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Chlorophyll and Total Carotenoid Contents in Microalgae Isolated from Local Industry Effluent in West Sumatera, Indonesia

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ABSTRACT

Chlorophylls and carotenoids are two main pigments contained in microalgae. The pigments have important function as they serve as bioactive compounds with high nutritional value. In this study, chlorophyll and carotenoids contents of four microalgae strains isolated from local industry effluent were evaluated. The pigments were extracted in methanol and determined based on the absorbance of three wavelength: 470, 652.4 and 665.2 nm using Spectrophotometer UV-Vis. The results showed that three of the strains which were eukaryotic microalgae contained chlorophyll (a+b) with content of 4.037 ± 0.0875%, 4.967 ± 0.1050% and 3.880 ± 0.1866% of dry biomass for Micractinium ehime IPOME-1, Micractinium sp. CCAP IPOME-2 and Mychonastes rotundus IPOME-3 strains repectively. Under nitrogen starvation medium, chlorophyll (a+b) content of prokaryotic microalgae (Uncultured Oscillatoria sp IPOME-4 strain) decreased from 2.096 ± 0.0209% to 1.684 ± 0.0391% (decrease of 0.412%) and total carotenoid content of the strain increased with increase of 0.308%, from 0.773 ± 0.0289% to 1.081 ± 0.0390%.

Keywords: Chlorophyll, Carotenoid, Microalgae

INTRODUCTION

Microalgae are microorganisms with a wide range of applications. Various bioactive compounds can be extracted from microalgae biomass which is beneficial for human health. Some high-value compounds with health benefits contained in microalgae are lipids, polyunsaturated fatty acids, proteins, vitamins, minerals, and pigments [1]. Microalgae pigments are classified into three types of pigments, consisting of chlorophylls, carotenoids and phycobilliproteins [2]. Chlorophyll is a green pigment found in all higher plants and algae [3]. Carotenoids, known as efficient antioxidants are pigments which are characterized by their yellow or red color [4]. Phycobiliproteins are brilliant coloured and water soluble pigment which are covalently bound to apoprotein [2].

Chlorophylls present abundantly in nature and play important role in photosynthesis. The pigments are recognized as one of bioactive compounds with high nutritional value. Chorophyll molecule consist of a ring porphyrin (head) containing a central magnesium atom and a long hydrocarbon phytol (tail), in which these both parts are bounded by an ester bond [5]. All chlorophylls have two major absorbtion bands, namely blue or green one (450-475 nm) and red one (630-675 nm) which results in their characteristic of green colour [2]. Two main types of chlorophylls are chlorophyll a and chlorophyll b [5]. Chlorophyll a ($C_{55}H_{72}O_4Mg$ with $-CH_3$ as its functional group) is the major light harvesting pigment which converts light energy into chemical energy. Chlorophyll b ($C_{55}H_{70}O_6N_4Mg$ with -CHO as its functional group) involves indirectly in photosynthesis by transferring the light it absorbs into Chlorophyll a [6,7]. Chlorophyll has a wide range of applications. With its characteristic of selective absorbance of light in certain wavelength which results in green color, it is potentially used as food coloring agent. Chlorophyll and its derivatives are also used widely in pharmaceutical products. Chlorophyll has antibacterial property, deodorizing function and nontoxic nature which enables it to be applied in the treatment of oral sepsis [4]. In tumor or cancer therapy, chlorophyll or chlorophyll derivatives can also be utilized as a photodynamic agent [3].

Carotenoids in algae mainly fuction as accessory pigments in photosynthesis. The compounds are synthesized through enzymatically polymerization from 5-carbon isoprene units to form 40-carbon polyene chain [4]. Carotenoids can be chemically devided into carotenes and xanthophylls. Carotenes represent a group of carotenoids with hydrocarbon lacking oxygen, consisting of α-carotene, β-carotene and lycopene. Xanthophylls belong to carotenoids with oxygen being present as –OH groups (e.g., lutein), as oxi-groups (e.g., cantaxanthin) or as a combination of both (e.g., astaxanthin) [8,9]. High reactive conjugated double bonds in carotenoids make them effective in trapping free radicals, thus function as antioxidants [10].

Some experimental evidences have confirmed that the compounds can play important roles in prevention and treatment of human diseases including degenerative diseases such as atherosclerosis, cardiovascular disease (CVD), cancer, inflammatory, neurological diseases and diabetes [11].

The aim of this research was to determine the chlorophyll and carotenoid pigments simultaneously from four strains of microalgae isolated from local industry effluent. Extraction was performed using methanol as solvent. Methanol is a very good extractant for chlorophylls due to less volatile and flammable [6]. The quantitative analysis was carried out using spectrophotometric method.

MATERIALS AND METHODS

Collection of microalgae strain

Microalgae strains were provided by Biotecnology Laboratorium of Andalas University, which were previously isolated from palm oil mill effluent of Mutiara Agam Company, West Sumatera, Indonesia. The strains were *Micractinium ehime* IPOME-1, *Micractinium* sp. CCAP IPOME-2 and *Mychonastes rotundus* IPOME-3 that belong to eukaryotic microalga and Uncultured *Oscillatoria* sp. IPOME-4, a prokaryotic one. Microalgae cells were prepared by culturing the strains in Bold Basal Medium (BBM) untill each strain reaches its respective optimum growth [12,13]. To investigate the effect of nitrogen starvation medium on chlorophyll and total carotenoid, one of the strain namely Uncultured *Oscillatoria* sp. IPOME-4 was chosen as a sample and cultured in nitrogen starvation medium of BBM. Uncultured *Oscillatoria* sp. IPOME-4 is a prokaryotic microalgae which demonstrated unique characteristic (the cells turning into yellow under nitrogen starvation medium) [13].

Extraction procedure

Microalga culture at the end of exponential phase was subjected for analysis. Extraction was carried out based on the procedure reported by Sartory & Grobbelar [14], with little modification. As much as 2 ml microalgae cells of each strain was centrifuged at 12500 rpm for 5 min. Pellet was taken and suspended with 2 ml methanol (90%). The mixture was incubated in waterbath at 64.7^{o}C for 5 min, followed by maceration for 20 h in the dark. The steps were continued by centrifugation at 12500 rpm for 5 min. The supernatant was transferred and measured by spectrophotometer UV-Vis at 470 nm, 652.4 nm and 665.2 nm against the solvent (methanol) blank.

Estimation of chlorophyll and total carotenoid contents

The concentration of chlorophyll a, chlorophyll b and total carotenoids were calculated using the equations reported by S. Nayek et al. [6], as follows :

Chlorophyll a (Ch-a)=16.72(A665.2)–9.16(A652.4)
Chlorophyll b (Ch-b)=34.09(A652.4)–15.28(A665.2)
Total carotenoid content=[1000(A470)–1.63Ch-a–104.96Ch-b]/221

RESULTS AND DISCUSSION

In this study, all the strains were cultured under normal BBM medium, except for Uncultured *Oscillatoria* sp. IPOME-4 (-N) which refers to strain Uncultured *Oscillatoria* sp. IPOME-4 which was cultured under nitrogen starvation of BBM medium. The data obtained from the spectrophometric measurement were substituted into the equations. The results were the concentration of chlorophyll a, chlorophyll b and total carotenoids (datas not shown). Furthermore, the graphic was constructed based on the datas of chlorophyll a, chlorophyll b and total carotenoid concentrations which showed the comparison of the pigments concentration in strains tested. Figure 1 demonstrates the graphic of chlorophyll a, chlorophyll b and carotenoid concentrations of the strains. It shows that strain *Micractinium* sp. CCAP IPOME-2 has the highest content of either chlorophyll a, chlorophyll b or total carotenoid concentrations which accounted for 21.44 ± 0.38 µgm/mL, 8.57 ± 0.26 µgm/mL and 6.04 ± 0.03 µg/mL respectively (datas not shown). It is in correlation with dry biomass content of the strain (1.2082 gm/L) which was also the highest figure among the strains investigated in the previous study [12,13].

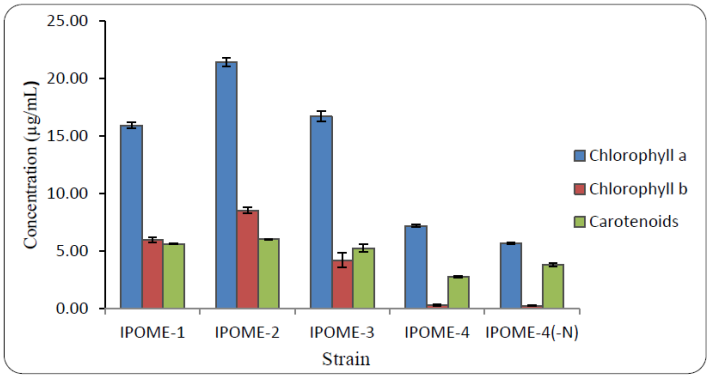

Figure 1: Comparison of chlorophyll a, chlorophyll b and total carotenoid concentrations in extract of four strains tested (including strain cultured in nitrogen starvation of BBM)

From the graphic, it can be noted that the strains *Micractinium ehime* IPOME-1, *Micractinium* sp. CCAP IPOME-2 and *Mychonastes rotundus* IPOME-3 that belong to eukaryotic microalga contain higher content of chlorophylls (a and b) compared to that of Uncultured *Oscillatoria* sp. IPOME-4 strain which is prokaryotic one. Those first three strains are green algae in which chlorophylls exist as the main pigment which is responsible for the green color of the cells. Total carotenoid content of the three strains are quite significant and the concentrations are comparable to each other. Strain Uncultured *Oscillatoria* sp. IPOME-4 shows different composition of the pigments.

As prokaryotic microalgae, beside chlorophylls and carotenoids, the strain also possesses phycocyanin pigment; a blue pigment which is together with chlorophyll determines the bright bluish green color of the cells [13]. The effect of culturing the strain under nitrogen starvation of BBM medium has caused the change in pigments of the strain. The concentration of either chlorophyll a or chlorophyll b decreased under nitrogen starvation of BBM medium. The decrease in chlorophyll caused by insufficient availability of nitrogen due to nitrogen function as one of significant element needed for the formation of chlorophyll as chlorophyll molecules contain four nitrogen atoms in its structure, therefore lack of nitrogen in medium results in the decrease of chlorophyll content [15]. On the other hand total carotenoids concentration under nitrogen starvation medium increased due to under stress condition additional production of carotenoid is caused by excessive formation of free radicals in which carotenoid is produced to protect the cells and to maintain the continuation of the growth [15].

In addition, the chlorophyll and carotenoids content in respective dry biomass of individual strains were also calculated based on the dry cell weight (dcw) determination of the strains in the previous research [12,13]. The content of chlorophyll (a+b) and total carotenoids in respective dry biomass of the strains are presented in Table 1.

Tabel 1: Chlorophyll (a+b) and total carotenoids content in respective dry biomass

Strains	Chlorophyl (a+b) (%)	Total carotenoid (%)
Micractinium ehime IPOME-*1*	4.037 ± 0.0875	1.040 ± 0.0036
Micractinium sp. CCAP IPOME-2	4.967 ± 0.1050	1.001 ± 0.0054
Mychonastes rotundus IPOME-3	3.880 ± 0.1866	0.980 ± 0.0555
Uncultured *Oscillatoria* sp. IPOME-4	2.096 ± 0.0209	0.773 ±0.0289
Uncultured *Oscillatoria* sp. IPOME-4(-N)	1.684 ± 0.0391	1.081 ± 0.0390

It can be noted that chlorophyll content in first three microalgae strains are in accordance with chlorophyll content in some eukaryotic microalgae species, like chlorophylls (a+b) content of *Cholrella* species which can contain the pigments up to 4.5% of dry weight [16]. As discussed above, chlorophyll content in strain Uncultured *Oscillatoria* sp. IPOME-4 under nitrogen starvation medium has reduced due to lack of nitrogen which is needed for the formation of chlorophyll, on the other hand carotenoids content has increased as a result of responding to unfavorable condition.

CONCLUSION

Micractinium ehime IPOME-1, *Micractinium* sp. CCAP IPOME-2 and *Mychonastes rotundus* IPOME-3 strains that belong to eukaryotic microalgae were found to contain higher content of chlorophylls, which accounted for 4.037 ± 0.0875%, 4.967 ± 0.1050% and 3.880 ± 0.1866% of respective dry biomass compared to that of Uncultured *Oscillatoria* sp. IPOME-4 strain (2.096 ± 0.0209%) which is known as prokaryotic microalgae. Carotenoid contents of eukaryotic microalga are comparable to each other, and also higher than that of Uncultured *Oscillatoria* sp. IPOME-4 strain. Under nitrogen starvation medium, chlorophyll content of Uncultured *Oscillatoria* sp. IPOME-4 strain decreased from 2.096 ± 0.0209% to 1.684 ± 0.0391% (decrease of 0.412%). While total carotenoid content of the strain increased from 0.773 ±0.0289% to 1.081 ± 0.0390% (increase of 0.308%). Thus the induction of nitrogen starvation medium can be applied to enhance total carotenoid content.

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