Int.J.Curr.Microbiol.App.Sci (2020) *9*(8): 3255-3261

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 8 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.908.372

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

Radha Gupta[1*] and P.S. Bisen[2]

[1]Department of Plant molecular Biology and Biotechnology, College of Agriculture, RVSKVV, Gwalior, M.P., India
[2]School of Biotechnology, Jiwaji University, Gwalior, India

**Corresponding author*

Keywords

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

Article Info

Accepted:
24 July 2020
Available Online:
10 August 2020

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.

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 ± 2^0C 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 rpmfor10 min) and prefixed in culture medium with an equal volume of 1% glutraldehyde in phosphate buffer. Cell were allowed to stand for 30 min on ice, pelleted, suspended in phosphate buffer with 2% glutraldehye and incubate for 1h at room temperature. Sample were washed with phosphate buffer and post fixed in 1% osmium tetraoxide 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^oC 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.

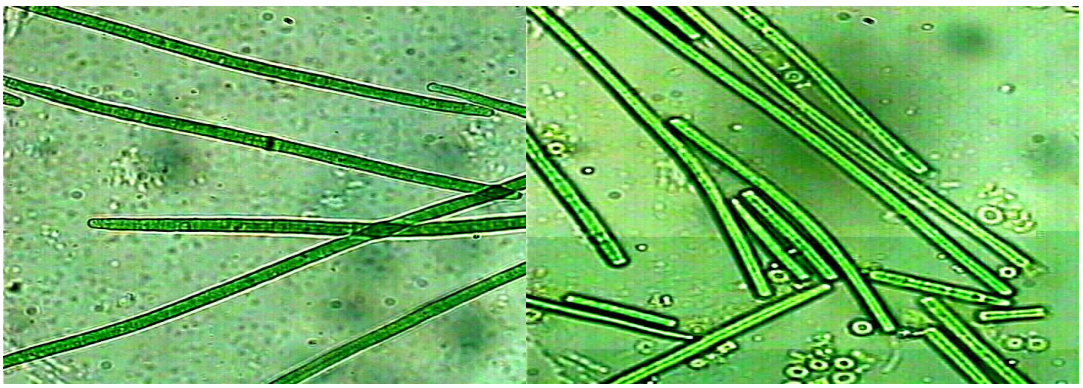
Results and Discussion:

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 filamentsi n 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

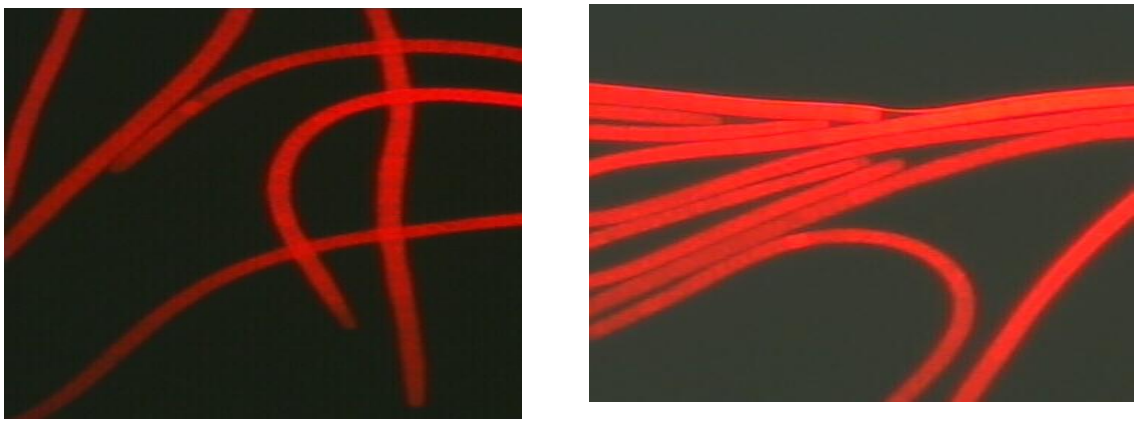
Parameters	UV-B Untreated	UV-B treated
Specific growth rate	0.065	0.049
Dry weight	1.25l	1.10
Chlorophyll content	1.82	1.41
Protein content	61.0	51.5
Carbohydrate content	21.97	34
NR activity	8	3.8
NiR activity	5	2.25

Fig.1 Bright field microscopically examination of a) Control *b)* UV-B treated *S. platensis*



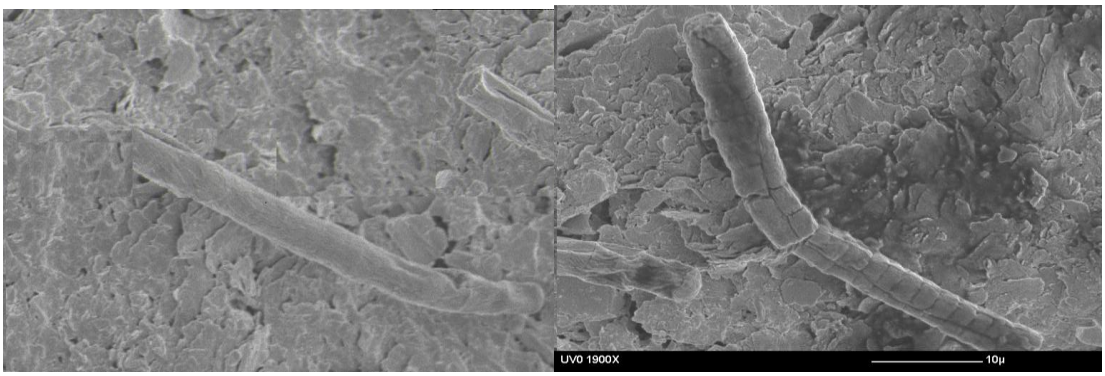
(a) **(b)**

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



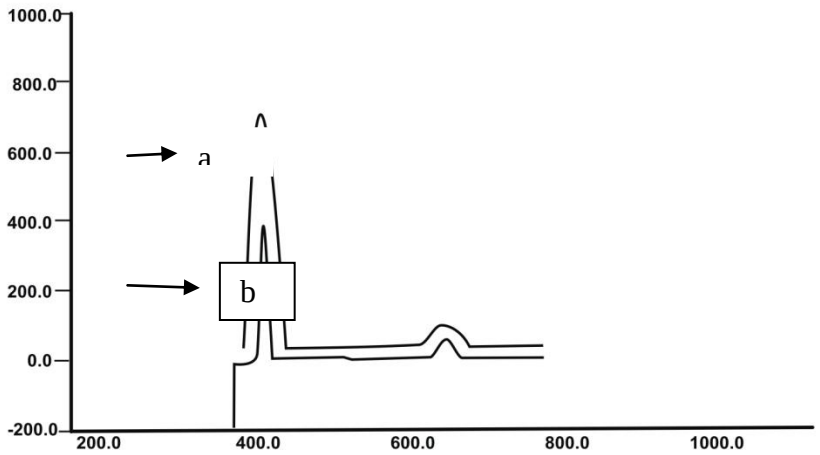
(a) (b)

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



(a) **(b)**

Fig.4 Fluorescence emission spectra of the chla a) Control *b)* UV-B treated *S. platensis*



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 (Tomaselli *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

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.

References

Abomohra, AEl-Fatah., Shouny, El- Wagih and Sharaf, Mona, Eleneen, MA Effect of Gamma Radiation on Growth and Metabolic Activities of *Arthrospira platensis* Braz. arch. biol. technol. vol.59 e16150476 (2016)

Bhadauriya P, Gupta R, Singh S and Bisen PS Physiological and biochemical alterations in a diazotrophic cyanobacterium *Anabaena cylindrica* under NaCl stress. Curr Microbiol 55:334–338 (2007).

Bisen PS, and Shanthy S Regulation of assimilatory nitrate reductase in the cyanobacterium *Anabaena doliolum*. Curr Microbiol 23:239–244 (1991).

Crutzen, P. J. (1992) Ultraviolet on the increase. Nature 356:104-105

Donkor, V., and D. P. Häder. Effects of solar and ultraviolet radiation on motility, photo movement and pigmentation in filamentous, gliding cyanobacterium. FEMS Microbiol. Ecol. 86:159-168 (1991).

Ferreira, K. N., T. M. Iverson, K. Maghlaoui, J. Barber, and S. Iwata. Architecture of the photosynthetic oxygen-evolving center. Science 303:1831-1838 (2004).

Gupta R., Bhadauriya P., Chauhan V.S. and P.S. BisenImpact of UV-B radiation on thylakoid membrane and fatty acid profile of *Spirulina platensis*. *Curr. Microbiol*., 56(2):156-161(2008).

Gupta,R. (2018) Evaluation of n-alkane contents in *Spirulina platensis* under UV-B stress by GC-MS. Int. J. Pure App. Biosci. 6 (3): 637-642.

Gupta,R. and Bisen, P.S. (2018) Optimization of biomass production of *Spirulina platensis* International Journal on Agricultural Sciences 9 (1) : 14-17.

Häder, D. P. (2000) Effects of solar UV-B radiation on aquatic ecosystems. Adv. Space Res. 26:2029-2040

Holzinger, AandLütz, C. Algae and UV irradiation: effects on ultrastructure and related metabolic functions Micron.; 37(3): 190-207. Epub 2005 Nov 28 (2006).

Hongyan W, Kunshan G, Virginia EV, Watanabe T, Walter HE Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis*. Appl Environ Microbiol 71:5004–5013 (2005)

Jadaun, P., Yadav, D., Bisen Prakash. *Spirulina platensis* prevents high glucose-induced oxidative stress mitochondrial damage mediated apoptosis in cardiomyoblast. *Cytotechnology* DOI 10.1007/s10616-017-0121-4 (2017).

Kaiqin, L and Alexander, N.G Ultraviolet-B photodestruction of a light-harvesting omplex. Proc. Natl. Acad. Sci. USA Vol. 93, pp. 5258-5263 (1996).

Kerr, J., and C. McElroy. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science262:1032-1034 (1993).

Kulshrestha, A. Zacharia, A., Jarouliya, U., Bhadauriya, P., Prasad, GBKS., Bisen P.S. Spirulina in health care management. *Curr. Pharma. Biotech.* 9,

400-405 (2008).

Lesser, M.P., Effects of ultraviolet radiation on productivity and nitrogen fixation in the cyanobacterium, *Anabaena* sp. (Newton's strain). *Hydrobiologia*598, 1-9 (2008).

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ Protein measurement with folin phenol reagent. J BiolChem193:265–275 (1951).

Mackinney, G. Absorption of light by chlorophyll solutions. J Biol Chem 140:315–322 (1941).

Rai, P.K. and Rai, L.C. Interactive effect of UV-B and Cu on photosynthesis, uptake and metabolism of nutrient in green alga *Chlorella vulgaris* under stimulated ozone column. J. Gen. Appl. Microbiol. 43 (5): 281-288 (1997).

Rastogi R, Prasad RP, Sinha SH, Moh TK, Lee S, Kottuparambil YJ, Kim JS, Rhee EM, Choi MT, Brown DP, Häder TH, Ultraviolet radiation and cyanobacteria Journal of Photochemistry and Photobiology B: Biology Volume 141pp 154-169(2014).

Safonova, E. and Reisser, W. Growth promoting and inhibiting effects of extracellular substances of soil microalgae and cyanobacteria on *Escherichia coli* and *Micrococcus luteus*. *Phycol. Res*., 53: 189 – 193 (2005).

Sarah A. Rashed AMM, Ibrahim AB, Gehan A, El-Gaaly, AS AS. and Ahmed ME, Evaluation of radical scavenging system in two microalgae in response to interactive stresses of UV-B radiation and nitrogen starvation Saudi J Biol Sci. 2016 Nov; 23(6): 706–712. Published online 2016 Jul 18. doi: 10.1016/j.sjbs.2016.06.010 (2016).

Singh, B.B., Pandey, P.K., Singh, S. and Bisen, P.S. Regulation of nitrate uptake and nitrite efflux in the *Cyanobacterium nostoc* MAC. J. Basic microbiol. 36: 433-438 (1996).

Singh, S. P., Hader, D. P., and Sinha, R. P. Cyanobacteria and ultraviolet radiation (UVR) stress: mitigation strategies. *Ageing Res. Rev*. 9, 79–90. doi: 10.1016/j.arr.2009.05.004 (2010).

Sinha, R. P., H. D. Kumar, A. Kumar, and D. P. Häder. Effects of UV-B irradiation on growth, survival, pigmentation and nitrogen metabolism enzymes in cyanobacteria. Acta Protozool. 34:187-192(1995).

Tomaselli L., Torzillo G., Giovannetti L, Pushparaj B., Materassi R. Recent research on Spirulina in ItalyHydrobiologia 151/152:79-82 M. A. Ragan and C. J. Bird (eds) 79Twelfth International Seaweed Symposium (1987).

Vonshak A *Spirulina platensis* (Arthrospira): physiology, cell biology and biotechnology. Taylor & Francis, London. ISBN 0-7484-0674-3(1997).

Vonshak, A.,Torzillo, G. and Tomaseli, L Use of chlorophyll fluorescence to estimate the effect of photoinhibition in outdoor cultures of *Spirulina platensis* Journal of Applied Phycology 6 (1): pp 31–34 (1994).

Xiong, F., J. Komenda, J. Kopecky, and L. Nedbal. Strategies of ultraviolet-B protection in microscopic algae. Physiol. Plant. 100:378-388 (1997).

How to cite this article:

Radha Gupta and Bisen, P.S. 2020. Physiological and Biochemical Changes in *Spirulina platensis* under UV-B Stress. *Int.J.Curr.Microbiol.App.Sci.* 9(08): 3255-3261.
doi: https://doi.org/10.20546/ijcmas.2020.908.372