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MINI REVIEW ARTICLE

Extremophile Microbial Communities and Enzymes for Bioenergetic Application Based on Multi-Omics Tools

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Abstract: Genomic and proteomic advances in extremophile microorganism studies are increasingly demonstrating their ability to produce a variety of enzymes capable of converting biomass into bioenergy. Such microorganisms are found in environments with nutritional restrictions, anaerobic environments, high salinity, varying pH conditions and extreme natural environments such as hydrothermal vents, soda lakes, and Antarctic sediments. As extremophile microorganisms and their enzymes are found in widely disparate locations, they generate new possibilities and opportunities to explore biotechnological prospecting, including biofuels (biogas, hydrogen and ethanol) with an aim toward using multi-omics tools that shed light on biotechnological breakthroughs.

Keywords: Biodiversity, microorganisms, enzymes, molecular methods, multi-omics, extremophiles.

1. MICROBIAL COMMUNITIES IN EXTREME ENVIRONMENTS – AN INTRODUCTION

From a human point of view, some habitats seem so harsh and inhospitable for life that they are commonly thought to be sterile. The > 85°C hot springs in the Yellowstone Park (USA), the ultra-arid Atacama Desert (Chile), and the permanent glacier ice of South Pole (Antarctica) represent some examples. These "extreme" environments, however, are dominated by microbial cells from all three domains of life: Bacteria, Archaea, and Eukarya. Microorganisms inhabiting such environments are called "extremophiles" because of their endemic (or restricted) nature to survive at the edge of life [1]. Extremophiles exhibit unusual biochemical and ecological adaptations not found by organisms dwelling in no-extreme habitats. Physical and chemical parameters in the extreme environments include a broad range of temperature (from –40 to >130°C), salinity (from rainwater to 5 M NaCl), pH (from virtually 0 to 13), pressure (from 0.3 atm at Mount Everest to 1200 atm in the deepest point of Mariana Trench), desiccation (from wet to the complete absence of water), and radiation (wavelengths of ultraviolet and gamma rays) [2, 3].

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Extremophiles are classified in categories based on environmental parameters, with sub-definitions created to accommodate organisms that present moderate, extreme, hyper-extreme and also obligate extremophile (Table **1**).

Comparative studies between extremophiles and their non-extreme counterparts have led to systematic descriptions of how growth and survival are possible under extreme conditions. In general, extremophiles adjust their membranes, proteins and nucleic acids to preserve the functional stability of their structures and molecular components [4, 5]. To achieve this stability, several strategies evolved as microorganisms were exposed to environmental stresses. For example, to maintain their metabolism under extreme cold, psychrophiles must resist freezing and, at the same time, conserve membrane flexibility. These cold-adapted microorganisms enriched their membranes with unsaturated fatty acids that provide flexibility, and present enzymes enriched in hydrophilic amino acids (*e.g.*, glycine) that help retains liquid water for metabolism [6].

Virtually all extremophiles present enzymatic adaptations to cope with their unusual environments. As enzymatic function depends on temperature, pH and water availability, for example, all intra- and extracellular reactions rely on adaptations of extremophilic enzymes-also known as "extremozymes". While performing the same enzymatic functions as their non-extreme counterparts, extremozymes catalyze

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chemical reactions in conditions that would completely inhibit or even denature other enzymes [7]. In fact, some of these enzymes display a phenomenon known as polyextremophilicity, *i.e*., they present activity in several extreme conditions. For example, biochemical analysis of an alcohol dehydrogenase from the hyperthermophilic archaea *Thermococcus sibiricus* showed activity at high temperatures (optimal close to 100°C) and high salt concentrations (4 M NaCl) [8, 9]. These unique features from extremophiles and their enzymes opened a myriad of applications in bioprocesses and bioenergy research [7, 10].

2. EXTREMOZYMES AND MULTI-OMIC TOOLS

Extremozymes are enzymes obtained from extremophile microorganisms that have several ecological roles in environment and succession, being a rich niche to be explored considering enzymes and other molecules [11]. The biodegradability and high stability of extremozymes have been exploited in a large range of industrial applications with various tolerances, including acid, cold, salt and alkali-tolerant enzymes [12, 13]. Due to its catalytic capability, enzymes have been widely applied for industrial purposes, the prospection of enzymes market is in increasing growth. In 2004, it was estimated at $2 billion dollars; in 2010, the value increased to $3.3 billion; and in 2018, the estimated value of this market was $7.1 million [14-16].

With the advancement of "omics" technologies, the development of new bioinformatics tools is possible to estimate the microbial composition communities and also understand the functional aspects of these communities and prospecting genes and enzymes with a desirable characteristic or functionality [17-19]. Thus, metagenomic [20, 21], metatranscriptomic [22] and metaproteomic [23, 24] are interesting strategies to prospect and study these enzymes and their role in extremophile communities. This variety of strategies can be used to identify molecular mechanisms or metabolic routes with possible industrial applications of microorganisms and enzymes (Fig. **1**).

Using metagenomic analysis is possible to evaluate the thermophile community composition by sequencing markers like 16S rRNA [25, 26], however, analyzing the whole DNA of these communities by shotgun metagenome strategy, the potential functionality of the microbiome may be addressed and also identify coding genes that play an important role in metabolic route with biotechnological interest or genes with unknown function [19, 27, 28].

Besides metagenomics data and complete genome sequences from different species widely available and led to discover new genes, transcriptome and proteome techniques are very useful not only to identify the expressed genes but also to understand their expression with certain conditions and the physiological responses of these conditions [29-31]. Although the metatranscriptome is very useful to provide insights about the transcriptional activity of certain environmental conditions [18, 32], the mRNA and protein expressions could not be directly correlated because of the post-transcription regulations [33]. However, metaproteomic analysis is usually less restrictive and can offer rapid physiological responses since protein expression is modulated very fast. In addition, metaproteomics analysis has some important bias, which depends on the protein extraction and separation methods [34].

Though the combination of metagenome, metatranscriptome and metaproteomics could bring more evidence of the functionality and expression of certain enzymes with biotechnological interest [18], these studies with extremophile microbial communities are scarce in the literature, especially because this is still a developing field. A study of Mandelli *et al.* [35] performed a multi-omic approach with the extremophile *Thermus filiformis* using DNA sequencing, RNA-Seq and mass spectrometry. They considered that the multi-omic analyses were complementary to each other and allowed them to identify the main changes in the physiological state of *T. filiformis* under varying temperatures. The authors also discovered several thermostable macromolecules produced by *T. filiformis*, including amylases, pyrophosphatases, glucosidases, and galactosidases, all of which have a wide range of biotechnological and industrial applications.

Table 1. Classification of extremophiles according to environmental parameters.

Environmental Parameter	Classification	Definition	Examples
Temperature	Hyperthermophile	Optimal growth > 85°C	*Pyrolobus fumarii*, (113°C)
	Thermophile	Optimal growth 45–85°C	*Synechococcus lividis*
	Psychrophile	Optimal growth < 15°C	*Psychrobacter* sp., *Polaromonas vacuolata*
Salinity	Halophile	Requires 2–5 M NaCl	*Halobacterium salinarum*, *Danaliella salina*
pH	Alkaliphile	pH > 8	*Natronobacterium*, *Bacillus firmus* OF4, *Spirulina* spp. (all pH 10.5)
	Acidophile	pH < 5	*Cyanidium caldarium*, *Ferroplasma* sp. (both pH 0)
Pressure	Piezophile	Growth > 400 atm	*Thermococcus piezophilus*, *Shewanella oneidensis*
Desiccation	Xerophile	$A_w < 0.8$	*Trichosporonoides nigrescens*
Radiation	Radiotolerant	Up to 60 Gy/hour	*Deinococcus radiodurans*

Adapted from Duarte *et al*. [3].

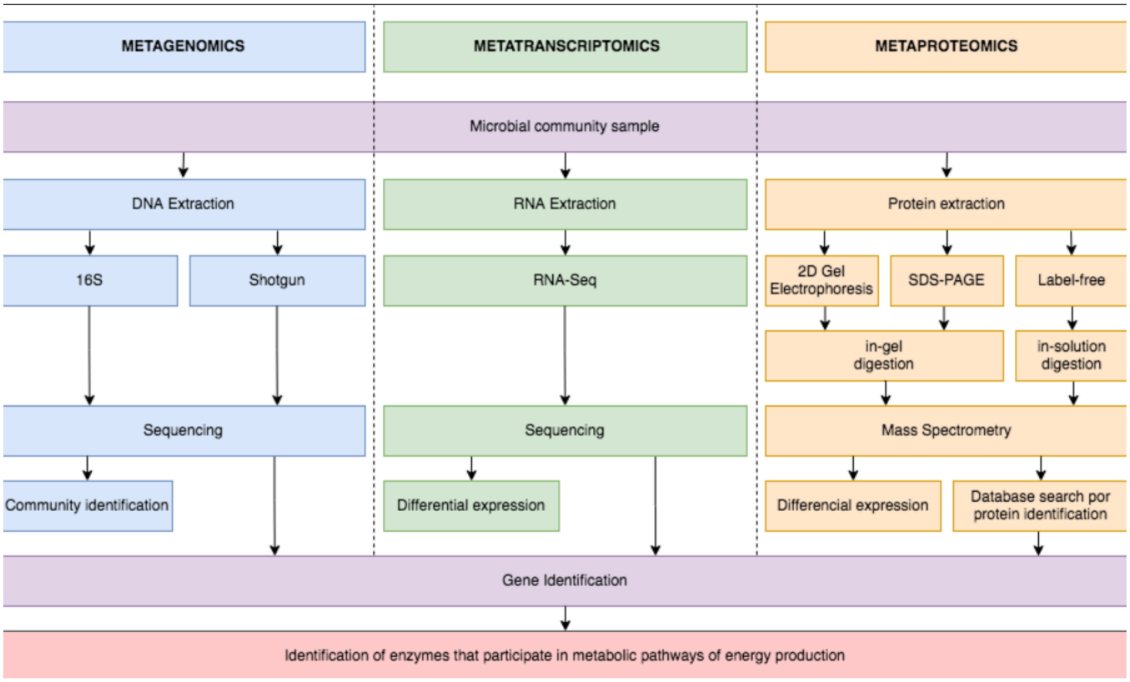

Fig. (1). "Multi-omics" strategies and basic workflows. Based on metagenomic, meta-transcriptomic and metaproteomic approaches to prospect genes and proteins for biotechnological application. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

More recently, Chignell, De Long, and Reardon [23] used label-free metaproteomics and 16S rRNA gene-based community analysis approaches to compare protein expression in acetate-fed anode biofilms before and after the onset of robust energy generation. They discovered a significant enrichment of protein categories specific to membrane and transport functions among proteins from electricity-producing biofilms. They discovered that proteins detected only in electricity-producing biofilms were associated with gluconeogenesis, the glyoxylate cycle, and fatty acid β-oxidation, as well as with denitrification and competitive inhibition.

2.1. Thermophilic Enzymes

Thermophilic enzymes (also called thermozymes) present industrial advantages, including resistance to chemical denaturants, thermostability, thermal activation and high catalytic activity, capable of optimal operation even in high-temperature processes [36, 37]. Thermozymes have low adhesiveness and contamination risk and also greater solubility of substrates [38]. These advantages can be attributed to a small number of specific modifications, including a high content of alanine and arginine acids, barrel folding, hydrophobic interactions and disulfide bonds [39, 40]. In addition, the introduction of disulfide and hydrogen bonds, along with hydrolytic changes, are being explored as mechanisms of improving these enzymes' performance [41-43].

When compared to mesophilic counterparts, thermozymes show more specific activity, better accessibility to the active site and compact oligomers [44]. In addition, these enzymes have characteristics suitable for industrial exploration, such as the maintenance of its thermal properties even when expressed in mesophilic hosts, and without of phylogenetic relationships among them. It suggests that these characteristics are genetically encoded and thus, these organisms are appropriated metabolic engineering platforms [45, 46]. Among the range of known thermozymes a wide number of enzymes have been characterized, such as pullulanase, amylases, xylanases, chitinases, pectinases, esterases, phytases, mannanase, proteases, lipase and cellulases [43-47]. Such diversity enables different applications on biotechnological demands, or even for genetic modifications with a wide range of purposes.

2.2. Psychrophilic Enzymes

Most ecosystems on Earth are exposed to temperatures permanently below 5°C, distributed among marine and terrestrial areas. Permanently frozen formations such as glacier ice and permafrost are a vast depository of ancient viable cells [48]. Successful survival of these microorganisms in cold environments depends on a number of adaptations in their metabolism, including cold-shock proteins, ice-binding molecules, and cold-adapted enzymes.

Low temperatures impose a strong negative effect on most biological reactions. As described by the Arrhenius equation, enzymatic reaction rates decrease exponentially with any decrease in the temperature. Psychrophilic and psychrotolerant microorganisms evolved their enzymes to cope with this thermodynamic problem [49]. Several structural adaptations are observed in cold-adapted enzymes, most of them increasing molecular flexibility. For example, reduced number of hydrogen bonds and salt bridges [48, 50], reduced use of proline and arginine [13, 51, 52] and increased surface-loaded residues [53] provide greater flexibility and less

enthalpic contribution to stability [51]. Other important structural adaptations, such as loop extensions, provide greater accessibility of the active site for substrate and cofactor binding [54, 55], and also improve electrostatics in the vicinity of the reactive center [54, 56], ensuring the efficiency of these enzymes in the cold.

According to the type of catalyzed reaction, enzymes are classified into six main classes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases [57]. Cold-adapted enzymes are found among all these classes (Table **2**).

In recent years, the search for cold-adapted enzymes was improved by metagenomic approaches. Arctic and Antarctic glaciers, snow, lakes, soil and marine sediments had their microbial community characterized and cold-adapted enzyme producing organisms were identified. Oh *et al.* [115] used metagenomics in Antarctic soils to search for microorganisms able to produce cold-adapted lignolytic and cellulolytic enzymes. The metagenomics analysis found that 162 (1.42%) of a total of 11,436 genes were annotated as active carbohydrate enzymes. Actinobacteria, the dominant phylum in that soil metagenome, harbored most of the genes related to catabolism of lignocellulose, including glycoside hydrolases (GH13, GH26 and GH5). On the other hand, degradation pathways of lignocellulose in the Antarctic soil metagenome have shown a synergistic role for several bacterial genera, including *Streptomyces*, *Streptosporangium* and *Amycolatopsis*.

There are several advantages to using cold-adapted enzymes in biotechnology. These enzymes are generally thermolabile and may be inactivated by heat-controlled procedures [11]. They catalyze the desired reactions at lower temperatures, present high catalytic efficiency at small amounts of the enzyme, and have lower activation energy cost [57].

2.3. Piezophilic Enzymes

Naturally, the Earth shows several environments with high-pressure, with 62% of the total biosphere characterized by pressures greater than 10 bars [116]. In this context, several evolutionary events occurred independently, selecting piezophilic organisms in the sea and vulcanos [117-120]. Initially, they were referred to as barophilic, later changed to piezophilic (*piezo* in Greek means pressure); they are characterized by several adaptations [121, 122]. The mechanisms of adaptation include polyunsaturated acid fatty acids, Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA), both being necessary to the maintenance and fluidity of lipid membranes, presence of electrostatic and hydrogen bond in place, beyond that expression of genes regulated by pressure such as *ompH* and *ompL* present in *Photobacterium spp.* [123, 124]. Some piezophilic organisms have no saline channels, distinguishing them from thermopiezophiles that are adapted to low temperature and pressure [125, 126].

Pressure usually has been assumed as denaturant of proteins, whereas structural characteristics can ensure resistance to different pressures. However, the activity and/or stability of enzymes can be potentialized utilizing hydrostatic pressure, as thermolysin and hydrogenase [127, 128]. *Sulfolobus solfataricus* is an example of a thermoacidophilic archaebacterial isolated from vulcanos that produce a ribonuclease with piezophilic adaptation, with possibilities of industry application. Thus, piezophilic enzymes show potential for biotechnology and industry applications. They can optimize processes such as the production of trisaccharide instead of maltobiose and tetrasaccharide using less energy of substrate or high efficiency as a detergent in chemical products [11, 71, 102, 129, 130].

2.4. Acidophilic Enzymes

Acidophilic microorganisms can be found in several environments containing pH between 5-3 or less, showing optimal growth in this range of pH. Among these microorganisms, there are prokaryotes and eukaryotes found growing in acid lakes. Acid tolerance differs from acidophilia; acid-tolerant organisms can resist variation of pH and do not necessarily depend on it, whereas acidophilia depends on a range of pH to growth [131, 132].

Several adaptations are required to permit their survival; the intracellular pH is neutral in general and cytoplasmic enzymes show an optimal function at pH 7 [94, 133]. Beyond that, the extracellular enzymes and redox-active proteins located at periplasm of gram-negative acidophilic show stability at low pH. These organisms are distributed into three domains. The major groups of archaeal acidophilic are *Euryarchaeota* and *Crenarchaeota*, within bacterial phyla exist some groups known by containing acidophiles, including *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Nitrospira* and *Aquifex* [132].

There are some industrial applications utilizing these microorganisms; one of the possibilities of biotechnology using acidophilic microorganisms includes biomining utilizing organisms to solubilize and extract minerals from ore [134, 135]. A comparison between archaea (*Acidianus brierleyi*) and bacteria (*Acidithiobacillus ferrooxidans*) demonstrated the solubilization of metals (chalcopyrite, sphalerite and pyrite), demonstrating a better result for archaea at 65ºC/pH 1.2 than for bacteria at 30°C/pH 2 [136]. Another application involves cellulolytic and xylanolytic enzymes combined with high temperature and pH promote processes of hydrolysis [94, 114].

2.5 Halophilic Enzymes

Halophilic microorganisms are found in the most saline environments, from natural salt lakes to solar salterns, found even in the Dead Sea [137]. They can be classified according to the optimal saline concentration, including extreme halophiles, (developing at 2,500-5,200 mM NaCl), moderate halophiles (developing at 500-2,500 mM NaCl) and the slight halophiles (developing at 200-500 mM NaCl) [138]. Halotolerant bacteria maintain the osmotic balance, excluding salt from the cytoplasm ('salt-out' strategy) and using organic solutes in energy costly strategies [139]. On the other hand, haloarchaea sustain osmotic balance in high salinities *via* equimolar salt accumulation in the cytoplasm ('salt-in' strategy), using potassium due to its lower hydrophilicity when compared to sodium [140, 141]. Although being aerobic heterotrophs, some haloarchaea have the potential for anaerobic growth [142].

Table 2. Extremozymes isolated from different microorganisms.

Enzyme Name	Adaptation	Host Organism	References
Oxidoreductases			
Alcohol dehydrogenase	Hot	*Thermococcus sibiricus*	[8, 9]
Catalase	Cold	*Bacillus* sp. N2a	[58, 59]
Glutathione reductase	Cold	*Colwellia psychrerythraea*	[60]
Transferases			
Aspartate aminotransferase	Cold	*Pseudoalteromonas haloplanktis* TAC125	[61]
Glutathione s-transferase	Cold	*Pseudoalteromonas* sp. ANT506	[62]
Glutathione s-transferase	Cold	*Halomonas* sp. ANT108	[63]
Hydrolases			
α-galactosidase	Hot	*Geobacillus stearothermophilus*	[44]
β-galactosidase	Cold	*Pseudoalteromonas* sp. 22b	[64, 65]
β-galactosidase	Cold	*Rahnella* sp.R3	[66]
β-galactosidase	Acid	*Teratosphaeria acidotherma* AIU BGA-1	[67]
α-amylase	Hot	*Anoxybacillus sp.* GXS-BL	[68]
α-amylase	Cold	*Pseudoalteromonas* sp. M175	[69]
α-amylase	Cold	*Luteimonas abyssi* XH031T	[70]
Xylanase	Hot	*Caldicellulosiruptor sp. Tok7B*	[47]
Xylanase	Hot	*Sulfolobus solfataricus strain* MT4	[71]
Xylanase	Cold	*Flavobacterium frigidarium*	[72]
Xylanase	Cold	*Flavobacterium johnsoniae*	[73]
Xylanase	Acid	*Aureobasidium pullulans* XynI	[74]
Chitinase	Hot	*Humicola grisea*	[75]
Chitinase	Hot	*Paenibacillus barengoltzii* CAU904	[76]
Protease	Hot	*Anoxybacillus kamchatkensis* M1V	[77]
Protease	Cold	*Pseudoalteromonas* sp. NJ276	[78]
Protease	Cold	*Flavobacterium xanthum* ANS4-15	[79]
Protease	High Salt	*Bacillus licheniformis* TD4	[80]
Asparaginase	High Salt	*Bacillus aryabhattai* GA5	[81]
Aminopeptidase	Cold	*Pseudoalteromonas haloplanktis* TAC125	[82]
Lipase	Hot	*Pyrococcus furiosus*	[83]
Lipase	Cold	*Bacillus pumilus* ArcL5	[84]
Lipase	Cold	*Pseudomonas* sp. LSK25	[85]
Lipase	Cold	*Pseudomonas vancouverensis*	[86]

(Table 2) contd….

Enzyme Name	Adaptation	Host Organism	References
Phytase	Hot	*Sporotrichum thermophile* BJTLR50	[87]
Phytase	Cold	*Rhodotorula mucilaginosa* JMUY14	[88]
Phytase	Cold	*Bacillus licheniformis* ATCC14580	[89]
Esterase	Hot	*Thermus thermophilus* HB27	[90]
Esterase	Cold	*Thalassospira* sp.	[91]
Esterase	Cold	*Oleispira antarctica*	[92]
Esterase	Cold	*Pseudomonas* sp.TB11	[93]
Esterase	Acid	*Ferroplasma acidiphilum* DSM 12658	[94]
α-glucosidase	Cold	*Leucosporidium antarcticum*	[65]
β-glucosidase	Cold	*Micrococcus antarcticus*	[95]
Cellulase	Hot	*Myceliophthora thermophila* ATCC 42464	[96]
Cellulase	Hot	*Geobacillus sp.* HTA426	[97]
Cellulase	Cold	*Flavobacterium* sp. AUG42	[98]
Cellulase	Cold	*Bacillus* sp. K-11	[99]
Cellulase	Alkaline	*Streptomyces thermoalkaliphilus* 4-2-13	[100]
Cellulase	High Salt	*Paenibacillus tarimensis* L88	[101]
Ribonuclease	Hot	*Sulfolobus solfataricus*	[102]
Ribonuclease	Cold	*Psychrobacter* sp. ANT206	[103]
Phosphatase	Cold	*Cobetia marina*	[104]
Lyases			
Alginate lyase	Hot	*Nitratiruptor sp.* SB155-2	[105]
Γ-carbonic anhydrase	Cold	*Colwellia psychrerythraea*	[106]
Glutamic acid decarboxylase	Cold	*Colwellia psychrerythraea*	[107, 108]
Pectate lyase	Cold	*Massilia eurypsychrophila*	[109]
Pectate lyase	Acid-Alkaline	*Bacillus amyloliquefaciens* S6	[110]
Isomerases			
Sedoheptulose 7 phosphate isomerase	Cold	*Colwellia psychrerythraea* 34H	[107]
Triose phosphate isomerase	Cold	*Pseudomonas* sp. π9	[111]
Ligases			
Glutathione synthetase	Cold	*Pseudoalteromonas haloplanktis*	[112]
DNA ligase	Cold	*Pseudoalteromonas haloplanktis* TAE72	[113]
DNA ligase	Acid	*Ferroplasma acidarmanus* Fer1	[114]

Some of the most studied halophiles genus include *Natronococcus, Haloferax, Halobacterium, Halobacillus, Halorhabdus, Halothermothrix, Micrococcus, Marinococcus, Acinetobacter*, *Bacillus*, producing different proteins, such as amylases, lipases, proteases, and xylanases [143-145]. Haloenzyme properties include their stability in the presence of organic solvents, high functionality through a large pH variation (3.0 to 10.5) and even high temperatures

(80°C) [101, 146]. Halophilic proteins are maintained in solutions through a network of hydrated cations coordinated by negative charges, and they also possess small hydrophilic residues on protein surfaces, unlike the common large hydrophobic residues [147, 148]. However, these characteristics give these enzymes low solubility in aqueous/organic and non-aqueous media [149, 150].

3. POTENTIAL USE OF EXTREMOZYMES IN BIOENERGY CHAIN

The use of extremozymes has provided the industry with a wide range of resistant biomolecules with various applications, meeting the main objectives in enzyme research, with novel extreme activities and improved stability [13, 38]. The large biotechnological interest and applications can be attributed to their consistency, selectivity, efficacy, reproducibility, diversity and the low production of by-products, contributing to environmental impact decreases as a greener solution for many industrial purposes [58, 151, 152].

There are various types of extremozymes suitable for the energy production process, including lipases, esterases, cellulases and xylanases [153-155]. The use of extremophile enzymes offers the advantages of working at high temperatures and eliminating some procedural steps, increasing sugar production, decreasing production cost and improving process efficiency [156].

Among the biotechnological applications of extremozymes, biofuels production has been consistently increasing. Crosby *et al.* [46] discuss the utilization of extremophile microbes in industrial processes. Focusing on extreme thermophiles from the genera *Caldicellulosiruptor*, *Pyrococcus*, *Thermococcus*, *Thermotoga* and *Sulfolobus*, the authors were able to engineer the metabolism of these microorganisms to increase the production of valuable industrial products, including biohydrogen, bioethanol, butanol and others. The main setback is the scalability of such systems is that these studies have been conducted in laboratory conditions and have yet to be tested on an industrial scale. Thus, the enzymes of extremophiles and themselves can be applied to the production of second-generation ethanol, microorganisms such as *Zymomonas mobilis, Bacillus subtilis, Geobacillus thermoglucosidasius, Clostridium thermocellum, Thermoanaerobacter ethanolicus* and *Thermoanaerobacter mathranii* have been investigated [157-163]. Their adaptations allow the fermentation and distillation concomitant, eliminating the step of cool utilized before the distillation [164]. Beyond that, some advantages such as shown by *Clostridium thermocellum* that can metabolize the pentose and hexose sugars resulting in the fermentation of cellulose to ethanol contain potential to application in industry or the tolerance of *Zymomonas mobilis* to the production of ethanol [161, 165, 166].

Other extremozymes that are under the interest of industry applications are glucosidases capable of hydrolyzing steryl glucosides that harm the ethanol production due to the formation of insoluble precipitates and thus cause aggregation, crystallization and precipitation of other molecules [167, 168]. These consequences can clog or block oil filters, hamper cold-flow conditions and cause engine failures, increasing production costs [169, 170]. In this sense, thermostable glucosidases capable of hydrolyzing steryl glucosides in biodiesel would produce free sugars and sterols (soluble in biodiesel) enabling water washing steps, due to the enzyme's thermostability and solvent tolerance [168, 171].

CONCLUSION

Extremophile microorganisms can produce enzymes capable of converting biomass into bioenergy (biogas, biohydrogen, and bioethanol). In this context, combined genomics, transcriptomics, and proteomics technologies open enormous possibilities for prospecting new extremophile microorganism and their noble extremozymes for biotechnological applications.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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