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Biotechnological and Industrial Applications of Enzymes Produced by Extremophilic Bacteria. A Mini Review

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Abstract

Extremophilic bacteria are important groups of extremophilic organisms that have been studied during the last years. They are considered as a source of enzymes due to great diversity and can survive under extreme conditions. Many enzymes produced by these microorganisms are of great importance and have found applications in several industries. Due to their activity and stability under extreme conditions, these enzymes offer new alternatives for current biotechnological and industrial applications. They have a wide range of potential uses and have been a nuclear subject of many different investigations. To date, some of the enzymes produced by extremophilic bacteria are currently being assessed thier industrials applications. Despite, benefits that present these enzymes, their potentials remain largely unexplored. These enzymes pose new opportunities for new line of research, and biotechnological applications. This review provides a summary on diversity and biotechnological and industrial applications of some enzymes produced by extremophilic bacteria.

Keywords: extromphiles; extremophilic bacteria; enzymes; biotechnology application

1. Introduction

The extremophiles are microorganisms that live in extreme conditions and adapt in environmental variables, such as at high and low temperatures, extremes of pH, high salt concentrations, high pressure, and chemical extremes [1-3]. Studies about the extremophilic microorganisms have increased considerably in the last decades due to potential biotechnological applications. Enzymes produced by extremophilic bacteria have been receiving more attention due to biotechnological and industrial applications, also their stability [4-6]. Many enzymes have been isolated from extremophilic bacteria and these enzymes to play a crucial role as metabolic catalysts [7]. Several enzymes have been isolated from genera such as *Bacillus*, *Corynebacterium glutamicum*, *Pseudomonas*, *Aletromonas, Halobacterium*, *Shewanella*, *Psychrobacter*, *Pseudoalteromonas*, *Arthrobacter*, *Colwellia, Gelidibacter, Marinobacter, Psychroflexus, Moritella, Halomonas, Photobacterium, Colwellia, Thioprofundum, Methanolobus* and *Methanococcoide* [8-15]. With the growth and development of biotechnology, the interest for enzymes has increased considerably as a strategy towards attaining a biobased economy [16, 17]. Enzymes from extremophilic bacteria have found major commercial applications. Enzymes such as polymerases, amylase, galactosidases, pullulanases, cellulase, xylanase, protease, esterase, ligases, pectinases, isomerases, dehydrogenases, chitinase, α-glucosidases and endoglucanases have great potential application for biotechnology such as in pharmaceuticals industries, biofuel, cosmetics, nutritional supplements, molecular probes, food industries, enzymes, fine chemicals, animal feed and bioprocessing sustainable agriculture production of antibiotics, anticancer, and antifungal drugs.

Other enzymes such as transaminases and dehalogenases, are currently being assessed their industrial applications [1]. Some of these enzymes have been purified and characterized in terms of their activity and stability profiles at different pH, temperature. The exploration of enzymes with novel extreme activities and improved stability continues to be a priority objective in enzyme research. In this review, we briefly describe the enzymes more important from extremophilic bacteria and the most recent reports on the biotechnological applications.

2. Enzymes produced by extremophilic bacteria.

2.1. Enzymes produced by acidophilic, alkaliphilic bacteria

Acidophiles are microorganisms that live at pH below 3-4 [18]. Numerous enzymes have been produced by acidophilic and alkaliphilic microorganisms. Enzymes from acidophiles possess stability in acidic milieus and are active at pH low. Extremozymes from acidophiles possess a great potential for biotechnological and industrial applications especially in biofuel and ethanol production [19]. Nevertheless, the adaptation of some acidiphilic enzymes has not been clearly understood [20]. Enzymes produced by these microorganisms such as amylases, proteases, ligases, cellulases, xylanases, α-glucosidases, endoglucanases, endo-β-glucanase and esterases possess a great potential for biotechnological and industrial applications [21, 22]. Enzyme as α-glucosidases play important roles in carbohydrate metabolism, energy processing and glycosylation of lipids. The α-glucosidase from *Ferroplasma acidiphilum* has demonstrated stability at low pH [23].

Proteases and esterases are enzymes with extensive applications in commercial and physiological fields, whereas, amylases are one of the most important groups of enzymes with extensive applications in the conversion of starch to sugar syrups, and in the bread and textile industries [24]. These enzymes are very stable in organic solvents. Currently, these enzymes are used in starch industry is active at pH 6.5. Alkaline enzymes have a dominant position in the global enzyme market especially in detergents [25]. These enzymes are stable, also, have good activity in saline conditions [26]. Despite, their stability alkaliphilic enzyme have not been exploited for biotechnological and industrial applications [27]. Some enzymes produced by alkaliphilic microorganisms such as xylanases, amylases, proteases, dextranases, xylosidases, chitinases, cellulase, alginases, catalase, RNase, DNase, cellulases, glucose dehydrogenase, α-galactosidase, β- galactosidase, pectinase, β-mannanase, uricase, oxidase, β- mannosidase and lipases have been used in several biotechnological applications such as industries of food, bioremediation, biosynthetic processes pharmaceutical industries detergents, leather processing, waste management and textile industry [8]. For example, protease, cellulase, lipase and amylase are generally put in detergents as additives, these enzymes contribute to an improvement of the washing power, they have activity and stability in detergents. Proteases are very important for industrial enzymes. In the market, their sales amount to 60% of the total enzymes [25]. Proteases produced by members of *Bacillus* uses mainly in detergent formulations, additionally, these enzymes use in contact lens solutions, in cheese production, processing of meat products, and for the recovery of silver from photographic films [28-30]. Numerous research have shown that lipases from bacteria are stable at pH between 4 to 11 [31].

Amylases are other enzymes that are very important for several industries. These enzymes produce manly by *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens,* and *Bacillus megaterium* [32]. In detergents, amylases have an activity at pH between 8-11, these enzymes have a practical use in the laundry industry [33]. Xylanases from alkaliphilic bacteria have various biotechnological and industrial applications such as in the pre-bleaching of pulp, conversion of lignocellulosic biomass to serve as source of biofuel, improvement of cereal food products and animal feedstocks, and degumming of plant fibers, these enzymes are stable at broad ranges of pH [34, 35]. Regarding alkaline pectinases have found various applications biotechnological mainly during fiber processing for usage in textiles and during the paper-making process [36, 37]. Pectinases have been produced from several microorganisms of *Bacillus* sp. For example pectinase from *Bacillus subtilis* SS can be to increase the brightness, whiteness, and fluorescence of paper pulp, thus improving characteristics of paper [38]. Some enzymes produced by extromophilic bacteria have been produced in great quantities at low cost, and have been commercialized [39].

2.2. Enzymes from psychrophilic and thermophilic bacteria

Psychrophiles are microorganisms that live in cold environments from the deep sea to mountain and polar regions [40]. They are a source of enzymes that can be use in different innovative reactions applicable to industry [41]. Enzymes from psychrophilic microorganisms have a structural flexibility and high catalytic efficiency [42]. There are numerous benefits to the application of psychrophilic enzymes such as the high diversity, high yield, immense stability, high catalytic activity and economic feasibility highlighted its biotechnological potential and industrial applications [17, 18].

In recent years, numerous research and various industrials have increased efforts to discover and develop novel cold-adapted enzymes. The cold adapted enzymes have been evolved in psychrophilic bacteria as a strategy for low temperature adaptation [43-45]. Enzymes produced by psychrophilic bacteria such as alkaline phosphatases, glycosylases, nucleases, lipases, proteases, amylases, cellulases, mannanases, pectate lyases, amylases, DNA ligase, endo-arabinanase, polygalacturonase, cellulases, pectinases and catalase have great potential application for biotechnology in detergent, textile, food, beverages and cosmetic applications including many techniques used in molecular biology. Proteases from psychrophilic bacteria have use in detergent industries [46, 47]. Other enzymes such as amylase, cellulases, dehydrogenases, β-glucanase, pectinases y DNA polymerases have been used to improve the efficacy of cold-water household, also, in textiles, biosensors, maturing cheeses, milk products, processing of fruit juices [48, 49]. Recently, a novel cold-adapted cellulase complex from an earthworm living in a cold environment was discovered that contained both endo-β-1,4-D-glucanase and β-1,4-glucosidase activities that could convert cellulose directly into glucose [47]. Cold-adapted enzymes have potential application in mixed aqueous-organic or non-aqueous solvents for the purpose of organic synthesis. Thus, cold adapted enzymes provide a strategy to psychrophilic microorganism for cold adaption at low temperature [50]. Genera such as *Caldicellulosiruptor, Thermotoga maritima* and *Thermus*, are among the most studied thermophiles to date [51]. Enzymes produced by thermophilic microorganisms such as cellulases, xylanases, mannanase, pectinases, chitinases, amylases, pullulanases, proteases, lipases, glucose isomerases, alcohol dehydrogenases, and esterases have great biotechnological applications [52-54].

For example, xylanases from *Thermus brockianus* and *Thermotoga thermarum* are considered appropriate for industrial applications, due to high optimal reaction temperature of 95 ºC, stability and high specific activity [55]. Currently, proteases, lipases and gylcosyl hydrolases, account for more than 70% of all enzymes sold. Proteases are the most sold and used of enzymes thermophilic. Proteases have numerous biotechnological and industrials applications, however, the largest application is in laundry detergents, are used to break apart and remove stains. Lipase is other important enzyme, has been used for various industrial applications such as paper industry, milk industry, in processing of dyed products, leather industry and in pharmaceuticals [1, 55]. Currently, various lipases have been widely purified and characterized from different microorganisms without losing their activity and stability profiles depending to pH, temperature and effects of metal ions and chelating agents. This purification is important for some industries such as fine chemicals, pharmaceuticals and cosmetics. The techniques more common used are nonspecific techniques, extraction, precipitation, hydrophobic interaction, chromatography, gel filtration, affinity chromatography, crystallization and ion exchange chromatography. The alcohol dehydrogenases (ADHs) are considered as one of the most interesting enzyme groups from thermophilic microorganisms. The ADHs was amplified by PCR and overexpressed in *Escherichia coli*. Currently, a new carboxyl esterase has been identified form *Thermogutta terrifontis* from and *phylum Planctomycetes*. This enzyme is very thermostable and retained 95% of its activity after incubation. The enzymes produced by thermophilic bacteria are stable to high temperature, presence of solvents, and resistance to proteolysis, are ideal features for industrial applications [56, 57].

2.3. Enzymes from halophilic bacteria

Halophilic bacteria are considered as one of the most important extremophiles, they can be found in saline or hypersaline environments. Enzymes from halophilic bacteria are considered as a novel alternative for use as biocatalysts in different industries. Currently, there are few studies on halophilic enzymes [58]. Due to unique properties, halophilic microorganisms have been explored for their biotechnological potential in different fields [59]. Enzymes produced by halophilic microorganisms offer important opportunities in biotechnological applications such as food processing, environmental bioremediation, biosynthetic processes fermented food, textile, pharmaceutical and leather industries [60]. These enzymes are stable at high salt concentrations, but also can withstand and carry out reactions efficiently under extreme conditions such as high pH values, high or low temperature, low oxygen availability, pressure, and toxic metals [61, 62]. Enzymes such as xylanases, dehydrogenase, amylases, proteases, α-amylases and lipases, have been produced by different genera of halophilic bacteria such as *Cyanobacteria, Proteobacteria, Firmicutes, Actinobacteria, Spirochaetes Salinivibrio, Halomonas, Bacillus-Salibacillus* and *Bacteroidetes, Pseudoalteromonas ruthenica* and *Bacillus, Halobacillus* and *Thalassobacillus*. These enzymes have been commercialized especially in the production of polyunsaturated fatty acids, food, biodiesel, baking, feed, chemical and pharmaceutical, paper and pulp, detergent, leather industries, fish sauce and soy sauce preparations, saline waste water, and oilfield waste treatment [63-66]. Lipases, proteases and amylases isolated from halophilic bacteria constitute an excellent alternative in the industrial processes due to their stability and versatility [67]. Hydrolases is other enzymes characterized from halophilic bacteria [68].

During the last years, the halophiles have developed of novel enzymes. These enzymes have unique structural features and catalytic power to sustain the metabolic and physiological processes under high salt conditions [69, 70]. Due to stability under high salt concentrations, the demand for enzymes produced by halophiles has increased considerably [71]. Various investigations have reported on production or purification of haloenzymes from halophilic bacteria, resistance of the enzymes toward different organic solvents has been examined [72]. Enzymes produced by halophilic bacteria show interesting properties for use in different biotechnological and industrial applications.

3. Conclusions

Currently, exists numerous publications that considerable diversity, biotechnological and industrial applications of enzymes produced by extremophilic bacteria, however, many remain to be explore. Despite, the advantages that offer these enzymes have not been exploited their potentials and, the actual number available is very limited. Extremophilic bacteria are a good source to produce enzymes, however, it presents some difficulties such as high and low pH, high temperatures or high concentration of salts, high pressure, chemicals, organic solvents normally require bioreactors under extreme conditions. Many enzymes produced by extremophilic bacteria are commercially available. Current, the market leaders in commercial enzyme production are Novozymes A/S, Genencor International, Inc. and DSM N.V, however, these market leaders need to develop of new industrial processes and new methods especially, in biochemistry, microbiology and genetic engineering based on enzymes are required. The increasing demand of enzymes are of great interest for extremophile investigation.

Enzymes produced by extremophiles especially from bacteria have great potential biotechnological applications such as in agriculture, food beverages, pharmaceutical, detergent, textile, leather, pulp and paper, and biomining industries. Currently, the use of enzymes requires the adaptation and creation of new methodologies, assays, and techniques that operate under conditions extreme. To date, the discovery of new enzymes based on genetic sequences require more research especially for extremophiles. The extremophilic microorganisms are sustainable sources that might be better exploited in several biotechnological areas towards the expansion of a bio-based economy. Extremozymes have had a large impact so far from a commercial and biotechnological perspective. In conclusion, the development of novel molecular tools, more efficient production processes, and novel technologies will further advance the application of enzymes in different industries.

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Table 1. Biotechnological and industrial applications of some enzymes more important from extremophilic bacteria.

Types	Growth characteristics	Environment/source/ Geographical location	Genus	Enzymes	Application	Reference
Acidophile	Organism that grows at pH optimum below 3-4	Acid Mine Drainage Volcanic Springs, USA	*Sulfolobus solfataricus Acetobacter*	Amylase Glucoamylase	Starch processing. Single-cell protein from shellfish waste.	[73]
				Proteases	Animal feed for the improvement of Digestibility.	[74, 75]
				Cellulases	Removal of hemicellulosic material from feed. Feed component.	[76]
				Oxidases	Desulfurization of coal.	
Alkaliphile	Organism that grows at pH optimum above 10	Soda Lakes, Utah USA.	*Bacillus, Micrococcus, Pseudomonas, Actinobacteria*	Proteases	Detergents, food, and feed.	[1, 41, 77]
				Cellulases	Fermentation of beer and wine, breadmaking, and fruit juice processing.	
Halophile	Organism that requires at least 1 M salt (manily NaCl) for	Salt Lakes, Utah USA	*Halobacterium Halococcus Bacillus*	Proteases	Peptides synthesis	[8, 78]

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	growth			Dehydrogenases	Biocatalysis in organic media. Asymmetric chemical Synthesis	[79]
Neoliths	Organism that grows and lives inside rocks	Upper subsurface to deep subterranean, Mediterranean and Japan Seas	*NI	*NI	*NI	[80]
Hyperthermophile	Organism that grows at temperature optimum of 80ºC or higher	Submarine Hydrothermal Vents, East Pacific, as Porto di Levante, Vulcano, Italy	*NI	*NI	*NI	[58]
Hypolith	Organism that grows and lives inside rocks in cold deserts	Desert, Rock, Cornwallis Island and Devon Island in the Canadian high Arctic	*NI	*NI	*NI	[81]
Metallophiles	Organisms with characteristics specially, is capable to tolerate high levels of heavy metals, such as copper, cadmium, arsenic, and zinc	Heavy metals, Latin America, and Europe	*NI	*NI	*NI	[82]
Oligotroph	Organism capable to grow in nutritionally deplete habitats	Carbon source, or carbon concentration, Antarctic	*NI	*NI	*NI	[83]
Piezophile	Organism that grows and lives with optimum at hydrostatic pressures of 40 MPa or higher	Deep Ocean eg. Mariana Trench, Antarctic ice	*Shewanella, Photobacterium, Colwellia hadaliensis Moritella,* and *Psychromonas*	To be defined	Food processing and antibiotic production.	[84]

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Psychrophile	Organism that grows at temperature between 10-20ºC	Ice, Snow, Antarctic ice and Arctic Ocean	*Bacillus, Clostridium, Actinomycetes, Pseudoalteromonas Betaproteobacteria*	Proteases	Detergents, food applications.	[85, 86]
				Amylase	Detergents and bakery.	
				Cellulases	Detergents feed and textiles.	
				Dehydrogenases	Biosensors	
Radioresistant	Organisms that is very resistant to high levels of ionizing radiation	Sunlight eg. High UV radiation, Brazil	*NI	*NI	*NI	[56]
Thermophile	Organism that can prosper at temperatures between 60ºC and 85ºC humidity	Hot Spring, Grand Prismatic Spring, Yellowstone National Park, USA		Lipase	Additive to detergents for washing at room temperature.	[8, 17, 87]
				Protease	Breaking down of lipid stains. Breaking down of protein stains. Detergents in food and feed, brewing, baking. Biodiesel production by transesterification of oils and alcohols. Flavor modification, optically active esters.	
				Amylases Pullulanase	Starch, cellulose, chitin, and pectin processing, textiles.	[67]
				Glucoamylases, Cellulases	Breakdown starch-based stains.	

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					Wash of cotton fabrics.	[47]
				Xylanases	Starch hydrolysis. Clarification of fruit, vegetable juices, and wine. Cheese ripening. Dough fermentation, bakery products. Chitin modifications for food and health products.	[88]
				Chitanases	Conversion of cellulose to ethanol.	
				Esterases	Paper bleaching. Bioremediation, degradation and removal of xenobiotics and toxic Compounds.	[76]
				DNA Polymerases	Detergents, stereospecific reactions.	[8]
				Dehydrogenases	Molecular biology. Oxydation reaction.	
				Mannanase	Degradation of mannan or gum	
Toxitolerant	Organisms capable to resist at high levels of damaging agents, such as organic solvents	Water saturated with benzene or Water-core of a nuclear reactor , Yellowstone National Park, USA	*NI	*NI	*NI	[89]
Xerophile	Organism capable to	Desert, Rock, Surfaces,	*NI	*NI	*NI	[7]

grow at low water activity and resistant to high desiccation	Atacama Desert in Chile

*NI: No Information

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