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Extremophiles as a Source of Unique Enzymes for Biotechnological Applications

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Abstract

Extremophiles are unique microorganisms that are adapted to survive in ecological niches such as high or low temperatures, extremes of pH, high salt concentrations and high pressure. These unusual microorganisms have unique biochemical features which can be exploited for use in the biotechnological industries. Due to the high biodiversity of extremophilic archaea and bacteria and their existence in various biotopes a variety of biocatalysts with different physicochemical properties have been discovered. The extreme molecular stability of their enzymes, membranes and the synthesis of unique organic compounds and polymers make extremophiles interesting candidates for basic and applied research.

Some of the enzymes from extremophiles, especially hyperthermophilic marine microorganisms (growth above 85 °C), have already been purified in our laboratory. These include the enzyme systems from *Pyrococcus, Pyrodictium, Thermococcus and Thermotoga sp.* that are involved in polysacharide modification and protein bioconversion. Only recently, the genome of the thermoalkaliphilic strain *Anaerobranca gottschalkii* has been completely sequenced providing a unique resource of novel biocatalysts that are active at high temperature and pH. The gene encoding the branching enzyme from this organism was cloned and expressed in a mesophilic host and finally characterized. A novel glucoamylase was purified from an aerobic archaeon which shows optimal activity at 90 °C and pH 2.0. This thermoacidophilic archaeon *Picrophilus oshimae* grows optimally at pH 0.7 and 60 °C. Furthermore, we were able to detect thermoactive proteases from two anaerobic isolates which are able to hydrolyze feather keratin completely at 80 °C forming amino acids and peptides. In addition, new marine psychrophilic isolates will be presented that are able to secrete enzymes such as lipases, proteases and amylases possessing high activity below the freezing point of water.

The role of enzymes in biotechnology

Enzymes (biocatalysts) are present in all biological systems including bacteria, archaea and yeast, which are needed to control vital cellular processes. The industrial application of biocatalysts began in 1915 with the introduction of the first detergent enzyme by Dr. Röhm. Since that time enzymes have found wider application in various industrial processes and production. The most important fields of enzyme application are nutrition, pharmaceuticals, diagnostics, detergents, textiles and leather industries. There are more than 3,000 enzymes known to date that catalyze different biochemical reactions among the estimated 7,000. Interestingly, only few hundred enzymes are being used industrially. The world market for industrial enzymes, which includes enzymes for research and diagnostic, is estimated to be around 1 billion US dollars. The products derived from these enzymes are estimated to represent a value of more than 100 billions US dollars. For various industrial applications,

there is a great demand for enzymes of high specificity and stability. In many cases microbial biocatalysts, especially of extremophiles, are superior to the traditional catalysts, because they allow to perform industrial processes even under harsh condition, under which conventional proteins are completely denaturated.

Extremozymes – novel tools for biocatalysis

Extremophiles are unique microorganisms that are adapted to survive in ecological niches such as high or low temperatures, extremes of pH, high salt concentrations and high pressure. Accordingly biological systems and enzymes can even function at temperatures between –5 and 130 °C, pH 0-12, salt 3-35 % and 1000 bar. The majority of the organisms that grow in these extreme environments belong to a group with distinct characteristics. Carl Woese named this group archaea, and postulated the archaea as the third domain of life on earth, different form bacteria and eukarya. Up to date more than 60 species of hyperthermophilic archaea (growth 80 to 110°C) have been isolated and characterized. By virtue of their positive properties, stability, specificity, selectivity and efficiency, enzymes already occupy a prominent position in modern biotechnology. For many processes in the chemical and pharmaceutical industries, suitable microbial enzymes can be found that have the potential to optimise or even replace chemical processes. By using robust enzymes in bio-technical processes one is often able to better utilise raw materials, minimise pollutant emissions and reduce energy consumption while simultaneously improving quality and purity of products. The additional benefits in performing industrial processes at high temperature include reduced risk of contamination, improved transfer rates, lower viscosity and higher solubility of substrates. The recent exciting results in the field of extremophile research, the high demands of the biotech industries for tailor-made novel biocatalysts and the simultaneous rapid development of new techniques e.g. genomics, proteomics, DNA-chip technology will stimulate to develop innovative processes on the basis of biocatalyst from extremophiles.

Characteristics of extracellular enzymes from extremophiles

Several enzymes from hyperthermophiles have been purified their genes cloned and expressed in mesophilic hosts e.g. *E. coli* and *B. subtilis* (Table 1). As a general rule, they show extraordinary heat stability, are resistant to chemical reagents, detergents, urea and guandinium hydrochloride. The hyperthermophilic archaeon *Pyrococcus woesei* (growth 100 °C) harbours an amylase that is active at 130°C. Even complex enzymes such as DNA-dependent RNA polymerases or glutamate dehydrogenases show a remarkable heat stability. The principles of heat stabilization of thermoactive enzymes are so far not elucidated. The overall amino acid composition is very similar to homologous mesophilic enzymes. However, trends commonly associated with elevated thermostability in proteins include relatively small solvent-exposed surface area, increased packing density that reduces cavities in the hydrophobic core, an increase in core hydrophobicity, decreased length of surface loops and increased hydrogen bonds between polar residues. The three dimensional structure of the first archaeal amylase from *Pyrococcus woesei* has been recently resolved. Due to the high biodiversity of extremophilic archaea and their existence in various biotopes a variety of other biocatalysts with

different physicochemical properties have been discovered. The thermoacidophilic archaeon *Picrophilus oshimae* produces a glucoamylase, which is even active at pH 0 and 90°C. A thermostable endoglucanase, which is able to hydrolyse cellulose, has been found in *Pyrococcus furiosus* (maximal enzyme activity at 105 °C). A xylanase with a maximal activity at 110 °C has been purified form the hyperthermophilic marine archaeon *Pyrodictium abysii*. Xylanase treatment of wood at elevated temperatures opens up the cell wall structure thereby facilitating lignin removal. Chitin with an annual world-wide formation rate of 100 billion tons is also a substrate for extremophilic archaea such as *Thermococcus chitinophagus* and *Pyrococcus kodakaraensis* (growth

Table 1- Enzymes with potential biotechnological application from extreme thermophilic and hyperthermophilic Archaea and Bacteria

Enzymes	Organism and growth temperature	Optimal temperature and pH of the enzymes	Biocatalysis	Applications
α-Amylase	*Pyrococcus woesei* (100°C)	100°C- pH 5.5	Hydrolysis α-1,4 glycosidic linkages in starch	Starch industry, bio-conversion of starch to glucose syrup.
Debranching Enzyme (pullulanase type I)	*Fervidobacterium pennivorans* Ven5 (75°C)	80°C- pH 6.0	Debranching of amylopectin to linear oligosaccharides	Starch bio-conversion glucose.
CGTase	*Thermococcus sp.* (75°C)	100°C- pH 2.0	Production of cyclodextrins	Gelling, thickening, stabilizing agents in food industry.
Cellulase	*Pyrococcus furiosus* (100°C)	100°C- pH 6.0	Hydrolysis of cellulose to glucose	Color extraction of juice, color bright-ening, improving nutritional quality.
Endoxylan-ases	*Thermotoga maritima MSB8* (80°C)	92°C- pH 6.2 105°C- pH 5.4	Degradation of xylan	Bleaching of paper.
Chitinase	*Pyrococcus kodakaraensis* (95°C)	85°C- pH 5.0	Chitin hydroly-sis	Utilization of biomass of marine environment.
Serine protease	*Fervidobacterium pennivorans* (70°C)	80°C- pH 10	Keratin hydrolysis	Soaking in leather industry. Produc-tion of amino acids and peptides from feathers.
DNA Polymerase	*Thermus aquaticus* (75°C)	72°C- pH 7.0	DNA synthesis	Taq polymerase. Polymerase chain reaction (PCR)
Glucose Isomerase	*Thermotoga maritima* (80°C)	105°C- pH 6.5	Isomerization glucose to fructose	Production of high fructose corn syrup
Alcohol deydrogenase	*Sulfolobus solfataricus* (88°C)	95°C- pH 7.0	Oxidation of secondary alcohols	Reduction of ketons

Esterase	*Pyrococcus furiosus* (100°C)	100°C- pH 7.6	Cleavage of esters	Organic bio-synthesis

85 °C). In addition, a number of heat-stable proteases has been identified in hyperthermophilic archaea such as *Pyrobaculum* sp. and *Staphylothermus* sp., and thermoacidophilic archaea (growth pH 3 and 75°C) such as *Sulfolobus* sp. and extreme thermophilic bacteria belonging to the order of Thermotogales. Some of these serine proteases have a residual activity even at 135 °C after 10 min of incubation. In order to be able to find application for various extremozymes it is essential, however, to ensure overexpression of these proteins in mesophilic hosts such as the gram-positive bacteria *Bacillus* sp. and *Staphylococcus*, or yeast (*Pichia* sp.) followed by the optimization of the cultivation process.

Extremophiles as a cell factory for intracellular enzymes and chemical compounds

Intracellular enzymes from thermophiles e.g. thermostable DNA polymerases (Table 1) play a key role in a variety of molecular biological applications e.g. PCR, polymerase chain reaction (PCR). *Taq* polymerase, the first thermostable DNA polymerase characterized and applied in PCR, has a 5′-3′-exonuclease activity, but no detectable 3′-5′-exonuclease activity. This enzyme, which is derived from the thermophilic bacterium *Thermus aquaticus*, is unable to excise mismatches and as a result, the base insertion fidelity is low. Archaeal proofreading polymerases such as *Pwo* pol from *Pyrococcus woesei*, *Pfu* pol from *Pyrococcus furiosus*, Deep Vent^{TM} pol from *Pyrococcus* strain GB-D or Vent^{TM} pol from *Thermococcus litoralis* have an error rate that is up to 10-fold lower than that of *Taq* polymerase.

In addition to the mentioned enzymes, thermophilic archaea and bacteria are a resource for unique chemical compounds such as ether-lipids and compatible solutes (ectoine, methylglycerate). Very little, however, is known on the ability of extremophiles to produce bioactive compounds that are of value for the pharmaceutical and food industries. There is a need to develop fast and intelligent screening techniques for the identification of new products from extremophiles. New far-reaching ideas and problem solving potential are expected to emerge in future from the sectors of genome sequence analysis, functional genomics, proteomics and directed evolution. These techniques are expected to give decisive impulses to extremophilic biotechnology in the near future.

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