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Characterization of UV-absorbing Mycosporine-like Amino Acids in Freshwater Cyanobacterium *Nostoc calcicola*

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Authors' contributions

This work was carried out in collaboration among all authors. Author MCMB conceived the study and wrote the manuscript, author KW isolated the cyanobacterial strain, authors MCMB and MMCB detected and characterized MAAs, authors SI, KKBK and MDS analyzed the data, authors GM and MA revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Mycosporine-like amino acids (MAAs) are gaining attention for their UV photoprotective properties and potential applications in biomedical fields, cosmetics, and toiletries. In this study, *Nostoc calcicola* a genus of cyanobacteria distributed in freshwater were tested for MAA production. This organism was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. It was isolated and cultured in the laboratory, in 500 ml glass flasks containing 300 ml of liquid medium BG11 treated on UV-B light 0.1 Wm^{-2}. At different time, cultures were exposed to UV-B light at intensity 0.1 W. m^{-1} to determine the Maximum PSII photochemical (Fv/Fm). MAAs were extracted using 100% HPLC grade methanol and separated through high-performance liquid chromatography and liquid chromatography/mass spectrometry. Based on liquid chromatography/mass spectrometry analysis, one MAA compound was identified as porphyra. Thus, UV-B treatment of *Nostoc calcicola* significantly enhanced the production of UV-absorbing mycosporine-like amino acids (MAAs). The content of porphyra could reach up to 8.5 mg/g dry weight in *Nostoc calcicola* under UV-B treatments for 2 days. The present cyanobacteria could be used for biotechnology research and the UV-absorbing compound porphyra may be of great value in the development of novel sunscreens.

Keywords: Cyanobacteria; mycosporine-like amino acids; Nostoc calcicola; porphyra; ultraviolet B.

1. INTRODUCTION

"The concerns are growing more and more about increasing ultraviolet (UV) radiation on the earth surface due to the depletion of stratospheric ozone layer in the past 40 years" [1,2,3]. The energetic UV radiation is considered to be very harmful on the living beings, such as inducing acute and chronic disorders on the skins, triggering DNA mutations in the cells, bursting reactive oxygen species, reduction in growth, death of the organism and so on [2,3,4]. "Mycosporine-like amino acids (MAAs) are the well-known photoprotectants to attenuate the harmful UV radiation by converting it into heat without generation of ROS" [5,6,7]. "Generally, MAAs represent a suite of low molecular weight (ranging from 188 to 756 Da), water-soluble and colorless UV-absorbing compounds" [8,9,10]. "Various amino acids were conjugated with a cyclohexenone or cyclohexeniminone rings resulting in the formation of diverse aminocyclohexenone-type MAAs or aminocyclohexene imine-type MAAs" [4,6,11]. "Different substituents on the central cyclohexenone or cyclohexeniminone rings conferred the variations of UV-absorbance with the maximum wavelength between 312 and 360 nm and high molar absorptivity from 2.81×10^4 to 5.00×10^4 $M^{-1}\cdot cm^{-1}$" [6]. "Nowadays, more than 40 kinds of structurally different MAAs or MAA derivatives have been reported, and are considered as the promising active ingredients in cosmetic industry" [4,11,12,13]. "Exactly, the formulation containing MAA shinorine as an active ingredient has been developed in two market sunscreen products (Helioguard 365 and Helionori)" [14]. Recent years scientists are exploring the kinds of MAAs especially in the photosynthetic organisms, which is fundamental to develop the next generation of sun care product formulation [15,16,17].

"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 (without the ozone shield), cyanobacteria have evolved sophisticated ways to synthesis diverse MAAs to cope with UV radiation for their ubiquitous adaptation on earth" [18]. "The abundance and composition of MAAs varied among cyanobacterial species and were also affected by the environmental factors such as light fluctuations and nutrient limitations" [4,9,19].

Nostoc is a filamentous cyanobacterium fixing atmospheric nitrogen which is needed for other plants to survive. This cyanobacterium has the ability to live in environmental with intense solar radiation and high temperatures. It can tolerate negative effect of UV-B radiation and can induce MAA under UV-B stress [2,10,20]. This present study will identify UV absorbing compounds mycosporine-like amino acids (MAAs) in *Nostoc calcicola* from freshwater.

2. MATERIALS AND METHODS

2.1 Experimental Organism and Growth Conditions

Nostoc calcicola FACHB 389 was provided by the institute of hydrobiology, the Chinese academy of sciences. It was isolated and cultured in the laboratory conditions. The strain was cultured in 500 mL glass flasks (Sterilized in an autoclave at 121 ^{0}C for 20 minutes) containing 300 mL of liquid medium BG11 (Table. 1). "This Organism was grown under white fluorescent lamps at intensity 15 µmol photons $m^{-2}s^{-1}$, and temperature 26 ± 2 °C. The culture was shaken three times per day. After 14 days, organism was transferred and exposed to fluorescent UV-B lamps (with irradiation 0.1 $W.m^{-2}$) in addition to white light illumination for 7 days. After growth, Samples were collected by centrifugation and kept at -80^{o}C until MAAs analysis" [21].

2.2 Microscopy Observation

The morphology of the cyanobacterium was observed using a laser scanning confocal microscope (LSM710, Carl Zeiss Microscopy, Germany) coupled with Zen 2 software (Carl Zeiss Microscopy). It is a filamentous, non heterocystous. The filaments show straight or waved or arcuate without branches (Fig. 1). The cells in the filaments are cylindrical and blunt (Fig. 1).

Table 1. Composition of BG11 Media

Reagents	BG11
$NaNO_3$	1.5 g
$MgSO_4.7H_2O$	0.075 g
Na_2CO_3	0.02 g
K_2HPO_4	0.04 g
$CaCl_2.2H_2O$	0.0027 g
Ferric ammonium citrate	0.006 g
Citric acid	0.006 g
$EDTANa_2$	0.001 g
Micronutrients	1.0 mL
Distilled water	1.0 L
pH	7.1
***Micronutrients* stock solution**	
H_3BO_3	2.86 g
ZnSO4.7H2O	0.22 g
$CuSO_4.5H_2O$	0.79 g
$CoCl_2$	0.049 g
$MnCl_2.4H_2O$	1.81 g
$Na_2MoO_4.2H_20$	0.39 g
Distilled water	1.0 L

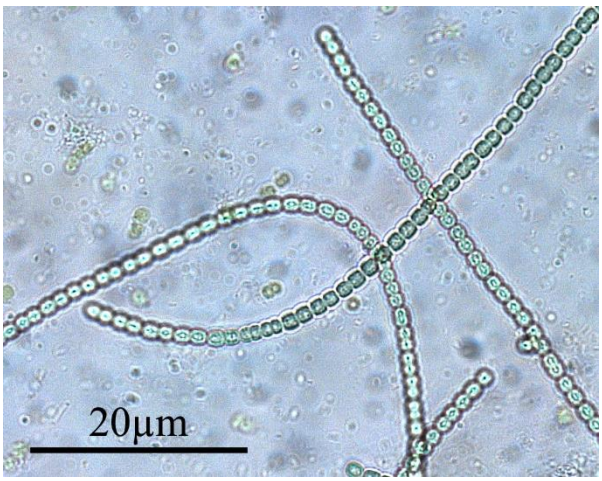

Fig. 1. Cell morphology of *Nostoc calcicola* under microscope

2.3 Effects of UV-B radiation on *Nostoc Calcicola*

"Samples were placed into petri dish glass and exposed to UV-B with intensity 0.1 $W \cdot m^{-2}$up for 6 h at 25 ^{0}C. UV-B non-exposed samples were considered as control. Each hour we determined the maximum PSII photochemical Fv/Fm by using a Plant Efficiency Analyser(PEA, Hansatech Instruments Ltd, King's Lynn and Norfolk, UK). Samples were dark adapted for 10 minutes before measurements. Each sample was replicated three times (n=3)" [21].

"For Induction of MAAs under UV-B stress, samples were exposed to UV-B lamp with radiation 0.1 $W.m^{-2}$ in petri dishes glass for 6, 24 and 48 hours. UV-B non exposed samples were used as control. After UVB treatment, samples were collected by centrifugation at 6000 rpm for 5 min and MAAs were extracted in 4 mL of 100% HPLC grade methanol overnight at 4^{o}C in a refrigerator. The methanol extracts were then centrifuged at 6000 rpm for 5 min, and Supernatant was transferred to a 2 mL Eppendorf tube. Supernatants were analyzed by UV-Visible spectrophotometer between 200 and 800 nm. Data and peaks were analyzed by probe software" [21].

2.4 Characterization, Identification and Quantification of MAAs in *Nostoc calcicola*

"The UV-B treated culture was harvested for MAA extraction by methanol as above. The methanolic extracts were dried in a vacuum concentrator (Labconco, UK) and ultrapure water (18.2 MΩ·cm) was added to resolve the samples. Equal volume of chloroform was used to remove pigments in the solution and MAAs were then characterized by liquid chromatography-mass spectrometry (LC-MS) analysis on the machine of Agilent technologies 6540 UHD Accurate-Mass Q-TOF. The solution sample was injected into a reversed-phase HPLC system coupled with in-line absorption spectral scans and equipped with column inertsil ODS-SP (5 µm, 4.6 mm × 250 mm, GL Sciences Inc, Japan). The MAAs were detected at 330 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 mass spectrometer" [21].

For MAA content analysis, the cyanobacterial culture was exposed to 0.1 $W \cdot m^{-2}$ UV-B. At each time point, the cyanobacterial pellets were harvested by centrifugation at 6000 rpm for 5 min. MAAs were extracted and separated by high performance liquid chromatography as above. The MAA eluent solution was collected from HPLC in EP-Tube and recorded for absorbance at 334 nm using spectrophotometer. The MAA content was finally calculated using its extinction coefficient 42300 $M^{-1} \cdot cm^{-1}$[11].

3. RESULTS

3.1 Effect of UV-B on the Maximum Photochemical PSII (Fv/Fm)

The Fv/Fm for *Nostoc calcicola* significantly decreased after 6 h to UV-B treatment (Fig. 2). The decrease of Fv/Fm value from non exposed UV-B (control) was less than from UV-B treatment. The decrease of Fv/Fm indicated negative effect of UV-B treatment in *Nostoc calcicola*.

3.2 Effects of UV-B Radiation on MAA Production

The cellular 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 considerable MAAs induction by UV-B treatments (Fig. 3). The quite low peak at 334 nm suggested MAA production in *Nostoc calcicola* (Fig. 3). MAAs production were slightly increased after 6 h exposure of 0.1 $W \cdot m^{-2}$ UV-B, but significantly enhanced after 24 h and 48 h UV-B treatments (Fig. 3). The changes of MAA contents were positively related to prolonged UV-B treatments, suggesting protective roles in *Nostoc calcicola* from UV-B radiation.

3.3 Characterization of Mycosporines like Amino Acids (MAAs) in *Nostoc calcicola*

After separation and detection by HPLC at 330 nm, a peak was observed at 2.6 min suggesting one type of dominant MAA production by UV-B induction in *Nostoc calcicola* (Fig. 4). The mass spectrum of the compound corresponding to this peak showed a prominent ion peak of protonated fragment $[M+H]^+$ at m/z 347.1556 suggesting the

molecular weight of 346 (Fig. 5). Besides, this compound also demonstrated in-line absorbance spectrum centered at 334 nm (Fig. 6). The results of molecular weight and profile of absorbance spectrum were consistent with the characteristics of previously recognized MAA porphyra [22]. These results also indicated porphyra was the dominant type or likely the only kind of MAA in *Nostoc calcicola* under continuous UV-B exposure.

3.4 Total amount of UV-absorbing MAAs in *Nostoc calcicola*

Liquid chromatography/mass spectrometry analysis revealed that porphyra, a UV-absorbing MAA, was significantly enhanced in Nostoc calcicola, with content reaching up to 8.5 mg/g dry weight after 2 days of UV-B treatment (Fig. 7).

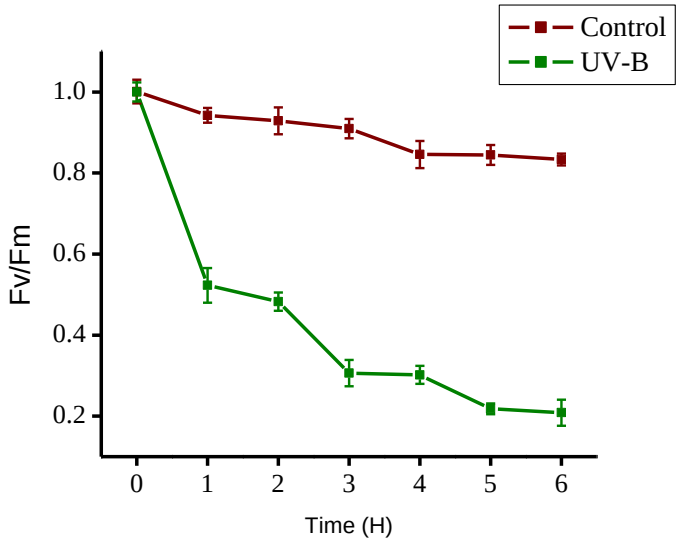

Fig. 2. Photosynthetic tolerance of *Nostoc calcicola* after UV-B treatment at 0.1 W·m^{-2} for several hours (h)

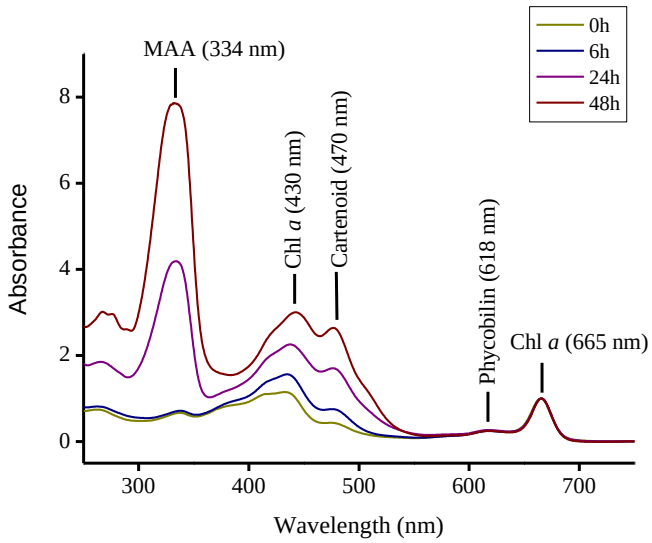

Fig. 3. MAA induction in *Nostoc calcicola* under 0.1 W·m^{-2} UV-B treatment

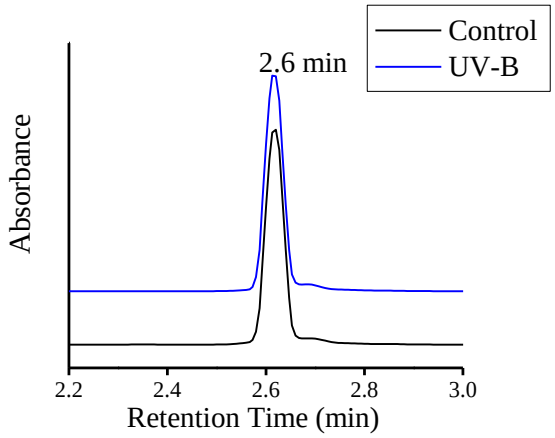

Fig. 4. HPLC profile of MAA from *Nostoc calcicola* showing the typical peaks at retention time 2.6 min

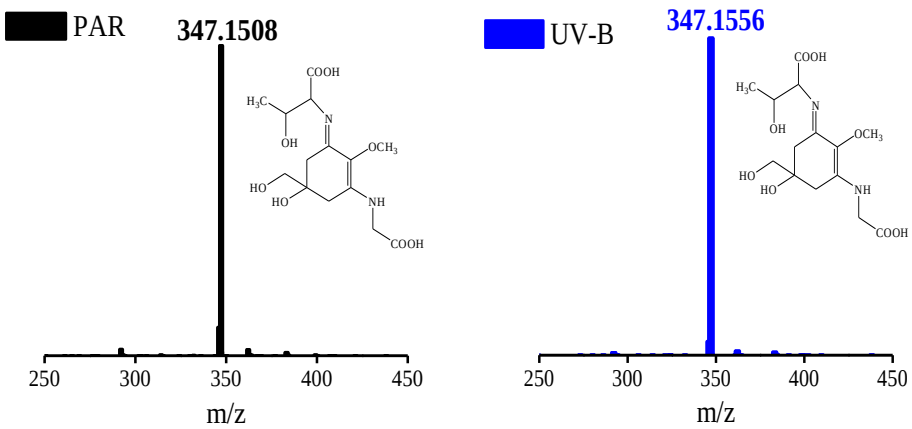

Fig. 5. Mass spectrometric analysis of MAA in *Nostoc calcicola.*

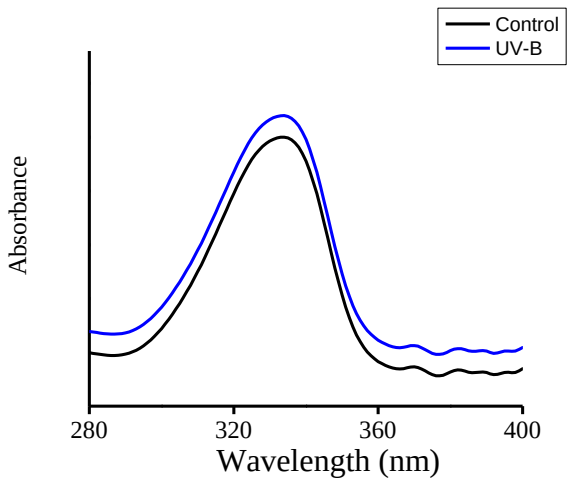

Fig. 6. In-line absorbance spectra of methanol extracts from *Nostoc calcicola*.

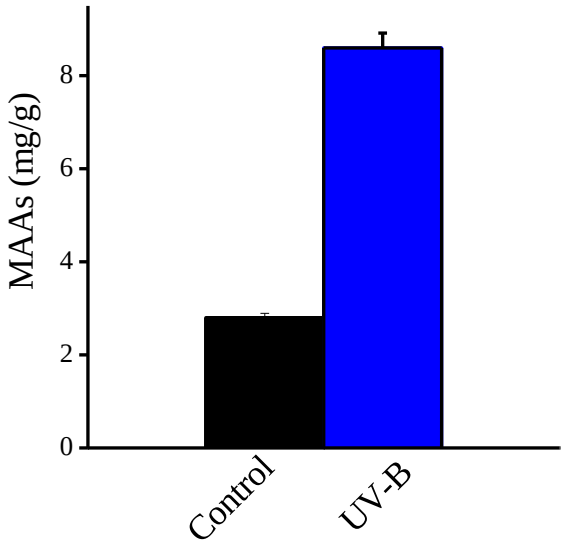

Fig. 7. MAA Concentration in *Nostoc calcicola* cultured under white light of 15 µmol photons $m^{-2}s^{-1}$ plus 0.1 W·m^{-2} UV-B for up to 2 days. There are three replicates (*n*= 3)

4. DISCUSSION

MAAs are the well-known UV-screening compounds and play pivotal roles in photoprotection of cells under UV radiation conditions [2]. Their outstanding features of strong UV absorption and high molar coextinction efficiency suggested promising application in cosmetic industries to reduce the harmful effects of UV radiation on human skin cells. Cyanobacteria are considered as a rich source of MAA production because of their unexpected diversity and ubiquitous distribution on earth [18]. In present study, we isolated a filamentous cyanobacterial *Nostoc calcicola* from the freshwater, and characterized the production of MAA porphyra. *Nostoc calcicola* is taxonomically belonged to the Nostocales order. MAA production varied a lot in Nostocales order. Some *Nodularia* isolates produced two types MAAs Shinorine and Porphyra [23]. "*Nostoc* sp HKAR-2 and *Nostoc* sp HKAR-6 isolated respectively from the hot springs of Rajgir and rice paddy fields were found to produce both shinorine and porphyra [22]. Recently, the terrestrial cyanobacteria *Nostoc flagelliforme* produced mycosporine-2-(4-deoxygadusolyl-ornithine), and an aquatic cyanobacterium *Nostoc verrucosum* produced shinorine and porphyra. The *Nostoc commune* from Kakuma campus of Kanazawa University produced porphyra" [24]. *Anabaena variabilis* PCC 7937 was also found to produce Shinorine, Palythine-serine and Mycosporine-glycine [25]. Multiple MAAs were observed in some organisms, such as palythine and asterina in *Lyngbya sp.* CU2555 and shinorine, asterina-332, mycosporine-glycine and porphyra-334 in *Trichodesmium* strains [26,27]. In this study, *Nostoc calcicola* produced a considerable amount of porphyra under UV-B radiation. MAA porphyra significantly increase after 48h of UV-B treatment. "The presence of high concentration of MAA porphyra in *Nostoc calcicola* cells is supposed to provide protection by absorbing lethal dose of UV-B. Cyanobacteria increase their MAA content in response to UV radiation and were able to adapt to a changing daily solar radiation in their natural habitat. MAA protect the cells by absorbing highly energetic UV radiation and then dissipating this energy in the form of harmless heat radiation to their surroundings" [28]. MAA content may play an important role in photoprotection.

More than 40 kinds of MAAs are reported in various organisms, and can be generally divided into aminocyclohexenone-type MAAs and aminocyclohexene imine-type MAAs and their derivatives [4,11]. The former MAAs have an amino acid group condensed on the central cyclohexenone ring and are typically presented by mycosporine-glycine and mycosporine-alanine [4]. While the latter MAAs either contain two amino acid substituents (e.g. shinorine, porphyra) or composed of one amino acid group (e.g. palythine and palythine-threonine) on the

central cyclohexeniminone rings. However, the genetic bases and biochemical pathways have mainly resolved for biosynthesis of mycosporine-glycine, and shinorine and porphyra in cyanobacteria [29,30,31]. The 4-deoxygadusol (4-DG) can be synthesized from shikimate pathway and pentose pathway as the central rings for various MAA productions [30]. “In the pentose pathway, desmethyl-4-deoxygadusol synthase and O-methyltransferase catalyze sedoheptulose-7-phosphate to form 4-DG, and ATP-grasp ligase exclusively ligated glycine moiety into 4-DG to produce mycosporine-glycine [29,31] Finally, porphyra was then yielded through condensing threonine with mycosporine-glycine by (non-ribosomal peptide synthase)-like enzyme or D-ala D-ala ligase” [29,31].

5. CONCLUSION

MAAs are considered as the efficient UV absorbing compounds to attenuate UV radiation in various organisms, and also have potential applications in cosmetic markets in the biotechnological perspectives. Cyanobacteria are effective MAA producers and have being explored for various MAA production.UV-B light seemed to play key role in regulation of MAA production in cyanobacteria from natural habitat. *Nostoc calcicola* produced porphyra as the only one type of MAA under UVB radiation. MAAs could be produced in *Nostoc calcicola* under low dose of UV-B treatment and have ability to protect cells against UV-B damage because they are able to increase their concentration. This study suggests that Nostoc calcicola could be a valuable source of UV-absorbing compounds like porphyra, which may have significant potential in developing novel sunscreens and other biotechnological applications.

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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