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OPTIMIZATION OF B-CAROTENE PRODUCTION IN *Dunaliella salina* USING LED AND DIFFERENT CULTURE MEDIA

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ABSTRACT

Dunaliella salina is a green microalgae that has the ability to produce β-carotene used in various fields, such as food supplements, natural colorant, antioxidants, anti-cancer, and anti-aging. *D. salina* is capable of producing large amounts of carotenoids under stressful conditions, including light and nutrient. Light is the main factor that stimulate the production of carotenoid pigments and media composition plays an important role for growth, biomass, and β-carotene production. The accumulation of β-carotene in microalgae is closely related to the type and quality of light and the composition of the culture media. This study aimed to determine the production of β-carotene in microalgae *D. salina* using LEDs and different culture media to select the best culture conditions for producing high value compounds. The results showed that *D. salina* cultured using red LED light and technical Walne media was able to produce β-carotene with the highest amount of 767,499 mg/100 g. In blue LED light and Walne Pro-analysis media, the β-carotene content was 380,522 mg/100 g, while the ZA+NPK media has the lowest value. In this study, Walne Pro-analysis media became the best culture medium for *D. salina*. Therefore, natural sources of β-carotene can be obtained from *D. salina*, so it could reduce the use of synthetic carotene in meeting global demand.

Keywords: β-caroten, *Dunaliella salina*, LED, culture media

INTRODUCTION

Dunaliella salina is a unicellular green microalgae, motile, and belonging to the class Chlorophyceae (Chen and Jiang, 2011, Moghadasi *et al*., 2011). Current research shows that *D. salina* containing the pigment β-carotene has potential as a dietary supplement (Lam and Lee, 2014). β-carotene is a source of nutrition because it can be converted into vitamin A. In addition, the pigment is widely used as natural colorant, food additives, heart disease prevention supplements, cosmetics (Del Campo *et al*., 2007; Prieto *et al*., 2011), antioxidants (Farouk *et al*., 2002; Sathasivam *et al*., 2012), anti-cancer and anti-aging (Zhang *et al*., 2014; Gong and Bassi, 2016).

The most research on the production of β-carotene from *D. salina* has been carried out compared to other marine microalgae species (Bonnefond *et al*., 2017). *D. salina* was able to produce carotenoids of 12.6% per dry weight of algae, -carotene (60.4%), β-carotene (17.7 %), zeaxanthin (13.4 %), lutein (4.6%) and cryptoxanthine (3.9%) (El-Baky *et al*., 2007). Meanwhile, the content of β-carotene can be increased under stress conditions (García-González *et al*., 2005; Raja *et al*., 2007; Sathasivam *et al*., 2015). Several studies have shown that the accumulation of β-carotene can be increased by salinity and temperature stress (Cheirsilp *et al*., 2011; Vilchez *et al*., 2011, Takaichi, 2011), as well as light intensity (Mendoza *et al*., 2008; Kusumaningrum and Zainuri, 2014). Light is the main factor that can stimulate the production of β-carotene (Lamers *et al*., 2010). The use of red LEDs as a light source can increase the synthesis of β-carotene by optimizing the entire carotenoid biosynthetic pathway (Xu and Harvey, 2019).

Mass cultivation of *D. salina* has been widely carried out due to the biomass produced is a source of important compounds, such as polysaccharides, fats, vitamins, proteins, and pigments, especially carotenoids and chlorophyll (Talebi *et al*., 2015; Srinivasan *et al*., 2017) that widely used in various biotechnological applications. In addition, commercial cultivation specifically for the production of β-carotene has also been carried out in many countries. This is because global demand reaches 1430 tons/year (Ramaraj *et al*., 2015). In 2017, the market price of β-carotene reached 1.5 billion USD and is expected to increase to 2 billion USD by 2022 (McWilliams, 2018). The price of natural β-carotene ranges from 300-3000 USD/kg, depending on the type of product and market demand (Mohammadi and Arashrad, 2016). However, mass-scale production of microalgae is still quite expensive to do, despite many efforts for cost efficiency. Various culture media have been designed (Zainuri *et al*., 2008) and media composition plays an important role for growth, biomass, and β-carotene production (Fazeli *et al*., 2006; Lamers *et al*., 2008). The use of Walne as a

cultivation medium can increase the growth of *D. salina* (Helena *et al*., 2016). *D. salina* requires complete macro and micro nutrients for growth and biomass production (Harrison & Berges, 2005). Ahuja and Roy *et al*. (2019) also cultured *D. salina* with optimized media which resulted in the production of β-carotene of 6.07 mg/g or an increase of 1.45 times compared to normal conditions.

The accumulation of β-carotene in microalgae is closely related to the type and quality of light and the composition of the culture media. Therefore, this study aimed to determine the production of β-carotene in microalgae *D. salina* using LEDs and different culture media to select the best culture conditions for producing high value compounds.

MATERIALS AND METHODS

Culture of *D. salina*

The cells of *D. salina* used in this study obtained from the Center for Brackish Water Aquaculture (BBPBAP) Jepara, Central Java. Prior to the experiment, cells were acclimatized for 5 days at the Marine Biology Laboratory, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang. Culture of *D. salina* was carried out using pure seawater which had been filtered using a membrane with a pore diameter of 0.22 µm and sterilized using an autoclave. Cells were cultured in a ratio of 1: 2 (seeds: media), then added 1 mL of Walne fertilizer for 1 L of seawater.

Experimental Design

D. salina was cultured for 5 days using red and blue LED lights, and different media types, namely Walne Pro-analysis (WP), Walne Technical (WT), and ZA+NPK (Fig. 1). The experiment was carried out in a glass container with a final volume of 5 L, temperature between 16-25 ºC, salinity 30 ppt, light using 2 fluorescent lamps of 32 watt with intensity between 3500-4000 lux, and ligh/dark cycle of 12/12 hours L/D. During the experiment, aeration was carried out continuously so that the microalgae cells were homogeneously distributed. The number of cells is counted daily. Each experiment was carried out with three replications.

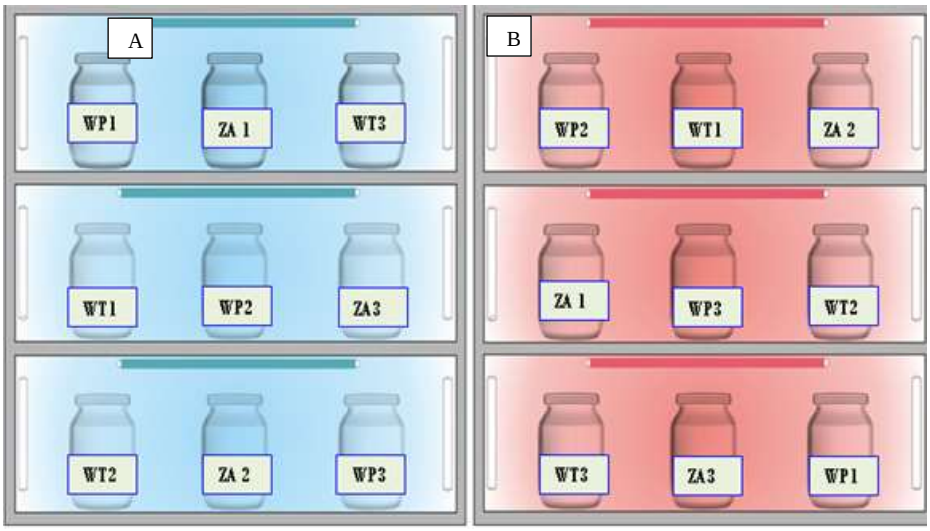

Figure 1. Experiment design illustration (A) blue LED, (B) red LED, WP (Walne Pro-analisis), WT (Walne Technic), ZA+NPK

Microalgae Biomass Harvesting

During the study, microalgae culture was carried out continuously (continuous mode culture) with dilution using media every 5 days. This was done to maintain the culture in the exponential phase, because in that phase the microalgae cells were in optimal conditions. Harvesting was done after getting ± 20L of biomass by deposited the culture for 24 hours using ice cubes. Thus, it was centrifuged at 4000 rpm for 2 minutes. *D. salina* was then dried by aerating at room temperature for 48 hours. The next step was to analyze the content of β-carotene.

β-Carotene Content Analysis

The content of β-carotene was analyzed using the method of UV Spectrophotometry Genesys 10. The sample was melted using a hot plate, then 0.5±0.0001 g was taken.

$$\beta\ karoten = \frac{Abs.\ 446\ x\ 3.38\ x\ volume\ of\ solvent\ (mL)}{sample\ weight\ (g)} x\ 100$$

After that, the sample was put into a 25 mL volumetric flask, dissolved using isoctan, and shaken until homogeneous. Put the solution into the cuvette 1 cm. Absorbance was calculated at the

wavelengths of 446 nm, 646 nm, and 663 nm. Furthermore, the content of β-carotene can be calculated using the formula:

RESULTS AND DISCUSSION

This study used a variety of media for culturing D. salina microalgae, such as Walne Pro-analysis (WP), Walne Technical (WT), and ZA+NPK. The nutrient content in the media decreased during the incubation period because there was no addition of nutrients. The nutrient content, which was still high at the beginning of culture, was utilized by each D. salina to carry out the growth process. In general, microalgae use inorganic CO2 as a carbon source to carry out photosynthesis and metabolism in their bodies.

Microalgae D. salina is known to produce high amounts of carotenoid pigments. Based on the results of the study, D. salina produced different β-carotene for each treatment (Fig. 2). Microalgae culture using red LED light and Walne Technical media was able to produce β-carotene with the highest amount of 767,499 mg/100 g. In blue LED light and Walne Pro-analysis media, the β-carotene content was 380,522 mg/100 g, while the ZA+NPK media gave the lowest yield. This indicates that different types of LED lights and culture media had a significant effect on the accumulation of β-carotene.

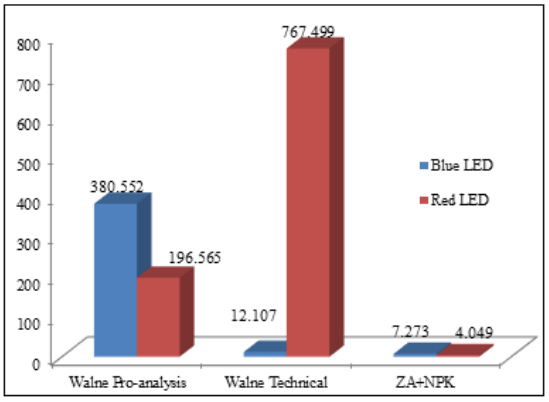

Figure 2. β-carotene content using different types of LEDs and culture media

Microalgae with red LED light source and Walne Technical media produced the highest β-carotene. These two parameters caused D. salina to experience environmental stress due to high light exposure and nutrient restriction. As a result, D. salina will produce secondary metabolites such as β-carotene (Helena et al., 2016). The use of red LEDs has been shown to increase β-carotene synthesis by optimizing all carotenoid biosynthetic pathways (Xu and Harvey, 2019). Carotenoids can be synthesized by microalgae through a non-mevalonate pathway, namely the methylerythritol 4-phosphate (MEP) (Barredo, 2012).

The use of red and blue LEDs shown different results in the production of β-carotene. This is because the red LED has a wavelength of 630-665 nm which can be absorbed by chlorophyll-a and -b, while the blue LED (430-465 nm) is absorbed by chlorophyll-b. The higher absorption of the red LED light source increase the growth of D. salina with the result that the biomass and β-carotene production is higher than the use of blue LEDs (Han et al., 2019). However, the use of red LED with Walne Pro-analysis media and ZA+NPK media in D. salina culture did not produce higher β-carotene than blue LED as a light source. This is probably because the microalgae culture only experienced light stress, but there was no nutrient restriction.

ZA+NPK media used in D. salina culture are rich in nutrients, especially N, P and K. Macronutrients N, P, K are needed for the growth and biomass production from microalgae (Elfiza et al., 2019). The use of ZA+NPK media for the cultivation of D. salina shown the lowest β-carotene production compared to other media. This indicates that there is no stress due to nutrient restriction so that the production of β-carotene was very low in this medium. Both technical and Pro-analysis Walne media showed high β-carotene production. The lower N and P content in both media compared to ZA+NPK caused microalgae experiencing nitrogen and phosphorus stress. The stress of this nutrient content will stimulate physiological responses so that the production of β-carotene secondary metabolites increases (Paliwal et al., 2017). Minhas et al. (2016) also showed the same results where D. salina produced β-carotene above 2.7% dry weight due to N deficiency in the culture medium.

The production of β-carotene is strongly influenced by environmental conditions such as light intensity, nutrition and aeration (Johnson and Schroeder, 1996). In addition, microalgae species will also affect the production of β-carotene because each species has a different ability to absorb light and use nutrients in culture media (Li et al., 2008; Mencfel, 2013). Microalgae D. salina produces 2 types of β-carotene, trans β-carotene and 9-cis-isomer-β-carotene. Trans β-carotene compounds can be synthesized simultaneously with the formation of chlorophyll at the same time as trans β-carotene from C. pyrendoidosa. The compound 9-cis-isomer-β-carotene in D. salina was synthesized in the logarithmic phase but would accumulate in the fat layer of the chloroplast as a response to environmental stress. This makes D. salina tolerant to salinity and high light intensity. Carotenoid production will continue until the death phase

(Kusumaningrum and Zainuri, 2014). The use of the right media supports the high content of β-carotene contained in D. salina. Media Walne Pro-analysis became the best media in this study. This is proven by the value of 380,522 mg/100 g of β-carotene content obtained. Therefore, natural sources of β-carotene can be obtained from D. salina so can reduce the use of synthetic carotene in meeting global demand.

CONCLUSION

In this study, Walne Pro-analysis media became the best culture medium for D. salina with β-carotene production of 380,522 mg/100 g. Therefore, natural sources of β-carotene can be obtained from D. salina so can reduce the use of synthetic carotene in meeting global demand.

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