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EXTREAMOZYMES : A Review

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ABSTRACT
A variety of microbes that can thrive in extreme environment are called extremophiles and they produce protein called extremozymes. Extreme environment include high temperature, pressure, pH, salt concentration and low temperature, Nutrient concentration and water availability and also condition having heavy metal, radiation. These organism have evolved several structural and chemical adaption, which allow them to survive in extreme environment. The enzymes of these microbes, which function in extreme environments (extremozymes), have several biotechnological applications. Use of such enzymes in increasing the reactions is achieved in food and paper industries, in removal of detergents, drugs, toxic wastes, drilling for oil has been included and studied extensively. Antimicrobials, compatible solutes and other microbial compounds are also finding new uses in recent days.
Keywords: Extemophiles, Extremo-Enzymes, Halophiles, Psychrophiles, Thermphiles.

I. INTRODUCTION

Microorganisms growing in extreme environments are called extremophiles and their enzymes produced are called as extremozymes. The term extremophile was first used by MacElroy [1], three decades ago. Extreme environments include those with either high (55 to 121 °C) or low (–2 to 20 °C) temperatures, high salinity (2–5 M NaCl) and either high alkalinity (pH>8) or high acidity (pH<4), reviewed by Madigan and Marrs [2]. and Rothschild and Manicinelli. Various extremophiles can tolerate other extreme conditions including high pressure, high levels of radiation or toxic compounds, or conditions that we consider unusual, such as living in rocks deep below the surface of the earth or living in extremely dry areas with very low water and nutrient supply [2]. Theproducts obtainable from extremophiles such as proteins,enzymes (extremozymes) and compatible solutes are ofgreat interest to biotechnology.

II. METHODS AND MATERIAL

Historical background and commercial prospect of enzymes

Enzymes are nature's own catalysts. They are proteins which accelerate the rate and specificity of chemical reactions by reducing the required activation energy. Early ideas about enzymes and bio-catalytic processes started to take form during the seventeenth and eighteenth centuries; however, the first major breakthroughs were not achieved until the nineteenth century. In 1833, the first enzyme was discovered, diastase, now known as amylase. The term enzyme was not adopted until 44 years later in 1877 by the German scientist Kühne. Nearly 40 years later, the first enzymatic preparation for a com- mercial application was developed by Otto Rohm in 1914. He isolated trypsin from animal pancreases and added it to washing detergents to degrade proteins. Industrial enzymes have since evolved into a multibillion dollar global market. The global market for industrial enzymes is expected to reach $7 billion by 2018 with a compound annual growth rate (CAGR) of 8.2% from 2013 to 2018 [3].

Enzymes have become important tools for diverse industrial markets, such as food and beverage, animal feed, detergents, and technical enzymes, including biofuels, leather, pulp and paper, and textile markets. Specialty enzyme use is also growing in markets, such as diagnostics, pharmaceuticals, and research and development.

From Enzymes to Extremozymes

Due to the intrinsic characteristics of enzymes, they have influenced almost every industrial market and their demand has constantly increased over the years. These natural catalysts are fast, efficient, and selective, in addition to producing low amounts of by-products. They are also fully biodegradable molecules, resulting in a low environmental impact and a greener solution to

many industrial challenges. The diversity, and unique properties of microbial enzymes, e.g., consistency, reproducibility, high yields, and economic feasibility among others, have elevated their biotechnological interest and application to different industrial areas [4]. In many cases, traditional chemical solutions are still the only viable option under such harsh conditions. There is a clear need for more sustainable and environmentally friendly methods to replace the current potentially harmful chemical processes. Extremophiles are organisms, mainly microorganisms, which belong to the domains Archaea and Bacteria. They thrive in environmental conditions considered by human standards to be extreme. Extremophiles grow and reproduce under high temperature in hot springs or thermal vents, low temperatures in glaciers or the deep sea, acidic and basic pH in industrial or mine waste water effluents, high concentration of salts in salt lakes, and high levels of radiation and extreme desiccation in deserts among other physical or chemical extreme conditions in various ecological niches [2, 5, 6]. In many cases, enzymes from extremophiles have adapted to withstand extreme conditions on their own. These adaptations correspond to key changes in the amino acid sequence, which are translated into variations in the structure, flexibility, charge, and/or hydrophobicity of the enzymes. These changes do not follow a pattern or a specific trend. Extremophilic proteins display substantial variability in adaptations for similar extreme physical or chemical conditions [7].

Halophiles

Halophilic microorganisms grow in very high sal (NaCl) concentrations. They are found in salterns and hyper saline lakes, such as the Great Salt Lake, the Dead Sea and solar lakes in Africa, Europe and the USA. They have even been found in Antarctic lakes. They accumulate salts such as NaCl or KCl up to concentrations that are isotonic with the environment. As a result, proteins from halophiles have to cope with very high salt concentrations (up to about 4 M KCl and over 5 M NaCl [8-10]. Halophilic proteins employ different adaptation mechanisms. Proteins from halophilic organisms have a biased amino acid composition in order to remain stable and active at high ionic strength. Halophilic proteins typically have an excess of acidic amino acids (*i.e.* glutamate and aspartate) on their surface. Negative charges on the halophilic proteins bind significant amounts of hydrated ions, thus reducing their surface hydrophobicity and decreasing the tendency to aggregate at high salt concentration. Halophilic proteins are distinguished from their non-halophilic homologous proteins by exhibiting remarkable instability in solutions with low salt concentrations and by maintaining soluble and active conformations in high concentrations of salt, for example, up to 5 M NaCl [11, 12]. Halophiles respond to increases in osmotic pressure in different ways. The extremely halophilic archaea, the *Halobacteriaceae*, accumulate K+, while other bacteria accumulate compatible solutes (*e.g.* glycine, betaine, sugars, polyols, amino acids and ectoines), which help them to maintain an environment isotonic with the growth medium [11, 13]. These substances also help to protect cells against stresses like high temperature, desiccation and freezing. Consequently, in surroundings with lower salt concentrations, the solubility of halophilic proteins is often very low [11, 12]. Halophiles from the archaeal domain provide the main source of extremely halophilic enzymes. Halophilic enzymes like such as xylanases, amylases, proteases and lipases, have been reported to be produced by some halophiles belonging to the genera *Acinetobacter, Haloferax, Halobacterium, Halorhabdus, Marinococcus, Micrococcus, Natronococcus, Bacillus, Halobacillus and Halothermothrix* [8, 10, 12-14]. High negative surface charge of halophilic proteins makes them more soluble and renders them more flexible at high salt concentrations, conditions under which non-halophilic proteins tend to aggregate and become rigid. This high surface charge is neutralized mainly by tightly bound water dipoles [12, 13]. Halophiles encapsulated in reverse-micelles can be used in bioremediation (to reduce or to eliminate environmental hazards resulting from accumulation of toxic chemicals or other hazardous wastes) [16].

Halophilic Extremozymes

Highly saline environments, such as the Dead Sea, Great Salt Lake or saltern pools, represent another habitat occupied by extremophilic microorganisms including two groups of halophilic archaea: aerobic haloarchaea and anaerobic halophilic methanoarchaea [17]. Fundamentally different approaches are employed by each in order to survive the osmotic challenges associated with life in saline environments [18]. Till now, the bulk of interest in applied halophilic archaeal

products have focussed on stabilizing agents like betains, ectoines, and polysaccharide, on secreted polymers for use of biodegradable plastics or halo-tolerant lipids from these strains [19]. In some cases, the high salt tolerance of haloarchaeal compounds is exploited, whereas in other instances, this property is secondary. For instance, retinal proteins such as bacteriorhodopsin have found uses in holographic films or in other light-sensitive or 'bioelectric' applications [20]. To date, the bulk of applied interest in products derived from halophilic archaea has focused on stabilizing agents such as betaines or ectoines, on polysaccharides, on secreted polymers for use as biodegradable plastics or on salt-tolerant lipids from these strains [19]. In some cases, the high salt tolerance of haloarchaeal compounds is exploited, whereas in other instances, this property is secondary. For instance, retinal proteins such as bacteriorhodopsin have found uses in holographic films or in other light-sensitive or 'bioelectric' applications [20]. A patent for production of a novel restriction enzyme of unusual specificity from a species of the genus Halococcus has been filed. *A chymotrypsinogen* B-like protease has been isolated from the haloalkaliphilic archaeon *Natronomonas pharaonis* and shown to act optimally at 61_C and pH 10 [21]. Unlike many of the previously reported halo-archaeal proteases which lose their catalytic activity at lower salt concentrations [22], the alkaliphilic *N. pharaonic* proteases could function at lower salt concentrations and thus have applications as detergent additive [23].

Psychrophilic extremozymes

Cold habitats such as Antarctica, where temperatures never exceed 5_C, are widespread. In fact, deep oceans, which cover over 70% of the Earth's surface, represent the major biosphere on the planet. Able to function with reduced energy requirements, extremozymes from psychrophilic or 'cold-loving' microorganisms could find use in numerous applications.

Psychrophilic polymer hydrolysing extremoenzymes like β-glycanases to detergents can be used for efficient washing in cold water. These enzymes also could find their applications in paer industry- in manipulation of pulp or in bioremediation efforts [24]. The food industry could exploit pectinases that act at lower temperatures in the processing of fruit juices or cheeses. Cold environments, however, present proteins with a number of physical challenges. Cold adapted proteins contain specific sequence modifications, fewer disulfide bonds and salt bridges, helix dipole structures of lower net charge, increased solvent interactions, a decreased number of hydrogen bonds at domain interfaces and a lower degree of hydrophobic interactions in the core of the protein [25]. Archaea have been detected in many low-temperature aquatic environments. 16S ribosomal RNA gene sequence analysis of Pacific Ocean plankton samples taken from depths of 100 and 500 m revealed the presence of various, currently unidentified archaeal strains [26]. Archaea account for over a third of the prokaryotic biomass in coastal Antarctic surface waters [27]. A novel crenarchaeal strain, *Cenarchaeum symbiosum*, lives symbiotically in marine sponge tissues and grows well at 10_C [28].

Thermophilic Extremozymes

Thermophilic enzymes possess certain advantages over their mesophilic counterparts. These enzymes are active and efficient under high temperatures, extreme pH values, high substrate concentrations and high pressure. They are also highly resistant to denaturing agents and organic sol- vents. In addition, hot extremozymes perform faster reactions and are easier to separate from other heat-labile proteins during purification steps. Due to their overall activity and stability at high temperatures, (hyper-) thermophilic enzymes are attractive for several important industrial activities. It is difficult, however, to obtain pure enzymes from source microorganisms and yields are typically low when cultivated on a large scale for industrial applications. Efforts to overcome the problems have focused on cloning and expressing (hyper) thermophilic enzymes in mesophilic hosts. Ideally, this can be done without losing their activity and thermostability. Thermostable enzymes expressed in mesophilic hosts can typically be purified easily and the degree of purity obtained is suitable for industrial applications. Enzymatic starch hydrolysis is used to form syrups through liquefaction, saccharification, and isomerization processes. Products of starch hydrolysis, such as glucose, maltose, and oligosaccharides, can be used for the production of a variety of non-food products including alcohols, polyols, ascorbic acid, lysine, etc. In the manufacture of syrups, liquefaction and saccharification processes are run at high temperatures, easily over 60–70°C [29]. Commonly, enzymes isolated from hyperthermophiles work optimally between 80 and

110°C and at pH levelsfrom4.0to7.5. These conditions correlate with the optimal conditions for starch liquefaction (100°C and pH4.0–5.0) [30]. Thus, characterization and development of novel hyper-thermophilic enzymes is essential for these industrial processes. The expression of a mutant thermostable α-amylase from *B. licheniformis*, with optimum activity at high temperature and lower pH, in *E. coli* and *P. pastoris*, was recently achieved. Because of their high-temperature activity profiles, β-amylases from *Thermo-anaero-bacterium thermosulfurigenes* and *Clostridiumthermo cellum* SS8 areal so good candidates for saccharification processes [31]. Amylases are also applied in processes, such as baking, brewing, the preparation of digestive aids, and in the production of cakes and fruit juices.

III. CONCLUSION

The potential conflict between the harsh conditions of an industrial process and the limited stability of enzymes has long been recognised. Extremoenzymes having higher stability in extreme conditions could provide a possible solution to this problem. In this review, we have described the biodiversity of extremophiles and the diversity of their metabolic organisation. Also, we have noted that only a small number of species from this large group of organisms have been isolated and characterised in detail. Thus, it is clear that a large number of extremozymes are as yet undiscovered. With an increase in number of extremophiles isolated and their enzymes characterized, their application possibilities in biocatalysis and biotransformation are bound to increase. It is possible that the boundaries defining the limits of life will be extended to even greater extremes. It is also clear that our understanding of the structural basis of enzyme stability and activity under extreme conditions is far from complete. Therefore, we do not yet have the theoretical basis for engineering enhanced stability into a mesophilic enzyme of choice, nor are we able to alter the specificity and catalytic activity of extremozymes in a predictable fashion. Thus with respect to traditional protein engineering methods, use of directed evolution approach involving random mutagenesis, DNA shuffling and screening for desired characteristics is much successful. This situation seems likely to continue well into the future, particularly as the mutations generated and selected in directed evolution experiments are not limited to those found in naturally occurring, stable enzymes.

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