

Psychrophiles as a novel and promising source of cold-adapted industrial enzymes

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Received January 23, 2023
Revised May 25, 2023
Accepted May 28, 2023
Published June 30, 2023



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Abstract: Psychrophiles are an exclusive group of microbes that thrive in extremely cold environments, such as polar regions and deep-sea. These cold-loving microbes have developed a range of adaptations that enable them to function at low temperatures, including the production of cold-adapted enzymes. These enzymes are highly active and stable in cold environments, making them valuable biocatalysts for various industrial processes. The potential applications of psychrophilic enzymes are vast, including in the food, pharmaceutical, and bioremediation industries. Cold-adapted enzymes are particularly useful in low-temperature applications, such as in the production of cold-processed foods and cold-water detergents. They can also be used in the production of antibiotics and other pharmaceuticals that require low-temperature conditions. Additionally, psychrophilic enzymes can be used in bioremediation processes, where low-temperature conditions are often encountered. Metagenomic studies have revealed the potential for discovering new psychrophilic enzymes from uncultivated microorganisms in cold environments. The use of recombinant DNA technology has enabled the production of large quantities of psychrophilic enzymes for industrial applications. Psychrophiles represent a novel and promising source of cold-adapted industrial enzymes. The use of these enzymes in various industries has the potential to significantly reduce energy consumption and environmental impact. With ongoing research and technological advancements, more diverse and efficient cold-adapted enzymes will likely be discovered from psychrophilic microorganisms, further expanding the array of applications for these enzymes in the future.

Keywords: Psychrophiles; extremophiles; cold-adapted enzyme; psychrozymes; extremozymes.

Introduction

High altitude and cold regions are distinctive in that they contribute significantly to ecological succession among different habitats and have mixed effects on biodiversity and soil physicochemical qualities. Multiple climatic and nutritional variations, like exposure to strong ultraviolet radiations, low partial pressure, low temperature, high wind velocity, soft water, and nutrient availability, substantially impact biodiversity in high-altitude environments.

Microbes can colonize and maintain metabolic activity at sub-zero temperatures despite adverse environmental conditions due to their ability to create specific enzymes that conduct specialized biological tasks [1, 2]. D' Amico *et al.* (2006) identified these harsh environmental conditions as a niche for isolating efficient novel enzymes/ genes that may be employed for essential industrial applications [3]. Because of their ability to sustain enzymatic activity and stability under such stressful conditions, these psychrophile bacteria are a potential source of cold-adapted enzymes, also known as psychrozymes.

Also known as cold-loving or cryophiles, psychrophiles thrive at -20 to 10 °C. They can survive their cell cycle by producing cold-adapted enzymes, also known as psychrophilic enzymes or psychrozymes [4]. The structural features of psychrozymes have evolved to allow for considerable structural flexibility, particularly around the active site, higher levels of activity at lower temperatures, low substrate affinity, and lower activation enthalpy.



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Enormous stability, high yield, economic feasibility, diversity, and increased catalytic activity are the only advantages of using psychrozymes in biotechnological and industrial applications. Glycosylases, cellulases, alkaline phosphatases, nucleases, proteases, polygalacturonase, lipases, mannanases, amylases, pectate lyases, endo-arabinase, DNA ligase pectinase, and catalase are just a few of the enzymes produced by psychrophilic bacteria. Psychrozymes have high catalytic efficiency and a straight forward purification procedure, at mild to moderate temperatures.

Cryo-Defense Strategies of Psychrophiles

Psychrophiles have a wide range of unique and adaptable traits at almost all cell architecture and function stages to stay alive in extreme and freezing conditions [4]. They are thought to go through three stages when exposed to cold shock. Phase I is known as the "acclimation phase", starting right after exposure to cold. During this phase, different cold-shock proteins are produced, and as a result, their development rate slows down due to decreased membrane fluidity. The second phase, sometimes known as the "recovery phase," At this point, cells are said to be "cold-adapted". Cells resume making proteins in great quantities at this stage and continue to expand. Phase III is referred to as the "stationary phase," in which cells develop significantly more slowly because non-cold shock proteins are produced. Psychrophiles typically credit cunning strategies for their capacity to adapt to such circumstances.

The enormous biotope volume of the oceans constitutes the largest reservoir of psychrophiles conducive to matter cycling, but psychrophiles and associated biomolecules have already discovered a variety of applications. Previous papers have reported on a variety of their physiology, ecology, and molecular alterations. A wide range of adaptive traits is needed for life in cold conditions at almost all cell design and functional levels. Living things are severely constrained by the physicochemical effects of cold. For instance, cold makes the water more viscous, slows biological reactions, reduces molecular diffusion rates, disturbs weak contacts that promote molecular recognition and interaction, and strengthens hydrogen bonds that maintain inhibitory nucleic acid structures. It also increases gas solubility, stabilizes toxic metabolites, and reduces the fluidity of cellular membrane systems [5, 6].

Commercially Significant Cold-Adapted Enzymes

Psychrozymes, enzymes resistant to low temperatures and other cold-related stressors, are produced by psychrophilic microorganisms. Pharmaceutical, food, paper, and pulp sectors all use these enzymes. Enzymes that are cold-adapted, or psychrozymes, can endure temperatures between 10 and 5 °C. Because of their ability to tolerate unfavorable environments, psychrozymes are highly demanded in industries. Proteins isolated from cold-loving microbes are increasingly being used because they act at their optimal temperature, boosting the recovery of enzymatic reaction products [3]. Amylose, cellulose, pectin, β -galactoside, protein, and lipid are a few polymeric compounds that psychrozymes can break down. Because of their enormous

promise, psychrozymes have attracted a lot of money from all over the world. Industrially necessary psychrozymes are used in food technology (like β -galactosidase and pectinase), bio-polishing of textiles goods, and detergent formulation industries. Additionally, these psychrozymes are employed in biotransformations (aminotransferases and methylases) as well as various pollution control approaches, including bioremediation (oxidases).

Psychrozymes provide a variety of advantages, including high specific activity at low temperatures. They can offer several other benefits, including energy savings, reducing volatile chemicals, preventing of contamination, and simply deactivating enzymes. To maintain food quality throughout transit and storage, most food sectors use psychrozymes. Psychrozymes are widely used in the detergent and textile sectors as well. Pectinases and cellulases are also employed to clarify fruit juices, while proteases aid in removing fish skin [7].

Psychrozymes are applied for low-temperature biodegradation in addition to the food industry. The greatest alternative to physicochemical approaches for cleaning up solids and wastewater contaminated by oils, hydrocarbons, and lipids is bioremediation. Psychrozyme biodegradation has many advantages over other existing traditional methods. It has been discovered that using psychrozymes to treat contaminated soil is far more cost-effective than storage, conventional incineration, and concentration procedures. Brun et al. (1997) looked into the recombinant Antarctic *Pseudoaeromonas haloplanktis*, responsible for producing TOMO (toluene-O-xylene monooxygenase) [8].

In order to preserve product quality throughout shipping and storage, the majority of the food industry prefers to treat products with cold-adapted enzymes. Two further industries that regularly use cold-adapted enzymes are the textile and detergent sectors as in Figure 1.

Lipase

Lipase is an enzyme that helps digest fats and catalyzes triglyceride hydrolysis to free fatty acids and glycerol. Triacylglycerol acyl hydrolases, sometimes referred to as lipases, can hydrolyze and create fatty acid esters in both aqueous and non-aqueous conditions. Studying cold-adapted lipases from psychrophilic and psychrotrophic microorganisms isolated from the deep-sea environments, polar regions, Antarctic, and refrigerated food samples, compared them to all psychrotrophic lipases and cold-adapted lipases that possess the canonical/ hydrolase fold. The structural modification of psychrophilic lipases allows for considerable flexibility and substrate accommodation at low temperatures, which is one of their distinguishing characteristics [9].

Alquati et al. (2002) studied the molecular structure of lipase from *Pseudomonas immobilis* and *P. fragis* and revealed some of the distinctive features of the cold-adapted lipases from their mesophilic counterparts [10]. Compared to their mesophilic counterparts, CLPs have fewer arginine residues than lysine residues, fewer proline residues, a weaker hydrophobic core, fewer salt bridges, and fewer aromatic-aromatic interactions. Psychrophilic lipase, according to Georlette et al. (2004) and Gomes and Steiner

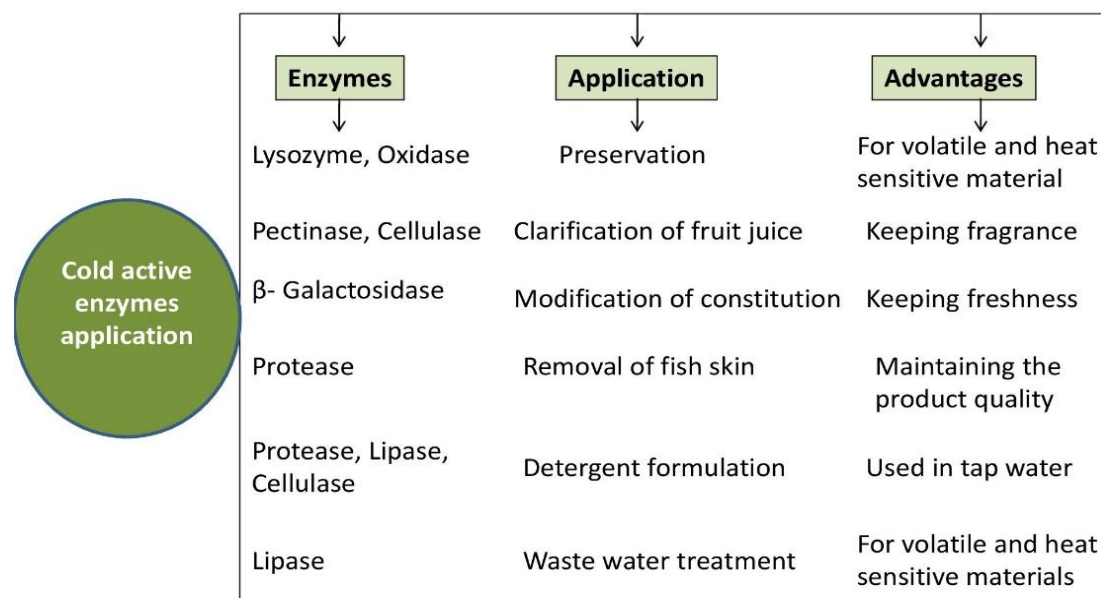


Figure 1. Cold-active enzymes and their applications.

(2004), likewise exhibits a relatively high concentration and accumulation of glycine remains (for local mobility), a decreased quantity of ion pairs, and poor charge-dipole interactions in helices [11, 12]. Its ability to put up substrates at low temperatures [13, 14] and its capacity to catalyze hydrolysis in a low water medium [15] are the reasons for psychrophilic lipase's adaptability. Psychrophilic lipase is a better choice than mesophilic and thermophilic lipase, as mesophilic and thermophilic have high rigidity and low flexibility.

Due to their use in detergents and the trend toward cold-washing detergents [16], innovatively adapted lipases are currently the subject of intensive research. The lipase known as Lipoclean®, created by Novozymes, is stable in multienzyme solutions and aims to stain from triglycerides that are active at low temperatures (20 °C). According to Buchon et al., in 2000, psychrophilic lipases offer tremendous promise for bioremediation, wastewater treatment in a cold environment that is polluted with fat, and the synthesis of active chemicals in cold temperatures [17]. In biotechnological processes such as detergent formulation, molecular biology applications, food industry additives, environmental bioremediations, biotransformation, and heterologous gene expression in psychrophilic hosts to avoid the generation of inclusion bodies [14], psychrophilic lipase has a wide range of applications. Its remarkable catalytic activity at lower temperatures makes it a unique possibility.

Protease

Psychrophilic proteases are extracted from microorganisms like the Arctic, cold desert soil, polar regions, ice permafrost, deep sea, alpine areas, sub-Antarctic sediments, and various colder parts of the planet. Cold-active proteases are isolated from *Azospirillum* sp., *Clostridium* sp. from the Antarctic region [18], *Exiguobacterium* sp. obtained from cold desert soil, *Pedobacter cryoconitis* from glacier ice [19, 20], *Penicillium chrysogenum* from the cold marine environment, *Pseudomonas* sp. from the deep sea [21],

Psychrobacter proteolyticus from Antarctic krill *Euphausia superba* [22], *Serratia* sp. from coastal water [23].

A marine protease from *Flavobacterium* YS-80 was compared to its mesophilic analog alkaline protease, which was isolated from *P. aeruginosa*, and a psychrophilic alkaline protease (PAP), which was recovered from an Antarctic *Pseudomonas* species. It was observed that the Zn²⁺-Tyrosine-OH bond is more flexible in the psychrophilic alkaline protease, which helps in facilitating substrate accessibility and also maintains the activity in low temperatures, i.e., the specific activity of the psychrophilic protease is higher than its mesophilic counterparts (4 folds higher in 4 and 25 °C), half time of denaturation multiple times shorter (10 times) and have excellent catalytic efficiency at low temperatures [21]. It can wash textiles at room temperature, and application dehairing of hides and skins saves energy and reduces the impact of harmful chemicals used in dehairing. The weakness of cold proteases at temperatures above 25 °C is a drawback. The heat stability and molecular adaptability of psychrophilic protease have been the subject of recent research [22, 23]. The limited understanding of psychrophilic protease is a setback to this day.

Amylase

Amylase is an enzyme that catalyzes the hydrolysis of starch, glycogen, and intermediate hydrolysis products for the synthesis of dextrin and glucose subunits (cleaving internal α-1, 4-glycosidic bonds). Three types of Amylases have been classified (α-, β-, and γ-amylases) based on their mechanism of action. Amylase constitutes 30% of the world's commercial enzyme production. Amylases are found in bacteria, fungi, animals, and plants. *Alteromonas haloplanktis*, an Antarctic bacterium, was used by Feller et al. in 1998 to isolate and express the first psychrophilic amylase. Novozymes patented a variation of the parent *B. licheniformis*' amylase that exhibits increased activity of the enzyme at temperatures between 10 and 60 °C [24].

Georges Feller (2013) gave a proteomic analysis of the Antarctic psychrophile and mentioned the missing disulfide linkage between A and B, its two domains. Amylase extracted from *Pseudoalteromonas haloplanktis* shows higher flexibility, catalytic activity, and higher specificity for its mesophilic counterpart [25]. Roohi et al. (2013) noted a novel active, stable psychrophilic α -amylase at alkaline pH (7 to 10) and low temperatures (4–37 °C), remarkably showing its optimal activity at 22 °C, which was isolated from *Bacillus cereus* GA6 [26]. Cold-adapted Amylase isolated from *Glaciozyma antarctica* PI12 has a main application in water treatment and bioremediation in a cold environment [27]. There have been reports of the synthesis of psychrophilic enzymes by several psychrophilic bacteria, including *Schwanniomyces alluvius*, *Lipomyces kononenkoae*, *Candida antarctica*, *Trichosporon pullulans*, and *C. flavus*. Cold-active amylases can catalyze these industrial processes, which not only helps to save energy but also lowers the risk of fouling [28]. The limitations of psychrophilic α -amylases are impaired due to the oxidizing conditions at low temperatures resulting in poor stability, which limits their usage for commercial purposes

Cellulase

A category of hydrolytic enzymes known as cellulases helps break down the polysaccharide cellulose into shorter polysaccharides and oligosaccharides, such as glucose, as well as monosaccharides. These enzymes are valuable economically as a result of their many applications. Cellulases are frequently employed in the agricultural, medicinal, food and feed, textile, pulp, paper, detergents, and laundry sectors of the economy. They can be used to soften clothing, take the extra color out of fabrics, biopolish denim, process coffee, pulp woody raw materials, bioremediate industrial wastes, and treat phytobezoars, among other things.

Fungi, bacteria, and protozoans are the leading producers of cellulases. These microbial cellulases consist of a linker region, a module for binding carbohydrates, and a catalytic module. Comparatively, to psychrophilic cellulases, mesophilic cellulases contain an easy-to-follow, repetitive linker region that is frequently rich in threonine, proline, valine, glycine, and serine residues. They contain plenty of cysteines, asparagine, serine, glycine, aspartate, and threonine in their linker region. Compared to mesophilic cellulases, they have a structural change that increases their versatility and enzyme activity at low temperatures [29]. Because of these characteristics, they can work in the abovementioned areas and get more significant rewards.

Pectinase

Plant cell walls include pectin, which is broken down by the enzyme pectinase into less complicated compounds like galacturonic acids. They can be classified into three groups: pectin lyases, polygalacturonases, and pectin esterases. The main issue facing the commercial (food and beverage) business is that the majority of the time, mesophilic proteins necessitate heating, which negatively affects the final product's organic properties and nutritional functions [29, 30].

The use of cold-adapted pectinase enables the reduction of natural product juice thickness at cold temperatures and increases the mash's squeezing capacity, resulting in the degradation of jam structure; as a result, the raw product juice is easily obtained with greater amounts [20].

Aspects of the current technology's psychrophilic acidic pectinases enzyme compositions include retting in the textile industry, degumming of fiber crops, and producing high-quality paper through fermentation, oil extraction, and wastewater treatment. The Novozymes-developed pectinase Xpect® breaks 1, 4-glycosidic linkages in polygalacturonic acid at 20 °C into water-soluble "simpler sugars".

β -galactosidase

The enzyme β -galactosidase (EC 3.2.1.23) catalyzes the hydrolysis of the β -galactosidic bonds in oligosaccharides under specific circumstances where the swap of a sugar moiety from a glycosyl donor to an acceptor. Cold-adapted β -galactosidases are found in microorganisms that are native to habitats with consistently low temperatures. In general, cold-active enzymes have lower catalytic maximum temperatures (T_{optimum} , 20–45 °C) and higher specific activity at low temperatures. However, some cold-adapted enzymes have a T_{optimum} that is identical to their thermophilic counterparts [31, 32], signifying that their truly distinguishing feature is their ability to sustain extremely low temperatures [33].

Psychrophilic β -galactosidases offer several benefits, together with the capability to catalyze lactose breakdowns at retention temperatures, speed up the reaction, and lower the process' overall cost by saving energy. Along with lowering the danger of infection, they also increase the taste, solubility, sweetness, and digestibility of dairy products. Excellent examples of cold-adapted enzymes include the usage of β -galactosidase to remove lactose from chilled milk and pectinase to reduce viscosity and turbidity in chilled juices.

Laccase

A multi-copper oxidase found in bacteria, fungi, and plants called laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) belongs to the superfamily of multi-copper oxidases [34]. Laccase is widely distributed among wood-degrading fungi like *Trametes ochracea*, *T. versicolor*, *T. hirsute*, *T. gallica*, and *T. villosa* [35]. Although most laccases produced by wood-degrading fungi are extracellular glycoproteins, intracellular laccases have also been discovered. Lignin degradation, morphogenesis, and pathogenicity are fungal laccase processes [36]. *Azospirillum lipoferum*, a plant-root-associated bacterium, was the first to discover bacterial laccase [37]. Most bacterial laccases researched are intracellular, such as those identified in *A. lipoferum* and *B. subtilis*; extracellular bacterial laccases were also discovered in *S. cyaneus* [38]. The structure of all fungal laccases is the same, consisting of three sequentially organized copper domains. Small two-domain laccases have recently been discovered in bacteria, in addition to the conventional three-domain laccases.

Laccase activity and role of mediators

Laccase is an enzyme that only targets phenolic compounds. Laccases often contain three different forms of copper, one of which is responsible for its distinctive blue color. Yellow or white laccases are similar enzymes that lack the Cu atom that causes the blue hue; however other researchers do not consider them true laccases. Laccases also depend on Cu atoms dispersed at one of three types of copper centers: type-1, or blue copper center, type-2, or typical copper center, and type-3, or coupled binuclear copper center, which vary in their properties of electronic paramagnetic resonance (EPR) signals. The active site of the laccase transfers one electron from the organic substrate to a reactive radical, which then performs a non-enzymatic process. The electron is taken in and transported to the trinuclear group at type-1 Cu, where it reduces oxygen to water. It is believed that mononuclear copper type 1 functions as a major electron acceptor, removing electrons from the reducing substrate and transferring them to the trinuclear site, where oxygen gets converted to water and the oxidized form of the enzyme is regenerated [39].

Laccase's substrate range can be expanded to include non-phenolic subunits using mediators, also known as enhancers, because their use significantly improves laccase's catalytic activity. Since the identification of mediators with high redox potential, laccases have assumed a significantly greater role in a variety of biotechnological applications. ABTS (2, 2 [Azino-bis-(3-ethylbonzthiazoline-6-sulphonic acid) diammonium salt] and HOBt are two well-known mediators (1-hydroxy benzotriazole). A perfect mediator can go through several cycles without degrading or causing adverse effects. The non-phenolic substrate can be oxidized non-enzymatically by the oxidized mediator from the laccase-catalyzed process. The driving power of the reaction is due to the difference between the substrate's redox potential to be oxidized and the T-1 Cu center. Mediators are a group of low-molecular-weight compounds having a high redox potential. Laccases are crucial for commercial applications because they can catalyze the breakdown of phenolic and non-phenolic compounds as well as the conversion of molecular oxygen to water [35].

Alkaline phosphatase

Alkaline phosphatase (AP; EC 3.1.3.1) is a commonly used enzyme that, when present in alkaline environments, catalyzes the hydrolytic cleavage of phosphate monoesters, liberating inorganic phosphate from a variety of phosphate-containing materials [40]. Alkaline phosphatase is used in molecular biology for dephosphorylating plasmid DNA before cloning to prevent circularization, dephosphorylating 5'-nucleic acid termini before polynucleotide kinase 5'-end labeling, and removing pyrophosphate and dNTPs from polymerase chain reaction assays. A novel thermolabile alkaline phosphatase was isolated and characterized from metagenomes utilizing the Genetic Enzyme Screening System (GESS). The nucleotide phosphodiesterases psychrophilic *Sphingopyxis* sp. MC1 (85% amino acid sequence identity, GenBank accession no. WP 003042625) exhibits the highest level of sequence identity. The metagenomic alkaline phosphatase showed

strong similarity with the alkaline phosphatase superfamily. Additionally, it was discovered that metagenome-derived AP (mAP) outperformed Aps from Antarctic, prawn, calf intestine, and *Sinorhizobium meliloti* as well as that from *S. meliloti* and *E. coli*, in terms of catalytic efficiency (k_{cat}/K_m) and turnover number (k_{cat}) [41].

The enzyme was incubated for 15 minutes at a range of temperatures (20-80 °C) to determine its thermal stability. The psychrophilic mAP demonstrated exceptional thermal instability after 15 minutes of incubation at 65 °C, completely losing its enzymatic activity and improving catalytic effectiveness at low and moderate temperatures. Therefore, AP generated from psychrophiles with such characteristics might be very useful in biotechnology. New England Biolabs Inc. (Ipswich, Massachusetts, USA) sells heat-labile alkaline phosphatase under the trade name Antarctic phosphatase. A heat-labile alkaline phosphatase made by the Arctic prawn *Pandalus borealis* can be bought from Biotec Pharmacon ASA in Troms, Norway, or GE Healthcare Life Sciences in Little Chalfont, United Kingdom [40].

Uracil-DNA N-glycosylase

Psychozymes employed in molecular biology includes double-strand-specific DNase and uracil-DNA N-glycosylase (UNG). The release of uracil from DNA that contains uracil is catalyzed by UNG. In site-directed mutagenesis, RT-PCR, PCR amplification, and SNP genotyping, this enzyme helps to prevent carry-over contamination. However, the enzyme DNase, which was created specifically for double-stranded DNA, catalyzes the digestion of this DNA type without causing damage to single-stranded DNA molecules like primers and probes. This enzyme detoxifies PCR mixtures by eliminating genomic DNA from RNA preparations. It is therefore possible to use the psychrophilic counterparts of these enzymes to facilitate their selective and irreversible shutdown by heat, eliminating their effect in later stages [42].

DNA ligase

In order to create a phosphodiester connection between the hydroxyl group at the 3' end of one DNA strand and the 5' phosphate of the opposite DNA strand, an enzyme called DNA ligase, needs either adenosine triphosphate (ATP) or oxidized nicotinamide adenine diphosphate (NAD^+) as a cofactor. The backbone of sugar-phosphate remains unaltered by this process. Recently, a novel temperature-sensitive tularemia vaccine was developed using a cold-adapted DNA ligase's thermolability [43]. Low temperature helps bridge double-strand breaks with cohesive ends because it supports base pairing between brief regions of complementary nucleotides at the break site. While ligase activity is decreased at lower temperatures, DNA overhangs can base-pair and remain annealed long enough for the ligase to join them. Compared to their mesophilic counterparts, cold-adapted enzymes are more vulnerable to thermal denaturation and have higher catalytic efficiency (k_{cat}/K_m) at low and moderate temperatures [44]. Both features appear to result from a more flexible protein structure [45, 46].

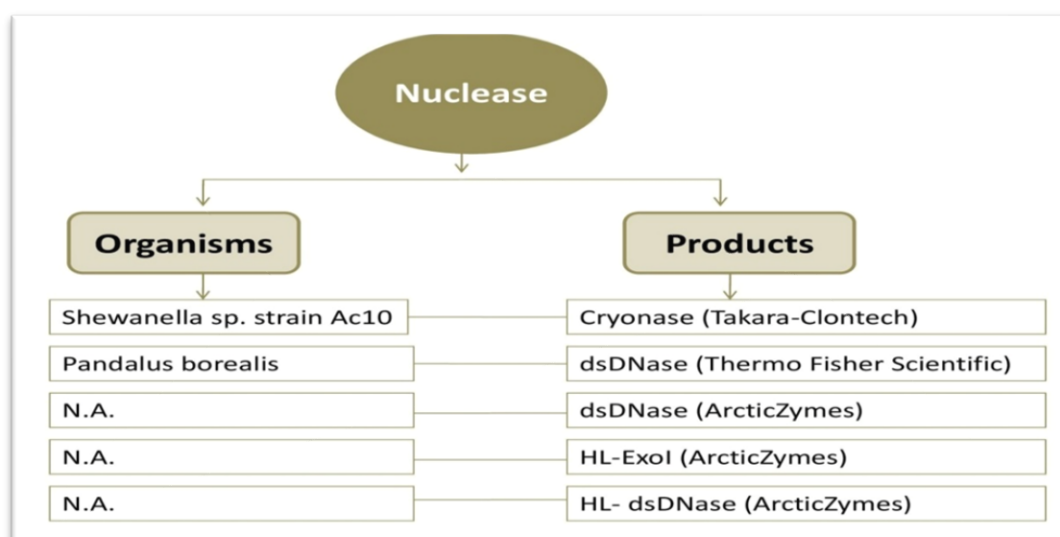


Figure 2. Nuclease cold-active enzymes that are commercially available for use in molecular biology.

Nuclease

Nucleases are a significant class of hydrolases that break down nucleic acids and have a wide range of uses in both research and business [47, 48]. An extremely significant molecular biology tool will be made possible by the synthesis and purifying of a nuclease from psychrophilic organisms. The discovery and characterization of the nuclease from the very psychrophilic bacterium *Psychromonas ingrahamii* can thrive at temperatures as low as 12 °C and grows exponentially at 5 °C. The putative endonuclease I gene was cloned and expressed in *Pichia pastoris* after being found in the *P. ingrahamii* genome. The pure recombinant protein's nucleolytic properties were investigated. There are currently three commercially available nucleases: Cryonase™ (Takara, Japan), Benzonase® (Merck), and HL-SAN (ArcticZymes). Cryonase™ was obtained from the psychrotrophic *Shewanella* sp. strain AC10, while Benzonase® was obtained from the mesophilic bacterium *Serratia marcescens* [33].

Nucleases (EC 3.1.21) PCR master mixes can be cleaned up with double-strand specific DNases, and RNA preparations can be cleaned up by removing genomic DNA. Another use is for ssDNA-specific enzymes, such as ExoI, to hydrolyze the target nucleic acid starting at its 3' end. The preference is for thermolabile nucleases because any subsequent stage in the technique requires the deactivation of nucleases. Figure 2 reports some instances of heat-labile nucleases.

PinNuc enzyme

The newly discovered enzyme, PinNuc, has characteristics of nonselective endonucleases. A variety of substrates that represented various nucleic acid shapes and kinds were used to assess the activity of the PinNuc enzyme after it was isolated from *Pichia pastoris* and homogeneously purified. It is extremely active at ambient temperature in a weak ion-strength solution even when magnesium ions are present in small amounts. A small amount of a reducing agent can effectively inactivate the enzyme, which has cysteine six residues, all of which are most likely involved in disulfide

bridges. The enzyme is only active in its oxidized state. The disclosed nuclease is anticipated to increase the variety of bioactive substances that may be used in cold environments and has a promising future for use in molecular biology and the biotechnology sector as an alternative to its already commercially available equivalents [49].

Xylanases

Xylanase and glycoside hydrolases also degrade hemicellulose, one of the main building blocks of plant cell walls, in addition to the polysaccharide beta 1,4-xylan. Industrial dough conditioners, which are employed to raise the quality of the bread, are largely made up of xylanases. Xylanases from an Antarctica bacterium were discovered to be a novel class of xylanases. By converting the dough's insoluble hemicellulose into soluble sugars before baking, xylanases in the process of making bread produce huge loaves of bread that are soft and elastic and have a fluffy but robust dough. This efficiency seems to be connected to the psychrophilic activity of xylanase during the cool temperature resting period required by the dough as well as to its particular mechanism of xylan hydrolysis [4].

A recent study found that when compared to mesophilic xylanases from *Aspergillus aculeatus* and *B. subtilis*, three psychrophilic xylanases from *Flavobacterium* sp. MSY-2, *P. haloplanktis* TAH3A, and one from an unidentified bacterial origin significantly improved dough qualities and final bread volume (up to 28%) [50]. It is said that this one has produced the most psychrophilic enzyme. New cold-adapted xylanases from a variety of organisms have been described recently [42].

Mannanase

Along with these usual detergent enzymes, the dishwashing/ laundry industry is actively searching to provide additional cold-active enzymes like mannanases. Mannan, often known as gum, is a common carbohydrate found in many foods and personal care items. Mannanase is used to break down mannan. In order to create smaller, more soluble carbohydrates, this enzyme hydrolyzes the 1, 4 links in mannose polymers. There aren't many

commercially available cold-active mannanases right now, however, Mannaway® (Novozymes) and Effectenz™ (DuPont) can both be employed at low temperatures (20 °C and 30 °C, respectively) [42].

Psychrophiles Are Used in Bioremediation

ZoBell (1946) recognized the ability of a wide range of microorganisms to use hydrocarbons as the only source of carbon and energy (biodegradation) [52], which served as the foundation for the creation of biological remediation techniques. An efficient technique from an ecological and financial standpoint, bioremediation aims to speed up the natural biodegradation rates by the optimization of limiting environmental conditions. Because of the psychrophilic microbial population's innate ability to degrade materials, organic pollutants in cold environments can be biodegraded at low temperatures.

They convert organic contaminants into less dangerous, non-hazardous compounds through alteration or mineralization, which are then incorporated into the planet's biogeochemical cycles. The majority of research on hydrocarbon biological remediation in cold climates has been on treating petroleum hydrocarbons since cold settings are more exposed to petroleum production, exploration, and transportation, which raises the potential of unintended oil spills. The evaluations of microbial growth in marine ice at temperatures approaching the freezing point of water and below -10 °C indicate that there is some hydrocarbon breakdown occurring in oil-contaminated ice [53]. A psychrophile of the same species was conjugated with a mesophile of the same species to transfer the TOL plasmid, which degraded toluene at temperatures as low as 0 °C. This process has been used to create psychrophiles with special degradative abilities for 20 years [54].

Recombinant Antarctic toluene-o-xylene monooxygenase (ToMO), which effectively converts several aromatic chemicals into their associated catechols over a wide temperature range, has been reported to be expressed by *P. haloplanktis*. The use of these modified Antarctic bacteria has been suggested for the biological restoration of chemically harmed cold effluents/ marine environments [8]. The research was conducted for examining the genetics, physiology, and ecology of bacteria that degrade in cold environments and to use this information to decontaminate the polluted area.

Effective Use of Anti-Freeze Proteins

One of the protective mechanisms created by organisms that thrive in cold environments is called AFPs (Anti-freezing Proteins). As was already noted, marine psychrophiles produce large amounts of AFPs to adjust to temperature stress and avoid the production of ice. Therefore, for AFPs activity, *P. fluorescens*, *P. putida*, *Marinomonas protea*, and a type of *Moraxella* were reported as numbers of cold-adapted bacteria. According to studies on AFPs, they will be helpful in cryosurgery and tissue and cell preservation. Additionally, AFPs can be utilized in the food sector to enhance the quality of frozen food and preserve taste and texture. In order to avoid the re-crystallization of ice during thawing and freezing and to retain ice slurry fluidity,

microbial degradation of frozen food can be reduced or prevented. Additionally, fish with direct AFP injections had improved cold tolerance [29].

Pharmaceuticals and Medical Uses of Psychrozymes

A considerable portion of bacteria suited to living in sea ice may produce PUFA, such as eicosapentaenoic acid and docosahexaenoic acid, according to research on psychrophilic bacteria from the Antarctic genera *Colwellia* and *Shewanella*. The conventional source of long-chain polyunsaturated fatty acids (LPUFA) provides a variety of health benefits, including the ability to prevent and treat diseases including high blood pressure, diabetes, and atherosclerosis. However, getting LPUFA from the oil of fish has drawbacks that outweigh the benefits of mass production and purification. Additionally, it is expected that commercial fish stocks would dwindle in the future. Algal-derived oils require a relatively high investment in terms of both technology and expense compared to the possibility of bacterial fermentation, despite bacteria having fewer lipids than algae. In the cell membrane of bacteria, PUFAs are fatty acids that are a component of particular phospholipids. In general, long-chain PUFAs consist of a long chain of carbon atoms (commonly C20-C22) with a variety of (usually 4 to 6) methylene-interrupted double bonds. One major benefit of producing PUFAs by bacteria is that only one PUFA is created, as opposed to the complicated mixture produced by algal oils or fish [29].

Biocatalyst

Psychrophilic enzymes' catabolic activity is being examined today and needs to be optimized to achieve the desired result [46]. The key characteristics that give psychrozymes their three main advantages in biotechnology are:

1. Since fewer enzymes are needed to achieve the activation energy requirement, they are economically advantageous.
2. Without extra heat assistance, psychrozymes are effective.
3. They can be selectively inactivated with low heat input thanks to their thermal lability.
4. The indigenous psychrophiles can be a valuable new supply of often-needed catalysts, such as xylanase from the bacterium *Pseudoalteromonas haloplanktis* or lipases from the yeast *Candida antarctica* [55].

Additionally, a variety of commercially valuable peptides, oligosaccharide derivatives, fatty acid esters, and other chemicals are produced from substrates with poor aqueous solubility, which may be improved by adding enzymes that require less water. Further research has shown that restricting water activities is the most effective way to control the hydration level. In this situation, a conflict occurs since decreased hydration typically results in decreased enzymatic efficacy, which is directly related to reaction kinetics. Because they are more adaptable and tolerant than mesophilic and thermophilic enzymes, psychrozymes may consequently have advantages in less watery environments. This will make it possible to use less water, which will increase yield [56].

Biofuels

As the world's population rises, demand for alternative energy sources is also rising. Psychrophiles have been successfully used to increase the efficiency of biogas digesters, which generate cooking and heating gas for Alaskan households. Warm environments are the only ones where methanogens that produce biogas can thrive. Psychrophilic methanogens have been identified to increase the biogas coming from biogas digesters in cold regions. Cold-adapted glycosyl hydrolases such as cellulases, xylanases, and glucosidase may be able to efficiently convert lignocellulosic biomass, which could lead to the creation of a fuel that is both commercially feasible and renewable to address the world's rising energy needs [57].

Commercially Available Psychrozymes

Scientific and industry efforts to find and produce novel psychrozymes have expanded significantly in recent years. As previously stated, these enzymes' intrinsic properties make them ideal for various biotechnological applications in multiple sectors. As a result, psychrozymes replace mesophilic enzymes in many industrial processes [42]. However, most of these enzymes have undergone genetic modification to increase their biotechnological potential.

Cryonase™ Cold-active Nuclease

Cryonase, cold-active endonuclease, is isolated from the psychrophile *Shewanella* sp. and isolated from, as well as expressed in, recombinant *E. coli*. Single-stranded, double-stranded, linear, and circularised DNA and RNA substrates can all be digested by *E. coli* at lower temperatures. This enzyme helps break down nucleic acids in samples that contain proteins or other heat-unstable materials since it can function even on ice. Based on the findings of a joint investigation between TaKaRa Bio and Professor Nobuyoshi Ezaki, et al. of Kyoto University's Institute for Chemical Research on a genetic analysis of *Shewanella* sp., this product was created and provided by Professor Ezaki's team is the Ac10 strain [58].

Subtilisin-like proteases or subtilases

Although findings are supporting the genesis of other organisms as well, subtilisin-like serine proteases are typically bacterial [59]. Typically, they are released extracellularly to scavenge resources. Tyrosine, phenylalanine, and leucine are examples of the aromatic or hydrophobic residues that this class of proteases prefers (at position P1). They are extremely sensitive to diisopropyl-fluorophosphate, sulphonyl fluoride, phenyl and methyl, and potato inhibitor. They can be distinguished from other serine proteases, such as chymotrypsin, carboxypeptidase, and peptidase A from *E. coli*, by their amino acid composition and three-dimensional structure. Serine, histidine, and aspartic acid make up the catalytic triad of subtilisins. Although subtilisins can range in size from 18 kDa to 90 kDa, all subtilisins utilized in detergents are roughly 27 kDa in size. The great stability and relatively limited substrate specificity of subtilisins—a feature typical of extracellular proteases—are two bases on which their effectiveness is predicated.

Several *Bacillus* species, including *B. amyloliquefaciens*, *B. licheniformis*, and *B. subtilis*, are highly represented in this family of proteases [60]. *Flavobacterium* also produces it. subtilisin BPN' from *B. amyloliquefaciens* was the subject of the first publication on DNA sequence determination. Subsequently, nucleotide sequences of subtilisins and other alkaline proteases were found, including subtilisin E from *B. subtilis*, subtilisin Carlsberg from *B. licheniformis*, subtilisin amylosacchariticus from *B. subtilis* var. *amylosacchariticus*. In computational molecular biology, these analyses of DNA and protein sequence similarity are commonplace since they are required for some reasons [60].

Novozymes Savinase®

Savinase® is a serine-type endoprotease that removes stains completely and is frequently used in liquid and powder laundry formulations. A powerful protease is known as Savinase® is used to dissolve soils that include proteins, including dairy products, chocolate, pork, eggs, grass, blood, and gravy. Savinase® should be kept chilled, between 3 and 5 °C, avoiding freezing. Its substrate specificity is fairly wide. In a nutshell, it can hydrolyze the majority of peptide bonds found within proteins. The resulting peptides and amino acids disintegrate or disperse in the washing water. Savinase® is active from pH 8 to 12 (pH 10 is ideal), which is the pH range of importance for the majority of detergent applications. The range from 20 - 60 °C temperature is where savinase is most effective at hydrolyzing peptide bonds. Savinase uses in dishwashing products and washing powders made to remove protein-based stains and residues (blood, food, and feces) because of its chemical capabilities that break down proteins. 55 °C is the ideal temperature for it. Protease inhibitors are present in some foods, including potatoes and eggs, which may prevent Savinase® from working properly [61, 62].

Furthermore, because savinase is a protein molecule, it may become inactive or denatured under specific circumstances, such as an unfavorable pH or temperature, which would result in a reduction in activity. Savinase, specifically, possesses an irreversible thermo-inactivation at 70 °C, which appears to cause the enzyme to digest itself. Savinase® is a high-quality, extremely broad-spectrum endo-protease that offers deep hydrolysis and is used for the extraction of animal proteins. It is a product of a technical caliber. An ACE-inhibitory lentil protein hydrolysate of Savinase (*Bacillus* sp. alkaline serine protease) yielded several peptide sequences [63, 64].

Alcalase®

A particular strain of *B. licheniformis* was used to create alcalase, a serine endopeptidase, using submerged fermentation, which sheds light on the well-known catalytic structure of the traditional catalytic amino acid triad. It is a single-chain polypeptide with a molecular weight of 27.3 kDa and contains 275 amino acid residues. The primary enzyme in it is subtilisin [65]. Store it in the refrigerator between 3 and 5 °C. Never freeze. Its substrate specificity is fairly wide. To put it another way, it can hydrolyze the majority of peptide bonds found within a protein molecule. There occurs in the formation of peptides and amino acids, which are either dissolved or disseminated in the washing water. From pH 6.5 and 8.5, it is functional. It operates

between 45 and 65 °C, peaking at about 60 °C, after which its activity rapidly decreases. Protease inhibitors, which may prevent its activity, are present in some foods, including blood, potatoes, and eggs. Compared to certain other proteases, it is kinder to silk and wool proteins.

Broad specificity characterizes this intricate enzymatic preparation. Leu, Tyr, and Val are the most common hydrophobic residues on the carboxylic side of peptide bonds that are broken. While maintaining their nutritional value, hydrolytic enzymes can improve the functional qualities of protein meals. Due to alcalase's great tolerance for alkaline pH levels, the detergent industry has employed it extensively. Numerous studies also describe its application for creating increased proteic meals. Enzymatic hydrolysis of proteins can enhance the physical, chemical, and organoleptic qualities of the original food if it is done correctly. Alcalase® is a detergent ingredient that helps in removing protein-derived stains including blood, grass, mucus, excrement, and other eggs or gravy-like foods. It was the first alkaline protease utilized in 1960s biological washing powders, and it is being utilized today. The food and beverage sectors both use alcalase extensively. Since it is simple to understand changes in the alcalase structure and activity, it is frequently utilized in the creation of bioactive peptides, cheese flavor development, and meat tenderization [65, 66].

The product is suitable for individuals with dysphagia because alcalase accelerated proteolysis, which softened surimi gel and boosted antioxidant activity. The hydrolyzate of the citric acid deamidation-alcalase breakdown of wheat gluten masks the initially bitter flavor of the grain by having a high concentration of umami-flavored amino acids (glutamic acid and aspartic acid). Furthermore, the ability of a specific enzyme to bind IgE was significantly reduced by the discovery of the hydrolyzed product of alkaline protease, a successful technique for generating hypoallergenic wheat products. Alcalase is a protease with wide specificity, and in production applications, the enzyme activity and the appropriate specific enzyme cutting sites are the major factors affecting product quality. Additionally, functional fish protein hydrolysate (FPH), one of the greatest enzymes that have been widely employed by numerous studies, is made with the help of the alcalase enzyme [67].

Studying Putative Marine Psychrophilic Enzymes

There is growing interest in psychrophilic enzymes due to the elevated activity of these cold-adapted enzymes at low as well as moderate temperatures. As an illustration, regular water can be used in place of 37°C while "peeling" leather using cold adapt protease. These enzymes are quickly, effectively, and selectively inactivated in complicated combinations thanks to their heat-lability. It demonstrated that a mesophilic homolog may be made to adapt to cold temperatures with relatively slight structural changes. According to the estimate, forty-three businesses are engaged in arctic region-sourced product research and development as well as sales. These businesses' operations can be divided into nine general specialization areas. Furthermore, 31 patents or filings for patents related to technologies derived from or produced by Arctic genetic

resources have been discovered in databases in the US and Europe [68]. According to the information, 34% of filings for patents or the patents listed from enzymes with life science research applications in DNA research, 23% of filings for patents or the patents listed in the pharmaceutical and medical categories, 10% of filings for patents or the patents listed within the groups of cosmetics, nutraceuticals, skin care, dietary supplements and other products involving food technology or aquaculture in case of animals.

Cellular Mechanism of Cold Adaptation

Low temperatures can impede transcription and translation because they make random secondary transcript structures more stable. RNA chaperones can disassemble or stop hostile secondary RNA constructions. According to Jones and Inouye, cold shock proteins (CSPs) are tiny proteins that bind to RNA to maintain its single-stranded structure [69]. Some psychrophiles have higher levels of DEAD-box RNA helicases, which can unfold extra structures in an ATP-dependent manner [70]. The amount of CSP genes in the genomes of psychrophiles varies greatly.

CSPs are RNA chaperones, but they also play other roles. Certain proteins that include CSD can regulate the cold shock reflex or greatly affect mesophile development in subfreezing temperatures [71]. Because they are consistently rather than transitorily produced at low temperatures, many Csp function as cold-adaptive proteins in psychrophiles [3]. *E. coli* exhibits increased cold tolerance at low temperatures when *cspA* from *Psychromonas arctica* is overexpressed. *Shewanella oneidensis* tends to develop best at low temperatures when one of the three CSPs is active [72]. There are not always known CSPs homologs in bacteria and archaea that can survive in cold temperatures.

Williams et al. (2010, 2011) postulated that small proteins of *M. burtonii*, which contain a single RNA-binding TRAM domain, function as RNA chaperones similarly to Csp by upregulating at low temperatures [73, 74]. For these hypothetical RNA chaperones, Ctr proteins have been designated, which are unique to a subgroup of archaea. There has been speculation that Ctr proteins, which are abundant in *M. burtonii* at very low growth temperatures (2 °C), may aid cell function under conditions of cold stress [73, 74].

Additional proof in favor of a larger role for Ctr proteins in the cell as stress response proteins comes from the overexpression of Ctr proteins in *M. burtonii* when subjected to growth in the presence of solvent methanol [73]. According to Maruyama et al. (1999), small RNA-binding proteins, like Csp, also serve additional beneficial functions in the cell [75]. Rbps are small proteins that include just one glycine-rich RNA binding motif and can assist cells in adjusting to the cold. According to Maruyama et al. (1999), other bacteria don't often have them, but cyanobacteria do [75].

Anabaena sp. PCC 7120 exhibits increased *rbp* gene expression for osmotic stress. Because they both reduce the amount of free water available, the reactions of cold and osmotic stressors overlap. Rbp proteins may also be involved in psychrophilic cyanobacteria's ability to adapt to

temperature changes, as evidenced by the Antarctic strain *Oscillatoria* sp. SU1's increased production of *rbp* genes in response to low temperatures [76]. tRNA stability may be impacted by nucleoside changes. Because of this, hyperthermophilic archaea and bacteria typically have a high degree of modification.

Interestingly, some psychrophilic bacteria and archaea have higher levels of dihydrouridine, which can improve tRNA flexibility. Several, psychrophilic archaea and bacteria up-regulate their production during low-temperature growth, like proteases and RNases from the permafrost bacteria *M. burtonii* and *Psychrobacter arcticus*. These enzymes are responsible for breaking down proteins and RNA. Although both interpretations are not mutually exclusive, it has also been characterized as a technique for protecting precursors from biosynthesis [77] or as a better monitoring of irreversibly degraded proteins and RNA.

In response to low-temperature growth, it is possible to regulate energy conservation and biosynthetic pathways. The Siberian permafrost eurypsychrophile *Psychrobacter cryohalolentis* rises the cytoplasmic reserve of ATP and ADP to compensate for lower ATP-dependent reaction rates. In *M. burtonii*, distinct carbon substrate utilization pathways are variably regulated with growth temperature (e.g., methanol versus trimethylamine). While *P. cryohalolentis* exhibits overexpression of glyoxylate cycle enzymes [78], *P. arcticus* exhibits downregulation of a huge volume of energy metabolism genes at cold temperatures [77].

These illustrations show how different and intricate each psychrophile's metabolic responses are. Cells are susceptible to additional stresses at temperatures low enough for ice to develop, including ice destruction, osmotic disequilibrium, and oxidative damage. Ice-induced mechanical damage to the cell membrane can be prevented by extracellular polymeric substances (EPS). *Colwellia psychrerythraea*, among other sea-ice bacteria, produces polysaccharide-rich EPS.

Psychrophilic archaea like *M. Burtonii* and *H. lacusprofundi* produces EPS aggregates in multicellular at lower temperatures. At lower temperatures, the reduction in the permeability and fluidity of membranes occurs. As a result, the cell membrane loses its ability to operate biologically, including its ability to transport molecules, and is converted from an elastic liquid crystalline to a gel phase. A more loosely packed array is produced as a result of growth in the percentage of unsaturated fatty acids in the lipid bilayer [79]. By producing less saturated fatty acids from scratch or by declining already-existing fatty acids saturation, it is possible to increase the amount of unsaturated fatty acids.

Exiguobacterium sibiricum, an eurypsychrophilic bacterium, expresses more fatty acid desaturase genes at lower temperatures. Fewer saturated isoprenoid lipid precursors are produced by *M. burtonii*, which is without a fatty acid desaturase, due to altered expression of many lipid biosynthesis genes. *H. lacusprofundi* has also been found to have unsaturated isoprenoid lipids. Numerous Gammaproteobacteria species that are psychrophilic, such as the *Marinomonas*, *Photobacterium*, *Moritella*,

Colwellia, *Shewanella*, and *Psychromonas* species, are distinguished by having an inclined percentage of unsaturated fatty acids in their cell membranes. A microbial grouping in glacial ice was discovered to have considerably elevated levels of genes related to the preservation of membrane fluidity in a metagenomic investigation [80]. Cellular response to the cold seems to generally conserve membrane lipid modifications.

Psychrophile Adaptation Revealed by Genomes and Global Gene Expression Profiles

Studies using psychrophile genetic sequences have greatly advanced our knowledge of adaptive processes. For psychrophiles, there are about 30 bacterial genome sequences and 4 archaeal genome sequences that have been collected from a diverse of cold samples, such as sediment, sea sponges, Antarctic lakes, permafrost, fish, and marshes [81].

Genomes also serve as the foundation for specialized and comprehensive functional research (such as proteomics and transcriptomics), which explains the capabilities of psychrophiles. Understanding adaptive processes is being obtained much more quickly because of the ability to see global responses, especially as researchers distinguish between the general qualities of psychrophilic bacteria and the unique traits of individual psychrophiles. Recent assessments of expression patterns across various growth temperatures serve as excellent examples of what can be determined by these methods. Transcriptomics was used to examine *P. arcticus* and multiplex proteomics was used to analyze *M. burtonii* to quantify changes over seven growth temperatures. Researchers were able to distinguish between stressful and non-stressful physiological conditions in the latter trial by adding growing extremes of temperature as well as temperatures in between [74].

It's interesting to note that both the temperature (up/ low) extremes up-regulated the expression of oxidative stress proteins, highlighting the vital yet diverse paths in which temp-causing oxidative stress appears in the cell. It also showed that the protein depicts at the temperature where *M. burtonii* grew the quickest and the temperature where growth was at its highest (T_{max}) were similar [82].

Reaction Mechanism of Cold-Adapted Enzymes

Chemical reactions proceed at extremely slow rates in lower-temperature environments because their kinetic energy isn't enough to get past the hurdles that prevent enzyme activation. A drop in temperature from 37 to 0 °C causes an enzyme's activity to decrease 20 to 80 times for a biochemical reaction that is taking place in a mesophile at 37 °C, stopping low temperatures from blooming the most. Fortunately, low-temperature-adapted organisms have evolved several methods to overcome this limitation, including the energy-intensive approach of increased release of enzymes and cyclical expression of isoenzymes [46].

The reaction rate (k_{cat}) of cold-active enzymes, which is frequently adaptive, is mainly temperature independent. For instance, increasing the activation energy for a psychrophilic α -amylase from 35 to 70 kJ mol⁻¹ for a

thermophilic α -amylase increased k_{cat} by 21 folds at 10 °C [83]. The active sites of cold-active enzymes are typically bigger and easier for substrates to reach, which promotes substrate binding at a low energy cost. Therefore, compared to their thermophilic counterparts, substrates for cold-active enzymes typically have lower binding affinities.

According to an activity-stability balance, cold-active enzymes typically have flexible structures and low stability as a result of this low stability at low temperatures. According to Siddiqui et al. (2005) and Feller (2008), many cold-active enzymes have a catalytic region that is more labile and flexible than the remaining protein structure known as localized flexibility [4, 84]. Accordingly, cold-active enzymes rely on an increased disorder to maintain molecular dynamics and, thus, function in a setting characterized by slow molecular motion and kinetic energy.

For instance, a hydrophilic region was found at a surface loop encircling the active site of a psychrophilic alanine racemase with exceptionally poor thermal stability and high low-temperature activity. Both the polar and hydrophilic regions of the enzyme's surface are expected to promote solvent interactions, which will reduce the enzyme's compactness, according to Okubo (1999) and Siddiqui and Cavicchioli (2006). The *P. haloplanktis* amylase, AHA, has developed into a model to investigate the relationship between form, stability, and activity in cold-adapted enzymes [85, 86].

Overall, the findings demonstrate that the structure of AHA grew to have few charges, allowing for adequate flexibility in conformation to permit action at cold temperatures while preserving an adequate degree of protein integrity as a whole. When the genomes of psychrophilic archaea were sequenced, it was discovered that their proteins had higher concentrations of hydrophobic residues, uncharged polar amino acids, lower concentrations of hydrophobic amino acids, and decreased charge that is linked with destabilizing the surface of psychrophilic proteins. Such adaptability was made possible by the selection of amino acid consumption during evolution. According to genome studies of marine Gammaproteobacteria, cold-adapted strains exhibit higher concentrations of Ile, Lys, and Asn than non-cold-adapted strains and lower concentrations of Ala, Arg, and Pro [87]. Two of them, Pro and Arg, are known to have the ability to increase stability by preventing structural rotations and, respectively, by forming many salt bridges and hydrogen bonds.

Psychophilic proteins have lesser and declined disulfide bridges, metal-binding locations, electrostatic interactions (H-bonds, salt bridged), a decreased ratio of lysine/arginine, lessened inter-subunit and inter-domain connections, higher and deeper repeats, less 2 \cdot structure, high residues of glycine, lesser prolines in loops and increased prolines in helices [88]. Some of these adaptable 5-turn and strand-2 \cdot configurations, as well as long gaps that are largely lined with acidic residues to create room for water molecules, are also frequently seen in cold-adapted proteins [88]. Even though psychrophilic proteins have the aforementioned structural characteristics, each protein will only have a small subset of these characteristics and a particular context for them [86].

Conclusion and Future Prospects

The creation of novel psychrozymes with specialized functionalities is required due to the rising demand for industrial enzymes. There appears to be a lack of fundamental knowledge about these psychrozymes, including their types, enzyme activity, extraction techniques, optimal circumstances for development for enhanced enzyme synthesis, and characterization. As a result, it is critical to focus research on this vast, unknown biodiversity to comprehend its functional potential better.

Furthermore, modern molecular tools such as genome sequencing and thorough proteome investigations of these enzymes would help researchers to better understand their biochemistry, the link between structure and function, and molecular biology. This type of research would advance our knowledge of psychrozymes. They would help categorize and understand their environmentally friendly role in the cold environment by adding details regarding their inducibility, secretions, types, and mode of action to the current information repository.

Enzymes like these are resilient and able to survive extreme circumstances, but genetic engineering or chemical mutagenesis can enhance their selectivity, stability, activity, half-life, and other characteristics to make them more suitable for particular industrial uses. Potential biotechnological opportunities have been shown by the development of technology for commercializing such enzymes. It can improve cost-effectiveness, yield, productivity, enzyme kinetics, and a variety of safety aspects. Because of the scarcity of information and the potential for numerous biotechnological achievements with the help of psychrozymes, they represent a path that must be studied and utilized for future industrial applications that benefit humanity.

Declarations

Author Contribution: VG and PB contributed to conceiving the presented idea for the article, performed the literature search, analyzed the data, and wrote the original draft; VG drew the figures; JT and SB performed the literature search and wrote the manuscript; AS and RT provided critical feedback and helped shape the final draft for submission.

Funding: There is no funding source involved in conducting this study.

Conflict of Interest: The authors declare no conflict of interest.

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