Research Article Biotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

UV-B PROTECTING ACTIVITY OF *SYNECHOCOCCUS SPP. PCC7942* BY ANTIOXIDATIVE DEFENSE SYSTEM

NIDA FATIMA[1], HERA CHAUDHRY[2] AND IFFAT ZAREEN AHMAD[1]*

[1] *Department of Bioengineering, Integral University, Dasauli, Kursi Road, Lucknow- 226026, Uttar Pradesh, India.*
[2] *Department of Biosciences, Integral University, Dasauli, Kursi Road, Lucknow- 226026, Uttar Pradesh, India.*

ABSTRACT

Cyanobacteria are the prokaryotes and the most primitive organism to evolve on the earth. They are autotrophs and perform oxygenic mode of photosynthesis. During the course of evolution cyanobacteria were exposed to extreme habitats and environmental conditions which developed an ability to synthesize an array of complex secondary metabolites in these cells. One such class of compounds is UVR protectants which include some photosynthetic pigments, antioxidant compounds and enzymes, mycosporine-like amino acids and scytonemin. Of these, antioxidant enzymes provide an important defense against the harmful effects of UVR. UV-B radiations are deleterious when it comes to human health because in recent times due to depletion of ozone layer more radiations reach the earth's surface. Therefore, there arises a need for novel and natural UV protectants. Cyanobacteria are suitable candidates for the isolation and purification of such compounds. For this, study should be conducted on the various antioxidant enzymes like SOD, CAT, POD and ASC-POD in cyanobacteria.

KEYWORDS: **Cyanobacteria,UV-B radiations,antioxidant enzymes, isozymes.**

IFFAT ZAREEN AHMAD
Department of Bioengineering, Integral University, Dasauli, Kursi Road, Lucknow- 226026, Uttar Pradesh, India.

***Corresponding author**

INTRODUCTION

A growing interest in natural bioactive compounds that bear antioxidant properties has been increased as they may provide relevant contributions to maintain health, e.g., via regular ingestion as part of formulated foods. Indeed, literature gathered in a large number of worldwide researches supports the role of antioxidants in the prevention of and in the growth control of certain tumours, as well as in the incidence and severity of cardiovascular and degenerative diseases[1–3]. There is a growing concern to obtain novel antioxidants which are less expensive than conventional sources and cyanobacteria presents itself to be the most suitable source for this purpose.Microalgae are the important component of the food chain in aquatic ecosystems. They can use sun's energy to convert H_2O and CO_2 into complex organic compounds and later accumulate and/or secrete many primary and secondary metabolites [4–6]. Furthermore, they exhibit adaptive strategies to oxidative stresses mostly by stimulation of their antioxidant defence system[7] that consists of both enzymatic and non-enzymatic mechanisms: superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase are key enzymes in the former, whereas the non-enzymatic counterpart includes mediator compounds like ascorbic acid, reduced glutathione, tocopherols, carotenoids and phycocyanin [8].It has been found that the detrimental effects of UVB radiation on cyanobacteria are always mediated by reactive oxygen species (ROS) [9-12].To quench ROS, organisms develop efficient antioxidant systems to scavenge them, including antioxidant molecules and antioxidant enzymes, which play obvious protective roles in organisms under oxidant stress [13-18]. Enzymatic components include superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), of which SOD is a major scavenger of O_2^- free radicals and converts them into O_2 and H_2O_2. The H_2O_2 is then scavenged by CAT and variety of POD into H_2O and O_2. In addition to SOD, CAT, and POD, another major component of the enzymatic defense is the ascorbate (ASC) glutathione (GSH) cycle that involves four enzymes including ascorbate peroxidase (ASC-POD), monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase. It is good for *Nostoc* protection if those antioxidant elements increase when cells are under stress. Among them ASC-POD is the major scavenger of H_2O_2, which requires reduced ASC provided by the ASC-GSH cycle for the reaction to occur[19].The research work presented in this paper deals with the exploration of the protective antioxidant defense mechanisms to counteract damaging effects of UV-B radiations which ultimately leads to the uses of cyanobacteria in the cosmetic industry. Therefore, the experimental work includes the study of growth behavior of cyanobacteria under UV-B stress. The extracts were considered, so as to comprehensively characterize the strains in terms of total antioxidant capacity.

MATERIALS AND METHODS

I. Test organisms and growth conditions

The axenic culture of unicellular and non-heterocystous yanobacterium, *Synechococcus spp. PCC7942* was obtained from Algal Laboratoy, Department of Botany, University of Allahabad, Uttar Pradesh, India. Cultures were grown in BG11 medium [20] supplemented with nitrogen source and no stress culture served as control. Cultures were maintained at 27°C±1°C under fluorescent illumination of 30-40 $\mu Em^{-2} s^{-1}$ provided by fluorescent tubes exposed to a 14 h light /10 h dark photoperiod and swirled manually for five minutes, thrice daily. The test cyanobacterial cultures were subjected to UV-B stress for 1 to 6 hours by using UV-B tube {PHILIPS TEK 40W, ACTINIC BL, 240 Volt (100V-300V) 50 Hz, of Range=280nm-350nm, Made in Germany. Fresh biomass was harvested in the logarithmic phase of incubation by centrifugation at 10,000 rpm for 5 minutes and the pellet was homogenized and the resulting supernatant was used for further assays. Biochemical characterization of cultures was carried out for growth behavior, specific growth rate and the growth of cyanobacteria in

This article can be downloaded from www.ijpbs.net

terms of protein content. The results were presented as means of triplicates after statistical analysis.

II. Growth behavior

Evaluation of growth behavior of different cyanobacterial strains subjected to various stresses was done by taking whole cell absorbance (A_{660nm}) after every alternate day and by plotting a graph of absorbance versus days.

III. Specific growth rate

To measure the survival of the acclimated cyanobacteria, the cells were treated with different durations of stress. The lethal dose was determined against the control. Growth was determined in terms of specific growth rate by using the following equation [21]

$K=2.303 (\log N2 - \log N1) / T2 -T1$

Where, N1=Initial OD / Protein concentration at time T1, and N2 = Final OD/ Protein concentration at time T2.

IV. Estimation of growth of cyanobacteria in terms of total protein content

Total proteins were extracted from the stress tolerant and reference cultures. The total protein content of the cyanobacterial cultures was estimated by following the method of Lowry *et al* [22] .

V. Total Antioxidant Potential

(i) DPPH Radical Scavenging Assay

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Shimada *et al* . [23] Various concentrations of methanol extract of the sample (4.0 ml) were mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The Mixture was shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517 nm. BHA was used as control .The percentage of DPPH decolorization of the sample was calculated according to the equation:

Scavenging activity (%) = [Abs (control) – Abs (standard)] ×100.

Where, Abs (control): Absorbance of DPPH radical + Methanol

Abs (standard): Absorbance of DPPH radical +extract/standard.

(ii) Ferric Reducing antioxidant Power Assay (FRAP)

Ferric Reducing antioxidant Power Assay was determined by the method described by Benzie and Strain [24].

(iii)Enzyme extraction

Extractions of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), and ASC-POD (EC 1.11.1.11) were as described by Lin and Kao [25] with few modifications. Fresh biomass was harvested after 11 days (logarithmic phase) of incubation by centrifugation at 10,000 rpm for 10 minutes and the pellet was suspended in ice-cold 0.1M sodium phosphate buffer (pH 6.8). The homogenized samples were centrifuged at 15,000 Xg for 30 min at 4°C, and the resulting supernatant was used for enzyme activity assays.SOD activity assay was based on the method described by Giannopotitis and Ries[26]. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitro blue tetrazolium reduction measured at 560 nm.CAT activity was determined according to Cakmak and Marschner [27] with slight modification. The reaction mixture in a total volume of 3ml contained 1.9 ml 50mM sodium phosphate buffer (pH 7.0) and 1ml 0.2% H_2O_2. The reaction was initiated by the addition of 0.1 ml enzyme extract and activity was determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm.POD activity was determined by measuring the rate of increase in absorbance at 470nm of a mixture containing 1ml 50mM sodium phosphate buffer (pH 7.0),

0.95 ml 0.2% 2-methoxyphenol, 1ml 0.2% hydrogen peroxide, and 0.05 ml enzyme extract or distilled water for control (total reaction volume 3 ml).ASC-POD activity was etermined by following the decrease of absorbance at 290nm using an extinction coefficient (e) 2.8mM^{-1} cm^{-1} [28].

VI.Differential expression of antioxidant proteins in Synechococcus spp. PCC 7942 under UV-B radiation

(i)Discontinuous polyacrylamide gel electrophoresis

The enzyme extracts underwent SDS-PAGE according to Sanchez' method [29] on vertical slab gels. The polyacrylamide gel consisted of a (1) 4.5%T/1.2%C_{bis} stacking gel in 0.125 M Tris-HCl buffer (pH 6.8) with 0.1% (w/v) SDS, and (2) 12.5%T/3.3%C_{bis} resolving gel in 0.375 M Tris-HCl buffer (pH 8.8) with 0.1% (w/v) SDS. Thereafter, 100µL enzyme solution was mixed with 10µL 0.025% bromophenol blue. Samples of 30-40µL were loaded in the wells. The voltage was regulated to 150V, stabilizing it to 200V after bromophenol blue come into a separation gel. The electrophoresis was ended when the distance from bromophenol blue to bottom was 1 cm [30].

(ii)Isozyme of SOD

Sample preparation: Fresh cyanobacterial culture was pellet down and dried. One gram of sample was ground in a precooled mortar with 1.5 ml 0.05 mol/L phosphate buffer (pH 7.8). The resulting material was centrifuged for 40 min at 12000 rpm at 4 °C. One milliliter 0.05 mol/L phosphate buffer (pH 7.8) was added to the precipitate. It was then ground and centrifuged for 40 min at 12000 rpm at 4 °C. This allowed us to obtain the enzyme sample.*Staining solution preparation* [31]: Nitroblue tetrazolium (NBT) 25 µmol/L, lactochrome 0.01%, 50 mmol/L phosphate buffer (pH 7.8) containing 1mmol/L EDTA. The gel was placed into 80ml NBT nitroblue tetrazolium solution for 15 min. It was then soaked in lactochrome solution for 5 min, and thereafter immersed in 50 mmol/L phosphate buffer (pH 7.8) containing 1 mmol/L EDTA. The gel was illuminated at 10 cm height using 40W fluorescent lamps until transparent bands emerged.

(iii)Isozyme of CAT

Sample preparation. Fresh cyanobacterial culture was pellet down and dried. One gram of the cells was ground in a precooled mortar with 5 ml of 1mol/L phosphate buffer (pH 7.8). Materials were stored at 4 °C in the refrigerator for 2 h. Thereafter, they were centrifuged for 10 min at 10000 rpm. The extracted material contained the enzyme sample, which was stored at -20 °C. The concentration of separation gel was 10-11%. Also, 0.5% soluble starch solution was added to the separation gel. *Staining.* It was made using two solutions: A and B. A Solution: 25 ml 3% H_2O_2, 5 ml 0.1mol/L phosphate buffer (pH 7.0) and 3.5 ml 0.1mol/L $Na_2S_2O_3$. B Solution: 25 ml 0.09mol/L KI was dissolved in 25ml double distilled water. The gel was soaked in the A solution for 15 min at room temperature, washed with double distilled water, and placed into the B solution. The resulting material was fixed in 10% glycerin.

VII. Statistical Analysis

Data were statistically analyzed and the results were expressed as means (±SD) of average of three replicates (n=3). p- Values (≤ 0.05) considered as significant compared to the respective controls.

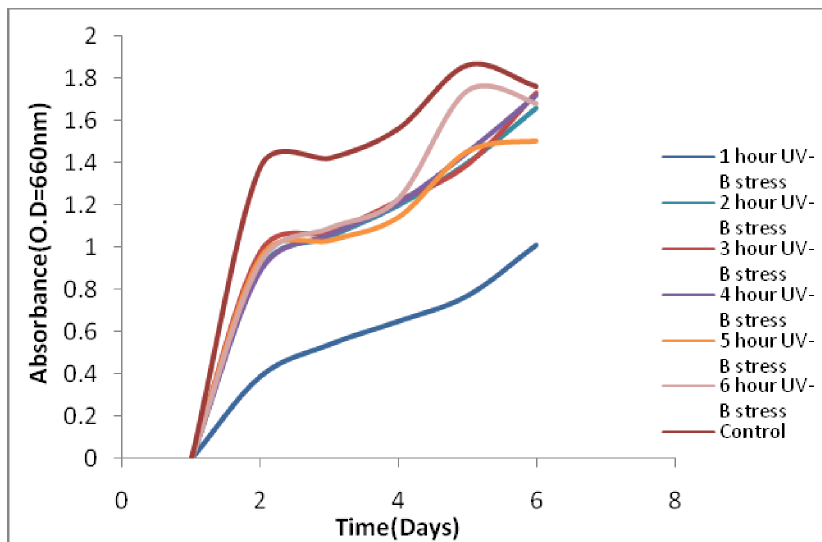
RESULTS AND DISCUSSION

I. Growth behavior and Specific growth rate of Synechococcus spp. PCC 7942

The growth of *Synechococcus spp. PCC 7942* increased during initial exposure with UV-B radiation for 2 hours then decreased till 5 hours of stress after which there was an increase as compared to control culture. Effect of UV-B exposure on specific growth rate of *Synechococcus spp. PCC7942* showed that at the first day of exposure growth rate decreased significantly after 1 hour of exposure and it was very less as compared to the control. The specific growth rate on 3rd day of exposure showed gradual increase in growth but lesser than control. The specific growth rate pattern of

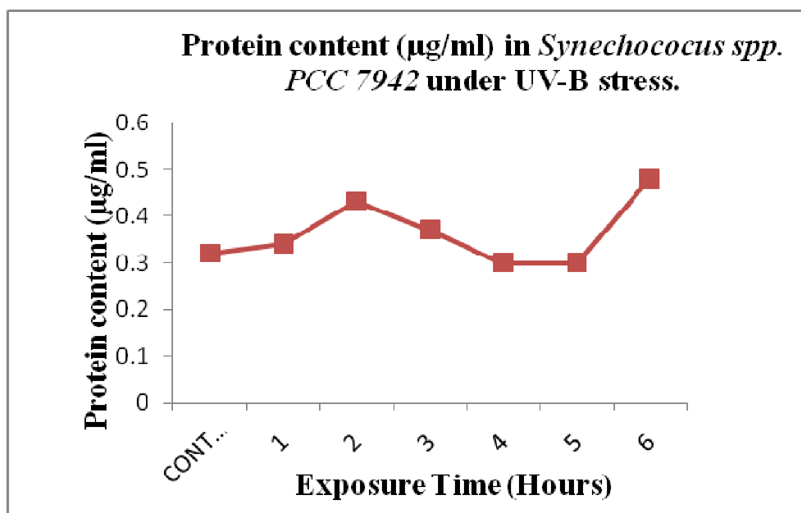
5th day was same as that of the 3rd day. The growth rate pattern of the 7th and 9th day showed increased growth. Thus, this could be concluded that in the initial hours and days of exposure there was adaptations in cyanobacterial cells which retarded the growth rate but as the time and days of exposure increased the cells became tolerant and the growth enhanced (Graph 1).

Graph 1
Specific growth rate of Synechococcus spp. PCC 7942 under UV-B stress.



II. Effect of UV-B radiation on protein content of Synechococus spp. PCC 7942.
The amount of protein in *Synechococcus spp. PCC 7942* increased initially till 2 hours of exposure after which it decreased after which it decreased till 5 hours of stress and then again showed an increase in content (Graph 2).

Graph 2
Protein content of Synechococcus spp. PCC 7942 under UV-B stress



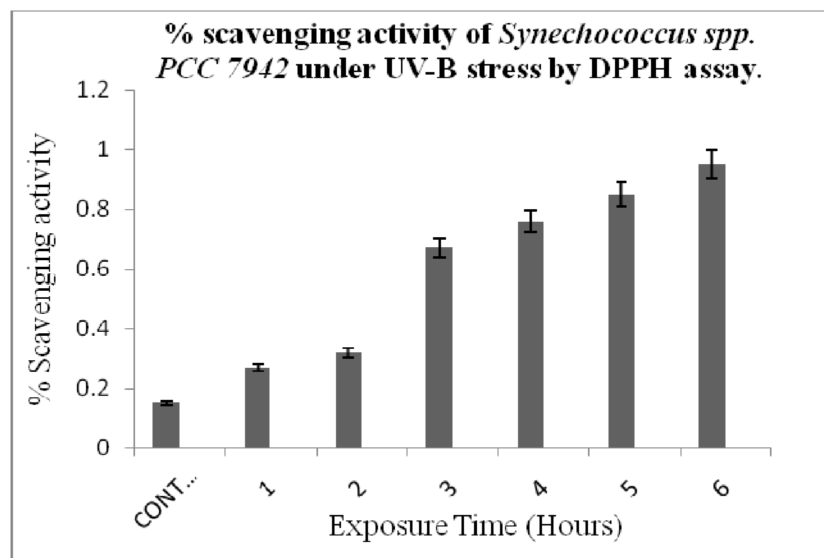
****Cont.-Control***

III. Total Antioxidant Assay

(i) DPPH Antioxidant assay

The DPPH assay has shown increased scavenging activity of antioxidant enzymes which continuously increased on increasing the exposure time (Graph 3).

Graph 3
Free radical scavenging activityof Synechococcus spp. PCC 7942 under UV-B stress by DPPH assay.

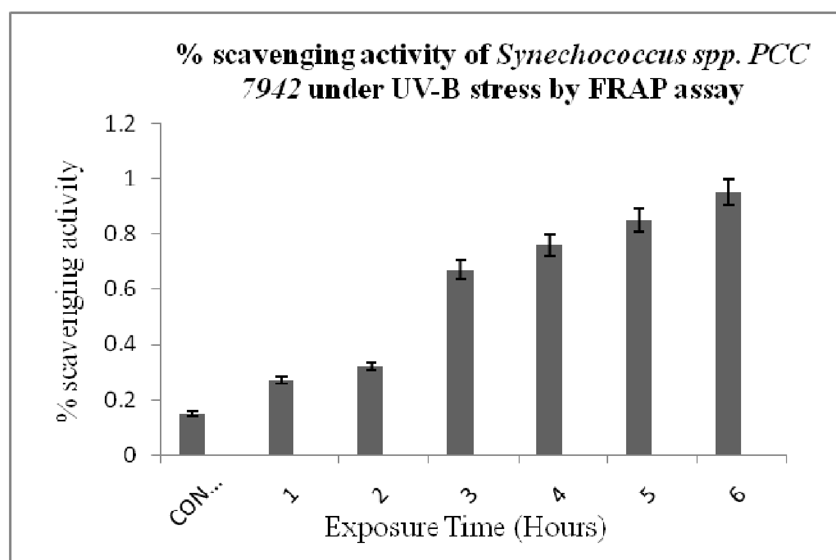


***Cont.-Control**

(ii) FRAP Antioxidant assay

A similar pattern in the result of FRAP was observed as in DPPH. The results of the FRAP assay showed production of antioxidants in the stressed cells. The % scavenging calculated from the absorbance clearly indicated that the scavenging activity in the cells has increased which occurred due to the presence of antioxidants, produced to neutralize the free radicals generated. The 6 hours of exposure showed visible increase in % scavenging (Graph 4).

Graph 4
Free radical scavenging activityof Synechococcus spp. PCC 7942 under UV-B stress by FRAP assay.

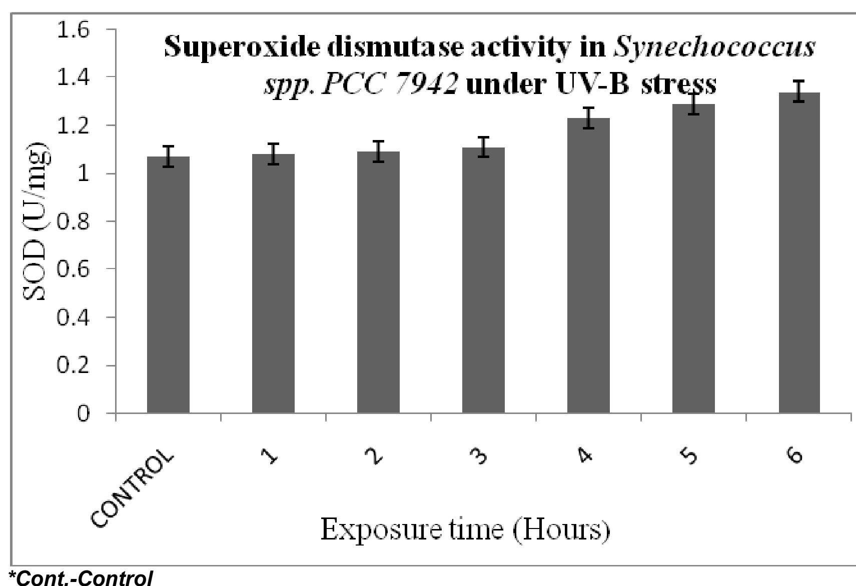


***Cont.-Control**

IV. Enzyme assays

Extracts of *Synechococcus spp. PCC 7942* grown in control and stress conditions exposed to UV-B radiation for different time durations (1-6 hours) were evaluated for the activity of antioxidant enzymes. The extracts of the stressed culture of cyanobacteria showed significant antioxidant activity as compared to control. The level of SOD increased gradually in the samples exposed to UV-B radiation as compared control. The activity of SOD significantly increased on increasing the duration of exposure. This indicated that as the stress increases, production of reactive oxygen species also increases, which leads to the significant increase in the level of enzymatic antioxidants in the cells. It was seen that SOD activity was highest in cells exposed to 6 hours UV-B radiations. The decrease in the levels of SOD during initial hours can be attributed to the increased utilization of these antioxidants to combat the reactive oxygen species produced excessively during the oxidative stress (Graph 5).

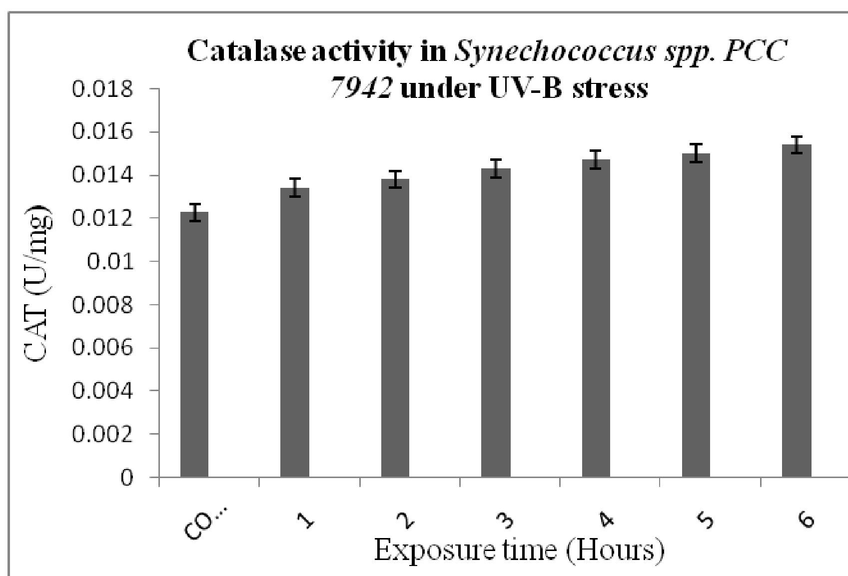
Graph 5
SOD activity in Synechococcus spp. PCC 7942 under UV-B stress.



***Cont.-Control**

CAT activity also showed the same pattern of activity as SOD. A continuous increase was observed as the exposure time increased reaching maximum in 6 hrs exposed samples. CAT showed its minimum activity in control sample. Increase in the level of CAT activity during exposure indicated that ROS level is deccreased due to higher activity of CAT (Graph 6).

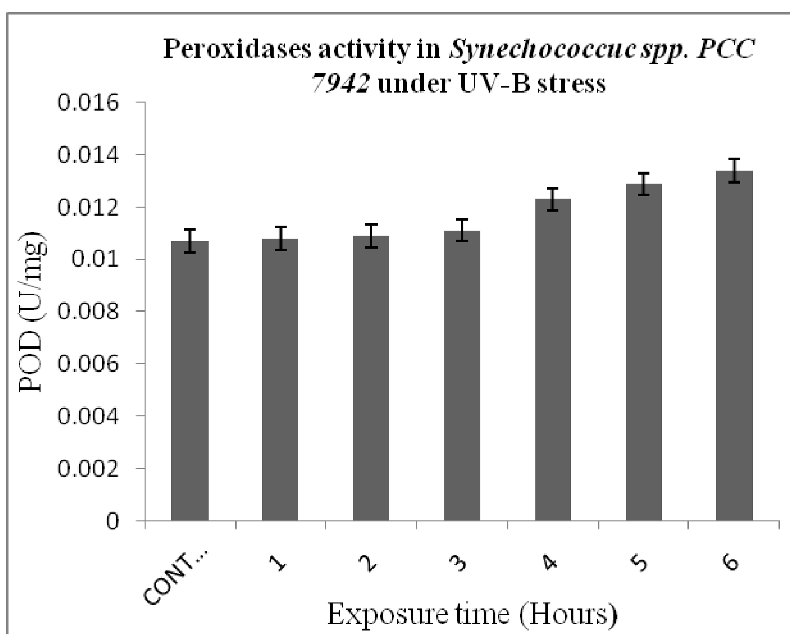
Graph 6
CAT activity in *Synechococcus spp. PCC 7942* under UV-B stress.



Cont.-Control

POD activity showed gradual increase as duration of stress increases. POD activity was again greater under stress conditions for all hours of exposure as compared to the normal condition. However, it was seen that the activity was maximum in 6 hours of stress; this indicated that highest oxidative stress was produced in 6 hours of stress (Graph 7).

Graph7
POD activity in *Synechococcus spp. PCC 7942* under UV-B stress.

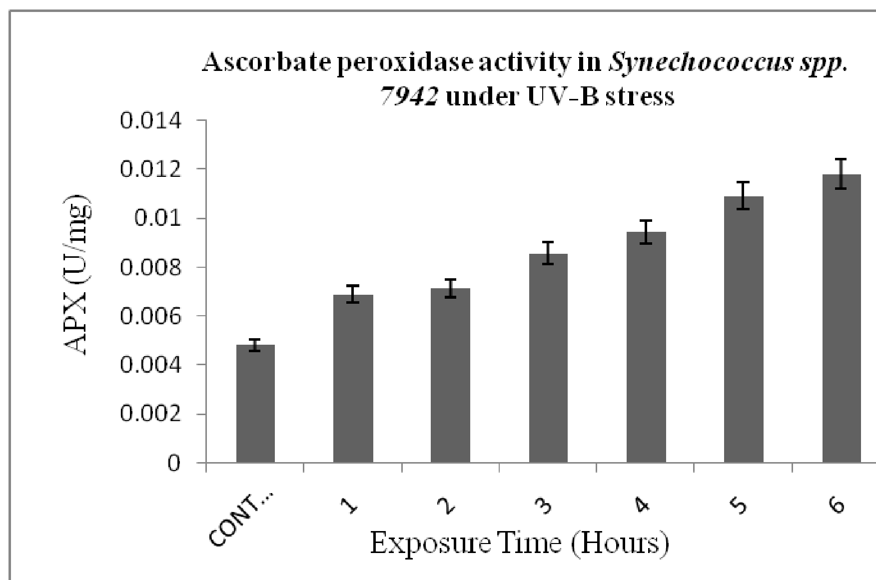


Cont.-Control

Ascorbate peroxidase activity was seen to increase markedly under stress condition reaching maximum after 6 hours of stress (Graph 8). APX showed its minimum enzymatic activity in control. It

was observed that APX activity was very high in stress condition. The results indicate that, more or less, andioxidant production is related to enhanced cellular capacity to detoxify H_2O_2. For this detoxification, the operation of ascorbate peroxidase together with the ascorbate-regenerating enzymes appears to be of particular importance [32].

Graph 8
ASC-POD activity in Synechococcus spp. PCC 7942 under UV-B stress.



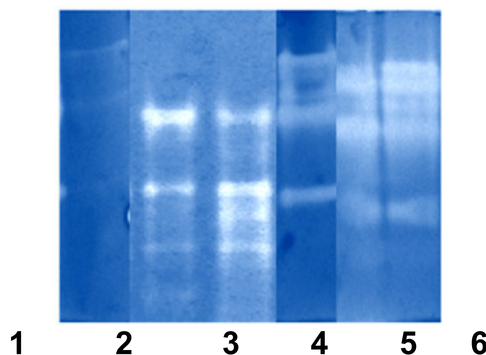
***Cont.-Control**

UV-B affected the growth and survival of cyanobacteria as compared to control. SOD activity continuously increased from 1 to 6 hour of exposure. However, its presence in all samples suggested that this enzyme may participate in protection against free superoxide radicals. Catalase activity showed the same pattern as peroxidase. APX showed its maximum activity in late stress hour. The similar effect of UV-B radiation on antioxidant enzymes has also been earlier reported [33].

Isozyme activity

The electrophoretic profiles of SOD and CAT isozymes showed that the activities of isozymes were affected during UV-B exposure. The effect of exposure was seen more on activity of constitutive enzyme pools. In the present study, we observed a differential SOD and CAT isozyme activities in different hours of UV-B exposure.

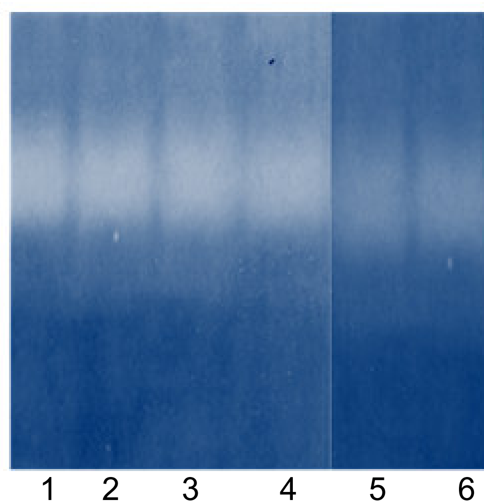
Figure 1
SOD activity in discontinuous polyacrylamide gel electrophoresis.



1 2 3 4 5 6

There were six SOD isozymes observed in different hours of exposure on electrogram of discontinuous polyacrylamide gel electrophoresis showing different mobilities. Some of them were stress specific and some had higher expression (as shown by the intensity of the band) during long hours of exposure. It was also observed that expression of these isozymes was significant. In the electrogram, six SOD isoenzymes were found, three of them being prominent while the others showed faint bands (Figure 1). The activity of the fastest moving isoenzyme increased as the exposure pursued. The sample which was exposed for 1 hour showed three faint bands while the samples after 2 and 3 hours of exposure showed three bands of high intensity in which one new band appeared. The samples which had exposure of 4, 5 and 6 hrs exhibited four prominent bands of which two were new. These results are in accordance with the previous findings where number and higher expression of SOD was observed in the sample which got long exposure of UV-B stress [34]. Cyanobacteria can have iron-containing SOD (cytosolic) and manganese-containing SOD (thylacoid-bound) isoforms[35]. The strains tested showed induction of both a Fe-SOD isoform and Mn-SOD due to the experimental conditions (Figures 1). SOD activities have been shown to change dramatically in response to conditions that favour the formation of superoxide[36].

Figure 2
CAT activity in discontinuous polyacrylamide gel electrophoresis.



The exposure time did not show a significant effect on the expression CAT of isozymes and was almost similar during exposure hours (Figure 2). Expression of CAT enzyme was higher during first four hours of exposure as shown by the intensity of the band. A decrease in the intensity of the isozyme band was observed during 5 and 6 hours of exposure. No significant change was observed in CAT isozyme pattern in all the samples. It is known that enzymes that have multiple intracellular distributions are present as different isozyme forms in different cell compartments [37]. Therefore, the analysis of the activity of individual isozymes of enzymes is important, because it can help to understand how each stress may affect the different sub-cellular compartments [38].

DISCUSSION

Cyanobacteria tend to produce a myriad of secondary metabolites with complex structures and extraordinary bioactivity [39]. Besides photoprotective mechanisms several other defence strategies such as avoidance and production of antioxidants are also operative in cyanobacteria against UVR. Cyanobacteria possess massive potential for serving as a source material for the production of antioxidative enzymes [40, 41].Our results

concerning the impact of the initial exposure degree on the growth of the host strain are in concert with the findings of Kagami *et al*. [42]. These results were in support of the proposition that cells response to stress depending on its duration and degree. This indicates that the change of SOD activity was very sensitive to the environment [43, 44]. Since Kong and Sang [45] have shown that SOD was one of the key enzymes to eliminate active oxygen in algal cells once the pathogen invasion increases, the cellular detoxification system was stimulated and the synthesis of SOD was started. As these changes took place at the molecular level in the cells, they happened much earlier than growth or reproduction. Catalase activity has been shown to be associated with all cyanobacterial species [46]. The alteration in protein profiles and in the activity of antioxidant enzymes are part of the process of survival and a proof that these biomolecules play a significant role in pathogen tolerance. The interpretation of plant responses to stress is exceedingly complex because the mechanisms involved differ depending on the species, tissues, stage of development, physiological status of the plant, and the degree and duration of the stress [47]. On the basis of the similarity of the isoenzyme profiles of SOD and CAT a conclusion could be drawn that the abiotic stress of the host alga was connected with oxidative stress. To mitigate the oxidative stress caused by ROS, organisms develop efficient antioxidant systems to scavenge them, including antioxidant molecules such as carotenoids, tocopherols, ASC, and reduced GSH, and antioxidant enzymes such as SOD, CAT, and POD [13-15, 17-18].

CONCLUSION

From these findings, we came to know that prolonged exposure of UV-B disrupts the cellular homeostasis and enhances the production of ROS in cyanobacteria to a certain extent. Radiation in the UV-B range of approximately 300 nm interferes with various metabolic reactions, primarily by generating free radicals and active oxygen species. These deleterious compounds are inactivated by antioxidants. Several natural products have the potential to exhibit antioxidative properties. Among them are the photosynthetic pigments, phycocyanin and carotenoids, antioxidant enzymes, MAAs and scytonemin. Thus, this study shows the photo-protective effects of these compounds in cyanobacterium, *Synechococcus spp. PCC7942* and of the antioxidant enzymes in particlar. The adaptation of the cyanobacteria under UV-B stress shows that they get adapted to the stress conditions by enhancing the activity of antioxidant enzymes. Improved understanding of these enzymes will be helpful in developing cyanobacteria with in-built capacity of enhanced levels of tolerance to ROS using biotechnological approach.

CONFLICT OF INTEREST

Conflict of Interest:
Conflict of interest declared none.

REFERENCES

1. Abe K, Hattor, Hiran HM, Accumulation and antioxidant activity of secondary carotenoids in the aerial microalga *Coelastrella striolata* var. multistriata. Food Chem, 100: 656–661, (2005).
2. Guerin M, Huntley ME, Olaizola M, *Haematococcus* astaxanthin: Applications for human health and nutrition. Trends Biotechnol, 21: 210–216, (2003).
3. Wang HM, Pan JL, Chen CY, Chiu CC, Yang MH, Chang HW, Chang JS, Identification of anti-lung cancer extract from *Chlorella vulgaris* C-C by antioxidant property using supercritical carbon dioxide extraction. Process Biochem, 45: 1865–1872, (2010).
4. Guedes AC, Amaro HM, Malcata FX, Microalgae as sources of high added-value compounds—A brief review of recent work. Biotechnol. Prog*,* 27: 597–613, (2011).
5. Tandeau-de-Marsac N, Houmard J, Adaptation of cyanobacteria to

environmental stimuli: New steps towards molecular mechanisms. FEMS Microbiol. Rev, 104: 119–190, (1993).
6. León R, Martín M, Vigara J, Vilchez C, Vega J, Microalgae-mediated photoproduction of β-carotene in aqueous organic two-phase systems. Biomol. Eng, 20: 177–182, (2003).
7. Srivastava A, Bhargava P, Rai L, Salinity and copper-induced oxidative damage and changes in the antioxidative defense systems of *Anabaena doliolum*. World J. Microbiol. Biotechnol, 21: 1291–1298, (2005).
8. Honga Y, Hua HY, Xiea X, Li FM, Responses of enzymatic antioxidants and non-enzymatic antioxidants in the cyanobacterium *Microcystis aeruginosa* to the allelochemical ethyl 2-methyl acetoacetate (EMA) isolated from reed (*Phragmites communis*). J. Plant Physiol, 165: 1264–1273, (2008).
9. Wendehenne D, Durner J, Klessig DF, Nitric oxide: a new player in plant signaling and defense responses. Curr. Opin. Plant Biol, 7: 449–455, (2004).
10. Babu TS, Akhtar TA, Lampi MA, Tripuranthakam S, Dixon DG, Greenberg BM, Similar stress responses are elicited by copper and ultraviolet radiation in the aquatic plant *Lemna gibba*: implication of reactive oxygen species as common signals. Plant Cell Physiol, 44: 1320–1329, (2003).
11. 11. He YY, Ha¨ der DP, Involvement of reactive oxygen species in the UV-B damage to the cyanobacterium *Anabaena sp*. J. Photochem. Photobiol. B: Biol, 66: 73–80, (2002b).
12. Agarwal S, Sairam RK, Srivastava GC, Tyagi A, Meena RC, Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant Sci, 169: 559–570, (2005).
13. Gong H, Zhu X, Chen K, Wang S, Zhang C, Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci, 169: 313–321, (2005).
14. Costa H, Gallego SM, Tomaro ML, Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. Plant Sci, 162: 939–945, (2002).
15. White AL, Jahnke LS, Contrasting effects of UV-A and UV-B on photosynthesis and photoprotection of X -carotene in two *Dunaliella* sp. Plant Cell Physiol, 43: 877–884, (2002).
16. Fedina IS, Grigorova ID, Georgieva KM, Response of barley seedlings to UV-B radiation as affected by NaCl. J. Plant Physiol, 160(2): 205–208, (2003).
17. Mallick N, Copper-induced oxidative stress in the chlorophycean microalga *Chlorella vulgaris*: response of the antioxidant system. J. Plant Physiol. 161: 591–597, (2004).
18. Sigaud-Kutner TCS, Neto AMP, Pinto E, Colepicolo P, Diel activities of antioxidant enzymes, photosynthetic pigments and malondialdehyde content in stationary-phase cells of *Tetraselmis gracilis* (Prasinophyceae). Aquat. Bot. 82: 239–249, (2005).
19. Ali MB, Yu KW, Hahn EJ, Paek KY, Differential responses of anti-oxidants enzymes, lipoxygenase activity, ascorbate content and the production of saponins in tissue cultured root of mountain Panax ginseng C.A. Mayer and *Panax quinquefolium* L. in bioreactor subjected to methyl jasmonate stress. Plant Sci. 169: 83–92, (2005).
20. Stainer RY, Kunisawa R, Mandel M, Cohin-Bazire G, Purification and properties of unicellular blue green algae (Order Chrococcales). Bact Rev, 35: 171–205, (1971).
21. Myers J and Kratz WA, A relationship between pigment content and photosynthetic characteristics in a blue green alga. J. Gen. Physiol. 39: 11-22, (1955).
22. Lowry OH, Rosehmugh NJ, Farr AL and Randall KJ, Protein measurelnent wltb the Folin phenol reagent. J. Biol Chem, 193: 265-275, (1951).
23. Shimada K, Fujikawa K, Yahara K and Nakamura T, Antioxidative properties of xanthan on the anti-oxidation of soybean

oil in cyclodextrin emulsion. J. Agric. Food Chem.,40: 945–948, (1992).
24. Benzie IFF, Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of ―antioxidant power: The FRAP Assay- Analytical Biochemistry, Vol. 239: 70–76, (1996).
25. Lin CC, Kao CH, Effect of NaCl stress on H_2O_2 metabolism in rice leaves. Plant Growth Regul, 30: 151–155, (2000).
26. Giannopotitis CN, Ries SK, Superoxide dismutase in higher plants. Plant Physiol, 59: 309–314, (1977).
27. Cakmak I, Marschner H, Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascrobate peroxidase, and glutathione reductase in bean leaves. Plant Physiol, 98: 1222–1227, (1992).
28. Chen GX, Asada K, Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol., 30: 987–998, (1989).
29. Sanchez I, Sesena S and Palop L, Identification of lactic acid bacteria from spontaneous fermentation of 'Almagro' eggplants by SDS-PAGE whole cell protein fingerprinting. Int J Food Microbiol., 82: 181-189, (2003).
30. Yang TX and Zeng MQ, The changes of isozyme, soluble protein and the dry matter accumulation during endosperm and embryo development in maize. Acta Photophysiologica Sinica, 10: 77-86, (1984).
31. Wang ZR, Plant Allozyme Analysis, Scientific Publishing House, Beijing, China, 95-112, (1996).
32. Cakmak I, Marschner H, Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascrobate peroxidase, and glutathione reductase in bean leaves. Plant Physiol, 98: 1222–1227, (1992).
33. Wang G, Chen K, Chen L, Hu C, Zhang D, Liu Y, The involvement of the antioxidant system in protection of desert cyanobacterium *Nostoc* sp. against UV-B radiation and the effects of exogenous antioxidants. Ecotox Environ Safe, 69: 150–157, (2008).
34. Yan PM, Zhang HF, Wang Q, Yan XY, Sun Y, Comparison of isozyme transformation in maize as a result of insertion of the chitinase gene. Int. J. of Exp. Bot., 79: 117-121, (2010).
35. K. Asada. Superoxide dismutase. In: S. Otsuka and T. Yamanaka (eds.), Metalloproteins, Elsevier. Amsterdam, pp. 331-41,(1988).
36. Obinger C, Günther C, Regelsberger M, Pircher A, Strasser G and Pescheck G, Scavenging of superoxide and hydrogen peroxide in blue-green algae (cyanobacteria). Physiologia Plantarum, 104: 693-698 (1998).
37. Damondara C, Venkaia B, Studies on isoenzymes of superoxide dismutase from mung bean (*Vigna radiate*) seedlings. J. Plant Physiol., 116:279-284, (1984).
38. Scandalios JG, Oxygen stress and superoxide dismutases. Plant Physiol, 101: 1-7, (1993).
39. Yadav S, Sinha RP, Tyagi MB and Kumar A, Cyanobacterial secondary metabolites. Int J Pharm Bio Sci, 2:144-167, (2011).
40. Rastogi RP and Sinha RP, Solar ultraviolet radiation-induced DNA damage and Protection/repair strategies in cyanobacteria. Int J Pharm Bio Sci, 2: 271-288. (2011).
41. Kesheri M, Richa and Sinha RP, Antioxidants as natural arsenal against multiple stresses in cyanobacteria. Int J Pharm Bio Sci, 2:168-187, (2011).
42. Kagami M, De Bruin A, Ibelings BW, van Donk E, Parasitic chytrids: their effects on phytoplankton community and food-web dynamics. Hydrobiologia, 578: 113–129, (2007).
43. Wu Z, Deng P, Wu X, Luo S, Gao Y, Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus*. Hydrobiologia, 592: 465-474, (2007).
44. Li X, Ping X, Xiumei Sh, Zhenbin W, Liqiang X, Toxicity of cypermethrin on growth, pigments, and superoxide dismutase of *Scenedesmus obliquus*.

Ecotoxicol. Environment. Safety, 60: 188-192, (2005).
45. Kong FX, Sang WL, Physiological and biochemical response of *Scenedesmus* obliquus to combined effect of Al, Ca, and low pH. Environ. Contam. Toxicol., 62: 179-186, (1999).
46. Obinger C, Regelsberger G, Pircher A, Str asser G, Peschek GA, Scavenging of superoxide and hydrogen peroxide in blue-green algae (cyanobacteria). Physiologia Plantarum, 104: 693–698, (1998).
47. Baek KH, Skinner DZ, Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. Plant Sci, 165: 1221-1227, (2003).