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Psychrophiles: Cold Adaptation

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Abstract

Almost three-quarters of the Earth's surface are covered by cold ecosystems, involving the depths of ocean, and Polar Regions. These continuous cold environments have been successfully covered by members of extremophiles which are known as psychrophiles (means cold-loving). To grow at temperatures that are close to, or below, the freezing point of water requires many adaptations. To survive in such cold weather, psychrophilic organisms developed structural, physiological and molecular adaptations. This includes the membrane fluidity, production of specialized proteins and cold-active enzymes. The cold adaptation of psychrophilic organisms is poorly understood. Psychrophilic products have generated considerable interest; they can be used to improve the efficiency of industrial processes and also for environmental applications. They may add new insights into the understanding of catalytic mechanism of enzymes. Because of their higher specificity and high catalytic efficiency, the cold-active enzymes are generating more interest in various fields.

Keywords: *Extremophiles, psychrophiles, cold adaptation, freezing point, cold-active enzymes*

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INTRODUCTION

Earth is a cold planet and ~ 85% of its surface is covered by ice permanently. The ice-covered regions include Arctic, Antarctic, Alpine and Himalayan regions which possess very low temperatures almost throughout the year [1]. These cold regions possess colonized habitats of cold-adapted organisms. Bacteria which grow at low temperatures are known as psychrophiles (cold-loving) [1]. The term psychrophiles was given by Schmidt-Nelson in 1902 for the organisms growing at 0 °C. Psychrophiles were characterized into strict or obligate and facultative by Morita [2]. Facultative psychrophiles do not prefer low temperature; they have optimum temperature about 15 °C and more, and are thus termed psychrotrophs [3]. To survive in extreme conditions, the organisms need to adapt to various stress factors like UV irradiations, heating weather for shorter period and low-temperature weather. To well adapt in such extreme cold environment, psychrophilic microorganisms mainly adapt through their lipid membrane and their cold-active enzymes [4]. The microflora of the Antarctic regions has not been studied well in the past [5]. The true Psychrophiles were isolated in 1960s. But due to industrial development psychrophilic microorganisms are creating great interest for the cold-active as well as biologically produced compounds [6]. Psychrophiles produce the cold catalytic enzymes which remain active at very low temperatures [3]. Various kinds of psychrophilic genera of microorganisms are found to produce cold-active lipases, proteases, phosphatases, amylases, oxidases and β-galactocidases [7]. The products of psychrophilic organisms are also useful in the food-processing, detergent, paper, pharmaceutical industries as well as in biotechnology for biotransformation, bioremediation, bioleaching, biosensors,

bioreporters, and disease diagnosis and in molecular studies [8].

In this article the idea is generated on the survival of psychrophilic organisms in such cold environment and the tools which are used by them to adapt and propagate in cold weather and also some of the biotechnological implications of these cold-adaptive microorganisms.

WHERE ARE PSYCHROPHILES PRESENT?

They are present in the cold environment having temperature ~ 0 °C or below [9]. Psychrophiles are present in cold ponds, lakes, deep sea water and snow of mountains and glaciers, polluted snow of cold regions, blue green algal mats of polar regions, fish skin surfaces, internal tissues of ascidians, pigeon face, snow animals' skin, milk, etc. [5, 10]. The majorly is found in the cryosphere where water is in solid state [1].

ADAPTATION AGAINST COLD TEMPERATURE

Temperature is considered as key environmental factor for life. Low temperature slows down and inhibits reaction rates catalyzed by enzymes which we can call as "workhorses" in cell metabolism. The effect of temperature on chemical reactions is basically described by the Arrhenius equation: $k = A\mathrm{e}\_Ea/RT$ [described by Arrhenius in 1889], where k is the rate constant, A is the pre-exponential factor that depends on the reaction, Ea is the so-called activation energy, R is the gas constant (8.31 kJ mol^{-1}) and T is the temperature in Kelvin. If we want to express this equation then we can explain it through Q10, which shows the ratio among the rates of reaction measured after each 10 °C. This Q10 is, usually, close to 3 so a gap of 30 °C of the temperature should induce a decrease of the rate of the reactions occurring in the organism by a factor near to 30. This will immediately lead to death or to a dormant state of the organism [3]. To prevent this, many higher organisms have systems to maintain their internal temperature stability from outer atmosphere and some are using hibernation to protect them from adverse temporary environmental condition. But how do these small psychrophilic organisms protect themselves from freezing is an interesting phenomenon [11]. The psychrophiles produce certain substances called "cryoprotectants." They are products such as small molecules like glycerol, glucose, sorbitol, trehalose, amino acids and various derivatives [12]. They depress the freezing point of water by colligative effect; they prevent the dehydration of cells when extracellular ice is present by their rapid redistribution across the membrane and protect proteins from cold-denaturation. They possess a special type of protein called "antifreeze protein" which helps these organisms to effectively survive in low temperature [4].

The antifreeze proteins are very common proteins which were isolated first from the fish of Antarctic and they are majorly glycoprotein in nature having three amino acids Ala-Ala-Thr, bound to a disaccharide [1]. These proteins do not possess common structural characteristics but they have a similar effect; many among them reduce the freezing point of water and this leads to a difference in the freezing point of water and in the melting point of ice, known as thermal hysteresis. They all bind to microcrystals of snow at particular sites and prevent the growth of crystals, preserving in this way fluidity of physiological materials. Other antifreezes (known as IR, for ice recrystallization) do not depress the freezing point but prevent recrystallization. Other than this, in case of fungi, there are extracellular as well as intracellular antifreezes which allow fungi to be active at subzero temperature. A fungus needs the maintenance of an aqueous environment for its growth and to secrete enzymes and absorb carbon and other nutrients. However, antifreezes may be useful for inhibiting the recrystallization of ice and promoting fungal survival during freeze-thaw cycles [9]. Though antifreeze works efficiently but some cold-adapted organisms like microorganisms, insects, plants and even some of the vertebrates, for example, some species of frogs, have developed additional tools that help to either avoid freezing or tolerate freezing [1]. The [INPs] ice nucleating proteins work when the temperature is above the routine cold temperature. In some psychrophiles to control the activity of INP the [ANPs] anti-nucleating proteins are there

which help to melt the ice as well as inhibit the activity by INP [7].

Lipid Fluidity at Low Temperature

To be well adapting in such an extreme cold environment, the membrane composition plays a vital role. Microorganisms have developed a number of strategies to maintain their membrane lipid fluidity and function at low-growth temperature. This membrane is necessary to prevent the diffusion of toxic compounds, and to regulate the entry of nutrients and to allow the excision of waste products from the cell. Lipid bilayer must have proper fluidity to support the permeability properties and motion of essential membrane proteins. The functional state of this bilayer is a liquid-crystalline phase, but cold temperature induces a gel-phase and loss of membrane [4]. The specific temperature in which the phase transition occurs essentially relies on the lipid composition, majorly the fatty acyl chains that are inside the membrane. Therefore, psychrophiles modify their composition in terms of lipid content to maintain their membrane fluidity at such low environmental temperatures. The fluidity of the membrane is adjusted by shortening the length of fatty acids and by the addition of unsaturation and branched fatty acyl chains [13]. As the growth temperature is decreased, the lipids contain an increasing proportion of unsaturated fatty acids [4].

Enzyme Activity at Low Temperature

Enzymes are referred to as the work horses of the cells for their metabolism. The growth of psychrophiles at low temperatures has promoted the discovery of new enzymes with cold-active properties. Low temperatures have a freezing effect on enzyme's structure that can affect the interaction of the enzyme with the substrates. Adjustments of enzyme's structure are, therefore, necessary [14]. Two very important and general properties of psychrophilic enzymes are:

1. They have a much higher specific activity at low and moderate temperatures than mesophilic.
2. They have higher thermo-sensitivity, less stable than their mesophilic counterparts.

Molecular Adaptations in Psychrophilic Enzymes

Structural Adaptations at the Active Site

It is useful to study the architecture of the active site of cold-active enzymes to reveal regarding its higher specificity and efficiency of working at very low temperatures. The information can be generated through X-ray crystallography of various cold-active enzymes [12]. Structures of various cold enzymes like α-amylase, citrate synthase, malate dehydrogenase, etc., are known now. The active sites of psychrophilic enzymes are almost identical with their mesophilic form. The amino acid compositions as well as inhibitor effect is also same for both forms. So, it is concluded that the cold adapting capacity is due to variations in the structure of protein apart from its active site [4].

Conformational Stability-Flexibility-Activity Relationships

Correlation between conformational flexibility and enzyme activity was the most widely accepted hypothesis accounting for adaptation of psychrophilic enzymes, i.e., their high activity and their weak stability. But the current accepted hypothesis suggests that psychrophilic enzymes have to increase their plasticity in order to perform catalysis at low temperatures. The enhanced plasticity is due to low stability of protein structure. The psychrophilic enzymes stabilize their structure by the weak interactions. The flexibility of proteins is directly co-related with the stability. Relative flexibility study is suggested for comparison of homologous proteins adapted for various temperature environments and thermo-stability of proteins is checked. The low stability of all psychrophilic enzymes is due to drastic shift of optimal temperature of activity, low resistance to protein denaturants, and susceptibility of secondary structures to moderate temperatures [15]. Circular dichroism spectra of psychrophilic and mesophilic α-amylases suggest that the conformation of the cold-adapted enzyme is less compact at all temperatures [8]. Both enzymes look to possess the same conformation at their physiological temperature. The difference in conformational stability between psychrophilic and mesophilic α-amylases has been estimated to 30 $kJmol^{-1}$

using heat-induced unfolding curves recorded by fluorescence (Figure 1). The adaptation to cold of the psychrophilic α-amylase has a weak intramolecular interaction which leads to decrease in the thermo-stability of the protein. This provides appropriate plasticity around the catalytic site which is necessary to adapt. The catalytic efficiency of the enzyme at low temperature is clear that the limit of stability of protein has to reach further to decrease stability, and therefore any further improvement of the flexibility of the protein has to follow this strategy [1, 16].

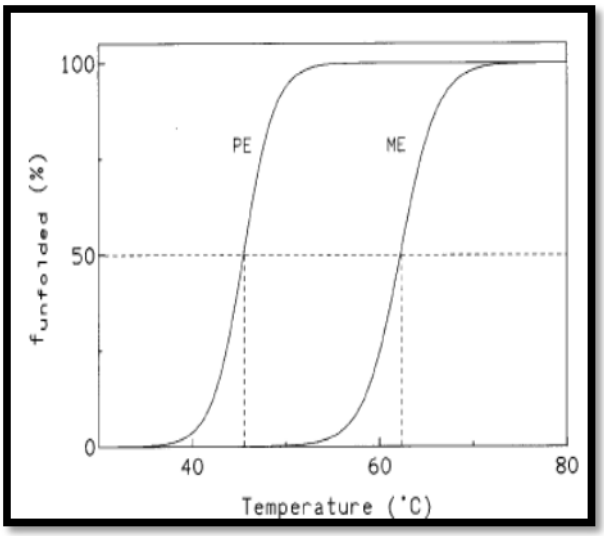

Fig. 1: *Heat-Induced Unfolding of Psychrophilic (PE) and Mesophilic (ME) Enzymes α-Amylases. The Tm is the, midpoint of the thermal unfolding Curve. The difference in melting point values usually ranges between 15 and 20 °C.*

Mutational Studies

Protein engineering clearly states mutation in a single amino acid in the native protein structure can affect the whole function of it. So to improve low catalytic efficiency of the psychrophilic enzymes towards change in temperature and weak interactions, scientists use point mutational studies in cold-active enzymes [17, 18] Apart from these molecular factors which help in cold adaptation, certain structural factors also take part in their stability and efficiency at low temperature, e.g., H-bonding patterns, aromatic interactions, ion binding, dipole interactions in α-helices, hydrophobic effects, etc. [8, 14, 19]

APPLICATIONS IN THE FIELD OF BIOTECHNOLOGY

There are vast applications of the psychrophilic organisms as well as their biological products.

Psychrophilic Organism

In Bioremediation

Two approaches are used: bio-stimulation in the supply of nutrients to indigenous microorganisms to stimulate their metabolic activities and bio-augmentation that is carried out by microorganisms specialized in specific degradation [20]. Psychrophilic microorganisms appear to be appropriate for the process of bio-augmentation due to their ability to efficiently work at low and moderate temperatures, and also produce exo-enzymes which are having much higher specific activity as compared to their temperate counterparts. They work for the decontamination of soil and water contaminated by more harmful components such as petroleum, hydrocarbons, etc. [3, 20, 21]

In Bioleaching

They can be useful for the bioleaching processes. *Thiobacillus ferroxidans* strains isolated from the cold environment was found useful in the bioleaching of uranium [2].

Psychrophilic Products

Antifreeze Proteins

The antifreeze proteins produced by psychrophiles are used for the preservation of tissue and organs at sub-zero temperature [21].

The antifreeze compound Antarticine-NF3 which is a glycoprotein extracted from *P. antarctica* is effective for the treatment of scar and re-epithelization of wounds [19]. Certain antifreeze proteins extracted from eel like fish is now an important component of ice-cream brands produced by Uniliver which helps in reduction of taste and texture quality improvement [1].

Ice Nucleating Protein [INP]:

Pseudomonas syringae which infects the plants, INP from them is used for ice formation in ski tracts. It converts the water droplets into snow and this product is called "snomax."

Anti-nucleating Protein [ANP]

ANPs from several strains are used for preservation of liver and other organs [1, 5].

Cold-Active Enzymes

This is a very useful application of psychrophilic organisms. Cold-active enzymes from psychrophiles are essentially applied in detergent, dairy, paper, and food-processing industries [17]. It is eco-friendly. People all over the world and especially in Asia and Africa are intolerant to milk. These people do not produce an enzyme, named lactase or β galactocidases, which splits common sugar present in milk, lactose, into its two well-digestible components: glucose and Galactose [14]. For intolerant people, it is necessary to remove the lactose from any milk-based product. This is achieved with pretreatment of milk using a mesophilic enzyme. But discovery of cold-active β-galactocidases is very active in removing lactose from milk. The cold-adapted enzyme is three times more active than commercial enzyme at 5 °C and can also be used during storage and transport of milk at low temperature.

CONCLUSIONS

Psychrophiles adapt effectively to survive and grow in extremely cold environment. Through the structural, physiological and molecular adaptations psychrophiles live below 0 °C. Cold-active enzymes of these psychrophiles are very much helpful in industrial field due to their high catalytic efficiency at lower as well as moderate temperature and high specificity. Modification in the molecular structure of these proteins can produce advantageous products. By knowledge of genetics and physiology of these organisms, we can enhance the current status of biotechnology.

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