IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Adaptation of halophiles at hyper saline environment

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ABSTRACT

Halophiles are salt loving microorganisms that flourish saline environments. Microbes adapted to life at high salt concentrations are found in all three domains of life: Archaea, Bacteria, and Eucarya. Halophilic microorganisms, either bacteria or archaea, flourish in media with salinity levels varying from negligible until to saturation in NaCl and thus are considered extremophiles. Halophiles are classified as slight halophiles, moderate halophiles and extreme halophiles Several strains of halophilic bacteria and archaea have been isolated from such environments having high salt content and characterized by their ability to produce enzymes having biocatalytic potential for use in several domains like industry, agriculture and biotechnology. The hunt for halophiles has been fuelled in the past several's years by indutrial's realization that the "survival kit" possessed by halophiles can potentially serve in an array of applications.

Keywords: Halophiles, Hypersaline, Adapation, Osmolyte,

I. INTRODUCTION

Microorganisms are present everywhere in the environment that includes thermal ducts, great depths of the oceans, international space station, and in general are heterogeneously distributed throughout the aggregates of soil particles and contribute significantly to the earth's biological diversity. Most microorganisms live in communities, or assemblages of more than one species. In these communities they carry out their own functions and contribute to and depend on the activities of other microorganisms.

Extremophiles are the microorganism that can grow in extreme environment. Members of these groups of organisms may hold secret for origin of life and answer many basic questions about the stability of the macromolecules under extreme conditions. Among extremophiles, halophiles have received growing attention for their potential applications as a source of salt-adapted enzymes. Halophiles are a group of halophillic microorganisms able to grow over a wide range of salinity varied from seawater to highly concentrated brines and optimally in media containing 3 to 15% NaCl [1]. Majority of halophiles can inhabit very extreme saline environments such as salt lakes and salt evaporation ponds. The saline content in halophilic environment is usually 10 times the saline/salt content of normal ocean water.

II. DIVERSITY AND PERSPECTIVES OF HALOPHILES

2.1 Halophiles

Halophiles are a group of microorganisms that live and grow in extreme saline environments and in many cases require brackish environment to survive [2]. Hypersaline environments have been reservoirs for the long term evolution of specifically adapted microbes. Halophilic bacteria are found in a variety of salt environments like marine ecosystems, salted meat, salt evaporation pools and salt mines. Halophiles include a great diversity of

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ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

organisms, like moderately halophilic aerobic bacteria, cyanobacteria, sulphur-oxidizing bacteria, heterotrophic bacteria, anaerobic bacteria, archaea, protozoa, fungi, algae and multicellular eukaryotes. Microorganisms that are able to grow in the absence as well as in the presence of salt are designated as halotolerant and these halotolerant grows optimally at 0.2-0.85mol/L (2 to 5%) NaCl concentration eg. Methanosalsum zhilinae followed by moderate halophiles that grows optimally in the range of 3–15% w/v NaCl eg. Halomonas almeriensis; and those that are able to grow above approximately 15% (w/v) NaCl eg. Halogeometricum borinquense are considered extremely halophillic [3], [2]. Ventosa classified many marine Halophilic organisms into slight halophiles, Moderate halophiles, extreme halophiles [4].

2.2 Moderately halophilic bacteria

Moderate halophiles extensively reviewed by Ventosa [1]. Moderately halophilic bacteria **are** the important groups of bacteria that are able to thrives in concentrations up to about 15% salt and others can adapt to conditions even at higher salt concentrations [5]. These halophiles form a versatile group and also adapted to life at low saline environment and have the ability to adjust rapidly to changes in the external salt concentration.

III. ADAPATION OF HALOPHILES IN SALINE ENVIRONMENT

Life is based on organic chemistry and the mechanisms of such chemistry must be allowed to function for life to continue. Extremophiles adopt two distinct approaches to living within extreme environments; they adapt to function within the physical and chemical bounds of their environment or they maintain mesophilic conditions intracellularly, guarding against the external pressures. Among them, halophiles are an interesting class of extremophilic organisms that have adapted to harsh, hypersaline environments. The organisms living in extreme conditions possess special adaptation strategies that make them interesting not only for fundamental research but also towards exploration of their applications, Horikoshi (2008). These organisms may hold secret for the origin of life, apart from that it will unfold many basic questions about the stability of the macromolecules under extreme conditions. Therefore, their studies would provide important clues for adaptation under salinity. To cope up with the high and often changing salinity of their environment, the aerobic halophilic bacteria, similar to all other microorganisms, need to balance their cytoplasm with the osmotic pressure exerted by the external medium [6], [7]. Osmotic balance can be achieved by the accumulation of salts, organic molecules or similar mechanism. Alternatively, the cell is able to control water movement in and out and maintain a hypo-osmotic state of their intracellular space. The extremely halophilic archaea and bacteria adapt various strategies, viz. molar concentrations of chloride is pumped into the cells by co-transport with sodium ions and/or using the light-driven primary chloride pump halorhodopsin [7].

Basically there are two different strategies adapted by halophilic and halotolerant microbes to survive in high salt concentrations. The “high-salt-in” strategy and the “low-salt, organic-solutes-in” strategy. The “high-salt-in” strategy requires all intracellular proteins to be stable and active in the presence of KCl and other salts. Mostly used by used by the Halobacteriaceae, Salinibacter, and the anaerobic Halanaerobiales. Haloarcula marismortui has been the most popular model organism for the study of the behavior of proteins active in a high-salt environment. On the other hand organic-solutes-in” strategy worked either by the accumulating organic solutes or by the biosynthesis of these solutes that do not interfere with the activity of normal enzymes.

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ISSN (O) 2319 - 8354
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Volker Muller (Frankfurt, Germany) uses Halobacillus halophiles as a model to understand the mechanisms of osmotic adaptation by a bacterium that accumulates organic compatible solutes.

3.1 Chloride Pumps

Two bacterial groups; anaerobic Halanaerobiales and the aerobic extremely halophilic Salinibacter rubber having high requirement of chloride ions that accumulate inorganic salts intracellularly rather than using organic osmotic solutes. Halobacillus is the first chloride-dependent bacterium reported, and several cellular functions depend on Chloride. From this, it is clearly understood that chloride has specific functions in halo-adaptation in different groups of halophilic microorganisms [8].

3.2 Osmoregulation in bacteria

The fundamental phenomenon to overcome osmotic stress is Osmoregulation. Bacteria adapts to high solute concentrations by accumulating intracellular organic compounds called osmolytes. Osmolytes are compatible solutes that get accumulated to high intracellular concentrations without adversely affecting cellular processes. Osmolytes can be either taken up from the environment or synthesized de novo, and they act by counterbalancing external osmotic strength, thus preventing water loss from the cell and plasmolysis. Bacteria respond to osmotic upshifts in three overlapping phases: dehydration (loss of some cell water), adjustment of cytoplasmic solvent composition and rehydration and cellular remodeling.

3.2.1 Compatible solutes

Compatible solutes are low-molecular weight osmoregulatory compounds, including highly water-soluble sugars, alcohols, amino acids, betaines, ectoines or their derivatives. All microorganisms maintain osmatic conditions across the cell membrane by accumulating organic solutes. Most fascinating is that some solutes are only produced in response to salt but some only to temperature stress. These solutes stabilize the biomolecules and whole cells and therefore act as salt antagonists or stress-protective agents. They have protein-stabilizing properties that help in the proper folding of polypeptide chains [9] and sometimes referred to as chemical chaperones [10]These compatible solute can be used for various research and in industries for different biotechnological application due to their stabilizing effects. Archaea synthesize unusual solutes such as β-amino acids, N-acetyl-β-lysine, mannosylglycerate and di-myo-inositol phosphate. Among all of them, uptake of solutes such as glycine betaine is preferred over de novo synthesis. They exert their effect not by changing the structure of protein but by changing the structure of solvent and dynamic properties of the protein [11]. They also interact with nucleic acids and influences the protein–DNA interactions [12], [13].

3.2.1.1 Glycine Betaine

Some microbes behave as halopsychrophiles that thrives both high salt concentrations and low Temperatures. This polyextremophiles nature is attributed mainly due to the accumulation of the compatible solute glycine betaine also referred as osmoprotectant. glycine betaine is the most extensively studied compounds related to the tolerance of salt stress. It contributes to the osmotic potential in the cytoplasm to maintain an appropriate water content. One of the most effective compatible solutes widely used by bacteria is glycine betaine, the N-trimethyl derivative of glycine, which can be accumulated intracellularly at high concentration through either synthesis or uptake or both. Betaine was shown to be a more effective cryo-protectant than serum albumin or trehalose/dextran, particularly under conditions stimulating long-term storage [14]. Bacillus subtilis has been

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ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

shown to possess three transport systems for glycine betaine: the secondary uptake system opuD and two binding-protein-dependent transport systems, opuA and opuC (proU).

3.2.1.2 Mannosylglycerate

It is a novel compatible solute mostly obtained from halotolerant microbes eg. Methanothermus fervidus, Pyrococcus furiosus and Rhodothermus marinus. This solute also isolated from many hyperthermophilic archaea and they accumulate concomitantly as the concentration of salt increases [15]. Mannosylglycerate osmolyte is restricted to thermophilic bacteria and hyperthermophilic archaea which led to the hypothesis that it plays a major role in thermal adaptation [16]. This compatible solute is used as enzyme protectants against physical or chemical stress and excipient in pharmaceuticals.

3.2.1.3 Diglycerol phosphate

Compatible solute Diglycerol phosphate accumulates under conditions of salt stress in the hyperthermophile eg. Archaeoglobus fulgidus. This new osmolyte has a strong protective effect and act as protein stabilizer as it exerted a considerable stabilizing effect against heat inactivation of various dehydrogenases [17].

3.2.1.4 Trehalose

Usually trehalose is used by organisms to counteract drying, but it also serves as an compatible solute [18]. It occurs in a wide variety of organisms, from bacteria and archaea to fungi, plants and invertebrates. Trehalose is not only useful as a cryoprotectant for the freeze–drying of biomolecules, but also for long-term conservation of microorganisms, as trehalose preserved the the membrane structure [16].

3.2.1.5 Ectoine

Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) is one of the most common compatible solutes in

the domain bacteria. First discovered in the *Ectothiorhodospira halochloris* which is haloalkaliphilic photosynthetic sulphur bacterium, but later found widely in halophilic and halotolerant bacteria [19]. This osmolyte have gained much attention in biotechnology as protective agents for enzymes, DNA and whole cells against stresses such as freezing, drying and heating. It is claimed that it counteracts the effects of skin ageing accelerated by UV-A-induced and therefore, is being used as a dermatological cosmetic additive in moisturizers for the care of aged, dry or irritated skin. Ectoines enhances the stability and freshness of foods by stabilizing food components. Ectoines also find applications in the treatment of the mucous membranes of the eye. Ophthalmologic preparations containing these molecules are useful for eye treatment to decrease the dryness syndrome. Introduction of ectoine

and its derivatives into preparations for oral care has also been suggested [20]

3.2.1.6 Distribution of amino acids

The cell wall of halophilic archaea eg. Halobacterium has a high proportion of the acidic amino acids such as aspartate and glutamate as sodium salts. Interestingly, this sodium binding is essential to maintain the cell wall.

3.2.1.7 Molecular aspects of salinity

Marine microbes play an essential role in the global cycling of nitrogen, carbon, oxygen, phosphorous, iron, sulfur and trace elements. Salinity tolerance comes from genes that regulate the rate of salt uptake from the soil or water and the transport of salt throughout the plant, adjust the ionic and osmotic balance of cells in roots and shoots and regulate leaf development and the onset of senescence. However, very little progress has been made

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ISSN (O) 2319 - 8354
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in this regard so far, as the gene expression pattern and analysis has been difficult. Most of the sequenced culturable microorganisms from the deep-sea are Alteromonadales from the Gammaproteobacteria. Sequencing of deep-sea microbes showed that they have unique properties. They all have a high ratio of rRNA operon copies per genome size, and that their intergenic regions are larger than average. These properties are characteristic of bacteria with an opportunistic lifestyle and a high degree of gene regulation to respond rapidly to environmental changes when searching for food. Study of the molecular basis of osmoadaptation and its osmatic regulation in archaea is still in its infancy, but genomics and functional genome analyses combined with classical biochemistry shed light on the processes that confer osmoadaptation in archaea. Furthermore, they showed that betS is constitutively expressed, whereas BetS activity depends on posttranslational activation by high osmolarity and is most likely the emergency system transporting betaines for immediate osmotic protection. Many microorganisms possess two or more glycine betaine transport systems. Salmonella typhimurium, for example, possesses two genetically distinct pathways, a constitutive low affinity system (ProP) and an osmotically induced high-affinity system (ProU), while *B. subtilis* has three glycine betaine transport systems, OpuD, OpuA, and OpuC.

3.3 pH

In *Bacteria* adaptation of intracellular to extracellular osmotic activity is coupled with a range of physiological properties like internal pH and ionic strength, along with heat- and cold tolerance. Especially Na+ and pH homeostasis play an important role in physiology and are closely linked. Internal pH is maintained by both active and passive regulation. Active regulation involves sodium ion channels that actively drive the entry of protons across the membrane through H+/Na+ antiporters, thus decreasing the overall pH of the cytoplasm and passive regulation done through cytoplasmic pools of polyamines and low membrane permeability. The cytoplasmic pools of polyamines are rich in amino acids with positively charged side groups. Besides this cell wall also play an important role in protecting the cell from alkaline environments. In addition to peptidoglycan they contains certain acidic polymers, such as galacturonic acid, gluconic acid, glutamic acid, aspartic acid, and phosphoric acid in their cell wall.

3.4 Light

Halophilic archaea thrives in shallow evaporation pond usually encounter with very high temperature and ultraviolet light. They have developed a special retinal pigments called carotenoid. These retinal pigments provide protective barrier to the ultraviolet light. These pigments not only found in halophilic archaea but also in haloalkaliphiles.

IV. POTENTIAL OF HALOPHILES

An important breakthrough occur with the advent of enzymology in the biotechnology industry with the use of nearly U.S. $ 1.5 billion enzymes in 2000 worldwide. Food industry is the largest consumer of the enzymes followed by detergent industry. The microorganisms, proven to be the single most comprehensive source of industrially important enzymes for commercial application. Halophiles brooding in environments of utmost temperature, pressure, pH and salinity, secrete extremozymes that show high stability and solubility under such extreme environments, are considered as a valuable source of novel biocatalysts. In many industrial processes,

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ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

including agricultural, chemical and pharmaceutical applications, extremozymes from halophiles have a great economic potential. Enzymes from different sources have wide range of application in different industries. Halophilic microorganisms find number of applications in biotechnology. Although produced by non-halophilic organisms, but halophilic products have distinct advantages. Halophilic media are less prone to contamination and produces product with unique properties. The interest to use extremozymes from halophiles in industrial applications is their resistance to organic solvents and extreme temperatures. The industrial enzyme market in greater number is busied by hydrolytic enzymes, such as proteases, amylases, amidases, esterases and lipases. A hydrolase is an enzyme that catalyse the hydrolysis of their substrates by adding water across the bond they split. The substrates include ester, glycosyl, ether, peptide, acid-anhydride, C-C, halide and P-N bonds.

V. CONCLUSION

Halophiles are able to survive in a wide range of salinities. To investigate the adaptations of halophiles it would requires to study the properties and structures of these microorganisms and their molecules. Compatible solutes from these microorganisms can be used for a wide range of applications, including protein and/or enzyme stabilizers, cosmetic actives, therapeutic agents, bioremediation and also in improving the salinity tolerance of crop plants by gene transfer. Overall, the review has generated information on adapataion of these halophilic microbes by salt in strategy and osmoregulation by compatible solutes and probed into their potential applications. Halophilic archaea usually employ a continuous influx of ions like K_+ in order to balance the hypersaline environment outside the cell. In such cases the intracellular protein machinery is dependent on high salt concentrations for function and stability. On the other hand, halophilic bacteria usually synthesize or accumulate osmolytes to maintain the osmotic equilibrium in response to the high-salt external environment. These osmolytes are compatible with the intracellular machinery even at molar concentrations and also named as compatible solutes. They maintain the cell volume, turgor and electrolyte concentrations within the cell system resulting in an appropriate hydration level of the cytoplasm and cell growth can proceed under osmotically unfavourable conditions. Compatible solutes have stabilizing effects and they are used as salt antagonists, stress protective agents, moisturizers and therapeutics. They stabilize enzymes, DNA and whole cells against stresses such as freezing, drying and heating. They increase freshness of foods by stabilizing components. Induction of osmolytes in cells can increase protein folding and thereby improve salt tolerance which could be useful in agriculture and xeriscaping.

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