

Biophysical Characterization of Scytonemin: A Sunscreening Pigment in Cyanobacterial Biofilms

A. Pandey and R. P. Sinha

Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005, India

Email: pandeyabhabhu@gmail.com, rpsinhabhu@gmail.com

(Received September 05, 2019)

Abstract: Scytonemin is a natural photoprotectant against high intensity ultraviolet radiation (UVR) which has been reported in more than 300 cyanobacteria. Cyanobacteria are Gram-negative prokaryotic microorganisms that constitute the greatest biodiversity in terrestrial, freshwater and marine environments. Cyanobacteria are exposed to potentially lethal levels of solar ultraviolet radiation and accumulate photoprotective compound (PPC) scytonemin in their extracellular polysaccharide sheath to counteract the damaging effects. UV-B radiation has deleterious effects on physiological and biochemical processes of living organisms. In this study, cyanobacterial epilithic biofilms collected from diverse habitat were screened to detect the presence of scytonemin. Scytonemin was partially separated and characterized by using high performance liquid chromatography [Retention time: 4.8 min]. Thereafter, scytonemin was further characterized by using various spectroscopic techniques such as UV-Vis [$UV\lambda_{max}$: 386 nm]; electrospray ionization-mass spectrometry [ESI-MS] ($[M+H]^+$ m/z: 545.2); and Fourier Transform Infrared (FTIR) [3431 cm^{-1} , 2940 cm^{-1} , 2920 cm^{-1} , 1585 , 1385 , 1173]. The photoprotective capability of scytonemin against UV-B radiation was assessed on survivability of *Escherichia coli*. Our results suggest that scytonemin plays a crucial role in the better survival of cyanobacteria in stressed habitats facing harsh environmental conditions. Scytonemin is an eco-friendly natural sunscreen with potential for dermatological or biomedical applications and a safe alternative to synthetic UV sunscreens.

Keywords: Cyanobacteria, *Scytonema*, photoprotection, scytonemin, ultraviolet radiation.

1. Introduction

The Cyanobacteria are phylogenetically a primitive and diverse group of Gram-negative prokaryotes possessing oxygenic photosynthesis, which is similar to that of algae and higher plants. The existence of cyanobacteria on the Earth dates back to 2.8 to 3.5×10^9 years ago during the Precambrian era. At that time they might have been the main primary producers of organic matter and the first organisms to release oxygen into the then oxygen-free atmosphere. Member of cyanobacteria also possess a central position in nutrient cycling largely owing to their inherent capacity to fix atmospheric N_2 with the help of the enzyme nitrogenase directly into ammonium (NH_4^+), a form through which nitrogen enters the food chain. Cyanobacteria are thought to have survived from a wide spectrum of environmental stresses such as extreme temperatures, drought, salinity, nitrogen starvation, photooxidation, anaerobiosis, osmotic and ultraviolet radiation stress¹. Members of cyanobacteria are also unique in having a cosmopolitan distribution from hot springs to arctic regions and are therefore expected to face various levels of ultraviolet radiation (UVR) in their natural habitat². Recent findings suggest that continuous accumulation of anthropogenically released atmospheric pollutants, such as chlorofluorocarbons (CFCs), chlorocarbons (CCs), organobromides (OBs) and reactive nitrogen species (RNS) including nitric oxide (NO), nitrous oxide (N_2O) and peroxyxynitrite (ONOO) in the stratosphere has resulted in the depletion of ozone layer which provides shield against UVR (100-400 nm)³⁻⁵. The decreased density of ozone layer results in increased intensity of harmful UV-B (280-315 nm) radiation reaching on the Earth's surface.^{6,7} UV-B radiation (<1% of total irradiance), being the most detrimental component of the solar spectrum, has the potential to exert adverse effects on photosynthetic terrestrial and aquatic organisms, thereby affecting the productivity of ecosystems⁸⁻¹⁰.

Harvesting of solar radiation exposes cyanobacteria simultaneously to harmful doses of UV-B and UV-A (315-400 nm) radiations in their natural habitats. The UV-B radiation has the greatest potential for cell damage caused by both direct effects on DNA and proteins and indirect effects *via* the production of reactive oxygen species (ROS). There are several targets for the potentially toxic ROS including lipids, DNA and proteins. Moreover, damage to the photosynthetic apparatus is also partially mediated by ROS resulting in inhibition of photosynthesis¹¹. Investigations have shown that UV-B irradiation adversely affects key physiological and biochemical life processes, such as morphology, cell differentiation, survival, growth,

pigmentation, motility and orientation, N₂ metabolism, phycobiliproteins composition, protein profile, DNA, CO₂ uptake and membrane permeability of various photosynthetic organisms as they are simultaneously exposed to visible and UVR in their natural habitats¹².

Cyanobacteria have evolved a number of mitigation strategies to overcome the damaging effects of UVR. These strategies include avoidance, scavenging of UV-induced ROS by enzymatic and non-enzymatic antioxidants¹³⁻¹⁵, and screening by certain UV-absorbing compounds that counteract the deleterious effects of UVR. Besides this, repair of UV-induced damage of DNA by photoreactivation and excision repair and resynthesis of proteins are also important mechanisms to prevent UV-induced photodamage^{16,17}. Certain photoprotective metabolites such as mycosporine-like amino acids (MAAs) and scytonemin play significant roles in photoprotection against UVR¹⁸.

Scytonemin is a highly stable, lipid-soluble, non-fluorescent, yellow-brown-coloured pigment that is accumulated in the extracellular polysaccharide sheath of different cyanobacteria. Scytonemin is an indole alkaloid constitute of two heterocyclic units symmetrically linked through an olefinic carbon-carbon bond. The special structure location in a cell as well as strong absorption maxima (370 nm *in vivo*) in UV-A-violet-blue region makes scytonemin a unique among natural products. Purified scytonemin shows absorption maxima at 386 nm, additionally, it also shows significant absorbance at a wavelength of 300, 278 and 252 nm. The photoprotective property of scytonemin is due to its large extinction coefficient ($\epsilon = 112.6 \text{ L g}^{-1} \text{ cm}^{-1}$ at 384 nm). Conventionally, scytonemin is a well-established photoprotective compound (PCC) against ultraviolet radiation. The accumulation of UV-screening compound scytonemin in the cyanobacterial sheath clearly represents a strategy adopted by several cyanobacteria to protect their cellular components against the harmful effects of UV radiation, which, especially the highly energetic UV-B radiation (280-315 nm).

Some cyanobacteria produce sunscreen scytonemin in response to UV-A induction¹⁹. Other abiotic stress factors such as salinity, heat, osmotic and oxidative stress alone or in addition to UV stress can also affect the biosynthesis of scytonemin²⁰. The genomics of scytonemin biosynthesis was studied in *Nostoc punctiforme* which indicated the involvement of 18 co-transcribed genes (NpR1276 to NpR1259). The construction of a scytonemin less mutant strain, *Nostoc punctiforme* ATCC 29133 (PCC 73102) by Soule et al. (2007), paved the way for thorough understanding of

the genetics and biosynthesis of this compound at molecular level²¹. Further analysis of these 18-gene clusters revealed some open reading frames (ORFs) such as six of these genes, *scy ABCDEF* (NpR1276 to NpR1271) are directly involved in biosynthesis of scytonemin and in *N. punctiforme*, NpR1273 plays central role in its biosynthesis in cytoplasm. In the early stages of scytonemin assembly, the involvement of *ScyA*, *ScyB*, and *ScyC* was effectively demonstrated *in vitro*²². The protein encoded by *scyE* is essential for catalyzing the oxidative dimerization of monomer moiety in the periplasm to finally produce the scytonemin molecules. Klicki et al. (2018) have reported that the Ebo complex is responsible for the transportation of monomer moieties of scytonemin from cytoplasm to periplasm²³.

Scytonemin acts as an antioxidant by preventing cellular damage occurring from ROS produced upon UVR exposure²⁴. Owing to its efficient UV-absorbing ability as well as its antiproliferative and non-toxic properties, scytonemin finds immense importance in cosmetic and pharmaceutical industries. Scytonemin is an interesting candidate for a sunscreen because of its outstanding UV-absorption coupled with a high photostability and non-toxicity. Scytonemin is an eco-friendly natural sunscreen with potential for dermatological or biomedical applications and a safe alternative to synthetic UV-sunscreens. In the present investigation, we have characterized the sunscreen scytonemin from the cyanobacterial epilithic biofilms dominated with *Scytonema* sp. This study may provide an insight for better selection of cyanobacteria to be used as a source for natural UV sunscreen compounds.

2. Materials and methods

2.1 Experimental organism and growth conditions: The cyanobacterium *Scytonema* sp. was collected from the cyanobacterial epilithic biofilms (Figure 1 A) on the surface of the rock around the hills region of Lakhaniya Dari, Mirzapur, Uttar Pradesh, India. Identification of the cyanobacterium was done with the help of standard monographs and taxonomic keys²⁵. *Scytonema* sp. is a member of family Scytonemataceae and an autotrophically growing heterocystous and filamentous cyanobacterium that has false branching in its filaments. The morphology of cyanobacterium was studied using a light microscope (CX21i, Olympus Corporation, Tokyo, Japan; Figure 1 B). *Scytonema* sp. was cultured utilizing standard microbiological techniques as done previously²⁶. Axenic culture of the cyanobacterium was grown in BG-11 medium²⁷ without nitrogen supplement at 20±2 °C with 14/10 light/dark cycle illuminated with cool

white fluorescent tubes (Philips TL 40 W/54, India) having photon flux density of $12 \pm 2 \text{ W m}^{-2}$ at the surface of vessels. For avoiding contamination, culture was sub-cultured at regular intervals and was hand-shaken five times daily. Exponentially growing cultures were used for all experiments having an initial optical density of 0.6 ± 2 at 750 nm.

2.2 Extraction and partial purification of scytonemin: The UV-absorbing biomolecule, scytonemin was extracted as described earlier¹⁸. Extraction of scytonemin was done by incubating the cyanobacterial biofilm (dry weight = 1 g) in 10 mL methanol: ethyl acetate (1:1 v/v) at 4°C overnight followed by sonication (2011-Sonic, cycle 30%, Power 40%) for 4 min. Centrifugation of resulting extract was done at 10,000g for 5 min and a vacuum evaporator was used for evaporating the supernatant at 38°C which was re-dissolved in methanol: ethyl acetate (1:1 v/v) (500 μL). Finally, the samples were filtered through 0.22 μm pore-sized sterilized microcentrifuge syringe-driven filter (Axiva Sicheem Biotech., New Delhi) before performing the UV/Vis and HPLC analyses.

2.3 Characterization of scytonemin:

2.3.1 UV-Vis spectroscopy: UV/Vis spectroscopic analysis between 200-700 nm was performed using a UV-Vis double beam spectrophotometer (U-2910, UV/VIS, Hitachi, Tokyo, Japan). The raw data were transferred to a microcomputer, and peaks were analyzed with the software provided by the manufacturer.

2.3.2 High-Performance Liquid Chromatography (HPLC): Scytonemin was purified using a reverse phase HPLC system (Waters 2998 with Photo diode-Array PDA detector, auto-injector 717 plus, 515 PUMP, Milford, USA) which was equipped with Empower-2 software. The HPLC system had an ODS-2 (RP 18) column (Water, Spherisorb analytical column, 5 μm , 4.6 x 250 mm diameter, Ireland) along with guard (4.6 x 10 mm inside diameter). An auto-injector (Waters 717 plus autosampler) was used for injecting samples (10 μL) in the HPLC column. Elution was maintained at flow rate of 1.0 mL min^{-1} utilizing the mobile phase (solvent A: ultra-pure water; solvent B: acetonitrile-methanol-tetrahydrofuran, 75:15:10, v/v). Gradient elution program of 30 min was set which increase linearly with 0-15 min from 15% solvent A to 100% solvent B, and 15-30 min at 100% solvent B. The detection wavelength was set at 386 nm and PDA scan wavelength was set in the range of 250-750 nm. Identification of scytonemin was done through its characteristic absorption maxima (386 nm) in the solvent corresponding to the appropriate retention time (RT) and scytonemin concentration was determined using an extinction coefficient of

112.6 L g⁻¹cm⁻¹ at 384 nm²⁸. Thereafter, we collected the pure scytonemin containing fraction and evaporated it by using a rotovapor, and further dried in a vacuum manifold for 30 min to form a powder. The pure scytonemin was further characterized by liquid chromatography-electrospray ionization mass spectrometry (ESI-MS) and fourier Transform Infrared (FTIR) spectroscopy.

2.3.3 Electrospray Ionization-Mass Spectrometry (ESI-MS): HPLC purified scytonemin was collected for the production of protonated molecules by ESI. Amazon SL mass spectrometer (Bruker Daltonics Inc., Bremen, Germany) was utilized for recording mass spectra. A cone voltage of 30 V was found to induce the formation of (M+H)¹⁺ with a scan range from 100 to 600 *m/z*. Precursor ions were specifically selected for MS/MS based on previously determined retention times and *m/z* values. Other MS settings were: capillary voltage (5500 V) and temperature (300⁰C). Manual mode was selected for carrying out MS/MS with fragmentation *via* collision-induced dissociation (CID) of the precursor ion using He as the collision gas at 40 V. Precursor ions were selected within an isolation width of 2 u (mass-to-charge ratio) and accumulation of scans was done with varying RF signal amplitudes. Calibration of the *m/z* scale of MS was done utilizing the external calibration standard electrospray ‘tuning mix’ (Agilent Technologies, Santa Rosa, USA). The data were analyzed using the software Data Analysis 4.0 (Bruker Daltonics Inc., Bremen, Germany).

2.3.4 Fourier Transform Infrared (FTIR): Infrared (IR) transmission measurements were performed between 4000 and 650 cm⁻¹ with an FTIR/FIR spectrophotometer version 10 (Perkin Elmer, Waltham, MA, USA) and a KBr beam splitter. The scytonemin was fused with oven-dried potassium bromide (stored in desiccator) in a 1:100 ratio. The samples were presented in the form of a transparent KBr disc and the spectra were recorded at a resolution of 4 cm⁻¹ with 64 scans.

2.4 UV-induction experiment: The exponentially growing cells of cyanobacteria were exposed to artificial UVR 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, Ultraviolet-B TL 40 W/12 fluorescence tubes (Cat No. G15T8E, Sankyo Denki, Japan) emitting its main output at 312 nm and Ultraviolet-A TL 15 W fluorescence tubes (Philips ACITINIC BL 15W UVA tubes) emitting its main output at 365 nm. The distance of the UV- tube in the chamber from the sample was adjusted to have a UV- intensity of 1 and 3 Wm⁻² for

UV-B and UV-A radiations, respectively. During UV treatment, samples were simultaneously irradiated with cool white fluorescent light ($12 \pm 2 \text{ W m}^{-2}$). Homogeneous cyanobacterial cultures (800 mL) were exposed to UVR in eight sterile Petri dishes (with open lids) for the desired time intervals (12, 24, 36, 48, 60 and 72 h). Petri dishes were covered with 295, 320 and 395 nm cut-off filter foils (Ultraphan; Digefra, Munich, Germany) to ensure PAR+UV-A+UV-B (PAB), PAR+UV-A (PA) and PAR exposure respectively. All experimental cultures were exposed with constant temperature of $23 \pm 2^\circ\text{C}$ and were shaken at regular intervals during exposure to avoid heating effects and self-shading of the cells. Cultures irradiated with white fluorescent light served as control. Equal amount of cells were removed after 72 h of continuous exposure and analysed for scytonemin induction.

2.5 Photoprotective role of scytonemin: The role of scytonemin in photoprotection was assessed using the bacterium *Escherichia coli*. Different concentrations of partially purified scytonemin were added into 200 μL of freshly cultured *E. coli* cells suspension (OD, 0.8 at 600nm) and gently vortexed. The *E. coli* cells with and without scytonemin were transferred into 1.5 ml sterile Eppendorf microcentrifuge tube and irradiated with UV-B (0.4 W m^{-2}) for 20 min and thereafter 30 μL of each sample was plated in triplicate on sterile Petri dishes containing solid LB medium. All the samples were incubated at 37°C for 12 h and colonies of *E. coli* cells appearing on each Petri dish were counted by colony counter and percentage survival of *E. coli* was determined.

3. Results

3.1 Analysis of scytonemin: In the present investigation, HPLC chromatogram as well as absorption spectrum of *Scytonema* sp., extracted with 1:1 (v/v) methanol: ethyl acetate revealed the presence of a single prominent peak of scytonemin having RT of 4.8 min (Figure 2A) and UV absorption maximum ($\text{UV}\lambda_{\text{max}}$) at 386 nm (Figure 2B). The concentration of total scytonemin was 0.6 mg g^{-1} dry wt of cyanobacterial mat, as calculated by using an extinction coefficient of $112.5 \text{ L g}^{-1}\text{cm}^{-1}$ at 384 nm. Eluted scytonemin was collected for further characterization. ESI-MS data showed a prominent ion peak of the protonated molecule ($[\text{M}+\text{H}]^+$) at m/z 545.1 (Figure 3).



Figure 1. Collection of samples from cyanobacterial epilithic biofilms (shown by arrow) (A), Microphotographs of dominant cyanobacterium *Scytonema* sp. (B) having heterocyst and false branching (shown by arrow).

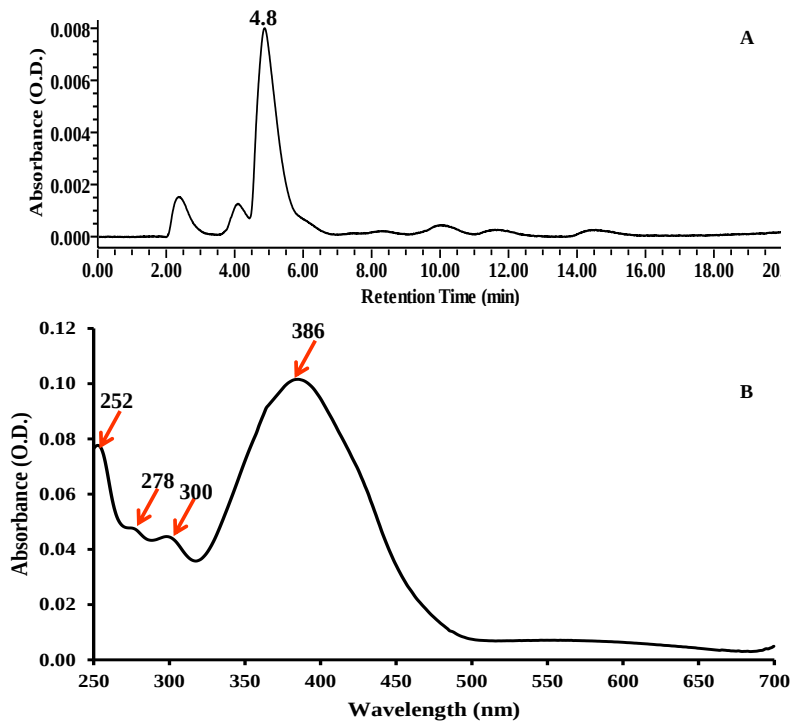


Figure 2. HPLC chromatogram and absorption spectrum of partially purified scytonemin.

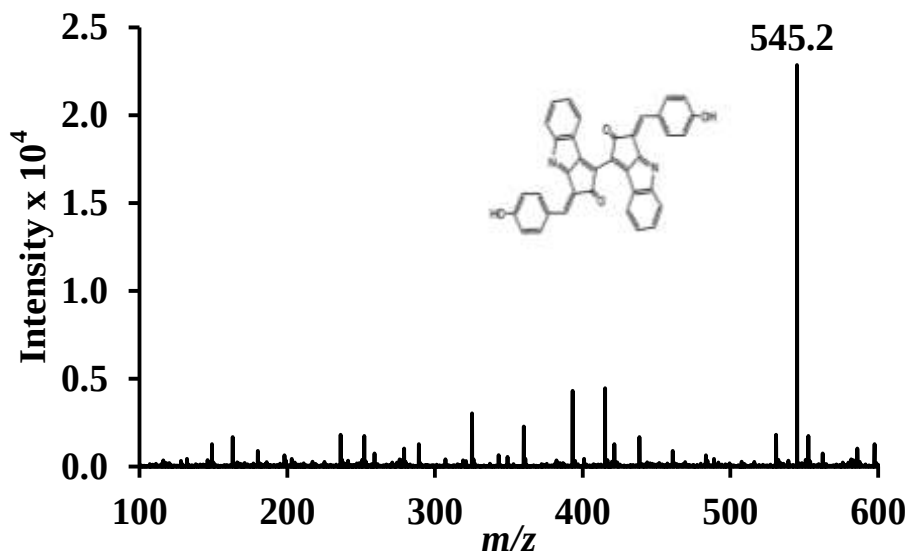


Figure 3. Mass spectrum of HPLC-purified scytonemin showing a prominent peak at m/z 545.2.

The FTIR spectrum of scytonemin (Figure 4) exhibited a broad absorption at 3431 cm^{-1} corresponds to the phenol group. Absorbance at 2940 cm^{-1} and 2920 cm^{-1} is typical of C-H stretching vibrations of alkanes. C-O stretching is generally observed at $1000\text{--}1320\text{ cm}^{-1}$. The spectral band at 1585 , 1513 , 1385 and 1173 cm^{-1} are tentatively assigned for (CCH) p-disubstituted aromatic ring, (N=C—C=C) ring mode, (CCN) indole ring and (CC) ring breathing pyrrole, respectively.

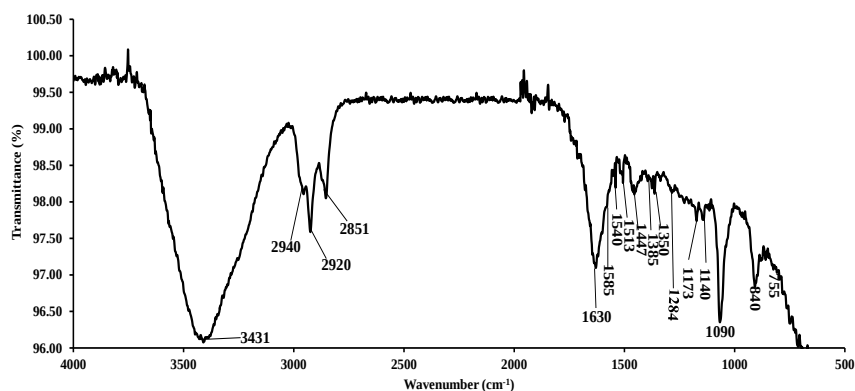


Figure 4. FTIR spectrum showing prominent spectral bands of scytonemin.

3.2 UV-Induction of scytonemin: Induction of scytonemin synthesis was observed under PAR, PA and PAB treatments (Figure 5). Significant ($P<0.05$) induction in scytonemin content was found under PAB exposure. Maximum induction was found in the cells irradiated under PAR+UV-A+UV-B (PAB), followed by PAR+UV-A (PA) and PAR in comparison to control in *Scytonema* sp. Induction increased in a dose-dependent manner and maximum scytonemin induction was found to be at 72 h of PAB treatment. After 72h scytonemin content remained constant, so we selected duration of 72 h for further studies.

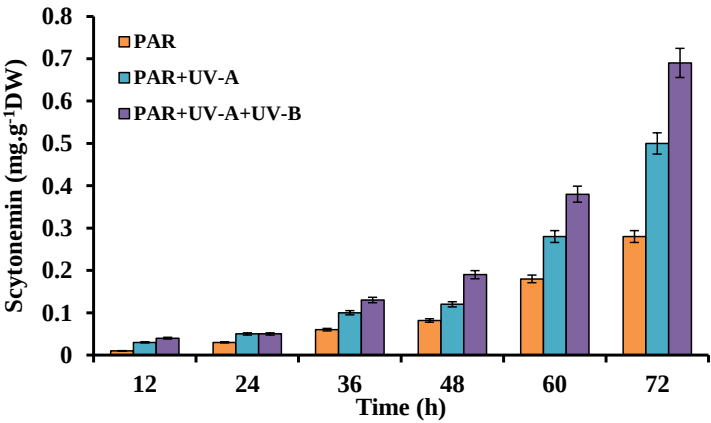


Figure 5. Scytonemin content in *Scytonema* sp. after different duration of exposure of PAR, PAR+UV-A, PAR+UV-A+UV-B.

3.3 Photoprotective role of scytonemin: Scytonemin from biofilms also exhibited efficient UV-photoprotective ability performed on *E. coli* cells (Figure 6). After UV irradiation, the survival rate of *E. coli* cells decreased to 21%. However, in the presence of different concentrations of scytonemin such as 0.2, 0.4 and 0.8 mM, the rates of survival of *E. coli* under UV radiation were decreased less than that of the control to about 25, 300, and 42 % respectively (Figure 6). The data obtained clearly indicate the efficacy of scytonemin in photoprotection against solar UV radiation.

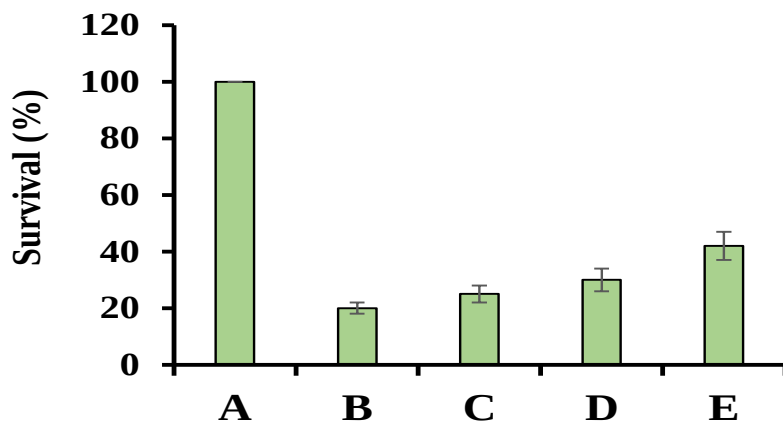


Figure 6. Photoprotective function of scytonemin on *Escherichia coli* against UV-B radiation. A, control sample (without any treatment); B (UV-B irradiated without scytonemin), C-E (UV-B irradiated without scytonemin. (C-E, 0.2, 0.4 and 0.8 mM). The error bars denote standard deviations of means (means $\pm$ SD, n = 3).

4. Discussion

Cyanobacteria are one of the most dominant photosynthetic prokaryotes in terms of ecological and economical point of view. In this study, we found that the phototrophic microbial biofilms were dominated by the cyanobacterial filaments of *Scytonema* sp. Cyanobacteria are capable of surviving in a broad range of harsh habitats including brightly lit environments having high UV fluxes. Aim of present study was to isolate and partially characterize scytonemin, the UV-screening cyanobacterial pigment from *Scytonema* sp. and to assess its UV-protective role against UV-B induced damages. HPLC chromatogram of scytonemin extracted from *Scytonema* sp. showed the presence of a prominent peak at RT of 4.8 min with a similar absorption maximum (UV λ_{max}) at 386 nm, which was consistent with previous reports²⁸. For quantification and identification of secondary metabolites such as scytonemin from cyanobacteria, extensive chromatographic separation of their extracts is required. In the mass spectrum of the extract from *Scytonema* sp. the presence of prominent ion at m/z 545.2 was found to be consistent with the data of scytonemin given in on-line MS spectra of extracts from cyanobacteria¹⁸.

The presence of UV-protecting pigment scytonemin and its UV-screening function have been demonstrated in several cyanobacterial strains

and varying levels of such UV-absorbing compounds in different species of cyanobacteria may be responsible for their different levels of sensitivity to UVR. The presence of sheath, antioxidative machinery and DNA repairing systems also determines such responses in cyanobacteria. However, UV-B + scytonemin had less deleterious effects in comparison to UV-B radiation alone, indicating the significant role of scytonemin. The photoprotective role of scytonemin has been demonstrated in a number of cyanobacteria inhabiting several extreme habitats, e.g. sub-aerial, mangrove, polar, etc.

5. Conclusion

Overall, increased solar UV radiation on the Earth's atmosphere may alter the normal physiology and biochemistry of all sun-exposed living systems including cyanobacteria, and any adverse effects of UV radiation on this dominant microflora could disrupt the balance of entire ecosystems. The biosynthesis of Scytonemin is an important defence mechanism adopted by cyanobacteria against short wavelength UV radiation. Moreover, scytonemin are ecologically important biomolecules having great potential in photoprotection by minimizing the cellular damage from UV-induced damages and help the cyanobacteria to thrive under harsh environments. Furthermore, due to their strong UV absorption characteristics, UV-induction and photoprotective capacity, these compounds may be used as an active ingredient in cosmetic and other pharmaceutical industries.

References

1. R. P. Sinha and D.-P. Häder, Photobiology and Ecophysiology of Rice Field Cyanobacteria, *Photochem Photobiol*, **64** (1996), 887-896.
2. R. Y. Stanier and G. Cohen-Bazire, Phototrophic Prokaryotes: the Cyanobacteria, *Ann Rev Microbiol*, **31** (1977), 225-274.
3. P. J. Crutzen, Ultraviolet on the Increase, *Nature*, **356** (1992), 104-105.
4. D. Lubin and E. H. Jensen, Effects of Clouds and Stratospheric Ozone Depletion on Ultraviolet Radiation Trends, *Nature*, **377** (1995), 710-713.
5. A. R. Ravishankara, J. S. Daniel and R. W. Portmann, Nitrous Oxide (N₂O): the Dominant Ozone-Depleting Substance Emitted in the 21st Century, *Science*, **326** (2009), 123-125.
6. D. J. Hofmann and T. Deshler, Evidence from Balloon Measurements for Chemical Depletion of Stratospheric Ozone in the Arctic Winter of 1989-90, *Nature*, **349** (1991), 300-305.

7. S. Madronich, R. McKenzie, R. Bjorn and M. Caldwell, Changes in Biologically Active Ultraviolet Radiation Reaching the Earth's Surface, *J Photochem Photobiol B Biol*, **46** (1998), 5-19.
8. W. F. Vincent and S. Roy, Solar Ultraviolet-B Radiation and Aquatic Primary Production: Damage, Protection, and Recovery, *Environ Rev*, **1** (1993), 1-12.
9. C. E. Williamson, What Role Does UV-B Radiation Play in Freshwater Ecosystems? *Limnol Oceanogr*, **40** (1995), 386-392.
10. S. P. Singh, D. P. Häder and R. P. Sinha, Cyanobacteria and Ultraviolet Radiation (UVR) Stress: Mitigation Strategies, *Ageing Res Rev*, **9** (2010), 79-90.
11. Y. Y. He and D. P. Häder, Reactive Oxygen Species and UV-B: Effect on Cyanobacteria, *Photochem Photobiol Sci*, **1** (2002) 729-736.
12. R. P. Sinha, S. Kumari and R. P. Rastogi, Impacts of Ultraviolet-B Radiation on Cyanobacteria: Photoprotection and Repair, *J Sci Res*, **52** (2008), 125-142.
13. E. M. Middleton and A. H. Teramura, Understanding Photosynthesis, Pigment and Growth Responses Induced by UV-B And UV-A Irradiances, *Photochem Photobiol*, **60** (1994), 38-45.
14. G. Singh, P. K. Babele, R. P. Sinha, M. B. Tyagi and A. Kumar, Enzymatic and Non-Enzymatic Defense Mechanisms Against Ultraviolet-B Radiation in Two *Anabaena* Species, *Process Biochem*, **48** (2013), 796-802.
15. J. Pathak, A. Pandey, P. K. Maurya, Rajneesh, R. P. Sinha and S. P. Singh, Cyanobacterial Secondary Metabolite Scytonemin: a Potential Photoprotective and Pharmaceutical Compound, *Proc Natl Acad Sci India Sect B Biol Sci*, (2019).
16. A. B. Britt, Repair of DNA Damage Induced by Ultraviolet Radiation, *Plant Physiol*, **108** (1995), 891-896.
17. S. T. Kim and A. Sancar, Photorepair of Nonadjacent Pyrimidine Dimers by DNA Photolyase, *Photochem Photobiol*, **61** (1995), 171-174.
18. R. P. Rastogi, R. P. Sinha, and A. Incharoensakdi, Partial Characterization, UV-Induction and Photoprotective Function of Sunscreen Pigment, Scytonem in from *Rivularia* Sp. HKAR-4, *Chemosphere*, **93** (2013), 1874-1878.
19. F. Garcia-Pichel and R. W. Castenholz, Characterization and Biological Implications of Scytonemin, a Cyanobacterial Sheath Pigment, *J Phyco*, **27** (1991), 395-409.
20. J. G. Dillon, C. M. Tatsumi, P. G. Tandingan and R. W. Castenholz, Effect of Environmental Factors on the Synthesis of Scytonemin, a UV-Screening Pigment, in a Cyanobacterium (*Chroococcidiopsis* Sp.), *Arch Microbiol*, **177** (2002), 322-331.
21. T. Soule, V. Stout, W. D. Swingley, J. C. Meeks and F. Garcia-Pichel, Molecular Genetics and Genomic Analysis of Scytonemin Biosynthesis in *Nostoc Punctiforme* ATCC 29133, *J Bacteriol*, **189** (2007), 4465-4472.
22. E. P. Balskus and C. T. Walsh, An Enzymatic Cyclopentyl[b]Indole Formation Involved in Scytonemin Biosynthesis, *J Am Chem Soc*, **13** (2009), 14648-14649.

23. K. Klicki, D. Ferreira, D. Hamill, The Widely Conserved Ebo Cluster is Involved in Precursor Transport to the Periplasm During Scytonemin Synthesis, *Nostoc Punctiforme*, *mBio*, **9** (2018), e02266-18.
24. K. Matsui, E. Nazifi, Y. Hirai, N. Wada, S. Matsugo And T. Sakamoto, The Cyanobacterial Uv-Absorbing Pigment Scytonemin Displays Radicals Scavenging Activity, *J Gen Appl Microbiol*, **58** (2012), 137-144.
25. T. Y. Desikachary, *Cyanophyta*, Indian Council of Agricultural Research, New Delhi, India, 1959.
26. R. P. Sinha, 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** (1995), 187-192.
27. R. Rippka, J. Deruelles, J. B. Waterbury, M. Herdman and R. Y. Stanier, Generic Assignments, Strain Histories and Properties of Pure Culture of Cyanobacteria, *J Gen Microbiol*, **11** (1979), 11-61.
28. J. Rath, S. Mandal and S. P. Adhikary, Salinity Induced Synthesis of UV-Screening Compound Scytonemin in the Cyanobacterium *Lyngbya aestuarii*, *J. Photochem Photobiol B Biol*, **115** (2012), 5-8.