

Mycosporine-like Amino Acids and Antioxidative Enzymes Activity in *Scytonema* sp. under Cumulative Stress of UV Radiation and Salinity

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Abstract: We have studied the cumulative effects of ultraviolet radiation (UVR), photosynthetically active radiation (PAR), and varying concentrations of NaCl (50, 150 and 200 mM) on the induction of mycosporine-like amino acids (MAAs) and antioxidative enzymes namely, ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) in a cyanobacterium *Scytonema* sp. isolated from agricultural field of Maunath Bhanjan, India. Spectroscopic analysis and high performance liquid chromatography (HPLC) revealed the presence of a single MAA ($\lambda_{\max}=334$ nm) at a retention time of 3.22 min. Based on the UV-VIS absorption spectrum, HPLC and mass spectrometry analyses, the MAA was identified as shinorine ($\lambda_{\max}334$, m/z: 333). Maximum induction of shinorine was observed in PAR+UV-A+UV-B treated samples having 200 mM of NaCl after 60 h of treatment. Production of antioxidative enzymes increased after PAR, PAR+UV-A and PAR+UV-A+UV-B treatments and highest level of production occurred in the cultures having 200 mM concentration of NaCl. These findings suggest that *Scytonema* sp. could serve as a suitable strain for bioremediation of saline environments and production of value added compound such as MAAs.

Index Terms: Antioxidative enzymes, cyanobacteria, mycosporine-like amino acids, photosynthetically active radiation, reactive oxygen species, salinity, ultraviolet radiation

Abbreviations: APX: Ascorbate peroxidase; CAT: Catalase; ESI-MS: Electrospray ionization-mass spectrometry; HPLC: High performance liquid chromatography; MAAs: Mycosporine-like amino acids; PAR: Photosynthetically active radiation; RT: Retention time; ROS: Reactive oxygen species; SOD: Superoxide dismutase; UVR: Ultraviolet radiation

I. INTRODUCTION

Cyanobacteria are the most ancient photoautotrophic prokaryotes, dominant in both aquatic and terrestrial ecosystems. During their long evolution, cyanobacteria have developed efficient mechanisms to adapt to a broad range of environmental stresses like high temperature, UVR and salinity (De Marsac and Houmard, 1993; Barnawal et al., 2014). Productivity of photosynthetic organisms is adversely affected by the increased salinity of soils and water (Epstein and Bloom, 2005). A number of factors promote salinization such as, anthropogenic use of agricultural fertilizers, road salts (Kaushal et al., 2005) and environmental factors such as global temperature shifts and drought (Cañedo-Argüelles et al., 2013). Current research interests are more inclined towards using brackish or non-arable environments for growing strains suitable for the potential production of biofuels and other value added products (Rajneesh et al., 2017). Hence, it will be advantageous to use strains that can grow in a wide range of habitats, including those that are tolerant to high concentration of salts (Singh and Montgomery, 2013). Cyanobacteria have attracted considerable attention in this regard, since they are inhabitants of biotopes which are characterized by changing salinities and can serve as model organisms for salt acclimation. Thus, cyanobacteria can help in understanding of more complex physiological processes of higher plants, which are grown in saline environments (Bohnert and Jensen, 1996). Salinity adversely affects many physio-chemical processes such as photosynthesis, lipid metabolism, nitrogen assimilation and ion homeostasis (Mittler, 2002; Pade

and Hagemann, 2015). Production of toxic ROS has been reported by many researchers during stressful environmental conditions (Rajneesh et al., 2019). Important macromolecules such as proteins, nucleic acids and photosynthetic pigments are also affected by increased level of ROS during salinity stress (Singh and Montgomery, 2013b). In response to salinity stress, cells increase the synthesis and accumulation of compatible organic osmolytes and genes encoding antioxidative enzymes get over expressed, which enable the survival of the cells during stressed conditions (Mittler, 2002). Antioxidant enzyme system include enzymes such as SOD, CAT and APX etc., which scavenge the toxic effects of ROS generated during oxidative stress (Richa and Sinha, 2015a; Rajneesh et al., 2019). In the past few decades, due to increase in anthropogenically released atmospheric pollutants such as chlorofluorocarbons, there is an increase in UVR (100-400 nm) on the Earth's surface (Williamson et al., 2014), which have pronounced negative impacts on both aquatic as well as terrestrial life forms (Häder et al., 2015). Harvesting of solar energy to perform photosynthesis and nitrogen fixation exposes these cyanobacteria to lethal doses of UV-B and UV-A radiations in their natural brightly lit habitats (Gao et al., 2008; Pathak et al., 2015). UV-B is thought to be the most active constituent of solar radiation and induces inflammation, DNA damage, oxidative stress, free radical production, sunburns and skin cancer in various living organisms (Halliday and Lyons, 2008; Häder et al., 2015; Rastogi et al., 2015). In particular, the resistance to UVR is generally associated with the biosynthesis of certain photoprotective compounds such as MAAs and scytonemin that may act as natural sunscreens against harmful UV-A and UV-B radiation (Garcia-Pichel et al., 1993; Singh et al., 2017; Pathak et al., 2019). MAAs are well known UV-absorbing compounds that provide photoprotection against UVR (Sinha and Häder, 2008; Singh et al., 2010; Richa and Sinha, 2015b). In the present study we investigated the cumulative effects of UVR and salinity (varying concentrations of NaCl) on the biosynthesis of MAAs and production of antioxidant enzymes such as APX, SOD and CAT on *Scytonema* sp. for their possible application in bioremediation of saline agricultural fields.

II. MATERIALS AND METHODS

Test organism and growth conditions: The cyanobacterium *Scytonema* sp. was isolated from agricultural field of Maunath Bhanjan, U.P., India. The organism (Fig. 1) was identified with the help of standard taxonomic keys and monographs using morphological characteristics (Desikachary, 1959). The cyanobacterial culture was grown at 20 ± 2 °C in an autoclaved BGA-11 medium without any nitrogen source under continuous fluorescent white light at an intensity of 12 ± 2 Wm⁻² for a 14/10 h light/dark cycle. To avoid clumping and self-shading cyanobacterial culture hand shaken five times daily. Growth was

analyzed by measuring optical density at 750 nm. Exponentially growing cultures were used for the experiment.

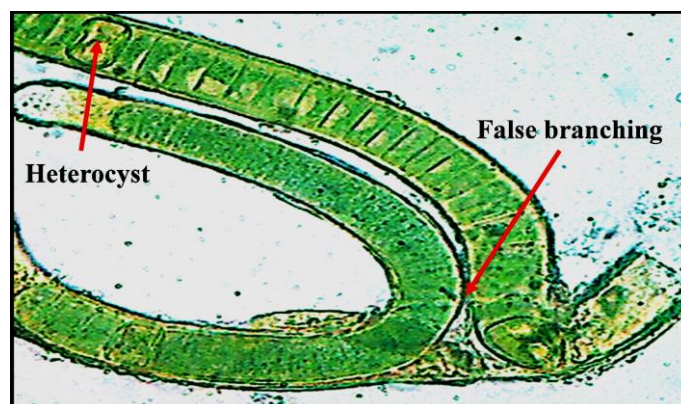


Figure 1. Morphological structure of filamentous cyanobacterium *Scytonema* sp., showing false branching and a heterocyst.

A. Experimental setup

All experiments were performed in triplicates in two sets. In the first set exponentially growing cells were exposed to PAR (P) and UVR inside induction chamber fitted with cool white fluorescent and UV/+98 lamps. Samples were transferred into sterile Petri dishes (75 mm) covered with the cut-off filter foils of 395, 320 and 295 nm (Ultraplan, UV Opak Digepra, Munich, Germany) to obtain the desired radiation regimes of P, PAR+UV-A (PA) and PAR+UV-A+UV-B (PAB) radiation. In the second set of experiment, samples were exposed to PAB along with varying concentration of NaCl (50, 150 and 200 mM). Equal amount of cells were removed from each Petri dish after 12, 24, 48 and 60 h of continuous exposure and analyzed for induction of MAAs and antioxidative enzymes. Untreated samples were taken as control. MAAs were analyzed to identify the most potent wavebands for their induction, and also the range of NaCl at which highest level of MAAs synthesis occurs. The intensities of P, UV-A and UV-B were 14 ± 2 , 6.5 and 0.56 Wm⁻² respectively. Temperature was maintained at 25 ± 2 °C during the entire period of exposure.

B. Extraction of MAAs

Exponentially growing cells were harvested by centrifugation and MAAs were extracted in 2 mL of 100% methanol (HPLC-grade) by overnight incubation at 4°C. After extraction, the aliquots were centrifuged (10,000g for 5 min) and supernatants i.e. methanolic extracts were evaporated to dryness at room temperature to avoid heat disintegration of MAAs and re-dissolved in 500 µL of double-distilled water and few drops of chloroform were added to the collected supernatant and vortexed. After centrifugation (10,000 rpm, 10 min), the uppermost aqueous phase (400 µL) was transferred carefully into a new Eppendorf tube. Thereafter, samples were filtered through

0.02 μm pore-sized microcentrifuge filters and subsequently analyzed with the HPLC system.

C. HPLC and ESI-MS

The filtered samples of MAAs (dissolved in water) were analyzed with a HPLC system (Waters 2998, Photodiode Array, pump L-7100, USA) equipped with a Licospher RP 18 column and guard 5 μm packing; 250 mm \times 4 mm inside diameter). The samples (10 μL) were injected into the HPLC column through a Waters 717 plus autosampler. Acetic acid (0.02%) in double-distilled water was used as a mobile phase, which was isocratically run at a flow rate of 1 mL \cdot min $^{-1}$. Absorption spectra of separated peaks were recorded for each second between 250 and 400 nm directly on the HPLC system. Purified MAAs collected by HPLC were used to produce protonated molecules by ESI-MS were recorded on an Amazon SL mass spectrometer (Bruker Daltonics Inc., Bremen, Germany). Cone voltage of 30V was found to induce the formation of (M+H) $^{1+}$ with a mass range of 100-1,000 m/z . Other MS settings were the following: capillary voltage (5,500 V) and temperature (300 $^{\circ}\text{C}$). The m/z scale of the MS was calibrated using the external calibration standard electrospray 'tuning mix' from Agilent Technologies (Santa Rosa, USA). Data were analyzed using the software Data Analysis 4.0 (Bruker Daltonics Inc., Bremen, Germany).

D. Determination of antioxidant enzyme activity

For determination of antioxidant enzymes assay, the samples were harvested by centrifugation at 10,000 rpm for 10 min at room temperature. Cell extracts were prepared by sonicating cells in 5 mL of extraction buffer under ice-cold conditions. The extraction buffer consisted of 50 mM potassium phosphate buffer (KPP) (pH 7.5), 1 mM ethylenediaminetetracetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVP), 1mM phenylmethylsulfonyl fluoride (PMSF) dissolved in ethanol, with the addition of 1 mM ascorbate in the APX (EC 1.11.1.11) assay. The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatants were collected and used for assays of CAT (EC 1.11.1.6), APX and SOD (EC 1.15.1.1). CAT activity was determined according to the method of Aebi (1984). Reaction mixtures contained 300 μM phosphate buffer (pH 7.2), 100 μM H $_2$ O $_2$ and 500 μL of enzyme extract. Activity was determined spectrophotometrically by recording O $_2$ released from enzymatic dissociation of H $_2$ O $_2$ in darkness for 1 min. O $_2$ released by enzymatic reaction was estimated by measuring the decrease in H $_2$ O $_2$ absorption at 240 nm using the extinction coefficient: $\Delta\epsilon=39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ for 1 min. SOD activity was measured by monitoring the inhibition in reduction of nitro blue tetrazolium (NBT) as described previously (Beyer Jr and Fridovich, 1987)

Reaction mixtures consisted of methionine (200 mM), nitrobluetetrazolium chloride (2.25 mM), EDTA (3 mM), phosphate buffer (0.5 M, pH 7.5), sodium carbonate (1.5 M) and then enzyme extract was added to make the volume 2 mL.

Reaction was initiated by adding riboflavin (100 μL). Reaction mixture without enzyme extract was considered to be 100% (served as blank), and enzyme activity was calculated by normalizing to the control and determining percent inhibition. Approximately, 50% inhibition was considered equivalent to 1 unit of SOD activity in comparison to tubes lacking enzymes. APX activity was determined by the method of Nakano and Asada (1981), by measuring ascorbate oxidation to monodehydroascorbate at 290 nm, $\Delta\epsilon=2.8 \text{ mM}^{-1} \text{ cm}^{-1}$. The activity was determined by measuring the decrease in absorbance at 290 nm for 1 min. Reaction mixtures consisted of 0.1 mM H $_2$ O $_2$, 0.1 mM EDTA, 0.5 mM ascorbate and 100 μL of enzyme extract.

E. Statistical Analysis

The experiments were repeated thrice for accuracy of the results. All results are presented as mean values of three replicates and statistical analyses were done by one-way analysis of variance. Once a significant difference was detected post-hoc multiple comparisons were made by using the Tukey test (SPSS 16.0). The level of significance was set at 0.05 for all tests.

III. RESULTS

A. Analysis of MAAs (Effects of PAR, UVR and NaCl on synthesis of MAAs)

The absorption spectra of methanolic extracts of all samples treated with P, PA and PAB along with varying concentration of NaCl showed the peaks for MAAs, (310-334 nm), Chl *a* (434 and 665 nm), carotenoids (473 nm) and biliproteins (616 nm) (Fig. 2). HPLC chromatograms (Fig. 3) of partially purified aqueous solutions of all samples revealed the presence of single MAA i.e. shinorine (334 nm), eluted at RT of 3.22 min respectively, in cyanobacterium *Scytonema* sp. Purified MAA isolated *via* HPLC were used to generate protonated molecules by ESI-MS analyses which showed the m/z value at 333 (Fig. 4), which further confirm the presence of shinorine (334 nm, m/z 333).

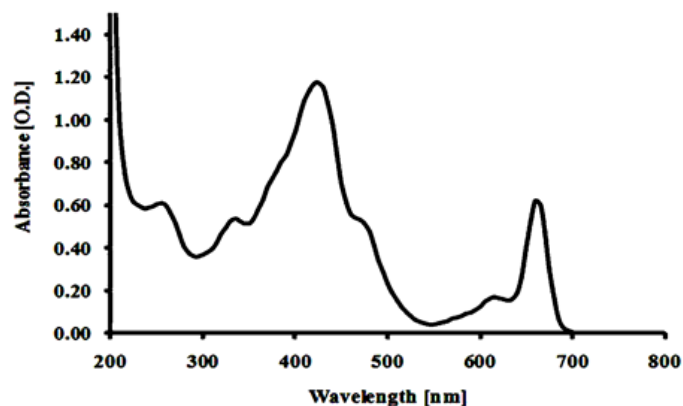


Figure 2. Absorption spectrum of the methanolic extract of *Scytonema* sp. showing the peaks of Chl *a* (434 and 665 nm),

biliproteins (616 nm), carotenoids (471 nm), and MAAs (310-334 nm) after 48 h of exposure to PAR+UV-A+UV-B with 200 mM NaCl.

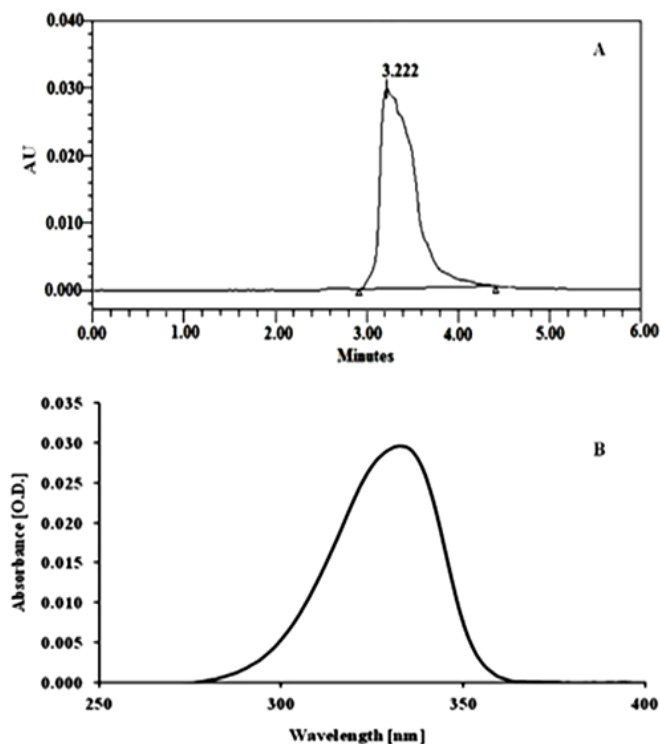


Figure 3. HPLC chromatogram of partially purified MAAs i.e. shinorine from *Scytonema* sp., showing the typical peak at a RT of 3.22 min (A) with an absorption maximum at 334 (B)

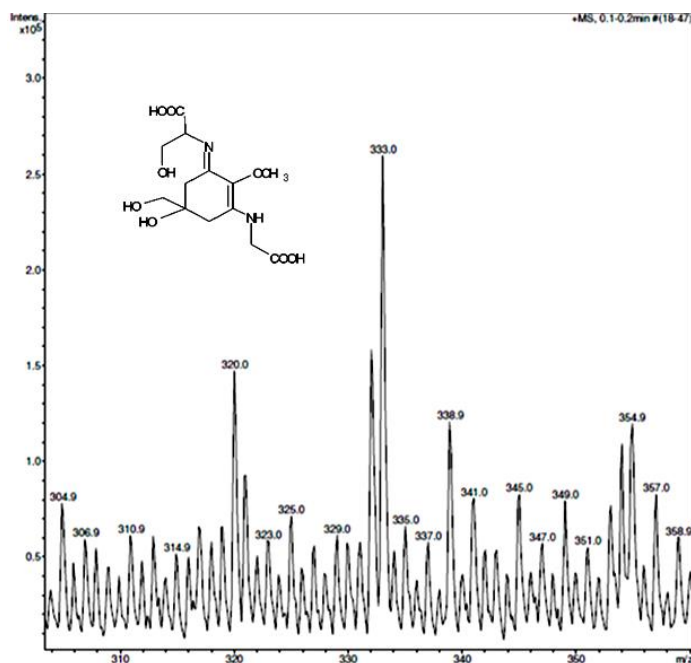


Figure 4. Mass spectra of the HPLC-purified MAA shinorine showing a prominent peak at m/z 333.

B. Induction of MAAs

To find the most appropriate wavelength for the induction of MAA shinorine, the organism was irradiated under three different cut-off filter foils as described above in materials and methods section. Biosynthesis of shinorine was not significant ($P>0.05$) in the cells irradiated under 395 (P) and 320 nm (PA) cut-off filters, suggesting that P and PA alone is less effective in inducing the biosynthesis of shinorine in the cyanobacterium *Scytonema* sp. There was not much difference in shinorine induction under P and PA radiation after 24 h of exposure, however, a significant ($P<0.05$) induction of shinorine was observed after 48 h of P and PA exposure with respect to the untreated samples. MAA content was high in the samples exposed under PA as compared to P irradiated samples. The increase in shinorine concentration (in term of peak area) under PAB was significantly ($P<0.05$) higher than P and PA exposure, indicating the potential role of UV-B radiation in shinorine induction in the cyanobacterium *Scytonema* sp., however, shinorine biosynthesis was significantly ($P<0.05$) higher after 48 h of exposure and being highest after 60 h of exposure under PAB exposure (Fig. 5A). The enhanced level of shinorine under PAB radiation might be due to some specific photoreceptor molecules in the cyanobacterial cells which are responsible for light dependent-induction of MAA biosynthesis (Portwich and Garcia-Pichel, 2000; Rastogi and Incharoensakdi, 2014). Once it became evident that PAB radiation have a major effect on the biosynthesis of shinorine, we further investigated effects of salt (NaCl) on MAA biosynthesis in samples exposed under 295 nm cut-off filter by adding varying (50, 150 and 200 mM) concentrations of NaCl (Fig. 5B). The samples having NaCl in the culture medium, under PAB, showed significant ($p>0.05$) increased in biosynthesis of shinorine, in comparison to the samples exposed to the PAB radiation only. The effect of NaCl was found to be more significant ($p<0.05$) after 12 h of treatment. NaCl (150 and 200 mM concentrations) along with PAB treatment results in increase in shinorine concentration upto 48 h of treatment, followed by significant decline ($p>0.05$) at 60 h of treatment, except for the sample having 50 mM concentration of NaCl. Sample having 50 mM NaCl showed increased concentration of shinorine till 60 h of exposure. Culture having 200 mM NaCl showed significant ($p>0.05$) induction i.e. 3.3, 3, 2 and 1 folds higher MAAs biosynthesis after 12, 24, 48 and 60 h of exposure respectively than culture having no NaCl in their culture medium. Samples having 150 mM NaCl showed significant ($p>0.05$) i.e. 3, 2, 1.5 and 1 fold better induction after 12, 24, 48 and 60 h of irradiation respectively than culture without NaCl. 50 mM of NaCl showed least effect on shinorine induction i.e. 2, 1.5, 1 and 1.2 folds MAA induction after 12, 24, 48 and 60 h of exposure than the samples without NaCl (Fig. 6A and B). It is evident that NaCl have concentration dependent effect on shinorine biosynthesis, moderate concentration of NaCl enhanced shinorine synthesis in

cyanobacterial culture treated for longer time, but after increasing salt concentration shinorine biosynthesis declined significantly ($p>0.05$) after certain hours of treatment.

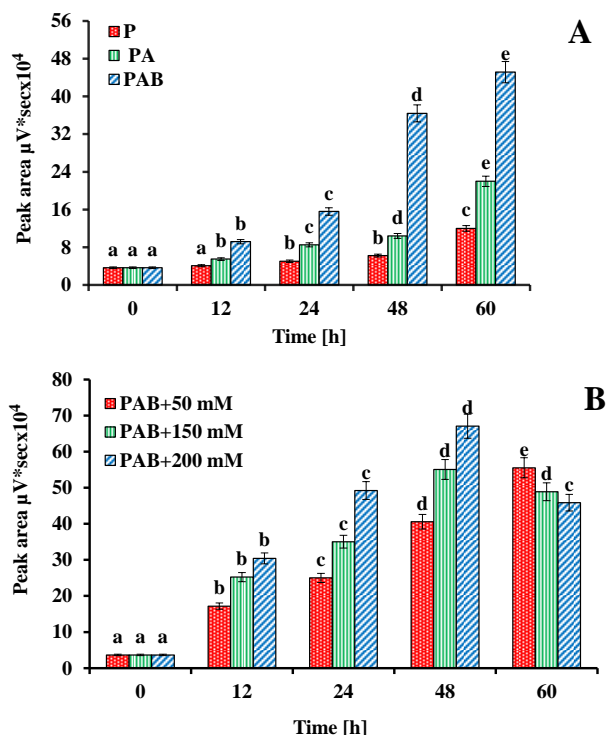


Figure 5. Induction of MAA (shinorine) during 12, 24, 48 and 60 h of exposure under P, PA and PAB exposure (A) and after using different concentrations of NaCl (50, 100 and 200 mM) under PAB exposure (B). The error bar denotes standard deviations of means ($n=3$) and significance differences of means designated as different alphabet letters ($p<0.05$) obtained by Tukey's multiple comparison test.

C. Antioxidant activity

After finding the effective cut-off filter i.e. 295 nm and concentration of NaCl (200 mM) for shinorine biosynthesis, we proceeded for the investigation of effect of P, PA and PAB radiation and 200 mM NaCl on the activity of antioxidant enzymes. In the present study we have chosen 200 mM concentration of NaCl because at this concentration maximum production of shinorine was observed. Significant ($p>0.05$) level of alteration in antioxidant enzymes activities was observed. Least APX activity was observed in P treated samples as compared to PA and PAB. In PAR, significant ($P<0.05$) increase in APX activity was reported after 60 h of exposure. Maximum APX activity was observed in PAB treated samples followed by PA and it increased upto 6 folds after 60 h of PAB treatment while 4.5 folds increase was observed in PA. After addition of NaCl in the culture exposed with PAB, resulted in significant ($p>0.05$) increase in APX activity upto 48 h (2 folds) followed by a significant decline ($p>0.05$). APX activity

increased upto 3 folds in P+NaCl, while in PA+NaCl increase was upto 2 folds after 60 h of treatment. The APX activity was significantly ($p>0.05$) higher in UVR+NaCl treated samples in comparison to UVR alone (Fig. 7).

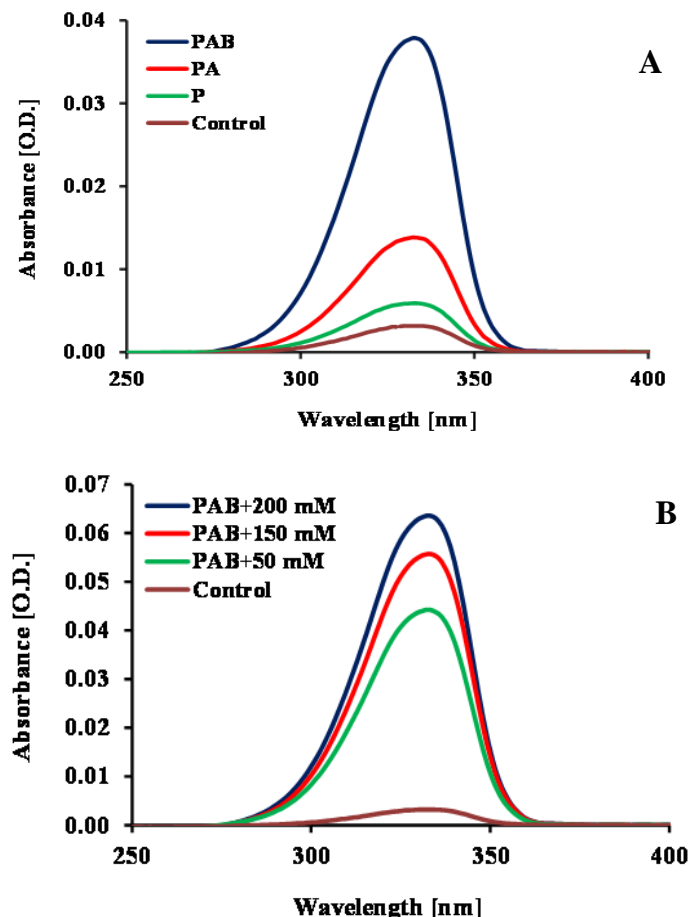


Figure 6. The absorption spectrum of partially purified MAA from *Scytonema* sp. under P, PA and PAB radiations after 48 h of exposure (A) with changing concentration of NaCl i.e., 200 mM, 150 mM and 50 mM under PAB after 48 h of exposure (B).

CAT activity increased significantly ($p>0.05$) as duration of treatment with UVR increased. After 60 h, 3 and 2 folds increase was observed under PAB and PA respectively. While in P, CAT activity almost remained constant till 48 h but thereafter 1.5 folds increase was recorded at 60 h in comparison to untreated control. After addition of NaCl, UVR exposure resulted in increased CAT activity in comparison to UVR alone. Exposure till 60 h resulted in significant ($p>0.05$) i.e. 2 and 1.5 folds increase in CAT activity in PAB+NaCl and PA+NaCl treated samples. While in P+NaCl also 1.5 folds increase was observed after 60 h of NaCl addition against the samples having no NaCl in culture medium (Fig. 8).

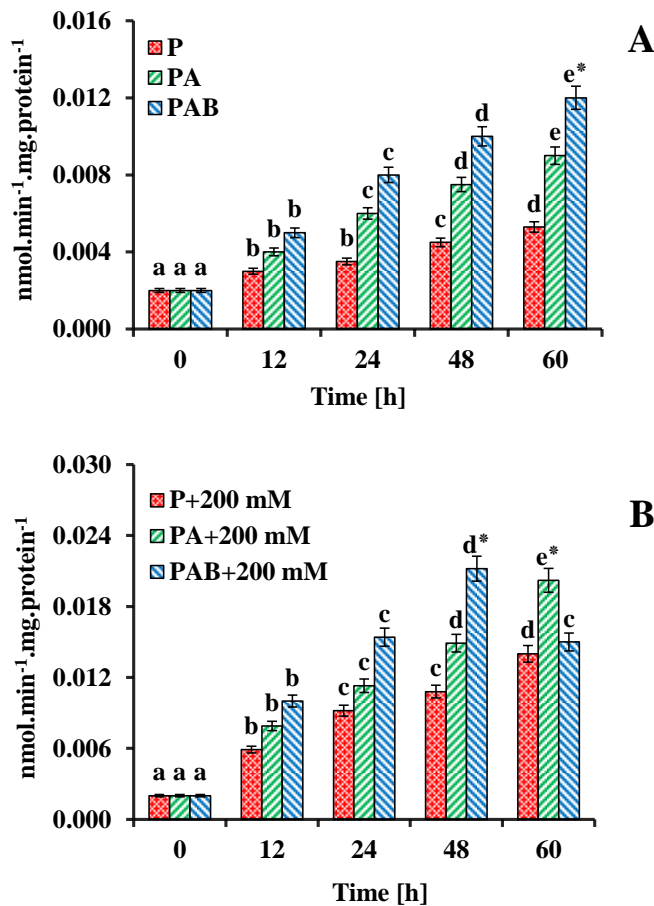


Figure 7. Activity of APX in cultures exposed to P, PA and PAB radiations without NaCl (A) and after the addition of 200 mM NaCl (B) for varying time periods. The error bar denotes standard deviations of means (n=3) and significance differences of means designated as different alphabet letters ($p < 0.05$) obtained by Tukey's multiple comparison test.

Similarly, SOD activity increased significantly ($p > 0.05$) in time dependent manner upon exposure of P and UVR than control samples. In PAB, PA and P treated samples 5, 3.5 and 3 folds increase was observed respectively after 60 h of treatment in comparison to control. While after addition of NaCl with UVR resulted in increase in SOD activity which was more pronounced than UVR alone and activity increased upto 48 h (2 folds) in PAB+NaCl thereafter, it declined significantly ($p > 0.05$). However, in PA+NaCl treatment, SOD activity increased upto 60 h (2 folds). While in P+NaCl treatment, SOD activity also increased and after 60 h it was 2 folds in comparison to control (Fig. 9).

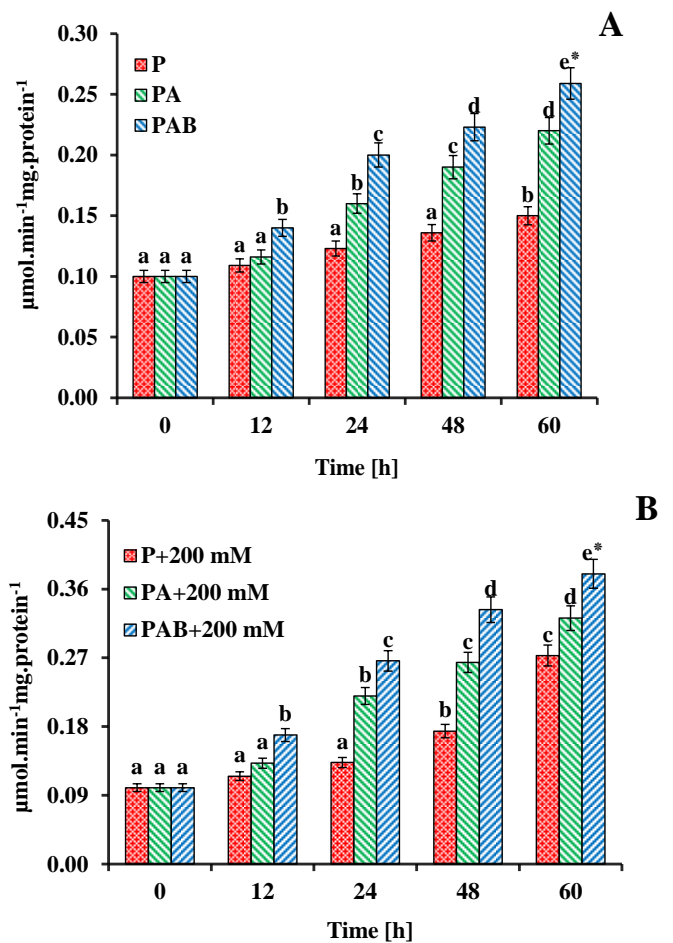


Figure 8. Activity of CAT in cultures exposed to P, PA and PAB radiation without NaCl (A) and after the addition of 200 mM NaCl (B) for varying time periods. The error bar denotes standard deviations of means (n=3) and significance differences of means designated as different alphabet letters ($p < 0.05$) obtained by Tukey's multiple comparison test

Among P, PA and PAB, highest activity was found under PAB treatment and NaCl in combination with PAB resulted in highest activity of all studied antioxidative enzymes. The studied *Scytonema* sp. showed potent antioxidant activity under UVR which increased to many folds after adding NaCl to the culture medium, this enabled the cyanobacterium to withstand harsh stress conditions of UVR and salinity. In our study we found that activity of APX under UVR stress was less in comparison to CAT and SOD in the *Scytonema* sp.

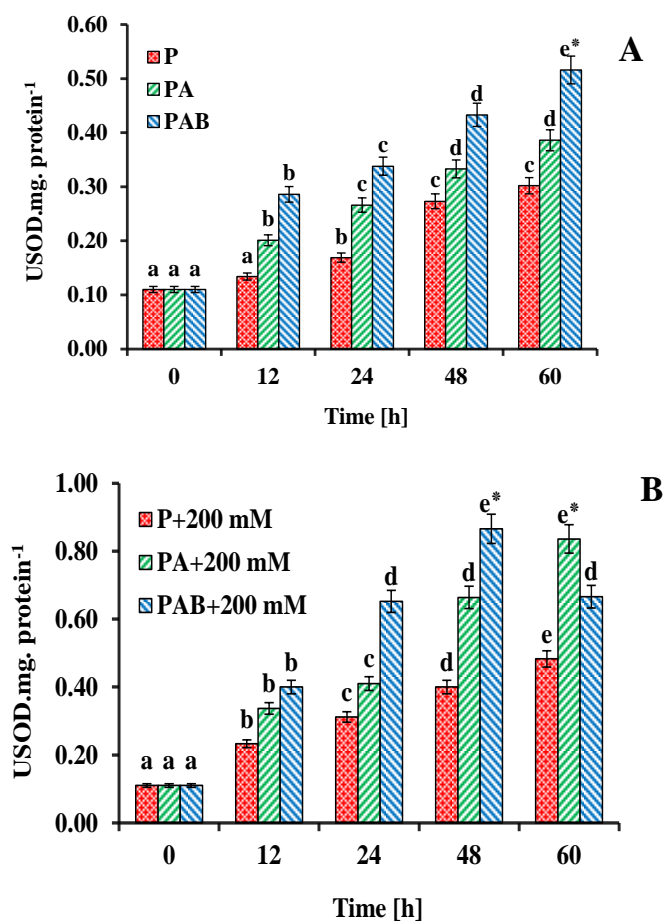


Figure 9. Activity of SOD in cultures exposed to P, PA and PAB radiation without NaCl (A) and after the addition of 200 mM NaCl (B) The error bar denotes standard deviations of means (n=3) and significance differences of means designated as different alphabet letters ($p < 0.05$) obtained by Tukey's multiple comparison test.

DISCUSSION AND CONCLUSIONS

The presence of high concentrations of MAAs in cells exposed to P, PA and PAB provide protection to the cyanobacteria from harmful effects of UVR. In this study we have shown the presence of MAA shinorine in *Scytonema* sp. PAB showed pronounced effect on shinorine production among P, PA and PAB. We studied the time-dependent induction of shinorine under PAB and varying concentration of salt (50, 150 and 200 mM). Maximum concentration of shinorine was reported after 48 h of PAB exposure along with 200 mM of NaCl in the cyanobacterial samples. Our findings are in agreement with earlier report where NaCl and UVR have synergetic effect on the biosynthesis of MAAs (Singh et al., 2008). Previous studies have showed that MAAs biosynthesis increases as a response of UV-B radiation in cyanobacteria (Sinha et al., 2003; Richa and Sinha, 2015a, b; Rastogi et al., 2016). There is clear evidence that the presence of MAAs

protects vital functions in phytoplankton and other aquatic organisms from deleterious short wavelength radiation (Klisch et al., 2001). According to previous findings, MAAs prevents three out of 10 photons from hitting cytoplasmic targets in cyanobacterium (Garcia-Pichel and Castenholz, 1993; Garcia-Pichel et al., 1993). They can act as antioxidants to prevent damage from ROS resulting from UVR (Dunlap and Yamamoto, 1995). MAAs biosynthesis also increase in response to salt stress in *Lyngbya aestuarii* (Portwich and Garcia-Pichel, 2000; Rath et al., 2014; Vale, 2015). The induction of MAAs under salt stress other than UVR indicates that MAAs might have some other functions besides UV-photoprotection (Rastogi et al., 2010). During salt stress, to provide the necessary osmotic balance, microorganisms accumulate low-molecular-weight, generally uncharged organic molecules, which serve as so-called 'osmotic solutes' or 'compatible solutes'. The MAAs that accumulate in the cells' cytoplasm are also small uncharged organic molecules, and thereby they contribute to the osmotic pressure within the cell (Vale, 2015).

The first report of the occurrence of MAAs was in the unicellular halophilic cyanobacterium *Eubhalotheca* sp. inhabiting a gypsum crust and when it was subjected to dilution with distilled water, loss of intracellular MAAs by excretion was observed which was approximately proportional to the extent of the dilution (Oren, 1997). Highest MAAs concentration (up to 0.8% of the cell dry weight) was measured in the cyanobacterium *Gloeocapsa* (Kogej et al., 2006). The observed decrease in the concentration of shinorine in our experiment after 60 h in salt and PAB treatment samples can be explained as after 48 h the shinorine concentration was probably sufficient to balance the salinity in the medium and further induction was not required (Singh et al., 2008).

In a study on the cyanobacterium *Chlorogloeopsis* strain PCC 6912, which can tolerate up to 70% sea water salinity, Portwich and Garcia-Pichel (2000) have reported that MAA synthesis can be induced by salt stress without PAR or UVR. Therefore, they distinguish between salt-dependent biochemical and light-dependent photosensory induction of MAA synthesis. It has been reported that shinorine, mycosporine-glycine, porphyra-334, and asterina-330 were the most abundant MAAs found in marine cyanobacteria (Carreto and Carignan, 2011; Volkmann and Gorbushina, 2006). Induction of transcripts of antioxidative enzyme systems by UV-B radiation has been reported by several researchers (Willekens et al., 1994; Kumari et al., 2009). It has been reported that higher plants have suppressed levels of APX activity which in turn induces higher level of SOD and CAT activity (Garcia-Pichel, 1993). Singh et al. (2013) reported antioxidant potential of two *Anabaena* sp. i.e. *Anabaena doliolum* and *Anabaena* strain L31, most common sp. of Indian rice field. Hanaa et al. (2008) reported that extract of *Spirulina maxima* ease the damage caused by the effect of salinity on wheat plants in addition to improving the antioxidant defence

abilities. Our report of increased activities of antioxidant enzymes like CAT, SOD and APX due to salt stress are in agreement with the previous findings such as in *Dunaliella tertiolecta* (Wang et al., 2007), *Dunaliella salina* (Tammam et al., 2011), *Nostoc sphaeroides* and *Nostoc Muscorum* (Hend et al., 2015).

Damage caused by production of ROS to important cellular components is prevented by increased synthesis of antioxidant enzymes. Against the ROS, first line of defense mechanism is provided by the SOD which scavenges superoxide radicals by dissociating them into H₂O₂ and water and H₂O₂ is further converted into water and oxygen either by CAT or APX (Mittler, 2002). Thus, increased level of SOD, CAT and APX during UVR+NaCl stress, might have resulted in the removal of toxic ROS to keep cellular process as usual (Joset, 1996). Additionally, bioprospection and use of salt-tolerant cyanobacterial strains is very crucial for the remediation of salt-impacted soils (Subhashini and Kaushik, 1981; Kaushik, 1989; Rao and Burns, 1991). Biosynthesis of photoprotectants is an important mechanism to prevent photodamage in cyanobacteria. Thus, it is necessary to endeavor new strains of cyanobacteria which are capable of tolerating harsh environmental stresses such as salinity and UVR and can be used for bioremediation of saline soils and water and at the same time could serve as source of value added products such as MAAs. UV-induced photoprotective function of MAAs may facilitate them to perform important ecological functions under harsh environmental conditions. There are very few reports on qualitative and quantitative characterization of shinorine in cyanobacteria in response to UVR and salinity stress and more work is required to elucidate the defense strategy of cyanobacteria during such stressful environment.

ACKNOWLEDGMENT

Vidya Singh, Jainendra Pathak, Deepak Kumar Singh and Abha Pandey are thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, India, for the financial support in the form of fellowships. Haseen Ahmed is thankful to University Grant commission (UGC), New Delhi, India, for the research fellowship. The work was also partially supported by DST-INSPIRE provided to DK in the form of JRF and SRF.

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