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Extremozymes: Unusual Biocatalysts with Distinct Genesis and Potential Applications in Industry and Research

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ABSTRACT

Enzymes, called biocatalysts, have demonstrated exceptional abilities in transforming the pharmaceutical, chemical, biotechnological, bioremediation and agricultural sectors. However, the limited stability of these enzymes prevents them from being used in a wide range of applications. These limitations can be circumvented by enzymes produced by extremophiles, which are microorganisms that flourish in hostile settings. These enzymes, often called extremozymes, are crucial for industrial processes and scientific research because they can operate in harsh environmental conditions with high temperatures, pressures, salinities and pH levels. Extremozymes like thermophilic enzymes, psychrophilic enzymes, acidophilic proteins, alkaline enzymes and barophilic enzymes have been studied during the last few years and have great potential applications for biotechnology, such as in agricultural, chemical, biomedical and biotechnological processes. Extremozymes will significantly increase biotechnology's potential for real-time applications. The objective of this article is to shed light on the origin, categorization and uses of extremozymes.

KEYWORDS

Extremozymes, thermophilic enzymes, psychrophilic enzymes acidophilic enzymes, barophilic enzymes

INTRODUCTION

Extremozymes are stable enzymes, which are capable of tolerating changes in environmental conditions such as pH, temperature, pressure, salinity, etc. They are obtained from microorganisms, called extremophiles, which survive under harsh environmental conditions such as temperature, pH, salinity, pressure, nutrients, oxygen, water and radiation levels[1]. Extremozymes can catalyze their specific reactions under a variety of non-aqueous conditions, in water/solvent mixes, at very high pressures, in alkali and acidic pH ranges, at temperatures as high as 140°C and near the freezing point of water. Extremozymes have a wide range of potential industrial applications[2]. This article aimed to explore the origin, classification and applications of extremozymes.

Types of extremozymes: Five types of extremozymes are discussed.

Thermophilic enzymes: Enzymes active at high temperatures are called thermophilic enzymes. These enzymes are thermostable because they have a high defined melting (transition) temperature (Temp.) or have a long half-life at a selected high temperature. These enzymes can be used to catalyze

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high-temperature chemical processes/reactions accomplished in the food and paper industry, detergents industry, pharmaceutical industry and toxic waste removal[3]. Performing an industrial process at elevated temperatures reduces the risk of contamination by common mesophilic organisms, improves the solubility of organic compounds and increases reaction rates due to a decrease in viscosity and increased diffusion coefficient of the substrates. Taq polymerases are isolated from the thermophilic bacterium *Thermus aquaticus*, found in hot springs and can tolerate high temperatures, making these enzymes heat-stable. This heat stability is crucial for Polymerase Chain Reaction (PCR), a technique used to amplify DNA, as it allows the reaction to be carried out at high temperatures without denaturing the enzyme. Amylases are produced by *Thermus* and *Bacillus* sp. and are used for starch hydrolysis, brewing, baking and detergents. Lipases are produced by thermophilic *Pyrococcus horikoshii* and *Pseudomonas* sp. and are used in the dairy, detergent, pulp, pharmaceuticals, cosmetics and leather industries. Thermophilic proteases produced by Bacillus brevis and *Pyrococcus* sp., are used in baking, brewing, detergents and the leather industry. Xylanases are produced by *Bacillus circulans* and are used for xylan degradation in the pulp and paper industry. Chitinase produced by *Bacillus* strain MH-1 is used in food, cosmetics, pharmaceuticals and agrochemicals. The γ-lactamase is derived by Sulfolobus solfataricus and is used for the production of optically pure gamma lactam. Alcohol dehydrogenase is produced by *Aeropyrum pernix* and is used for the production of optically pure alcohols. Taq polymerases are isolated from the thermophilic bacterium *Thermus aquaticus*, found in hot springs and can tolerate high temperatures, making these enzymes heat-stable. This heat stability is crucial for Polymerase Chain Reaction (PCR), a technique used to amplify DNA, as it allows the reaction to be carried out at high temperatures without denaturing the enzyme[4]. The Taq polymerase is an example of a low-fidelity enzyme that cannot repair misincorporated nucleotides because it exhibits a 5'-3' exonuclease activity, but no 3'-5' exonuclease activity was found. The Pfu DNA polymerase has 3'-5' exonuclease proofreading activity, which corrects nucleotide misincorporation mistakes, in contrast to Taq polymerase. Amylases are produced by *Thermus* and *Bacillus* sp. and are used for starch hydrolysis, brewing, baking and detergents. Lipases are produced by thermophilic *Pyrococcus horikoshii* and *Pseudomonas* sp. and are used in the dairy, detergent, pulp, pharmaceuticals, cosmetics and leather industries. Thermophilic proteases produced by *Bacillus brevis* and *Pyrococcus* sp. are used in baking, brewing, detergents and the leather industry. Xylanases are produced by *Bacillus circulans* and are used for xylan degradation in the pulp and paper industry. Chitinase produced by *Bacillus* strain MH-1 is used in food, cosmetics, pharmaceuticals and agrochemicals. The γ-lactamase is derived by *Sulfolobus solfataricus* and is used for the production of optically pure gamma lactam. Alcohol dehydrogenase is produced by *Aeropyrum pernix* and is used for the production of optically pure alcohols.

Psychrophilic enzymes: Enzymes produced by psychrophilic organisms that survive at very low temperatures (below 5°C) are called psychrophilic enzymes. These enzymes are cold-active and thermolabile. The main advantages of using cold-active enzymes are: (i) Their high catalytic activity (high kcat) at low temperatures, (ii) The cost of enzyme preparation is lower. Further, due to their heat lability, they can be selectively deactivated easily by a slight increase in temperatures. Psychrophilic enzymes have found vast applications in food industry (for improving the digestibility and removing hemicellulose from feed, meat tenderizing, ripening of cheese, dough fermenting, stabilizing wine and beverage), detergent industry, pharmaceutical industry, environmental biotechnology for bioremediation and chemical synthesis (of peptides, epoxides, oligosaccharides and other organic compounds) by reverse hydrolysis in organic solvents[5]. Alkaline phosphatase is the most useful psychrophilic DNA-modifying enzyme in molecular biology. This enzyme is used to remove phosphate groups from the 5' end of DNA strands before end labeling by T4 polynucleotide kinase and to dephosphorylate DNA vectors before cloning to avoid self-ligation. Some of the commonly used psychrophilic enzymes include β-galactosidase, DNA ligase, alanine racemase and pectinases. The β-galactosidase produced by *Carnobacterium piscicola* is used in the dairy industry for lactose reduction and yogurt production. Psychrophilic DNA ligase derived from *pseudoalteromonas haloplanktis* is used in molecular biology for cloning, DNA sequencing and genomic analysis. Alanine racemase from psychrophilic *pseudomonas fluorescens* TM5-2 bacteria is a fascinating enzyme with significant potential for biotechnological applications like modification of amino acid composition in the food industry and synthesis of chiral compounds in the pharmaceutical industry. Pectinase from the *Sclerotinia borealis* fungus is used in the food industry for juice clarification and fruit preservation.

Acidophilic enzymes: These enzymes are derived from acidophilic microorganisms which are adapted to work under low pH. In other words, the optimum pH for their activity lies in the acidic range. The optimum pH for the activity of these enzymes lies in the acidic range. These enzymes can be exploited in industrial processes under harsh conditions[6]. Glucoamylase derived from various fungi and bacteria is used in the production of high-fructose corn syrup (HFCS) and bioethanol and to improve bread quality in the baking industry. Xylanase derived from *Penicillium oxalicum is* used in the baking industry to improve bread quality by reducing dough viscosity and Used in the brewing industry to improve beer clarity. In addition psychrophilic proteases, β-galactosidase and β-mannanase are all valuable enzymes with applications in the food industry.

Alkaline enzymes: Alkaline enzymes are obtained from organisms that can grow in alkaline environments, i.e., pH>9.0 and can function under high alkaline pH values because of their stability under these conditions. Alkaline enzymes often show activities in a broad pH range, thermostability and tolerance to oxidants compared to neutral enzymes. Alkaline enzymes are widely used in food and tannery industries, pharmaceutical formulations, waste treatment, silver recovery and amino acid combination resolution[7,8]. Alkaline proteases have been utilized to create high-nutrient protein hydrolysates that are utilized in baby food formulations and have a significant impact on blood pressure regulation. Alkaline proteases, alkaline cellulases, alkaline amylases and alkaline lipases are examples of alkaline enzymes that are primarily employed as detergents to increase cleaning effectiveness.

Barophilic enzymes: These enzymes are derived from piezophilic/barophilic microorganisms that are stable at high pressures and can be found in the deep sea. They can be applied to high-pressure enzymatic processes, including those found in the food processing sector for catabolic activities, deep-sea waste disposal, sterilisation of food and the creation of new natural products. These enzymes are also used in high-pressure bioreactors. Barophilic dihydrofolate reductase (DHFR) derived from facultative psychropiezophile, *moritella profunda*, can thrive under 220 bar at 6°C[9].

CONCLUSION

Extremozymes are enzymes that can function in unfavorable environments, such as extreme pressure and temperature swings, intense radiation and unfavorable pH levels. These enzymes can be extracted from a variety of bacteria, including hyperthermophiles, psychrophiles, acidophiles and alkaliphiles. Extremozymes exhibit great potential for use in the medicinal, chemical and agricultural sectors. New discoveries of extremozymes with good stability from various organisms could lead to an increase in the number of biotechnological products. In conclusion, extremozymes will undoubtedly greatly expand the potential of biotechnology for real-time applications.

SIGNIFICANCE STATEMENT

Extremozymes are extremely important because of their special qualities and potential uses. These enzymes provide several benefits because they are designed to perform best in harsh conditions. Extremozymes are remarkably stable in extreme environments, including high salinity, low pH, high temperatures and organic solvents. In industrial operations, this consistency translates into greater longevity and efficiency. Extremozymes support ecologically beneficial and sustainable technologies. For a variety of sectors, they provide less polluting and energy-intensive alternatives. Furthermore, extremozymes are used in a wide range of sectors, such as biotechnology, the food industry, environmental remediation and the textile sector. In conclusion, their unique properties and diverse applications make them a key focus area in biotechnology research and development.

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