COLD-ACTIVE ENZYMES AS NEW TOOLS IN BIOTECHNOLOGY

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Summary

Cold-adapted organisms, able to proliferate in cold environments, and their enzymes offer a multitude of actual and potential applications in various fields of biotechnology. Cold-active enzymes are characterized by high catalytic efficiency at low and moderate temperatures at which homologous mesophilic enzymes are not active. The possibility to improve the low thermal stability of cold-active enzymes without altering the specificity opens prospects for industrial applications. The transfer of enzymes with the desired properties to suitable host organisms is also an important aspect for the industrial large-scale production. The use of cold-active enzymes in detergents allows colder washing cycles and thus energy saving by lowering the temperature without a loss of enzyme activity. In the food industry, cold-active, heat-labile enzymes are desirable for several processes in order to improve food quality and preservation. Cold-active enzymes operating in presence of organic solvents are of interest for the synthesis of high value products. The synthesis of volatile and heat-sensitive compounds, such as flavors and fragrances, is facilitated at low temperatures. In molecular biology, heat-labile enzymes are advantageous to obtain irreversible enzyme inactivation by mild heat treatment without interference with subsequent reactions. Biosensors functional at low temperatures are useful for the on-line monitoring of bioprocesses and quality control. Bioremediation and low-energy wastewater treatment in cold climates is based on the ability of cold-adapted microorganisms to degrade organic contaminants.

1. Introduction

As a result of the adaptation to their natural environment, extremophilic microorganisms have evolved unique properties, which are of considerable biotechnological and, therefore, commercial significance. The objective of this article is to summarize the potential of cold-active enzymes originating from organisms that live in permanently cold environments, such as fresh and marine waters, snow, glacier and sea ice, polar and high alpine soils, cold deserts, and permafrost sediments. Adaptation strategies to cold with regard to growth, enzyme production, and enzyme activity enable them to compensate for the negative effects of low temperatures on biochemical reactions.

2. Advantages of Cold-Active Enzymes in Biotechnology

Cold-active enzymes are characterized by high catalytic efficiency at low and moderate temperatures at which homologous mesophilic enzymes are not active. This property is useful in biotechnology in order to

- shorten process times for processes operated at low temperatures
- save energy costs
- decrease the enzyme concentration required
- obtain high yields from reactions involving thermosensitive components
- prevent undesired chemical transformations
- prevent the loss of volatile compounds
- perform on-line monitoring under environmental temperature conditions.

Cold-active enzymes are thermolabile. Rapid enzyme inactivation by a mild heat treatment

- does not affect product quality and prevents the materials from damage
- permits selective enzyme inactivation in a complex medium

- does not require expensive heating/cooling systems.

3. Improvement of Enzyme Yield and Thermostability

The optimal temperature for the production of cold-active extracellular enzymes is usually significantly below the optimal growth temperature of the producing strains. The highest quantities of various enzymes (protease, lipase, phosphatase, amylase, cellulase, chitinase, pectinase, etc.) are obtained when the strains are cultivated at temperatures that correspond to that of their natural environment. Most microorganisms with an optimal growth temperature around 20–25 °C produce the highest enzyme yields when cultivated at 4–10 °C. For example, the maximum activity of amylase from Antarctic *Alteromonas haloplanktis* recorded in cultures grown at 18 and 25 °C was only 13 and 6% of the activity recorded in cultures grown at 4 °C. A psychrotrophic *Pseudomonas fluorescens* with comparable growth at temperatures from 10 to 30 °C produced the highest amount of protease when cultivated at 10 °C, but protease production was reduced by 50% at 20 °C and did not occur at 30 °C. Thermal characteristics of enzymes secreted at low or moderate temperatures (e.g., at 4 and 20 °C) are not affected by growth temperature.

Low-temperature fermentation prevents or limits the risk of contamination with mesophilic bacteria or fungi, which is especially advantageous in continuous systems. However, as a consequence of the cold-dependent extracellular enzyme production, cold-adapted isolates are not appropriate candidates for large-scale fermentation, because fermentation below 10 °C would require energy-consuming cooling systems. Another drawback of cold-adapted microorganisms is the low production level of wild strains. These disadvantages can be circumvented by overexpressing the genes coding for cold-active enzymes in appropriate mesophilic host bacteria, such as *Escherichia coli*, for which efficient expression systems have been designed. It has been demonstrated with various enzymes (amylase, lipase, protease, ß-galactosidase) that the recombinant enzymes conserve the main character of the wild-type enzyme (i.e., a shift of the optimal temperature of activity towards low temperatures and pronounced heat lability). Recombinant lipases had an even lower optimum temperature for activity (35 °C versus 40–45 °C) and a lower thermostability than the wild-type enzyme. Concerning enzyme production, a good compromise has to be found between the growth rate of the transformed mesophilic host and the denaturation rate of the gene product. A cultivation temperature of 18 or 25 °C has been found appropriate; an incubation at 37 °C inactivated the enzymes. In other cases, recombinant enzymes were only expressed in *E. coli* if the host was grown at 25 °C and incubated for two days at 4 °C. The transfer of enzymes with the desired properties to suitable (e.g., food-grade) host organisms is an important aspect for the industrial large-scale production.

The major drawback in the use of cold-active enzymes for some applications is their weak thermostability. Enzyme stability is required for storability reasons. The challenge of finding stable proteases that function in cold water has been the focus of recent studies. Protein engineering is now commonly used to increase the stability of industrially important enzymes and has been successfully applied for the enhancement of the thermostability of cold-active proteases. Stability and catalytic efficiency of a cold-active subtilisin could be increased simultaneously. The enzyme from Antarctic

Bacillus sp. was expressed in a mesophilic *Bacillus* strain. After site-directed mutagenesis, all mutants had higher catalytic constants than the wild-type enzyme. At 50 °C, the half-life of one mutant was 60 min compared to 6 min for the wild-type enzyme. One mutant was even more stable than the wild-type enzyme, it displayed at 4 °C a specific activity twice that of the wild-type enzyme and 20 times that of subtilisin Carlsberg. Site-directed mutagenesis was also applied to increase the thermal stability of euphauserase, a cold-active multifunctional serine protease from Antarctic krill. The enzyme was expressed in yeast (*Pichia pastoris*). The mutant enzyme retained 20% more of activity after 10 min at 40 and 45 °C (95 and 50% residual activity, respectively) than the wild-type enzyme, but both mutant and wild-type enzyme were inactivated at 50 °C.

Another method to enhance thermostability is evolution engineering. Artificial cold adaptation of the mesophilic subtilisin BPN´ was obtained by means of random mutagenesis. One mutant acquired higher proteolytic activities at low temperatures; activity increases of 10 and 30% were observed at 10 and 1 °C, respectively. There was no difference in activity at temperatures above 25 °C, nor was a difference in thermostability between mutant and wild-type subtilisin.

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Biographical Sketch

Rosa Margesin was born in 1962. In 1982 she commenced her study of biology, subdivision microbiology, at the University of Innsbruck (A). In 1993 she received a fellowship of the European Environmental Research Organization; and since 1998 has been associate professor of microbiology at the Institute of Microbiology, University of Innsbruck, Austria. She is coeditor of six books. Scientific topics include cold-adapted microorganisms (characterization of proteases from psychrophilic bacteria, biodegradation and bioremediation of organic contaminants at low temperatures, ecological characterization of cryoconite on glacier ice) and soil biology (monitoring of bioremediation of hydrocarbon-polluted soil and water by biological methods).