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## Original Research Article

# Isolation and characterization of terrestrial cyanobacterium producing commercially important UV-B absorbing compound

### ABSTRACT

Mycosporine-like amino acids (MAAs) constitute an important class of small water-soluble ultraviolet radiation (UV)-absorbing compounds to counteract UV damages in algae and other organisms, and have potential applications in sun care products as the active ingredients. In this study, one filamentous cyanobacterium strain was isolated from the soil in southeast of Niger (Diffa), which received strong UV radiation in summer. The 16S rDNA phylogenetic analysis classified the new isolate as *Leptolyngbya* sp. CN1. MAA production was low in *Leptolyngbya* sp. CN1 under white light condition but was significantly enhanced by prolonged UV-B treatments. Liquid chromatography revealed one dominant type of MAAs in *Leptolyngbya* sp. CN1, and this kind of MAA was characterized as shinorine based on special characteristics of in line absorption spectrum and mass spectrum. The shinorine content was as high as 940 ug.mg<sup>-1</sup> dry weight after 4 days of UV-B treatment. The present investigation indicates considerable shinorine is induced to protect *Leptolyngbya* sp. CN1 from UV-B radiation, and also provide an avenue to explore the source of various MAAs from the biotechnology perspective.

**Keywords:** *Cyanobacteria*, *Leptolyngbya*, *mycosporine-like amino acids*, *shinorine*, *ultraviolet B*

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## 1. INTRODUCTION

Mycosporine-like amino acids (MAAs) are low molecular weight (ranging from 188 to 756), water-soluble, colorless secondary metabolites found in a wide variety of organisms, such as fungi, cyanobacteria, algae, and corals [1,2,3, 28]. More than 40 kinds of MAAs have been reported [4]. In general, they structurally compose a substituted cyclohexenone or an imino cyclohexene ring [5,6,7]. The different substituent of amino acids or its imino alcohol is conjugated with a cyclohexenone or an imino cyclohexene ring to form diverse MAAs, and also determine the differences of their absorbance spectra [4,8]. These special structures confer MAAs to efficiently absorb UV radiation with the maximum wavelength between 312 and 360 nm and high molar absorptivity from  $2.81 \times 10^4$  to  $5.00 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$  [7]. MAAs attenuate the harmful UV radiation by converting it into heat to dissipate the energy into environments without generate ROS [9,10]. Studies have also suggested other functions of MAA, such as scavenging oxygen radicals as antioxidant molecules, managing salt stress as osmotic solutes, etc. [11]. In recent years, MAAs have attracted considerable attention as the promising additives for application in pharmaceutical and cosmetic industries [4]. Indeed, the formulation containing shinorine as an active ingredient has been developed in two market sunscreen products (Helioguard 365 and Helionori) [12]. Thus, it is highly significant to explore the new source of MAA from natural organisms.

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. Since approximate 2.6-3.5 billion years ago before the present ozone shield, cyanobacteria have evolved sophisticated ways to synthesis diverse MAAs to cope with UV radiation for their ubiquitous adaptation on the earth [13]. Studies have showed that many taxonomically different cyanobacteria from marine and terrestrial environments can produce and accumulate MAAs [7,13]. The abundance and composition of MAAs varied among cyanobacterium species and were also affected by the environmental factors such as light fluctuations and nutrient limitations [2,4]. In this study, we try to isolate a cyanobacterium strain for novel MAA production from terrestrial soil in southeast of Niger (Diffa), which received strong UV radiation and periodic desiccation in most of the time during all the year.

## 2. MATERIALS AND METHODS

### 2.1 Sample collection and isolation

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The soil was collected in southeastern Niger (Diffa), considered to have a desert climate where the average air temperature is 34 °C, with rainfall 141 mm in August.

The soil was transferred to flasks and immersed in BG11 liquid medium for cyanobacterial enrichment under 30  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  at 25°C. The upper solution turned slightly green after three weeks and some aliquots were then transferred to new flasks with fresh BG11 liquid medium. After growth for 20 days, the culture was serially diluted by the gradients of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  and spread on BG11 plates containing 1% agar. The single green clones were picked up and cultivated in new flasks containing liquid BG11 media for next classification. The morphology of cyanobacterium culture was observed using a laser scanning confocal microscopy (TCS SP5 Leica).

## **2.2 Preparation of Partial gene DNA, 16S rDNA amplification and phylogenetic analysis**

Cells (1 mL) were harvested by Centrifugation at 12000 rpm for 2 min. the supernatant was removed and the cell pellet was rinsed twice by pure water and resuspended in 100  $\mu\text{l}$  water. The cells were incubated at 100°C water bath for up 3 min. Thereafter cell debris were removed by centrifugation at 12000 rpm for 2 min.

Rinsed cells pellet was resuspended in 250  $\mu\text{l}$  Tris-EDTA buffer (pH= 8.0) containing 20 mg/ml), and incubated at 37°C for 20 min. Proteinase K (20 mg/ml) and SDS were added to the cell mixture to final concentrations of 50-100  $\mu\text{g/ml}$  and 2% (w/v) respectively. This mixture was incubated at 60 °C for about 50 min. Subsequently, RNase A (20mg/ml) was added to a final concentration about 10  $\mu\text{g/ml}$  and incubated at 65°C for 40 min. when samples cooled to room temperature Promega protein precipitation solution was added and incubated on ice for 5 min. Precipitated protein was removed by centrifugation at 12000 rpm for 3 min, and the clear aqueous supernatant was mixed with equal volume of prechilled 100% isopropanol (HPLC grade). This mixture was kept at 4°C. The precipitated DNA pellets were washed in cold 70% (v/v) ethanol twice and air dried at room temperature for 5-10 min until the pellets became transparent. Isolated DNA were resuspended in water and kept at 20°C. The partial gene of 16S rRNA was amplified by PCR using the genomic DNA and the primers of 16S\_27F, 5'-AGAGTTTGATCCTGGCTCAG-3' and 16S\_1494R, 5'-TACGGCTACCTTGTTACGAC-3'. The PCR condition was set as the initial denaturation at 95°C for 5 min, 35 cycles of denaturation for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. The PCR products were extracted after gel electrophoresis and sequenced. The sequence was subjected to BLAST analysis and further used to construct phylogenetic tree by

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using MEGA (version 6.0) software for cyanobacterium classification.

### 2.3 Induction and detection of MAAs

After the culture growth for nearly three weeks under white light of  $30 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  at  $25^\circ\text{C}$ , the culture was divided into several open petri dishes to induce MAA production under  $0.1 \text{ W.m}^{-2}$  UV-B treatments for 6, 24, and 48 hours. The cyanobacterium cells were harvested by centrifugation at 6000 rpm for 5 min. The pellets were mixed with methanol at  $4^\circ\text{C}$  overnight to extract MAAs. After the mixture was centrifuged at 6000 rpm for 10 min, the MAAs in supernatant were detected by spectroscopic analysis between 300-800 nm using a UV/Vis spectrophotometer.

### 2.4 Characterization of MAAs

The cyanobacterium cells were harvested for MAA extraction by methanol as above. The methanolic extracts were dried in a vacuum concentrator (Labconco, UK) and water was added to resolve the MAA samples. Equal volume of chloroform was used to remove pigments in the MAA solution and MAAs were characterized by liquid chromatography- mass spectrometry (LC-MS) analysis on the machine of Agilent technologies 6540 UHD Accurate-Mass Q-TOF. The samples of  $50 \mu\text{L}$  solution were injected into a reversed-phase HPLC system coupled with in-line absorption spectral scans and equipped with column inertsil ODS-SP ( $5 \mu\text{m}$ ,  $4.6 \text{ mm X } 250 \text{ mm}$  GL Sciences Inc Japan). The MAAs were detected at  $330 \text{ nm}$  after separation by  $1 \text{ mL.min}^{-1}$  of binary gradient elution of mobile A (methanol) and mobile B (water) (0-7min, 1%-20% mobile A; 7-9 min, 20%-50% mobile A; 9-17min, 50%-80% mobile A; 17-22 min, 80% mobile A). The electrospray interface (ESI) source and positive mode was used for mass spectrometer. MAAs could be characterized from the features of in-line spectra and mass spectra. The concentration of MAAs was determined by the value of absorption spectra maximum at  $334 \text{ nm}$ , the extinction coefficient ( $\text{M}^{-1}\text{Cm}^{-1}$ ) and Molecular Weight.

## 3. RESULTS

### 3.1 Classification of the isolated cyanobacterium

one filamentous cyanobacterium strain was finally isolated. The filaments show straight or slightly waved or arcuate without branches and sheaths. The cells in the filaments are cylindrical and blunt at both ends with the size of  $2.3\text{-}7.6 \mu\text{m}$  in length and  $2.7\text{-}4.4 \mu\text{m}$  in width (Fig.1). The constrictions were observed at the crosswalls between adjacent cells. Based on the 16S rDNA phylogenetic tree, the isolated strain

was classified into the clade of *Leptolyngbya* genus (Fig.2). Thus, this strain can be named as *Leptolyngbya* sp.CN1. In consistence with the classification, *Leptolyngbya* sp.CN1 showed 98% and 97% identity of their 16s rDNA sequence with *Leptolyngbya* sp. JSC-1 and *Leptolyngbya* antarctica ANT.LAC.1, respectively. However, the similarities of the 16S rDNA sequences between *Leptolyngbya* sp.CN1 strain and the other related strains are less than 95% (Table 1).

Table 1. The 16S rDNA comparisons between *Leptolyngbya* sp. CN1 and other cyanobacteria.

Sequence Identity Matrix	1	2	3	4	5	6	7	8	9	10
1 <i>Leptolyngbya</i> _sp._CN1	ID									
2 <i>Leptolyngbya</i> _sp._JSC-1	0.98	ID								
3 <i>Leptolyngbya</i> _antarctica_ANT.LAC.1	0.97	0.97	ID							
4 <i>Leptolyngbya</i> _sp._PCC7104	0.94	0.93	0.92	ID						
5 <i>Leptolyngbya</i> _lammosa_ETS-08	0.93	0.92	0.93	0.91	ID					
6 <i>Leptolyngbya</i> _frigida_ANT.L53B.2	0.93	0.92	0.93	0.91	0.93	ID				
7 <i>Leptolyngbya</i> _sp._Doroninskoye	0.94	0.93	0.93	0.92	0.92	0.92	ID			
8 <i>Leptolyngbya</i> _sp._LEGE_06070	0.94	0.93	0.93	0.92	0.92	0.92	1.00	ID		
9 <i>Leptolyngbya</i> _valderiana_SABC022801	0.94	0.93	0.93	0.92	0.92	0.92	1.00	1.00	ID	
10 <i>Leptolyngbya</i> _sp._HBC1	0.93	0.92	0.92	0.92	0.90	0.92	0.92	0.92	0.92	ID

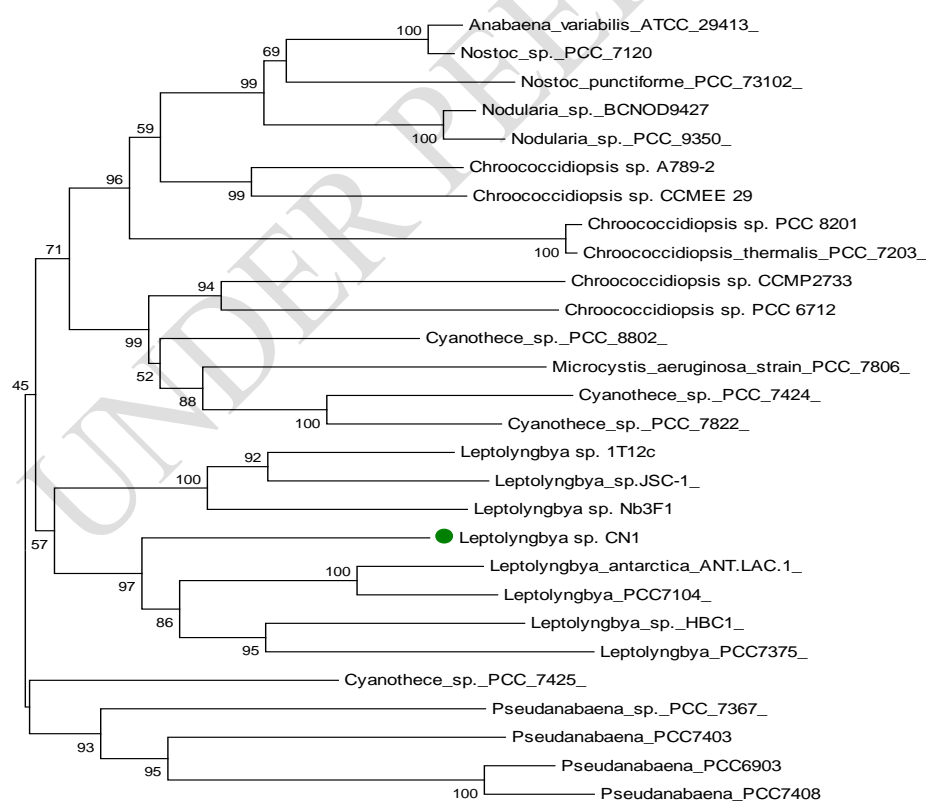


Fig. 1. Maximum-likelihood phylogeny of cyanobacteria based on 16S rRNA gene sequences



Fig. 2. Cell morphology of *Leptolyngbya* sp. CN1 under white field of confocal microscopy, the scale bar presents 20  $\mu\text{m}$ .

### 3.2 Induction of MAAs in *Leptolyngbya* sp.CN1

Chlorophyll a (Chl a) contributed to absorbance peak at 665 nm and carotenoids gave rise to the absorbance in the range of 400 nm to 600 nm. The strong absorbance from 300 nm to 400 nm indicated MAA production by UV-B treatments (Fig.3). The quite low peak at 334 nm suggested MAA was produced in *Leptolyngbya* sp.CN1. MAA production was positively related to prolonged UV-B treatments, suggesting protective roles of MAAs in *Leptolyngbya* sp.CN1 from UV-B radiation (Fig.3).

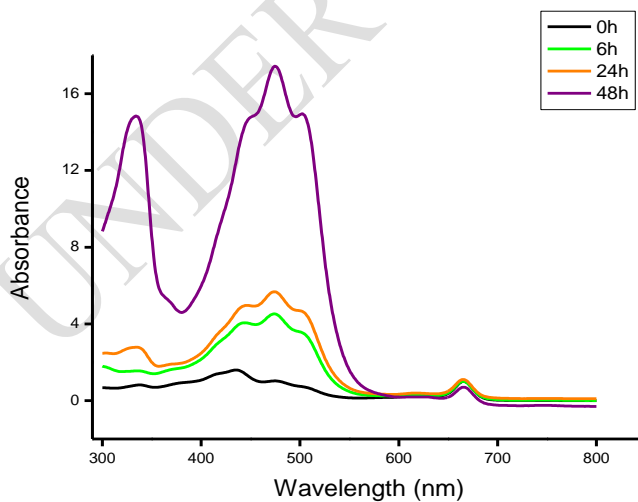


Fig. 3. Absorption spectra of methanol extract from *Leptolyngbya* sp. CN1 treated by  $0.1 \text{ W.m}^{-2}$  UV-B for different hours.

### 3.3 Identification of MAAs in *Leptolyngbya* sp.CN1

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MAAs are well-known for their strong absorbance in UV regions. In consistence, the extracted MAA solution from UV-B treated *Leptolyngbya sp.*CN1 showed maximal absorbance at 334 nm. The extracted MAAs were then separated and characterized by the technology of LC-MS. The HPLC profile at detecting wavelength of 330 nm showed an obvious peak at 2.6 min, possibly suggesting one type of MAA production by UV-B induction (Fig.4A). The mass spectrum of the compound corresponding to this peak showed a prominent ion peak of protonated fragment [M+H] at m/z 333.1269 suggesting the molecular weight of 332 (Fig.4B). Besides, this compound also demonstrated in-line absorbance spectrum centered at 334 nm (Fig.4C). The results of molecular weight and profile of absorbance spectrum were consistent with the characteristics of previously recognized MAA shinorine. These results also indicated shinorine was the dominant type or likely the only one type of MAAs in *Leptolyngbya sp.*CN1.

The shinorine content reached up to 940  $\mu\text{g}\cdot\text{mg}^{-1}$  dry weight after 4 days of UV-B treatment. There were no significant changes in shinorine production in *Leptolyngbya sp. CN1* at the subsequent time points (Fig. 5).

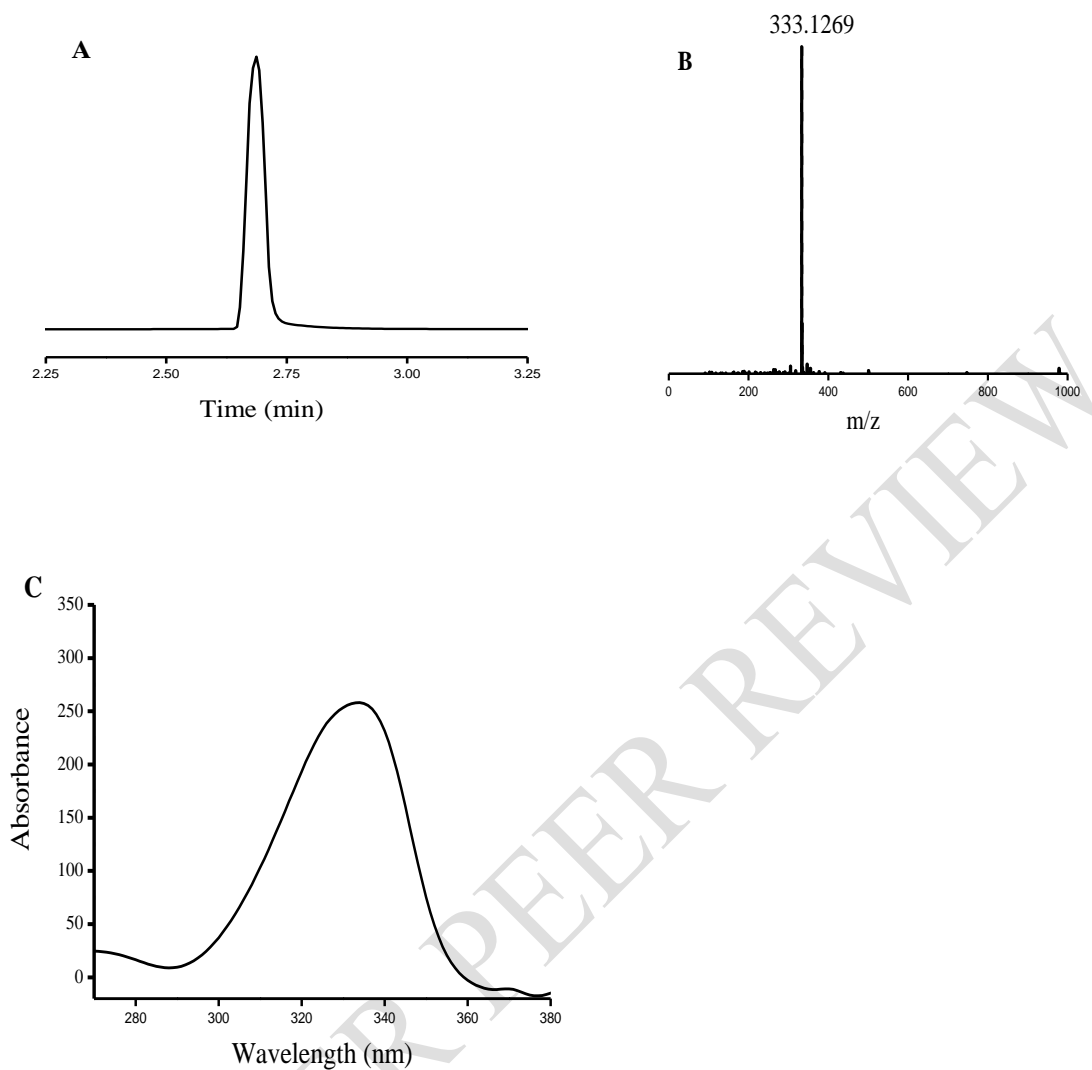


Fig. 4. Characterization of MAA in *Leptolyngbya* sp. CN1, HPLC profile (A), LC-MS analysis (B) and in line absorption spectrum (C) for methanol extract of *Leptolyngbya* sp. CN1 with 0.1 W.m<sup>-2</sup> UV-B treatments for 7 days.

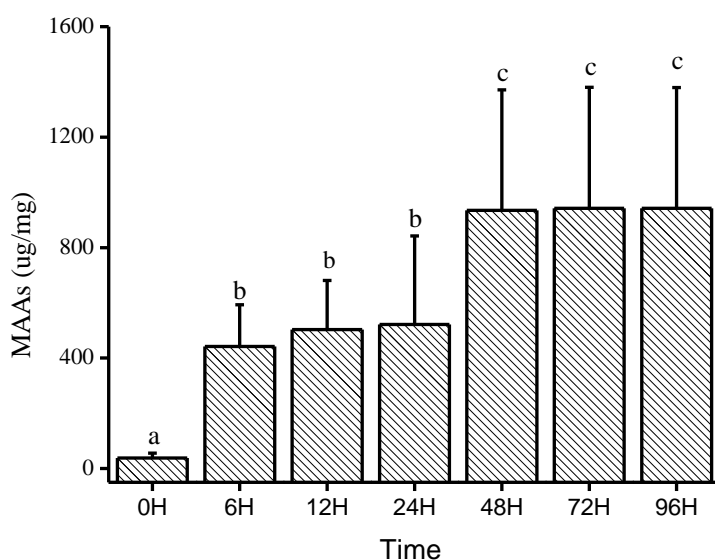


Fig.5. Concentration of MAAs in *Leptolyngbya* sp. CN1 exposed to  $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  photosynthetically active radiation and  $0.1 \text{ W m}^{-2}$  UV-B radiation for up to 96 H. Different letters on the error bars indicate significant differences (Tukey's HSD,  $p \leq 0.05$ ). Data are presented as the mean  $\pm$  standard deviation ( $n = 3$ ). [31]

#### 4. DISCUSSION

MAAs are well-known as UV-protective compounds and play an essential role in photoprotection of cells under UV radiation conditions.[14]. Their strong UV absorption and high molar coextinction efficiency suggested a promising application in cosmetic industries to reduce the harmful effects of UV radiation on human skin cells. Cyanobacteria are considered a rich source for the production of novel MAAs because of their unexpected diversity and ubiquitous distribution on the earth [13, 29]. Although more than 40 kinds of MAAs have been reported, scientists have explored novel MAAs in various cyanobacteria from different environments [3,15,16,17,18 ].

In the present study, we isolated a filamentous cyanobacterium strain, *Leptolyngbya* sp. CN1, from soil in the southeast of Niger and characterized the production of the mycosporine-like amino acid (MAA) shinorine in *Leptolyngbya* sp. CN1. *Leptolyngbya* sp. CN1 taxonomically belongs to the order Oscillatoriales. To our knowledge, only a few cyanobacterial strains in the Oscillatoriales order have been reported to produce MAAs, including *Arthrospira* sp. CU2556 [19, 21], *Lyngbya* sp. CU2555 [20], *Microcoleus chthonoplastes* [22], *Oscillatoria spongeliidae* [23], *Trichodesmium* spp. [24]. MAA production varied widely among genus, species, and even strains. Some *Microcoleus* isolates produced one type MAA shinorine while *Microcoleus chthonoplastes* and *Microcoleus paludosus* strains biosynthesized four other

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unidentified MAAs in addition to shinorine[22]. *Arthrospira sp.CU2556* was found to produce one type MAA mycosporine-glycine [21]. Further detection showed that several MAAs were observed in other organisms, such as palythine and asterina in *Lyngbya sp. CU2555* and shinorine, asterina-332, mycosporine-glycine and porphyra-334 in *Trichodesmium* strains [20, 24]. Other cyanobacteria frequently studied for MAA production mainly belong to the orders Synechococcales, Chroococcales, and Nostocales. Generally, shinorine, mycosporine-glycine, porphyra-334, and asterina-330 are the most abundant of MAAs among these cyanobacteria [13, 19].

The genetic bases and biochemical pathway have exclusively resolved for shinorine biosynthesis in cyanobacteria [14, 25, 26, 27]. The 4-Deoxygadusol 4-DG can be synthesized from shikimate pathway and pentose pathway as the precursor for various MAA production [26]. In the pentose pathway Desmethyl-4-Deoxygadusol Synthase DDGS and O-Methyltransferase O-MT catalyze Sedoheptulose-7-Phosphate SH7P to form 4-DG, which was used as the substrate with glycine to produce mycosporine-glycine [25]. Shinorine was then yielded through condensing serine with mycosporine-glycine by non-ribosomal peptide synthase (NRPS) or D-ala D-ala ligase [3, 25, 27, 30].

## 5. CONCLUSION

*Leptolyngbya sp.CN1* was identified as a organism that produces a considerable abundance of shinorine to protect against UV-B radiation. Present study shown that *Leptolyngbya sp.CN1* may be useful as a producer of UV absorbing compound MAA. It will be the next exciting research to elucidate the genetic bases and biosynthetic pathway for shinorine in *Leptolyngbya sp.CN1* in the future.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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