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UNLOCKING COLD SECRETS; PSYCHROPHILES AND THEIR BENEFITS IN FOOD TECHNOLOGY

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ABSTRACT: Psychrozymes, a diverse set of cold-active enzymes produced by psychrophiles including β-galactosidases, pectinases, and amylases which are examined for their specific benefits across various food processing domains. An in-depth exploration of psychrophiles in cold fermentation processes reveals their pivotal role in shaping the sensory attributes of fermented foods and beverages. Psychrophiles contribute to nuanced flavour profiles and distinctive textures, positioning them as catalysts for innovation in cold fermentation. Psychrophiles in bio-preservation have led to advancements in cold-chain technologies, which extend the shelf life of refrigerated and frozen foods while minimizing energy consumption, providing sustainable solutions. This paper highlights food biotechnological applications, showcasing psychrophiles role in advancing brewing and bioconversion of agricultural byproducts, their influence in baking, flavouring, meat tenderization, cheese production, and animal feed formulation. Additionally, this paper also deals with the mechanism and factors contributing to survival of psychrophiles in cold temperatures. Psychrophiles transcend traditional boundaries, impacting sensory attributes and nutritional profiles across a variety of food products.

INTRODUCTION: Psychrophiles have shown immense potential in the degradation of substances essential not only in the food industry, but also in pharmaceuticals, textile industry, bioremediation, biotransformation, and detergent formulation to name a few. Their ability to produce enzymes capable of withstanding extremely cold temperatures proves to be advantageous to industries. Due to this, researchers from all around the globe have invested time, money and effort in the better utilization of the organisms and making complete use of their benefits.

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Currently, the commercially available enzymes, tend to work poorly in cold temperatures, due to which arises the need for enzymes stable at cold temperatures [1]. In order to prevent the proliferation of unwanted microorganisms, preserve delicate and volatile flavor components, and reduce energy consumption, it is preferable to carry out this process in low temperatures [1].

Cold-adapted enzymes have gained significant interest in the food processing industry because of their ability to maintain high catalytic activity at low temperatures, which prevent spoilage and preserve taste and nutritional value. Moreover, their natural lack of structural stability allows for easy inactivation once the desired product is achieved [2]. Cold-active proteins are highly desirable in the food industry as there is a growing trend to process food under milder conditions in order to prevent

spoilage and maintain taste and nutritional value. Consequently, there has been a significant increase in interest in cold-active compounds over the years, with a corresponding rise in literature documenting their applications in food industries. Among the most underutilized resources are psychrophiles, which are microorganisms that thrive in colder regions of the planet where temperatures remain below 5°C for most of the year [3]. These psychrophilic microorganisms offer great potential for various applications.

Enzymes obtained from psychrophiles, have attracted considerable attention due to their distinct properties and wide-ranging applications in various industries, particularly the food industry. These enzymes, such as pectinase, β-galactosidase, protease, amylase, and xylanase, possess cold-active characteristics that make them valuable assets in food processing and production. One specific enzyme, pectinase, which is derived from cold-adapted species like *Cladosporium* and *Tetracladium*, plays a vital role in the production of fruit juice by breaking down pectin, a polysaccharide present in the cell walls of fruits. This enzymatic process reduces viscosity, enhances juice yield, and improves the overall quality of the final product. Similarly, β-galactosidase from psychrophiles aids in lactose hydrolysis, providing solutions for individuals with lactose intolerance and contributing to the production of lactose-free dairy products.

Psychrophilic proteases, amylases, and xylanases are highly efficient at low temperatures. They play a significant role in processes such as tenderizing meat, enhancing flavor, improving bread quality, and reducing haze in juices and beers. These enzymes undergo structural changes to remain active and stable in cold conditions, demonstrating the complex mechanisms that psychrophiles employ to survive and thrive in freezing environments. The survival strategies of psychrophiles go beyond enzymatic adaptations and include modifications to their membranes, adjustments in metabolism, regulation of RNA synthesis, mechanisms for protein folding, control of gene expression, and variations in their genomic makeup. These mechanisms collectively enable psychrophiles to maintain cellular functions, metabolic activities, and environmental adaptations even at subzero temperatures. Exploring the enzymes derived from psychrophiles and comprehending their cold adaptation mechanisms not only contributes to the progress of biotechnological applications but also provides insights into the intriguing adaptations of microorganisms to extreme surroundings. This investigation into cold-active enzymes paves the way for groundbreaking solutions in food processing, preservation, and improving quality. It emphasizes the convergence of biology, technology, and industry in tackling global challenges.

Psychrophilic Enzymes and their Applications:

Pectinase: Pectinases encompass a category of enzymes that facilitate the breakdown of pectic compounds. Pectin, a crucial element found in the middle lamella and primary cell wall of higher plants, is composed of high molecular weight acidic heteropolysaccharides, primarily consisting of α (1−4) linked D-galacturonic acid residues. There are three main groups of pectic polysaccharides, each containing varying amounts of D-galacturonic acid [4].

As of 2023, three previously unknown species of *Cladosporium*, namely *Cladosporium para-sphaerospermum*, *Cladosporium chlamydos-porigenum*, and *Cladosporium compactisporum*, were identified and classified. These newly discovered species were found to produce pectinases that are active in cold temperatures. Specifically, *C. parasphaerospermum* exhibited the highest activity at pH 6.0 and 10 °C, followed by *C. chlamydosporigenum* at pH 6.0 and 15 °C, and C. compactisporum at pH 5.0 and 15 °C [5] *Tetracladium sp.* also produced pectinase [6].

Pectin is a polysaccharide found in the cell walls of fruits. When fruits are subjected to grinding or processing, pectin is released from the cell walls into the liquid phase making it soluble in the fruit juice. Since, pectin has the ability to bind with water molecules, it forms a gel-like consistency making it thick and viscous. Additionally, fruit pulp, the solid part of the fruit flesh is also obtained enhancing the sensory experience of the juice. Therefore, incorporation of pectinase can reduce viscosity and provide with more fruit juice due to the disintegration of jelly-like structure [7].

Also known as pectic enzymes, pectinases are used in industries where the degradation of pectin is essential. The applications of pectinase in the food industry include fruit juice production, processing of coffee and tea, maceration of plants and vegetable tissues, degumming plant fibers, extracting vegetable oil, clarifying wine by removing haze, and manufacturing low methoxy pectin for diabetic-friendly food [7]. Godfrey observed that adding pectinases into mashed fruit, typically at a rate of 40-200 grams of enzyme/ton, for a duration of 30-60 minutes at temperatures ranging from 15 to 30°C, results in a reduction in viscosity through pectin hydrolysis, consequently enhancing the yield [7]. Psychrophilic organisms have sought immense attention in the food industry, especially for the production of fruit juices. The use of cold adapted pectinases for hydrolysis of fruits have proven to be efficient in reducing the risk of contamination by maintaining sugar concentration, clarifying fruit juices by avoiding formation of gel-like consistency, increase storage capacity, minimise viscosity, preserves the product for prolonged duration, maintains product quality and lowers processing cost [7].

In the process of making wine, pectinases can be utilized not only to enhance mash filtering but also to improve the extraction of juice from grapes. Additionally, these enzymes aid in the release of compounds that contribute to the color and aroma of wines. Among the microorganisms capable of breaking down pectin, filamentous fungi are highly effective. They possess the ability to secrete a wide range of pectin-degrading enzymes. Currently, the majority of commercially available pectinolytic enzymes are produced by filamentous fungi, specifically from the genera *Aspergillus, Trichoderma, and Penicillium* [8].

β-Galactosidase: Glycosidases, otherwise known as glycoside hydrolase, are enzymes that catalyse the hydrolysis of glycosidic bonds in carbohydrates to yield monosaccharides and oligosaccharides. One such glycosidase is β-Galactosidase, commonly referred to as lactase [9], which catalyses the hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides. β-Galactosidases have proven to showcase their applications in food technology, and dairy technology for the preparation of lactose-free milk. *Arthrobacter psychrolactophilus, Guehomyces pullulans, Bacillus subtilis KL88, Carnobacterium piscicola BA, Pseudoalteromonas haloplanktis*, and *Planococcus* are a few examples of psychrophiles that secrete cold-active β –galactosidase [6, 7]. Generation after generation, the number of individuals being lactose-intolerant have spiked, and has been predicted to only increase in the future. So, treating milk with psychrophilic β -galactosidase can aid in the degradation of lactose. Commercially available β -galactosidases have an optimal temperature of 37°C at which they exhibit maximum activity and efficiency in breaking down lactose into its constituent sugars, glucose, and galactose. Since most dairy processes are carried out at different temperatures, the enzyme activity is lowered by 5-10 times [10].

On the other hand, psychrophilic β-Galactosidase could accelerate the lactose hydrolysis process. The primary products obtained from cold-adapted β-galactosidase, which reduce lactose content during processing, include: low-lactose milk, concentrated low-lactose bases for ice cream, syrups and sweeteners for food production, low-lactose yogurt, and sweetened yogurt made from acid and sweet whey [7]. Kur's study revealed that the recombinant cold-adapted β-galactosidase from the Antarctic bacterium *Pseudoalteromonaus haloplankis* exhibited significant efficiency in lactose hydrolysis within the temperature span of 0°C to 30°C. About 90% of the lactose in milk was hydrolyzed by this enzyme after 6 hours at 30°C and after 28 hours at 5°C [7]. β-galactosidase enzyme from this organism is cold-adapted and at 10 degrees C retains 20% of maximum activity [11].

β-galactosidases that function effectively in cold conditions and at a neutral pH have the potential to improve the digestibility of dairy products for individuals who are lactose-intolerant. Additionally, these enzymes can enhance sweetness while minimizing the risk of contamination. By operating at an acidic pH, cold-adapted β-galactosidases can also have a positive impact on reducing pollution and increasing the practical utility of whey, a byproduct of the cheese industry. These enzymes are capable of producing syrups rich in glucose and galactose, which can serve as sweeteners in various food products. Furthermore, these syrups are easily fermentable by

microorganisms that produce alcohol. Cold-adapted β-galactosidases have also demonstrated the ability to perform transglycosylation, wherein lactose hydrolysis occurs alongside the transfer of monosaccharides to form higher oligosaccharides like tri- and tetrasaccharides. These galacto-oligosaccharides can be produced and utilized as additives in probiotic food products to promote the growth of bifidobacteria in the large intestine. Additionally, they can serve as low-calorie sweeteners since they are not easily metabolized in the small intestine. This makes them suitable for direct addition to milk or as an ingredient in dairy products derived from whey [2].

During the process of baking, several enzymes such as xylanases, proteases, amylases, lipases, and glucose oxidases have the ability to alter the properties of hemicellulose, gluten, starch, and free sulfhydryl groups. This modification occurs during dough preparation and processing, which typically occurs at temperatures below 35°C. By working together, these enzymes can enhance the elasticity and ease of handling of the dough, resulting in a larger volume and improved structure of the final product. Recent studies have shown that a cold-adapted family 8 xylanase has proven to be more effective in baking. When compared to a widely-used commercial mesophilic enzyme preparation, the cold-adapted xylanase yielded a larger volume loaf, highlighting yet another advantage of utilizing cold-adapted enzymes in the baking industry [2].

Protease: Proteases, which are also referred to as proteinases or proteolytic enzymes, encompass a vast category of enzymes that facilitate the breakdown of peptide bonds in proteins and polypeptides through hydrolysis. These enzymes exhibit variations in characteristics such as their preference for specific substrates, the structure of their active sites, their catalytic mechanisms, optimal pH and temperature conditions, and their stability profiles [12].

Alkaliphilus transvaalensis, Penicillium nalgiovense, Pseudoalteromonas sp., Arsukibacteriumikkense, Teredinobacter turnirae, Trichoderma atroviride, Vibrio sp. Curtobacterium luteum, Escherichia freundii, Pedobacter cryoconitis, Alcaligenes faecalis, Bacillus amyloliquefaciens, Engyodontium album, Escherichia freundii, Serratia marcescens, Pseudomonas aeruginosa, Serratia marcescens are few of the psychrophilic organisms that produce cold-active protease [6, 13].

Using cold-active proteases can lead to economic benefits as they enable working at low temperatures, even on an industrial scale. For instance, instead of using conventional proteases from mesophilic or thermophilic microbes to heat and raise the temperature during the industrial peeling process of leather, the same process can be carried out at the temperature of tap water by utilizing cold-active proteases. This not only saves energy but also allows for cost-effective operations.

Proteases, as a group, have found application in various fields such as baking, brewing, cheese making, and in the preparation of protein concentrates. Furthermore, they possess specific applications in various processes, such as the fermentation of fish and soy sauce. These applications do not alter the nutritional value or flavor. They can also serve as alternatives to rennet, accelerating the maturation process of slow-ripening cheeses in conjunction with lipase. Additionally, cold-active proteases can be advantageous in taste enhancement and tenderization of frozen meat products. A cold-active protease obtained from the psychrophilic *Pseudoalteromonas sp.* has been found to secrete essential amino acids that selectively enhance the flavor of frozen meat. The psychrophilic serine protease belonging to *Chryseobacterium sp.* has been suggested for its potential to improve meat quality due to its salt tolerance and optimal activity at low temperatures [13].

Amylase: Amylase, an enzyme responsible for digestion, is primarily secreted by the pancreas and salivary glands. Although it can also be found in other tissues, its presence is minimal. The main function of amylases is to break down the glycosidic bonds in starch molecules, converting complex carbohydrates into simpler sugars. These enzymes are classified into three categories: alpha-, beta-, and gamma amylases. Each type targets specific parts of the carbohydrate molecule. Alpha amylase is present in humans, animals, plants, and microbes, while beta amylase is predominantly found in microbes and plants. Gamma amylase,

however, can be found in both animals and plants [14]. *Micrococcus antarcticus, Arthrobacter psychrolactophilus, Pseudoalteromonas sp., Zunongwangia profunda, Exiguobacterium sp., Geomycespannorum, Bifidobacterium longum* are some examples of the cold-active amylase producing psychrophiles [6].

Maltose, a disaccharide derived from glucose molecules, serves as the primary component of maltosugar syrup. It is widely utilized as a sweetener and holds significant value in the food industry due to its non-hygroscopic nature and resistance to crystallization. When producing maltose from starches like potato, sweet potato, corn, and cassava, cold-active α-amylase can be employed. The action of this enzyme breaks down the starch into maltotetraose, resulting in the production of maltotetraose syrup. This syrup can effectively substitute sucrose as a sweetener. Additionally, it has the ability to lower the freezing point of water more than sucrose or high fructose syrup, making it useful for controlling the freezing points of frozen foods [6].

Cold-active α-amylases have the potential to be employed in the food industry to minimize haze formation in juices. In addition, these enzymes can also be utilized in the brewing industry to accelerate the mashing phase, particularly at low temperatures. By incorporating enzymes with a high level of activity below 20°C during food processing, it becomes possible to restrict the growth of unwanted microorganisms, reduce process durations, and eliminate the need for costly heating procedures [6].

Xylanase: Xylanases are enzymes that break down the β-1, 4 bonds of the complex polysaccharide xylan, which is found in the cell walls of plants. Xylan is a major component of both soft and hard food, and is the second most abundant renewable polysaccharide after cellulose. Various microorganisms, such as bacteria, actinomycetes, yeast, and fungi, produce xylanases and other related enzymes that facilitate the hydrolysis of hemicelluloses. *Penicillium canesence, Truncatella angustata, and Pseudogymnoascus roseus, Bacillus sp., Sorangium cellulosum and Zunongwangia profunda, Aspergillus sp.*15, *Luteimonas sp.*[16], *Flavobacterium sp.*[17], *Pseudoalteromonas haloplanktis TAH3a., C. adeliea* [18] have been found to actively produce cold-active xylanase. Xylanases possess a multitude of potential applications in various industrial processes, including the food industry. For instance, in brewing, they are employed to enhance wort filterability and minimize haze in the end product. Xylanases are also utilized in coffee extraction and the production of soluble coffee. While xylanases can be used independently, they are often combined with other enzymes, particularly other hydrolases, as well as proteases, oxidases, isomerases, and more [19].

The use of cold-adapted xylanases can provide significant benefits in processes that require low temperatures or where heating is not economically feasible. These enzymes are particularly advantageous in situations where maintaining ingredient and product quality is crucial, such as preserving flavor and color, preventing microbial growth and fermentation, and avoiding product denaturation. Compared to currently used xylanases, cold-adapted xylanases exhibit higher activity levels at low to moderate temperatures, making them ideal for various applications in the food industry. In the baking industry, for instance, cold-adapted xylanases are well-suited for tasks like dough preparation and proofing, which typically take place at temperatures below 35°C. Their ability to function optimally under these conditions makes them a desirable choice for improving the efficiency and quality of baked goods. By utilizing cold-adapted xylanases, manufacturers can achieve better results in processes where high temperatures are impractical or detrimental. These enzymes offer a promising alternative for enhancing performance and maintaining the integrity of products in a wide range of temperature-sensitive applications [19].

TABLE 1: PSYCHROZYMES AND THEIR APPLICATIONS IN FOOD INDUSTRY

Source	Enzyme extracted	Application in food industry	Author, Journal, Year
Pseudoalteromonaus haloplankis	β-Galactosidase	Lactose hydrolysis	Shanshan *et al*. 2022. Microbial Pathogenesis
Cystofilobasidium	Pectinase	Extraction of grape juice during	Saavedra-Bouza *et al. Curr*

capitatum		winemaking	*Res Biotechnol.* 2023
Pseudoalteromonas sp. DY3, P. haloplanktis, V. salmonicida, V. rumoiensis S-1	Catalase	Cheese production	Yusof. J Fungi (Basel) 2021
Paracoccus sp., Cystofilobasidium capitatum SPY11, Rhodotorula sp.	Lactases β-galactosidase	Lactose hydrolysis	Hamid *et al.* Molecules 2022
Pseudoalteromonas haloplanktis TAH3A, *Flavobacterium* sp. MSY-2, *Rhodococcus* sp., *Pseudomonas* sp., *Flammeovirga pacifica* WPAGA1, *Cryptococcus adeliensis*	Xylanases		Hamid *et al.* Molecules 2022
Geomycespannorum, Bacillus subtilis N8, *Geomyces pannorum Microbacterium foliorum Zunongwangia profunda*	α-Amylases	Flavour enhancement and improve bread quality	Hamid *et al.* Molecules 2022
Pyrococcus sp	Cellulases	Ethanol fermentation	Hamid *et al.* Molecules 2022
Cystofilobasidium capitatum PPY-1, Rhodotorula mucilaginosa PT1, Cystofilobasidium capitatum SPY11, Leucosporidium drummii, Sporobolomyces salmonicolor, Penicillium chrysogenum F46	Pectinases	Pectin degradation, juice extraction	Hamid *et al.* Molecules 2022
Pseudoalteromonas haloplanktis TAC125, Penicillium canesense, Pseudomonas sp. VITCLP4	Lipases	Animal feed	Hamid *et al.* Molecules 2022
Flavobacterium limicola, Acinetobacter sp., Geomyces pannorum, Naganishia albida	Proteases	Fermentation of fish and soy sauce	Ojha *et al.* Food Biotechnology 2019

There are several theories that have been proposed to explain the low temperature surviving instincts of psychrophiles. These microorganisms have evolved specific mechanisms in order to adapt and thrive in such freezing conditions, typically below 15°C (59°F). These adaptations involve physiological, biochemical, and genetic changes that allow them to maintain cellular functions, membrane integrity, and metabolic activity at low temperatures [20].

Membrane Composition, Fluidity and integrity in Psychrophiles: Psychrophiles undergo specific modifications in the composition and fluidity of their cell membranes to ensure survival and functionality in low temperatures. One of the key adaptations involves increasing the proportion of unsaturated fatty acids in their membrane lipids. Unsaturated fatty acids have double bonds in their carbon chains, which introduce kinks in the lipid tails, preventing close packing and thereby enhancing membrane fluidity.

This adjustment in membrane composition allows psychrophiles to maintain the fluidity of their membranes even at low temperatures, which is essential for various cellular processes [21].

The reason for this increased proportion of unsaturated fatty acids is due to low temperatures, phospholipid bilayers tend to become more rigid due to closer packing of lipid molecules. Saturated fatty acids, with straight carbon chains and no double bonds, exacerbate membrane rigidity at low temperatures. In contrast, unsaturated fatty acids introduce bends and kinks in the lipid tails, preventing tight packing and maintaining membrane fluidity. By increasing the proportion of unsaturated fatty acids, psychrophiles counteract the tendency for membrane rigidity, ensuring the proper functioning of membrane-bound proteins and enzymes. This facilitates cellular processes even in cold conditions [21, 22].

Membrane fluidity is crucial for various cellular processes, including nutrient uptake, signalling, and membrane-bound enzyme activity [20, 23]. In cold environments, where temperatures are below the optimal range for many biological processes, maintaining membrane fluidity becomes particularly challenging. Proper membrane fluidity allows membrane-bound proteins and enzymes to move freely within the lipid bilayer, facilitating their interactions and ensuring optimal enzymatic activity. By adjusting membrane composition to enhance fluidity, psychrophiles ensure the continuity of essential cellular functions even in cold conditions.

In addition to altering lipid composition, psychrophiles may employ other mechanisms to regulate membrane fluidity. For example, they may modulate the synthesis of membrane proteins or adjust the ratios of specific lipid classes (e.g., phospholipids, glycolipids) to further optimize membrane properties for cold adaptation. Furthermore, psychrophiles may produce extracellular polysaccharides or exopoly-saccharides that interact with membrane components, influencing membrane structure and fluidity [23].

Cold-Active Enzymes: Research findings suggest that cold-adapted enzymes commonly display heightened catalytic efficiency (kcat/KM) at lower temperatures compared to mesophilic enzymes, albeit with compromised thermal stability [24]. Psychrophiles have evolved enzymes with structural adaptations that enhance their stability and activity at low temperatures, minimizing denaturation. These cold-adapted enzymes allow psychrophiles to maintain metabolic activity and cellular functions even in cold environments where reaction rates are typically slower [25].

The strategies employed by proteins, especially enzymes, to adapt to cold environments typically involve augmenting flexibility in protein architecture, either at the active site or in distant structural regions crucial for conformational changes. This increased flexibility often results from amino acid substitutions, particularly glycine, predominantly found in loop regions. Moreover, cold-adapted proteins tend to exhibit decreased stabilizing interactions, such as hydrogen bonds, salt bridges, and ionic and aromatic interactions. Nonetheless, these enzymes undergo structural adaptations, including the presence of flexible regions and surface loops, which significantly enhance their flexibility and catalytic efficiency at low temperatures. These structural modifications allow cold-active enzymes to maintain their active conformation and catalytic activity despite the challenges posed by cold conditions [26]. As a result, psychrophiles rely on these producing these enzymes to carry out crucial metabolic processes, not only for the adjustment of enzyme production to compensate for the effect of temperature on growth but also for, nutrient uptake, energy production, and biosynthesis of macromolecules, even when temperatures are low. Essentially, cold-active enzymes serve as indispensable biological tools for psychrophiles, enabling them to thrive and sustain vital cellular functions in cold environments. Therefore, there exists no universal blueprint for cold adaptation, as each protein adopts distinct mechanisms to bolster catalytic activity in cold conditions.

Enzymes integrated within the cellular structure may be partially protected from temperature-induced denaturation compared to isolated enzymes. Cellular integrity, including membrane structure and composition, provides additional protection to thermolabile enzymes by maintaining proper enzyme-substrate interactions. Despite these protective mechanisms, enzyme activity still decreases at super optimal temperatures, indicating the overall sensitivity of cellular components to temperature fluctuations [3, 25].

Metabolic Adjustments: Metabolic adjustments are fundamental mechanisms through which psychrophiles adapt to cold temperatures, ensuring the efficient operation of cellular processes. These adjustments involve intricate modifications in metabolic pathways to sustain essential functions even in chilly environments. One significant adaptation is the upregulation of enzymes participating in key metabolic pathways, such as glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport chain. By increasing the expression of these enzymes, psychrophiles enhance ATP production, which serves as a vital energy source for various cellular activities [25].

Moreover, psychrophiles employ specific metabolic pathways to cope with the challenges of cold temperatures. For instance, they engage in the synthesis of compatible solutes, such as polyols and amino acids, which play crucial roles in maintaining osmotic balance and safeguarding cellular structures from cold-induced damage. These compatible solutes act as Osmo protectants, helping psychrophiles counteract osmotic stress caused by external environmental factors. Additionally, they contribute to cellular cryoprotection by preventing the formation of ice crystals within cells, thereby preserving cellular integrity in freezing conditions [3].

The proteolytic activity also displays a key role in maintaining homeostasis for psychrophiles by production of proteases, enzymes responsible for protein degradation, play a crucial role in maintaining protein homeostasis in psychrophiles. The quantitative and qualitative changes in intracellular proteolytic activities in response to growth temperature indicate the adaptive capacity of psychrophiles to cold environment. Different sets of proteases may be synthesized by psychrophiles to cope with temperature fluctuations, ensuring proper protein turnover and function at low temperatures [21].

In cold environments, certain psychrophiles produce antifreeze proteins (AFPs) or ice-binding proteins (IBPs) as a crucial survival strategy [30]. These proteins serve to prevent the formation and growth of ice crystals within cells, thereby protecting cellular structures from damage. When ice crystals begin to form, AFPs bind to them, effectively inhibiting their growth. This inhibition prevents the expansion of ice crystals, which, if left unchecked, could lead to cellular damage and lysis. By employing AFPs, psychrophiles can thrive in subzero temperatures, avoiding the potentially lethal consequences of freezing-induced cellular disruption.

RNA Synthesis Inhibition: The inability of psychrophiles to synthesize RNA at super optimal temperatures limits their growth potential beyond a certain temperature threshold[21]. RNA is essential for protein synthesis and gene expression, and its inhibition at high temperatures prevents the production of critical cellular components required for growth and survival. This temperature-dependent inhibition of RNA synthesis serves as a key factor in fixing the maximum growth temperature for psychrophiles.

Protein Folding and Stability: Psychrophiles possess sophisticated mechanisms to ensure proper protein folding and stability, even in cold environments. Chaperone proteins play a vital role in this process by assisting in the folding of newly synthesized proteins and facilitating the refolding of misfolded proteins under cold stress conditions [25]. Additionally, cold shock proteins and RNA chaperones contribute to maintaining stability by stabilizing RNA molecules and optimizing translation efficiency at low temperatures. These mechanisms collectively help psychrophiles maintain the integrity and functionality of their proteome, essential for their survival in cold habitats [3].

Regulation of Gene Expression: Psychrophiles employ precise regulation of gene expression to adapt to cold stress and ensure cellular homeostasis [26]. Transcriptional regulators, including cold shock proteins and transcription factors, orchestrate the expression of cold-responsive genes involved in various aspects of adaptation, including metabolic adjustments and stress responses. Furthermore, post-transcriptional and post-translational modifications play crucial roles in fine-tuning protein activity and stability in cold environments. Together, these regulatory mechanisms enable psychrophiles to dynamically adjust their gene expression profiles, allowing them to efficiently

respond to cold stress and thrive in challenging cold habitats.

Genome Analysis: Different genome sizes also suggests that psychrophiles may employ different genetic strategies to thrive in cold environments. Psychrophilic organisms with larger genomes may possess a greater repertoire of genes involved in cold adaptation, stress response, metabolic flexibility, and environmental sensing. Genomic analyses of psychrophiles can identify candidate genes and pathways associated with cold adaptation, providing insights into their mechanisms of survival in cold environments [3].

One study aimed to investigate variations in genome size in relation to the capacity for survival in cold conditions and illustrated the disparities observed among different organisms. However, there hasn't been any demonstration of a direct correlation between psychrophiles and the GC content of their genomes. Nevertheless, Analysis of genome mining has revealed the presence of 12 primary clusters of chaperones, encompassing a total of 89 genes responsible for encoding heat-shock proteins or molecular chaperones. These clusters play an important role in the mechanism of cold adaptation through diverse intracellular processes, including molecular functions, cellular components, and biological processes.

CONCLUSION: Psychrophilic enzymes, have emerged as invaluable biocatalysts with immense potential across diverse industries. Their unique properties, such as optimal activity at low temperatures, exceptional stability, and specificity, render them superior to their mesophilic counterparts in numerous applications. From the food and detergent industries to bioremediation and biotechnology, these enzymes are revolutionizing processes by enhancing efficiency, reducing energy consumption, and minimizing environmental impact.

While significant advancements have been made in understanding and harnessing psychrophilic enzymes, further research is imperative to unravel the complexities of their structure-function relationships. This knowledge will facilitate protein engineering for tailored enzyme properties and expand their applicability to new frontiers. As our planet faces increasing environmental challenges, psychrophilic enzymes stand as promising tools for sustainable development and innovation.

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