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Review Article

Adaptations of Psychrophilic Microorganism to Low-Temperature Environments

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Abstract: Earth's surface has varied environmental conditions. The cold climate can be a rich source of cold-friendly microorganisms known as psychrophiles. They play an important role in global biogeochemical cycles. However, continuous survival at low temperatures is generally considered inhumane to life but is very good for psychrophilic organisms to survive in these environments. Biochemical and physical management attributed to its innate adaptive ability to withstand the cold and the stresses associated with it. This review focuses on biochemical and physiological adaptations that use psychrophilic microorganisms under adverse conditions.

***Keywords*:** psychrophiles, cold adaptation, microorganism, environments

1. Introduction

Living organisms become sensitive to drastic changes in their environments. Extreme conditions/situations of temperature, pressure, drought, salinity, and pH disrupt the crucial interactions that keep biological molecules folded and serviceable, thereby speedily destroying cellular integrity [1, 2]. The biotechnological research conducted within Antarctica's confines illuminates the potential solutions these endeavors hold for contemporary environmental dilemmas. The Antarctic continent stands as a pristine realm, untouched by human habitation, distinguished by its extreme conditions-extreme cold, aridity, and sparse population. Remarkably, it harbors approximately 70% of the world's fresh water in its frozen expanse. Over millennia, its inhabitants, though few and limited in species diversity have adapted through natural selection. This unique ecosystem serves as an unparalleled laboratory for scientific inquiry, spanning the continent's entirety. Within this isolated expanse, a myriad of species, including specialized fauna, algae, lichen, and microorganisms, thrive. Scientists leverage this rich biodiversity to confront pressing global challenges, such as climate change and environmental degradation. Through meticulous investigation, utilizing species, enzymes, and genes endemic to Antarctica, researchers delve into various realms of biotechnology, including bioremediation and biological control [3]. Therefore, many scientists have tended to assume that there are so many strict boundaries to the biosphere imposed by terrestrial life demands for a rather narrow and specific range of environmental conditions. In the past few decades, discoveries have shown that life is not always as sensitive as we might have assumed and that the limits of life on Earth are very far [4-6].

Microbial diversity profoundly influences global processes essential for life due to their omnipresence and

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decomposing capabilities. Microorganism distribution in soil is affected by temperature, water saturation, oxygen levels [5, 6], and nutrient availability. As we understand microbial diversity better, conserving these microbes and their gene pools becomes crucial. Over 75% of Earth's biosphere experiences cold stress, which is consistently below 5 °C [7, 8]. This stress presents challenges, particularly in higher altitudes, where low oxygen levels, temperatures, aridity, intense Ultraviolet (UV) radiation, and soil deficiencies create a hostile environment for many life forms, including plants [9-11]. Cold-adapted microbes thrive in these harsh environments despite frozen and inhospitable conditions, suggesting that complex microbial communities inhabit cold climates [7]. Cold stress significantly alters the chemical and physical characteristics of living cells. Studying altitudinal gradients and microbial dynamics helps us understand how these organisms survive at higher elevations [12]. Historical views on adverse environments have been challenged by microorganisms that not only tolerate but often require extreme conditions for survival [13]. The psychrophilic isolates, from Deception Island, noting their resilience in adverse conditions that extremophilic cells remain viable due to natural forces such as temperature fluctuations, radiation, and pressure differentials, which stimulate growth and adaptation [14]. Geochemical extremes, including desiccation, salinity fluctuations, pH variations, and redox potential shifts, also shape the survival and development of these organisms [15, 16]. Studies on the photosynthesis pathway of Ferredoxins (Fds) enzymes from *Chlamydomonas sp*. UWO241 (unicellular green alga) isolated 17 m below the surface of the permanently ice-covered Lake Bonney in Antarctica and concluded that cold-adapted provides an advantage for life at low temperatures, likewise *Cyanobacteria* are the dominant photosynthetic prokaryotes of the polar regions contain high levels of *Canthaxanthin*, *myxoxanthophyll*, and other carotenoids with the ratio carotenoids/chlorophyll a help to maintain high catbolic rate at low temperature [13]. There have been also a number of metabolic pathways based upon methane, sulfur, and even iron [17] may be a key mechanism that helps a microorganism to exist at low temperatures.

Therefore, these findings indicate that microbial adaptation at metabolic pathways is coupled with the extraordinary physiological capacities of many microorganisms to survive in extreme environmental conditions [4, 18]. Recent advancements in space technologies enable the study of terrestrial organisms' adaptations and survival under new categories of extreme conditions in space, such as simulations of meteoritic impacts or space conditions. Exploring the diversity of microorganisms and understanding their adaptive mechanisms allows for the development of hypotheses regarding the conditions necessary for the origin and early diversification of life on Earth [19, 20]. Microorganisms can inhabit diverse environments, from depths of 6.7 km inside the Earth to more than 10 km deep in the ocean at pressures up to 110 MPa, and from hydrothermal vents at 122 °C to -20 °C in frozen seawater (4). Among various physical extremes, temperature stands out as a critical factor for bacterial survival and growth, with cold environments being prevalent on Earth. Significant insights have been gained from studying bacterial adaptations to low-temperature conditions [21].

1.1 *Microorganisms in extreme cold environmental condition (psychrophiles)*

Psychrotrophic bacteria pose a significant challenge to the dairy industry due to their ability to persist despite pasteurization, primarily due to inadequate sanitation practices. Psychrotrophs, capable of thriving at temperatures of 7 °C or lower, can continue to grow in refrigerated milk, though their growth significantly slows or stops at freezing temperatures. Psychrophiles, on the other hand, are organisms adapted to thrive in very low-temperature environments, capable of growth at 0 °C or below, with an optimal range typically between 15 °C and 20 °C. In the identification of cold-adapted bacteria, blast tools were employed, using *Moritellaprofunda* (Taxoid: 111291) as the queried domain, followed by analysis with Pfam. This analysis encompassed a total of 7,782 species, of which 7,034 were bacteria and few name listed in Table 1 and Figure 1. Initially studied in deep-sea environments where temperatures hover around 3 °C, psychrophiles and psychrotolerant bacteria were isolated from deep-sea mud samples, demonstrating their adaptation to cold conditions. Many psychrophiles also exhibit halophilic traits, enabling survival in cold and saline environments by lowering the freezing point of water. For example, *Psychrobacter cryopegella* can thrive at temperatures as low as -10 °C and maintain metabolic activity at -20 °C. These microorganisms play crucial roles in global ecology, particularly in environments below 5 °C, influencing food spoilage and offering potential for biotechnological applications in low-temperature conditions [4, 22-25].

Table 1. List of the various microbes adapted to cold environments

Microbes	Reference
Gram-negative *α*-, *β*- and *γ*-proteobacteria (*Pseudomonas* spp. and *Vibrio* spp.) and the *Cytophaga-Flavobacterium-Bacteriodes* phylum	D'Amico et al. [31]
Methanogeniumfrigidum and *Methanococcoidesburtonii*	Saunders et al. [110]
Cyanobacteria, *Oscillatoria, Phormidium* and *Nostoc commune*	Pandey et al. [111]
Psychrophilic yeasts, particularly *Cryptococcus* spp.	D'Amico et al. [31]
Bacillus psychrosaccharolyticus	Seo et al. [112]
Arthrobacterglobiformis	Berger et al. [113]
Shewanella sp. Ac10, *Escherichia coli*	Yoshimune et al. [114]
Moritellaprofunda sp. nov. and *Moritellaabyssi* sp. nov.	Xu Yet al. [115]
Shewanella sp. SIB1	Suzuki et al. [116]
Pseudoalteromonashaloplanktis	Tosco. [117]
Desulfotaleapsychrophila	Rabus. [118]
Algae: *Chlamydomonasnivalis* (Common), *Cynobacteria: Oscillatorian cyanobacteria*(Prokaryotic photosynthetic microorganism)	Rabus. [118]
Psychrophilic Bacteria/fungi identified by National Centre for Biotechnology Information (NCBI)	
Nonlabens sp. Ci31	Taxonomy ID: 2608253
Eutreptiella pomquetensis	Taxonomy ID: 215699
Chlamydomonas sp. ICE-L	Taxonomy ID: 309537
Fragilarisa sp. HGC-2007-2	
Chlamydomonas sp.CCMPS 681	Taxonomy ID: 2812568
Chlamydomonas sp. CCMP 1619	Taxonomy ID: 2812568
Grammonema striatula	Taxonomy ID: 33645
Pseudoalteromonas raudensis	-
Navicula sp	Taxonomy ID: 1923966
Nitzschila sp.	-
Porosira sp.	Taxonomy ID: 2315415
Chaetoceros sp	Taxonomy ID: 49240
Penicillium sp CMV 2018	Taxonomy ID: 5081

The molecular mechanisms of psychrophiles, the adaptation of organisms to cold environments, are intricately tied to biochemical processes [26]. Psychrophiles and psychrotrophs exhibit lower growth temperature limits influenced by the freezing properties of aqueous solutions inside and outside cells. Adaptive changes in cellular proteins and lipids enable growth at low temperatures while restricting growth at moderate temperatures. Genotypic changes in proteins affect enzyme and translation system characteristics crucial for cold adaptation, while lipid modifications regulate membrane fluidity and permeability, potentially distinguishing psychrophiles from psychrotrophic. The upper growth temperature limit is determined by the inactivation of specific enzymes or systems essential for protein synthesis and energy generation. Psychrophilic enzymes, such as *α*-amylases, proteases, lipases, DNA polymerases, and cellulases, play pivotal roles in cold environments by facilitating starch hydrolysis, protein degradation, lipid metabolism, DNA replication and repair, and cellulose degradation, respectively [27-30]. These enzymes are vital for the adaptation and survival of organisms in cold habitats, ensuring the execution of essential metabolic processes despite the challenges posed by low temperatures.

The advent of genome sequencing has revolutionized our understanding of psychrophile biology, revealing insights into their adaptations to cold environments. Reviews by D'Amico et al. [31] and Bowman, [32] have highlighted significant findings, including the sequencing of three complete genomes of psychrophilic bacteria and draft genomes of cold-adapted Archaea. Next-generation sequencing (NGS) technologies have expanded this knowledge base, with

the GOLD database listing 83 complete or permanent draft genomes and 102 targeted or incomplete genomes of psychrophiles, encompassing bacteria, archaea, and eukaryotes. A majority of these organisms (43.4%) originate from marine environments, particularly around Antarctica's Pacific and Southern Oceans. Psychrophiles undergo permanent adaptations to cope with the harsh conditions of low temperatures, where enzyme activity is affected, and high solute concentrations can become potentially toxic. Comparative genomic studies between psychrophilic and mesophilic organisms highlight specific genetic features enabling cold adaptation. For instance, comparisons between cold-adapted and mesophilic *Alteromonas* sp strains reveal 15 unique genetic regions in SN2, aiding its survival in cold marine tidal flats genetic regions in the cold-adapted variants, enhancing their survival in cold marine environments. Similar studies with Antarctic *Halobacterium* and *Halorubrum* species demonstrate genetic adaptations related to gas vesicles, light-harvesting proteins, and biochemical changes crucial for thriving in extreme cold. These insights underscore the physiological and biochemical strategies that enable psychrophiles to endure and flourish in challenging cold environments [31-33].

2. Biochemical adaptation of cold-adapted microorganism (psychrophilic)

The biochemical adaptation of psychrophiles, or cold-adapted microorganisms, is essential for their survival in cold environments. Unlike higher organisms, microbes cannot insulate themselves or move to avoid freezing [34, 35]. Instead, they rely on biochemical strategies to maintain functionality at low temperatures. Microbes have several advantages: they are small, grow rapidly, and can proliferate in large quantities [36]. Under extremely cold conditions, microorganisms undergo numerous biochemical changes to enhance survival [37]. These changes include alterations in protein structure and folding, reduced biochemical reaction rates, reinforcement of weak molecular interactions, stabilization of hydrogen bonds, inhibitory nucleic acid structures, increased gas solubility, and stability of toxic metabolites. Additionally, there is a reduction in cellular membrane fluidity and the induction of stress proteins such as antifreeze proteins (AFPs) and ice-nucleating proteins (INPs) [37, 38]. Cold-adapted enzymes are pivotal for the survival and growth of psychrophilic microorganisms due to their high specific activities at low temperatures [39]. These enzymes play essential roles in various metabolic processes tailored for cold environments, facilitating the biochemical adaptations necessary for successful adaptation and thriving in harsh cold conditions [40].

2.1 *Protein synthesis*

Researchers [41, 42] suggest that cold shocks have profound effects on cell growth by affecting ribosomal synthesis and subsequent protein synthesis in psychrophilic bacteria. These bacteria have developed special properties that allow them to function efficiently despite the initiation of protein synthesis being particularly susceptible to inhibition by temperature changes [43]. Studies have also observed that cold shock is followed by the suppression of housekeeping gene expression and synthesis of the cold shock gene family [44, 45]. Additionally, some cold shock proteins have been reported as transcriptional regulators, potentially influencing DNA supercoiling. For example, CspA, a major cold shock protein of *E. coli*, serves as a transcriptional regulator of other cold shock genes. Homologous proteins with similar functions have been identified in *Bacillus subtilis* and Antarctic psychrotrophic bacteria [43, 44].

In a similar vein, several other cold shock proteins have been identified that play a role in preventing cold shock, even if they are not essential for cell growth. One such example is the nucleoid-associated DNA-binding protein H-NS in *E. coli*, which helps mitigate the effects of low temperatures [40]. Scientist [40] highlights that freezing conditions lead to a limited rate of protein synthesis within cells, which serves to reduce damage and trigger cellular responses aimed at compensating for impaired growth. For example, in the Antarctic Bacterium *P. haloplanktis*, about 30% of the proteins that are upregulated at low temperatures (4 °C) are ribosomal proteins and RNA chaperones. Similarly, in archaea such as *M. burtonii, P. haloplanktis, C. psychrerythraea*, and *P. ingrahamii*, there is a notable increase in the stimulation of rRNA and tRNA genes, with up to 106 genes being influenced. Additionally, enzymes like helicases and aconitase are upregulated at low temperatures, aiding in the unwinding of RNA secondary structures to facilitate efficient translation in cold conditions. These examples highlight the adaptive mechanisms of psychrophiles to optimize protein synthesis in cold environments [46]. In the study by [47], the relative abundance of twenty amino acids in eight psychrophilic proteins was investigated. It was found that psychrophilic proteins exhibit higher levels of isoleucine and

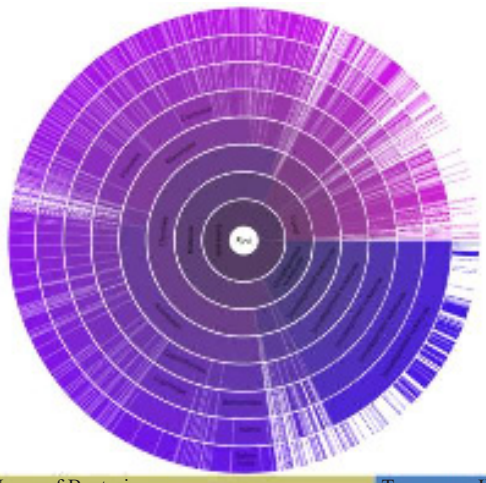
proline. Additionally, neutral hydrophilic amino acids such as asparagine and serine were prevalent, along with slightly elevated levels of hydrophobic, non-polar, aromatic amino acids like tryptophan and phenylalanine in their polypeptide chains. The increased hydrophobicity contributes to the stability of psychrophilic proteins during cold denaturation processes [47]. However, it's noteworthy that psychrophilic proteins containing catalytic multi-domains are reported to be heat-labile. This could be attributed to the presence of fewer salt bridges on the outer surface of the protein, resulting in reduced conformational flexibility [48, 49].

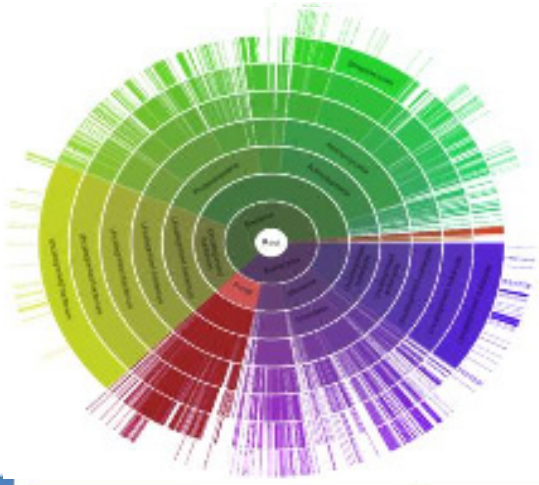
2.2 *Protein folding*

In psychrophiles, low temperatures compromise the protein folding process, creating various physicochemical barriers such as a decreased folding rate, reduced molecular diffusion rate, and increased solvent viscosity. These disruptions affect the conformational structure of proteins and enhance their interaction with substrates. Protein folding is often a rate-limiting step for psychrophile growth [50]. Newly synthesized nascent polypeptides must undergo precise folding into tertiary and quaternary structures to become fully functional [43]. Psychrophilic proteins are characterized by high flexibility, contributing to their extreme stability. They exhibit a higher number of hydrogen bonds and van der Waals interactions, along with reduced electrostatic interactions and proline content. For example, lipases from Candida antarctica are highly stable due to their rigidity, increased van der Waals forces, reduced electrostatic interactions, and enhanced hydrogen bonding [51]. Recent research by [52, 43] underscores the importance of protein flexibility in psychrophilic organisms, highlighting the role of specific amino acid residues in enhancing protein stability at low temperatures. These findings strongly suggest that protein folding represents a rate-limiting step for psychrophiles, leading to the preferential synthesis of certain proteins known as Cold Shock Proteins (CSPs) [50]. This phenomenon modulates the properties of the chaperone machinery, explaining the occurrence of both cold-adapted and non-cold-adapted protein chaperones in psychrophiles. Enzymatic processes in psychrophiles are also influenced by pH, particularly at low temperatures. Psychrophilic microorganisms exhibit maximum enzyme activity at alkaline pH levels, especially for proteases. For instance, protease production in *Cryptococcus victoriae* was optimal at pH 8.0, while *Chryseobacterium sp*. showed the highest specific enzyme activity at pH 7.0. *Bacillus sp*. and *Bacillus subtilis* secreted maximum protease at alkaline pH values of 9.0 and 10.0, respectively. *Stenotrophomonas maltophilia* achieved its highest protease yield of 62.2 U/ml at pH 9.0 [53]. Further underscore the importance of pH regulation in enzymatic processes in psychrophilic microorganisms, providing insights into the adaptive mechanisms of these organisms in cold environments [54].

2.3 *Cold shock proteins, anti-freeze protein and chaperones*

Cold-induced proteins, including cold shock proteins (CSPs) and cold acclimatization proteins (Caps), are pivotal for the growth and survival of psychrophilic bacteria, facilitating RNA stabilization and preventing secondary structure formation at low temperatures [55, 56]. For example, CspA from *Escherichia coli* (PDB: 1A04) and cold-shock protein HZB mutants from *Bacillus caldolyticus* (PDB: 1HZB) underscore their roles in responding to abrupt temperature changes and maintaining cellular integrity through electrostatic interactions. Antifreeze proteins (AFPs), 5ANP such as those from *Hypogastrura harveyi* (PDB: 5ANP) and 1AI7 *Pseudopleuronectes americanus* (PDB: 1AI7), safeguard against ice crystal growth by binding to ice surfaces, thereby shielding cells from cold-induced damage [60-62]. The histidine-containing phosphotransfer (HPt) domain of ArcB from *E. coli* 1A0B (PDB: 1A0B) plays a critical role in bacterial adaptive responses to environmental changes, including anaerobic conditions (Figure A1 and supplementary Table A1). Molecular studies reveal highly conserved sequences rich in glycine within the RNA-binding cold-shock domain (CSD) of CSPs, ubiquitous among psychrotrophic bacteria (Figure A2). CSPs, small proteins approximately 50 amino acids long [42, 57], exhibit specific activity at low to moderate temperatures but display low thermostability [58, 43]. Protein domain analyses using NCBI data and tools like GeneDoc illuminate how AFPs aid in cold adaptation in psychrophilic bacteria, identifying highly conserved protein domains extending up to position 280 [56] (Figure A3). Cells in active growth phases are more vulnerable to freezing damage, with -20 °C posing a greater threat than liquid nitrogen (-196 °C). AFPs, in glycosylated or lipidated forms, vary in molecular weight from 2 to 50 kDa, with the largest AFP reported at 1.5 MDa from Antarctic bacterium *Marinomonas primoryensis* [61] (Figure A3).





Name of Bacteria	Taxonomy ID
Eutreptiella pomquetensis	215699
Chlamydomonas sp. ICE-L	309537
Chlamydomonas sp.CCMPS 681	2812568
Chlamydomonas sp. CCMP 1619	2812568
Grammonema striatula	33645
Pseudoalteromonas raudensis	1923966
Navicula sp	
Nitzschila sp.	2315415
Porosira sp.	49240
Chaetoceros sp	2608253
Nonlabens sp. Ci31	

Name of Fungi	Taxonomy ID
Penicillium swiecickii	1295096
Penicilliun christenseniae	1131548
Penicilliunvirgatumn	282147
Penicilliumjamesonlandense	357992
Helotiales	5178
Tetracladium globosum	1413240
Glaciozyma antarctica	105987
Sclerotinia borealis	77105
Tetracladium ellipsoideumn	1413239
Pseudogymnoascus destructans	655981

Figure 1. List of Different Microorganism adapted to Low temperature designed by NCBI blast tools sing *Moritella profunda* (Taxoid: 111291) as the query domain, followed by analysis with pfam

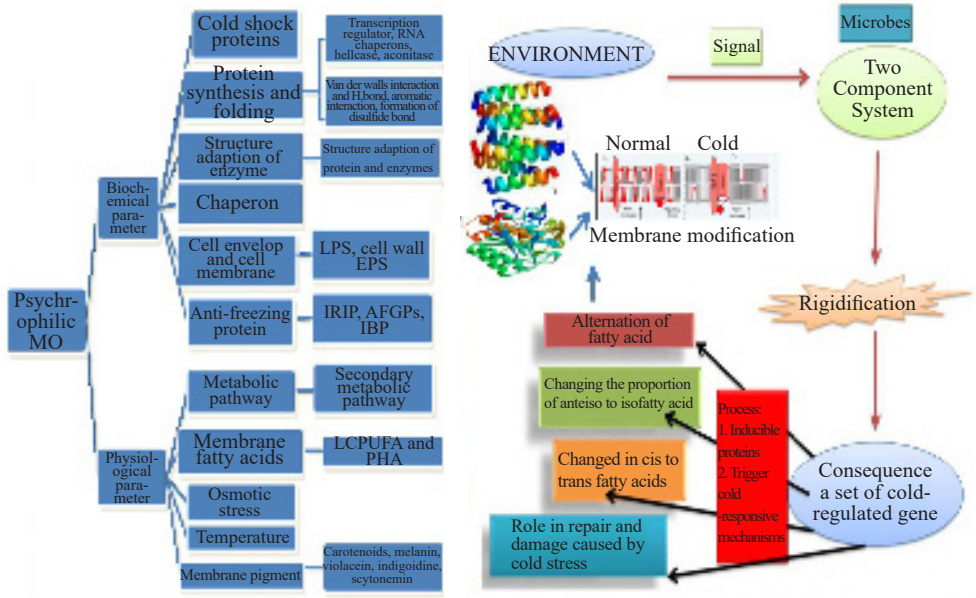


Figure 2. Diagrammatic representation and survival mechanisms of psychrophilic bacteria in cold environments, focusing on the key molecules and pathways activated during cold stress to modify the membrane structural properties

AFPs, also known as Ice Binding Proteins (IBPs), play crucial roles in protecting cells by binding to ice crystal surfaces, encompassing antifreeze glycoproteins (AFGPs) and ice recrystallization inhibiting proteins (IRIPs). Psychrophilic organisms harbor diverse IBPs, including Type I IBPs characterized by Domain of Unknown Function 3494 (DUF3494) found in Antarctic green algae like *Chlamydomonas raudensis* UWO241, *Chloromonas brevispina*, and *Chlamydomonas* ICE-MDV [24], potentially transferred horizontally among various organisms [63]. Type II IBPs are identified in Antarctic snow alga CCMP681 and Antarctic *Chloromonas sp*. [24]. Research on Antarctic bacterium Efc IBPs by Mangiagalli et al. [64] underscores their roles in thermal hysteresis and ice recrystallization inhibition critical for survival at subzero temperatures. Studies on *Rhodococcus sp. JG3* highlights adaptation mechanisms under cold and salty conditions, potentially involving IBPs or analogous molecules to cope with environmental stress and modulate freezing properties [24].

Protein and RNA/DNA chaperones play critical roles in maintaining cellular function under cold conditions by ensuring proper folding of proteins and stabilization of nucleic acids, thereby preventing misfolding, aggregation, and maintaining RNA and DNA secondary structures [65]. In psychrophilic microorganisms, these chaperones are continuously overexpressed or upregulated at low temperatures to counteract cold denaturation, which weakens hydrophobic interactions and increases the risk of protein misfolding compared to mesophilic and thermophilic counterparts [66, 61].

In the realm of recombinant protein production, molecular chaperones like caseinolytic proteases (Clps), Transcription Factor (TF), GroEL, DnaK, and GroES play crucial roles. These chaperones are upregulated in *Escherichia coli* during cold shock, and their expression supports efficient protein folding even at low temperatures. For instance, the expression of cpn60 (GroEL) and cpn10 (GroES) from the Antarctic bacterium *Oleispira antarctica* facilitates *E. coli* growth at 4 °C, highlighting their importance in mediating protein folding and adaptation to cold environments [67]. Microorganisms thriving in permanently cold environments, such as deep-sea, polar, and mountainous regions, have evolved enzymes tailored to function optimally under frigid conditions [68]. The Arrhenius law governs enzyme activity, showing that reaction rates decrease exponentially with decreasing temperature [69]. Psychrophiles have adapted their enzymes, such as DNA-dependent RNA polymerase, ribonuclease, and alkaline phosphatase, with distinct activities and amino acid compositions compared to mesophilic enzymes [55]. Cold-adapted enzymes typically exhibit lower proline or arginine content, altered ratios of arginine to lysine, reduced hydrophobic residues, increased polar residues, and fewer disulfide bonds [55]. Despite lower overall sequence identity, these enzymes retain conserved active site regions critical for catalytic function across different temperature ranges [71]. Enzyme catalytic activity in psychrophiles is categorized into phases sensitive to temperature variations: substrate recognition and binding, conformational changes inducing transition states and product formation, and product release [70]. Psychrophilic enzymes often have markedly different optimal temperatures for activity compared to their mesophilic counterparts, with enhanced activity observed at low temperatures and lower activation energies [72]. This adaptation ensures enzyme functionality even at near-freezing temperatures, reflecting their finely tuned structural and functional adaptations to cold environments.

Psychrophilic enzymes also exhibit a correlation between thermostability and specific activity at low temperatures, which is more pronounced compared to mesophilic enzymes [59]. Studies on various enzymes like protease, α-amylase, DNA ligase, and xylanase demonstrate this temperature-dependent correlation through techniques such as acrylamide quenching of tryptophan fluorescence.

Cold shock proteins (CSPs), induced at high levels during temperature shifts below 20 °C in organisms like *E. coli*, play essential roles as stress proteins in psychrotolerant and psychrophilic bacteria [56]. These proteins, identified in *Bacillus psychrophilus*, *Bacillus cereus*, *Pseudomonas fragi*, and other cold-adapted species, aid in cellular viability under cold stress conditions by regulating essential processes like unsaturated fatty acid synthesis (Table 2). Thus the adaptive strategies of cold-adapted organisms underscore the intricate interplay between protein structure, function, and environmental conditions, highlighting the specialized mechanisms psychrophilic microorganisms employ to thrive in extreme cold.

Table 2. Psychrotolerant and Psychrophilic bacteria have Cold-shock proteins (Csps) and cold-acclimation proteins (Caps)

S. No.	Protein	Putative function	Reference
1.	CspA Family, CspA, CspB, CspG	all these proteins help organisms, particularly bacteria, adapt to cold environments by stabilizing nucleic acids and ensuring essential cellular processes can proceed despite the stress-induced changes.	Gounot and Russell [56]
2.	CsdA (Cold shock DEAD-box protein A)	CsdA is a multifaceted RNA helicase crucial for cold shock adaptation, ensuring proper RNA structure and function in bacteria under low-temperature conditions. Its activity helps maintain protein synthesis and general cellular function during stress.	
3.	RbfA (Ribosome-binding factor A)	RbfA is essential for the final maturation of the 30S ribosomal subunit in bacteria, particularly under conditions of cold stress. Its primary role is to assist in the processing of 16S rRNA, ensuring that the ribosome can function efficiently in protein synthesis.	
4.	NusA (N-utilization substance A)	NusA is a versatile transcription factor that regulates transcription elongation, termination, and antitermination. It interacts with RNA polymerase and other proteins to ensure proper gene expression, affecting transcription rates, RNA stability, and the balance between terminating or continuing transcription under different cellular conditions.	
5.	PNPase (Polynuclotide phosphorylase)	Polynucleotide phosphorylase (PNPase) is an essential enzyme in bacteria that plays a major role in RNA degradation and processing. It is a phosphorolytic exoribonuclease involved in the degradation of mRNA, trimming of rRNA and tRNA, and, in some cases, RNA polyadenylation. PNPase is also part of the RNA degradosome, contributing to overall RNA metabolism and adapting to cold stress conditions.	
6.	H-NS (Histone-like Nucleoid Structuring protein)	H-NS is a key DNA-binding protein found in many bacteria, including Escherichia coli. It plays a critical role in organizing the bacterial nucleoid and regulating gene expression, particularly in response to environmental changes.	
7.	RecA DNA-repair, Recombination	RecA is an essential protein involved in DNA repair, homologous recombination, and the bacterial SOS response to DNA damage. It facilitates the repair of broken DNA by promoting homologous recombination, activates the SOS response to upregulate DNA repair proteins, and helps bacteria survive DNA damage through tolerance mechanisms.	
8.	GyrA (DNA gyrase)	GyrA is an essential bacterial enzyme that introduces negative supercoils into DNA, which is critical for DNA replication, transcription, and maintaining DNA topology.	
9.	If2 (Initiation factor)	If2 is a bacterial protein involved in the initiation phase of protein synthesis. It plays a key role in the formation of the ribosomal initiation complex, which is crucial for starting translation.	
10.	Dihydrolipoamide acetyltransferase	Dihydrolipoamide acetyltransferase is an enzyme that plays a critical role in the oxidative decarboxylation of alpha-keto acids in cellular metabolism. It is part of the pyruvate dehydrogenase complex and other multienzyme complexes involved in energy production.	
11.	PDH (Pyruvate dehydrodenase)	PDH is an enzyme complex that converts pyruvate into acetyl-CoA, a critical step linking glycolysis to the citric acid cycle (Krebs cycle). This process is essential for energy production and cellular metabolism.	
12.	Hsc66 (Heat shock cognate 66))	Hsc66 is a molecular chaperone protein that assists in protein folding, stabilization, and maintenance within the cell. It is part of the larger family of heat shock proteins (HSPs), specifically the Hsp70 family.	
13.	IBPs (Microalgal ice-binding proteins)	IBPs are specialized proteins found in certain microalgae that help them survive in freezing environments. These proteins play a crucial role in ice crystal regulation and protection against ice damage.	Cho et al. [63]
14.	INPs (Ice nucleating proteins)	INPs are specialized proteins that promote ice formation in supercooled water. These proteins are found in various organisms, including bacteria, fungi, and plants, and play a role in processes ranging from environmental adaptation to influencing weather patterns.	
15.	Cold adapted enzymes (psychrophilic enzymes)	Cold-adapted enzymes, are enzymes that are adapted to function efficiently at low temperatures. These enzymes are produced by organisms that live in cold environments, such as Arctic and Antarctic regions, deep-sea habitats, and high-altitude areas.	Collins and Margesin [61]
16.	AFPs (Antifreeze protein)	Antifreeze proteins are specialized proteins produced by bacteria to prevent the formation and growth of ice crystals within their bodies. These proteins are crucial for survival in extremely cold environments, where they help organisms avoid freezing and the associated cellular damage.	

2.4 *Cell envelop and cell membrane*

The cell envelope is essential for microorganisms adapting to cold environments, protecting against stressors, maintaining turgor pressure, and facilitating nutrient uptake and cellular processes like signaling and adhesion [61, 62]. Cold conditions reduce membrane fluidity, increase permeability, and impair protein function, potentially causing cell rupture due to ice formation or freeze-thaw cycles [66]. To cope with these challenges, microorganisms modify the lipid

composition of their cell membranes. They favor shorter chain lengths, methyl-branched, and/or cis-isomeric lipids, while decreasing lipid saturation, to maintain optimal membrane fluidity under cold conditions (Figure 2) [66]. Genes involved in membrane biogenesis, fatty acid synthesis, fatty acid desaturation, and production of branched-chain fatty acids are upregulated in cold-adapted organisms to support these lipid modifications [62].

In Gram-negative bacteria like *Pseudomonas* species, the cell envelope consists of an inner and outer membrane separated by a periplasmic space containing a thin peptidoglycan layer. The outer membrane includes phospholipids, proteins, and lipopolysaccharides (LPS) composed of lipid A, a core oligosaccharide, and an O-polysaccharide [21]. Cold-adapted bacteria, such as *Planococcus halocryophilus* Or1, may further enhance their cell envelope by thickening the outer surface with hydrophobic encrustations containing peptidoglycan, calcium carbonate, and choline [63]. The lipid A component of LPS in these bacteria often comprises higher proportions of short-chain and/or unsaturated fatty acids, which contribute to improved membrane fluidity in cold environments [21]. Moreover, exopolysaccharides (EPS) surrounding bacterial cells also play a critical role in cold adaptation by providing protection against freeze-thaw cycles and acting as cryoprotectants. EPS production, particularly with mannose as a major component, is common among cold-adapted bacteria, further illustrating their adaptive strategies to thrive in extreme cold [21]. The adaptation of the cell envelope and membrane lipid composition are fundamental strategies employed by microorganisms to maintain cellular integrity and function in cold environments, highlighting their remarkable ability to survive and thrive in extreme conditions.

3. Physiological adaptation of psychrophilic microorganism

Psychrophiles, from a physiological standpoint, can thrive at temperatures up to 20 °C, with optimal growth occurring at 15 °C or below. In response to cold stress conditions, bacteria have developed protective mechanisms that involve alterations in morphology and metabolism. In the chilly environment, microbial life experiences reduced biochemical reaction rates, heightened solvent viscosity, and increased gas solubility, including oxygen molecules and reactive oxygen species (ROS). These factors negatively impact solute transport and diffusion, leading to phenomena such as ice formation and osmotic stress, which can have detrimental effects on biological processes [73] (Table 3). These adaptive transformations are genetically regulated and can be passed down from one generation to the next.

Table 3. Summary of metabolic pathway up regulated (↑) and down regulated (↓) in cold adapted MO

S.No	Metabolic pathway	Psychrophilic MO
1.	Glycolysis	↑
2.	TCA	↓
3.	Electron chain reaction	↓
4.	Beat oxidation of fatty acid	↑
5.	Pentose phosphate pathway	↓
6.	Molybdopter in metabolism	↓
7.	Reactive oxygen species (ROS)	↑
8.	Acetyl-CoA metabolism	↑
9.	Methylcitrate pathway	↑
10.	Branched amino acid degradation	↑
11.	Glyoxylate,	↑
12.	Methyglyoxal or 2-methylcitrate cycle	↑
13.	Ethanol oxidation Pathway	↑
14.	Propionyl-CoA catabolism	↑

3.1 *Role of temperature*

The Arrhenius equation effectively describes how temperature impacts microbial growth rates, showing an inverse proportionality to absolute temperature (Kelvin). In the Arrhenius plot, the linear section represents temperatures suitable for physiological growth, while deviations occur under stress-inducing temperatures. Psychrophiles maintain linearity down to 0 °C, psychrotolerants between 5-10 °C, and mesophiles around 20 °C. Psychrophiles and psychrotolerants grow faster at lower temperatures than mesophiles, reflecting lower activation energy in psychrophiles. Temperature affects various physiological and biochemical processes, including photosynthesis and gene expression [74]. Adaptation to low temperatures involves lipid and fatty acid modifications, favoring unsaturated fatty acids to maintain membrane fluidity and prevent rigidity. Additionally, psychrophiles have evolved cold shock and antifreeze proteins and modified their photosynthetic electron transport chains to survive and complete life cycles in cold environments. Research into gene expression patterns related to photosynthesis, carbohydrate metabolism, electron transfer, and cell maintenance has shed light on cold adaptation mechanisms (Table 3). For instance, Chong et al. [75] discovered six ice-binding proteins (IBPs) in Antarctic algae Chlorella UMACC 234, homologous to IBPs from Antarctic snow algae, which were highly expressed at low temperatures, highlighting their role in cold adaptation.

3.2 *Change in metabolic activity*

Under cold conditions, primary metabolic pathways are typically down-regulated, while non-secondary pathways activate to support cold-adapted lifestyles. The increased solubility of oxygen at low temperatures leads to enhanced production of reactive oxygen species (ROS) and oxidative stress, often depressing pathways like glycolysis, the pentose phosphate pathway, the tricarboxylic acid cycle (TCA), and the electron transport chain [21]. Certain heterotrophic bacteria, such as *Psychrobacter arcticus*, exhibit unique metabolic features, lacking glycolysis genes but possessing gluconeogenic enzymes and favoring oxidized carbon sources like acetate [55]. Genomic and proteomic studies of *Psychrobacter* sp. PAMC 21119 from Antarctic permafrost soil shows upregulation of acetyl-CoA metabolism and downregulation of energy production proteins [21]. *Nesterenkonia* sp. AN1 and *Exiguobacterium sibiricum* also demonstrate increased expression of enzymes for the glyoxylate cycle and energy metabolism at low temperatures, respectively [76]. Secondary pathways become essential for growth under low temperatures. For example, marine bacteria like *Sphingopyxis alaskensis* upregulate fatty acid degradation pathways at low temperatures to generate acetyl-CoA for the TCA cycle. Similarly, *Pseudomonas putida* KT2440 exhibits upregulation of the 2-methylcitrate pathway and branched amino acid degradation at 10°C, allowing it to catabolize propionate and propionyl-CoA for TCA cycle replenishment [21]. Even bacteria not native to cold environments, such as *Pseudomonas putida* KT2440, adapt through metabolic reprogramming, substituting primary pathways with alternative ones like the glyoxylate, methylglyoxal, or 2-methylcitrate cycles, ethanol oxidation, acetate metabolism, or propionyl-CoA catabolism. This strategy helps alleviate oxidative stress and conserve energy [21]. Certain compounds, including glycine, betaine, glycerol, trehalose, sucrose, mannitol, and sorbitol, play a critical role in cold adaptation by reducing the freezing point of the cytoplasm, preventing macromolecule aggregation, scavenging free radicals, and stabilizing cellular membranes. Studies on Antarctic *Pseudoalteromonas strains* revealed protein S-thiolation regulated by glutathione as a potential cold adaptation mechanism. *Mesorhizobium sp*. *Strain N33* accumulated sarcosine, threonine, and valine at 4 °C, likely acting as cryoprotectants. *Colwellia psychrerythraea* showed genes for polyamide synthesis and degradation, indicating a unique adaptation to cold environments [78]. Therefore, cold adaptation involves metabolic reprogramming, upregulation of non-classical pathways, and the use of specific compounds for cryoprotection, demonstrating the diverse strategies employed by cold-adapted microorganisms.

3.3 *Osmotic stress*

Osmotic stress, often accompanying solute concentration increases in freezing environments, is mitigated by microorganisms producing or accumulating compatible solutes. These non-toxic organic osmolytes, such as glycine betaine, trehalose, glycerol, sucrose, sarcosine, mannitol, and sorbitol, can accumulate to molar concentrations [80, 81]. Compatible solutes depress the freezing point of solutions, lower the colloidal glass transition temperature (Tg), stabilize proteins and membranes, restore osmotic balance, and counteract water loss and cell shrinkage during

low temperatures [83]. Additionally, they scavenge free radicals, prevent protein aggregation, enhance protein folding, and stabilize proteins and membranes [83]. Several bacteria indigenous to cold environments can synthesize polyhydroxyalkanoates (PHAs), a family of microbial polyesters with significant physiological roles. PHAs are non-toxic, biodegradable, and biocompatible biopolymers that can replace petrol-based polymers. These compounds serve as dynamic reserves of carbon, nitrogen, reducing equivalents, and energy in cells, helping overcome low-temperature challenges to carbon and nitrogen uptake [21]. PHAs play roles in cryoprotection, oxidative stress resistance, cellular redox balance, and cell motility. They enhance bacterial survival and resistance to environmental stress. In freezing conditions, PHA depolymerase increases significantly after 24 hours at 10 °C. Phasins, major PHA granule-associated proteins, have multiple functions, including stress protection and fitness enhancement. The PhaP phasin isolated from Sphingopyxis alaskensis, P. extremaustralis, and Pseudomonas sp. increases at low temperatures [21]. The bacterial cell wall serves a crucial role in providing structural integrity to the cell, particularly under stress conditions like freezing-thawing pressure, ice formation, and osmotic imbalance at low temperatures [63, 84]. At lower temperatures, the fluidity of bacterial cell membranes is significantly affected, necessitating adaptations to maintain normal membrane function. Psychrophilic bacteria employ mechanisms to adjust membrane fluidity, such as the conversion of saturated fatty acids into unsaturated fatty acids. Hassan et al. [85] involved isolating 42 bacterial strains from glaciers in the Hunza Valley and studying the distribution of cell membrane fatty acids. They found that monounsaturated fatty acids (n-MUFAs) and branched fatty acids (br-FAs) are major components of cell membrane fatty acids, and their distribution is significantly influenced by temperature. This study sheds light on the role of br-FAs in maintaining cell membrane fluidity in bacteria inhabiting non-polar habitats (Table 3).

3.4 *Lipid and membrane fluidity and membrane pigments*

Similarly, research on Antarctic *flavobacteria* by Kralova [86] highlighted the critical role of lipid structures' semi-liquid state in bacterial biomembranes. Alterations in membrane composition can significantly impact membrane fluidity. Additionally, microorganisms thriving at low temperatures are known to produce bioactive compounds like polyunsaturated fatty acids (PUFAs), particularly long-chain PUFAs (LCPUFAs) with carbon chain lengths of C20 and above [87]. These PUFAs, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA), contribute to membrane fluidity maintenance by forming hydrophobic edges between lipid bilayers, shielding cells from reactive oxygen species and ensuring membrane flexibility. Furthermore, mutants of *Shewanella livingstonensis* from Antarctic seawater have been found to produce eicosapentaenoic acid (EPA), a precursor of PUFAs, enhancing the growth of psychrophilic bacteria. Suzuki et al. [87] also identified *Koliella Antarctica*, isolated from ice-free seawater in Antarctica, as a potential producer of PUFA, LC-PUFAs, with considerable nutritional and pharmaceutical value. PUFAs play various roles in human health, regulating cardiovascular and immune systems, inflammation, and contributing to brain, eye, and central nervous system development and functioning. Essential fatty acids like linoleic acid (LA) and α-linolenic acid (ALA) must be obtained from the diet, while LC-PUFAs such as DHA and ARA are recommended in infant diets Figure 2.

Pigment production is a common trait among psychrophilic microorganisms, aiding their survival in extreme environments. These pigments serve multiple purposes, including energy for photosynthesis, defense against stressors like extreme temperatures, desiccation, and UV irradiation, and enhancing immune responses against other bacteria [88]. The pigments contribute significantly to the ability of these microbes to thrive in low-temperature environments. Psychrophilic microorganisms produce a diverse array of pigments, including carotenoids, prodigiosin, melanin, violacein, indigoidine, and scytonemin [73, 89]. Studies by Shen et al. [88] identified various pigments such as α- and β-carotene, diatoxanthin, peridinin, zeaxanthin, lutein, butanoyloxy, and fucoxanthin in representative colonies. Carotenoids, particularly α-carotene, were dominant. The proportion of pigmented bacteria increased with depth in ice cores, with yellow-colored colonies being prevalent. Carotenoids and other pigments are believed to modulate cell membrane fluidity and enhance membrane rigidity, advantageous for cold-adapted organisms. The concentration of zeaxanthin-like pigments increased by 26-65% after freeze-thaw cycles, suggesting these pigments confer better cryoprotection by regulating membrane fluidity [90]. Similarly, *Serratia marcescens* produces red pigments (prodigiosin) at lower temperatures (25 °C) compared to higher temperatures (37 °C), indicating a role in temperature adaptation [89]. The bacteria, *Flavobacterium* strains, produce carotenoids and flexirubin-type pigments, giving them a yellowish or orange appearance [91]. These bacteria inhabit various environments across temperate and polar regions, including

lakes, oceans, glaciers, plants, and animals (Table 3).

4. Industrial applications

Cold-adapted microbes employ various strategies to cope with freezing temperatures and other stressors. These strategies include the synthesis of cryoprotectants and stress-protectant molecules, the production of cold-active proteins and enzymes, and the regulation of membrane fluidity. Extremophiles, including psychrophiles, have garnered attention for their potential applications in producing antibiotics, drugs, enzymes, and other biotechnological products [92]. Buzzini et al. [93] categorized psychrophiles into obligate psychrophiles and facultative psychrophiles based on their response to temperature. These microbes adapt to stress conditions by modulating membrane fluidity and producing a diverse array of metabolites. Psychrophilic bacteria have diverse applications across various industries. In the food industry, proteases from *Pseudoaltermonas sp. SM9913*, *Penicillium nalgiovense PNA9*, *Arsukibacterium ikkense*, *Chryseobacterium polytrichastri*, *Serratia sp. WJ39* and Antarctic yeast *Glaciozyma antarctica PI12* are used as additives in baking, food processing, and preservation [94-96]. Lipases from *Pseudomonas*, *Aeromicrobium sp. SCSIO 25071*, *Halocynthiibacter arcticus*, *Streptomyces coelicolor A3(2)*, *Malassezia globose*, *Staphylococcus epidermidis AT2*, *Rhodococcus sp. AW25M09*, *Neisseria meningitides*, *Lactobacillus acidophilus NCFM*, *Bacillus licheniformis ATCC 14580*, and *Rhodosporidium kratochvilovae* strain YM25235 are applied in food processing, dairy, organic ester production, and flavor compounds [95, 97-99]. Pectinase from *Pseudoalteromonas haloplanktis* and *Tetracladium sp*. is used in fruit juice and fermented beverage production, while mannanase from *Bacillus subtilis* Bs5 serves the food processing industry [95]. In pharmaceuticals, antifreeze glycoprotein Antarticine-NF3 from *Pseudoalteromonas* is effective in scar treatment, and Colwellia psychrerythraea and *Antarctic actinomycete Nocardioides sp*. produce unique bioactive antimicrobial compounds [98, 100]. For bioremediation, genera like *Nitrosopumilus*, *Nitrosoarchaeum*, *Pseudomonas*, *Aeromonas*, *Oceanisphaera*, *Shewanella*, *Paeniglutamicibacter*, and *Rhodococcus*, along with classes like *Bacteroidies, Clostridia, Acidimicrobiia, Planctomycetes*, and *Deltaproteobacteria*, are effective in hydrocarbon pollutant degradation and biosurfactant production. Additionally, phages such as *Paraglaciecola* Antarctic GD virus 1 (PANV1), *Paraglaciecola Antarctic JLT* virus 2 (PANV2), *Octadecabacter*, Antarctic BD virus 1 (OANV1), and *Octadecabacter Antarctic* DB virus 2 (OANV2) infect common sea ice bacteria, aiding in bioremediation efforts [101, 98]. Psychrophilic microorganisms have several practical applications due to their production of thermally adapted enzymes. For instance, *P. haloplanktis* produces *α*-amylase and Family 8 glucanases at 10 °C, *Colwellia psychrerythraea* generates aminopeptidase at 10 °C, *Alteromonas haloplanktis* secretes amylase, *Pseudomonas fluorescens* synthesizes alanine racemase, *Carnobacterium piscicola BA* produces *β*-galactosidase, and *Pseudoalteromonas haloplanktis* makes DNA ligase. In biofuel production, *Psychrobacter cryohalolentis* strain F5-6 ferments biomass at low temperatures to produce bioethanol. In the textile industry, *Psychrobacter proteolyticus* produces cold-active cellulases for bio-polishing denim. In agriculture, *Pseudomonas putida* aids in the decomposition of organic matter and nutrient cycling at low temperatures, promoting plant growth and improving soil fertility. Their applications span various industries including pollution control, cosmetics, food production, and scientific research, as summarized in Table 4

Table 4. Industrial application of Psychrophilic Bacteria

Name of psychrophilic bacteria's	Applications	References
Food industry		
Proteases use in food industry		
Pseudoaltermonas sp. SM9913, *Penicillium nalgiovense* PNA9, *Arsukibacteriumikkense Chryseobacterium polytrichastri, Serratia sp. WJ39 Antarctic yeast Glaciozyma antarctica PI12*	Application is in as additives in food, As additives in baking industry, food processing and preservation industries	[94-96]

Table 4. (cont.)

Name of psychrophilic bacteria's	Applications	References
Food industry		
Lipases use in food industry		
Pseudomonas Aeromicrobium sp. SCSIO 25071 *Halocynthiibacter arcticus Streptomyces coelicolor* A3(2) *Malassezia globose Staphylococcus epidermidis* AT2 *Rhodococcus* sp. AW25M09 *Neisseria meningitides Lactobacillus acidophilus* NCFM *Bacillus licheniformis* ATCC 14580 *Rhodosporidium kratochvilovae strain* YM25235	Broad range of application is in as Food processing industry, Dairy and food processing industry, Production of organic esters for use in food industry, Production of flavored compounds, Biosynthesis of polyunsaturated fatty acids (PUFAs) and glycerol with its cold adaptation	[95, 97-99]
Pectinase use in food industry		
Pseudoalteromonas haloplanktis; Tetracladium sp.	Production of fruit juice and fermented beverages	[95]
Mannanase *Bacillus subtilis* Bs5	Food processing industry	
Pharmaceutical Industries		
Antarticine-NF3(Antifreeze glycoprotein from Pseudoalteromonas)	Effective in scar treatment	[98, 100]
Colwellia psychrerythraea, Antarctic actinomycete Nocardioides sp	Produces unique bioactive compounds at low temperatures that have antimicrobial properties.	[98, 101]
Bioremediation		
Nitrosopumilus and *Nitrosoarchaeum* and to the *Nitrososphaeraceae* family; *Pseudomonas, Aeromonas, Oceanisphaera, Shewanella, Paeniglutamicibacter*, and *Rhodococcus*; bacteria of the classes Bacteroidia, Clostridia, Acidimicrobiia, Planctomycetia, and Deltaproteobacteria were less represented. FB4_88, *Anoxybacillus amylolyticus* DSM 15939T, Chlorobium antarcticum *Paraglaciecola* Antarctic GD virus 1 (PANV1), *Paraglaciecola* Antarctic JLT virus 2 (PANV2), *Octadecabacter* Antarctic BD virus 1 (OANV1), and *Octadecabacter* Antarctic DB virus 2 (OANV2). The phages infect common sea ice bacteria belonging to the genera *Paraglaciecola* or *Octadecabacter.*	The Potential for from Hydrocarbon Pollutants, Oil-degradation and Biosurfactant	[102-105]
Thermally adapted enzymes		
P. haloplanktis	*α-amylase (10 °C)*	[106]
P. haloplanktis	*Family 8 glucanases (10 °C)*	
Colwellia psychrerythraea	*Aminopeptidase (10 °C)*	
Alteromonas haloplanktis	*Amylase*	
Pseudomonas fluorescens	Alanine racemase	
Carnobacterium piscicola BA	*β-galactosidase*	
Pseudoalteromonas haloplanktis	*DNA ligase*	
Biofuel Production		
Psychrobacter cryohalolentis strain F5-6	*Ferments biomass at low temperatures to produce bioethanol*	[107]
Textile Industry		
Psychrobacter proteolyticus	Produces cold-active cellulases that help in bio-polishing denim	[108]
Agriculture		
Pseudomonas putida	Psychrophilic bacterium helps in the decomposition of organic matter and nutrient cycling at low temperatures, promoting plant growth and improving soil fertility	[109]

5. Conclusions

All free-living bacteria face regular environmental changes, requiring them to quickly adapt to sudden disturbances. Microorganisms inhabiting extremely low-temperature environments have developed a range of sophisticated adaptations at all levels of their cells to survive in their harsh habitats. Many of these adaptive mechanisms involve biochemical and physiological changes, as depicted in Figure 2 and Table 3, although understanding their underlying mechanisms remains an ongoing challenge. Psychrophilic bacteria thrive at temperatures at or below 45 °F (7.2 °C) and can form visible colonies on plates within ten days when incubated at 7 ± 0.5 °C. Their ability to grow at refrigeration temperatures makes them a primary concern for the spoilage of milk and various dairy products, where they can cause a range of defects. While *Pseudomonas* species are commonly encountered among psychrophiles, many bacterial genera contain psychrophilic species. They are typically found in water, soil, and contaminated equipment, necessitating efforts to remove these sources of contamination from milk and dairy products. Key areas of study include understanding the structure-function relationships and roles of cell membranes and their chemical compositions, such as EPS, PUFA, LPS, and membrane pigments. Additionally, there is a need to gain a better understanding of the metabolic adjustments employed by psychrophiles, including the accumulation of compounds like PHA in cold adaptation, and the effects of cold denaturation on proteins at various temperatures. Advancements in these areas will further our understanding of adaptive trends and novel metabolic peculiarities utilized by cold-adapted microorganisms, potentially leading to the identification of novel biomolecules through biotechnological tools. Already, there is ongoing commercialization of equipment derived from psychrophile innovations.

Conflict of interest

I declare there is no conflict of interest and the entire responsibility for the published results data is up to the author.

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Appendix A

Table A1. Antifreeze proteins and Cold shock proteins

SN	Antifreeze protein	Microbes and sources
1	1AI7	Escherichia coli (PDB: 1AI7)
2	5ANP	Bizioniaargentinensis JUB59 (PDB: 5ANP)
3	1A04	Escherichia coli (PDB: 1A04)
4	1A0B	Escherichia coli (PDB: 1A0B)
5	1HZB	(PDB: 1HZB)

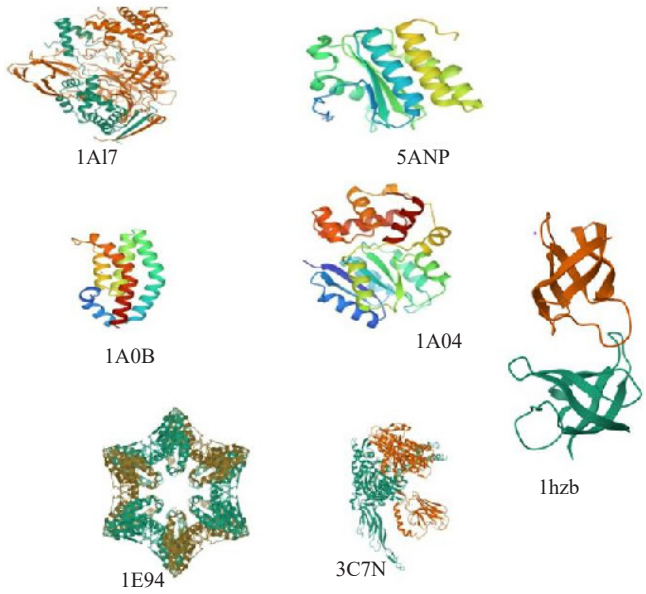

Figure A1. Different cold shock protein motif to low temperature in psychrophilic microorganisms

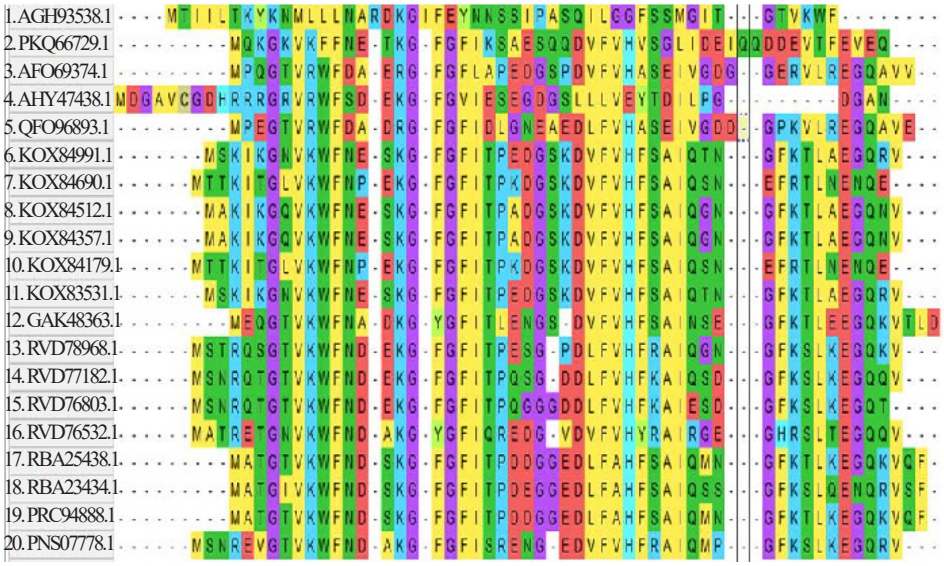

Figure A2. Different cold-shock protein domain in psychrophilic

Figure A3. Different Antifreezing protein in psychrophilic microorganism