Chapter 1
Introduction to Extremozymes

R. Navanietha Krishnaraj and Rajesh K. Sani

What Will You Learn from This Chapter?
This chapter introduces the basic concepts of enzymes, applications of enzymes and advantages of microbial bioprocesses over the enzymatic bioprocesses. The chapter gives an introduction about the extremozymes, its sources, and advantages of extremozymes over other enzymatic processes. This chapter also explains the different types of extremozymes from thermophilic, hyperthermophilic, psychrophilic, barophilic, acidophilic, alkaliphilic, xenophilic, halophilic as well as metal-resistant microorganisms. This chapter gives a broad outline about extremophilic enzymatic processes which is a prerequisite for the readers to understand the following chapters.

Biotechnology and bioprocess engineering are a boon to mankind. Biochemical engineers make use of the microorganisms as catalysts for the wide range of applications including food processing, water treatment, solid waste disposal, and production of organic acids, vitamins, antibiotics, and therapeutic molecules. Microorganisms utilizes the substrates as the source of energy and produce primary and secondary metabolites. They convert the substrate to product either in a single reaction or a linear/complex series of reactions. Each of these reactions are carried out by a single or a set of enzymes.

Biotechnology research and bioprocess industry have grown at rapid pace to incredible heights that they have developed bioprocesses for almost all traditional chemical processes. The bioprocesses, which are ecofriendly and economical, are green alternatives to the chemical processes. They can operate at ambient physical conditions such as temperature, pH and pressure unlike chemical processes which demands very high temperature, pressure, or a specific pH. The biological processes

R. Navanietha Krishnaraj • R.K. Sani (✉)
Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, 501 East St. Joseph Street, Rapid City, SD 57701-3995, USA
e-mail: Rajesh.Sani@sdsmt.edu

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also do not require any special apparatus or sophisticated processes for the production of desired product as in the case of chemical process. Besides these, the microbial processes can make use of the waste organic materials such as effluent or solid waste from agri-food or any other industry as the substrate. This helps greatly in reducing the costs of operation besides bioremediation/disposal of wastes from the environment. The major issue with the microbial processes is that their metabolic pathways are very complex leading to several undesired products. Therefore purification of the products especially in the case of therapeutic molecules/foods/single cell proteins is very difficult. In some cases, microorganisms can also release some toxins into the reaction system. These major limitations can be circumvent by the use of specific enzymes which can confer sensitivity and selectivity to the reaction.

Enzymes, also known as biocatalysts, produced by the microorganisms that can catalyze a particular reaction or a set of reactions. Enzymes are generally proteins, however, ribozyme is an exception. Some enzymes need cofactors or coenzymes for their catalytic activity. The use of enzymes for different applications have been explored well. Technologies have improved in such a way that there is innumerable number of products based on enzymes. Enzymes are indispensable to research as well as modern life. However, these enzymes have also certain limitations. They are so fragile they get denatured easily because they are mainly composed of proteins. Few enzymes can only be operated under very narrow operating conditions. In addition, purification of enzymes is a tedious job. The use of extremophilic enzymes can help to overcome some of the limitations (Rothschild and Mancinelli 2001).

Extremophilic enzymes can be operated under adverse conditions such as a high or low temperature, pressure, extreme radiations and pH conditions. The operating conditions of most enzymes depend on the microorganisms from which they are isolated. These enzymes can be isolated from thermophiles, hyperthermophiles, psychrophiles, barophiles, acidophiles, alkaliphiles, xenophiles, halophiles as well as metal-resistant microorganisms. Figure 1.1 shows the various sites in USA (South Dakota, Wyoming, and Washington) and India (Himachal Pradesh and Haryana) where extremophiles are present. The extremophilic enzymes have several advantages over the mesophilic enzymes. These extremophilic enzymes can operate a much broader range of conditions besides being stable, and have much longer shelf life. These enzymes also possess higher activity and high rate the catalysis when compared with the normal enzymes. They are more resistant to proteolysis and are more robust to organic solvents. They can be overexpressed to very high levels using heterologous host-vector systems. The high structural stability of the extremozyme also helps in engineering the enzymes by genetic engineering or site directed mutagenesis/protein engineering approaches. It also helps in improving the immobilization processes onto the wide range of carriers either by surface immobilization by functionalization/covalent bonding/adsorption by weak Vander walls forces or entrapment/encapsulation avoiding the mass transfer limitations leading to improvement in effectiveness. The extremozymes can be produced from extremophilic microorganisms including bacteria, algae, fungi or even from plants growing in adverse conditions (Anitori 2012; Atomi 2005).

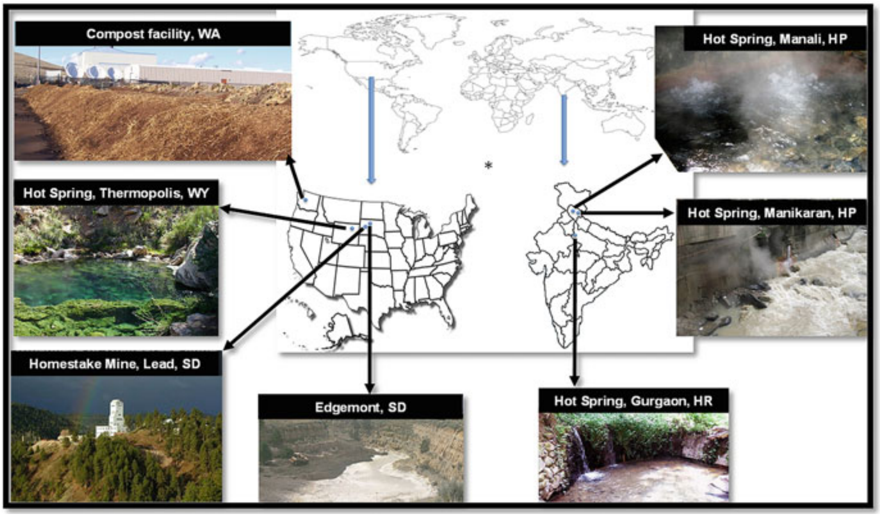

Fig. 1.1 Presence of extremophiles at various sites in USA (South Dakota, Wyoming, and Washington) and India (Himachal Pradesh and Haryana)

The extremophilic enzymes have wide range of applications when compared with the normal enzymes. For example, thermostable enzymes have several potential advantages e.g., higher specific activity and greater half-lives. Carrying out hydrolysis at higher temperature can ultimately lead to improved performance through decreased enzyme dosage and reduced hydrolysis time, thus, resulting in decreased hydrolysis costs. Besides these, high temperature can also help in avoiding the mesophilic contamination and thus prevent from undesired reactions. Thermophilic proteases find its applications in hydrolysis in food, feed, brewing, and baking. Thermophilic glycosyl hydrolases namely amylases, pullulanase, glucoamylases, glucosidases, cellulases and xylanases are shown to have applications in processing the polysaccharides such as starch, cellulose, chitin, pectin, and textiles. Thermophilic lipases, proteases and esterases have been widely used in detergent industry. Thermophilic xylanases are used for bleaching paper. Thermophilic DNA polymerases has been used in molecular biology for PCR. Like thermophilic enzymes, the psychrophilic proteases, amylases and cellulases have also been used in detergent industry. Extremophilic oxidoreductases are widely used for the real time development of electrochemical biosensors. Halophilic peptidases have been used for peptide synthesis. Acidophilic proteases have been used for detergent, food, and feed industry.

Several investigations have been carried out to understand the structural features that confer better stability to thermophilic enzymes when compared with mesophilic enzymes. literature suggest that the increased surface charge, increased protein core hydrophobicity, and replacement of exposed ‘thermolabile’ amino acids together can lead to the increase in the stability of the thermophilic enzymes. Halophilic enzymes can exhibit catalysis at a very high salt concentration (e.g. KCl concentrations of 4 M and NaCl concentrations of >5 M). The halophilic enzymes have a relatively large number of negatively charged amino acid residues on their surfaces which helps them to adapt to this environmental pressure without getting precipitated. However, halophilic enzymes have several limitations that they are not soluble in surroundings with lower salt concentrations which hinders the use of halophiles in these environment (Egorova and Antranikian 2005; Demirjian et al. 2001).

Extremozymes have immense potential for applications in industries including agricultural, chemical, and pharmaceutical. So far, a very small percentage of the extremozymes have been explored for industrial applications. With research advancements in highly stable extremozymes from different organisms, the number of biotechnology products may also increase. In summary, there is no doubt that extremozymes will significantly improve the scope of biotechnology towards real time applications.

Take Home Message

- The bioprocesses have several advantages over chemical processes. The bioprocess operations can operate at ambient physical conditions such as temperature, pH and pressure whereas chemical processes require very high temperature, pressure, or a specific pH. The bioprocesses are also ecofriendly and economical.
- The bioprocess operations can be mediated by enzymes or microorganisms. Enzymatic processes have advantages such as high rate of catalysis, specificity and selectivity; however, suffers from limitations such as high cost and narrow range of operating conditions. Enzymatic processes are prone to denature at high temperature. The use of extremozymes will help to circumvent these shortcomings.
- Extremozymes are those enzymes which can be operated under adverse conditions such as a high or low temperature and pressure and extreme radiations and pH conditions. These enzymes can be isolated from thermophiles, hyperthermophiles, psychrophiles, barophiles, acidophiles, alkaliphiles, xenophiles, halophiles as well as metal-resistant microorganisms. The extremozymes can be used for wide range of applications including agricultural, chemical, and pharmaceutical sectors.

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