*Journal of Photoscience* (2002), Vol. 9(2), pp. 439–441

# Impacts of Ultraviolet-B Radiation on Rice-Field Cyanobacteria

Rajeshwar P. Sinha* and Donat-P. Häder
Institute for Botany 1, Friedrich-Alexander University, Staudtstr. 5, D-91058 Erlangen, Germany

Cyanobacteria are the dominant microflora in rice-fields, contributing significantly to fertility as a natural biofertilizer. Recent studies show a continuous depletion of the stratospheric ozone layer, and the consequent increase in solar UV-B (280-315 nm) radiation reaching the Earth's surface. UV-B radiation causes reduction in growth, survival, protein content, heterocyst frequency and fixation of carbon and nitrogen in many cyanobacteria. UV-B induced bleaching of pigments, disassembly of phycobilisomal complexes, thymine dimer formation and alterations in membrane permeability have also been encounterd in a number of cyanobacteria. However, certain cyanobacteria produce photoprotective compounds such as water soluble colorless mycosporine-like amino acids (MAAs) and the lipid soluble yellow-brown colored sheath pigment, scytonemin, to counteract the damaging effects of UV-B. Cyanobacteria, such as *Anabaena* sp., *Nostoc commune*, *Scytonema* sp. and *Lyngbya* sp. were isolated from rice fields and other habitats in India and screened for the presence of photoprotective compounds. A circadian induction of the synthesis of MAAs by UV-B was noted in a number of cyanobacteria. Polychromatic action spectra for the induction of MAAs in *Anabaena* sp. and *Nostoc commune* also show the induction to be UV-B dependent peaking at 290 nm. Another photoprotective compound, scytonemin, with an absorption maximum at 386 nm (also absorbs at 300, 278, 252 and 212 nm), was detected in many cyanobacteria. In conclusion, a particular cyanobacterium having photoprotective compounds may be a potent candidate as biofertilizer for crop plants.

**Key words:** rice-field cyanobacteria, mycosporine-like amino acids, action spectrum, scytonemin, ultraviolet-B radiation

## INTRODUCTION

There is mounting evidence that the solar flux of UV-B has increased at the Earth's surface due to the depletion of the stratospheric ozone layer by anthropogenically released atmospheric pollutants such as chlorofluorocarbons (CFCs) [1]. Cyanobacteria with a cosmopolitan distribution are the most common photosynthetic prokaryotes on Earth playing an important role as atmospheric nitrogen fixers in both aquatic as well as terrestrial ecosystems. The role of cyanobacteria as natural biofertilizers in rice paddy fields is well documented [2]. Light is one of the most important factors determining the growth of cyanobacteria in their natural habitats. As biologically effective doses of UV-B radiation can penetrate deep to ecologically significant depths in natural waters, the observed increases in surface UV-B radiation may adversely affect the productivity of cyanobacteria [3]. UV-B has the potential to cause wide ranging effects, including alteration in the structure of proteins, DNA and other biologically relevant molecules, chronic depression of key physiological processes leading to either reduction in growth and cell division rates or death of the organism [2,3].

*To whom correspondence should be addressed.
E-mail : r.p.sinha@gmx.net

Although cyanobacteria are more or less susceptible to UV-B, they are not defenseless. They can limit the damage of DNA and other chromophoric molecules by synthesizing photoprotective compounds such as mycosporine-like amino acids (MAAs) and scytonemin [4,5]. Below we discuss the impacts of UV-B radiation on rice-field cyanobacteria and the photoprotective compounds synthesized by them in order to mitigate the negative effects of UV-B.

## IMPACTS OF UV-B ON CYANOBACTERIA

*Survival and growth.* The survival and growth of several cyanobacteria was found to be severely affected following UV-B irradiation for different durations. Growth and survival cease within 120 - 180 min of UV-B irradiation, depending upon the species type. Strains such as *Scytonema* sp. and *Nostoc commune*, filaments of which are embedded in mucilagenous sheaths, were more tolerant in comparison to filaments which do not have such coverings such as *Anabaena* sp. and *Nostoc* sp. [6].

*Pigmentation.* The effects of UV-B on pigmentation of various cyanobacteria have revealed that all types of photosynthetic pigments such as chlorophyll *a* (absorption maxima at 437 and 672 nm) as well as the accessory light

harvesting pigments such as phycocyanin (620 nm) and phycoerythrin (560 nm) are susceptible to UV-B. In some cases the accessory light harvesting pigment, phycocyanin, was bleached more rapidly and drastically than any other pigment such as Chl *a* or the carotenoids [6]. A decrease in the phycobiliprotein contents and the disassembly of phycobilisomal complexes following UV-B irradiation was recorded in a number of cyanobacteria, indicating impaired energy transfer from the accessory light harvesting pigments to the photosynthetic reaction centers [7].

*Heterocysts and enzymes of nitrogen metabolism.* Differentiation of vegetative cells into heterocysts was severely affected by UV-B in a number of cyanobacteria. Major heterocyst polypeptides of around 26, 54 and 55 kDa were decreased following UV-B irradiation, suggesting that the multilayered thick wall of heterocysts may be disrupted resulting in the inactivation of the nitrogen fixing enzyme nitrogenase [8]. UV-B induced membrane disruption leading to changes in membrane permeability and release of $^{14}C$-labelled compounds have been observed in a number of cyanobacteria [9]. UV-B induced inactivation of the nitrogen-fixing enzyme nitrogenase was observed in many cyanobacteria [8].

*Photosynthetic enzymes.* The activity of the ribulose 1,5-bisphosphate carboxylase (RuBISCO) was inhibited by UV-B irradiation in a number of cyanobacteria which may be due to protein destruction or enzyme inactivation [13]. UV-B-induced inhibition of $^{14}CO_2$ uptake was recorded in various rice-field cyanobacteria which could be due to the effect on the photosynthetic apparatus leading to the reduction in the supply of ATP and $NADPH_2$ [8]. UV-B causes opening of the membrane-bound calcium channels in the cyanobacterium *Anabaena* sp. [10].

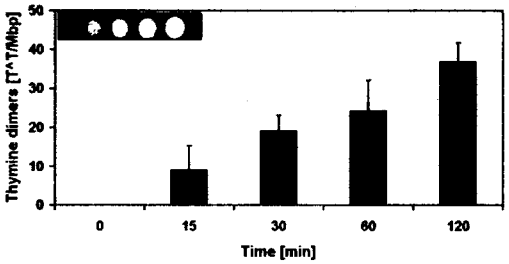
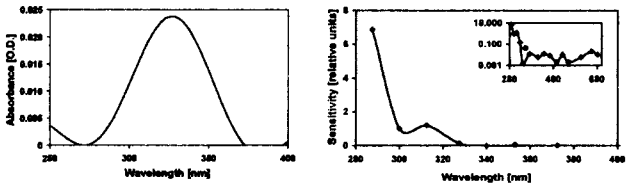



**Figure 1** UV-B induced DNA thymine dimer formation in *Nostoc* sp. as detected by the dot-blot and chemiluminescence method using thymine dimer specific antibody.

*Protein and DNA damage.* Total protein profiles of several cyanobacteria as evidenced by SDS-PAGE show a decrease in protein content with increasing UV-B exposure time, indicating that cellular proteins are among the main targets of UV-B [6,8]. UV-B-induced formation of thymine dimers (Fig. 1), the most cytotoxic and mutagenic lesions was recorded in many cyanobacteria [11].

## PHOTOPROTECTIVE COMPOUNDS

*Mycosporine-like amino acids (MAAs).* MAAs are water-soluble substances characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol, having absorption maxima ranging from 310 to 360 nm and an average molecular weight of around 300 [4-5]. In cyanobacteria MAAs prevent 3 out of 10 photons from hitting cytoplasmic targets. Cells with high concentrations of MAAs are approximately 25 % more resistant to UV radiation centered at 320 nm than those with no or low concentrations [12]. Experiments with rice-field cyanobacteria, *Anabaena* sp., *Scytonema* sp. and *Nostoc commune*, have revealed the existence and circadian induction by UV-B radiation of a single MAA, shinorine, with an absorption maximum at 334 nm (Fig. 2a) [13]. Polychromatic action spectrum for the induction of MAAs in *Anabaena* sp. and *Nostoc commune* had a single prominent peak at 290 nm (Fig. 2b) [14].




**(a)** **(b)**

**Figure 2.** (a) Absorption spectrum of the MAA shinorine, (b) polychromatic action spectrum for the induction of shinorine in *Nostoc commune*.

*Scytonemin.* It is a yellow-brown, lipid soluble dimeric pigment located in the extracellular polysaccharide sheath of some cyanobacteria. It has a molecular mass of 544 Da and a structure based on indolic and phenolic subunits. Purified scytonemin has an absorption maximum at 386 nm, but it also absorbs significantly at 252, 278 and 300 nm (Fig. 3) [15]. Strong evidence for the role of scytonemin as UV-shielding compound has been presented in several cyanobacterial isolates and collected materials from various harsh habitats, mostly exposed to high light intensities [16]. Studies indicate that the incident UV-A radiation entering the cells may be reduced by around 90 % due to the presence of scytonemin in the cyanobacterial sheaths [17]. Once synthesized, it remains highly stable and carries out its screening activity without further metabolic investment from the cell.

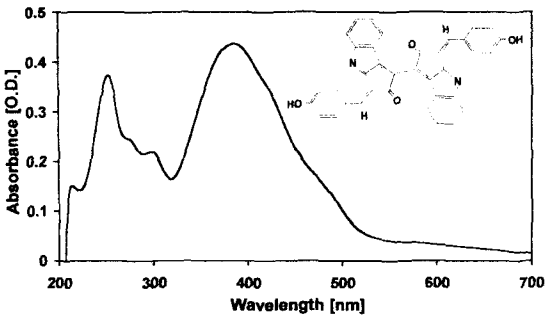



**Figure 3.** Absorption spectrum and molecular structure of the cyanobacterial sheath pigment, scytonemin.

Rapid photodegradation of scytonemin does not occur which is evidenced by its long persistence in terrestrial cyanobacterial crusts or dried mats. This strategy may be invaluable to several scytonemin containing cyanobacteria inhabiting harsh habitats, where they experience intermittent physiological inactivity (e.g., desiccation). During these metabolically inactive periods, other ultraviolet protective mechanisms such as active repair or biosynthesis of damaged cellular components would be inaffective.

## CONCLUSIONS

Increases in UV-B radiation are likely to induce changes in cyanobacterial community composition since there are differential susceptibility of species to UV-B-induced damage. Species having the ability to synthesize UV protective compounds will likely be favored. A particular cyanobacterium capable of synthesizing photoprotective compounds may be a potent candidate for use as biofertilizer in tropical rice-growing countries.

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