Physiological and Biochemical Changes in *Spirulina platensis* under UV-B Stress

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A B S T R A C T

Physiological and biochemical variation have been investigated under UV-B stress on cyanobacterium *Spirulina platensis*. Morphological examinations showed significant change in terms of granulation, pigmentation, both apical and terminal end of filaments, showing altered emission intensity of pigment and changes in morphological structure of sheath in UV B treated as compared to untreated counterpart could observed respectively by bright field, fluorescent and scanning electron microscopy. UV B radiation decreased the, dry weight, Chl a, protein, but carbohydrate content is recorded to increase. UV-B treatment inhibited the both NR and NiR activity by more than 50% as compared to control. Fluorescence spectroscopy of the Chl a demonstrates that exposure of UV-B radiation significantly altered the fluorescence emission spectral profile up to 33% as compared to control. The results showed that owing to adaptation morphological and biochemical changes occur in the cyanobacterium *S. Platensis* subjected under UV-B treated conditions.

Keywords

Fluorescence spectroscopy, Microscopy, *Spirulina* cyanobacteria, UV B stress, Microbial physiology and growth

Article Info

Accepted: 24 July 2020
Available Online: 10 August 2020

Introduction

Cyanobacteria are the largest, widely distributed group of photosynthetic prokaryotes on the Earth and is the most appropriate model system for studying various stresses due to its ability to adapt a wide range of environmental stress such as light, radiation, temperature, and salinity (Ferreira *et al.*, 2004). UV-B radiation is potentially detrimental to all forms of life but is more detrimental to photosynthetic organisms, including cyanobacteria (Hader, 2000; Rastogi *et al.*, 2014) which are increasing in the solar radiation due to depletion of ozone layer and global climate change (Crutzen, 1992; Kerr and McElroy, 1993; Gupta, 2018). In cyanobacteria UV-B
stress not only impairs the motility and photo orientation (Donkor and Häder, 1991) but also affects a number of physiological and biochemical processes, such as growth, survival, morphology, pigmentation, nitrogen metabolism etc. (Sinha et al., 1995). There are several targets for the deleterious effect of UV-B radiation in photosynthetic organisms like cyanobacteria, such as proteins, fatty acids, and thylakoid membranes (Gupta et al., 2008; Gupta and Bisen, 2018) and also posses a variety of defense strategies, such as production of UV-absorbing compounds (Xiong et al., 1997; Sarah et al., 2016: Gupta, 2018).

Spirulina is a free-floating filamentous cyanobacterium characterized by cylindrical, multicellular trichome and occurs naturally in tropical and subtropical lakes with alkaline pH. S. platensis also have long history of being used as food supplement and rich in ingredients like essential fatty acids, high protein content with nutritional and biomedical values (Kulshreshtha et al., 2008; Jadaun et al., 2017). The responses of cyanobacteria to changing environmental patterns associated with global climate change are important subjects for research. Considering above importance in response to UV-B stress physiological and biochemical variation has been studied in a cyanobacterium S. platensis.

Materials and Methods

Organism and growth conditions

Axenic culture of Spirulina platensis was maintained in Zarrouk’s medium. The culture was grown at a temperature of 30 ± 2°C and light intensity of 3000 lux with light and dark cycles of 10 and 14 h, respectively. During the process of growth, the flask was shaken for 3 to 4 times (Gupta et al., 2008).

UV-B treatment

Exponentially growing culture were harvested and transferred to sterile Petri dishes (25-mm diameter) for exposure to artificial UV-B (280–315 nm) radiation, generated from a UV-B lamp (TL 12 20W fluorescent tubes, Phillips, Holland). The intensity of UV-B radiation falling upon the cells was measured by photometer (Type IL 1350, Japan) (Gupta et al., 2008).

Photomicroscopy

Photomicroscopy of untreated and UV-B treated S. platensis were performed by using Lieca compound microscope, fluorescent microscopy and scanning electron microscopy (SEM).

Scanning electron microscopy

S. platensis cells were harvested by centrifugation (6,000 rpm for 10 min) and prefixed in culture medium with an equal volume of 1% glutaraldehyde in phosphate buffer. Cell were allowed to stand for 30 min on ice, pelleted, suspended in phosphate buffer with 2% glutaraldehyde and incubate for 1h at room temperature. Sample were washed with phosphate buffer and post fixed in 1% osmium tetroxide in the same buffer and washed once in distilled water. Then sample were kept on carbon stubs and gold coating were done with fine coat ion sputter (JFC 1100). Sample observed with scanning electron microscope (JEOL JSM-840).

Analytical methods

Chlorophyll-a (chl a) was assayed following the method of Mackinney [16], and cellular protein was estimated by the method of Lowry et al., [15] using bovine serum albumin (BSA) as the standard. Carbohydrate was estimated by the phenol-sulfuric acid method.
**NO$_3$/NO$_2$ Uptake assay**

Uptake of NO$_3^-$ / NO$_2^-$ was assayed by measuring their depletion from the external medium (Bisen and Shanthy, 1991; Singh et al., 1996). Uptake was carried out by addition of 5 mM KNO$_3$/ KNO$_2$ to cell suspension at zero time. Samples were collected at regular time intervals and cell-free supernatants were analyzed for residual NO$_3^-$ / NO$_2^-$. Linear portions of the curves were used to calculate uptake rate.

**Fluorescence spectroscopy**

**Sample preparation of pigments**

Sample of chlorophyll a from UV-B treated and UV-B untreated cells of *Spirulina platensis* was suspended equal volume of 100% acetone and sonicated for 30-60 sec in a ice slurry to reduce heating. Tubes are wrapped in aluminum foil, placed in freezer – 20°C and extracted overnight 12 hr. After extraction the supernatant is filtered and dispensed into a fresh vial.

Fluorescence spectrum of chl a of the UV-B treated and UV-B untreated *S. platensis* were studied at an excitation wavelength of 435 nm using a fluorescent spectrophotometer (F-5000, Hitachi, Japan) having continuous light supply of tungsten lamp at room temperature.

**Results and Discussion:**

Morphological studies of cyanobacteria would be of great importance in better understanding the biology and diversity of these organisms, and in providing insights into their ecological responses. In present investigation morphological examinations of *S. platensis* were carried through bright field, fluorescent and scanning electron microscopy. A bright field microscopy shows significant morphological difference in terms of granulation, pigmentation and both apical and terminal end of filaments in UV-B treated *S. platensis* (Fig. 1 a, b). Fluorescent microscopy (Fig. 2 a, b) revealed that fluorescent emission of pigment was higher in UV-B untreated *S. platensis* as compared to UV-B treated cells due to the high level pigments. The surface scanning electron micrograph (Fig. 3 a) shows the smooth morphological structure with the appearance of ridges due to coverage of sheath of control *Spirulina* but UV-B treated *Spirulina* shows distorted and straight morphological structure (Fig. 3b).

**Table 1** Specific growth (µh$^{-1}$), dry weight (g/l), protein (%), chl a (%), carbohydrate (%), NR (µmol NO$_3$ - produced mg-1 protein min$^{-1}$) and NiR (µmol NO$_2$ - produced mg-1 protein min$^{-1}$) activities of *S. platensis* under UV-B treated and untreated conditions

| Parameters            | UV-B Untreated | UV-B treated |
|-----------------------|----------------|--------------|
| Specific growth rate  | 0.065          | 0.049        |
| Dry weight            | 1.251          | 1.10         |
| Chlorophyll content   | 1.82           | 1.41         |
| Protein content       | 61.0           | 51.5         |
| Carbohydrate content  | 21.97          | 34           |
| NR activity           | 8              | 3.8          |
| NiR activity          | 5              | 2.25         |
Fig. 1 Bright field microscopically examination of a) Control b) UV-B treated *S. platensis*

![Fig. 1](image1.png)

Fig. 2 Fluorescent microscopically examination a) Control b) UV-B treated *S. platensis*

![Fig. 2](image2.png)

Fig. 3 Scanning electron micrograph of a) Control b) UV-B treated *S. platensis*

![Fig. 3](image3.png)
Earlier studies also suggested that UV-B treatment damaged photosynthetic light-harvesting complex of cyanobacteria and also affect the ultrastructure (Kaiqin and Alexander, 1996; Holzinger and Lutz, 2006). Morphological changes in cyanobacteria *Anabaena cylindrica* under NaCl stress were also recorded by Bhadauriya *et al.*, (2007). Hongyan *et al.*, (2005) performed similar studies where UV-B radiation not only affected photosynthesis but also altered the morphological development of filamentous cyanobacteria.

Data in each column are mean from two independent experiments with four replicates each. The maximum variation from the mean value was 5%.

It is evident from the data of Table 1 that specific growth, dry weight, Chl a, protein content, NR and NiR activity decreased respectively under UV-B stress but carbohydrate content was increased. Nitrate uptake was found to be more as compared to nitrite under similar conditions and UV-B treatment inhibited the both NR and NiR activity by more than 50% in UV-B treated cells of *Spirulina platensis*. Vonshak (1997) recorded that protein content and chlorophyll content is reduced under stress. Stress cells have a lower biosynthesis capacity for protein but higher biosynthesis capacity for carbohydrate (Tomasselli *et al.*, 1987). Abomohra *et al.*, (2016) reported that low doses of gamma radiation stimulated carbohydrates contents and inhibited protein production in *Arthrospira platensis*. Carbohydrates are used as cellular energy source and consumed more under stresses. Rai and Rai (1997) also suggested that high light stress induced alterations in the nitrogen assimilatory enzymes in *Spirulina*. Growth promoting and inhibiting effect of carbohydrates secreted as extracellular substances by some species of cyanobacteria was reported by Safonova and Reisser (2005).

The fluorescence emission spectrum of the chla was monitored in *Spirulina* cells at room temperature (Fig. 4) demonstrate that prolonged exposure of *Spirulina* cells to moderate levels of UV-B significantly altered the fluorescence emission spectral profile of pigment of *S. platensis*. Vonshak *et al.*, (1994) demonstrated that fluorescence

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**Fig. 4** Fluorescence emission spectra of the chla. a) Control b) UV-B treated *S. platensis*
measurements can be used as a fast reliable indication for photoinhibition in algal cultures. Lesser, (2008) reported that morphology, cell differentiation, growth, survival, pigmentation, and N2 metabolism, affected by UV. Physiological responses at cellular level in cyanobacterial populations affected by UV radiation is important to understand the connection of environmental changes and persistence of species in aquatic ecosystems.

Acknowledgements

We would like to thank Defense Research Development Establishment, Gwalior, M.P., India for providing instrumentation facility and Prof. N. K. Sah, Head, Department of Biotechnology, MITS, Gwalior, M.P., India for his support.

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