

Some like it cold: understanding the survival strategies of psychrophiles

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Abstract

Much of the Earth's surface, both marine and terrestrial, is either periodically or permanently cold. Although habitats that are largely or continuously frozen are generally considered to be inhospitable to life, psychrophilic organisms have managed to survive in these environments. This is attributed to their innate adaptive capacity to cope with cold and its associated stresses. Here, we review the various environmental, physiological and molecular adaptations that psychrophilic microorganisms use to thrive under adverse conditions. We also discuss the impact of modern “omic” technologies in developing an improved understanding of these adaptations, highlighting recent work in this growing field.

Keywords genomics; metagenomics; molecular adaptations; omic technologies; psychrophile

Introduction

Approximately 80% of our planet's biosphere is permanently cold, that is, at temperatures below 5°C. This includes much of the world's oceans—which cover 70% of the Earth's surface—the polar regions, which encompass Antarctica and parts of North America and Europe that are within the Arctic circle, montane regions (Alps, Himalayas and Rocky Mountains), the mesosphere and stratosphere, and to a lesser extent, man-made habitats such as fridges and freezers [1–3]. Taken together, this makes low temperature the most wide-spread “extreme” environment and, as such, psychrophiles represent the most abundant, diverse and widely distributed extremophiles on Earth [4,5]. The diversity of psychrophiles that is associated with various aquatic and terrestrial cold environments has recently been comprehensively reviewed [1].

Organisms that inhabit cold environments have been subdivided into psychrophiles *sensu stricto*, which grow optimally at less than 15°C (upper limit of 20°C), and psychrotolerant organisms, which survive at temperatures below 0°C but grow optimally at 20–25°C

[6]. Psychrophiles *sensu stricto* predominate in marine ecosystems, where the abyssal oceanic waters are permanently cold (< 5°C), whereas cold-adapted microorganisms isolated from terrestrial environments—which are much more prone to extreme temperature fluctuations—are mostly considered as psychrotolerant [7,8]. As such, there is some bias in research on cold survival and adaptation mechanisms in psychrophilic/psychrotolerant microorganisms, as evidenced by the observed preponderance of genome and metagenome sequences derived from marine environments, and transcriptome profiling of psychrotolerant microorganisms across wider and experimentally viable temperature spectra. The lack of adequate temperature data also makes it difficult in many cases to assign studied strains as psychrotolerant or psychrophilic. Therefore, we will employ the generic term *psychrophiles* to encompass both groups, making reference to the psychrophilic/psychrotolerant nature of the strains studied where possible.

The lower temperature limit for psychrophiles is not clearly defined, although a limit of –12°C for reproduction and –20°C for metabolic function has been proposed [9]. Photosynthesis in the Antarctic lichen *Umbilicaria aprina* has been reported to occur at –17°C [10], and the yeast *Rhodotolura glutinis* can cause frozen food spoilage at –18°C [11]. Continued cellular functioning has recently been proposed to exist at temperatures below –20°C [12,13].

Cold temperatures place severe physicochemical constraints on cellular function by negatively influencing cell integrity, water viscosity, solute diffusion rates, membrane fluidity, enzyme kinetics and macromolecular interactions [3,5]. The ability of an organism to survive and grow in cold conditions is therefore dependent on a number of adaptive strategies in order to maintain vital cellular functions at cold temperatures [3]. As such, psychrophiles have evolved mechanisms to successfully counteract additional stress factors associated with cold environments, such as desiccation, radiation, excessive UV, high or low pH, high osmotic pressure and low nutrient availability [14,15].

The mechanisms of adaptation to cold stress have received considerable attention in the last few decades, particularly in the light of the perceived biotechnological potential of these organisms and their biomolecules [16]. Research in this field has also been bolstered by the development of “omic” technologies. In this review, we discuss the environmental, molecular and physiological

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| Glossary | |
|-----------------------|--|
| Chasmoendolith | Organism that colonizes fissures and cracks in rocks |
| Cryptoendolith | Organism that colonizes structural cavities in porous rocks |
| EPS | Extracellular polymeric substance |
| GOLD | Genomes OnLine Database. Comprehensive information database on complete and ongoing metagenome and genome projects |
| HGT | Horizontal gene transfer, the process of transfer of genes between organisms in a manner other than vertical transmission from parent to progeny |
| NGS | Next-generation sequencing technologies that allow the accurate, rapid and cost-effective sequencing of genomes, transcriptomes and metagenomes |
| ROS | Reactive oxygen species. Chemically reactive molecules containing oxygen |
| MG-RAST | MetaGenomes – Rapid Annotation using Subsystems Technology. Online metagenome repository and analysis pipeline |

adaptation strategies employed by psychrophilic microorganisms to survive in cold extremes, highlighting the recent body of knowledge contributed by “omic”-based research.

Psychrophiles in the next-generation sequencing era

Our understanding of psychrophile biology has been greatly enhanced by the advent of genome sequencing. A review by D’Amico *et al* [4] described the availability of complete genomes of three psychrophilic bacteria and draft genomes of two cold-adapted Archaea. The subsequent development of NGS technologies has resulted in an “explosion” of new psychrophile genome sequence data sets. A keyword search for “psychrophile” against the GOLD database [17] shows that 83 complete or permanent draft psychrophile genomes have been sequenced, with another 102 genomes targeted or incomplete (Table 1). The complete/permanent draft genomes include those of 78 bacteria, four archaea and one eukaryote. The majority of sequenced psychrophiles (43.4%) have been isolated from marine environments, predominantly the Pacific Ocean and Southern Ocean surrounding the Antarctic continent (Fig 1A). The availability of a large number of genomes of psychrophilic organisms and mesophilic phylogenetic relatives has strongly stimulated comparative genomic analyses, whereby the molecular determinants of psychrophile

can be elucidated through the presence/absence of genes in organisms across the temperature spectrum. For example, a comparison of the *Alteromonas* sp. SN2 genome with those of two mesophilic *Alteromonas macleodii* strains revealed the presence of 15 genomic islands specific to SN2, which are thought to confer ecological fitness to this strain in the cold marine tidal flat environment [18]. Similarly, comparative analyses of *Halobacterium* sp. tADL isolated from Deep Lake in Antarctica showed unique genomic features, including gas vesicle, bacteriorhodopsin and polyhydroxyalkanoate biosynthesis genes, which may contribute to its dominance in this environment [19], whereas a comparison of the Antarctic halophilic archaeon *Halorubrum lacus profundus* with various mesophilic haloarchaea showed amino acid substitutions in 7.85% of positions in *H. lacus profundus* proteins invariant in the mesophiles [20].

The power of genomics has been bolstered by other “omic” technologies, such as transcriptomics and proteomics. These technologies can be used to study the differential expression of genes and proteins, respectively, in microorganisms exposed to a wide range of temperatures *in vitro*. For example, transcriptome profiling of the psychrotroph *Exiguobacterium sibiricum* 255-15, which can grow at temperatures ranging from -5 to 40°C , identified a large number of genes—involved in DNA replication, transcription and translation, carbohydrate and amino acid metabolism and cell membrane adaptation—that are differentially expressed when the strain is grown at -2.5°C and 39°C [21]. Similarly, proteomic approaches have, for example, been employed to determine the effects of temperature—in a range of -2 to 28°C —on protein abundance profiles, as a measure of adapted cellular processes in the Antarctic archaeon *Methanococcus burtonii* [22]. The combined use of these “omic” technologies may allow us to understand the global cellular response of psychrophilic microorganisms to lower temperatures, which is currently poorly understood (see Sidebar A).

It is widely accepted that less than 1% of all microorganisms can be cultured [23]. However, the availability of a pure culture was, until recently, a prerequisite for genome sequencing, biasing the sequence information available to culturable organisms. This requirement has, however, been surmounted by the development of metagenomic strategies, which entail DNA extraction from the entire community and subsequent analyses [24]. Sequencing and functional screening of the total metagenomic library can be utilized to unravel the molecular determinants underlying a given phenotype, such as psychrophile, of unculturable organisms. There are 315 psychrophilic metagenomes for which temperature metadata exists (sampling temperature lower than 15°C) deposited in the MG-RAST database, and the majority of samples are from marine and soil/sediment environments (Fig 1B). The power of metagenomic strategies has been combined with other “omic” technologies, such as comparative metagenomics, transcriptomics, proteomics and metabolomics, allowing the parallel analysis of metagenome sequences from different environments. Comparison of microbial mat metagenomes from ice shelf ponds in Antarctica and the high Arctic identified many common genes of the environmental stress response, in particular genes for exopolysaccharide biosynthesis and membrane adaptations. The abundant copper homeostasis genes were seen as evidence of the higher exposure of the Arctic communities to pollutants, whereas the

Table 1. Current status of genome sequencing of psychrophiles

| | Psychrophile | Psychrotolerant/ troph | Total |
|------------------------|--------------|---------------------------|-------|
| Complete and published | 26 | 20 | 46 |
| Permanent draft | 21 | 16 | 37 |
| Incomplete | 47 | 32 | 79 |
| Targeted | 6 | 17 | 23 |
| Total | 100 | 85 | 185 |

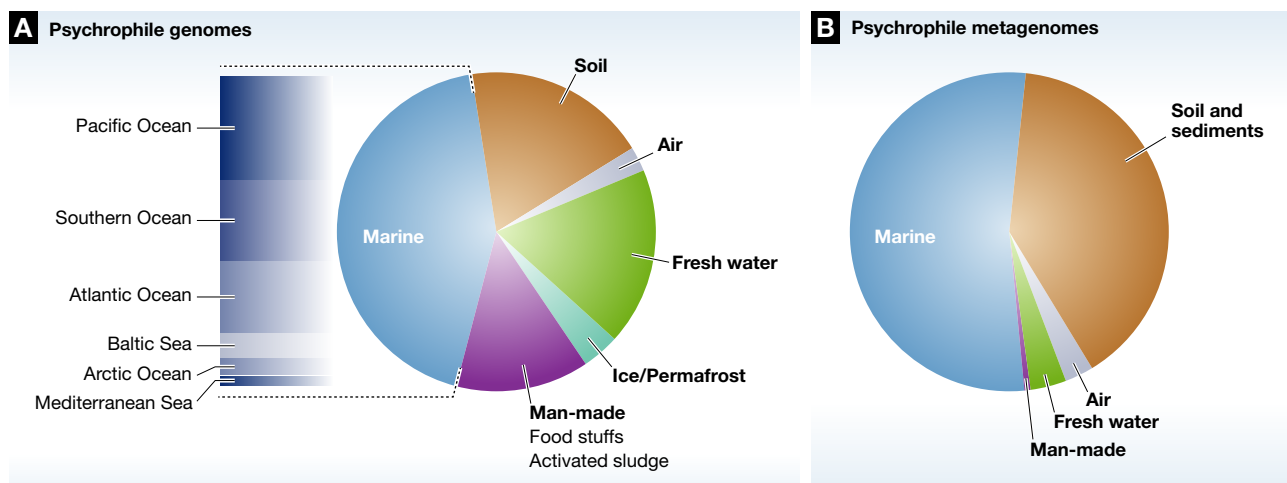


Figure 1. Distribution of psychrophile genomes and metagenomes in different cold ecosystems.

(A) Pie chart of the relative proportions of sequenced psychrophile genomes per ecological niche. Psychrophile genome statistics were determined by key word search against the GOLD database. The geographic distribution of marine genomes is given in the chart. (B) Pie chart of the relative proportions of psychrophile metagenomes derived from different ecological niches. The psychrophile metagenomes include all datasets submitted to the MG-RAST database for which temperature data are available (lower than 15°C).

dominance sigma B genes in the Antarctic metagenome was thought to indicate that this community was exposed to higher osmotic stresses associated with the freezing of Antarctic ponds [25]. Metagenome sequencing and functional annotation methods have been used to study seasonal differences in the bacterioplankton communities associated with Antarctic Peninsula coastal waters. The summer community has a predominant chemoheterotrophic and photoheterotrophic metabolism, whereas winter communities are dominated by chemolithoautotrophic bacteria and archaea [26]. Such “systems biology” approaches [27] are still in their infancy, but will undoubtedly facilitate future research into the complex adaptive strategies employed by psychrophilic communities (see Sidebar A).

Environmental adaptation

Habitat selection

Ecological limiting factors such as nutrient and water availability, salinity, pressure, UV irradiation and temperature are all characteristics of cold environments. In some terrestrial habitats, these stresses dictate that psychrophilic communities develop most effectively in protected niches or “refugia” [28,29]. Key drivers of habitat selection in cold terrestrial environments include light (for autotrophic growth and avoidance of UV damage), water availability and physical stability [28]. Psychrophilic communities in these environments are frequently associated with lithic habitats. For example, hypolithic communities, which grow on the underside of translucent rocks, are protected from physical instability, desiccation and UV fluxes [30]. These specialized communities are typically dominated by autotrophic cyanobacteria that make use of photosynthetically active radiation, which penetrates the rock and thereby supports complex heterotrophic communities [31]. Terrestrial psychrophilic communities are also associated with cryptoendolithic and chasmolithic environments [28].

Psychrophilic microorganisms in glacial ice reside primarily in veins or liquid films containing metabolic substrates [32,33]. Sea ice is characterized by highly variable salinity, pH, dissolved gases and in/organic nutrients and light [34]. Psychrophilic communities are localized in hypersaline pockets and channels within the ice. These brine pockets present a microenvironment rich in dissolved organic matter and support extensive populations of heterotrophic bacteria and algae [34].

Gas vacuolate prokaryotes have been isolated from sea ice, as well as from marine and fresh water ecosystems. Gas vacuoles provide buoyancy and allow the microorganisms to move to a zone of favorable temperature, in thermally stratified water columns and during summer thawing [19,35].

Viable but non-culturable state

Some microorganisms seem to disappear at cold temperatures, only to resurface when more favorable temperatures return. This has been observed for Gram-negative bacteria such as marine *Vibrio* and *Aeromonas* spp. and various bacterial isolates from Antarctic lakes [36–38], and is ascribed to a transition into a dormant viable but non-culturable state (VBNC), in which the organisms remain capable of respiration and substrate uptake but cannot replicate [37,39]. This behavior may also explain the anomaly of viable photoautotrophic cyanobacteria found in dark Siberian permafrost [40]. Laboratory propagation at higher temperatures demonstrated the readily reversible nature of this VBNC state, with resuscitated bacteria maintaining their photosynthetic capabilities [40]. However, whether the VBNC state represents an active survival strategy or cells in this state become increasingly attenuated and eventually lose their ability to be revived remains a matter of debate [38]. Metabolically active, high GC Gram-positive Actinobacteria have been recently shown to exist in permafrost samples from Antarctica, Canada and Siberia with an estimated age of 500,000 years [41]. Given that ancient DNA is thought to be completely sheared into short (< 100 bp)

fragments within 100,000 years, these results suggest that dormant cells retain effective DNA repair mechanisms over such timescales [41].

Molecular adaptation

Genome structure

Comparative genome analysis of *Desulfotalea psychrophila* and *Archaeoglobus fulgidus* (optimum growth temperature difference of 73°C) indicated that the G+C contents of these microorganisms is similar [42]. Although the general opinion is that overall genomic G+C content cannot be used to distinguish between microbial thermal classes, some psychrophilic microorganisms contain distinctly high G+C genomic regions, which mainly code for informational proteins (tRNAs, elongation factors, RNA polymerases) [42,43]. In addition, a high level of redundancy occurs in the genomes of psychrophilic microorganisms, which encode multiple copies of tRNA species for biosynthesis of all amino acids, as well as an increased variety and number of chaperones [18]. For example, four copies of the chaperone DnaJ are encoded on the genome of *Psychromonas ingrahamii*, and the psychrophilic *Alteromonas* sp. SN2 has a higher number and diversity of tRNA species than mesophilic members of the genus [18,44]. This suggests that a high capacity for translation and post-translational processing may be vital for growth at low temperatures. In addition, the analysis of numerous psychrophilic genomes and metagenomes has indicated the presence of a large number of features contributing to genome plasticity, such as plasmids, transposable and other mobile genetic elements. Many of these mobile genetic elements and the genes they carry can be directly linked to cold-adaptive traits, such as unsaturated fatty acid biosynthesis [18,45]. Ultimately, the cold survival traits that are acquired by HGT may also explain both the similarity and diversity observed between numerous organisms growing in low temperature environments. Genomic analyses of cold-adapted *Shewanella* strains have provided evidence for genetic exchange from the marine gene pool, with coding sequences in *S. halifaxensis* and *S. sediminis* showing higher levels of homology to *P. profundum* SS9 and *C. psychrerythraea* [46]. In addition, cold sensitivity is linked to transposon inactivation in *P. profundum* SS9, clearly indicating that these elements play a role in the adaptation to low temperature [47]. Furthermore, clustered regularly interspaced short palindromic repeats (CRISPRs)—which are associated with reverse transcriptases—are more abundant in the genome of cold-adapted *Alteromonas* sp. SN2 [18].

Proteins and enzymes

Temperature is one of the factors governing biochemical reactions and, as predicted by Arrhenius Law, reaction rates are greatly reduced at low temperatures. Psychrophilic enzymes must therefore be suitably adapted to maintain adequate catalytic rates for cellular function [48], and the topic of psychrophilic protein structure and function has been extensively reviewed [4,48,49]. Psychrophilic enzymes are generally characterized by a higher degree of structural flexibility, lower thermostability and higher specific activity at low temperatures than their mesophilic counterparts. The increased structural flexibility of cold-adapted enzymes may be global, or

restricted to the catalytic regions, and allows them to exist in a more disordered ground state [4,50]. This increased flexibility enhances the degree of complementarity between the catalytic site and substrate, thereby reducing activation energies and increasing substrate turnover rates [3,4].

Comparative genomic, protein structure and crystallography studies have revealed several trends in amino acid composition, protein sequence and structure, and disorder across homologous proteins from different thermal classes (Fig 2) [51,52]. Notably, multiple mechanisms are used to increase enzyme flexibility and activity, as well as decrease thermostability, and not all mechanisms are applicable to a given psychrophilic protein [49].

One of these mechanisms is to reduce arginine and proline content. These amino acids form multiple hydrogen bonds and salt bridges and reduce conformational flexibility, and reduced levels have been observed in a number of psychrophilic enzymes [53,54]. Reduced alanine contents have been observed in proteins from psychrophilic *Shewanella* spp., while lower proline/arginine content was detected at the genome level for *Psychrobacter arcticus*, particularly in proteins involved in reproduction and cell division [43,46]. Other compositional biases observed in psychrophilic proteins include increased asparagine, methionine and glycine contents, glycine clustering at the enzyme catalytic site—which increases local mobility—and increased lysine-to-arginine ratios, which lower hydrogen bonding and salt bridge formation [54–56].

A comparative analysis of 2,816 mesophilic and 3,665 psychrophilic proteins, encoded on the genomes of six psychrophiles and six mesophiles, respectively, showed a predominance of amino

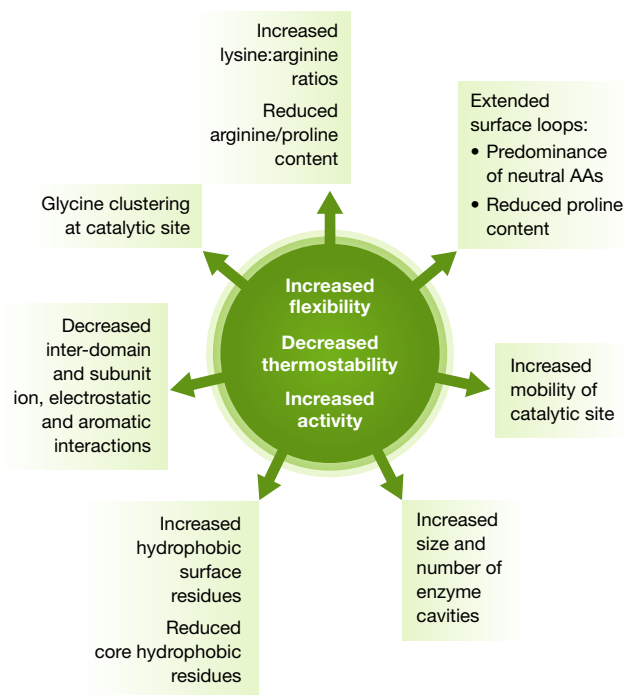


Figure 2. Common structural modifications of psychrophilic enzymes resulting in decreased thermostability, increased flexibility and increased specific activity.

acids with small/neutral side chains in the loop regions of secondary structures in psychrophilic proteins, [51]. This contributes to protein flexibility within these loops, whereas helical regions contain fewer amino acids capable of inter-domain and inter-subunit interactions than mesophilic proteins [51]. An increase in amino acids with hydrophobic side chains on the solvent-exposed regions of the protein, and fewer hydrophobic residues in the enzyme core, have also been observed [53].

Variations in the three-dimensional structures of psychrophilic proteins compared with their mesophilic counterparts have also been identified. Longer external loops with reduced proline content result in less compact and stable proteins. Indeed, the catalytic site and surrounding molecular structures have been shown to have more flexibility and mobility [57,58]. This is thought to enhance the accessibility of substrates to the active site, possibly reducing catalytic energy costs [59,60]. High-resolution models of psychrophilic proteins have shown that both the number and size of cavities in cold-adapted proteins is greater than in mesophilic orthologs [50]. Cavities appear to retain a high number of hydrophilic groups, binding a greater number of water molecules, which increases enzyme flexibility by enhancing the internal solvation [50]. For example, a region in close proximity to the lid helix of the cold-adapted M37 lipase from *Photobacterium lipolyticum* contains a surface cavity [61]. The destabilizing effects of such surface cavities may confer flexibility to the helical lid, allowing increased lateral movement upon substrate binding. Comparison of M37 to the orthologous lipase from the mesophile *Rhizomucor miehei* also revealed a wider oxy-anion hole in the former structure. This modification allows the binding of additional water molecules, which may assist in lowering the energy required to obtain the transient tetrahedral intermediate, subsequently decreasing the optimum temperature [61].

Cold acclimation through differential gene expression

Cold temperatures reduce the activity of transcriptional and translational enzymes, increase DNA/RNA secondary structure stability and slow the kinetics of protein folding. In response to sudden exposure to lower temperatures, both mesophiles and psychrophiles up- or down-regulate the expression of a significant number of genes, a process termed the cold-shock response. Recently, the concept of cold-shock response in psychrophiles *sensu stricto* has been called into question, with proteomic profiles of the true psychrophile *Pseudoalteromonas haloplanktis* TAC125 showing that no cold-induced proteins were synthesized in response to a temperature shift from 18 to 4°C and that cold-repressed proteins matched those observed when this strain was maintained at 4°C [62]. However, it is noted that true psychrophiles such as *P. haloplanktis* may survive at temperatures as low as -20°C, and the technical challenges with replicating rapid temperature downshifts under laboratory conditions, make it difficult to test the plausibility of a cold-shock response with a temperature downshift over this extreme temperature range [62]. In light of this current debate, we will focus on cold acclimation by differential gene expression as an adaptive function in psychrotolerant microorganisms.

A total of 1295 genes (944 up-regulated/351 down-regulated; ~40% of total genes) were differentially expressed in the archaeon *Methanobus psychrophilus* grown at 4°C versus 18°C [63]. Similarly, 785 (320/465 induced/repressed) and 546 (217/329

induced/repressed) genes were differentially expressed in the psychrotolerant bacterium *S. oidenensis* grown at 8 and 15°C, respectively [64]. The rapid nature of this effect has been demonstrated in *Pseudomonas putida*, in which 2337 genes are differentially expressed within 2 h of a temperature downshift from 30 to 10°C [65].

The most prominently up-regulated genes are those encoding cold-shock proteins (CSPs), a family of small, single-stranded nucleic acid binding proteins that regulate a variety of cellular processes, including transcription, translation, protein folding and membrane fluidity [1,4]. Notably, a number of CSPs that are expressed in response to cold exposure in mesophiles are constitutively expressed in psychrophiles, and are classified as cold acclimation proteins (CAPs) [4]. Additional genes that are strongly up-regulated in response to cold exposure include a number of cold-induced RNA helicases—which can destabilize secondary DNA and RNA structures—molecular chaperones, heat shock proteins and genes associated with sugar transport and metabolism and cell envelope biogenesis [63,64].

The solubility of gases increases at lower temperatures, resulting in increased concentrations of reactive oxygen species and thus the potential for oxidative damage. As a result, the expression of genes encoding antioxidative enzymes—such as catalases and superoxide dismutases—is increased at low temperatures, whereas ROS-producing pathways are generally down-regulated [2,63]. A variety of genes encoding proteins involved in amino acid, nucleotide and protein synthesis, flagellar motility and energy metabolism are also down-regulated [64–66]. This information obtained initially from transcriptome analyses has largely been corroborated by proteomic studies, as described for the psychrophiles *Methanococcoides burtonii*, *Psychrobacter arcticus* and *Photobacterium profundum* [66–68]. However, higher levels of proteins involved in energy metabolism (glycolysis and the acetate kinase A-phosphotransacetylase pathway) and flagellar motility have been observed in proteomic analysis of *L. monocytogenes* and *Shewanella livingstonensis* grown at 4°C, respectively, whereas oxidative stress-related proteins were repressed in the *P. haloplanktis* grown at low temperatures [5,69,70].

As is the case for organisms living in warmer habitats, it should be noted that some studies have shown poor or, at best, moderate correlations between transcript and protein levels (Sidebar A). Coupled transcriptomic and proteomic analyses of *S. livingstonensis* revealed an increase in the abundance of 12 proteins, in the absence of an increase in gene expression [70]. Caution should thus be exercised when interpreting transcriptome/proteome data [71]. Furthermore, inter-specific and inter-strain differences must be taken into account when developing a global picture of the mechanisms employed for cold adaptation.

Physiological adaptations

Membrane function

The fluidity of the membrane is essential for its structural integrity and, thus, cellular functioning [72]. It has long been known that one of the most significant impacts of low temperature is on membrane fluidity and that organisms growing at both ends of the biotic thermal range have evolved a range of mechanisms designed to alter

membrane fluidity [72,73]. It should be noted that extensive differences exist in the physiologies of Gram-negative and Gram-positive bacteria and archaea, particularly in terms of their cell membrane compositions and responses to temperature changes. Here, we discuss membrane adaptations in psychrophilic microorganisms generically. Psychrophile membrane adaptations include increased polyunsaturated to saturated fatty acid ratios in membrane phospholipids, changes in lipid class composition, reduced size and charge of lipid head groups, which affects phospholipid packing, and conversion of trans- to cis-isomeric fatty acids (Fig 3), and have been extensively reviewed [1,4,72–74].

Recent transcriptome analyses corroborate earlier physiological work and have shown that exposure to cold temperatures induces a rapid up-regulation of genes involved in membrane biogenesis, such as fatty acid and LPS biosynthesis, peptidoglycan biosynthesis, glycosyltransferases and outer membrane proteins [64,65,75]. Comparative genomic studies have also revealed that genes involved in cell membrane biogenesis are overrepresented in the genomes of psychrophilic microorganisms [47,48]. Proteomic and transcriptomic studies have shown that general membrane transport proteins are also up-regulated, which serves as a counteractive measure against the lower diffusion rates across the cellular membranes experienced at colder temperature [69,76]. In particular, the up-regulation of peptide transporters facilitates cold and

hyperosmotic stress acclimatization by enhancing the uptake of nutrients, compatible solutes and recycling of membrane peptides for peptidoglycan biosynthesis [69,75,76]. By contrast, the expression of genes encoding other outer membrane proteins and structures, such as flagella, chemotaxis proteins and iron uptake receptors, is generally suppressed at cold temperatures (Fig 3) [5,75].

Carotenoid pigments represent another class of membrane fluidity modulators. Both polar and non-polar carotenoid pigments are produced by various Antarctic bacteria and have been postulated to buffer membrane fluidity and assist in maintaining homeoviscosity during temperature fluctuations (Fig 3) [3,49]. Wax esters are also believed to play an important role in cold-adjusted membrane fluidity. In *Psychrobacter urativorans*, they may account for up to 14% of the cell lipid content, and in *P. arcticus*, the wax ester synthase is constitutively expressed, regardless of the growth temperature [43].

Cryoprotectants and antifreeze proteins

Cellular freezing induces the formation of cytoplasmic ice crystals, resulting in cellular damage and osmotic imbalance [31]. The accumulation of compatible solutes—such as glycine, betaine, sucrose and mannitol—results in the lowering of the cytoplasmic freezing point thereby providing protection against freezing—as well as against desiccation and hyper-osmolality (Fig 3) [1,30]. The trehalose disaccharide may prevent denaturation and aggregation of

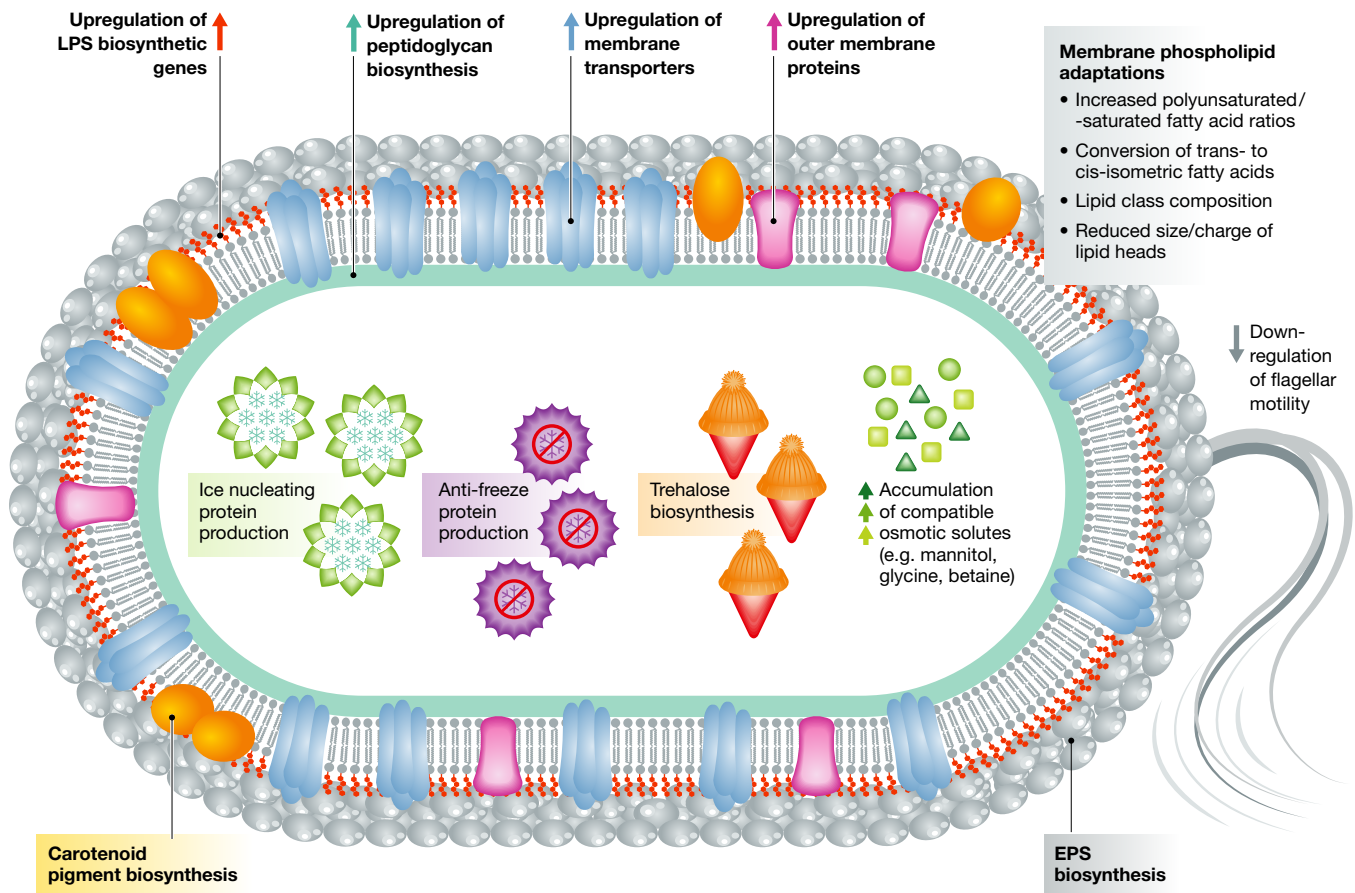


Figure 3. Common physiological adaptations in a psychrophilic prokaryote.

Sidebar A: In need of answers

Much of the amassed data on psychrophilic adaptation results from studies of individual proteins and genes, and from physiological analysis of individual strains. As a result, a major gap in our current knowledge is the global cellular or community response to low temperature exposure. This gap is only very slowly being filled by comparative genomic, metagenomic and other 'omic' approaches. The correlation of genomic and transcriptomic data with what truly occurs at the protein and functional level also remains open to debate, but an increasing use of polyphasic approaches, combining multiple "omic" techniques, will contribute toward stream-lined, consensus answers to questions relating to psychrophilic adaptations. This is expected to have a major impact on our understanding of the biology of psychrophilic communities.

proteins, scavenge free radicals and stabilize cellular membranes under cold conditions [77], and a transcriptome analysis of *Escherichia coli* has shown that the trehalose biosynthetic genes *otsA* and *otsB* are induced under cold conditions [78].

Some psychrophiles produce antifreeze or ice-binding (AFP) proteins (Fig 3), which bind to and control ice crystal growth and recrystallization by lowering the freezing point (thermal hysteresis) [79]. Ice-nucleating (IN) proteins can prevent supercooling of water by facilitating ice crystal formation at temperatures close to melting point [80]. The cryoprotective mechanisms employed may differ depending on the environment and microbial community structure, as demonstrated by a metagenomic study of temperate lakes that revealed a predominance of isolates with high cytoplasmic osmolyte content, with negligible ice-association (IN/AFP) phenotypes, whereas half of the epiphytic isolates from a frost-exposed chrysanthemum phyllosphere community showed IN activity [81,82].

Exopolysaccharide (EPS) production represents another potential cryoprotection mechanism and high levels of EPS are produced by psychrophiles under cold conditions [83–85]. The high polyhydroxyl content of EPS lowers the freezing point and ice nucleation temperature of water. In addition, EPS can trap water, nutrients and metal ions and facilitate surface adhesion, cellular aggregation and biofilm formation and may also play a role in protecting extracellular enzymes against cold denaturation and autolysis [84–86]. The exopolymeric substances of the psychrophilic diatom *Melosira arctica* and of cold-tolerant bacterium *Colwellia psychrerythraea* have been shown to cause alterations in the desalination and microstructure of growing ice, by increasing ice crystal disorder and pore density [87,88]. This results in a reduction in the permeability of ice, which subsequently leads to salt retention. Biologically active EPS may therefore effect the colonization and survival of organisms in the sea ice habitat by reducing ice growth due to increased salinity [87,88].

Conclusions

From the growing body of scientific research that focuses on the adaptation of psychrophiles to their cold environments, it is evident that multiple adaptive mechanisms have evolved to support their survival in such "inhospitable" environments. Environmental adaptations such as habitat selection allow these organisms to effectively

"avoid" some aspects of the cold environment, and physiological and genomic adaptations such as cryoprotectant biosynthesis and membrane composition provide mechanisms to compensate for the kinetic and thermodynamic effects of these extremes. Genetic and "omic" strategies have contributed substantially to and validated our understanding of the molecular strategies underlying cold adaptation. Given the many different adaptive mechanisms that are used by different psychrophilic organisms, and given that relatively few psychrophilic organisms have been studied in detail, it can be expected that other novel strategies for survival at cold temperatures are yet to be discovered. The isolation of new psychrophilic organisms from cold environments, the sequencing of their genomes and increasing integration of "omics" approaches into systems biological platforms will broaden our understanding of what lies beneath the tip of the psychrophile iceberg.

Author contributions

PDM, DA, CC and DAC wrote the original manuscript. All authors contributed to the final version.

Conflict of interest

The authors declare that they have no conflict of interest.

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