Send Orders for Reprints to reprints@benthamscience.net

REVIEW ARTICLE

Extremophile – An Adaptive Strategy for Extreme Conditions and Applications

Isha Kohli[1], Naveen C. Joshi[1], Swati Mohapatra[1] and Ajit Varma[1,*]

[1]*Amity Institute of Microbial Technology, Amity University, Noida, India*

ARTICLE HISTORY

Received: January 14, 2020
Revised: February 27, 2020
Accepted: March 19, 2020

DOI: 10.2174/1389202921666200401105908

Abstract: The concurrence of microorganisms in niches that are hostile like extremes of temperature, pH, salt concentration and high pressure depends upon novel molecular mechanisms to enhance the stability of their proteins, nucleic acids, lipids and cell membranes. The structural, physiological and genomic features of extremophiles that make them capable of withstanding extremely selective environmental conditions are particularly fascinating. Highly stable enzymes exhibiting several industrial and biotechnological properties are being isolated and purified from these extremophiles. Successful gene cloning of the purified extremozymes in the mesophilic hosts has already been done. Various extremozymes such as amylase, lipase, xylanase, cellulase and protease from thermophiles, halothermophiles and psychrophiles are of industrial interests due to their enhanced stability at forbidding conditions. In this review, we made an attempt to point out the unique features of extremophiles, particularly thermophiles and psychrophiles, at the structural, genomic and proteomic levels, which allow for functionality at harsh conditions focusing on the temperature tolerance by them.

Keywords: Thermophiles, psychrophiles, extremozymes, adaptations, applications, extremophiles.

1. INTRODUCTION

The earth has a wide area of niches and ecosystems that are generally not habitable and referred to as extremes where microbial life systems sustain that need immediate attention of research and development. Organisms, commonly called extremophiles, thrive best under these extreme environmental conditions and represent the unique adaptability of primitive life-forms [1]. Discussing particularly about thermophiles and psychrophiles, thermophiles are the organisms accustomed to its survival at uplifted temperature conditions. In contrast, psychrophiles or cryophiles are the organisms that proliferate at frigid temperature conditions. These organisms are difficult to study because of the isolation, maintenance and preservation at their optimum extrinsic temperatures. It is important to mention that irrespective of the temperature variations, all the macromolecules of the cell such as DNA, RNA, proteins, *etc*. should be operational and stable. One of the exciting features of these magical extremophiles lies hidden in the enzymes they secrete. The greater intrinsic stability of extremozymes than the enzymes isolated from its mesophilic counterparts makes them wonderful candidates to be used for application based industrial work. Although the genomic, proteomic and molecular basis of their stability is not fully understood, significant research is underway to explain their mechanism of adaptability to variations in temperatures [2]. Many reports in this area have suggested that the factors responsible for the stability could be various bondings and interactions such as H-bond [3, 4], covalent bond, the composition of amino acid [5], the G+C content [6], tRNA composition and its folding patterns [7]. Pioneer efforts of some early research [8, 9] have made insight into the study of the factors responsible for the thermostability of proteins. The complete genome sequences of many hyperthermophilic archaeons, thermophiles and psychrophiles have already been reported, thus determining the aspects of thermophilicity and psychrophilicity [10]. It has been studied that the structural stability of an organism depends on the wide range of interactions [11], such as hydrogen bonding, hydrophobic and van der Waals [10]. To adjust the thermostability, various proteins use certain combinations and permutations of these interactions [12], though these interactions are sometimes temperature-dependent. The stability of proteins in extremophiles is seen primarily through changes in the residues of amino acid. It has been observed that on the molecular surface of proteins charged residues (*i.e.,* Glu, Arg and Lys) increase in thermophiles, while the same is in lesser quantity in case of psychrophiles [13]. The subsequent rise in Gly residues in psychrophiles increases protein stability. Genomic studies on extremophiles revealed that the structural composition of DNA [14] and the arrangements of codon-anticodon interactions [15] could be the promising stability factors for their stability. tRNA sequence and its folding patterns in the complete genome of an organism could be co-related with its optimum growth temperature [7] and hence would account for organism thermostability. G+C content in the tRNA of extremophiles has also been investigated for analyzing the differ-

*Address correspondence to this author at the Amity Institute of Microbial Technology, E3 block, 4th floor, Amity University, Noida, India;
Tel: 9810899562; E-mail: ajitvarma@amity.edu

1389-2029/20 $65.00+.00

ences associated with stability at extremes of temperature [6, 16]. Experimental evidence has suggested that the enhanced stability of proteins and cell membranes of thermophiles or stability of unique cold shock proteins and cell membrane/ cell wall of psychrophiles could depend on interactions within proteins, DNA or on their structural stability [17-20] (Fig. **1**). Thus, it can be said that there exists an interesting relationship between OGT and various intra- and intermolecular interactions [12].

Many biochemical mechanisms can also be related to the existence of extremophiles to adverse conditions, some of which are involvement of lipids and membranes, various stabilizing factors, rapid resynthesis of heat-inactivated molecules as in case of thermophiles, hyperthermophiles or halothermophiles, allostery and highly charged macromolecular environment [21].

Environmental stress (high, low or moderate) also controls the constitution of lipid membranes in the extremophiles as well as non-extremophiles. Koga, Y. (2012) reviewed that the proportion of some lipids increases with the increase in temperature. It was investigated in his report that in thermophiles and archaea ester linkages are not heat resistant, whereas the ether lipids are stable at high temperatures. Moreover, on the increase in the growth temperature, isofatty acids increased, whereas on lowering the temperature, anteiso fatty acids increased in the case of *Bacillus* spp. [22]. As lipids generally function in the membrane as a group of molecules and not as a single molecule, it is reported that on changing the temperature, the organisms adapt by changing the acyl or alkyl side chain of their lipid membrane rather than changing to different lipid class [23].

As mentioned above, thermostable biomolecules have found notable importance in the field of biotechnology; and this has spurred research into an organism's capability to thrive best at exotic temperatures [24, 25] (Fig. **2**). Extremozymes have been employed in diverse industrial applications, some of which are starch processing, cellulose degradation, leather, baking and detergent industry [17]. These organisms also connect to the primitive forms of life present billion years before on our planet and help to study the evolutionary process [26].

2. STRUCTURAL DEPENDENCY ON EXTREMOPHILE STABILITY

Structure and protein sequence studies of extremophiles relate to its stability in comparison to its non-extremophilic counterparts. The sequence generally alters as a part of evolution when the organism inhabits different habitats. As explained in the introduction, various structural factors such as hydrophobicity [10], lengthening or shortening of loops, the pattern of atom packing, increased or decreased surface area, hydrogen bonding [4] and salt bridges [10] attribute to the structural stability of an organism (Fig. **1**). The Boltzmann factor defines the strength of any type of interaction relates to the organism's stability as analyzed by Miyazawa and Jernigan (1985), the advantage of this approach is that the large database of the structures and the strength of temperature-dependent interactions could be studied [27].

For studying the stability in extremophilic protein, hydrophobicity is one of the main stabilizing factors in proteins. It has a direct relationship with the enthalpy and entropy of the system, as, at room temperature where hydrophobicity is found to be maximum, the solvent molecules that are in contact with the non-polar molecules organize themselves in such a way that the enthalpy of the system is reduced and hence the entropy of the solvent decreases [28]. The enthalpy and entropy together contribute to the free energy of stabilization, which has a relationship with the optimum growth temperature of an organism [29]. The relevance of entropy and enthalpy in thermophiles and psychrophiles is mentioned below.

2.1. Thermophiles and Stability

When the temperature is increased above room temperature, as in the case of thermophilicity, hydrophobicity is one of the dominant factors that stabilize proteins of thermophiles [12]. The stability can be provided by a hydrophobic factor of a single protein or due to multiple protein chains. Razvi and Scholtz (2006) found that most thermophiles use the simple method that raises the ΔG at all temperatures as the principal way to increase their melting temperature [30] (Fig. **1**). Perutz and Raidt (1975) compared the structure of ferredoxin from the thermophiles and mesophiles and found that in between the polar groups, extra salt bridges were present in the structure of ferredoxin from thermophiles. Besides this, there were more side chain-hydrogen bonds in thermophilic ferredoxin that jointly increased stability [8]. In glutamate dehydrogenase isolated from thermophile *Pyrococcus furiosus,* salt bridges form a highly stable network and account for the stability of the protein in contrast to the glutamate dehydrogenase from non-thermophile *Clostridium symbiosum*, where the salt bridges form fewer networks [10]. It has been reported that deleted or shortened loops [31], greater rigidity, small surface area to volume ratio, more disulphide bonds, increased intracellular ionic concentrations, increased cationic proteins and supercoiling are proposed mechanisms of increased thermostability [32].

2.2. Psychrophiles and Stability

Similar to thermophiles, psychrophiles also have a sequence and structure relationship. The entropy of the system decreases with the decrease in temperature, explaining the phenomenon of psychrophilicity. For adjusting membrane fluidity in psychrophiles, there should be the presence of unsaturated and branched fatty acids chains, and the length of fatty acids has to be shortened [33]. Psychrophiles synthesize heat-labile and cold-active enzymes that have high enzymatic efficiency and activity at low temperatures. Experimental evidence proves that psychrophilic enzymes catalyze the reaction at lower ΔG values than the reaction catalyzed by its mesophilic counterpart, suggesting that psychrophilic enzymes are more active [34]. It is also found that the active site for the binding of enzymes in the case of psychrophiles is heat-labile in comparison to the mesophilic enzymes. Another assumed structural adaptability for the catalytic action of the psychrophilic enzyme is that the catalytic cavity of psychrophile is comparatively more than mesophiles or thermophiles by deletions of certain residues in loops bordering active site [35] or by the replacement of bulky side

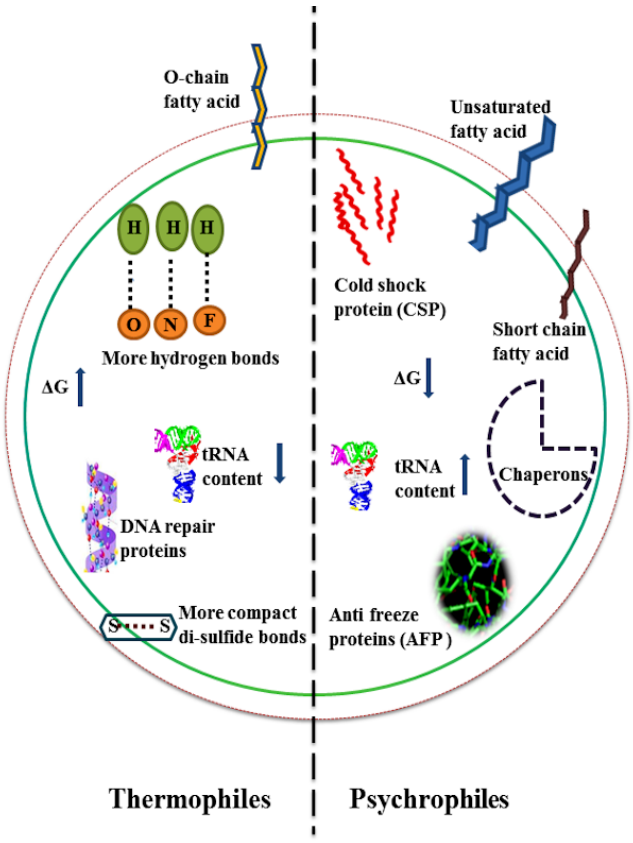

Fig. (1). Stability pattern of thermophiles *vs* psychrophiles. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

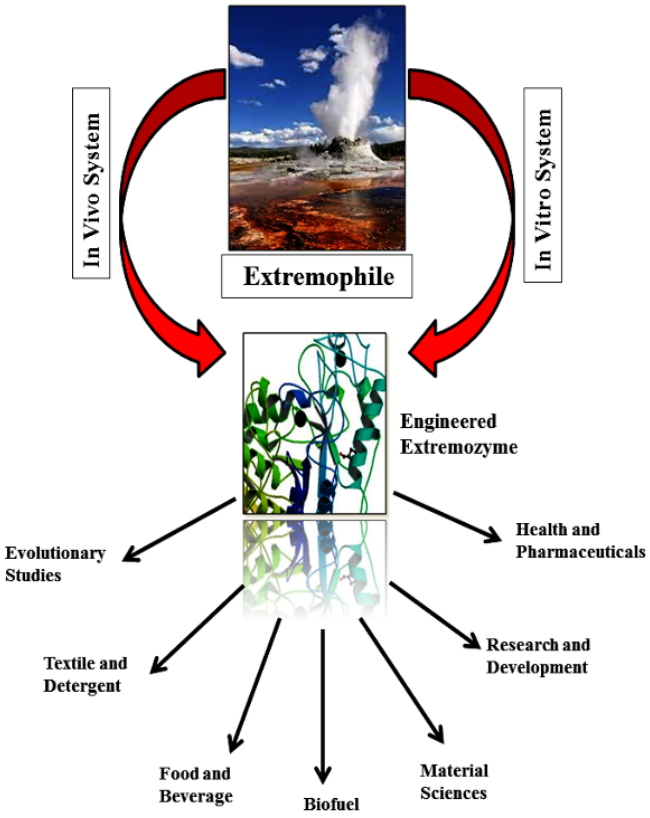

Fig. (2). Applications of extremophiles. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

chains with smaller groups [36], thus making the active site more accessible to ligands. Papaleo *et al.* (2006) noted that the sites of cold stable enzymes not responsible for catalysis are more rigid than the mesophilic homologs [37]. All the weak interactions such as hydrogen bonds, proline content, ion pairs, *etc*. are minimally found and have non-polar core clusters, with weaker hydrophobicity to make the protein interior less compact.

3. AMINO ACID COMPOSITION AND EXTREMOPHILE STABILITY

The composition of amino acid, as reported by several studies, is linearly related to the OGT of an organism [38]. It has been found that thermophilic proteins prefer to contain charged, aromatic and hydrophobic residues compared with its mesophilic proteins. Goldstein, R.A. (2007) analyzed the database of proteins for studying the interaction of amino acids from psychrophiles, thermophiles and hyperthermophiles [12]. When analyzed on the Ramachandran plot, the authors reported that the amino acids of the thermostable protein occur more on the helical and the sheet region [39].

3.1. Thermophiles and Stability

Jaenicke and Bohm (1998) studied the relationship of amino acids and stability of protein at extrinsic temperature. They observed in their study that when thermophiles/hyperthermophiles are kept at a temperature of 100°C or more, Val and Leu are the more thermostable amino acids followed by Ile, Tyr, Lys, His and apparently Asp, Glu, Arg and Cys are the least thermostable of all the common amino acids. They also compared the genome of thermophiles with the specific genes from mesophiles, and it was found that the thermophilic genome encodes for higher charged amino acids and lower polar/uncharged residues. They also observed that at a higher temperature, deamidation of glutamine increased [40]. Goldstein, R.A. (2007) supported the study of Jaenicke and Bohm (1998) and reported that a marked increase in the frequency of aromatic and charged amino acids (Tyr, Phe, Glu, Lys and Arg) was found in thermophiles and hyperthermophiles whereas the frequency of uncharged residues significantly decreased (Gln, Asn, His, Thr) [12]. Similar results were obtained by other workers [41, 42]. Zeldovich *et al.* (2007) also observed a significant rise in the presence of Val, Tyr, Trp, Arg, Glu and Leu in thermophiles [5]. The increase in the frequency of charged residues defines the role of salt bridges in protein stability and its dielectric properties in the case of extremophiles [43]. Russell *et al.* (1994) studied the comparison of the amino acid of citrate synthase from *Thermoplasma acidophilum* which is a thermophile and *Pyrococcus furiosus,* a mesophile and found that as optimum temperature increased Ile, Tyr, Lys and Glu content increased, but Asn, Gln and Cys residue decreased [35].

3.2. Psychrophiles and Stability

The frequency of uncharged polar residues is declined in psychrophiles. Unlike thermophiles, a lesser number of charged and aromatic residues are observed in psychrophiles. Violot *et al.* (2005) observed that when the strength of various interactions is decreased, the flexibility of psychrophilic protein increased [44]. The subsequent rise in the frequency of Gly is notified, which increases the stability of the protein by raising the entropy of the protein system. Metpally and Reddy (2009) found that Ala, Asp, Ser and Thr were preferred significantly while Glu and Leu were less preferred in psychrophiles when compared to mesophilic counterparts [45]. If the frequency of occurrence and alignment of proteins are compared in psychrophiles, it is reported that over aromatic, charged and hydrophilic groups, the tiny and neutral groups of amino acids are preferred. While considering the secondary structural elements, it is analyzed that α-helices of psychrophilic proteomes have fewer residues in comparison to coil regions. Psychrophiles, when grown at low temperature, show the production of cold-acclimation proteins (CAPs) whereas no CAPs are seen when grown at a milder temperature [46]. These CAPs function in maintaining cell cycle and growth of the organism at low temperature, though their functions are not yet fully understood.

4. GENOMIC ADAPTATIONS FOR EXTREMOPHILE STABILITY

Comparative genomics studies on thermophiles, psychrophiles and mesophiles disclose that a series of coordinated changes are linked with an organism's genome thriving at extreme conditions. OGT of an organism could be correlated with the arrangements and frequency of the presence of tRNAs, the G+C content of tRNA genes, secondary structures and its folding patterns. As studied before, the sequence and structure data study for any bacteria (be it hyperthermophile, thermophile, mesophile or psychrophile), revealed proportional linkages between OGT and DNA's dinucleotide compositions.

4.1. Thermophiles and Stability

It is known that the tRNA content of thermophilic organisms is less as compared to the frequency of tRNAs in mesophiles and psychrophiles (Fig. **1**). Dutta and Chaudhuri (2010) reported that tRNAs that were found to be reduced in number amongst thermophiles had the anticodons of hydrophilic (*i.e.,* Asp, Gln, Tyr, Val, Asn, Gly) and few had hydrophobic residues (*i.e.,* Met, Ile, Leu) [7]. Though there was no increase in the number of tRNA amongst any thermophile studied, some tRNAs did not alter (Ala^-, Ser^-, Thr^-, Cys^-, His^-). tRNAs of thermophiles showed greater structural stability than psychrophiles and mesophiles [47]. Experimental results with a group of psychrophilic and thermophilic genomes confirmed that there was a directly proportional correlation between the G+C content of tRNAs and OGT of an organism, though thermophilic genes corresponding to tRNA have been reported to exhibit a much higher G+C content in comparison to psychrophiles and mesophiles [14]. With the increase in temperature, tRNA of psychrophiles tends to fold its structure in such a way that it results in increasing more loops than stems whereas thermophiles remain folded into a structure that is stable at all temperature ranges, and this tendency of extremophiles might be a reason for the stability of RNA's secondary structure at adverse temperatures. Moreover, important to note that the G+C content of ribosomal RNA was linearly correlated with growth temperature, while the G+C content of genomic DNA hardly shows any difference in hyperthermophiles, thermophiles as well as in mesophiles [7, 14]. It is believed that the higher G+C content in

Table 1. Enzymes isolated from thermophilic bacteria and their industrial applications.

Enzyme	Industries Wherein Enzyme is Used	Bacterial Species	References
Lipase	Baking industry, Cosmetic industry, Dairy industry, Detergent industry, Leather industry, Paper industry, Pharmaceutical industry, Pulp industry	*Anoxybacillus flavithermus* WK1 *Geobacillus stearothermophilus* 5 *Geobacillus* sp. SBS-4S *Geobacillus zalihae* sp. Nov *Pseudomonas aeruginosa* BTS-2 *Thermosyntropha lipolytica*	[60] [61] [62] [63] [64] [65]
Protease	Baking industry, Brewing industry, Dairy industry, Detergent industry, Food and Feed, Pharmaceutical and Biotech industry	*Bacillus brevis* *Bacillus* HUTBS62 *Bacillus* HUTBS71 *Bacillus* sp. JB-99 *Bacillus stearothermophilus* *Chaetomium thermophilum* *Geobacillus collagenovorans* MO-1 *Paenibacillus tezpurensis* sp. nov. AS-S24-II *Pyrodictium* sp. *Thermococcus onnurineus* NA1 *Thermus aquaticus* YT-1	[66] [67] [68] [69] [70] [71] [72] [73] [74] [75]
Xylanase	Baking industry, Bioprocessing of fabrics, Biobleaching of pulp, Detergent industry, Paper industry, Pulp industry, Waste paper recycling industry	*Actinomadura* sp. strain Cpt20 *Anoxybacillus kaynarcensis* sp. *Bacillus halodurans* *Bacillus* sp. *Caldocellum saccharolyticum* *Dictyoglomus thermophilum* *Dictyoglomus thermophilum* Rt46B.1 *Geobacillus* sp. MT-1 *Thermoanaerobacterium saccharolyicum* NTOU 1 *Thermomyces lanuginosus*	[76] [77] [78] [79] [80] [81] [82] [83] [84] [85]
α- Amylase	Bakery industry, Cellulose and chitin processing industry, Detergent industry, Textile industry	*Bacillus* sp. *isolate A3-15* *Bacillus thermooleovorans* NP54 *Bacillus stearothermophilus* *Geobacillus stearothermophilus* *Halothermothrix orenii* *Streptomyces* sp. TO1	[86] [87] [88] [89] [90] [91]
β- Glucosidase	Biofuel industry, Biorefining industry, Brewing industry, Chemical industry	*Aureobasidium pullulans* *Bacillus thuringiensis* *Fervidobacterium islandicum* *Thermoanaerobacter brockii*	[92] [93] [94] [95]

(Table 1) contd….

Enzyme	Industries Wherein Enzyme is Used	Bacterial Species	References
Chitinase	Agriculture industry,	*Aeromonas* sp. DYU-Too7	[96]
	Chitin modification for food and health products	*Bacillus licheniformis*	[97]
		Bacillus sp. HSA,3-1a	[98]
		Bacillus sp. Hu1	[99]
		Microbispora sp. V2	[100]
		Ralstonia sp. A-471	[101]
		Silanimonas lenta	[102]
		Streptomyces roseolilacinus	
Cellulase	Agriculture industry,	*Acidothermus cellulolyticus*	[103]
	Animal Feed industry,	*Clostridium thermocellum*	[104]
	Bioethanol industry,	*Clostridium thermocellum* Cel9I	[105]
	Brewing industry,		
	Paper	*Geobacillus pallidus*	[106]
	processing industry,	*Moorella* sp.F21	[107]
	Pulp industry,	*Rhodothermus marinus*	[108]
	Textile industry,	*Thermobifida fusca* Cel9A	[105]
	Wine industry		
Esterase	Detergent industry,	*Alicyclobacillus acidocaldarius*	[109]
	Food industry,	*Anoxybacillus gonensis* A4	[110]
	Paper industry,	*Anoxybacillus gonensis* G2	[111]
	Pharmaceutical industry	*Bacillus circulans*	[112]
		Caldocellum saccharolyticum	[113]
		Fervidobacterium nodosum Rt17-B1	[114]
		Thermobacillus xylanilyticus	[115]
		Thermus sp. P1074	[116]
		Thermus thermophilus HB27	[117]
Urease	Agriculture industry,	*Bacillus* sp. strain TB-90	[118]
	Automobile industry,	*Campylobacter laridis*	[119]
	Biofuel and	*Campylobacter larus*	[120]
	Chemical industry,	*Campylobacter sputorum biovar paraureolyticus*	[121]
	Crop, Biorefinig	*Campylobacter* sp.	[122]
		Streptococcus sp.	[123]

thermophiles might be a mechanism by which the organism facilitates intramolecular stabilization of the RNA secondary structure. As discussed, a direct connection subsists between DNA's dinucleotide compositions and growth temperature, so changes in dinucleotides are way good than alterations in mononucleotides (G+C content). The reason for this is that altering the dinucleotide sequence may produce different sets of DNA sequences, those codes for amino acids [14]. It is also proposed that all the genes and proteins might acquire stability toward temperature in bacteria, and for this, the dinucleotide composition of DNA would be biased in such a direction that an overall increase in charged residues occurred at the protein level.

When the uracil content of 16S rRNA was considered, it was found that it had an inverse correlation with OGT, *i.e.,* as the temperature increased, A:U base pair content of RNA decreased [48]. Thus, 16S rRNA's uracil content acted as a useful reader of OGT in thermophiles and psychrophiles. It is found that rRNA stems also had some mismatched base pairs and on the thermodynamic grounds, as the temperature increased, G:U mismatches that are less stable are selected

Table 2. Enzymes isolated from psychrophilic bacteria and their industrial applications.

Enzyme	Industries Wherein it is Used	Bacterial Species	References
Lipase	Baking industry,	*Acinetobacter calcoaceticus* LP009	[124]
	Cosmetic industry,	*Acinetobacter* sp. RAG-1	[125]
	Dairy industry,	*Moraxella* sp.	[126]
	Detergent industry,	*Pseudomonas aeruginosa NCIM 2036*	[127]
	Leather industry,	*Pseudomonas* sp. B11-1	[128]
	Paper industry,		
	Pharmaceutical industry,	*Psychrobacter glacincola*	[129]
	Pulp industry	*Psychrobacter immobilis* B10	[130]
		Psychrobacter sp. TA144	[131]
		Psychrobacter okhotskensis	[132]
α-Amylase	Bakery industry,	*Aeromonas veronii* NS07	[133]
	Cellulose and chitin processing industry,	*Alteromonas haloplanktis*	[134]
	Detergent industry,		
	Textile industry,	*Pseudoalteromonas haloplanktis*	[135, 136]
	Wine industry	*Pseudoalteromonas haloplanktis* TAC 125	[137]
Protease	Baking industry,	*Alteromonas* sp.	[138]
	Brewing industry,	*Bacillus sp.*	[126]
	Dairy industry,	*Bacillus sp. 158*	[139]
	Detergent industry,	*Clostridium* sp. LP3	[140]
	Food and feed,	*Colwellia* sp. NJ341	[141]
	Pharmaceutical and Biotech industry	*Clostridium schirmacherense*	[140]
		Flavobacterium YS-80	[142]
		Halomonas sp.	[138]
		Pseudoalteromonas sp. NJ276	[141]
		Pseudomonas strain DY-A	[143]
		Pseudomonas fluorescens 114	[144]
		Streptomyces sp.	[145]
		Rheinheimera sp.	[138]
β-galactosidase	Biofuel,	*Arthrobacter psychrolactophilus*	[146]
	Biorefining,	*Arthrobacter sp. SB*	[147]
	Brewing industry,	*Bacillus* sp.	[54]
	Chemical industry	*Bacillus subtilis* KL88	[148]
		Carnobacterium piscicola BA	[149]
		Paenibacillus sp. strain C7	[150]
		Pedobacter cryoconitis	[151]
		Pseudoalteromonas haloplanktis	[152]
		Shewanella atlantica	[153]
		Shewanella canadensis	

(Table 2) contd….

Enzyme	Industries Wherein it is Used	Bacterial Species	References
Xylanase	Baking industry, Bioprocessing of fabrics, Biobleaching of pulp, Detergent industry, Fruit juice processing, Paper industry, Pulp industry, Waste paper recycling industry	*Clostridium* strain PXYL1 *Flavobacterium* sp. *Flavobacterium frigidarium* *Glaciecola mesophila* KMM 241 *Paenibacillus curdlanolyticus* B6 *Paenibacillus* sp. KIJ1 *Pseudoalteromonas haloplanktis*	[154] [155] [156] [157] [158] [159] [160]
Cellulase	Agriculture industry, Animal feed industry, Bioethanol industry, Brewing industry, Fruit juice processing, Paper processing industry, Pulp industry, Textile industry, Wine industry	*Arthrobacter* sp. *Cadophora malorum* *Fibrobacter succinogene* *Flavobacterium* sp. *Geomyces* sp. *Paenibacillus* sp. *Pedobacter* sp. *Pseudoalteromonas haloplanktis* *Rhodotorula glutinis* *Shewanella* sp. *G5*	[161] [162] [163]
Chitinase	Chitin modification for food and health products	*Aeromonas veronii* CD3 *Alteromonas* sp. strain O-7 *Arthrobacter* sp. TAD20 *Glaciozyma antarctica* PI12 *Moritella marina* *Verticillium lecanii* A3 *Vibrio* sp. Fi:7	[164] [165] [166] [167] [168] [169] [170]
Esterase	Detergent industry, Food industry, Paper industry, Pharmaceutical industry,	*Oleispira antarctica* RB8 *Oleispira antarctica* *Pseudoalteromonas arctica* *Pseudomonas* sp. B11-1 *Psychrobacter* sp. Ant 300 *Streptomyces coelicolor* A3	[171] [172] [173] [174] [175] [176]

over A:U pairs, which are more stable [49]. Studies have been conducted to see if changing the content of uracil has any relationship with rRNA stability as RNAs show sensitivity to chemical hydrolysis at a temperature of more than 50ºC [50]. For the stability of RNAs at low temperature, Dalluge *et al*. [51] observed various modifications that maintained the conformational flexibility of RNA.

4.2. Psychrophiles and Stability

In psychrophiles, it is found that the tRNA content is higher in comparison to its thermophilic counterpart (Fig. **1**). As the temperature of the psychrophilic organism is increased, its tRNA folds in an unstable structure that has more loops than stems. It is also observed that the low temperature strengthens the relationship between the double helix and the supercoiled state of DNA. The presence of nucleic acid-binding protein, which plays a central role in relieving the adverse effects of low temperature on psychrophiles, has been analyzed [51, 52].

5. APPLICATIONS REVEALED BY EXPLORING EXTREMOPHILES

The isolation and characterization of the extremophilic prokaryotes have proven that these organisms have engrossing metabolic features and fascinating evolutionary past [53]. The novel methods for extremophile's isolation, characterization and the utilization of molecular tools for analyzing and understanding their phylogeny and diversity paved the way for searching organisms with diverse applications in research, health, evolution and biotech industries (Fig. **2**).

Lately, a novel perspective towards genome sequencing has opened the doors for the investigation of these magical organisms for various academic, researches, industrial and biotechnological applications. Most organisms, specifically pointing hyperthermophiles and thermophiles, inhabit closely to "universal ancestor" of all extant of life on Earth. Thus, their origin under stressed conditions would give useful hints on the evolution of our planet and, likewise, others in the universe. Extremophiles, as explained in the introduction, produce extremely stable enzymes known as extremozymes, whose catalytic properties are used in conditions that were initially nominated as unsuited and harsh [54]. Extremozymes are of particular interest in several industrial processes like baking, saccharification [55], detergent, pharmaceutical, food, beverage or textile industries [56] (Fig. **2**). These enzymes are evolved by nature and do not require manipulations to get adapt to temperature, solvent-tolerance, or other extremities in comparison to enzymes tailored synthetically from non-extremophiles. Some examples of enzymes isolated from thermophiles and psychrophiles, along with their sources and the industries wherein they are used, are listed in Tables **1** and **2**, respectively.

Extremophiles have also shown applications in the production of single-cell protein (SCP), bioremediation, petroleum industry, biomining, biosensors, medicines and antibiotic production. Raja *et al.*, (2010), isolated *Dactyl sporangium* from Rohtang hill soil and found that it produces a proteinaceous substance, which showed antimicrobial activity against streptomyces and thus acted as an antibiotic [57]. The species of *Bacillus* and *Geobacillus* that can degrade hydrocarbon can be effectively used for bioremediation.

FUTURE WORK AND CONCLUDING REMARKS

In summary, current knowledge of extremophiles is scarce, and very few of them are cultivable in the defined environment. More development in gene expression studies, like developing new heterologous systems, will increase the investigation of microbial diversity [58]. More novel extremozymes with diverse catalytic activity can be isolated, characterized and purified if their gene expression libraries are screened with fast and accurate detection technologies. Studies are going on to discover microbial communities in the environment, once considered to be harsh for any form of life to exist [59]. With the advancements of new techniques, remote areas such as ice-covered ocean and deep-sea could be explored; organism could be tapped at structural, genomic and proteomic levels, and could be exploited to provide a precious resource for biotechnology and industry.

LIST OF ABBREVIATIONS

OGT	=	Optimum Growth Temperature
DNA	=	Deoxy Ribonucleic Acid
RNA	=	Ribonucleic Acid

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The study was supported by the Department of Science and Technology (DST) SERB (Grant no. PDF/2017/001367) and DST-FIST (Grant no. SR/FST/LSI-590/2014), Govt. of India.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

The authors thanks Science and Engineering Research Board, Department of Science and Technology, Government of India and Amity University, Noida, Uttar Pradesh for the assistance provided.

REFERENCES

[1] Singh, P.; Jain, K.; Desai, C. *Microbial Community Dynamics of Extremophiles/Extreme Environment*; Elsevier Inc., **2019**. http://dx.doi.org/10.1016/B978-0-12-814849-5.00018-6

[2] Orellana, R.; Macaya, C.; Bravo, G.; Dorochesi, F.; Cumsille, A.; Valencia, R.; Rojas, C.; Seeger, M. Living at the frontiers of life: extremophiles in chile and their potential for bioremediation. *Front. Microbiol.,* **2018**, *9*, 2309. http://dx.doi.org/10.3389/fmicb.2018.02309 PMID: 30425685

[3] Kumar, S.; Dangi, A.K.; Shukla, P.; Baishya, D.; Khare, S.K. Thermozymes: adaptive strategies and tools for their biotechnological applications. *Bioresour. Technol.,* **2019**, *278*, 372-382. http://dx.doi.org/10.1016/j.biortech.2019.01.088 PMID: 30709766

[4] Vogt, G.; Woell, S.; Argos, P. Protein thermal stability, hydrogen bonds, and ion pairs. *J. Mol. Biol.,* **1997**, *269*(4), 631-643. http://dx.doi.org/10.1006/jmbi.1997.1042 PMID: 9217266

[5] Zeldovich, K.B.; Berezovsky, I.N.; Shakhnovich, E.I. Protein and DNA sequence determinants of thermophilic adaptation. *PLoS Comput Biol,* **2007**, *3*, 0062-0072. http://dx.doi.org/10.1371/journal.pcbi.0030005

[6] Satapathy, S.S.; Dutta, M.; Ray, S.K. Higher tRNA diversity in thermophilic bacteria: a possible adaptation to growth at high temperature. *Microbiol. Res.,* **2010**, *165*(8), 609-616. http://dx.doi.org/10.1016/j.micres.2009.12.003 PMID: 20172701

[7] Dutta, A.; Chaudhuri, K. Analysis of tRNA composition and folding in psychrophilic, mesophilic and thermophilic genomes: indications for thermal adaptation. *FEMS Microbiol. Lett.,* **2010**, *305*(2), 100-108. http://dx.doi.org/10.1111/j.1574-6968.2010.01922.x PMID: 20659165

[8] Perutz, M.F.; Raidt, H. Stereochemical basis of heat stability in bacterial ferredoxins and in haemoglobin A2. *Nature,* **1975**, *255*(5505), 256-259. http://dx.doi.org/10.1038/255256a0 PMID: 1143325

[9] Weaver, L.H.; Kester, W.R.; Ten Eyck, L.F.; Matthews, B.W. *The Structure and Stability of Thermolysin*. *Experientia Suppl.*, **1976**, *26*, 31-39. http://dx.doi.org/10.1007/978-3-0348-7675-9_2

[10] Kumar, S.; Tsai, C.J.; Nussinov, R. Factors enhancing protein thermostability. *Protein Eng.,* **2000**, *13*(3), 179-191. http://dx.doi.org/10.1093/protein/13.3.179 PMID: 10775659

[11] Jaenicke, R. Stability and stabilization of globular proteins in solution. *J. Biotechnol.,* **2000**, *79*(3), 193-203. http://dx.doi.org/10.1016/S0168-1656(00)00236-4 PMID: 10867180

[12] Goldstein, R.A. Amino-acid interactions in psychrophiles, mesophiles, thermophiles, and hyperthermophiles: insights from the quasi-chemical approximation. *Protein Sci.,* **2007**, *16*(9), 1887-1895. http://dx.doi.org/10.1110/ps.072947007 PMID: 17766385

[13] Kreil, D.P.; Ouzounis, C.A. Identification of thermophilic species by the amino acid compositions deduced from their genomes. *Nucleic Acids Res.,* **2001**, *29*(7), 1608-1615. http://dx.doi.org/10.1093/nar/29.7.1608 PMID: 11266564

[14] Nakashima, H.; Fukuchi, S.; Nishikawa, K. Compositional changes in RNA, DNA and proteins for bacterial adaptation to higher and lower temperatures. *J. Biochem.,* **2003**, *133*(4), 507-513. http://dx.doi.org/10.1093/jb/mvg067 PMID: 12761299

[15] Singer, G.A.C.; Hickey, D.A. Thermophilic prokaryotes have characteristic patterns of codon usage, amino acid composition and nucleotide content. *Gene,* **2003**, *317*(1-2), 39-47. http://dx.doi.org/10.1016/S0378-1119(03)00660-7 PMID: 14604790

[16] Galtier, N.; Lobry, J.R. Relationships between genomic G+C content, RNA secondary structures, and optimal growth temperature in prokaryotes. *J. Mol. Evol.,* **1997**, *44*(6), 632-636. http://dx.doi.org/10.1007/PL00006186 PMID: 9169555

[17] Unsworth, L.D.; van der Oost, J.; Koutsopoulos, S. Hyperthermophilic enzymes--stability, activity and implementation strategies for high temperature applications. *FEBS J.,* **2007**, *274*(16), 4044-4056. http://dx.doi.org/10.1111/j.1742-4658.2007.05954.x PMID: 17683334

[18] Hollien, J.; Marqusee, S. Structural distribution of stability in a thermophilic enzyme. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*(24), 13674-13678. http://dx.doi.org/10.1073/pnas.96.24.13674 PMID: 10570131

[19] Gerday, C. Psychrophily and catalysis. *Biology (Basel),* **2013**, *2*(2), 719-741. http://dx.doi.org/10.3390/biology2020719 PMID: 24832805

[20] Moon, S.; Kim, J.; Koo, J.; Bae, E. Structural and mutational analyses of psychrophilic and mesophilic adenylate kinases highlight the role of hydrophobic interactions in protein thermal stability. *Struct. Dyn.,* **2019**, *6*(2), 024702. http://dx.doi.org/10.1063/1.5089707 PMID: 31111079

[21] Hawwa, R.; Aikens, J.; Turner, R.J.; Santarsiero, B.D.; Mesecar, A.D. Structural basis for thermostability revealed through the identification and characterization of a highly thermostable phosphotriesterase-like lactonase from Geobacillus stearothermophilus. *Arch. Biochem. Biophys.,* **2009**, *488*(2), 109-120. http://dx.doi.org/10.1016/j.abb.2009.06.005 PMID: 19615330

[22] Koga, Y. Thermal adaptation of the archaeal and bacterial lipid membranes. *Archaea,* **2012**, *2012*, 789652. http://dx.doi.org/10.1155/2012/789652 PMID: 22927779

[23] Vinçon-Laugier, A.; Cravo-Laureau, C.; Mitteau, I.; Grossi, V. Temperature-dependent alkyl glycerol ether lipid composition of mesophilic and thermophilic sulfate-reducing bacteria. *Front. Microbiol.,* **2017**, *8*, 1532. http://dx.doi.org/10.3389/fmicb.2017.01532 PMID: 28848536

[24] Javed, M.M.; Zahoor, S.; Sabar, H. Thermophilic bacteria from the hot springs of Gilgit (Pakistan). *J. Anim. Plant Sci.,* **2012**, *22*, 83-87.

[25] Friedrich, A.B.; Antranikian, G. Keratin degradation by *Fervidobacterium pennavomns*, a novel thermophilic anaerobic species of the order thermotogales. *Appl. Environ. Microbiol.,* **1996**, *62*(8), 2875-2882. http://dx.doi.org/10.1128/AEM.62.8.2875-2882.1996 PMID: 16535379

[26] Arora, N.K.; Panosyan, H. Extremophiles: applications and roles in environmental sustainability. *Environ Sustain,* **2019**, *2*, 217-218. http://dx.doi.org/10.1007/s42398-019-00082-0

[27] Finkelstein, A.V.; Badretdinov AYa, ; Gutin, A.M. Why do protein architectures have Boltzmann-like statistics? *Proteins,* **1995**, *23*(2), 142-150. http://dx.doi.org/10.1002/prot.340230204 PMID: 8592696

[28] Greaves, R.B.; Warwicker, J. Mechanisms for stabilisation and the maintenance of solubility in proteins from thermophiles. *BMC Struct. Biol.,* **2007**, *7*, 18. http://dx.doi.org/10.1186/1472-6807-7-18 PMID: 17394655

[29] Gianese, G.; Bossa, F.; Pascarella, S. Comparative structural analysis of psychrophilic and meso- and thermophilic enzymes. *Proteins,* **2002**, *47*(2), 236-249. http://dx.doi.org/10.1002/prot.10084 PMID: 11933070

[30] Razvi, A.; Scholtz, J.M. Lessons in stability from thermophilic proteins. *Protein Sci.,* **2006**, *15*(7), 1569-1578. http://dx.doi.org/10.1110/ps.062130306 PMID: 16815912

[31] Thompson, M.J.; Eisenberg, D. Transproteomic evidence of a loop-deletion mechanism for enhancing protein thermostability. *J. Mol. Biol.,* **1999**, *290*(2), 595-604. http://dx.doi.org/10.1006/jmbi.1999.2889 PMID: 10390356

[32] Daniel, R.M.; Cowan, D.A. Biomolecular stability and life at high temperatures. *Cell. Mol. Life Sci.,* **2000**, *57*(2), 250-264. http://dx.doi.org/10.1007/PL00000688 PMID: 10766021

[33] van den Burg, B. Extremophiles as a source for novel enzymes. *Curr. Opin. Microbiol.,* **2003**, *6*(3), 213-218. http://dx.doi.org/10.1016/S1369-5274(03)00060-2 PMID: 12831896

[34] Lonhienne, T.; Gerday, C.; Feller, G. Psychrophilic enzymes: revisiting the thermodynamic parameters of activation may explain local flexibility. *Biochim. Biophys. Acta,* **2000**, *1543*(1), 1-10. http://dx.doi.org/10.1016/S0167-4838(00)00210-7 PMID: 11087936

[35] Russell, R.J.; Hough, D.W.; Danson, M.J.; Taylor, G.L. The crystal structure of citrate synthase from the thermophilic archaeon, *Thermoplasma acidophilum*. *Structure,* **1994**, *2*(12), 1157-1167. http://dx.doi.org/10.1016/S0969-2126(94)00118-9 PMID: 7704526

[36] Aghajari, N.; Van Petegem, F.; Villeret, V.; Chessa, J.P.; Gerday, C.; Haser, R.; Van Beeumen, J. Crystal structures of a psychrophilic metalloprotease reveal new insights into catalysis by cold-adapted proteases. *Proteins,* **2003**, *50*(4), 636-647. http://dx.doi.org/10.1002/prot.10264 PMID: 12577270

[37] Papaleo, E.; Riccardi, L.; Villa, C.; Fantucci, P.; De Gioia, L. Flexibility and enzymatic cold-adaptation: a comparative molecular dynamics investigation of the elastase family. *Biochim. Biophys. Acta,* **2006**, *1764*(8), 1397-1406. http://dx.doi.org/10.1016/j.bbapap.2006.06.005 PMID: 16920043

[38] Szilágyi, A.; Závodszky, P. Structural differences between mesophilic, moderately thermophilic and extremely thermophilic protein subunits: results of a comprehensive survey. Structure, **2000**, *8*(5), 493-504.

[39] Panja, A.S.; Maiti, S.; Bandyopadhyay, B. Protein stability governed by its structural plasticity is inferred by physicochemical factors and salt bridges. *Sci. Rep.,* **2020**, *10*(1), 1822. http://dx.doi.org/10.1038/s41598-020-58825-7 PMID: 32020026

[40] Jaenicke, R.; Böhm, G. The stability of proteins in extreme environments. *Curr. Opin. Struct. Biol.,* **1998**, *8*(6), 738-748. http://dx.doi.org/10.1016/S0959-440X(98)80094-8 PMID: 9914256

[41] Haney, P.J.; Badger, J.H.; Buldak, G.L.; Reich, C.I.; Woese, C.R.; Olsen, G.J. Thermal adaptation analyzed by comparison of protein sequences from mesophilic and extremely thermophilic *Methanococcus* species. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*(7), 3578-3583. http://dx.doi.org/10.1073/pnas.96.7.3578 PMID: 10097079

[42] Chakravarty, S.; Varadarajan, R. Elucidation of factors responsible for enhanced thermal stability of proteins: a structural genomics based study. *Biochemistry,* **2002**, *41*(25), 8152-8161. http://dx.doi.org/10.1021/bi025523t PMID: 12069608

[43] Zhou, H.X.; Dong, F. Electrostatic contributions to the stability of a thermophilic cold shock protein. *Biophys. J.,* **2003**, *84*(4), 2216-2222. http://dx.doi.org/10.1016/S0006-3495(03)75027-9 PMID: 12668430

[44] Violot, S.; Aghajari, N.; Czjzek, M.; Feller, G.; Sonan, G.K.; Gouet, P.; Gerday, C.; Haser, R.; Receveur-Bréchot, V. Structure of a full length psychrophilic cellulase from *Pseudoalteromonas haloplanktis* revealed by X-ray diffraction and small angle X-ray scattering. *J. Mol. Biol.,* **2005**, *348*(5), 1211-1224. http://dx.doi.org/10.1016/j.jmb.2005.03.026 PMID: 15854656

[45] Metpally, R.P.R.; Reddy, B.V.B. Comparative proteome analysis of psychrophilic *versus* mesophilic bacterial species: Insights into the molecular basis of cold adaptation of proteins. *BMC Genomics,* **2009**, *10*, 11. http://dx.doi.org/10.1186/1471-2164-10-11 PMID: 19133128

[46] Berger, F.; Morellet, N.; Menu, F.; Potier, P. Cold shock and cold acclimation proteins in the psychrotrophic bacterium *Arthrobacter globiformis* SI55. *J. Bacteriol.,* **1996**, *178*(11), 2999-3007. http://dx.doi.org/10.1128/JB.178.11.2999-3007.1996 PMID: 8655472

[47] Stepanov, V.G.; Nyborg, J. Thermal stability of aminoacyl-tRNAs in aqueous solutions. *Extremophiles,* **2002**, *6*(6), 485-490. http://dx.doi.org/10.1007/s00792-002-0285-4 PMID: 12486457

[48] Khachane, A.N.; Timmis, K.N.; dos Santos, V.A.P.M. Uracil content of 16S rRNA of thermophilic and psychrophilic prokaryotes correlates inversely with their optimal growth temperatures. *Nucleic Acids Res.,* **2005**, *33*(13), 4016-4022. http://dx.doi.org/10.1093/nar/gki714 PMID: 16030352

[49] Varani, G.; Mcclain, W.H. The G-U wobble base pair diverse biological systems. *EMBO Rep.,* **2000**, *1*, 18-23. http://dx.doi.org/10.1093/embo-reports/kvd001 PMID: 11256617

[50] Poole, A.M.; Jeffares, D.C.; Penny, D. The path from the RNA world. *J. Mol. Evol.,* **1998**, *46*(1), 1-17. http://dx.doi.org/10.1007/PL00006275 PMID: 9419221

[51] Dalluge, J.J.; Hamamoto, T.; Horikoshi, K.; Morita, R.Y.; Stetter, K.O.; McCloskey, J.A. Posttranscriptional modification of tRNA in psychrophilic bacteria. *J. Bacteriol.,* **1997**, *179*(6), 1918-1923. http://dx.doi.org/10.1128/JB.179.6.1918-1923.1997 PMID: 9068636

[52] Michel, V.; Lehoux, I.; Depret, G.; Anglade, P.; Labadie, J.; Hebraud, M. The cold shock response of the psychrotrophic bacterium Pseudomonas fragi involves four low-molecular-mass nucleic acid-binding proteins. *J. Bacteriol.,* **1997**, *179*(23), 7331-7342. http://dx.doi.org/10.1128/JB.179.23.7331-7342.1997 PMID: 9393697

[53] Podar, M.; Reysenbach, A.L. New opportunities revealed by biotechnological explorations of extremophiles. *Curr. Opin. Biotechnol.,* **2006**, *17*(3), 250-255. http://dx.doi.org/10.1016/j.copbio.2006.05.002 PMID: 16701993

[54] Dhaked, R.K.; Alam, S.I.; Singh, L. Characterization of β-galactosidase from an Antarctic *Bacillus* sp. *Indian J. Biotechnol.,* **2005**, *4*, 227-231.

[55] Kohli, I.; Rakesh Tuli, V.P.S. Purification and characterization of maltose forming thermostable alkaline α-Amylase from *Bacillus gibsonii* S213. *Int. J. Adv. Res.,* **2016**, *4*, 356-366.

[56] Elleuche, S.; Schröder, C.; Sahm, K.; Antranikian, G. Extremozymes--biocatalysts with unique properties from extremophilic microorganisms. *Curr. Opin. Biotechnol.,* **2014**, *29*, 116-123. http://dx.doi.org/10.1016/j.copbio.2014.04.003 PMID: 24780224

[57] Raja, A.; Prabakaran, P.; Gajalakshmi, P. Isolation and screening of antibiotic producing psychrophilic actinomycetes and its nature from rothang hill soil against viridans *Streptococcus* sp. *Res. J. Microbiol.,* **2010**, *5*, 44-49. http://dx.doi.org/10.3923/jm.2010.44.49

[58] Tung, H.C.; Bramall, N.E.; Price, P.B. Microbial origin of excess methane in glacial ice and implications for life on Mars. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*(51), 18292-18296. http://dx.doi.org/10.1073/pnas.0507601102 PMID: 16339015

[59] Steven, B.; Léveillé, R.; Pollard, W.H.; Whyte, L.G. Microbial ecology and biodiversity in permafrost. *Extremophiles,* **2006**, *10*(4), 259-267. http://dx.doi.org/10.1007/s00792-006-0506-3 PMID: 16550305

[60] Zuridah, H. Identification of lipase producing thermophilic bacteria from Malaysian hot springs. *Afr. J. Microbiol. Res.,* **2011**, *5*, 3569-3573. http://dx.doi.org/10.5897/AJMR11.777

[61] Sifour, M.; Saeed, H.M.; Zaghloul, T.L. Isolation of lipase gene of the thermophilic *Geobacillus stearothermophilus* strain-5. *Biotechnology (Faisalabad),* **2010**, *9*, 55-60. http://dx.doi.org/10.3923/biotech.2010.55.60

[62] Tayyab, M.; Rashid, N.; Akhtar, M. Isolation and identification of lipase producing thermophilic *Geobacillus* sp. SBS-4S: cloning and characterization of the lipase. *J. Biosci. Bioeng.,* **2011**, *111*(3), 272-278. http://dx.doi.org/10.1016/j.jbiosc.2010.11.015 PMID: 21185780

[63] Abd Rahman, R.N.; Leow, T.C.; Salleh, A.B.; Basri, M. *Geobacillus zalihae* sp. nov., a thermophilic lipolytic bacterium isolated from palm oil mill effluent in Malaysia. *BMC Microbiol.,* **2007**, *7*, 77. http://dx.doi.org/10.1186/1471-2180-7-77 PMID: 17692114

[64] Kanwar, S.S.; Gupta, M.; Gupta, R. Properties of hydrogel-entrapped lipase of thermophilic *Pseudomonas aeruginosa* BTS-2. *Indian J. Biotechnol.,* **2006**, *5*, 292-297.

[65] Salameh, M.A.; Wiegel, J. Purification and characterization of two highly thermophilic alkaline lipases from *Thermosyntropha lipolytica*. *Appl. Environ. Microbiol.,* **2007**, *73*(23), 7725-7731. http://dx.doi.org/10.1128/AEM.01509-07 PMID: 17933930

[66] Banerjee, U.C.; Sani, R.K.; Azmi, W.; Soni, R. Thermostable alkaline protease from *Bacillus brevis* and its characterization as a laundry detergent additive. *Process Biochem.,* **1999**, *35*, 213-219. http://dx.doi.org/10.1016/S0032-9592(99)00053-9

[67] Program, S.; Arabia, S. Phenotypic and protease purification of two different thermophilic Bacillus strains *HUTBS71* and *HUTBS62*. *Ann. Biol. Res.,* **2012**, *3*(4)*,* 1747-1756.

[68] Johnvesly, B.; Naik, G.R. Studies on production of thermostable alkaline protease from thermophilic and alkaliphilic *Bacillus* sp. JB-99 in a chemically defined medium. *Process Biochem.,* **2001**, *37*, 139-144. http://dx.doi.org/10.1016/S0032-9592(01)00191-1

[69] do Nascimento, W.C.A.; Martins, M.L.L. Production and properties of an extracellular protease from thermophilic *Bacillus* sp. *Braz. J. Microbiol.,* **2004**, *35*, 91-96. http://dx.doi.org/10.1590/S1517-83822004000100015

[70] Li, A.N.; Li, D.C. Cloning, expression and characterization of the serine protease gene from *Chaetomium thermophilum*. *J. Appl. Microbiol.,* **2009**, *106*(2), 369-380. http://dx.doi.org/10.1111/j.1365-2672.2008.04042.x PMID: 19200305

[71] Itoi, Y.; Horinaka, M.; Tsujimoto, Y.; Matsui, H.; Watanabe, K. Characteristic features in the structure and collagen-binding ability of a thermophilic collagenolytic protease from the thermophile *Geobacillus collagenovorans* MO-1. *J. Bacteriol.,* **2006**, *188*(18), 6572-6579. http://dx.doi.org/10.1128/JB.00767-06 PMID: 16952949

[72] Rai, S.K.; Roy, J.K.; Mukherjee, A.K. Characterisation of a detergent-stable alkaline protease from a novel thermophilic strain *Paenibacillus tezpurensis* sp. nov. AS-S24-II. *Appl. Microbiol. Biotechnol.,* **2010**, *85*(5), 1437-1450. http://dx.doi.org/10.1007/s00253-009-2145-y PMID: 19669756

[73] Antranikan, G.; Bertoldo, C. Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. *Curr. Opin. Chem. Biol.,* **2002**, *6*(2), 151-160. https://doi.org/10.1016/S1367-5931(02)00311-3

[74] Choi, S.Y.C.; Oh, S.; Yeol, K. Enrichment and proteome analysis of a hyperthermostable protein set of archaeon Thermococcus onnurineus NA1. **2011**, *15*, 451-461. http://dx.doi.org/10.1007/s00792-011-0376-1

[75] Sakaguchi, M.; Takezawa, M.; Nakazawa, R.; Nozawa, K.; Kusakawa, T.; Nagasawa, T.; Sugahara, Y.; Kawakita, M. Role of disulphide bonds in a thermophilic serine protease aqualysin I from *Thermus aquaticus* YT-1. *J. Biochem.,* **2008**, *143*(5), 625-632. http://dx.doi.org/10.1093/jb/mvn007 PMID: 18216068

[76] Taibi, Z.; Saoudi, B.; Boudelaa, M.; Trigui, H.; Belghith, H.; Gargouri, A.; Ladjama, A. Purification and biochemical characterization of a highly thermostable xylanase from Actinomadura sp. strain Cpt20 isolated from poultry compost. *Appl. Biochem. Biotechnol.,* **2012**, *166*(3), 663-679. http://dx.doi.org/10.1007/s12010-011-9457-y PMID: 22161140

[77] Inan, K.; Belduz, A.O.; Canakci, S. *Anoxybacillus kaynarcensis* sp. nov., a moderately thermophilic, xylanase producing bacterium. *J. Basic Microbiol.,* **2013**, *53*(5), 410-419. http://dx.doi.org/10.1002/jobm.201100638 PMID: 22736500

[78] Kumar, V.; Satyanarayana, T. Applicability of thermo-alkali-stable and cellulase-free xylanase from a novel thermo-halo-alkaliphilic *Bacillus halodurans* in producing xylooligosaccharides. *Biotechnol. Lett.,* **2011**, *33*(11), 2279-2285. http://dx.doi.org/10.1007/s10529-011-0698-1 PMID: 21750994

[79] Annamalai, N.; Thavasi, R.; Jayalakshmi, S.; Balasubramanian, T. Thermostable and alkaline tolerant xylanase production by *Bacillus subtilis* isolated form marine environment. *Indian J. Biotechnol.,* **2009**, *8*, 291-297.

[80] Lüthi, E.; Reif, K.; Jasmat, N.B.; Bergquist, P.L. *In vitro* mutagenesis of a xylanase from the extreme thermophile *Caldocellum saccharolyticum*. *Appl. Microbiol. Biotechnol.,* **1992**, *36*(4), 503-506. http://dx.doi.org/10.1007/BF00170192 PMID: 1368204

[81] Borkhardt, B.; Harholt, J.; Ulvskov, P.; Ahring, B.K.; Jørgensen, B.; Brinch-Pedersen, H. Autohydrolysis of plant xylans by apoplastic expression of thermophilic bacterial endo-xylanases. *Plant Biotechnol. J.,* **2010**, *8*(3), 363-374. http://dx.doi.org/10.1111/j.1467-7652.2010.00506.x PMID: 20384855

[82] Zhang, W.; Lou, K.; Li, G. Expression and characterization of the *Dictyoglomus thermophilum* Rt46B.1 xylanase gene (xynB) in *Bacillus subtilis*. *Appl. Biochem. Biotechnol.,* **2010**, *160*(5), 1484-1495. http://dx.doi.org/10.1007/s12010-009-8634-8 PMID: 19430737

[83] Wu, S.; Liu, B.; Zhang, X. Characterization of a recombinant thermostable xylanase from deep-sea thermophilic *Geobacillus* sp. MT-1 in East Pacific. *Appl. Microbiol. Biotechnol.,* **2006**, *72*(6), 1210-1216. http://dx.doi.org/10.1007/s00253-006-0416-4 PMID: 16607523

[84] Hung, K.S.; Liu, S.M.; Fang, T.Y.; Tzou, W.S.; Lin, F.P.; Sun, K.H.; Tang, S.J. Characterization of a salt-tolerant xylanase from *Thermoanaerobacterium saccharolyticum* NTOU1. *Biotechnol. Lett.,* **2011**, *33*(7), 1441-1447. http://dx.doi.org/10.1007/s10529-011-0579-7 PMID: 21380775

[85] Khucharoenphaisan, K.; Tokuyama, S.; Kitpreechavanich, V. Characterization of the thermostability of xylanase produced by new isolates of *Thermomyces lanuginosus*. *Sci. Asia,* **2008**, *34*, 187-192. http://dx.doi.org/10.2306/scienceasia1513-1874.2008.34.187

[86] Arikan, B. Highly thermostable, thermophilic, alkaline, SDS and chelator resistant amylase from a thermophilic *Bacillus* sp. isolate A3-15. *Bioresour. Technol.,* **2008**, *99*(8), 3071-3076. http://dx.doi.org/10.1016/j.biortech.2007.06.019 PMID: 17689242

[87] Malhotra, R.; Noorwez, S.M.; Satyanarayana, T. Production and partial characterization of thermostable and calcium-independent α-amylase of an extreme thermophile *Bacillus thermooleovorans* NP54. *Lett. Appl. Microbiol.,* **2000**, *31*(5), 378-384. http://dx.doi.org/10.1046/j.1472-765x.2000.00830.x PMID: 11069641

[88] Tsukagoshi, N.; Iritani, S.; Sasaki, T.; Takemura, T.; Ihara, H.; Idota, Y.; Yamagata, H.; Udaka, S. Efficient synthesis and secretion of a thermophilic α-amylase by protein-producing *Bacillus brevis* 47 carrying the *Bacillus stearothermophilus* amylase gene. *J. Bacteriol.,* **1985**, *164*(3), 1182-1187. http://dx.doi.org/10.1128/JB.164.3.1182-1187.1985 PMID: 2999073

[89] Al-Qodah, Z. Production and characterization of thermostable α-amylase by thermophilic *Geobacillus stearothermophilus*. *Biotechnol. J.,* **2006**, *1*(7-8), 850-857. http://dx.doi.org/10.1002/biot.200600033 PMID: 16927263

[90] Mijts, B.N.; Patel, B.K.C. Cloning, sequencing and expression of an α-amylase gene, amyA, from the thermophilic halophile *Halothermothrix orenii* and purification and biochemical characterization of the recombinant enzyme. *Microbiology,* **2002**, *148*(Pt 8), 2343-2349. http://dx.doi.org/10.1099/00221287-148-8-2343 PMID: 12177328

[91] Mellouli, L.; Ghorbel, R.; Virolle, M.J.; Bejar, S. alpha-Amylase gene of thermophilic *Streptomyces* sp. TO1: nucleotide sequence, transcriptional and amino acid sequence analysis. *FEMS Microbiol. Lett.,* **1998**, *160*(1), 17-23. http://dx.doi.org/10.1111/j.1574-6968.1998.tb12884.x PMID: 9495007

[92] Saha, B.C.; Freer, S.N.; Bothast, R.J. Production, purification, and properties of a thermostable β-glucosidase from a color variant strain of *Aureobasidium pullulans*. *Appl. Environ. Microbiol.,* **1994**, *60*(10), 3774-3780. http://dx.doi.org/10.1128/AEM.60.10.3774-3780.1994 PMID: 16349415

[93] Papalazaridou, A.; Charitidou, L.; Sivropoulou, A. β-glucosidase enzymatic activity of crystal polypeptide of the *Bacillus thuringiensis* strain 1.1. *J. Endotoxin Res.,* **2003**, *9*(4), 215-224. http://dx.doi.org/10.1177/09680519030090040201 PMID: 12935352

[94] Jabbour, D.; Klippel, B.; Antranikian, G. A novel thermostable and glucose-tolerant β-glucosidase from *Fervidobacterium islandicum*. *Appl. Microbiol. Biotechnol.,* **2012**, *93*(5), 1947-1956. http://dx.doi.org/10.1007/s00253-011-3406-0 PMID: 22146852

[95] Breves, R.; Bronnenmeier, K.; Wild, N.; Lottspeich, F.; Staudenbauer, W.L.; Hofemeister, J. Genes encoding two different β-glucosidases of Thermoanaerobacter brockii are clustered in a common operon. *Appl. Environ. Microbiol.,* **1997**, *63*(10), 3902-3910. http://dx.doi.org/10.1128/AEM.63.10.3902-3910.1997 PMID: 9327554

[96] Lien, T.S.; Yu, S.T.; Wu, S.T.; Too, J.R. Induction and purification of a thermophilic chitinase produced by *Aeromonas* sp. DYU-Too7 using glucosamine. *Biotechnol. Bioprocess Eng.; BBE,* **2007**, *12*, 610-617. http://dx.doi.org/10.1007/BF02931076

[97] Songsiriritthigul, C.; Lapboonrueng, S.; Pechsrichuang, P.; Pesatcha, P.; Yamabhai, M. Expression and characterization of *Bacillus licheniformis* chitinase (ChiA), suitable for bioconversion of chitin waste. *Bioresour. Technol.,* **2010**, *101*(11), 4096-4103. http://dx.doi.org/10.1016/j.biortech.2010.01.036 PMID: 20133129

[98] Natsir, H.; Patong, A.R.; Suhartono, M.T.; Ahmad, A. Production and characterization of chitinase enzymes from sulili hot spring in south sulawesi, *Bacillus* sp. HSA,3-1a. *Indones. J. Chem.,* **2010**, *10*, 256-260. http://dx.doi.org/10.22146/ijc.21470

[99] Dai, D.H.; Hu, W.L.; Huang, G.R.; Li, W. Purification and characterization of a novel extracellular chitinase from thermophilic *Bacillus* sp. Hu1. *Afr. J. Biotechnol.,* **2011**, *10*, 2476-2485. http://dx.doi.org/10.5897/AJB10.1029

[100] Nawani, N.N.; Kapadnis, B.P.; Das, A.D.; Rao, A.S.; Mahajan, S.K. Purification and characterization of a thermophilic and acidophilic chitinase from *Microbispora* sp. V2. *J. Appl. Microbiol.,* **2002**, *93*(6), 965-975. http://dx.doi.org/10.1046/j.1365-2672.2002.01766.x PMID: 12452952

[101] Ueda, M.; Kotani, Y.; Sutrisno, A.; Nakazawa, M.; Miyatake, K. Purification and characterization of chitinase B from moderately thermophilic bacterium Ralstonia sp. A-471. *Biosci. Biotechnol. Biochem.,* **2005**, *69*(4), 842-844. http://dx.doi.org/10.1271/bbb.69.842 PMID: 15849428

[102] Manucharova, N.A.; Vlasenko, A.N.; Turova, T.P.; Panteleeva, A.N.; Stepanov, A.L.; Zenova, G.M. [Thermophilic chitinolytic microorganisms of brown semidesert soil]. *Mikrobiologiia,* **2008**, *77*(5), 683-688. http://dx.doi.org/10.1134/S0026261708050159 PMID: 19004351

[103] Mohagheghi, A.; Grohmann, K.; Himmel, M. Isolation and characterization of *Acidothermus cellulolyticus* gen. nov., sp. nov., a new genus of thermophilic, acidophilic, cellulolytic bacteria. *Int. J. Syst. Bacteriol.,* **1986**, *36*, 435-443. http://dx.doi.org/10.1099/00207713-36-3-435

[104] Ji, S.; Wang, S.; Tan, Y.; Chen, X.; Schwarz, W.; Li, F. An untapped bacterial cellulolytic community enriched from coastal marine sediment under anaerobic and thermophilic conditions. *FEMS Microbiol. Lett.,* **2012**, *335*(1), 39-46. http://dx.doi.org/10.1111/j.1574-6968.2012.02636.x PMID: 22788522

[105] Mingardon, F.; Bagert, J.D.; Maisonnier, C.; Trudeau, D.L.; Arnold, F.H. Comparison of family 9 cellulases from mesophilic and thermophilic bacteria. *Appl. Environ. Microbiol.,* **2011**, *77*(4), 1436-1442. http://dx.doi.org/10.1128/AEM.01802-10 PMID: 21169454

[106] Baharuddin, A.S.; Razak, M.N.A.; Hock, L.S. Isolation and characterization of thermophilic cellulase-producing bacteria from empty fruit bunches-palm oil mill effluent compost. *Am. J. Appl. Sci.,* **2010**, *7*, 56-62. http://dx.doi.org/10.3844/ajassp.2010.56.62

[107] Karita, S.; Nakayama, K.; Goto, M.; Sakka, K.; Kim, W.J.; Ogawa, S. A novel cellulolytic, anaerobic, and thermophilic bacterium, *Moorella* sp. strain F21. *Biosci. Biotechnol. Biochem.,* **2003**, *67*(1), 183-185. http://dx.doi.org/10.1271/bbb.67.183 PMID: 12619693

[108] Hreggvidsson, G.O.; Kaiste, E.; Holst, O. An extremely thermostable cellulase from the thermophilic eubacterium *Rhodothermus marinus. Appl. Environ. Microbiol.,* **1996**, *62*, 3047-3049. http://dx.doi.org/10.1128/.62.8.3047-3049.1996

[109] Agafonov, D.E.; Rabe, K.S.; Grote, M.; Huang, Y.; Sprinzl, M. The esterase from *Alicyclobacillus acidocaldarius* as a reporter enzyme and affinity tag for protein biosynthesis. *FEBS Lett.,* **2005**, *579*(10), 2082-2086. http://dx.doi.org/10.1016/j.febslet.2005.02.059 PMID: 15811322

[110] Faiz, O.; Colak, A.; Saglam, N.; Canakçi, S.; Beldüz, A.O. Determination and characterization of thermostable esterolytic activity from a novel thermophilic bacterium *Anoxybacillus gonensis* A4. *J. Biochem. Mol. Biol.,* **2007**, *40*(4), 588-594. http://dx.doi.org/10.5483/bmbrep.2007.40.4.588 PMID: 17669276

[111] Çolak, A.; Sişik, D.; Saglam, N.; Güner, S.; Canakçi, S.; Beldüz, A.O. Characterization of a thermoalkalophilic esterase from a novel thermophilic bacterium, *Anoxybacillus gonensis* G2. *Bioresour. Technol.,* **2005**, *96*(5), 625-631. http://dx.doi.org/10.1016/j.biortech.2004.06.003 PMID: 15501671

[112] Kademi, A.; Aït-Abdelkader, N.; Fakhreddine, L.; Baratti, J.C. Characterization of a new thermostable esterase from the moderate thermophilic bacterium *Bacillus circulans*. *J. Mol. Catal., B Enzym.,* **2000**, *10*, 395-401. http://dx.doi.org/10.1016/S1381-1177(99)00111-3

[113] Lüthi, E.; Jasmat, N.B.; Bergquist, P.L. Xylanase from the extremely thermophilic bacterium "*Caldocellum saccharolyticum*": overexpression of the gene in *Escherichia coli* and characterization

of the gene product. *Appl. Environ. Microbiol.,* **1990**, *56*(9), 2677-2683.
http://dx.doi.org/10.1128/AEM.56.9.2677-2683.1990 PMID: 2275529

[114] Yu, S.; Zheng, B.; Zhao, X.; Feng, Y. Gene cloning and characterization of a novel thermophilic esterase from *Fervidobacterium nodosum* Rt17-B1. *Acta Biochim. Biophys. Sin. (Shanghai),* **2010**, *42*(4), 288-295.
http://dx.doi.org/10.1093/abbs/gmq020 PMID: 20383468

[115] Rakotoarivonina, H.; Hermant, B.; Chabbert, B.; Touzel, J.P.; Remond, C. A thermostable feruloyl-esterase from the hemicellulolytic bacterium *Thermobacillus xylanilyticus* releases phenolic acids from non-pretreated plant cell walls. *Appl. Microbiol. Biotechnol.,* **2011**, *90*(2), 541-552.
http://dx.doi.org/10.1007/s00253-011-3103-z PMID: 21279344

[116] Lagarde, D.; Nguyen, H.K.; Ravot, G. High-throughput screening of thermostable esterases for industrial bioconversions. *Org. Process Res. Dev.,* **2002**, *6*, 441-445.
http://dx.doi.org/10.1021/op020019h

[117] Fuciños, P.; González, R.; Atanes, E. Lipases and esterases from extremophiles : overview and case example of the production and purifi cation of an esterase from thermus thermophilus HB27. *Methods Mol. Biol., 861,* 239-266.
http://dx.doi.org/10.1007/978-1-61779-600-5

[118] Maeda, M.; Hidaka, M.; Nakamura, A.; Masaki, H.; Uozumi, T. Cloning, sequencing, and expression of thermophilic *Bacillus* sp. strain TB-90 urease gene complex in *Escherichia coli*. *J. Bacteriol.,* **1994**, *176*(2), 432-442.
http://dx.doi.org/10.1128/JB.176.2.432-442.1994 PMID: 8288539

[119] Mégraud, F.; Chevrier, D.; Desplaces, N.; Sedallian, A.; Guesdon, J.L. Urease-positive thermophilic Campylobacter (*Campylobacter laridis* variant) isolated from an appendix and from human feces. *J. Clin. Microbiol.,* **1988**, *26*(5), 1050-1051.
http://dx.doi.org/10.1128/JCM.26.5.1050-1051.1988 PMID: 3384898

[120] Kaneko, A.; Matsuda, M.; Miyajima, M.; Moore, J.E.; Murphy, P.G. Urease-positive thermophilic strains of Campylobacter isolated from seagulls (Larus spp.). *Lett. Appl. Microbiol.,* **1999**, *29*(1), 7-9.
http://dx.doi.org/10.1046/j.1365-2672.1999.00565.x PMID: 10432626

[121] Kakinuma, Y.; Hayashi, K.; Tazumi, A.; Hirayama, J.; Moore, J.E.; Millar, B.C.; Kuribayashi, T.; Matsuda, M. Molecular analysis and characterization of a urease gene operon from *Campylobacter sputorum* biovar paraureolyticus. *Folia Microbiol. (Praha),* **2011**, *56*(2), 159-165.
http://dx.doi.org/10.1007/s12223-011-0020-6 PMID: 21431911

[122] Matsuda, M.; Moore, J.E. Urease-positive thermophilic Campylobacter species. *Appl. Environ. Microbiol.,* **2004**, *70*(8), 4415-4418.
http://dx.doi.org/10.1128/AEM.70.8.4415-4418.2004 PMID: 15294767

[123] Zotta, T.; Ricciardi, A.; Rossano, R.; Parente, E. Urease production by *Streptococcus thermophilus*. *Food Microbiol.,* **2008**, *25*(1), 113-119.
http://dx.doi.org/10.1016/j.fm.2007.07.001 PMID: 17993384

[124] Pratuangdejkul, J.; Dharmsthiti, S. Purification and characterization of lipase from psychrophilic *Acinetobacter calcoaceticus* LP009. *Microbiol. Res.,* **2000**, *155*(2), 95-100.
http://dx.doi.org/10.1016/S0944-5013(00)80043-9 PMID: 10950191

[125] Snellman, E.A.; Sullivan, E.R.; Colwell, R.R. Purification and properties of the extracellular lipase, LipA, of *Acinetobacter* sp. RAG-1. *Eur. J. Biochem.,* **2002**, *269*(23), 5771-5779.
http://dx.doi.org/10.1046/j.1432-1033.2002.03235.x PMID: 12444965

[126] Arpigny, J.L.; Zekhnini, Z.; Swings, J. Temperature dependence of growth, enzyme secretion and activity of psychrophilic Antarctic bacteria. *Appl. Microbiol. Biotechnol.,* **1994**, *41*, 477-479.
http://dx.doi.org/10.1007/s002530050176

[127] Suryavanshi, M.V.; Ghosh, J.S. Spoilage of white unsalted butter by psychrophilic lipolysis of *Pseudomonas aeruginosa NCIM 2036. British J. Dairy Sci.,* **2010**, *1*, 26-29.

[128] Choo, D.W.; Kurihara, T.; Suzuki, T.; Soda, K.; Esaki, N. A cold-adapted lipase of an Alaskan psychrotroph, *Pseudomonas* sp. strain B11-1: gene cloning and enzyme purification and characterization. *Appl. Environ. Microbiol.,* **1998**, *64*(2), 486-491.
http://dx.doi.org/10.1128/AEM.64.2.486-491.1998 PMID: 9464382

[129] Bowman, J.P.; Nichols, D.S.; McMeekin, T.A. *Psychrobacter glacincola* sp. nov., a halotolerant, psychrophilic bacterium isolated from Antarctic Sea ice. *Syst. Appl. Microbiol.,* **1997**, *20*, 209-215.
http://dx.doi.org/10.1016/S0723-2020(97)80067-7

[130] Arpigny, J.L.; Feller, G.; Gerday, C. Cloning, sequence and structural features of a lipase from the antarctic facultative psychrophile *Psychrobacter immobilis* B10. *BBA - Gene Struct. Expr.,* **1993**, *1171*, 331-333.
http://dx.doi.org/10.1016/0167-4781(93)90078-R

[131] De Santi, C.; Tutino, M.L.; Mandrich, L.; Giuliani, M.; Parrilli, E.; Del Vecchio, P.; de Pascale, D. The hormone-sensitive lipase from *Psychrobacter* sp. TA144: new insight in the structural/functional characterization. *Biochimie,* **2010**, *92*(8), 949-957.
http://dx.doi.org/10.1016/j.biochi.2010.04.001 PMID: 20382198

[132] Yumoto, I.; Hirota, K.; Sogabe, Y.; Nodasaka, Y.; Yokota, Y.; Hoshino, T. *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea. *Int. J. Syst. Evol. Microbiol.,* **2003**, *53*(Pt 6), 1985-1989.
http://dx.doi.org/10.1099/ijs.0.02686-0 PMID: 14657134

[133] Samie, N.; Noghabi, K.A.; Gharegozloo, Z. Psychrophilic α-amylase from *Aeromonas veronii* NS07 isolated from farm soils. *Process Biochem.,* **2012**, *47*, 1381-1387.
http://dx.doi.org/10.1016/j.procbio.2012.05.007

[134] Aghajari, N.; Feller, G.; Gerday, C.; Haser, R. Crystal structures of the psychrophilic α-amylase from *Alteromonas haloplanctis* in its native form and complexed with an inhibitor. *Protein Sci.,* **1998**, *7*(3), 564-572.
http://dx.doi.org/10.1002/pro.5560070304 PMID: 9541387

[135] Claverie, P.; Vigano, C.; Ruysschaert, J.M.; Gerday, C.; Feller, G. The precursor of a psychrophilic α-amylase: structural characterization and insights into cold adaptation. *Biochim. Biophys. Acta,* **2003**, *1649*(2), 119-122.
http://dx.doi.org/10.1016/S1570-9639(03)00184-5 PMID: 12878029

[136] Parrilli, E.; Giuliani, M.; Pezzella, C.; Danchin, A.; Marino, G.; Tutino, M.L. PssA is required for α-amylase secretion in Antarctic *Pseudoalteromonas haloplanktis*. *Microbiology,* **2010**, *156*(Pt 1), 211-219.
http://dx.doi.org/10.1099/mic.0.032342-0 PMID: 19778966

[137] Tutino, M.L.; Duilio, A.; Parrilli, R.; Remaut, E.; Sannia, G.; Marino, G. A novel replication element from an Antarctic plasmid as a tool for the expression of proteins at low temperature. *Extremophiles,* **2001**, *5*(4), 257-264.
http://dx.doi.org/10.1007/s007920100203 PMID: 11523895

[138] Zhou, M.Y.; Chen, X.L.; Zhao, H.L.; Dang, H.Y.; Luan, X.W.; Zhang, X.Y.; He, H.L.; Zhou, B.C.; Zhang, Y.Z. Diversity of both the cultivable protease-producing bacteria and their extracellular proteases in the sediments of the South China sea. *Microb. Ecol.,* **2009**, *58*(3), 582-590.
http://dx.doi.org/10.1007/s00248-009-9506-z PMID: 19301066

[139] Pawar, R.; Zambare, V.; Barve, S.; Paratkar, G. Protease as cleansing of contact lenses. *Asian Netw. Sci. Inf.,* **2009**, *8*, 276-280.
http://dx.doi.org/10.3923/biotech.2009.276.280

[140] Alam, S.I.; Dixit, A.; Reddy, G.S.N.; Dube, S.; Palit, M.; Shivaji, S.; Singh, L. *Clostridium schirmacherense* sp. nov., an obligately anaerobic, proteolytic, psychrophilic bacterium isolated from lake sediment of Schirmacher Oasis, Antarctica. *Int. J. Syst. Evol. Microbiol.,* **2006**, *56*(Pt 4), 715-720.
http://dx.doi.org/10.1099/ijs.0.63808-0 PMID: 16585682

[141] Wang, Q.; Hou, Y.; Xu, Z.; Miao, J.; Li, G. Optimization of cold-active protease production by the psychrophilic bacterium *Colwellia* sp. NJ341 with response surface methodology. *Bioresour. Technol.,* **2008**, *99*(6), 1926-1931.
http://dx.doi.org/10.1016/j.biortech.2007.03.028 PMID: 17499500

[142] Zhang, S.C.; Sun, M.; Li, T.; Wang, Q.H.; Hao, J.H.; Han, Y.; Hu, X.J.; Zhou, M.; Lin, S.X. Structure analysis of a new psychrophilic marine protease. *PLoS One,* **2011**, *6*(11), e26939.
http://dx.doi.org/10.1371/journal.pone.0026939 PMID: 22132082

[143] Zeng, R.; Zhang, R.; Zhao, J.; Lin, N. Cold-active serine alkaline protease from the psychrophilic bacterium Pseudomonas strain DY-A: enzyme purification and characterization. *Extremophiles,* **2003**, *7*(4), 335-337.

http://dx.doi.org/10.1007/s00792-003-0323-x PMID: 12910392

[144] Hamamoto, T.; Kaneda, M.; Horikoshi, K.; Kudo, T. Characterization of a protease from a psychrotroph, Pseudomonas fluorescens 114. Appl. Environ. Microbiol., **1994**, 60(10), 3878-3880. PMID: 16349422

[145] Cotârlet, M.; Negoitâ, T.; Bahrim, G.; Stougaard, P. Cold adapted amylase and protease from new Streptomyces 4alga antarctic strain. *Polar Res.,* **2009**, *5*(12), 23-30.

[146] Nakagawa, T.; Fujimoto, Y.; Uchino, M.; Miyaji, T.; Takano, K.; Tomizuka, N. Isolation and characterization of psychrophiles producing cold-active β-galactosidase. *Lett. Appl. Microbiol.,* **2003**, *37*(2), 154-157. http://dx.doi.org/10.1046/j.1472-765X.2003.01369.x PMID: 12859659

[147] Coker, J.A.; Brenchley, J.E. Protein engineering of a cold-active β-galactosidase from *Arthrobacter* sp. SB to increase lactose hydrolysis reveals new sites affecting low temperature activity. *Extremophiles,* **2006**, *10*(6), 515-524. http://dx.doi.org/10.1007/s00792-006-0526-z PMID: 16736094

[148] Nam, E.; Ahn, J. Antarctic marine bacterium *Pseudoalteromonas* sp. KNOUC808 as a source of cold-adapted lactose hydrolyzing enzyme. *Braz. J. Microbiol.,* **2011**, *42*(3), 927-936. http://dx.doi.org/10.1590/S1517-83822011000300011 PMID: 24031708

[149] Coombs, J.; Brenchley, J.E. Characterization of two new glycosyl hydrolases from the lactic acid bacterium *Carnobacterium piscicola* strain BA. *Appl. Environ. Microbiol.,* **2001**, *67*(11), 5094-5099. http://dx.doi.org/10.1128/AEM.67.11.5094-5099.2001 PMID: 11679331

[150] Shipkowski, S.; Brenchley, J.E. Characterization of an unusual cold-active β-glucosidase belonging to family 3 of the glycoside hydrolases from the psychrophilic isolate *Paenibacillus* sp. strain C7. *Appl. Environ. Microbiol.,* **2005**, *71*(8), 4225-4232. http://dx.doi.org/10.1128/AEM.71.8.4225-4232.2005 PMID: 16085807

[151] Margesin, R.; Spröer, C.; Schumann, P.; Schinner, F. *Pedobacter cryoconitis* sp. nov., a facultative psychrophile from alpine glacier cryoconite. *Int. J. Syst. Evol. Microbiol.,* **2003**, *53*(Pt 5), 1291-1296. http://dx.doi.org/10.1099/ijs.0.02436-0 PMID: 13130009

[152] Hoyoux, A.; Jennes, I.; Dubois, P.; Genicot, S.; Dubail, F.; François, J.M.; Baise, E.; Feller, G.; Gerday, C. Cold-adapted β-galactosidase from the Antarctic psychrophile *Pseudoalteromonas haloplanktis*. *Appl. Environ. Microbiol.,* **2001**, *67*(4), 1529-1535. http://dx.doi.org/10.1128/AEM.67.4.1529-1535.2001 PMID: 11282601

[153] Zhao, J.S.; Manno, D.; Thiboutot, S.; Ampleman, G.; Hawari, J. *Shewanella canadensis* sp. nov. and *Shewanella atlantica* sp. nov., manganese dioxide- and hexahydro-1,3,5-trinitro-1,3,5-triazine-reducing, psychrophilic marine bacteria. *Int. J. Syst. Evol. Microbiol.,* **2007**, *57*(Pt 9), 2155-2162. http://dx.doi.org/10.1099/ijs.0.64596-0 PMID: 17766891

[154] Collins, T.; Gerday, C.; Feller, G. Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol. Rev.,* **2005**, *29*(1), 3-23. http://dx.doi.org/10.1016/j.femsre.2004.06.005 PMID: 15652973

[155] Lee, C.C.; Smith, M.; Kibblewhite-Accinelli, R.E.; Williams, T.G.; Wagschal, K.; Robertson, G.H.; Wong, D.W. Isolation and characterization of a cold-active xylanase enzyme from *Flavobacterium* sp. *Curr. Microbiol.,* **2006**, *52*(2), 112-116. http://dx.doi.org/10.1007/s00284-005-4583-9 PMID: 16450065

[156] Humphry, D.R.; George, A.; Black, G.W.; Cummings, S.P. *Flavobacterium frigidarium* sp. nov., an aerobic, psychrophilic, xylanolytic and laminarinolytic bacterium from Antarctica. *Int. J. Syst. Evol. Microbiol.,* **2001**, *51*(Pt 4), 1235-1243. http://dx.doi.org/10.1099/00207713-51-4-1235 PMID: 11491319

[157] Guo, B.; Chen, X.L.; Sun, C.Y.; Zhou, B.C.; Zhang, Y.Z. Gene cloning, expression and characterization of a new cold-active and salt-tolerant endo-β-1,4-xylanase from marine *Glaciecola mesophila* KMM 241. *Appl. Microbiol. Biotechnol.,* **2009**, *84*(6), 1107-1115. http://dx.doi.org/10.1007/s00253-009-2056-y PMID: 19506861

[158] Waeonukul, R.; Pason, P.; Kyu, K.L.; Sakka, K.; Kosugi, A.; Mori, Y.; Ratanakhanokchai, K. Cloning, sequencing, and expression of the gene encoding a multidomain endo-β-1,4-xylanase from *Paenibacillus curdlanolyticus* B-6, and characterization of the recombinant enzyme. *J. Microbiol. Biotechnol.,* **2009**, *19*(3), 277-285. http://dx.doi.org/10.4014/jmb.0804.293 PMID: 19349753

[159] Park, I.; Cho, J. Partial characterization of extracellular xylanolytic activity derived from *Paenibacillus* sp. KIJ1. *Afr. J. Microbiol. Res.,* **2010**, *4*, 1257-1264.

[160] Dornez, E.; Verjans, P.; Arnaut, F.; Delcour, J.A.; Courtin, C.M. Use of psychrophilic xylanases provides insight into the xylanase functionality in bread making. *J. Agric. Food Chem.,* **2011**, *59*(17), 9553-9562. http://dx.doi.org/10.1021/jf201752g PMID: 21806059

[161] Benešová, E.; Marková, M.; Králová, B. α-glucosidase and β-glucosidase from psychrotrophic strain *Arthrobacter* sp. C2-2. *Czech J. Food Sci.,* **2005**, *23*, 116-120. http://dx.doi.org/10.17221/3380-CJFS

[162] Li, L.; Wang, Y.; Shen, W. Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. *Biometals,* **2012**, *25*(3), 617-631. http://dx.doi.org/10.1007/s10534-012-9551-9 PMID: 22538639

[163] Kasana, R.C.; Gulati, A. Cellulases from psychrophilic microorganisms: a review. *J. Basic Microbiol.,* **2011**, *51*(6), 572-579. http://dx.doi.org/10.1002/jobm.201000385 PMID: 21656807

[164] Liu, Y.; Zhou, Z.; Miao, W.; Zhang, Y.; Cao, Y.; He, S.; Bai, D.; Yao, B. A Chitinase from *Aeromonas veronii* CD3 with the potential to control myxozoan disease. *PLoS One,* **2011**, *6*(12), e29091. http://dx.doi.org/10.1371/journal.pone.0029091 PMID: 22205999

[165] Orikoshi, H.; Baba, N.; Nakayama, S.; Kashu, H.; Miyamoto, K.; Yasuda, M.; Inamori, Y.; Tsujibo, H. Molecular analysis of the gene encoding a novel cold-adapted chitinase (ChiB) from a marine bacterium, *Alteromonas* sp. strain O-7. *J. Bacteriol.,* **2003**, *185*(4), 1153-1160. http://dx.doi.org/10.1128/JB.185.4.1153-1160.2003 PMID: 12562783

[166] Mavromatis, K.; Feller, G.; Kokkinidis, M.; Bouriotis, V. Cold adaptation of a psychrophilic chitinase: a mutagenesis study. *Protein Eng.,* **2003**, *16*(7), 497-503. http://dx.doi.org/10.1093/protein/gzg069 PMID: 12915727

[167] Ramli, A.N.M.; Mahadi, N.M.; Shamsir, M.S.; Rabu, A.; Joyce-Tan, K.H.; Murad, A.M.; Illias, R.M. Structural prediction of a novel chitinase from the psychrophilic *Glaciozyma antarctica* PI12 and an analysis of its structural properties and function. *J. Comput. Aided Mol. Des.,* **2012**, *26*(8), 947-961. http://dx.doi.org/10.1007/s10822-012-9585-7 PMID: 22710891

[168] Stefanidi, E.; Vorgias, C.E. Molecular analysis of the gene encoding a new chitinase from the marine psychrophilic bacterium *Moritella marina* and biochemical characterization of the recombinant enzyme. *Extremophiles,* **2008**, *12*(4), 541-552. http://dx.doi.org/10.1007/s00792-008-0155-9 PMID: 18368288

[169] Fenice, M.; Selbmann, L.; Di Giambattista, R.; Federici, F. Chitinolytic activity at low temperature of an Antarctic strain (A3) of *Verticillium lecanii*. *Res. Microbiol.,* **1998**, *149*(4), 289-300. http://dx.doi.org/10.1016/S0923-2508(98)80304-5 PMID: 9766230

[170] Bendt, A.; Hüller, H.; Kammel, U.; Helmke, E.; Schweder, T. Cloning, expression, and characterization of a chitinase gene from the Antarctic psychrotolerant bacterium *Vibrio* sp. strain Fi:7. *Extremophiles,* **2001**, *5*(2), 119-126. http://dx.doi.org/10.1007/s007920100179 PMID: 11354455

[171] Ferrer, M.; Chernikova, T.N.; Timmis, K.N.; Golyshin, P.N. Expression of a temperature-sensitive esterase in a novel chaperone-based *Escherichia coli* strain. *Appl. Environ. Microbiol.,* **2004**, *70*(8), 4499-4504. http://dx.doi.org/10.1128/AEM.70.8.4499-4504.2004 PMID: 15294778

[172] Lemak, S.; Tchigvintsev, A.; Petit, P.; Flick, R.; Singer, A.U.; Brown, G.; Evdokimova, E.; Egorova, O.; Gonzalez, C.F.; Chernikova, T.N.; Yakimov, M.M.; Kube, M.; Reinhardt, R.; Golyshin, P.N.; Savchenko, A.; Yakunin, A.F. Structure and activity of the cold-active and anion-activated carboxyl esterase OLEI01171 from the oil-degrading marine bacterium *Oleispira antarctica*. *Biochem. J.,* **2012**, *445*(2), 193-203. http://dx.doi.org/10.1042/BJ20112113 PMID: 22519667

[173] Al Khudary, R.; Venkatachalam, R.; Katzer, M.; Elleuche, S.; Antranikian, G. A cold-adapted esterase of a novel marine isolate,

Pseudoalteromonas arctica: gene cloning, enzyme purification and characterization. *Extremophiles,* **2010**, *14*(3), 273-285. http://dx.doi.org/10.1007/s00792-010-0306-7 PMID: 20217440

[174] Suzuki, T.; Nakayama, T.; Choo, D.W.; Hirano, Y.; Kurihara, T.; Nishino, T.; Esaki, N. Cloning, heterologous expression, renaturation, and characterization of a cold-adapted esterase with unique primary structure from a psychrotroph *Pseudomonas* sp. strain B11-1. *Protein Expr. Purif.,* **2003**, *30*(2), 171-178. http://dx.doi.org/10.1016/S1046-5928(03)00128-1 PMID: 12880765

[175] Kulakova, L.; Galkin, A.; Nakayama, T.; Nishino, T.; Esaki, N. Cold-active esterase from *Psychrobacter* sp. Ant300: gene cloning, characterization, and the effects of Gly-->Pro substitution near the active site on its catalytic activity and stability. *Biochim. Biophys. Acta,* **2004**, *1696*(1), 59-65. http://dx.doi.org/10.1016/j.bbapap.2003.09.008 PMID: 14726205

[176] Brault, G.; Shareck, F.; Hurtubise, Y.; Lépine, F.; Doucet, N. Isolation and characterization of EstC, a new cold-active esterase from *Streptomyces coelicolor* A3(2). *PLoS One,* **2012**, *7*(3), e32041. http://dx.doi.org/10.1371/journal.pone.0032041 PMID: 22396747