Charles University
Faculty of Science

Study Programme: Biology

Branch of Study: Ecology and Evolutionary Biology

Matouš Jimel

Algal and Cyanobacterial Adaptations to Low Temperature and Desiccation

Adaptace řas a sinic na nízké teploty a vysychání

Bachelor Thesis

Supervisor: prof. Ing. Josef Elster, CSc.

Consultant: doc. RNDr. Linda Nedbalová, Ph.D.

Prague, 2020

Acknowledgement

I would like to extend my gratitude to my supervisor, prof. Ing. Josef Elster, CSc., for all his advices, corrections and patience, but also the inspiration and opportunity to study the subject of polar ecology both at home and in field. Furthermore, I would like to thank my consultant, doc. RNDr. Linda Nedbalová, Ph.D., for all her help and attentiveness, which proved invaluable. Lastly, I would like to thank my family, friends, and significant other for their incessant support.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 8. 1. 2020

Podpis

Abstract:

Algae and cyanobacteria, due of their evolutionary antiquity, are widely distributed primary producers that can withstand extreme environmental stresses. Low temperature, freezing and melting, and desiccation and rewetting, are common stresses prevalent mainly in polar regions and in winter seasons of temperate areas. In terms of physiological effects, these types of stresses share similar effects or are closely related to one another.

Low temperatures and desiccation exert a variety of stresses that need to be negated or lessened by adaptations. Specifically, adaptations to chill, freeze, and desiccation stresses will be discussed, as well as strategies that allow for stress avoidance or resistant morphological adaptations.

In this thesis, characteristics, functions and mechanisms of these adaptations and stresses are reviewed, as well as potential biotechnological uses of said adaptations.

Key words: algae, cyanobacteria, freezing, chill, desiccation, abiotic stress, cryoprotectants, osmoprotectants, akinetes, cryoinjury

Abstrakt:

Řasy a sinice, díky své dlouhé evoluční historii, jsou velmi rozšíření primární producenti, kteří dokážou odolat extrémním environmentálním stresům. Nízká teplota, vymrzání a tání, vysychání a opětovné zavodňování, jsou stresy, které jsou obzvlášť běžné v polárních regionech a zimních sezónách oblastí mírného pásu. Z hlediska fyziologických dopadů jsou si tyto stresy podobné či spolu úzce souvisí.

Nízké teploty a vysychání vyvíjí spoustu typů stresů, které musí být vyloučeny či zmírněny adaptacemi. Konkrétně budou shrnuty adaptace na chlad, vymrzání a vysychání, spolu se strategiemi, které umožňují vyhnutí se stresu či tvorbu odolných morfologických stádií.

Práce shrnuje vlastnosti, funkce a mechanismy těchto adaptací a stresů i potenciálních biotechnologických využití.

Klíčová slova: řasy, sinice, vymrzání, chlad, vysychání, abiotický stres, kryoprotektanty, osmoprotektanty, akinety, kryopoškození

Contents

1 Introduction

Algae and cyanobacteria are two major oxyphotosynthetic microorganisms that share similar ecological role and are often coupled together due to their role in global ecosystems as primary producers. Both are widely distributed and able to survive in extreme conditions such as extremely cold environments to an extent that allows them to produce visible biomass (Elster & Benson, 2004).

Among all the extreme environments, permanently cold areas are the most widespread and include permafrost regions, mountain ranges and deep oceans (Glaring et al., 2015). Microorganisms, including cyanobacteria and algae, are dominant in these areas and serve as the main primary producers. The average temperatures of these areas do not rise over 5 °C and often, the temperature is below zero (Glaring et al., 2015). Cold adapted organisms that live in these areas are usually classified as either psychrophiles or psychrotrophs. While both groups are defined as having the ability to grow at low temperatures, psychrophiles are those that have their growth optimum at ~15 °C or lower, and psychrotrophs at ~15 °C or higher (Morita, 1975).

Permanently cold areas share a specific set of stresses exerted on its biota. The most crucial of these is the low temperature hindering basic cellular functions, and lack of liquid water and consequential potential desiccation. Deformations of the cell wall and important organelles caused by the presence of ice is another prominent factor, which may occur both intracellularly and extracellularly (Storey & Storey, 2013). Furthermore, the presence of ice exerts more stresses. Namely, osmotic stress is in aquatic environment considered inherent to freeze stress (Tanghe et al., 2003), as water osmolarity in channels around sea ice increases to very high levels even in freshwater environments (Schmidt, 1991). Likewise, oxidative stress is accompanying almost any abiotic stress, freeze and desiccation included (Kvíderová et al., 2019; Tanghe et al., 2003).

The ability to overcome stresses of sub-zero temperatures, cyanobacteria and algae have developed various adaptations that include intracellular and extracellular substance production and whole cell transformation into morphologically specific (pre-)akinetes and other stages that possess increased resistance to abiotic stresses (Kvíderová et al., 2019; Fuller, 2013). These can be divided into groups based on which stress they target primarily - chill, desiccation, and freeze. It should be noted, however, that these adaptations are not necessarily specialized for just one stress, just like the stresses themselves, they often overlap and complement each other in their functions.

This thesis will summarize known mechanisms of algal and cyanobacterial injuries associated with low temperatures and desiccation, as well as all varieties of their adaptations and their mechanisms to overcome or avoid them and successfully colonize permanently cold areas. The importance of understanding these mechanisms is crucial for further development of future cryobiological methods, primarily within the study of cryopreservation, food and agricultural industries and biofuel production.

2 Effects of Low Temperatures and Desiccation on Microorganisms

For organisms to survive in low or sub-zero temperatures, the set of stresses exerted should be neutralized or, alternatively, avoided. Inability to compensate negative effects related to "phase change of water in both extra- and intracellular environments", which is associated with sub-zero temperatures, leads to cellular damage called cryoinjury (Gao & Critser, 2000). Cryoinjury is generally divided into two mechanisms depending on the rate of cooling at which the injury occurs. Portrayed on Figure 1, slow cooling is associated with increased intracellular and extracellular solute concentration, which leads to excessive turgor loss and shrinkage; whereas rapid cooling is related to extracellular ice formation that causes intracellular ice formation (Mazur, 1984) through excessive exosmosis. There does not seem to be a singular definition of rapid or slow cooling rate in terms of degrees Celsius, although Mazur (1984) hints at 2 °C of cooling per minute being slow rate of cooling and 4 °C as a fast rate. However, this could be only applicable to the type of cells (embryo) used in the study.

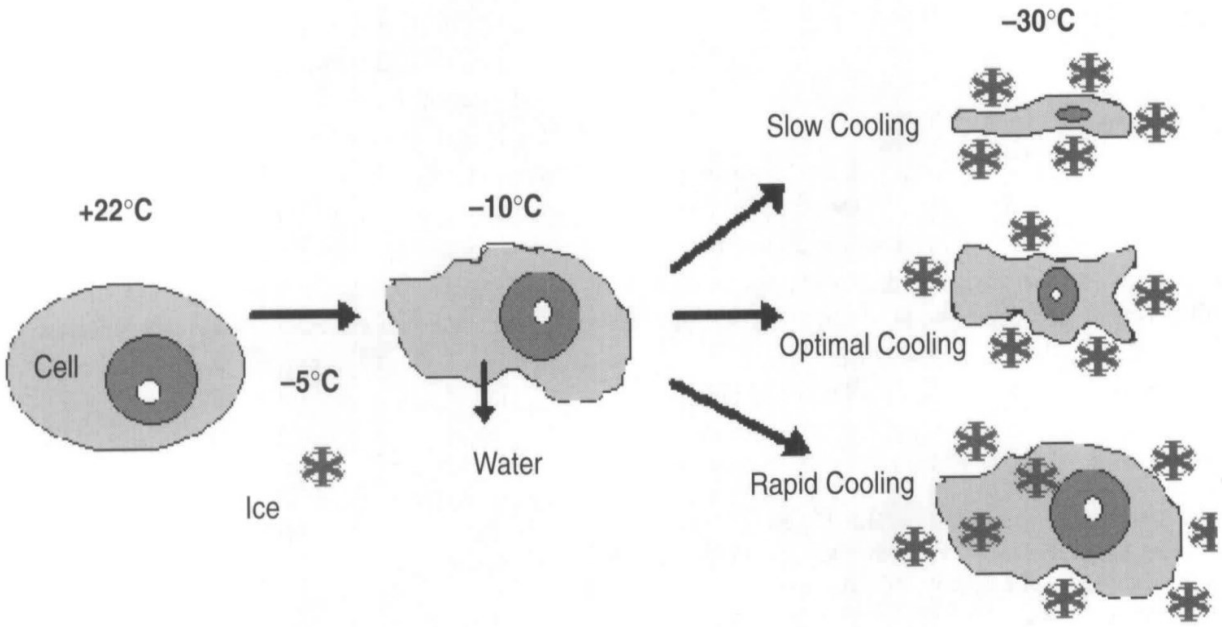

Figure 1: Schematic drawing of physical events in cells during freezing (Gao & Critser, 2000, adapted)

The first major principal phenomenon is that low temperatures decrease molecular kinetic energy of the environment, which has multiple implications for organisms, some of which are sublethal and some lethal. Enzyme activity is widely known to negatively correlate with low temperatures due to decreased kinetic energy that lowers the amount of collisions between enzymes and their appropriate substrate molecule, which slows down or halts entirely crucial metabolic processes. While not strictly lethal, the ability itself of individual cells to survive in extremely low temperatures does not grant them the ability to fully complete its life cycle, which includes reproduction (Clarke et al., 2013).

Second principal effect is that freezing temperature lowers the amount of liquid water. This principle is very similar to desiccation and the closely related osmotic pressure. With little to no liquid water, main macromolecules' functions are compromised, which endangers biochemical processes of the entire cell. Most importantly, the fluidity of membranes is compromised due to phospholipid degradation (Potts, 1999). Membrane fluidity facilitates maintaining its integrity, without which the cell is not able to maintain functions that are reliant on membrane bound proteins and electrochemical potentials. Nucleic degradation via depurination and loss of vital cellular materials through compromised membranes are also known to occur (Potts, 1999).

Formation of ice crystals in the extracellular environment causes the solute concentration to increase as the ice crystal expels any solutes. This further induces water efflux through osmotic gradient across the plasma membrane, which dehydrates the cell, causes its shrinkage and increases the viscosity of the intracellular environment. Dehydration induced cell shrinkage causes membrane damage due to its vesiculation, triggered by insufficient elasticity of the membrane (Steponkus & Lynch, 1989).

High viscosity reduces the molecular movement and, therefore, the movement of reactive compounds (Black & Pritchard, 2002). If, however, the membrane permeability does not allow for a high enough efflux rate, the cytoplasm can become supercooled, and form intracellular ice which tends to be lethal, as it compromises vital intracellular structures (Muldrew & McGann, 1994).

Intracellular ice formation generally requires a fast rate of cooling that is uncharacteristic to natural conditions (Clarke et al., 2013). Instead, slower rate of cooling allows for intracellular vitrification—a process, during which liquid properties of the intracellular environment are replaced by solid ones, while molecular change or thermodynamic properties remain largely unchanged. Under such conditions, most of metabolic and respiratory processes are almost entirely halted,

however, a diminutive amount of molecular movement permits contraction and the release of heat (Wowk, 2010). Because of small difference between vitrified and normal states, vitrification is not lethal, and cells are known to fully recover (Clarke et al., 2013).

Extracellular ice also poses danger to cells due to potential damage caused by the passage of ice crystals through the membrane as well as mechanical pressure (Mazur, 1984). However, it is generally accepted extracellular ice formation is usually not the direct source of freezing injury (Day & Fleck, 2015).

3 Stress Definitions

In this overview, several stresses and their corresponding adaptations will be discussed. Depending on the definition, cryoinjury can be caused by a range of stresses merely associated with low temperature exposure. Furthermore, many of said stresses are very similar, with some being strictly tied to one another, however, they can be distinguished.

Chilling Stress

While hard to precisely define in terms of specific numbers, chilling or low temperature stress is exerted on organisms that are in an environment of suboptimally low non-freezing temperature. In plants, chilling stress occurs when the temperature drops to between 10 to 15 °C and chill sensitive plants are considered those that are damaged when exposed to temperatures of 15 °C to 0 °C (Chen, 1994).

Freezing Stress

Like chill stress, freezing stress is caused by low temperature. In this case, the temperature that drops below 0 °C. This leads to a wide set of potential damaging events due to the formation of ice in the tissue of organisms (Chen, 1994) or around cells of microorganisms, both of which can be damaging or lethal. Freezing stress also directly causes other stresses due to the elimination of liquid water (Mazur 1970).

Desiccation Stress

Desiccation stress occurs when an organism gets extremely dried out and is left devoid of moisture. This has further implications of the viability of desiccated cells as biochemical processes rely largely on water.

Osmotic Stress

Osmotic stress describes the detrimental drawing out of water from cells due to an increase or decrease of osmolarity of the cell's environment. This may cause either hyperosmotic or hypoosmotic stresses. A change of osmolarity around a cell is common in environments, where ice crystals are present and is, therefore, often associated with freezing stress.

Salt Stress

Salt stress is the accumulation of salt in the cellular environment. While similar to osmotic stress in that it may cause osmotic unbalance (we may even say that salt stress leads to osmotic stress), it is specific in that it also encompasses salt toxicity and ionic unbalance, which may further incentivize the cell to increase the intake of other ions (Zhu, 2001).

Oxidative Stress

All previous stresses lead in one way or another to also produce oxidative stress, which produces reactive oxygen species (ROS), which damage essential cellular macromolecules like lipids, proteins and nucleic acids (Betteridge, 2000).

4 General Stress Resistance Adaptations

Microorganisms in general have developed a range of adaptations to stresses, some of which do not strictly aim at lessening the negative effects of any one specific stress. Instead, these adaptations lessen any potential damage caused by a combination of stresses or stress side effects.

4.1 Molecular Chaperones

Molecular chaperones are protein families associated with the control of folding of proteins. Specifically, they prevent or refold the aggregation of proteins which would remove the proteins' functionality. The expression of molecular chaperones occurs upon exertion of an environmental stress like a rapid change in temperatures or osmotic pressure (Papp et al., 2003, Bhagwat & Apte, 1989). Their mode of function entails binding to proteins with nonnative structure and restoring them to their native form (Buchner, 1996). While protein denaturation is not the most prominent source of cryoinjury in a cell (Mazur, 1970), molecular chaperones could facilitate disaggregation during thawing (Tanghe et al., 2003).

A major group of proteins with the function of a molecular chaperone are heat shock proteins. According to Bhagwat & Apte (1989), they are known to play a role during protection in a number of different stresses, including cold shocks, osmotic and oxidative stresses. While their presence in polar cyanobacteria remains unconfirmed (Kvíderová et al., 2019), its presence in polar algae is confirmed in both macroalgae (Smolina et al., 2016) and microalgae (Mock & Thomas, 2008).

4.2 Saccharides

Sucrose, and more importantly, trehalose function as molecular chaperones as well as a response to osmotic and desiccation stresses by acting as compatible solutes (Bougouffa et al., 2014; Potts, 1999). Sucrose and trehalose are two nonreducing disaccharides that are very commonly used by both algae and cyanobacteria (Bremauntz et al., 2011; Lunn et al., 2014; Potts, 1999). According to Erdmann & Hagemann (2001) both sucrose and trehalose have the highest energetic cost (at 109 ATP equivalents) and lowest solubility of all compatible solutes. This suggests that their importance lies primarily in their other functions. For example, Crowe et al. (1987) discovered that under certain conditions, trehalose is the most efficient membrane stabilizer during freeze-drying cycles among sugars, lagged slightly by sucrose. Even though this discovery was undercut by the finding that this is the only case under suboptimal conditions for freeze-drying, (Crowe et al., 2005) reaffirms their importance as an efficient membrane and protein stabilizer in at least some cases (Leslie et al., 1995). Furthermore, during desiccation, trehalose showed DNA - protective properties (Tashyreva & Elster, 2013; Zhang et al., 2017).

4.3 Antioxidant Substances

Abiotic stresses usually exert additional stress that may further damage the cell due to production of dangerous free oxygen radicals - oxidative stress. Betteridge (2000) describes oxidative stress as "a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, which may lead to tissue injury". While in low concentrations they can serve as important signaling molecules, in higher concentrations, they become toxic (Betteridge, 2000).

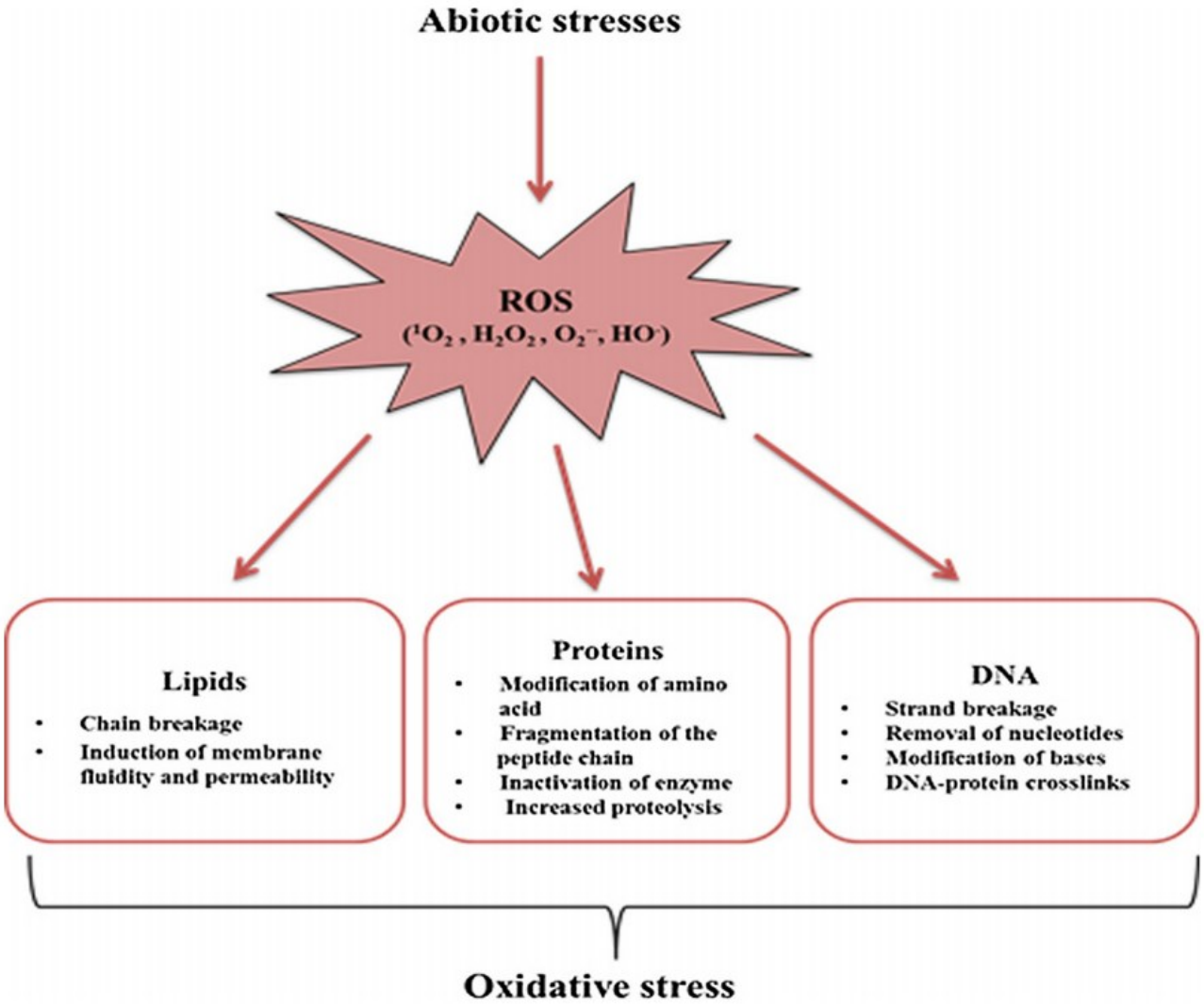

Figure 2: Reactive oxygen species (ROS) induce oxidative damage to proteins, lipids and DNA (Rezayian et al., 2019, adapted)

Reactive oxygen species (ROS) are not produced only as a result of abiotic stress, but also many usual biochemical processes, and in cases of extreme freezing, background ionizing radiation and cosmic rays (Ozkavukcu, 2002). This means that cells need to have a sufficiently developed

antioxidant responses to prevent increasing ROS concentrations that could damage their structures and key substances like DNA, lipids and proteins (Jamieson, 1998). The specific damage caused by ROS for every group is shown in Figure 2. During aerobic freeze-thaw cycle, an oxidative burst was observed in yeast cells, causing a lot of damage. This could partially explain the mechanism of freezing injury (Park et al., 1998).

Oxygen is a highly reactive element that serves many important functions within the metabolism of all aerobic organisms. In respiration, its primary function is that of terminal electron acceptor, which, at the end of the electron transport chain process, is prematurely reduced and a superoxide molecule (O_2^-) is produced. Superoxide is then usually transformed into a hydrogen peroxide and then into water or hydroxyl radical. In algae, it is generally produced in chloroplasts, cytosol, mitochondria and peroxisomes (Rezayian et al., 2019). However, other ROS exist and appear as a result of many different abiotic stresses like freezing (Tanghe, 2003) and desiccation (Potts, 1999). These include hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals (HO).

As a response to an increased presence of ROS, microorganisms developed antioxidant defense substances. These can be divided into two main groups - enzymatic and non-enzymatic antioxidants. There are three major enzymatic antioxidants that appear in both cyanobacteria and algae: superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Ismaiel et al., 2014). Non-enzymatic antioxidants include a very wide range of substances including carotenoids, tocopherols, phycobiliproteins and phenolic compounds (Ismaiel et al., 2014; Rezayian et al., 2019).

5 Chill Stress Adaptations

Chill stress adaptations aim at lessening negative effects of low temperatures and preventing potential cryoinjuries, should temperature drop below 0 °C caused by freeze stress (and any other stresses caused by it) by preserving the structures and functions of cells.

5.1 Plasma Membrane Adaptations

Membranes are the most basic and crucial parts of any living cell. Aside of the basic purpose of physical division of two different environments, many microorganismal membranes also have other key membrane bound functions, like transport of substances between intracellular and extracellular environments, respiration and photosynthesis. These functions and other membranal properties are regulated by cold and desiccation adapted algae and cyanobacteria if affected by said stresses. The most represented substances within membranes are lipids, which form the lipid bilayer. The bilayer is formed by two main parts - the fatty acid hydrophobic tails and phosphate group

associated hydrophilic heads, with other components like proteins and sterols are embedded into it (Alberts et al., 2002).

5.1.1 Fluidity

Membrane fluidity is one of the key properties that roughly describes the viscosity and the ability to let substances pass through the bilayer. This is valid for both passively permeating substances and those associated with active transport methods such as membrane proteins (Alberts et al., 2002).

Fluidity is dependent on several factors like temperature, which affects algae and cyanobacteria greatly due to their poikilotherm nature. It is determined by the composition of the bilayer, namely the representation of lipids and proteins and their placement within the structure. Cold-adapted organisms can change the composition based on temperature, but also surrounding water content (Singh et al., 2002). Bonding conformation also plays a role in control of fluidity. Using all these processes, cold adapted organisms aim to achieve "homeoviscous adaptation of membrane fluidity" (Suutari & Laakso, 1994).

Table 1: The cold-dependent lipid changes in different groups of microorganisms (Russel, 2008)

Type of lipid change	Group of microorganism
Increased FA unsaturation	Bacteria, archaea, yeasts, filamentous fungi, algae
Increased FA methyl branching	Bacteria
Increased *anteiso/iso*-branched ratio	Bacteria
Decreased FA average chain length	Bacteria, yeasts, filamentous fungi, algae
Decreased sterol/phospholipid ratio	Bacteria, yeasts, filamentous fungi, algae

FA, fatty acid (acyl)

5.1.2 Fluidity and Permeability Enhancing Adaptations

Microorganisms change fatty acid composition and properties of their membranes as a response to cold and heat shocks. The most important changes are listed in Table 1. These occur in complex glycero-acyl lipids, which are the predominant membrane lipids of most microorganisms (Russel, 2008). They are also precursors to many lipid related components like long-chain hydrocarbons and wax esters, and they are frequently bound to alcohols and amines, which confer a wide range of cellular properties (Suutari & Laakso, 1994).

5.1.3 Fatty Acid Desaturation

Degree of unsaturation of fatty acids greatly affects membrane fluidity, and a wide variety of organisms including both algae and cyanobacteria, use denaturases to change it. Desaturases are a

group of membrane bound enzymes that are able to channel two atoms from fatty acids to an acceptor, which usually happens to be oxygen (Russel, 2008). This process creates a water molecule and a fatty acid chain with a double bond. This configuration reduces the integrity of the membrane by shortening of the distances between atoms in the double bond, as well as their angle, which leads to a reduction of interactions between molecules and subsequent increased fluidity (Los & Murata, 1998).

It is likely that decreased fluidity associated with low temperatures increases denaturase activity, which in turn increases the fluidity, making it an autoregulatory system (Russel, 2008). Algae and some cyanobacteria use denaturases that produce fatty acids with multiple double bonds. These are called polyunsaturated fatty acids (PUFA). Among microorganisms, they are known to be produced primarily in green algae, diatoms and dinoflagellates, as well as some bacteria (Lyon & Mock, 2014; Ganesan et al., 2013).

5.1.4 Fatty Acid Branching

Methyl branching of acids is another commonly observed response to cold shocks in adapted prokaryotic organisms (Poger, 2014). The two major conformations of methyl branching are anteiso and iso, meaning the methylation occurs at the “antepenultimate” - third last - and penultimate carbon atoms, respectively. In some microorganisms, iso branching is dramatically reduced in favor of anteiso branching. This is due to anteiso branching being more potent in reducing the “gel to liquid-crystalline phase transition” temperature (Suutari & Laakso, 1994). While this phenomenon has been largely observed in gram-positive bacteria (Kaneda 1991), a significant proportion of anteiso very long chain fatty acids (those containing 22 or more carbon atoms) branched lipids were found in an Antarctic cyanobacterium *Calothrix* sp. (Řezanka et al., 2009).

5.1.5 Other Fluidity Increasing Alterations

The length of fatty acid chains is generally, though not always, negatively correlated with membrane fluidity (Quinn, 1991). This is due to decreasing van der Waals forces between the molecules. As individual chains spread further apart, the attractive effect of these forces decreases. This is an effect intensified if only alternate chains are shortened, as longer chains’ distal ends do not interact with van der Waals forces and have more space to move around in, increasing fluidity (Russel, 2008).

There are several other factors that further affect membrane fluidity in relation to decreasing temperatures. Sterols are another group of membrane protein that have a stabilizing and reinforcing effect on the lipid bilayer. As such, an increased presence of sterols is not required in cold adapted

cells. They are common and abundant in algae and eukaryotes in general, and much less so in prokaryotes. Instead, prokaryotes, and cyanobacteria specifically, produce functionally analogous hopanoids (Russel, 2008).

5.1.6 Aquaporins

Aquaporins, belonging to the major intrinsic protein (MIPs) family, are a major group of proteins that appear in almost all organisms. Their role in the passive membrane transport of water and similar non-polar substances like glycerol through the membrane (Tanghe et al., 2002). Tanghe et al. (2002) also suggests that an overexpression of aquaporin genes increases freeze tolerance in yeast cells and their deletion decreases it. This is likely due to faster water efflux rate facilitated by aquaporins. At freezing temperatures, the water efflux rate is impeded, which may be dangerous to cells as water presence may increase the presence of damaging ice crystals (Tanghe et al., 2002).

5.2 Enzymatic and Protein Production

At low temperatures, the structure and function of essential proteins become compromised, lowering the viability of the cells. Because of this, enzymes adapted to become better suited at functioning under chill stress, together with proteins whose presence helps retain basic cellular functions.

5.2.1 Cold Adapted Enzymes

As defined by Arrhenius equation, temperature affects all rate of reactions, including the enzymatic activities of cold-adapted organisms. Enzymatic activity is crucial for any life, and thus, for the continued survival of cells in suboptimally low temperatures, enzymes need to have increased structural flexibility, decreased thermostability and increased specific activity at low temperatures (De Maayer et al., 2014). As shown in Figure 3, there is a trade-off for all these properties as cold adapted enzymes have decreased stability and are more sensitive towards heat (Gianese et al., 2002; Devos et al., 1998). Devos et al., (1998) also discovered that two *Chloromonas* species, one mesophilic and one psychrophilic, differed in the RUBISCO enzyme activity - it was higher in the mesophilic species. Psychrophilic species, however, compensated for reduced activity by having double the amount of the enzyme compared to other mesophilic algae.

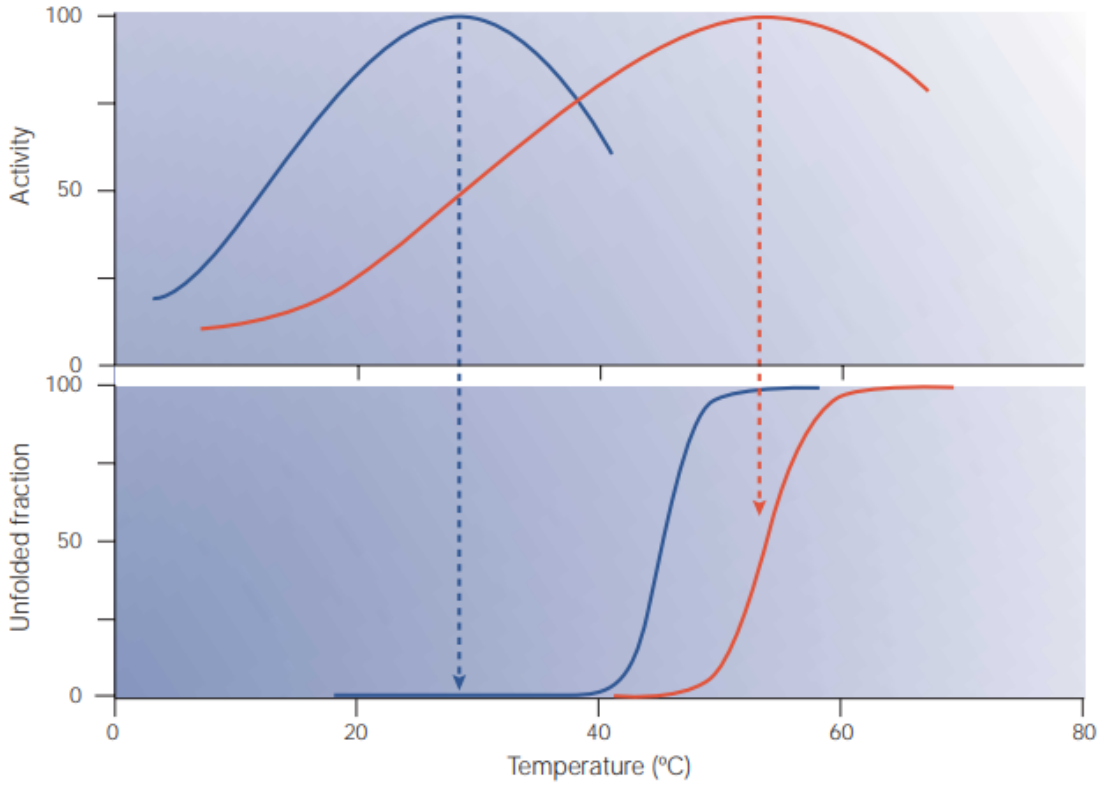

Figure 3: Psychrophilic (blue) and mesophilic (red) enzymatic activity (upper graph) and change of enzymatic conformation (lower). (Feller & Gerday, 2003, adapted)

5.2.2 Protein Production

An important group of proteins produced as a part of cold shock response are the cold shock proteins (CSPs). These bind to nucleic acids and facilitate continued functions of folding, transcriptions, translations and RNA degradation prevention. They have been described as having a chaperone function (Raymond-Bouchard & Whyte, 2017). Their production allows for prolonged growth at suboptimally low temperatures (Berger, 1996). They are known to be produced by algae (Kim, 2010), however, cyanobacteria produce analogous proteins with a similar function (Los & Murata, 1998).

Cold acclimation proteins (CAPs) are produced in similar conditions as CSPs, but they are distinct in that while CSPs are produced in response to cold shock, CAPs are produced continuously in organisms growing in low temperature environments (Panoff, 1997). They seem to be crucial for growth in suboptimally low temperatures (Berger, 1996), however, their precise function has not been fully investigated.

6 Desiccation Adaptations

Desiccation is very closely associated with osmotic stress and freezing stresses. For the purposes of this thesis, osmoprotectants and cryoprotectants, while primarily focused on osmotic stress, are considered a major group of substances that prevent desiccation, since osmotic stress usually precedes desiccation as water disappears from the environment (Potts, 1994). Other adaptations play a role in the stabilization of the extracellular and intracellular environments during desiccation.

6.1 Osmoprotectants and Cryoprotectants

Osmoprotectants, also known as compatible solutes (Bougouffa et al., 2014), are “small molecules that act as osmolytes and help organisms survive under extreme saline conditions” (Rajesh et al., 2014). As such, these organic compounds provide protection to cells that are trying to survive in conditions that exert high osmotic pressure. Especially in cold environments, freezing water very commonly exerts this pressure on microorganisms, primarily due to ice expelling solutes from its forming lattice into surrounding water, increasing the salinity of cells’ surroundings and creating an osmotic gradient that either dehydrates and shrinks the cells (Storey & Storey., 2013) or causes salt stress by inducing an influx of salts into the cell, inflicting damage to intracellular macromolecules (Bougouffa et al., 2014). This can be remedied partly by the accumulation of osmoprotectants, although possibly active Na^+ removal by antiports is required (Joset et al., 1996).

Microorganisms are capable of both producing (via biosynthesis) and accumulating osmoprotectants from their surroundings (Kempf & Bremer, 1998), although cyanobacteria are capable of biosynthesizing (and transporting) all their osmotically active substances (Joset et al., 1996). Since abiotic factors may require high accumulation of these substances, osmoprotectants have a proclivity towards having a neutral net charge at physiological pH; they also tend to have low molecular mass and they are highly soluble (Kempf & Bremer, 1998). Hence, the accumulation does not have significant adverse effects on cellular processes (Yancey, 1993). Nevertheless, some are known to have a charge at physiological pH, like glucosylglycerate (Empadinhas & da Costa, 2008).

6.1.1 Osmoprotectant and Cryoprotectant Classification

The main groups of osmoprotectants as described by Kempf & Bremer (1998) include amino acids and derivatives, quaternary amino compounds, tertiary sulfonium compounds, sugars and derivatives, sulfate esters and N-acetylated diamino acids and small peptides. Among algae, all of these were found, whereas cyanobacteria only accumulate some of these—specifically glucosylglycerol (absent in algae), glutamate betaine, glycine betaine, proline, sucrose and trehalose. Those that accumulate glycerol (primarily algae) or glycine betaine (primarily cyanobacteria) tolerate the highest levels of salt concentrations, whereas those that accumulate disaccharides tolerate the lowest levels. This correlates with the substances' solubilities and biosynthesis ATP requirements (Erdmann & Hagemann, 2001). Different types of osmoprotectants also imply different needs in the organisms in which they are aggregated (Hagemann, 2016).

Some of these substances can also be called cryoprotectants. Cryoprotectants are considered to be any substance that increases viability of a cell that undergoes freezing and thawing. There is an overlap of the functions of these compounds, due to the physiologically similar effects freezing and desiccation have (Karlsson & Toner, 1996).

6.1.2 Amino Acids and Derivatives

Among amino acids, proline, sucrose, alanine, glycine and glutamate are widely known to accumulate in algae (Yancey, 1993) and their accumulation tends to correlate with osmotic stress resistance (Brown & Hellebust, 1978). Proline appears to be the most widespread amino acid acting as an osmolyte (Yoshiba et al., 1997) and is deemed to be present particularly in green algae and diatoms (Hagemann, 2016). Interestingly, it is also a rare example of an osmolyte, that is being further metabolically active (Yoshiba et al., 1997). Its importance also extends beyond its function as an osmolyte - it is also able to stabilize DNA and improve membrane and enzyme integrity, all of which is endangered in highly saline environments (Rajendrakumar et al., 1997).

Glycine betaine (GB) or trimethylglycine is a widespread amino acid derivative commonly found in algae and cyanobacteria (Erdmann & Hagemann, 2001), but also archaea (Yancey, 1993) and higher plants (Hong-Bo, 2005). As a betaine, GB contains a nitrogen atom that is fully methylated and is considered a quaternary amino compound (McNeil et al., 2001). In most microorganisms that produce GB, the biosynthesis takes place as a two-step process of oxidations—first, it is oxidized into betaine aldehyde, which is further oxidized to form GB. In terms of function, GB is known to conserve the activity of photosystem II in highly saline environments, which are chemically similar to freezing ones, as well as to improve membrane and enzyme integrities (Khristin & Simonova, 1998).

6.1.3 Polyols

Polyols are a large group of osmoprotectants that is widely present in algae, and to a lesser extent in cyanobacteria (Empadinhas & da Costa, 2008). Glycerol, sorbitol, mannitol and inositol are recognized to be major compatible solutes in algae (Hagemann, 2016; Jackson & Seppelt, 2006).

One of the most efficient compatible solutes in terms of energy production and solubility is glycerol (Erdmann & Hagemann, 2001). It is the presence of sterols in eukaryotic organisms that allows cells to retain glycerol, the absence of which prevents most prokaryotic organisms to utilize it (Empadinhas & da Costa, 2008). Glycerol is a well-known osmolyte and cryoprotectant present primarily in halophilic algae (e.g. *Dunaliella* sp.), but also appearing in green algae (e.g. *Chlorococcum submarinum*) (Blackwell & Gilmour, 1991), including polar algae (e.g. *Prasiola crispa*) (Empadinhas & da Costa, 2008). This is most likely due to its properties as a remarkably hydrophilic compound whose production requirements are extremely low (comparatively 30 ATP equivalents to 57 of other polyols or 109 of sucrose and trehalose), which can facilitate survival at high concentrations of Na^+ (Erdmann & Hagemann, 2001).

Floridoside and its isomer isofloridoside are widespread heteroside osmoprotectants acting as the major compatible solutes primarily in red algae (e. g. *Rhodymenia palmate*, *Mastocarpus stellatus*) (Courtois et al., 2008), excluding Ceramiales, which accumulate mainly dulcitol, mannitol and sorbitol (Karsten et al., 1992; West et al., 1992). They are accumulated primarily as a result of photosynthesis and plays a role in cell wall resistance (Courtois et al., 2008). In terms of structure, they seem to be structurally similar to the major cyanobacterial compatible solute, glucosylglycerol. This compatible solute is produced, rather than accumulated from the environment (Pade et al., 2015).

Another major compatible solute from the polyol group is mannitol. While this compatible solute also appears in some red and green algae, it is most prominent among brown algae (e.g. *Eiseniea* sp., *Dictyota* sp.) (Hagemann, 2016). Like (iso)floridosides in red algae, brown algae are capable of mannitol biosynthesis via photosynthesis, where mannitol is its main product. Considering that its osmoregulatory function is principal (Davison & Reed, 2004), mannitol has several other functions like antioxidant and storage (Iwamoto & Shiraiwa, 2005).

6.1.4 Sulphuric Compounds

Dimethylsulfoniopropionate (DMSP) is a tertiary sulfonium compound functioning as a compatible solute that is commonly found in marine algae and rarely in cyanobacteria (Bucciarelli et al., 2013). Due to the compound missing a nitrogen atom, it is thought that DMSP can be the compatible solute of choice in nitrogen-limited areas. Another distinctive attribute of DMSP is that it is regulated by microorganisms much more slowly, even after a hyperosmotic shock, which normally induces compatible solute production or accumulation; or hypoosmotic shock, which induces compatible solute release (applies only for some algae like *Phaeocystis* sp.) (Stefels, 2000; Stefels et al., 1996). Due to this phenomenon Stefels (2000), hypothesizes that DMSP is not a conventional osmolyte, but rather a "constitutive compatible solute". Aside of its function as a "constitutive compatible solute", DMSP is also a known cryoprotectant that is capable of not only preserving functionality of some enzymes of polar algae, but to further stimulate them by up to 150% (Karsten et al., 1996).

Dimethyl sulfoxide (DMSO) is an important cryoprotectant that acts extracellularly. It is even widely used in the process of cryopreservation. Nonetheless, unlike compatible solutes, DMSO is harmful to cells in high concentrations (Hubálek, 2003). Due to its low molecular weight and hydrophilicity, it can permeate through the cell membrane. It is thought that DMSO creates hydrogen bonds with water and inhibits extracellular ice formation (Bui et al., 2013).

6.2 LEA Proteins

Late embryogenesis abundant proteins known as LEA proteins are known to be present in all life domains (Mertens et al., 2018). They are most abundant in plants, but they also have been discovered in algal phyla *Chlorophyta* and *Streptophyta* (Campos et al., 2013) and cyanobacterium *Nostoc commune* (Close, 1993). Due to this fact, it is possible that despite its relative abundance in vascular plants, LEA proteins predate them. This is further implied by LEA-like proteins found in

bacterial and archaeal taxa, although their presence may be attributed to convergent evolution or horizontal transfer (Campos et al., 2013).

These proteins are associated with abiotic stress resistance and tolerance—specifically, water deficit stress. They can be characterized as highly diverse hydrophobic proteins whose main function is the conservation of enzymatic function brought on by dehydration. Amara et al., (2014) describes LEA proteins as "a subset of gene products, seen to accumulate to high levels during the maturation phase of cotton seed development". Since they do not have a common structure, and are, as such, considered a part of intrinsically disordered proteins (IDPs) ("The Role of LEA - late embryogenesis abundant Proteins in Cellular Stress Tolerance", n. d.), they are categorized into several families that share similar coding sequence.

Overall, all LEA proteins lack one specific unifying trait in terms of chemical structure. Most are characteristically hydrophilic and rich in polar amino acids. However, the majority of LEA proteins can be branded "hydrophilins". These are defined as having a high content of non-polar amino acids, specifically glycine (above 6%), and a hydrophilicity index higher than 1 (Battaglia et al., 2008; Amara et al., 2014)

Although specific mechanisms on molecular level are unknown, LEA proteins are closely associated with an assortment of functions. Principal of these is the ability to synergistically act as molecular chaperones (Kovacs et al., 2008). Freezing and desiccation induced aggregation of citrate synthase, which is responsible for the functioning of the Krebs cycle, causes impairs the functioning of the enzyme. In the presence of a disaccharide sugar acting as a chaperone called trehalose, LEA proteins can prevent citrate synthase from aggregating (Goyal et al. 2015).

6.3 Exopolymeric Substances

Exopolymeric substances (EPSs) are an important group of organic ligands that are frequently produced by microorganisms (Norman et al., 2015). They form a mucilaginous mass taking the form of sheaths, slimes and capsules (Edwards et al., 2002) that cover microbial, often filamentous, biofilms (Kumar et al., 2018). EPSs do not have to tightly adhere to cells, they can also be produced freely around the filaments, creating "slime" (de Philippis, 2002). They are known to increase tolerance to low temperature and desiccation stresses in both cyanobacteria and algae (Tashyreva & Elster, 2013). Additionally, they have several more functions like predation, protection against antibacterial agents, sediment adherence, hydration, nitrogenase protection by reducing oxidative stress (de Philippis, 2002; Rossi et al., 2012; Tamaru et al., 2005). These properties allow

cyanobacteria to provide a microenvironment, which allows other species to survive, making them the optimal first colonizers of inhospitable environments (Rossi & de Philippis, 2015).

6.3.1 EPSs Contents

A study by Edwards et al. (2002) revealed that EPSs are mainly formed by five carbohydrates: ribose, xylose, glucose, galactose and uronic acid—with less significant amounts of mannose and glucuronic acid. Arabinose and rhamnose were also found to be dominant in some cases. EPSs further contain methyl, sulphate groups, rhamnose, fucose and urionic acids (de Philippis, 2002). The latter three confer a hydrophobic and hydrophilic characteristic to EPSs, which makes them amphiphilic. On the one hand, rhamnose and fucose are responsible for the hydrophobicity, which allows EPSs to adhere to its solid substrate (Bellezza et al., 2003). On the other hand, uronic acid contributes to increased anionic density in external layers of EPSs making them hydrophilic which allows for the storage of water and important materials and minerals like calcium, iron and magnesium, which increases desiccation tolerance (Rossi et al., 2012).

6.3.2 Function of EPSs

EPSs have several mechanisms of facilitating survival of desiccation. For example, EPS can increase liposome retention during desiccation. In a study by Hill et al. (1997), the EPSs of *Nostoc commune* were examined. While their retention capabilities are quite poor, in the presence of sucrose and trehalose, they were found to increase the total amount of liposomes retained from 80 to 90%.

Long-term desiccation increases the damage done by high intensities of light and also UV-B exposure. Reactive oxygen species can inflict damage that is intensified by UV light exposure (Castenholz & Garcia-Pichel, 2013). Exposure to UV-B light increased production of EPSs by up to three times, which is analogical to leaf thickening in higher plants. The EPSs contain mycosporines—amino acids that are produced by photosynthetic organisms to protect themselves from UV-B light (Bhatia et al., 2011; Ehling-Schulz & Scherer, 1999).

7 Freeze Stress Adaptations

Freeze stress adaptations are concerned with the limitation and controlling of ice crystal growth in the cellular environment and even preventing the formation of ice.

7.1 Ice Binding Proteins

Ice binding proteins (IPBs) are used by organisms to prevent freezing damage in the vicinity of ice crystals by binding to them and keeping their growth and shape in check and “inhibit ice

recrystallization" (Davies, 2014). They are known to be produced by taxa of many kingdoms—mainly fungi, plants, bacteria, animals and protists (Zalis et al., 2013). Depending on their function and mechanism thereof, IBPs are classified into several groups: high thermal hysteresis antifreeze proteins (AFPs), low thermal hysteresis IPBs and ice nucleating proteins (INPs) (Duman, 2015). Moreover, IPBs, relatively to their seemingly singular function of ice adsorption, have a large structural diversity. This implies that IPBs have evolved many times among several kingdoms (Davies, 2014). According to Zalis et al. (2013), IBPs include "single α-helices, single β-solenoids, four helix bundles, polyproline type II helix bundles, and small globular proteins".

This diversity blurs the understanding of how so many disparate proteins evolved with the sole unifying purpose of binding to ice, although there are some traits characteristic of IBPs in general. They are generally considered small (Zalis et al., 2013), freely soluble at millimolar concentrations in aqueous solutions and have a hydrophilic and a hydrophobic side, making them amphipathic. Their binding site is described as "extensive, relatively flat, and hydrophobic, largely devoid of charged residues, and often have some repeating motifs" (Davies, 2014).

7.1.1 Hystereses

The principal ability of all IBPs acting as AFPs is that they facilitate thermal hysteresis (TH). TH is often described as lowering the nonequilibrium freezing temperature below melting point, but this definition is more appropriate for freezing hysteresis Zalis et al., (2013). TH is rather more precisely described as combination of two separate hystereses: freezing (FH) and melting (MH). Melting hysteresis is the increase of nonequilibrium melting point temperature and is frequently omitted due to the value being relatively low (in an experiment done by Celik et al., (2010) only one tenth of TH was MH). Zalis et al. (2013), however, notes that it is unlikely that the importance of IBPs is in TH facilitation due to the temperature change being too small at only a few tenths of a degree. The value of TH is dependent on the type and concentration of the IBP (Zalis et al. 2013; Scotter et al., 2006).

7.1.2 IBP Function and Mechanism

Ice recrystallization is a process of larger crystals growing faster at the expense of smaller ones, which are thermodynamically unstable (Janech et al., 2006; Zalis et al., 2013). It is very common in natural conditions with temperature fluctuations, partially frozen environment or high subzero temperatures in general (Zalis et al., 2013). This process causes damage to the surrounding cells due to increased osmotic pressure, dehydration and structural disruptions. Portrayed in Figure 4B, IBPs that function as AFPs adsorb to the surface of ice crystals and restrict further water addition to the ice crystal to the spaces between each bound AFP. While ice can continue to grow in said spaces, the newly grown ice begins to curve and after mere nanometers, growth is inhibited. This is graphically represented in Figure 4A. The curvature increases to the extent that makes it energetically unfeasible to continue growing unless the temperature drops further. This is called the adsorption-inhibition hypothesis (Raymond & Devries, 1977). AFPs also reduce water loss through melting inhibition, a similar mechanism at a higher temperature (Davies, 2014).

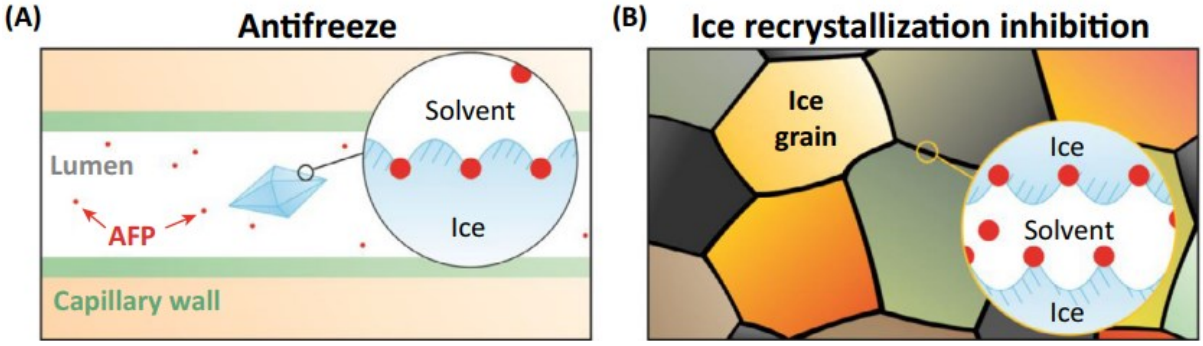

Figure 4:

(A) Diagram of an ice crystal in a blood vessel. Red dots represent AFPs, the inset shows their binding to the surface of the ice crystal, which causes it to form curvatures.

(B) Ice recrystallization between ice grains. AFPs (red dots) insets (grain boundaries), which inhibit movement that allows for ice recrystallization. (Davies, 2014, adapted)

An example of this mechanism was found in Antarctic Chlamydomonad algae, which can slow down the brine efflux from sea ice, which most likely increases nutrient availability and waste product removal (Raymond et al., 2007). Additionally, an Antarctic sea ice bacterium (*Colwellia* sp.) was found to do the same and some diatoms (*Fragilariopsis cylindrus, Navicula glaciei*) possibly as well (Janech et al., 2006; Raymond et al., 2007). As such, these discoveries imply that this mechanism could be widespread among polar Antarctic microorganisms. Furthermore, the genes for IBPs and IBP-homologues were found to be absent in some mesophilic diatoms, which may suggest that horizontal transfer of genes for IBP production is possible, making it even more widespread (Raymond & Kim, 2012).

7.1.3 IBP Anchoring Mechanism

Originally, hydrogen bonds and AFP IBS hydrophobicity were considered to be the main driving forces of AFP binding to ice lattice (Devries & Lin, 1977). However, incongruencies between the first hypothesis premise and the actual AFP behavior (preferential binding to ice at 55 M solvation) disproved it (Garnham et al., 2011). The second was disproved by several simulations of molecular dynamics, which indicated that AFPs are capable of preorganizing water into a "quasi ice-like structure" that facilitates the binding to an ice lattice. After that, a "zipper mechanism" is used to fully bind the protein—the ice-binding surface of an AFP stabilizes the growing ice lattice and fully merges with it (Nutt & Smith, 2008). The formation of ice in extracellular environment is not always detrimental to present organisms. In fact, it can help increase survival due to a phenomenon known as supercooling.

7.1.4 Ice Nucleation

In the absence of any impurities within an aqueous environment, the freezing point of water can be reduced, or supercooled, down to -40 °C. For the water to start freezing if no impurity, ice crystal or solid surface is present, an aggregate of water molecules formed into an ice-like pattern is required. However, in most cases, a solid surface or an impurity is present and thus, they are potential "ice nucleators" (Lundheim et al., 2002). The phenomenon of supercooling is quite frequent within cells, even in the presence of extracellular ice. (Mazur, 1984) claims that due to the absence of effective ice nucleators and the presence of surface membrane cells that blocks ice exogenous ice nucleation, they can get intracellularly supercooled to -15 °C.

As such, there are two imperatives for microorganisms to increase their survivability. The first one is that they must not allow any penetration of their membrane by ice crystals small enough to enter the cell through its pores, as it would trigger intracellular ice nucleation, which is usually lethal. Due to ice producing smaller crystals at lower temperatures, some microorganisms produce ice-nucleating proteins to increase the temperature at which ice forms, which tends to produce larger ice crystals (Knight, 1968, cited by Lundheim et al., 2002) that cannot pass through the cells' membrane (Lundheim et al., 2002; Mazur, 1984). Second is the osmotically induced reduction of intracellular water until any water remains unfreezable, which can constitute as little as 9% of the original content (Nei, 1973). Holmstrup & Westh, (1994) called this the "protective dehydration mechanism".

7.1.5 Ice Nucleation Proteins

Ice nucleation proteins are produced by some bacteria and fungi, but their production has been found in neither algae nor cyanobacteria. Regardless, in an algal natural (non-axenic) sample of species of *Prasiola crispa*, the freezing point has been found to be about 10 °C higher than in an axenic sample (Norman et al., 2015). The explanation for this could be that polar microalgae and cyanobacteria often live in mat-like formations with many different groups of organisms, including bacteria (Tashyreva & Elster, 2013)

8 Stress Avoidance

In some cases, mentioned adaptations of stress tolerance are not sufficient, and in some, escaping or lasting out unfavorable conditions in dormant stages is preferable. To that end, both algae and cyanobacteria have developed a wide range of morphologically distinct forms and multicellular consortia, which increase their resistance, as well as stages, which allow for spreading into more favorable environments.

8.1 Dormancy

Dormancy is divided into diapause and quiescence. Taking into account that both of these are similar in that they describe a reduction of metabolic rate and developmental processes, there is a difference between these concepts inasmuch as, while dormancy is endogenously controlled, quiescence is controlled by exogenous stresses (Evans & Womersley, 1980; Rebecchi, 2007) and dormancy is endogenously controlled. Moreover, quiescence can be further distinguished by that its state is quickly returned to original after the stresses disappear, whereas dormant stages can persist even after favorable conditions return (Womersley, 1981). In this context, diapause refers to morphological adaptations like akinete formation and quiescence non-morphological adaptations.

8.2 Morphological Alterations

The prime example of a dormant stage of microorganisms are akinetes and pre-akinetes, which are non-motile, thick cell walled stages with accumulated storage substances (Morison & Sheath, 1985; Nagao et al., 1999; Sukenik et al., 2007). Akinetes and pre-akinetes are produced primarily by cyanobacteria and, despite being commonly implied to be only produced by cyanobacterial orders of Nostocales and Stigonematales (Kaplan-Levy et al., 2010; Meeks et al., 2002; Sukenik et al., 2007; Sutherland & Stewart, 2009), they are also formed by algae, for example, by the members of the Xanthopyceae and Zygnematophyceae classes (Fuller, 2013; Nagao et al., 1999; Pichrtová et. al., 2013).

The literature is not unified in the usage of terms of akinetes and pre-akinetes. Fuller (2013) describes pre-akinetes as having thick cell walls and sheath layers, accumulated lipid bodies that obscure nucleus and non-visible pyrenoids, which is congruent with (Pichrtová et al., 2016) definition of “modified vegetative cells with thick cell walls and mucilaginous pectic layers, reduced chloroplast lobes, lower physiological activity and accumulated storage compounds”. However, (Nagao et al., 1999) and (Stancheva et al., 2012) do not use the term ‘pre-akinetes’ at all, even though the term describing an intermediate stage between vegetative cells and akinetes would be fitting. Furthermore, (Herburger et al., 2015; Pichrtová et al., 2013) consider akinetes single-celled, while pre-akinetes are still connected in filaments. This seems to contradict (Fuller, 2013; Nagao et al., 1999) who clearly show ‘mature akinetes’ as a part of a filament. The key distinction seems to be that while pre-akinetes may return to their vegetative form after rehydration, akinetes must burst open and germinate (Fuller, 2013; Pichrtová et al., 2013).

Dramatic decrease in biochemical processes like oxygen evolution are known to continue (Thiel & Wolk, 1983). They are morphologically and structurally distinct and are highly tolerant of desiccation and freezing stresses, as well as low light stress. This allows these structures to serve as a means of surviving periods of extreme stress like winter or droughts, when the viability of vegetative cells is dramatically decreased (Adams & Duggan, 1999).

8.2.1 Akinete Formation

There are several morphological changes that occur in vegetative algal cells during their transition to akinetes and pre-akinetes. As seen in Figure 5, Nagao et al., (1999) reported that upon prolonged starvation, *Tribonema bombycinum* cells reduced in length by ~50%, increased their weight by over 70 times, drastically reduced reproduction rate and changed in color from greenish to more yellowish during their transition to akinetes. Gradual extension of chloroplasts, wall thickening, increase in the number of smaller vacuoles (vacuolization) and oil droplet (storage product) accumulation were also reported.

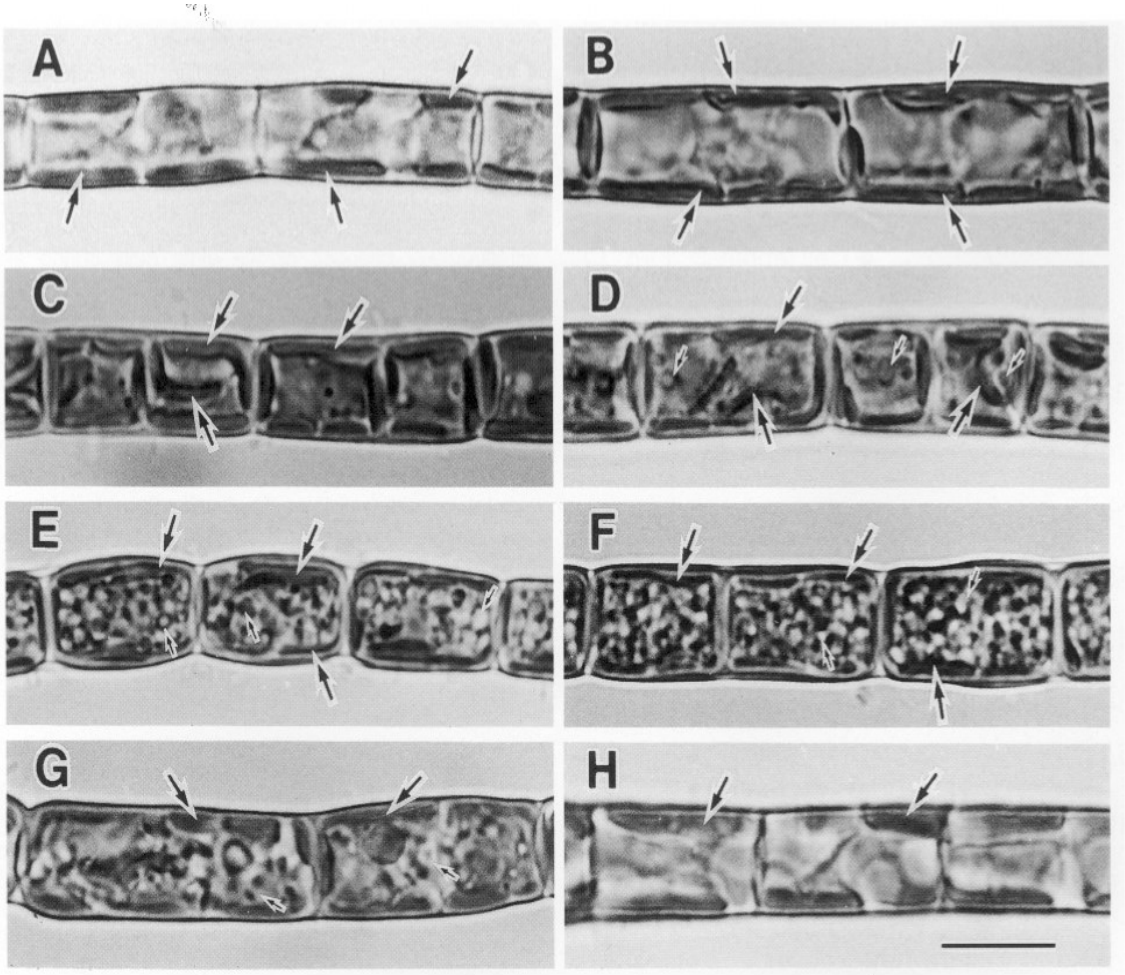

Figure 5: Light microscopy photographs showing Tribonema bombycinum progressing due to starvation from a vegetative stage (A) to an akinete (F) and its subsequent transformation back through (G) to (H) as fresh medium is introduced. (Nagao et al., 1999, adapted)

In cyanobacterial cells, the akinete tends to be much larger than that of a vegetative cell (Kaplan-Levy et al., 2010). In its transition, Sutherland et al. (2009) describes that in *Nostoc* sp. the process starts with the production of cyanophycin granules, the production of which spreads towards the heterocysts. Then, they increase in size along with their cell thickness, the intermediate layer of

which thickens from 10 to 70 nm which doubles the total cell wall thickness. The cell is then covered in a fibrillar layer which creates an envelope, upon which the filament fragments. It has also been discovered that fatty acid and lipid content is lower than in vegetative cells, although lipids were more saturated by up to 30% with oleic and linoleic acid proportion found to be higher than in vegetative cells (Yamamoto, 1972).

8.2.2 Akinete Production Triggers

The differentiation into akinetes is induced by prolonged limitations of nutrients or energy sources like light. While triggers for akinete formation seem to be species-specific, some are more universal (Kvíderová et al., 2019). Most notably, phosphates are deemed to trigger akinete formation, but some species also form akinetes in the absence of nitrates and sulfates (Nagao et al., 1999). Desiccation can also induce akinete production (Fuller, 2013; Nagao et al., 1999). Usually upon the reappearance of the limiting factor in the environment, the cells can start germinating, growing and producing vegetative cells in case of akinetes (van den Hoek et al., 1995), or return to vegetative state in the case of pre-akinetes (Pichrtová et al., 2013).

The production of akinetes - specifically, the storage product accumulation and cell wall thickening - is most likely energy intensive and, as a result, does not appear abundantly in polar regions, where other morphological stages can be found (Elster, 1999; Tashyreva & Elster, 2013). Instead, stages like hormogonia and hormocysts that do not have to go through aforementioned processes are found (Kvíderová et al., 2019).

8.2.3 Other Morphological Adaptations

Hormogonia are motile parts of fragmented cyanobacterial trichomes into smaller filaments. They can be told apart from a mature trichome by the absence of differentiated cells like the heterocyst. Instead, they produce gas vesicles, and which grant them buoyancy and motility, respectively (Damerval et. al., 2007). Thanks to these properties, hormogonia can easily escape unfavorable environment and colonize a more favorable one (Tashyreva & Elster, 2013). There does not seem to be a specific trigger that would induce hormogonia production, it seems a combination of stresses, like nutrient and light deficiency, is the production stimulant (Campbell et. al., 2007). Despite not being an adaptation that would directly help cyanobacteria survive freezing or desiccation, they are closely tied to akinetes which, as (Tamulonis et. al., 2011) claims, develop into hormogonia and can facilitate avoidance of said stresses. Furthermore, *Phormidium* sp. and *Pinnularia* sp. were found to be able to escape desiccation and light stresses through vertical motility (Davey & Clarke, 1991; Ehling-Schulz & Scherer, 1999).

Several other morphological types of resting stages exist, although they tend to be species specific. *Zygnema* spp. produce zygospores which are sexually produced cells that are resistant to stress and whose cell walls are enhanced by algaenan (Hull et. al., 2010; Poulíčková et. al., 2007). It is also known, along with many other algae (Stancheva et al., 2012), to produce asexual aplanospores and parthenospores. *Chlamydomonas nivalis* can produce hypnoblasts during which it fulfills most of its life-cycle. It is characterized by a huge accumulation of sugars, lipids and most importantly carotenoids, namely astaxanthin (Remias et. al., 2005).

8.2.4 Non-morphological Adaptations

Not all microorganisms are able to produce morphological stress resistant stages. But even those without such adaptations are capable of surviving in extreme conditions via changes in cellular ultrastructure and minor biochemical alterations (Tashyreva & Elster, 2013). For example, the cyanobacterium *Chroococcidiopsis* sp. can selectively stop CO_2 incorporation whenever water potential is too low and essentially halt its metabolism (Palmer & Friedmann, 1990). Additionally, it can form multilayered envelopes that are similar to that of *Nostoc* sp. (Caiola, et. al. 1996).

Both algae and cyanobacteria are also able to form algal mats or consortia (Vincent et al. 1993, Arrigo et al., 2014). These are multilayered dense aggregates that resistant to various stresses associated with freeze-thaw cycles (Elster & Benson, 2004). They have a basic structure, with uppermost regions containing increased concentration of certain pigments like scytonemin and cantaxanthin in cyanobacteria (Vincent et al., 1993) or xanthophylls in algae (Arrigo et al., 2014). The pigments provide protection from ultraviolet light, but they are more resistant to various stresses associated with freeze-thaw cycles. (Elster & Benson, 2004).

9 Biotechnological Potential of Antifreeze and Antidesiccant Adaptations

Thanks to diverse strategies and products used by many algae and cyanobacteria, it is possible to utilize these adaptations in various ways. Many of these products are rare or hard to harvest in an efficient manner which is why phototrophic organisms like cyanobacteria and algae could help in their production. Since their maintenance is usually very low, no fermentable sugars are required, and they do not need large areas of arable land (Abishek et al., 2014). Indeed, many algae and cyanobacteria have already been used for such purposes, most notoriously Rhodophyta and Phaeophyta have been used to produce agar and alginates. As seen in Table 2, most products are associated with nutrition, seeing as these uses do not require any preparation, and have been

explored for many centuries (Pulz & Gross, 2004). However, technological development now allows us to diversify the uses and the number of areas in which they are helpful. For example, products preventing freezing and desiccation injuries that are being utilized by algae and cyanobacteria are also used many different fields, mainly medical and food and agricultural industry.

Table 2: Microalgal species with high relevance for biotechnological applications (Pulz & Gross, 2004)

Species/group	Product	Application areas
Spirulina platensis/Cyanobacteria	Phycocyanin, biomass	Health food, cosmetics
Chlorella vulgaris/Chlorophyta	Biomass	Health food, food supplement, feed surrogates
Dunaliella salina/Chlorophyta	Carotenoids, β-carotene	Health food, food supplement, feed
Haematococcus pluvialis/Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives
Odontella aurita/Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food
Porphyridium cruentum/Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition
Isochrysis galbana/Chlorophyta	Fatty acids	Animal nutrition
Phaedactylum tricornutum/Bacillariohyta	Lipids, fatty acids	Nutrition, fuel production
Lyngbya majuscule/Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition

9.1 Ice-Binding Proteins Uses

IBPs are notably important due to their ability to manipulate ice recrystallization and induce thermal hysteresis. These properties are utilized in reducing blood cell haemolysis during thawing, after a human blood sample is frozen in liquid nitrogen. In a study by (Kang & Raymond, 2004), an IBP from an Antarctic diatom *Navicula glaciei* was used to reduce haemolysis by ~50% which is attributed to the IBPs ability to inhibit ice recrystallization. It should be noted that the reduction was only achieved in the presence of glycerol, which likely reduces the amount of water able to crystallize. IBPs are also used to help safely preserve tissues and organs (Bakhach, 2009).

IBPs in food and agricultural industry are used for a wide variety of purposes, some of which are minor, like smooth texture preservation in ice cream or drip loss reduction in lamb meat (Payne & Young, 1995), and some major, like the increase in freezing tolerance of some plants (Duman & Wisniewski, 2014), which, unfortunately, has been largely unsuccessful and a tolerance increase of only up to 3 °C was measured. An extensive list of uses of IBPs in food and agricultural history has been reviewed in (Voets, 2017).

9.2 Biofuel Production

Biofuels can be harvested from algae and cyanobacteria (Mata et al., 2010; Nozzi et al., 2013; Georgianna & Mayfield, 2012). Species that produce a high amount of lipids and carbohydrates are considered to be used for the production. While there are many algae and cyanobacteria that are candidates for an efficient and cheap biofuel production, only some are considered related to products of freezing and desiccation tolerance mechanisms.

Some algae that appear in polar regions with extreme environment are known to produce a significant amount of substances that can be converted into biofuels through transesterification (Demirbas & Demirbas, 2011). These are often substances that are used by microorganisms as storage compounds (Nagao et al., 1999). Some of these, such as *Chlorella antarctica* (Ahn et al., 2012), produce a large amount of lipids and carbohydrates, while being relatively eurythermal in terms of growth rates—the temperature range of growth for the former is between 4 and 30 °C (Ahn et al., 2012). Other algae like *Tribonema bombycinum* (Wang et al., 2014), and a large number of diatoms (Bozarth et al, 2009) are also known to produce a high amount of lipids and carbohydrates while being able to grow at lower temperatures, making them candidates for biofuel production that could continue through colder seasons without majorly increasing expenditure for heating (Ahn et al., 2012).

However, some more stenothermic algae with high lipid and carbohydrate yields could still be utilized. The cyanobacterium *Nodularia spumigena* has been examined with the purpose of adapting its tolerance to cold enable some algae to effectively produce biofuel throughout the year, including in winter (Hong et al., 2010). Moreover, some cyanobacteria, like *Synechococcus* spp. and *Synechocystis* spp. can be considered effective biofuel producers too (Nozzi et al., 2013).

9.3 Polyunsaturated Fatty Acids

Polyunsaturated Fatty Acids (PUFAs) already described in previous chapters are essential for human development. The most important of these are the α-linolenic, linoleic, eicosapentaenoic (EPA), docosahexaenoic acids (DHA) (Ganesan et al., 2013). They are abundant in many different foods like walnuts, fish oil and flaxseed oil, however, microalgal PUFAs have their own set of benefits that make them biotechnologically relevant.

For example, fish oil, while rich in EPA and DHA, has an unappealing smell, increased presence of heavy metals and cholesterol (Medina et al., 1998). Furthermore, fish oil is unsuitable for vegan consumers. Seed oils have a high content of linoleic acid and ALA; however, they are lacking in EPA and DHA. On the other hand, microalgal oils from certain algal species contain very high amounts of EPA and DHA, especially *Chlorella minutissima* and *Gonyaulox caterella*, respectively (Ganesan et al., 2013).

10 Conclusion

This work encompasses all significant algal and cyanobacterial adaptations to environmental extreme stresses, their mechanisms and varieties. They lessen or fully negate the negative effects of low temperatures and desiccation stresses. Thanks to these adaptation, cyanobacteria and algae, the most important primary producers in polar areas can survive in extreme conditions and allow survival of other species in the same area.

Stresses like low and sub-zero temperature, desiccation, osmotic and oxidative, are often lethal driving forces primarily in permanently cold areas. Chill stress is answered by modifying fluidity and permeability of membranes, as well as production of special proteins and enzymes that allow psychrotrophs and psychrophiles to survive at much lower temperatures. Osmoprotectants and cryoprotectants help alleviate osmotic stress and potential desiccation it leads to. Desiccation is further prevented by LEA proteins that help stabilize proteins during the process and exopolymeric substances that help conserve water. Freezing adaptations alter the structure of ice crystals to be less dangerous to surrounding cells and prevent the formation of new ice crystals. Lastly, algae and cyanobacteria can entirely avoid some stresses by forming highly resistant stages or creating stages that are able to spread more easily to favorable locations.

A lot of these adaptations have the potential to be further successfully harnessed in biotechnological research, as it already has, to some extent. Biofuel production seems especially lucrative, with food, agricultural and medical industries also benefiting from the knowledge of these adaptations. Further study of specific adaptations to low temperatures is needed and will likely prove remarkably beneficial in the light of ongoing changing climate.

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