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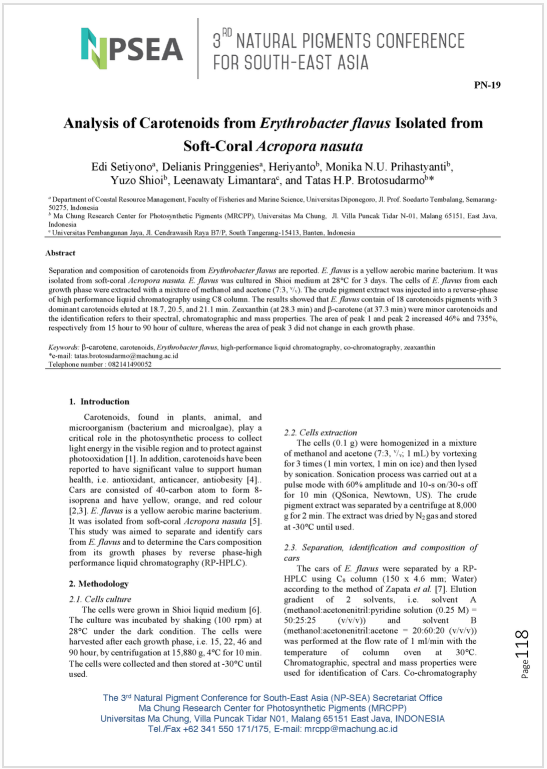

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Analysis of Carotenoids from *Erythrobacter flavus* Isolated from Soft-Coral *Acropora nasuta*

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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
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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 centrifuge 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

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