ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 5, pp. 511–522. © Pleiades Publishing, Ltd., 2007.
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REVIEW PAPER

Osmoadaptation of Haloalkaliphilic Bacteria: Role of Osmoregulators and Their Possible Practical Application

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Received May 03, 2007

Abstract—The review discusses osmoadaptation of haloalkaliphilic bacteria from diverse taxonomic and physiological groups, inhabiting soda lakes. Our experimental research has confirmed the similarity of the osmoregulation strategies in neutrophilic and alkaliphilic halophiles, independent of their pH homeostasis mechanism. The external osmotic pressure is equilibrated either due to accumulation of ions from the environment, or by accumulation or synthesis of cytoplasmic osmoregulatory compounds. The alkaliphiles following the "compatible solutes" strategy contain low or moderate concentrations of salts in their cytoplasm; their proteins do not require adaptation to salts. Those that follow the "salt-in" strategy do not synthesize osmoregulators; they accumulate high levels of salts within the cell and thus equilibrate the osmotic pressures of the cell and the environment. The proteins of these bacteria contain more acidic amino acid residues compared to the proteins of neutrophiles. The functions of bacterial organic osmoregulatory compounds are discussed, as well as their characteristics of possible practical value. Applications for ectoine and betaine are discussed based on the published data.

Key words: osmoadaptation, haloalkaliphiles, osmoregulatory compounds, ectoine, betaine.

DOI: 10.1134/S0026261707050013

Living organisms exist practically everywhere on the surface of the Earth, including extreme environments. Since the physical and chemical parameters of most habitats vary constantly, organisms must adapt to changing environmental conditions in order to support life. In such extreme biotopes as saline soils, salt marshes, and saline or soda lakes, the availability of water is among the most important parameters. Frequent climatic fluctuations (drought or rain) result in changes in water availability and cause osmotic stress to the organisms inhabiting these environments. Increased osmotic pressure can cause cell dehydration and decrease viability; capability to adapt to osmotic stress is therefore of utmost importance for living organisms in order to protect cells from changes in water activity and in the content of dissolved compounds in water. Both pro- and eukaryotes possess mechanisms for adaptation to abrupt environmental changes. The cell response aimed at the maintenance of their volume, turgor, and normal biological activity is termed osmotic regulation. For example, when lack of water in soil leads to decreased water content within plant cells, their turgor is maintained due to accumulation of inorganic [1] or organic [2] compounds (osmoregulators). The latter are capable of stabilizing the cellular structures not interfering with cellular metabolism; they are therefore termed compatible solutes. The biochemical mechanisms of their action are as yet unknown. They are hydrophilic in nature; it is therefore supposed that they can prevent the dehydration of protein complexes and membranes [2]. Other eukaryotes (halotolerant fungi, yeasts, and microalgae) are known to utilize organic osmoregulatory compounds to compensate for external osmotic pressure.

Similar osmoregulation strategies have been revealed in prokaryotes. In order to adapt to an increase in external osmotic pressure, bacteria either accumulate ions from the environment, or accumulate or synthesize osmoregulators. Presently, the mechanisms of osmoadaptation are of intense interest, partly due to the prospects of biotechnological application for halophilic microorganisms and their enzymes and osmoregulatory compounds.

A number of publications consider the mechanisms of osmoadaptation in halophilic microorganisms. Information concerning haloalkaliphilic microorganisms (which undergo osmotic stress under conditions of high carbonate alkalinity) is, however, scarce; it is mostly related to the study of the osmoregulator types [3–7]. Research on osmoadaptation in haloalkaliphiles is of both theoretical and practical interest. The first implies the study of the specific characteristics of the osmoadaptation mechanisms of these organisms as compared

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Table 1. Characteristics of halophilic bacteria

Microorganism	Order, family	pH range/optimum	Relation to Na^+	Na^+, M, growth range/optimum	Reference
Halomonas campisalis Z-7398-2	*Oceanospirillales* *Halomonadaceae*	7.5–10.4/8.8–9.5 alkaliphilic	Moderate halophile	0.16–3.1/1.0	[12]
'Halomonas kenyensis'	*Oceanospirillales* *Halomonadaceae*	7.5–10.6/9.5 alkaliphilic	Facultative, moderate halophile	0.04–2.2/0.5–1.2	[13]
Tindallia magadiensis	*Clostridiales* *Clostridiaceae*	7.5–10.5/8.5 alkaliphilic	Moderate halophile	0.17–1.7/0.51–1.0	[44]
Tindallia californiensis	*Clostridiales* *Clostridiaceae*	8.0–10.5/9.5 alkaliphilic	Moderate halophile	0.17–3.4/0.51–0.85	[11]
Natroniella acetigena	*Halanaerobiales* *Halobacteroidaceae*	8.1–10.7/9.7–10.0 alkaliphilic	Extreme halophile	1.7–4.4/2.0–2.56	[22]

to neutrophilic halophiles. The second implies the prospects of the practical application of osmoregulatory compounds. Their production by haloalkaliphilic microorganisms can be carried out on highly selective media (at high pH and salinity); the risk of contamination is therefore significantly reduced.

A number of haloalkaliphilic microorganisms are presently known; they belong to novel taxa of the species and genus levels. Russian microbiologists, in particular workers at the Institute of Microbiology, Russian Academy of Sciences, have contributed greatly to the study of the diversity of haloalkaliphilic microorganisms; to our knowledge, they have described ca. 50 new species and 20 new genera. The present review summarizes the results of our investigations on the osmoadaptation of the haloalkaliphilic bacterial isolates from soda lakes, which have been obtained and described in the Laboratory of Relic Microbial Communities, Institute of Microbiology [8–13]. The practical application of the organic osmoregulators revealed in the haloalkaliphiles are under study.

STRATEGIES OF OSMOADAPTATION

The existence of two fundamental strategies of microbial adaptation to osmotic stress has long been established [14]. The cells can maintain high intracellular concentrations of salts, which are osmotically equivalent to external concentrations (the "salt-in" strategy). The main intracellular cation is K^+, which is usually equilibrated with Cl^-. Due to its significantly lower water binding, the potassium ion is preferable to Na^+ [15]. In the case of this strategy, all the intracellular systems are adapted to high concentrations of salts. Alternatively, the cells can remove the salts actively and maintain their low cytoplasmic concentration. The external osmotic pressure is then equilibrated by means of small organic molecules (the "compatible solutes" strategy); these are either synthesized within the cell or transported inside from the environment. Compared to ionic compounds, uncharged and zwitterionic ones are preferable for protein stabilization. In the case of this strategy, the intracellular systems do not require specific adaptation to salts. This strategy and the types of osmoregulators are discussed in more detail in the relevant section of this review.

The "salt-in" strategy is usually applied by two microbial groups: extremely halophilic archaea of the order *Halobacteriales* and anaerobic halophilic bacteria of the order *Halanaerobiales* [16–18]. This strategy has also been recently revealed in an aerobic extremely halophilic bacterium *Salinibacter ruber* (order *Cytophagales*) [19, 20]. The overall content of salts in the cells of these bacteria is approximately equivalent to their external concentration. The intracellular concentration of chloride is usually close to that in the medium; the sodium concentration is somewhat lower and the potassium concentration several times higher than in the medium. Most of the remaining halophilic and halotolerant microorganisms apply the "compatible solutes" strategy. However, some halophiles that accumulate organic osmoregulators also contain moderate intracellular concentrations of Na^+, K^+, and Cl^-; they, therefore, apply both strategies [21]. The majority of the haloalkaliphilic microorganisms discussed in this review use the "compatible solutes" strategy.

THE "SALT-IN" STRATEGY

Intracellular concentrations of Na^+, K^+, and Cl^-. Determination of the exact concentrations of ions is hindered by the difficulty of determination of the cell volume. Moreover, Na^+ and other ions can bind to external cell layers; their amount then increases with increasing salinity [21]. Strictly speaking, the existing methods provide an estimate of the cytoplasmic ion concentrations, rather than their exact values. This estimate, however, is sufficient to determine which osmoadaptation strategy is used.

The acetogenic haloaenaerobic *Natroniella acetigena* is one of the haloalkaliphiles that apply the "salt-in" strategy [22] (Table 1). This bacterium was shown to maintain very high intracellular concentrations of Na^+, K^+, and Cl^-; these concentrations increase with increas-

Table 2. Intracellular concentrations of Na^+, K^+, and Cl^- ions and the transmembrane concentration gradients in haloalkaliphilic bacteria

Microorganism and cultivation conditions	Na^+ ext (M)	Na^+ int (M)	K^+ ext (M)	K^+ int (M)	Cl^- ext (M)	Cl^- int (M)	ΔpNa^+	ΔpK^+ (rev)	ΔpCl^-
Natroniella acetigena									
1% NaCl (0.17 M NaCl) 1.91 M Na^+_{Total}	2.07	0.91	0.013	0.83	0.18	0.29	2.3	63.8	0.62
5% NaCl (0.85 M NaCl) 2.59 M Na^+_{Total}	2.78	1.98	0.013	0.94	0.86	0.89	1.4	72.3	0.97
***Halomonas campisalis* Z-7398-2**									
3% NaCl (0.51 M NaCl) 0.70 M Na^+_{Total}	0.75	0.23	0.015	0.12	0.42	0.22	3.3	8.0	1.9
10% NaCl (1.71 M NaCl) 1.90 M Na^+_{Total}	2.06	0.24	0.015	0.26	1.47	0.22	8.6	17.3	6.7
'Halomonas kenyensis'									
2% NaCl (0.34 M NaCl), 0.53 M Na^+_{Total}	0.60	0.49	0.015	0.2	0.34	0.08	1.2	13.3	4.25
8% NaCl (1.37 M NaCl), 1.56 M Na^+_{Total}	1.73	0.58	0.015	0.34	1.27	0.16	3.0	22.7	7.9
Tindallia californiensis									
Without NaCl, 0.39 M Na^+_{Total}	0.31	0.14	0.004	0.03	ND	ND	2.2	7.5	ND
Without NaCl, 1.20 M Na^+_{Total}	1.26	0.92	0.004	0.17	0.007	0.008	1.4	42.5	0.88
Tindallia magadiensis									
Without NaCl, 0.48 M Na^+_{Total}	0.49	0.40	0.004	0.17	0.012	0.009	1.23	42.5	1.3
Without NaCl, 1.03 M Na^+_{Total}	1.15	0.65	0.004	0.22	0.015	0.014	1.77	55.0	1.1
4% NaCl (0.68 M NaCl), 1.16 M Na^+_{Total}	1.19	1.09[a]	0.004	0.33	0.66	0.39	1.1	82.5	1.7

Note: Ext, ion concentration in the supernatant after cell precipitation; int, intracellular ion concentration; ΔpNa^+, ΔpK^+, ΔpCl^-, concentration gradients across the cytoplasmic membrane; ΔpK^+ (rev), reverse gradient of potassium; a, concentration is possibly overestimated due to the superimposition of errors of each stage of the procedure; ND, not determined.

ing NaCl content in the medium. Thus, the organism balances internal against external osmotic pressure and provides for cell turgor (Table 2) [23]. In *N. acetigena*, the intracellular concentrations of Na^+, K^+, and Cl^- are close to those known for other haloanaerobes [18]. The intracellular concentration of potassium ions (ca. 0.9 M) is almost two orders of magnitude higher than in the medium; K^+ can therefore participate in osmoregulation in *N. acetigena*. The cytoplasmic ionic composition and the concentration gradients across the cytoplasmic membrane are determined by the combined action of a number of ion pumps, antiporters, and other transport proteins [18]. In *N. acetigena*, very high ΔpK^+ and significant ΔpNa^+ values are the result of bioenergetic processes. The proton electrochemical transmembrane gradient ($\Delta\mu_{H^+}$) is known to be the primary energy source for Na^+ removal and K^+ accumulation in the cells of most halophiles. The proton gradient has been previously shown to be the major factor of ATP

Table 3. Amino acid composition of the bulk cell protein of haloalkaliphilic, neutriophilic halophilic, and nonhalophilic bacteria

Amino acid	*Acetobacterium paludosum*	*Halomonas campisalis*	*'Halomonas kenyensis'*	*Tindallia magadiensis*	*Halanaerobium saccharolyticum*	*Natroniella acetigena*
Ala	11.94	13.35	12.57	11.17	10.73	10.02
Gly	10.95	12.14	10.66	9.60	9.80	9.84
Thr	6.94	5.61	5.39	5.69	5.69	4.71
Ser	6.54	5.00	5.03	4.88	5.33	3.92
Val	9.56	8.71	9.11	9.54	9.36	9.39
Leu	7.70	8.82	9.12	8.16	8.66	8.35
Ile	7.73	4.95	5.83	7.37	8.47	7.33
Pro	3.89	5.15	4.71	4.06	3.66	3.88
Asx	**10.50**	**10.94**	**10.87**	**12.31**	**13.33**	**13.66**
Glx	**10.27**	**12.21**	**12.71**	**12.05**	**12.36**	**17.25**
Phe	3.21	3.37	3.88	3.64	3.54	3.01
Lys	**5.58**	**4.42**	**3.03**	**5.26**	**4.02**	**4.84**
Tyr	2.41	2.26	2.17	2.16	2.35	1.70
Arg	**2.78**	**3.06**	**4.93**	**4.10**	**2.70**	**2.08**
Glx + Asx	**20.77**	**23.15**	**23.58**	**24.36**	**25.69**	**30.91**
Lys + Arg	**8.36**	**7.48**	**7.96**	**9.36**	**6.72**	**6.92**
(Glx + Asx)–(Lys + Arg)	**12.41**	**15.67**	**15.62**	**15.00**	**18.97**	**23.99**

Note: The data of at least three experiments are presented. Glx and Asx, total concentrations of Glu + Gln and Asp + Asn, respectively.

synthesis in *N. acetigena* [24, 25]. High intracellular salt concentrations in *N. acetigena* imply the absence of organic osmoregulators [23]; this is common to all previously known members of the order *Halanaerobiales* [17, 18]. Thus, the ions K^+ and, possibly, Cl^- perform an osmoprotective function; under high external concentrations of salts this is energetically more advantageous than synthesis of organic osmoregulators [26].

Amino acid composition of the bulk cell protein and salt tolerance of the enzymes. High concentrations of salts in the cytoplasm are usually very unfavorable for proteins and other macromolecules. Salt causes protein aggregation due to enhanced hydrophobic interactions; due to the hydration of ions, it decreases the availability of free water; it also prevents intra- and intermolecular electrostatic interactions [15].

In order to maintain the activity of the cellular metabolic systems under high concentration of salts in the cytoplasm, specific adaptation of all proteins is required [27, 28]. Salt resistance of the proteins of halophilic bacteria is achieved due to significant changes in their amino acid composition. Increased content of acidic amino acid residues (glutamate and aspartate) as compared to the proteins of neutrophiles is the general direction of evolution of these proteins [15]. The acidic residues, located mainly on the surface of the protein globule, are highly hydrated. Their increased amount makes a protein more hydrophilic and its structural collapse or conglomeration is thus prevented. In order to maintain their metabolically active state, the proteins with enhanced content of acidic amino acids require, however, anti-ions in the cytoplasmic space, which can neutralize their charges [14, 27]. A number of enzymatic reactions therefore depend on high K^+ concentrations; they are also tolerant to NaCl [29]. Moreover, the stability of these proteins is determined by the conditions when hydrophobic interactions intensify due to high NaCl or KCl concentrations.

Although data on the amino acid composition of the bulk cell protein of neutrophilic halophiles exist [20, 21, 30], such research on haloalkaliphiles has not been performed. Analysis of the amino acid composition of the bulk cell protein of a number of haloalkaliphilic bacteria revealed a much higher content of acidic amino acids in *N. acetigena* compared to microorganisms, which apply the "compatible solutes" strategy (Table 3) [31]. In *N. acetigena*, the surplus of acidic amino acids compared to the basic ones is close to that observed in archaea (order *Halobacteriales*) [20]. The results of amino acid analysis of *N. acetigena* proteins suggest salt resistance of the proteins of this organism. CO dehydrogenase, the key enzyme of its metabolism,

Table 4. Enzymes of haloalkaliphilic bacteria

Microorganism	Enzyme	Activity range	Optimal concentration
Natroniella acetigena	CO dehydrogenase	0–4.1 M NaCl	0.7 M NaCl
		0–0.95 M $NaHCO_3$	0–0.7 M $NaHCO_3$
Halomonas campisalis	Lactate dehydrogenase	0–2.3 M NaCl	0.3 M NaCl
		0–1.8 M KCl	0.24 M KCl
		0–1.1 M $NaHCO_3$	0.4 M $NaHCO_3$
	Glyceraldehyde-3-phosphate dehydrogenase	0–2.2 M NaCl	Without NaCl
		0–2.2 M KCl	Without KCl
		0–1.2 M $NaHCO_3$	Without $NaHCO_3$
	Nitrate reductase	0–4.1 M NaCl	0.3–2.0 M NaCl
		0–3.2 M KCl	0.2–2.1 M KCl
'Halomonas kenyensis'	Nitrate reductase	0–4.1 M NaCl	0.3–2.0 M NaCl
		0–3.2 M KCl	0.2–1.6 M KCl
Tindallia magadiensis	Hydrogenase	0–4.3 M NaCl	Without NaCl
		0–2.6 M KCl	0–2.0 M KCl
		0–1.2 M $NaHCO_3$	0.24 M $NaHCO_3$
	CO dehydrogenase	0–4.3 M NaCl	Without NaCl
		0–3.4 M KCl	0.2–2.2 M KCl
		0–1.2 M $NaHCO_3$	0.25 M $NaHCO_3$
Tindallia californiensis	Hydrogenase	0–4.3 M NaCl	Without NaCl
		0–1.2 M $NaHCO_3$	Without $NaHCO_3$
	CO dehydrogenase	0–4.3 M NaCl	Without NaCl
		0–1.2 M $NaHCO_3$	Without $NaHCO_3$

was indeed exceptionally resistant to NaCl (Table 4). Under salt concentrations from 0 to 3.5 M, its activity remained practically constant; higher concentrations caused an insignificant decrease in activity. The high salt resistance of *N. acetigena* CO hydrogenase is similar to that of the enzymes of other haloanaerobes [17, 32].

Comparison of two haloanaerobes which apply the "salt-in" strategy (an alkaliphilic *N. acetigena* and a neutrophilic *Halanaerobium saccharolyticum* [33]) revealed a similar percentage of basic amino acids in their proteins (Table 3). The percentage of acidic amino acids is, however, higher in *N. acetigena* than in *H. saccharolyticum.* Such an excess of acidic amino acids is possibly required not only for osmoadaptation, but also for the regulation of intracellular pH in this haloalkaliphilic organism. The values of intracellular pH in obligate alkaliphiles are known to be within the range 8.0–9.0, i.e., at least two units lower than in the environment [34].

THE "COMPATIBLE SOLUTES" STRATEGY

Since organic intracellular osmoregulators maintain the osmotic balance without interfering with the metabolic functions, they are termed compatible solutes. Even at molar concentrations, they do not inhibit most cellular processes, but rather stabilize proteins and lipids [35]. Accumulation of osmoregulatory compounds promotes the maintenance of cell volume, turgor, and electrolyte concentration [36]. Moreover, osmoregulators protect proteins and membranes against such damaging factors as decreased water activity, changes in the concentration of salts, freezing, heating, and drying [37].

Osmoregulatory compounds can be accumulated in the cell either by synthesis or by transport from the medium; the latter is more preferable energetically. The transport systems responsible for the transport of osmoregulators from the environment depend on ATP or are coupled to $H^+(Na^+)$ symport [38].

Most of the various compounds used as osmoregulators belong to one of the following four groups: polyols (usually glycerol), sugars (sucrose, trehalose), amino acids (proline, glutamic acid), and quaternary amines (glycine betaine, ectoine, hydroxyectoine) [39, 40]. Of these compounds, glycine betaine and ectoine are the most widespread. Glycine betaine is the preferable osmolyte for most prokaryotes and plants and for some animals [1]. Ectoine, which was originally isolated from the phototrophic bacterium *Ectothiorhodospira halochloris* [40], is the most common osmolyte among aerobic chemoheterotrophic eubacteria. Hydroxyectoine, its derivative, is more widespread among halophilic and halotolerant gram-positive bacteria [41]. Ectoine and especially hydroxyectoine provide much more efficient enzyme protection against diverse stressing factors than other osmoregulators.

The presence of osmoregulatory compounds increases the salt resistance of the cells. Thus, their accumulation can be the limiting factor in salt tolerance. The chemical nature of the major osmolyte is important for the degree of salt resistance of the organism. Glycine betaine provides for higher osmotolerance than proline, which, in turn, is more preferable than glutamate [42]. The type of the osmoregulatory compound depends on the bacterial species and, to a lesser extent, on the growth conditions. Nonhalophilic and weakly halophilic bacteria usually accumulate amino acids (glutamate, proline, etc.), K^+, and certain sugars (sucrose, fructose, glucose, or trehalose). Moderately and extremely halophilic and halotolerant bacteria accumulate mainly glycine betaine or glutamate betaine, glycozyl glycerol, ectoine, and sometimes glutamate, K^+, trehalose, or sucrose as minor components.

Intracellular concentrations of Na^+, K^+, and Cl^-. The microorganisms which use the “compatible solutes” strategy do not maintain the cytoplasmic concentration of salts sufficient to equilibrate external osmotic pressure. Unlike the haloanaerobe *N. acetigena*, haloalkaliphilic members of the genera *Halomonas* and *Tindallia* maintain low or moderate intracellular concentrations of Na^+, K^+, and Cl^- [23, 43] (Table 2).

Haloalkaliphilic *Halomonas* species [12, 13] had low intracellular concentrations of Na^+ and Cl^-, which either did not change or increased insignificantly with increasing salinity of the medium. In the cells of both species, the K^+ concentration was approximately an order of magnitude higher than in the medium; it increased with increasing concentrations of external Na^+ (Table 2). Such accumulation weakly correlates with the increasing salinity of the medium and is insufficient to equilibrate external osmotic pressure. Thus, potassium ions play a secondary role in the osmoadaptation of these bacteria. Potassium probably plays a certain part in osmoadaptation on low-mineral media. The results of our investigation are in accordance with the known data concerning this group of halophilic microorganisms [21]. Although the intracellular ion concentrations vary somewhat between the *Halomonas* species, they are never sufficiently high to equilibrate osmotic pressure under high salinity.

In the haloalkaliphilic *Tindallia magadiensis* [44], intracellular concentrations of Na^+, K^+, and Cl^- were determined on media containing $NaHCO_3$ alone or $NaHCO_3 + NaCl$ [43]. Importantly, the intracellular ion concentrations were significantly lower in the first case, in spite of the approximately equal molar concentration of Na^+ (Table 2). In both cases, Na^+ and K^+ concentrations increased with increasing salinity of the medium. When Cl^- was present in the medium in trace amounts, its intracellular accumulation was insignificant. We have revealed similar patterns of intracellular Na^+, K^+, and Cl^- concentrations in the newly described species *T. californiensis* [11] grown on bicarbonate-containing media.

The membranes of most halophiles are known to exhibit high activity of Na^+/H^+ antiporters, which utilize the proton electrochemical gradient to remove Na^+ from the cell [45]. Alkaliphilic halomonads probably also use the Na^+/H^+ antiporter in order to maintain low intracellular concentrations of inorganic ions. This sodium gradient can be used to power certain endoergic processes in the cells. The higher sodium gradient in these organisms as compared to the *N. acetigena* ΔpNa^+ may indicate higher efficiency of the sodium pumps which remove Na^+ from the cytoplasm. Increase of the reverse potassium gradient with increasing medium salinity indicates the growing role of K^+ in the osmoadaptation of these microorganisms.

Osmoregulators. Since the low (*Halomonas* species) or moderate (*Tindallia* species) concentrations of inorganic ions are insufficient for an osmotic balance, the possibility of accumulation of organic osmolytes was to be verified. Ectoine and glutamate were revealed in the cells of *Halomonas campisalis* Z-7398-2 by means of ^{13}C-NMR [23]; when the growth medium contained yeast extract, betaine was also detected. The cells of *Halomonas* species usually contain more than one osmolyte; their composition and ratio depend on both the species and the growth conditions. In all cases, however, ectoine is the main component of the osmoprotective cocktail. Glutamate is also known to perform the osmoprotective function [21]. Since this is an anionic compound, its accumulation depends on the presence of equivalent amounts of cations in the cytoplasm. For example, accumulation of glutamate (and of other weak acids) is stimulated by potassium [14]; thus, they are probably accumulated together as potassium glutamate. For *H. elongata*, the key role of potassium glutamate in osmoadaptation was shown for salt concentrations below 0.51 M Na^+; at higher salinity, however, ectoine becomes the major osmolyte [46]. In the case of *H. campisalis*, the pattern is probably the same. Since yeast extract contains compounds which are betaine precursors, detection of betaine in the cells grown in its presence also conforms to the published data.

Glycine betaine, the osmoregulator typical for chemotrophic bacteria, was revealed when similar research methods were applied to haloalkaliphilic *Tindallia magadiensis*; glycine betaine is, like ectoine, a promising biologically active compound.

Amino acid composition of the bulk cell protein and salt-resistance of the enzymes. Comparative study of various haloalkaliphilic bacteria revealed that the ratio of acidic amino acids (glutamate and aspartate) in bulk cell protein and their excess compared to the basic amino acids (lysine and arginine) increased in more halophilic microorganisms. In *Acetobacterium paludosum*, a typical freshwater organism [47], which was used as the reference strain, these values were the lowest; the highest values were recorded for extremely halophilic *Natroniella acetigena*, which uses the "salt-in" strategy (Table 3). Moderately halophilic *Tindallia magadiensis, Halomonas campisalis*, and *'H. kenyensis'* exhibited intermediate values of these parameters (Table 3).

Since the moderately halophilic *T. magadiensis* and *H. campisalis* (and possibly *'H. kenyensis'* and *T. californiensis*, as well) contain organic osmolytes, which provide for the major part of the osmotic equilibrium, their proteins do not need significant adaptation to salts. The cytoplasmic enzymes are usually most active in the absence of salts. However, since the concentrations of salts in the intracellular space are moderate, halophilic properties can be expected from some enzymes, especially from the membrane-bound ones [21]. In our case, salt tolerance correlated with the amino acid composition of the cell protein and with the intracellular concentrations of salts. Although the investigated enzymes are salt-tolerant, their sensitivity to salts varies significantly. For instance, CO dehydrogenase and hydrogenase from *T. magadiensis* were most active in the absence of NaCl; $NaHCO_3$, however, did not suppress their activity (Table 4). Although NaCl had an inhibitory effect, the enzymes retained up to 40% of their maximal activity at the salt concentration above 4.0 M [31]. *T. californiensis* CO dehydrogenase and hydrogenase were less resistant to salts; both NaCl and $NaHCO_3$ suppressed the enzymatic activity. The activities of these enzymes were maximal in the absence of salts; at near-saturating concentrations of NaCl or $NaHCO_3$, their residual activity was 10–20%. In *H. campisalis*, lactate dehydrogenase was most active at moderate concentrations of NaCl, KCl, and $NaHCO_3$; compared to salt-free conditions, its activity increased by 10–12%. However, further increase in the concentrations of salts resulted in the inhibition of the enzyme. In the case of glyceraldehyde-3-phosphate dehydrogenase, these three salts had a similar effect: the enzymatic activity was the highest in their absence and was completely suppressed by 2.2 M NaCl or 1.8 M KCl [48]. Nitrate reductases of *H. campisalis* and *'H. kenyensis'* were more resistant to both NaCl and KCl; their highest activities were revealed at moderate concentrations of these salts (Table 4). The enzymatic activity was retained throughout the whole experimental range of concentrations (0–4.1 M NaCl and 0–3.2 M KCl).

PRACTICAL APPLICATION OF OSMOREGULATORS

Unlike neutrophilic halophiles, haloalkaliphiles can act as producers of both alkaline enzymes and organic osmoregulatory compounds. Discussion of the biotechnological potential of certain organic osmoregulators is one of the goals of this review. The specific characteristics of these compounds make them valuable in various fields, including biotechnology, cosmetology, pharmacy, medicine, and agriculture.

Biotechnological application for osmoregulatory compounds. 1. A significant number of in vitro biological applications for osmoregulators are concentrated on the development of advanced buffer systems for the preservation of activity of commercially important enzymes. Both ectoine and hydroxyectoine stabilize nucleic acids and a number of labile enzymes in vitro and thus increase their working life. They can also protect them against high or low temperatures, salts, and desiccation [49]. Large amounts of purified osmolytes are required for in vitro applications; the search for easily cultivable producers is therefore important. *Halomonas elongata* is presently one of the most important producers of ectoine and hydroxyectoine [50]; when grown on media with 10% NaCl, it accumulates intracellularly up to 2.25 M ectoine per liter of osmotic volume [21, 51]. The species of haloalkaliphilic halomonads are also among the promising producers, due to their ease of cultivation, selectivity of growth media, and extensive organotrophic potential. They do not require specific growth factors and can grow in a wide range of pH, salinity, and temperature.

2. Organic osmolytes are used as cryoprotectors. Betaine was shown to be a more effective protective agent for freezing and long-term storage of microbial cells than such known compositions as albumin serum or the mixture of trehalose and dextran [52]. Betaine provides for the longer preservation of microbial viability.

3. Osmolytes are chemical chaperones; their functions include participation in protein folding. Protein folding was long believed to be a spontaneous process; however, since macromolecular aggregation or formation of incorrect structures is possible, specific protein molecules, molecular chaperones, are often involved in this process [53]. Moreover, molecular chaperones can maintain the native conformation of a protein under stresses of various nature. Physicochemical interactions between the chaperones and the proteins they protect prevent unfolding and subsequent aggregation of protein molecules and thus preserve their native organization. Chemical chaperones can also protect native structures from damaging influences by forming complexes with protein macromolecules. As a result of the diversity and broad spectrum of action of chaperones, they are objects of constant interest; the number of newly discovered chaperones is still increasing. It was also found that insoluble hyperexpressed or heterologous proteins or those with an incorrect tertiary structure can be refolded in the presence of osmolytes [36, 54].

4. PCR optimization. Some osmolytes can be used for PCR amplification of DNA with high G+C content

and therefore with a high melting point [55]. Betaine and ectoine, for example, have been shown to decrease the DNA melting temperature; ectoine was more efficient in this respect. Homoectoine, a synthetic derivative of ectoine, is, however, the most suitable agent for this purpose [56].

Cosmetology. Osmolytes are compatible with cellular metabolic processes but do not interfere with them; they can also stabilize biomolecules, cells, and tissues. These features have found application in cosmetology. Ectoine and hydroxyectoine are the most commonly used compounds. Ectoine has been found to protect human skin against harmful ultraviolet irradiation [57]; it also protects the skin microflora against unfavorable environmental factors. Introduction of ectoine or its derivatives into cosmetic preparations increases their humidifying activity and provides for the stabilization of the skin [58].

Since osmolytes can suppress the liberation of ceramides from skin cells, cosmetic preparations containing osmoregulators are used for protection of the skin against premature aging and wrinkling [59].

Medicine and pharmacy. The main metabolic function of ectoine is to be a donor of methyl groups; these are transported to homocysteine by betaine–homocysteine methyltransferase. Methionine is formed, the precursor of S-adenosyl methionine (SAM), which is an important participant in a number of biochemical processes, including the synthesis of nucleic acids, lipids, and proteins [60, 61]. In the course of certain diseases, the SAM level decreases. Successful application of SAM is known as a therapeutic agent for homocystinuria (this condition is related to the inhibition of homocysteine decomposition) and for liver disorders resulting from alcohol abuse. Formation of methionine and SAM is inhibited in the course of alcohol assimilation. SAM, however, is a rare natural compound; its high price limits its applications for therapeutic purposes. Since betaine is the precursor of SAM, it has been suggested for application in such cases. In fact, betaine can be used to protect the liver from such hepatotoxic compounds as ethanol or CCl_4. Introduction of betaine into the ration of rats has been found to stimulate the formation of methionine and SAM in the liver [62, 63]. Therefore, betaine can be expected to have therapeutic potential for the treatment and prophylaxis of the adipose infiltration of the liver; this disease is accompanied by decreased SAM levels and the initial stages of cirrhosis. A similar effect can be expected in the case of treatment of homocystinuria and other diseases which are accompanied by decreased SAM levels [64, 65].

Cardiovascular diseases [66] and neurological and psychological disorders [67] can be indirectly linked to impaired homocysteine metabolism. Betaine is used in the complex therapy of such diseases [68]. Betaine decreases the probability of heart attacks, infarctions, strokes, and diseases of peripheral arteries. Its anticoagulant properties help to prevent thrombus formation and proliferation [69]. The combination used in medicine contains vitamin B_6 and betaine, choline, or lecithin, as well as folic acid or vitamin B_{12} [68]. Betaine can be used as an anticoagulant for the storage of blood preparations. It has also been shown to decrease the side effects of anti-inflammatory preparations (diclophenac, indometacin). Moreover, this compound blocks the spasms caused by high homocysteine levels [70]. The cause of this anticonvulsant activity is not known; it is assumed to be related to the specific structure of the betaine molecule, which can compete with neurotransmitters for binding to synaptic receptors.

Ophthalmologic preparations containing osmoregulators and polyanionic components are useful for eye treatment to decrease the dryness syndrome, to protect the mucous membranes of the eye against unfavorable factors, and for more rapid healing after surgery [71]. Introduction of ectoine and its derivatives into preparations for oral care has also been suggested [72]. Effects of betaine on lipid metabolism, including cholesterol metabolism have been demonstrated in experiments on rabbits; it has also been shown to be applicable for the treatment of atherosclerosis, which results from high cholesterol levels [73].

Agriculture. Agricultural productivity depends on soil salinity. The negative effect of salts on plants is caused by water deficiency (the result of osmotic stress) and by the effect of excessive Na^+ ions on important biochemical processes. Under these conditions, nitrogen consumption, the efficiency of its transport from root to stem, and nitrate assimilation in leaves decrease [74]. In response to salt stress, plants accumulate such low-molecular osmoregulatory compounds as sugar alcohols and amino acids. Accumulation of these compounds is the basic mechanism of salt tolerance and adaptation to osmotic stress in plants; in some cases, however, the amount of osmoregulators is insufficient. Since the effect of the osmoregulators of bacterial origin is not species-specific, alien systems of their biosynthesis can be introduced into plants to protect the new host [75].

The introduction of genes responsible for the synthesis of osmolytes into nonhalophilic organisms increases their resistance to salt stress. Since plants are often subject to droughty conditions, which increase soil salinity, they are suitable objects for such experiments. For example, the introduction of the *Halomonas elongata* genes responsible for ectoine biosynthesis [76] increased the salt tolerance of the plants. Water delivery from roots to stems was improved, as was nitrogen supply to the leaves; the latter was the result of enhanced transpiration. The rates of photosynthesis increased due to the protection of RuBisCO against salts.

Expression of the system of glycine betaine synthesis has also contributed to the development of stress-resistant commercially important plant cultures, for example, transgenic tobacco plants exhibiting enhanced salt resistance. Experiments on the leaves of such plants have revealed that the damage caused by intense illumination and salt stress is repaired more quickly; resistance to photoinhibition at low temperatures also increases [77]. Agricultural plants, especially those in the reproductive phase, are known to be highly sensitive to low temperatures. Cold results in significant productivity losses. Expression in the genome of tomato plants of the genes responsible for glycine betaine synthesis has been shown to increase the cold resistance of both plants and seeds [78].

Direct treatment of plants with osmolyte-containing preparations is an alternative to the expression of systems of osmoregulator synthesis. Glycine betaine is successfully used to alleviate the low-temperature stress in a number of agricultural plants [79–81]. Plants treated with glycine betaine proved to be more resistant to decreased temperatures; the decrease in their productivity was insignificant. Use of betaine treatment in order to increase the cold resistance of seeds has also been suggested [82].

CONCLUSIONS

Detailed analysis of the bacterial mechanisms which regulate the system of response to salt stresses improves our understanding of bacterial growth and survival in natural environments and enables the development of new technological processes in various fields of industry. The data presented in this review confirm the productivity of industrial application of osmoregulators; the presented results on haloalkaliphilic organisms suggest the possibility of their use as producers of such compounds.

ACKNOWLEDGMENTS

The work was supported by the President of the Russian Federation Leading Scientific Schools grant no. RI-12/001/027 and the Presidium of the Russian Academy of Sciences Molecular and Cell Biology grant (supervisor, G.A. Zavarzin).

REFERENCES

1. Räsänen, L.A., Saijets, S., Jokinen, K., and Lindstrm, K., Evaluation of the Roles of Two Compatible Solutes, Glycine Betaine and Trehalose, for the *Acacia senegal–Sinorhizobium* Symbiosis Exposed To Drought Stress, *Plant and Soil*, 2004, vol. 260, pp. 237–251.
2. Bohnert, H.J. and Shen, B., Transformations and Compatible Solutes, *Scientia Horticulturae*, 1999, vol. 78, pp. 237–260.
3. Khmelenina, V.N., Kalyuzhnaya, M.G., Sakharovsky, V.G., Suzina, N.E., Trotsenko, Y.A., and Gottschalk, G., Osmoadaptation in Halophilic and Alkaliphilic Methanotrophs, *Arch. Microbiol.*, 1999, vol. 172, pp. 321–329.
4. Trotsenko, Yu.A. and Khmelenina, V.N., The Biology and Osmoadaptation of Haloalkaliphilic Methanotrophs, *Mikrobiologiya*, 2002, vol. 71, pp. 149–159 [*Microbiology* (Engl. Transl.), vol. 71, no. 2, pp. 123–132].
5. Trotsenko, Yu.A. and Khmelenina, V.N., Biology of Extremophilic and Extremotolerant Methanotrophs, *Arch. Microbiol.*, 2002, vol. 177, pp. 123–131.
6. Banciu, H., Sorokin, D.Yu., Kleerebezem, R., Muyzer, G., Galinski, E.A., and Kuenen, J.G., *Thioalkalivubrio halophilus* sp. nov., a Novel Obligately Chemolithoautotriphic, Facultalively Alkaliphilic, and Extremely Salt-Tolerant, Sulfur-Oxidizing Bacterium from a Hypersaline Alkaline Lake, *Extremophiles*, 2004, vol. 8, pp. 185–192.
7. Romano, I., Giordano, A., Lama, L., Nicolaus, B., and Gambacorta, A., *Halomonas campaniensis* sp. nov., a Haloalkaliphilic Bacterium Isolated from a Mineral Pool of Campania Region, Italy, *Syst. Appl. Microbiol.*, 2005, vol. 28, pp. 610–618.
8. Zhilina, T.N. and Zavarzin, G.A., Alkaliphilic Anaerobic Community at pH 10, *Curr. Microbiol.*, 1994, vol. 29, pp. 109–112.
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 [*Microbiology* (Engl. Transl.), vol. 68, no. 5, pp. 503–521].
10. Zavarzin, G.A. and Zhilina, T.N., Anaerobic Chemotrophic Alkaliphiles, *Journey to diverse microbial worlds*, Seckbach, J., Ed., Kluwer, 2000, pp. 191–208.
11. Pikuta, E.V., Hoover, R.B., Bej, A.K., Marsic, D., Detkova, E.N., Whitman, W.B., and Krader, P., *Tindallia californiensis* sp. nov., a New Anaerobic, Haloalkaliphilic, Spore-Forming Acetogen Isolated from Mono Lake in California, *Extremophiles*, 2003, vol. 7, pp. 327–334.
12. Boltyanskaya, Yu.V., Antipov, A.N., Kolganova, T.V., Lysenko, A.M., Kostrikina, N.A., and Zhilina, T.N., *Halomonas campisalis*, an Obligatorily Alkaliphilic, Nitrous Oxide-Reducing Denitrifier with a Molybdenum Cofactor-Lacking Nitrate Reductase, *Mikrobiologiya*, 2004, vol. 73, pp. 326–334 [*Microbiology* (Engl. Transl.), vol. 73, no. 3, pp. 271–278].
13. Boltyanskaya, Yu.V., Kevbrin, V.V., Lysenko, A.M., Kolganova, T.V., Turova, T.P., Osipov, G.A., and Zhilina, T.N., *Halomonas mongoliensis* sp. nov. and *Halomonas kenyensis* sp. nov., Novel Haloalkaliphilic Denitrifiers from Soda Lakes capable of N_2O Reduction, *Mikrobiologiya*, 2007, vol. 76 (accepted for publication).
14. Imhoff, J.F., Survival Strategies of Microorganisms in Extreme Saline Environments, *Adv. Space Res.*, 1986, vol. 6, pp. 299–306.
15. Dennis, P.P. and Shimmin, L.C., Evolutionary Divergence and Salinity-Mediated Selection in Halophilic Archaea, *Microbiol. Mol. Biol. Rev.*, 1997, vol. 61, pp. 90–104.
16. Oren, A., Intracellular Salt Concentrations of the Anaerobic Halophilic Eubacteria *Haloanaerobium praevalens* and *Halobacteroides halobius, Can. J. Microbiol.*, 1986, vol. 32, pp. 4–9.
17. Rengpipat, S., Lowe, S.E., and Zeikus, J.G., Effect of Extreme Salt Concentrations on the Physiology and Bio-

chemistry of *Halobacteroides acetoethylicus, J. Bacteriol.*, 1988, vol. 170, pp. 3065–3071.

18. Oren, A., Life at High Salt Concentrations, *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, Dworkin, M., Falkow, S., Rosenberg, E., Scheifer, K.-H., and Stackebrandt, E., Eds., New York: Springer, 2000, 3rd ed. (electronic publication).
19. Oren, A., Heldal, M., Norland, S., and Galinski, E.A., Intracellular Ion and Organic Solute Concentrations of the Extremely Halophilic Bacterium *Salinibacter ruber, Extremophiles*, 2002, vol. 6, pp. 491–498.
20. Oren, A. and Mana, L., Amino Acid Composition of Bulk Protein and Salt Relationships of Selected Enzymes of *Salinibacter ruber*, an Extremely Halophilic Bacterium, *Extremophiles*, 2002, vol. 6, pp. 217–223.
21. Ventosa, A., Nieto, J.J., and Oren, A., Biology of Moderately Halophilic Aerobic Bacteria, *Microbiol. Mol. Biol. Rev.*, 1998, vol. 62, pp. 504–544.
22. Zhilina, T.N., Zavarzin, G.A., Detkova, E.N., and Rainey, F.A., *Natroniella acetigena* gen. nov., sp. nov., an Extremely Haloalkaliphilic, Homoacetic Bacterium: a New Member of *Haloanaerobiales, Curr. Microbiol.*, 1996, vol. 32, pp. 320–326.
23. Boltyanskaya, Yu.V., Detkova, E.N., Shumskii, A.N., Dulov, L.E., and Pusheva, M.A., Osmoadaptation in Representatives of Haloalkaliphilic Bacteria from Soda Lakes, *Mikrobiologiya*, 2005, vol. 74, pp. 738–744 [*Microbiology* (Engl. Transl.), vol. 74, no. 6, pp. 640–645].
24. Pusheva, M.A., Pitryuk, A.V., and Zavarzin, G.A., Na^+- and H^+-Dependent ATP Synthesis in Extremely Alkaliphilic Anaerobes, *Dokl. Akad. Nauk*, 2000, vol. 374, pp. 833–835 [*Dokl.* (Engl. Transl.), vol. 374, pp. 546–548].
25. Pitryuk, A.V. and Pusheva, M.A., Different Ionic Specificities of ATP Synthesis in Extremely Alkaliphilic Sulfate-Reducing and Acetogenic Bacteria, *Mikrobiologiya*, 2001, vol. 70, pp. 459–464 [*Microbiology* (Engl. Transl.), vol. 70, no. 4, pp. 398–402].
26. Oren, A., Bioenergetic Aspects of Halophilism, *Microbiol. Mol. Biol. Rev.*, 1999, vol. 63, pp. 334–348.
27. Lanyi, J.K., Salt-Dependent Properties of Proteins from Extremely Halophilic Bacteria, *Bacteriol. Rev.*, 1974, vol. 38, pp. 272–290.
28. Eisenberg, H., Life in Unusual Environments: Progress in Understanding the Structure and Function of Enzymes from Extreme Halophilic Bacteria, *Arch. Biochem. Biophys.*, 1995, vol. 318, pp. 1–5.
29. Oren, A. and Gurevich, P., The Fatty Acid Synthetase of *Haloanaerobium praevalens* Is Not Inhibited by Salt, *FEMS Microbiol. Letts.*, 1993, vol. 108, pp. 287–290.
30. Gandbhir, M., Rasched, I., Marlire, P., and Mutzel, R., Convergent Evolution of Amino Acid Usage in Archaebacterial and Eubacterial Lineages Adapted to High Salt, *Res. Microbiol.*, 1995, vol. 146, pp. 113–120.
31. Detkova, E.N. and Boltyanskaya, Yu.V., Relationships between the Osmoadaptation Strategy, Amino Acid Composition of Bulk Protein, and Properties of Certain Enzymes of Haloalkaliphilic Bacteria, *Mikrobiologiya*, 2006, vol. 75, pp. 312–319 [*Microbiology* (Engl. Transl.), vol. 75, no. 3, pp. 259–265].
32. Pusheva, M.A., Detkova, E.N., Bolotina, N.P., and Zhilina, T.N., Characteristics of the Periplasmic Hydrogenase of the Extremely Halophilic Homoacetic Bacterium *Acetohalobium arabaticum, Mikrobiologiya*, 1992, vol. 61, pp. 933–938.
33. Zhilina, T.N., Zavarzin, G.A., Bulygina, E.S., Kevbrin, V.V., Osipov, G.A., and Chumakov, K.M., Ecology, Physiology and Taxonomy Studies on a New Taxon of *Haloanaerobiaceae, Haloincola saccharolytica* gen. nov., sp. nov, *Syst. Appl. Microbiol.*, 1992, vol. 15, pp. 275–284.
34. Krulwich, T.A., Ito, M., Hicks, D.B., Gilmour, R., and Guffanti, A.A., pH Homeostasis and ATP Synthesis: Studies of Two Processes That Necessitate Inward Proton Translocation in Extremely Alkaliphilic Bacillus Species, *Extremophiles*, 1998, vol. 2, pp. 217–222.
35. Galinski, E.A., Compatible Solutes of Halophilic Eubacteria: Molecular Principles, Water-Solute Interaction, Stress-Protection, *Experientia*, 1993, vol. 49, pp. 487–496.
36. Roberts, M.F., Organic Compatible Solutes of Halotolerant and Halophilic Microorganisms, *Saline systems*, 2005, vol. 1.
37. Göller, K. and Galinski, E.A., Protection of a Model Enzyme (Lactate Dehydrogenase) Against Heat, Urea and Freeze-Thaw Treatment by Compatible Solute Additives, *J. Mol. Catalysis B: Enzymatic*, 1999, vol. 7, pp. 37–45.
38. Pflüger, K. and Müller, V., Transport of Compatible Solutes in Extremophiles, *J. Bioenerg. Biomembr.*, 2004, vol. 36, pp. 17–24.
39. Galinski, E.A. and Trüper, H.G., Betaine, a Compatible Solute in the Extremely Halophilic Phototrophic Bacterium *Ectothiorhodospira halochloris, FEMS Microbiol. Letts.*, 1982, vol. 13, pp. 357–360.
40. Galinski, E.A., Pfeiffer, H.P., and Trüper, H.G., 1,4,5,6-Tetrahydro-2-Methyl-4-Pyrimidinecarboxylic Acid. A Novel Cyclic Amino Acid from Halophilic Phototrophic Bacteria of the Genus *Ectothiorhodospira, Eur. J. Biochem.*, 1985, vol. 149, pp. 135–139.
41. Severin, J., Wohlfarth, A., and Galinski, E.A., The Predominant Role of Recently Discovered Tetrahydropyrimidines for the Osmoadaptation of Halophilic Eubacteria, *J. Gen. Microbiol.*, 1992, vol. 138, pp. 1629–1638.
42. Imhoff, J.F., Osmoregulation and Compatible Solutes in Eubacteria, *FEMS Microbiol. Rev.*, 1986, vol. 39, pp. 57–66.
43. Detkova, E.N., Osmoadaptation of Halophilic Bacteria from Soda Lakes, Proc. Winogradsky Institute of Microbiology, Russian Acad. Sci., (in press).
44. Kevbrin, V.V., Zhilina, T.N., Rainey, F.A., and Zavarzin, G.A., *Tindallia magadii* gen. nov., sp. nov.: an Alkaliphilic Anaerobic Ammonifier from Soda Lake Deposits, *Curr. Microbiol.*, 1998, vol. 37, pp. 94–100.
45. Padan, E., Venturi, M., Gerchman, Y., and Dover, N., Na^+/H^+ Antiporters, *Biochim. Biophys. Acta, Bioenergetics*, 2001, vol. 1505, pp. 144–157.

46. Kraegeloh, A. and Kunte, H.J., Novel Insights Into the Role of Potassium for Osmoregulation in *Halomonas elongata, Extremophiles*, 2002, vol. 6, pp. 453–462.
47. Kotsyurbenko, O.R., Simankova, M.V., Nozhevnikova, A.N., Zhilina, T.N., Bolotina, N.P., Lysenko, A.M., and Osipov, G.A., New Species of Psychrophilic Acetogens: *Acetobacterium bakii* sp. nov., *A. paludosum* sp. nov., *A. fimetarium* sp. nov, *Arch. Microbiol.*, 1995, vol. 163, pp. 29–34.
48. Boltyanskaya, Yu.V., Physiological and Biochemical Characteristics of Some Haloalkaliphilic Bacteria, *Cand. Sci. (Biol.) Dissertation*, Moscow, 2006.
49. Lippert, K. and Galinski, E.A., Enzyme Stabilization by Ectoine-Type Compatible Solutes: Protection Against Heating, Freezing and Drying, *Appl. Microbiol. Biotechnol.*, 1992, vol. 37, pp. 61–65.
50. Sauer, T. and Galinski, E.A., Bacterial Milking: a Novel Bioprocess for Production of Compatible Solutes, *Biotechnol. Bioeng.*, 1998, vol. 57, pp. 306–313.
51. Wohlfarth, A., Severin, J., and Galinski, E.A., The Spectrum of Compatible Solutes in Heterotrophic Halophilic Eubacteria of the Family *Halomonadaceae, J. Gen. Microbiol.*, 1990, vol. 136, pp. 705–712.
52. Cleland, D., Krader, P., McCree, C., Tang, J., and Emerson, D., Glycine Betaine As a Cryoprotectant for Prokaryotes, *J. Microbiol. Methods*, 2004, vol. 58, pp. 31–38.
53. Lund, P.A., Microbial Molecular Chaperones, *Adv. Microb. Physiol.*, 2001, vol. 44, pp. 93–140.
54. Barth, S., Huhn, M., Matthey, B., Klimka, A., Galinski, E.A., and Engert, A., Compatible-Solute-Supported Periplasmic Expression of Functional Recombinant Proteins Under Stress Conditions, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 1572–1579.
55. Henke, W., Herdel, K., Jung, K., Schnorr, D., and Loening, S., Betaine Improves the PCR Amplification of GC-Rich DNA Sequences, *Nucleic Acids Res.*, 1997, vol. 25, pp. 3957–3958.
56. Schnoor, M., Voss, P., Cullen, P., Boking, T., Galla, H.J., Galinski, E.A., and Lorkowski, S., Characterization of the Synthetic Compatible Solute Homoectoine As a Potent PCR Enhancer, *Biochem. Biophys. Res. Commun.*, 2004, vol. 322, pp. 867–872.
57. Bünger, J. and Driller, H., Ectoin: An Effective Natural Substance To Prevent UVA-Induced Premature Photoaging, *Skin Pharmacol. Physiol*, 2004, vol. 17, pp. 232–237.
58. Martin, R., Bünger, J., and Driller, H., Use of Ectoine Or Ectoine Derivatives in Cosmetic Formulations, US Patent, 6602514, 2003
59. Bünger, J. and Krutmann, J., Use of Compatible Solutes for Inhibiting the Release of Ceramides, *US Patent Application*, 20050201955, 2005.
60. Finkelstein, J.D., Pathways and Regulation of Homocysteine Metabolism in Mammals, *Semin. Thromb. Hemost*, 2000, vol. 26, pp. 219–225.
61. Schwahn, B.C., Chen, Z., Laryea, M.D., Wendel, U., Lussier-Cacan, S., and Genest, J., Mar MH., Zeisel S.H., Castro C., Garrow T., Rozen R. Homocysteine-Betaine Interactions in a Murine Model of 5,10-Methylenetetrahydrofolate Reductase Deficiency, *Faseb J.*, 2003, vol. 17, pp. 512–514.
62. Barak, A.J., Beckenhauer, H.C., Junnila, M., and Tuma, D.J., Dietary Betaine Promotes Generation of Hepatic S-Adenosylmethionine and Protects the Liver from Ethanol-Induced Fatty Infiltration, *Alcohol. Clin. Exp. Res.*, 1993, vol. 17, pp. 552–555.
63. Barak, A.J., Beckenhauer, H.C., Mailliard, M.E., Kharbanda, K.K., and Tuma, D.J., Betaine Lowers Elevated S-Adenosylhomocysteine Levels in Hepatocytes from Ethanol-Fed Rats, *J. Nutr.*, 2003, vol. 133, pp. 2845–2848.
64. Smolin, L.A., Benevenga, N.J., and Berlow, S., The Use of Betaine for the Treatment of Homocystinuria, *J. Pediatr*, 1981, vol. 99, pp. 467–472.
65. Schwahn, B.C., Hafner, D., Hohlfeld, T., Balkenhol, N., Laryea, M.D., and Wendel, U., Pharmacokinetics of Oral Betaine in Healthy Subjects and Patients with Homocystinuria, *Br. J. Clin. Pharmacol.*, 2003, vol. 55, pp. 6–13.
66. Eikelboom, J.W., Lonn, E., Genest, J.., Hankey, G., and Yusuf, S., Homocyst(e)ine and Cardiovascular Disease: a Critical Review of the Epidemiologic Evidence, *Ann. Int. Med.*, 1999, vol. 131, pp. 363–375.
67. Regland, B., Johansson, B.V., Grenfeldt, B., Hjelmgren, L.T., and Medhus, M., Homocysteinemia Is a Common Feature of Schizophrenia, *J. Neural. Transm.*, 1995, vol. 100, pp. 165–169.
68. Wilcken, D.E. and Wilcken, B., B Vitamins and Homocysteine in Cardiovascular Disease and Aging, *Ann. NY Acad. Sci.*, 1998, vol. 854, pp. 361–70.
69. Messadek, J., Glycine Betaine and Its Use, US Patent, 6855734, 2005.
70. Freed, W.J., Gillin, J.C., and Wyatt, R.J., Anticonvulsant Properties of Betaine, *Epilepsia*, 1979, vol. 20, pp. 209–213.
71. Vehige, J.G., Simmons, P.A., and Chang-Lin, J.-E., Ophthalmic Compositions and Methods for Treating Eyes, *US Patent Application*, 2006, p. 20060106104.
72. Buenger, J. and Driller, H., and den Hollander, O., Use of Ectoine Or Ectoine Derivatives for Oral Care, US Patent, 7048910, 2006.
73. Panteleimonova, T.N. and Zapadniuk, V.I., Effect of Trimethylglycine on Lipid Metabolism in Experimental Atherosclerosis in Rabbits, *Farmakol. Toksikol.*, 1983, vol. 46, pp. 83–85.
74. Peuke, A.D., Glaab, J., Kaiser, W.M., and Jeschke, W.D., The Uptake and Flow of C, N and Ions between Roots and Shoots in *Ricinus communis* L.. IV. Flow and Metabolism of Organic Nitrogen and Malate Depending on Nitrogen Nutrition and Salt Treatment, *J. Exp. Bot.*, 1966, vol. 47, pp. 377–385.
75. Rontein, D., Basset, G., and Hanson, A.D., Metabolic Engineering of Osmoprotectant Accumulation in Plants, *Metab. Eng.*, 2002, vol. 4, pp. 49–56.
76. Moghaieb, R.E., Tanaka, N., Saneoka, H., Murooka, Y., Ono, H., Morikawa, H., Nakamura, A., Nguyen, N.T., Suwa, R., and Fujita, K., Characterization of Salt Tolerance in Ectoine-Transformed Tobacco Plants (*Nicotiana tabaccum*): Photosynthesis, Osmotic Adjustment, and

Nitrogen Partitioning, *Plant, Cell Environ.*, 2006, vol. 29, pp. 173–182.

77. Holmstrom, K.O., Somersalo, S., Mandal, A., Palva, T.E., and Welin, B., Improved Tolerance to Salinity and Low Temperature in Transgenic Tobacco Producing Glycine Betaine, *J. Exp. Bot.*, 2000, vol. 51, pp. 177–185.
78. Park, E.J., Jeknic, Z., Sakamoto, A., DeNoma, J., Yuwansiri, R., Murata, N., and Chen, T.H., Genetic Engineering of Glycinebetaine Synthesis in Tomato Protects Seeds, Plants, and Flowers from Chilling Damage, *Plant J.*, 2004, vol. 40, pp. 474–487.
79. Rajashekar, C.B., Zhou, H., Marcum, K.B., and Prakash, O., Glycine Betaine Accumulation and Induction of Cold Tolerance in Strawberry (*Fragaria ananassa* Duch.) Plants, *Plant Sci.*, 1999, vol. 148, pp. 175–183.
80. Nayyar, H., Chander, K., Kumar, S., and Bains, T., Glycine Betaine Mitigates Cold Stress Damage in Chickpea, *Agron. Sustain. Dev.*, 2005, vol. 25, pp. 381–388.
81. Park, E.J., Jeknic, Z., and Chen, T.H., Exogenous Application of Glycinebetaine Increases Chilling Tolerance in Tomato Plants, *Plant Cell Physiol.*, 2006, vol. 47, pp. 706–714.
82. Naidu, B.P., Method for the Treatment of Seed with Betaines To Increase Stress Tolerance, Seedling Vigour and Yield, *Australian Patent Application*, no. 27071/95.