Extreme Adaptations of Extremophiles: Extremozymes

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Abstract
Extremozymes are the enzymes that are derived from extremophiles, microorganisms that thrive well inextreme environments such as extremes of salinity, acidity, alkalinity, temperature or pressure as well as other seemingly drastic surroundings. Extremozymes provide new possibilities for biocatalysis and biotransformation due to their extreme thermostability.Extremozymessuch as cellulases, amylases, xylanases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases and phytases have useful applications in molecular biology, medical research, industrial food or feed technology, detergents and cosmetics. These enzymes are highly stable at extreme conditions.
Keywords: Extremophiles, extremozymes, enzymes.

Introduction
Extremophiles are the organisms which permanently experience environmental conditions which may be considered as extreme in comparison to the physio-chemical characteristics of the normal environment of human cells. These organisms thrive in habitats which are intolerably hostile or even lethal to other forms of terrestrial life. They thrive in extreme hot niches, ice and salt solutions, as well as acid and alkaline conditions, some may grow in toxic waste, organic solvents, heavy metals, or in several other habitats that were previously considered inhospitable for life. They are classified according to the conditions in which they grow. Extremophiles include members of all three domains of life, i.e., bacteria, archaea and eukarya. Most extremophiles are microorganisms, but this group also includes eukaryotes such as protists (e.g., algae, fungi and protozoa) and multicellular organisms. Archaea is the main group to thrive in extreme environments. Although members of this group are generally less versatile then bacteria and eukaryotes.

Psychrophiles
Psychrophiles (psychrotolerant or psychrotroph) are the microorganism that prefers permanently cold environments, but can also tolerate a wide range of temperatures reaching up into the mesophilic range (Cavicchioli and Siddiqui, 2006). Psychrophilic microorganisms have successfully colonized all permanently cold environments from deep sea to mountains. Psychrotolerent microbes are extremely important since they survive and retain their functionality in cold temperature conditions, while growing optimally at warm temperatures. High-altitude cold habitats of the Himalayas are little explored with respect to bacterial diversity. Soil formation and primary microbial succession can be well studied in recently deglaciated areas where phototrophic microorganisms may play a role as primary producers (Frey *et al*., 2013). Psychrophilic microorganisms provide an enormous natural resource of enzymes that function effectively in the cold, and these cold-adapted enzymes have been targeted for their biotechnological potential (Table 1). Cold-adapted enzymes provide economic benefit by being more productive than mesophilic or thermophilic homologues at low temperature, thereby providing energy savings to the processes for which these enzymes are used. Cold-adapted enzymes have found application in industries like household detergents, molecular biology and baking. The biotechnological value of cold-adapted

enzymes stems from their high k_{cat} at low to moderate temperatures, their high thermo-lability at elevated temperatures and their ability to function in organic solvents (Margesin and Feller, 2010).

Table 1: Potential application of extremophiles in biotechnology and industry.

S. No.	Source	Enzymes	Use	Reference
1.	Psychrophiles	Protease	Contact lens cleaning solution, meat tenderizing	Cavicchioli and Siddiqui (2006); Wang *et al.*, (2010)
		Protease, lipase, cellulases, amylases	Detergent	Joseph *etal*. (2008); Collins *etal*. (2005); Wang *et al.*, (2010)
		Alkaline phosphatase	Molecular biology	Dahiya *etal*., (2006)
		Lipases and proteases	Cheese manufacture	Cavicchioli and Siddiqui (2006)
		β - Galactosidase	Lactose hydrolysis in milk products	Cavicchioli and Siddiqui. (2006); Joseph *etal*. (2008)
2.	Thermophiles	Cellulases	Production of alcohol, fruit industry, household chemistry	Antranikian *et al.* (2005)
		Amylases, Pullulanases	Starch processing, glucose and fructose for sweeteners	Alqueres *et al.* (2007); Antranikian *et al.* (2005); Eichler J. (2001)
		Proteases and lipases	Dairy products	Egorova *et al.* (2005); Eichler J. (2001)
		Xylanases	Paper bleaching	Egorova *et al.* (2005); Eichler J. (2001); Andrade *et al.* (2001)
		Protease	Amino acid production from keratins, food processing, baking, brewing, detergents	Egorova *et al.* (2005); Eichler J. (2001)
3.	Halophiles	Carotene	Food colouring	Raj *et al.*, (2007); DasSarma *et al.*, (2010)
		Glycerol	Pharmaceuticals	Lentzen *et al.*, (2006); DasSarma *et al.*, (2010)
		Lipids	Heating oil and cosmetic packing	Bestvater *et al.* (2008)
		Nucleases, amylases and proteases	Flavouring agents	Amoozergar *et al.*, (2008); Fukushima *et al.*(2005); Souza, (2010)
		Membranes	Surfactants for pharmaceuticals	DasSarma *et al.*, (2010)
4.	Alkaliphiles	Proteases, cellulases, xylanases and lipases	Detergents	Ito *et al.* (1998); Horikoshi *et al.* (1973)
		Proteases	Gelatin removal on x-ray film	Fujiwara *et al.* (1991); Ishikawa *et al.* (1993)
		Pectinases	Fine papers, waste treatment and degamming	Yoshihara *et al.* (1982)
5.	Acidophiles	Amylases	Degradation of starch	(Buonocore *et al.* 1976).
		Proteases	Non-allergic preservatives for medicines and cosmetics	Gaffney *et al.* 1996; Honda 1998)
6.	Barophiles	Proteases	High pressure bioreactor systems	Michels *et al.* (1997)

Thermophiles

Thermophiles are the microorganisms which optimally grow between temperatures 60-110^{o}C. The ability of thermophilic bacteria to grow and propagate at elevated temperature and to produce extracellular enzymes with unique and valuable properties was due to their ability to manipulate their genetic composition. Thermophilic microorganisms may have tremendous potential in future microbial and enzyme technology because their unique ability to function at high temperature enables development of improved or new biotechnology.Thermophiles mostly belong to two phylogenetically very different domains of life which include bacteria and archaea (Stetter *et al.,* 1993 and Stetter, 2006). Generally, it is agreed that in most hydrothermal environments where temperatures range between 50 and 90^{o}C bacteria is dominating in the communities of microorganisms. In environments where temperatures are above 90^{o}C archaea are dominating (Reysenbach and Yernool, 2002). Among the enzymes microbial esterases and lipases are of substantial interest because of their prospective biotechnological application such as the modification of triglycerides for fat and oil industry as shown in Table 1.

Halophiles

Halophiles (from the Greek, hal, meaning sea or salt, and philos, meaning loving) are distinguished by their requirement of high salinity conditions for growth. Halophiles are salt-loving organisms that flourish in saline environments and can be classified as slightly, moderately or extremely halophilic, depending on their requirement for sodium chloride (DasSarma*etal*., 2012). Although most marine organisms are slightly halophilic, but moderate and extreme halophiles which inhabit hypersaline environments with salinity higher than in the sea are generally more specialized microbes. Many halophiles and halotolerant microorganisms can grow over a wide range of salt concentrations, with requirement or tolerance for salts sometimes dependent on environmental and nutritional factors. High osmolarity in hypersaline conditions is deleterious to most cells since water is lost to the external medium. Halophiles generally accumulate high solute concentrations within the cytoplasm to prevent loss of cellular water (Roberts, 2005; Yancey, 2005). These organisms produceacidicproteinsthatcanfunctioninhigh salinity byleft oversolvatedandreducingaggregation, precipitation anddenaturation(Madern *et al.*, 2000). Halophilic microorganisms also produce many stable enzymes including hydrolytic enzymes such as DNAses, lipases, amylases, gelatinases and proteases which are capable of functioning under high saline conditions, which would lead to precipitation or denaturation of most other proteins. Compatible solutes of halophilic bacteria are used in cosmetics and improving hydration properties generally (Bestvater *et al.*, 2008). Industrial uses of compounds present in halophiles such as β-carotene, poly-β-hydroxyalkanoate, exopolysaccharides,etc as shown in Table 1.

Alkaliphiles

Alkaliphiles are the organisms that usually grow between pH 10 and 12 with optimum pH for growth being above 9. Alkaliphilic microorganisms are widely distributed and can be found in almost any environment, even in environments where the overall pH may not be particularly alkaline. The main industrial application of alkaliphilic enzymes (Table 1) is in the detergent industry (Ito *et al.*, 1998). Alkaline protease is used to decompose the gelatinuous coating of x-ray films from where silver is recovered (Fujiwara *et al.*, 1991; Ishikawa *et al.*, 1993). Alkaliphilic microorganisms are divided into two groups: ones that grow only at alkaline pHabove 8 but not at pH 7, and ones that grow not only at alkaline pH but also at neutral ph. For convenience, the former is called an absolute alkaliphile and the latter as facultative alkaliphile.

Acidophiles

Acidophiles are the microorganisms having optimum pH for growth less than 3.Both natural and man-made acidic environments occur in the biosphere, including sulfidic mine areas and marine volcanic vents, the microorganisms that inhabit these habitats are termed ‘acidophiles’. Acidophiles seem to share distinctive structural and functional characteristics including a reversed membrane potential, highly impermeable cell membranes and a predominance of secondary transporters. Once protons enter the cytoplasm, methods are required to alleviate effects of a lowered internal pH. Acidophiles are most widely distributed in the bacterial and archaeal domains (Johnson and Hallberg, 2003). Acidophiles have a highly impermeable cell membrane to restrict proton influx into the cytoplasm to help maintain change in Ph (Konings *et al.*, 2002). Permeability ofprotons through the membrane determines the rate at which protons leak inward, the balance between proton permeability, proton influx through energetic and transport systems, and the rate of outward proton pumping determines whether a cell can sustain an appropriate PMF. Number of enzymes from acidophiles have application in pharmaceutical and food industry as shown in Table 1.

Basophiles

Barophilic (piezophilic) microorganisms display elevated growth rates at pressures above 1 atmosphere (Zobell and Morita 1957; Yayanos A. A. 1995). From high-pressure, low-temperature deep-sea sediments numerous barophilic microorganisms havebeen isolated and analyzed for their growth characteristics (Kato *et al.*, 1996, 1995). Studies have shown that these extremophiles have been found to modulate gene expression in response to pressure (Bartlett *et al.*, 1989; Kato *et al.*, 1997; Welch *et al.*, 1996). The enzymes like proteases find their industrial application in high pressure bioreactor systems.

Radiophiles

Radiophiles are the microorganisms that are highly resistant to high levels of ionizing and ultraviolet radiation. The genetic engineering and environmental biotechnology aspects of radiophiles have been reviewed by Daly (Daly M.J., 2000). Examplesinclude*Deinococcusradiodurans* (Sandigursky *et al.*, 2004), *Deinococcusradiophilus* (Yun and Lee, 2004), *Thermococcusmarinus* sp. And *Thermococcusradiotolerans* (Jolivet *et al.*, 2004). The remarkable bacterium thatwas first isolated in 1956 is highly resistant to chemicals, oxidative damage, high levels of radiation*Deinococcusradiodurans* (5 Mrad, 3000 times higher than what would kill a human) and dehydration. It contains a spectrum of genes that encode for multiple activities that repair DNA damage. The genes of three putative uracil-DNA glycosylases have been cloned and expressed to determine their biochemical function (Sandigursky *et al.*, 2004).

Conclusion

Extremozymes have great economic potential in many industrial processes (e.g. agriculture, food, feed and drinks, detergents, textile, leather, pulp and paper).The state of Jammu and Kashmir experiences very harsh climatic conditions and presence of number of glaciers and hot springs can provide numerous opportunities to explore the extremozymes from psychrophilic and thermophilic microorganisms which can prove useful for industries. It is strongly believed that discoveries of new extremophiles and genetic engineering of the newly isolated as well as of the currently available extreme microbes will offer novel opportunities for biocatalysis and biotransformations.

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