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EXPLORING THE PLETHORA OF HIDDEN POTENTIAL IN THE QUEST FOR SUSTAINABLE DEVELOPMENT: IMPACT OF ECOLOGICAL NICHE ON THE ENZYMES FROM EXTREMOPHILES

S. Navya Prasanna[1], Venkatesh Kamath B.[2,✉], Aravinda Pai[3], Rutwik Garge[1] and M. Pranathi Priya[1]
[1] PG Student, Department of Pharmaceutical Biotechnology,
Manipal College of Pharmaceutical Sciences, MAHE, Manipal-576104, Karnataka, India,
[2] Department of Pharmaceutical Biotechnology,
Manipal College of Pharmaceutical Sciences, MAHE, Manipal-576104, Karnataka, India,
[3] Department of Pharmaceutical Chemistry,
Manipal College of Pharmaceutical Sciences, MAHE, Manipal-576104, Karnataka, India
✉Corresponding Author: venkatesh.kamath@manipal.edu

ABSTRACT

It is well known that microorganisms are omnipresent. It would be fascinating for scientists to encounter organisms that not only grow in normal conditions but are able to thrive in extreme conditions. Such organisms are suitably termed extremophiles including thermophiles, psychrophiles, alkaliphiles, piezophiles, and marine extremophiles, to name a few. The UN General Assembly has proposed 17 goals with 169 targets to attain sustainable development by 2030 with a motto of health for all. Microorganisms play a vital role in the well-being of not just humans but animals as well as plants. Of the 17 goals, goals 14 and 15 address life in water and life on land and the need for protecting our Biodiversity, both on land and sea. The soil on land is a hub for various microbes, likewise, microorganisms show equal presence in marine habitats, able to survive the extremes of conditions. The ability of these microbes to cope with such extremes of conditions makes them an ideal candidate in search of metabolites that would be suitable candidates for various industrial and pharmaceutical applications. It thus becomes significant to analyze and study extremophiles for their properties that include primary and secondary metabolites obtained from them. Enzymes from these extremophiles, known as extremozymes, could potentially be more stable which is an important aspect for various industrial aspects including pharmaceutical purposes. The present article dwells on extremophiles and the extremozymes obtained from them and how these could play a role towards achieving goals 14 and 15 that ultimately would also cater to goal 3, that being the health and wellbeing of all.

Keywords: Extremophiles, Enzymes, Sustainable development, Health, Environment.
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INTRODUCTION

Fuelled by increasing industrial needs, the quest for biocatalysts that can withstand harsh industrial processes has been on the rise. This has also to do with the fact that biocatalysts provide an added advantage in being a cost-effective method for catalyzing multiple reactions. Extremophiles offer researchers valuable insights into this aspect and are a source of unique enzymes that could also possess the property of being stable during tough environmental conditions that it could encounter. It is with this background that, microbes that are able to survive and thrive in extremes of environmental conditions with their metabolites included, have gained rapid attention among the scientific community. Geochemical extremes such as pH, salinity as well as physical extremes including temperature, pressure, or radiation could be some of the parameters that a microbe could encounter. Extremophiles, falling under such a diverse group of microbes, have progressed through evolution to live in diverse habitats and include thermophiles, acidophiles, psychrophiles, barophiles, and halophiles to name a few. Biocatalysts derived from these extremophiles could be used in a wide range of situations, thus opening a plethora of fascinating possibilities for a wide range of applications. Extremophiles include microbes from bacteria, archaea as well as eukarya which form three major domains of life. The mechanisms through which these microbes acclimatize to harsh

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settings offer a distinctive viewpoint on vital biological processes including biochemical limits to macromolecular stability along with genetic instructions for building macromolecules that are stable under extreme environmental settings. Table-1 gives an account of some groups of extremophiles indicating their growth characteristics, enzymes obtained as well as major applications.

Table-1- Major Classification and Applications of Extremophiles

Extremophiles type	Growth characteristics	Enzymes	Applications
Thermophiles	Thermophiles (45-65°C), Hyperthermophiles (65-90°C), Extreme thermophiles (>90°C)	Proteases, Xylanases, Chitinases, Lipases, Esterases	Detergents, food and feed hydrolysis, Brewing, Textiles, Baking
Psychrophiles	Temperature <15°C	DNA polymerases, Dehydrogenases, Proteases, Amylases, Cellulases, Lipases	Molecular biology, Oxidation reactions, Detergents, Food applications, Bakery, Textiles
Halophiles	High salt for example, 2-5M NaCl	Proteases, Dehydrogenases	Synthesis of peptides, Speeding up reactions involving organic media
Alkaliphiles	pH greater than 9	Proteases, Cellulases	Detergents, Food as well as feed
Acidophiles	2-3 being the optimal pH range	Amylases, Glucoamylases, Proteases, Cellulases, Oxidases	Starch processing, Feed component, coal Desulfurization
Piezophiles	Fond of pressure upto a range of 130MPa	Not sufficiently defined	Antibiotics, food processing
Marine extremophiles	Temperature, 2-20°C	Hydrolases	Production of single-isomer chiral medicines

Thermophiles

Microorganisms have the potential to survive in extreme environments in which, factors such as p^H, temperature, pressure, and salt conditions have higher values than those that are regarded as the standard for microorganisms. Among the various factors, the temperature has the most vital impact on the biological components and molecules that function. It is well known that most organisms thrive at normal temperatures between 25-45°C but there are some microorganisms that grow at higher temperatures that are regarded as thermophiles. Based on the temperature range, they are further categorized into thermophiles (45-65°C), hyperthermophiles (65-90°C), and extreme thermophiles (>90°C). As these microorganisms are able to adapt to higher temperatures, they have various advantages in industrial fermentation and various other processes.[2] Some of the examples include *Brevundimonas bullata, Thermus aquaticus, Geobacillus stearothermophilus,* and *Paenibacillus glycanilyticus* to name a few.

Psychrophiles

Microbes that are able to adjust, grow, and thrive in cold environments are termed psychrophiles. They are often regarded as cold-loving organisms for their adaptation to cold environments. These microbes possess a complex set of machinery in place to adapt to such extremes and include protein synthesis machinery, biodegradative enzymes, membrane energy-generating systems, and nutrient uptake mechanisms to name a few. Enzymes from psychrophiles, also known as psychrozymes, have lately gained attention for their industrial applications which are mainly attributed to the fact that there have been continuous efforts to reduce energy consumption. For instance, enzymes from psychrophiles are increasingly being used in detergents as these enzymes can develop laundry applications that can be attained at lesser temperatures. Enzymes like amylases, proteases, and lipases from psychrophiles have been often used. Industries dealing with pulp and paper are showing interest in the degrading polymer as they are active at lower temperatures.[3] *Bacillus psychrosaccharolyticus, Exiguobacterium antarcticum, Moraxella, Flavobacterium* and *Planococcus antarcticus* are some examples.

Halophiles

Halophiles are those organisms that survive in hypersaline habitats as they can maintain osmotic balance. These organisms are inhabitants of an environment that is hypersaline. In general, these are the organisms that can show optimum growth at 0.2M NaCl concentration and above. In these halophiles, there is an accumulation of salts, mainly sodium chloride or potassium chloride and these are present in such concentration to maintain isotonicity with the environment. There have been reports of extreme halophiles that can tolerate salt concentrations of 30% and above. To avoid precipitation, the enzymes have evolved to this environmental extreme by accumulating amino acid residues in high numbers which are negatively charged on their surfaces. As a result, halophilic enzyme solubility is generally poor in environments with lower salt concentrations, potentially limiting their application. When compared with the thermophilic and alkaliphilic extremophiles, halophilic microorganisms are yet to find uses in biotechnological applications but still have major exploration potential.[4] *Bacillus horikoshii, Halobacterium salinarum, Bacillus marisflavi,* and *Halomonas halophila,* are some of the microbes that fall under this class of extremophiles.

Alkaliphiles/Acidophiles

Microorganism-derived enzymes that can live at pH levels from extremely acidic to alkaline environments could be principally valuable for applications involving reaction conditions having such extreme conditions for instance, in the manufacture of detergents. However, because acidophilic and alkaliphilic microbes can maintain a neutral internal pH, the intracellular enzymes from these microbes need not be adapted to harsh growth conditions. Alkaliphiles are those, which are able to maintain an internal pH lower than that of the external one. Soil that is alkaline, salt lake, and oceans are some of the natural habitats of these alkaliphiles. On the other hand, acidophiles have adapted themselves by maintaining their cellular pH around the neutral range and are inhabitants of conditions that are highly acidic which could include acid rock and mine drainage, and vents from volcanoes to mention a few. Proteases, amylases, lipases, and such enzymes which are resistant as well as active in current detergents with pH on the higher side and chelator concentrations have a higher degree of desirability.[5] Some examples of acidophiles include *Bacillus aerophilus, Bacillus atrophaeus* and *Bacillus nanhaiensisArthrobacter ramosus, Bacillus alcalophillus, Bacillus halodurans, and Burkholderia cepacian* are all examples of alkaliphiles.

Piezophiles

Food processing and sterilization processes use high pressure.[6] These organisms are able to withstand extreme hydrostatic pressure and are also referred to as barophiles. In such a scenario, pressure-resistant proteins could be of use in these setups. Microbes that have been isolated from the deep sea are often exposed to high-pressure environments. Metabolites and extremozymes derived from such microbes could be very useful under circumstances that demand high-pressure reactions and can reach in excess of 40MPa. The majority of piezophiles are bacteria and can be grouped into one of five genera that include *Shewanella, Colwellia, Photobacterium, Moritella*, and *Psychromonas*.

Biocatalysis of Extremophilic Enzymes

Research has been done on structural proteins as well as crucial metabolic enzymes which account for organisms' rare properties. Major extremozymes comprise lipases, esterases, glycosidases, aldolases, nitrilases as well as phosphatases. Thermophilic enzymes, particularly proteases, and halophilic enzymes which demonstrate good stability at higher saline concentrations act as models for improving the rate of reaction in low-aquatic conditions.

Esterases and Lipases

Esterases and lipases are extensively employed extremozymes in fine chemical applications owing to their benefits in the generation of optically pure compounds. As per various studies, lipase from *Bacillus thermocatenulatus* has been produced in the presence of a promoter located upstream of the lipase gene up to that point, the enzyme remained stable. Maximum activity was discovered at pH 10-11 and temperatures of 60–70°C. This lipase was also extremely stable under various organic solvents and detergents. An *E. coli* recombinant strain was used to express an esterase from *Bacillus licheniformis*. At pH 7–8.5, the enzyme was stable, with optimum activity at pH 8–8.5. The ideal temperature is 45°C, and the activity was 45 minutes, while at 64°C, the half-life was 1 hour.[7]

Proteases and Peptidases

Most of the extremophilic enzymes were studied as they are used in the laundry and detergent industry. Low temperature encourages high yields due to the reduction in the hydrolysis of acyl-enzyme in peptidyl synthesis with mesophilic enzymes, according to research.

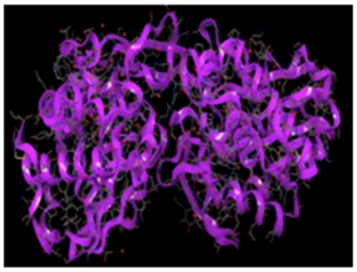

Fig.-1: Structure of Acetyl Esterase

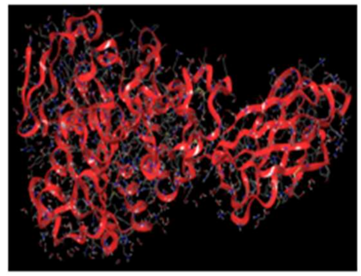

Fig.-2: Structure of Lipases

Oligopeptides possessing glycine were synthesized with a yield of up to 76% using *Halobacterium halobium*, the reaction being catalyzed by extracellular protease and in the process providing an added advantage of improved stability in organic environments. Solvents although combining high saline concentration with an organic solvent appears to improve activity, the salt's solvability confines the concentration of the solvent, which can be controlled by the immobilization of enzymes.[8]

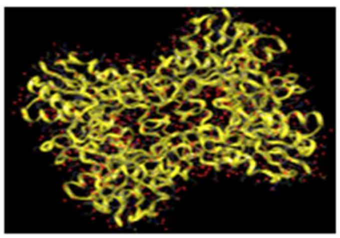

Fig.-3: Structure of Peptidases

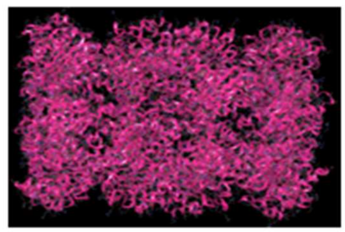

Fig.-4: Structure of Proteases

Production of Extremozymes from Extremophiles

The identification of valuable enzymes is aided by functional activity screening and DNA data mining. The discovery of potentially beneficial enzymes, however, is merely the initial step to getting the most out of the discovered enzymes must be made based on this knowledge available for a thorough examination of the biocatalytic tests on properties and applications. There are two approaches for the extremophiles are used to produce enzymes. First, biocatalysts production can be improved by enhancing extremophiles biomass production. Alternatively, the biocatalyst gene could be mutated, replicated, and then expressed in an appropriate host.

Biomass Production

Biomass production can be enhanced by modifying specific fermentation techniques (e.g., changing the medium composition), continuous cultivation, cell recycling, or fed-batch cultivation). Special bioreactors, like gas-lift fermenters, are used. For instance, certain extreme halophiles are cultivated for the manufacture of polyglutamic acid (PGA). Additional unique necessities that must frequently be met are those connected to the large volume of traffic stresses that can be found in a variety of situations. Extremophiles are secluded from the environment, such as deep oceans.[9]

Production of Enzymes by Gene Cloning

Nowadays fermentation technology is not considered for the production of extremophiles. To make useful biocatalysts from extremophiles other ways have been developed. Cloning and expression of coding genes in microbial hosts growing or thriving at moderate temperatures is a method. Several plasmid vectors as well as inducible gene expression methods are existing and genetic tools for *E. coli* expression are widely established. Effective disruption of most *E. coli* proteins after a brief treatment involving heat facilitates the production of thermophilic enzymes. *Bacillus, Pseudomonas,* and *Lactobacillus* as well as eukaryotic systems like *Pichia, Candida,* and *Hansenula* are among the many alternative hosts now available. For biological activity, if glycosylation is needed, bacterial hosts should be avoided; nevertheless, if the enzyme does not require glycosylation for biological activity, bacterial hosts should be used as an extracellular enzyme. *Bacillus,* for example, is likely to be the obvious pick. Protein engineering and the accessibility of efficient cloning and expression tools could also be used to improve and tune the biocatalytic characteristics of enzymes from extremophiles.[10]

Marine Extremophiles

Several marine extremophiles are adept at overcoming harsh conditions and are a source of enzymes having beneficial properties. That is why marine extremophiles are used in industrial processes mainly in biocatalysis. Due to the wide range of maritime ecosystems, new hydrolases have been created with unique specificities and features, including the ability to withstand harsh circumstances and be employed in industrial operations. Extremophile prokaryotes available through maritime settings are a source of unique genes, which in turn can help form new bioproducts like enzymes and other active metabolites, according to metagenomic research. Therefore, it is crucial to research and comprehend these microbes in order to utilize their ecological, evolutionary, biochemical as well as industrial prospect. Hydrolases from these extremophiles have many advantages. Their catalysts are eco-friendly, highly precise, and operate under benign reaction circumstances. The ability of these hydrolases to function in the form of organic solvents is essential for the production of single-isomer chiral medicines.[11]

CONCLUSION

Extremophiles produce a wide range of extracellular hydrolytic enzymes which are beneficial to mankind. These enzymes are mainly proteases, lipases, amylases, cellulases, etc. Extremophiles are in demand to treat cancers involving intense heat. Protein folding errors and the insertion of structures into neurons in the brain are hallmarks of major neurodegenerative disorders such as Huntington's and Parkinson's. Extremozymes may be present in thermophiles which includes characteristics such as the suppression of native or foreign conformational alteration mutant proteins, which could be important in the development of neurodegenerative disease therapies. Drugs and vaccinations have been delivered using lipids produced from halophilic bacteria. Siderophores, which are found in halophilic archaea, are iron-chelating agents that can be utilized to treat iron deficiency or to boost antibiotic action. Acidophiles are commonly utilized for medicinal purposes and have even had a role in medical evolution, they are usually used to prevent gastric cancer and stomach ulcers. Psychrophile-derived polyunsaturated fatty acids possess a varied array of applications in pharmaceuticals. Proteases have been used in medicine for many years to treat blood disorders, and they now have intriguing indications for other diseases as well as cosmeceuticals, are used to treat pancrelipase and muscle spasms. The use of extremozymes is vast and opens a wider arena for further research. Careful and meaningful use of life on land and life on the water, which these extremophiles are known for would enable the path towards goals 14 & 15 for sustainable development. Goals 14 & 15 would in turn have a synergistic effect on goal 3 with the focus being on health for all.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the faculty authors can be verified from their ORCID ids, given below:

S. Navya Prasanna https://orcid.org/0000-0001-7144-2322
Venkatesh Kamath B. https://orcid.org/0000-0001-6508-782X
Aravinda Pai https://orcid.org/0000-0003-2010-9479
Rutwik Garge https://orcid.org/0000-0002-6544-3828
M. Pranathi Priya https://orcid.org/0000-0003-4052-6050

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