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Characterization of Mycosporine-like Amino Acids in Chlorophyll f Producing Cyanobacteria from Shaded Niches

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Abstract

Cyanobacteria are the oldest photoautotrophic prokaryotes that can perform plant-like oxygenic photosynthesis. The obligate requirement of sunlight for photosynthesis inevitably exposes cyanobacteria to UV radiation. Mycosporine-like amino acids (MAAs) played necessary roles in protecting cyanobacteria from UV radiation and were frequently reported in cyanobacteria exposed to high light radiation. Here, the MAA production was

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tried in the chlorophyll f-producing cyanobacterial strains isolated from the shaded environments. Four Chroococcidiopsis strains were finally induced to produce MAAs under 0.15 W·m-2 of UV-B exposure, and the MAA contents increased along with the prolonged UV-B treatments in these four Chroococcidiopsis strains. After separation by HPLC system, one MAA type was detected at similar retention times in the methanol extracts of Chroococcidiopsis strains, and all the MAA compounds showed in-line absorption at 310 nm and mass spectra 246 m/z. The absorption spectra and mass spectra matched well the characteristics of the simplest MAA mycosporine-glycine. Chroococcidiopsis had the simplest MAA gene clusters for mycosporine-glycine. MAAs could also be produced in the cyanobacteria even distributed in the light-deficient niches. These results suggested other roles of MAAs in addition to UV-B protection in the special cyanobacteria from shaded environments.

Keywords: Chroococcidiopsis; cyanobacteria; mycosporine-like amino acids; mycosporine-glycine; ultraviolet B

Introduction

Cyanobacteria are a group of photosynthetic oxygen prokaryotes found in variety of habitats ranging from freshwater to oceans, soil, bare rocks, deserts, and lakes (Whitton and Potts, 2000; Vincent, 2009). Chlorophylls (Chls) play important roles in light harvesting, energy transfer, and electron-transfer processes in oxygenic photosynthesis. To date five types have been found such as Chl *a*, *b*, *c*, *d*, and *f* in oxygenic photosynthetic organisms. Chl *d* and Chl *f* were the red-shifted Chls, which absorb longer wavelengths into far-red light in comparison to other Chls (Chen et al., 2012; Li and Chen, 2015). Both Chl *d* and Chl *f* were just produced in certain cyanobacteria, which are distributed in far-red light replete and visible light-deficient niches (Zhang et al., 2019). Although Mycosporine-like amino acids (MAAs) have been reported in diverse cyanobacteria from marine and terrestrial environments to counteract UV damage (Jain et al., 2017; Shick and Dunlap, 2002; Chrapusta et al., 2017), the synthesis of MAAs is not reported in the Chl *d* and Chl *f*-producing cyanobacteria yet, which preferentially habited the shaded environments. In the following study, we report the characterization of MAAs in Chl *f*-producing cyanobacteria isolated from shaded environments (Zhang et al., 2019).

Materials And Methods

Materials and culture conditions

Four Chl *f*-producing cyanobacterial strains were recently reported from the current laboratory (Zhang et al., 2019). They were originally isolated

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from various shaded habitats of mosses on arid and humid limestones, macrophytes and freshwater in the forest ecosystems (Table 1; Zhang et al., 2019). They were cultured in 500 mL glass flasks containing 200 mL of BG11 medium at 25 ºC. The white light was provided with white fluorescent lamps (PAR, 400-700 nm) at an intensity of 30 μmol photons m-2s-1. After growth for 14 days, samples were illuminated by fluorescent UVB lamps (TL 40W/12 RS; Philips, Germany) in addition to white light of 30 μmol photons m-2s-1 PAR. Finally, the 0.15 W·m-2 of UVB radiation was provided. After one week of treatment for UVB radiation, samples were collected and frozen in liquid nitrogen and kept at -80 °C for MAA characterization.

Table 1. The chlorophyll *f*-producing cyanobacterial strains and habitats (Zhang et al, 2019)

Taxonomy	Niches	Locations
Chroococcidiopsis sp. CCNUC1	*Haplocladium angustifolium* (Hampe & Müll. Hal.) Broth. on the arid limestone	CCNU
	Haplocladium angustifolium (Hampe & Müll. Hal.) Broth. on the arid limestone	CCNU
	Entodon challengeri (Paris) Cardot on the humid limestone	CCNU
	Vallisneria natans (Lour.) Hara in the pond	WBG
	Vallisneria natans (Lour.) Hara in the pond	WBG
	Cabomba caroliniana A. Gray (cabomba) in the pond	WBG
Chroococcidiopsis sp. CCNUC2	*Haplocladium angustifolium* (Hampe & Müll. Hal.) Broth. on the arid limestone	CCNU
Chroococcidiopsis sp. CCNUC3	*Hyophila involute* (Hook.) A. Jaeger on the arid limestone	CCNU
Chroococcidiopsis sp. CCNUM1	*Bryoerythrophyllum gymnostomum* (Broth.) P. C. Chen on the humid limestone	MTSA
	Cabomba caroliniana A. Gray (cabomba) in the pond	WBG

CCNU, Central China Normal University; MTSA, Mulan Tianchi Scenic Area; and WBG, Wuhan Botanical Garden, Chinese Academy of Sciences.

Microscopic observation

The morphological images of cyanobacteria were captured directly under the bright field observation on a laser scanning confocal microscope (LSM710, Carl Zeiss Microscopy, Germany) coupled with Zen 2 software (Carl Zeiss Microscopy).

Chlorophyll fluorescence measurements

Samples were placed into open Petri dishes and exposed to UV-B at intensity 0.15 $W \cdot m^{-2}$with 30 μmol photons $m^{-2}s^{-1}$ PAR up to 36 h at 25°C. The maximal PSII photochemical efficiency (Fv/Fm) was determined by a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd, King's Lynn and

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Norfolk, UK). All samples were dark-adapted for 10 min before measurements.

MAA extraction, induction, and characterization

Cyanobacteria culture were exposed to UV-B treatments at intensity of 0.15 $W \cdot m^{-2}$ for 6h, 24h and 48 h, respectively. At each time point, samples were immediately collected and centrifuged at 6000 rpm for 5 min. The cyanobacterial pellets were mixed with methanol at 4ºC overnight in a refrigerator to extract MAAs. The extracts were then centrifuged at 6000 rpm for 5 min. After centrifugation, supernatant was transferred in a new tube and scanned to detect MAA induction by spectroscopic analysis between 300-800 nm using a UV/Vis spectrophotometer (UV-2700, Shimadzu).

MAAs were extracted and characterized by LC–MS (Agilent technologies 6540 UHD Accurate-Mass Q-TOF). The MAAs were detected at 310 nm after separation by 1 $ml \cdot min^{-1}$ of binary gradient elution of mobile A (methanol) and mobile B (water) (0-7 min, 1%-20% mobile A; 7-9 min, 20%-50% mobile A; 9-17 min, 50%-80% mobile A; 17-22 min, 80% mobile A). The electrospray interface (ESI) source and positive mode was used for the mass spectrometer.

Results

Morphologies of *Chroococcidiopsis* strains

All the four Chl *f*-producing cyanobacteria of *Chroococcidiopsis* strains showed most aggregates with more than two cells in the liquid culture under white light conditions (Fig. 1). Amongst them, it seemed that the aggregates were formed with the least cells in *Chroococcidiopsis* sp. CCNUM1 culture (Fig. 1).

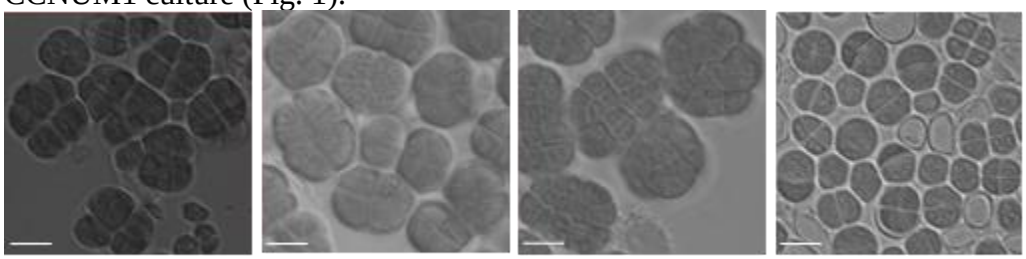

Fig.1. Morphologies of *Chroococcidiopsis* strains from white light cultures. CCNUC1, CCNUC2, CCNUC3 and CCNUM1 were abbreviated for *Chroococcidiopsis* sp. CCNUC1, *Chroococcidiopsis* sp. CCNUC2, *Chroococcidiopsis* sp. CCNUC3 and *Chroococcidiopsis* sp. CCNUM1, respectively. Scale bar represented 5 μm.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

UV-B tolerance assays

The Fv/Fm significantly decreased in the four cyanobacterial strains during UVB treatments (Fig. 2). *Chroococcidiopsis* CCNUM1 was most sensitive to UV-B radiation whereas *Chroococcidiopsis* CCNUC3 was most tolerant to UV-B. The decrease of Fv/Fm indicated negative effects of UV-B in *Chroococcidiopsis* strains.

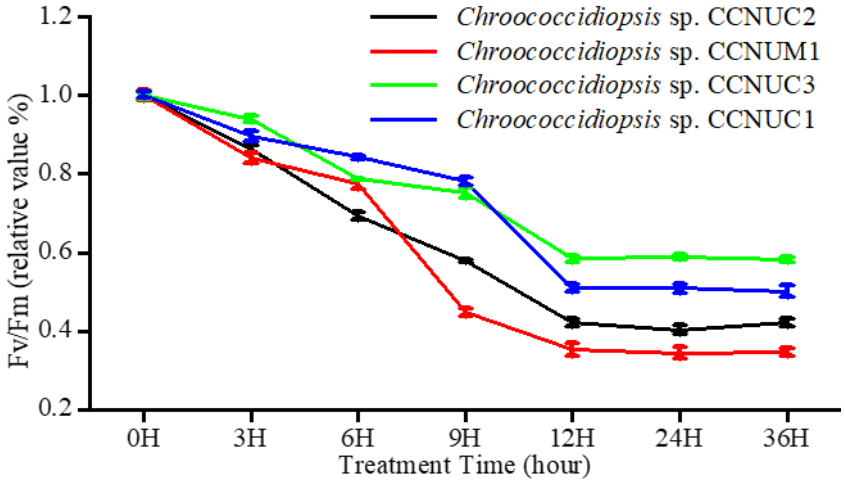

Fig.2. Photosynthetic tolerance of *Chroococcidiopsis* strains after UV-B treatments at 0.15W·m^{-2} for several hours (H). There are three replicates for each treatment.

MAA Induction

The absorption spectra at the range of 200 to 800 nm were recorded for the methanolic extracts from cyanobacteria. The peaks at 435 nm and 665 nm represented the presence of Chl *a*. The absorbance between 300 and 340 nm with maximum at 310 nm typically represented the presence of MAAs (Fig. 3). MAA content gradually increases in response to prolonged UVB treatments in these four *Chroococcidiopsis* strains (Fig. 3). The content of MAAs was most induced in *Chroococcidiopsis* sp. CCNUC3 amongst all the four strains, and the other three strains produced similar content of MAA after UVB treatments for 48 h (Fig. 3).

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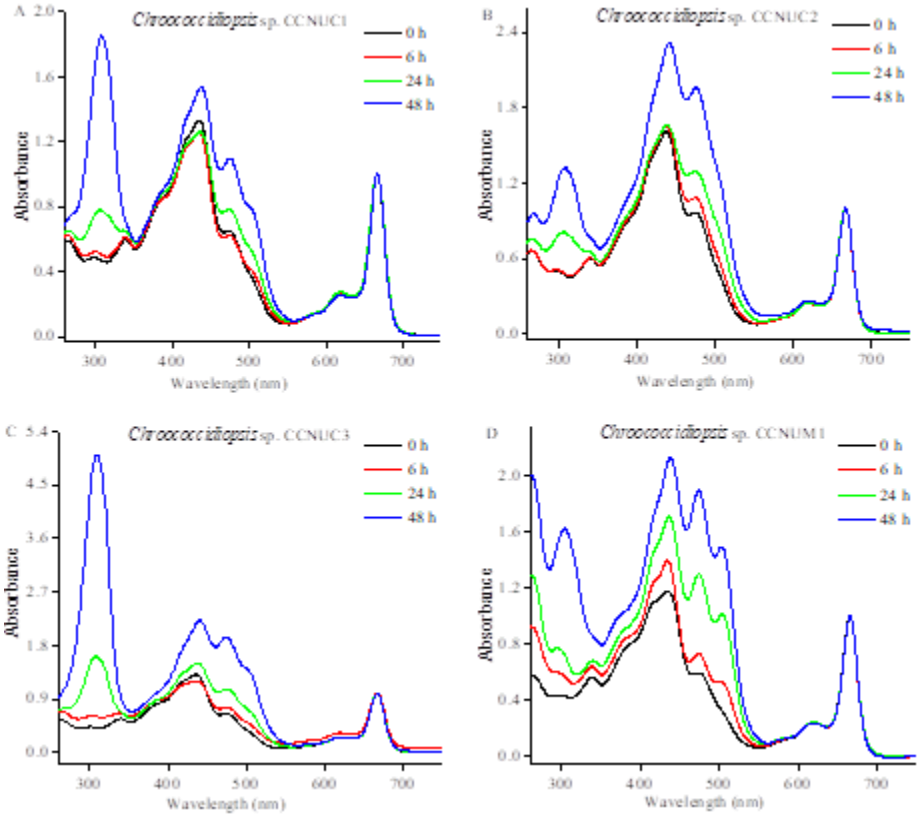

Fig. 3. MAA induction in *Chroococcidiopsis* strains under 0.15 $W \cdot m^{-2}$ UV-B treatments. The difference of MAA content was shown by normalized absorbance spectra of methanol extracts by the chlorophyll *a* peak at 665 nm. A, *Chroococcidiopsis* sp. CCNUC1; B, *Chroococcidiopsis* sp. CCNUC2; C, *Chroococcidiopsis* sp. CCNUC3 and D, *Chroococcidiopsis* sp. CCNUM1

MAA identification in *Chroococcidiopsis* strains

MAAs in the methanolic extraction was separated by HPLC and then subjected to mass spectra analysis for characterization of the compounds. HPLC profiles at detecting wavelength of 310 nm showed one prominent peak at 2.7 min with a main absorption peak centered 310 nm for the sample from *Chroococcidiopsis* sp. CCNUC1 (Fig. 4A). The mass spectrum further showed protonated molecule $[M+H]^+$ at *m/z* 246.0977 suggesting the molecular weight of 245 (Fig. 4A). In the sample from *Chroococcidiopsis* sp. CCNUC2, HPLC profile showed a peak at 2.7 min with a main absorption peak centered 310 nm (Fig. 4B). The mass spectrum showed the protonated molecule $[M+H]^+$ at *m/z* 246.0970 suggesting the molecular weight of 245 (Fig. 4B). In the sample from *Chroococcidiopsis* sp. CCNUC3, HPLC profile shows a peak at 2.7 min with a main absorption peak centered 310 nm (Fig. 4C). The mass spectrum show the protonated molecule $[M+H]^+$ at m/z 246.0974 suggesting the

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molecular weight of 245 (Fig. 4C). In the sample from *Chroococcidiopsis* sp. CCNUM1, HPLC profile shows a peak at 2.7 min with a main absorption peak centered 310 nm (Fig. 4D). The mass spectrum show the protonated molecule $[M+H]^+$ at *m/z* 246.0974 suggesting the molecular weight of 245 (Fig. 4D). All HPLC profiles showed one peak at 2.7 min and the compounds corresponding to the peaks also showed the similar absorption spectra and mass spectra. The detected absorption spectra and mass spectra matched well with previously known MAA mycosporine-glycine (Fig. 4 and Fig. 5) (Shukla et al., 2015; Rosic et al., 2019). These results indicated mycosporine-glycine was the dominant type or the only kind of MAA in the four *Chroococcidiopsis* strains.

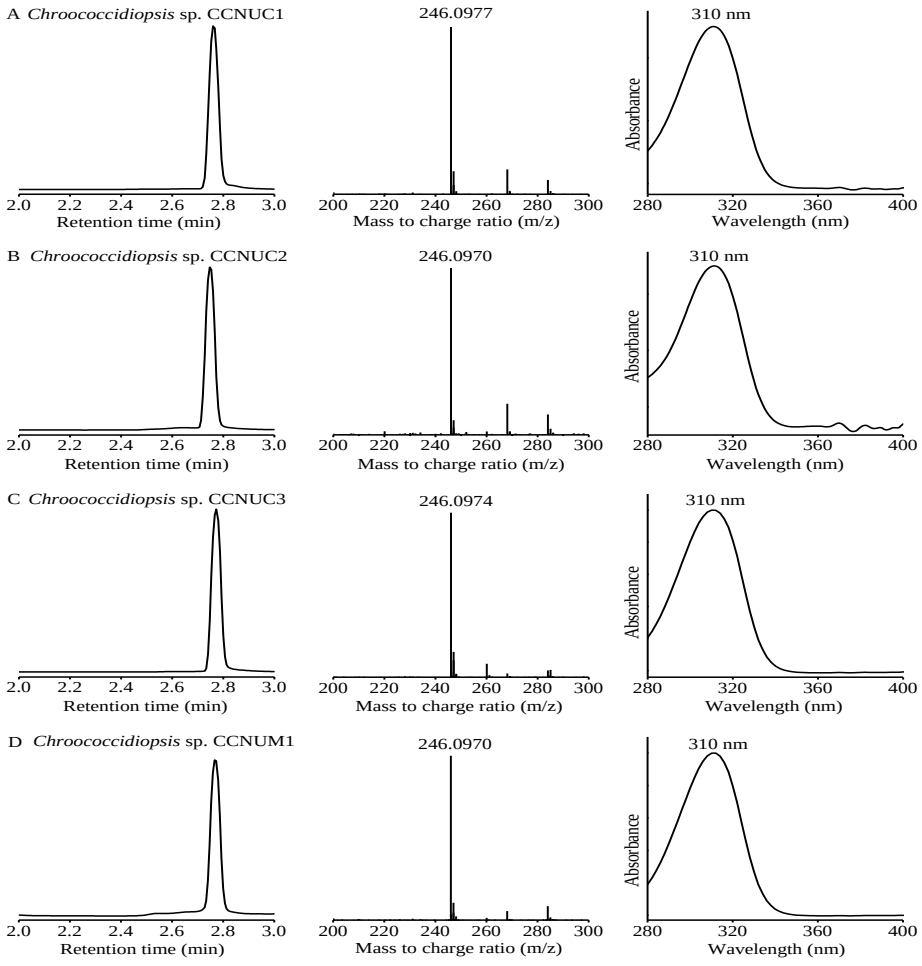

Fig. 4. MAA Characterization in *Chroococcidiopsis* strains. LC-MS analysis and in-line absorbance spectra of methanol extracts from 0.15 $W \cdot m^{-2}$ UV-B treated *Chroococcidiopsis* sp. CCNUC1 (A), *Chroococcidiopsis* sp. CCNUC2 (B), *Chroococcidiopsis* sp. CCNUC3 (C) and *Chroococcidiopsis* sp. CCNUM1 (D) for 7 days. Each LC profile was normalized by maximum MAA absorption.

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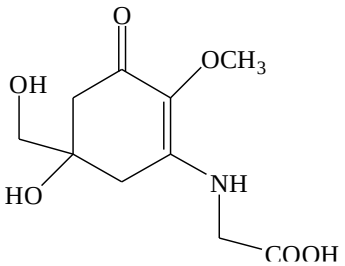

Fig. 5. Molecular structure of mycosporine-glycine

Discussion

Cyanobacteria were considered as the efficient MAA producers. The compositions of MAA production varied a lot in different kinds of cyanobacteria (Jain et al., 2017). Generally, the most reported MAAs included shinorine, porphyra-334 and mycosporine-glycine in cyanobacteria (Jain et al., 2017). The orders of Synechococcales, Chroococcales, Oscillatoriales, and Nostocales are frequently explored for MAA production (Jain et al., 2017). But almost no reports of MAA production have been studied in the orders of Gloeobacterales, Spirulinales, Pleurocapsales, and Chroococcidiopsidales. Present investigation firstly showed that *Chroococcidiopsis* strains belong to Chroococcidiopsidales have the abilities to produce the MAA mycosporine-glycine. To our knowledge, *Arthrospira sp.* CU2556 isolated from brightly lit habitat, produced mycosporine-glycine as the only one type MAA under solar UV radiation (Rastogi and Incharoensakdi, 2014). Most cyanobacteria produced mycosporine-glycine in addition to other kinds of MAAs. For examples, *Trichodesmium* spp. produced mycosporine-glycine plus with shinorine, asterina-332 and porphyra (Subramaniam et al., 1999). Multiple MAA production of mycosporine-glycine, porphyra and shinorine were also observed in *Anabaena doliolum* (Singh et al., 2008). Mycosporine-glycine was the simplest MAA and was supposed as the precursor to synthesis other bi-substituted MAAs (Carreto and Carignan, 2011). This was likely the case that mycosporine-glycine was often detected in cyanobacteria.

MAAs were usually synthesized to copy with UVR in cyanobacteria (Carreto and Carignan, 2011). However, these four *Chroococcidiopsis* strains inhabited the shaded environments of rock, soil, stream and pond, where almost no UVR reached (Table. 1; Zhang et al., 2019). It was known that *Chroococcidiopsis* were very old cyanobacteria and were widely distributed in harsh environments exposed to strong solar radiation (Friedmann and Ocampo 1976; Cockell and Stokes 2004; Warren-Rhodes et al., 2006, 2007). The early *Chroococcidiopsis* likely possessed the abilities of MAA production to protect themselves from solar radiation. But the environments probably changed and shaded the niches for *Chroococcidiopsis* later in the evolutionary

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history. In the shaded niches where far-red light enriched but visible light lacked, *Chroococcidiopsis* gained the abilities to synthesis chl *f* for survival, but also kept the capacities to synthesize MAA for other functions such as osmotic regulation, roles in desiccation and thermal stress in cyanobacteria (Jain et al., 2017; Chrapusta et al., 2017). It is also possible that MAA production suggested protective roles of MAAs in *Chroococcidiopsis* strains to adapt daily fluctuations of sunlight on their natural habitat.

In summary, the *Chroococcidiopsis* strains produced mycosporine-glycine as the only one type of MAA under UVB radiation. It was the first report of MAA production in *Chroococcidiopsis* genus to cope with UVB radiation. These results suggested other roles of MAAs in addition to UVB protection in the special cyanobacteria from shaded environments. Furthermore, the results were also helpful to explore the new sources of MAAs for potential application in industry.

Author contributions

B.S. Qiu conceived the study; M.C.M. Boukar and C. Zhang isolated the cyanobacterial strain and wrote the manuscript; M.C.M. Boukar and K. Wang detected and characterized the MAAs; M.D. Saley and M.C.M. Boukar analyzed the data; B.S. Qiu and M. Ali revised the manuscript.

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Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All data are included in the content of the paper.

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