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Kinetics of Halophilic Enzymes

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Abstract

Hypersaline environments are those with salt concentrations 9–10 times higher (30–35% of NaCl) than sea water (3.5% of NaCl). At high concentrations of soluble salts, cytoplasm—mainly of bacteria and archaea—is exposed to high ionic strength and achieves osmotic equilibrium by maintaining a cytoplasmic salt concentration similar to that of the surrounding media. Halophilic enzymes are extremozymes produced by halophilic microorganisms; they have similar characteristics to regular enzymes but different properties, mainly structural. Among these properties is a high requirement of salt for biological functions. Furthermore, the discovery of enzymes capable of degrading biopolymers offer a new perspective in the treatment of residues from oil deposits, under typically high conditions of salt and temperature, while giving valuable information on heterotrophic processes in saline environments.

Keywords: halotolerants, halophiles, *salt-in*, synthesis intracellular compounds, extremozymes

1. Introduction

Extreme environments involve a wide range of extreme conditions (pH, temperature, pressure, light intensity, oxygen, nutrient conditions, heavy metals, and salinity). Hypersaline environments are those with salt concentrations 9–10 times higher (30–35% of NaCl) than sea water (3.5% of NaCl). These sites are widely distributed around the world and can harbor microorganisms from three different life domains (archaea, bacteria, and eukaryota); together, these microorganisms are known as halophiles, which survive or even thrive in saline environments [1].

2. Classification of halophiles and halophile environments

Nowadays, several classifications of halophiles have been suggested; the classification proposed by Ollivier et al. [2] considers those microorganisms capable of growing in salt concentrations $\geq 150 \text{ g L}^{-1}$ (15% w/v, 2.5 M) as halophiles. Another classification considers the optimum growth salinity as follows: mild halophiles (1–6%, w/v NaCl), moderate halophiles (7–15%) and extreme halophiles (15–30%) [3]. On the other hand, Ventosa and Arahal [4] defines halophiles as organisms that have an optimal growth above 3% salt concentration; if the optimal growth occurs between 3 and 15% salt, they are regarded as moderate halophiles; and when it occurs above 15% and up to halite saturation (34%), they are regarded as extreme halophiles. In addition, DasSarma and DasSarma [5] described halophiles as those organisms that thrive from sea salinity ($\sim 0.6 \text{ M}$) up to saturation salinity ($>5 \text{ M NaCl}$). However, the most complete and widely used classification scheme was proposed by Kushner and Kamekura [6], in which halophilic microorganisms are separated into six groups based on their salt requirement and tolerance (**Table 1**): non-halophiles are those that have optimal growth in culture media containing less than 0.2 M NaCl ; slight halophiles (marine bacteria) grow best in media with $0.2\text{--}0.5 \text{ M NaCl}$; moderate halophiles grow best with $0.5\text{--}2.5 \text{ M NaCl}$; borderline extreme halophile that growth best at $2.5\text{--}4.0 \text{ M}$; extreme halophiles show optimal growth in culture media containing NaCl concentration between 4 and 5.9 M ; and finally halo-tolerant microorganisms, which are non-halophiles that can tolerate high salt concentrations but do not require salt to survive; any microorganism viable at 2.5 M of NaCl is considered extremely halotolerant. Archaea and bacteria are the most widely distributed organisms in hypersaline environments [7], especially in those in which salinities exceed 1.5 M (about 10%). In recent years, halophilic organisms are mainly isolated from saline environments, such as salt lakes, marine solar salterns, saline soils, and marine sediments (see **Table 1**). However, halophile bacteria have also been isolated from some non-common places, for example, textile effluents, halophytes, mine tailings as well as processed foods (**Table 1**).

2.1. Hypersaline environments

Hypersaline environments are extreme habitats with limited microbial diversity as result of high salt concentrations and other environmental factors. Nowadays, most environmental studies have been carried out on aquatic habitats, such as saline lakes and solar salterns used for the production of salt for commercial purposes [8]. Nevertheless, halophilic bacteria can be found in other habitats including saline soils, salted foods and other products, hides, and deep-sea brine pools [7, 9–11]. Depending on whether they originated or not from seawater, hypersaline environments are classified as thalassohaline and athalassohaline, respectively.

2.1.1. Thalassohaline environments

The thalassohaline environments are saline environments of marine origin, which contain the following ions: Cl^{-1} , Na^{+} , Mg^{2+} , SO_4^{2-} , K^{+} , Ca^{2+} , Br^{-} , HCO_3^{-} , and F^{-} [4]. Some examples of thalassohaline as explained as follows.

Category	Salt tolerance (M)	Example	Isolation site	References
Non-halophile	<0.2	<i>Vibrio palustris</i> EAdo9 ^T and <i>Vibrio spartinae</i> SMJ221 ^T	Salt-marsh plants	[106]
Slight halophile	0.2–0.5	<i>Paracoccus</i> sp. GSM2	Textile mill effluent	[107]
		<i>Bacillus</i> sp. NY6	Saline wastewater	[108]
		<i>Zunongwangia endophytica</i> CPA58 ^T	Tissues of the halophyte <i>Halimione portulacoides</i>	[109]
Moderate halophile	0.5–2.5	<i>Salinispora arenicola</i> CNH-643 ^T and <i>Salinispora tropica</i> CNB-440 ^T	Marine sediments	[110]
		<i>Salinispora pacifica</i> CNR-114 ^T	Marine sediments	[111]
		<i>Martelella endophytica</i> YC6887 ^T	Root of <i>Rosa rugosa</i>	[112]
		<i>Streptomyces halophyticola</i> KLBMP 1284 ^T	Stems of <i>Tamarix chinensis</i>	[113]
		<i>Labrenzia suaedae</i> YC6927 ^T	Root of <i>Sauceda maritima</i>	[114]
		<i>Kocuria arsenatis</i> CM1E1 ^T	<i>Prosopis laevigata</i>	[115]
		<i>Proteus</i> sp. NA6	Textile effluent drain	[116]
		<i>Candidatus Desulfonatrobullus propionicus</i>	Hypersaline soda lakes	[117]
		<i>Novosphingobium pokkali</i> L3E4 ^T	Rhizosphere of saline-tolerant pokkali rice	[118]
		<i>Marinobacter aquaticus</i> M6-53 ^T	Marine saltern located in Huelva, Spain	[119]
		<i>Agrobacterium salinitolerans</i> YIC 5082 ^T	Root nodules of <i>Sesbania cannabina</i> grown in a high-salt and alkaline environment	[120]
		<i>Salinicola tamaricis</i> F01 ^T	Leaves of <i>Tamarix chinensis</i>	[121]
		<i>Salinirubellus salinus</i> ZS-35-S2 ^T	Marine solar saltern	[122]
		<i>Aliifodiniibius halophilus</i> 2W32 ^T	Marine solar saltern	[123]
		<i>Desulfosalsimona propionica</i> PropA ^T	Hypersaline sediment of the Great Salt Lake	[124]
Borderline extreme halophile	2.5–4.0	<i>Salinibacter iranica</i> CB7 ^T and <i>Salinibacter luteus</i> DGO ^T	Aran-Bidgol salt lake, Iran	[125]
		<i>Halanaerobium sehlinense</i> 1Sehel ^T	Sediments of the hypersaline lake Sehline Sebkha	[126]
		<i>Sporohalobacter salinus</i> CEJFT1B ^T	Under the salt crust of El-Jerid hypersaline lake in southern Tunisia	[127]
		<i>Lentibacillus kimchii</i> K9 ^T	Korean fermented food (kimchi)	[128]
		<i>Marinobacter salexigens</i> HJR7 ^T	Marine sediment	[129]
		<i>Gracilimonas halophila</i> WDS2C40 ^T	Marine solar saltern	[130]
		<i>Salinifilum proteinilyticum</i> Miq-12 ^T	Wetland in Iran	[131]
		<i>Natronospira proteinivora</i> Bsker1 ^T	Marine solar saltern	[132]

Category	Salt tolerance (M)	Example	Isolation site	References
Extreme halophile	4–5.9	<i>Salinibacter ruber</i> M31 ^T	Saltern crystallizer ponds in Alicante and Mallorca, Spain	[12]
		<i>Limimonas halophila</i> IA16 ^T	Mud of the hypersaline Lake Aran-Bidgol, Iran	[133]
		<i>Desulfonatrobacter acetoxydans</i> APT3	Hypersaline soda lake	[47]
Halo-tolerant	A non-halophile that tolerant salt; if it is viable 2.5 M, in is considered extremely halotolerant	<i>Brevibacterium salitolerans</i> TRM 415 ^T	Sediment from a salt lake	[134]
		<i>Kineococcus endophytica</i> KLMMP 1274 ^T	Halophytic plant (<i>Limonium sinense</i>)	[135]
		<i>Anditalea andensis</i> ANESC-S ^T	Alkali-saline soil	[136]
		<i>Brevibacterium jeotgali</i> SJ5-8 ^T	Traditional Korean fermented seafood	[137]
		<i>Salimicrobium</i> sp. LY19	Saline soil	[138]
		<i>Brevibacterium metallicus</i> NM3E2 ^T	Edge of mine tailings	[139]
		<i>Bacillus subtilis</i> BLK-1.5	Salt mines	[76]
		<i>Halomonas nigrificans</i> MB G8645 ^T	Acid curd cheese called Quargel	[140]

Table 1. Classification of bacteria based on their salinity tolerance according with criteria proposed by Kushner and Kamekura [6].

2.1.1.1. Solar salterns

These sites have a similar composition to seawater and they are used for salt production by evaporation. They generally consist of several ponds interconnected to form the so-called multipond system. Seawater is pumped or allowed to flow into the first ponds, and as a consequence of solar evaporation, the concentration of salts increases slightly and the water is moved to the next ponds, where it will concentrate further. Finally, in the last pond (called crystallizer), common salt is precipitated [4]. Many studies have focused on the isolation of bacteria harbored in hypersaline environments, identifying the following major groups: Bacteroidetes [12], Firmicutes [13–15], γ -Proteobacteria [13, 16–17], and γ -Proteobacteria being the most abundant.

2.1.1.2. Soils

Saline soils are those with an electrical conductivity (EC) higher than 4 dS mL⁻¹, approximately 40 mM NaCl [18]. Nowadays, salinized areas are increasing at rate of 10% annually for various reasons, including low precipitation, high surface evaporation, weathering of native

rocks, irrigation with saline water, and poor cultural species practices [19]. Several studies on hypersaline soils mainly isolated moderate halophiles and non-halophilic bacteria affiliated with different genera of the following taxonomic groups: Firmicutes [20–22], actinobacteria [23, 24], and proteobacteria [25, 26].

2.1.1.3. Great Salt Lake

In 1957, a rock-filled railroad causeway was completed across the lake, dividing it into a northern and a southern basin. The northern arm presented high salinity (33%), while the southern arm separated by a semipermeable rock causeway contains a moderate concentration of salt (12%) [27]. A number of studies on the isolation of bacteria from the sediment from Great Salt Lake have been carried out [28, 29].

2.1.2. Athalassohaline environments

These are environments that do not have a marine origin and their ionic proportions are quite different from that of the dissolved salts in seawater [30]. They reflect the composition of the surrounding geology, topography, and climate conditions, often particularly influenced by the dissolution of mineral deposits [31].

2.1.2.1. Dead Sea

The Dead Sea, which is actually an inland lake, is famous for being so saline that people can float with ease on its surface. The site is composed mainly of divalent ions like Mg^{2+} [32]. The Dead Sea is a hypersaline lake with 34% salinity, and its name is due to the lack of any living macroscopic creatures. The lake consists of a deeper northern basin and a shallow southern basin, which has been recently dried up and used for commercial mineral production [33]. The water level is dependent on the balance between amount of freshwater inflow and evaporation [32]. The Jordan River is the main source of freshwater inflow, in addition to several water springs and the complex system of underwater springs, which has been recently discovered [34]. Metagenomic studies demonstrated the presence of Halobacterium-like sequences and Mg^{2+} transport-related proteins, suggesting a potential adaptation to the high magnesium concentration by Dead Sea halophiles [35]. In addition, metagenomic sequence analysis and amino acid profiling also demonstrated the presence of halophiles never previously isolated or sequenced in the Dead Sea [35, 36]. It was recently discovered that the Dead Sea harbors some bacteria with biotechnological properties, such as *Bacillus persicus* 24-DSM, which showed antimicrobial activity [33].

2.1.2.2. Soda lakes

Athalassohaline alkaline salt lakes (or soda lakes), rich in NaCl, $NaHCO_3$, and Na_2CO_3 , are usually formed by dissolution of rocks that are low in magnesium and calcium, which would

otherwise cause carbonate to precipitate [37, 38]. The most studied lakes are those in the East African Rift Valley, continental Russia, and the USA. In addition to being able to tolerate high pH values and elevated salinities, microbes inhabiting soda lakes have to cope with low availability of NH_4^+ , caused by weak dissociation of ammonia at high pH. An accumulation of stressful but volatile NH_3 may occur in enclosed alkaline-saline systems such as sea ice or locally in soda lakes [32]. Several studies about bacteria isolation from soda lakes from different continents are available [39–47].

Halophilic microorganisms have several biotechnological applications, such as β -carotene production of fermented foods. In recent years, uses of halophilic microorganisms have significantly increased. Many enzymes, stabilizers, and valuable compounds from halophiles may present advantages for the development of biotechnological production processes.

2.2. Biology and adaptation of halophilic bacteria

The first chemical stress encountered during the evolution of life on earth may have been salt stress. Thus, from the beginning, organisms must have evolved strategies and effective mechanisms for the stabilization of protoplasmic structures and ion regulation [48]. At high concentrations of soluble salts, cytoplasm—mainly of bacteria and archaea—is exposed to high ionic strength and achieves osmotic equilibrium by maintaining a cytoplasmic salt concentration similar to that of the surrounding media. This can affect microbes via two primary mechanisms: osmotic effect and specific ion effects. Soluble salts increase the osmotic potential (more negative) of the soil water, drawing water out of cells which may kill microbes and roots through plasmolysis [49, 50].

To thrive in the hypersaline environment, halophiles have two main adaptation mechanisms to prevent NaCl from diffusing into the cells. The first mechanism is accumulation of inorganic ions (mainly KCl) for balancing osmotic pressure. This mechanism is mainly utilized by aerobic and extremely halophilic archaea and some anaerobic halophilic bacteria [32, 49, 51]. In contrast, most halophilic bacteria accumulate water soluble organic compounds of low molecular weight, which are referred to as compatible solutes or osmolytes, to maintain low intracellular salt concentration [52–54].

2.2.1. Salt-in mechanisms

As mentioned above, microorganisms that grow optimally in the presence of extremely high salinities (up to 5 M NaCl), accumulate intracellular potassium and chloride ions in concentrations higher than the external NaCl concentration to maintain a turgor pressure. This so-called “salt-in” strategy is observed in *Halobacteriales* (archaea) and *Halanaerobiales* (anaerobic halophilic bacteria) [55, 56]. The mechanism (“salt-in” to balance “salt-out”) requires far-reaching adaptations of the entire intracellular machinery, as all enzymes and functions in the cytoplasm have to be functional in the presence of molar concentrations of KCl [57]. A characteristic feature of halophilic proteins from microorganisms that accumulate KCl for osmotic balance is their highly acidic nature, with a great excess of acidic amino acids (glutamate and

aspartate) over basic amino acids (lysine and arginine). Such proteins are highly negatively charged compared to their non-halophilic equivalents. In addition, halophilic proteins generally have a low content of hydrophobic amino acids [58, 59].

2.2.2. Synthesis of intracellular compounds

As explained above, microorganisms have the ability to adapt to or tolerate stress caused by salinity by accumulating osmolytes, also known as compatible solutes. The compatible solute strategy is broadly known in domain archaea, bacteria, as well as eukarya. Organisms accumulate organic solutes by uptake from the environment or *de novo* synthesis of organic compounds, such as sugars and polyols, amino acids and their derivatives, and other compatible solutes for protection against salinity stress [60–62].

Organic solutes act as stabilizers for biological structures and allow the cells to adapt not only to salts but also to heat, desiccation, cold, or even freezing conditions [63]. Many halophilic bacteria accumulate ectoine or hydroxyectoine as the predominant compatible solutes. Other intracellular compatible solutes include amino acids, glycine betaine and other compounds accumulated in small amounts [54].

Mei et al. [64] describe the physiology of a *Natrinema* sp. strain J7–2, an extremely halophilic archaea isolated from a salt mine in China, under salt stress conditions (15, 25, and 30% NaCl). This strain showed the highest growth rate at 25–30% of NaCl, while at 15% cells were more fragile. Furthermore, the glycerolipid and amino acidic metabolism showed a significant difference in cellular transcripts levels, perhaps playing a role in membrane production/alteration or in accumulation of specific amino acids (glutamate family—Glu, Arg and Pro; aspartate family—Asp; and aromatic amino acids—Phe and Trp), especially Glu and Asp as carbon substrates and energy resources or compatible solutes.

The most common inorganic solutes used as osmolytes by salinity tolerant microbes are potassium cations, while proline and glycine betaine are the main organic osmolytes [65]. However, the synthesis of these compounds requires high amounts of energy [50, 66]. Given these high energetic requirements, there are few reports of halophilic microorganisms that can produce compatible solutes to mitigate the stress by variable concentrations of salts. The capacity of two halophilic strains is noteworthy: *Planococcus* sp. VITP21 and *Bacillus* sp. VITP4, which are capable of *de novo* synthesis of two rarely occurring diamino acids, Nε-acetyl α-lysine and Nδ-acetyl ornithine, respectively; besides the well-known ectoine and proline [67] as simple diamino acidic molecules to tolerate salt stress.

2.3. Production of extremozymes

Halophilic enzymes are extremozymes produced by halophilic microorganisms; they have similar characteristics to regular enzymes but different properties, mainly structural. Among these properties is a high requirement of salt for biological functions. In recent years, different studies have focused on the detection of halophiles in saline environments in order to isolate and characterize new enzymatic activities. This resulted in several halophile hydrolases being

described, including amylases, lipases, and proteases. Furthermore, the discovery of enzymes capable of degrading biopolymers offer a new perspective in the treatment of residues from oil deposits, under typically high conditions of salt and temperature, while giving valuable information on heterotrophic processes in saline environments.

2.3.1. Extremozymes-producing halophiles

Nowadays, investigation on the production of extremozymes from different bacterial genus and halophilic archaea has intensified. This interest is due to their capacity to efficiently catalyze a process and show optimal activities at different salt concentrations. Halophiles are the most probable source of extremozymes, since they are also capable of tolerating alkaline pH and high temperatures, as reported by several authors [68–79].

Most of the evaluation studies on the enzymatic capacities of halophiles begin with the isolation of these microorganisms from environments considered extreme due to specific characteristics such as high salt concentrations, high pH values, and extreme temperature conditions. Sánchez-Porro et al. [80] report the isolation of moderately halophile strains from water and salterns in different areas of southern Spain: Almería (Cabo de Gata), Cádiz (San Vicente and San Fernando), and Huelva (Isla Bacuta, Río Tinto and Isla Cristina). Isolates have been identified as members of the genera *Salinivibrio*, *Bacillus*, *Salibacillus*, *Halomonas*, *Chromohalobacter*, *Salinicoccus*, and *Marinococcus* and they showed amylase, protease, lipase, and DNase activities.

In 2007, Vidyasagar et al. [68] isolated the extreme halophile *Chromohalobacter* sp. from solar lanterns, and subsequently produced and partially purified a halo-thermophile protease extracellular enzyme. *Chromohalobacter* sp. required a 4 M concentration of NaCl for optimal growth and protease secretion, and no growth was observed under 1 M NaCl. The initial pH of the medium for growth and enzyme production was in the interval of 7.0–8.0, with an optimum value of 7.2. Halophile *Salinivibrio* sp. isolated from Bakhtegan Lake in southern Iran also produced an extracellular protease [81].

Rohban et al. [82] studied extremophiles in Howz Soltan, a hypersaline lake located in central Iran. The organisms successfully isolated produced a wide variety of extracellular enzymes, where 84.4% had lipase activity, 76.6% amylase, 43.2% protease, 41.1% inulinase, 39.8% xylanase, 29.4% cellulase, 14.2% DNase, and 12.1% pectinase. Halophile strains were identified as members of the following genera: *Salicola*, *Halovibrio*, *Halomonas*, *Oceanobacillus*, *Thalassobacillus*, *Halobacillus*, *Virgibacillus*, *Gracilibacillus*, *Salinicoccus*, and *Piscibacillus*. Most of the lipase and DNase producers belonged to the *Gracilibacillus* and *Halomonas* genera, respectively, while most of the organisms capable of producing hydrolytic enzymes (amylase, protease, cellulase, and inulinase) were part of Gram-positive genera, such as *Gracilibacillus*, *Thalassobacillus*, *Virgibacillus*, and *Halobacillus*.

In 2011, Perez et al. [83] reported the isolation and purification of a lipase obtained from the *Marinobacter lipolyticus* SM19 halophile, isolated from a saline habitat in southern Spain. The properties of this enzyme are of great potential for the food industry. Li and Yu [84] isolated the halophile strain LY9, which has amylolytic properties, from soil samples obtained in Yuncheng, China. The strain LY9 was identified as a member of the *Halobacillus* genus and it was discovered that the production of amylase secreted for this strain depended on the salinity

of the growth medium. The maximum production of amylase was observed in the presence of 10% KCl or 10% NaCl. Maltose was the main product from hydrolysis of soluble starch, pointing out to β -amylase activity.

In agreement with previous studies from 2003, 2007, and 2009, Shahbazi and Karbalaeei-Heidari [85] reported the capacity of *Salinivibrio* sp. to produce extracellular low molecular weight proteases, and Jayachandra et al. [86] reported the isolation and identification of extracellular activity of hydrolytic enzymes from bacteria in the *Salinicoccus* sp. genus. Strain JAS4 was isolated from the Arabal soil in the west coast of Karnataka, India. These bacteria showed great potential to produce extracellular enzymes such as amylase, protease, inulinase, and gelatinase. Also in 2012, Kumar et al. [70] isolated halophiles from different saline environments in India, by means of morphological, biochemical, and 16S rRNA analyses. The authors identified the genera *Marinobacter*, *Virgibacillus*, *Halobacillus*, *Geomicrobium*, *Chromohalobacter*, *Oceanobacillus*, *Bacillus*, *Halomonas*, and *Staphylococcus* as having hydrolase activities of industrial relevance, pointing out the presence of amylases, lipases, and proteases. A new genus of marine bacteria is included among halophiles that are capable of producing extracellular hydrolytic enzymes, according to the research by Ardakani et al. [69], who isolated extracellular hydrolytic enzymes from the water and sediments of the Persian Gulf, in that site the isolation of bacteria that produced enzymes belong to the *Pseudoalteromonas* genera, and the activities include amylase, protease, and lipase.

During 2013, studies were made on enzyme-producing halophilic archaea capable of synthesizing two new alcohol-dehydrogenases, amylase and a thermostable halo-alkaliphile α -amylase; the producing organisms were identified as *Haloferax volcanii*, *Natrialba aegyptiaca* and *Halorubrum xinjiangense*, respectively. Hagaggi et al. [87] reported the isolation of the extremely halophilic archaea *Natrialba aegyptiaca*, from a salty soil near Aswan in Egypt. This organism is capable of producing an extracellular halophilic amylase that digests raw starch; therefore the enzyme may be used to efficiently process different vegetable sources. Moshfegh et al. [71] isolated a thermostable halo-alkaliphile α -amylase from an archaea located in the salty water of the Urmia Lake, which lies in northeast Iran. The producing organism was identified as *Halorubrum xinjiangense*, based on its morphological, biochemical, and molecular properties. Nigam et al. (2013) [72] tested the alkaline proteases produced by halophilic bacteria isolated from the Sambar Lake in Rajasthan for keratolytic activity. Moreno et al. [88] have shown that some microorganisms from hypersaline environments in Spain are able to produce hydrolytic enzymes; these have been related to the genera *Salinivibrio*, *Halomonas*, *Chromohalobacter*, *Bacillus-Salibacillus*, *Salinicoccus*, *Marinococcus*, *Halorubrum*, *Haloarcula*, *Halobacterium*, *Salicola*, *Salinibacter*, and *Pseudomonas*.

The bacteria isolated from a saline lake in Iran produced lipases, these bacteria belonging to the genera *Salicola*, *Halovibrio*, *Halomonas*, *Oceanobacillus*, *Thalassobacillus*, *Halobacillus*, *Virgibacillus*, *Gracilibacillus*, *Salinicoccus*, and *Piscibacillus*. On the other hand, sediments of deep waters in China have been found to contain amylase-producing organisms from the genera *Alcanivorax*, *Bacillus*, *Cobetia*, *Halomonas*, *Methyloarcula*, *Micrococcus*, *Myroides*, *Paracoccus*, *Planococcus*, *Pseudomonas*, *Psychrobacter*, *Sporosarcina*, *Sufflavibacter*, and *Wangia*. In a desert in Chile, enzymes with DNase activity were related to the genera *Bacillus*, *Halobacillus*, *Pseudomonas*, *Halomonas*, and *Staphylococcus* [89].

A halo-alkaliphile, thermostable extracellular protease was reported by Selim et al. [74] produced by *Natronolimnobius innermongolicus* WN18 (HQ658997), an organism that belongs to the genus *Natronolimnobius* and was isolated from the sodium lake of An-Natron, Egypt. Another halophile studied in the same year was the marine bacterium *Zunongwangia profunda*, due to its production of a new α -amylase resistant to low temperatures and tolerant to high concentrations of NaCl (4 M) [90].

Gupta et al. [75] reported a halo-alkaliphile isolated from a soil sample collected from the Sambhar Lake in Rajasthan, northern India, which produced an extracellular alkaline protease; the results of the analysis of gene 16S rRNA showed a 98% match with *Halobiforma* sp. Del Campo et al. [91] and Kumar and Khare [92] used fermentation in a solid medium to produce an esterase from halophilic archaea (*Natronococcus* sp. TC6, *Halobacterium* sp. NRC-1, and *Haloarcula marismortui*). These authors also optimized the production and nano-immobilization of *Marinobacter* sp. for an efficient hydrolysis of starch.

The *Kocuria* is mentioned as an example of a genus capable of producing extracellular amylases [77]. On the other hand, archaea *Halobacterium* sp. was isolated from samples of fermented fish and was considered a strong source of halophilic protease [93]; the bacterium *Bacillus licheniformis* isolated from sea water and sediments in Alexandria Eastern Harbor, Egypt, together with *Bacillus subtilis* isolated from the salt mines in Karak, Pakistan, were found to be producers of extracellular amylases and proteases, respectively [76, 94].

Dumorné et al. [95] stated that the halophiles *Acinetobacter*, *Haloferax*, *Halobacterium*, *Halorhabdus*, *Marinococcus*, *Micrococcus*, *Natronococcus*, *Bacillus*, *Halobacillus*, and *Halotheothrix* produce extremozymes such as xylanases, amylases, proteases, and lipases. Halophile bacterium *Idiomarina* produces two extracellular proteases and was isolated in Badab-Sourt, Iran [79]. The same year, Hosseini et al. [96] described the isolation of bacteria capable of nitrite reduction that belonged to five different genera: *Bacillus*, *Halobacillus*, *Idiomarina*, *Oceanobacillus*, and *Virgibacillus*, in a paper regarding denitrifying halophile bacteria. Isolates capable of producing nitrate reductase were found among the genera *Halobacillus* and *Halomonas*. Another study on soils was carried out by Bhatt et al. [78], who isolated halo-alkaliphile bacteria from the saline desert soil in Little Rann of Kutch, India. Phylogenetic analysis indicated that isolates belong to phylum Firmicutes, which comprises lower G + C Gram-positive bacteria of different genera. Most of the halophilic isolates produced proteases (30% of isolates), followed by cellulases (24% isolates), CMCase (24% of isolates), and amylases (20% of isolates).

2.4. Physicochemical parameters and kinetic properties of extremozymes from halophilic microorganisms

Halophilic enzymes have specific mechanisms for solubility at high salt concentrations, such as a highly negative superficial charge given by carboxylic groups that depend on high salt concentrations to remain soluble. Halophilic archaea are known to secrete active proteases at high concentrations of NaCl (4 M), and to accumulate high concentrations of KCl in their cytoplasm in order to face osmotic stress, while maintaining the conformation of their proteins. The study made by Akolkar and Desai [97] suggests that proteases from haloarchaea may be active and stable in the presence of osmolytes different from NaCl/KCl at different degrees, as shown by the kinetics and thermodynamic analyses of casein hydrolysis produced by *Halobacterium* sp., in the presence of a compatible solute (sodium glutamate). In 2012, Zhang et al. [98] demonstrated

that NaCl may improve the thermal stability of enzymes, and the presence of NaCl or KCl increases enzymatic activity 10-fold, approximately; this agrees with other investigations that demonstrate that enzyme activity depends on the concentration of NaCl or KCl, as well as on the substrate, pH, and presence of ions. Moshfegh et al. [71] demonstrated that the concentration of NaCl 4 M or KCl 4.5 M determines the maximum activity of halo-alkaliphile α -amylase produced by archaea *Halorubrum xinjiangense*, besides improving its thermal stability. In a similar manner, Selim et al. [74] showed that the protease activity of *Natronolimnobius innermongolicus* depends on high concentrations of salt to remain active and stable. Proteases purified by Faghihi et al. [79] increased their activity in the presence of metallic ions such as Mn^{2+} and Cu^{2+} , while decreasing activity when exposed to Hg^{2+} and Fe^{2+} . Both proteases were strongly inhibited by SDS, while DDT, EDTA, and 2-mercaptoethanol may stimulate their activity.

The affinity of an enzyme to hydrolyze a substrate is determined by the Michaelis constant (K_m), which is the concentration of substrate at which the reaction velocity is half the maximum velocity (V_{max}). V_{max} is the maximum velocity when the system is saturated with substrate. The value of K_m is a measure of the enzyme-substrate affinity, and at the moment there are very few determinations made on extremozymes from halophilic organisms. **Table 2** shows the values of kinetic constants for extremozymes currently available.

Nowadays, recombinant DNA techniques and genetic engineering are used to obtain customized extremozymes to be used for specific purposes, greatly improving their catalytic ability, as demonstrated by Kui et al. [99] with the expression of genes from extremozyme β -1,4-xylanase, which was cloned from *Nesterenkonia xinjiangensis* and expressed in *Escherichia coli*. This enzyme was thermostable, retaining more than 80% of the initial activity after incubation at 60°C for 1 h, and more than 40% activity at 90°C for 15 min. In the same way, Qin et al. [90] cloned a novel gene that codifies a new α -amylase, which is active at low temperatures and tolerant to salt (AmyZ), from the marine bacterium *Zunongwangia profunda*, this protein was also expressed in *Escherichia coli*. It was observed that AmyZ is one of the few α -amylases that tolerate both low temperatures and high salinity, which makes it a potential candidate for research in basic and applied biology.

2.5. Halophile extremozyme applications

As mentioned above, halophiles are good sources of several extremozymes, and among them hydrolases have been the most studied, mainly amylases, proteases, lipases, xylanases, cellulases, and DNases. Some extremozymes from halophiles exhibit extraordinary biochemical properties, which show the potential for industrial applications. It has been demonstrated that extremozymes derived from halophiles are able to function under harsh conditions and remain

Microorganism	Extremozyme	K_m (mg/mL)	V_{max}	Reference
<i>Nesterenkonia xinjiangensis</i>	Xilanasa	16.08	45.66 μ mol/min-mg	[99]
<i>Bacillus</i> sp.	Celulasa	3.18		[98]
<i>Halorubrum xinjiangense</i>	α -amilasa	3.8	12.4 U/mg	[71]
<i>Aspergillus gracilis</i>	α -amilasa	6.33	8.36 U/mg	[87]
<i>Kocuria</i> sp.	Amilasa	3.0	90.09 U/ml	[77]

Table 2. Kinetics parameters of extreme-enzymes produced by halophiles microorganisms.

stable and active with different properties than conventional enzymes, offering opportunities in several applications such as environmental bioremediation, food processing, and residual water treatment. Recent research points out the application of halophilic extremozymes in the production of biofuels. Since several halophiles are also alkaliphiles, their enzymes are of interest for the textile and detergent industries, and some have been explored as raw materials in the production of commercial enzymes, particularly proteases and amylases [5, 100–105].

3. Conclusions

Halophile microorganisms have the ability to adapt to or tolerate stress caused by salinity by accumulating osmolytes. Halophilic microorganisms have several biotechnological applications, in recent years, uses of halophilic microorganisms have significantly increased. Many enzymes, stabilizers, and valuable compounds from halophiles may present advantages for the development of biotechnological production processes. Halophiles are the most probable source of extremozymes, since they are also capable of tolerating alkaline pH and high temperatures.

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References

- [1] DasSarma S, DasSarma P. Halophiles. In: eLS. Chichester: John Wiley & Sons, Ltd. p. 2012. DOI: 10.1002/9780470015902.a0000394.pub3
- [2] Ollivier B, Caumette P, Garcia JL, Mah RA. Anaerobic bacteria from hypersaline environments. Microbiological Reviews. 1994;**58**:27-38
- [3] Madigan MT, Martinko JM, Parker J. Brock Biology of Microorganisms. Upper Saddle River, NJ: Prentice Hall; 1997. p. 11
- [4] Ventosa A, Arahal DR. Physico-chemical characteristics of hypersaline environments and their biodiversity. Extremophiles. 2009;**2**:247-262
- [5] DasSarma S, DasSarma P. Halophiles and their enzymes: Negativity put to good use. Current Opinion in Microbiology. 2015;**25**:120-126. DOI: 10.1016/j.mib.2015.05.009
- [6] Kushner DJ, Kamekura M. Physiology of halophilic eubacteria. In: Rodriguez-Valera R, editor. Halophilic Bacteria. Boca Raton, Fla: CRC Press; 1988
- [7] Ventosa A. Unusual micro-organisms from unusual habitats: Hypersaline environments. In: Symposia-Society for General Microbiology. Cambridge: Cambridge University Press; 1999
- [8] Ventosa A, de la Haba RR, Sánchez-Porro C, Papke RT. Microbial diversity of hypersaline environments: A metagenomic approach. Current Opinion in Microbiology. 2015;**25**:80-87. DOI: 10.1016/j.mib.2015.05.002
- [9] La Cono V, Smedile F, Bortoluzzi G, Arcadi E, Maimone G, Messina E, et al. Unveiling microbial life in new deep-sea hypersaline Lake Thetis. Part I: Prokaryotes and environmental settings. Environmental Microbiology. 2011;**13**(8):2250-2268
- [10] Oren A. Ecology of halophiles. In: Horikoshi K, Antranikian G, Bull AT, Robb FT, Stetter KO, editors. Extremophiles Handbook. Japan: Springer. DOI: 10.1007/978-4-431-53898-1_3.2
- [11] Yakimov MM, La Cono V, Spada GL, Bortoluzzi G, Messina E, Smedile F, et al. Microbial community of the deep-sea brine Lake Kryos seawater–brine interface is active below the chaotropicity limit of life as revealed by recovery of mRNA. Environmental Microbiology. 2015;**17**(2):364-382. DOI: 10.1111/1462-2920
- [12] Antón J, Oren A, Benlloch S, Rodríguez-Valera F, Amann R, Rosselló-Mora R. *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the bacteria from saltern crystallizer ponds. International Journal of Systematic and Evolutionary Microbiology. 2002;**52**(2):485-491. DOI: 10.1099/00207713-52-2-485
- [13] Yeon SH, Jeong WJ, Park JS. The diversity of culturable organotrophic bacteria from local solar salterns. Journal of Microbiology. 2005;**43**(1):1-10
- [14] Lim JM, Jeon CO, Kim CJ. *Bacillus taeanensis* sp. nov., a halophilic gram-positive bacterium from a solar saltern in Korea. International Journal of Systematic and Evolutionary Microbiology. 2006;**56**(12):2903-2908. DOI: 10.1099/ijs.0.64036-0

- [15] Pappa A, Sánchez-Porro C, Lazoura P, Kallimanis A, Perisynakis A, Ventosa A, et al. *Bacillus halochares* sp. nov., a halophilic bacterium isolated from a solar saltern. International Journal of Systematic and Evolutionary Microbiology. 2010;**60**(6):1432-1436. DOI: 10.1099/ijs.0.014233-0
- [16] Maturrano L, Santos F, Rosselló-Mora R, Antón J. Microbial diversity in Maras salterns, a hypersaline environment in the Peruvian Andes. Applied and Environmental Microbiology. 2006;**72**(6):3887-3895. DOI: 10.1128/AEM.02214-05
- [17] Baati H, Amdouni R, Gharsallah N, Sghir A, Ammar E. Isolation and characterization of moderately halophilic bacteria from Tunisian solar saltern. Current Microbiology. 2010;**60**(3):157-161. DOI: 10.1007/s00284-009-9516-6
- [18] Shrivastava P, Kumar R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences. 2015;**22**(2):123-131. DOI: 10.1016/j.sjbs.2014.12.001
- [19] Jamil A, Riaz S, Ashraf M, Foolad MR. Gene expression profiling of plants under salt stress. Critical Reviews in Plant Sciences. 2011;**30**(5):435-458. DOI: 10.1080/07352689.2011.605739
- [20] Li WJ, Zhang YQ, Schumann P, Tian XP, Zhang YQ, Xu LH, et al. *Sinococcus qinghaiensis* gen. nov., sp. nov., a novel member of the order Bacillales from a saline soil in China. International Journal of Systematic and Evolutionary Microbiology. 2006;**56**(6):1189-1192. DOI: 10.1099/ijs.0.64111-0
- [21] Tian XP, Dastager SG, Lee JC, Tang SK, Zhang YQ, Park DJ, et al. *Alkalibacillus halophilus* sp. nov., a new halophilic species isolated from hypersaline soil in Xin-Jiang province, China. Systematic and Applied Microbiology. 2007;**30**(4):268-272. DOI: 10.1016/j.syapm.2006.08.003
- [22] Chen YG, Cui XL, Li WJ, Xu LH, Wen ML, Peng Q, et al. *Salinicoccus salitudinis* sp. nov., a new moderately halophilic bacterium isolated from a saline soil sample. Extremophiles. 2008;**12**(2):197-203. DOI: 10.1007/s00792-007-0116-8
- [23] Li WJ, Park DJ, Tang SK, Wang D, Lee JC, Xu LH, et al. *Nocardiopsis salina* sp. nov., a novel halophilic actinomycete isolated from saline soil in China. International Journal of Systematic and Evolutionary Microbiology. 2004;**54**(5):1805-1809. DOI: 10.1099/ijs.0.63127-0
- [24] Meklat A, Bouras N, Riba A, Zitouni A, Mathieu F, Rohde M, et al. *Streptomonospora algeriensis* sp. nov., a halophilic actinomycete isolated from soil in Algeria. Antonie Van Leeuwenhoek. 2014;**106**(2):287-292. DOI: 10.1007/s10482-014-0195-3
- [25] Martínez-Cánovas MJ, Quesada E, Martínez-Checa F, del Moral A, Bejar V. *Salipiger muce-scens* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium isolated from hypersaline soil, belonging to the α -Proteobacteria. International Journal of Systematic and Evolutionary Microbiology. 2004;**54**(5):1735-1740. DOI: 10.1099/ijs.0.63166-0
- [26] Martínez-Checa F, Quesada E, Martínez-Cánovas MJ, Llamas I, Bejar V. *Palleronia maris-minoris* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium belonging to the 'Alphaproteobacteria', isolated from a saline soil. International

- Journal of Systematic and Evolutionary Microbiology. 2005;**55**(6):2525-2530. DOI: 10.1099/ijs.0.63906-0
- [27] Larsen H. Ecology of hypersaline environments. Developments in Sedimentology. 1980;**28**:23-39. DOI: 10.1016/S0070-4571(08)70227-9
- [28] Jakobsen TF, Kjeldsen KU, Ingvorsen K. *Desulfohalobium utahense* sp. nov., a moderately halophilic, sulfate-reducing bacterium isolated from Great Salt Lake. International Journal of Systematic and Evolutionary Microbiology. 2006;**56**(9):2063-2069. DOI: 10.1099/ijs.0.64323-0
- [29] Almeida-Dalmet S, Sikaroodi M, Gillevet PM, Litchfield CD, Baxter BK. Temporal study of the microbial diversity of the north arm of Great Salt Lake, Utah, US. Microorganisms. 2015;**3**(3):310-326. DOI: 10.3390/microorganisms3030310
- [30] Demergasso C, Casamayor EO, Chong G, Galleguillos P, Escudero L, Pedrós-Alió C. Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, northern Chile. FEMS Microbiology Ecology. 2004;**48**(1):57-69. DOI: 10.1016/j.femsec.2003.12.013
- [31] Rodríguez-Valera F. Characteristics and microbial ecology of hypersaline environments. In: Rodríguez-Valera F, editor. Halophilic Bacteria. Vol. 1. Boca Raton, FL: CRC Press. pp. 3-30
- [32] McGenity TJ, Oren A. Life in saline environments. In: Bell EM, editor. Life at Extremes. Environments, Organisms and Strategies for Survival. UK: CABI International; 2012. pp. 402-437
- [33] Al-Karablieh N. Antimicrobial activity of *Bacillus persicus* 24-DSM isolated from dead sea mud. The Open Microbiology Journal. 2017;**11**:372. DOI: 10.2174/1874285801711010372
- [34] Ionescu D, Siebert C, Polerecky L, Munwes YY, Lott C, Häusler S, et al. Microbial and chemical characterization of underwater freshwater springs in the Dead Sea. PLoS One. 2012;**7**(6):e38319. DOI: 10.1371/journal.pone.0038319
- [35] Bodaker I, Sharon I, Suzuki MT, Feingersh R, Shmoish M, Andreishcheva E, et al. Comparative community genomics in the Dead Sea: An increasingly extreme environment. The ISME Journal. 2010;**4**(3):399. DOI: 10.1038/ismej.2009.141
- [36] van der Wielen PW, Bolhuis H, Borin S, Daffonchio D, Corselli C, Giuliano L, et al. The enigma of prokaryotic life in deep hypersaline anoxic basins. Science. 2005;**307**(5706):121-123. DOI: 10.1126/science.1103569
- [37] Grant WD, Jones BE. Alkaline environments. Encyclopedia of Microbiology. 2000;**1 A-C**: 126-133
- [38] Grant WD. Introductory chapter: Half a lifetime in soda lakes. In: Halophilic Micro organisms. Vol. 1. Berlin, Heidelberg: Springer; 2004. pp. 17-31. DOI: 10.1007/978-3-662-07656-9
- [39] Ma Y, Zhang W, Xue Y, Zhou P, Ventosa A, Grant WD. Bacterial diversity of the inner Mongolian Baer soda Lake as revealed by 16SrRNA gene sequence analyses. Extremophiles. 2004;**8**(1):45-51. DOI: 10.1007/s00792-003-0358-z

- [40] Rees HC, Grant WD, Jones BE, Heaphy S. Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. *Extremophiles*. 2004;**8**(1):63-71. DOI: 10.1007/s00792-003-0361-4
- [41] Mesbah NM, Hedrick DB, Peacock AD, Rohde M, Wiegel J. *Natranaerobius thermophilus* gen. nov., sp. nov., a halophilic, alkalithermophilic bacterium from soda lakes of the Wadi an Natrun, Egypt, and proposal of *Natranaerobiaceae* fam. nov. and *Natranaerobiales* ord. nov. *International Journal of Systematic and Evolutionary Microbiology*. 2007;**57**(11):2507-2512. DOI: 10.1099/ijs.0.65068-0
- [42] Dimitriu PA, Pinkart HC, Peyton BM, Mormile MR. Spatial and temporal patterns in the microbial diversity of a meromictic soda lake in Washington state. *Applied and Environmental Microbiology*. 2008;**74**(15):4877-4888. DOI: 10.1128/AEM.00455-08
- [43] Sorokin DY, Rusanov II, Pimenov NV, Tourova TP, Abbas B, Muyzer G. Sulfidogenesis under extremely haloalkaline conditions in soda lakes of Kulunda steppe (Altai, Russia). *FEMS Microbiology Ecology*. 2010;**73**(2):278-290. DOI: 10.1111/j.1574-6941.2010.00901.x
- [44] Sorokin DY, Detkova EN, Muyzer G. Sulfur-dependent respiration under extremely haloalkaline conditions in soda lake 'acetogens' and the description of *Natroniella sulfidigena* sp. nov. *FEMS Microbiology Letters*. 2011;**319**(1):88-95. DOI: 10.1111/j.1574-6968.2011.02272.x
- [45] Blum JS, Kulp TR, Han S, Lanoil B, Saltikov CW, Stolz JF, et al. *Desulfohalophilus alkaliarsenatis* gen. nov., sp. nov., an extremely halophilic sulfate- and arsenate-respiring bacterium from Searles Lake, California. *Extremophiles*. 2012;**16**(5):727-742. DOI: 10.1007/s00792-012-0468-6
- [46] Sorokin DY, Tourova TP, Sukhacheva MV, Muyzer G. *Desulfuribacillus alkaliarsenatis* gen. nov. sp. nov., a deep-lineage, obligately anaerobic, dissimilatory sulfur and arsenate-reducing, haloalkaliphilic representative of the order Bacillales from soda lakes. *Extremophiles*. 2012;**16**(4):597-605. DOI: 10.1007/s00792-012-0459-7
- [47] Sorokin DY, Chernyh NA, Poroshina MN. *Desulfonatronobacter acetoxydans* sp. nov.: A first acetate-oxidizing, extremely salt-tolerant alkaliphilic SRB from a hypersaline soda lake. *Extremophiles*. 2015;**19**(5):899-907. DOI: 10.1007/s00792-015-0765-y
- [48] Sarwar MK, Azam I, Iqbal T. Biology and applications of halophilic bacteria and archaea: A review. *Electronic Journal of Biology*. 2015;**11**(3):98-103. ISSN: 1860-3122
- [49] Oren A. Bioenergetic aspects of halophilism. *Microbiology and Molecular Biology Reviews*. 1999;**63**:334-340. DOI: 10.92-2172/99/\$04.0010
- [50] Yan N, Marschner P, Cao W, Zuo C, Qin W. Influence of salinity and water content on soil microorganisms. *The International Soil and Water Conservation Research (ISWCR)*. 2015:3316-3323. DOI: 10.1016/j.iswcr.2015.11.003
- [51] Edbeib MF, Wahab RA, Huyop F. Halophiles: Biology, adaptation, and their role in decontamination of hypersaline environments. *World Journal of Microbiology and Biotechnology*. 2016;**32**:135. DOI: 10.1007/s11274-016-2081-9
- [52] Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline Systems*. 2005;**1**:5. DOI: 10.1186/1746-1448-1-5

- [53] Oren A. Microbial life at high salt concentrations: Phylogenetic and metabolic diversity. *Saline Systems*. 2008;**4**:2. DOI: 10.1186/1746-1448-4-2
- [54] Yin J, Chen JC, Wu Q, Chen GQ. Halophiles, coming stars for industrial biotechnology. *Biotechnology Advances | Industrial Biotechnology: Tools and Applications*. 2015;**33**:1433-1442. DOI: 10.1016/j.biotechadv.2014.10.008
- [55] Hanelt I, Muller V. Molecular mechanisms of adaptation of the moderately halophilic bacterium *Halobacillus halophilus* to its environment. *Lifestyles*. 2013;**3**:234-243. DOI: 10.3390/life3010234
- [56] Sharma A, Vaishnav A, Jamali H, Kumar SA, Saxena AK, Kumar SA. Halophilic bacteria: Potential bioinoculants for sustainable agriculture and environment management under salt stress. In: *Plant-Microbe Interaction: An Approach to Sustainable Agriculture*. Singapore: Springer; 2016. pp. 297-325. DOI: 10.1007/978-981-10-2854-0_14
- [57] Kunte HJ, Trüper HG, Stan-Lotter H. Halophilic microorganisms. In: *Astrobiology*. Berlin, Heidelberg: Springer; 2002. pp. 185-200. DOI: 10.1007/978-3-642-59,381-9_13
- [58] Lanyi JK. Salt-dependent properties of proteins from extremely halophilic bacteria. *Bacteriological Reviews*. 1974;**38**:272-290
- [59] Oren A. Diversity of halophiles. In: Horikoshi K, Antranikian G, Bull AT, Robb FT, Stetter KO, editors. *Extremophiles Handbook*. Japan: Springer; 2011. pp. 309-325. DOI: 10.1007/978-4-431-53,898-1_3.2
- [60] Imhoff JF, Rodriguez-Valera F. Betaine is the main compatible solute of halophilic eubacteria. *Journal of Bacteriology*. 1984;**160**(1):478-479. DOI: 0021-9193/84/100478-02\$02.00/0
- [61] Robert MF. Osmoadaptation and osmoregulation in archaea: Update 2004. *Frontiers in Bioscience*. 2004;**9**:1999-2019. DOI: 10.2741/1366
- [62] Ma Y, Galinski EA, Grant WD, Oren A, Ventosa A. Halophiles 2010: Life in saline environments. *Applied and Environmental Microbiology*. 2010;**76**:6971-6981. DOI: 10.1128/AEM.01868-10
- [63] Delgado-García M, Valdivia-Urdiales B, Aguilar-González C, Contreras-Esquivel J, Rodríguez-Herrera R. Halophilic hydrolases as a new tool for the biotechnological industries. *Journal of the Science of Food and Agriculture*. 2012;**92**:2575-2580. DOI: 10.1002/jsfa.5860
- [64] Mei Y, Liu H, Zhang S, Yang M, Hu C, Zhang J, et al. Effects of salinity on the cellular physiological responses of *Natrinema* sp. J7-2. *PLoS One*. 2017;**12**:e0184974. DOI: 10.1371/journal.pone.0184974
- [65] Csonka LN. Physiological and genetic responses of bacteria to osmotic-stress. *Microbiological Reviews*. 1989;**53**:121-147. DOI: 0146-0749/89/010121-27\$02.00/0
- [66] Oren A. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: Implications for the functioning of salt lake ecosystems. *Hydrobiologia*. 2001;**466**:61-72. DOI: 10.1023/A:1014557116838

- [67] Joghee NN, Jayaraman G. Metabolomic characterization of halophilic bacterial isolates reveals strains synthesizing rare diaminoacids under salt stress. *Biochimie*. 2014;**102**:102-111. DOI: 10.1016/j.biochi.2014.02.015
- [68] Vidyasagar M, Prakash S, Jayalakshmi SK, Sreeramulu K. Optimization of culture conditions for the production of halothermophilic protease from halophilic bacterium *Chromohalobacter* sp. *World Journal of Microbiology and Biotechnology*. 2007;**23**:655-662. DOI: 10.1111/j.1472-765X.2006.01980.x
- [69] Ardakani MR, Poshtkoughian A, Amoozegar MA, Zolgharnein H. Isolation of moderately halophilic *Pseudoalteromonas* producing extracellular hydrolytic enzymes from Persian gulf. *Indian Journal of Microbiology*. 2012;**52**:94-98. DOI: 10.1007/s12088-011-0243-x
- [70] Kumar S, Karan R, Kapoor S, Singh SP, Khare SK. Screening and isolation of halophilic bacteria producing industrially important enzymes. *Brazilian Journal of Microbiology*. 2012;**43**:1595-1603. DOI: 10.1590/S1517-83822012000400044
- [71] Moshfegh M, Shahverdi AR, Zarrini G, Faramarzi MA. Biochemical characterization of an extracellular polyextremophilic α -amylase from the halophilic archaeon *Halorubrum xinjiangense*. *Extremophiles*. 2013;**17**:677-687. DOI: 10.1007/s00792-013-0551-7
- [72] Nigam VK, Singhal P, Vidyarthi AS, Mohan MK, Ghosh P. Studies on keratinolytic activity of alkaline proteases from halophilic bacteria. *International Journal of Pharma and Bio Sciences*. 2013;**4**:389-399
- [73] Timpson LM, Liliensiek AK, Alsafadi D, Cassidy J, Sharkey MA, Liddell S, et al. A comparison of two novel alcohol dehydrogenase enzymes (ADH1 and ADH2) from the extreme halophile *Haloferax volcanii*. *Applied Microbiology and Biotechnology*. 2013;**97**:195-203. DOI: 10.1007/s00253-012-4074-4
- [74] Selim S, Hagagy N, Aziz MA, El-Meleigy ES, Pessione E. Thermostable alkaline halophilic-protease production by *Natronolimnobius innermongolicus* WN18. *Natural Product Research*. 2014;**28**:1476-1479. DOI: 10.1080/14786419.2014.907288
- [75] Gupta M, Aggarwal S, Navani NK, Choudhury B. Isolation and characterization of a protease-producing novel haloalkaliphilic bacterium *Halobiforma* sp. strain BNMIITR from Sambhar lake in Rajasthan, India. *Annals of Microbiology*. 2015;**65**:677-686. DOI: 10.1007/s13213-014-0906-z
- [76] Ali N, Ullah N, Qasim M, Rahman H, Khan SN, Sadiq A, et al. Molecular characterization and growth optimization of halo-tolerant protease producing *Bacillus subtilis* strain BLK-1.5 isolated from salt mines of Karak, Pakistan. *Extremophiles*. 2016;**20**:395-402. DOI: 10.1007/s00792-016-0830-1
- [77] Soto-Padilla MY, Gortáres-Moroyoqui P, Cira-Chávez LA, Levasseur A, Dendooven L, Estrada-Alvarado MI. Characterization of extracellular amylase produced by haloalkaliphilic strain *Kocuria* sp. HJ014. *International Journal of Environmental Health Research*. 2016;**26**:396-404. DOI: 10.1080/09603123.2015.1135310

- [78] Bhatt HB, Gohel SD, Singh SP. Phylogeny, novel bacterial lineage and enzymatic potential of haloalkaliphilic bacteria from the saline coastal desert of Little Rann of Kutch, Gujarat, India. *3 Biotech*. 2018;**8**:53. DOI: 10.1007/s13205-017-1075-0
- [79] Faghihi LS, Seyedalipour B, Riazi G, Ahmady-Asbchin S. Introduction of two haloalkali-thermo-stable biocatalysts: Purification and characterization. *Catalysis Letters*. 2018;**148**:831-842. DOI: 10.1007/s10562-018-2295-6
- [80] Sánchez-Porro C, Martín S, Mellado E, Ventosa A. Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. *Journal of Applied Microbiology*. 2003;**94**:295-300. DOI: 10.1046/j.1365-2672.2003.01834.x
- [81] Karbalaeei-Heidari HR, Ziaee AA, Schaller J, Amoozegar MA. Purification and characterization of an extracellular haloalkaline protease produced by the moderately halophilic bacterium, *Salinivibrio* sp. strain AF-2004. *Enzyme and Microbial Technology*. 2007;**40**:266-272. DOI: 10.1016/j.enzmictec.2006.04.006
- [82] Rohban R, Amoozegar MA, Ventosa A. Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. *Journal of Industrial Microbiology & Biotechnology*. 2009;**36**:333-340. DOI: 10.1007/s10295-008-0500-0
- [83] Pérez D, Martín S, Fernández-Lorente G, Filice M, Guisán JM, Ventosa A, et al. A novel halophilic lipase, LipBL, showing high efficiency in the production of eicosapentaenoic acid (EPA). *PLoS One*. 2011;**6**:e23325. DOI: 10.1371/journal.pone.0023325
- [84] Li X, Yu HY. Extracellular production of beta-amylase by a halophilic isolate, *Halobacillus* sp. LY9. *Journal of Industrial Microbiology & Biotechnology*. 2011;**38**:1837-1843. DOI: 10.1007/s10295-011-0972-1
- [85] Shahbazi M, Karbalaeei-Heidari HR. A novel low molecular weight extracellular protease from a moderately halophilic bacterium *Salinivibrio* sp. strain MS-7: Production and biochemical properties. *Molecular Biology Research Communications*. **2012**(1):45-56. DOI: 10.22099/mbrc.2012.576
- [86] Jayachandra SY, Kumar A, Merley DP, Sulochana MB. Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes. *Recent Research in Science and Technology*. 2012;**4**:46-49
- [87] Hagaggi NS, Hezayen FF, Abdul-Raouf UM. Production of an extracellular halophilic amylase from the extremely halophilic archaeon *Natrialba aegyptiaca* strain 40 T. *Al-Azhar Bulletin of Science*. 2013;**24**:93-107
- [88] Moreno ML, Pérez D, García MT, Mellado E. Halophilic bacteria as a source of novel hydrolytic enzymes. *Lifestyles*. **2013**(3):38-51. DOI: 10.3390/life3010038
- [89] Moreno ML, Piubeli F, Bonfá MR, García MT, Durrant LR, Mellado E. Analysis and characterization of cultivable extremophilic hydrolytic bacterial community in heavy-metal-contaminated soils from the Atacama Desert and their biotechnological potentials. *Journal of Applied Microbiology*. 2012;**113**:550-559

- [90] Qin Y, Huang Z, Liu Z. A novel cold-active and salt-tolerant α -amylase from marine bacterium *Zunongwangia profunda*: Molecular cloning, heterologous expression and biochemical characterization. *Extremophiles*. 2014;**18**:271-281. DOI: 10.1007/s00792-013-0614-9
- [91] Del Campo MM, Camacho RM, Mateos-Díaz JC, Müller-Santos M, Córdova J, Rodríguez JA. Solid-state fermentation as a potential technique for esterase/lipase production by halophilic archaea. *Extremophiles*. 2015;**19**:1121-1132. DOI: 10.1007/s00792-015-0784-8
- [92] Kumar S, Khare SK. Chloride activated halophilic α -amylase from *Marinobacter* sp. EMB8: Production optimization and nanoimmobilization for efficient starch hydrolysis. *Enzyme Research*. 2015:1-9. DOI: 10.1155/2015/859485
- [93] Chuprom J, Bovornreungroj P, Ahmad M, Kantachote D, Dueramae S. Approach toward enhancement of halophilic protease production by *Halobacterium* sp. strain LBU50301 using statistical design response surface methodology. *Biotechnology Reports*. 2016;**10**: 17-28. DOI: 10.1016/j.btre.2016.02.004
- [94] Abel-Nabey HM, Farag AM. Production, optimization and characterization of extracellular amylase from halophilic *Bacillus licheniformis* AH214. *African Journal of Biotechnology*. 2016;**2016**, **15**:670-683. DOI: 10.5897/AJB2015.15073
- [95] Dumorné K, Camacho Córdova D, Astorga-Eló M, Renganathan P. Extremozymes: A potential source for industrial applications. *Journal of Microbiology and Biotechnology*. 2017;**27**(4):649-659. DOI: 10.4014/jmb.1611.11006
- [96] Hosseini M, Al-Rubaye MTS, Fakhari J, Babaha F. Isolation and characterization of denitrifying halophilic bacteria from Bahr Al-Milh Salt Lake, Karbala, Iraq. *Journal of Applied Biology & Biotechnology*. 2018;**6**:32-36. DOI: 10.7324/JABB.2018.60406
- [97] Akolkar AV, Desai AJ. Catalytic and thermodynamic characterization of protease from *Halobacterium* SP. SP1 (1). *Research in Microbiology*. 2010;**161**:355-362. DOI: 10.1016/j.resmic.2010.04.005
- [98] Zhang G, Li S, Xue Y, Mao L, Ma Y. Effects of salts on activity of halophilic cellulase with glucomannanase activity isolated from alkaliphilic and halophilic *Bacillus* sp. BG-CS10. *Extremophiles*. 2012;**16**:35-43. DOI: 10.1007/s00792-011-0403-2
- [99] Kui H, Luo H, Shi P, Bai Y, Yuan T, Wang Y, et al. Gene cloning, expression, and characterization of a thermostable xylanase from *Nesterenkonia xinjiangensis* CCTCC AA001025. *Applied Biochemistry and Biotechnology*. 2010;**162**:953-965. DOI: 10.1007/s12010-009-8815-5
- [100] DasSarma P, Coker JA, Huse V, DasSarma S. Halophiles, industrial applications. In: Flickinger MC, editor. *Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology*. 2010. pp. 1-43
- [101] Ma Y, Galinski EA, Grant WD, Oren A, Ventosa A. Halophiles 2010: Life in saline environments. *Applied and Environmental Microbiology*. 2010;**76**:6971-6981. DOI: 10.1128/AEM.01868-10
- [102] Oren A. Industrial and environmental applications of halophilic microorganisms. *Environmental Technology*. 2010;**31**:825-834. DOI: 10.1080/09593330903370026

- [103] Oliart-Ros RM, Manresa-Presas Á, Sánchez-Otero MG. Utilización de microorganismos de ambientes extremos y sus productos en el desarrollo biotecnológico. *Ciencia UAT*. 2016;**11**:79-90
- [104] Waditee-Sirisattha R, Kageyama H, Takabe T. Halophilic microorganism resources and their applications in industrial and environmental biotechnology. *AIMS Microbiology*. 2016;**2**:42-54. DOI: 10.3934/microbiol.2016.1.42
- [105] Javed S, Azeem F, Hussain S, Rasul I, Siddique MH, Riaz M, et al. Bacterial lipases: A review on purification and characterization. *Progress in Biophysics and Molecular Biology*. 2017;**132**:23-34. DOI: 10.1016/j.pbiomolbio.2017.07.014
- [106] Lucena T, Arahal DR, Ruvira MA, Navarro-Torre S, Mesa J, Pajuelo E, et al. *Vibrio palustris* sp. nov. and *Vibrio spartinae* sp. nov., two novel members of the Gazogenes clade, isolated from salt-marsh plants (*Arthrocnemum macrostachyum* and *Spartina maritima*). *International Journal of Systematic and Evolutionary Microbiology*. 2017;**67**(9):3506-3512. DOI: 10.1099/ijsem.0.002155
- [107] Bheemaraddi MC, Patil S, Shivannavar CT, Gaddad SM. Isolation and characterization of *Paracoccus* sp. GSM2 capable of degrading textile azo dye reactive violet 5. *The Scientific World Journal*. 2014:410704. DOI: 10.1155/2014/410704
- [108] Zhang X, Gao J, Zhao F, Zhao Y, Li. Characterization of a salt-tolerant bacterium *Bacillus* sp. from a membrane bioreactor for saline wastewater treatment. *Journal of Environmental Sciences*. 2014;**26**(6):1369, 1374. DOI: 10.1016/S1001-0742(13)60613-0
- [109] Fidalgo C, Martins R, Proença DN, Morais PV, Alves A. Henriques: *Zunongwangia endophytica* sp. nov., an endophyte isolated from the salt marsh plant, *Halimione portulacoides*, and emended description of the genus *Zunongwangia*. *International Journal of Systematic and Evolutionary Microbiology*. 2017;**67**(8):3004, 3009. DOI: 10.1099/ijsem.0.002069
- [110] Maldonado LA, Fenical W, Jensen PR, Kauffman CA, Mincer TJ, Ward AC, et al. *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family Micromonosporaceae. *International Journal of Systematic and Evolutionary Microbiology*. 2005;**55**(5):1759-1766
- [111] Ahmed L, Jensen PR, Freel KC, Brown R, Jones AL, Kim BY, et al. *Salinispora pacifica* sp. nov., an actinomycete from marine sediments. *Antonie Van Leeuwenhoek*. 2013;**103**(5):1069-1078. DOI: 10.1007/s10482-013-9886-4
- [112] Bibi F, Chung EJ, Khan A, Jeon CO, Chung YR. *Martellella endophytica* sp. nov., an anti-fungal bacterium associated with a halophyte. *International Journal of Systematic and Evolutionary Microbiology*. **63**(8):2914-2919. DOI: 10.1099/ijms.0.048785-0
- [113] Qin S, Bian GK, Tamura T, Zhang YJ, Zhang WD, Cao CL, et al. *Streptomyces halophytocola* sp. nov., an endophytic actinomycete isolated from the surface-sterilized stems of a coastal halophyte *Tamarix chinensis* Lour. *International Journal of Systematic and Evolutionary Microbiology*. 2013;**63**(8):2770-2775. DOI: 10.1099/ijms.0.047456-0
- [114] Bibi F, Jeong JH, Chung EJ, Jeon CO, Chung YR. *Labrenzia suaedae* sp. nov., a marine bacterium isolated from a halophyte, and emended description of the genus *Labrenzia*.

- International Journal of Systematic and Evolutionary Microbiology. **64**(4):1116-1122. DOI: 10.1099/ijms.0.047456-0
- [115] Roman-Ponce B, Wang D, Vásquez-Murrieta MS, Chen WF, Estrada-de los Santos P, Sui XH, et al. *Kocuria arsenatis* sp. nov., an arsenic-resistant endophytic actinobacterium associated with *Prosopis laevis* grown on high-arsenic-polluted mine tailing. International Journal of Systematic and Evolutionary Microbiology. 2016;**66**(2):1027-1033. DOI: 10.1099/ijsem.0.000830
- [116] Abbas N, Hussain S, Azeem F, Shahzad T, Bhatti SH, Imran M, et al. Characterization of a salt resistant bacterial strain *Proteus* sp. NA6 capable of decolorizing reactive dyes in presence of multi-metal stress. World Journal of Microbiology and Biotechnology. 2017;**32**(11):181. DOI: 10.1007/s11274-016-2141-1
- [117] Sorokin DY, Chernyh NA. 'Candidatus Desulfonatronobulbus propionicus': A first halo-alkaliphilic member of the order Syntrophobacterales from soda lakes. Extremophiles. 2016;**20**(6):895-901. DOI: 10.1007/s00792-016-0881-3
- [118] Krishnan R, Menon RR, Busse HJ, Tanaka N, Krishnamurthi S, Rameshkumar N. *Novosphingobium pokkali* sp. nov, a novel rhizosphere-associated bacterium with plant beneficial properties isolated from saline-tolerant pokkali rice. Research in Microbiology. 2017;**168**(2):113-121. DOI: 10.1016/j.resmic.2016.09.001
- [119] León MJ, Sánchez-Porro C, Ventosa A. *Marinobacter aquaticus* sp. nov., a moderately halophilic bacterium from a solar saltern. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(8):2622-2627. DOI: 10.1099/ijsem.0.001984
- [120] Yan J, Li Y, Yan H, Chen WF, Zhang X, Wang ET, et al. *Agrobacterium salinitolerans* sp. nov., a saline-alkaline-tolerant bacterium isolated from root nodule of *Sesbania cannabina*. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(6):1906-1911. DOI: 10.1099/ijsem.0.001885
- [121] Zhao GY, Zhao LY, Xia ZJ, Zhu JL, Liu D, Liu CY, et al. *Salinicola tamaricis* sp. nov., a heavy-metal-tolerant, endophytic bacterium isolated from the halophyte *tamarix chinensis* Lour. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(6):1813-1819. DOI: 10.1099/ijsem.0.001868
- [122] Hou J, Zhao YJ, Zhu L, Cui HL. *Salinirubellus salinus* gen nov, sp. nov, isolated from a marine solar saltern. International Journal of Systematic and Evolutionary Microbiology. 2018;**68**(6):1874-1878. DOI: 10.1099/ijsem.0.002757
- [123] Xia J, Ling SK, Wang XQ, Chen GJ, Du ZJ. *Aliifodinibius halophilus* sp. nov, a moderately halophilic member of the genus *Aliifodinibius*, and proposal of Balneolaceae fam nov. International Journal of Systematic and Evolutionary Microbiology. 2016;**66**(6):2225-2233. DOI: 10.1099/ijsem.0.001012
- [124] Kjeldsen KU, Jakobsen TF, Glastrup J, Ingvorsen K. *Desulfosalsimonas propionica* gen nov, sp. nov, a halophilic, sulfate-reducing member of the family Desulfobacteraceae isolated from a salt-lake sediment. International Journal of Systematic and Evolutionary Microbiology. 2010;**60**(5):1060-1065. DOI: 10.1099/ijms.0.014746-0

- [125] Makhdoumi-Kakhki A, Amoozegar MA, Ventosa A. *Salinibacter iranicus* sp. nov and *Salinibacter luteus* sp. nov, isolated from a salt lake, and emended descriptions of the genus *Salinibacter* and of *Salinibacter ruber*. International Journal of Systematic and Evolutionary Microbiology. 2012;**62**(7):1521-1527. DOI: 10.1099/ijs.0.031971-0
- [126] Abdeljabbar H, Cayol JL, Hania WB, Boudabous A, Sadfi N, Fardeau ML. *Halanaerobium sehlinense* sp. nov, an extremely halophilic, fermentative, strictly anaerobic bacterium from sediments of the hypersaline lake Sehline Sebkha. International Journal of Systematic and Evolutionary Microbiology. 2013;**63**(6):2069-2074. DOI: 10.1099/ijs.0.040139-0
- [127] Abdallah MB, Karray F, Mhiri N, Cayol JL, Tholozan JL, Alazard D, et al. Characterization of *Sporohalobacter salinus* sp. nov, an anaerobic, halophilic, fermentative bacterium isolated from a hypersaline lake. International Journal of Systematic and Evolutionary Microbiology. 2015;**65**(2):543-548. DOI: 10.1099/ijs.0.066845-0
- [128] Oh YJ, Lee HW, Lim SK, Kwon MS, Lee J, Jang JY, et al. *Lentibacillus kimchii* sp. nov, an extremely halophilic bacterium isolated from kimchi, a Korean fermented vegetable. Antonie Van Leeuwenhoek. **109**(6):869-876. DOI: 10.1007/s10482-016-0686-5
- [129] Han JR, Ling SK, Yu WN, Chen GJ, Du ZJ. *Marinobacter salexigens* sp. nov, isolated from marine sediment. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(11):4595-4600. DOI: 10.1099/ijsem.0.002337
- [130] Lu DC, Xia J, Dunlap CA, Rooney AP, Du ZJ. *Gracilimonas halophila* sp. nov, isolated from a marine solar saltern. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(9):3251-3255. DOI: 10.1099/ijsem.0.002093
- [131] Nikou MM, Ramezani M, Harirchi S, Makzoom S, Amoozegar MA, Fazeli SAS, et al. *Salinifilum* gen nov, with description of *Salinifilum proteinilyticum* sp. nov, an extremely halophilic actinomycete isolated from Meighan wetland, Iran, and reclassification of *Saccharopolyspora aidingensis* as *Salinifilum aidingensis* comb nov and *Saccharopolyspora ghardaiensis* as *Salinifilum ghardaiensis* comb nov. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(10):4221-4227. DOI: 10.1099/ijsem.0.002286
- [132] Sorokin DY, Kublanov IV, Khijniak TV. *Natronospira proteinivora* gen nov, sp. nov, an extremely salt-tolerant, alkaliphilic gammaproteobacterium from hypersaline soda lakes. International Journal of Systematic and Evolutionary Microbiology. 2017;**67**(8):2604-2608. DOI: 10.1099/ijsem.0.001983
- [133] Amoozegar MA, Makhdoumi-Kakhki A, Ramezani M, Nikou MM, SAS F, Schumann P, et al. *Limimonas halophila* gen nov, sp. nov, an extremely halophilic bacterium in the family Rhodospirillaceae. International Journal of Systematic and Evolutionary Microbiology. 2013;**63**(4):1562-1567. DOI: 10.1099/ijs.0.041236-0
- [134] Guan TW, Zhao K, Xiao J, Liu Y, Xia ZF, Zhang XP, et al. *Brevibacterium salitolerans* sp. nov, an *Actinobacterium* isolated from salt-lake sediment. International Journal of Systematic and Evolutionary Microbiology. 2010;**60**(12):2991-2995. DOI: 10.1099/ijs.0.020214-0
- [135] Bian GK, Feng ZZ, Qin S, Xing K, Wang Z, Cao CL, et al. *Kineococcus endophytica* sp. nov, a novel endophytic actinomycete isolated from a coastal halophyte in Jiangsu, China. Antonie Van Leeuwenhoek. 2012;**102**(4):621-628. DOI: 10.1007/s10482-012-9757-4

- [136] Shi W, Takano T, Liu S. *Anditalea andensis* gen nov, sp. nov, an alkaliphilic, halotolerant bacterium isolated from extreme alkali-saline soil. *Antonie Van Leeuwenhoek*. 2012;**102**(4):703-710. DOI: 10.1007/s10482-012-9770-7
- [137] Choi EJ, Lee SH, Jung JY, Jeon CO. *Brevibacterium jeotgali* sp. nov, isolated from jeotgal, a traditional Korean fermented seafood. *International Journal of Systematic and Evolutionary Microbiology*. 2013;**63**(9):3430-3436. DOI: 10.1099/ijms.0.049197-0
- [138] Xin L, Hui-Ying Y. Purification and characterization of an extracellular esterase with organic solvent tolerance from a halotolerant isolate, *Salimicrobium* sp. LY19. *BMC Biotechnology*; **13**(1):08. DOI: 10.1186/1472-6750-13-108
- [139] Román-Ponce B, Li YH, Vásquez-Murrieta MS, Sui XH, Chen WF, Estrada-De Los Santos P, et al. *Brevibacterium metallicus* sp. nov, an endophytic bacterium isolated from roots of *Prosopis laegivata* grown at the edge of a mine tailing in Mexico. *Archives of Microbiology*. 2015;**197**(10):1151-1158. DOI: 10.1007/s00203-015-1156-6
- [140] Oguntoyinbo FA, Cnockaert M, Cho GS, Kabisch J, Neve H, Bockelmann W, et al. *Halomonas nigrificans* sp. nov, isolated from cheese. *International Journal of Systematic and Evolutionary Microbiology*. 2017;**68**(1):371-376. DOI: 10.1099/ijsem.0.002515