EFFECT OF ENHANCED ULTRAVIOLET-B RADIATION ON GROWTH, PIGMENTATION, NITROGEN METABOLISM AND POLYAMINE CONTENTS IN A CYANOBACTERIUM NOSTOC MUSCORUM

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AGRAWAL S. B. *Effect of enhanced ultraviolet-b radiation on growth, pigmentation, nitrogen metabolism and polyamine contents in a cyanobacterium* Nostoc muscorum. BIOTRONICS **25**, 1–9. 1996. The effects of enhanced UV–B radiation was seen on growth, pigmentation, polyamines and some physiological processes of a nitrogen fixing cyanobacterium *Nostoc muscorum*. UV–B exposure of 90 min having intensity of $3.5\,Wm^{-2}$ proved to be lethal for this alga. Amongst all the pigments, phycocyanin was most affected. Nitrate reductase activity was also decreased but not completely inhibited even after 60 min of UV–B exposure. The test alga contains a higher amount of unusual polyamine homo–spermidine in comparison to other usual polyamines (putrescine, spermidine and spermine). Enhanced UV–B exposure decreased the nitrogenase activity *vis a vis* the homo–spermidine contents, showing a direct correlation between the enzyme nitrogenase and homo–spermidine. These results suggest that possibly homo–spermidine plays some specific role in the regulation of nitrogen–fixation in the cyanobacterium under investigation.

Key words : Cyanobacterium, *Nostoc*, ultraviolet-B, pigments, nitrogenase, polyamines.

INTRODUCTION

Several anthropogenic human activities have led to the release of halogenated hydrocarbons and many trace gases in the atmosphere resulting in the depletion of the stratospheric ozone layer (*3, 20*). Such a depletion is expected to increase the amount of solar UV–B (290–320 nm) radiation reaching the surface of earth which can damage the biological ecosystems. Numerous studies have been conducted on the effects of enhanced UV–B on plant growth and physiological and biochemical processes (*1, 20*). Photosynthetic enzymes, metabolic pathways, photosynthetic pigments and stomatal function are found to be adversely affected directly or indirectly by UV–B radiation, as a result photosynthetic activity decreased (*29*). Tevini (*29*) has reported that in some sensitive plants carotenoids as well as membrane lipids such as galactolipids principally found in chloroplasts have been affected. Since chlorophyll pigments are membrane bound, any change in chloroplast membrane may destroy the

chlorophylls.

Solar UV–B can penetrate deep into the photic zone of water columns and can damage planktonic organisms (31). UV–B radiation also affected motility, orientation, pigmentation and community composition, and several metabolic processes of algal systems (3, 30). Some field studies in aquatic systems indicated that photosynthesis is impaired first, followed by decreases in protein concentration and changes in pigment composition resulting in a dramatic decrease in photosynthetic oxygen production after exposure to solar radiation (15, 26). Cyanobacteria–a phylogenetically old group with prokaryotic cellular organisation have probably faced more intense ambient solar UV–B radiation than other algae or higher plants appeared at a later period (9).

Supply of nitrogen fertilizers has been a big constraint in rice production in many third world countries. The nitrogen fixing cyanobacteria occur abundantly in rice field of tropics and their contribution towards the fertility of such soils has been well documented (25). Cyanobacteria alone are estimated to produce 35 million tons of nitrogen annually (15). Newton et al. (24) and Tiyagi et al. (30) showed that nitrogenase, the main N_2-fixing enzyme in cyanobacteria is more sensitive to UV–B than other metabolic processes.

Polyamines have a number of antisenescent properties and their induction in response to UV–B stress in plant system is well documented (17, 19). They can bind to membrane via ionic interactions and inhibit lipid peroxidation. Polyamine putrescine, spermidine, and spermine are widely distributed in biological materials and have been linked mainly to the biosynthesis of proteins and nucleic acids. In addition to these some other 'unusual' or 'uncommon' polyamine have been demonstrated in certain plants. Sym–homo–spermidine, an unusual polyamine has been detected in thermophilic bacteria, algae, higher plants and animals (3, 16). They also observed that sym–homo–spermidine was present as major polyamines in some nitrogen fixing species of cyanobacteria. The authors also suggested that it might play some role in the regulation of nitrogen fixation.

Higher plants cannot assimilate atmospheric nitrogen themselves and large share of nitrogen consumed by higher plants (e. g. in tropical rice paddies) is made available by cyanobacteria. As these cyanobacteria are highly sensitive to solar UV–B radiation so losses in nitrogen fixation by prokaryotic microorganisms due to UV–B may need to be compensated by artificial nitrogen fertilization (15). Considering the consequences of enhanced UV–B levels and the role of cyanobacteria in nitrogen economy, the present study was undertaken to characterize the effect of UV–B radiation on growth, pigmentation and activities of the nitrogenase and nitrate reductase enzymes of Nostoc muscorum as all the parameters are directly or indirectly related to nitrogen metabolism. This study for the first time reports the effect of UV–B on polyamine titres of a nitrogen fixing cyanobacterium and relationship of unusual polyamine homo–spermidine with the nitrogenase activity.

MATERIALS AND METHODS

The organism was isolated from a rice field in Varanasi (India) and grown axenically in Allen & Arnon's (5) medium under $72\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ of light provided by fluorescent lamps and a 14/10–hr photoperiod at $27\pm1°C$. This also was selected mainly due to its abundance in rice fields, for its faster growth rate and ability to form discrete colonies on agar plates. The cultures were harvested during exponential growth (8 days old) and exposed for different periods to supplemental UV–B radiation. One group of Petriplates containing algal cultures were exposed to $3.5\,\text{Wm}^{-2}$ dose of UV–B from Q–Panel UV–B 313 lamps (Cleveland OH, USA) at a distance of 30 cm (+ UV–B) covered with either 0.14 mm cellulose diacetate to transmit UV–B or 0.13 mm polyester to exclude UV–B. Triplicate experiments were performed for each parameter. The cell density used for UV–B treatment to study various parameters were the same.

Heterocyst frequency was determined by counting the number of heterocysts per hundred vegetative cells in at least 25–30 filaments of apporoximately equal lengths. Nitrogenase activity was measured by the acetylene reduction technique as described by Tiyagi *et al.* (*30*). Two ml UV–B treated cultures were placed in 7 ml vacutainer tubes and acetylene was added to a final concentration of 10% (v/v). The tubes were incubated in fluorescent light at 27°C. The ethylene formed was determined at intervals lasting up to 3 h using a gas chromatograph fitted with a porapak R column and flame ionization detector.

Nitrate reductase activity was estimated by the methods of Camm & Stein (*8*) and nitrite formed was determined by the diazocoupling method of Lowe & Evans (*22*). Enzyme activity was expressed as $\mu\text{g}\,NO_2^-\cdot\mu\text{g}\,\text{protein}^{-1}$.

Chlorophyll *a* and carotenoids were extracted and measured in acetone according to Myers & Krantz (*23*). Phycocyanin was extracted in 2.5 mM potassium phosphate buffer (pH 7.0) and the content was measured by the method of Bennett & Bogorad (*6*).

Free soluble polyamine content was determined by HPLC using the method described by Agrawal *et al.* (*3*).

RESULTS

The *Nostoc muscorum* cultures were exposed to different time periods with UV–B. Table 1 shows 100% killing at 90 min of UV–B exposure and 50% killing at 40 min exposure.

Table 2 shows the cellular contents of photosynthetic pigments in cultures treated with UV–B for 35 and 70 min durations. UV–B treated cultures had lower chlorophyll, phycocyanin and carotenoid contents as compared to –UV–B treatment. It was found that pigment content showed significant decrease even at 35 min UV–B treatment and all the pigments were severely affected by higher UV–B exposure of 70 min. Maximum % reduction was observed in phycocyanin content (Table 2). At 70 min UV–B exposure reduction was 86.4% for phycocyanin, 81.25% for carotenoids and 76.8% for chlorophyll *a* compared to

Table 1. Survival of exponentially grown culture[1,2] of *N. muscorum* exposed to 3.5 wm[-2] UV-B (mean±S. E.)

Time (min)	Survival (%)
0 (-UV-B)	100
15	92±1.8
30	79±0.8
45	38±1.2
60	16±0.6
75	7±0.3
90	0

1. Cultures having an initial dry weight of 0.1 mg/ml were exposed to UV-B ;
2. Equal numbers of cells were plated for each UV-B exposure and colony counts were made after 8 days of incubation in fluorescent light.

Table 2. Effect of UV-B radiation on photosynthetic pigment content of *Nostoc muscorum** (mean±S. E.)

Photosynthetic pigments		UV-B exposure duration (min)		
		0 (-UV-B)	35	70
Chlorophyll *a*	μg ml^{-1}	0.95±0.12	0.68±0.16	0.22±0.12
Phycocyanin	%	100.0	71.6	23.2
	μg ml^{-1}	38.2±0.08	27.60±0.04	5.20±0.06
Carotenoids	%	100.0	72.3	13.6
	μg ml^{-1}	0.64±0.22	0.48±0.12	0.12±0.08
	%	100.0	75.0	18.75

*Pigments were extracted after 3 days incubation.

control.

There was no significant change in heterocyst differentiation at various exposures of UV-B (Table 3). Exposure of cultures to even 4 min UV-B exposure resulted in a significant decrease in nitrogenase activity i. e. nearly 55% inhibition of nitrogenase activity (Table 3). The activity was reduced with the increase of UV-B exposure and complete loss of activity was noticed beyond 16 min of UV-B exposure. At 16 min the nitrogenase activity was only 2.9% of the control. As both nitrogenase and nitrate reductase are molybdoenzymes and require ATP and reductant for normal enzyme activity, they both should behave similarly to UV-B treatment. Like nitrogenase, nitrate reductase activity was also affected after UV-B exposure, but the nitrate reductase activity was not completely inhibited even after 60 min of UV-B exposure (Table 4).

The alga contained polyamines viz., putrescine, spermidine spermine and homo-spermidine (Table 5). Homo-spermidine was the most abundant poly-

Table 3. Effects of UV-B on heterocyst differentiation and nitrogenase activity of *Nostoc muscorum* (mean±S. E.)

UV-B exposure (min)	Heterocyst frequency (% of vegetative cells)	Nitrogenase activity* (nmol $C_2H_4/\mu g$ protein/hr)
0 (-UV-B)	5.2±0.06	0.560±0.003
4	5.2±0.06	0.254±0.031
8	5.2±0.11	0.125±0.011
12	5.0±0.06	0.094±0.001
16	4.9±0.17	0.016±0.002

*After incubation of 3 h.

Table 4. Effect of UV-B on nitrate reductase activity of NO_3^- grown culture of *N. muscorum* (mean±S. E.)

Time (min)	Nitrate reductase activity (%)
0 (-UV-B)	100
4	96±2.4
8	87±1.8
12	81±1.6
16	76±1.8
20	65±1.2
24	60±0.8
28	52±1.2
36	38±0.6
44	30±0.8
52	25±0.2
60	22±0.4

Table 5. Effects of UV-B on polyamine content of *N. muscorum* (mean±S. E.)

UV-B dose (min)	Polyamines (μmol/g fresh weight)			
	Putrecine	Spermidine	Spermine	Homo-spermidine
0 (-UV-B)	0.106±0.22	0.090±0.02	0.079±0.008	4.354±0.46
4	0.324±0.12	0.094±0.08	0.072±0.004	3.126±0.32
8	0.378±0.02	0.098±0.06	0.068±0.01	1.056±0.12
12	0.383±0.36	0.104±0.02	0.062±0.004	0.882±0.22
16	0.376±0.26	0.098±0.04	0.060±0.006	0.412±0.08

amine in this alga. Treatment of cultures with 4, 8 and 12 min UV–B exposure resulted in a 3.1 to 3.6–fold increase in putrescine level and slight increase in spermidine level. The increase in putrescine and spermidine was accompanied by a relatively small reduction in spermine content. An exposure time dependent significant decrease in homo–spermidine content was observed in response to UV–B exposure. Exposure to 16 min UV–B resulted in decrease of homo–spermidine by more than 90% (Table 5).

It is interesting to note that increase in UV–B dosage caused reduction in the homo–spermidine content *vis a vis* the nitrogenase activity (Tables 3, 5). It is of special interest to note that occurrence of homo–spermidine in significant amount in this cyanobacterium is closely associated with nitrogen fixation.

DISCUSSION

The findings of present investigation show that the test organism, *N. muscorum* is sensitive to UV–B radiation. Inhibition of growth and killing of other cyanobacteria have already been demonstrated by various workers (*10, 24, 30*). Findings of Döhler *et al.* (*11*) showed that marine diatoms were more affected by UV–B than cyanobacteria. In the present study, phycocyanin was found to be more sensitive to UV–B treatment than chlorophyll *a* and carotenoids. Our findings are in agreement with the earlier findings of Döhler *et al.* (*10*) and Tiyagi *et al.* (*30*). Agrawal (*2, 4*) also reported reduction in chlorophylls and carotenoids in some green algae. According to Döhler *et al.* (*10*), the phycobilisomes attached to the thylakoids are more exposed to UV–B radiation than chlorophyll *a* and carotenoids, therefore the damage be seen more in phycocyanin. More damage of carotenoids may be because it protects chlorophylls from photo–oxidative destruction (*18*). The reductions in pigment contents due to UV–B radiation have been ascribed either to an inhibition of chlorophyll biosynthesis or to a breakdown of pigments or their precursors (*12, 28*).

Our data suggest that UV–B damages nitrogenase and 54.6% inhibition in C_2H_2 reduction occurred within 4 min of exposure and completely lost after 16 min of treatment. Similar observations were also made by some other workers in *Anabaena* and *Nostoc* spp. (*24, 30*) with different exposure times of UV–B. Heterocyst frequency was not changed significantly after UV–B exposure so it was not responsible for the decline in nitrogenase activity. In the present study nitrogenase activity was not lost instantly and growth of cyanobacteria was not severely affected at 16 min UV–B exposure. Nitrate reductase activity also decreased but not completely lost even after 60 min UV–B exposure. Döhler (*11*) has also reported UV–B induced inhibition of this enzyme in marine diatoms. Among prokaryotes, cyanobacteria are unique in possessing photosynthesis closely associated with nitrate reduction (*21*). Thus inhibition of nitrate reduction in the test alga by UV–B may be attributed due to reduced carbon fixation as already reported by Döhler (*11*) and Tiyagi *et al.* (*30*). The reduction in photosynthetic pigments was also noticed in present study due to UV–B radiation might be responsible for inhibition of nitrate reductase activity. The

adverse effects of UV-B on nitrogenase may act in two different ways a) there might be direct action of UV-B on enzyme complexes or b) there could be depletion of ATP and reductant pools which are pre-requisites for nitrogenase mediated reaction as also suggested by Tiyagi *et al.* (*30*). As main source of ATP and reductants is photosynthesis in cyanobacteria (*14*), inhibition of photosynthesis might reduce the amount of ATP and reductant for nitrogenase activity. Döhler *et al.* (*10*) reported that the reduction in photosynthesis is followed by change in nucleic acid and protein composition, which ultimately affects the enzyme activity.

Among polyamines, putrescine and spermidine titres were increased in response to UV-B radiation in the test alga *N. muscorum*. This finding is consistent with the earlier finding of Agrawal (*4*) in a green alga *Scenedesmus obliquus* and Kramer *et al.* (*17*) in cucumber cultivars. Such effects may be related to the ability of polyamines to stabilize membrane structures and inhibit lipid peroxidation (*17*). There are some reports that polyamines have radical scavenging properties amongst oxidative stress (*7, 19*). In plants, free radicals are formed during normal metabolism as well as during environmental and osmotic stress. Accumulation of putrescine may be a biochemical response to increased UV-B radiation as a protective mechanism. Putrescine and other polyamines were observed to accumulate in plants including the green algae subjected to various environmental stress (*3, 13*). The increase in putrescine and spermidine was linked with the decrease of spermine content. This reduction may be due to inhibition of enzyme, spermine synthase responsible for the conversion of spermidine to spermine.

Homo-spermidine, an unusual polyamine has been shown to occur not only in some angiosperms, eukaryotic algae, bacteria but also in cyanobacteria (*16*). It is absent or present in small quantity in most of the green algae and non-nitrogen fixing cyanobacteria. Hamana *et al.* (*16*) showed polyamine distribution patterns in 16 species of cyanobacteria and found that major polyamine in nitrogen fixing cyanobacteria is homo-spermidine. The results seem to suggest that homo-spermidine plays some specific role in nitrogen metabolism. In the present study a positive correlation was observed between the amount of unusual polyamine homo-spermidine and nitrogenase activity after UV-B exposure. The nitrogenase activity decreased by UV-B along with the homo-spermidine titre. This also suggests that homo-spermidine plays some definite role in the regulation of nitrogen fixation. Presence of homo-spermidine was also reported in nitrogen fixing *Rhizobium* and legume root nodules (*27*).

This study indicates that this cyanobacterium rich with phycocyanin and carotenoids may tolerate high levels of UV-B and have some protective device by an increase in putrescine levels. Further, detailed investigations are required at various levels to understand the possible role of homo-spermidine on the nitrogenase activity of this cyanobacterium.

ACKNOWLEDGEMENTS

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