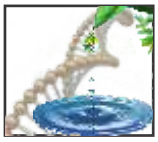

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Halophiles and their Important Enzymes Used for Biotechnology Application

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ABSTRACT: *Halophilic bacteria can grow in hypersaline environments like salt mines, solar salterns, hydrothermal, marshy lagoons and hypersaline. They have ability to adapt salt stress conditions by accumulating different water soluble organic molecules such as ectoine, glutamate, glycine betaine and proline. Mostly Haloarchaea can flourish in hypersaline environments by accumulating potassium chloride ions to maintain their internal environments. Bacterial proteins confer osmoregulation, toxic heavy metal resistance and enzymes that expand the nutritional ability of the cell. Metaproteomics (environmental proteomics study deals with all the proteins estimated directly from environmental samples (soil, water, plant or animal tissues). The field of environmental biotechnology has been widely extended by the recent advances in metagenomics levels. It also extended the post genomic area of research.*

Key Words: *Halophiles, Enzymes, osmo-adaptative mechanisms*

INTRODUCTION

Halophiles are salt loving organisms and are found in saline conditions. These organisms can be classified in to slight, moderate and extreme halophilic organisms (Shivanand and Mugeraya, 2011). Their classification depends on the requirement of sodium. Hypersaline environments are widely found in coastal, deep sea, arid and salt mines. Halophilic microorganisms include a variety of phototrophic, heterotrophic and photosynthetic organisms (DasSarma and DasSarma 2012).

The ability of microorganism to adapt the changes in the osmolality of the external milieu is of fundamental importance for growth and survival, and thus prokaryotic cells have evolved a number of osmoadaptative mechanisms to cope with elevated osmolality. One hundred bacterial isolates were isolated from soil from Egypt and screened for salt tolerance (Fernandez-Aunión et al., 2010).

Salt is required for all forms of life. Halophiles are unique by their requirement high salt conditions for growth. They can also be distinguished by their salt requirement. Some grow optimally at 0.2-0.85 M sodium chloride. Moderate halophiles grow at 0.85- 3.4 M and extreme halophiles grow up to 3.4-5.1 M (Jamadar et al.,2016). Halotolerant organisms can grow in high salinity or in the absence of high salt concentrations. Hypersaline conditions are lethal to most cells because of the loss of high water content from the cell. Halophiles accumulate high salt concentrations in their cytoplasm to prevent any loss of cellular water. Cell volume is maintained by isosmotic balance. The osmolytes accumulate in halophiles are sugars, polyols and different amino acids (Ventosa et al., 1998). These osmolytes do not interfere with intracellular metabolism and lack any net charge at physiological pH. Halotolerant yeast and green algae accumulate polyols, halotolerant bacteria accumulate zwitterionic species such as glycine betaine and ectoine (Abbas et al., 2006).

Solute accumulation may take place by biosynthesis or from the storage material. Haloarchea and extreme halophilic bacteria accumulate potassium chloride equal to external concentration of NaCl (Deole et al., 2013). These organisms produce acidic proteins that can function

in high salinity by remaining solvated and reducing aggregation, precipitation and denaturation.

A great biodiversity of prokaryotic halophiles exists naturally. They have been studied widely by both culturing and non culturing techniques. Phylogenetic analysis and taxonomic classification are widely used to identify the prokaryotes (Felsenstein, 1985). A method ofmultilocus sequencing typing is used to sequence the nucleotides of multiple genes. For nonculture halophiles, environmental metagenomics studies making use of 16S ribosomal ribonucleic acid genes for sequencing are used to study the diversity among halophiles (Abbas et al., 2006).

Enzymes of Halophiles

Hydrolases is a class of enzymes that is widely distributed naturally from bacteria to eukaryotes. Different screening techniques were used in recent years to isolate the important enzymes of halophiles. Several hydrolases were isolated from hyper saline conditions, including amylases, lipases and proteases and are used for biotechnological approaches (Dalmaso et al., 2015). The lipolytic (LiPBL) enzymes have advantages over other lipases, it active over a wide range of pH and temperature. The immobilized LipBL derivatives obtained and tested in regio- and enantioselective reactions, showed an excellent behavior in the production of free polyunsaturated fatty acids (PUFAs). On the other hand, the extremely halophilic bacterium, *Salicola marasensis* sp. IC10 showing lipase and protease activities, was studied for its ability to produce promising enzymes in terms of its resistance to temperature and salinity (Akhtar et al., 2008)

Marinobacter lipolyticus SM19, an important intracellular enzyme produced by halophilic bacterium was isolated and characterized. LipBL Lipolytic enzyme was assigned to the family VIII of lipolytic enzymes. Its expression was measured in E.coli. The molecular weight of LipBL (lipolytic enzyme) protein is 45.3 kDa and it is 404 amino acids long. LipBL was purified and biochemically characterized. The temperature for its maximal activity was 80 qC and the pH optimum determined at 25 qC was pH 7.0, showing optimal activity without sodium chloride, while maintaining 20% activity in a wide range of NaCl concentrations (AnbuRajan et al., 2008).

Proteases

The most important group of enzymes that are used in industry are proteases. Food industry and in washing detergents, these enzymes has a worldwide commercial importance. Halophilic proteases are good for industry because of their stability and properties. Haloprotease CP1 has been isolated from the moderately halophilic bacterium *Pseudoalteromonas ruthenica*, it is an extracellular protease. This enzyme was purified by using ion exchange gel filtration chromatography. The protease was also extracted from Halobacillus karajensis strain MA-2 and characterized. This enzyme belongs to the class serine metalloproteases. These findings suggested that this enzyme has great biotechnological application and potential during alkaline conditions (Moreno et al., 2009).

Amylase

Amylases are considered important enzymes in industry. They have a wide spread applications in starch sachharification, clinical and analytical chemistry. Many D-amylases are were purified from moderate halophiles e.g Nesterenkonia sp. strain F. the amylase extracted from Nesterenkonia sp. strain F have molecular weight of 110 kDa, it was determined by using SDS-PAGE. This enzyme show maximum activity at pH 7-7.5. The temperature at which it show best activity is 45 °C. The extracted enzyme is highly stable under the saline conditions ranging from 0 to 4%, its activity is not affected by the presence of Ca^{2+}, Rb^{+}, Li^{+}, Cs^{+}, Mg^{2+} and Hg^{2+} but its activity is stop or effected by Fe^{2+}, Cu^{2+}, Zn^{2+} and Al^{2+}. These amylases can also stop activity when effected to EDTA.

Another D-amylases was extracted and purified from halophilic *Thalassobacillus* sp. LY18 .This enzyme showed a molecular mass of 31 kDa and its optimal enzyme activity was found to be at 70 qC (Pinar et al., 2014).

Lipolytic and Proteolytic Enzymes

Some hydrolases are also extracted from *Salicola marasensis* IC10, it produces an extracellular protease. These bacteria are extreme

halophiles, intracellular lipase LipL was also purified from this bacterium. This enzyme is active in presence of different compounds as substrates: p-nitrophenyl butyrate, p-nitrophenylvalerate, p-nitrophenylcaprilate and p-nitrophenyldecanoate as well as 4-methylumbelliferone and the enzyme production is maximal at the end of the exponential phase (Hu et al., 2005).

The characterization of the intracellular fraction of *Salicola* sp. IC10 during growth was performed, finding the optimal conditions at pH 8.0, 40 qC and a medium with 15%±20% (w/v) NaCl. Thus, there is a correlation between the optimal conditions for cultivation of the strain and the maximum production of the proteolytic enzyme. This protease showed the capability to effectively catalyze the hydrolysis of various proteins. The most specific substrate to the enzyme was egg albumin, followed by gelatine (97% relative activity). Therefore, we can conclude that enzymes produced by halophilic bacteria show interesting properties for use in different industries (Moreno et al., 2013).

Salt stress is one of the most significant factors that negatively affect plant growth and development. Proline has been reported to be an osmoprotectant that confers tolerance to salinity in various plant species. A cDNA for Δ1-pyrroline-5-carboxylate synthetase (P5CS), a key enzyme involved in proline biosynthesis was isolated and characterized from *E. camaldulensis*, now designated EuP5CS. The full-length EuP5CS gene has 2,944 bp containing an open reading frame of 2,142-bp that encodes for 713 amino acids. The deduced EuP5CS protein structure exhibited a high homology to the P5CS of other plant species, and was predicted to possess a glutamate 5-kinase domain at its N-terminal and a gamma-glutamyl phosphate reductase domain at its C-terminal. Semi quantitative reverse transcription polymerase chain reaction analysis revealed that the transcriptional expression level of the EuP5CS gene was considerably up-regulated by up to about 50% in response to NaCl treatments. This result indicated that EuP5CS is a salt-inducible gene and plays an important role in proline biosynthesis in *E. camaldulensis* clones subjected to salt stress (Ekchaweng et al., 2012)

CONCLUSION

Halophiles are organisms that prefer to live in highly saline conditions. Halophiles are excellent sources of enzymes that are not only salt stable but also can withstand and carry out reactions efficiently under extreme conditions. The aim of the study was to study the diversity among halophilic bacteria producing enzymes of industrial value.

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