Cold-Adapted Microorganisms: Survival Strategies and Biotechnological Significance

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Abstract

Thermal stress either cold or heat stress is one of the major factors that influence microorganisms, and to survive from these adverse conditions, microorganisms have to adapt different survival strategies. Some major survival strategies adapted by the microorganisms in response to cold stress are metabolic adaptations, change in cell membrane structure and functions such as membrane fluidity, molecular adaptations that includes change in gene expression, production of cold-adaptive enzymes, and the production of compounds like cryoprotectants that protects microorganisms from these adverse effects. These survival strategies represent bacterial adaptations, and those microorganisms that have potential to adapt better in these survival conditions are most likely to survive in cold stress. Cold-adaptive microorganisms possess high survival instincts and simultaneously offer numerous advantages in pursuits of biotechnological advances. This advancement includes production of cold-adaptive enzymes and proteins, and these proteins and enzymes are very important for commercial purpose that contribute to Indian economy too.

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16.1 Introduction

To survive in different conditions, microorganisms have to adapt different mechanisms to counter any environmental stress. These stresses may include thermal stress, osmotic stress, and nutrient stress. Temperature plays an important role in bacterial growth, and their survival can severely affect functioning and productivity of an ecosystem. Temperature plays a very important role in regulating the growth of microorganisms as it directly affects the cellular constituents, for instance, macromolecules like proteins and enzymes, which are the key players in determining the rate of a metabolic reactions. Arrhenius equation provides the significant relationship between temperature and rate of reaction:

$$k = Ae^{-Ea/RT}$$

where K = rate constant, Ea = activation energy, R = universal gas constant, A = constant of steric collision and frequency, and T = absolute temperature.

Generally, for most of the enzymes, activation energy is about 420 KJ mol^{-1}, so when temperature drops from 20 °C to 0 °C, there is fourfold decrease in enzymatic activity. Thus, temperature plays a very important role in regulating cell overall biochemistry (Russell 1984). Freezing temperatures can cause decreased growth rate, reduced enzymatic activity, and altered physiological and cellular properties of microorganisms.

In most part of the earth, cold environment is predominant in nature contributing about 80% of total aquatic and terrestrial area (Yadav et al. 2017). A vast amount of the information regarding bacterial adaptation to low temperatures can be generated by analyzing bacterial population with molecular tools and techniques such as MALDI-TOF, metagenomics, genome mining, etc. (Pandey et al. 2019). In cold stress several modifications occur in microorganisms that include changes at cellular, physiological, metabolic, and molecular level (Phadtare 2004). To thrive in such inhospitable conditions, cold-adapted microorganisms use a vast array of strategies that include maintenance of membrane fluidity, accumulation and synthesis of compatible solutes, antifreezing compounds, differential gene expression, change in protein as well as total soluble protein within the cell, RNA degradasomes, and ice nucleator proteins (Mishra et al. 2010). Microorganisms that are mostly predominant in cold environment are categorized as psychrophiles and psychrotolerant. According to Schmidt-Nielsen (1902), term psychrophile is used for those microorganisms which are able to grow at 0 °C. Stokes, in 1963, termed psychrophiles as those microorganisms which are able to grow at 0 °C and

macroscopically visible within 1 week. Psychrophiles are those which can grow at temperatures ranging from −20 to 25 °C. These groups of microorganisms however are unable to grow at temperatures above 15 °C, whereas psychrotolerant microorganisms grow optimally at 20–25 °C and are metabolically active even at 0 °C (Santiago et al. 2016). Terms like *Stenopsychrophiles* and *Eurypsychrophiles* are often used to indicate narrow and wide range of temperature tolerance of microorganisms, respectively (Atlas and Bartha 1998). Obligate psychrophiles can be classified as stenothermal psychrophiles, whereas facultative psychrophiles, often termed as psychrotrophs, are generally classified as eurythermal psychrophiles (Feller and Gerday 2003).

Due to their broad range of temperature tolerance, psychrotolerant microorganisms are more abundant than psychrophilic microorganisms (Yadav et al. 2017). Extreme environments like temperature regions are hotspot for microbial diversity. Cold-tolerant microbial communities include archaea, fungi, algae, and different phyla of domain bacteria (Zachariah and Das 2017). While bacteria dominate and are present in greater diversity than archaea in polar environments, archaea are widespread in cold, deep ocean waters (Mishra et al. 2010). *Flavobacterium*, *Janthinobacterium*, *Kocuria*, *Lysinibacillus*, *Methylobacterium*, *Microbacterium*, *Pseudomonas*, *Psychroflexus*, *Paenibacillus*, *Halorubrum*, *Arthrobacter*, *Providencia*, *Brevundimonas*, *Serratia*, *Citricoccus*, *Azotobacter*, *Clostridium*, *Exiguobacterium*, *Hydrogenophaga*, *Burkholderia*, *Enterobacter*, *Azospirillum*, *Pseudoalteromonas*, *Moraxella*, *Psychrobacter*, *Polaromonas*, *Polaribacter*, *Moritella*, *Vibrio*, *Bacillus* and *Micrococcus*, *Methanogenium*, *Methanococcoides*, *Candida*, *Cryptococcus*, *Penicillium*, *Cladosporium*, and *Chloromonas* species are some of the microorganisms that are well adapted to colder regions (Feller and Gerday 2003; Bhandari et al. 2020). Yeasts have been reported to be better adapted to low temperatures in comparison to bacteria (Buzzini and Margesin 2014). These cold-adapted microorganisms are gaining attention as they are a treasure trove of bioactive compounds and enzymes of biotechnological and industrial significance (Santiago et al. 2016).

16.2 How Does Psychrophiles and Psychrotrophs Grow at Lower Temperature?

Adaptive changes in cellular constituents, viz., proteins and lipids, are key determinants that are used by psychrophiles and psychrotrophs. These adaptations may be phenotypic or genotypic. Genotypic adaptations are those adaptations that arise over an evolutionary time scale which are easily observed in interspecies, and phenotypic adaptations are those that occur within the lifetime of organisms which may be seasonal or not. Genotypic adaptations of organisms are the results of Darwinian selection that are adapted by organisms in continued stress. Genotypic adaptation of psychrophiles and psychrotrophs represents sum of end points in phenotypic adaptations also, and different organisms reach to this end point, viz., combination of phenotypic as well as genotypic adaptation in different time and

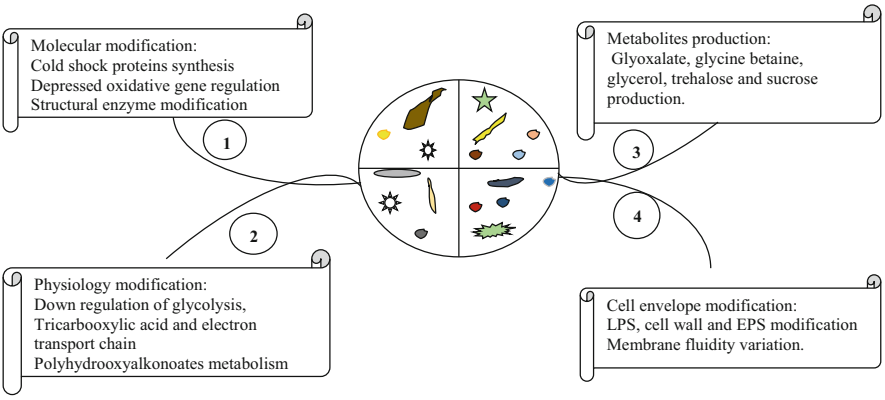

Fig. 16.1 Survival strategies adopted by cold native microorganisms

different ways, and their extent level is also different. However phenotypic adaptations provide an advantage by providing the mechanisms that are involved (Hochachka and Somero 1984). Various changes at structural, physiological, and molecular level occur such as regulation of metabolism in response to the low temperature conditions, maintenance of cellular integrity, etc. An increased expression of enzymes and pigments has been reported in microorganisms native to cold climates. Low temperatures also induce a shift in carbon source utilization as well increase their susceptibility toward antibiotics (Mishra et al. 2010). *Cryptococcus* genus has been reported to be dominantly present in cold regions due to its ability to produce a polysaccharide capsule which serves as an additional survival strategy for the organism (Buzzini and Margesin 2014) (Fig. 16.1).

16.3 Role of Metabolic Pathway in Cold Adaptation

With decrease in temperature, growth rate decreases as lag phase of bacteria extends before growth which finally results in decrease in cell number. During lag phase some major physiological changes occur that include decrease in saturation of fatty acid and synthesis of DNA, RNA, and proteins stops (Berry and Foegeding 1997). With decrease in temperature, major pathway like glycolysis, tricarboxylic acid (TCA), and electron transport chain get depressed. Analysis of psychrophile *Psychrobacter arcticus* 273–4 revealed that it can grow at temperature of 10 °C and lacks major genes that are responsible for glycolysis and lacks phosphotransferase system but possesses enzymes for gluconeogenesis pathway. Phenotypic analysis of this bacteria revealed that major energy metabolism pathway depends upon acetate pathway as acetate can easily diffuse through the membrane. Metabolic activity at given temperature is a function of two variables, viz., temperature function (Q_{10}) and temperature characteristics (μ). Most of the substrates have temperature coefficient value lower for psychrophiles as compared to mesophiles

(Ingraham and Bailey 1959). Microorganisms have been isolated from cold stress condition, and their metabolic activity has been checked to find out basic and advanced changes that are occurring during thermal adaptability. In a given research, a strain has been isolated from Arctic sea, e.g., *Psychromonas ingrahamii*, and its generation time was measured and was found to be 24 h at −12 °C which is much higher when compared to generation time at 5 °C that was 12 h, and this was the first case of growth analysis at −12 °C (Breezee et al. 2004). Using cell-free system study, it was found that rate of transcription and translation is relatively slow when compared to its optimum temperature.

16.4 Polyhydroxyalkonoate Metabolisms

Several microorganisms seem to synthesize polyhydroxyalkonoates (PHA) that are considered to be a reserve polymer under cold stress. This PHA seems to play a very important role in survival strategy adapted by microorganisms under cold stress. Genomic analysis of *C. psychrerythraea* revealed that there is a duplication of gene like enoyl-CoA hydratase, and acyl CoA dehydrogenase occurs that shows number of versatile compounds that can be synthesized by PHAs (Methe et al. 2005). Proteome analysis of *S. alaskensis* suggests that there is increase in number of enzymes at low temperature that are related to PHA metabolism, and these enzymes seem to compensate the reduced enzymatic activity or poor nutritional transport as a survival strategy acquired by *S. alaskensis* under cold stress (Ting et al. 2010). Among all the proteins, the major protein was found to be phasin which is a granule-associated protein that plays an important role in stress protection (Mezzina and Pettinari 2016). This finding suggests that cold adaptation in *S. alaskensis* not only involves de novo synthesis of PHA but also involves enzyme secretion for scavenging extracellular PHA (Ting et al. 2010).

16.5 Temperature-Induced Modifications in Cell Membrane

Cell membrane consists of phospholipids that are arranged in the form of bilayer with polar head groups which are at periphery and thus can interact with aqueous phase inside and outside the cell (Neidhart et al. 1990). It is a very well-established fact that microorganisms are able to adjust their lipid composition within membrane in response to fluctuation in temperature. This helps bacteria to maintain the solute transport rate and enzymatic activity in such extreme conditions (Brown and Minnikin 1973). At low temperatures, changes tend to occur in fatty acid and glycolipid compositions that lead to change in membrane fluidity (Berry and Foegeding 1997). These changes cause decreased membrane permeability and impaired membrane functions due to low temperature-induced gel-phase transitions; therefore, in order to counter these effects, the lipid bilayer must have proper fluidity under freezing conditions (Feller and Gerday 2003). Generally, at low temperatures, unsaturation of fatty acid within the cell membrane and reduced fatty acid chain

length are observed which result in an increased membrane fluidity as increased unsaturation destabilizes the steric constraints and improves their mobility by decreasing packing density. In *Clostridium botulinum* with drop in temperature from 37 °C to 8 °C, level of unsaturation in fatty acid chain occurs from 27% to 40% with the help of desaturase enzyme that is localized within the membrane itself (Berry and Foegeding 1997). Average fatty acid chain length is also shortened that results in fewer carbon to carbon interaction which allow membrane fluidity to increase in the cell membrane (Russell 1990). Increased rate of unsaturation of fatty acids in cold stress has been reported in one of the Gram-positive strain, e.g., *Micrococcus roseus*, and a Gram-negative bacterial strain, e.g., *Sphingobacterium antarcticus* (Chattopadhyay and Jagannadham 2001).

Another mechanism for cold adaptation was observed in *Salmonella* spp. and *C. botulinum*, where an increase in branched chain fatty acids and decreased numbers of cyclic fatty acids were observed (Russell 1984; Evans et al. 1998). To maintain the content of fatty acids in response to cold stress, increase in one fatty acid content may result in decrease of other fatty acid contents and vice versa. For example, as there is a decreased amount of laurate in the cell in response to cold stress, an increase in palmitoleate content was observed (Carty et al. 1999). Since palmitoleate is an unsaturated fatty acid, this increase clearly indicates that unsaturation in fatty acid content is directly linked to membrane fluidity as it lowers the phase transition. Certain research reported that in response to cold stress, acyltransferase Lpx P gets activated, and this seems to be the responsible for attaching palmitoleate to lipid A (Vorachek-Warren et al. 2002). In *Bacillus subtilis* desaturation of membrane fatty acid occurs in already existing phospholipids, and this was done by desaturase (Des) which is regulated by DesK sensor kinase and DesR response regulators (Aguilar and De Mendoza 2006). When there is a shift toward cold temperature, there is activation of DesK kinase; DesK phosphorylates the Des R which is a transcriptional activator which further activates des gene promoter, and in return this des gene promoter expresses D5-desaturase. This D5-desaturase catalyzes the addition of double bond in already existing fatty acid chain inside the cell (Aguilar and De Mendoza 2006; Albanesi et al. 2004).

Microorganisms (*Psychrotolerant* and *Psychrophiles*) that are adapted to low temperature tend to have reduced number of branched chain lipid and increased number of unsaturated fatty acid as compared to mesophiles and thermophiles (Morita 1975). In thermophiles, there is high level of saturated and branched chain fatty acid to make membrane stiff at higher temperatures. Membrane transport proteins also play an important role during survival of microorganisms at different temperatures, and *Psychrophiles* thus have much more efficient mechanisms of adaptation than mesophiles (Jay 1986). Envelope of Gram-negative bacteria consists of inner and outer membrane separated via periplasmic space. Outer membrane of Gram-negative consists of lipopolysaccharide, proteins, and phospholipids. LPS contain lipid A anchored to the membrane, external O-polysaccharide, and intermediate oligosaccharide component. In case of *P. haloplanktis*, high percentage of cell envelope gene is observed in the genome and concluded that it might somehow help in countering the effect of cold (Médigue et al. 2005). A similar result was observed

when *S. alakensis* drops to temperature of 10 °C. Its proteome analysis revealed that there is upregulation severalfold of genes that are related to envelope biogenesis and exopolysachharide biosynthesis (Ting et al. 2010).

In the case of Gram-positive bacteria, the cell envelope bacteria consist of cell wall and inner membrane. Under cold stress thickening of cell wall occurs like increase in cell wall biosynthesis gene observed in *E. sibiricum* at 2.5 °C, which suggests that thickening of cell wall prevents the cell from disruption during ice formation (Rodrigues et al. 2008). *Planococcus halocryophilus* has an unusual cell envelope which consists of characteristic surrounding encrustation when put under cold stress. Transcriptomic analysis of this microorganisms also revealed that genes that are relevant to peptidoglycan synthesis and its precursor compounds were upregulated. Microscopic analysis results show that there is increase in hydrophobicity content with 20% calcium carbonate, 29% choline, and 50% increase in peptidoglycan along with increase of several copies of gene encoding carbonic anhydrase which is responsible for mineralization of calcium carbonate. There is another surprising feature of *P. halocryophilus* at zero temperature which is an increase in fatty acid saturation percentage with decrease in temperature (Mykytczuk et al. 2013). It has been postulated that *P. halocryophilus* adapts a different mechanism for preserving membrane fluidity (Ronholm et al. 2015). EPS (exopolysaccharide) is a constituent of extracellular polymers surrounding bacterial cells and also one of the important factors that are required to cope with cold environments (Mancuso Nichols et al. 2004). *Pseudoalteromonas* sp. that is isolated from Arctic marine regions produces a complex EPS that contains mannose as one of the main components. This finding suggests that EPS production along with some modification in it is the key feature shared by cold-adaptive microorganisms (Corsaro et al. 2004).

16.6 Enzyme Adaptations in Cold Stress

Enzyme plays a very crucial role in all the metabolic and molecular activities that are occurring inside the cells under low temperature conditions. Cold stress hampers the normal metabolic functioning and chemical reactions in microorganisms; thus it becomes important for the psychrophiles and psychrotolerant microorganisms to maintain an optimally working enzymatic machinery for catalyzing the reactions at normal rate. The microorganisms occupying these places express cold-active enzymes that are heat labile and maintain high activity, i.e., ten times higher than their mesophilic homologues under low temperature conditions (Feller and Gerday 2003). For instance, a cold-active superoxide dismutase was isolated from *Deschampsia antarctica.* The enzyme was found to be highly thermostable, showing its optimal activity at 20 °C. However, it retains 80% of its activity when temperature is reduced to 0 °C (Rojas-Conteras et al. 2015). High activity is generally attained in these enzymes by destabilization of their active sites which increases the flexibility of its catalytic center under freezing conditions. Thus, particular enzymes for a particular physiological process are expressed under cold stress, and ribonuclease

(Reddy et al. 2004), alkaline phosphatase (Chattopadhyay et al. 1995), and DNA-dependent RNA polymerase (Uma et al. 1999) have been identified already from Antarctic regions. The nature of both thermal adaptive enzymes and cold-adaptive enzymes is different, viz., generally thermophilic enzymes are poor catalyst, whereas cold stress enzymes require greater structural flexibility (Gerday et al. 2000). Attempts have been made to find out the difference between nature and functionality of enzymes that are isolated from mesophilic bacteria and cold stress bacteria. Amino acid profiles of cold-adapted enzymes indicate that there is a lower content of proline and arginine that cause restrictions in free backbone movements. In addition to that, there is a decreased hydrophobic amino acid content in comparison to polar amino acid with fewer disulfide bond residues (D′ Amico et al. 2002). All the weak interactions are minimized, and protein interior is generally less compact due to decreased hydrophobicity of the nonpolar core (Feller and Gerday 2003). β-Galactosidase isolated from psychrotolerant bacteria, e.g., *Arthrobacter*, retain a 50% activity when it is fed from 18 °C to 0 °C, and this activity was surprisingly 5.0 times higher than mesophilic bacteria, e.g., *E.coli* at 20 °C and 10 °C, respectively (Coker et al. 2003). This shows the adaptability of microorganisms with respect to cold stress. Besides, in comparison to their mesophilic counterparts, ΔG and ΔH values are lower in the case of reactions catalyzed by enzymes that are active under cold stress (Feller and Gerday 2003). Research postulates that there is a cooperative as well as synergistic role of intramolecular interaction that somehow promotes the thermal adaptability in microorganisms (Wintrode et al. 2000; Zartler et al. 2001). Low stability of cold-active enzymes is indicated by the fact that they show cooperativity during unfolding of the enzyme. Besides, these show high degree of unfolding reversibility as well (Feller and Gerday 2003).

To maintain the demand supply of cold-active enzymes for biotechnological and industrial purposes, various methods are being used for production such as use of molecular chaperones, stimulation of cold-active promoters, optimized heterologous host, etc. (Santiago et al. 2016). Cold-active enzymes that are active at moderate as well as high temperatures are beneficial for economic purposes and thus are helpful in various industrial processes. For instance, adding enzymes in detergents suitable for washing clothes at low temperature in order to protect their color, texture, and quality is more profitable as mesophilic enzymes are not optimally active at low temperatures. Similarly, using cold-active proteases helps to perform the process of peeling in leather industry by using tap water instead of heating at 37 °C which is required while working with mesophilic proteases (Feller and Gerday 2003).

16.7 Compounds Involved in Cold Adaptation

Many microorganisms that are adapted to cold stress tend to have accumulation of compatible solutes. These compatible solutes not only play an important role in cryoprotection and osmoprotection but also serve as nitrogen, carbon, and energy sources (Methe et al. 2005). Some important compatible solutes include trehalose,

sucrose, glycine, betaine, glycerol, sorbitol, and mannitol. These compatible solutes tend to decrease freezing point of the cytoplasm and also prevent macromolecular aggregation and stabilize cellular membrane under cold stress (Collins and Deming 2013). Glycine betaine was detected in *L. monocytogenes*, a food-borne pathogen which survives at low temperatures (Angelidis and Smith 2003). Glycine betaine acts as a molecular chaperone that prevents protein aggregation and also helps in maintaining membrane fluidity at low temperatures (Chattopadhyay 2002a). Trehalose, another cryoprotectant used by microorganisms native to low temperature regions, is a nonreducing sugar that stabilizes the membrane and proteins. Presence of exogenous trehalose is also helpful in providing protection against freezing conditions. Enzymes, trehalose-6-phosphate synthases, and trehalose-6-phosphatase are key players in trehalose biosynthesis which are encoded by genes *ots*A and *ots*B (Mishra et al. 2010). Cryoprotectants, viz., chemical substances, also protect the microorganisms from cold stress. These substances include sugars (fructose, glucose), amino acids (proline, alanine), and sugar alcohol (glycerol, mannitol). Cryoprotective role of glycine betaine has been observed in bacteria (Chattopadhyay 2002b). Glycine betaine seems to prevent aggregations of proteins that otherwise accumulate in cold temperature and promotes membrane fluidity. Two strains isolated from Antarctica, viz., *Pseudomonas haloplanktis* TAC125 and *Pseudoalteromonas* sp. TB 41, show remarkable difference in glutathione metabolism in cold stress, and this glutathione seems to promote cryotolerance in *Pseudomonas haloplanktis* TAC125 at much higher efficient rate by serving as compatible solute (Mocali et al. 2017). Strain N33 also accumulates large amount of compatible solutes like valine, threonine, and sarcosine which seems to work as cryoprotectant (Ghobakhlou et al. 2015).

16.8 Carotenoids' Role in Cold Adaptations

Carotenoids have been reported to occur in plants and some bacteria as well as fungi. Most unique feature of these molecules is presence of conjugated double bonds in the backbone structure. Due to the presence of highly delocalized π-electrons, these compounds are able to absorb the radiation in the wavelength region ranging from 400 to 500 nm. Major role of carotenoids in microorganisms is to prevent oxidative damage from reactive oxygen species (ROS) (Moliné et al. 2014). Role of carotenoids is also one of the important mechanisms adapted by microorganisms to counter cold stress. Strains like *Sphingobacterium antarcticus* and *Micrococcus roseus* isolated from Antarctic regions when subjected to fractionation seem to have carotenoids attached to membrane and promote the membrane fluidity. There is also increase in the polar carotenoids content, but amount of nonpolar carotenoid decreases. Role of polar carotenoids is somehow not clear, but it was postulated that in response to increase in unsaturated fatty acid content to increase membrane fluidity in cold stress, polar carotenoid amount increases to rigidify membrane (Chattopadhyay and Jagannadham 2001). *Phaffia rhodozyma*, a cold-adapted yeast, has been reported to be the only known species to produce astaxanthin,

Table 16.1 Some important survival strategy adapted by microorganisms with representations of microorganisms

Cold microorganism adaptations	Example	References
1. Enzyme adaptations. (a) Amino acid composition change. (b) Production of cold adaptive enzyme	*Arthrobacter psychrolactophilus* • aminopeptidase • β-galactosidase • ribonuclease • protein-tyrosine phosphatase • alkaline phosphatase • DNA-dependent RNA polymerase	Coker et al. (2003) Huston (2008) Groudieva et al. (2004) Reddy et al. (2004) Tsuruta et al. (2004) Chattopadhyay et al. (1995) Uma et al. (1999)
2. Fatty acid composition. (a) Increase in unsaturation of fatty acid in the membrane. (b) Shortening of fatty acid chain length	Gram-positive *Micrococcus roseus* Gram-negative *Sphingobacterium antarcticus* *Listeria* sp.	Chattopadhyay and Jagannadham (2001) Russell (2002)
3. Structural change. (a) Cell size increases. (b) Filament formation	*Candida utilis* *Pseudomonas putida*	Herbert (1986) Phillips et al. (1998)
4. Carotenoid production	Dihydroxycarotenoids Zeaxanthin Violaxanthin	Subczynski et al. (1992)
5. Cold shock protein. (a) CspA. (b) Hsc 25. (c) CspC. (d) CspD. (e) CspE	*E. coli* *Pantoea ananas* *C. Botulinum* *E. coli* *E. coli*	Ray et al. (1994) Kawahara et al. (2000) Derman et al. (2015) Uppal et al. (2008) Feng et al. (2001)
6. Cryoprotectant production. Glycine betaine	*L. monocytogenes*	Koo et al. (2016)
7. Antifreeze protein production (AFP)	*Marinomonas primoryensis*	Gilbert et al. (2005)
8. Heat shock protein. ClpB Htp G	*Synechococcus* PCC 7942	Porankiewicz and Clarke (1997) Hossain and Nakamoto (2003)

representing 80% of the total carotenoids produced by it (Moliné et al. 2014) (Table 16.1).

16.9 Potential Role of Cold Microorganisms in Biotechnology

Several studies are ongoing to find out the important role of cold-adaptive microorganisms in industry sector. Cold-adaptive microorganisms offer so many advantages to different industry sectors, and one of the main sectors is biotechnology

sector. Cold-adaptive microorganisms are employed in different biotechnology sectors to produce some important cold-adaptive proteins, some important biotechnology vectors that are associated with bioremediation and disease control. This property of cold-adaptive microorganisms is also very important for agriculture sector which directly contributes to countries' economy too. Some important properties of cold-adaptive microorganisms are as follows.

16.9.1 Saving Energy

Microorganisms that are adapted to low temperature (0–20 °C) are able to grow and perform various enzymatic activities at this range only; this turns out to be an advantage because other microorganisms are not able to interfere; as a result source of contamination is less. This offers an advantage to various biotechnology processes by reducing process time in removing contamination source and thus plays a very important role.

16.9.2 Promoting Plant Growth in Cold Regions

Due to poor soil nutrient availability, frost and freezing conditions, low soil fertility, and moisture content, productivity and yield of agriculturally important crops in cold regions are hampered. Plants suffer chilling, wilting chlorosis, etc. due to low temperatures. Some cold-adapted microorganisms colonize the plant rhizosphere under cold stress conditions and help the plant to combat the stress in direct or indirect manner like other plant growth-promoting microorganisms. Thus, microbial inoculants from these extreme conditions possessing beneficial attributes can be efficiently utilized as biofertilizers or biocontrol agents for promoting growth and yield of crops grown in cold regions (Bhandari et al. 2020). These microorganisms promote plant health by either frost injury protection, growth stimulation, or protection from pests and diseases. For instance, Sinorhizobium meliloti was reported to improve growth of under cold and anaerobic conditions, i.e., ice encasement (Prévost et al. 2003). Similarly, Pseudomonas lurida isolated from rhizosphere Himalayan plants was found to protect plant from chilling stress (Bisht et al. 2014). Some members of genus Pseudomonas can cause frost damage to crops, and this could be prevented by inoculating ice mutant strains, as these strains are further prevented from ice-positive strains, viz., Pseudomonas syringae (Herbert 1992).

16.9.3 Enzyme Production

Psychrophiles or psychrotrophs seemed to have very potential role in having higher number of lipase and protease activity. Lipases are involved in recovery of silver from X-ray film and integral part of food industry. Proteases are used in beer

treatment, in bakeries, in production and maturation of cheese, and in production of fermented foods. Production of both enzymes can be enhanced by cold-adaptive microorganisms; thus they became a very important part of food industry and are also used as detergent additives. The first cold-adapted enzyme was isolated from Antarctic bacteria which are further sequenced, cloned, and expressed in a recombinant host for production of subtilisins, α-amylase, and lipases, and all these enzymes are well characterized as true representative of industrial enzymes (Hoag 2008).

16.9.4 Biodegradation and Bioremediation

Biodegradation of organic contaminant in cold environment is stimulated by indigenous psychrophiles. These Psychrophiles can degrade organic contaminant to less harmful substances which are on later stage integrated to biogeochemical cycles. In most cases in cold environments, petroleum hydrocarbon bioremediation is focused because these cold regions are very much exposed to petroleum transportation and its production. These environments include alpine, permafrost, sea, sediments, and polar regions where temperature rarely exceed 10 °C. There are large number of bacteria and fungi strains that have been reported to have a potential role to bioremediate petroleum hydrocarbon (Yeargeau et al. 2009). Microorganisms that are adapted to these cold environments face many challenges like increased viscosity of liquid hydrocarbon, reduced rate of enzyme catalyzed reaction, limited bioavailability of contaminants, poor nutrient supply, and extreme pH and salinity (Aislabie et al. 2006). The most common widely used method of bioremediation in cold soil is biostimulation of indigenous microorganisms with good supply of appropriate nutrients (Walworth et al. 2007). Bioaugmentation is another method to treat petroleum contaminant where addition of cultured microorganisms is done at subsurface of soil or ground water to remove contaminants, and several studies have been done in Alaska, Greenland, and Canada, but this strategy is not well established as it gives no better results than fertilization. Bioaugmentation with nonindigenous microorganisms or genetically engineered microorganisms has already been banned in Sweden, Norway, Iceland, and Antarctica (Filler et al. 2009). Biodegradation under low temperature of another major pollutant (phenol is one of the major representatives of toxic aromatic compound in water bodies) removed by Psychrophilic bacteria (Rhodococcus spp.) and yeast (Rhodotorula psychrophenolica). They are able to remove 12.5–15 mM of phenol at 10 °C (Margesin et al. 2005).

16.9.5 Recombinant Protein System

Recombinant protein secretion system at low temperature is absolutely necessary for facilitating biotechnological application of psychrophiles. Production of cold-active enzymes by wild-type strain is usually very low. The first recombinant protein isolated was α-amylase from Antarctic Pseudomonas haloplanktis. This method of

cold gene expression system was further optimized and developed for extracellular secretion of heterologous protein in P. haloplanktis (Cusano et al. 2006). Another recombinant protein system that works at low temperature was developed by using Shewanella sp. strain (Miyake et al. 2007) by selecting a suitable promoter and a plasmid having broad host range. Much higher production of β-lactamase was observed when strain is grown at 4 °C around 64% more than that when strain is grown at 18 °C. Expression vectors are also designed in such a manner that transcript sequences which are of microorganism interest, for example, enable microorganisms to survive in response to cold stress. A number of experiments have been designed to build a series of expression vectors which are based on cold shock protein transcript sequences, e.g., CspA in E. coli as a host to produce an improved quality of proteins and also in some research CspA transcript sequence used as a promoter sequence too (Vasina and Baneyx 1996).

16.9.6 Psychrophiles and Enzymes at Industry Level

At industry level in polar regions, the most well-characterized microorganism is Candida antarctica. This microorganism is known to produce two lipases A and B. Lipase B reported to be involved in large number of organosynthesis application which seems to be very important for pharmaceutical, food processing, or cosmetics (Babu et al. 2008). In a survey it was established that in most of the patents that are registered related to Antarctica, patents of lipases produced by C. antarctica by far are most of them in product base or processed based. This is a very good example of potential biocatalyst that is isolated from genetic resources in polar regions. The market for enzymes that are commonly used as detergents comprises of 30–40% worldwide. Among all the enzymes used in detergents, subtilisins are by far large in number. Bacillus spp. for the first time reported to produce to psychrophilic subtilisins in Antarctica (Narinx et al. 1997). Pseudoalteromonas haloplanktis reported to produce xylanases, and it is a classic example of successful biotechnological transfer of academic research to application-based industry production. Xylanases are enzymes that are responsible of breaking down β-1,4 xylan to hemicelluloses which is one of the major components in plant cell wall. It is also the most important ingredient in dough conditioner and thus seems to improve bread quality. Further careful optimization of this psychrophilic xylanase results in production of highest known psychrophilic enzyme up to date, and its products are now sold by Puratos (Belgium) (Collins et al. 2003).

16.10 Molecular Adaptation in Response to Cold Stress

Jones and others (1987) for the first time reported a change in gene expression pattern in response to cold stress in *E. coli*, and this leads to induction of those proteins that are very crucial for cold adaptation in microorganisms, and these proteins are said to be cold shock proteins (Jones et al. 1992a). Cold shock proteins

can be defined as set of proteins that are involved in various important cellular process especially made at 10 °C rather than at 27 °C. Both machinery (transcription and translation) regulated in such manner that microorganisms that are exposed to low temperature get enough amount of cold shock proteins. Although the role of cold shock proteins is not well understood, the major role of cold shock protein is to recover the microorganisms from the partial block in protein synthesis during cold stress, and therefore it increases the rate of protein synthesis of those proteins that are engaged in cold stress adaptation. Mihoub et al. (2003) also reported a major shift in gene expression in *E. coli* under cold stress by using proteomics analysis. Nature of polypeptide chain in cold shock proteins can be same or different in different microorganisms. For example, cold shock proteins CspA identified in *E. coli* have a sequence similarity with two different microorganisms *Streptomyces clavuligerus* and *Bacillus subtilis*. Sequence homology of *E. coli* CspA with *B. subtilis* cold shock protein, viz., CspB, was found to be 61% (Willimsky et al. 1992), and isolated protein from *S. clavuligerus* of around 7.0 KDa has the sequence similarity of around 56% (Jones and Inouye 1994). In response to shift in temperature, many components (transcription and translation factors) of molecular machinery are still synthesized which normally block in case of those microorganisms that are not able to adapt with cold temperature stress. During lag phase when *E. coli* was subjected to a temperature of 10 °C, synthesis of cold shock proteins gets affected by regulators like guanosine 5'diphosphate 3'diphosphate (ppGpp) and 5'triphosphate 3′ diphosphate (pppGpp). Variation in the level of these regulators has been observed in low temperature. In *E.coli* trace amount of pppGpp has been found when *E. coli* is subjected to low temperature and variation in this pppGpp is directly proportional to extent of low temperature can proceed. Mutant experiment analysis provides the evidence that mutant microorganisms that do not contain a detectable amount of pppGpp when exposed to cold stress (10 °C) seem to promote induction of many cold shock proteins and increase level of many transcriptional and translational proteins. Jones et al. (1992b) suggested that there is an inverse relationship between pppGpp and cold shock proteins, viz., downregulation of pppGpp somehow upregulates the cold shock proteins at 10 °C, and it is somehow related to adaptation to cold stress. Not only cold stress but many chemicals like tetracycline and chloramphenicol which are known to inhibit bacterial protein synthesis also seem to induce many cold shock proteins. These compounds are responsible for downregulating the pppGpp level, and this reduction in pppGpp level upregulates the cold shock proteins (Van Bogelen and Neidhardt 1990).

Cold shock proteins have been found in many genera of microorganisms including *Bacillus cereus* (Berry and Foegeding 1996), *Pseudomonas fragi* (Berry and Foegeding 1997), *Trichosporon pullulans* (Juleseth and Inniss 1990), and *Vibrio vulnificus* (McGovern and Oliver 1995). More than 50 different cold shock proteins have been identified, and their number and their level of expression vary from species to species. The number of cold shock proteins and level of their expression depend upon the extent of temperature drop. For example, *Pseudomonas fragii* is known to produce 15 cold shock proteins when temperature drops from 20 °C to 5 °C (Russell 2002). Cold shock responses of psychrotrophic bacteria when fed to

temperature close to freezing are different from the cold shock responses of mesophilic bacteria (Hebraud and Potier 1999). The second group of cold-induced proteins was identified as cold acclimation proteins (caps) that seem to be very similar with Csps (cold shock proteins) and continuously synthesized during prolonged growth at low temperature which can be easily differentiated from mesophiles and psychrophiles. At DNA level when subjecting *E. coli* to cold stress, DNA gets more negatively super coiled, and negative supercoiling is also somehow related to environmental factors such as change in osmolarity (Higgins et al. 1988). In cold stress stabilization of secondary structure is necessary other than it can affect the process of transcription and translation. A main function of these cold shock proteins is to prevent any such secondary structure and also facilitates the degradation of structured RNA. Csps family includes nucleic acid chaperones which target any unnecessary secondary structures. Helicase DeaD also falls under Csps family, and they are also responsible for removing any such secondary structure with the help of exoribonuclease RNase R and PNPase. RNase R have been isolated from *E. coli* that contain 3–59 exonuclease that efficiently removes double-stranded RNA (Matos et al. 2009; Phadtre 2004). The role of these cold shock proteins can be similar or different from each other in terms of their chaperone activity, and chaperone activity of these Csps is very crucial for maintaining mRNA stability. CspsA is one of the major cold shock proteins as it destabilizes secondary structure. CspE acts in an opposite manner as it binds to poly-A tail of mRNA which prevents its degradation by exoribonuclease like PNPase and RNase E (Feng et al. 2001). Thus, CspA and CspE act antagonistically but at last promote microorganism's survival under cold stress.

16.11 Bacterial Adaptations to Cold Stress

Microbiomes of the extreme environment impart important information about the critical limits for survival and adaptability of microorganisms. Bacterial communities are also adapted to cold regions in such manner that there is a functional abundance of those genes that are very important for survival in cold stress. Metagenomics analysis of cyanobacterial mats from the Antarctic and Arctic revealed that there is an abundance of genes that are involved in membrane modification, cold shock protein synthesis, and exopolysachharide synthesis (Varin et al. 2012). Several metagenomics datasets that are taken from ice-covered regions of Antarctic Lake Joyce showed that there is an induction of antifreeze proteins, cold shock proteins (CspA, CspB, CspC, CspD, CspE, and CspG), cold shock DEAD-box protein A, trehalose synthase, and ice nuclear protein (Koo et al. 2016). Although there are many research in progress to discover the variation in microbial community and adaptation in cold stress, coexistence of many metabolic groups that are yet to be identified results in complexity of understanding microbial communities. Recent study also revealed permafrost communities typically use highly diverse and complex type of biochemical process that are involved in organic matter decomposition, carbon processing, methane generation, and nitrogen cycling.

This is also one of the big reasons of complexity that arise in microbial communities which are adapted to cold stress (MacKelprang et al. 2017).

16.11.1 Heat Shock Protein Role in Cold Stress

Heat shock proteins (HSP) are ubiquitously expressed protein in microorganisms which promote growth of microorganisms against thermal stress, but also these heat shock proteins promote survival of microorganisms in cold stress. Some known heat shock proteins are expressed in *E. coli* during heating of these microorganisms at 42 °C before putting it to −80 °C, and this heating somehow promotes survival of these microorganisms at such extreme cold temperature because there is an accumulation of heat shock proteins (Chow and Tung 1998). Another HSP called Clp B tends to increase in *Synechococcus* PCC 7942, a cyanobacterial strain, when this strain is transferred from 37 °C to 25 °C. In mutant experiment where Clp B is knocked out, there is a repression in photosynthetic activity of these microorganisms observed (Porankiewicz and Clarke 1997). Another HSP, viz., Htp G from *Synechococcus* PCC 7942, is also under cold stress, and this was too confirmed by mutation experiments. This protein helps microorganism in photosynthetic activity, and this was confirmed by Western blotting (Hossain and Nakamoto 2003). Although these HSP help microorganisms to counter cold stress, these HSP not only induce cold stress condition, but also other environmental factors can initiate these HSP. These HSP seem to promote correct protein folding which gets distorted during thermal stress.

Other compounds termed as cold acclimation proteins (caps) are a set of 20 proteins that are present in cold-adapted microorganisms. These proteins ensure improved protein synthesis, continued growth, and cell cycle regulation at low temperatures (Margesin et al. 2005). Hsc 25, an example of Caps, is produced by *Pantoea ananas* KUIN-3, an ice-nucleating bacterium, that was found to be useful in refolding of cold-denatured enzymes (Kawahara et al. 2000). Cold shock proteins (Csps) are expressed in microorganisms when they are exposed to a downshift in temperature. This cold shock response is not only specific to psychrophiles or psychrotolerant microorganisms but all the microorganisms that are exposed to such temperature shifts (Mishra et al. 2010). Csps are involved in RNA folding and stabilization of secondary structures of macromolecules and regulate protein synthesis in low temperature stress. Regulation of Csps occurs at the level of transcription and translation (Horn et al. 2007). Ice nucleator proteins prevent supercooling at temperatures below 0 °C by forming crystal-like arrangements on water molecules, thus reducing the energy required for ice formation (Zachariassen and Kristiansen 2000). Ice-nucleating agents perform cellular protection by either establishing extracellular freezing instead of intracellular freezing that is lethal toward the cell or releasing the heat of fusion (Mishra et al. 2010). Based on presence of Ina protein, on bacterial cell wall microorganisms are categorized into ice-plus and ice-minus. Ina acts as a nucleating center for the formation of ice crystals.

Erwinia herbicola, *Pseudomonas*, and *Xanthomonas* have been reported as potent ice nucleators (Lindow et al. 1978; Maki et al. 1974; Obata et al. 1990).

16.11.2 Antifreeze Protein Role in Cold Stress

Antifreeze proteins (AFPs) get accumulated in cold stress and promote survival of microorganisms in cold stress. The role of (AFPs) has been already observed in blood of fishes. Not only in fishes but also in plants and in insects role of AFPs has been identified. The role of thermal hysteresis for the first time in bacteria is observed in strain of *Moraxella* sp., and not only that, the first AFPs were also found in this strain (Duman et al. 2004). Thermal hysteresis value of AFPs that are isolated from microorganisms is lower as compared to thermal value of AFPs isolated from animals. Calcium-dependent AFPs were also found in isolates of *Marimonas primoryensis* that are isolated from Antarctic regions (Gilbert et al. 2005).

16.11.3 Ribonuclease Role in Cold Stress

Degradosome is a multisubunit protein complex which is responsible for maintaining stability of RNA. This stability of RNA is somehow regulatory mechanisms in protein synthesis and in protein degradations. Degradosome protein isolated from *Pseudomonas syringae* of Antarctic regions has similar kind of degradosome that has been isolated from *E. coli*. But in addition to that, degradosome protein complex of *P. syringae* has an addition of another endoribonucleases, e.g., RNase E, RNase R, and RNA helicase. The role of RNase E is somehow not clear, but role of RNase R is to degrade any protein secondary structure that can be seen during cold stress (Purusharth et al. 2005).

16.12 Conclusion

Low temperature conditions strongly influence growth and metabolism of organisms that limit their growth. But metabolic and molecular adaptations by the microorganisms provide a very significant prospectus in terms of modification in metabolic pathway and switch on and off of particular gene through operon system or just by regulating gene at post-transcription and post-translation modification. Cold-adaptive microorganisms possess different patterns of gene expression and accumulation of cold-adaptive proteins which offer so many advantages to biotechnology sectors that result into ongoing research to identify more and more cold-accumulated proteins. Different numbers of cold-adaptive protein are yet to be identified that will unlock role of various mysterious players in cold adaptation strategy.

References

Aguilar PS, De Mendoza D (2006) Control of fatty acid desaturation: a mechanism conserved from bacteria to humans. Mol Microbiol 62(6):1507–1514

Aislabie J, Saul DJ, Foght JM (2006) Bioremediation of hydrocarbon-contaminated polar soils. Extremophiles 10:171–179

Albanesi D, Mansilla MC, De Mendoza D (2004) The membrane fluidity sensor DesK of Bacillus subtilis controls the signal decay of its cognate response regulator. J Bacteriol 186 (9):2655–2663

Angelidis AS, Smith GM (2003) Role of glycine betaine and carnitine transporters in adaptation of Listeria monocytogenes to chill stress in defined medium. Appl Environ Microbiol 69:7492–7498

Atlas RM, Bartha R (1998) Microbial ecology: fundamentals and applications, 4th edn. Benjamin/ Cummings Science Publishers, Menlo Park, CA, pp 1–704

Babu E, Ramteke PW, Thomas G (2008) Cold active microbial lipases: some hot issues and recent developments. Biotechnol Adv 26:457–470

Berry ED, Foegeding PM (1996) Cold shock proteins and cold shock domains in Bacillus cereus. In Proceedings of the 96th General Meeting of American Society of Microbiology, Washington, DC, pp 19–23

Berry ED, Foegeding PM (1997) Cold temperature adaptation and growth of microorganisms. J Food Prot 60(12):1583–1594

Bhandari G, Nautiyal N, Sharma M (2020) Assessment of genes and enzymes of microorganisms of high altitudes and their application in agriculture. In: Microbiological advancements for higher altitude agro-ecosystems & sustainability. Springer, Singapore, pp 307–326

Bisht SC, Joshi GK, Mishra PK (2014) CspA encodes a major cold shock protein in Himalayan psychrotolerant Pseudomonas strains. Interdiscip Sci Comput Life Sci 6(2):140–148

Breezee JN, Cady JT, Staley (2004) Subfreezing growth of the sea ice bacterium "Psychromonas ingrahamii". Microbial Ecol 47:300–304

Brown CM, Minnikin DE (1973) Effect of growth temperature on fatty-acid composition of some psychrophilic marine pseudomonads. J General Microbiol 75:R9

Buzzini P, Margesin R (2014) Cold-adapted yeasts. In: Miscellaneous cold-active yeast enzymes of industrial importance. New York, Springer

Carty SM, Sreekumar KR, Raetz CR (1999) Effect of cold shock on lipid a biosynthesis in Escherichia coli induction at 12° C of an acyltransferase specific for palmitoleoyl-acyl carrier protein. J Biol Chem 274(14):9677–9685

Chattopadhyay MK (2002a) Glycine betaine is a bacterial cryoprotectant and is believed to act by stabilizing cellular proteins and membranes at low temperature. Resonance 7:59–63

Chattopadhyay MK (2002b) Bacterial cryoprotectants. Resonance 7(11):59–63

Chattopadhyay MK, Devi KU, Gopishankar Y, Shivaji S (1995) Thermolable alkaline phosphatase from *Sphingobacterium antarcticus*, a psychrotrophic bacterium from Antarctica. Polar Biol 15:215–219

Chattopadhyay MK, Jagannadham MV (2001) Maintenance of membrane fluidity in Antarctic bacteria. Polar Biol 24:386–388

Chow KC, Tung WL (1998) Overexpression of dnaK/dnaJ and groEL confers freeze tolerance to Escherichia coli. Biochem Biophys Res Commun 253:502–505

Coker JA, Sheridan PP, Loveland-Curtze J, Gutshall KR, Auman AJ, Brenchley JE (2003) Biochemical characterization of a β-galactosidase with a low temperature optimum obtained from an Antarctic *Arthrobacter* isolate. J Bacteriol 185:5473–5482

Collins RE, Deming JW (2013) An inter-order horizontal gene transfer event enables the catabolism of compatible solutes by Colwellia psychrerythraea 34H. Extremophiles 17:601–610

Collins T, Meuwis MA, Gerday C, Feller G (2003) Activity, stability and flexibility in glycosidases adapted to extreme thermal environments. J Mol Biol 328:419–428

Corsaro MM, Lanzetta R, Parrilli E, Parrilli M, Tutino ML, Ummarino S (2004) Influence of growth temperature on lipid and phosphate contents of surface polysaccharides from the antarctic bacterium Pseudoalteromonas haloplanktis TAC 125. J Bacteriol 186:29–34

Cusano AM, Parrilli E, Duilio A, Sannia G, Marino G, Tutino ML (2006) Secretion of psychrophilic alpha-amylase deletion mutants in Pseudoalteromonas haloplanktis TAC125. FEMS Microbiol Lett 258:67–71

D'Amico S, Claverie P, Collins T, Georlette D, Gratia E, Hoyoux A, Meuwis M-A, Feller G, Gerday C (2002) Molecular basis of cold adaptation. Philos Trans R Soc London B Biol Sci 357:917–925

Derman Y, Söderholm H, Lindström M, Korkeala H (2015) Role of csp genes in NaCl, pH, and ethanol stress response and motility in *Clostridium botulinum* ATCC3502. Food Microbiol 46:463–470. https://doi.org/10.1016/j.fm.2014.09.004

Duman JG, Bennett V, Sformo T, Hochstrasser R, Barnes BM (2004) Antifreeze proteins in Alaskan insects and spiders. J Insect Physiol 50(4):259–266

Evans RI, McClure PJ, Gould GW, Russell NJ (1998) The effect of growth temperature on the phospholipid and fatty acyl compositions of non-proteolytic Clostridium botulinum. Int J Food Microbiol 40(3):159–167

Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1(3):200–208

Feng P, Everly DN, Read GS (2001) mRNA decay during herpesvirus infections: interaction between a putative viral nuclease and a cellular translation factor. J Virol 75(21):10272–10280

Filler DM, van Stempvoort DR, Leigh MB (2009) Remediation of frozen ground contaminants with petroleum hydrocarbons: feasibility and limits. In: Margesin R (ed) Permafrost soils. Springer, Berlin, pp 279–301

Gerday C, Aittaleb M, Bentahir M, Chessa J-P, Claverie P, Collins T, D'Amico S, Dumont J, Garsoux G, Georlette D, Hoyoux A, Lonhienne T, Meuwis M-A, Feller G (2000) Cold-adapted enzymes:from fundamentals to biotechnology. Trends Biotechnol 18:103–107

Ghobakhlou AF, Johnston A, Harris L, Antoun H, Laberge S (2015) Microarray transcriptional profiling of Arctic Mesorhizobium strain N33 at low temperature provides insights into cold adaption strategies. BMC Genomics 16:383

Gilbert JA, Davies PL, Laybourn-Parry J (2005) A hyperactive, Ca2+−dependent antifreeze protein in an Antarctic bacterium. FEMS Microbiol Lett 245(1):67–72

Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, Antranikian G (2004) Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 8(6):475–488

Hébraud M, Potier P (1999) Cold shock response and low temperature adaptation in psychrotrophic bacteria. J Mol Microbiol Biotechnol 1(2):211–219

Herbert RA (1986) The ecology and physiology of psychrophilic microorganisms. In: Herbert RA, Codd GA (eds) Microbes in extreme environments. The Society for General Microbiology, Academic Press, London, pp 1–24

Herbert RA (1992) A perspective on the biotechnological potential of extremophiles. Trends Biotechnol 10:395–401

Higgins CF, Dorman CJ, Stirling DA, Waddell L, Booth IR, May G, Bremer E (1988) A physiological role for DNA supercoiling in the osmotic regulation of gene expression in S. typhimurium and E. coli. Cell 52(4):569–584

Hoag H (2008) Polar biotech. Nature Biotechnol 26:1204

Hochachka PW, Somero GN (1984) Biochemical adaptation. Princeton University Press, Princeton, NJ, p 538

Horn G, Hofweber R, Kremer W, Kalbitzer HR (2007) Structure and function of bacterial cold shock proteins. Cell Mol Life Sci 64(12):1457–1470

Hossain MM, Nakamoto H (2003) Role for the cyanobacterial HtpG in protection from oxidative stress. Curr Microbiol 46(1):0070–0076

Huston AL (2008) Biotechnological aspects of cold-adapted enzymes. In: Psychrophiles: from biodiversity to biotechnology, pp 347–363
Ingraham JL, Bailey GF (1959) Comparative study of effect of temperature on metabolism of psychrophilic and mesophilic bacteria. J Bacteriol 77(5):609
Jay JM (1986) Modern food microbiology, 3rd edn. Van Nostrand Reinhold, Wayne, UK, p 214
Jones PG, Cashel M, Glaser G, Neidhardt FC (1992a) Function of a relaxed-like state following temperature downshifts in Escherichia coli. J Bacteriol 174(12):3903–3914
Jones PG, Inouye M (1994) The cold-shock response—a hot topic. Mol Microbiol 11(5):811–818
Jones PG, Krah R, Tafuri SR, Wolffe AP (1992b) DNA gyrase, CS7. 4, and the cold shock response in Escherichia coli. J Bacteriol 174(18):5798–5802
Julseth CR, Inniss WE (1990) Induction of protein synthesis in response to cold shock in the psychrotrophic yeast Trichosporon pullulans. Can J Microbiol 36(8):519–524
Kawahara H, Koda N, Oshio M, Obata H (2000) A cold acclimation protein with refolding activity on frozen denatured enzymes. Biosci Biotechnol Biochem 64(12):2668–2674
Koo H, Hakim JA, Fisher PR, Grueneberg A, Andersen DT, Bej AK (2016) Distribution of cold adaptation proteins in microbial mats in Lake Joyce, Antarctica: analysis of metagenomic data by using two bioinformatics tools. J Microbiol Methods 120:23–28
Lindow SE, Arny DC, Upper CD (1978) Erwinia herbicola: a bacterial ice nucleus active in increasing frost injury to corn. Phytopathology 68:523–527
Mackelprang R, Burkert A, Haw M, Mahendrarajah T, Conaway CH, Douglas TA, Waldrop MP (2017) Microbial survival strategies in ancient permafrost: insights from metagenomics. ISME J 11(10):2305–2318
Maki IR, Galyon EL, Chang-Chien M, Cald WDR (1974) Ice nucleation induced by Pseudomonas syringae. Appl Microbiol 28:456–460
Mancuso Nichols CA, Garon S, Bowman JP, Raguénès G, Guézennec J (2004) Production of exopolysaccharides by Antarctic marine bacterial isolates. J Appl Microbiol 96:1057–1066
Margesin R, Fonteyne PA, Redl B (2005) Low-temperature biodegradation of high amounts of phenol by Rhodococcus spp. and basidiomycetous yeasts. Res Microbiol 156:68–75
Matos RG, Barbas A, Arraiano CM (2009) RNase R mutants elucidate the catalysis of structured RNA: RNA-binding domains select the RNAs targeted for degradation. Biochem J 423:291–301
McGovern VP, Oliver JD (1995) Induction of cold-responsive proteins in Vibrio vulnificus. J Bacteriol 177(14):4131–4133
Médigue C, Krin E, Pascal G, Barbe V, Bernsel A, Bertin PN, Cheung F, Cruveiller S, D'Amico S, Duilio A et al (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium Pseudoalteromonas haloplanktis TAC125. Genome Res 15:1325–1335
Methe BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang X, Moult J, Madupu R, Nelson WC, Dodson RJ et al (2005) The psychrophilic lifestyle as revealed by the genome sequence of Colwellia psychrerythraea 34H through genomic and proteomic analyses. Proc Natl Acad Sci U S A 102:10913–10918
Mezzina MP, Pettinari MJ (2016) Phasins, multifaceted polyhydroxyalkanoate granule-associated proteins. Appl Environ Microbiol 82(17):5060–5067
Mihoub F, Mistou MY, Guillot A, Leveau JY, Boubetra A, Billaux F (2003) Cold adaptation of Escherichia coli: microbiological and proteomic approaches. Int J Food Microbiol 89 (2–3):171–184
Mishra PK, Joshi P, Bisht SC, Bisht JK, Selvakumar G (2010) Cold-tolerant agriculturally important microorganisms. In: Plant growth and health promoting bacteria. Springer, Berlin, pp 273–296
Miyake R, Kawamoto J, Wei YL, Kitagawa M, Kato I, Kurihara T, Esaki N (2007) Construction of a low-temperature protein expression system using a coldadapted bacterium, Shewanella sp. strain Ac10, as the host. Appl Environ Microbiol 73(15):4849–4856

Mocali S, Chiellini C, Fabiani A, Decuzzi S, Pascale D, Parrilli E, Tutino ML, Perrin E, Bosi E, Fondi M et al (2017) Ecology of cold environments: new insights of bacterial metabolic adaptation through an integrated genomic-phenomic approach. Sci Rep 7:839

Moliné M, Libkind D, de Garcia V, Giraudo MR (2014) Production of pigments and photoprotective compounds by cold-adapted yeasts. In: Cold-adapted Yeasts. Springer, Berlin, pp 193–224

Morita RY (1975) Psychrophilic Bacteria. Bacteriol Rev 39:144–167

Mykytczuk NCS, Foote SJ, Omelon CR, Southam G, Greer CW, Whyte LG (2013) Bacterial growth at 15 °C; molecular insights from the permafrost bacterium Planococcus halocryophilus Or1. ISME J 7:1211–1226

Narinx E, Baise E, Gerday C (1997) Subtilisin from psychrophilic Antarctic bacteria: characterization and site-directed mutagenesis of residues possibly involved in the adaptation to cold. Protein Eng 10:1271–1279

Neidhardt FC, Ingraham JL, Schaechter M (1990) Physiology of the bacterial cell: a molecular approach, vol 20. Sinauer Associates, Sunderland, MA

Obata H, Kakinami K, Tanishita J, Hasegawa Y (1990) Identification of new ice-nucleating bacterium and its ice nucleation properties. Agric Biol Chem 54:725–730

Pandey A, Jain R, Sharma A, Dhakar K, Kaira GS, Rahi P, Shouche YS (2019) 16S rRNA gene sequencing and MALDI-TOF mass spectrometry based comparative assessment and bioprospection of psychrotolerant bacteria isolated from high altitudes under mountain ecosystem. SN Appl Sci 1(3):1–12

Phadtare S (2004) Recent developments in bacterial cold-shock response. Curr Issues Mol Biol 6:125–136

Phillips LE, Humphrey TJ, Lappin-Scott HM (1998) Chilling invokes different morphologies in two Salmonella enteritidis PT4 strains. J Appl Microbiol 84(5):820–826

Porankiewicz J, Clarke AK (1997) Induction of a heat shock protein, ClpB, affects cold acclimation in the cyanobacterium Synechococcus sp. strain PCC 7942. J Bacteriol 179:5111–5117

Prevost D, Drouin P, Laberge S, Bertrand A, Cloutier J, Levesque G (2003) Cold-adapted rhizobia for nitrogen fixation in temperate regions. Can J Bot 81:1153–1161

Purusharth RI, Klein F, Sulthana S, Jäger S, Jagannadham MV, Evguenieva-Hackenberg E, Klug G (2005) Exoribonuclease R interacts with endoribonuclease E and an RNA helicase in the psychrotrophic bacterium Pseudomonas syringae Lz4W. J Biol Chem 280(15):14572–14578

Ray MK, Sitaramamma T, Ghandhi S, Shivaji S (1994) Occurrence and expression of cspA, a cold shock gene, in Antarctic psychrotropic bacteria. FEMS Microbiol Lett 116(1):55–60

Reddy GS, Matsumoto GI, Schumann P, Stackebrandt E, Shivaji S (2004) Psychrophilic pseudomonads from Antarctica: *Pseudomonas antarctica* sp nov., *Pseudomonas meridiana* sp. nov. and *Pseudomonas proteolytica* sp. nov. Int J Syst Evol Microbiol 54:713–719

Rodrigues DF, Ivanova N, He Z, Huebner M, Zhou J, Tiedje JM (2008) Architecture of thermal adaptation in an Exiguobacterium sibiricum strain isolated from 3 million year old permafrost: a genome and transcriptome approach. BMC Genomics 9:547

Rojas-Contreras JA, de la Rosa APB, De León-Rodríguez A (2015) Expression and characterization of a recombinant psychrophilic Cu/Zn superoxide dismutase from Deschampsia antarctica E. Desv.[Poaceae]. Appl Biochem Biotechnol 175(7):3287–3296

Ronholm J, Raymond-Bouchard I, Creskey M, Cyr T, Cloutis EA, Whyte LG (2015) Characterizing the surface-exposed proteome of Planococcus halocryophilus during cryophilic growth. Extremophiles 19:619–629

Russell NJ (1984) Mechanisms of thermal adaptation in bacteria: blueprints for survival. Trends Biochem Sci 9(3):108–112

Russell NJ (1990) Cold adaptation of microorganisms. Philosophical transactions of the royal society of London. B Biol Sci 326(1237):595–611

Russell NJ (2002) Bacterial membranes: the effects of chill storage and food processing. An overview. Int J Food Microbiol 79(1–2):27–34

Santiago M, Ramírez-Sarmiento CA, Zamora RA, Parra LP (2016) Discovery, molecular mechanisms, and industrial applications of cold-active enzymes. Front Microbiol 7:1408

Schmidt-Nielsen S (1902) Ueber einige psychrophile Mikroorganismen und ihr Vorkommen. Zentr Bakteriol Parasitenkd Infektionsk Hyg Abt II 9:145–147

Subczynski WK, Markowska E, Gruszecki WI, Sielewiesiuk J (1992) Effects of polar carotenoids on dimyristoylphosphatidylcholine membranes: a spin-label study. Biochimica et Biophysica Acta (BBA)-Biomembranes 1105(1):97–108

Ting L, Williams TJ, Cowley MJ, Lauro FM, Guilhaus M, Raftery MJ, Cavicchioli R (2010) Cold adaptation in the marine bacterium, Sphingopyxis alaskensis, assessed using quantitative proteomics. Environ Microbiol 12:2658–2676

Tsuruta H, Tamura J, Yamagata H, Aizono Y (2004) Specification of amino acid residues essential for the catalytic reaction of cold-active protein-tyrosine phosphatase of a psychrophile, *Shewanella* sp. Biosci Biotechnol Biochem 68:440–443

Uma S, Jadhav RS, Kumar GS, Shivaji S, Ray MK (1999) A RNA polymerase with transcriptional activity at 0°C from the Ant-arctic bacterium *Pseudomonas syringae*. FEBS Lett 453:313–317

Uppal S, Akkipeddi VS, Jawali N (2008) Posttranscriptional regulation of cspE in *Escherichia coli*: involvement of the short 5′-untranslated region. *FEMS Microbiol*. Lett 279:83–91. https://doi.org/10.1111/j.1574-6968.2007.01009

VanBogelen RA, Neidhardt FC (1990) Ribosomes as sensors of heat and cold shock in Escherichia coli. Proc Natl Acad Sci 87(15):5589–5593

Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J (2012) Metagenomic analysis of stress genes in microbial mat communities from Antarctica and the high Arctic. Appl Environ Microbiol 78(2):549–559

Vasina JA, Baneyx F (1996) Recombinant protein expression at low temperatures under the transcriptional control of the major Escherichia coli cold shock promoter CspA. Appl Environ Microbiol 62:1444–1447

Vorachek-Warren MK, Carty SM, Lin S, Cotter RJ, Raetz CR (2002) An Escherichia coli mutant lacking the cold shock-induced Palmitoleoyltransferase of lipid a biosynthesis absence of unsaturated acyl chains and antibiotic hypersensitivity at 12° C. J Biol Chem 277 (16):14186–14193

Walworth JL, Pond A, Snape I, Rayner J, Ferguson S, Harvey P (2007) Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil. Cold Regions Sci Tech 48:84–91

Willimsky G, Bang H, Fischer G, Marahiel MA (1992) Characterization of cspB, a Bacillus subtilis inducible cold shock gene affecting cell viability at low temperatures. J Bacteriol 174 (20):6326–6335

Wintrode PL, Miyazaki K, Arnold FH (2000) Cold-adaptation of a mesophilic subtilisin-like protease by laboratory evolution. J Biol Chem 275:31635–31640

Yadav AN, Verma P, Kumar V, Sachan SG, Saxena AK (2017) Extreme cold environments: a suitable niche for selection of novel psychrotrophic microbes for biotechnological applications. Adv Biotechnol Microbiol 2:1–4

Yergeau E, Arbour M, Brousseau R, Juck D, Lawrence JR, Masson L, Whyte LG, Greer CW (2009) Microarray and real-time PCR analyses of the responses of high-arctic soil bacteria to hydrocarbon pollution and bioremediation treatments. Appl Environ Microbiol 75:6258–6267

Zachariassen KE, Kristiansen E (2000) Ice nucleation and anti-nucleation in nature. Cryobiology 41:257–279

Zachariah S, Das SK (2017) Idiomarina andamanensis sp. nov., an alkalitolerant bacterium isolated from Andaman Sea water. Antonie Van Leeuwenhoek 110(12):1581–1592

Zartler ER, Jenney FE Jr, Terrell M, Eidsness MK, Adams MW, Prestegard JH (2001) Structural basis for thermostability in aporubredoxins from *Pyrococcus furiosus* and *Clostridium pasteurianum*. Biochemistry 40:7279–7290