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# Role of Lipids and Fatty Acids in Stress Tolerance in Cyanobacteria

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**Summary**. Lipids are the most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes. They also have a vital role in tolerance to several physiological stressors in a variety of organisms including cyanobacteria. The mechanism of desiccation tolerance relies on phospholipid bilayers which are stabilized during water stress by sugars, especially by trehalose. Unsaturation of fatty acids also counteracts water or salt stress. Hydrogen atoms adjacent to olefinic bonds are susceptible to oxidative attack. Lipids are rich in these bonds and are a primary target for oxidative reactions. Lipid oxidation is problematic as enzymes do not control many oxidative chemical reactions and some of the products of the attack are highly reactive species that modify proteins and DNA. This review deals with the role of lipids and fatty acids in stress tolerance in cyanobacteria.

**Key words:** cyanobacteria, desiccation, fatty acids, lipids, salinity, temperature stress.

## INTRODUCTION

Cyanobacteria are gram-negative photoautotrophic prokaryotes having ´higher plant-type‘ oxygenic photosynthesis (Stewart 1980, Sinha and Häder 1996a). Certain cyanobacteria differentiate a small fraction of their cells into heterocysts, the site of aerobic nitrogen fixation. The significant role of these $N_2$-fixing microorganisms in improving the fertility of wetlands such as rice paddy fields, at the sole expense of photosynthetic energy produced on their own, is well documented (Sinha and Häder 1996a; Sinha *et al*. 1998a; Vaishampayan *et al*. 1998, 2001).

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The cyanobacteria such as *Spirulina* and *Nostoc* have been used as a source of protein and vitamin for humans and animals (Ciferri 1983, Kay 1991, Gao 1998, Takenaka *et al*. 1998). *Spirulina* has an unusually high protein (up to 70 % of the dry weight) content for photosynthetic organisms and is being also used as a source of natural colorants in food, and as a dietary supplement (Kay 1991). *Nostoc flagelliforme* and *Nostoc commune* is considered a delicacy in China (Gao 1998, Takenaka *et al*. 1998) and Philippines (Martinez 1988) respectively. The availability of powerful genetic techniques allow the biotechnological application of cyanobacteria to produce specific products, to biodegrade organic pollutants in surface waters, to control mosquitoes and for many different other purposes (Koksharova and Wolk 2002). The medicinal value of cyanobacteria was appreciated as early as 1500 BC,

when strains of *Nostoc* were used to treat gout, fistula and several forms of cancer (Pietra 1990). The very high incidence of novel, biologically active compounds isolated so far indicates that cyanobacteria are a rich source of potentially useful natural products (Moore 1996). Over 40 different Nostocales species, the majority of which are *Anabaena* and *Nostoc* spp. produce over 120 natural products (secondary metabolites) having activities such as anti-HIV, anticancer, antifungal, antimalarial and antimicrobial. Cyanovirin (CV-N, cyanovirin-N), a 101 amino acid protein extracted from *Nostoc ellipsosporum* was found to have potent activity against all immunodeficiency viruses such as HIV-1, M-and T-tropic stains of HIV-1, HIV-2, SIV (simian) and FIV (feline) (for a review see Burja *et al*. 2001).

Complete genomic sequences have been obtained for the unicellular cyanobacterium, *Synechocystis* sp. strain PCC 6803 (3.57 Mb plus sequenced plasmids) and the filamentous, heterocyst-forming cyanobacterium, *Anabaena* spp. strain PCC 7120 (6.41 Mb plus sequenced plasmids) (Kaneko and Tabata 1997, Kaneko *et al*. 2001a; see http://www.kazusa.or.jp/cyano/). Extensive sequence data are available for two ecotypes (MED4, a high light adapted ecotype from the Mediterranean Sea, and MIT 9313, a low light adapted ecotype from the Gulf Stream) of the unicellular cyanobacterium *Prochlorococcus* (http://www.jgi.doe.gov/JGI-microbial/html/prochlorococcus/prochloro-pickastrain.html), *Nostoc punctiforme* strain PCC 73102 (ATCC 29133) (http://www.jgi.doe.gov/JGI-microbial/html/nostoc/nostoc homepage.html), *Gloeobacter* and several strains of *Synechococcus* (Bryant *et al*. 2001, Holtman *et al*. 2001, Kaneko *et al*. 2001b). Genomic sequence data provide the opportunity for global monitoring of changes in genetic expression at transcriptional and translational levels in response to varying environmental conditions. The available genomic sequence data may be extremely useful in identifying regulatory and structural genes (Ochoa de Alda and Houmard 2000, Zhulin 2000), investigating molecular mechanisms of natural genetic transformation (Yoshihara *et al*. 2001) and analyzing evolutionary events (Herdman *et al*. 2000, Rujan and Martin 2001).

Lipids are esters of fatty acids and alcohols that comprise a large group of structurally distinct organic compounds including fats, waxes, phospholipids, glycolipids etc. Cyanobacteria may contain significant quantities of lipids (fats and oil) with compositions similar to those of vegetable oils. The lipids of some cyanobacterial species are also rich in essential fatty acids such as the $C_{18}$ linoleic (18:2ω6) and y-linolenic (18:3ω3) acids and their $C_{20}$ derivatives, eicosapentaenoic acids (20:5ω3) and arachidonic acid (20:4ω6). These fatty acids are essential components of the diet of humans and animals and are becoming important feed additives in aquaculture (Borowitzka 1988). The lipids of cyanobacteria are generally esters of glycerol and fatty acids (Table 1). They may be either saturated or unsaturated. Some of the filamentous cyanobacteria tend to have large quantities (25 to 60 % of the total) of polyunsaturated fatty acids (Parker *et al*. 1967, Holton and Blecker 1972, Kenyon *et al*. 1972). A few cyanobacterial strains, which show facultative anoxygenic $CO_2$ photoassimilation with sulphite as electron donor, lack polyunsaturated fatty acids in their lipids (Oren *et al*. 1985).

The cosmopolitan distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as heat, cold, desiccation, salinity, nitrogen starvation, photo-oxidation, anaerobiosis and osmotic stress etc. (Fay 1992, Tandeau de Marsac and Houmard 1993, Sinha and Häder 1996b). They have developed a number of mechanisms by which cyanobacteria defend themselves against environmental stressors. Important among them are the production of photoprotective compounds such as mycosporine-like amino acids (MAAs) and scytonemin (Sinha *et al*. 1998b, 1999a, b, 2001), enzymes such as superoxide dismutase, catalases and peroxidases (Burton and Ingold 1984, Canini *et al*. 2001), repair of DNA damage (Sinha and Häder 2002) and synthesis of shock proteins (Borbély and Surányi 1988, Bhagwat and Apte 1989, Sinha and Häder 1996b). In this review, we discuss only the role of lipids and fatty acids in stress tolerance in cyanobacteria.

## STRUCTURE OF LIPIDS

Triglycerides are the most common storage lipids and may constitute up to 80 % of the total lipid fraction in cyanobacteria (Klyachko-Gurvich 1974, Tornabene *et al*. 1983). Besides triglycerides, the other major lipids are sulphoquinovosyl diglycerides (SQDG), monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG) and phosphatidyl glycerol (PG). These four major lipids (Fig. 1) can be identified on the basis of their Rf values in TLC, $^{1}H$ NMR and $^{14}C$ NMR (Döhler and Datz 1980, Sato and Murata 1981, Piorreck and Pohl 1984, Harwood *et al*. 1988, Singh 2001).

**Table 1.** Composition of fatty acids in cyanobacteria

| Fatty acid | Position of double bond | Percentage of total fatty acids |
|---|---|---|
| 14:0 | - | 40 |
| 16:0 | - | 60 |
| 16:1 | 9 | 50 |
| 16:2 | 6, 9 | 20 |
| 16:2 | 9, 12 | 50* |
| 17:1 | - | 10 |
| 18:0 | - | 30 |
| 18:1 | 9 | 40 |
| 18:2 | 9, 12 | 40 |
| 18:3 | 6, 9, 12 | 30 |
| 18:3 | 9, 12, 15 | 40 |
| 18:4 | 6, 9, 12, 15 | 30 |
| 20:1 | 11 | 10 |

*Present only in few strains of cyanobacteria. References: Kates and Volcani 1966; Harrington *et al*. 1970; Wood 1974; Mercer and Davies 1975; Paoletti *et al*. 1976a,b; Yurieva *et al*. 1984; Borowitzka 1988

## ROLE OF LIPIDS AND FATTY ACIDS IN DESICCATION TOLERANCE

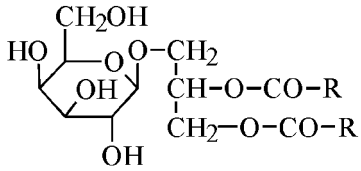
Water is most essential for life. Removal of water from a cell creates a severe, often lethal stress. Desiccation-tolerant cells implement structural, physiological and molecular mechanisms to survive acute water deficit. These mechanisms, and the components and pathways, which facilitate them, are poorly understood in cyanobacteria (Potts 2001). Mechanisms, which maintain the structural integrity of membranes, appear to be of importance. Some sugars, particularly trehalose, prevent damage from dehydration not only by inhibiting fusion between adjacent membrane vesicles during drying, but also by maintaining membrane lipids in the fluid phase in the absence of water (Crowe *et al*. 1987, 1992). Water molecules are critical components of the reaction mechanism; they contribute to the stability of proteins, DNA and lipids. Water may have played a determinative role in the origin and evolution of the genetic code (Wolfenden *et al*. 1979, Potts 1994).

Some desiccation-tolerant cells accumulate large amounts of either one or both of the disaccharides trehalose and sucrose (Crowe *et al*. 1992). Such observations have led to the conclusion that these disaccharides are effective at protecting enzymes during both freeze-drying and air-drying. Recent evidence show that the P=O stretch of the phospholipid increases in frequency by about 30 $cm^{-1}$ when the protein is dried without trehalose but is decreases to or below the frequency of hydrated P=O when the protein is dried with trehalose. Molecular modelling shows that trehalose can fit between the phosphate of adjacent phospholipids (Rudolph *et al*. 1990). At low trehalose/lipid ratios trehalose is not available to bind water thus showing a direct interaction between the sugar and lipid.

When the membranes are isolated, free radicals cause fatty acids deesterification from phospholipids. Free fatty acids typically accumulate in desiccation sensitive cells during aging and are a cause of reduced membrane integrity. The respiratory rate prior to desiccation correlates well with the number of free radicals in the dry state, which suggests that the curtailment of the respiratory metabolism prior to rehydration may be essential for the retention of membrane integrity and desiccation tolerance (Hoekstra 1993). The imibition of viable, dry cells may result in extensive leakage and death, particularly when it occurs at low temperature, because rehydration may involve a reverse phase change of membrane lipids from the gel to liquid-crystal phase which occurs in the presence of water (Crowe *et al*. 1987).

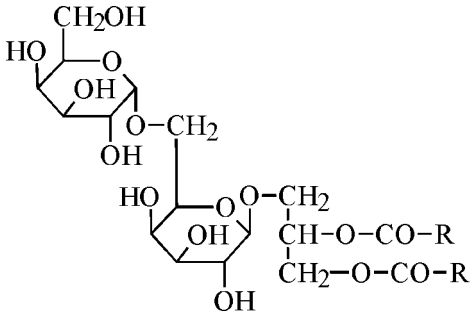
Alteration in the lipid content of membranes of an organism is of major importance in response to environmental stresses (Olie and Potts 1986, Ritter and Yopp 1993). Maintenance of membrane integrity in anhydrobiotic organisms represents a central mechanism of desiccation tolerance (Carpenter and Crowe 1989, Crowe and Crowe 1992a). The role of membrane fluidity and lipid composition on survival of bacteria at extreme temperatures, salinity and drying has been reported (Russell and Fukunga 1990, Oliver *et al*. 1998). Almost 60 % of the total phospholipids in the purified cytoplasmic membrane of *N. commune* UTEX 584 was found to be 20:3ω3 fatty acid (Olie and Potts 1986). Rehydration of dry mats of *Scytonema geitleri* resulted in a slight increase in the amount of total lipids (Singh 2001). Trehalose can stabilize membranes (Crowe and Crowe 1986, 1992a,b; Crowe *et al*. 1987; Leslie *et al*. 1994). Membranes dried without trehalose undergo vesicle fusion, change in morphology and loss of calcium transport activity upon subsequent rehydration (Crowe *et al*. 1992). Oliver *et al*. (1998) developed the water replacement hypothesis to describe how the non-reducing sugar trehalose protects cells, membranes, proteins, and nucleic acids when they are dried. Trehalose seems to depress the phase transition temperature of the dry lipids after desiccation and maintains them in the liquid-crystal state (Crowe *et al*. 1992, Leslie *et al*. 1994).

**(a) Monogalactosyldiacylglycerol (MGDG)**



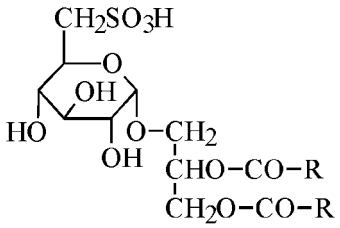
1,2-di-O-acyl-3-O-β-D-galactopyranosyl-sn-glycerol

**(b) Digalactosyldiacylglycerol (DGDG)**



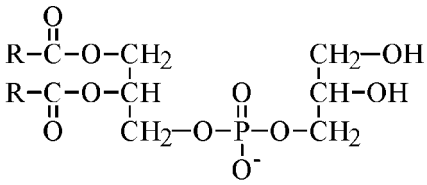
1,2-di-O-acyl-3-O[-α-D-galactopyranosyl-(1'-6')-
O-β-D-galactopyranosyl]-sn-glycerol

**(c) Sulfoquinovosyl-diacylglycerol (SQDG)**



6-sulfo-α-D-quinovopyranosyl-(1-43')-1',2'-diacyl-sn-glycerol

**(d) Phosphatidyl glycerol (PG)**



3-sn-phosphatidyl-1'-sn-glycerol

**Fig.1.** Structural formula based on $^{13}C$ and $^{1}H$ NMR of lipids of *Scytonema geitleri* as separated on silica gel TLC

NMR measurements of dry mixtures of trehalose and 1,2-dipalmitoyl-*sn*-phosphatidylcholine (DPPC) indicate that the sugar is in close proximity to the hydrophilic region from the phosphate head group to the interfacial regions (Lee *et al*. 1986). The eight hydroxyls on each trehalose are all available for hydrogen bonding to the phosphate and carbonyl groups of the lipids. The sugar is thought to occupy some space between the lipid molecules.

A number of desiccation tolerant cyanobacteria have highly pigmented sheaths. One pigment that is unique and restricted to only a few cyanobacteria is the yellow-brown lipid-soluble pigment scytonemin. It is an optically inactive dimeric pigment located in the extracellular polysaccharide sheath with a molecular mass of 544 Da and a structure based on indolic and phenolic subunits (Proteau *et al*. 1993). Scytonemin with absorption maximum at 386 nm (but also absorbs significantly at 252, 278, and 300 nm) has been proposed to serve as an ultraviolet (UV) sunscreen (Sinha *et al*. 1998b, 1999b). Once synthesized, scytonemin remains highly stable and carries out its screening activity without further metabolic investment from the cell. Rapid photodegradation of scytonemin does not occur which is evidenced by its long persistence in terrestrial cyanobacterial crusts or dried mats. This strategy may be invaluable to several scytonemin containing cyanobacteria inhabiting harsh habitats and subject to regular cycles of desiccation and rewetting and must survive long periods of metabolic inactivity (Sinha *et al*. 1999b). Thus the mechanism of desiccation tolerance by lipids and fatty acids in cyanobacteria seems to be complex interactions, which will not be easily resolved through genetic analysis (Potts 1999).

## ROLE OF LIPIDS AND FATTY ACIDS IN TOLERANCE TO SALT-INDUCED DAMAGE IN CYANOBACTERIA

There are many environmental factors that limit growth and productivity of microorganisms; salt stress is one of them. The mechanisms of the hyperosmotic stress-induced and the salt stress-induced inactivation of the photosynthetic machinery, particularly the oxygen evolving machinery of the photosystem II complex, have been investigated in *Synechococcus* sp. PCC 7942 (Allakhverdiev *et al*. 2000a,b; 2001). Hyperosmotic stress due to 1 M sorbitol induces the efflux of water through water channels and reduces the volume of cells by more than 50 %. This loss of water from the cytosol might be expected to increase the intracellular concentration of salts and leads to the rapid but reversible inactivation of the oxygen evolving machinery (Allakhverdiev *et al*. 2000b). Salt stress due to 0.5 M NaCl has both osmotic and ionic effects (Allakhverdiev *et al*. 2000a). The osmotic effect due to 0.5 M NaCl is not as strong as the effect of 1 M sorbitol and inactivates reversibly the photolytic water splitting. The ionic effect of 0.5 M NaCl is caused by the influx of $Na^+$ ions through $K^+$ ($Na^+$) channels and the resultant increase in the intracellular concentration of $Na^+$ ions and counterpart anions that are mostly $Cl^-$ ions (Allakhverdiev *et al*. 2000a). These changes result in the irreversible inactivation of the oxygen evolving machinery. As a consequence salt stress appears to be much more damaging to the oxygen evolving machinery than osmotic stress. Cyanobacteria have several kinds of mechanisms that allow them to acclimate to salt stress. For example, the inducible synthesis of compatible solutes such as sucrose is synthesized in salt-sensitive strains of cyanobacteria such as *Synechococcus* (Mackay *et al*. 1984, Reed *et al*. 1986, Joset *et al*. 1996, Hagemann and Erdmann 1997); glucosylglycerol is synthesized in strains with intermediary tolerance such as *Synechocystis* sp. PCC 6803 (Hagemann *et al*. 1987, 2001; Erdmann *et al*. 1992; Joset *et al*. 1996; Hagemann and Erdmann 1997; Mikkat and Hagemann 2000); glycinebetaine is synthesized in salt tolerant *Synechococcus* sp. PCC 7418 (Mackay *et al*. 1984, Reed *et al*. 1986, Joset *et al*. 1996, Hagemann and Erdmann 1997). Direct evidence for the ability of these compatible solutes to protect the cyanobacterial cells may be seen from studies of transgenic systems (Deshnium *et al*. 1995, 1997; Ishitani *et al*. 1995; Nakamura *et al*. 1997). Genes encoding a substrate-binding protein (ggtB) and two integral membrane proteins (ggtC and ggtD) of the binding-protein-dependent ABC transporter for osmoprotective compound glucosylglycerol have been identified in the genome of *Synechocystis* sp. PCC 6803. These genes are clustered on the chromosome about 220 kb away from the previously identified *ggtA* gene, which encodes the ATP-binding protein of this transport system (Mikkat and Hagemann 2000). Changes in matrix water potential (a term generally applied to considerations of water interactions at surfaces and interfaces; Nobel 1983) have been reported to affect total lipids. Total lipids have been shown to be decreased on lowering of the matrix water potential in a cyanobacterium *Scytonema geitleri*. The maximum decrease was observed on

- 2.8 megaPascals (MPa). There was no apparent change between - 14.5 and - 21 MPa (Singh 2001).

Many reports have suggested that lipids might be involved in the protection against salt stress (Huflejt *et al*. 1990, Khamutov *et al*. 1990, Ritter and Yopp 1993). When photosynthetic organisms are exposed to salt stress, the fatty acids of membrane lipids are desaturated. Allakhverdiev *et al*. (2001) have used targeted mutagenesis to alter genes for fatty acid desaturases in *Synechocystis* sp. PCC 6803, and they have produced strains with decreased levels of unsaturated fatty acids in their membrane lipids (Tasaka *et al*. 1996) as well as decreased the tolerance to salt (Allakhverdiev *et al*. 1999). Their results demonstrate that an increase in the unsaturation of fatty acids in membrane lipids enhances the tolerance to salt stress of the photosynthetic and $Na^+/H^+$ antiport systems of *Synechococcus*. Wild type cells are more sensitive to NaCl and less able to recover from its effects than *desA*$^+$ cells. It can be explained by the following four mechanisms. (i) The activity of water channels is responsible for the sorbitol-induced inactivation (Allakhverdiev *et al*. 2000b) and the rapid phase of the NaCl-induced inactivation (Allakhverdiev *et al*. 2000a). Therefore, it is quite possible that their activity might be affected by the unsaturation of membrane lipids or by changes in the fluidity of the membrane, (ii) $K^+$ ($Na^+$) channels, as well as the water channels, are located in the plasma membrane, and their activities might be depressed by the unsaturation of fatty acids of membrane lipids (Allakhverdiev *et al*. 2000b, 2001), (iii) The $Na^+/H^+$ antiport system, consisting of $Na^+/H^+$ antiporter(s) and $H^+$ ATPase(s), is also located in the plasma membrane. The unsaturation of fatty acids in membrane lipids might activate the $Na^+/H^+$antiport system *via* enhanced fluidity of the membrane with resultant protection of PSII and PSI activities (Blumwald *et al*. 1984, Padan and Schuldiner 1994, Allakhverdiev *et al*. 2001). The activities of several membrane bound enzymes are known to be affected by changes in membrane fluidity (Kates *et al*. 1984, Kamada *et al*. 1995). (iv) The unsaturation of fatty acids might stimulate the synthesis of the $Na^+/H^+$ antiporter(s) and/or $H^+$ ATPase(s). The increased density in the membrane of these components of the antiport system might result in a decrease in the concentration of $Na^+$ in the cytosol, which would tend to protect PSII and PSI against NaCl-induced inactivation and to accelerate the recovery of PSII and PSI activities (Allakhverdiev *et al*. 2001).

## DESATURATION OF MEMBRANE LIPIDS FAVORS CYANOBACTERIA TO ACCLIMATIZE IN LOW TEMPERATURE

The environmental factors compel organisms to acclimatize to the external conditions. The physical properties of a biological membrane depend on the fatty acid composition of its component membrane lipids. Unsaturated fatty acids are essential constituents of polar glycerolipids of biological membranes and the unsaturation level of membrane lipids is important in controlling the fluidity of membranes (Chapman 1975).

Cyanobacteria respond to a decrease in ambient growth temperature by desaturating the fatty acids of membrane lipids to compensate for the decrease in membrane fluidity at low temperatures (Murata and Nishida 1987). Fatty acid desaturases are the enzymes that introduce the double bonds into the hydrocarbon chains of fatty acids, and thus these enzymes play an important role during the process of cold acclimation of cyanobacteria (Sato and Murata 1980, 1981, 1982; Wada and Murata 1990). The unsaturation of fatty acids occurs without *de novo* synthesis of fatty acids during low temperature acclimation of cyanobacterial cells (Sato and Murata 1981, Wada and Murata 1990). Most of the cyanobacterial desaturases are intrinsic membrane proteins that act on acyl-lipid substrates. It has been demonstrated that the unsaturation of membrane lipids is essential for low temperature tolerance of cyanobacteria by genetic manipulation of the *desA* ($\Delta$12 desaturase) gene, which was isolated from the transformable cyanobacterium *Synechocystis* sp. strain PCC 6803 (Wada *et al*. 1990, 1992, 1994; Gombos *et al*. 1992, 1994; Sakamoto *et al*. 1998). In *Synechocystis* sp. strain PCC 6803, the mRNA level for the *desA* gene, which was shown to be regulated in response to temperature (Los *et al*. 1993, 1997), and *desB* ($\omega$3 or $\Delta$15 desaturase) transcripts accumulated in cells grown below 26°C (Sakamoto *et al*. 1994). Chemical hydrogenation of the cytoplasmic membrane stimulated accumulation of the *desA* transcripts, implying that a change in membrane fluidity might be the primary signal for the onset of expression of the desaturase genes (Vigh *et al*. 1993). It has been suggested that the accumulation of *desA* transcripts at low temperatures might be explained by an acceleration of transcription and by stabilization of the *desA* mRNA (Los and Murata 1994). It should be stressed that cold-sensitive *Synechocystis* with monounsaturated fatty acids only become cold-tolerant

by introduction of the gene for Δ12 desaturase that allows cells to synthesize diunsaturated fatty acids (Wada *et al*. 1990). On the other hand, directed mutations of desaturases in cold-tolerant *Synechocystis* that lead to production of monounsaturated fatty acids make this strain cold-sensitive (Tasaka *et al*. 1996). However, the temperature-sensing mechanism(s) and the control mechanism(s) regulating the expression of the desaturase genes are still unknown.

## LIPID DESATURATION: REGULATION AND CHARACTERIZATION OF GENE EXPRESSION AS A FUNCTION OF TEMPERATURE

The unicellular marine cyanobacterium *Synechococcus* sp. strain PCC 7002 is classified as member of Group 2, based upon the fatty acid composition of its lipids and their pattern of desaturation (Murata *et al*. 1992). This cyanobacterium synthesizes lipids containing $C_{18}$ fatty acids with none, one, two or three double bonds at the Δ9, Δ12, and *w*3 or Δ15 positions at the *sn*-1 position and $C_{16}$ fatty acids containing none or one double bond at the Δ9 position of the *sn*-2 fatty acid (Murata and Wada 1995). Double bonds in the *sn*-1 fatty acid are added sequentially starting with desaturation at the Δ9 position and proceeding to the *w*3 position. Three desaturases, DesC (Δ9 desaturase) DesA (Δ12 desaturase) DesB (*w*3 or Δ15 desaturase) are responsible for the conversion of stearate to α-linoleate. Desaturation at the Δ9 and Δ12 positions occurs both at 34°C and at 22°C, while desaturation at the *w*3 (or Δ15) position only occurs in cells grown at low temperatures (Murata *et al*. 1992). Sakamoto and Bryant (1997) isolated and characterized the DesB (*w*3 desaturase) and DesC (Δ9 desaturase) genes from *Synechococcus* sp. strain PCC 7002 and studied the patterns of expression of the three desaturase genes in response to change in ambient temperature. They also demonstrate that changes in the stabilities of mRNAs for the desaturase genes contribute the overall regulation of the desaturase gene expression. The study of Sakamoto and Bryant (1997) revealed that transcription of the three desaturase genes in *Synechococcus* sp. strain PCC 7002 is differently regulated in response to changes in temperature in terms of mRNA synthesis and mRNA stabilization. Moreover, based upon a kinetic analysis with a time resolution of 5 min, they demonstrate that accumulation of mRNAs from these genes occurs very quickly during the process of the acclimation to low temperature. It has been proposed that specific temperature-sensor and signal transduction mechanisms might be involved in the regulation of the desaturase genes during cold acclimation of cyanobacteria (Murata and Wada 1995). However, it is possible that changes in the transcriptional and translational activities at lower temperatures as well as inherent differences in mRNA stability might have a direct influence on the up-regulation of the desaturase genes immediately following a temperature shift-down. However, the rates of mRNA synthesis from the *desA*, *desB* and *desC* genes as a function of temperature remain to be examined, and the mechanism(s) responsible for the transient increase of mRNA synthesis from the *desC* genes has not yet been identified.

## ROLE OF LIPIDS IN TOLERANCE TO HIGH LIGHT-INDUCED PHOTOINHIBITION

Exposure of cyanobacteria to high PAR (photosynthetically active radiation) or UV radiation leads to photoinhibition of photosynthesis thereby limiting the efficient fixation of light energy (Han *et al*. 2001, Nishiyama *et al*. 2001). Photoinhibition occurs due to two basic mechanisms: (i) photoinduced, nonphotochemical quenching of excitation energy and (ii) photoinduced damage to the photosynthetic machinery (Krause 1988). In cyanobacterial photosynthesis, the nonphotochemical quenching particularly measured by $O_2$ evolution is not induced by light, indicating that the photoinhibition is mainly due to the photoinduced damage to the photosynthetic machinery. The molecular mechanism of photoinhibition revealed that the light-induced damage is caused by inactivation of the D1 protein of the PSII complex (Aro *et al*. 1993, Kanervo *et al*. 1993, Tyystjärvi *et al*. 2001). The damaged D1 protein is degraded proteolytically leaving the PSII complex depleted of the D1 protein. In the recovery process the precursor of the D1 protein is synthesized *de novo*, incorporated into the PSII complex, and then processed to yield the active D1 protein, with resultant generation of the active PSII complex (Andersson *et al*. 1992). The extent of the photoinhibition depends on the balance between the inactivation of the PSII complex and the recovery of the complex from the inactivated state (Gombos *et al*. 1994). Transformation of the cyanobacterium *Synechococcus* sp. PCC 7942 with the *desA* gene for a Δ12 desaturase have been reported to increase the unsaturation of membrane lipids and thereby enhance the tolerance of cyanobacterium to high light

(Gombos *et al*. 1997). These findings demonstrate that the ability of membrane lipids to desaturate fatty acids is important for the photosynthetic organisms to tolerate high light stress, by accelerating the synthesis of the D1 protein *de novo*.

## LIPID PEROXIDATION

Oxidants and free radicals are deleterious in many ways. The organisms employ numerous approaches to block their production or limit their damage. Hydrogen atoms adjacent to olefinic bonds are susceptible to oxidative attack and especially those between unconjugated olefinic bonds. Lipids are rich in these bonds and thus are primary targets for oxidative reactions. Lipids oxidation is problematic as many oxidative chemical reactions are not controlled and constrained by enzymes and may show exponential reaction rates. Some of the products of the attack are highly reactive species that modify proteins and DNA (McIntyre *et al*. 1999). Lipid peroxidation has been shown to increase under drought conditions. Generally, in organisms undergoing a stress response, the enzymes of the Halliwell-Asada pathway and their main substrates have relatively higher activities and levels than those encountered under normal conditions (Elstner and Osswald 1994, Foyer *et al*. 1994). Hydroperoxides and MDA were often considered as indicators of membrane damage (Hagege *et al*. 1990a, b). Hydroperoxides are the initial products of lipid oxidation and usually account for the majority of bound oxygen measured by the peroxide value. MDA and a variety of aldehydes have long been recognized as secondary products derived from the degradation of lipid hydroperoxides.

Reactive oxygen species (ROS) such as $O_2^-$, $H_2O_2$ and $^{\cdot}OH$ are highly toxic to cells. Cellular antioxidant enzymes, and the free radical scavengers normally protect a cell from toxic effects of the ROS. There are reports that a decrease in PUFA (polyunsaturated fatty acid) content coincides with the increased levels of MDA in response to high osmotic stress. These responses, which are temporarily associated with an increase in electrolyte leakage, suggest that in fact water stress induces damage at the cellular and subcellular membrane levels *via* lipid peroxidation (Asada 1992, Aziz and Larher 1998). Cell membranes, which are structurally made up of large amounts of PUFA, are highly susceptible to oxidative attack and consequently changes in membrane fluidity, permeability, and cellular metabolic functions (Bandopadhyay *et al*. 1999).

## CONCLUSIONS

Most cyanobacteria are a common source of a wide range of fats, oils, hydrocarbons and sterols with potential not only as a renewable source of liquid fuels but also for the production of a range of pharmacologically and industrially important products. The application of cyanobacteria in the production of these latter compounds is only just being explored and their importance has yet to be determined. New developments in the chemical industries, particularly in the area of converting natural products to industrial feedstocks, will further enhance the range of commercially important products synthesized by cyanobacteria.

In cyanobacterial cells, lipids are typically found only in the membranes. Hence the increased desaturation of lipids at low temperature must represent an environmentally triggered acclimation to improve membrane functionally at low temperature. Earlier studies have suggested that irreversible damage to cyanobacterial cells in the dark is initiated at low temperature by a phase separation of plasma membrane lipids. The phase separation of thylakoid membrane lipids occurs at a higher temperature. In most cyanobacteria, phase separation of thylakoid membrane lipids causes reversible loss of photosynthetic activity, and this depression of photosynthetic activity is reversed when cells are returned to their growth temperature. Increased desaturation of the lipids of the thylakoid membrane might be less important in the overall acclimation of cells to low temperature than desaturation of the lipids of the plasma membrane. The uptake of nutrients has an important role of the plasma membrane. It has been proposed that the decreased rate of the nutrient uptake from the environment could be the rate-limiting step for growth of cyanobacteria at low temperature. All three enzymes for nitrate assimilation are associated with membranes: the nitrate transporter is integrated in the plasma membrane, and nitrate and nitrite reductases are components of the thylakoid membranes. It is possible that membrane lipid unsaturation protects these enzymes from inactivation at low temperature. Covalent modification with lipids is a common feature of many membrane associated proteins, and the acyl groups function to anchor such proteins to membranes.

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