



## Review

# Psychrophiles: A journey of hope

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Psychrophiles are organisms living in extremely cold conditions within the temperature range of  $-20^{\circ}\text{C}$  to  $+10^{\circ}\text{C}$ . These organisms survive in harsh environment by modulating their genetic make-up to thrive in extremely cold conditions. These cold-adaptations are closely associated with changes in the life forms, gene expression, and proteins, enzymes, lipids, etc. This review gives a brief description of the life and genetic adaptations of psychrophiles for their survival in extreme conditions as well as the bioactive compounds that are potential antimicrobials.

**Keywords.** Antibiotics; antimicrobial; cold-adaptations; CRISPR-Cas; metagenomics; psychrophiles

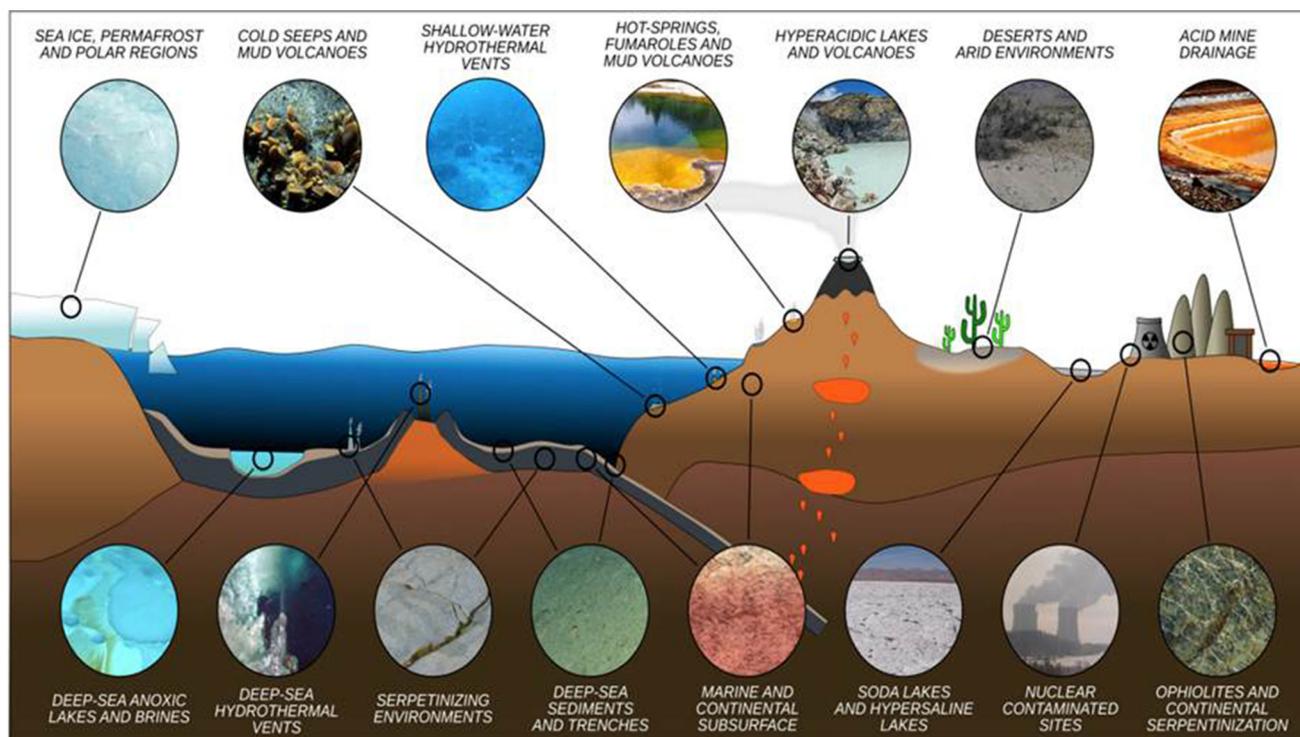
## 1. Introduction

A few organisms can grow in drastic conditions such as the high temperature of volcanic springs, polar regions at lower temperatures, high and low pH levels, high or low concentrations of salts, and deep sea at high pressures. These have three groups: psychrophiles, mesophiles, and thermophiles, depending on their growing conditions. Psychrophiles mostly occupy larger space in the ecosystem, especially marine psychrophiles cover  $\sim 70\%$  of the oceans below the depth of 1000 m at the temperature of  $4-5^{\circ}\text{C}$  (Kumar *et al.* 2014). Psychrophiles are also present in  $-10^{\circ}\text{C}$  at the Arctic permafrost, porous dry rocks in Antarctic valleys at  $-60^{\circ}\text{C}$ , and glacier surfaces of cryoconite holes (Cameron *et al.* 2012) (figure 1).

When it comes to organisms existing in snow or icebergs, for example, *Chlamydomonas nivalis*, *Raphidonema nivale*, and *Chloromonas nival* produce carotenoids and astaxanthin add coloration to the snow and hence it is called 'red snow' (Remias *et al.* 2005). Another example is the *Janthinobacterium*, found in the

supraglacial water has been reported to have a defense mechanism against viral genomes. This bacterium enters into the dominant phase and the outer membrane forms vesicles. These vesicles recognize the viral host and inject the virus into itself and block the replication (Rassner 2017). The psychropiezophilic bacterium such as *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, *Psychromonas*, *Alphaproteobacteria*, and *Firmicutes* sp. are found in deep-sea beds. These species do not include PUFAs, like EPA and DHA, but contain unsaturated fatty acids for their growth in higher temperatures (Nogi 2017). Li and Kato have reported *Antarcticum vesiculatum*, *Cytophaga fermentans*, *Cytophaga diffluens*, *Coxiella bumetii*, *Eubacterium angustum*, *Haliscomenobacter hydrossis*, *Microcilla aggregans*, and *Psychroserpens burtonensis* found in the deep-sea sediments and sequenced for small-subunit ribosomal RNA genes (Li and Kato 1999).

*Psychrophilic* microorganism has two important physical challenges: low thermal energy and high viscosity. The main objectives of adaptations are completely depending on the production of proteins. The



**Figure 1.** Representation of extremophiles and their approximate location. Reprinted with permission from Copyright © Merino *et al.* (2019).

proteins control entry of nutrients, waste product out-flow, maintain stability between products and substrate, folding of nucleic acids, and assemblies of macromolecules (Amico *et al.* 2006). These organisms are predominantly represented in domains such as algae, archaea, bacteria, yeast, and few eukaryotes. Psychrophiles are also present underneath the icepacks of polar fish. These characteristics represent that psychrophiles are most abundant in relationships with distribution and diversity (Feller 2013).

## 2. Lifestyle and adaptations

Psychrophiles can adapt to harsh environmental conditions (sub-zero temperature) such as in Arctic permafrost at  $-10^{\circ}\text{C}$  and in salty cryopegs polar ice crystals at  $-20^{\circ}\text{C}$ . These organisms are exposed to various challenges in cold habitats and develop adaptation strategies to survive in the environment. *Planococcus halocryophilus* a bacterium was reported to sustain at  $-25^{\circ}\text{C}$  and cause division at  $-15^{\circ}\text{C}$  before quiescence. In Antarctic basins, fungi and crypto endolytic communities are found which is underneath  $-2^{\circ}\text{C}$  (Cavicchioli *et al.* 2011; Feller 2017).

The cell envelope provides the functions such as shape, protection, support, and regulation of movement in and out of the cells. During lower temperatures, few functions are compromised such as reducing the fluidity of the membrane, permeability, and diffusion rate. Also, weaken the function of embedded proteins and upsurge turgor pressure. The cell membrane provides mechanical support to the cell and monitors the molecules that transport inside and outside the cells. But during cold-adaptations, there is overexpression of encoding genes of different proteins that are included in the biogenesis and fatty acid synthesis and desaturation. An increase in these proteins may degrade the membrane molecules entering the cell membrane. In few cases, proteins present in the lipid layer lessen the diffusion rate and upregulation of protein transport (Collins and Margesin 2019). At lower temperatures, long-chain polyunsaturated fatty acids (LCPFAs) such as eicosapentaenoic, arachidonic, and docosahexaenoic are produced at greater rates (Okuyama *et al.* 2008). LUPFAs protect the cell membrane against reactive oxygen species (ROS) as anti-oxidative functions. The LUPFAs form hydrophobic boundaries between lipid bilayers preventing ROS and the intake of  $\text{H}_2\text{O}_2$  into cells act. Hence, LUPFAs act as shield from the

oxidative stress at lower temperature (Yoshida *et al.* 2016).

Pigments and carotenoids (pigmented hydrocarbon) maintain the fluidity of the cell membrane in microorganisms. At low temperatures, there is augmented membrane rigidity in polar carotenoids that lead to better survival of the microorganisms. These pigments play a significant role such as photoprotection (protecting against UV radiations and high light at lower temperatures), as light harvesters (photosynthetic microorganisms), and as antimicrobials. These findings can suggest that pigment levels and functions may have a conflicting mechanism in few microorganisms (Pandey *et al.* 2018). The pigments have various health benefits such as maintaining and developing the immune system, anti-microbial agent, anti-oxidants, protecting the in-eye tissues, act as a food emulsifying agent, used in skincare products, and so on. Also, *Flexibacter* and *Sporocytophaga* produce the violet pigment that is used to examine drinking water samples. *Vogesella indigofera* is used to analyze chromium contamination at various locations. The pigment Astaxanthin obtained from *Agrobacterium auranticum*, *Paracoccus carotinifaciens*, *Mycobacterium lacticola* is used as a colorant in food. Also, Astaxanthin produced from *Xanthophyllomyces dendrorhous* is used as a food supplement for salmon, crabs, and shrimps. *Bradyrhizobium sp.*, *Halobacterium sp.*, *Cantharellus cinnabarinus*, and *Mycobacterium brevicale* are used in the pigment canthaxanthin in poultry and fish feeds (Kirti *et al.* 2014).

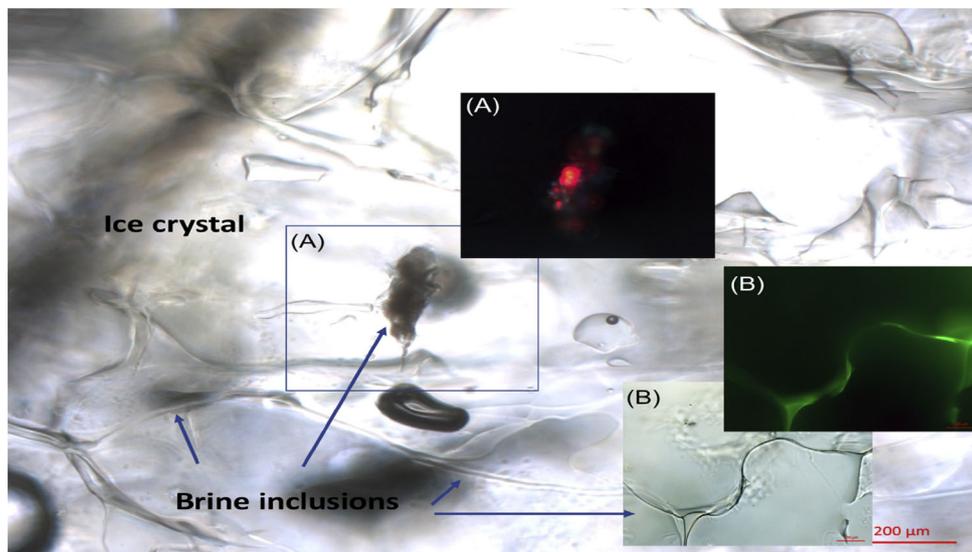
In addition to the cell wall adaptation, the microorganisms undergo an increase in biosynthesis of peptidoglycan that in turn thickened the peptidoglycan layer to sustain to low temperature. For example, *Planococcus halocryophilus* possesses thickened outer cell wall by a distinctive mechanism composed of calcium carbonate, choline, and peptidoglycan (Mykytczuk *et al.* 2013). Few bacteria also contain lipopolysaccharide (LPS) structure in the outer layer of the cell to acclimatize to the cold environment. LPS component A contains short-chain unsaturated fatty acids upsurge fluidity in cold environments. In recent transcriptome findings, higher levels of glycosyltransferases and outer layer proteins were observed in certain organisms (Casillo *et al.* 2016). One example is the mutation in the glycosyltransferase gene (*wapH*) of LPS causes impaired growth at low temperatures in an Antarctic bacterium (Benforte *et al.* 2018).

Another characteristic is the anti-freeze proteins (AFP) present in the microorganism interact with the ice thereby preventing recrystallization and inhibition

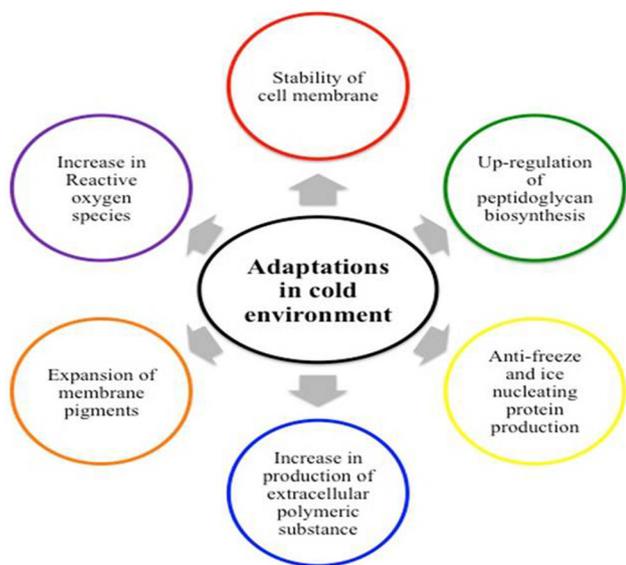
of ice growth in the cells. One of the major AFP discovered from *Marinomonas primoryensis*, found in the Antarctic, contains 1.5 MDa ice adhesion AFP. It has been reported that Antarctic algae and glacier ice bacteria safeguard the cell vicinity, protect the cell membrane and structural integrity, and stabilize the brine pockets (figure 2). At lower temperatures, several organisms help to enhance oxygen and nutrients uptake and help to position the cell on the ice surface (Voets 2017). On the other hand, fall the ice-nucleating proteins (INPs) that accelerate the formation of ice. INPs are less destructive to the cell membrane when present in minute crystals and provide crystallization quicker. These INPs frigidify damage the plants and facilitate access to nutrients (Pummer *et al.* 2015). Numerous INPs are currently used in the manufacturing of artificial snow, which is cost-effective and encloses other commercial applications (figure 3).

In consideration of the molecular adaptations, microorganisms at lower temperatures have advanced capacity to undergo translation and post-translation modifications. Many genomes and metagenomes of psychrophiles are a source of large features such as conserving plasticity of the genome concerning transposable or mobile genetic materials. The mobile genetic material transport genes form the biosynthesis of unsaturated fatty acids in cold-adaptive microorganisms. Few of these organisms may also experience horizontal gene transfer to familiarize themselves with lower temperatures (De Maayer *et al.* 2014). Zhao and colleagues have shown *Shewanella* strains have been confirmed to have genetic exchange with coding sequence in *S. halifaxensis* and *S. sediminis*. These organisms have greater homology with *P. profundum* SS9 and *C. psychrerythraea* that play a vital role in adapting to lower temperatures (Zhao *et al.* 2010). Considering the gene expressions, there is an increase in the amount of secondary structure of DNA/RNA while protein-folding kinetics decelerates. In Mesophiles and Psychrophiles, there is down-regulation of various genes to respond to cold-shock. Piette and colleagues reported that *Pseudoalteromonas haloplanktis* showed no synthesis of cold-shock protein from a temperature shift from 18 to 4°C. But, *P. haloplanktis* can survive up to -20°C in the Antarctic drift (Piette *et al.* 2012).

At lower temperatures, ROS production and the associated gas solubility is greater, which is commonly linked with cellular damage. Along with ROS, lower temperature promotes activities like solubility of nutrients, diffusion reduction, osmotic stress expansion, and formation and desiccation of ice. Due to the



**Figure 2.** Picture showing Arctic Sea ice of (A) ice algae cluster in ice brine pocket (Chukchi sea ice; with an epifluorescent image of the same cluster—note the brightly red chloroplasts) (B) brine pockets stained with green fluorescing polymer stain CTC (K. Junge). CTC, 5-cyano-2,3-ditolyl-tetrazolium chloride. Reprinted with permission from Karen Junge, Karen Cameron, and Brook Nunn Microbial Diversity in the Genomic Era. DOI: <https://doi.org/10.1016/B978-0-12-814849-5.00012-5>. Copyright © 2019 Elsevier.



**Figure 3.** Representation of psychrophilic proteins and enzymes that help to modify the according to the demands of the environment.

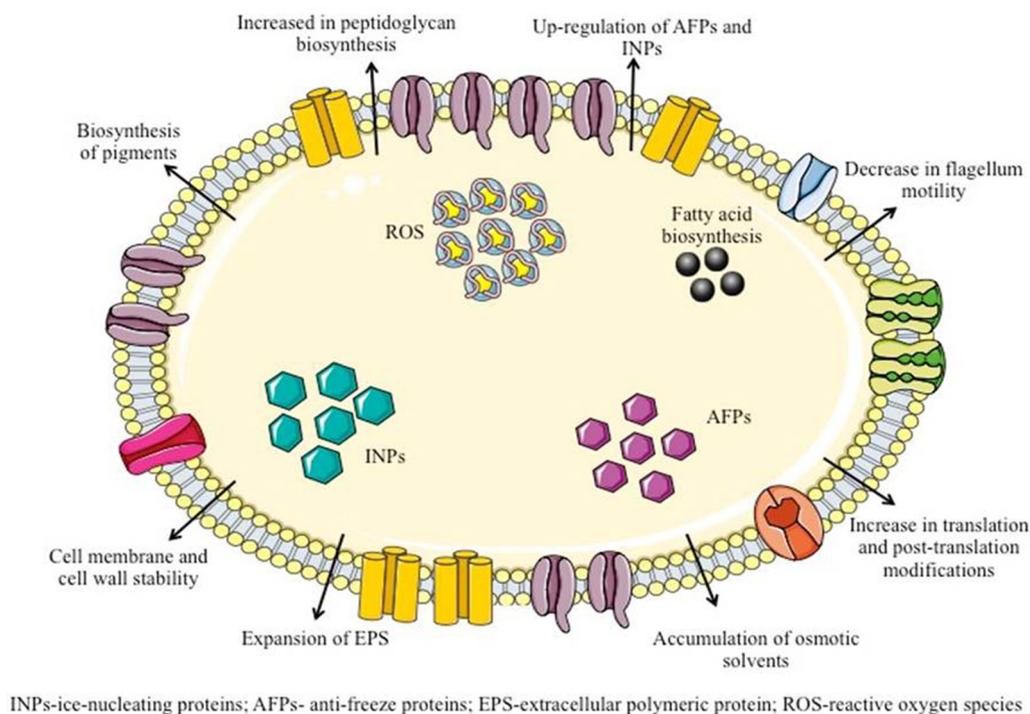
increase in ROS, there is a high production of catalases and superoxide dismutases that are considered to turn down ROS assembly. Considering the above, there is an accumulation of osmotic solvents, up-regulation of AFPs and INPs, the stability of cell wall and membrane, increased in peptidoglycan biosynthesis and post and translation modification along with changes in encoding proteins, nucleotide, and amino acids. *Methanococcoides burtonii*, *Psychrobacter arcticus*,

and *Photobacterium profundum* proved the above concept in the transcriptomic analysis (Campanaro et al. 2011). Similarly, *Listeria monocytogenes* and *Shewanella livingstonensis* found in Antarctic water, produce more than 30 proteins at 4°C and 18°C to maintain cell membrane, motility and increase energy metabolism (glycolysis and acetate kinase A-phosphotransacetylase pathway). On the other hand, *P. haloplanktis* showed reduced activity of ROS at low temperature (Cacace et al. 2010; Kawamoto et al. 2007; Piette et al. 2012) (figure 4).

### 3. Physiological adaptations and biodiversity of psychrophiles

Psychrophiles have a mucous-like protective layer called named as exopolymers that allow them to sustain in extreme temperatures. This mucous helps in maintaining the inner temperature of the microorganisms and prevents freezing or ice formation. In undeniable cases, microbial life at low temperatures is restricted to unfrozen water inside the solid ice cover and brine channels. These organisms contain an ample amount of salts, exopolymers and the temperature system is maintained by fluid flow (Genilloud 2018).

The capability of psychrophiles to survive and reproduce at freezing temperatures implies that these organisms have overcome limited and extreme conditions of environments. These perplexing conditions



**Figure 4.** Most observed adaptations in psychrophiles.

included instability of enzyme system, reduced membrane fluidity, and decreased physiological activities such as transcription, translation, cell division, protein activity, and its slowdowns cellular growth. The organism that is adapted to the environment of subzero temperature possesses evolved genotypic and phenotypic characteristics, which support its growth in extreme conditions. These extremophiles (Psychrophiles) have occupied a permanent space in cold environments from deep sea, arctic, Himalayan mountain to polar regions and hence called by the name psychrotolerant or psychrotroph (Morita 1975).

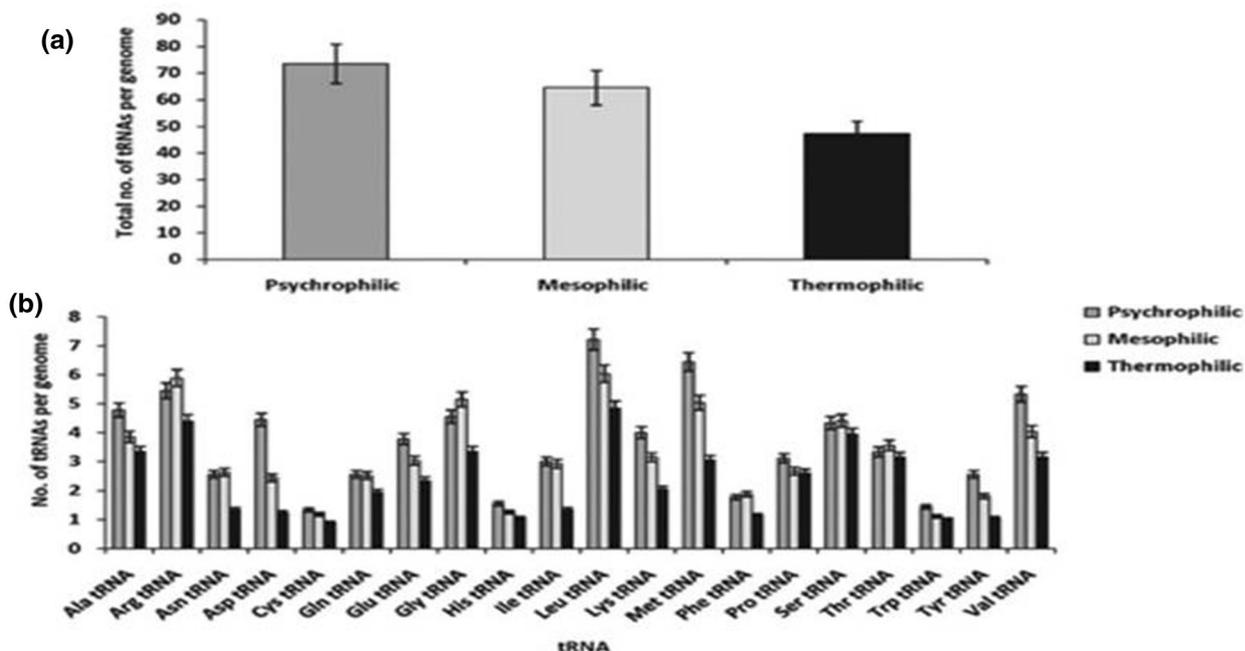
The environments at subzero temperatures have the various growing condition and hence aerobic and anaerobic bacteria are found at these temperatures. Other factors such as osmotic and hydrostatic pressure, solar, earth and cosmic radiation, oxidative stress, and nutrient availability also strongly affect living conditions. Consequently, adaptation to cold is often combined with acclimatization with various survival factors. Despite all of these challenges, life thrives in these environments with remarkable microbial biodiversity of main bacteria, fungi (in particular yeasts), and microalgae. Among the bacteria that have been reported, the most common microorganisms are the Gram-negative  $\alpha$ -,  $\beta$ - and  $\gamma$ -proteobacteria (*Pseudomonas* spp. and *Vibrio* spp.) and the *Cytophaga-Flavobacterium-Bacteroides* phylum and diversity of Archaea, although, in some areas such

as deep-sea waters, these are found in equivalent numbers, with Methanogenium and Methanococcus being the most cited genera. Among identified cyanobacteria, Oscillatoria, Phormidium, and Nostoc commune are dominant in most of the Antarctic habitats (Deo Pandey *et al.* 2004). Psychrophilic yeasts, particularly *Cryptococcus* spp., have been isolated repeatedly from soil samples and some researchers have described these organisms as the most important life form in Antarctic desert soils (Vishniac and Klinger 1986). Refer to Krembs *et al.* (2002) for a detailed description of the types of communities associated with specific cold environments.

## 4. Genomic and proteomic studies

### 4.1 Genetic modulations of psychrophiles for stress-response

The genes encoding for cold-adaptation and survival in stressful conditions dominate the genome of psychrophiles. The ‘omics’ explains various strategies and the responsible genes for stress-response, which include psychrophiles from extreme cold marine niches. Recently, Jin-Cheng Rong and the team sequenced the whole genome of CGMCC 1.6503<sup>T</sup> strain of *Paenisporosarcina antarctica*, a marine



FEMS Microbiol Lett, Volume 305, Issue 2, April 2010, Pages 100–108, <https://doi.org/10.1111/j.1574-6968.2010.01922.x>

**Figure 5.** Distribution of tRNAs among the psychrophiles, mesophiles, and thermophiles (a) all tRNAs, and (b) individual tRNAs. Reprinted with permission from Avirup Dutta and Keya Chaudhuri. Copyright © 2010. Federation of European Microbiological Societies FEMS Microbiol. Lett. 305 (2010). Published by Blackwell Publishing Ltd. All rights reserved.

psychrophilic bacterial species from Antarctica. The study revealed the presence of a set of cold-adaptation genes that help the survival of the bacteria in the cold environment (Rong *et al.* 2020). The genomic analysis of *Halobacterium* from Antarctica's Deep Lake shows genes encoding for bacteriorhodopsin, polyhydroxyalkanoate biosynthesis, gas vesicles, etc. which help in cold-adaptation (De Maayer *et al.* 2014). Genomic studies show a high content of GC regions in the psychrophiles, mainly comprising of genes coding for abundant amino acids and chaperonins. This data shows the occurrence of heavy translation and post-translation modifications, largely associated with traits required for cold-adaptation (De Maayer *et al.* 2014). The sigma factor that is elemental in transcription initiation also takes part in stress-response (Liu *et al.* 2019). *Methanococcoides burtonii*, like other psychrophiles, has shown an increase in fatty acids content of the cell membrane modulates cellular process, tRNA modification, and increases ribosomal proteins and fluidity. The differentially expressed proteins assigned to Cluster Orthologous groups are involved in cellular functions like translation, protein turnover, chaperones, energy production and conversion, carbohydrate transport and metabolism, etc. (Yusof *et al.* 2019).

The GC content of tRNAs and rRNAs is believed to be positively dependent on the optimum growth temperature of psychrophiles but is not true for the GC content of the genomic DNA. This supports the suggestion that rRNA and tRNA correlate to each other than the genomic DNA. The DNA is also believed to be intrinsically stable, as it does not denature at higher temperatures, which in turn seems to be due to the increased GC content. This being said, the tRNAs of psychrophiles are stable at lower temperatures and destabilize at higher temperatures, when checked with the folding energy minimization values. The adaptive changes of tRNA folding may be responsible for their stability at specific temperatures (Dutta and Chaudhuri 2010) (figure 5).

Comparative sequence analysis of psychrophilic algae with a mesophile shows that they modulate their physiological systems and metabolism depending on their surrounding environment and the nutrition available around them (Cook *et al.* 2019). The fact that psychrophiles are better equipped with oxidative stress countering genes is well understood in this study that compares such genes in the genomes of *psychrophilic methanogens* and the *non-psychrophilic methanogens* and is upregulated at lower temperatures (Chen *et al.* 2015).

## 5. Cold-adaptations

The genes related to cold-adaptations, such as EPS, putative anti-freeze proteins, and PUFA biosynthesis genes are responsible for cold-adaptations in psychrophiles (Vollmers *et al.* 2013). The cold-adaptations further explained by Collins *et al.* (2010) had depicted that there is more amount of extracellular DNA in winter and hence cells are compactly arranged and in very closely or tightly arranged each other with the high amount of extracellular DNA (Collins *et al.* 2010). The particulars of this environment, where cells are concentrated and in close proximity with each other along with high concentrations of eDNA, viruses, and other organisms and a host of chemicals likely favors the frequency of HGT processes and therefore the exchange of traits that could enhance function in it. The organism like *Marinomonas primoryensis* Ca<sup>2+</sup>-dependent and hyperactive (Gilbert *et al.* 2005) and rhizobacterium *Pseudomonas putida* GR12-2 shows both antifreeze and ice-nucleating activities. The high level of exopolysaccharides (EPS) in bacteria has been found in subzero temperature areas of Antarctic and Arctic sea ice. The main role of EPS is the retention of water, protect against cold-shock, act as cryoprotectants (Nichols *et al.* 2005; Krembs *et al.* 2002) (table 1).

### 5.1 Cold-shock and oxidative stress

Modification of DNA also plays a role in cold-adaptation and stress-response. The differential DNA methylation did in a *psychrotolerant psychrophile*, *Naganishia* yeast induced changes in the elongation factor G and chitin synthase export chaperon associated genes and are said to be a cold-shock response (Turchetti *et al.* 2020). To cope with the oxidative stress produced in response to the increased heat and respiration in cold conditions, there is an increased biosynthesis of PutA, the proline utilization protein, and KatG, the catalase-peroxidase; which eventually induce increased production of H<sub>2</sub>O<sub>2</sub> that will activate proteins against reactive oxygen species (ROS) (Gregson *et al.* 2020). Genes like *vicK* and *vicR* are believed to be responsible for coping with environmental pressure; *resE* and *resD* for oxygen stress; *nreABC* and *narGHIIJ* operon genes for respiration; *envZ* and *ompR* for osmotic upshift and operon genes *osmXY* and *osmVW* for osmotic stress (Rong *et al.* 2020).

**Table 1.** Antifreeze proteins and cryoprotectants

Protein/metabolite	Physiological role	References
Antifreeze proteins	Bind to ice crystals and create thermal hysteresis	Jia and Davies (2002)
Trehalose	The colligative effect helps in preventing protein denaturation and aggregation	Phadtare (2004)
Exopolysaccharides	Promote physico-chemical environment of bacterial cells, cell adhesion to surfaces, retention of water, sequestration, and concentration of nutrients, retain and protect extracellular enzymes against cold denaturation	Mancuso Nichols et al. (2005)

Some of the genes responsible for ROS response under oxidative stress are *sodA* (encoding for superoxide dismutase), peroxiredoxin *bcp* (encoding for thiol peroxidases), *trxA* (encoding for thiol-disulfide isomerase and thioredoxins), *osmC/ohr* (encoding for organic hydroperoxide reductase), *trxB* (encoding for thioredoxin reductase), *katE* and *katG* (encoding for catalase), etc. These genes are present in multiple copies in psychrophiles which contribute to their survival in cold as well as when exposed to high UV radiation (De Maayer *et al.* 2014).

### 5.2 Genes are acquired as an evolutionary adaptation

The genes pertaining to cold-adaptation in the CGMCC 1.6503<sup>T</sup> strain of *Paenisporsarcina antarctica* were found to be encoded by chromosomes rather than the plasmids, indicating these are not inherited and instead could be evolutionary strategies (Rong *et al.* 2020). Plasmids exhibit the genetic diversity of the microorganisms while increasing their genome's plasticity (Dziewit and Bartosik 2014). A study mentions the use of horizontal gene transfer for such acquired genes. This is seen in the case of the aerobic bacteria, *Oleispira antarctica* RB-8 as evidenced by the genes for alkane monooxygenase, located in the transposase-rich region. The study involving a phage-host relation of a *psychrophile*, indicates gene transfer to be mediated by the phage; while some evidence of plasmid-mediated

transfer was also found (Gregson *et al.* 2020). The phages in psychrophiles also carry the *psychrophilic* characteristics, for instance, the proteins DNA adenine methylase, transglycosylase SLT domain protein-1 and RNase HI, as seen in a study involving *Colwellia psychrerythraea*. Furthermore, psychrophilic ORFs have been found in phage-specific gene regions and BLASTN analysis suggests lateral gene transfer of ribonucleotide reductase genes between the phage and *psychrophile* genomes and are located close to each other (Colangelo-Lillis and Deming 2013). A similar recently reported NGS (next-generation sequencing) study in *Marinomonas* strain from an Antarctic psychrophilic ciliate shows acquisition of certain genomic islands through horizontal gene transfer (John, *et al.* 2020a, b). In a comparative study of psychrophiles, it was found that the horizontal gene transfer takes place through conjugative plasmids or mobilizable replicons engaging in the conjugal transfer. These plasmids may also be responsible for the host metabolism and functioning and encode for proteins responsible for energy production (Dziewit and Bartosik 2014).

As we are talking about the involvement of phages and NGS studies, NGS has also proven to be a diagnostic tool, as explained by Maria *et al.* (2016) to diagnose a meningitis patient. The infection caused by *Psychrobacter* sp. was checked with metagenomic analysis using next-generation sequencing in the patient's CSF. This study gives a lot of insight into the virulence of the species, which interestingly shared some characteristics with pathogenic bacteria having antimicrobial resistance. These resistant genes express ATP-binding permease, macrolide-specific ABC-type efflux related proteins, a peripheral membrane protein of the fusion protein family, and Zn-dependent metallo- $\beta$ -lactamase. The ABC transporter ATPase gene might be responsible for the heme-iron uptake. The OmpA gene for outer membrane proteins found here is believed to take part in the invasion of the brain microvascular endothelial cells and could be crucial in the pathogenesis. The type 4 pilin proteins encoding genes could be involved in DNA uptake, surface adhesion, motility, and aggregation; which would also cause pathogenesis (Maria *et al.* 2016).

### 5.3 Cell structure adaptations

Cell membranes in psychrophiles are modified to adapt to cold conditions, as some of the 'omics' studies show increased biosynthesis of the membrane components (De Maayer *et al.* 2014). Studies have shown the

presence of a couple of genes involved in the unsaturated fatty acid synthesis and the maintenance of cell membrane fluidity. For instance, the histidine kinase sensor gene, *desK*, and a response regulator gene, *desR* are believed to be important to help the bacteria thrive in cold stress conditions. These genes could be responsible for maintaining membrane fluidity and activation of the phospholipid desaturase in the membrane (Rong *et al.* 2020). Other studies show increased production of lipopolysaccharides, peptidoglycans, and glycosyltransferases in the *psychrophile* membranes (De Maayer *et al.* 2014). NGS studies and transcriptomic data of a *psychrophilic* copepod, *Tigriopus kingsejongensis* showed two stearyl-CoA desaturase genes *TkSCD-1* and *TkSCD-2* which are responsible for fatty acid biosynthesis, which is believed to be produced for cold-adaptation of the organism. The conserved histidine-rich motifs provide sites for ferric iron-binding. These desaturases play an important role in the conversion of saturated fatty acids into monounsaturated fatty acids that are responsible for membrane fluidity and biosynthesis, for survival in cold stress environments (Jung *et al.* 2016). The movement of the bacteria in such a highly viscous environment is powered by the flagellar protein FLiL, which is produced by the transcriptional regulator FleQ. This structural integrity of the flagella is enhanced by an alteration in the ratio of flagellin subunits such as an increase in FlaB protein levels (Gregson *et al.* 2020). Although such structural changes are reported to take place, some studies show that the genes for flagella and iron uptake receptors are generally down-regulated at cold temperatures (De Maayer *et al.* 2014).

## 6. Enzymes and proteins play a crucial role in cold-adaptation and stress-response

The transcriptomic analysis shows the presence of superoxide reductase, 3-Cys thioredoxin peroxidase, and other proteins like anti-oxidation stress proteins and electron-transfer enzymes at lower temperatures (Chen *et al.* 2015). Marine psychrophiles produce certain cold-active enzymes like extracellular aminopeptidase ColAP, which are believed to contribute to structural stability and fluidity at cold temperatures (Huston *et al.* 2004). It is interesting to note that some of these enzymes like the isoaspartyl dipeptidases extracted from the psychrophiles are thermostable, which is attributed to their octameric structures (Park *et al.* 2017). As for the response to the cold-shock, certain genes are down-regulated or

differentially expressed. For instance, antioxidant enzymes like catalase and superoxide dismutase are increasingly produced, while the genes in the ROS pathway are down-regulated (De Maayer *et al.* 2014). The catalase genes enable the psychrophiles to survive in oxidizing environments (Bendia *et al.* 2018). Some of the proteins and enzymes like hydroxylases play an important role in the degradation of long-chain hydrocarbons and are found at sites polluted with oil and certain psychrophiles are also resistant to heavy metals (Orellana *et al.* 2018).

The cold shock proteins have a nucleic acid-binding domain called the cold shock domains that preserve their RNA's single-strand conformation. For cold-adaptation, RNA-binding proteins, RNA helicases, dihydrouridine help to make changes in the RNA. The nucleic acid-binding proteins are crucial for DNA replication, transcription, translation, ribosome synthesis structures (Dalmaso *et al.* 2015). Temperature-based studies reveal that ribosome biogenesis, the proteins involved in energy production, chaperones, and archaeal protein folding are temperature sensitive. Likewise, the expressions of certain methanogenic proteins are increasingly expressed at higher temperatures as compared to the cold ones. However, some proteins like the trimethylamine corrinoid proteins are not significantly sensitive to temperature, although they decreased at 4°C. Other proteins involved in the methanogenic pathway like the tetrahydromethanopterin S-methyltransferase subunit H, methyl coenzyme M reductase alpha subunit, and tetrahydromethanopterin formyl transferase alpha subunit are again temperature sensitive. The ice-binding proteins and antifreeze protein also help the cells to survive in cold-stress conditions (Liu *et al.* 2019; Rong *et al.* 2020).

The genomic analysis through 16S rRNA and rRNA based NGS sequence information revealed the presence and its possible biological applications in identifying microbial phylogeny. The physiological activity like methane cycling, iron reduction, sulfide reduction, and sulfur oxidation. The mechanism of mineral utilization (FeS<sub>2</sub> oxidation and formation of a reactive Fe oxide) and metabolism microbes. Further Dieser *et al.* (2014), Cameron *et al.* (2015) have demonstrated that the production of FeS<sub>2</sub> oxidation and formation of a reactive Fe-oxide is mediated by microbes. These techniques are being used to measure the production of protein and enzymes, which help in the understanding of active subglacial metabolisms.

## 7. Amino acid modifications, chaperones, and chaperonins

Some species express the chaperonins Cpn60 and Cpn10, which help them to adapt to the cold environment (Gregson *et al.* 2020). The protein chaperones TF and Hsc66 play an important role in cold shock response along with other proteins responsible for trehalose synthesis, lipid biosynthesis, and cellular metabolism (Turchetti *et al.* 2020). *Psychrobacter arcticus* has reduced expression of Trigger Factor, which is a chaperone bound to the ribosome, and has increased expression of the GroEL/ES chaperonins in cold conditions (Chen *et al.* 2015). The proteins in psychrophiles have reduced synthesis of amino acids like alanine, proline, and arginine, and increased synthesis of methionine, asparagine, and glycine to enable flexibility by a reduction in hydrogen bond and salt bridge formation. Most of these proteins are long with external loops, which ensure their stability. A higher number of hydrophilic groups on these proteins ensures high solvation and enzyme flexibility (De Maayer *et al.* 2014). A study on TRiC chaperonin of *Glaciozyma antarctica*, a psychrophilic yeast showed that the genes are expressed constantly, regardless of heat or cold shock. It is also known to restore denatured luciferase to its functional state at 4°C (Yusof *et al.* 2019).

For the proteins to efficiently fold, the electrostatic interactions of ion pairs are reduced. The hydrophobic interactions are also altered for cold-adaptation through residue substitutions in the TRiC (TCP1-1 ring complex (TRiC) chaperonin) subunits. Non-polar amino acids like Cysteine, Tryptophan, Leucine, Phenylalanine, and Isoleucine are the ones involved in the hydrophobic residues. The substitution of such hydrophobic residues with hydrophilic residues like serine, arginine, aspartic acid, lysine, histidine, and glutamic acid alter the hydrophobic interactions by increasing the residue distance which ultimately helps with cold-adaptation. An example of such substitution is Isoleucine to Valine at TRiC $\alpha$  at position 372 in *G. antarctica*. The isoleucine is placed into hydrophobic pockets formed by the distanced residues. The aromatic amino acids are also involved in interactions responsible for cold-adaptation (Yusof *et al.* 2019). It might be interesting to note that the genes responsible for proline, which acts as a protective osmolyte are also found in some *psychrophile* genomes. The osmolytes help the cells to maintain integrity and viscosity. Lysine has been used increasingly by psychrophiles to ensure protein flexibility at cold temperatures (Liu *et al.* 2019).

## 8. Other strategies for cold-adaptation

The exposure of psychrophiles to sudden changing environments will certainly affect the expression of proteins and thus produced proteins are called heat shock proteins or cold shock proteins that take part in several cellular processes such as transcription, translation and ultimately help in regulating membrane fluidity (Phadtare 2004). Carotenoid pigments are produced by psychrophiles that help them maintain the membrane fluidity and viscosity in the events of temperature changes (De Maayer et al. 2014). The carotenoids believed to stabilize the cell membrane at extremely cold temperatures include genes like *crtB* (phytoene synthase), *crtI* (phytoene dehydrogenase), *crtY* (lycopene beta cyclase), *crtZ* (betacarotene hydroxylase), and *idi* (isopentenyl-diphosphate delta-isomerase) which are found in the carotenoid biosynthesis pathways in the psychrophiles (Liu et al. 2019). Proteorhodopsin, the light-dependent proton pump is believed to play a role in the physiological adaptation under stress (Liu et al. 2020). The extracellular polysaccharides support the cells as cryoprotectants (Liu et al. 2020). To counter the cytoplasmic freezing, glycine, mannitol, betaine, and sucrose are produced that also prevent UV radiation mediated cell damage; or the antifreeze proteins and exopolysaccharides are produced (De Maayer et al. 2014). Glycogen is also believed to help the psychrophiles resist stressful temperatures. The genes involved in glycogenesis—*glgA* (encoding for glycogen synthase), *glgB* (encoding for glycogen branching enzyme), and *glgC* (encoding for glucose-1-phosphate adenylyltransferase) were found in *psychrophile* genomes, which are probably involved in energy accumulation. Adaptation to stressful stimuli show the presence of genes responsible for copper homeostasis, dominance sigma B genes, etc. (De Maayer et al. 2014).

## 9. Antibiotics and new-age antibiotic-like compounds from *psychrophilic* marine microorganisms

Marine microorganisms are the leading source for the development and discovery of new antibiotics. This is due to wilder adaptability to the punitive environment and genetic capability to produce distinctive metabolites. Researchers are extensively studying and aiming to unravel newer antibiotics from psychrophiles, mainly due to their temperature resistance and subsequent applications in human health. Antibiotics and

antibiotic-like, or collectively termed antimicrobial compounds developed from alternate sources in the wake of antibiotic resistance and multi-drug resistance, provide more reason to study this aspect. Psychrophiles show a huge potential for the bioactive compounds that can be used for this purpose. Species discovered by scientific teams dating back to the late 1990s and early 2000s showed secretions of antibiotics by marine microorganisms, of which many were *psychrophilic* bacteria and algae (Stonik et al. 2020). NGS done on permafrost samples from lakes in Canada showed the presence of new psychrophiles along with some of their exopolymers, which include commercially relevant enzymes and polymers. Some of these have potential therapeutic activities like anti-viral, anti-bacterial, anti-cancer, drug-delivery, etc. (Finore et al. 2020). Studies in metabolites from marine psychrophiles have made them potential candidates and stand in the list of commercial bioactive compounds.

Secondary metabolites are abundantly produced in *Streptomyces* species like *Streptomyces griseus* and *Streptomyces hygroscopicus*, which include potential candidate compounds for antibiotics (Goodfellow and Fiedler 2010). Such heat-stable antibiotics produced from *Streptomyces* species show a higher efficiency at the acidic range, against gram-positive bacteria (Ogata et al. 1971). It has been reported that most of the antibiotics are found in gram-positive bacteria during biosynthesis and have pharmacological and clinical uses. Jiang and teammates have reported spiro-tetronate antibiotics Lobophorins B from actinomycetes, while Lobophorins F and H were obtained from *Streptomyces* spp. in the South China Sea. Lobophorins F and H work against *B. subtilis* and is a potential drug candidate against many species (Jiang et al. 1999). Another actinomycete *Verrucosipora* found in the Japanese sea, polycyclic antibiotics called abyssomicins B, C, and D revealed activity against *S. aureus* (Bister et al. 2004). A new antibiotic caboxamycin found in *Streptomyces* spp. found in deep sediments of the Atlantic Ocean. Caboxamycin displayed inhibitory action against *B. subtilis* and *Staphylococcus lentus* and devious activity against *S. epidermidis* (Hohmann et al. 2009). Engyodontiumone H belonging to the xanthone class obtained from the *Engyodontium album* in the China Sea show inhibitory activities against *E. coli* and *B. subtilis* (Huang et al. 2013). Having said this, antibiotics and antibiotic-like compounds are abundantly found in *actinobacteria*, which includes microorganisms belonging to the genera *Acanabacterium*, *streptomyces*, *Streptomyces*, *Actinobaculum*, etc. As *actinomycetes* from terrestrial sources have

been a primary source of antibiotics for decades, it is only logical that the species from other sources like marine ecosystems be studied for the commercial application.

The psychrophiles portray an added advantage of having heat-stable bioactive metabolites with a higher efficiency even against antibiotic-resistant species like methicillin-resistant *Escherichia coli*, vancomycin-resistant *Enterococcus faecium*, etc., as well as other pathogenic microorganisms like fungi and viruses (Stonik *et al.* 2020). Moreover, the genes involved in adaptation in extreme conditions like high temperature and high pressure are instrumental in the industrial processes and provide novel commercially viable by-products. For instance, hydrolases produced by extremophiles enhance the catalysis process and are efficient to use with other solvents. The products of catalysis provide the basic constituents of antibiotics and other drugs; for instance, psychrophiles produce lipases that are used to synthesize amino based antibiotics (Dalmaso *et al.* 2015).

Antimicrobials from bacteria called bacteriocins have been in the spotlight lately, for their abundance and efficiency. A similar compound isolated from a psychrophile found in Argentina exhibits antimicrobial activity in pathogenic strains like *Escherichia coli* by blocking DNA replication (Borchert *et al.* 2017; Sánchez *et al.* 2010). Subtilomycin, another bacteriocin or widely known as lantibiotics produced by *Bacillus subtilis* isolated from psychrophilic sponge show activity against pathogens including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Staphylococcus aureus* (Phelan *et al.* 2013; Borchert *et al.* 2017). 16s rDNA analysis of Antarctic psychrophiles done by a team at the University of Messina in Italy show antibacterial activities (Lo Giudice *et al.* 2007). Similar activity was observed in bacterial isolates from Antarctic sponges as well as in arctic lichens that showed activity against gram-positive and gram-negative bacteria (Borchert *et al.* 2017).

Apart from the metabolites, the genetic sequences involved in cold-adaptation have also been instrumental in building recombinant plasmids or nanoparticle synthesis that exhibit antibiotic-like properties or the development of antimicrobial formulations. For instance, the genes from a mesophilic microorganism were transfected into a psychrophile to construct a biocatalyst which was used to produce reuterin. This compound is a potent antimicrobial that is generally found to be produced by *Lactobacillus reuteri*. However, the construct from a psychrophilic system would

make it heat-stable (Tajima *et al.* 2013). The cold-adaptations studied in psychrophilic microorganisms suggest the genes in question are capable of a horizontal gene transfer (HGT), which probably enable these microorganisms to acquire the cold-adaptation characteristics (Vollmers *et al.* 2013). A study of marine psychrophiles shows the presence of acquired omega-3 and omega-6-type PUFA synthesizing genes from Gamma proteobacteria and Bacteroidetes through Horizontal gene transfer (Bowman 2017). Synthesis of PUFA such as eicosapentaenoic acid and docosahexaenoic acid (DHA) have also been found in *Psychroflexus torquis* through HGT which apparently help increase membrane fluidity and are also involved in thermo-sensory regulation of the bacteria (Feng *et al.* 2014). In this context, a *Marinomonas* species associated with a marine psychrophile *Euplotes focardii*, have been used to produce silver nanoparticles, exploiting the HGT mechanism. The nanoparticles exhibited strong antimicrobial activities against pathogens like *Staphylococcus aureus* (John, *et al.* 2020a, b). The nanoparticles produced by *Pseudomonas* associated with the psychrophile show an enhanced antimicrobial activity when compared to a chemically synthesized one (John, *et al.* 2020a, b), thereby suggesting the activity exhibited by the protozoan metabolites.

Many species show an abundance of clustered regularly interspaced short palindromic repeats (CRISPRs) in their genomes (De Maayer *et al.* 2014). A new psychrophile from Arctic soil whose closest phylogenetic member is *Flavobacterium*, shows cold-adaptation proteins in its genome, along with a possible CRISPR-Cas system and certain secondary metabolite biosynthesis gene clusters (Chaudhary *et al.* 2020). Bacteriophages detected in association with psychrophiles in some studies also show a vast CRISPR-Cas system, indicating the presence of viral microorganisms in the niche (Liu *et al.* 2019). The CRISPR editing system comes in handy to construct recombinant plasmids for antibiotic applications as mentioned here. Moreover, it is being studied as an emerging tool in techniques like bacteriophage therapies to overcome antimicrobial resistance (Gholizadeh *et al.* 2020; Aslam *et al.* 2020).

## 10. Conclusions

The psychrophilic species are adapted to a cold environment by modulating their genetic information according to their surrounding environment. The cold-adaptive enzymes and proteins, DNA modifications,

exopolysaccharides, anti-freeze proteins, and other secretions mentioned in the above sections tell us how prepared the organisms are to combat the stressful conditions. The plasmids and phage-based studies explaining the accounts of acquired genes also tell us that the genetic makeup also depends on their niches, rather than just the habitat. Therefore, these adaptations allow the psychrophiles to produce secondary metabolites that would be commercially relevant, especially for antibiotic synthesis. With the current battle of drug-resistance, novel antimicrobial and antiviral candidates, preferably of biological sources are in the spotlight.

Antimicrobial peptides from various sources are being studied for this purpose with special attention to bacteriocins or antimicrobial peptides of bacterial origin, due to their abundance and ease of modification. However, large scale productions result in these bioactive compounds being subjected to varying temperatures and pressure. Therefore, the compounds isolated from extremophiles like psychrophiles help cope with this and also provide a novel range of genes to experiment with. However, very few studies and links have been established in this aspect. Looking at the available genomic and cold-adaptation data on these psychrophiles can open up avenues to more bioactive compounds that may be directly or indirectly used for antimicrobial activities.

More studies on phage-host genomics might shed some light on the gene transfer mechanisms and explain some of the adaptive mechanisms acquired from their surrounding environment. Since bioactive compounds are explored for their application in pharmaceuticals and other industries, a database of the *psychrophile* genomes will be beneficial to study more about the enzymes and proteins produced by these microorganisms, especially in the unculturable ones. This being said, most of the genomic studies have a major paucity in the knowledge of the survival of the microorganisms in extremely hot and extremely cold environments, even if some sequences in both temperatures have been discovered (Dalmaso et al. 2015). Despite multiple studies suggesting multiple gene copies in the *psychrophile* genome for cold-adaptation, Liu et al. (2020) say this might not be the case (Liu et al. 2020). Certain sequences from psychrophiles like *Arthrobacter* and *Psychrobacter* are found in both cold and hot environments (Bendia et al. 2018). These shortcomings need to be addressed with more extensive genetic analysis, only then will the door to novel bioactive compounds will be opened. In the current review, we tried to link the available data on

psychrophiles and the potential antimicrobial activities of their metabolites.

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