ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE BIOTECHNOLOGY

ANTIOXIDANTS AS NATURAL ARSENAL AGAINST MULTIPLE STRESSES IN CYANOBACTERIA

MINU KESHERI, RICHA AND RAJESHWAR P. SINHA*

Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India

RAJESHWAR P. SINHA
Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India

***Corresponding author**

ABSTRACT

Cyanobacteria evolved as ubiquitous photosynthetic oxygen evolving prokaryotes, which owing to their limited motility are compelled to be exposed to the extremes of environmental stress conditions including oxidative stress prevailing at their natural habitats. Cyanobacteria possess an array of protective and repair mechanisms comprising of enzymatic and non-enzymatic antioxidants as natural arsenal to alleviate the damages caused by reactive oxygen species (ROS), thereby bestowing them with high adaptability to endure the hazards of endogenous and exogenous stresses. Cyanobacteria possess immense potential for serving as a source material for the production of antioxidative enzymes and pigments in addition to their role as biofertilizers. The responses and mechanisms employed by cyanobacteria may be implemented to design strategies for understanding their metabolic and genetic plasticity for further development of technologies relevant to enhancing the stress tolerance capacity of living organisms.

KEYWORDS

Cyanobacteria, Reactive oxygen species (ROS), Antioxidants, Enzymes.

INTRODUCTION

Cyanobacteria, a group of photosynthetic organisms, supposed to have evolved around 3.5 billion years ago are the first oxygen evolving organisms to release oxygen into the then oxygen free atmosphere leading to the development of aerobic metabolism and the subsequent rise of higher plants and animal forms[1]. Cyanobacteria can occupy various ecotopes by virtue of having high ecological adaptivity[2]. Recovery of photochemical reactions, ^{14}C-fixation and C_2H_2 reduction on rehydration reflects their capacity to survive certain extreme conditions in general and desiccation in particular[3]. Owing to their ability to survive in warm temperatures, high light and low CO_2 concentrations cyanobacteria have radiated into a broad range of habitats thus being cosmopolitan in distribution and forming a prominent component of microbial population in aquatic as well as terrestrial ecosystem. They play a central role in successional processes, global photosynthetic biomass production and nutrient cycling. In addition, nitrogen fixing cyanobacteria are often the dominant microflora in wetland soils, especially in rice paddy fields, contributing significantly to fertility as natural biofertilizer.

Oxidative signaling is an important and critical function associated with the mechanisms by which organisms sense the environment and make appropriate adjustments to gene expression, metabolism and physiology[4]. Despite of extremely adverse environmental conditions posed for their growth, cyanobacteria are found to be widely distributed in the terrestrial habitats. Endolithic organisms are subjected to much more severe environmental stress than those in cold deserts due to the sudden changes between warm-humid and hot-dry conditions. Studies have shown abundance of cyanobacterial growth in such habitats facing frequent wetting and drying in Indian continent[5-10]. The day temperature in the tropics varies from 20 ^oC to 50 ^oC[11] with surface temperature of building tops reaching as high as 70 ^oC[12] mainly in summers. The cyanobacterial flora on the surfaces of bark and buildings facing high degree of variability in humidity (20 - 80% RH), temperature (6 - 44 ^oC) and light intensity (250 - 436 cal cm^{-2} day^{-1}) comprises of non spore forming species of *Aphanocapsa, Aphanothece, Chroococcus, Tolypothrix, Gleocapsa*, *Gleothece, Lyngbya, Microcoleus, Symploca, Scytonema*[13,14].

Reactive Oxygen Species (ROS)

ROS are molecules like hydrogen peroxide; radicals like the hydroxyl radical, the most reactive of all ROS having the capacity to oxidize and react with organic molecules and the superoxide anion which is both ion and radical.

Stresses Responsible For the Generation of ROS

(A) Endogenous Stresses

ROS are commonly generated during growth and development as well as during normal cell metabolism related with oxidation and reduction processes, especially in chloroplasts during photosynthesis and mitochondria during respiration. It is only relatively recently that mitochondrial reactive oxygen species generation and protein oxidation have been perceived as contributing factors to the "Oxidative Stress" syndrome[15,16].

ROS are formed as a result of thylakoids, mitochondria and plasma

membrane linked transport and subsequent leaking of electron to the molecular oxygen in the cell. When oxygen comes in contact with metabolic systems it can be transformed into more reactive and toxic form of superoxide ion, hydrogen peroxide, hydroxyl radical and singlet oxygen. Formation of singlet oxygen ($^1O_2$) subsequently stimulates the production of other reactive oxygen species such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl (OH^-), and perhydroxyl radicals (O_2H). Plant cells produce ROS particularly hydrogen peroxide and super oxide as second messengers in many processes associated with plant growth and development[17]. Resistance or sensitivity to stress depends on the species, the genotype and the developmental age of the organism. Cyanobacteria are the only prokaryotes known so far, for possessing regulation of physiological functions with approximate daily periodicity, or circadian rhythms that are controlled by a cluster of three genes, *kaiA, kaiB* and *kaiC*[18] .

ROS are also produced by root nodules of N_2-fixing plants. The enhancement of metabolism and growth by nitrogen may require the presence of additional defense mechanism against ROS[19]. Development of symbiosis and nodule are also triggered by ROS[20-22]. All nitrogen-fixing organisms face a critical dilemma. Nitrogenase, the enzyme that catalyses the conversion of nitrogen into ammonium, is quickly and irreversibly inactivated by oxygen, yet nitrogen fixation is an extremely energy demanding process with at least 16 mol. of ATP being consumed per mol of nitrogen fixed[23]. Therefore, relying on aerobic respiration to supply this energy puts the organism at risk of inactivating nitrogenase. Presence of SOD and catalase enzyme in *Nostoc cycadae* colonizing the coralloid roots of *Cycas* sp. may be attributed to the involvement of these enzymes in a type of respiratory protection that limits the entry of oxygen into the nodule interior. The presence of high levels of antioxidants suggests that respiratory consumptions of oxygen in the endodermis or nodule parenchyma may be an essential component of oxygen diffusion barrier that regulates the entry of oxygen into the central region of nodules and ensures optimal functioning of nitrogenase[24].

(B) Exogenous or Environmental Stresses

One of the major ways in which plants transmit information concerning changes in the environment is *via* the production of bursts of super oxide at the plasma membrane[25]. Environmental factors that cause oxidative stress include air pollution (increased amounts of O_3 or SO_2), oxidant forming herbicides such as paraquat dichloride (methyl viologen, 1, 1'-dimethyl-4, 4'-bipyridinium), heavy metals, heat stress, chilling[26, 27], freezing[28], ice-encasement[29], ozone[30] and highly intense light conditions that stimulate photoinhibition, and pathogen infection during senescence.

Water stress conditions in particular may trigger an increased formation of superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which can directly attack membrane lipids and inactivate SH-containing enzymes[31]. During water depletion, superoxide radical may also react non-enzymatically with H_2O_2, giving rise to products such as hydroxyl radicals and singlet oxygen, which are even more reactive than superoxide itself [32]. This formation is a consequence of the Mehler reaction, which provides a pathway for the excess removal of electrochemical energy determined by drought stress[33]. Decrease in polyunsaturated fatty acid content coinciding with the increased levels of MDA in response to high osmotic stress is temporarily associated with the increase in electrolyte leakage, suggesting that water stress induces damage at the cellular and subcellular membrane levels *via* lipid peroxidation[34].

The harmful effects of photooxidative stress defined as the generation of reactive oxygen species by light dependent processes have been extensively studied since it has long been appreciated that reactions associated with photosynthesis and photorespiration are major sources of ROS within plant cells[35]. The

photoreduction of dioxygen to produce active oxygen in cyanobacteria and eukaryotic algae has been demonstrated in intact cells[36-38] and in isolated thylakoids[39,40]. It is expected that free radicals accumulate during drying, especially in the light, when the cells are exposed to high incident solar radiations. It has been proposed that heat treatment in the light leads to an overproduction of free radicals which causes protein cross-linking and precludes the removal of the D-1 protein of the PS II from thylakoids which occurs during photodamage at normal temperature.

It is well documented that axenically grown cultures of many cyanobacterial species (e.g., *Gleocapsa* sp., *Oscillatoria redekei*) are sensitive to high light intensities[41]. It was observed that field tests of different laboratory strains of cyanobacteria, including species of *Microcystis, Scynechococcus, Plectonema and Nostoc* showed that condition for photooxidative death exists in blooming ponds[42].

Anthropogenic activities leading to the release of atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs) and organobromides (OBs) owe to the continued depletion of stratospheric ozone and has resulted in an increase in ultraviolet-B (UV-B; 280-315 nm) radiation on Earth's surface[43-46]. Cyanobacteria in their natural habitats are exposed to harmful ultraviolet radiation (UVR) owing to the absorption of solar energy to drive photosynthesis and nitrogen fixation. Lethal doses of UVR reach deep into water column[47, 48]; down to a depth of 20 m in the clearest oceanic water and to a few centimeters in brown humic lakes and rivers[49]. Because of its high energy, UV-B radiation leads to the production of ROS in photosynthetic organisms[50].

Under salt stress plants have to cope up with water stress imposed by the low external water potential, and with ion toxicity, due to accumulation inside the plant[51]. Salt stress in addition to the known component of osmotic stress & ion toxicity is also manifested as an oxidative stress[52]. However, ion content and salt tolerance are not often correlated and several studies indicate that acquisition of salt tolerance may also be a consequence of improving resistance to oxidative stress[53-56].

Damages Caused By ROS

UV irradiation presumed to be an elicitor of multilevel oxidative stress, may cause morphological changes, alter pigment composition, influence adaptive mechanisms, impair photosynthesis and decrease bioproductivity[57-60]. UV-B irradiation disturbs the balance of monodehydroascorbate radical (MDA) production and reduction, resulting in increased light-induced MDA signal. The enhancement of ascorbate photooxidation at the UV-B damaged donor site of PS II appears as a major factor in this process[61]. Reactive oxygen species produced due to UV-B radiation easily destroys proteins, DNA and other biological molecules[62]. A number of physiological and biochemical processes such as growth survival, pigmentation, photosynthetic oxygen production and phycobiliproteins composition have been reported to be susceptible to UV-B radiation[63]. DNA breaks accumulate during exposure of bacterial cells to superoxide radical and H_2O_2.

DNA seems to be a weak link in a cell's ability to tolerate oxygen free radical attack. Since DNA is effective in binding metals that are involved in Fenton reactions, and can tolerate less damage than other macromolecules, therefore the cell has a number of DNA repair enzymes[64]. One reason why eukaryotic organisms have compartmentalized DNA in the nucleus, away from sites of redox cycling that are high in NAD(P)H and other reductants, may be to avoid oxidative damage. Activated oxygen and agents that generate oxygen free radicals, such as ionizing radiation, are responsible for inducing numerous lesions in DNA due to which deletions, mutations and other lethal genetic effects are caused. Characterization of this damage to DNA indicates that both the sugar and the base moieties are susceptible to oxidation thereby causing base degradation,

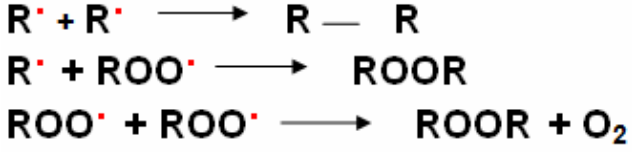
single strand breakage, and cross-linking to protein[65]. Degradation of the base produces numerous products, including 8-hydroxyguanine, hydroxymethyl urea, urea, thymine glycol, thymine and adenine ring-opened and -saturated products. The principle cause of single strand breaks is the oxidation of the sugar moiety by the hydroxyl radical. Cross-linking of DNA to protein serves to be one of the consequences of hydroxyl radical attack on either DNA or its associated proteins[66].

Oxidative attack on proteins result in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis. Primary, secondary and tertiary protein structures alter the relative susceptibility of certain amino acids. Sulphur containing amino acids, and thiol groups specifically, are very susceptible sites. Activated oxygen can abstract an H atom from cysteine residues to form a thiyl radical that will cross-link to a second thiyl radical to form disulphide bridges. Alternatively, oxygen can add to a methionine residue to form methionine sulphoxide derivatives. Reduction of both of these may be accomplished in microbial systems by thioredoxin and thioredoxin reductase[67]. A protein-methionine-S-oxide reductase has been measured in pea chloroplasts[68]. This enzyme reduces the methionyl sulfoxide back to methionyl residues in the presence of thioredoxin[69]. Other forms of free radical attack on proteins are irreversible. For example, the oxidation of iron-sulphur centres by superoxide destroys enzymatic function[70]. Many amino acids undergo specific irreversible modifications when a protein is oxidised. For example, tryptophan is readily cross-linked to form bityrosine products[71]. Various peptide components of photosystem II turnover at different frequencies whereby the D1 protein specifically is observed for its high rate of turnover, as a consequence of oxidative attack at specific sites on the protein[72].

When photosynthetic organisms are exposed to salt stress, the fatty acids of membrane lipids are desaturated[73]. The reactions of oxygen free radicals with polyunsaturated lipids are the most frequent consequence of oxygen radical production in plant cells. The lipid bilayer membrane is composed of a mixture of phospholipids and glycolipids that have fatty acid chains attached to carbon 1 and 2 of the glycerol backbone by an ester linkage. The peroxidation reactions differ among these fatty acids depending on the number and position of the double bonds on the acyl chain[74].

The lipid hydroperoxide (ROOH) is unstable in the presence of Fe or other metal catalysts because ROOH will participate in a Fenton reaction leading to the formation of reactive alkoxy radicals:

Therefore, in the presence of Fe, the chain reactions not only propagate but get amplified too. Among the degradation products of ROOH are aldehydes, such as malondialdehyde, and hydrocarbons, such as ethane and ethylene that are commonly measured end products of lipid peroxidation. The peroxidation reactions in membrane lipids are terminated when the carbon or peroxy radicals cross-link to form conjugated products that are not radicals, such as those shown in the following reactions:



Typically high molecular weight, cross-linked fatty acids and phospholipids accumulate in peroxidised membrane lipid samples. Singlet oxygen can react readily with unsaturated fatty acids producing a complex mixture of hydroperoxides. Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products than the hydroxyl radical[75]. The effect of free radical reactions on the presence of specific lipid-soluble, membrane antioxidants (phenols, flavonoids and quinones), are poorly understood due to differences in their composition.

Protection against Damages caused by ROS

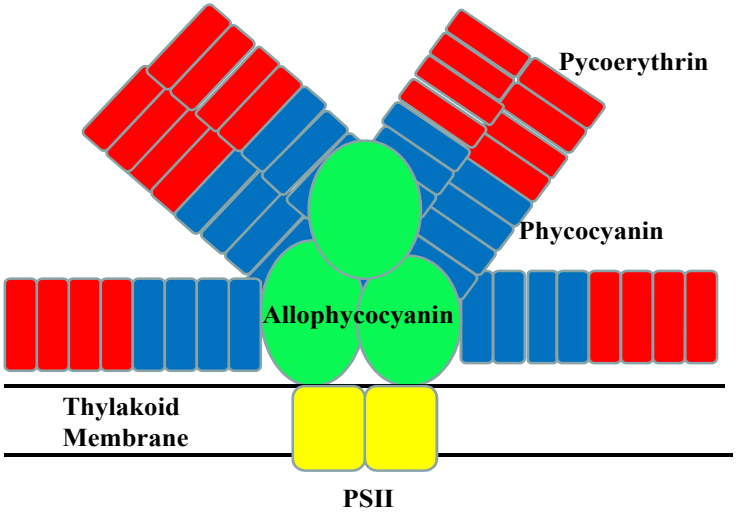
Photosynthetic organisms can tolerate elevated oxygen levels because of endogenous mechanisms that effectively scavenge and remove the toxic products before cellular damage occurs. An array of protective and repair mechanisms to combat the menace posed by the presence of ROS has been reported in many organisms including cyanobacteria[76-80]. Response to stress is subjected to interspecific variations and therefore different organisms respond differently and specifically to the environmental stress conditions[81]. Antioxidants can be divided into three general classes; (i) lipid soluble and membrane associated tocopherols, (ii) water soluble reductants such as ascorbic acid and glutathione and (iii) enzymes such as superoxide dismutase (SOD), catalase, peroxidase, ascorbate peroxidase and glutathione reductase[82].

Non Enzymatic Antioxidants

PHYCOBILIPROTEINS

Phycobiliproteins are the predominant accessory light-harvesting pigments in cyanobacteria[83]. They absorb maximally in the 470 - 650 nm region, in the valley between the blue and far-red absorption peaks of chlorophyll *a*. Phycobilisomes are very large assemblages of many phycobiliproteins subunit each containing many covalently attached bilin prosthetic groups as well as linker polypeptides (Fig.1).

Structure showing phycobilisome subunits



Structure of Phycobilisomes

Figure 1

The multimeric protein complex phycobilisomes as major light-harvesting complex in cyanobacteria.

Phycobiliproteins are reported to be used in the measurement of peroxy radical damage[84]. In *Spirulina* the phycocyanobilin has been shown to have antioxidizing activity and therefore may act as an effective antioxidant in humans[85]. Based on their response to light quantity, cyanobacteria can alter their phycobilisomes size and number; it can also change the phycoerythrin and/or phycocyanin levels[86] by a process called as chromatic adaptation. Low light intensities may stimulate the synthesis of phycobilisomes and may cause increase in the size of rod structure[87, 88]. Phycoerythrins are highly sensitive to variations in pH, salt, temperature, water stress and light[89-91].

TOCOPHEROL

Tocopherol is a non-polar solvent soluble, organic molecule that is only synthesized by oxygen-evolving phototrophs including some cyanobacteria and all green algae and plants[92-94]. Alpha, beta, gamma and delta tocopherols differing in the number and position of methyl groups on the chromanol ring form a group of lipophilic molecules that are collectively termed as vitamin E. Tocopherols scavenge and quench various reactive oxygen species and lipid oxidation byproducts, which would otherwise propagate lipid peroxidation chain reactions in membranes[95]. A protective role of tocopherol against photooxidative damage in photosystem II has been reported in *Chlamydomonas reinhardtii*[96]. Antioxidation, membrane stabilization, intracellular signaling and cyclic electron transport around photosystem II are suggested functions of tocopherol[97-99]. The antioxidant property of tocopherol is the result of its ability to quench both singlet oxygen and peroxides.

CAROTENOIDS

Carotenoids act as a competitive inhibitor for the formation of singlet oxygen and this is aided considerably by their proximity to chlorophyll in the light harvesting complex. This method of protection is especially critical as light intensity increases above saturation levels[100]. In terms of its antioxidant properties carotenoids can protect the photosystems in one of four ways; (i) by reacting with lipid peroxidation products to terminate chain reactions, (ii) by scavenging singlet oxygen and dissipating the energy as heat[101], (iii) by reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen and (iv) by the dissipation of excess excitation energy through the xanthophyll cycle. Carotenoids may augment α-tocopherol in scavenging peroxy radicals[102]. About 600 different carotenoids have been identified from different organisms such as cyanobacteria, bacteria, fungi, phytoplankton, macroalgae, plants and animals[103,104]. Animals lack the ability to synthesize carotenoids endogenously and thus have to take up these compounds through their diets[105]. A considerable increase (≈ 50 %) in the outer-membrane carotenoids echinenone and myxoxanthophyll was found in the cyanobacterium *Nostoc commune* after few hours of UV-B irradiation[106]. The photosynthetic activity of cyanobacterium *Synechococcus* sp. was found to be more tolerant against UV-B radiation when having increased amounts of endogenous carotenoids caused by genetic manipulation[107]. A small increase in carotenoid content was found in a marine cyanobacterium, *Oscillatoria* sp. strain BG 091600, after irradiation with UV-A at an irradiance of 10 W/m^2[108]. Carotenoids such as diadinoxanthin, diatoxanthin and β-carotene were found to be induced in phytoplankton under high PAR and UVR[109] and they may play a role in reducing photoinhibition of photosynthesis[110]. The occurrence and role of the orange carotenoid protein (OCP) in photoprotective mechanisms in various cyanobacteria has recently been reported[111].

ß-carotene is formed by the cyclization of lycopene and the xanthophylls are formed by mixed function oxidases that introduce hydroxyl groups to the carotene molecule[112]. Another carotenoid, zeaxanthin, has been implicated in the dissipation of thermal energy. Zeaxanthin apparently facilitates the

conversion of triplet to singlet chlorophyll in more efficient manner than ß-carotene. The xanthophyll cycle involves the reversible conversion of the xanthophylls between two forms, violaxanthin and zeaxanthin.

Enzymatic Antioxidants

SUPEROXIDE DISMUTASE (SOD)

SOD might be the first antioxidant arsenal against nascent oxygen species. SOD is found to play a critical role in mitigating the toxic effect of superoxide ion. The first implication on the protective role of cyanobacterial SOD in photo-oxidative damage was shown in *Anacystis nidulans*[113]. Subsequently, several studies on protective role of SOD of cyanobacteria in response to various physiological processes/stresses like photosynthesis[114], desiccation[115], chilling[116], nitrogen starvation[117] and with azo dyes have been reported. SOD was first isolated as a copper storage protein[118]. Subsequently, the enzyme was identified by a number of names; erythrocuprein, indophenol oxidase and tetrazolium oxidase until its catalytic function was discovered[119]. SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen.

There are three distinct types of SOD classified on the basis of the metal cofactor: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD) isozymes[120]. These isozymes can be separated by native polyacrylamide gel electrophoresis, their activity detected by negative staining and identified on the basis of their sensitivity to KCN and H_2O_2. The Mn-SOD is resistant to both inhibitors, whereas the Cu/Zn-SOD is sensitive to both inhibitors; Fe-SOD is resistant to KCN, and sensitive to H_2O_2.The subcellular distribution of these isozymes is also distinctive. The Mn-SOD is found in the mitochondria of eukaryotic cells; some Cu/Zn-SOD isozymes are found in the cytosol, others in the chloroplasts of higher plants. The Fe-SOD isozymes are often not detected in plants, but when detected, Fe-SOD is usually associated with the chloroplast compartment[121]. The prokaryotic Mn-SOD and Fe-SOD, and the eukaryotic Cu/Zn-SOD enzymes are dimers, whereas the Mn-SOD of mitochondria is tetramer[122].

Prokaryotic cells and many eukaryotic algae contain only the Mn-SOD and Fe-SOD isozymes and are believed to be more ancient forms. In *E. coli*, SOD activity is transcriptionally regulated by the SOX RS operon[123]. The presence of SOD is a prominent biomarker of defense against oxidative stress[124, 125] and its activity increases with stress as a direct consequence. Natural cyanobacterial samples when subjected to native PAGE and stained for SOD activity showed the presence of two isoforms in *Nostoc cycadae,* five isoforms in *Scytonema* sp., three isoforms in *Microcystis* sp., single isoform in *Oscillatoria* sp., and two isoforms in *Aulosira* sp. (Fig 2).

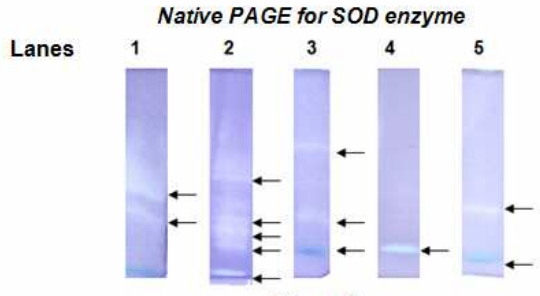


Figure 2

Native PAGE showing profile for superoxide dismutase enzyme in cyanobacteria. Lanes 1. Nostoc cycadae, 2. Scytonema sp., 3. Microcystis sp., 4. Oscillatoria sp. and 5. Aulosira sp. (Arrows indicate isoforms).

Moreover superoxide generated by extracellular polysaccharide (glycan) of *Nostoc commune* DRH1 upon exposure to UV-A irradiation were scavenged by SOD and it was proposed that

high levels of sodF and its release from dried cells upon rehydration counter the effects of oxidative stress imposed by multiple cycles of desiccation and rehydration during irradiation in situ[126]. It has been reported that ROS produced in heterocysts under aerobic conditions cause the inactivation of nitrogenase in absence of Mn-Sod[127]. SOD is probably required in heterocysts to protect against cellular damage by superoxide ion[128].

CATALASE

Known for being the first enzyme to be isolated in a highly purified state, catalase is a tetrameric heme-containing enzyme which is responsible for the dismutation of hydrogen peroxide (H_2O_2) into water and oxygen:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Multiple forms of catalase have been described and cloned from maize[129]. Maize has three isoforms termed cat-1, cat-2 and cat-3, which are on separate chromosomes and are differentially expressed and independently regulated[130]. Potato is the only plant catalase examined that is devoid of NADPH[131]. NADPH functions in animal catalase to protect against inactivation by hydrogen peroxide[132]. Catalase is very sensitive to light and therefore has a rapid turnover rate similar to that of the D1 protein of PSII[133].

Stresses such as salinity, heat shock or cold, known to reduce the rate of protein turnover, causes depletion of catalase activity[134]. Desiccation tolerant cyanobacteria implement structural, physiological and molecular mechanisms to survive acute water deficit. Cytoplasmic catalase plays a role in maintenance of intracellular redox balance during dehydration and therefore tolerance against water stress. Catalase prevents dehydration related oxidative damage to membranes and also helps in complete recovery of cells[135].

It has been reported that several antioxidant enzymes act jointly to maintain redox homeostasis which was shown by the presence of five, two and three isoforms of SOD, ascorbate peroxidase and catalase enzymes respectively in the crude extracts of freshly collected mats of desiccation tolerant cyanobacterium *Lyngbya arbicola* on native PAGE[136]. Moreover presence of two and four bands of catalase and SOD as detected by native PAGE has also been reported in *Tolypothrix*[115]. Crude extracts of green growing cyanobacterial samples collected from their natural habitats when subjected to native PAGE and stained for catalase activity showed the presence of single isoform each in *Scytonema* sp., *Oscillatoria* sp., and *Nostoc cycadae,* while *Aulosira* sp. *and Microcystis* sp. showed presence of three isoforms of catalase (Fig 3).

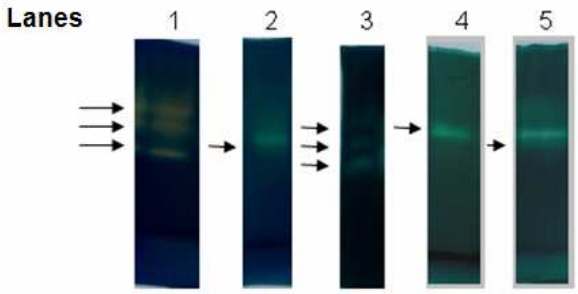


Figure 3
Native PAGE showing profile for catalase enzyme in cyanobacteria. Lanes: 1. Aulosira sp., 2. Oscillatoria sp., 3. Microcystis sp., 4. Nostoc cycadae and 5. Scytonema sp. (Arrows indicate isoforms).

ASCORBATE PEROXIDASE

Scavenging of ROS has been reported to follow Halliwell-Asada cycle[137]. Generally in organisms undergoing a stress response the enzymes of the Halliwell-Asada pathway (Fig 4) and their main substrates have relatively higher activities and levels than those encountered during normal conditions. However, activity of some of the enzymes of the cycle has been found to be absent in cyanobacteria[138]. On the basis of presence and absence of ascorbate peroxidase, the cyanobacteria may be classified into two groups: (i) the one that scavenges hydrogen peroxide with the peroxidase using a photoreductant as the electron donor, and (ii) the group which only scavenges hydrogen peroxide with catalase. It was found that the cells of ascorbate peroxidase-containing *Synechocystis* 6803 supported the quenching of chlorophyll fluorescence induced by hydrogen peroxide whereas it was completely absent in case of the cells of *Anacystis nidulans* which were devoid of ascorbate peroxidase.[139] Ascorbate peroxidase was found to be the major enzyme involved in the removal of hydrogen peroxide under oxidative stresses in *Synechococcus* PCC 9742 (R2) cells[140]. The ascorbate peroxidase active component was later purified and characterized in *Synechococcus* PCC 9742 (R2) cells[141]. Higher affinity of ascorbate peroxidase for H_2O_2 than that of catalase was observed in *Nostoc muscorum* when H_2O_2 production was enhanced by photorespiration[142]. The antioxidative potential of *Nostoc muscorum* 7119 and *Synechococcus* 6311 to scavenge hydroperoxides formed as by-products of photosynthetic activity was investigated and it was concluded that in cyanobacteria an effective reaction sequence for removal of hydroperoxides involves ascorbate peroxidase and recycling of glutathione and ascorbate[143].

Halliwell-Asada pathway

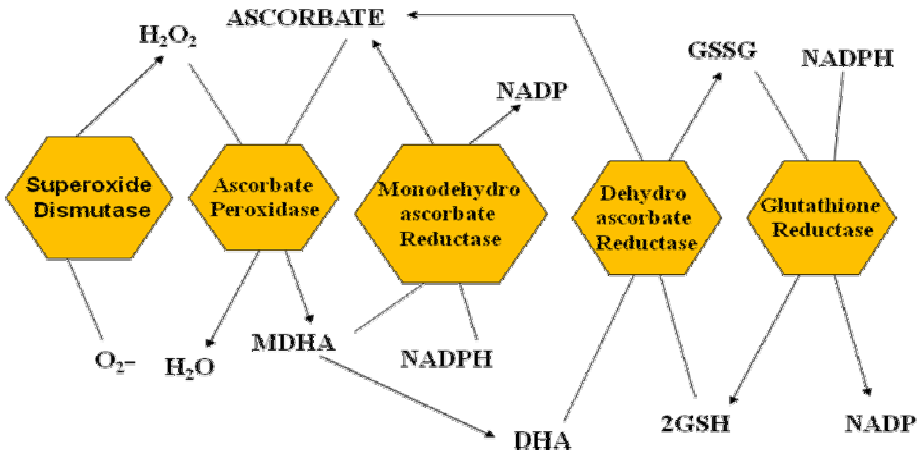


Figure 4

Redox cycling of ascorbate in the chloroplast via Halliwell-Asada pathway. Superoxide and hydrogen peroxide are produced by the illuminated chloroplasts from sites on the thylakoids, most commonly PSI. Superoxide is converted into hydrogen peroxide by either spontaneous dismutation or by the SOD enzyme. Hydrogen peroxide is scavenged by ascorbate in the presence of enzyme ascorbate peroxidase. Ascorbate is regenerated either via monodehydroascorbate reductase, or glutathione and dehydroascorbate reductase. The terminal electron donor is NADPH.

GLUTATHIONE REDUCTASE

Glutathione reductase (GR), a widespread enzyme catalyzing the reduction of GSSG to GSH with NADPH as the reducing cofactor, is necessary for maintaining high GSH/GSSG ratios in cells[144]. In particular, it is a key enzyme in the glutathione-ascorbate cycle, which functions in peroxide scavenging and protection against other oxidative processes[145]. A protective function, analogous to that of the chloroplast enzyme was assigned to NADPH-glutathione reductase (EC 1.6.4.2) that was purified from the filamentous cyanobacterium *Anabaena* sp. strain 7119[146]. Glutathione reductase (GR) has been shown to play a role in the protection mechanism which removes oxygen radical in N_2-fixing cyanobacterium *Nostoc muscorum*[147]. Cyanobacterial GR has been found to show remarkable thermostability by retaining secondary structure when heated to temperatures as high as 80°C in *Spirulina maxima*[148]. GR has already been purified from the cyanobacterium *Anabaena* PCC 7120 and a 3-kilobase genomic DNA fragment containing the coding sequence for the GR gene (*gor*) was identified and cloned by polymerase chain reaction based on sequences of selected peptides isolated from proteolyzed GR[149].

Glutathione (GSH), a nonribosomal thiol tripeptide, is found in virtually all eukaryotic cells, however, its production in prokaryotes is restricted to cyanobacteria and proteobacteria and a few strains of Gram-positive bacteria. GSH plays an important role in many cellular functions, including protection against oxidative stress. It has been shown to be involved in the protection against (ROS), osmotic shock, acidic conditions, toxic chemicals, and heavy metals. The investigations on *Synechocystis* sp. indicates a strong similarity of the subcellular distribution of glutathione and cysteine in cyanobacteria and plastids of plants and provides a deeper insight into glutathione metabolism in bacteria[150]. Although little is known about the roles of glutathione in cyanobacteria, it was reported that conditions promoting growth stimulate glutathione

biosynthesis in *Synechocystis* sp. PCC 6803[151]. It has been reported that the structural gene for glutathione synthetase (*gshB* gene) is involved in the biosynthesis of glutathione in *Synechococcus* sp. PCC 7942[152].

FUTURE PROSPECTS

Antioxidants serve to provide structural and functional stability to the cells at extreme conditions. Screening of algal strains is essential for their exploitation as potential source of antioxidants forming a substitute for chemicals being used as food additives, beverages, nutraceuticals and cosmeceuticals. Due to the toxicity problems caused by synthetic colors, cyanobacterial pigments such as carotenoids and phycobiliproteins have recently been favored in the food industries and as fluorescent marker in biochemical assays. β carotene has been reported to be medicinally significant being antimutagenic and protective against breast cancer[153-155]. Porphyran and shinorine traced from cyanobacteria have found to play role in delaying aging process[156]. Phycoerythrin has been crucial in amelioration of diabetic complications[157]. It has been observed that a diet high in antioxidants foods decreases the risk of cardiovascular disease mortality[158]. Besides direct health benefits, antioxidants may serve significant role in processing and storage of food and other medicinally important preparations such as dry vaccines. Further study is required to elucidate the mechanisms behind the stability of antioxidant enzymes in various systems. Moreover, peculiar antioxidative isozymes may be widely used as molecular markers for the studies of population genetics and genetic variations within and between populations.

Since biological systems do not normally function in isolation at any metabolic level, they strive for an intricate balance of electronically charged molecules in order to cope with the hazards of reactive oxygen species generated thereof. Fortunately, cyanobacteria with an array of antioxidative systems comprising of enzymatic and non enzymatic antioxidants efficiently combat the cellular impairments caused as a result of oxidative stress. Thus, establishing the bioavailability of antioxidants requires further research crucial in maintaining cellular homeostasis and its biotechnological applications.

ACKNOWLEDGEMENT

MK is thankful to UGC, New Delhi, for providing financial assistance in the form of a fellowship.

REFERENCES

1. M.R. Walter, J. Bauld, D.J. Des Marais and J.W. Schopf. A general comparison of microbial mats and microbial stromatolites: bridging the gap between the modern and the fossil. In: J. Schopf and C. Klein, (eds), *The Proterozoic Biosphere*, Cambridge University Press, New Dehli, India (1992), pp. 335-338.
2. B.A.Whitton. Survival and dormancy of blue-green algae. In: Y. Hennis (ed.), *Survival and Dormancy of Microorganisms*, Wiley, New York, 1987, pp. 109-167.
3. Tiwari BS and Tripathi SN, Effect of hydration and dehydration on initiation and dynamics of some physiological reactions in desiccation tolerant cyanobacterium *Scytonema geitleri*. Indian J Biochem Biophys, 35:72-178, (1998).
4. Foyer CH, Noctor G, Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ, 28:1056-1071, (2005).
5. Biswas K, The subaerial algae of the Berkuds island in the Chilka Lake, Ganjan

District, Madras Presidency. J Proc Asiatic Soc Bengal N S, 20:359-365, (1924).
6. Ghose SL, Subaerial blue-green algae of Rangoon. J Indian Bot Soc, 6:79-84, (1927).
7. Bharadwaja Y, Contribution to our knowledge of Myxophyceae of India. Ann Bot, 47:117-143, (1933).
8. Bharadwaja Y, Some aspects of the study of Myxophyceae. 27th Indian Science Congress, Botany Section, Madras, 1940, pp. 1-51.
9. Rao CB, The Myxophyceae of the United Provinces, India. II. Proc Ind Acad Sci B, 2:535-538, (1936).
10. Rao CB, The Myxophyceae of the United Provinces, India. III. Proc Ind Acad Sci B, 5:101-108, (1937).
11. Zehnder A, Beitrag zur kenntnis van mikroklima und algan vegetation des nackten gesteins in der tropen. Ber Schwein Bot Ges, 63:307-312, (1953).
12. Tripathi SN, Talpasayi ERS, Sulphydryls and survival of blue-green algae. Curr Sci, 52:550-552, (1980).
13. Tripathi SN, Tiwari BS, Talpasayi ERS, Growth of cyanobacteria (blue-green algae) on urban buildings. Energ Build, 15-16:449-505, (1991).
14. Vaishampayan A, Sinha RP, Häder DP, Dey T, Gupta AK, Bhan U, Rao AL, Cyanobacterial biofertilizers in rice agriculture. Bot Rev, 67:453-516, (2001).
15. Sweetlove L, Heazlewood JL, Herald V, Holtzapffil R, Day DA, Leaver CJ, Millar AH, The impact of oxidative stress on *Arabidopsis* mitochondria. Plant J, 32:891-904, (2002).
16. Møller IM, Kristensen BK, Protein oxidation in plant mitochondria as a stress indicator. Photochem Photobiol Sci, 3:730-735, (2004).
17. Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torresk MA, Linstead P, Costa S, Brownlee C, Jonathan Jones DG, Davies JM, Dolan L, Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature, 27: 442–446, (2003).
18. Dvornyk V, Vinogradova O and Nevo E, Long term microclimatic stress causes rapid adaptive radiation of kaiABC clock gene family in a cyanobacterium, *Nostoc linckia*, from “Evolution Canyons” I and II, Israel. Pro Natl Acad Sci, Ukraine, 99: 2082-2087, (2002).
19. P.M. Mullineaux, G.P. Criessen, Glutathione reductase: regulation and role in oxidative stress. In: J.G. Scandalios (ed.), *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*, (Monograph Series vol.34), Cold Spring Harbor Laboratory press, Cold Spring Harbor, 1997, pp. 667-713.
20. Becana M, Dalton DA, Moran JF, Iturbe-Ormaetxe I, Matamoros MA, Rubio MC, Reactive oxygen species and antioxidants in legume root nodules. Physiol Plant, 109:372-381, (2000).
21. Baudouin E, Frendo P, Le Gleuher M, Puppo A, A *Medicago sativa* haem oxygenase gene is preferentially expressed in root nodules. J Exp Bot, 55:43-47, (2004).
22. Frendo P, Harrison J, Norman C, Herandaez-Jimenez MJ, Van de Sype G, Gilabert A, Puppo A, Glutathione and homoglutathione play a critical role in the nodulation process of *Medicago truncatula*. Mol Plant-Microb Interac, 18:254-259, (2005).
23. Ljones T, Burris RH, ATP hydrolysis and electron transfer in the nitrogenase reaction with different combinations of the iron protein and the molybdenum-iron protein. Biochim Biophys Acta, 275:93-101, (1972).
24. Dalton DA, Joyner SL, Becana M, Iturbe-Ormaetxe I and Chatfield JM, Antioxidant defenses in the peripheral cell layers of legume root nodules. Plant Physiol, 116:37-43, (1998).

25. N. Doke, Y. Miura, L. Sanchez, K. Kawakita, Involvement of superoxide in signal transduction: Responses to attack by pathogens, physical and chemical shocks, and UV irradiation. In: C.H. Foyer and P. Mullineaux (eds.), *Causes of Photooxidative Stresses and Amelioration of Defense Systems in Plants*, CRC Press, Boca Raton, FL, USA, (1994), pp. 177-198.
26. Fadzillah NM, Gill V, Finch BP, Burdon RH, Chilling, oxidative stress and antioxidant responses in shoot cultures of rice. Planta, 199:552-556, (1996).
27. O'Kane D, Gill V, Boyd PL, Burdon R, Chilling, oxidative stress and antioxidant reponses in *Arabidopsis thaliana* callus. Planta, 198:371-377, (1996).
28. Kendall EJ, McKersie BD, Free radical and freezing injury to cell membranes of winter wheat. Physiol Plant, 76:86-94, **(**1989**).**
29. Hetherington PR, McKersie BD, Borochov A, Ice-encasement injury to microsomal membranes from winter wheat crowns. I. Comparison of membrane properties after lethal ice-encasement and during a post-thaw period. Plant Physiol, 85:1068-1072, (1987).
30. Rao MV, Paliyath G, Ormrod DP, Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol, 110:125-136, (1996).
31. Navari-Izzo F and Izzo R, Induction of enzyme activities and antioxidant production in barley plants as a result of SO_2 fumigation, Plant Sci, 96:31-40, (1994).
32. Elstner EF, Osswald W, Mechanisms of oxygen activation during plant stress. Proc Roy Soc Edinburgh, 102:131-154, (1994).
33. Gamble P, Burke J, Effect of water stress on the chloroplast antioxidant system. I: Alterations in glutathione reductase activity. Plant Physiol, 76:615-621, (1984).
34. Aziz A, Larher F, Osmotic stress induced changes in lipid composition and peroxidation in leaf discs of *Brassica napus* L, J Plant Physiol, 153:754-762, (1998).
35. Foyer CH, Noctor G, Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant, 119:355-364, (2003).
36. Patterson CO, Mayers J, Photosynthetic production of hydrogen peroxide by *Anacystis nidulans*. Plant Physiol, 51:104-109, (1973).
37. Radmer RJ, Kok B, Photoreduction of O_2 primes and replaces CO_2 assimilation. Plant Physiol, 58: 336-340, (1976).
38. Peltier G, Thibault P, O_2 uptake in the light in *Chlamydomonas*: evidence for persistent mitochondrial respiration. Plant Physiol, 79:225-230, (1985).
39. Honeycutt R, Krogmann DW, Further studies on the oxygen-reducing system of *Anabaena virabilis*. Biochem Biophys Acta 256:467-476, (1972).
40. Hammans JWK, Hendricks GM, Teerlink T, Light dependent oxygen uptake by the blue green alga *Anacystis nidulans*. Biochem Biophys Res Commun, 74:1560-1565, (1977).
41. Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G, Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol Rev, 35:171-205, (1971).
42. Eloff JN, Steinitz Y, Shilo M, Photooxidation of cyanobacteria in natural conditions. Appl Environ Microbiol, 31:119-126, (1976).
43. Crutzen PJ, Ultraviolet on the increase. Nature, 356:104, (1992).
44. Kerr JB, McElory CT, Evidence for large upward trend of ultraviolet-B radiation linked to ozone depletion. Science, 262:1032, (1993).
45. Lubin D, Jensen EH, Effects of clouds and stratospheric ozone depletion on

ultraviolet radiation trends. Nature, 377:710, (1995).
46. Weatherhead EC, Andersen SB, The search for signs of recovery of the ozone layer. Nature, 441:39, (2006).
47. Smith RC, Baker KS, Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem Photobiol, 29:311-323, (1979).
48. Häder DP, Kumar HD, Smith RC, Worrest RC, Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci, 6:267-285, (2007).
49. Kirk JTO, Optics of UV-B radiation in natural waters. Arch Hydrobiol 43:1-16,(1994).
50. Apel K, Hirt H, Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol, 55:373-399, (2004)
51. Greenway H, Munns R, Mechanisms of salt tolerance in non halophytes. Ann Rev Plat Physiol, 31:149-190, (1980).
52. Gueta-Dahan Y, Yaniv Z, Zilinskas A, Ben-Hayyim G, Saltand oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. Planta, 203:460-469, (1997).
53. Hernández P, Dorado G, Laurie DA, Martin A, Snape JW, Microsatellites and RFLP probes from maize are efficient sources of molecular markers for the biomass energy crop. *Miscanthus*. Theor App Genet, 102: 616-622, (2001).
54. Gosset DR, Banks SW, Millhollon EP, Lucas MC, Antioxidant response to NaCl stress in a control and an NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. Plant Physiol, 112:803-809, (1996).
55. Streb P, Feierabend J, Oxidative stress-responses accompanying photoinactivation of catalase in NaCl-treated rye leaves. Bot Acta, 109:125-132, (1996).
56. Gómez JM, Hernández JA, Jiménez A, del Río LA, Sevilla F, Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Rad Res, 31:S11-S18, (1999).
57. Tevini M, Teramura AH, UV-B effects on terrestrial plants. Photochem Photobiol, 50:479-487, (1989).
58. J.F. Borman, A.H. Teramura. Effects of ultraviolet-B radiation on terrestrial plants. In: A. R. Young, L. O. Björn, J. Moan, and W. Nultsch (eds.), *Environmental UV Photobiology*, Plenum Press, New York, USA, 1993, pp. 427-471.
59. Vass I, Sass L, Spetea C, Bakou A, Ghanatakis DF, Petrouleas V, UV-B inhibition of photosystem II electron transport system studied by EPR and chlorophyll florescence. Impairment of donor and acceptor side components. Biochemistry, 35:8964-8973, (1996).
60. Bornman JF, Sundby-Emanuelsson C, Response of plants to UV-B radiation: some biochemical and physiological effects, BIOS Scientific Publishers: Oxford, 245-262, (1995).
61. Hideg E, Junichi M, Chiaki O, Kozi A, Increased levels of monodehydroascorbate radical in UV-B-irradiated broad bean leaves. Plant Cell Physiol, 38:684-690, (1997).
62. Sinha RP, Klisch M, Gröniger A, Häder D-P, Ultraviolet absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. J Photochem Photobiol B: Biol, 47: 83-94, (1998).
63. Wang JG, Zhang CL, DNA damage and repair of two ecotypes of *Phragmites communis* subjected to water stress. Acta Bot Sin, 43:490-494, (2001).
64. Beyer W, Imlay J, Fridovich I, Superoxide dismutases. Prog Nucl Acid Res, 40:221-253, (1991).

65. Imlay JA, Linn S, DNA damage and oxygen radical toxicity. Science, 240:1302-1309, (1986).
66. Oleinick NL, Chiu S, Ramakrishnan N, Xue L, The formation, identification, and significance of DNA-protein cross-links in mammalian cells. Brit J Cancer, 55:135-140, (1986).
67. Farr SB, Kogoma T, Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol Rev, 55:561-585, (1991).
68. Ferguson DL, Burke JJ, A new method of measuring protein-methionine-S-oxide reductase activity. Plant Physiol, 100:529-532, (1992).
69. Brot N, Weissbach H, The biochemistry of methionine sulfoxide residues in proteins. Trends Biochem Sci, 7:137-139, (1982).
70. Gardner PR, Fridovich I, Superoxide sensitivity of *Escherichia coli* 6-phosphogluconate dehydratose. J Biol Chem, 266:1478-1483, (1991).
71. Davies KJA, Protein damage and degradation by oxygen radicals. I. General aspects. J Biol Chem, 162:9895-9901, (1987).
72. Stadtman ER, Oxidation of proteins by mixed-function oxidation systems: implication in protein turnover, aging and neutrophil function. Trends Biochem Sci, 11:11-12, (1986).
73. Allakhverdiev SI, Kinoshita M, Inaba M, Suzuki I, Murata N, Unsaturated fatty acids in membrane lipids protect the photosynth**et**ic machinery against salt-induced damage in *Synechococcus*. Plant Physiol, 125:1842-1853, (2001).
74. Frankel EN, Chemistry of free radical and singlet oxidation of lipids. Prog Lipid Res, 23:197-221, (1985).
75. Bradley DE, Min DB, Singlet oxygen oxidation of foods. Cat Rev Food Sci Nutri, 31:211-236, (1992).
76. Smirnoff N, The role of active oxygen in response of plants to water deficit and desiccation. New Phytol, 125:27-58, (1993)
77. Potts M, Desiccation tolerance of prokaryotes. Microbiol Rev, 58:755- 805, (1994).
78. Takeda T, Yokota A, Shigeoka S, Resistance of photosystem to hydrogen peroxide in algae. Plant Cell Physiol, 36:1089-1095, (1995).
79. Alscher RG, Donahue JL, Cramer CL, Reactive oxygen species and antioxidants: Relationships in green cells. Plant Physiol, 100:224-233, (1997).
80. Foyer CH, Humberto LD, Dat JF, Scott IM, Hydrogen peroxide and glutathione associated mechanism of acclamatory stress tolerance and signaling. Plant Physiol, 100:241-254, (1997).
81. Abeliovich A, Shilo M, Photooxidative death in blue-green algae. J Bacteriol, 111:682-689, (1972).
82. C. H. Foyer. Ascorbic acid. In: R.G. Alscher, and J.L. Hess (eds.), *Antioxidants in Higher Plants*, CRC Press, Boca Raton, 1993, pp. 31-58.
83. A. L. Glazer, Phycobiliproteins. In: L. Packer and A. N.Glazer (eds.), *Methods in Enzymology*, San Diego, Academic, 1988, Vol. 167, pp. 291-303.
84. DeLange RL, Glazer AN, Phycoerthrin fluorescence based assay for peroxy radicals: A screen for biologically relevant protective agents. Anal Biochem, 177:300-306, (1989).
85. Hirata T, Tanaka M, Ooike M, Tsunomura T, Sakaguchi M, Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis.* J App Phycol, 12:435-439, (2000).
86. Tandeu de Marsac N, Houmard J, Complementary chromatic adaptation: physiological conditions and action spectra. Methods Enzymol, 167:318-328, (1988).
87. Lonneborg A, Lind LK, Kalla SR, Gustafsson P, Öquist G, Acclimatation process in the light harvesting system of

cyanobacterium *Anacystis nidulans* following a light shift from white light to red light. Plant Physiol, 78:110-114, (1985).
88. Melis A, Mamdori A, Glick RE, Ghirardi ML, McCauly SW, Neal PJ, The mechanism of photosynthetic membrane adaptation to environmental stress conditions; a hypothesis on the role of electron transport capacity and of ATP/NADPH pool in regulation of thylakoid membrane organization and function. Physiol Veg, 23:757-765, (1985).
89. Sarada R, Pillai MG, Ravishankar GA, Phycocyanin from *Spirullina* sp.: influence of processing of biomass on phycocyanin yield, analysis of efficiency of extraction methods and stability studies on phycoyanin. Process Biochem, 34:795-801, (1999).
90. Sun L, Wang S, Chen L, Gong X, Promising fluorescent probes from phycobiliproteins. IEEE. J Select Topics Quant Electron, 9:177-188, (2003).
91. Martinez-Oyanedel J, Contreras-Martel C, Bruna C, Bunster M, Stuctural-functional analysis of the oligomeric protein R-phycoerythrin. Biol Res, 37:733-745, (2004).
92. D.R. Threlfall, G.R.Whistance. Biosynthesis of isoprenoid quinones and chromanols. In: T. W. Goodwin (ed.), *Aspects of Terpenoid Chemistry and Biochemistry*, Academic Press, London, 1971, pp. 357-404.
93. Collins MD, Jones D, Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol Rev, 45:316-354, (1981).
94. Y. Sakuragi, D.A. Bryant. Genetic manipulation of the quinone pathway in cyanobacteria. In: J. H.Golbeck (ed.), *Photosystem I: The Plastocyanin:Ferredoxin Oxidoreductase in Photosynthesis*, 2005.
95. Kamal-Eldin A, Appelqvist L, The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 31:671-701, (1996).
96. Trebst A, Depka B, Jager J, Oettmeier W, Reversal of the inhibition of photosynthesis by herbicides affecting hydroxyphenylpyruvate dioxygenase by plastoquinone and tocopheryl derivatives in *Chlamydomonas reinhardtii*. Pest Manage Sci, 60:669-674, (2004).
97. Munné-Bosch S, Alegre L, The function of tocopherols and tocotrienols in plants. Crit Rev Plant Sci, 21:31-57, (2002).
98. J.L. Hess, Vitamin E, α-tocopherol. In: R.G. Alscher and J.L. Hess (eds.), Antioxidants in Higher Plants, CRC Press, Boca Raton, 1993, pp.111-134.
99. Fryer MJ, The antioxidant effects of thylakoid vitamin E (α-tocopherol). Plant Cell Environ, 15:381-392, (1992).
100. B. Demmig-Adams, W.W. Adams, The xanthophyll cycle. *In:* R.G. Alscher and J.L. Hess (eds.) *Antioxidants in Higher Plants*. CRC Press, Baco Raton , (1993) pp. 59 -90.
101. Mathis P, Kleo J. The triplet state of ß-carotene and of analog polyenes of different length. Photochem. Photobiol. 18:343-346, (1973).
102. Burton GW, Ingold KU, ß-carotene: an unusual type of lipid antioxidant. Science, 224:569-573, (1984).
103. Grynbaum MD, Hentschel P, Putzbach K, Rehbein J, Krucker M, Nicholson G, Albert K, Unambiguous detection of astaxanthin and astaxanthin fatty acid esters in krill (*Euphausia superba* Dana). J Sep Sci, 28:1685-1693, (2005).
104. Polivka T, Sundström V, Ultrafast dynamics of carotenoid excited states-from solution to natural and artificial systems. Chem Rev, 104:2021-2071, (2004).
105. Moeller RE, Gilroy S, Williamson CE, Grad G, Dietary acquisition of photoprotective compounds

(mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod. Limnol Oceanogr, 50:427-439, (2005).
106. Ehling-Schulz M, Bilger W, Scherer S, UV-B induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. J Bacteriol, 179:1940-1945, (1997).
107. Götz T, Windhovel U, Böger P, Sandmann G, Protection of photosynthesis against UV-B radiation by carotenoids in transformants of the cyanobacterium *Synechococcus* PCC 7942. Plant Physiol, 120:599-604, (1999).
108. Wachi Y, Burgess JG, Iwamoto K, Yamada N, Nakamura N, Matsunaga T, Effect of ultraviolet-A (UV-A) light on growth, photosynthetic activity and production of biopterin glucoside by the marine UV-A resistant cyanobacterium *Oscillatoria* sp. Biochimica et Biophysics Acta, 1244:165-168, (1995).
109. Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255: 952-959, (1992).
110. Brown BE, Ambarsari I, Warner ME, Fitt WK, Dunne RP, Gibb SW, Cummings DG, Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. Coral Reefs, 18:99-105, (1999).
111. Boulay C, Abasova L, Six C, Vass I, Kirilovsky D, Occurrence and function of the orange carotenoid protein in photoprotective mechanisms in various cyanobacteria. Biochim Biophy Acta, 1777:1344-1354, (2008).
112. K.E. Pallett, A.J.Young. Caratenoids. In: R.G. Alscher and J.L. Hess. (eds.), *Antioxidants in Higher Plants*, CRC Press, Boca Raton, 1993, pp. 91-110.
113. Herbert SK, Samson G, Fork DC, Laudenbach DE, Characterization of damage to photosystems I and II in a cyanobacterium lacking detectable iron superoxide dismutase activity. Proc Natl Acad Sci USA, 89:8716-8720, (1992).
114. Kim JH, Suh KH, Light-dependent expression of superoxide dismutase from cyanobacterium *Synechocystis* sp. strain PCC 6803. Arch Microbiol, 183:218-223, (2005).
115. Rajendran UM, Kathirvel E, Anand N, Desiccation-induced Changes in Antioxidant Enzymes, Fatty Acids, and Amino Acids in the Cyanobacterium *Tolypothrix scytonemoides*. World J Microbiol Biotechnol, 23:251-257, (2007).
116. Thomas DJ, Avenson TJ, Thomas JB, Herbert SK, A cyanobacterium lacking iron superoxide dismutase is sensitized to oxidative stress induced with methyl viologen but not sensitized to oxidative stress induced with norflurazon. Plant Physiol, 116:1593-1602, (1998).
117. Saha SK, Uma L, Subramanian G, Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. FEMS Microbiol Ecol, 45:263-272, (2003).
118. Mann T, Kleilin D, Homocuprein and heptacuprein, copper-protein compounds of blood and liver in mammals. Proc R Soc London B, 126:303-315, (1938).
119. McCord JM, Fridovich I, Superoxide dismutase, an enzymatic function for erythrocuprein. J Biol Chem, 244:6049-6055, (1969).
120. Bannister JV, Bannister WH, Rotils G, Aspects of the structure, function and applications of superoxide dismutase. CRC Crit Rev Biochem, 22:110-180, (1987).
121. Bowler C, Van Montague M, Inzé D, Superoxide dismutase and stress tolerance. Ann Rev Plant Physiol Plant Mol Biol, 43:83-116, (1992).

122. Scandalias JG, Oxygen stress and superoxide dismutase. Plant Physiol, 101:7-12, (1993).
123. Farr SB, Kogoma T, Oxidative stress responses in *Escherichia coli* and *Typhimurium*. Microbiol Rev, 55:561-585, (1991).
124. Balakrishnan P, Reddi KS, Vincent DJ, Lakshmanan U, Gopalkrishnan S Dharmar P, Characterization of manganese superoxide dismutase from a marine cyanobacterium *Leptolyngbya valderiana* BDU20041, Saline Systems, 6:6 (2010).
125. Chongpraditnum P, Mori S, Chino M, Excess copper induces copper/zinc superoxide dismutase in soybean root. Plant Cell Physiol, 33:239-244, (1992).
126. Shirkey B, Kovarcik DP, Wright DJ, Wilmoth G, Prickett TF, Helm RF, Gregori EM, Potts M, Active Fe containing SOD and abundant sodF mRNA in *Nostoc commune* (cyanobacteria) after years of desiccation. J Bacteriol, 182:189-197, (2000).
127. Zhao W, Ze Ye, Zhao J, RbrA a cyanobacterial rubrerythin, functions as a FNR-dependent peroxidase in heterocysts in protection of nitrogenase from damage by hydrogen peroxide in *Anabaena* sp. PCC 7120. Mol Microbiol, 66:1219-1230, (2007).
128. Fay P, Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol Rev, 56:340-373, (1992).
129. Redinbaugh MG, Wadsworth GJ, Scandalias JG, Characterization of catalase transcripts and their differential expression in maize. Biochim Biophys Acta, 951:104-116, (1988).
130. Scandalias JG, Response of plant antioxidant defense genes to environmental stress. Adv Genet, 28:1-41, (1990).
131. Beaumont F, Jouvc HM, Cagnan J, Gillard J, Pelment J, Purification and properties of a catalase from potato tubers (*Solanum tuberosum*). Plant Sci, 72:19-26, (1990).
132. Kirkman HN, Galiano S, Gaetani, The function of catalase-bound NADPH. J Biol Chem, 262:660-666, (1987).
133. Hertwig B, Steb P, Feierabend J, Light dependence of catalase synthesis and degradation in leaves and the influence of interferring stress conditions. Plant Physiol, 100:1547-1553, (1992).
134. Feierabend J, Schaan C, Hertwig B, Photoinactivation of catalase occurs under both high- and low-temperature stress conditions and accompanies photoinhibition of photosystem II. Plant Physiol, 100:1554-1561, (1992).
135. Wai HH, Va L, Oakes M, Nomura M, Complete detection of yeast rDNA repeats and integration of a new rDNA repeat: Use of rDNA deletion strains for functional analysis of rDNA promoter elements in vivo. Nucleic Acid Rev, 28:3524, (2000).
136. Tripathi SN, Srivastava P, Presence of stable oxygen scavenging enzymes superoxide dismutase, ascorbate peroxidase and catalase in a desiccation tolerant cyanobacterium *Lyngbya arbicola* under dry state. Curr Sci, 81:197-200, (2001).
137. Halliwell B and Gutteridge JMC, Ed. Free Rad Biol Med, 2nd Edn, Clarendon Press, Oxford, 86-187, (1989).
138. Obinger C, Regelsberger G, Pircher A, Strasser G, Peschek GA, Scavenging of superoxide and hydrogen peroxide in blue-green algae (cyanobacteria). Physiol Plant, 140:693-698, (1998).
139. Miyake C, Michihata F, Asada K, Scavenging of hydrogen peroxide in prokaryotic and eukaryotic algae: Acquisition of ascorbate peroxidase during the evolution of cyanobacteria. Plant Cell Physiol, 32:33-43, (1990).
140. Mittler R, Tel-or E, Oxidative stress responses and shock protein in unicellular cyanobacterium *Synechococcus* PCC

7942 (R2), Arch Microbiol, 155:125-130, (1991).
141. Rozen A, Mittler R, Burstein Y, Tel-Or E, A unique ascorbate peroxidase active component in the cyanobacterium *Synechococcus* PCC 7942 (R2). Free Radical Res Commun, 17:1-8, (1992).
142. Tel-or E, Huflejt ME, Packer L, Hydroperoxide metabolism in cyanobacteria. Arch Biochem Biophys, 246:396-402, (1986).
143. Tel-Or E, Huflejt M, Packer L, The role of glutathione and ascorbate in hydroperoxide removal in cyanobacteria. Biochem Biophys Res Commun, 132:533-539, (1985).
144. Williams CH Jr., Flavin containing dehydrogenases. In Boyer PD, (ed.), *The Enzymes*, Academic Press, New York, 3rd Edn, 13: 89-173 (1976).
145. Foyer CH, Halliwell B, The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta, 133:21-25, (1976).
146. Serrano A, Rivas J, Losada M, Purification and properties of glutathione reductase from the cyanobacterium *Anabaena* sp. strain 7119, J Bacteriology, 158:317-324, (1984).
147. Karni L, Moss SJ, Tel-Or E, Glutathione reductase activity in heterocysts and vegetative cells of *Nostoc muscorum*, Arch Microbiol, 140:215-217, (1984).
148. Domínguez AR, Hernández-Arana A, Mendoza- Hernández G, Rendón JL, Thermal denaturation of glutathione reductase from cyanobacterium *Spirulina maxima*, Biochem Mol Biol Internl, 42: 631-639, (1997).
149. Jiang F, Hellman U, Sroga GE, Bergman B, Mannervik B, Cloning, sequencing, and regulation of the glutathione reductase gene from the cyanobacterium *Anabaena* PCC 7120, J Biol Chem, 270: 22882-22889, (1995).
150. Bernd Z, Ana T, Lucija H, Hrvoje F, Subcellular distribution of glutathione and cysteine in cyanobacteria, Protoplasma, 246:65-72, (2010).
151. Cameron JC, Pakrasi HB, Essential Role of glutathione in acclimation to environmental and redox perturbations in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol, 154:1672-1685, (2010).
152. Okumura N, Masamoto N, Wada H, The *gshB* gene in the cyanobacterium *Synechococcus* sp. PCC 7942 encodes a functional glutathione synthetase. Microbiology, 143:2883-2890, (1997).
153. Okai Y, Higashi-Okai K, Yano Y, Otani S, Identification of antimutagenic substances in an extract of edible red alga, *Porphyra tenera* (Asakusa-nori), Cancer Lett 100:235-240, (1996).
154. Maruyama H, Watanabe K, Yamamato I, Effect of dietary kelp on lipid peroxidation and glutathione peroxidase activity in livers of rats given breast carcinogen DMBA. Nutr Cancer, 15:221-228, (1991).
155. Yang YJ, Nam S-J, Kong G, Kim MK, A case control study on seaweed consumption and the risk of breast cancer, Br J Nutr, 103:1345-1353, (2010).
156. Rastogi RP, Richa, Sinha RP, Singh SP, Häder D-P, Photoprotective compounds from marine organisms, J Ind Microbiol Biotecnol, 37:537-558, (2010).
157. Yabuta Y, Fujimura H, Kwak CS, Enomoto T, Watanabe E, Antioxidant activity of the phycoerythrobilin compound formed from a dried Korean purple laver (*Porphyra* sp.) during in vivo digestion, Food Sci Technol Res, 16:347-351, (2010).
158. Shimazu T, Kuriyama S, Hozawa A, Ohmori K, Sato Y, Nakaya N, Nishino Y, Tsubono Y, Tsuji I, Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study, Int J Epidemiol, 36:600-609, (2007).