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Changes of Carotenoids in *Haematococcus pluvialis* With the Presence of Light Metals

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ABSTRACT

In this paper, the effects of light metals (Al, Li, and Mg) to the content of carotenoids in microalgae *Haematococcus pluvialis* was reported. In this study, *H. pluvialis* was exposed to different concentrations of light metals, and the changes of carotenoids were measured with spectrophotometer with wavelength 482 nm. The results showed the concentrations ranged from 0.01 – 10.00 mg/l and the presence of all three light metals caused the increase of carotenoids content compared to negative control. The results also showed that the cells at exponential growth phase and pH 6.5 – 7.0 produced the highest content of carotenoids exposed to Al. The results confirmed that the presence of light metals would increase the carotenoids content in cells, which might indicate the increase of reactive oxygen species and oxidative stress.

Key words : *Light metals, Oxidative stress, Carotenoids production, Microalgae*

Introduction

Microalgae *H. Pluvialis* produce high concentration of carotenoids. Recent studies show that the accumulation of carotenoids can be related to the presence of oxidative stress (González-Peña *et al.*, 2021; Malook *et al.*, 2017). The accumulate in of carotenoids reduces the oxidative stress by quenching the reactive oxygen species (ROS) and free radicals. The presence of metals in the environment, especially heavy metals lead to the increase of oxidative stress (AYDOĐAN *et al.*, 2017; Djearamane *et al.*, 2020).

Light metals, such as aluminium (Al), lithium (Li), and magnesium (Mg) are widely used in industries, e.g. food and beverage packaging and automotive industries, battery and power storage industries, and build material industries.The light metals might bring negative impact to the environment. Studies by Ajibade *et al.* (2019) and Exley and Mold (Exley and Mold, 2019) confirmed that the presence of Al affects kidney and brain, while Mezni *et al.* (2017) confirmed that the presence of high concentration of lithium may cause heart diseases. In plants, the presence of high concentrations of Al and Li lead to the increase of oxidative stress and affect the growth of the plants (Baran, 2019; Silva, 2012). Mg is required as nutrient by many organisms. However, exposure of plants to high concentration of Mg will cause the increase of oxidative stress and can be deleterious (Gao *et al.*, 2015; W. Guo *et al.*, 2015).

To date, the toxic effect of heavy metals to environment, especially on microalgae, has been studied regularly but the presence of light metals as a source of oxidative stress in environment is not much re-

ported. In this paper, the effects of light metals (Al, Li, and Mg) towards the content of carotenoids in the microalgae, *H. pluvialis* have been reported. The effects of the cell age and pH to the content of carotenoids were studied as well.

Methodology

Cell culture

Stock culture of *H. pluvialis* was obtained from Culture Collection of Algae at the University of Texas at Austin, United States of America. The culture medium was prepared from the Bold's Basal medium stock medium provided by Sigma-Aldrich, Malaysia. The culture was incubated in room temperature, with light and dark conditions maintained at 16 hours and 8 hours, respectively. Subculture of cells were conducted at stationary phase.

Immobilization of cells

The agarose was obtained in powder form from Sigma-Aldrich, Malaysia. One percent agarose solution was prepared by dissolving 1 g agarose solution to 100 mL water.Microalgae cells were mixed with agarose solution with ratio of 1:1 in 4-sided clear cuvette. The mixture was left in room temperature for 5 minutes. The cells were immobilized in solidified agarose.

Exposure to light metals

Aluminium in the form of $Al_2(SO4)_3$, lithium in the form of $LiNO_2$, and magnesium in the form of $MgSO_4$ were purchased from Sigma-Aldrich (Malaysia). Different concentrations of light metal solutions (0.001 – 10.000 mg/ml) were added to the cuvettes with the immobilized cells.The amount of *in vivo* carotenoids were measured with wavelength 482 nm using spectrophotometer (Gene Quant 1300, GE) at different duration of exposure. The cells obtained from exponential growth phase were exposed for 15 to 75 minutes to light metals, with approximate number of cells of 3.7×10^5 in each exposure. The time and number of cells for the exposure tests werepre-determined in preliminary studies.

The changes were compared to the negative control, as shown in Equation 1 (Wong and Chong, 2014). All exposure tests were conducted in triplicate (n = 3). The results obtained from exposure tests were analysed using Microsoft Excel 2016.

OD increase (%) = $[OD_0\text{-}OD_1/OD_0] \times 100$ % (Eq. 1)

Where; OD_0 = absorbance reading before addition of light metal, OD_1=Absorbance reading after addition of light metal.

Effect of culture age and pH to the response of cells

In order to study the effect of culture age, immobilized *H. pluvialis* cells with different culture age (day 3 to day 30) were exposed to 0.1 mg/l aluminium for 90 min. The changes of carotenoids and chlorophyll were recorded.

For the study on the effect of pH to the cells' response, and to immobilized *H. pluvialis* cells were exposed to 0.1 mg/l aluminium with the solution adjusted to different pH (4.5 – 8.0), for 90 min. The value of pH was adjusted using HCl and NaOH (Sigma-Aldrich, Malaysia) and determined using pH meter (Starter 3000, Ohaus).

Results and Discussion

Different growth phases can be clearly seen in the growth of *H. pluvialis.* Results showed the growth of cells was slow for the first 15 days of culture, and entering the exponential growth phase on culture day 15 to day 24. The reduction of growth observed after day 24. Experimental results confirmed that the amount of carotenoids was proportionally correlated to the number of cells. In line with the studies done by (Machado and Soares, 2019) and (Wong *et al.*, 2018), cells from exponential growth phase were used in all toxicity tests due to the sensitivity of the cells to the changes of environment.

The responses of *H. pluvialis* to different light metals are shown in Fig. 1. The highest responses were obtained from the exposure of the highest concentrations of the metals, which might indicate the production of carotenoids to remediate the changes in short period after the exposure (between 15 – 75 minutes). Aluminium are toxic to microalgae that causes changes of enzymatic activities and lipid metabolisms, degradation of organelles, changes in cell structure (Ameri *et al.*, 2020). The presence of Al increases the oxidative stress of microalgae as well. Several studies identified the synthesis of carotenoids upon exposure to different oxidative stresses (Chen *et al.*, 2020; Ramos-Parra *et al.*, 2019; Zoufan *et al.*, 2018). The presence of oxidative stress might induce the genes responsible for the production of carotenoids. The level of change in carotenoids are determined by the sensitivity of the spe-

cies to the stress (. Guo *et al.*, 2020). Lithium is a non-essential element for life. The toxicity effect of Li is exhibited in different ways (Hawrylak-Nowak *et al.*, 2012; Jiang *et al.*, 2014), with the production of oxidative stress in the cells is evident (Baran, 2019). Therefore, increase of carotenoids content as a response to the increase of Li is expected.

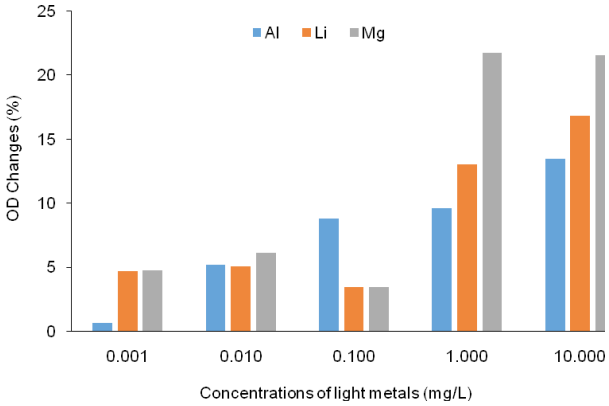

Fig. 1. Responses of cells toward the exposure of different light metals. The standard deviations of all tests were < 1 %.

Magnesium is a nutrient required for the growth of many microalgae. However, higher dosage of Mg might increase both oxidative stress and the production of reactive oxygen species, and affect both enzymatic and physiology of the plants (Guo *et al.*, 2015). Therefore, the changes of magnesium concentration might cause the changes in the content of carotenoids in *H. pluvialis*.

The content of carotenoids in *H. pluvialis* cells increased after the exposure to Al, for all cells from culture day 3 to day 30 (Figure 2). Cells from the culture day 12 to 21 produced the highest increase in carotenoids content. The high carotenoids content might be related to the high cell growth during exponential growth phase on day 15 to day 24, when the cell cycle was active and the cells were sensitive to the change of the environment.

The increase of carotenoids affected by the changes of pH as shown in Figure 3. With the presence of Al, the highest carotenoids were recorded at pH 6.5. The value was near to the pH value of the medium used for the culture. Han *et al.* (2020) reported that *Haematococcus lacustris* produced more astaxanthin, a kind of carotenoids under short period of pH shock. Study by Dos *et. al.* (Dos Santos and Lombardi, 2017) identified that the production of carotenoids at pH 6.3 was the highest for *H. pluvialis*. In this study, the carotenoids content in *H. pluvialis*was the highest at pH 7with additional Al as stressor.

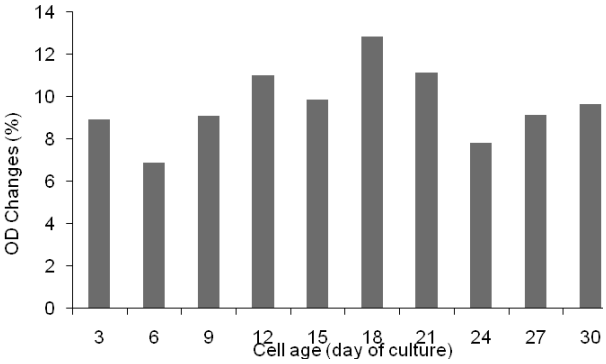

Fig. 2. Responses of cells with different day of culture toward the exposure to 0.1 mg/L of aluminium, with exposure time of 15 – 75 minutes. The standard deviations of all tests were < 1 %.

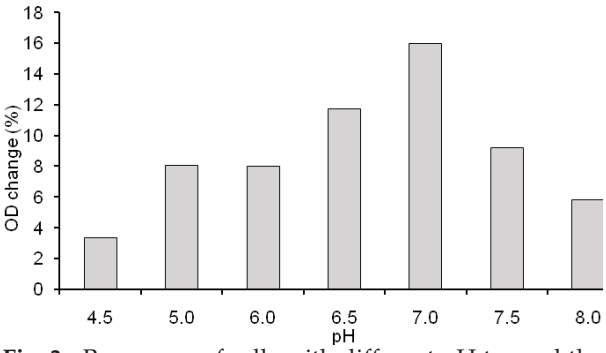

Fig. 3. Responses of cells with different pH toward the exposure to 0.1 mg/l of aluminium. The standard deviations of all tests were < 1 %.

Conclusion

The study shows the presence of light metals (Al, Li, and Mg) 0.01–10.00 mg/l on top of required amount for the growth of microalgae *H. pluvialis* increases the content of carotenoids in the microalgae. The results might cause by the increase of oxidative stress brought by the presence of the light metals. The study also confirmed that cell age and pH affect the content of the carotenoids with the presence of Al, with the highest content of carotenoids determined within the exponential phase and pH 6.5 – 7.0.

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