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Microbiology

Extremophiles: An Overview of Microorganism from Extreme Environment

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Abstract

Extremophilic organisms are primarily prokaryotic (archaea and bacteria), with few eukaryotic examples. Extremophiles are defined by the environmental conditions in which they grow optimally. The organisms may be described as acidophilic (optimal growth between pH 1 and pH 5); alkaliphilic (optimal growth above pH 9); halophilic (optimal growth in environments with high concentrations of salt); thermophilic (optimal growth between 60 and 80 °C); hyperthermophilic (optimal growth above 80 °C); psychrophilic (optimal growth at 15 °C or lower, with a maximum tolerant temperature of 20 °C and minimal growth at or below 0 °C); piezophilic, or barophilic (optimal growth at high hydrostatic pressure); oligotrophic (growth in nutritionally limited environments); endolithic (growth within rock or within pores of mineral grains); and xerophilic (growth in dry conditions, with low water availability). Some extremophiles are adapted simultaneously to multiple stresses (polyextremophile); common examples include thermoacidophiles and haloalkaliphiles. Extremophiles are of biotechnological interest, as they produce extremozymes, defined as enzymes that are functional under extreme conditions. Extremozymes are useful in industrial production procedures and research applications because of their ability to remain active under the severe conditions typically employed in these processes. The study of extremophiles provides an understanding of the physicochemical parameters defining life on Earth and may provide insight into how life on Earth originated. The postulations that extreme environmental conditions existed on primitive Earth and that life arose in hot environments have led to the theory that extremophiles are vestiges of primordial organisms and thus are models of ancient life.

Highlights

Extremophiles are micro-organisms that inhabit some of earth's most hostile environments which produce extremozymes useful in industrial production procedures and research applications because of their ability to remain active under the severe conditions typically employed in these processes.

Keywords: Extremophiles, extremozymes, psychrophiles, thermophiles, mesophiles, halophiles, acidophiles

An extremophile (from Latin *extremus* meaning "extreme" and Greek *philia* (öéëßá) meaning "love") is an organism that thrives in and may even require physically or geochemically extreme conditions that are detrimental to the majority of life on Earth. (In contrast, organisms that live in more moderate environments may be termed mesophiles or neutrophiles.) In the 1980s and 1990s, biologists found that microbial life has an amazing flexibility for surviving in extreme environments - niches that are extraordinarily hot, or acidic, for example - that would be completely inhospitable to complex organisms. Some scientists even concluded that life may have begun on Earth in hydrothermal vents far under the ocean's surface (NASA, 2003).

Most known extremophiles are microbes. The domain Archaea contains renowned examples, but extremophiles are present in numerous and diverse genetic lineages of both bacteria and archaeans. Furthermore, it is erroneous to use the term extremophile to encompass all archaeans, as some are mesophilic. Neither are all extremophiles unicellular; protostome animals found in similar environments include the Pompeii worm, the psychrophilic Grylloblattodea (insects), Antarctic krill (a crustacean), and the "water bear".

The history of the human race has been intimately entwined with the discovery and utilization of new enzymes and their sources. Compared with organic synthesis, biocatalysis is considered better as it can lead to more efficient production of specific products, fewer side reactions and lower environmental burden (Rozzell, 1999). Hence, research is going on to modify existing chemical processes and make them more biological by using enzymes as biocatalysts. Natural diversity is being increasingly exploited to access enzymes with greater industrial utility. Despite the fact that more than 3000 different enzymes have been identified to date, the present enzyme tool is not sufficient to meet all biotechnological and industrial demands. One major reason for this is the fact that many of these enzymes, attuned to work in their native cellular environment, are not well suited for the harsh reaction conditions encountered in industrial processes. Thus, since their discovery, enzymes are being modified/ engineered and screened from various sources to have desired properties, required for industrial and biotechnological applications (Demirijian *et al.*, 2001).

Several physical and chemical methods have been employed for the stabilization of enzymes suiting to harsh environment viz. protein engineering (Martinez and Arnold, 1991; Gerike *et al.*, 2001), chemical modification (Tyagi and Gupta, 1998; Gaur and Khare, 2009; Gupta and Khare, 2009), directed evolution (Arnold and Moore, 1998) and immobilization (Gaur *et al.*, 2008; Datta *et al.*, 2013). Some of these are also promising but loss in enzyme activity and cost of stabilization are major drawbacks. However, if enzymes are naturally stable and exhibit high activities under extreme conditions, such drawbacks can be rejected. Screening the enzymes from extremophiles, the organisms growing under extreme environments, in recent years, has become a subject of considerable interest. The unique properties of these biocatalysts have resulted in several novel applications of enzymes in industrial processes.

The term, extremophile was first introduced by McElroy in 1974 (Macelroy, 1974). Most extremophiles belong to the domains of archaea, bacteria and eukarya (Rothschild and Mancinelli, 2001). Although ten years ago, extremophiles were exotic organisms, explored by only a few research groups throughout the world. Now, it has emerged as a prospective area for enzymologists to exploit these microbes for various industries (Van den Burg, 2003). In the last decade, studies on extremophiles have progressed to the extent that the First International Congress on Extremophiles was convened in Portugal (1996) and the scientific journal, "Extremophiles" was established in 1997. An international society, "The International Society for Extremophiles (ISE)" was also founded in 2002 for the exchange of information and experience in the rapidly growing field of research on extremophiles.

The discovery of new extremophilic microorganisms and their enzymes have a great impact on the field of biocatalysis. An extremophile is an organism, which thrives in or requires "extreme" conditions i.e. adapted to survive in diverse ecological niches. These (extreme) conditions can refer to physical as well as geochemical extremes such as temperature, pressure, radiation, salinity, pH, presence of toxic compounds and water availability. Accordingly, there are many different classes of extremopdhiles, each corresponding to different environments (conditions) to which microorganisms have adapted (Table-1) [http://en.wikipedia.org/wiki/extremophile].

Usefulness of extremophiles in various industrial and other applications is due to their wide spectrum of unique properties. A major impetus driving research on extremophiles is the biotechnological potential associated with the microorganisms and their cellular products. Metabolic processes and specific biological functions of these microorganisms are mediated by enzymes and proteins that are responsible for the organisms' unusual properties. With the notion that extremophiles are capable of surviving in extreme environments, it is assumed that their enzymes are adapted to function optimally under such conditions. Indeed, data collected for the enzymes that have been isolated recently from these exotic microorganisms support this assumption. These enzymes show unique features as extreme thermal stability, resistance against chemical denaturants such as detergents, chaotropic agents, organic solvents and extremes of pH (Gaur *et al*., 2010; Karan *et al.*, 2011). Dubbed as 'extremozymes' these hold tremendous potential as industrial biocatalysts to work under harsh and rugged operational conditions, wherein normal enzymes get denatured and lose their activity (Hough and Danson, 1999; Niehaus *et al.*, 1999; Van den Burg, 2003). They are and being also used as a model for designing and constructing proteins with new properties that are of interest for various industrial applications. Researchers are surveying the natural world with renewed vigor and finding rapid new ways to identify and produce extremozymes relevant for industrial biocatalysis (Table 1).

Much work is going on into developing methods by which extremophiles can be effectively cultivated for increased production of extremophilic biomasses, enzymes and biomolecules. To improve biomass production, different research groups have adopted different techniques such as: optimization of the medium composition (Gomes *et al.*, 2000, Patel *et al.*, 2006), use of different modes of fermentation (e.g. fed-batch, cell-recycling or continuous cultivation) (Schiraldi and Rosa, 2002). Due to the difficulties associated with large-scale culture of extremophiles and downstream processing of extremozymes, attempts have been underway to express corresponding genes from extremophiles into mesophilic hosts (Eichler, 2001).

Thermophiles

Thermophilic microorganisms (optimum growth temperature of 50^0C or above) have attracted great attention among extremophiles because they are sources of thermostable enzymes (Singh *et al.*, 2011). They can be generally classified into moderate thermophiles (growth optimum; 50–60^0C), extreme thermophiles (growth optimum; 60–80^0C) and hyperthermophiles (growth optimum; 80–110^0C). Thermophiles have been isolated from the different ecological zones (e.g. hot springs, deep sea) of the earth. The organisms with the highest growth temperatures (103–110^0C) are members of the genera *Pyrobaculum*, *Pyrodictium*, *Pyrococcus* and *Melanopyrus* belonging to archea, within fungi the *Ascomycete* and *Zygomycete* family have high growth temperature (Busk and Lange, 2013) while in case of bacteria, *Thermotoga maritime* and *Aquifex pyrophilus* exhibit the highest growth temperatures of 90 and 95^0C respectively (Haki and Rakshit, 2003; Kumar *et al.*, 2011). These properties imply extremely important implications because enzymes that have been isolated from such microorganisms show unique features are extremely thermostable and usually resistant against some chemical denaturants.

The reasons to exploit enzymes stable/active at higher temperatures are mainly due to the fact that they are better suited for running biotechnological processes at elevated temperatures that have many advantages. The increase of temperature has a significant influence on the bioavailability and solubility of many polymeric compounds, resulting in faster reaction rates, efficient bioremediation and decreased risk of microbial contamination and undesired complications (Van den Burg, 2003, Sinha *et al.*, 2012). To date, a large number of polymer degrading enzymes (e.g. amylases, cellulases, chitinases, pectinases, pullulanases and xylanases), proteases, isomerases, esterases, lipases, phytases, dehydrogenases and DNA-modifying enzymes have been characterized from extremely thermophilic and hyperthermophilic microorganisms (Bertoldo and Antranikian, 2002; Haki and Rakshit, 2003; Roy *et al.*, 2003). Thermostable DNA polymerases, isolated from hyperthermophiles, have led to a tremendous advance in molecular biology due to their capacity to amplify DNA, in the polymerase chain reaction (PCR), *Taq* polymerase from the bacterium *Thermus aquaticus*, being the well-known success story (Chien *et al.*, 1976; Kaledin *et al.*, 1980).

The enzymes from thermophiles show high potential in detergent, food, feed, starch, textile, leather, pulp and paper and pharmaceutical industries (Fujiwara, 2002). They have also been used as models for understanding the reasons imparting thermostability and thermoactivity, to provide useful leads for protein engineering (Kumar and Nussinov, 2001; Sterner and Liebel, 2001). Several three-dimensional structures have been solved and compared with those of mesophilic counterparts, with the ultimate goal of elucidating the mechanisms underlying thermostability (Vieille and Zeikus, 2001; Van den Burg and Eijsink, 2002). In a comprehensive study, it was found that increased ionic interaction and hydrogen bonds (surface charge), increased protein core hydrophobicity, and less exposed thermolabile amino acids confer stability on the thermophilic proteins (Scandurra *et al.*, 1998; Gomes and Steiner, 2004).

Psychrophiles

Psychrophilic (growth temperature of 15^0C or lower) or psychrotolerant (able to grow at temperature close to the freezing point of water but fastest growth rate at above 20^0C) microorganisms are found inhabiting the low temperature environments of the Earth, including polar

Table 1: Enzymes from various types of extremophiles and their applications.

Extremophile	Habitat	Enzymes	Representative applications
Thermophile	High temperature	Amylases	Glucose, fructose for sweeteners
	Moderate thermophiles	Xylanases	Paper bleaching
	(50–60°C) Thermophiles	Proteases	Baking, brewing, detergents,
	(60–80°C) Hyper-	Lipases, esterases	hydrolysis in food and feed
	thermophiles (>80°C)	Alkaline phosphatase	Detergents, stereo-specific reactions,
		Glycosyl hydrolases	dairy productsDiagnosticsStarch, cellulose,
		(amylases, pullulanase,	chitin, pectin processing, textiles
		glucoamylases, gluco-sidases,	Hydrolysis of starch,
		cellulases, xylanases)	Cellulose and related poly- and oligosaccharides
		Dehydrogenases	Oxidation reactions
		DNA polymerases	Genetic engineering
Psychrophile	Low temperature (<15 ^{0}C)	Proteases Dehydrogenases	Cheese maturation, dairy production, detergents
			Biotransformations, biosensors
		Amylases	Detergents,
		Cellulases	Detergents, feed, textiles
		Lipases	Detergents, food, cosmetics
		Alkaline phosphatase	Molecular biology
		β-galactosidase	Lactose hydrolysis
		Methanogens	Methane production
Acidophile	Low pH (pH <2–3)	Amylases, glucoamylases	Starch processing
		Proteases, cellulases	Feed component
		Sulfur oxidation	Desulfurization of coal
		Chalcopyrite concentrate	Valuable metals recovery
Alkaliphile	High pH (pH >9)	Cellulases, proteases	Polymer degradation in detergents,
		Proteases	food and feed
		Elastases, keratinases	Gelatin removal on X-ray film
		Cyclodextrins	Hide dehairing
		Xylanases	Foodstuffs, chemicals and pharmaceuticals
		Pectinases	Pulp bleaching
			Fine papers and degumming
Halophile	High salt concentration	Proteases	Peptide synthesis
	(e.g. 2–5 M NaCl)	Dehydrogenases	Biocatalysis in organic media
		Bacteriorhodopsin	Optical switches
		Polyhydroxyalkanoates	Medical plastics
		Rheological polymers	Oil recovery
		Compatible solutes	Protein and cell protectants in a variety
			of industrial uses, e.g. freezing, heating
		Whole microorganism	Hypersaline waste transformation and degradation.
			Ion exchange resin regenerant disposal, producing poly
			(β -glutamic acid; PGA) & poly
			(β-hydroxy butyric acid; PHB)
Piezophile	High pressure	Whole microorganism	Formation of gels and starch granules.
	(up to 130 Mpa)		Food processing and antibiotic production
Metalophile	High metal concentration	Whole microorganism	Ore-bioleaching, bioreme-diation, biomineralization
Radiophile	High radiation levels	Whole microorganism	Bioremediation of radio-nuclide contaminated sites
Toxitolerant	High levels of toxic	Proteases Lipases	Peptide synthesis, enzymatic catalysis
	reagents/ organic solvents		in non-aqueous solvents
			Esterification/ transesterification
		Cyclodextrin glucanotransferase	Synthesis of non-reducing cyclodextrins
			from starch, utilized in food, pharmaceutical,
			and chemical industries
		Whole microorganism	Solvent bioremediation and biotransformation
Micro-aerophile	Growth in <21% O_2	Catalase, Superoxide Dismutase	Produce toxic substances like superoxide
			free radicals and peroxides

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regions, glaciers, ocean deeps, shallow subterranean regions, upper atmosphere, refrigerated appliances and on and in plants and animals inhabiting cold regions (Cavicchioli *et al.*, 2002). These microorganisms mainly belong to the family of bacteria (e.g. *Pseudoalteromonas*, *Vibrio, Pseudomonas, Arthrobacter* and *Bacillus*) (Collins *et al.*, 2002; Zeng *et al.*, 2004; Okuda *et al.*, 2004), archea (e.g. *Methanogenium* and *Halorubrum*), fungi (such as *Penicillium* and *Cladosporium*) (Sakamoto *et al.*, 2003) and yeast (such as *Candida and Crystococcus*) (Nakagawa *et al.*, 2004).

Psychrophilic enzymes produced by such cold-adapted microorganisms display a high catalytic efficiency at low temperature that offers considerable potential in detergent, textile, food, pharmaceutical, leather, brewing and wine, paper and pulp industries (Kumar *et al.*, 2011). The use of psychrophiles and their enzymes have also been proposed in the bioremediation of soils and waste waters, as an alternative to physiochemical methods (Singh *et al.*, 2011). This adaptation of the enzymes to low temperature is related to the flexible structures of cold-active enzymes, which is achieved by a combination of structural features including reduction in core hydrophobicity, decreased ionic interactions, increased surface charge and longer surface loops. Due to these modifications, psychrophilic proteins loose their rigidity and gain increased structural flexibility (Gerday *et al.*, 2000).

Alkaliphiles/ Acidophiles

Alkaliphiles are the class of extremophiles that thrive in alkaline environments at a pH of 8 or more such in soda lakes and carbonate-rich soils. Acidophiles on the other hand, tend towards acidic conditions with a pH optimum for growth at, or below, pH 3 (Rothschild and Mancinelli, 2001; Gomes and Steiner, 2004). One of the most striking properties of such organisms is their use of proton pumps to maintain a neutral pH internally. Thus, intracellular enzymes from these microorganisms do not need to be adapted to extreme growth conditions, while their extracellular enzymes need to function at low or high pH environments depending upon their source (Kumar *et al.*, 2011). For pH adaptation, alkaliphiles and acidophiles utilize several strategies. Active mechanisms to achieve this may involve secondary proton uptake by membrane-associated antiporters. Passive mechanisms include negatively charged cell-wall polymers in alkaliphiles and unusual bioenergetics, unusual permeability properties, positive surface charges, high internal buffer capacity, overexpression of H^+ export enzymes and unique transporters in acidophiles (Horikoshi, 1999).

The alkaliphiles/acidophiles have great potential for biotechnological exploitation (Van den Burg, 2003; Wiegel and Kevbrin, 2004). Alkaliphiles are good source of pH stable enzymes like proteases, amylases, cellulases, lipases, xylanases, pullulanases, pectinases and chitinases (Kumar, 2002). The main industrial applications of these enzymes are in processes that are performed at high pH such as in the detergent industry, hide-dehairing, pulp and paper industry, starch-hydrolysis and food processing (Horikoshi, 1999; Hasan *et al*., 2010). Acidophiles have found a niche in the bioprocessing of minerals as mineral-sulfide oxidizing acidophiles find applications in the development of industrial mineral processing bioreactor (Norris *et al.*, 2000). Polymer-hydrolysis related processes have also initiated the search for biocatalysts from acidophiles.

Piezophiles

Microorganisms that prefer high-pressure conditions are termed piezophiles (barophiles). Oceans or deep-sea are home to these microorganisms and they are mainly distributed among the genera *Shewanella*, *Methanococcus*, *Pyrococcus* and *Moritella* (Kato *et al.*, 1998; Kato and Nogi, 2001). It is believed that enzymes isolated from piezophiles are stable at high pressure and do not need specific pressure-related adaptations. Piezophilic microorganisms and their enzymes have considerable potential for use in biotechnology, in particular for food industries, where high pressure is applied for processing and the sterilization of food materials.

Protein stabilization by high pressure during processing and sterilization of food materials, high pressures of a few hundred MPa can be used to induce the formation of gels or starch granules, the denaturation/coagulation of proteins or the transition of lipid phases. The use of high pressure leads to better flavor and color preservation than the use of high temperature to achieve the same ends. Moreover, enzymes that can operate at increased pressure and temperature like chymotrypsin have great advantages in biotechnological applications (Kumar *et al.*, 2011). The molecular basis of piezophily is now being investigated extensively. The focus has mainly been on the identification of pressure-regulated operons showing the relationship between pressure and microbial growth and function of certain proteins (Abe and Horikoshi, 2001; Yano and Poulos, 2003).

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Radiophiles

Microorganisms that are highly resistant to high levels of ionizing and ultraviolet radiation are called radiophiles. These radiation-resistant microorganisms show high potential in the treatment of radioactive environmental wastes. Numerous radiation-resistant bacteria have been isolated by incubating the culture in the presence of high gamma radiation. Radiophiles are receiving lot of attention recently, because of their ability to survive under conditions of starvation, oxidative stress and high amounts of DNA damage (Daly, 2000). *Deinococcus radiodurans*, the most radiation resistant organism known and the only one for which a system of genetic transformation and manipulation has been developed, is currently being engineered for remediation of radioactive wastes (Brim *et al.*, 2000).

Xerophiles

The organisms, which have the ability to grow in extremely dry conditions or in the presence of very low water activity, are considered to be xerophiles. However, only some specialized genera among bacteria, yeasts, fungi, lichens, algae are able to survive under such environment (Rothschild and Mancinelli, 2001). Xerophiles are considered to be responsible for spoiling of dry foods and stored grains, spices, nuts and oilseeds.

Metallophiles

Microorganisms that can grow in the presence of high metal concentrations (otherwise essential as trace elements) are called metallophiles. Since pollution by heavy metals (Cu, Cr, Zn, Cd, Co, Pb, Ag, Hg) posses a threat to public health, fishery and wild-life, there has been an increasing interest in metallophiles for removal of the toxic heavy metals from soils, sediments and wastewaters (Valls and de Lorenzo, 2002). Metallophiles also show high potential in bio-mining of expensive metals from effluents of industrial processes (Nies, 1999). To this end, a vast diversity of microbe-metal interactions is being exploited, ranging from reduction for anaerobic respiration to reduction for detoxification, as well as biosorption, bioleaching, bioaccumulation and biomineralization (Pieper and Reineke, 2000; Gadd, 2010). Heavy metals generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions or modifying the active conformation of biological molecules (Wood and Wang, 1983; Giller *et al.*, 1998; Rawlings, 2002). However, certain microorganisms are able to undo these effects and show resistance either by accumulating the metals in the form of particular protein-metal association, or heavy metal efflux systems (Wehrheim and Wettern, 1994; Nies and Silver, 1995; Naz *et al.*, 2005).

Halophiles

Halophiles are extremophilic microorganisms that can grow optimally in saline environment (media containing 0.5–5.2 M NaCl). In this system, non-halophiles are those that grow best in media containing <0.2 M NaCl, slight halophiles grow best with 0.2 to 0.5 M NaCl, moderate halophiles grow best with 0.2 to 0.5 M NaCl and extreme halophiles show optimal growth in media containing 1.5 to 5.2 M NaCl (Kumar and Khare, 2012). Such microorganisms are found in all three domains of life: Archea, Bacteria and Eukarya and have received considerable attention because of their potential use in biotechnology (Margesin and Schinner, 2001). There is an increasing interest in enzymes produced by these halophiles because they are expected to show optimal activities in the presence of salts and surfactants.

Halophiles employ different adaptation mechanisms to survive in hyper saline habitats. Halophilic archea accumulate salts (NaCl or KCl) up to the concentrations, isotonic with the environment, to maintain an osmotic balance. In order to cope with such high salt concentration, their enzymes acquire a relatively large number of negatively charged amino acid residues on their surface to prevent precipitation. Whereas in case of halophilic eubacteria, intracellular salt concentration is low and they maintain an osmotic balance between their cytoplasm and external medium by accumulating high concentrations of various organic osmotic solutes namely ecotines and hydroxyecotines (Margesin and Schinner, 2001). Operating via this mechanism, their intracellular enzymes have no special features for salt tolerance (Madern *et al.*, 2000; Margesin and Schinner, 2001; Kumar *et al.*, 2011).

Microaerophiles

Microaerophiles are microorganisms which are unable to grow when oxygen concentrations reach those found in air (20%) but nevertheless whose growth requires the presence of some oxygen (e.g., 2 to 10%). "Microaerophiles appear to grow best in the presence of a small amount of free oxygen. They grow below the surface of the medium in a culture tube at the level where oxygen availability matches their needs.

The respiratory chain enzymes of microaerophilic bacteria

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should play a major role in their adaptation to growth at low oxygen tensions. The genes encoding the putative NADH:quinone reductases (NDH-1), the ubiquinol: cytochrome c oxidoreductases (bc1 complex) and the terminal oxidases of the microaerophiles Campylobacter jejuni and Helicobacter pylori were analysed to identify structural elements that may be required for their unique energy metabolism. The gene clusters encoding NDH-1 in both *C. jejuni* and *H. pylori* lacked nuoE and nuoF, and in their place were genes encoding two unknown proteins. The NuoG subunit in these microaerophilic bacteria appeared to have an additional Fe-S cluster that is not present in NDH-1 from other organisms; but *C. jejuni* and *H. pylori* differed from each other in a cysteine-rich segment in this subunit, which is present in some but not all NDH-1. Both organisms lacked genes orthologous to those encoding NDH-2. The subunits of the bc1 complex of both bacteria were similar, and the Rieske Fe-S and cytochrome b subunits had significant similarity to those of Paracoccus denitrificans and Rhodobacter capsulatus, well-studied bacterial bc1 complexes. The composition of the terminal oxidases of *C. jejuni* and *H. pylori* was different; both bacteria had cytochrome cbb3 oxidases, but *C. jejuni* also contained a bd-type quinol oxidase (Smith *et al.*, 2000)

Conclusion

Extremozymes obtained from extremophiles have a great economic potential in many industrial processes, including agricultural, chemical and pharmaceutical applications. Many consumer products will increasingly benefit from the addition or exploitation of extremozymes. It has been suggested that less than 10% of the organism in a defined environment will be cultivatable and so further improvement of gene expression technologies (e.g., by the development of novel and improved heterologous host systems) will accelerate the exploration of microbial diversity. These extremozymes will be used in novel biocatalytic processes that are faster, more accurate specific and environmentally friendly. Concurrent developments of protein engineering and directed evolution technologies will result in further tailoring and improving biocatalytic traits which will increase the application of enzymes from extremophiles in industry.

References

Abe F. and K. Horikoshi. 2001. The biotechnological potential of piezophiles. *Trends in Biotechnology* **19**(3): 102-108.

Arnold, F.H. and J.C. Moore. 1998. Para-Nitrobenzyl Esterases with Enhanced Activity in Aqueous and Nonaqueous Media, *U.S. Patents* **5**: 741-691.

Bertoldo, C. and G. Antranikian. 2002. Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. *Current Opinion in Chemical Biology* **6**: 151-160.

Brim, H., S. McFarlan, L. Wackett, K.W. Minton, M. Zhai, J. Fredrickson and J.D. Michael. 2000. Engineering Deinococcus radiodurans for metal remediation in radioactive mixed waste environments. *Nature Biotechnology* **18**: 85-90.

Busk, P.K. and L. Lange. 2013. Cellulolytic potential of thermophilic species from four fungal orders. *AMB Express* **3**:47-56.

Cavicchioli, R., N. Saunders and T. Thomas. 2002. In Encyclopedia of life support systems (EOLSS), Eolss Publishers, Oxford, UK.

Chien A., D.B. Edgar and J.M. Trela. 1976. Deoxyribonucleic acid polymerase from the extreme thermophile Thermus aquaticus. *Journal of Bacteriology* **127**: 1550–1557.

Collins, T., M.A. Meuwis, I. Stals, M. Claeyssens, G. Feller and C. Gerday. 2002. A novel Family 8 Xylanase. Functional and Physicochemical Characterization. *Journal of Biological Chemistry* **277**: 35133-35139.

Daly, M.J. 2000. Engineering radiation-resistant bacteria for environmental biotechnology. *Current Opinion in Biotechnology* **11**: 280-285.

Datta, S., L.R. Christena and Y.R.S. Rajaram. 2013. Enzyme immobilization: an overview on techniques and support materials. *3 Biotech* **3**: 1–9. DOI 10.1007/s13205-012-0071-7

Demirjiana, D.C., F. Morýs-Varas and C.S. Cassidy. 2001. Enzymes from extremophiles. *Current Opinion in Chemical Biology* **5**(2): 144–151.

Eichler, J. 2001. Biotechnological uses of archaeal extremozymes. *Biotechnology Advances* **19**: 261–278.

Gadd, G.M. 2010. Metals, minerals and microbes: Geomicrobiology and Bioremediation. *Microbiology* **156**: 609–643.

Gaur, R., T. Grover, S. Kapoor and S.K. Khare. 2010. Purification and characterization of a solvent stable aminopeptidase from Pseudomonas aeruginosa: Cloning and analysis of aminopeptidase gene conferring solvent stability. *Process Biochemistry* **45**: 757-764.

Gaur, R. and S.K. Khare. 2009. Cellular response mechanisms in Pseudomonas aeruginosa PseA during growth in organic solvents. *Letters in Applied Microbiology* **49 (3)**: 372-377.

Gaur, R., G.N. Gupta, Vamsikrishnan and S.K. Khare. 2008. Protein-Coated Microcrystals of Pseudomonas aeruginosa PseA lipase. *Applied Biochemistry and Biotechnology* **151**: 160-166.

Gerday, C., M. Aittaleb, M. Bentahir, J.P. Chessa, P. Claverie, T. Collins, S. D'Amico, J. Dumont, G. Garsoux, D. Georlette, A. Hoyoux, T. Lonhienne, M. Meuwis and G. Feller. 2000. Cold adapted enzymes: from fundamentals to biotechnology. *Trends in Biotechnology* **18**: 103-107.

Gerike, U., M.J. Danson and D.W. Hough. 2001. Cold-active citrate synthase: mutagenesis of active-site residues. *Protein Engineering* **14(9)**: 655-661.

Giller, K.E., E. Witter, S.P. Mcgrath. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil Biology and Biochemistry* **30**:1389–1414

Gomes, J. and W. Steiner. 2004. The Biocatalytic Potential of Extremophiles and Extremozymes. *Food Technology and Biotechnology* **42**: 223-235.

Gupta, A. and S.K. Khare. 2009. Enzymes from solvent tolerant microbes: Useful biocatalysts for non-aqueous enzymology, *Critical Review of Biotechnology* **29(1)**: 44-54.

Haki, D.J. and S.K. Rakshit. 2003. Developments in industrially important thermostable enzymes: a review. *Bioresource Technology* **89**: 17-34.

Hasan, F., A.A. Shah, S. Javed and A. Hameed. 2010. Enzymes used in detergents: Lipases. *African Journal of Biotechnology* **9**(31): 4836-4844.

Horikoshi, K. 1999. Alkaliphiles: some applications of their products for biotechnology. *Microbiology & Molecular Biology Reviews* **63**: 735–50.

Hough, D.W. and M.J. Danson. 1999. Extremozymes. *Current Opinion in Chemical Biology* **3**: 39–46.

Kaledin, A.S., A.G. Sliusarenko and S.I. Gorodetski-. 1980. Isolation and properties of DNA polymerase from extreme thermophylic bacteria Thermus aquaticus YT-1. *Biokhimiia* **45**(4): 644-51.

Karan, R., R.K.M. Singh, S. Kapoor and S.K. Khare. 2011. Gene identification and molecular characterization of solvent stable protease from a moderately haloalkaliphilic bacterium Geomicrobium sp. EMB2. *Journal of Microbiology and Biotechnology* **21**:129-135.

Kato, C. and Y. Nogi. 2001. Correlation between phylogenetic structure and function: examples from deep-sea Shewanella. *FEMS Microbiology Ecology* **35**: 223–230.

Kato, C., L. Li, Y. Nogi, Y. Nakamura, J. Tamaoka and K. Horikoshi. 1998**.** Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters. *Applied & Environmental Microbiology* **64**: 1510–1513.

Kumar, L., G. Awasthi and B. Singh. 2011. Extremophiles: A novel source of industrially important enzymes. *Biotechnology*, **10**: 121-135.

Kumar, S. and S.K. Khare. 2012. Purification and characterization of maltooligosaccharide-forming á-amylase from moderately halophilic Marinobacter sp. EMB8. *Bioresource Technology* **116:** 247–251

Kumar, S. and R. Nussinov. 2001. How do thermophilic proteins deal with heat? *Cellular & Molecular Life Sciences* **58**(9): 1216–1233.

Kumar, C.G. 2002. Purification and characterization of a thermostable alkaline protease from alkalophilic *Bacillus pumilus*. *Letters in Applied Microbiology, **34***: 13–17.

Madern, D., C. Ebel and G. Zaccai. 2000. Halophilic adaptation of enzymes. *Extremophiles* **4**: 91–8.

Margesin, R. and F. Schinner. 1994. Properties of cold-adapted microorganisms and their potential role in biotechnology. *Journal of Bacteriology* **33**: 1–14.

Martinez, A. and G. Soberon-Chavez. 2001. Characterization of the lip A gene encoding the major lipase from *Pseudomonas aeruginosa* strain IGB 83. *Applied Microbiology & Biotechnology* **56:** 731-735.

Macelroy, R. 1974. Some comments on the evolution of extremophiles. *Biosystems* **6**(1): 74-75, DOI:10.1016/0303-2647(74)90026-4

Nakagawa, T., T. Nagaoka, S. Taniguchi, T. Miyaji and N. Tomizuka. 2004. Isolation and characterization of psychrophilic yeasts producing cold-adapted pectinolytic enzymes, *Letters in Applied Microbiology* **38**: 383–387.

NASA 2003. Retrieved 2009. Mars Exploration – Press kit. 07-14.

Naz, N., H.K. Young, N. Ahmed and G.M. Gadd. 2005. Cadmium accumulation and DNA homology with metal resistance genes in sulfate-reducing bacteria. *Applied & Environmental Microbiology* **71**(8): 4610-8.

Niehaus, F., C. Bertoldo, M. Ka¨hler and G. Antranikian. 1999. Extremophiles as a source of novel enzymes for industrial application. *Applied Microbiology and Biotechnology* **51**: 711 – 29.

Nies, D.H. and S. Silver. 1995. Ion efflux systems involved in bacterial metal resistances. *Journal of Industrial Microbiology* **14**: 186–199.

Nies, D.H. 1999. Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology* **51**(6): 730-50.

Norris, P.R., N.P. Burton and N.A.M. Faulis. 2000. Acidophiles in bioreactor mineral processing. *Extremophiles* **4**(2):71-76.

Okuda, M., N. Sumitomo, Y. Takimura, A. Ogawa, K. Saeki, S. Kawai, T. Kobayashi and S. Ito. 2004. A new subtilisin family: nucleotide and deduced amino acid sequences of new high- -molecular-mass alkaline proteases from *Bacillus* spp. *Extremophiles* **8**: 229–235.

Patel, R.K., M.S. Dodia, R.H. Joshi and S.P. Singh. 2006. Purification and characterization of alkaline protease from a newly isolated haloalkaliphilic *Bacillus* sp. *Process Biochemistry* **41**: 2002-2009.

Pieper, D.H. and W. Reineke. 2000. Engineering bacteria for bioremediation. *Current Opinion in Biotechnology* **11**(3): 262-270.

Rawlings, DE. 2002. Heavy metal mining using microbes. Annual Review of Microbiology **56**: 65-91.

Rothschild, L.J. and R.L. Manicinelli. 2001. Life in extreme environments. *Nature* **409**: 1092–101.

Roy, I., M. Sardar and M.N. Gupta. 2003. Hydrolysis of chitin by PectinexTM. *Enzyme & Microbial Technology* **32**(5): 582-588.

Rozell, J.D. 1999. Commercial scale biocatalysis myths and realities. *Bioorganic Medicinal Chemistry* **7**: 2253-2261.

Sakamoto, T., H. Ihara, S. Kozakic and H. Kawasaki. 2003. A cold-adapted endo-arabinanase from *Penicillium chrysogenum*. *Biochimica et Biophysica Acta - General Subject* **1624**: 70–75.

Scandurra, R., V. Consalvi, R. Chiaraluce, L. Politi and P.C. Engel. 1998. Protein thermostability in extremophiles. *Biochimie* **80**: 933-941.

Schiraldi, C. and M. De Rosa. 2002. The production of biocatalysts and biomolecules from extremophiles. *Trends in Biotechnology* **20**(12): 515-21.

Singh, S.K., S.K. Singh, V.R. Tripathi, S.K. Khare and S.K. Garg. 2011. A novel sychrotrophic, solvent tolerant Pseudomonas putida SKG-1 and solvent stability of its psychro-thermoalkalistable protease. *Process Biochemistry* **46**: 1430-1435.

Singh, S., L. Shukla, S.K. Khare and L. Nain. 2011. Detection and characterization of new thermostable endoglucanase from Aspergillus awamori strain F18. Journal of Mycology and Plant Pathology **41(1)**:97-103.

Sinha, A., K.K. Pant and S.K. Khare. 2012. Studies on mercury bioremediation by alginate immobilized mercury tolerant

PRINT ISSN.: 0974-1712 ONLINE ISSN.: 2230-732X

Bacillus sp. cells. *International Biodeterioration and Biodegradation* **71**:1-8

Smith, M.A., M. Finel, V. Korolik and G.L. Mendz. 2000. Characteristics of the aerobic respiratory chains of the microaerophiles *Campylobacter jejuni* and *Helicobacter pylori*. *Archives of Microbiology* **174**(1-2): 1-10.

Sterner, R. and W. Liebl. 2001. Thermophilic adaptation of proteins. *Critical Reviews in Biochemistry and Molecular Biology* **36**: 39-106.

Tyagi, R. and M.N. Gupta. 1998. Chemical modification and chemical cross-linking for protein/enzyme stabilization. *Biochemistry - Moscow* **63**: 334-344.

Valls, M. and V. de Lorenzo. 2002. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS Microbiology Reviews* **26**: 327-338.

Van den Burg, B. and V.G. Eijsink. 2002. Selection of mutations for increased protein stability. *Current Opinion in Biotechnology* **13**: 333–337.

Van den Burg, B. 2003. Extremophiles as a source for novel enzymes. *Current Opinion in Microbiology* **6**: 213-218.

Vieille, C. and G.J. Zeikus. 2001. Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. *Microbiology & Molecular Biology Reviews* **65**: 1-43.

Wehrheim, B. and M. Wettern. 1994. Biosorption of cadmium, copper and lead by isolated mother cell walls and whole cells of *Chlorella fusca*. *Applied Microbiology and Biotechnology* **41**: 725-728.

Wiegel, J. and V.V. Kevbrin. 2004. Alkalithermophiles. *Biochemical Society Transactions* **32**: 193-198.

Wood JM and Wang H.K 1983. Microbial resistance to heavy metals. *Environmental Science & Technology* **17**: 82a–90a.

Yano, J.K. and T.L. Poulos. 2003. Review. New understandings of thermostable and peizostable enzymes. *Current Opinion in Microbiology* **14**(4): 360-5.

Zeng, X., X. Xiao, P. Wang and F. Wang. 2004. Screening and Characterization of psychrotrophic lipolytic bacteria from deep sea sediments. *Journal of Microbiology and Biotechnology* **14**: 952-958.

PRINT ISSN.: 0974-1712 ONLINE ISSN.: 2230-732X