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by Tatas Hardo Panintingjati Brotosudarmo

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Analysis of Carotenoids from *Erythrobacter flavus* Isolated from Soft-Coral *Acropora nasuta*

Edi Setiyono[a], Delianis Pringgenies[a], Heriyanto[b], Monika N.U. Prihastyanti[b], Yuzo Shioi[b], Leenawaty Limantara[c], and Tatas H.P. Brotosudarmo[b]*

[a] Department of Coastal Resource Management, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Jl. Prof. Soedarto Tembalang, Semarang-50275, Indonesia
[b] Ma Chung Research Center for Photosynthetic Pigments (MRCPP), Universitas Ma Chung, Jl. Villa Puncak Tidar N-01, Malang 65151, East Java, Indonesia
[c] Universitas Pembangunan Jaya, Jl. Cendrawasih Raya B7/P, South Tangerang-15413, Banten, Indonesia

Abstract

Separation and composition of carotenoids from *Erythrobacter flavus* are reported. *E. flavus* is a yellow aerobic marine bacterium. It was isolated from soft-coral *Acropora nasuta*. *E. flavus* was cultured in Shioi medium at 28°C for 3 days. The cells of *E. flavus* from each growth phase were extracted with a mixture of methanol and acetone (7:3, v/v). The crude pigment extract was injected into a reverse-phase of high performance liquid chromatography using C8 column. The results showed that *E. flavus* contain of 18 carotenoids pigments with 3 dominant carotenoids eluted at 18.7, 20.5, and 21.1 min. Zeaxanthin (at 28.3 min) and β-carotene (at 37.3 min) were minor carotenoids and the identification refers to their spectral, chromatographic and mass properties. The area of peak 1 and peak 2 increased 46% and 735%, respectively from 15 hour to 90 hour of culture, whereas the area of peak 3 did not change in each growth phase.

Keywords: β-carotene, carotenoids, *Erythrobacter flavus*, high-performance liquid chromatography, co-chromatography, zeaxanthin
*e-mail: tatas.brotosudarmo@machung.ac.id
Telephone number : 082141490052

1. Introduction

Carotenoids, found in plants, animal and microorganism (bacterium and microalgae), play a critical role in the photosynthetic process to collect light energy in the visible region and to protect against photooxidation [1]. In addition, carotenoids have been reported to have significant value to support human health, i.e. antioxidant, anticancer, antiobesity [4].. Cars are consisted of 40-carbon atom to form 8-isoprena and have yellow, orange, and red colour [2,3]. *E. flavus* is a yellow aerobic marine bacterium. It was isolated from soft-coral *Acropora nasuta* [5]. This study was aimed to separate and identify cars from *E. flavus* and to determine the Cars composition from its growth phases by reverse phase-high performance liquid chromatography (RP-HPLC).

2. Methodology

2.1. Cells culture

The cells were grown in Shioi liquid medium [6]. The culture was incubated by shaking (100 rpm) at 28°C under the dark condition. The cells were harvested after each growth phase, i.e. 15, 22, 46 and 90 hour, by centrifugation at 15,880 g, 4°C for 10 min. The cells were collected and then stored at -30°C until used.

2.2. Cells extraction

The cells (0.1 g) were homogenized in a mixture of methanol and acetone (7:3, v/v; 1 mL) by vortexing for 3 times (1 min vortex, 1 min on ice) and then lysed by sonication. Sonication process was carried out at a pulse mode with 60% amplitude and 10-s on/30-s off for 10 min (QSonica, Newtown, US). The crude pigment extract was separated by a centrifugation at 8,000 g for 2 min. The extract was dried by N_2 gas and stored at -30°C until used.

2.3. Separation, identification and composition of cars

The cars of *E. flavus* were separated by a RP-HPLC using C_8 column (150 x 4.6 mm; Water) according to the method of Zapata *et al.* [7]. Elution gradient of 2 solvents, i.e. solvent A (methanol:acetonenitril:pyridine solution (0.25 M) = 50:25:25 (v/v/v)) and solvent B (methanol:acetonenitril:acetone = 20:60:20 (v/v/v)) was performed at the flow rate of 1 ml/min with the temperature of column oven at 30°C. Chromatographic, spectral and mass properties were used for identification of Cars. Co-chromatography with the standard pigments and the saponification

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process were done to support the identification. The content of Cars for each growth phase was determined from the peak area of the dominant Cars detected at 450 nm.

3. Results and discussion

Cars separation of crude pigment extract from *E. flavus* is shown in Figure 1. At least 18 Cars have been well separated by RP-HPLC with three dominant Cars appeared at retention time (t_R) of 18.7 (peak 1), 20.5 (peak 2), and 21.1 (peak 3) min. Most of the Cars of this marine bacterium have a similar of maximum absorption wavelength (λ_{max}) at around 451-453 nm. This similarity of λ_{max} indicates that Cars of *E. flavus* have the same number of conjugated double bonds in their chromophore. Juliadiningtyas *et al* (2016) reported most of the Cars of this bacterium have similar core chemical structure.

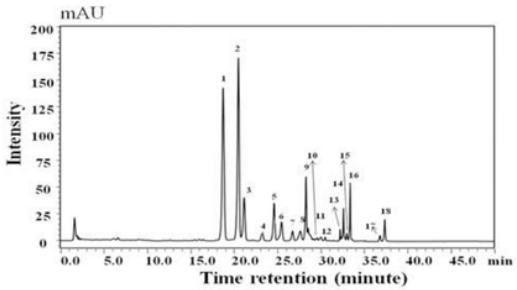

Figure 1. HPLC Chromatogram of crude pigment extract from *E. flavus* detected at 450 nm. The number of peak is described in the text.

Co-chromatography results with zeaxanthin and β-carotene standards suggested peak 9 at 28.3 min and peak 18 at 37.3 min were identified as zeaxanthin and β-carotene, respectively. Mass spectra of these Cars confirm the identification. In addition, the λ_{max} of zeaxanthin (at 453) and β-carotene (at 452 nm) is in agreement with the values in Zapata *et al* [7]. Zeaxanthin is one of baterial Cars produced by several marine bacteria, such as *Staphylococcus aureus, Vibrio psychroerythrus, Streptomyces* sp., and *Hahella chejuensis* [9]. Peak 1 and peak 3 were identified as the esterified Car according to the HPLC result of saponificated pigment extract. These peaks decreased and on other hand other peak increased as a free Car compared to the result of unsaponificated sample. The other dominant Car (peak 2) was a free pigment due to no effect on the saponification treatment.

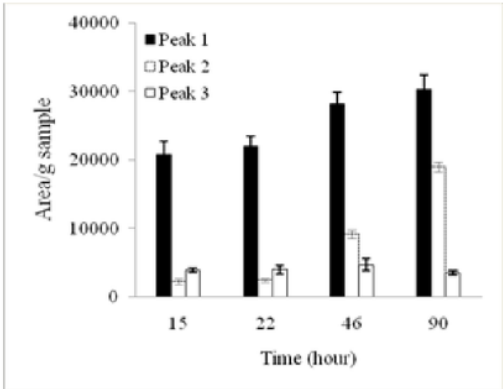

Figure 2. The Cars concentration (area per 1 g of the cell) of three dominant Cars in every growth phase

In this study Cars biosynthesis of *E. flavus* was determined from the peak area of the dominant Cars. The result of Cars composition from peak 1, 2, 3 at each growth phase is shown in Figure 2. The growth phase of *E. flavus* culture was classified into three phases, such as lag phase (0-15 hour), exponential phase (22-46 hour), and stationary phase (90 hour). The total area (per 1 g of the cells) of peak 1 increased 46% from 15 hour to 90 hour, whereas peak 2 accumulated 735% and it was higher than the peak 1. The total area of peak 3 was relatively same in each growth phase. The cars biosynthesis of *E. flavus* was continuously occurred to the peak 1 and peak 2, whereas peak 3 did not change. The cars composition from purple bacterium was influenced by species, age of the cell, and culture condition [10]. Taylor *et al* reported total Cars lineary increased with the curve of growth in *Streptococcus faecium* [11].

4. Conclusion

At least 18 Cars have been separated by RP-HPLC from the crude pigment extract of *E. flavus*. Zeaxanthin and β-carotene were identified as the minor Cars, while the three dominant Cars were eluted in front of the zeaxanthin peak and have the same λ_{max} as those minor Cars. Cars at peak 1 and peak 2 were extensively biosynthesized by *E. flavus*.

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