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Life at extreme conditions: Extremophiles and their Biocatalytic potential

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ABSTRACT

Extremophiles are the organisms that can survive in extreme conditions. The extreme conditions include high pH , high temperature, high salinity, high concentration and low oxygen tension. The organisms that are included in extremophiles are maximum prokaryotes (archae and bacteria) and others include Eukaryotes. These organisms may be classified as acidophilic (optimal growth between pH 1-5), alkaliphilic (optimal growth above pH 9), halophilic (grows in highly saline environments), thermophilic (grows in a temperature range of 60-80°C), hyperthermophilic (can grow in a temperature above 80°C), psychrophilic (can survive at very low temperature range), barophilic(can survive at high hydrostatic pressure), oligotrophic(can grow in nutritionally deficient environment), endolithic(which can grow within rocks or within pores of mineral grains) and xerophilc (can grow in dry conditions with less water availability). Extremophiles produce extremozymes that are functional under highly extreme conditions. Examples of extremozymes are cellulases, amylases, proteases, xylanases, keratinases, pectinases, lipases, catalases, esterases, peroxidases, and phytases. These enzymes can be applied for various biotechnological processes. The study of these organisms helps us to understand the physiochemical parameters which define life on this earth and can help us to find how life on the earth originated.

Keywords: Extremophiles, extremozyme, polyextremophiles, Biocatalytic potential

I. INTRODUCTION

There are a variety of microorganisms that can survive in extreme conditions like salinity, pH, temperature, pressure, light intensity, oxygen and nutrient conditions and such microorganisms are known as extremophiles. Thus these are the organisms that can survive in physically and geochemically extreme conditions. There are 3 domains of such organisms are bacteria, archaea and eukaryotes. The most widely distributed of these organisms are Bacteria and Archaea. Extremophiles are found in niches & have potential to produce biocatalysts that function under these conditions. These are mainly classified on basis of temperature and chemical, these are thermophiles (which can survive in high temperature), psychrophiles (which can survive in low temperatures), acidophiles (which can survive in extreme acidic conditions), alkaliphiles (which can survive in extreme alkaline conditions) and halophiles (which can survive in highly saline conditions). Extreme environments refer to those conditions to which microorganisms have adapted. The diversity of environments to which different extremophiles have adapted offers many exciting opportunities for a variety of applications. Microbial life shows great flexibility for surviving in harsh conditions. These organisms secrete a variety of enzymes that are helpful in various industrial processes eg: the themophilic catalase isolated from an organism *Thermus* *brockianus* helps in the initiation of breakdown of hydrogen peroxide into oxygen and water. This catalase can work efficiently in a temperature range from 30°C-94°C and a ph range from 6-10. This catalase is used in industries for the removal of hydrogen peroxide in processes like pulp, paper, bleaching, textile bleaching, food pasteurization and surface decontamination of food packaging.

II. CLASSIFICATION OF EXTREMOPHILES

They are classified according to the conditions in which they grow: as thermophiles, hyperthermophiles and psychrophiles (organism growing at extreme temperature), acidophhiles and alkaliphiles (organism growing at extreme pH), halophiles (organism growing at extreme salt concentration), barophiles (organism growing best under pressure), polyextremophiles (organism adapted more than one extreme conditions)

2.1 On the basis of extreme tempertature

2.1.1 Thermophiles

A thermophile is a type of extremophile- that can survive at very high temperatures, between 41 and 122 °C (106 and 252 °F). Various geothermally heated regions of the Earth, such as hot springs like those in Yellowstone National Park and deep sea hydrothermal vents, as well as decaying plant matter, such as peat bogs and compost shows the occurance of such extremophiles . Thermophilic microbes can survive at high temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures. Thermophiles on the basis of degree of temperature can be generally classified into moderate thermophiles (which shows growth in a temperature range of 50-60°C), extreme thermophiles (which shows growth in temperature range of 60-80°C) and hyperthermophiles (which shows growth in a temperature range of 80-110°C). Thermophilic extremophiles have attracted attention particularly in extremophilic proteases, lipases and polymer degrading enzymes, such as cellulases, chitinases and amylases. The biocatalytic potential of thermophiles and their enzymes has been reviewed by Adams [1], D.W Hough [2], C.M Andrade [3], F Niehaus [4], D.C Dermiorijan [5], J Eichler [6], C Vieille [7], Damico [8] , C Vieille [9], S Fujiwara [10]. At high temperature the solubility of many reaction components is significantly improved. And the risk of contamination which causes undesired complications are also reduced at higher temperature. From Pyrococcus woesei, Pyrococcus furiosus and Thermococcus profundus, thermostable amylase have been characterised. Hyperthermophilic archaea of the genera Sulfolobus, Desulfurococcus, Thermococcus and Staphylothermus shows amylolytic activity.

2.1.2 Psychrophiles

Psychrophiles or cryophiles are the extremophilic organisms that can show growth and reproduction in cold temperatures, ranging from −20 °C to +10 °C. These organisms include bacteria, but psychrophiles also include eukaryotes such as lichens, snow algae, fungi, and wingless midges. Desication and vitrification (glass transition) protects the psychrophiles from freezing and expansion of ice. More recently, enzymes from psychrophiles are very useful for industrial applications, partly because of ongoing efforts to decrease energy consumption. For example, psychrophilic enzymes can be used in detergents. With such enzymes it becomes possible to develop laundry applications that can be performed at lower temperatures. Psychrophilic proteases, amylases or lipases have great commercial potential for such processes. Polymer degrading enzymes that are active at lower temperatures are used in pulp and paper industry. Various food processing applications also

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found benefit from the availability of low temperature enzymes such as L-glutaminase and L-asparaginase. A characteristic feature of many enzymes derived from psychrophiles is that they show catalytic activity and low thermal stability at moderate temperatures which can be explained by the increased flexibility of the molecule, compared with mesophilic and thermophilic enzymes.

Adaptation of microbes at low temperature have been studied by different scientists as E Leveque [11], C.Bertoldo [12], J Kamakshi [13], L Kumar [14], G. Feller [15] .The potentials of psychrophiles and psychrophilic enzymes have been reviewed by S Y Kim [16], A O Smal [17], R Cavicchioli [18], J W Deming [19], R Margesin [20]. A diverse range of psychrophilic microorganisms, belonging to Gram-negative bacteria (e.g., *Pseudoalteromonas*, *Moraxella*, *Psychrobacter*, *Polaromonas*, *Psychroflexus*, *Polaribacter*, *Moritella*, *Vibrio* and *Pseudomonas*) Gram-positivebacteria (e.g., *Arthrobacter*, *Bacillus* and *Micrococcus*) archaea (e.g., *Methanogenium*, *Methanococcoides* and *Halorubrum*) yeast (*Candida* and *Cryptococcus*) and fungi (*Penicillium* and *Cladosporium*) have been isolated from these cold environments [16],[17],[18],[19],[20]. A diverse range of psychrophilic microorganisms, belonging to Gram-negative bacteria (e.g., *Pseudoalteromonas*, Moraxella, Psychrobacter, Polaromonas, Psychroflexus, *Polaribacter*, *Moritella*, *Vibrio* and *Pseudomonas*) Gram-positive bacteria (e.g., *Arthrobacter*, *Bacillus* and *Micrococcus*) archaea (e.g., *Methanogenium*, *Methanococcoides* and *Halorubrum*) yeast (*Candida* and *Cryptococcus*) and fungi (*Penicillium* and *Cladosporium*) have been isolated from these cold environments [16],[17],[18],[19],[20]. The use of cold-active hydrolytic enzymes such as proteases, lipases, amylases and cellulases in the formulation of detergents would be of great advantage for cold washing. Apart from these examples, cold-active enzymes have potential for other interesting applications such as the hydrolysis of lactose in milk using-galactosidase, biopolishing and stone washing of textile products using cellulases, extraction and clarification of fruit juices using pectinases, tenderization of meat or taste improvement of refrigerated meat using proteases [21] improvement of bakery products using glycosidases (e.g., amylases, proteases and xylanases) softening of wool or cleaning of contact lenses using proteases.

2.2 On the basis of chemical extremes

2.2.1 Halophiles

They have the ability to survive in hypersaline conditions that's why they are also called as halophytic organisms. Salt is required by all life forms but halophiles need high concentration of salt for their survival. Halophiles maintain the osmotic pressure with the environment they absorbing sodium and potassium chloride salts. Their proteins cope with high salt concentration. by acquiring a relatively large number of negatively charged amino acid residues on their surfaces to prevent precipitation,their enzymes have adapted to the environment. There are a number of ways by which halophiles respond to increasing osmotic pressure. Extremely halophilic organism, Halobacteriaceae accumulate K^+, while other bacteria accumulate compatible solutes (e.g., glycine, betaine, sugars, polyols, amino acids and ectoines) with which help they can maintain an environment isotonic with the growth medium. These compatible solutes provide protection to the cells against stresses such as high temperature, desiccation and freezing. Consequently, in surroundings with lower salt concentrations, the solubility of halophilic proteins is often very low [22], [23]. Extremozyme from these halophiles are having potential biotechnological application. Halophilc enzymes are active in environment having low water activity. Enzymes remain active by predominance of negatively charged residues on the

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solvent exposed surface of protein. These negative charges attract water molecules & keep the protein hydrated so that they do not precipitate. The enzymes produced by the halophiles are: Proteases, Glycosyl, Hydrolases, beta Galactosidases, Restriction enzymes, Esterase and lipases. The halophilic enzymes, such as xylanases, amylases, proteases and lipases are produced from halophiles belonging to the genera *Acinetobacter*, *Haloferax*,*Halobacterium*,*Halorhabdus*, *Marinococcus*, *Micrococcus*, *Natronococcus*, *Bacillus*, *Halobacillus* an d *Halothermothrix* shows some recent reports on these enzymes.

2.2.2 Acidophiles

Acidophiles or acidophilic organisms are the organisms that can survive under highly acidic conditions (which is at pH 2.0 or below) Archaea, Bacteria, and Eukaryotes comes under acidophiles Some of the examples of acidophiles are *acetobacter aceti,helocobacter pylori,acidobactetrium* etc. The enzymes derived from acidophiles which can survive under highly acidic conditions can be used for various industrial purposes for example, in the production of detergents. However, one of the striking properties of acidophiles is their tendency to maintain a neutral pH internally and so the intracellular enzymes derived from these microorganisms do not need to be adapted to harsh growth conditions. However, for extracellular proteins which have to function in low pH environments in the case of acidophiles does not account for this. In order to maintain a neutral pH internally, acidophiles use proton pumps and so the intracellular enzymes from these microorganisms do not need to be adapted to harsh growth conditions. However, the extracellular enzyme proteins of acidophiles function at low pH. Acidophiles utilize various strategies in order to survive in the extreme conditions of pH. For biobleaching of pulp and paper, Cellulase free xylanases are used. For the degumming of ramie fibers, pectinases are used. For the removal of residual hydrogen peroxide from effluent streams of the textile processing industry peroxidase or oxidoreductase may be used.

2.2.3 Alkaliphiles

Alkaliphiles are a class of extremophilic microbes which can survive in alkaline (at a pH roughly 8.5–11) environments, growing optimally around a pH of 10. Alkaliphiles have negatively charged cell wall polymers in addition to peptidoglycan which may reduce the charge density at the cell surface and help to stabilize the cell .Cellular fatty acids in alkaliphilic bacterial strains contain predominantly saturated and mono-unsaturated straight-chain fatty acids. Alkaliphiles consist of two main physiological groups of microorganisms; alkaliphiles and haloalkaliphiles. Alkaliphiles require an alkaline pH of 9 or more for their growth and have an optimal growth pH of around 10, whereas haloalkaliphiles require both an alkaline pH (>pH 9) and high salinity (up to 33% (w/v) NaCl) [24]. Thermoalkaliphiles and alkaliphiles are good sources of alkaliphilic enzymes like cellulases, xylanases, amylases, proteases, lipases, pectinases, chitinase, catalase, peroxidase and oxidoreductase. Thermoalkaliphilic enzymes have great biocatalytic potential in processes that are performed at alkaline pH and higher temperatures. For example, proteases, lipases and cellulases are used as additives in laundry and dishwashing detergents, proteases are also used for dehairing of hides and skins and to improve smoothness and dye affinity of wool [25] as well as in detergent industries as a additives [26], [27] Horikoshi have spent the major part of his research career to investigate thephysiology, ecology, taxonomy, enzymology, molecular biology and genetics of the alkaliphiles.

2.3 Other extremophiles

At present, it is clear that no matter how extreme the conditions are at defined locations on Earth there is a fair chance that microbes will be able to survive. Additional examples further to those described above are microorganisms that grow in the presence of high metal concentrations (metallophiles) at high radiation levels (radiophiles) or under oxygen deprivation (microaerophiles). The biotechnological application of enzymes from such industry extremophiles is not always obvious. Nevertheless, in view of the great potential of biocatalysis it is very likely that new concepts will be developed that will result in the application of enzymes from these and other extremophiles in industrial processes..

2.3.1 Xerophiles

The driest regions in the world can support life, even dry stones and deserts. However, only specialized microbes belonging to fungi, lichens and algae have the ability to grow in such extremely dry conditions. These specialized organisms are called xerophiles [28], [29]. Xerophiles also referred as Osmophiles is an extremophilic organism that can grow and reproduce in conditions having a low availability of water. Many osmophiles are responsible for spoiling of dried foods and stored grains, spices, nuts and oilseeds as they thrives in dry conditions. Some xerophiles (osmophiles) spoil salty foods, and others spoil sugary foods. Example: *Wallemia sebi* is a xerophile mold that grows in dried fruit, salted meats and even the evaporation beds where sea salt is produced. Mold growth on bread is an example of food spoilage caused by xerophiles. As very limited studies have been conducted on xerophiles, the biocatalytic potential of these microbes and their enzymes is not yet known.

2.3.2 Radiophiles

Radiophiles also called as radioresistant microbe. These microbes are highly resistant to high levels of ionizing and ultraviolet radiation. These organisms consistently survive doses of radiation that are 500 times greater than the lethal dose for humans. Daly [30] reviewed the genetic engineering and environmental biotechnology aspects of these radioresistant microbe. Examples of radiophiles are *Deinococcus radiodurans* [31] is listed by the Guinness Book of world records as “the world’s toughest bacterium”, *Deinococcus radiophilus* [32] *Thermococcus marinus* sp. nov. and *Thermococcus radiotoleran* [33]. *Deinococcus radiodurans* is a remarkable bacterium that is highly resistant to chemicals, oxidative damage, high levels of radiation and dehydration. It contains a spectrum of genes that encode for multiple activities that repair DNA damage. The biochemical function of the genes encoding three putative uracil-DNA glycosylases determined by their cloning and expression analysis [31]. Many nuclear waste materials contains toxic chemicals, heavy metals, halogenated solvents and radionuclides and the main challenge arise is their disposal and separation of different species. *Deinococcus radiodurans* strains and other detoxifying microorganisms may be utilized to detoxify halogenated organics and toxic metals such as mercury, and theoretically could be used to remove these classes of compounds selectively from mixed wastes under mild conditions.

2.3.3 Barophiles

Microorganisms that thrives high-pressure environments such as bottom of the ocean for growth are termed barophiles formerly known as piezophiles. Piezophiles are distributed among the genera Shewanella, Colwellia, Moritella, Methanococcus, Pyrococcus and Thermus (28,29). The biocatalytic potentials of piezoenzymes from piezophiles have recently been reviewed by Abe and Horikoshi (28) and Yano and Poulos (29).

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2.3.4 Metallophiles

Microorganisms that can grow in the presence of high metal concentrations are called metallophiles. These organisms, including several members of the genus Ralstonia, colonize industrial sediments, soils or wastes with high contents of heavy metals. Metal-resistant Ralstonia have adapted well to the harsh environments created by extreme anthropogenic activities or biotopes [34]. Ralstonia metallidurans, a gram-negative, non-spore forming Bacillus, thrives in millimolar concentrations of toxic heavy metals. It was first isolated in 1976 from the sludge of a zinc decantation tank in Belgium that was polluted with high concentrations of several heavy metals. A typical feature of these metal-resistant Ralstonia is the presence of one or two large megaplasmids that contain genes for multiple resistances to heavy metals. These plasmids confer resistance to Zn, Cd, Co, Pb, Cu, Hg, Ni and Cr. Since pollution by heavy metals poses a threat to public health, fishery and wildlife, there has been an increased interest in developing systems that can remove or neutralize the toxic effects of heavy metals in soils, sediments and wastewaters. Many microorganisms, including Ralstonia, could be used in heavy metal bioremediation. In addition to their use as biosorbents, bacteria can be used to immobilize certain heavy metals efficiently, this being mediated by their capacity to reduce these elements to a lower redox state, producing metal species that have a lower bioactivity. Bacteria ex

III. CONCLUSIONS

Extremophiles are bizarre microorganisms that can grow and thrive in extreme environments. The steady increase in the number of newly isolated thermophillic and hyperthermophillic microorganism and the related discoveries of their enzymes document the enormous potential within scientific field. Extremozymes from extremophiles have great biotechnological potential in many industrial processes (e.g. agriculture, food, feed and drinks, detergents, textile, leather, pulp and paper). These extremozymes will be used in novel biocatalytic processes that are faster, more accurate specific and environmentally friendly.This renewed confidence in enzyme biotechnology may have emerged as a result of the success of genome-based technologies that are currently in use. It has been suggested that less than 10% of the organism in a defined environment will be cultivatable and so further improvement of gene expression technologies will accelerate the exploration of microbial diversity. Concurrent developments of protein engineering and directed evolution technologies will result in further tailoring and improving biocatalytic traits which will increase the application of enzymes from extremophiles in industry. Our experience with extremophiles leads us to caution that it will take a lot of research to turn extremozymes into industrial products. We strongly believe that discoveries of new extremophiles and genetic engineering of the newly isolated as well as of the currently available extreme microbes will offer novel opportunities for industrially important enzymes.

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