Journal of Tropical Biodiversity and Biotechnology
Volume 08, Issue 03 (2023): jtbb74986
DOI: 10.22146/jtbb.74986

Review Article

Extremophilic Cellulases: A Comprehensive Review

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Keywords:
Cellulases
Extremophiles
Extremozymes
Acidophile
Halophiles
Thermophiles
Submitted:
02 June 2022
Accepted:
17 May 2023
Published:
08 November 2023
Editor:
Miftahul Ilmi

ABSTRACT

Microbial cellulases are an important industrial enzyme having diverse applications in biotechnology, environmental challenges, industrial products and processes. Extremophiles like thermophillic bacteria are a good source of industrially important cellulases as these can withstand industrially rigorous procedures like paper deinking, fabric material softening, bio stoning, paper and pulp, biopolishing cloth material, animal feed and juice. Identification of novel cellulases or improving them through biotechnological interventions has remained a challenge for researchers. Genetic manipulation of thermophilic bacteria for increased cellulase production or synthetic biology approaches for cellulase gene/gene cluster extraction from thermophilic bacteria and expression in appropriate hosts for improved cellulase synthesis. The classic and high -throughput technologies like genomics, metagenomics and bioinformatics could be exploited to isolate cellulase genes from a variety of thermophilic bacteria and further processing. Keeping in view the ultimate requirement of extremophilic cellulases in industries, present study is a compilation of various aspects related to extremophilic cellulases their sources, production, biotechnological interventions and challenges.

INTRODUCTION

Bio molecules originating from natural resources are playing an important role in production of everyday items. Enzymes are one of those compounds that are well-known across the world for their numerous industrial uses. For example, they are widely used in dairy products, food and feed, paper and pulp, brewing, pharmaceutical manufacturing, and detergents industry. Cellulase is a widely used enzyme. The need for cellulase enzyme is expanding quickly, according to current worldwide cellulase market study studies. Cellulose, the cellulase substrate, is the most prevalent carbohydrate on the planet. It is the most important element in plant matter. (O'sullivan 1997).

Extremophiles are important research subjects for many scientific disciplines, from adaptation studies to extreme environments to biogeochemical cycles. Extremophiles, especially those that can survive in a variety of extremes, are a key topic of study for many disciplines. Research on extremophiles has an impact on both the study of quest for life origin and extraterrestrial life. In modern times by producing extremozymes, extremophiles are playing an important role in biotechnology sector. The frequent use of extremozymes in industrial production processes and research under extreme conditions (high temperature, high pressure, and extreme pH ranges) makes them important entities. Present work is a pragmatic approach to discuss current state of "Extremophilic Cellulases" knowledge and applications of extremozymes in many industries, identifying knowledge gaps and potential study areas.

CELLULASES

Cellulose is a biodegradable material found in large quantities in agricultural waste. Microbial cellulases have a significant approach nowadays due to their wide industrial utilization. Also, cellulases are an important part of the second-generation biofuel production as the hydrolytic action of the enzyme converts complex cellulose into simple monomer units (Jayasekara & Ratnayake 2019). The generated reducing sugars are then utilized to make ethanol, which is used as a biofuel. Endoglucanases, cellobiohydrolases or exoglycanases, and alpha glucosidases are the three primary types of cellulases. These enzymes can be found in anaerobic cellulolytic bacteria as single enzymes or as part of a multicomponent enzyme complex (cellulosome). Thermophiles and thermotolerant enzymes have diverse industrial applications.

Cellulose- a linear polymer of D-glucose connected through 1,4-glucosidic linkage is the most common carbohydrate found in nature and an important structural cell wall component of plants. It resists enzymatic breakdown better than other plant cell wall polysaccharides because of its partly crystalline form (Patyshakuliyeva 2016). Cellulases are inducible enzymes generated during the growth of cellulosic materials by a variety of microorganisms, including fungus and bacteria. (Kuhad et al. 2011). There are bacteria that are anaerobic, aerobic, thermophilic, and mesophilic. The most researched cellulase manufacturers include Trichoderma, Cellulomonas, Clostridium, Thermomonospora and Aspergillus (Table 1).

Table 1. Different types of genus and species of micro-organisms involved in the production of cellulases enzyme.

Group	Genus	Species
Fungi	*Aspergillus*	*Aspergillus niger* *Aspergillus oryzae*
	Fusarium	*Fusarium solani* *Fusarium oxysporum*
	Trichoderma	*Trichoderma Reesei* *Trichoderma. harzianum*
Bacteria	*Acidothermus*	*Acidothermus cellulolyticus*
	Bacillus	*Bacillus* sp. *Bacillus subtilis*
	Clostridium	*C. acetobutylicum*
Actinomycetes	*Cellulomonas*	*Cellulomonas. fimi* *Cellulomonas uda*
	Streptomyces	*Streptomyces* sp.

EXTREMOPHILES- NICHES (HABITATS, ISOLATED)

When it comes to temperature, pH, and salinity, the anthropocentric word "extremophile" was coined more than 30 years ago to characterise a microorganism capable of surviving and thriving under conditions that are extremely harsh and difficult for humans and the majority of known microbes (Canganella & Wiegel 2011) to survive. Extremophiles thrive in harsh conditions like extremes pH, high salt and temperature, radiation, high metal concentrations and extreme pressures. Extreme pH, temperature, high salinity, radiation acidic and basic, low water activity are only a few of the environmental difficulties for which these bacteria have developed unique systems and molecular responses (Sarmiento et al. 2015). These extremophiles have developed different molecular strategies for survival (Neifar 2015).

Alkalophiles live in very alkaline pH habitats, such as sodic lakes, and thrive in extremely alkaline pH conditions. Other extremophilic forms, known as pollution-loving microorganisms, may live and develop in the presence of high quantities of nuclides, pollutants such as polyhydroxylalkanoates (PAHs), pesticides, and other contaminants.

Acidophiles are microbes in very acidic conditions, often with a pH of 2. Acidophilic bacteria flourish in acidic lakes, certain hydrothermal systems, acid sulphate soils, sulfidic regoliths, and ores, as well as metal and coal mine-affected environments. The oxidation of the metal and other sulfidic minerals produces very acidic conditions that are home to a wide range of prokaryotic and eukaryotic life forms that are acidophilic and acid tolerant.

Halophiles are a type of extremophile that needs a lot of salt to survive and flourish. Halophiles are divided into two types: obligatory halophiles, which require a NaCl content of 3% or above, and halotolerant, which may thrive at both average and higher salt concentrations. Compatible solutes, also known as osmolytes, are metabolites that help cells maintain osmotic equilibrium. Producing or acquiring compatible solutes is a frequent adaptation for living in high-salt habitats (Charlesworth & Burns 2016). Halophilic bacteria are the major microbial communities present in hypersaline environments all over the planet. Halophilic bacteria have low nutritional needs, are resistant to high salt concentrations, and can regulate the osmotic pressure of their surroundings. The salt requirements of halophiles are divided into three categories: low (1-3%), moderate (3-15%), and extreme (3-15%). (15-30 percent). Temperature, pH, and growth media all influence salt requirements.

Adaptations

Extremophiles have developed unique methods to survive in their harsh surroundings by modifying their natural machinery. These methods and mechanisms, on the other hand, are extremely complicated. For example, in extreme alkali concentrations, extremophiles maintain an osmotic balance by enhancing appropriate solutes inside the cells or using ion pumps; in low pH conditions, an adequate pH is maintained by proton pump inside the cell; and in reduced or extreme heats, they change the design of their cytoplasmic membrane.

Exopolysaccharides (EPSs) surrounding most microbial systems in extreme ecosystems like deep-sea hydrothermal vents, Antarctic region, salty lagoons and hot springs constitutes an important component of extracellular polymers. Extremophiles have developed many adaptations to withstand and survive the harshness of extreme conditions, for instance, high temperatures, reduced pH or temperature, extreme salt and radioactivity (Nicolaus 2010).

The cytoplasmic membrane of bacteria determines the makeup of the cytoplasm to a great extent because ion electrochemical gradients across membranes, particularly proton and sodium ion electrochemical gradients, are critical for these microorganism's bioenergetic conditions, techniques to limit ion permeability across their cytoplasmic membrane are required. All biological membranes proton and sodium permeabilities increase as the temperature rises. Psychrophilic (cold-suitable) organisms populations have potential uses in a broad range of technical, agricultural and therapeutic processes. In order for progress to occur in reduced-hotness atmospheres, all cellular parts must fit to the cold (Elleuche 2014). Mesophilic & psychrophilic bacteria, as well as hyperthermophilic and halophilic archaea can change their membrane lipid contents to keep constant and low proton permeability at their specific growth temperatures. Thermophilic microorganisms, in another way, have a harder time confining proton infiltration through their membrane at extreme hotness, and these organisms must depend on less penetrable sodium ions to maintain an extreme sodium-cause in order to drive their strength-intensive membrane-bound movements. Enzymes from thermophilic animals have found ultimate efficient commercial use to date by way of their overall genetic stability (Demirjian 2001). Basically, to maintain function at extreme hotness, thermophilic proteins have an important hydrophobic gist and reinforced electrostatic contacts (Stetter 1999). Basic ATP-compelled transport systems mainly mediate the solute transport across the bacterial and archaeal sheath and or subordinate transport methods compelled by proton or sodium motive forces drive it. Hyperthermophilic microorganisms and archaea favour primary ATP-compelled assimilation processes for carbon and energy. Several ABC transporters accompanying high similarity for sugars from hyperthermophiles have been labelled and characterized. ABC transporters help these individuals to develop in a nutrient-inadequate environment. Different microorganisms like bacteria, yeast cyanobacteria, and algae from specific surroundings specify a valuable reserve that not only can be exploited sustainably in novel biotechnological processes but also as study models for identifying the biomolecules helping them to survive through extreme conditions (Herbert 1992).

Extremozymes – Industrial relevance

Extremozymes and extremolytes produced from extremophiles are having applications in different sectors of biotechnology including white, red and grey biotechnologies with potential to increase the biobased economy (Raddadi 2015).

Currently, very small number of microorganisms (1-2%) on the earth have been commercially exploited and most efficient ones among these are from extreme environments (Gomes & Steiner 2004). Extremozymes have been used and found very important in processes of biofuel production, pharmaceutical and chemical compound synthesis & food industries. The understanding of particular biochemical or metabolite that is responsible for adaptation to extreme habitats like specific enzymes have been targeted for biotechnological uses and applications (Dalmaso 2015). Currently and in the past also biocatalysis has been improved using natural enzymes but there are few reports available on uses of extremozymes in industria developments. Microbial extremozymes and their genetic consistency, reproducibility and increased yields in harsh environments, have led their use in a variety of industrial procedures.

Extremophile-derivative enzymes, or extremozymes, can proceed biochemical reactions in extreme circumstances, such as those visualized in technical processes, where enzymatic action was earlier thought to be impossible. Extremozymes offer novel catalytic options for current mechanical applications on account of their superior action and balance under harsh surroundings. For example, most of the universal industrial strains including bacterial spp (Bacillus, Escherichia coli, Corynebacterium glutamicum and Pseudomonas spp.) and yeast are grown in mild environments (pH of 5–7 and 30–37 °C) and medium enriched with yeast extract. These temperate conditions favours growth of most of the microorganisms in air, water and soils (Shrestha 2018).

Enzymes are employed in the dairy business for cheese production and creamery product preparation; in the baking production, enzymes increase bread condition; in beverage manufacturing, enzyme is used to maintain especially of wine purity and colour while lowering Sulphur levels. Extremophillic hemicellulases (β-mannanase, β-mannosidase, glucoronidase, galactosidase, feruloyl esterase, acetyl xylan esterase and α-arabinofuranosidase) are effective enzymes for the complete plant cell wall saccharification (Antranikian & Egorova 2007). Some modern enzymes can further be employed to boost the produced things filterability and flavour.

Arthrobacter, *Acidithiobacillus*, *Micrococcus*, *Bacillus*, *Geobacillus*, *Caldicellulosiruptor*, *Clostridium*, *Enterobacter*, *Coprothermobacter*, *Paenibacillus*, *Picrophilus*, *Pseudoalteromonas*, *Penicillium*, and *Thermobifida* are just a few of the extremophile sources for enzymes that have been isolated and tried for biomass processing. Table 2 enlists some of important extremophiles with their classification and extremozymes produced. Tetrathionate hydrolase, decarboxylase, -galactosidase, subtilase, xylanase, dehydrogenase, endoglucanase, α-amylase, β-glucosidase, enzymes were reported (Zhu 2020).

Many industries will more and more be benefitted from the exploitation of extremozymes. It has been recommended that low proportion of these organisms (below 10%) could be cultivatable and further improvement of molecular biology and gene expression studies will help in understanding the microbial diversity for sustainable exploitation (Gupta 2014).

ISOLATION OF THERMOPHILIC CELLULOLYTIC BACTERIA

Many workers have reported the isolation of thermophiles in past. Based on the reports we have tried to compile the important aspects of isolations. According to a published method, sterile containers with various compost samples (temperature > 50 °C) were taken. To minimize mesophiles and anaerobic isolates, the compost piles were air-dried and heated to a temperature of 55°C for one week before being dried. Two techniques were employed to isolate thermophilic bacteria. After serial dilution, both straight spreads improved or plated on CMC (Carboxy methyl

Table 2. Classification of extremophiles and modern use of few enzymes.

Types	Growth Characteristics	Source	Enzymes	Applications
Acidophiles	can growth at or beneath pH 3-4	Volcanic springs, Acid mine drainage, USA	Amylase	Single cell protein, starch processing
			Cellulases	Removal of hemi cellulosic material from feed
Halophile	can grow in elevated salt concentartions	Salty Lakes, saline soils	Proteases	Peptide synthesis
Alkaliphile	Growth at elevated pH value (more than 10)	salty lakes	Cellulases	Fermentation
Thermophile	Can survive between 60 degrees Celsius and 85 degrees Celsius	Hot springs	Lipases, cellulases	Additive to detergents for washing at room condition Breaking lipid stains, to breakdown lignocellulose

cellulose) agar using cellulose broth. All incubations were carried out in a controlled atmosphere at 55°C for two-four weeks with shaking at 120 rpm (Ibrahim & El-diwany 2007).

SELECTION OF CELLULOLYTIC THERMOPHILIC ISOLATES

The cellulolytic bacterial isolates were screened using a 1% congo red indicator. The plates were soaked with 1% Congo red indicator for 15 minutes before being soaked with 1M NaCl solution for another 15 minutes. The presence of a halo zone around the colony indicated that cellulose hydrolysis had occurred. If the zone was too cloudy, 0.1N HCL was added (Wood & Bhat 1998)

Effect of pH & Temperature

The temperature profile for cellulase activity can be determined between 30°C and 80°C. At different temperatures (30-80°C), the soluble enzyme extract along with the substrate (CMC) are subjected to assay the maximum activity. Various studies have been carried out at different pH by different workers to find the best assay pH for cellulase activity: 0.05 M sodium acetate (pH 3-4.5), 0.05 M sodium citrate (pH 5-5.5), and 0.05 M sodium phosphate buffer (pH 5-5.5) (Oyekola 2003; Ariffin et al. 2006; Ray et al. 2007)

Thermal Stability and Production of Cellulases

Thermal stability is important for industrial applications. One study indicated that *Bacillus subtilis* cellulase enzyme activity was highest at 50°C. The selection and formulation of an ideal cellulose basal medium is research-intensive and highly required for industrial production. One such study for the optimization using *Bacillus subtilis* in four distinct cellulose basal media for enzyme production has been described Ray et al. (2007). *Bacillus subtilis* produced the greatest enzyme activity.

Applications

For many decades, cellulases have been employed as key biocatalysts in variety of industries. Fabrics, pulp & paper, laundry and detergents, agriculture, pharmaceuticals, and food industry are just a few examples that employbacterial cellulases. CMI (Coherent market insights) reported textile sector was the largest market for cellulases in 2017. Further enzyme market research findings included food and beverages, livestock, textile industry and bioenergy as the important application areas. Extremophiles, especially archaea and bacteria, provide an excellent platform for treating industrial waste streams that were previously thought to be hostile to the model organisms in microbial electrochemical systems (MESs) expanding the application from industry to environmental remediation (Shrestha 2018).

Fabrication Manufacturing

The fabrication industry is one of the world's most important industries. Cellulase enzymes are flexible enzymes that may be used to efficiently replace non-eco-friendly chemical treatments in textile manufacturing (Shah 2013). Customers are increasingly demanding individuality in terms of styles, colours, and the clothing they wear. As a consequence of rising client demand, this business has experienced tremendous expansion in recent decades. In these applications, this enzyme is currently the third most often used group of enzymes. Manufacturers that are constantly seeking for environmentally friendly methods to differentiate their products will find this to be a very competitive market platform. In the industrial sector, cellulase is utilised for a number of purposes. Some of the primary uses of this enzyme in the industry, notably for textile wet processing, include bio stoning of textiles, biopolishing of fabric fibres, smoothing of cloths, and excess textile colour removal. Cellulases from *Trichoderma reesei* are the most often used enzyme in the textile industry. *Streptomyces* and *Thermobifida* are two genera of actinomycetes., as well as bacteria from the genera *Pseudomonas* and *Sphingomonas*, are further sources of enzymes for textile dye decolorization and deterioration.

Paper and Pulp Industry

According to the World Wildlife Fund (WWF), the paper sector consumes more than 40% of all industrial wood marketed worldwide, including items such as office and catalogue paper, glossy paper, tissue, and paper-based packaging. Paper and pulp are both renewable natural resources (Shah 2013). As a result, two common ideas in this industry are recycling and reuse. The employment of microbial cellulases is commonly used to accomplish this. In this industry, cellulases are employed in a number of ways. From the 1980s to the present, the variety of possible applications has grown significantly. Deinking, pulping, industrial waste bioremediation, bleaching, and fibre enhancement (Kuhad 2011) are some key processes of this industry where cellulases are employed. Considering these processes as harsh conditioned reactions, these will be more reproducible if extremophillic cellulases that can be more effective in these conditions are employed.

Laundries & Detergents

Enzymes have been used to generate enzymatic washing agents or biological detergents since the 1960s. Enzymes are often employed in detergent compositions generally. According to market data, the largest single market for enzymes was the detergent sector in 2014, accounting for 25–30% of total sales (Zhang & colleagues 2013). To date, cellulases from fungi such as *Humicola (H. insolens and H. griseathermoidea)*, *Aspergillus niger*, *Trichoderma* sp. (*T. longibrachiatum*, *T. reesei*, *T. viride*, and *T. harzianum*) and *Bacillus* sp. have been intensively investigated for use in detergents. The greatest addition to conventional detergents is alkaline cellulases. It's due to their capacity to remove dirt and soil particles from the fabric's interfibrillar regions. Cellulases break down the rough projections of cellulose fibres or cellulose aggregates on the cloth. As a result, the material's shine and smoothness could be improved.

Agriculture

In agriculture, cellulases are often used to increase crop growth or in disease management. Combination of cellulases, hemicellulases, and pectinases is commonly used for this purpose. Certain fungal cellulases have the capacity to break down plant pathogen cell walls. Several studies using bacteria to increase plant performance, such as plant growth-promoting rhizobacteria (PGPR) has been reported in the past and still counting. These bacteria are said to serve a key role in lowering the use of artificial fertilisers, promoting plant development, and regulating possible plant infections and disease protection. In addition, numerous fungi, such as *Geocladium* sp., *Trichoderma* sp., *Chaetomium* sp. and *Penicillium* sp. promote seed germination, quick plant growth, faster flowering, stronger root systems, and increased crop yield. However, the specific mechanisms behind these reactions remain uncertain. All of these species, however, may produce cellulase and other enzymes, which may play a direct part in these processes. Some studies have found probable synergies between the development of bacterial cellulase and the production of bacterial antibiotics against plant harmful fungi. Extremozymes are of great interest for a variety of industrial processes, in addition to starch and lignocellulose degradation. Chitinases are enzymes that work together to degrade the -1,4-glycosidic linked N-acetylglucosamine units of chitin. Chitinolytic enzymes are used as bio fungicides and bioinsecticides since this structural polysaccharide is found in the exoskeletons of fungus, insects, and crustaceans (Elleuche 2014).

In terms of climate change, microbial communities and plants from harsh habitats are being tested as biotechnological tools to increase cereal crop production and development of tree species tolerant to unfavourable climate events (droughts, flooding, frosts, heat, and cold waves, and so on) (Jorquera 2019).

Applications in Medicine

Medical pharmacology is a very busy field of research right now, with new findings being made all the time. One such sector is development and use of cellulases for medicinal purposes. The survival mechanisms of extremophiles are being studied in order to address difficulties related to human health; knowing these processes might be beneficial in management of human ailments (Babu 2015). Many extremophilic bio-products have already been employed as life-saving medications (Singh 2012). Although humans cannot produce cellulases, a recent study in health and medicine has found that ingesting enzyme blends that include cellulase offers health advantages. Cellulases produced from natural fermentation processes of *Trichoderma reesei* and *Bacillus licheniformis* has been added to commercially available enzyme mixes in response to global demand for enzyme blends. Fruits and vegetables, cereals, legumes, grains, nuts and seeds, soy, dairy, nutritious greens, sprouts, and herbs, as well as fats (lipids), sugars, proteins, carbs, and gluten, are all targets for these enzyme combinations. VeganZyme is one such example. Aside from that, there are digestive aids (such as Digestin, P-A-L Plus Enzymes, Polyenzyme Plus, and others) gaining global attention as viable medical treatment for metabolic illnesses. Antimicrobial peptides have been discovered in both *Halobacteriaceae* and *Sulfolobus* species (phylogenetic family including all halophilic archaea). Halophilic archaea peptides (halocins) are considered to be found in all members of the family. Although halocins have been proven to destroy archaeal cells, there is no evidence that they kill bacteria that are dangerous to people (Coker 2016). DNA polymerases from thermophiles are being used in PCR-based diagnostics for a wide spectrum of animal diseases, considered as most well-known use of an extremophile product in veterinary medicine (Irwin 2010).

Biofuels and Bioenergy Production

Biofuels are a renewable source of energy. Second-generation bioethanol production is becoming increasingly popular due to the availability of low-cost raw ingredients. Pre-treatment is an important stage in the production of bioethanol from lignocellulosic biomass (Sindhu et al. 2016). Lignocellulosic biomass includes agricultural waste (corn stover, crop straws, and bagasse), herbaceous and weed crops (alfalfa, switch grass), short-rotation woody crops, forestry residues, wastepaper, etc. is among the most promising raw material for fuel production. The lignocellulosic biomass is the most abundant renewable biomass. Global lignocellulosic cellulose output is expected to exceed 200 billion metric tonnes per year (Sindhu et al. 2016). The benefits of producing bioethanol from these feedstocks are numerous. Removal of lignocellulosic materials, which are non-edible plant parts is one such benefit. It is eco-friendly and most importantly as no food crops are eaten before harvesting, bioethanol production from lignocellulosic biomass does not contribute to food insecurity or food vs fuel controversy. Further its availability throughout the year as a raw material is beneficial for industrial production.

The structural complexity of lignocellulosic biomass, on the other hand, is a significant constraint of this production approach. Lignin cellulose, and hemicellulose form a very stable and complex structure when united. This makes it difficult to break the stable structure and support fermentation., Therefore the substrate must be pre-treated for efficient production. However, the pre-treatment procedures are neither eco-friendly nor cost-effective. But the novel or extremophillic cellulases can be used to de-stabilise this lignocellulosic biomass. Researchers are finding such efficient cellulose which can alone or in combination can be useful to degrade lignocellulosic biomass and bioethanol production.

SOURCE

Cellulases are enzymes that break down cellulose chains' -1,4 bonds. Fungi, bacteria, protozoans, plants, and mammals all make them. Based on their amino acid sequences and transparent constructions, cellulase catalytic modules are differentiated into many classifications. At the N- or C-end of catalytic modules in cellulases, noncatalytic carbohydrate-binding modules (CBMs) and/or other operationally recognised or mysterious modules are created.

Many cellulose-degrading enzymes have existed cloned, and signified from a variety of cellulolytic thermophilic bacteria. Conversely, a search for cellulose-degrading enzymes in hyperthermophilic bacteria indicated that such enzymes are uncommon in this group. Furthermore, only the Archaea genera *Pyro coccus* and *Sulfolobus* have been discovered to metabolise thermoactivated cellulases. In comparison to anaerobic microbes, aerobic thermophilic microorganisms have also been found to produce cellulases. *Rhodothermus marinus*, an aerobic thermophile isolated from a subsurface natural hot water spring in Reykjanes, NW Iceland produced thermostable cellulase (Cel12A) showing 50% activity after 3.5 h hours at 100°C.

The thermophilic filamentous bacteria *Thermobifida fusca* (previously *Thermomonospora fusca*) are a prominent cellulose degrader. Cel9B, Cel6A, Cel5A (earlier E1, E2, and E5), two exoglucanases Cel6B and Cel48A (already E3 and E6), and an endo/exoglucanase Cel9A are all secreted by this actinomycete and have been well described. More in-depth research is required to understand better the catalytic mechanism using computational and experimental methods. The crystal structure of this enzyme in association with substrate and inhibitor has also been determined. Table 3 compiles the different temperature rhymes and environmental conditions.

FUNGAL CELLULASES

Few thermophilic fungi have been identified as cellulase producers and listed in table 4.

Properties of Some (energetic) thermophilic cellulolytic microorganisms.

Microorganism	**Enyme**	**Mol mass** (kDa)	**Optimal T** (°C)	**Optimal pH**
Acidothermus cellulolyticus	E1	72.0	81	5.0
Anaerocellum thermophilum	CelA	230.0	85-95	5.0-6.0
Caldibacillus cellulovorans	CMCase	174.0	80	6.5-7.0
Clostridium thermocellum	CelI	98.5	70	5.5
Pyrococcus furiosus	EglA	35.9	100	6.0

Table 3. Thermophilic cellulolytic Bacteria and Archaea showing growth at different temperature and in different environmental conditions.

Microbe Name	**Domain**	**Gram Reaction**	**Growth T** (°C)	**Growth conditions**
Acidothermus cellulolyticus	Bacteria	+	55	Aerobic
Alicyclobacillus acidocaldarius	Bacteria	+	60	Aerobic
Caldibacillus cellulovorans	Bacteria	+	68	Aerobic
Clostridium stercorarium	Bacteria	+	65	Anaerobic
Clostridium thermocellum	Bacteria	+	60	Anaerobic
Dictyoglomus thermophilus	Bacteria	-	73	Anaerobic
Dictyoglomus turgidus	Bacteria	-	72	Anaerobic
Pyrococcus abyssi	Archaea	-	96	Anaerobic
Pyrococcus furiosus	Archaea	-	98	Anaerobic

Table 4. Properties of different thermophilic fungi.

Microorganism	**Enzyme**	**Mol mass** (kDa)	**Optimal T** (°C)	**Optimal pH (pH)**
Chaetomium thermophile	EG	67.8	60	4.0
Humicola grisea var.*thermoidea*	EGI	58.0	55-60	5.0
Myceliophthora thermophile	EG	100.0	65	4.8
Talaromyces emersonii	EGI-III	68.0	75-80	5.5-5.8
Thermoascus aurantiacus	EGI	78.0	75	5.0
	EGII	49.0	68	5.0

Several cellulases have been reported to beproduced by the thermophilic filamentous fungus *Humicola* sp., and several of the genes have been cloned, sequenced, and expressed. The thermophilic fungus *Humicola insolens* has a cellulase system with enzymatic machinery for effective cellulose consumption. This order, that is related to *T. reesei's*, has five endoglucanases: EGI (Cel7B), EGII (Cel5), EGIII (Cel12), EGV (Cel45A), and EGVI (Cel6B), in addition to two cellobiohydrolases: CBHI (Cel7A) and CBHII (Cel6A) showed optimum activity between pH 5.5-9.0. Among these Cel7B was found to be highly active across a wide pH range showing over 60% activity between pH 5.0 -10.0. Cel45A exhibited a pH optimum of 9.0, while the other three cellulases had pH optimums of 6.0 to 8.0. There was no information regarding the ideal temperature and stability. The explanation for the abundance of enzymes produced by *H. insolens*, *T. reesei*, and many other cellulolytic microbes is unknown. According to the most widely accepted view, each enzyme plays a singular role concerning the substrate's assortment (solubility, degree of substitution or polymerization).

Biotechnological interventions to increase the production

Developing and establishing routes to enhance the cellulase production at industrial level involved different microorganisms and different methods. Every step in the production of cellulase is important, isolation of the suitable microorganism, pre-treatment, inoculation, fermentation, extraction of the enzyme and enzyme assay. Biotechnology is particularly interested in salt and cold-active enzymes (Karan 2012). Most of the industrial enzymes are obtained from using submerged fermentation (SmF) as it is easier to handle the environment such as pH and temperature. Enzyme production cost can be reduced, and yield can be improved by using Solid state fermentation Optimal conditions are very important for enhanced production, *A. niger* was proved to be productive at pH of 6, and temperature of 30°C.

Inducible ion outflow systems reduce the intracellular accumulation of a particular ion through active transport, which is how heavy metal ions are usually detoxified. Because these bacterium's metal resistance determinants are all involving reasoning from facts, their regulatory structures maybe exploited to assemble biosensors that assess biologically appropriate heavy metal concentrations in the environment. Only the cytoplasm of the relevant cell is detoxified when resistance is based on metal ion efflux. As a result, this resistance mechanism can't be directly used to the development of biotechnological methods. (Nies 2000).

Soil microorganisms such as *Pseudomonas fluorescens*, *Bacillus subtilIs*, *E. coli*, and *Serratia marcescens* are potential cellulase produces (Sethi 2013). For maximum cellulase production culture medium and culture conditions were was optimized for such as pH 10 and temperature 40°C was reported as efficient for production n. Among all microorganisms *Pseudomonas fluorescens* was found to be better cellulase producer.

Adaptive development is an effective approach to customize the strains and genes that includes the degeneration of biomass, this method has been used to produce the mutant strain (N402) of *Aspergillus niger*, the transcriptomic study revealed that the expression of noxR, encoding the regulatory subunit of the NADPH oxidase complex, was lowered in the mutant version in contrast to the maternal strain (Patyshakuliyeva 2016). The properties of this mutant showed that it has five-time higher potential of producing cellulase than its parent strain.

Challenges

Biotechnological production and development possess greater challenges and vary between every isolation technique, development strategy and so on. Understanding existence under extreme environments is challenging on account of the troubles of artificial sophistication and observation because most of the structures cannot be refined. Many challenges are faced when we work with extremophilic microorganisms as these organisms live in extreme environments that are not suitable for our human survival. Extreme environments involve high pH, temperatures, salt concentrations, pressures and low temperatures, pH and salt concentrations, and pressures. Such harsh environments are intolerably hostile and can also be lethal to earthly life forms. These microorganisms thrive in extreme hot slots, ice, and alkali fluids, as well as acid and salty conditions, few may evolve in hazardous waste, organic solvent, and several other habitats. They have happened to be at 6.7 km inside the Earth's coating, in addition 10 km deep inside the sea at pressures of until 110 MPa from extreme acid (pH 0) to extreme fundamental environments (pH 12.8); and from hydrothermal vents at 122 °C to iced salt solution, at −20 °C. Extremophiles have a great phylogenetic diversity and are difficult to investigate in general. Only extremophiles are found in some orders or genera, whereas together non-extremophiles and extremophiles are found in others (Rampelotto 2013).

The optimum temperatures, pH and pressures of extremophillic organisms are very challenging, so for the survival they should meet the optimum conditions. Some extremophilic organisms need more than two extreme conditions for their growth and some can tolerate one though they are growing in normal conditions. For example, the archaeal *Methano pyruskandleri* strain 116 evolves at 122$^{\circ C}$ (the maximal recorded hotness), while the type *Picrophilus* (for instance, *Picrophilus torridus*) include ultimate acidophilic now known, accompanying the strength to evolve at a pH of 0-6. In conditions of extreme resistance to extreme environments, individuals of the most influential eukaryotic polyextremophiles are tardigrade. Tardigrades can participate through an inaction mode, named the tun state, whereby it can survive the conditions from −272 °C to 151 °C, vacuum environments (imposing extreme aridity), pressure of

6,000 ATM plus disclosure to X-rays and gamma-beams (Rampelotto 2013).

As studying these extremophiles is challenging one, research on these gives a key to research of new areas like astrobiology or outer terrestrial environments. These organisms can be considered as model organisms when research goes on extra-terrestrial existence of life. These possess remarkable adaptation mechanisms for their survival. To allow growth at extreme heats, nucleic acids may be shielded by various designs. Nucleotides are the construction blocks of deoxyribonucleic acid molecules, and the bases adenine and thymine link through two hydrogen bonds, while cytosine and guanine are affiliated by three hydrogen bonds. Since extreme genomic GC content, particularly at the tertiary codon position, confers better establishment, it is assumed that this maybe the main method of protecting double-stranded DNA from denaturation in extremophiles (Wang 2006).

Recent Advances

The microbial capability to live in harsh and extreme environments led many investigators to study the adaptive characteristics of these microorganisms to further exploit them sustainably (Tango & Islam 2002). Many industries and researchers are committed to work on microorganisms and to engineer its genetic makeup to produce desirable characteristics. Mostly commercially available common enzymes have less industrial applicability as they could not withstand pH, high temperatures and pressures. In recent times extremozymes have received more attention. Cold-suitable proteases and amylases with potential to remove starch stains have been commercialized by industrial giants like Genesco & Novozymes. Industrial enzymes with application in biorefineries are being considered as specialized enzymes with an estimated value of $ 1 billion in 2010 to $5.0 billion in 2021 at a rate of 4.0% every year. This has surpassed the former, as they attained $5.5 billion in 2018 and are presently calculated to reach $7.0 billion by 2023 (Zhu 2020).

Extremozymes having application in delignification and subsequent usage of lignocellulosic biomass for bioenergy have been isolated and identified (Zhu 2020). Thermophilic microorganisms and their enzymes have been reported for excellent use in bioenergy production and anaerobic digestion. Some of these include *Caldicellulosiruptor bescii, Geobacillu sproteiniphilus, Thermoanaerobacterium, Pyrococcus, and Caldicellulosiruptor* (Zhu 2020).

A gene cluster for biosynthesis of glycocin from a thermophilic *Aeribacillus pallidus* has been transferred and expressed heterologously in *E. coli* for the increased production of glycocin with potential in biofuel manufacturing (Kaunietis 2019).

In another research bioaugmentation process has been exploited for the improvement of a *Clostridium* sp. strain WST. This has considerably enhanced the butanol yield to 0.54 g/g by 98-fold. This hike offers an eco -friendly and cost effective approach to use lignocellulose for production of biofuels and bioenergy by reducing the manufacturing cost (Shanmugham et al. 2019).

Arsenic immune nematodes have existed found in Mono reservoir Researchers revived eight variety from the salted, alkaline environment—increasing the acknowledged biodiversity of organisms in the California pond five-fold.

CONCLUSIONS

Microbial communities are still being found in conditions that are unfriendly for life to exist. The viruses of several of these extremophiles are frequently neglected, despite their importance in influencing microbial community or as extremozyme developers. Studying unreachable locations such as the deep sea and ice-covered oceans has never happened smoothly because of the growth of revolutionary culturing sciences and new designs for exploring improbable regions such as the deepest oceans and ice-covered oceans (Podar & Reysenbach 2006).

Extremophiles – microorganisms that evolve in extreme salt or heavy metal concentrations, or at limits of heat, pressure, or pH — may soon play a bigger role in biotechnology. Microorganisms and microbial consortia have a considerable impact on pH, from the nano- to macro-scale. (Merino 2019). These creatures and their natural components are attractive as they allow process working in more circumstances than their classical counterparts (Ludlow & Clark 1991). Among the possible enzyme technologies conversion to glucose from cellulose by process of hydrolysis is salient of all processes. The range of industries impacted by the application these elements are innumerable. This is true when we evaluate plant-based industries being that at least 60% of plant mass is cellulose, thus developing other means of breaking it down are paramount to advancements. Extremophile research has yielded huge scientific discoveries, but it is now time to assess whether these results are sustainable and can be used to improve industrial operations to get new products or to undergo biotransformatics (Schiraldi & De Rosa 2002). Nowadays research on extremophiles focuses on screening and isolation of potential strains which are sources of novel enzymes with increased industrial potential and viability in critical conditions. Further extremophiles play a key function in biorefinery applications by providing novel metabolic pathways and catalytically resistant/strong enzymes functional under rough industrial conditions.

The application of extremophiles and their enzymatic elements has brought forth a range of versatile applications which are otherwise perceived to be difficult to perform. The most remarkable observation is that extremophiles exist in environments too strident for most organisms (these include but are not limited to extreme temperatures, pressures, pH, etc.), thus making it even more interesting to observe how some extremophilically derived enzymes turn out to be polyextremophilic exhibiting stability and activity in one or more extreme states, including high salt concentrations and alkaline pH, low temperatures, and non-aqueous environments. Keeping aforementioned in mind, the future applications of many of these enzymatic elements derived from extremophiles open new insights to the realm of modern enzymology and many industries further perpetuating and incentivizing the transition toward sustainable economies and industries. These proteins, which have adapted to function within extreme sets of conditions, are less prone to denaturing, opening doors to applications like the use of anaerobic and aerobic extremophiles second hand either as whole cells or their enzymes alone in the result of different biofuels, containing biodiesel, biomethane, bio butane, and bio hide in more recent times with promising long-term applications. The primary industries that advanced as a result of blending extremophile-based processes into their framework initially were food, agriculture, cosmetic, synthetic and fabric industries. Modern endeavours have also seen the fields of bioremediation of contaminated environments where much research considers extremophiles as a promising candidate due to their specific metabolic activities and the strength to indulge harsh habitats. Recently, various extremophiles have researched for their capability to produce extremolytes like biopterin, mycosporine like amino acids (MAA), palythine, porphyra-344 and phlorotannin that play pertinent roles in several applications in relation to agents that block cell cycle, drugs of anti-cancer, antioxidants, and commercial products like sunscreen. In addition to that, multiple extremozymes were clarified and recognized from extremophiles for their industrial and biotechnological applications. This illustrates to some extent the vast and versatile extent to which extremophiles can be utilized in a means move toward a bioeconomy, while at the same time providing new aspects to researching the origin of life and astrobiology. With the advancement of biological tools, such as CRISPR-CAS9, the boundaries of extremophile-based utilities and research are practically endless.

FUTURE PROSPECTIVES

To address the depleting fossil fuel resources and the need for environmental protection and alternative energy resources, a number of biorefinery based applications and methods have been developed and proposed by researchers globally, but most of them are still in the pilot stage. Extremophiles and their enzymes have a significant market share that is anticipated to continue expanding because of their proven commercial success in the diverse biotechnology fields. To further use this immense potential sustainably, however, a lot of research has to be done to address problems associated with their industrial scale production, activity enhancement, novel sources etc.

AUTHOR CONTRIBUTION

S.M. written and prepared the manuscript, M.B. written and reviewed the manuscript, J.D.B. written and reviewed the manuscript, S.S. edited and reviewed the manuscript; V.S. designed and conceptualized the research and supervised all the process.

ACKNOWLEDGMENTS

The authors acknowledge the support of Head and Faculties of Molecular Biology and Genetic Engineering domain, School of Bioengineering and Biosciences, Lovely Professional University, Jalandhar, India.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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