig. 3).

The described properties of cell suspensions (their response to changes in pH) characterize the resistance of the alkaliphilic bacteria to the environmental conditions.

We have previously shown that the energy metabolism of the alkaliphilic acetogen *N. acetigena* is based on a combination of the proton and sodium cycles accompanied by ATP synthesis with the involvement of the proton-translocating F_1F_0 ATPase [11]. In the present work, we studied, in short-term experiments, the effect of ionophores and inhibitors of the energy-generating processes on the ratio of chemical elements in nongrowing bacterial cells. In *N. acetigena* cells incubated in the presence of rhodamine G (3 μM), an agent inhibiting proton ATPase, or in the presence of valinomycin (20 μM), an agent causing elimination of potassium and, in part, sodium gradients, the P/S ratio was 2.5-fold lower than in control cells. In the presence of the protonophore SF-6847 (3 μM), the P/S ratio decreased ninefold.

Thus, elimination of the ionic electrochemical gradients, as well as inhibition of proton ATPase, resulted in a significant decrease in the P/S ratio. These results are consistent with our previous data on the effect of inhibitors on suspensions and cultures of *N. acetigena*: in the presence of inhibitors and ionophores, growth arrest and blockage of acetogenesis were observed in the bacterium [11].

In *D. lacustre* cells, ionophores and inhibitors produced a different effect on the P/S ratio. Monensin and rhodamine G did not diminish the P/S ratio, whereas this parameter was reduced twofold after the elimination of the proton gradient in the presence of SF-6847 (Fig. 4b). These results support the previously obtained evidence indicating that *D. lacustre* contains the sodium-translocated ATPase and lacks the proton-translocating ATPase [11].

Thus, the P/S ratio seems to characterize the energetic state of the alkaliphilic cells studied.

The K/Ca ratio was reduced by different inhibitors to a different degree in *D. lacustre* cells (Fig. 5). The effects of amiloride and monensin, which affect the transmembrane sodium gradient, were the most pronounced. In the presence of the same inhibitors and ionophores, K and Ca were not detectable in the *N. acetigena* cells, although the cell integrity was preserved.

Content of Mg and microelements in *N. acetigena* cells and in the medium

Element	Intracellular concentration, μmol/l of osmotic volume	Concentration in the medium*, μmol/l
Mg	8000	520
Co	78	0.84
Se	70	Contamination
Cu	<53	0.08
Mo	<18	0.08
Mn	16	3.63
Zn	<8	0.7

* Element concentrations are calculated from the nutrient medium composition.

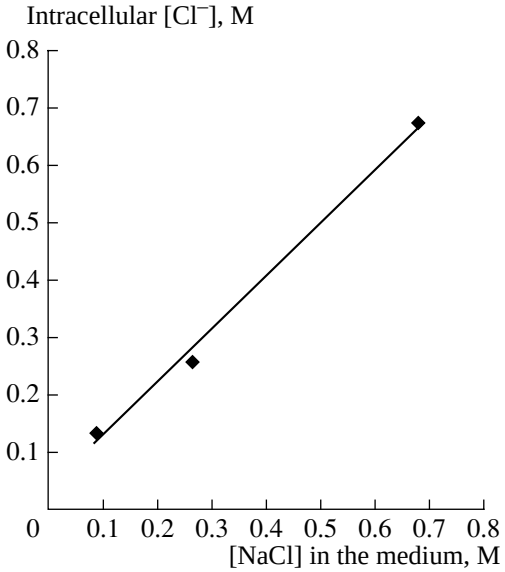

Fig. 6. Changes in intracellular Cl^- concentration of *N. acetigena* as dependent on the content of NaCl in the medium.

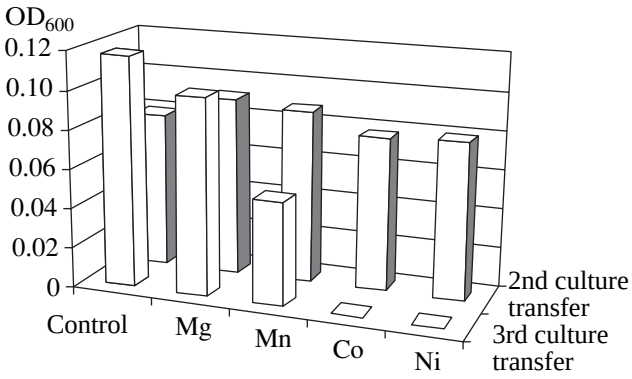

Fig. 7. Accumulation of *N. acetigena* biomass in the Mg, Mn, Ni, or Co-deficient media.

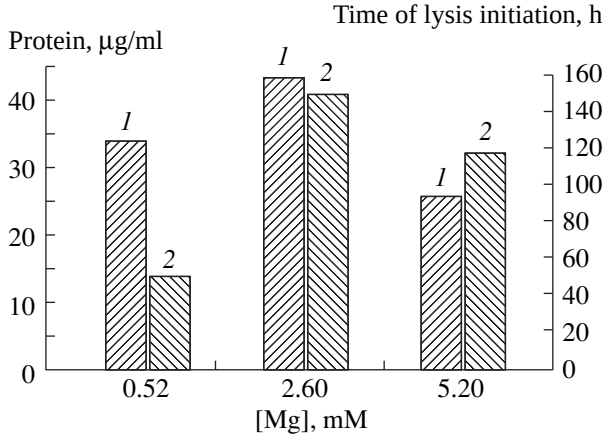

Fig. 8. Effect of $MgCl_2$ on (*1*) biomass accumulation and (*2*) the moment of cell lysis onset in a culture of *N. acetigena.*

Unlike *D. lacustre*, the haloalkaliphilic cells of *N. acetigena* contain significant amounts of chlorine, and, therefore, the effect of NaCl concentration in the medium on the intracellular chloride concentration was studied. In the *N. acetigena* cells grown on a medium containing 0.26 M NaCl the intracellular chloride concentration was 0.258 M. With an increase in the medium salinity, the amount of intracellular chloride grows linearly to reach 0.675 M on the medium containing 0.68 M salt (Fig. 6). Thus, the intra- and extracellular Cl^- concentrations were correlated, which suggests the osmotic function of the anion, as well as its possible role in maintaining the charge balance. In the aerobic marine *Halobacillus halophilus*, with an increase in the medium chloride concentration, the intracellular Cl^- concentration also increased, as well as the rate of cell growth. In this bacterium, chloride uptake was found to be energy-dependent [12]. Since *H. halophilus* is a moderately halophilic organism, the uptake of chloride by its cells was proposed to occur during osmoadaptation, when the cells accumulate salts from the medium until equalizing the intra- and extracellular concentrations.

A series of chemical elements was quantified in the *N. acetigena* cells by using mass spectrometry (table). In the *N. acetigena* cells grown on a medium containing 0.52 mM magnesium chloride, the Mg content was 8 mmol per 1 l of osmotic cell volume. As shown previously, in most bacteria, the content of magnesium ranges from 6 to 30 mM. However, in halophilic microorganisms, it can reach 500 mM [6].

Alkaliphilic bacterium *N. acetigena* was isolated from the soda Lake Magadi with a high content of carbonates and high pH. Hence, the bivalent ion concentration, primarily that of magnesium, is low; magnesium precipitates in the form of magnesite, $MgCO_3$ [1]. Theoretical calculations showed that soluble Mg concentration in the medium supplemented with 0.52 mM $MgCl_2$ may comprise 4.2 μM. After several transfers of *N. acetigena* on the Mg-deficient medium, no growth inhibition was observed (Fig. 7). Measurement of the magnesium content by the trilonometric method revealed less than 10 μM Mg in the deficient medium. Nevertheless, the *N. acetigena* growth did depend on the content of magnesium salts in the medium. A fivefold increase of magnesium concentration in the medium stimulated *N. acetigena* growth and delayed cell lysis (Fig. 8), although calculations showed that the amount of soluble Mg changed insignificantly when the content of magnesium salts in the medium was five- or tenfold increased. Magnesium is known to stabilize cell wall in addition to being involved in the catalysis of various reactions in the cell. It was demonstrated that lysis of various bacterial cells depends on the magnesium content in the medium [13]. The results obtained suggest that the growth of *N. acetigena* depends on the magnesium content in the medium, although the bacterium requirement for magnesium is low. Similar data were obtained by Tindall *et al.* [14]. They have isolated from

the soda Lake Magadi the alkaliphilic archaea *Halobacterium* sp., the growth of which was observed in the magnesium-deficient medium (less than 50 μM), although the optimal magnesium concentration ranged from 0.1 to 2 mM at pH 9.5.

The elemental analysis of cells showed that *N. acetigena* accumulated from the medium about 70 μmol of Se, 18 μmol of Mo, 53 μmol of Cu, and 8 μmol of Zn per 1l of the osmotic cell volume (table). Selenium and molybdenum are known to be incorporated into the active centers of many enzymes, such as formate dehydrogenase, hydrogenase, molybdopteryns. Although the medium was lacking selenium, it was detected by mass spectrometry. Copper is the important constituent of enzymes involved in the electron transport chain [8]. Zinc participates in the activation of carbonic anhydrase necessary for carbon dioxide assimilation in many organisms, including acetogens [15]. Intracellular Co and Mn content in the *N. acetigena* cells was 78 and 16 μM, respectively (table). In the bacterial cells, the Mn concentration is known to vary from 10 μM in *Bacillus subtilis* to 370 μM in *Escherichia coli*. Manganese is required for activation and stabilization of many enzymes [8]. As seen from the table, the alkaliphilic cells accumulate large amounts of elements as compared with the content of the latter in the medium. This suggests that active transport systems for metal ions exist in the bacteria.

Metals displaying changes in valence, especially Ni, Cu, and Co, participate in electron transport and redox reactions in acetogens. For instance, Co is a cofactor of corrinoid proteins involved in acetogenesis, and Ni is a constituent of metal-containing hydrogenase and CO-dehydrogenase [16].

The alkaliphilic acetogen *N. acetigena* was grown on media deficient for Mn, Co, Ni to study the demand for microelements. After the third culture transfer on medium deficient for cobalt and nickel, the growth of *N. acetigena* was arrested, whereas after the third culture transfer on the manganese-deficient medium, the *N. acetigena* growth was only twice inhibited.

Spectral X-ray microanalysis of the dominant ions and elements showed that the P/S ratio reflecting the level of cell energy was pH-dependent. Under conditions of cell deenergization in the presence of ionophores with ion-selective activity, this parameter was reduced in *N. acetigena*. The potassium spectra in *N. acetigena* and *D. lacustre* depended on pH and on the presence of ionophores, suggesting the involvement of the K^+/Na^+ antiport in the maintenance of pH homeostasis. The intracellular Cl concentration was directly correlated with the concentration of NaCl in the medium. Mass spectrometry revealed a series of metals necessary for bacterial activity. *N. acetigena* was found to have an obligate requirement for Ni and Co. The demand for Mg was insignificant; nevertheless, increased Mg concentration in the medium led to growth stimulation and higher cell resistance to lysis.

Thus, cell physiology of the alkaliphilic bacteria studied depends on various ions necessary for cell growth, metabolism, and energy generation. The demands for definite ions and elements may result from the bacterial adaptation to the environmental conditions typical of soda lakes.

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REFERENCES

1. Zavarzin, G.A., Zhilina, T.N., and Kevbrin, V.V., The Alkaliphilic Microbial Community and Its Functional Diversity, *Mikrobiologiya*, 1999, vol. 68, no. 5, pp. 579–599.
2. Zavarzin, G.A. and Zhilina, T.N., Anaerobic Chemotrophic Alkaliphiles, *Journey to Diverse Microbial Worlds*, Seckbach, J., Ed., Dordrecht: Kluwer Academic, 2000, pp. 191–208.
3. Zhilina, T.N., Zavarzin, G.A., Detkova, E.N., and Rainey, F.A., *Natroniella acetigena* gen. nov., sp. nov., an Extremely Haloalkalophilic, Homoacetic Bacterium: A New Member of *Haloanaerobiales*, *Curr. Microbiol.*, 1996, vol. 32, pp. 320–326.
4. Pikuta, E.V., Zhilina, T.N., Zavarzin, G.A., Kostrikina, N.A., Osipov, G.A., and Rainey, F.A., *Desulfonatronovibrio lacustre* gen. nov., sp. nov.: A New Alkaliphilic Sulfate-Reducing Bacterium Utilizing Ethanol, *Mikrobiologiya*, 1998, vol. 67, no. 1, pp. 127–135.
5. Nikitin, D.I., Sorokin, V.V., Pitryuk, I.A., and Nikitina, E.S., Elemental Composition of Cells of Bacteria Belonging to Various Taxa, *Prikl. Biokhim. Mikrobiol.*, 1998, vol. 34, no. 2, pp. 180–182.
6. Fagerbakke, K.M., Norland, S., and Heldal, M., The Inorganic Ion Content of Native Aquatic Bacteria, *Can. J. Microbiol.*, 1999, vol. 45, pp. 301–311.
7. Reznikov, A.A., Mulikovskaya, E.P., and Sokolov, I.Yu., *Metody analiza prirodnykh vod* (Methods for the Analysis of Natural Waters), Moscow: Nedra.
8. *Metals and Microorganisms*, Hughes, M.N. and Poole, R.K., Eds., London: Chapman and Hall, 1989.
9. Ni, S., Boone, J.E., and Boone, D.R., Potassium Extrusion by the Moderately Halophilic and Alkaliphilic Methanogen *Methanolobus taylorii* GS-16 and Homeostasis of Cytosolic pH, *J. Bacteriol.*, 1994, vol. 176, no. 23, pp. 7274–7279.
10. Tisa, L.S., Calcium Transport by *Frankia* sp. Strain EAN1pec, *Curr. Microbiol.*, 1998, vol. 37, pp. 12–16.
11. Pusheva, M.A., Pitryuk, A.V., and Zavarzin, G.A., Na^+- and H^+-Dependent ATP Synthesis in Extremely Alka-

liphilic Anaerobes, *Dokl. Akad. Nauk*, 2000, vol. 374, no. 6, pp. 833–835.

12. Roéler, M. and Müller, V., Quantitative and Physiological Analyses of Chloride Dependence of Growth of *Halobacillus halophilus*, *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 10, pp. 3813–3817.
13. Murray, T., Popham, D.L., and Setlow, P., *Bacillus subtilis* Cells Lacking Penicillin-Binding Protein 1 Require Increased Levels of Divalent Cations for Growth, *J. Bacteriol.*, 1998, vol. 180, no. 17, pp. 4555–4563.
14. Tindall, B.J., Mills, A.A., and Grant, W.D., An Alkalophilic Red Halophilic Bacterium with a Low Magnesium Requirement from a Kenyan Soda Lake, *J. Gen. Microbiol.*, 1980, vol. 116, pp. 257–260.
15. Braus-Stromeyer, S.A., Schnappauf, G., Braus, G.H., Göner, A.S., and Drake, H.L., Carbonic Anhydrase in *Acetobacterium woodii* and Other Acetogenic Bacteria, *J. Bacteriol.*, 1997, vol. 179, no. 22, pp. 7197–7200.
16. Pusheva, M.A., Berestovskaya, Yu.Y., and Borodulina, N.P., The Effect of Nickel on the Metabolism of Homoacetic Bacteria, *Mikrobiologiya*, 1989, vol. 58, no. 2, pp. 206–211.