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The Responses of Natural Cell-bounded Carotenoids to Short Term Exposure of Heavy Metals

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Abstract. In this paper, the responses of carotenoids in *Daucus carota* cell suspension to short term exposure of copper (Cu) and lead (Pb) are reported. With the increment of OD measured at λ= 450 nm, the responses of the cells to 0.01, 0.10, and 1.00 part per million (ppm) of Cu and 1.00 ppm of Pb were confirmed respectively. The presence of oxidative stress by the heavy metals might increase the synthesis of carotenoids, which served as antioxidants in the cells. The sensitivity of the cells to the heavy metals could not be compared due to the difference in experimental designs. The responses of carotenoids within 60 minutes suggest that these natural cell-bounded pigments are good candidates for biosensor applications.

Keywords: Biosensor, Carotenoids, Heavy metals, *Daucus carota*.

1. Introduction

Living cells have been widely recognized as a tool for environmental pollutants evaluation. As biochemical responses of cells reflect real effects of the toxicity of pollutants to living organisms [1], many of the biochemical responses of whole cells have been utilized in biosensors for the detection of environmental pollutants. For instance, response of chlorophylls in photosynthetic cells has been used to evaluate the presence of environmental pollutants through changes of fluorescence emission [2] and oxygen release [3,4]. The presence of environmental pollutants can also be measured by enzyme activity [5] and the production of specific marker protein by cells [6]. However, one of the widely available pigments – natural cell-bounded carotenoids, has not been reported to be utilized in biosensor. This paper reports the initial findings of the carotenoids' responses to short term exposures of heavy metals. The findings of this research might be useful for the development of a novel whole cell biosensor to detection of environmental pollutants.

Carotenoids are light-harvesting pigments in photosynthetic organisms [7]. The pigments play two important roles in photosynthetic plants, which are harvesting light for photosynthesis and protecting the plants from reactive oxygen species. Heavy metals, pesticides, and herbicides, which are the major environmental pollutants inhibit photosynthesis in many ways [8-12] and at the same time, increase the oxidative stress in plants [13]. Thus, these environmental pollutants are expected to induce carotenoids responses, which can be utilized in biosensor design.

Dar et al. [14] and Pinto et al. [15] reported different responses of carotenoids in different photosynthetic plants after a long period of exposures to heavy metals. The changes in the amount of carotenoids, which is quantifiable, had been utilized as a measuring method in several biosensors [16-18]. By far, the attempts to couple the responses of carotenoids to biosensor applications are limited either to long term exposures, or transgenic organisms. In this paper, the responses of carotenoids in *D. carota* cell suspension to copper (Cu) and lead (Pb) in short term exposures are reported.

2. Methodology

2.1. Cell Culture

Taproot of *D. carota* was peeled and sliced into discs with thickness of approximately 1 cm, immersed in 70% ethanol for 30 s, and agitated in sterilant (0.525% NaOCl, 0.05% Triton X-100) for 25 min. The discs

were then washed in distilled water with agitation. After that, the discs were excised into explants of approximately 1 cm in dimensions, with cambium in the middle of the explants. These explants were cultured on MS medium [19] with 25 g/L sucrose, 2.2 g/L gelrite and supplemented with 1 mg/ml 2,4-dichlorophenoxyacetic acid for 60 days in dark at 25°C.

The explants were sub-cultured after 60 days onto the same medium overlaid with MS broth medium, followed by the incubation at 25°C for 30 days in dark. After that, the resulting liquid with cell suspension was filtered through a sterile mesh filter and centrifuged at 500 g for 10 minutes at 25°C to collect the cells. The cell pellet was resuspended in MS broth medium.

2.2. OD Wavelength of Carotenoids

A total of 2 mL of *D. carota* cell suspension was transferred into cuvette for spectrophotometry examination. The OD reading was taken from wavelengths 300 – 700 nm with 50 nm of interval. The wavelength with the highest OD output was then chosen to be used in this experiment.

2.3. Exposure to Heavy Metals

The heavy metals (Cu and Pb) stock solutions with the concentration of 10.0 part per million (ppm) and 0.1 ppm were prepared respectively. Other concentration of heavy metals were obtained through the dilution of the stock solutions.

A volume of 0.2 mL of Cu stock solution with concentration 10 ppm was added into 1.8 mL medium containing *D. carota* cell suspension, to make a final concentration of 1 ppm of Cu. OD reading was taken before the exposure. Subsequent readings were taken again after the cells had been exposed to Cu for 20, 40 and 60 minutes respectively. The experiment was repeated using different concentrations of Cu. The same procedure was then applied using 1 ppm Pb exposure.

2.4. Analysis of Results

The results obtained from the exposure of *D. carota* to heavy metals were compared to the responses of cells without heavy metals (blank). The following equation is used to calculate the percentage of the OD increment:

$$\text{OD increment} = [(OD_1 - OD_0) / OD_0] \times 100\%$$

Where,

OD_0= OD before the exposure to heavy metals

OD_1= OD after the exposure to heavy metals

3. Results and Discussion

The *D. carota* taproot cells were cultured in dark to minimize the production of chlorophylls and maximize the synthesis of carotenoids [20,21]. The cell suspension was filtered to reduce the number of clumping cells. The presence of clumping cells decreases the surface of contact between the cells and the heavy metals, hence affect the sensitivity of the cells. The presence of carotenoids in *D. carota* cells were confirmed by the highest yield of OD at λ= 450 nm (Fig. 1). The result obtained is in agreement with the research carried out by Ortiz et al. [22] and Oliveira et al. [23].

The responses of *D. carota* to different concentrations of Cu are depicted in Fig. 2. Absorbance of the cells at λ= 450 nm was found to increase within 60 minutes of exposure to Cu. The test with Pb showed increment of A_{450} in response to 1 ppm of Pb as well (Fig. 3). The increment of the absorbance suggests that the synthesis of carotenoids in the cells increased due to the presence of heavy metals, a response to counteract the presence of oxidative stress [24,25]. However, the reasons for this increase has yet to be studied. The results obtained from the cells' exposures to different heavy metals cannot be compared due to the varied source of cells, as well as the density of cells used in the experiment.

According to Roger [26], for biosensors to act as environmental monitoring tools, they have to be sensitive, inexpensive to produce, and can be used to improve the efficiency of monitoring processes. The cells used in this experiment had a good response towards Cu exposure, which can detect the presence of Cu qualitatively from the range 0.01 – 1.0 ppm. The reproducibility of cells was calculated with average

standard deviation of ± 3.03%, ± 16.78%, and ± 14.45% for 0.01 ppm, 0.10 ppm, and 1.00 ppm of Cu respectively. The results showed that the cells can be potentially used for qualitative measurement in low Cu concentration. The cells had shown a fast responses to both Cu and Pb, within 60 minutes of exposure. These results confirmed the potential of carotenoids in *D. carota* to be used as biosensor.

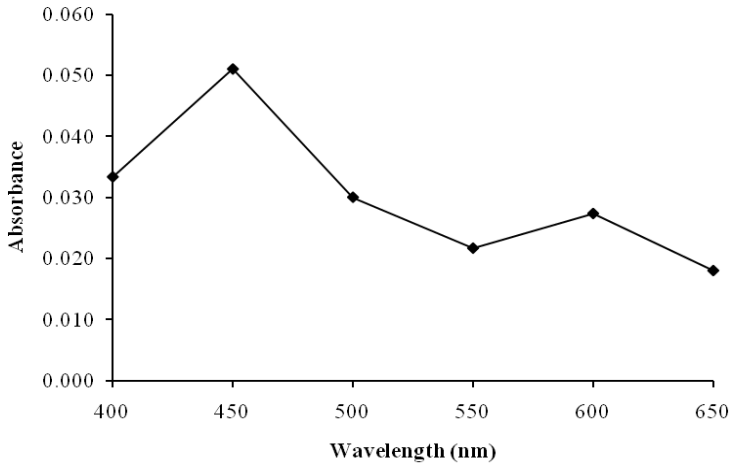

Fig. 1: The absorbance of *D. carota* cell in suspension under the wavelength 400 – 650 nm.

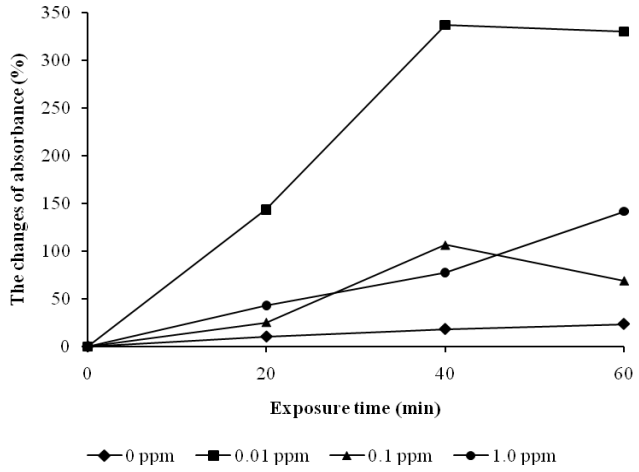

Fig. 2: The changes of absorbance in *D. carota* exposed to different concentration of Cu for 60 minutes, with n= 3.

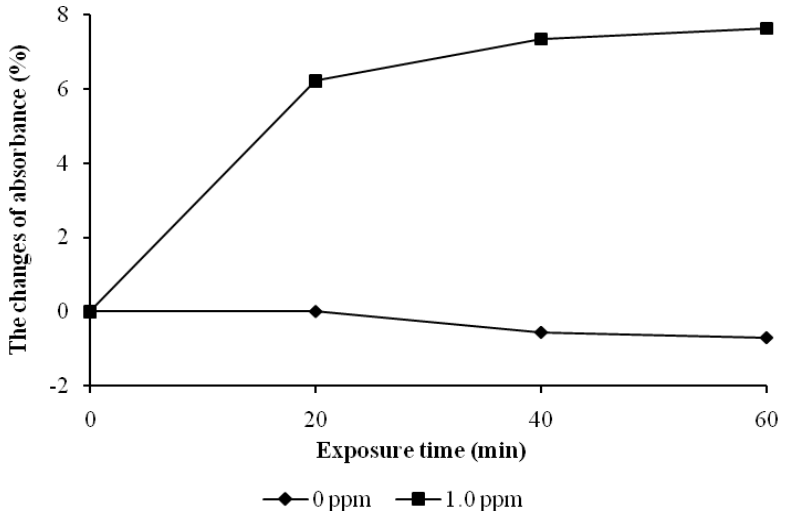

Fig. 3: The changes of absorbance in *D. carota* exposed to 1 ppm of Pb for 60 minutes, with n= 2.

Yoshida et al. [18] utilized carotenoids in transgenic *Rhodopseudomonas palustris* to detect arsenite in 24 hours with naked eyes. In another biosensor designed by Rahman et al. [16], the reduction of carotenoids in whole cells *Nostoc muscorum* and *Synechococcus* PCC 7942 could be detected after 10 days of heavy metals incubation. So far, the utilization of cell-bounded carotenoids in biosensor application is limited either to the usage of transgenic organisms, or to long periods of exposure. The responses of natural cell-bounded carotenoids to heavy metals within 60 minutes in this study might elevate the potential of these cell-bounded pigments to be used as biosensor.

4. References

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