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Review

Halophilic Micro-Organisms Resources and its Application in Industrial and Environmental Biotechnology

Melissa Menie[1] and Ratnakar Deole[1*]

[1]*Department of Natural Sciences, Northeastern State University, Broken Arrow, Oklahoma, USA*

Introduction to Halophilic Organisms

Extremophiles are microorganisms which are characterized by the ability to survive and thrive in environments that are often considered too extreme to sustain life. These extreme environments may include but are not limited to abnormal pH, temperature, salinity and toxicity [1]. Halophiles are extremophiles that have adapted to hypersaline environments. Halophiles are often classified as slightly, moderately or extremely halophilic; this classification is based on the NaCl concentration range at which the organism exhibits optimum growth [2]. According to the classification proposed by Kushner and Kamekura [3], slight, moderate and extreme halophiles grow optimally at 0.2–0.85M (1–5%) NaCl, 0.85–3.4 M (5–20%) NaCl; and 3.4–5.1 M (20–30%) NaCl respectively [3]. In comparison, non-halophiles grow optimally at less than 0.2M (>1%) NaCl. Halophiles can be found in hypersaline environments where salt concentration may exceed, up to five times the concentration of salt in the ocean [4].

Ecology of Halophilic Organisms

Aquatic saline environments compose approximately 97% of water on earth and salt deposits may be found in over one fourth of the land [5]. Slight halophiles are often found in marine environments, where moderate and extreme halophiles are found in areas of higher salt concentration such as saline lakes, evaporation ponds, groundwater and salt deposits [6]. Halophiles compose large microbial communities in well-studied areas such as the Great Salt Lake in Utah and the Dead Sea in the Middle East [2,7]. Small areas, known as evaporation ponds, where sea or ocean water drains inland, establish a saline environment that has a continuously changing salt concentration due to the rate of evaporation. Halophiles have been sampled from many different evaporation ponds including Solar Lake near the Red Sea and Guerrero Negro on the Baja California coast. Soda brines are alkaline hypersaline lakes and sustain a large population of extreme and moderate halophiles; the Great Basin lakes located in the western United States and the Wadi Natrun lakes of Egypt are soda brines with a diverse populous of halophiles [2]. A common ecological phenomenon throughout most hypersaline environments is the stratification of the water column due to salinity and density gradients which prevent mixing of the water. As a result of stratified salinity ranges, a diverse array of halophiles corresponds with each layer. Microbial mats are found at the bottom of many hypersaline lakes and pond and exhibit a high salt concentration [5]. Thus, extreme halophiles often comprise these mats; however organisms that use osmoadaptive strategies which allow them to flourish in a wide range of salt concentrations have the best chance of survival.

Halophilic Adaptations "High-Salt-in" Strategy

The majority of biological membranes are permeable to water which leads to water loss by osmosis in high-salt conditions. To withstand this process the cell must pump water back in, against the concentration gradient, or the cytoplasm must be iso-osmotic with the surrounding environment to avoid water loss all together. Halophiles have developed two different adaptive strategies that allow them to avoid water loss and thrive high-salt environments; "high-salt-in" (KCl) strategy and accumulation of compatible solutes [8].

The "high-salt-in" or KCl strategy requires the accumulation of potassium and chloride ions within the cell. Potassium and chloride ions are pumped across the membrane and into the cytoplasm until it reaches a molar concentration that is equivalent to the salt concentration outside the cell, preventing an osmotic movement of water [9]. This is the most common strategy among halophilic Archaea and halophilic anaerobic bacteria. Though efficient, the KCl strategy requires extensive adaptations to the intercellular machinery responsible for transport and proper protein folding in the presence of salt [8]. It has also been concluded that the organisms using the KCl strategy have a highly acidic proteome. It is proposed that the use of KCl as an osmoprotectant and proteome acidity are related due to the requirement of stabilization between K^+ cations and acidic side chains on the protein surfaces, resulting in proteome acidity [10]. Due to the need for K^+ cations to stabilize

***Corresponding author:** Ratnakar Deole, Department of Natural Sciences, Northeastern State University, Broken Arrow, Oklahoma, USA, E- mail: deole@nsuok.edu

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proteins, in areas of low salt concentration denaturation of these proteins is likely. As a result, the majority of halophiles using the "high-salt-in" strategy cannot survive in low salt environments and are considered inflexible in their ability to adapt to changing salt concentrations [8,11]. The accumulation of KCl has been noted in the family *Halobacteriaceae*, which includes extremely halophilic Archaea such as *Halobacterium salinarium*, *Haloarcula marismortui*, *Halobacterium NRC-1, Natronomonas pharaonis,* and *Halquadratum walsbyi*. However, the "high-salt-in" strategy has also been noted in halophilic bacterial species belonging to the family *Halanaerobiales* and the extremely halophilic bacterium *Salinibacter ruber,* within the family *Bacteroidetes* [8,12].

Accumulation of Compatible Solutes: "Low-Salt, Organic Solute-in" Strategy

The second osmoadaptive strategy, accumulation of compatible solutes or 'low-salt, organic solute-in' strategy, is more readily found in nature and is commonly used among halophilic bacteria and eukaryotes. It requires the *de novo* synthesis of compatible solutes or uptake of organic solutes from the environment and exclusion of salt within the cytoplasm. These compatible solutes, also known as osmolytes, balance the osmotic pressure inside the cell with the pressure surrounding the cell and allow the organism to maintain cell turgor [8]. Compatible solutes acquired within the cell may also aide in stabilization of enzymatic functions, protecting against denaturation due to changing salt concentrations [11]. The list of compatible solutes used by halophiles is long; however each solute has many common properties. Compatible solutes may include sugars, polyols, amino acids and derivatives of each, alcohols, betaines, ectoines and peptides [8,13]. The use of organic solutes has a wide phylogenic distribution and spans all three domains of life. Although, the type of osmolyte synthesized seems to correspond with the organisms place on the phylogenetic tree [8]. Polyols are commonly used by halophilic eukarya, such as algae and fungi, though they are not exclusive to the eukarya domain. In contrast, halophilic Archaea often use osmolytes that are exclusive to their domain. For example, the *Methanohalophilus* species uses β-amino acids and derivatives which are rare in nature. Halophilic bacteria exhibit the most diverse use of osmolytes and some are used by a broad range of organisms. Osmolytes are used by multiple bacterial species such as; *Chromohalobacter salexigens, Halobacterioides acetoethylicus, Halorhodaspira halochloris,* and *Halorhodaspira halophila* [2, 8]. Glycine betaine is used by many halopilic bacteria, but in most cases it is taken up from the environment, as most organisms are incapable of biosynthesis of the compound. Furthermore, some species have the ability to both synthesize and retrieve compatible solutes from the environment. For example, moderate halophile, *C. salexigens* adjusts compatible solutes within the cytoplasm to cope with high salinity and temperatures changes. This adjustment is achieved by an accumulation of solutes based on importance, along with the uptake of osmoprotectants such as glycine betaine or choline, succeeded by *de novo* synthesis of solutes including ectoines and small amounts of glutamate, glutamine, trehalose and glucosylglycerate [14,15].

Biotechnological and Industrial Applications

In recent years the use of extremophiles has become increasingly common in biotechnological and industrial applications. Thermophiles, which survive in high temperatures, and alkaliphiles, which survive in varying pH, are in high industrial demand. These organisms and other extremophiles produce specialized enzymes that will function in extreme environments. Compared with catalysts commonly used by industries in the past, these specialized "extremophilic" enzymes may be produced more efficiently, may be more environmentally friendly and may have less net loss due to interfering reactions. These enzymes are currently being used in detergents, food processing, bleaching and more [16]. Though in high demand, many extremophiles and their associated enzymes have yet to be studied and commercially applied. Halophiles exhibit a wide diversity and synthesize many useful enzymes, yet have been used very little in commercial applications.

According to Oren [17], "Applications (current and potential) of halophilic microorganisms can be divided into a number of categories," these categories include:

1. Manufacturing of solar salt from seawater.
2. Catalysis of industrial processes in high salt environments.
3. Exploitation of the properties of osmolytes.
4. Applications of compounds unique to certain halophiles (not salt tolerant related). i.e. bacteriorhodopsin produced by *Halobacterium salinarum*
5. Industrial uses of compounds produced by both halophiles and non-halophiles. i.e. β- carotene.
6. Textile and tanning industry produces a large quantity of polluted wastewater containing azo dyes, phenol, and toxic anions. These effluents are also highly saline. Halophiles have been used to decolorize azo dyes and use phenol as a primary source of carbon and energy.
7. Halophilic gas vesicles and liposomes have been used to develop vaccines with potentially lesser adverse reactions.

The biotechnological and industrial demands for halophiles are growing. New approaches to understanding this diverse group of extremophiles are being developed and leading to the discovery of novel applications for halophiles and their enzymes.

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