PROGRESS REPORT

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MAJOR RESEARCH PROJECT

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Project Title

"Screening and isolation of UV-B protecting compounds from fresh water cyanobacteria"

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PRINCIPAL INVESTIGATOR

Prof. Shanthy Sundaram
Coordinator
Centre of Biotechnology
University of Allahabad
Allahabad. U.P
211002

Introduction:

Cyanobacteria are phylogenetically a group of Gram negative photosynthetic prokaryotes having cosmopolitan distribution ranging from hot springs to the Antarctic and Arctic regions. The role of cyanobacteria in nitrogen fixation and in the maintenance of the fertility of rice is well documented. They are also significant constituents of marine ecosystems and account for a high percentage of oceanic primary productivity representing an estimated 40% of the biomass. Absorption of solar energy to drive photosynthesis and nitrogen fixation exposes cyanobacteria to harmful ultraviolet-B (UV-B; 280–315 nm) radiation resulting from continued depletion of stratospheric ozone layer due to anthropogenically released atmospheric pollutants such as CFCs, chlorocarbons and organobromides. This increased UV-B radiation induces hazardous effect on physiological and morphological traits of plants. Cyanobacteria are most widespread and abundant oxygenic photosynthetic prokaryotes with a dominant contribution to phytoplankton primary productivity. The absorption of solar energy to drive photosynthesis and nitrogen fixation exposes cyanobacteria to hazardous ultraviolet radiation (UVR) in their natural habitats. The cyanobacteria are exposed to various types of environmental stresses, hence effect on their survival in habitats rich of UV-B light is interesting.

UV radiations are one of the most harmful exogenous agents and may affect a number of biological functions in all sun exposed living organisms. Solar radiation exposes the organisms to harmful doses of UV-B and UV-A (315-400 nm) radiation in their natural habitats. In response to intense solar radiation, organisms have evolved certain mechanisms such as avoidance, repair and protection by synthesizing or accumulating photoprotective compounds. One type or class includes a variety of colorless, water-soluble compounds with narrow absorption bands and maxima between310 and 334 nm, such as MAAs. Furthermore, MAAs is the most common compounds with a potential role as UV sunscreens in marine organisms. Mycosporine-like amino acids have been reported in diverse organisms; they are a family of secondary metabolites that directly or indirectly absorb the energy of solar radiation and protect organisms exposed to enhanced solar UV radiations. MAAs are intracellular, small (400 Da), colorless and water-soluble compounds that consist of cyclohexenone or cyclohexenimine chromophores conjugated with the nitrogen substituent of amino acids or its imino alcohol. MAAs are favored as photoprotective compounds because they have maximum UV absorption

between 310 and 362 nm. MAAs are cyclohexane or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol.MAAs are synthesized by shikimate pathway (prokaryotes) and by pentose phosphate pathway (eukaryotes). The UV radiation in cyanobacteria alters surviving rates, pigmentation, motility, oxygen photoevolution, carbon dioxide, and nitrogen fixation and phycobilliprotein content. Therefore MAA production reduces all the above changes to some extent. The number of MAAs compound like M-tau, dehydroxylusujirene, M-343, Mycosporine glycine, Shinorine etc. are found in number of cyanobacterial species like *Anabaena* species, *Synechocystis* species, *Nostoc* species*, Plectonema* species, *Aphanothece* species etc. We investigated shinorine compound in *Anabaena cylindrica* and mycosporine glycine compound in *Synechocystis* PCC6803 in our experiment.

The presence of substantial amounts of UV-absorbing compounds in cyanobacteria has been documented in several instances and it has frequently been hypothesized that these substances may be playing a UV-photoprotective role in the organisms that contain them. Other type for which a sunscreen role has been established is the extracellular sheath pigment scytonemin. Scytonemin is a colored (i.e., it is a pigment), lipid-soluble compound with broad absorption in the UV and violet wavelengths (280 to 450 nm) and an in vivo maximum at 370 nm. The pigment was first reported by Negeli (1849) in some terrestrial cyanobacteria and later termed Scytonemin. It is a dimer composed of indolic and phenolic subunits having a molecular mass of 544Da. Scytonemin exists in oxidized (green) and reduced (red) form which was named as fuscochlorin and fuscorhodin, respectively.

The role of any of the water-soluble compounds as sunscreens has not yet been determined rigorously, but some circumstantial evidence supporting the sunscreen hypothesis has been gathered in certain cases.

To assess how common and diverse these substances are among cyanobacteria from habitats exposed to intense solar radiation, we analyzed a variety of isolates of cyanobacteria for MAA-like, UV-absorbing, water-soluble substances. We also investigated the cellular locations of the substances and the effect of UV radiation on their specific contents.

Biosynthesis of MAAs:

Preliminary genomic studies in fungi and cyano-bacteria (Favre-Bonvin *et al.*, 1987; Portwich and Garcia-Pichel, 2003) suggested that the biosynthesis of MAAs originates from the first part of the shikimate pathway, possibly at the level of 3-dehydroquinate synthase (DHQ synthase, encoded by *aroB*) (Portwich and Garcia-Pichel, 2003) (Fig. 2). Tracer experiments in *Chlorogloeopsis* sp. revealed that the amino acid condensed directly on the cyclohexenone ring and resulted in the origin of the variable substituents. Most probably mycosporine-gly was the first MAA synthesized and acted as a precursor for other bi-substituted imino-mycosporines, such as shinorine (Portwich and Garcia-Pichel, 2003). Recently, comparative genomics of cyanobacteria has helped to identify a region of potential interest as the locus for MAA biosynthesis (Singh *et al.*, 2010a). This was based on the presence of a DHQ synthase homologue (ORF Ava_3858) flanked by a putative O-methyltransferase (O-MT), which might potentially lead to the cyclohexenone core. The gene products of *N. punctiforme* also formed 4-deoxygadusol (4-DG) when expressed in tandem *in vitro* if supplied with sedoheptulose -6-phosphate (SHP) as substrate, but not when supplied with 3-DHQ. Details of biosynthetic pathway of MAA and tentative organization of genes involved in its synthesis are well described in the review of Gao and Garcia-Pichel (2011). However, much work is needed to decipher the biosynthetic pathway especially the role of various genes in synthesis of MAA in different species of cyanobacteria (Balskus and Walsh, 2010).

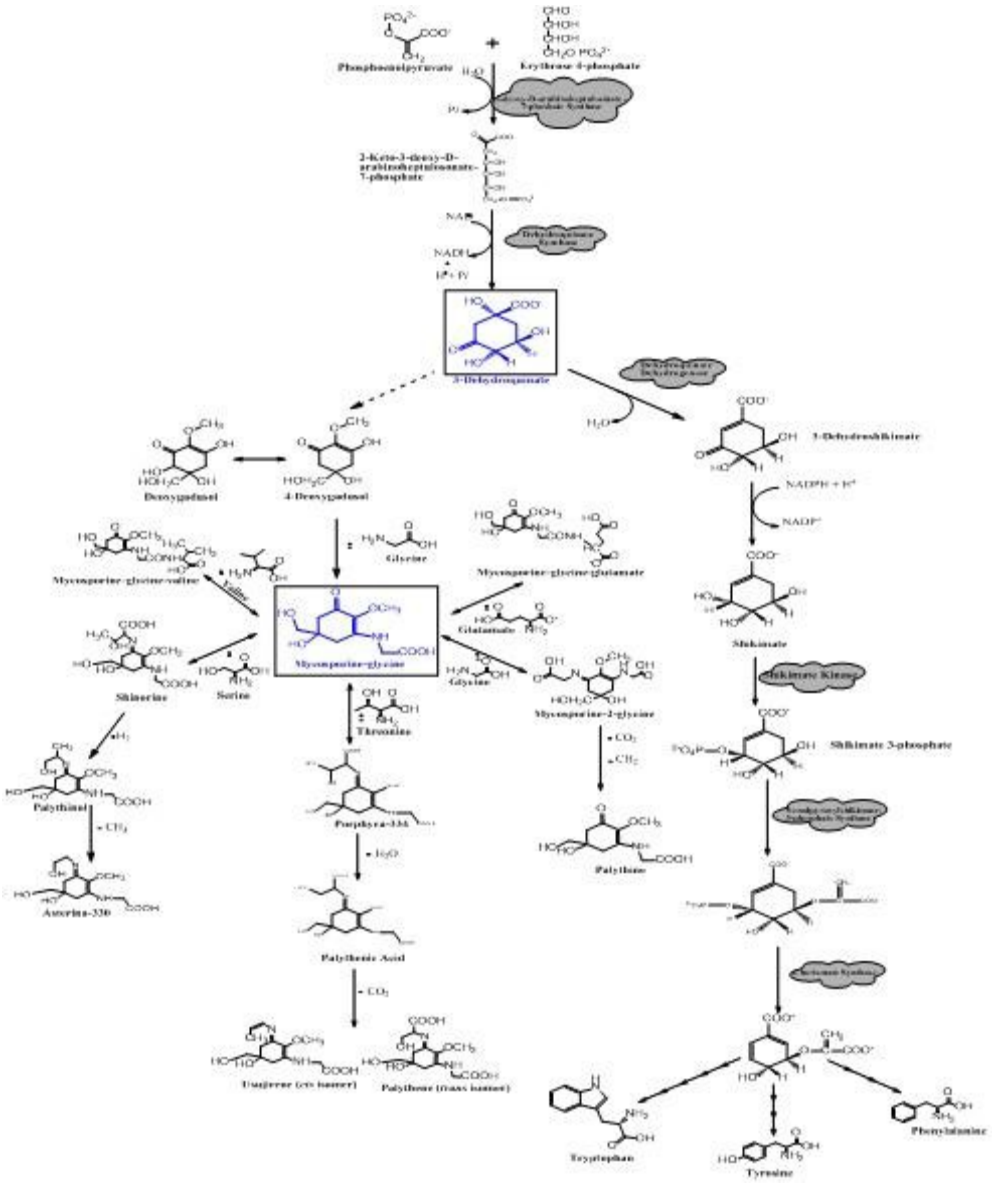

Fig. Biosynthetic pathways for mycosporine-like amino acids.

Literature Search:

Cyanobacteria are ubiquitous in distribution ranging from hot spring to the Antarctic and Arctic regions. The role of cyanobacteria in nitrogen fixation and thereby maintaining the fertility of rice paddy fields and other soils is well documented (Vaishampayan *et al*., 1992). They are also significant constituents of marine ecosystems and account for a high percentage of oceanic primary productivity. Absorption of solar energy to drive photosynthesis and nitrogen fixation exposes cyanobacteria to harmful ultraviolet radiation (UVR) in their natural habitats. Lethal doses of UVR reach deep into water column (Smith and Baker, 1979; Häder *et al*., 2007); down to a depth of 20 m in the clearest oceanic water and to a few centimeters in brown humic lakes and rivers (Kirk, 1994).

It has been found that MAAs provides protection from UV radiations not only for their producers, but also to primary and secondary consumers through the food chain (Helbling *et al*., 2002). MAAs has been reported extensively from taxonomically diverse organisms, including many marine groups such as heterotrophic bacteria (Arai *et al*., 1992), cyanobacteria and micro/macroalgae (Rastogi *et al*., 2010).

Much information on UV-B absorbing/sun-screening compounds from diverse organisms has been gathered over the past few decades. This has led to the development of a database on photoprotective compounds. Readers may find detailed information on MAAs in various organisms. MAAs may also act as antioxidants, preventing cellular damage resulting from UV-induced production of active oxygen species. Protection against UV radiation provided by scytonemin may have been an important ecophysiological factor in cyanobacterial evolutionary history. In addition to the important adaptive role of scytonemin to the cyanobacteria which produce it, this pigment must certainly still play an important role in microbial communities exposed to high solar radiation.

Cyanobacteria which are simultaneously exposed to visible and UV radiation have evolved certain mechanisms such as light dependent repair of UV-induced damage of DNA (Britt, 1995; Kim and Sancar, 1995; Pakker *et al*., 2000; Sinha *et al*., 2002; Häder and Sinha, 2005), accumulation of carotenoids and detoxifying enzymes or radical quenchers and antioxidants (Mittler and Tel-Or, 1991), and synthesis of photoprotective compounds such as mycosporine-like amino acids (MAAs) (Singh *et al*., 2008) and scytonemin (Karsten *et al*., 1998a, b; Sinha *et al*., 1998; Sinha *et al*., 1999; Richter *et al*., 2006; Sinha and Häder, 2008; Sinha *et al*., 2008;

Rastogi and Sinha, 2009) to counteract the damaging effects of UVR. Cyanobacteria were present on the early earth when there was no oxygen in the atmosphere (Fischer *et al*., 2008) and thus the presence of UV-screening compounds such as MAAs and scytonemin might have played an important role in protecting these organisms from the lethal UVR (Cockell and Knowland, 1999). Thus, screening of UV-B and UV-A radiation is an important mitigation strategy in brightly lit habitats where organisms encounter intense solar radiation.

The gene cluster responsible for scytonemin biosynthesis in the filamentous, heterocystous cyanobacterium *Nostoc punctiforme*, was identified in 2007 (Soule et al., 2007). This disclosure has been followed by a series of studies seeking to understand the regulation of biosynthetic gene expression (Sorrels et al., 2009; Soule et al., 2009) and to elucidate the molecular logic underlying pigment assembly (Balskus & Walsh, 2008, 2009).

Aims and Objective

1. To analyze the effect of prolonged UV-B radiation (12-72hr) on growth, photosynthetic pigments (chlorophyll, carotenoid, phycocyanin, phycoerythrin), protein, proline, and lipid peroxidation.

2. To analyze the presence of MAAs compound from various cyanobacterial strains.

3. To find out the level of conservation and variability regions in the other cyanobacterial strains along with these 2 strains (*Anabaena cylindrica* and *Synechocystis* PCC 6803) for protein evolved in MAA production pathway.

Material and Methods

Organism and Growth condition

The study focused on *Anabaena cylindrica* and *Synechocystis* PCC 6803 which differ in their morphological features and responses to UV stress condition. *Anabaena cylindrica* is a filamentous cyanobacteria known for its nitrogen fixation capability that's why grown in BG-11 negative medium where as *Synechocystis* PCC 6803 is an unicellular organism devoid of any heterocyst organ for nitrogen fixation that's why culture in BG-11 +ve medium supplemented with Nitrate(1.5g/l $NaNO_3$) (Stanier *et al.,* 1971). Both requires pH 7.8 and temperature 27+_2 C in culture room under fluorescent light of 72 μmol photon/m/sec Photosynthetically Active Radiation (PAR) with a photoperiod of 14:10 (light: dark) .The culture were shaken at regular interval by hand. The cultures were harvested during exponential growth (8 days old) and exposed for different periods to supplemental UV-B radiation. Makeup this media to 1 L.

Growth medium

To provide all desired macro and micronutrients for the proper growth of *Anabaena cylindrica* and *Synechocystis* PCC 6803, BG-11 inorganic liquid media was prepared as stock having the composition as follows:

Composition of BG-11 medium (pH – 7.8)

Macronutrients	***g/L***
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (disodium salt)	0.001
Na_2CO_3	0.020

Micronutrients	*mg/L*
H_3BO_3	2.860
$MnCl_2.2H_2O$	1.810
$Na_2MoO_4.2H_2O$	0.391
$ZnSO_4.7H_2O$	0.222
$CuSO_4.5H_2O$	0.079
$Ca(NO_3)_2.6H_2O$	0.049

To make an inorganic liquid media, take 10 ml of each macronutrient in 1 L flask and add 1 ml of micronutrient, maintain pH of the media at 7.8, makeup this media to 1 L. Medium supplemented with 1.5g/l $NaNO_3$.

This is the composition of BG-11 (+ve) liquid media.

Mode of UV-B Treatment:

Cyanobacterial cultures were exposed to UV-B radiation for 0 to 72 hrs in open petridishes under constant stirring at 25±2 °C. UV-B tubes having maximal emission at 300 nm with 40 nm half band width were used to provide UV-B radiation source.

Growth and photosynthetic pigment measurement:

Growth experiments were performed in liquid medium and sample were taken for measurement of growth by spectrophotometer at 750nm. The photosynthetic pigment (chlorophyll-a) was estimated by spectrophotometrically at 660nm following the method of McKinney (1941). The carotenoid content was estimated (spectrophotometrically) at 480 nm following the method of Davis (1976). Phycocyanin content was estimated at 460 nm according to Brody and Brody (1961).

Protein Estimation:

Protein concentration was measured by the method developed by Lowry *et al*. (1951).

Reagents Required

1. Analytical reagents:

A. 1 N NaOH

B. (a) 5% sodium carbonate mixed in 1 N NaOH solution.

(b) 0.5% copper sulfate solution mixed with 1% sodium potassium tartarate solution. Prepare analytical reagents by mixing 2 ml of (b) with 48 ml of (a)

2. Folin - Ciocalteau reagent solution (1N) Dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water).

Procedure

1. take 0.5 ml culture suspensions
2. add equivalent amount (0.5ml) of NaOH solution in culture
3. keep in dark room for 10 minutes for digesting
4. now adding reagent B 2.5 ml
5. After 10 minutes, centrifuge the solutions then transfer the solutions un another tube.
6. Add 0.5ml diluted Folin Ciocalteau solution (reagent solutions) to each tube and incubated for 15 min.
7. The intensity of the resulting blue color was determined by spectrophotometer after 15 minutes at 650nm.

Extraction of Cyanobacterial Protein:

In order to efficiently purify and analyze proteins, they must first be released from their host cell in a soluble form. Therefore, the first step in protein purification and analysis is cell lysis. Cyanobacteria possess a rigid cell wall composed of peptidoglycans that must be disrupted to access cellular proteins. This is accomplished by mechanical means, for example by subjecting cells to shear forces in a French press and sonication. Here we reported the freeze and thaw method for the cyanobacterial proteins extraction (Wanarskaet *et al*., 2007).

Isolation of Soluble Protein Extracts from Cyanobacterial Cell (By Freeze and Thaw method):

Requirements:

1. Tris HCl (20 mM)

2. Vortex mixer
3. -20^{o}C Deep freezer
4. Water bath
5. Centrifuge

Protocol:

1. Harvest bacterial cultures at 10,000 rpm for 10 minutes in a micro centrifuge.
2. Remove all the media by aspiration.
3. Resuspend the pallet in 1 ml of 20 Mm Tris HCl by gently pipetting up and down until the cell suspension is homogenous. Vortex the tube at room temperature.
4. Kept the tube in -20^{o}C Deep freezer for 20 minutes.
5. After 20 minutes place the tube in water bath at 100^{o}C for 5 minutes.
6. Repeat the freeze and thaw steps 4 and 5 for the 5 times that causes the disruption of the cell wall and release of cytoplasmic materials.
7. After completing above steps centrifuge the tubes at 10,000 rpm for 5 minutes to separate the soluble and insoluble fractions. The soluble protein in the supernatant.
8. Transfer the supernatant to clean tube and stored at -4^{o}C.
9. Add equivalent amount of sample buffer and boil for 5 minutes before running the gel.

Determination of Lipid Peroxidation (MDA) (Heath and Packer; 1968):

Reagent Preparation –

- 1% TCA in double distilled water
- 20% TCA in double distilled water
- 0.5% TBA in 20%TCA (Trichloro acetic acid)

Procedure

Harvested cyanobacterial cell (50mg) were homogenized in 1%Trichloro acetic acid (TCA) and centrifuged at 10000 rpm for 10 minutes at room temp. Equal volumes of supernatant and 0.5% Thiobarbituric acid (TBA) in 20% TCA solution (freshly prepared) were added into a new test tube and incubated at 95^{0} for 30 minutes in water bath .The supernatant was transferred into ice bath & then centrifuged at 10,000 rpm for 5 minutes .The absorbance of the supernatant was recorded at 532 nm & corrected on non-specific turbidity by subtracting the absorbance at 600nm. 0.5%TBA in 20% TCA was used as the blank. MDA contents were determined using the coefficient of 15mM/cm.

Proline estimation by Ninhydrin method (Bates *et al*; 1973):

Reagent Preparation –

- 3% sulphosalicyclic acid
- 3% Ninhydrin
- 3% Glacial acetic acid

Procedure:

Cells were suspended in 10 ml of 3% sulphosalicyclic acid & centrifuged at 5000g for 10 minutes to remove cell debris. In 2 ml of supernatant, 2 ml of ninhydrin was added followed by addition of 2 ml glacial acetic acid and incubated at boiling temperature for 1 hour. The mixture was extracted with toluene. Proline was quantified spectrophotometrically at 520 nm from organic phase.

Primary screening of UV protectants by UV- Vis. Spectroscopic method.

Spectroscopic analysis:

UV-B treated and control samples of 1.5 ml cell suspension were centrifuged for10 min at 9000 x g. The pellet was extracted over night in darkness in 750 µl 100 % methanol at 4^0C. Absorption spectra were recorded in the range between 250 nm and 750 nm using a single beam spectrophotometer (DU 70, Beckman, Palo Alto, USA).

MAAs extraction and measurement.

For determination of MAAs content, cyanobacterial biomass was washed twice with distilled water. Dried Cells were homogenized in 20% (vol/vol) aqueous methanol and kept at 45ºC in water bath for 2 h. After centrifugation supernatant was filtered through Whatman filters (no. 1). The absorbance of filtrate was measured at (260, 310, 320, 330, 332, 334 and 360 nm) and corrections were made according to the following expression (Garcia-Pichel and Castenholz, 1993).

HPLC analysis:

Cells were harvested by centrifugation at 5000 x g for 10 min at room temperature. Samples were extracted in 5 ml of 20 % (v/v) aqueous methanol (HPLC grade) by incubating at 45ºC for 2.5 h. After centrifugation (5000 x g; GP centrifuge, Beckman, Palo Alto, USA) the supernatant was lyophilized and redissolved in 750 µl 100 % methanol, vortexed and centrifuged at 10 000 x g for 10 min .Thereafter a 700 µl aliquot of the supernatant was evaporated to dryness at 45ºC and the residue redissolved in 250 µl of double distilled water. The samples were filtered through 0.2 µm pore-sized micro centrifuge filters (Mikro-Spin Zentrifugenfilter, Roth, Karlsruhe,

Germany). Further analysis was performed by HPLC using a LiCrospher RP 18 column and guard (5 &µm packing; 250 4 mm I.D.) and a mobile phase of 0.02 % acetic acid at a flow rate of 1.0 ml min-1. Detection was done using a photodiode array detector (Waters 990, Waters, USA) in the wavelength range between 280 and 400 nm. Identification of the MAAs was done by comparing the absorption spectra and retention times with several standards kindly provided by Prof. R.P.Sinha, Centre of Advance Study in Botany, Banaras Hindu University, Varanasi. U.P. India.

RESULTS AND DISCUSSION:

Two cyanobacterial species *Synechocystis* PCC 6803 and *Anabaena cylindrica* were treated for 0-72 hours of UV-B radiation. The cells were observed for their growth, photosynthetic pigment contents, proline content, lipid peroxidation, total protein and production of UV-B protecting compounds after UV radiation.

Growth

The growth response of the cyanobacterium *Synechocystis* PCC 6803 and *Anabaena cylindrica* to UV-B was inhibitory and the effect varied with different UV-B dose. The decreasing trend in growth in cyanobacterium *Synechocystis* PCC 6803 was 30% of control while *Anabaena cylindrica* decreases 42% of control with UV-B exposure time up to 72 hrs, caused several damage to cellular system (membrane integrity, light harvesting system and activity of photo system. The decreasing trend in survival might be due to damage of the cellular constitute or inactivation of various vital processes, eventually causing death of the cells. This result reveals that UV-B induced inhibition on growth of *Syenechocystis* PCC 6803 and *Anabaena cylindrica* might be due to irreparable damage to DNA, Protein and photosynthetic apparatus.

Photosynthetic Pigment content:

The decreasing trend in Photosynthetic pigments, Chl a, carotenoids and phycocyanin was shown with UV-B exposure time. The experimental organism *Synechocystis* PCC 6803 showed maximum % decreased in chlorophyll (45%) at 72 hrs UV exposure while *Anabaena cylindrica* showed up to 40% decreased in chlorophyll at 72 hrs UV exposure. Carotenoids showed varied response of UV-B exposure for 0 to 72 hrs radiation in starting up to 48 hrs its concentration

increases up to 30% in comparison to control of *Synechocystis* PCC 6803 and after that drastically decreases where as in *Anabaena cylindrica* up to 36 hrs its concentration increases up to 20% in comparison to control and after that drastically decreases. Phycocyanin was severally affected in both species in comparison to chlorophyll and carotenoid. Phycocyanin content of *Synechocystis* PCC6803 showed decrease up to 32% and *Anabaena cylindrica* showed decrease up to 25% .The damaging effect on photosynthetic pigments was due to bleaching caused by UV-B radiation reported by (Nultsch and Agel, 1986). A decrease in photosynthetic pigment content particularly Chl a, Carotinoids, Phycocynin, was reported in cyanobacteria following enhanced UV-B irradiation. Carotinoids were less affected than Chl a, phycocynin, indicating the role of Carotenoids in cyanobacteria as a general defence against photo oxidation under stress.

Protein content

Protein was estimated of control and UV-B stress culture of both species. UV-B stress was given for 12-72 hrs. Decrease in protein content is a common phenomenon in UV stress. The reason for this is that the amino acid of proteins will absorb UV and will be degraded. But in this study, we observed an increase in protein content in UV-B treatments. This increase is probably due to the synthesis of defense proteins and enzymes. The maximum percent of Protein increase (34-63%) was shown in *Synechocystis* PCC 6803 up to 72 hr of UV radiation.

SDS–PAGE protein profile

The SDS protein profile of *Synechocystis* PCC 6803 was done of different hours of UV-B irradiation was analyzed. Many proteins were repressed in response to UV-B irradiation. Protein profile of the control showed that 78, 68, 42, 33, 21 and 16 kDa were the major proteins. Of these 74, 54, and 43 kDa proteins were very sensitive to UV-B irradiation up to 48 h. However, 30 and 21 kDa proteins were induced after exposure to 36-72 hrs of UV-B which possibly were the key proteins protecting *Synechocystis* PCC 6803 and UV-B for the extended duration up to 72 hrs of UV radiation.

Total protein profile of cyanobacteria showed significant alterations following exposure of cultures to UV-B radiation (Sinha *et al.,* 2005). Several protein bands disappeared and a few new protein bands appeared in the gel. Total proteome analysis of *Synechocystis* sp. PCC 6803 by 2-dimensional (2-D) gel electrophoresis showed different expression level of proteins in the cytoplasm under short and long-term UV-B stress (Gao *et al.,* 2009). The above study focused on

amino acid biosynthesis, photosynthesis and respiration, energy metabolism, protein biosynthesis, cell defense, and other functional groups (Gao *et al.,* 2009).

Lipid peroxidation

The experimental result indicates high degree of lipid peroxidation in *Synechocystis* PCC 6803 it enhances 6-22% in comparison to control and 5-15% in *Anabaena cylindrica*. However, an increase in MDA formation was induced when UV-A was included in the irradiation conditions, while much more MDA was observed with UV-B included. The induction of lipid peroxidation was related to the irradiation time and the irradiation intensity. Lipid peroxidation increased with irradiation time and intensity within the lower range of UV-B irradiance.

Proline content:

Proline functions as an important osmoregulator, protein structure stabilizer, metal chelator, redox potential stabilizer, lipid peroxidation inhibitor, stabilizer of key cellular detoxification mechanisms, and scavenger of Reactive Oxygen Species, thereby reducing the damage of oxidative stress. Result shows that after UV-B radiation proline concentration greater influence in *Synechocystis* PCC 6803 just double of control where as in *Anabaena cylindrica* it enhances up to 40% of control sample. Intracellular proline content has been reported to be an important index for stress tolerance capacity due to its function as a hydroxyl radical and singlet oxygen scavenger (Fatma *et al*., 2007).

Spectroscopic analysis:

Results shows some major peaks in the absorption spectrum between 250 -700 nm. The peak at 680 nm is due to the absorption of chlorophyll a and peak at 480 nm is of carotenoids. An important interesting result is induction of a peak at **310 nm** is of UV protecting compound named **mycosporine glycine** in *Synechocystis* PCC 6803 which increases with increasing UV radiation at certain periods, where as in *Anabaena cylindrica* a peak at **334 nm** is of UV protecting compound named **shinorine.** This results shows that cyanobacteria produces protecting compound due to UV-B stress. Thus, from this findings we can say that MAAs provide protection from UV radiation in their producer and also in primary and secondary metabolites.

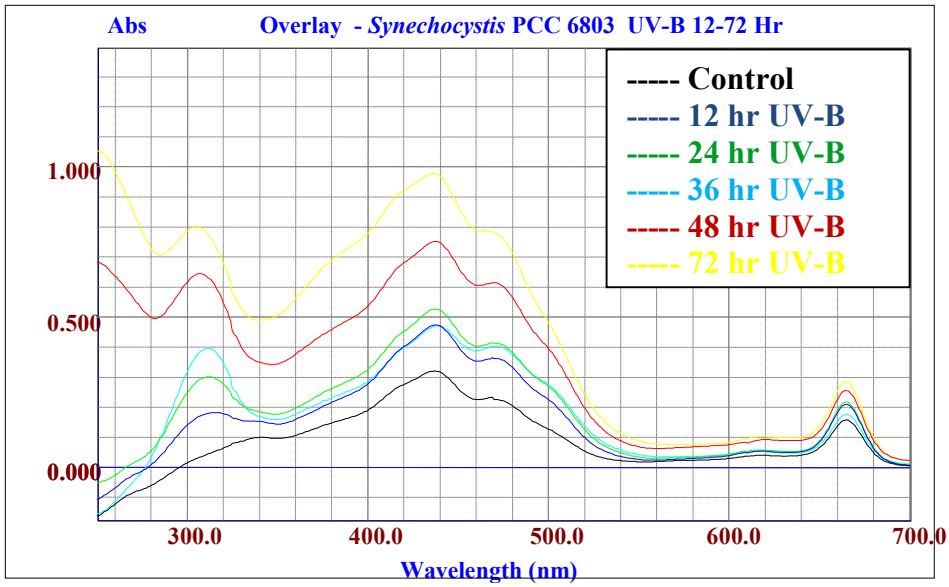

Fig 1. UV-visible scanning of UV-B treaeated cyanobacteria *Synechocystis* PCC 6803

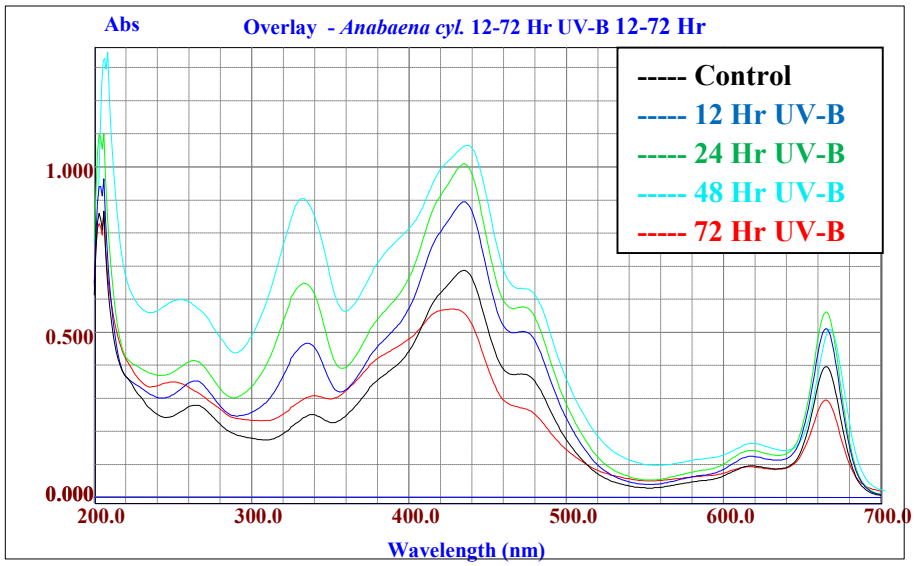

Fig 2. UV-visible Scanning of UV-B treaeated cyanobacteria *Anabaena cylindrica*

HPLC analysis of MAAs:

HPLC analysis revealed that the samples contain a complex mixture of MAAs. Three of the compounds present could be tentatively identified by comparison of retention times and absorption spectra as shinorine (fraction 1, RT 2.1 min), porphyra-334 (fraction 2, RT 2.6 min), and palythine (fraction 4, RT 4.1 min). The peak at 3.1 min (fraction 3) had an absorption maximum at the same wavelength as mycosporine-glycine (310 nm) but a different retention time. The shape of the peak at 9.5 min indicates that it results from an incompletely separated mixture of at least three compounds. Shinorine (fraction 1) was not completely separated from compounds with strong absorption below 300 nm tailing into the longer wavelength range.

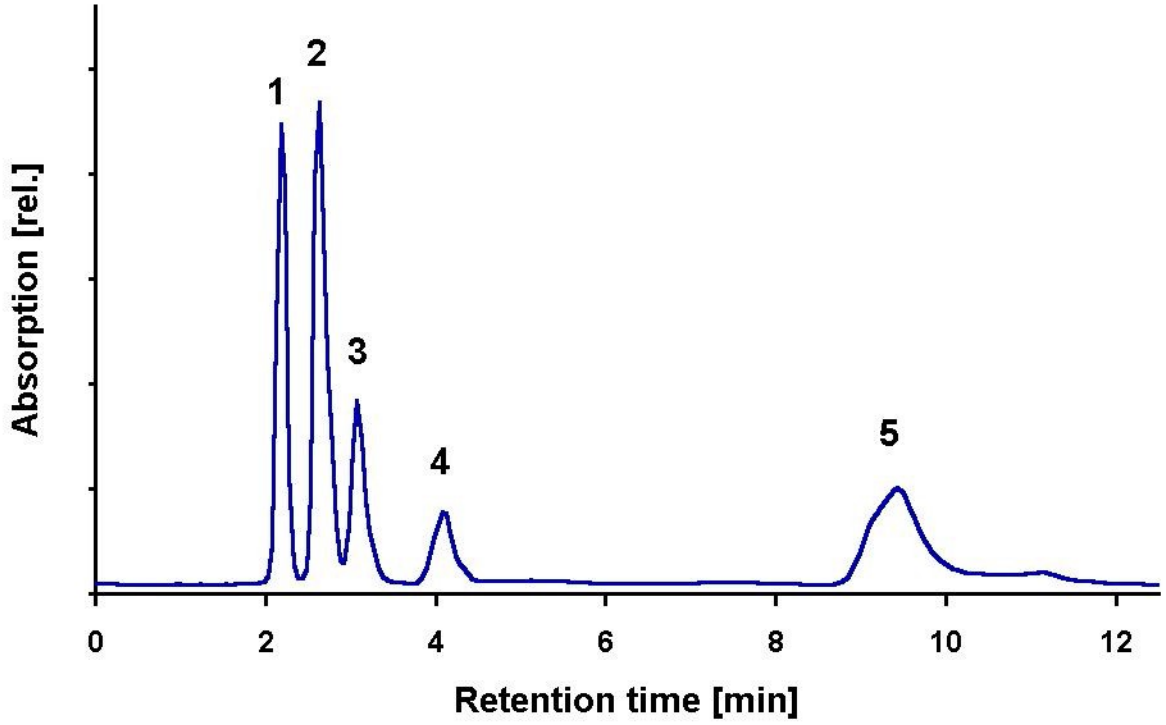

Fig 3. Chromatographic separation of MAAs from *Anabaena cylindrica* exposed to UV-B radiation under a WG 305 cut-off filter for 72 h.

Conclusions

In conclusion, this study has demonstrated that UV-B stress caused significant reduction in growth, contents of photosynthetic pigments like Chlorophyll 'a' and Phycocyanin of *Anabaena cylindrica* and *Synechocystis* PCC 6803. In contrast to this lipid peroxidation, Proline, Total protein amount were enhanced. An important interesting result is induction of a peak at **334 nm** is of UV protecting compound named **shinorine** in *Anabaena cylindrica* and a peak at 310nm of **mycosporine glycine** in *Synechocystis* PCC 6803 which increases with increasing UV radiation at certain periods. This results shows that cyanobacteria produces protecting compound due to UV-B stress. Thus, present findings suggest that UV-B stress; alter the key physiological and biochemical processes of *Anabaena cylindrica* and *Synechocystis* PCC 6803.

Future Prospects:

- Elucidations of pathway of UV screening Compounds (such as MAA production by the shikimic acid pathway).
- Use of mutants to study the physiological value of UV- screening compounds in a promising area of development.
- Commercial scale production and tie up with cosmetic industries.

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