Maspari Journal 03 (2011) 20-23

MASPARI JOURNAL

http://masparijournal.blogspot.com

Dynamic Response of Ultra Violet Absorbing in *Dunaliella* sp

Moh. Muhaemin
Jurusan Budidaya Perikanan, Universitas Lampung, Lampung

Received 06 January 2011; received in revised form 08 April 2011;
accepted 12 April 2011

ABSTRACT

Micosporine-like Amino Acid (MAA) which can be found in fresh water and marine microalgae. its accumulation may have a role as a response to UV exposure. The accumulation of MAA was found in both artificial and solar UV radiation. No clear correlation between response of MAA to UV exposure.

Keywords: MAA, UV, *Dunaliella* sp

1. INTRODUCTION

Some species showed considerable UV tolerance, and the other species might extremely UV-sensitive. Such a species-specific variability in UV sensitivity was found not only in terrestrial plants (Tevini and Teramura, 1989; Teramura and Sullivan, 1993), and also in terrestrial and freshwater microalgae (Williamson and Zagarese, 1994; Xiong et al., 1999).

However, the mechanisms that underlie such a wide variability have yet to be described well. The mechanisms such as photolyase-mediated photo reactivation that repairs UVdamaged DNA, UV screening of the leaf epidermis that can effectively reduce the amount of UV radiation reaching the target site in mesophyll, and the D1-turnover-mediated repair cycle of UV-damaged photosynthetic machinery were hypothesized (Britt, 1995; Day, 1993; Lesser et al., 1994). Similarly, aquatic organisms have been shown to accumulate UV-absorbing compounds when irradiated with UV radiation (Carreto et al., 1990; Karentz et al., 1991; GarciaPichel and Castenholz, 1993). These compounds, collectively called as mycosporine-like amino acids (MAAs), have absorption maxima in the UV region between 300-400 nm.

Although the importance of UV-absorbing compounds in UV protection in fresh water microalgae has been well confirmed (Williamson and Zagarese, 1994; Xiong et al, 1999), the role of MAAs in the UV protection in marine microalgae is poorly understood. Furthermore, less information on the occurrence of MAAs in marine microalgae is available since most studies have addressed marine organisms or cyanobacteria (Boham et al., 1995; Karentz et al., 1991; Nakamura et al., 1982). In this work, we used Dunaleilla sp. We observed the occurrence of MAAs in the marine microalgae species, and evaluated the

Corresponden number: Tel. +62711581118; Fax. +62711581118
E-mail address: masparijournal@gmail.com

correlation between UV tolerance and M-Gly accumulation in response to UVB exposure.

II. MATERIALS AND METHODS

The research was conducted on September 2010 to February 2011 in Aquaculture Laboratory, University of Lampung. Pure isolate of Dunaliella sp were selected as experimental materials in this study. The algal species originated from The Marine-culture Developing Centre Laboratory, Hanura-Lampung. The algae were grown in batch cultures under the laboratory conditions as describe elsewhere (Muhaemin, 2008).

The exponentially growing cultures were diluted to 10 mM Chl. 50 ml of the diluted cell suspension were subjected to cellulose acetate-filtered artificial UV radiation (2 W m^{-2}) over 2 h. For long-term exposure to artificial UV radiation, the diluted cell suspension (ca. 0.25 of OD753) was placed in a UV-transparent vessel, and was irradiated with 15 Wm^{-2} of PAR supplemented with artificial UV radiation that was provided by UV lamps (Philips TL 20W/12). UV radiation was measured with SED-240 detector/IL 1400 A radiometer calibrated with a spectroradiometer. The incident UV at the surface of the culture was adjusted to 1.2 Wm^{-2} by altering the distance between the vessel and the lamps. The dose rate was equivalent to the ambient level of UV radiation monitored at high noon at the experimental location. For the control, the UV component was removed by filtering the vessel with Mylar film (0.25 mm) (Xiong et al, 1999).

Chlorophyll fluorescence was measured with a PAM fluorometer, while the photochemical efficiency of PS II was calculated according to Genty et al. (1989). Before measurement, both the control and the UV exposed samples were dark adapted for 10 min.

Microalgae cells suspensions were pelleted by centrifugation at 2500 rpm for 15 min. The pellet was mixed with 2 ml of 100% methanol and the same volume of glass beads (0.25 mm, Sigma). After 5 min of vigorous mixing, the cell debris was spun down and the supernatant was collected.

Aliquots were taken from the culture at intervals, during cultivation, pelleted and stored at -70°C for HPLC analysis of UV-absorbing compounds which was conducted within the following 4-5 days. The frozen sample was extracted with 70% (v/v) methanol at 45°C for 2.5 h with occasional vortex mixing. The maximum absorption (λ_{max}=326) of methanol extracts was used for quantitative estimate of MAAs in the tested algal species. Due to the absence of absorption coefficients for individual MAAs, only the total content of MAAs was quantified. The content was expressed as A_{λ} mg^{-1} (DW), where, A_{λ} was derived by multiplying the maximum absorbance measured with a 1 cm path length cuvette with the total volume of the extract. For HPLC, the methanol extract was dried under vacuum on a rotary evaporator at 45°C. The resulting dry matter was dissolved in the mixture (0.2% v/v acetic acid). The insoluble residue was removed firstly by centrifugation at 3000 rpm for 30 min, and then by filtration through Whatman GFF filters. The 25 ml of aliquots were injected for HPLC analyses. The mobile phase was 0.2% (v/v) acetic acid±4% (v/v) methanol in water. Separation was carried out with a flow rate of 1 ml min^{-1} at room temperature (ca. 20°C). Absorption spectra of MAAs in the elution were detected in the range of 280±340 nm.

One way ANOVAs were used to examine UV effects. The LSD

(least significant difference) means test was used to compare difference between non-UV and UV treatment.

III. RESULTS AND DISCUSSIONS

Slight degradation of chlorophyll in the UV-resistant can caused by 40 min UV exposure. To verify the contribution of UV absorbing compounds to the observed increase in UV region of in vivo absorption spectra, aqueous extracts of the cultures were examined with HPLC. The results revealed widespread occurrence of UV-absorbing M-Gly in the tested microalgae (Figure 1) In Dunaliella sp. HPLC also illustrated that UV-induced accumulation of MAAs was largely individual MAA-specific.

In tropical and temperate marine organisms the occurrence of UV-absorbing MAAs and their accumulation has been postulated as a potentially protective way against deleterious UV radiation (Xiong et al., 1999). Over a dozen MAAs have been identified in marine algae (Gealson, 1993). The total content of MAAs among the tested and different microalgae ranges between 0.05±0.09 A_{326} mg $^{-1}$ DW with controlled cultures (Table 1).

Table 1. Total specific contents of MAA among microalgae (*Xiong et al., 1999)

Microalgae Species	MAA Specific Content	
	Without UV	UV irradiated
Chlorella sp (*)	0.06±0.003	0.09±0.005
Scenedesmus sp (*)	0.06±0.004	0.06±0.003
Dunaliella	0.08±0.002	0.18±0.003

The correllation between occurence of MAA accumulation in response to UV exposure cannot be found significantly based on the result. A large variability (Table 1) in screening effectiveness was assumed that those attributed to the spatial location of UV absorbing; mainly in the epidermis or in the mesophyll (Day et al, 1994). Garcia-Pichel and Castenholz's (1993) shoed a similar phenomena in the magnitude of the sunscreen factor, as a fraction of incident radiation, which can be largely stimulated by the cell size. The accumulation of UV absorbing coumpound does occur in response to elevated UV exposure; it was sufficient to completely offset UV caused damage.

The result showed that no correlation existed between accumulation of UV absorbing compound and UV tolerance in Dunaliella. The result showed that the difference of UV absorbing cannot be solely correlated with the differences in UV capacity. UV radiation insensitivity may a result from other protection mechanism. UV protective strategies of microalgae may need more assessment to provide deep understanding the importance of each mechanism.

REFERENCES

Britt, A.B. 1995. Repair of DNA damage induced by ultraviolet radiation. Plant. Physiol. 108: 891-896.

Boham, G.A., Pfleider, W., Boger, P., and Sherer, S. 1995. Structure of a novel oligosaccaride-mycosporine amino acid ultraviolet A/B sunscreen pigment from the terestrial cyanobacterium Nostoc commune. J. Biol. Chem. 270: 8536-8539.

Carreto, J.I., Carignan, M.O., Daleo, G., and De Marco, S.G. 1990. Occurence of mycosporine-like amino acid in the red tide dinoflagellate Alexandrium excavatum: UV-protective compound?. J. Plankton. Res. 12:909-921.

Day, T.A. 1993. Relatin UV-B radiation screening effectiveness of foliage to absorbing-compound concentration and anatomical characteristics in a diverse groups of plant. Oecologia. 95: 542-550.

Day, T.A., Howells, W., and Rice, W.J. 1994. Ultra violet absorption and epidermal transmittance spectra in foliage. Physiol. Plant. 92: 207-218.

Garcia-Pichel, F., and Castenholz, R.W. 1993. Occurence of UV absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl. Environ. Microbiol. 59: 163-169

Gealson, D.F. 1993. Differential effects of ultraviolet radiation on green and brown morphs of Carribean coral Porites astreroides. Limnol. Oceanogra. 38: 1452-1463.

Genty, B., Briantais, J.M., and Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quencing of chlorophyll fluorescence. Biochim. Biophys. Acta. 990: 87-92

Karentz, D., McEuen, F.S., Land, M.C., and Dunlap, W.C. 1991. Survey of mycosporine-like amino acid compounds in Antartic marine organisms: Potential protection from UV exposure. Marine Biol. 108: 157-166.

Lesser, M.P., Cullen, J.J., and Neale, P.J. 1994. Carbon uptake in a marine diatom during acute exposure to ultraviolet B radiation: Relative importance of damage and repair. J. Phycol. 30: 183-192.

Muhaemin, M. Glutathione diurnal cycling in Dunaliella salina. J. Coastal Development. Vol 12 (1) : 41-46.