ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 3, pp. 398–407. © Pleiades Publishing, Ltd., 2015.

EXPERIMENTAL ARTICLES

Sensitivity of Two *Nostoc* Species Harbouring Diverse Habitats to Ultraviolet-B Radiation[1]

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August 6, 2014

Abstract—The effects of ultraviolet-B (UV-B; 280–315 nm) radiation on certain key physiological and biochemical processes were studied in two *Nostoc* species harbouring diverse habitats. *Nostoc* sp. strain HKAR-2, a thermophilic cyanobacterium, was isolated from the hot-spring, Rajgir, whereas, *Nostoc* sp. HKAR-6 was a rice-field isolate from Banaras Hindu University, Varanasi. Complete killing of the cells occurred after 48 h of UV-B exposure in *Nostoc* sp. strain HKAR-6, whereas in case of *Nostoc* sp. strain HKAR-2 death occurred only after 72 h. Chlorophyll *a* and phycocyanin content were found to be adversely affected by UV-B irradiation in both the test organisms. However, a progressive increase in carotenoids content was observed upto 10 h of UV-B exposure but subsequently declined after 12 h in both the *Nostoc* sp. The antioxidative enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and peroxidase that scavenge the UV-B generated harmful reactive oxygen species were found to have multifold induction in their content in both the strains following UV-B exposure in comparison to non-irradiated control cultures. In addition, these organisms also synthesize mycosporine-like amino acids (MAAs); able to carry out UV-screening. Porphyra-334 and shinorine were found to be the common MAAs in both the *Nostoc* sp., while an unknown MAAs with a retention time of 6.9 min (λ_{max}—334 nm) was found in *Nostoc* sp. strain HKAR-2. Findings from this study suggest that both enzymatic and non-enzymatic defense mechanisms are being employed by *Nostoc* sp. strain HKAR-2 and *Nostoc* sp. strain HKAR-6 to counteract the damaging effects of UV-B radiation.

Keywords: ascorbate peroxidases, catalase, peroxidases, superoxide dismutase, ultraviolet-B radiation

DOI: 10.1134/S0026261715030169

Cyanobacteria are gram-negative, cosmopolitan, photolysis mediated oxygen evolving prokaryotes that can survive and fluorish in almost every habitat ranging from hot-springs to Arctic and Antarctic regions as well as in the form of symbionts in plants, lichens and several protists [1, 2]. They play a significant role in global photosynthetic biomass production, CO_2 fixation, successional processes, nutrient cycling and as a potent natural biofertilizer in rice paddy fields [3, 4]. Cyanobacteria are also important from biotechnological and pharmaceutical point of view [5], being harvested for biofuels such as alcohols and hydrogen [6].

Hot-springs are well-isolated habitats formed as a result of volcanic or tectonic activities, occurring as clusters in globally outlying areas and are characterized by high temperature and abundant reduced compounds which favors their enrichement with thermophilic microorganisms [7]. Papke et al. [8] have reported that the extremophiles inhabiting the hot-springs are adapted to conditions quite different from the ambient milieu (e.g., temperate lake, air, water, soil, paddy fields) through which they would have to disperse and hence the geographical isolation may be an important component in the diversification of hot-spring microorganisms. The cyanobacteria living in thermal springs of the world have been studied by many workers [9–11]. Castenholz [9] observed irregular distributions of cyanobacterial morphotypes inhabiting mats in hot- springs around the world.

Among the diverse habitats, rice-fields also constitute one of the favourable ecologies for the growth and proliferation of cyanobacteria [3]. Rice-fields are artificial shallow aquatic ecosystems in which the land management and the agricultural practices together with the rice plant growth govern the major environmental variables affecting the aquatic biota and its relationships [12]. Studies on cyanobacterial and algal successions have been performed in different rice-fields all over the world [13]. Nitrogen cycle in this ecosystem is particularly dominated by N_2-fixation, since the aquatic biota is dominated by N_2-fixing cyanobacteria. *Nostoc* sp. is one the most abundant

[1] The article is published in the original.

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[3] *Abbreviations*: APXs—ascorbate peroxidases; PODs—peroxidases; ROS—reactive oxygen species; SOD—superoxide dismutase; UV-B—ultraviolet-B.

flora in the majority of rice-fields, contributing significantly to their fertility [14].

Rapid increase in anthropogenically released atmospheric pollutants such as chlorofluorocarbons, chlorocarbons and organobromides has resulted in depletion of the stratospheric ultraviolet radiation (UVR)-protecting ozone layer [15, 16]. Consequently, increased levels of UVR (280–400 nm) reach the Earth's surface, and harvesting of solar radiation for photosynthesis exposes cyanobacteria to UV-B (280–315 nm) and UV-A (315–400 nm) radiations. The high-energetic UV-B radiation has the greatest potential for cell damage caused by both direct effects on DNA and proteins as they absorb maximally in the UV region and indirect effects via the production of reactive oxygen species [17–21]. Morphogenesis, pigmentation, motility and orientation, nitrogen and CO_2 fixation, phycobiliproteins, enzymatic activity proteome, genome and photosynthesis are well-known targets of short wavelength UV-B radiation that eventually affect the growth and survival of organisms [22]. However, to counteract the damaging effects of this highly energetic radiation these photosynthetic organisms have developed several lines of mitigation strategies such as avoidance, scavenging, screening, DNA repair systems and programmed cell death (apoptosis) that facilitate them to grow and acclimatize in adverse environments with high UV fluxes [22, 23].

Cyanobacteria have developed both enzymatic as well as non-enzymatic defense mechanisms to overcome the oxidative stress caused by UV-induced generation of free radicals [24, 25]. DNA, proteins and lipids are prime targets of reactive oxygen species (ROS). ROS-mediated damage to photosynthetic apparatus followed by the inhibition of photosynthesis has also been observed in cyanobacteria [26]. Antioxidative enzymes such as superoxide-dismutase (SOD), catalase (CAT), peroxidases (PODs) and ascorbate peroxidases (APXs) can scavenge reactive oxygen species [24, 27]. SOD catalyses the dismutation of highly reactive superoxide to hydrogen peroxide and oxygen via the reaction: $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$, and catalase is one of the main tetrameric enzymes that is responsible for the dismutation of hydrogen peroxide (H_2O_2) into water and oxygen. Peroxidases detoxify harmful H_2O_2 present in almost every organism [28]. APX catalyzes the reduction of H_2O_2 to water using the reducing power of ascorbate [29]. These enzymes counteract damage caused by UV-B stress and thus contribute to the survival of cyanobacteria under UV-B stress.

The non-enzymatic defense mechanisms, such as the presence of photoprotectants, e.g., mycosporine-like amino acids (MAAs) and scytonemin, absorb mainly in the UV region of the spectrum and help these organisms to grow and survive in habitats exposed to intense solar radiation. MAAs are small, water-soluble, colourless secondary metabolites having absorption maxima between 310 and 362 nm and are composed of a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol [30–32]. Several characteristics such as, high molar extinction coefficients (e = 28,100–50,000 M^{-1} cm^{-1}), strong UV absorption and stability against several abiotic stressors give strong evidence for the photoprotective role of MAAs [33, 34]. MAAs are known to dissipate absorbed energy as heat into their surroundings, without producing ROS [35]. In addition to their role as photoprotectant, MAAs are also proposed to act as antioxidants and osmoprotectants [36, 37]. Till date, 22 MAAs have been reported in cyanobacteria [38, 39].

In the present study, we have compared the enzymatic and non-enzymatic defense mechanisms of two *Nostoc* species (one isolated from hot-springs and another from rice-fields) in conferring protection against UV-B stress and contributing to the survival of the organisms. Any adverse effects on these ecologically and economically important cyanobacteria will adversely affect the productivity of ecosystems and differential sensitivity of individual species may result in change in species composition in a given ecosystem.

MATERIALS AND METHODS

Experimental Organisms and Growth Conditions

The experimental organisms *Nostoc* sp. strain HKAR-2 isolated from hot- springs of Rajgir [31] and *Nostoc* sp. strain HKAR-6 from rice-fields, BHU, Varanasi, India, were identified with the help of standard taxonomic keys and monographs using morphological characteristics [40] and *16S rRNA* gene amplification [41]. The cyanobacterium *Nostoc* sp. strain HKAR-2 shows long trichomes having moniliform (constricted at cross walls) dark green cells and heterocysts larger than the vegetative cells and *Nostoc* sp. strain HKAR-6 was heterocystous with frothy thallus, gelatinous, sheath absent and ellipsoidal cells [41]. The thermophilic and the rice-field strain were routinely grown in a culture room under axenic conditions in an autoclaved BGA (without nitrogen sources) medium [42] at a temperature of 28 ± 2°C and under continuous fluorescent white light of 12 W m^{-2}. The thermophilic strain HKAR-2 has been acclimatized in laboratory conditions since last four years at a temperature of 28 ± 2°C. Since this study only deals with the effects of UV-B radiation therefore both the cultures were maintained under similar laboratory conditions in order to avoid any temperature related differences among them. The cultures were hand shaken 5 times daily to avoid shelf shading and clumping. All experiments were performed with exponentially growing cultures.

Source and Mode of UV Irradiation

The cyanobacterial samples were exposed to artificial UV-B radiation in a UV-chamber in open glass petri dishes (120 mm in diameter). The UV-chamber was fitted with Osram L 36 W/32 Lumilux de luxe warm white and radium NL 36 W/26 Universal white tubes (Osram, Munich, Germany) for visible light and Ultraviolet-B TL 40 W/12 fluorescence tubes (Cat no. G15T8E, Sankyo Denki, Japan), emitting its main output at 312 nm. The distance of the UV-B tube in the chamber from the sample was adjusted to have a UV-B intensity of 1 W m^{-2}. During UV-B treatment, samples were simultaneously irradiated with cool white fluorescent light (12 W m^{-2}). Homogeneous cyanobacterial cultures (800 mL) were exposed to UV-B radiation in eight sterile Petri dishes (with open lids) for the desired time intervals (2, 4, 6, 8, 10, 12, 24 and 48 h). Petri dishes were covered with 295 nm cut-off filter foils (Ultraphan; Digefra, Munich, Germany) to avoid any UV-C radiation. All experimental cultures were exposed with constant temperature of 28 ± 2°C and were shaken at regular intervals during exposure to avoid heating effects and self-shading of the cells. Hundred mL of cultures were withdrawn after each time interval of which 100 μL was spreaded on agar plates to determine the percent survival. The remaining culture volume was immediately used for the estimation of photosynthetic pigments, MAAs and antioxidant enzymes. Cultures irradiated with white fluorescent light served as control.

Determination of Percent Survival

100 μL aliquots were withdrawn at desired time intervals after UV-B exposure and plated on agar plates. Plates were kept in the dark for 48 h and thereafter transferred to light in the culture room. Colonies appearing after 15 days of growth were counted in a colony counter and percentage survival was calculated.

Estimation of Pigments

Known volume of culture was centrifuged at 10000 *g* for 10 min and pellet was suspended in desired volume of 100% methanol and kept overnight in a refrigerator at 4°C for Chl *a* extraction. After centrifugation, the absorbance of clear supernatant was measured at 663 nm in a Hitachi UV-visible spectrophotometer (Hitachi 2900, Japan). The amount of Chl a was calculated using the method of Porra [43]. For the estimation of phycocyanin a known volume of culture suspension was taken and centrifuged to obtain the pellet. Phycocyanin was extracted from pellet using 2.5 mM phosphate buffer (pH 7.0) by repeated freezing and thawing. The absorbance was read at 615 nm against phosphate buffer as blank. Estimation of phycocyanin was done as per the method of Bennett and Bogorad [44]. Similarly, for the estimation of carotenoids a known volume of culture suspension was taken, centrifuged and the pellet was washed 2–3 times with distilled water to remove traces of adhering salts. To the pellet, 2 mL of acetone (80%) was added and stored overnight at 4°C. The extract was centrifuged and the supernatant containing carotenoids was collected. The process was repeated until the last supernatant became colorless. All the fractions of supernatants were pooled together and absorbance was taken at 450 nm using 80% acetone as blank and the carotenoids content was calculated according to Jensen [45] by taking the absorbance at 450 nm.

Determination of Antioxidant Enzyme Activity

For assaying antioxidant enzymes, cell extracts were prepared by sonicating the cells in 2 mL of extraction buffer consisting of 50 mM potassium phosphate buffer (pH 7.5), 1 mM ethylenediaminetetracetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVP), 0.5% (w/v) Triton X-100 with the addition of 1 mM ascorbate in the APX assay under ice-cold conditions. The homogenate was centrifuged at 10000 *g* for 10 min at 4°C, and the supernatants were collected and used for assays of catalase, ascorbate peroxidase, superoxide dismutase and peroxidases. The non-irradiated cells were used as control.

Catalase activity was determined according to the method of Aebi [46]. Reaction mixtures contained 300 μM phosphate buffer (pH 7.2), 100 μM H_2O_2 and 500 μL of enzyme extract. Catalase activity was determined spectrophotometrically at 240 nm by recording O_2 release from enzymatic dissociation of H_2O_2 in darkness for 1 min. SOD activity was measured by recording the inhibition in reduction of nitro blue tetrazolium (NBT) [47, 48]. Reaction mixtures consisted of methionine (200 mM), nitroblue tetrazolium 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 3 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. Ascorbate peroxidase (APX) activity was determined by measuring the decrease in absorbance of ascorbate oxidation to mono-dehydroascorbate at 290 nm for 1 min [49]. Reaction mixtures consisted of 0.1 mM H_2O_2, 0.1 mM EDTA, 0.5 mM ascorbate and 100 μL of enzyme extract. Peroxidase activity was measured by taking the absorbance at 420 nm, every 20 s for 2 min. Reaction mixtures contained 0.1 M phosphate buffer pH 6.0, 5.33% pyrogallol solution (M/V), 3% H_2O_2 as substrate and 100 μL of enzyme extract. One unit of peroxidase activity is defined as the amount of enzyme required to catalyze the production of 1 mg of

purpurogallin from pyrogallol in 20 s at 20°C under assay conditions [50]. Bradford method [51] was used for the estimation of total soluble protein.

MAAs Extraction and Spectrophotometric Analysis

Cyanobacterial cells from control and UV-B irradiated were harvested by centrifugation (Remi Centrifuge CM-12 Plus, Vasai, India) and MAAs were extracted in 2 mL of 100% HPLC grade methanol overnight at 4°C. Thereafter, extracts were centrifuged at 10000 *g* for 10 min and supernatants were subjected to spectroscopic analysis between 250 and 700 nm in a double-beam UV-VIS spectrophotometer (UV-VIS 2900, Hitachi, Japan). The raw spectra (peaks) were analyzed using UV Probe version software (Hitachi, Japan). After initial characterization of MAAs by spectroscopic analysis, methanolic extracts were evaporated to dryness at 45°C, and the dried product was dissolved in 1 mL Milli Q water in an Eppendorf tube. After adding a few drops of chloroform, the suspension was subjected to centrifugation, and the water phase was carefully transferred into a fresh Eppendorf tube to remove contaminating lipophillic compounds. Finally, the resulting suspension was filtered through a 0.2 μm pore size syringe filters (Axiva Sichem Biotech., New Delhi) and further subjected to high performance liquid chromatography analysis.

High-Performance Liquid Chromatographic Analysis of MAAs

Partially purified MAAs were further analyzed by an HPLC system (Waters 2998, Photodiode Array, pump L-7100, United States), using a reverse phase semi-preparative column equipped with a Licrospher RP 18 column and guard (5 μm packing; 250 × 4 mm inside diameter). The samples (100 μL) were injected into the HPLC column through a Waters 717 Plus Autosampler. 0.02% acetic acid (v/v) in double-distilled water was used as mobile phase, which was isocratically run at a flow rate of 1 mL min^{-1}. MAAs were detected at the wavelength of 330 nm, and absorption spectra were recorded between 250 and 400 nm directly on the HPLC-separated peaks. Quantification of MAAs was performed by using peak area, and values are expressed as nmol g^{-1} dry weight.

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.

RESULTS AND DISCUSSION

The effect of UV-B radiation on the survival of *Nostoc* sp. strain HKAR-2 and *Nostoc* sp. strain HKAR-6 were determined by measuring the percent survival after 2, 4, 6, 8, 10, 12, 24, and 48 h of UV-B exposure and compared the values to those of the control (untreated) cultures. It was evident from the results that survival was not significantly affected even after 4 h of exposure; however, with increasing duration of exposure a progressive decrease in survival was observed. Almost 50% inhibition was recorded after 10 h of UV-B exposure in both the test organisms. Complete killing of the cells occurred after 48 h of exposure in *Nostoc* sp. strain HKAR-6, whereas in case of *Nostoc* sp. strain HKAR-2 100% killing occurred after 72 h of irradiation (Fig. 1a).

Effects of UV-B were also studied on pigment content by measuring the concentrations of chlorophyll *a*, phycocyanin and carotenoids. It was observed that chlorophyll *a* levels in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 (initial values 0.887 and 0.995 mg mL^{-1} respectively) were maintained for up to 10 h of UV-B exposure and thereafter declined to approximately 37% (0.314 mg mL^{-1}) and 35% (0.376 mg mL^{-1}) respectively, after 48 h of continuous UV-B exposure (Fig. 1b). The initial phycocyanin content in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 (3.612 and 3.758 mg mL^{-1} respectively) was found to be adversely affected by UV-B radiation. Approximately 3.5 fold (0.943 and 1.05 mg mL^{-1} respectively) decrease in the phycocyanin content was observed after 48 h of UV-B radiation (Fig. 1c). Initial level of carotenoids content was recorded to be 0.613 and 0.641 mg mL^{-1} in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 respectively. However, a progressive increase in carotenoids content was observed after increasing duration of UV-B exposure and a 37% (0.843 mg mL^{-1}) in *Nostoc* sp. strain HKAR-6 and 38% (0.887 mg mL^{-1}) in *Nostoc* sp. strain HKAR-2 was recorded after 10 h of UV-B exposure. However, a subsequent decline in the level of carotenoids content was observed after 12 h of UV-B irradiation in both the *Nostoc* sp. (Fig. 1d). Carotenoids content approximately declined upto 67% (0.269 mg mL^{-1}) and 53% (0.305 mg mL^{-1}) after 48 h of exposure in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 respectively (Fig. 1d).

UV-B stress had significant effect on the levels of studied antioxidative enzymes. There was a significant increase in SOD activity in *Nostoc* sp. strain HKAR-2, which reached a maximum of 0.70 U mg^{-1} of protein after 10 h of treatment, and then, the activity declined after 12 h of exposure. We observed a 2.3-fold induction of SOD activity in *Nostoc* sp. strain HKAR-6 and a 2.8-fold induction in *Nostoc* sp. strain HKAR-2 after 10 h of exposure, but after 12 h, the activity began to decline (Fig. 2a). However, 0.213 U mg^{-1} and 0.32 U mg^{-1} of protein remained in

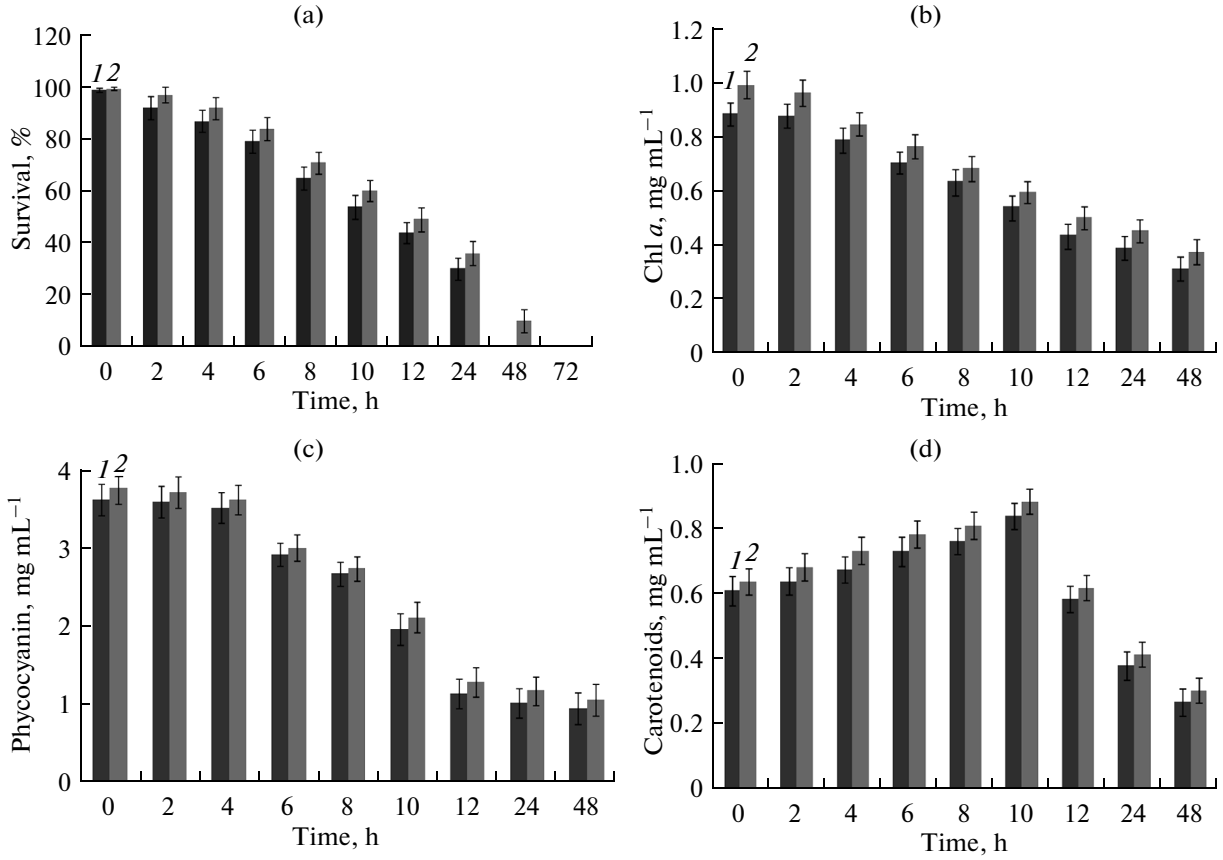

Fig. 1. Effects of UV-B radiation on percent survival (a), content of chlorophyll *a* (b), phycocyanin (c) and carotenoids (d) on *Nostoc* sp. strain HKAR-2 and *Nostoc* sp. strain HKAR-6. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means. *Nostoc* sp. strain HKAR-6 (*1*); *Nostoc* sp. strain HKAR-2 (*2*).

Nostoc sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 respectively, even after 48 h of UV-B exposure. Catalase activity also showed the similar trend. Initially an increase in the catalase activity in comparison to the untreated control culture was observed. After 10 h of UV-B exposure the catalase activity was found to be 0.99 and 1.45 μmol min^{-1} mg^{-1} of protein in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 respectively. The catalase activity thereafter slightly decreased, maintaining a basal level of 0.21 and 0.34 μmol min^{-1} mg^{-1} of protein in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 respectively after 48 h of continuous UV-B exposure (Fig. 2b). We observed a 4.8-fold (0.0318 U POD mgg^{-1}) increase in the peroxidase activity of *Nostoc* sp. strain HKAR-6 and a 5.0-fold induction (0.0410 U POD mg^{-1}) in *Nostoc* sp. strain HKAR-2 after 10 h in comparison to the control. But, after 12 h of UV-B exposure the peroxidase activity declined to 0.0113 and 0.0116 U POD mg^{-1} in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2 respectively (Fig. 2c).The activity of APX was found to be lower as compared to SOD, catalase and POD in both the selected test organisms. In *Nostoc* sp. strain HKAR-6, control cultures showed an initial activity of 0.00079 nmol min^{-1} mg^{-1} of protein and reached to 0.00234 nmol min^{-1} mg^{-1} of protein at 10 h of UV-B exposure and thereafter declined to 0.00083 nmol min^{-1} mg^{-1} of protein after 48 h of exposure. Similarly, *Nostoc* sp. strain HKAR-2 showed a maximum activity of 0.00279 nmol min^{-1} mg^{-1} of protein at 10 h, but to 0.0011 nmol min^{-1} mg^{-1} of protein after 48 h (Fig. 2d). It was observed that the activity of all four enzyme studied increased upto 10 h and a slight decrease was observed after that. However, the levels of all the enzymes were still higher than the untreated control cultures even after the prolonged exposure of 48 h.

Spectroscopic analysis of methanolic extracts of both the *Nostoc* sp. showed prominent peaks having absorption maxima in the range of 310–362 nm, which suggested the presence of UV-B screening, colourless mycosporine-like amino acids. HPLC chromatogram of the aqueous solutions confirmed the presence of MAAs in both the *Nostoc* species, showing two common peaks at 334 nm (retention time, RT 2.6 min in *Nostoc* sp. strain HKAR-6 and 3.1–3.8 min in *Nostoc* sp. strain HKAR-2). Furthermore one new, unknown peak with retention time 6.9 min (λ_{max}—334 nm) was also recorded in *Nostoc* sp. strain HKAR-2. Quantitative estimation showed

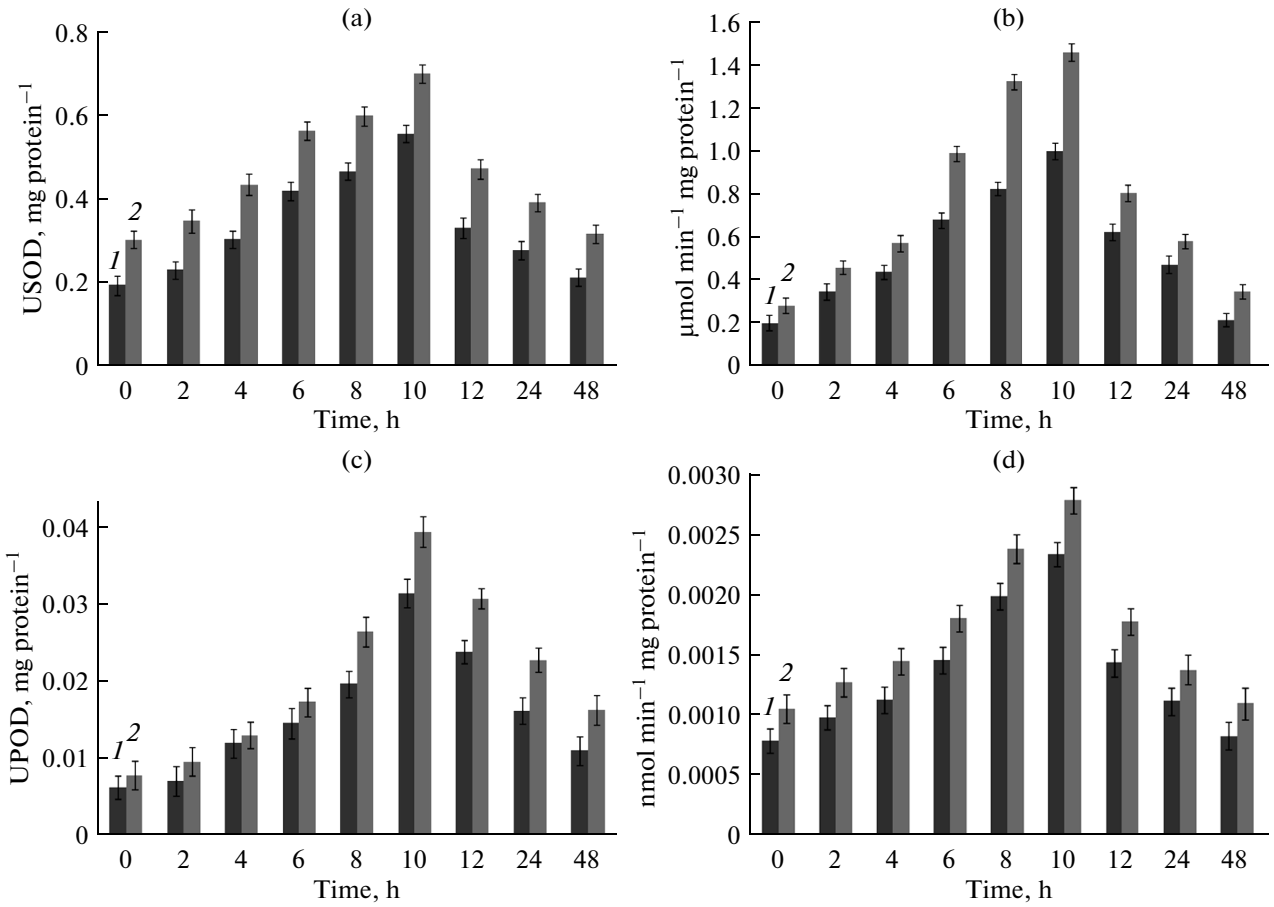

Fig. 2. Activity of antioxidative enzymes in *Nostoc* sp. strain HKAR-2 and *Nostoc* sp. strain HKAR-6 when exposed to UV-B radiation for varying duration of time. SOD (a), CAT (b), POD (c), and APX (d). Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means. Experimental conditions were identical for both the test organisms. *Nostoc* sp. strain HKAR-6 (*1*); *Nostoc* sp. strain HKAR-2 (*2*).

4.2-fold induction of MAAs in *Nostoc* sp. strain HKAR-6 and 5.0-fold induction in *Nostoc* sp. strain HKAR-2 after 10 h of UV-B exposure. Concentration of MAAs was 1510 and 1900 nmol g^{-1} dry wt in *Nostoc* sp. strain HKAR-6 and *Nostoc* sp. strain HKAR-2, respectively, after 10 h of UV-B exposure. However there was decline in the level of MAAs content after 12 h of continuous UV-B exposure in both the *Nostoc* species. But, in the hot-spring cyanobacterium *Nostoc* sp. strain HKAR-2, MAAs concentration did not drop below the basal level even after 48 h of UV-B stress as found in the rice-field isolate (Fig. 3).

The increased level of solar UV-B radiation on the Earth's surface may alter the normal physiology and biochemistry of the cell. Several workers have shown that UV-B radiation induces oxidative stress in cyanobacteria [19, 52, 53]. In the present study, an attempt has been made to compare the sensitivity of two strains of *Nostoc* sp. isolated from diverse habitats against UV-B radiation. Percent survival studied clearly indicates that the hot-spring isolate *Nostoc* sp. strain HKAR-2 has developed a better survival strategy in comparison to the rice-field isolate *Nostoc* sp. strain HKAR-6. The inhibition of growth or complete loss of cell's viability might be due to inactivation/damage of a number of cellular processes incited by UVR [54]. Chlorophyll *a* and phycocyanin content was found to be maintained in both the test organisms following UV-B exposure for almost 10 h. Thereafter, a significant decrease was observed in both the pigment content. Phycocyanin was found to be more adversely affected by prolonged UV-B exposure. The photoreduction of protochlorophyllide to chlorophyllide under UV-B stress attributing to the decrease in chlorophyll *a* content has been reported earlier [55]. UV-B radiation-induced bleaching of photosynthetic pigments particularly the accessory light harvesting complex phycobiliproteins has been reported by several workers [55, 56]. Strong inhibition of phycocyanin by UV-B radiation has also been reported and it seems that proteinaceous pigments rich in aromatic amino acids are the primary target of UV-B radiation [55, 57].

The present study reveals that two lines of defense mechanisms are operative in both the test organisms

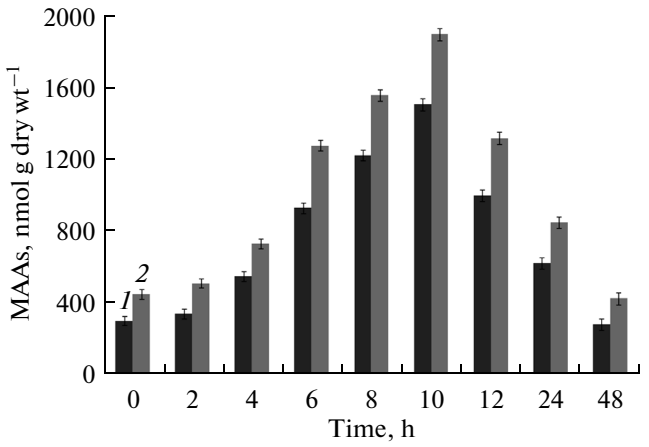

Fig. 3. MAAs concentration in *Nostoc* sp. strain HKAR-2 and *Nostoc* sp. strain HKAR-6 after exposure to UV-B radiation for different periods of time. Results are expressed as means of three replicates. Vertical bars indicate standard deviation of the means. *Nostoc* sp. strain HKAR-6 (*1*); *Nostoc* sp. strain HKAR-2 (*2*).

under UV-B stress. Our study suggests that both enzymatic and non-enzymatic defense mechanisms confer protection to the organisms under UV-B stress. A multifold induction in the antioxidative enzymes was observed. The role of UV-B radiation in induction of antioxidative enzyme systems has been reported earlier [58–61]. The hot-spring isolate *Nostoc* sp. strain HKAR-2 was found to be more potent in the induction of SOD after 10 h of UV-B exposure as compared to the rice-field isolate *Nostoc* sp. strain HKAR-6. Furthermore, a high basal level of SOD activity was observed in *Nostoc* sp. strain HKAR-2 as compared to *Nostoc* sp. strain HKAR-6 even after 48 h of continuous UV-B exposure. Similar trends were observed in case of other antioxidative enzymes such as catalase (CAT), peroxidise (POD) and ascorbate peroxidise (APX). However, the activity of APX under UV-B stress was not significant as compared to the other studied antioxidative enzymes. Probably, relatively higher levels of antioxidant enzyme activity even after 48 h of prolonged UV-B exposure might contribute to the higher survival of the hot-spring cyanobacterium *Nostoc* sp. strain HKAR-2 in comparison to the rice-field isolate. Willekens et al. [62] reported that the plants have suppressed levels of APX activity which in turn induces higher level of SOD, CAT and POD activity. We observed moderate levels of APX activity in both the *Nostoc* species, and low levels of induction of SOD, CAT and POD activity, which suggests the existence of a differentially regulated defense mechanism in the studied cyanobacteria and its absence in higher plants.

The non-enzymatic defense mechanism is mediated through synthesis of a special class of secondary metabolites namely mycosporine-like amino acids (MAAs) in cyanobacteria that absorbs mainly in the UV range. The porphyra-334 and shinorine was found to be the common MAAs in both the *Nostoc* sp. induced by UV-B stress. Furthermore, induction in the shinorine content was found to be less as compared to porphyra-334 in both the isolates. An unknown MAA with retention time 6.9 min (λ_{max}—334 nm) was also found to be synthesized after UV-B exposure in *Nostoc* sp. strain HKAR-2 [41]. This observation is in accordance with the previously reported data on the occurrence of MAAs such as, shinorine, porphyra-334 and mycosporine-glycine in certain other terrestrial and aquatic cyanobacteria [30, 63]. The potential of *Nostoc* species to synthesize and induce MAAs under UV-B stress may be partly responsible for the presence and low level of induction of antioxidative enzymes as compared to higher plants where the existence of MAAs has not been demonstrated so far. Furthermore, the higher concentration of MAAs and their profound induction under UV-B stress in the hot-spring isolate may partly contribute to their better survival as compared to the rice-field isolate.

The present study clearly suggests the role of both enzymatic and non-enzymatic defense mechanisms in conferring protection under UV-B stress in both the *Nostoc* species harbouring diverse habitats. The antioxidative enzymes such as SOD, CAT, POD and APX show a multifold induction upto 10 h of UV-B exposure and were responsible for the survival even after 48 h of continuous UV-B stress in both the *Nostoc* species. However, different species may show varying degree of induction in the level of the above enzymes under UV-B stress and that may govern the degree of survival [64]. The non-enzymatic MAAs synthesized by both the *Nostoc* sp. was found to exclude the harmful effects of UV-B as they have the potential to prevent 3 out of 10 photons from hitting cytoplasmic targets [31, 65] in cyanobacteria. A drastic decrease in their concentration was noticed after 10 h of exposure suggesting that they may not be effective for longer duration. In contrast, the antioxidative enzymes played a crucial role as they maintained a basal level even after prolonged exposure to UV-B irradiation upto 48 h. The hot-spring isolate *Nostoc* sp. strain HKAR-2 synthesizes three types of MAAs and showed high antioxidative enzymatic activity conferring them a better survival in comparison to the rice-field cyanobacterium *Nostoc* sp. strain HKAR-6.The increasing duration of UV-B irradiation resulted in the decreased survival of both the test organisms. Hence, lower values of all the studied parameter were recorded with increasing duration of exposure time. However, the cells maintained a basal level of the studied parameters even after prolonged UV-B exposure upto 48 h and were able to cope up the detrimental effects of the highly energetic radiation. The results obtained are in accordance with the earlier reports [64, 66]. It may be concluded that both enzymatic and non-enzymatic defense mechanisms are operative and important in protecting damages caused by UV-B stress in cyano-

bacteria. Our work suggests that *Nostoc* sp. strain HKAR-2 is more tolerant to UV-B radiation and would be a better candidate to be exploited for the production of natural sunscreens such as MAAs to be used in various biotechnological and biomedical applications.

ACKNOWLEDGMENTS

Richa is thankful to Department of Science and Technology, Govt. of India, for the financial assistance in the form of a fellowship under the project (no. SR/WOS-A/LS-140/2011).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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