# Exploiting extremophile machinery for industrial applications

## *Abstract*

Extremophiles live in harsh environments that tend to be at the ends of the pH, temperature, pressure, and salinity spectrums. In order to live in these harsh environments, these organisms must contain proteins that are structurally adapted to maintain a native state in the environments in which they live. These modified proteins can be used in industrial settings to streamline certain processes. These proteins have also been used for their capacity to degrade toxic waste compounds. In this review, the features of these proteins that allow them to maintain structural integrity in these environments are discussed, as well as the potential applications of such proteins in industrial settings.

## *Introduction*

Since the discovery of thermophiles in the early 1960s, researchers have been trying to utilize extremophile machinery in order to advance and perfect biotechnological processes. The capacity of the machinery of these organisms to regulate metabolic processes under such harsh conditions makes these organisms viable candidates for reactions that occur outside of moderate environmental conditions. Perhaps the most noteworthy use of these organisms in a laboratory setting is the use of DNA polymerase isolated from the thermophile *Thermus aquaticus* ; the use of this protein over DNA polymerase isolated from a mesophile allowed for the automation of polymerase chain reaction (PCR), which greatly reduced the amount of work required to run such an experiment and concurrently increased the potential efficiency of labs that perform these experiments on a regular basis. Furthermore, it made reactions such as quantitative PCR, a very valuable tool for not only quantifying relative amounts of DNA but also for identifying the presence of DNA that codes for a protein of interest. While the aforementioned example utilizes this machinery for the sake of amplifying DNA, extremophile proteins have potential to be used in many different processes such as breaking down hazardous wastes, creating biofuels, and have many different potential uses in the food industry. These proteins have potential to replace or modify many large scale industrial processes in the near future as more is learned about these organisms and the proteins that drive their metabolic processes. The purpose of this review is to explore the current understanding of what makes the proteins of these organisms stable under harsh conditions, as well as to explore the potential uses of these proteins in industry.

### *Protein stabilization under extreme conditions*

Extremophiles fall into several different categories, each of which are capable of surviving under conditions that would easily kill bacteria that grow in moderate environments. Extremophiles are typically divided into four different groups: bacteria that thrive in extreme temperatures, bacteria that thrive in low or high pH conditions, bacteria that thrive in high salinity, and bacteria that thrive under extremely high pressures. Amongst these categories, there tends to be a wide variety of conditions in which these organisms can survive, furthering the potential applications of these organisms in industrial processes.

Amongst the bacteria that live in extreme temperatures, there are several different classifications. Psychrophiles survive at temperatures ranging from 20°C to -20°C, while thermophiles optimally grow at temperatures ranging from 50°C to 70°C. There are also organisms known as hyperthermophiles that are capable of functioning at temperatures above 100° C (Stetter, 2006). One of the main factors contributing to the stability of organisms that exist in extremely high temperatures is increased binding interactions. There is an increased number of disulfide bonds in these proteins as well as an increase in the amount of salt bridging. In addition to increased bonding, there is greater oligomerization with more subunit rigidity; this ultimately results in a hydrophobic core that is more tightly packed that its mesophilic counterparts (Boutz, et al., 2008). This is critical to protein integrity as, at higher temperatures, hydrophobic interactions tend to weaken as the temperature increases. There is also a tendency to have increased polar charged molecules on the outer surface of the protein whereas mesophilic proteins tend to have polar uncharged residues on the outer surface; at higher temperatures, uncharged molecules face deamination, which subsequently leads to denaturation of the protein. These charged residues have also proposed to serve as a mechanism for preventing protein aggregation as temperatures increase (Vieille & Zeikus, 2001).

Contrary to thermophiles, psychrophilic organisms tend to have proteins with a more flexible structure and, as a consequence, weaker binding interactions. The structural adaptations required to remain stable in such an environment also give these proteins a low substrate affinity as these modifications alter how substrates bind at the active site; these modifications, however, also tend to give these proteins higher substrate specificity with a specific activity that is approximately ten times higher than that of its mesophilic counterparts (Georlette, et al., 2003).

Similarly, the primary adaptations seen with piezophiles tend to be more densely packed structures with a tightly packed hydrophobic core. However, the hydrophobic core of piezophiles tends to be structured with small hydrophobic residues as opposed to the larger hydrophobic residues as seen in thermophiles; ultimately, these smaller residues allow the structure to be more tightly packed which, intuitively, would be required of a protein that exists under extreme pressure. One study performed a statistical analysis on the amino acid substitution rate of two different piezophiles that reside deep within the ocean and found that, when compared to relatives that live under far more shallow waters, there is a positive selection force. This selection force is primarily directed towards inorganic ion transport, defense mechanisms, and intracellular trafficking, all processes that are related to function of the plasma membrane (Campanaro, et al., 2008). Contrary to the belief that there is a high-pressure adaption of these proteins, one study found that dihydrofolate reductase of *Moritella profunda,* a psychropiezophile, showed no significant difference when compared to the dihydrofolate reductase of *Escherichia coli* . *M. profunda* is an organism that optimally lives at a temperature of 2°C and a pressure of 280 bar, which is very different from the mesophilic environment of *E. coli* (Hay, et al., 2009). Although the current knowledge of pressure adaptations is limited, a possible explanation for this discrepancy could be that some proteins are suitable for wide ranges of pressures.

Halophilic organisms have one major modification that allows them to survive in high saline environments. Halophiles have proteins with surfaces covered in acidic residues. Currently, there are two potential explanations for this modification: the negative charges could serve as a mechanism for interacting with water molecules, and therefore act to keep these proteins in solution, or these charges interact with hydrated cations and therefore create a shell of hydration around the protein. Halophilic proteins also have lower relative amounts of hydrophobic residues. The modifications in halophilic proteins are made largely so that these proteins have more favorable interactions with water, likely to compete with the large concentrations of ions present in the environments in which they grow.

In organisms that live in pH extremes, proteins tend to also make modifications in the surface charges. Alkaliphiles tend to have a higher composition of arginine and lysine on the outer surface of their proteins, resulting in an overall positive charge (Siddiqui & Thomas, 2008). Acidophiles, however, tend to have higher numbers of glutamic acid and aspartic acid on their proteins imbuing an overall negative charge on the outer surface (Huang, et al., 2005). The structural modifications of these proteins may largely be attributed to the nature of the environments in which they live; acids have high concentrations of protons, which are positively charged, and alkaline solutions have high concentrations of the negatively charged hydroxide ions in solution.

It is important to specify that these types of adaptations are not completely independent of one another; in other words, some organisms are capable of living in multiple extreme conditions such as deep-sea organisms that live in cold, high pressure environments. These sorts of adaptations that allow these proteins to exist in multifaceted environments make them all the better for use in industrial settings.

*Commercially relevant compounds*

Perhaps one of the more popular uses of thermophiles in recent years is the utilization of these organisms in biofuel production. Since the utilization of fossil fuels as a fuel source has raised many concerns in recent years for a number of reasons, there has been a large push create new ways of producing fuel on a large scale. Many labs have pushed toward engineering strains of bacteria that are have been genetically altered to maximized production of ethanol. One study found that a high ethanol titer could be produced growing *Thermoanaerobacterium saccharolyticum* with wood, more specifically cellulose and hemicellulose, as a food source. By removing the genes that code for lactate dehydrogenase, acetate kinase, and phosphotransacetylase, the relative output of ethanol can be significantly increased while decreasing byproducts such as lactic acid and acetic acid. Using this method, this lab was able to obtain an ethanol yield of over 90%. However, this process was much less efficient when using pretreated hardwood; if this were to be used as a method for recycling old building materials, provisions to remove these harsh chemicals would have to be made in order for this process to be effective (Herring, et al., 2016). Another lab that was utilizing the same organism, but instead used simply xylose as a nutrient source, found that the relative concentrations of ethanol produced were very close to the theoretical yield. When compared to other organisms' ethanol conversion efficiency, *T. saccharolyticum* was more efficient than both *E. coli* and *Saccharomyces Cerevisiae* in terms of the relative concentrations of xylose put into the reaction and the ethanol yield (Shaw, et al., 2008). In addition to having a high yield, this method of ethanol production is continuous whereas most ethanol production processes rely on batch processing; this continuous method can be sustained as thermophilic bacteria can survive the increased temperature required to burn off the ethanol, whereas an organism such as a yeast could not.

Thermophilic bacteria have potential to be incredibly useful in the food industry as well. For example, the breakdown of starch into simpler sugars has two primary steps: a liquification step, which is the process of breaking bonds between starch molecules and requires temperatures of 105°C to 110°C and saccharification, which is the process of breaking down starch molecules into simple sugars and requires temperatures of 55° C to 60° C (Shivlata & Tulasi, 2015). Of course, using mesophilic bacteria, the proteins involved would ultimately become denatured at the end of this process, requiring a new bacterial culture to have the proper materials; the distinct advantage of using extremophile bacteria is that it is ultimately a more efficient process. Another food industry significant enzyme includes dextranase, which is very important in sugar cane processing. Processing sugar cane juice is an operation that occurs at high temperatures and an alkaline pH. Some organisms that tend to be resident in this juice produce dextran, which will block filters over time if it is not broken down; adding a dextranase that can remain stable at under these conditions solves this problem without having to take extensive steps to either clean the filters or find a way around these issues. Using this enzyme on test batches of sugar cane juice showed a 67% reduction in the content of dextran over a 24-hour period, proving to be very effective (Purushe, et al., 2012).

### *Degradation of waste materials*

Another potential application of these thermophile proteins is in degradation of waste materials. One study that explored the potential use of vegetable wastes as a nutrient source for extremophiles found that these organisms could be sustained solely on these waste materials. These waste materials are the byproducts of other processes, such as canning, and include the seeds, peels, and pulp of vegetables and fruits. This process not only utilizes waste materials, but it has potential to be used as a way to cultivate extremophile proteins and molecules that could be used in other industrially relevant processes (Di Donato, et al., 2011).

Extremophile proteins also tend to be associated with the production of keratinases. The food industry, particularly the meat industry, generates keratin rich wastes such as feathers, hair, nails and horns. Once these products are broken down, they can be used in other products such as fertilizers or feed. They also have potential use in the leather industry where hair must be removed from the leather products before they may be processed (Brandelli, 2008).

### *Conclusions*

While research about extremophiles is still developing, these organisms clearly have potential value from a commercial industry standpoint. A greater understanding of how these organisms and their proteins adapt to these types of environments will give researchers a better understanding of how to exploit these proteins and potentially apply them in an industrial setting.

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