EFFECT OF NITRATE CONCENTRATION ON CELL GROWTH AND ASTAXANTHIN ACCUMULATION IN FOUR DIFFERENT SPECIES OF ISOLATED MICROALGAE IN MALAYSIA

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SUMMARY:. Astaxanthin possess a red color pigment, a type of xanthophylls carotenoid and is mainly found in algae, fish and bird. In this study, cell growth and astaxanthin accumulation from four different isolated microalgae species based on their morphology were investigated. Isolated microalgae were cultured at three different nitrate concentration (1.64, 0.82, and 0.41 mM) and cell growth and astaxanthin were measured spectrophotometrically at 750 and 475 nm respectively. All species showed high growth rates at 1.64 mM nitrate concentration except for *Pediastrum* sp that is favorable in 0.82 mM nitrate. Among the four species, *Ankistrodesmus* sp showed the fastest growth rate. For the astaxanthin production, *Ankistrodesmus* and *Pediastrum* showed the highest yield. Thus it have a potential in production of astaxanthin in the future work.

***Keywords*—**Astaxanthin. Cell growth. Isolated microalgae. Nitrogen concentration. Spectrophotometrically.

INTRODUCTION

In recent years, there is currently much attention to extract bioactive compounds from natural resources due to its efficacy towards human diseases. The antioxidant properties are one of the most important properties of astaxanthin which has been noted to surpass the β-carotene and also α-tocopherol [1]. This antioxidant activity is very beneficial in blocking the activity of free radical species chemical that have found highly reactive and have potential in causing cell damages that may lead some cancer.

Microalgae, the microscopic organism seems to be the one of the major source for natural astaxanthin production. Microalgae can be classified into prokaryotic and eukaryotic photosynthetic microorganism that can grow rapidly without requirement of agricultural land, can live in harsh conditions and possess a high value of biological derivatives.

Main recipes for the growth of microalgae are light, temperature, nutrient, pH, carbon dioxide supply and salinity [2]. Besides, the biochemical composition in microalgae can be influenced by several factors such as environmental condition, growth rate and life cycle of the microalgae itself. Some stress for example nutrient starvation and high light intensity will increase the accumulation of astaxanthin in the microalgae [3]. Thus, the objective of this study is to investigate the effect of nitrate concentration on four species of isolated microalgae and to investigate the microalgae species that accumulate high amount of astaxanthin.

2. MATERIALS AND METHODS

2.1 Isolation and culture conditions

Four microalgae species based on its morphological identification (*Ankistrodesmus*, *Scenedesmus*, *Coelastrum*, and *Pediastrum*) were isolated from brackish water and river located at Hulu Langat Selangor by picking up single cells with a pipette using Olympus BH-12 microscope (Fig. 1). The microalgae was cultivated in AF-6 medium with different $NaNO_3$ concentration (1.64, 0.82, 0.41 mM) with 40 µmol photon m^{-2} s^{-1}light intensity at 24 h light.

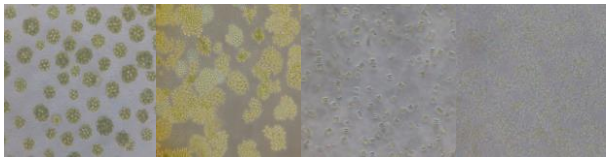
2.2 Growth measurement

Cell growth was measured everyday at 750 nm by Shimadzu 1800 UV-VIS.

2.3 Astaxanthin quantification

5 mL of microalgae samples were centrifuged at 4,000 rpm at 4 ^{O}C for 5 min. 5 % of KOH in 30 % methanol was added at 70 ^{O}C for 5 min. Then the samples was added with a few drops of acetic acid to remove the chlorophyll and treated with 5 mL acetone for 5 min in 70 ^{O}C. Then, the astaxanthin was measured at 480 nm by UV-VIS [4].

3. RESULTS



(a) (b) (c) (d)

Fig. 1. Isolated microalgae species (a) *Coelastrum* (b) *Pediastrum* (c) *Scenedesmus* (d) *Ankistrodesmus*

The effect of nitrate concentration on growth and astaxanthin accumulation were studied by adding different concentration of $NaNO_3$ (1.64, 0.82, 0.41 mM) to the AF-6 medium. Fig. 2 shows the cell growth of four different species of microalgae. The *Ankistrodesmus* shows a fast growth rate followed by *Scenedesmus*, *Coelastrum* and *Pediastrum*. All the species shows the high growth in 1.64 mM $NaNO_3$ concentration except for *Pediastrum* that is favorable in 0.82 mM $NaNO_3$. Astaxanthin accumulation show high yield in *Ankistrodesmus* in 0.41 mM and *Pediastrum* in 1.64 mM whereas in 0.41 mM of *Pediastrum* does not show the accumulation of astaxanthin.

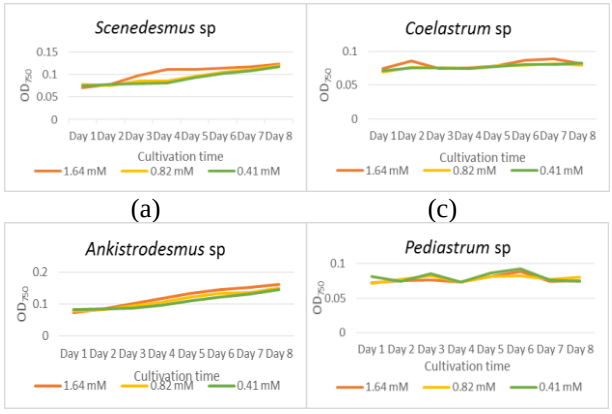


Fig. 2. Cell growth of four different species (a) *Scenedesmus*, (b) *Coelastrum*, (c) *Ankistrodesmus*, (d) *Pediastrum*

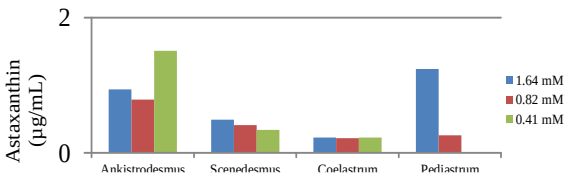


Fig. 3. Astaxanthin accumulation in four different species

4. DISCUSSION

The nutrient stress which is the nutrient starvation can generate free radicals species in the cell and influenced the antioxidant contains in the microalgae [5]. In this study, we can see the growth of four microalgae species should be until day 8 which means the nutrient is still not in starvation condition. Thus, the experiment was continuosly conducted until it reach the stationary phase. *Ankistrodesmus* species shows the high cell growth rate and high yield of astaxanthin accumulation compared to other species. Because of the time consuming, the identification of algal strains by 18 s rRNA sequence will be done as the future work. Furthermore, the microalgae will be cultivated at high light intensity such as 200 µmol photon m^{-2} s^{-1} in order to increase the stress on microalgae.

The astaxanthin are known as important antioxidant properties for human health and protect human skin against UV-induced damage [6]. The present study show the higher accumulation of astaxanthin higher in *Ankistrodesmus* (0.41 mM) and *Pediastrum* (1.64 mM) measured by UV spectrophotometer. For the future work, the accuracy of astaxanthin accumulation will be analyzed by HPLC. Then, antioxidant activity for the astaxanthin will be measured by using DPPH (1,1' diphenyl-2-picrylhydrazyl) assay.

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