

Research Article

Mycosporine-Like Amino Acids (MAAs) Profile of cyanobacteria from Different Historical Kunds of Varanasi, India

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Abstract

Copious facts have demonstrated that UV radiation is harmful to cyanobacteria. Sun-screening compounds such as mycosporine-like amino acids (MAAs) protect these organisms from deleterious UV radiation. MAAs absorb UV radiation in the range of 310 to 362 nm. These natural sunscreens obtained from cyanobacteria are excellent alternative to present day synthetic UV filters. In the present study, several cyanobacteria were collected from six historical Kunds of Varanasi, India. MAAs profile of these cyanobacteria was recorded with the help of UV-Vis spectroscopy, High performance liquid chromatography (HPLC) and Fourier transform infrared (FTIR) analysis. Various MAAs were identified as Porphyra-334 ($\lambda_{max} = 334$ nm), Palythine ($\lambda_{max} = 320$ nm), Asterina-330 ($\lambda_{max} = 330$ nm), Mycosporineglycine (λ_{max} =310 nm) and Mycosporine-methylamine-serine (λ_{max} =327 nm) having retention times (RT) of 3.62, 1.65, 1.53, 2.55 and 1.51 min, respectively, obtained from different cyanobacteria (Microcystis aeruginosa, Anabaenopsis sp., Merismopedia sp., Aulosira fertilissima, Rivularia sp., Phormidium sp., Nostoc sp. and Stigonema sp.). It is concluded that these MAAs from different historical Kunds may provide protection to the cyanobacteria growing thereof from the adverse effect of harmful UV radiation. MAAs are considered to be potential bioactive compounds that are highly intriguing from biotechnological perspective.

Keywords: Kunds; Mycosporine-like amino acids (MAAs); High performance liquid chromatography (HPLC); Fourier transform infrared (FTIR) spectroscopy; Ultraviolet radiation (UVR).

Introduction

Ultraviolet radiation (UVR) is a highly energetic shortwavelength radiation that can penetrate up to a depth of 70 m in water (Smith *et al.*, 1992; Häder *et al.*, 2007). Because of high penetration efficacy, UVR can negatively affect the aquatic organisms by damaging a number of physiological, biochemical and molecular processes (Häder *et al.*, 2015). UVR can be divided into three spectral regions such as UV-A (315 - 400 nm), UV-B (280 - 315 nm) and UV-C (100 - 400 nm), UV-B (280 - 315 nm) and UV-C (100 - 400 nm).

280 nm). Cyanobacteria are Gram-negative photosynthetic prokaryotes having cosmopolitan distribution in both aquatic as well as terrestrial habitats. Cyanobacteria are especially susceptible to negative effects of UVR due to their obligation for harvesting light energy to produce chemical energy during photosynthesis. Cyanobacteria have developed various defence mechanisms to reduce the impacts of UVR (Singh *et al.*, 2013) which is primarily associated with the biosynthesis of cyanobacterial photoprotective compounds such as MAAs and scytonemin (Garcia-Pichel and Castenholz, 1993; Rastogi *et al.*, 2010; Rastogi and Incharoensakdi, 2014).

Mycosporine-like amino acids (MAAs) are UV-absorbing intracellular secondary metabolites. They are colourless and water soluble substances with the molecular weight ranging from 188 to 1050 Da (Wada et al., 2015). Production of natural photoprotective compounds MAAs from cyanobacteria increases in response to UV-B radiation (Sinha et al., 1999; Singh et al., 2008). Synthesis of MAAs is possible by taxonomically diverse organisms except animals. In animals, MAAs is supposed to be accumulated through the food chain (Sinha et al., 2007). They show resistance against abiotic stresses such as temperature, UVR, pH and various solvents (Gröniger and Häder, 2000; Zhang et al., 2005). There are around forty MAAs reported so far, but chemical validation of a number of other MAAs is still awaited (Shick and Dunlap, 2002; Řezanka et al., 2004; Wada et al., 2015). They are characterized by cyclohexenone or cyclohexenimine chromophore confederate with the nitrogen substituent of an amino acid or its imino alcohol, having absorption maxima ranging from 310 to 362 nm (Favre-Bonvin et al., 1976; Wada et al., 2015). MAAs have several roles against various environmental stresses, and their synthesis can be affected by osmotic, desiccation and thermal stresses (Oren, 1997; Michalek-Wagner, 2001; Shick and Dunlap, 2002; Feng et al., 2012; Olsson-Francis et al., 2013; Waditee-Sirisattha et al., 2014).

Synthesis and accumulation of various types of MAAs have been reported in cyanobacteria. MAAs such as Porphyra-334 and shinorine were found to be present in *Aulosira fertilissima* (Mushir and Fatma, 2011) and *Microcystis aeruginosa* (Liu *et al.*, 2004). MAA such as Porphyra-334 and mycosporine-glycine were found to be present in *Nostoc* sp. (Inoue-Sakamoto *et al.*, 2018) and *Rivularia* sp. (Rastogi *et al.*, 2014) respectively. MAAs have the ability to avert UV-induced formation of thymine dimers owing to their photostability in both fresh and saline water in the presence of photosensitizers as well as having high molar extinction coefficient (ε =28,100-50,000 M⁻¹ cm⁻¹) (Misonou *et al.*, 2003). MAAs are supposed to be multifunctional secondary metabolites produced by a number of cyanobacteria from freshwater, marine, or terrestrial habitats (Garcia-Pichel and Castenholz, 1993; Karsten and Garcia-Pichel, 1996). They have extensive cosmeceutical and pharmaceutical applications due to their potent UV-screening and antioxidant properties (Carreto and Carignan, 2011). In the present study, cyanobacteria collected from six historical Kunds were examined and characterized for their ability to synthesize MAAs.

Materials and Methods

Collection Sites

Our collection sites are located in the Eastern part of Uttar Pradesh, Varanasi (25°28' N, 82°96' E), India. Collection of samples was done from six different historical sites such as Kurukshetra Kund, Ram Kund, Laxmi Kund, Suraj Kund, Ramkatora Kund and Ishwargangi Kund (Fig. 1). All of these historical and religious Kunds have their own importance and belief. Samples were filtered by using planktonic net of 10 μ m mesh size (Plankton Sampling Kit, Fieldmaster®, Wildco). These water samples were rich in cyanobacteria. Sampling sites were photographed by using digital camera (Fig. 1). UV-A and UV-B radiation, temperature, pH and relative humidity were recorded at sites accompanied by solarimeter, digital thermometer, pH meter and hygrometer respectively.

Identification of Microorganisms

The collected samples were centrifuged at 7000 rpm for 20 min. Pellets were washed with sterile distilled water repeatedly and examined for the presence of cyanobacteria by using light microscope (OLYMPUS, Model number: CX21i-TR). Photographs were taken by using Dewinter-2011 scientific digital camera and analyzed with Dewinter software.

Extraction and Partial Purification of MAAs

Samples of different Kunds were centrifuged at 7000 rpm for 20 min. Pellets were dissolved in 4 ml of 100% (v/v) methanol (HPLC-grade) and incubated overnight at 4 °C. After extraction, aliquots were centrifuged (5000 rpm for 10 min), and methanolic extracts were subjected to spectroscopic analysis between 200 -700 nm in a UV-Vis double beam spectrophotometer (U-2600, Shimadzu, Japan). The raw spectra were transferred to computer and peaks were analyzed by UV-probe software (Version-2, Japan). After scanning, methanol was evaporated into watch glass to complete dryness at 42 °C and redissolved in 2 ml double distilled water. It was centrifuged at 10,000 rpm for 10 min and the aqueous solution was filtered through 0.2 µm pore-sized sterilized microcentrifuge syringe-driven filters (Axiva Sichem Biotech., New Delhi). Thereafter, partially purified MAAs were subjected to HPLC analysis.



Fig. 1: Historical Kunds of Varanasi, India. (A) Ram Kund, (B) Suraj Kund, (C) Ishwargangi Kund, (D) Ramkatora Kund, (E) Laxmi Kund and (F) Kurukshetra Kund.

HPLC Analysis

The partially purified MAAs were analyzed by HPLC system equipped with a Photodiode Array (PDA) Detector, Waters 2998, 515 HPLC pump, Nova-Pak® C18 reverse phase guard column (4.6×150 mm inside diameter). A 40µL sample was injected into the HPLC column with the help of an autoinjector with a Waters 717 plus Autosampler. Wavelength was set at 330 nm in detector, scanning of wavelength from 250 to 400 nm by PDA to achieve the separate peaks. A 0.02 % (v/v) acetic acid was used as mobile phase, which eluted isocratically with a flow rate of 1 mL min⁻¹. Identification of MAAs was done by its characteristic absorption maxima and retention time. Eluted samples were collected with the help of Fraction Collector III and these purified fractions of MAAs were further used for identification and characterization.

MAAs Characterization by Fourier Transform Infrared (FTIR) Spectroscopy

Fraction of MAAs were purified by HPLC and lyophilized to perform FTIR by fusing with oven-dried potassium bromide, stored in desiccator in 1:100 ratio. Transparent disk was prepared by using hydraulic press and inserted in a Perkin Elmer FTIR/FIR Spectrometer Frontier version 10, Perkin Elmer, Waltham, MA, USA, to record the spectra.

Results

In the present study, we have investigated the presence of photoprotective compounds such as MAAs in cyanobacterial samples form Kunds by spectroscopic analysis. As the presence was confirmed, MAAs were further characterized by HPLC analysis on the basis of similarity to the retention times (RT) and absorption maxima (λ_{max}) of individual MAA accompanied by known standards.

Environmental Parameters and Organisms Identification Most of our sampling sites (Kunds) were exposed to the solar radiation for longer period of time. Stagnant water system was dominated by blue-green mats of cyanobacteria. These mats of cyanobacteria produce photoprotective compounds. At the time of sampling, relative humidity was found within the range of 71-85%, tropospheric ozone within the range of 59.94 -128.58 μ g/m³, UV-A within the range of 5-8 KJ/m² and UV-B within the range of 13.225-31.856 KJ/m².

Eight species of cyanobacteria (Table 1) were recorded from the six historical Kunds (Fig. 2). These Kunds were dominated by *Microcystis aeruginosa*, *Anabaenopsis* sp., *Merismopedia* sp., *Aulosira fertilissima*, *Rivularia* sp., *Phormidium* sp., *Nostoc* sp. and *Stigonema* sp. Bloomforming cyanobacteria such as *Microcystis aeruginosa* and *Merismopedia* sp. were also present in some Kunds but in very small number.

UV-Vis Spectroscopic Analysis

The absorption spectra of methanolic extracts of all cyanobacteria showed a peak in the range of 310-327 nm, which designated the presence of MAAs. In addition to the peaks for MAAs, methanolic extracts of all cyanobacterial samples also showed the peaks for chlorophyll *a* (443 and 660 nm), carotenoids (474 nm) and phycobiliproteins (620 nm) (Fig. 3).



Fig. 2: Cyanobacteria collected from different historical Kunds of Varanasi, India. (A) Aulosira fertilissima (B) Stigonema sp., (C) Microcystis aeruginosa, (D) Phormidium sp., (E) Rivularia sp., (F) Merismopedia sp., (G) Anabaenopsis sp. and (H) Nostoc sp.

Table 1: Cyanobacterial colonization (+ sign shows the presence and – sign shows the absence) in the different historical Kunds of Varanasi, India.

Cyanobacteria	Samples						
	Ram Kund	Suraj Kund	Ishwargangi Kund	Ramkatora Kund	Laxmi Kund	Kurukshetra Kund	
Aulosira fertilissima	+	+	+	-	+	+	
Stigonema sp.	+	-	+	+	-	-	
Microcystis aeruginosa	+	-	+	-	-	-	
Phormidium sp.	+	+	+	+	-	-	
<i>Rivularia</i> sp.	-	+	+	+	-	+	
Merismopedia sp.	-	+	-	-	+	-	
Anabaenopsis sp.	+	-	+	+	-	+	
Nostoc sp.	+	+	-	+	+	+	



Fig. 3: Absorption spectra of methanolic extracts of cyanobacterial samples from several historical Kunds of Varanasi, India, showing the presence of MAAs, Chl *a*, carotenoids and phycobiliproteins.

HPLC Analysis of MAAs

HPLC chromatograms showed various peaks with varying retention time (RT) corresponding to different MAAs (Fig. 4 A-F). On the basis of absorption maxima and retention times, various MAAs such as Porphyra-334 (λ_{max} -334 nm; RT-3.62 min); Palythine (λ_{max} -320 nm; RT-1.65 min); Asterina-330 (λ_{max} -330 nm; RT-1.53 min); Mycosporine-glycine (λ_{max} -310 nm; RT-2.55 min) and Mycosporine-methylamine-serine (λ_{max} -327 nm; RT-1.51 min) were identified in these Kunds (Table 2). Concentration of MAAs (nmol/gdw) have been shown in Fig. 5. Porphyra-334 was

present in all samples and its content was maximum in Laxmi Kund and minimum in Ishwargangi Kund. Palythine was present in Ramkatora Kund, Laxmi Kund and Kurukshetra Kund and its content was maximum in Kurukshetra Kund and minimum in Laxmi Kund. Asterina-330 was present only in Ram Kund. Mycosporine-glycine was present in Suraj Kund, Ram Kund and Ishwargangi Kund and its content was maximum in Ram Kund and minimum in Suraj Kund. Mycosporine-methylamine-serine was present in Suraj Kund and Ishwargangi Kund and its content was maximum in Suraj Kund and minimum in Ishwargangi Kund.



Fig. 4: HPLC chromatograms of partially purified MAAs and its corresponding absorption spectra. (A) Suraj Kund, (B) Ram Kund, (C) Ramkatora Kund, (D) Laxmi Kund, (E) Ishwargangi Kund and (F) Kurukshetra Kund.

	MYCOSPORINE-LIKE AMINO ACIDS (MAAs)							
Kunds/ Samples	MAAs	λ_{max} (nm)	Retention Time (min)	MAAs with their molecular structure				
	Porphyra-334 Mycosporine-methylamine- serine	334 327	3.62 1.51	H ₃ C HOOC				
Suraj Kund	Mycosporine-glycine	310	2.55	HO NH				
	Porphyra-334	334	3.64	Porphyra-334				
Ram Kund	Mycosporine-glycine	310	2.53	HO OCH3				
	Asterina-330	330	1.53	Palythine				
Ramkatora Kund	Porphyra-334	334	3.61	он оснз				
	Palythine	320	1.62					
	Porphyra-334	334	3.66	Asterina-330				
Laxmi Kund	Palythine	320	1.63					
	Porphyra-334	334	3.63	соон Mycosporine-glycine				
Ishwargangi Kund	Mycosporine-methylamine- serine	327	1.54	H ₃ C NH				
	Mycosporine-glycine	310	2.52	HO HO NH				
	Porphyra-334	334	3.61	н ₃ с соон				
Kurukshetra Kund	Palythine	320	1.65	о́н Mycosporine-methylamine- serine				

Table 2: Photoprotective compounds from different kunds samples with their corresponding absorption maxima and retention times.

Characterization of MAAs by FTIR

FTIR collected fractions containing KBr pellets revealed the presence of four prominent bands (shown by arrows) in all the six samples (Fig. 6). Band of 3343 cm^{-1} may be allocated to OH group. Band of 2936 cm⁻¹ for side chain

vibrations consisting of C-H stretching indicates the presence of NH_2^+ . Band of 2846 cm⁻¹ indicated the presence of OH stretching in COOH (carboxylic group). Band of 1640 cm⁻¹ may be assigned to the presence of an NH_2 group.



Fig. 5: Concentration of MAAs (nmol/gdw) in different historical Kunds of Varanasi, India. (PR: Porphyra-334, PT: Palythine, AS: Asterina-330, MG: Mycosporine-glycine, MMAS: Mycosporine-methylamine-serine). The error bar represents standard deviation of mean (means ± S.D., n = 3).



Fig. 6: Fourier transform infrared (FTIR) radiation spectroscopy showing four (see arrows) prominent spectral bands to exhibit the presence of MAAs.

Discussion

Cyanobacteria are the earliest photosynthetic microorganisms that have got the ability to synthesize UVabsorbing compounds such as MAAs to deal with the deleterious effects of UVR in their natural habitats. We have reported eight species of cyanobacteria from six historical Kunds. Aulosira fertilissima and Nostoc sp. were the dominant cyanobacterial taxa in most of the Kunds. Phormidium sp., Rivularia sp. and Anabaenopsis sp. were also present in most of the Kunds but their numbers were less. The cyanobacterium Sitgonema sp. was present in three Kunds. Microcystis aeruginosa and Merismopedia sp. were present in only two Kunds. All these cyanobacteria were growing as a blue-green mat on the surface of water. Variation in growth and pigmentation of cyanobacteria in all Kunds were due to changing environmental conditions such as light intensity, temperature, pH and nutrient availability.

Cyanobacteria are a rich source of photoprotective compounds MAAs, which were partially purified through HPLC and characterized through FTIR spectroscopy. MAAs provide protection from harmful UVR (Singh et al., 2010) by absorbing short wavelength radiation and dissipate energy in the form of heat. According to previous reports, palythine and asterina-330 were present in limited number of microalgae or macroalgae (Sinha et al., 2007; Kannaujiya et al., 2014; Pandey et al., 2017). High temperature (> 30 $^{\circ}$ C) and low pH (< 6.0) does not favour luxuriant cyanobacterial growth. Cyanobacterial growth rate increases at 25 °C owing to change in climate and supremacy of cyanobacteria in temperate water bodies (Joehnk et al., 2008; Paerl and Huisman, 2009; Davis et al., 2009). Five types of MAAs present in our samples were porphyra-334, palythine, asterin-330, mycosporine-glycine and mycosporine-methylamine-serine. MAAs such as shinorine, porphyra-334 and mycosporine-glycine shows protective effect on human fibroblast cells from harmful UVR. It was observed that all of three scrutinized MAAs protect the cells from UV-induced cell death, of which mycosporine-glycine was more effective (Oyamada et al., 2008). Along with photoprotective properties MAAs have pharmaceutical and biotechnological importance.

Together with anticancerous, antiaging and antiinflammatory activity, MAAs give protection from sunburn, erythema and edema (Richa and Sinha, 2013). MAAs shows commercial potential for food, cosmetics and biomedical research. Microcystins obtained from Microcystis sp., Anabaenopsis sp. and Nostoc sp. play role in inhibition of serine/ threonine protein phosphatases 1 and 2a (PP1/2a) (Dittmann and Wiegand, 2006). MAAs play role in the inhibition of formation of both 6-4 photoproduct and cyclobutane pyrimidine dimer (CPD) (Misonou et al., 2003). MAAs have free radical quenching and antioxidant property that are helpful in the prevention of cellular

damage arising from UV-induced production of reactive oxygen species (ROS) (Suh *et al.*, 2003; Oren and Gunde-Cimerman, 2007). These compounds are conceivably of significant value in the development of natural sunscreen due to reduction of approximately 90 % of UVR entering inside the cells. Further studies are still needed to find out other applications of MAAs apart from their photoprotective nature.

Author's Contribution

Rajeshwar P. Sinha designed the research plan; Sonal Mishra, & Deepak Kumar performed experimental works & collected the required data. Abha Pandey, Haseen Ahmed, Vidya Singh & Rajeshwar P. Sinha analysed the data; Sonal Mishra & Abha Pandey prepared the manuscript. Critical revision and finalization of manuscript was done by Rajeshwar P. Sinha, Deepak Kumar and Vidya Singh. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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