ADVANCED ENZYME TECHNOLOGY

Extremozymes (part 1)

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Chapter Description

- Expected Outcomes
  - To describe the principle of extremozymes
  - To explain about thermophiles and its enzyme adaptation
  - To discuss about the unique properties of thermozymes

Content

- Introduction
- Thermophiles
- Thermozymes
- Applications of thermozymes in industrial processes
- Possible mechanisms of thermostability
- Industrial Applications of thermozymes

Introduction

- Previously, a number of chemical industry sectors were more restrained in implementing enzymes technology, mainly due to enzymes were observed being too delicate to survive the extreme conditions in the reaction vessels.

- Generally, enzymes required particular conditions for example pH and temperature prior to perform their catalysis. However, frequently, these specific condition are quite different from the condition for which an industrial plant was designed.

- Nowadays, a new awareness of the microbial life diversity has pushed microbiology from a rather academic subject to the fore-front of biotechnology.

Introduction

- In a few decades, microbial communities was reported to thrive in a wide range of environments, including extremes of **temperature**, **pressure, salinity** and **pH**.

- These special microorganisms is known as **extremophiles**, that can produce **biocatalysts or enzymes** that can perform catalysis **in extreme environments**.

Introduction

- It is predicted that so far the number of microorganism that have been identified is less than 1%.
- there are probably in excess of 50 million bacterial species still undiscovered!
- Now, no need to adjust industry to accommodate an enzyme (costly); but can concentrates on searching enzymes which can work in existing industrial processes.

Examples of industrial processes special requirements:

- ✓ Enzymes that active in cold temperature for refrigerated food.
- ✓ Enzymes that can be put during perfumes processes –don't tolerate high temperatures
- ✓ Reduce the emission of CO_2 and save energy - Cold-wash detergents
- ✓ Enzyme that function and survive high temperature during PCR reactions

- What permits an organism to survive in extreme habitats (e.g: very high or very cold temperature and etc) ?
- The ability lies in its adapted biocatalysts known as extremozymes: enzymes that have capability to be active in extremely high environment.
- The amino acids of these extremozymes have special adaptation to preserve their functional folded 3D structures in extreme environment, where other enzymes would become insoluble and inactive.

THERMOPHILES

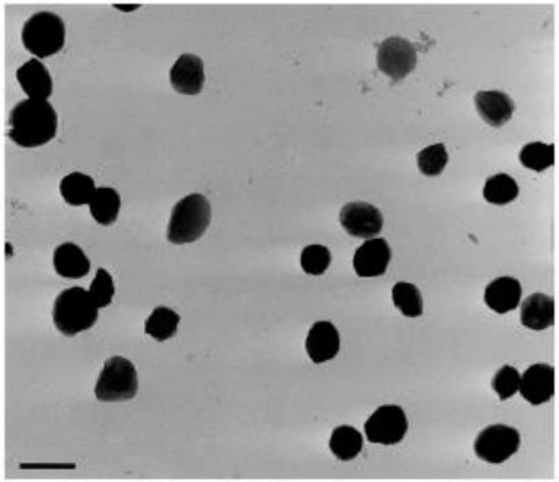


Fig. 1. Low-magnification electron micrograph of cells from isolate 1A, stained with uranyl acetate. *Bar*, 2µm

Pyrolobus fumarii is a champion (at least, at present time), H_2/O_2, NO_3^{2-}, $S_2O_3^{2-}$ 90-113 C (opt = 106 C). Stetter et al, Extremophiles, 1997.

Methanopyrus kandleri strain 116 is growing up to **122 C** (opt = 105 C) at 40 MPa. Kurr et al, Arch. Microbiol.,1991. Takai et al, PNAS, 2008.

Optimal temperature

106 C

hyperthermophiles

80 C

extreme thermophiles

70 C

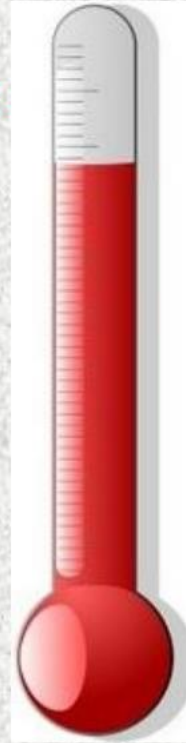
moderate thermophiles

40 C

mesophiles

15 C

psychrophiles



"Bacteria are able to grow… at any temperature at which there is liquid water…" Brock, 1967.

However: "More than 110 °C amino acids and other metabolites become highly unstable => unable to survive.

temperature limit is not far away from 113 C" Jaenicke, 1998

Thermozymes

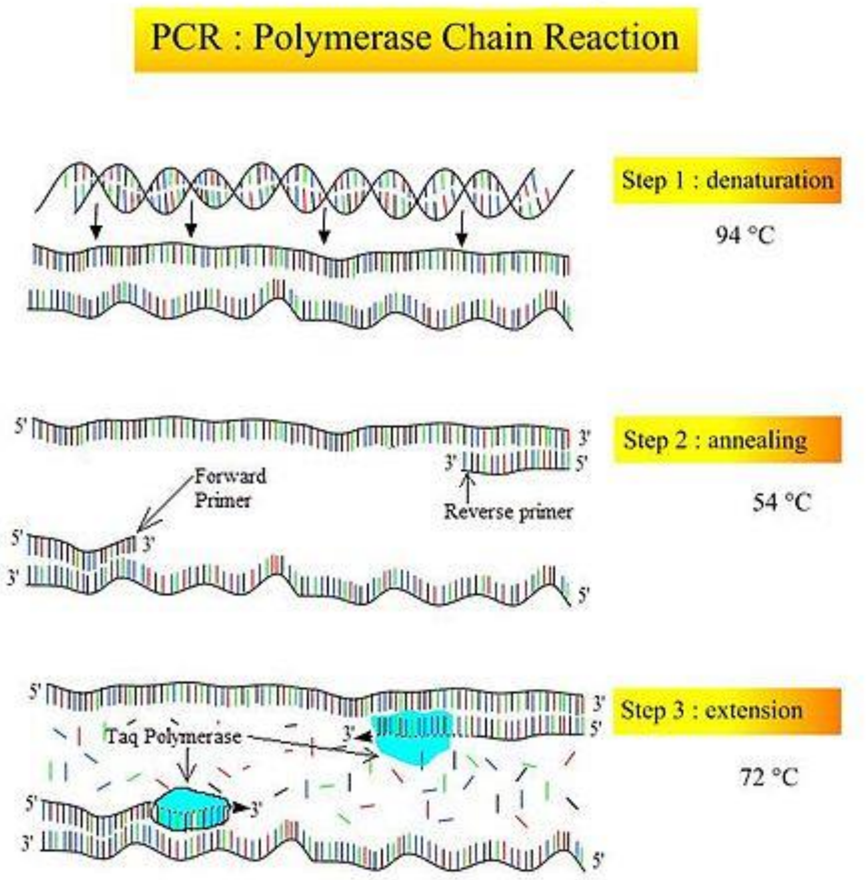
- **Thermozymes or thermophilic enzymes is the biocatalyst which is isolated from thermophiles.**

- Thermozymes have found applications in biotechnology where their uncommon thermal stability allow them to be soluble and active under environment that usually lead mesophilic enzymes denature.

Polymerase chain reaction

- Developed by the chemist Kary Mullis in 1993.
- Can be used to perform nucleic acids amplification.
- Involve reaction cycles between high temperature of about 95 °C and medium temperature range from 50 to 75 °C.
- Require special DNA polymerase enzyme for that purpose which is those than can survive at elevated temperatures environment:
  - Taq DNA polymerase - isolated from *Thermus aquaticus*
  - Pfu DNA polymerase - isolated from Pyrococcus furiosus

What happen inside PCR tube?



APPLICATIONS OF THERMOZYMES IN INDUSTRIAL PROCESSES

Property	Advantages
Increased thermostability	• Enhance enzymes half life. • Ease enzyme purification.
Resistance against various chemical agents	• Able to withstand extreme conditions such as a large number of organic solvents and a wide range of diverse pH.
High optimal temperature	• Inactive at temperate temperature. • Reduce capital cost –no active cooling during fermentation. • Lead to increase diffusion levels of substrates and products.
High Solubility	• Amount of substrates can be improved at elevated temperature except gases.
Low Viscosity	• Mixing and pumping can be enhanced.
Reduce Microbial contamination	• Low risk of contamination at elevated temperature. • Unwanted enzymes also can be inactivated.

Thermozymes Are Highly Identical to Their Mesophilic Homologues

- What make the thermophilic and mesophilic enzymes are so different is that the ranges of temperature in which they are soluble and functional.

- Other than that, thermophilic and mesophilic enzymes are highly identical:

  (i) the primary structure (sequences) of closely related thermophilic and mesophilic proteins are normally between 40 to 85% identical.

  (ii) three-dimensional structures of thermophilic and mesophilic proteins are superimposable .

  (iii) thermophilic and mesophilic proteins posses similar the same catalytic mechanisms

Citrate synthase

acetyl-CoA + oxaloacetate + $H_2O \rightarrow$ citrate + CoA-SH

growth temp

31 °C

37 °C

55 °C

85 °C

100 °C

The structure of citrate synthase from organisms that live at five different temperatures has been determined

Source organism	Optimum growth temperature (°C)
Arthrobacter DS23R	31 [a]
Pig	37
Thermoplasma acidophilum	55
Sulfolobus solfataricus	85
Pyrococcus furiosus	100

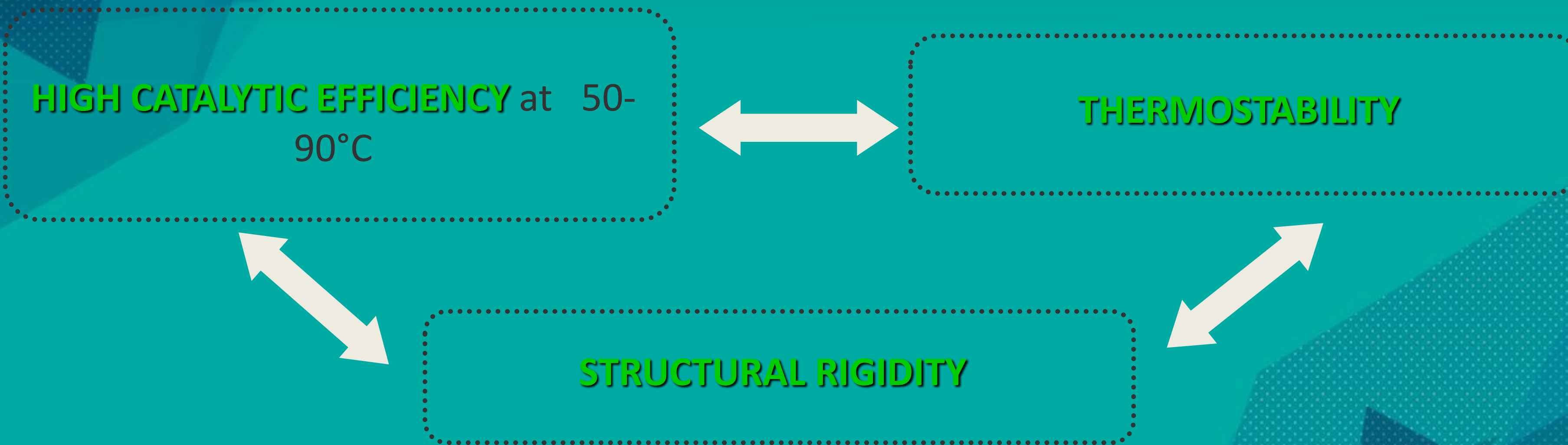
All CS are alpha helical homodimeric proteins with the active site located at the interface with residues from both subunits—maintaining the dimer structure is essential for enzymatic activity

Bell et al, Eur J Biochem 269, 6250 (2002)

Rigidity and Thermostability

- Stability of thermozymes at hiher temperature is related with a improved resistance to chemical denaturants (including solvent or guanidinium hydrochloride).
- Recent validated finding reported that thermozymes consist of extra rigid structure compared to their mesophilic homologues.
- This rigidity properties is a main requirement for high protein thermostability.
- This rigidity however explains why thermophilic enzymes are often inactive at low temperatures (i.e., around 20 to 37°C).

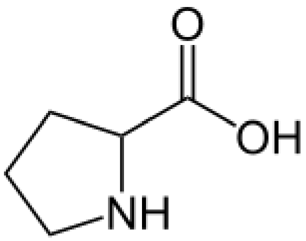
THERMOPHILIC ENZYMES: an OVERVIEW



Possible mechanisms of thermostability

- **More stable protein core**
  - Having low amount and volume of cavities
  - Higher number of hydrophobic residues - hydrophobic interaction improve with temperature
  - reduction in size of surface loop to minimize entropy
- **Number of hydrogen bonds**
  - Increased

- **Reduce conformational degrees of freedom**
  - Increase Proline residue



- **Increase number of electrostatic interactions,**
  - i.e. salt bridges
- **Metal binding**
  - Increased to improve stability

Industrial Applications of thermozymes

1. pulp and paper industry

- Xylanases will breakdown xylan, the major hemicelluloses constituent, during wood treatment processes for the production of pulp.
- The treatment of wood to obtain pulp is performed at extreme temperatures and at basic pH to help disrupt the cell wall structure.
- Thus, a thermostable xylanase is preferable from the industrial point of view and active at neutral to alkaline pH.

- Thermostable xyanases can decrease the chlorine usage during bleaching process. As a consequent, utilization of this enzyme type can reduce the environmental damage due to the presence of organic halogens.

- Hyperthermophilic xylanases from Thermotoga, Dyctio glomus, Sulfolobus, Pyrococcus and *Pyrodictium* were characterized with optimal temperatures above 90°.

- Among them, the xylanase from *Thermotoga* sp. strain FjSS3-B1 is the most hyperthermophilic xylanase, with an optimal temperature of 115°C

2. Starch bioconversion process.

- hyperthermophilic Enzymes, which are active above 100 °C and active at wide range of pH are considered as in the compatible pH range, are regarded as attracting applicants for starch bioconversion process.

- Thermostable a-amylases have previously been identified from *Pyrococcus woesei, Pyrococcus furiosus and Thermococcus profundus*

- The optimum temperatures for the activity of these enzymes are 100 °C, 100 °C and 80 °C respectively.

3. Detergent industry

- Among the many applications of lipases and proteases as hydrolases is their use as additives in the detergent industry.
- Thermostable lipases from hyperthermophilic archaea have been identified from *Pyrobaculum calidifontis, Pyrococ cus furiosus* and *Pyrococcus horikoshii* and cloned in *E. coli*.
- The pyrolysin, serine protease which reach maximal activity at 100°C was isolated from hyperthermophilic archaeon *Pyrococcus furiosus, Thermus aquaticus and Thermus thermophilus* .