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SURVIVING ATTRIBUTE OF PSYCHROTOLERANT LIFE FORMS: ICE BINDING PROTEINS

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Abstract– Periodic or continuous exposure to very low temperatures in psychrotolerant enhances production of few specific molecules that prevent them from freezing, called Ice binding proteins. Psychrophiles is capable of synthesizing some important proteins & peptides that can regulate growth of ice crystals and these are named Ice Binding Proteins (IBPs). Ice binding proteins are specialized proteins that are less popular but extremely crucial. Antifreeze proteins (AFP) are among them only that enhance the formation of large grains of ice inside cells that damage cellular organs or cause cell death. The unique ice recrystallization inhibition properties (IRI) and thermal hysteresis (TH) have become one of the promising tools in industrial applications such as cryobiology, food storage and others. This article summarizes the functions and applications of the large group of IBPs.

INTRODUCTION

The Earth's surface records very low temperatures below 5°C during the year. North Pole and Antarctica, mountain glaciers, deep water, permafrost etc. are recorded as some low temperature zones. Despite being under these extreme conditions, some permafrost areas have rich biodiversity which are called Psychrophiles which are in to 3 domains (Archaea, Bacteria, and Eukarya including yeast, lichens, small invertebrates, filamentous fungi, and plants). Psychrophiles have the special feature and capability of adaptations that helps them to meet the challenges of cold. Cold Adaption requires many changes at molecular level which include higher fluidity of lipid membrane, special proteins-chaperonins formation, cryoprotectants synthesis, growth factors reduction, synthesis of enzymes which are cold-active, and metabolic changes (Amico *et al*., 2006).

Ice binding proteins (IBPs) made by microorganisms which are cold-adapted, as a process of overcoming lower temperature, result in an increasing specificity interest. The properties such as lowering the melting point of water or protection against recrystallization during storage has opened the way of IBPs to become very useful in commercialization. Now importance is given to AFPs (antifreeze proteins), which are found as benefitting biomolecules for the Agriculture and food industry (Ustan *et al*., 2015), cryosurgery and cryopreservation of cells (Lee *et al*., 2012), tissues (Lee *et al*., 2015) and organs (Bakhach, 2009). This review discusses the importance and key applications of IBPs with special reference to potential industrial applications.

Functions

1. Antifreeze Protection: Freezing might cause death for most of the organisms. Similarly, water is the essential component for life, but it may also lead to cell death. Ice crystals formation can damage membrane of the cell, which may damage to cells. Psychrophiles develop adaptation to life in harsh conditions for their survival:1.1) By Avoiding freezing (FA) – By moving to warmer areas, Hibernation in mud. Organisms remove agents which nucleate ice and help in undercooling body fluids, with the help of FA strategy. Organisms which live in the areas with continuous extreme temperatures use this strategy, i.e., areas where longer and known temperature drops, e.g.northern hemisphere, flash freezing, high stress etc. 1.2) By Freezing tolerance(FT) - By supercooling, which inhibits the process of ice formation inside the tissue by ice nucleation and allows cells to keep maintain the water in liquid state. Cell solutions are kept in the state of equilibrium freezing point and the water homogeneous ice nucleation temperature (normally between -1 and -41 °C). As a result, the water inside the cell remains separate from the extracellular ice. This Freeze tolerance (FT) process enhances controlled and premature ice formation in extracellular spaces; as intracellular freezing is harmful. The tolerance limit is considered as two third fluids of the frozen body. Such approach is harmful to thawing and freezing damage to organisms. But also, it allows the use of small energy for to survive temperatures below 5 to 40 °C, using FA strategy. It is common in terrestrial regions, shallow waters and southern hemisphere, due to extreme variable cold and less extreme periods. Freeze Tolerance increases due to two key properties of AFPs: Ice recrystallization inhibition (IRI) & Thermal hysteresis (TH). The AFPs TH and IRI activities interact to makes the ice crystals small and harmless to cells. This process stops the liquids to get freeze inside the cells, and cause the death of cell. TH causes the freezing point decrease of fluid of the body and it was described in some polar fishes living in shallow water, by DeVries *et al.,* 1969.
2. Access to water and nutrition: Constant access to water and nutrition is done by many microorganisms by synthesizing secreted AFPs. Extracellular IBPs create water micro channels in many microorganisms in large sea ice, Like - Algae, Fungi Bacteria, and Yeast; grow and live in these microchannels.
3. Access to Oxygen : (*Marinomonas primoryensis*) an arctic bacteria maintains oxygen access by binding to ice surface through ice-binding domains of large Ca2+-dependent adhesion proteins (MpAFP, 1.5 MDa), which is an extracellular, multidomain "train-like" protein which at one end connects to bacterium and at the other end it binds to ice. Hence, MpAFP helps the strictly aerobic *M. primoryensis* remain in the upper reaches of the ice-covered lake where oxygen, nutrients, and light are most abundant (Vance *et al*., 2014).
4. Ice Nucleation: Ability to Ice Nucleation is one of the important function of IBPs. Bacteria which are pathogenic, e.g., *Pseudomonas syringae* produce INPs, in temperature from- 5 to -12 °C, improve the tolerance to freezing, e.g., by formation of ice crystals outside the cell, by using the generated heat as heat source. Also, INPs cause injuries in the epithelium of vegetables and fruits, to give access to nutrient, to bacteria (Xu *et al*., 1998)

Significant Applications Ice binding proteins:

1. Agriculture: Plants produce their own AFPs, however, with low IRI and TH activities which do not protect from formation of ice crystals, opposite to AFPs in insects orfishes. In plants these are produced by plants immune to temperature fluctuations for better survival during extracellular freezing. Over years, many plants are made to boost efficiency of fish AFPs in plants. Earlier also many attempts were made to increase the resistance of plants to lower temperature. In 2010, Zhang *et al*. created the *A. thaliana* with English rye grass cv. Caddy shack (*Lolium perenne*) gene which is able to survive the temperature between 4 and -8 °C (Zang *et al*., 2010). One more team in 2016 achieved *A. thaliana* resistance to temperatures from -5 to -8 °C. Results were satisfactory because the expression of AFPs enabled a reduction in dehydration of the cell and damage to the intracellular membrane caused by freezing. Increasing higher freezing plants resistance can be a great challenge for research community against the global climate change (Strong and MaCabe, 2017).
2. Food Processing: AFPs used as a food supplement improve the standard of stored frozen products, e.g., by keeping a smooth texture without the ice grains of frozen dessert or

by increasing the time of storage of meat, fish, fruits, dough, and vegetables. AFPs prevents recrystallization of ice because of temperature difference while storage of product. Small ice crystals melt and switch to large ones when the temperature decreases and improves organoleptic characteristic of frozen dessert. AFPs type III derived from psychrophilic fish were allowed for consumption in frozen dessert in 2008 by Food Safety Authority, Europe and in 2013 by Food and Drug Administration, USA (Regand and Goff 2006). AFPs reduce the dimensions of those crystals, in order that the feel is best within the mouthfeel. Because of the need of prolonging the time period of products, within the 1960s frozen dough was launched. Although frozen dough allows for extended storage and transport of the baked products further than the local bakeries, finished products, e.g., bread, lose the qualities like uniform crumb, volume, and qualities like odor, taste, and crust crispiness (Omedi *et al.*, 2019).

3. Cryobiology: Cryopreservation is the next major application of AFPs. Cryosurgery and Transplantation require new natural processes which promotes and provides the storage of organs and tissues at low temperatures. To extend Cell lifespan and protect them from damage caused by ice crystals, cell-penetrating cryoprotectants (ethylene glycol (EG), dimethylsulfoxide (DMSO), and glycerol) and non-penetrating cryoprotectants (glucose, sucrose, trehalose), polivinylpyrrolidone and polyvinyl alcohol) are widely used.Due to their lesser toxic and non-penetrating nature AFPs could be potential replacement for these. Since 1990s when AFPs are used as cryoprotectants firstly proteins derived from cold-blooded fish were used in numerous studies involving cryopreservation (Rubinsky *et al.*, 1991). Different proteins: LeIBP (Glaciozyma sp. AY30), FfIBP (Flavobacterium frigoris), and type III AFP (Zoarces americanus) being used in cryopreservation of ovaries frozen by vitrification (Lee, 2015).

Conclusion and Future Remarks: Applications of psychrophiles are not restricted to natural applications but it has wide scope in various fields such as food industry food technology, biology, medicine, biomedical field etc. Much is claimed about extremophiles biomolecule applications in differing kinds of industries. Numerous structural and kinetics adaptations that will be valuable from a technological point. IBPs known as cryoprotectants are increasingly desirable for: Coatings, Agri-food industry, Biomedical applications, Material science etc. Potential of these IBPs is much greater, but still it's not fully known, Although this is clear progress which has been observed in last few years in the functional and structural characteristics of such biomolecules. But still many related questions are unanswered, relating to IBP relationship types, activities and mechanisms of association. Great hopes associated with this are found within the intensive development of gene-splicing, synthetic biology, and bioinformatics. The last goal should be the manufacture of IBPs in high yields at low cost for scientific purposes and successful commercialization.

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