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The intracellular photopigment and glutathione (GSH) dynamics in *symbiodinium* natural population during light stress and recovery

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Abstract. It is already proven by scientists, that mass coral bleaching is an early signal for increasing global temperature. Over the last decades, there was a lack of studies aimed at photoinhibition in *Symbiodinium* during laboratory treatments. It was important to predict the initial adaptation in relation to photo-inhibitory of photosynthesis accompanied by slight photo-protective mechanisms and gradual recovery of *Symbiodinium*. This information is important to understand the recovery mechanism of mass coral bleaching, which can help to develop sustainable management of the ecosystem. In our manuscript, we applied medium light stress [600 μmol (photon).$m^{-2}.s^{-1}$] to trigger changes in intracellular photo-pigments (chlorophyll a, chlorophyll c, carotenoid) and glutathione (GSH) contents in *Symbiodinium*. The result showed that light-induced generated various depletion in chlorophyll (chl) *a* and chlorophyll (chl) *c* contents, and more production in intracellular carotenoid and GSH. The total intracellular GSH increased during the first 10 min of culture at high light treatment and followed by a decrease (at 60 min). The GSH recovery finished in *Symbiodinium* even after 6 h indicated the high ability of photo-protective processes in the species as another photo-inhibition effect and its photosynthetic performance in the initial environment.

Keywords: photopigment, glutathione (GSH), light, photoprotective, recovery

1. Introduction

Climate change as scientific phenomena has been a major issue for decades with a wide ecological diversity effect included coral reef ecosystem [1]. Coral reef ecosystem is most sensitive to climate change due to coral bleaching mechanism which may induce the severe effect of the dynamic mutualistic relationship between host and *Symbiodinium* [2]. *Symbiodinium* as major endo-symbiont of cnidarian group dominate widely in shallow marine tropical habitats. *Symbiodinium* as photo-eukaryotic microorganism found specifically in endodermal host tissue at various dense [3] or associates with anemones, flatworms, sponges, jellyfish, nudibranchs, tridacnid bivalves, and protozoa [4]. In associates with coral, they maintain mutualistic symbiosis by translocating fixed organic carbon and other valuable metabolites compounds from *Symbiodinium* to host and transporting inorganic compounds from host to *Symbiodinium* [5, 6]. *Symbiodinium* also plays a vital role in maintaining the ecological achievement of coral reef and constructing the tropical marine ecosystem [7] and a key of coral reef in maintaining their life. The stability of symbioses between coral host and endo-symbiont may be affected by a major environmental condition such as light and nutrient [4,6,9]. *Symbiodinium*

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could adapt with UV stress as described in specific coral growth model [10] and photon flux density (PFD) exposes by producing more intracellular glutathione (GSH) [9].

Light is crucial for *Symbiodinium* as driving force of photosynthetic to generate the initial intracellular energy [6], growth and survival [11]. Although excessive light can be harmful, the quantity of photons absorbed depends on the capacity of the photosynthetic activity or the dark reactions utilize them (over-excitation). The components of the electron transport chain could reduce with the increase of excessive light, which may lead to irreversible damage and decline in the photosynthetic efficiency and/or photosynthetic rate. Photosynthesis is the most prominent target of excessive light radiation which is triggering various possible cell damages such as the depletion of D1 and or D2 protein of light-dependent reaction, deterioration of intracellular photo-pigment, perturbation of electron flow between light and dark reactions, or reducing genes expression correlated with photosynthesis [11]. The photosynthesis decreasing and production reducing of symbiont may cause by the loss of photo-pigment, and generate the death of the host [10] or triggered high production of Reactive Oxygen Species (ROS) [9]. On the other hand, the intracellular photo-pigment may contribute to increasing the photosynthetic activity of symbiont, protect the host from solar radiation and provide phenotypical host characteristic [13].

The excessive light stress exposed to auto-phototropic organisms induces instability in the intracellular metabolism of oxygen, which may exceed intracellular oxidative stress. The excessive light may generate ROS through intracellular photo-acclimation reactions in phototropic microorganisms [14]. Acute intracellular oxidative stress may induce by an imbalance of photosynthetic efficiency, and deflect the generation of photosynthetic microorganisms, or tend to indicate DNA damage [15]. Conversely, microalgae might evolve specific internal photoprotective mechanism, photo recovery of DNA damage from UV radiation and the re-synthesis of damaged light dependent reaction proteins, ROS scavenging, and the production of UV sensitive compounds (mycosporine-like amino acids (MAAs), scytonemin and sporopollenin) [16,14,17]. Partially, Glutathione (GSH) regularly produced by *Symbiodinium*, it may show the cell protective mechanism by producing GSH which can reduce the damaging effect of light stress event the cell density was decreasing [9].

Because of the lack of study focused on the effect of photo inhibitation in *Symbiodinium*, in this research, we would like to analyze the effect of photoinhibition on the sensitivity of intracellular photosynthetic photo-pigment, and GSH response in relation with stress and recovery mechanism of *Symbiodinium* natural population.

2. Related works

Intracellular chemical compound instability in *Symbiodinium* indicated the unstable metabolism during environmental stress exposed. The variability of photopigment and GSH contents might be reflected in the initial recovery and adaptation mechanism of *Symbiodnium* cells [9,12]. GSH might be used as a chemical indicator to verify the *Symbiodinium* stress during an unusual environment condition [13,16]. The sensitivity of GSH as an indicator should be tested related to the specific stress exposed to *Symbiodinium*. GSH as a chemical indicator of light stress proposed by providing the change of intracellular GSH production indicated the specific photo adaptation mechanism of *Symbiodinium* [9].

3. Materials and Methods

The specimens, soft coral *Zoanthus* sp, were collected from Pahawang Island waters, Lampung and transported to Aquaculture Research Laboratory, the University of Lampung within less than 8 h after collecting. The acclimation was conducted at 20±2 ^{0}C. The specimens were cultured in semi-outdoor semi-running seawater tank and fully exposed with natural solar radiation 12:12 light:dark photoperiod ratio. The range of photosynthetic wavelength exposed to the specimens was 400-600 nm (400±50 $\mu mol.m^{-2}.s^{-1}$). After the acclimation (before experiments), the specimens were tested for photo-inhibitory effect by exposing to 600 $\mu mol.m^{-2}.s^{-1}$ PAR for 2h used Philips Lumileds warm white LED as a source of homogenous continuous light. After photo-inhibitory treatments, immediately, the

specimens were placed in three different light intensity defined low radiance (R1, 60-70 µmol.m^{-2}.s^{-1}), medium radiance (R2, 90-110 µmol.m^{-2}.s^{-1}), and high radiance (R3, 120-130 µmol.m^{-2}.s^{-1}). The partial samples were taken for analysis in specific intervals during the exposition (after 5, 10, 15, 20, 25, 30, 35, 40, 60, 120 and 360 min of the predicted recovery phase).

Isolated *Symbiodinium* from *Zoanthus sp* were homogenized in tissue homogenizer by using artificial seawater (calcium free) with ± 0.03 % (w/v) sodium dodecyl sulfate [4]. The contamination with host tissue and various unexpected microalgae minimized by preparing a half concentrated media of solid agar plates and tested with and without active aeration [4]. The Whatman GF/F filters (35 µm) used to remove clumps from homogenate samples and washed twice gently. The samples centrifuged for 15 min with 110 mM phosphate buffer pH 7.0. The advanced content and preparation of culture media based on the formula given in [9]. The various media was added with precision combination of expanded trace elements and organic supplements, a nitrogen source (NO_3^-/NH_4^-), and chelating agents. Silica and tris-base were prohibited for all culture media to minimize the bio-contamination by diatom. The *Symbiodinium* biomass was calculated by using Neubauer Counter Haemocytometer.

The photo-accessories pigments were analyzed by using HPLC (ChemStation 1100, Agilent Technologies-Germany with gradient system). Carotenoids and Chlorophylls were compared with standards and separated with a Lichrospher Reverse Phase C16 (250 × 4.6 mm) column by using a diode array detector. Light microscopy and cell vitality staining with fluorescein diacetate (FDA, Sigma) were conducted. Chl concentration was analyzed with a Handy PEA (Hansatech Instrument, pulse-modulated). Harvested microalgae (±0.04 ml) were layered into a leaf clip, equipped with a moistened paper filter, and kept in the dark for 45 min. The average of five measurements were calculated with respect of standard deviation.

Table 1. The stepwise gradient of HPLC for GSH analyses. Solvent A consists of H_2O, methanol, acetic acid with ratio 1000:50:2.5 (v/v/v). Solvent B consists of H_2O and methanol with a ratio of 1:9 (v/v). The combination of 26.3 ml solvent A and 9.8 ml solvent B was used for single analysis. The stability of pH was maintained at 3.9 by using a 10 M NaOH/HCl for both solvents. The temperature of the column and a sample were kept constant at 27 ºC and 4 ºC [19]

HPLC running time (min)	Flow rate (ml/min)	Solvent A (ml)	Solvent B (ml)
0	1	95	5
20	1	85	15
26	1	0	100
34	1	95	5
36	1	95	5

Table 1 showed the partial steps of GSH analysis by using HPLC. The particulate thiols (Glutathione, GSH) analyzed by preparing three 30 ml and three 10 ml samples of *Symbiodinium* monoculture and filtered with a Whatman GF/F filters. Sample filters heated at ±70 ^{0}C in 10 mmol.l^{-1} methano-sulfonic acid for 2 min and homogenized on ice [9]. The methano-sulfonic acid extract was retained for derivatization with the fluorescence tag monobromobimane. Derivatized homogenate analyzed on a Beckman HPLC (RP C-16 amide column and a 100 µl injection loop). The result were exceed quantified using a fluorescence detector post column (emission 474-650 nm and excitation 310-410 nm). The stepwise elution and buffers used are described elsewhere (Method C) [18]. Standard curves of peak area calibration were used to verify elution times which were made by using pure stock solutions of cysteine and glutathione. The detection limit for samples and standards is ± 210 femtomole per injection [9].

The statistical analysis was conducted to explore the differences in photo-pigments (chl-a, chl-c, and carotenoid) contents and glutathione before and after the photo-inhibitory treatment by using LSD test (SPSS v. 17 software package, p=0.05).

4. Results and Discussion

4.1 Intracellular Photo-pigments contents

The photo-inhibitory treatment has different effects on intracellular chl a, chl b, and carotenoid which was **decreased** 35.68% of chl a and 25.00% of chl b; and **increased** 22.95% of carotenoid contents of *Symbiodinium* (figure 1). Statistically, significant differences showed in chl a and carotenoid content, and neither was in chl b. Generally, a significant light treatment-induced changed the intracellular chl a and carotenoid contents of *Symbiodinium*.

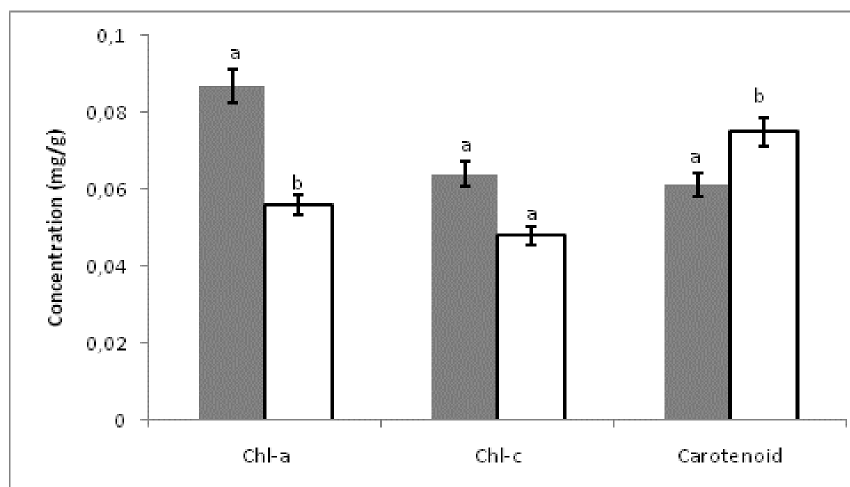

Figure 1. Photosynthetic pigment contents in *Symbiodinium* biomass. The *blackish columns* represented photo-pigments content analyzed before photo-inhibitory treatment. *White columns* represented photo-pigments content analyzed after photo-inhibitory treatment. Characters (a, b) described the result of significant difference (α=0.05) correlated to photo-inhibitory effect in *Symbiodinium* biomass.

4.2 Intracellular GSH content

Figure 2 described the intracellular GSH contents of *Symbiodinium* which were placed parallelly on each treatment after photo-inhibitory. The intracellular GSH reached the interval of 46-102 nmol.g^{-1} for R_1 treatment, 55-102 nmol.g^{-1} for R_2 treatment, and 70-105 nmol.g^{-1} for R_3 treatment. Intracellular GSH contents showed a statistically insignificant difference in the beginning for all treatments (R_1, R_2, and R_3). The medium and high treatments started a statistically significant difference in 10 min after placed in the initial treatment.

Figure 2 showed that intracellular GSH content tends to decrease during the observations, although those have different trends in creating new stability of GSH content. The R_1 treatment has the lowest new stability of GSH content (46±1 nmol.g^{-1}, approximately), followed by R_2 (55±2 nmol.g^{-1}, approximately), and highest in R_3 (70±2 nmol.g^{-1}, approximately). The stabile GSH content occurred in ±60 minutes after exposed with initial treatments.

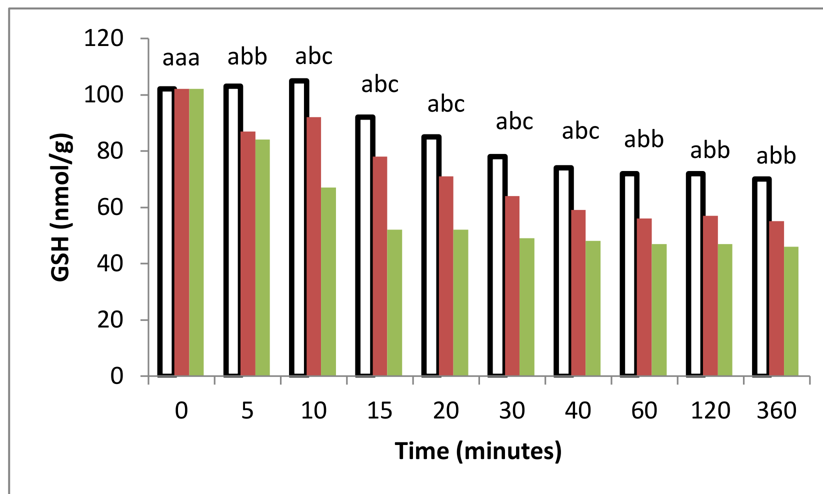

Figure 2. Intracellular Glutathione (GSH) contents in *Symbiodinium* after photo-inhibition treatment (600 µmol.m^{-2}.s^{-1} of PAR). The white columns indicate GSH contents analyzed in R_3 treatment, red columns indicate GSH contents analyzed in R_2 treatment, and green columns indicate GSH contents analyzed in R_1 treatment. Small character placed above the histograms (a, b, c) denote statistically significant difference (α=0.05) in *Symbiodinium*.

5. Discussion

Total chl c content was found lower than chl a and carotenoid. The photoinhibition treatment brought a significant effect to chl a and carotenoid of *Symbiodinium* (figure 1), while chl c was unaffected. This assumed indicates the destruction effect of extreme light intensity on chl a structure, while chl c remained unchanged. The change of chl a would be correlated with the destruction effect of light on intracellular chl a content due to ROS produced during extreme light exposed [20]. The significant effect of extreme light in carotenoid and GSH content indicated the early protective mechanism of *Symbiodinium* [21]. The short extreme light exposure to 600 µmol.m^{-2}.s^{-1} of PAR tend to lead the increase of intracellular GSH content of *Symbiodinium*. The high intracellular GSH content (102 nmol.g^{-1}) after photoinhibition treatment (600 µmol.m^{-2}.s^{-1} of PAR) showed high capacity of GSH pool in *Symbiodinium* cell. The such of light intensity of about 600 µmol.m^{-2}.s^{-1} of PAR might be assumed as high-intensity level and affected the production of intracellular GSH in *Symbiodinium*.

The different depletion trend of GSH under such light treatments after photoinhibition showed that the synthesis of GSH was higher than its degradation in the cell. This phenomenon was comparable with [19] which was found the increase of intracellular GSH content by the increasing of light intensity exposed to *Symbiodinium*. Therefore, the fluctuation of GSH during the treatments might be indicated a dynamic response of *Symbiodinium* related with high light stress exposed or the synthesis of intracellular GSH as a fact that light-dependent stress was not strong enough to restabilizing the intracellular GSH pool to the normal condition.

The time stated in the early lag phase indicated the recovery mechanism in *Sybiodinium*. The time of recovery might be more depending on the light intensity exposed after photoinhibition. The shortest time of recovery might be found in the lowest light intensity. *Symbiodinium* treated in low intensity (R_1) led to has the fast phase of recovery within the first 30 minutes after the photoinhibition treatment. *Symbiodinium* treated in medium intensity (R_2) and high intensity (R_3) has a similar time of recovery within 60 minutes after the photoinhibition treatment. This might be closely indicated to the more effective photoprotective mechanism of *Symbiodinium* which was treated with low light intensity. The GSH content in the lag phase might be closely correlated with the adaptation mechanism

of *Symbiodinium* to the initial treatment. The highest GSH content found in the highest light intensity (R_3), and the lowest GSH content found in the lowest light intensity (R_1).

6. Conclusion

The photoinhibition might be generated to the production of intracellular chl a, carotenoid, and GSH contents of *Symbiodinium*. The low light intensity might reduce photoinhibition effect in the recovery phase. The lag phase of GSH content might be used to predict the photo-recovery and photo-adaptation mechanisms of *Symbiodinium*.

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