Thermal Diversity of Bacteria and Their Secrets to Cold Survival

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ABSTRACT

Temperature is a critical physical factor influencing the survival, growth, and metabolic activity of organisms on Earth. Changes in temperature alter molecular kinetic energy, thereby affecting chemical reaction rates and essential cellular processes. While most organisms exhibit optimal growth within a narrow temperature range, many bacteria display remarkable adaptability across diverse thermal conditions. Exposure to sudden decreases in ambient temperature triggers cold shock responses that enable bacterial adaptation to new growth conditions. Previous studies have shown that bacteria share common cold-adaptation strategies, including the upregulation of cold stress genes encoding proteins that facilitate translation by preventing the formation of inhibitory mRNA secondary structures. In addition, bacteria modify the lipid composition of their cell membranes by adjusting fatty acid chain length, saturation, and cis–trans configuration to maintain membrane fluidity under cold stress. Genomic GC content has also been implicated in thermal adaptation, as it influences DNA stability and growth capacity at different temperatures. Importantly, psychrophilic and psychrotolerant bacteria produce cold-active enzymes that remain functional at low temperatures. This review highlights current knowledge on bacterial cold adaptation mechanisms and discusses future research opportunities enabled by advanced multi-omics technologies.

Keywords: Bacteria, Cold adaptation, Cold shock proteins, Extremophiles, GC content, Lipid membrane composition.

INTRODUCTION

Organisms that can survive and thrive at extreme temperatures are termed extremophiles, along with those that can live in extreme pH, pressure, and radiation. Most of these temperature extremophiles are made up of bacteria and archaea; however, there are a few eukaryotes that have evolved to adapt towards extreme temperatures as well (Singh, 2013; Ahmad et al., 2025). Each extremophile has different traits to help it survive and grow optimally at temperatures that are either too high or too low for most organisms to endure, and those traits have been studied for the past few decades, since they could be a breakthrough that would eventually enhance and improve human life. In general, these extremophiles can be divided into two thermal groups, namely psychrophiles and thermophiles, whereas organisms that live at moderate temperatures are known as mesophiles (Schiraldi & De Rose, 2002; Ahmad et al., 2025). However, some researchers would further divide the psychrophiles into true psychrophiles and psychrotrophs, and thermophiles into hyperthermophiles and normal thermophiles, based on their optimal growth temperature, as shown in Figure 1.

The core strategy that most extremophiles adopted to adapt to extreme temperatures is the unique metabolic activities of extremophiles that lead to the production of special enzymes, proteins, or even primary and secondary products (extremolytes) that would help other essential enzymes to maintain proper functionality at extreme temperatures (Lentzen & Schwarz, 2006; Rajawat et al., 2022). Some of these extremolytes can even affect the physiological properties of the cells in different ways to adapt to high or low temperatures (Tronelli et al., 2007). Further research on these metabolic strategies and extremolytes would eventually lead to the development in the field of pharmaceuticals as well as for biotechnology (Sandle & Skinner, 2012), reducing environmental

pollution (O'Driscoll et al., 2014; Chu et al., 2001; Hamdan, 2018), the discovery of new energy sources (Curvers et al., 2014; Hamdan, 2018), and boosting the commercial value of various industrial products such as sugar processing, milk production and alcohol production (Mehta et al., 2016).

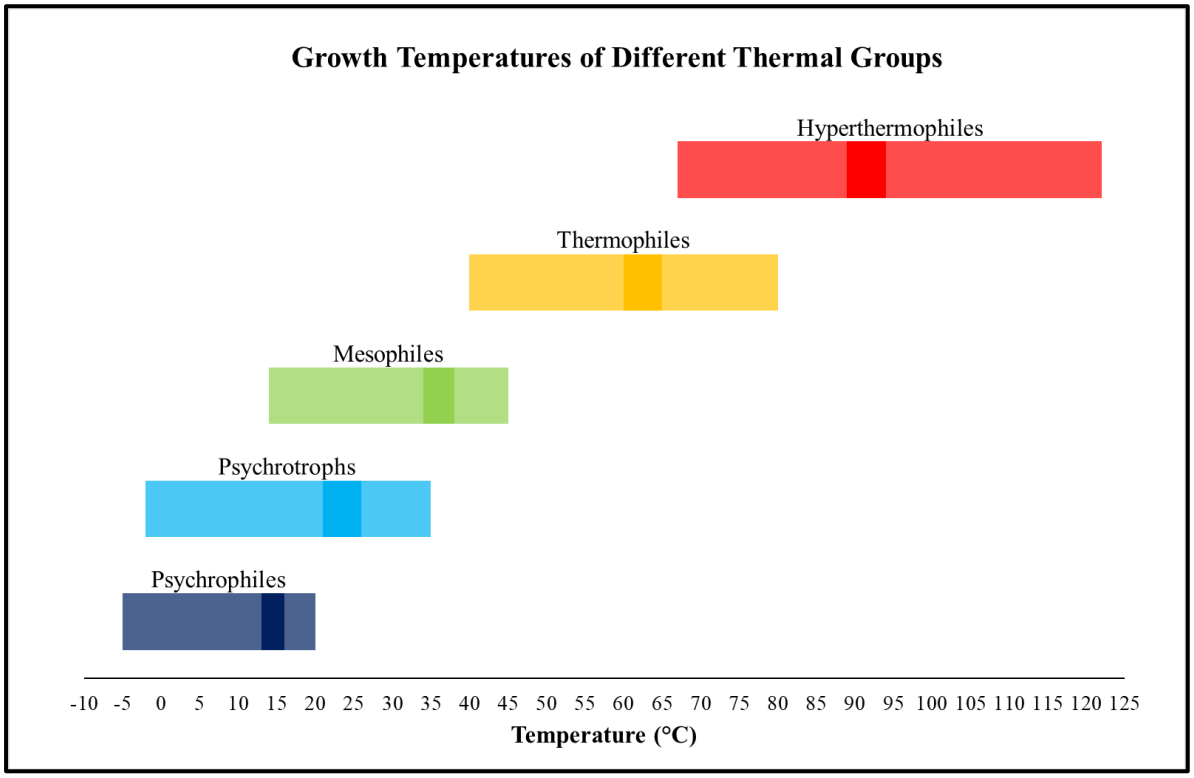

Figure 1: Growth temperature range for each thermal group. The darker region in each growth temperature bar represents the range of optimal growth temperature. Psychrophiles, including true psychrophiles and psychrotrophs, grow optimally at low temperatures, while thermophiles and hyperthermophiles have high optimal growth temperatures.

Psychrophiles, or cold-loving organisms, according to Morita (1975), are defined as "organisms having optimal temperature for growth at about 15 ˚C or lower, a maximal temperature for growth at about 20 ˚C, and a minimal temperature for growth at 0 ˚C or below". The cold environment made up the largest area on Earth. 70% of Earth is covered by the ocean with a temperature around 5 ˚C, whereas the polar region occupies another 15% (Singh, 2013).

One of the earliest studies on psychrophilic bacteria was reported in 1932 (Hampil, 1932). In 1959, Ingraham and Stokes came up with an article to discuss psychrophilic bacteria in detail, including ecology, physiology, and biochemistry of the bacteria (Ingraham & Stokes, 1959). The discovery of psychrophilic bacteria has provided insight into how life can be maintained under extreme cold. For example, Listeria monocytogenes, which can survive at refrigeration temperatures, is the main cause of food spoilage and contamination, even when foods are stored in cold temperatures (Farber & Peterkin, 1991). How the bacterium survives in such conditions leads to the study of cold adaptation in bacteria, to improve food quality and the health of consumers. Besides, further studies on psychrophilic bacteria led to the discovery of psychrophilic enzymes, which offer potential economic benefits (Feller & Gerday, 2003; Wang et al., 2024).

There is another thermal group of microorganisms that is always confused with psychrophiles, the psychrotrophs. Psychrotrophs are also referred to as facultative psychrophiles or psychrotolerant microorganisms. Psychrotrophs can grow at low temperatures, even down to 0 ˚C (Gounot, 1986), but unlike true psychrophiles, they have optimal growth temperatures above 15 ˚C (Moyer & Morita, 2007). Most of the psychrotrophs are determined as the main factor in food spoilage, particularly in the dairy industry (Champagne et al., 1994).

Organisms that live at intermediate temperatures are called mesophiles. They grow well at a moderate temperature ranging from 20 ˚C to 45 ˚C (Willey et al., 2008). When a mesophile is exposed to extreme temperature, its functional proteins or enzymes would either denature or turn inactive, which would lead to the mesophile's death. Most of the identified bacteria up until today are mesophilic bacteria. It is important to know that mesophilic bacteria have been involved in the preparation of beverages since ancient times, particularly in food fermentation. Mass production of beverages such as cheese, yoghurt, beers, and wine is possible thanks to the industrial application of bacteria. Mesophilic

Streptococci, Lactobacilli, and *Bacilli*, for instance, can produce lactic acid (Rajvaidya & Markandey, 2006), which is particularly important in dairy products. *Escherichia coli* is probably the most important mesophilic bacterium to mankind. It is the most studied bacterium, and the understanding of it is much broader than that of other bacteria. Besides being economically valuable, *E. coli* is also very important in the field of research, such as bacteriology, biotechnology, and genetic engineering. Cloning technology involving *E. coli* is probably the most significant contribution of this bacterium in the realm of research (Lodish et al., 2000).

Despite being beneficial in many ways, some mesophilic bacteria are human pathogens. Since human body temperature is always around 37 ˚C, it makes a perfect place for the growth of most mesophilic bacteria, including pathogens. *Mycobacterium tuberculosis,* which grows best at around 35 ˚C to 37 ˚C, is one of the most well-known mesophilic bacteria that are pathogenic to the mammalian respiratory system (Plorde, 2004).

A scorching environment can be found easily on Earth. Locations like hot springs, deserts, geothermal vents, and volcanic areas are extremely hot (Huber et al., 2000). These places are not habitable for most organisms, as heat will easily cause proteins and enzymes to denature, thus killing the organisms. However, thermophiles can adapt and survive in such environments with high temperatures. The term "thermophiles" generally denotes organisms that thrive at maximal growth temperatures between 70 °C and 80 °C. Only the hyperthermophiles reach and exceed 100°C, with 122°C being the current record for growth (Stetter, 2006; Madigan & Martino, 2006; De Maayer, 2022; Stetter, 2022).

Thermophilic bacteria have been identified to have optimum temperature from 55 ˚C to 105 ˚C, and many temperatures in between (Brock, 1986). *Thermus aquaticus* is the first thermophilic bacterium to be discovered living in a hot spring in Yellowstone National Park, United States of America, by Brock (1997). Since then, thermophilic bacteria have been studied extensively due to their industrial potential. Taq polymerase, the crucial enzyme that makes polymerase chain reaction (PCR) amplification,

is one of the successful products isolated from *T. aquaticus* (Chien et al., 1976).

Hyperthermophiles were once categorised as thermophiles. Like thermophiles, hyperthermophile lives in environments with high temperatures, except that the favoured temperatures are much higher than those tolerable by normal thermophiles. It is simply defined as an extreme thermophile, a bacterium that can live at a temperature higher than 90 ˚C (Brock, 2001). Although most of the hyperthermophiles are made up of Archaea, some non-Archaea bacteria can tolerate the extremely high temperature too. *Thermotoga maritime* is one of the most studied hyperthermophiles. It tolerates temperatures around 80 ˚C, which is too high for normal thermophilic bacteria to live in (Huber et al., 1986).

Cold Shock Response in Bacteria

Temperature is one of the best-defined environmental parameters that limit the activity, distribution, and survival of organisms. Temperature changes would affect biological systems, particularly in accelerating or decelerating the rate of biochemical reactions and shifting the reaction equilibrium in organisms (Rowbury, 2003; Wang et al., 2022). Proteins are one of the most important biomolecules that perform a vast array of functions in living organisms. However, the efficiency of proteins is greatly influenced by temperature. The high temperature would easily disrupt the non-covalent interactions that keep the protein in shape, leading to protein denaturation, while at low temperature, it would render most proteins inactive (Somero, 1995). Most organisms have evolved unique strategies to cope with different temperatures, for instance, by encoding proteins and enzymes that may withstand a range of temperatures or having isozymes that work at different temperatures (Vieille & Zeikus, 2001; Aouar et al., 2024; Ahmad et al., 2025). The adaptations are important to keep the function of the protein alive. Thermal stress occurs under heat or cold

conditions; however, in this study, the focus is mainly on the response of bacteria under cold shock.

Cold Acclimation and Cold Adaptation

To successfully colonise low-temperature or high-temperature environments, living organisms have evolved at the molecular level, the whole cell, or even the ecosystem level. The process of these genetic and phenotypic change that accumulates over several generations in response to an organism's specific environmental niche is termed "adaptation" (Morgan-Kiss et al., 2006). Thermal adaptation refers to a prolonged period of exposure to a temperature above or below the organism's optimal growth temperature, or the period of resumed exponential growth after a temperature shift (Frank et al., 2011; Dai et al., 2025). It takes a long time to achieve adaptation as it is part of an evolutionary process; however, changes in adaptation tend to be permanent, and the adaptive traits would be passed down to the offspring until the next adaptation happens.

Acclimation, in contrast to adaptation, which involves modification at the molecular level in response to environmental stress, refers to reversible, non-genetic changes in the phenotype of an organism that are induced by specific environmental conditions (Bennett & Lenski, 1997). It refers to temporary alterations, particularly physical reactions, that occur during a lifetime in response to transitory changes in surrounding conditions (Morgan-Kiss et al., 2006). It occurs during cold or heat shock, which is defined as the period immediately following a drop or rise in temperature of at least 10 ˚C until the organism re-enters the exponential stage of growth. Acclimation is often confused with the term "acclimatisation" (Barria et al., 2013). Both acclimation and acclimatisation refer to the same process; however, acclimation differs from acclimatisation in that, rather than the adaptive changes being induced by natural climate or environment, artificially induced environments are the ones that stimulate the modification or alteration.

Thermal adaptation and acclimation (or acclimatisation) are very crucial in the evolutionary process of bacteria. They work in continuation to ensure the survival of bacterial species during temperature shifts. Acclimation takes place right after an abrupt shift to a relatively low or high temperature, just to make sure that some of the bacteria survive the sudden temperature change. Then, adaptation starts working, generating some long-lasting adaptive traits that enable the bacteria to truly adapt to the new environment (Bradford, 2013). For instance, in response to cold shock, cold-induction protein, CIP, such as cold shock proteins, CSPs and cold acclimation proteins, CAPs will be highly expressed temporarily to sustain the viability of bacteria (Berger et al., 1996). Non-CIP would undergo some modification so that these proteins could function equally well at their optimal temperature. The process of adaptation will take some time, probably over several generations, to complete. CIP will then decrease gradually once bacteria have completely evolved the adaptive traits to adapt themselves towards the new temperature (Horn et al., 2007; Mittal et al., 2022).

Common Cold Stress Strategies in Bacteria

The fact that bacteria can thrive in such a wide range of temperatures shows that they have adopted some very effective strategies to cope with a sudden downshift in temperature. The three major thermal adaptations of bacteria include genomic Guanine (G) Cytosine (C) content, alteration of membrane lipids, and the production of extremolytes that could protect the bacteria from external stress (Russell, 1984).

G+C content

The Guanine-Cytosine (GC) content varies widely, from as low as 16.5% to 75% in different bacterial species (Belozersky & Spirin, 1958), and several studies have proven that such an extreme range occurred due to mutational bias (Freese, 1962; Cox & Yanofsky, 1967). GC content in the genome has always been expected to be closely related to thermal adaptation in organisms, including bacteria (Musto et al., 2004; Zheng and Wu, 2010). For instance, when the samples were restricted to the Family level in prokaryotes, positive correlations were shown between genomic GC content and their respective optimal growth temperatures, supporting the idea of genomic base composition selection in response to different growth temperatures (Musto et al., 2004; Musto et al., 2006).

The speculation behind the selectionist hypothesis that GC content affects the growth temperature was established since the GC pair is bound by three hydrogen bonds, which confer higher thermal stability to the DNA itself (Yakovchuk et al., 2006; Aliperti et al., 2024), as well as higher-order structures of DNA and RNA transcripts (Biro, 2008). In another word, more energy or higher temperature is required to break the triple GC hydrogen bonds as compared to that of double bonds from Adenine-thymine base pairing, thus, genomes with higher GC content would tend to be thermally stable and the organisms itself is expected to be able to withstand higher growth temperature (Nishio et al., 2003; Musto et al., 2006). This thermodynamic hypothesis was further supported by a recent study involving 364 non-redundant bacterial genomes that shows evidence of a correlation between genomic GC content and optimal growth temperatures (Wu et al., 2012).

Despite all the studies supporting the correlation between genomic GC content and growth temperatures, this idea is still being debated by scientists. For instance, in 1997, Galtier and co-workers (1997) presented their findings, revealing that the correlation between GC content and optimal growth temperature is true only in ribosomal and transfer RNA

stem, but not in the genomic DNA (Galtier & Lobry, 1997). These findings are then further strengthened in 2001, suggesting that only the GC content in structural RNA is strongly correlated with optimal temperature, while the genomic GC content and GC content in protein-coding genes (even at freely evolving sites) are not considered to take part in any form of thermal adaptation (Hurst & Merchant, 2001). Besides, a study in 2006 that reanalysed the data from Musto et al. (2004) proposed that the correlation is insignificant by claiming the use of more profound statistical methods rather than using a simple two-factor correlation analytical method (Wong et al., 2006). Furthermore, it is also proven that the GC content is also affected by multiple factors, including environment (Kembel & Jones 2022; Foerstner et al., 2005), nitrogen utilization (McEwan et al., 1998), the genome size (Heddi et al., 1998; Moran, 2002; Rocha & Danchin, 2002), aerobiosis (Naya et al., 2002), and state of free-living (Rocha and Danchin, 2002; Woolfit and Bromham, 2003), thus ruling out the idea that temperature alone can affect the G+C content in bacteria (Basak et al., 2005; Teng et al., 2023).

While the correlation between G+C content at the whole-genome level and optimal growth temperature remains ambiguous, many studies have used G+C content as a preliminary indicator that certain genes do have a significant correlation with optimal growth temperatures (Galtier and Lobry, 1997; Zheng and Wu, 2010). The genomic G+C content in prokaryotes is mainly influenced by the G+C content of protein-coding sequences, which occupy the majority of the genome due to the absence of introns (Brocchieri, 2014). That is why looking closer into those specific genes in the genome separately would yield a different perspective of functional biology than just looking at the whole genome alone.

Aid from cold stress proteins

The fact that bacteria can thrive in such a wide range of temperatures suggests that they have adopted some very effective

strategies to cope with the extremes in temperature, other than just modifying themselves. One of the major thermal adaptations of bacteria is the production of extremolytes that could protect the bacteria from external stress (Russell, 1984). These extremolytes are termed cold shock proteins, and they are highly produced after a rapid temperature shift, even though some of them are still being produced at relatively low levels under normal circumstances to regulate other biological functions.

Cold shock proteins (CSPs), or cold shock domains (CSDs), are protein domains of about 70 amino acids that have been found in prokaryotic and eukaryotic DNA-binding proteins (Doniger *et al*., 1992). The members of this CSP family are commonly found in the domain of eubacteria and appear to have various functions, such as transcriptional activators, RNA chaperones, and antifreeze protein (Graumann and Maraheil, 1998). Of all the CSPs, Csp-A-like protein (Csp-A) is the most common among bacteria, and its function is as an RNA chaperone (Yamanaka, 1999). This highlights the significance of CSPs as RNA chaperones as compared to other functions. CSPs are postulated to be one of the most important elements in the cold adaptation of bacteria, particularly psychrophiles and mesophiles (D'Amico et al., 2006; De Maayer et al., 2015). Normal cellular activities decreased at low temperatures due to the inactivation of the working proteins or enzymes. However, bacteria would induce CSPs, which could function normally to maintain their viability under cold conditions (De Maayer et al., 2015; Mittal et al., 2022).

Cold adaptation mechanisms of bacteria, especially psychrophilic bacteria, have always been of interest to researchers around the world. Many studies have been carried out, and several CSPs were identified and described in detail. For instance, CSPs from psychrophilic bacteria, *Shewanella livingstonensis* Ac10, were identified by different groups of researchers and were well described (Yoshimune et al., 2013). There are also a lot of studies on the CSPs from mesophilic bacteria. One of the earliest studies on mesophilic CSPs involved the induction of two classes of CSPs in *E. coli* (Jones et al., 1987). In 1997, *Enterococcus faecalis* JH2-2

was subjected to a cold-shock experiment, and results showed increased expression of 11 CSPs (Panoff et al., 1997). Expression CSPs do not just restrict to psychrophiles and mesophiles but are also demonstrated on thermophilic bacteria. Studies showed that thermophilic bacteria such as *Streptococcus thermophilic* (Wouters et al., 1999) and *Thermus aquaticus* (Jin et al., 2014) can produce CSPs as well.

Structural and dynamic features of CSPs have also been studied frequently to further investigate how the functional structure can affect the thermostability of CSPs of bacteria. As reported by Lee et al. in 2013, research on the Listeria monocytogenes (a psychrophilic bacterium) CspA (Lm-CspA) shows that it has a five-stranded β-barrel structure with hydrophobic core packing and two salt bridges. The study ended up concluding that the structural flexibility of Lm-CspA is one of the key factors that cause Lm-CspA to be less stable than mesophilic and thermophilic CSPs in terms of thermostability (Lee et al., 2013). However, there are no common CSPs except for CspA-like proteins that have been identified among bacteria so far, thus serving as a limitation to compare the functional structures of CSPs in different bacteria (Yamanaka, 1999).

Structural adaptation of cold-active enzymes

Most Antarctic psychrophiles and psychrotolerants that thrive at sub-zero temperatures need to continue to carry out cell function to survive and continue to grow. The main challenges for life at these temperatures are the exponential decrease in the chemical reaction rates. Hence, to maintain metabolic activities, they need to produce cold-active enzymes that exhibit high catalytic activities (k_{cat}) at low temperatures, often at a trade-off of thermal stability (Collins & Feller, 2023). The key structural adaptation to maintain high activity at low temperatures is to have increased conformational flexibility, to allow the enzymes to use a minimal amount of energy to carry out their tasks (Sarmiento et al., 2015). To achieve these, cold-adapted enzymes lower the number of salt bridges, hydrogen bonds, and ion pairs within the active site region, and harbour longer and

more glycine-rich loops, which increase the local entropy and flexibility of the protein backbone (Collins & Feller, 2023). Those enzymes also undergo amino acid substitution to maintain their flexibility, for instance, by replacing large hydrophobic residues, such as isoleucine or phenylalanine, with smaller ones, namely, valine or alanine. This creates small internal cavities that increase the mobility of the internal groups, thereby reducing the enthalpy of activation for catalysis (Feller, 2013). All these adaptations allow them to have distinct thermodynamic profiles. Overall, cold-active enzymes, unlike enzymes from mesophilic and thermophilic counterparts, typically have a reduced $\Delta H^{\ddagger}$ and a greater negative activation entropy ($\Delta S^{\ddagger}$). Nonetheless, this frequently results in a reduction in substrate affinity (higher K_m), as the highly adaptable active site may not engage the substrate as firmly as a rigid one (Collins & Feller, 2023), but is sufficient to allow psychrophiles and psychrotolerants to adapt well to the extreme cold.

Manipulation of Membrane Composition

Another mechanism adopted by bacteria to adapt to a cold environment is the alteration of membrane composition. The plasma membrane is a selective barrier made up of a phospholipid bilayer, which is susceptible to changes in environmental temperature (Mansilla et al., 2004). The effect of temperatures on membranes has been studied extensively to find out the role of membrane lipid composition in thermal adaptation. Previous studies suggested that bacteria, in general, can modulate membrane fluidity by altering their fatty acid composition (Chintalapati et al., 2004). Russell, in 1984, has described the interaction between bacterial membrane and temperature in detail (Russell, 1984).

These alterations of membrane composition mostly happen in the fatty acyl constituents of phospholipids and glycolipids (Russell, 1984). Although it happens less frequently, bacteria could also achieve the alteration by altering lipid head group, the protein content of the

membrane, the length of fatty acid chain, the cis-trans proportion of fatty acid, and the synthesis of various carotenoids (Chintalapati et al., 2004).

Rapid temperature shift would lead to phase separation of a phospholipid, and thus, decrease membrane fluidity and increase permeability (Cao-Hoanget et al., 2010; Sun et al., 2025). For example, membrane fluidity of *Bacillus subtilis* at temperatures lower than its optimal growth temperature revealed two distinct adaptation mechanisms, including the increase in the ratio of anteiso- to iso- branched fatty acids, and rapid desaturation of phospholipids' fatty acid chains by fatty acid desaturase (Beranová et al., 2008). The membrane alteration process involves a chain reaction between sensor kinase DesK and response regulator DesR, which eventually induces synthesis of D5-desaturase (Beranová et al., 2008; Barria et al., 2013).

Studies on the membrane also found that temperature could affect the proteins embedded in the membrane as well. Investigation of 66 membrane proteins during cold shock suggested that there are changes in amino acid composition and hydrophobicity in these proteins (Kahlke & Thorvaldsen, 2012). The results undoubtedly linked the membrane proteins down to the amino acid level to the cold adaptation strategies of bacteria.

Cold Stress Response in Thermophiles

Although there are significantly fewer cold shock studies on thermophiles as compared to mesophiles and psychrophiles, cold stress responses were documented from a few thermophilic species (Ranawat & Rawat, 2017; De Maayer, 2022). Cold shock exposed on thermophilic bacterium *Thermoanaerobacter tengcogensis* MB4 revealed that the bacterium uses some of the above mentioned cold stress strategies, including maintenance of bacterial cell membrane fluidity by regulation of several fatty acids and glycerophospholipid biosynthesis genes, as well as high expression of CSP encoded by gene TteCspC which helped to regulate

the expression of various genes while act as RNA chaperone to maintain transcriptional processes in the cell (Liu et al., 2014).

Other than these common cold shock responses that can be seen in other thermal groups, the same study by Liu et al. (2014) further discovered several cellular processes that were involved in the thermophilic cold acclimation. These include DNA replication, recombination, and repair to defend against cold-induced damage, flagellar biosynthesis and motility as an escape attempt from the environmental stress, induction of signal transduction to detect, respond and adapt to cold stress, and increasing energy production to synthesise more specific proteins to cope with the cold stress. Besides, cold adaptation strategies such as endospore formation and natural competence were also reported as measures for long-term survival (Liu et al., 2014; Molle and Lemos, 2025). Similar responses were also reported on *Thermus* sp. GH5 and *Bacillus stearothermophilus* TLS33, where CSPs were related to signal transduction that would initiate bacterial sporulation (Topanurak et al., 2005), the energy metabolism pathway was reconfigured, and different amino acids were synthesised in response to different cold shock conditions (Yousefi-Nejad et al., 2011).

General Discussions

Bacteria play important roles as the primary agents for nutrient cycling in every corner of the earth, and this is even more important in the polar or cold region that lacks higher organisms, which are also important contributors to nutrient cycling. Thus, in the polar regions, the roles depend mainly on the lower organisms, especially the psychrophiles and psychrotolerants (Deming, 2002; Cavicchioli, 2006) that are well adapted to the local environments, and their ecological roles are vital for maintaining the polar biogeochemical balance. The above review provides an overview of bacteria from different thermal groups, their characteristics and how they adapt to their thermal niches, with and special focus on the psychrophiles and psychrotolerants. These adaptations are closely related

to their dominance in the habitats they live in, and their active roles in the cycling of carbon, nitrogen, and other elements. At the same time, they also carry out decomposition of penguins, seals and birds' carcasses to enrich the nutrient-deficient terrestrial environments (Zdanowski, et al., 2005; Schaefer, et al., 2023; Zaini, et al., 2024). In summary, microbes, especially the bacteria that can withstand cold temperatures and perform decomposition and nutrient cycling, ensure that there are sufficient nutrients to sustain the life of microorganisms and some higher organisms, such as lichen and mosses, in the harsh Antarctic continent.

General Conclusion

Temperature is one of the most important environmental parameters that would affect the vitality of living organisms; thus, adaptation to different growth temperatures or sudden changes in surrounding temperature is crucial, especially for extremophiles (Barria et al., 2013). Besides the above common cold acclimation and cold adaptation strategies, many bacteria have also adopted their own unique strategies in order to cope with different cold stresses. Some of these strategies still remain undiscovered today, as many hypothetical proteins with unknown functions were found to be involved in the process of cold adaptation (da Costa *et al*., 2018). Moreover, many genes might seem irrelevant to cold stress response, but they are involved in metabolism pathways that are actually part of the cold stress response as well. Bacterial cells comprise a complex metabolic network, but the interacting genes and metabolic reactions have been approached and studied as separate systems most of the time, despite knowing that many of these metabolic pathways are interdependent on one another.

The investigation of cold adaptation in bacteria requires a comprehensive multi-omics approach to encompass the complete intricacy of the biological response. While RNA-sequencing may offer a detailed perspective of the expressed genes, it needs collaborative information at proteomics and metabolomics levels to address post-transcriptional

controls and actual metabolic fluxes. These will provide a holistic system biology coverage in revealing how the genome, transcriptome, and proteome interact to shift the metabolome toward a cold-adaptation state. These multi-omics are very important to resolve the functions of genes, especially in establishing the function of hypothetical genes and genes of unknown functions, considering that there are many genes involved in cold-adaptation that have yet to be discovered. Only through a multi-approach system biology analysis can we eventually understand the whole cold-adaptation strategies of the Antarctic microbes better (Govil et al., 2021). This multi-omics approach can also be capitalised to research on psychrophiles, especially obligate psychrophiles from deep-sea and sub-glacial environments that are currently difficult to culture. By combining the use of culture-independent metagenomic techniques, meta-proteomics, and metatranscriptomic and other new technologies, it is hoped that the adaptation mechanisms and strategies of obligate psychrophiles can be revealed and provide us with a better picture of how microscopic living things can live in a near-impossible cold place for many other organisms.

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