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Bioprocess Potentials of Aurantiochytrium Microalgae from Kulonprogo Mangrove Forest Yogyakarta, Indonesia

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Abstract. *Aurantiochytrium sp.* is a marine protist that is highly ecologically relevant in the mangrove environment. This species of microalgae has long been known to produce high concentration of Docohexanoic acid (DHA, omega-3, 22:6n-3). DHA has a beneficial role for maintenance of normal brain function, visual acuity as well as prevention of cardiovascular and neurological diseases in humans. Although Indonesia is known to have the largest mangrove forest in the world, unfortunately, research on the isolation, potential observation and use of *Aurantiochytrium* microalgae has been still rarely published. Therefore, this research aims to promote the importance of research field using microalgae *Aurantiochytrium* from Indonesian strain for future biochemical production processes. In this work, the samples of targeting microalgae were isolated from Kulonprogo mangrove, Yogyakarta using the direct plating method. The isolation medium contained 50% (v/v) seawater, 1% (w/v) agar, 0.5% (w/v) glucose, 0.1% (w/v) peptone, and 0.1% (w/v) yeast extract, without the addition of streptomycin and penicillin. Morphological characteristics were observed. The observation results confirmed the availability of *Aurantiochytrium* isolates in prepared medium. Therefore, future isolation of *Aurantiochytrium* microalgae from other Indonesian mangrove forests can use the direct planting methods presented in this paper. In addition, this paper presented future important applicability of Indonesian Aurantiochytrium strains for uses in strategic industrial sectors, including pharmaceuticals, nutraceuticals and biofuel companies.

INTRODUCTION

Conventional source of docosahexaenoic acid (DHA omega-3 22:6 n-3) for human nutrition, nutraceuticals and pharmaceutical products is marine fatty fish such as salmon and mackerel [1]. However, omega-3 extraction from fish oil arises some threatening problems such as heavy metals contamination, environmental issues related to illegal fishing and overfishing of the oceans. In addition, fish oil is not suitable for vegetarians and the odor makes it unattractive.

Therefore, recent trend of omega-3 sources shifts into omega-3 producing microorganisms [2]. Among the alternatives of marine microorganisms, Aurantiochytrium species is the most promising candidate for producing omega-3 PUFAs [3], [4]. This microalgae species has been chosen as the raw material to produce DHA-containing oil by Martek Biosciences Corporation (now part of the Royal DSM (Dutch State Mines) [2]. The superiorities of Aurantiochytrium species, among others, are high oil contents of 50-77% (dry weight), high DHA productivity with more than 30% DHA within the total oil produced and shorter cultivation time [5]. A comparison shows that Aurantiochytrium strains can produce high amount of DHA and biomass in a relatively short cultivation time (Table 1). In addition, tabel 1 also shows that Aurantiochytrium sp. can achieve DHA production of 5,5 g/ L until 20,5 g/ L and the productivity rate of those strains is more than 100 g/L / day within 4 days.

Aurantiochytrium sp. is ubiquitous in marine and estuarine environments, in both tropical and sub-tropical areas and is also reported to be associated with mangrove habitats. Followings description are taxonomy of Aurantiochytrium microalgae [6] :

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Domain : Eukaryota
Kingdom : Chromophyta
Phylum : Heterokonta
Family : Thraustochytriaceae
Order : Thraustochytriales
Genera : Aurantiochytrium

In addition to the potentials of high lipid production, Aurantiochytrium microalgae has also potentials usage in producing astaxanthin [7]–[9]. Recent potential of Aurantiochytrium is to fulfill a great demand by industry on squalene supply for adjuvant vaccine covid-19 [10], [11]. It is obvious that further study concerning of Aurantiochytrium microalgae shall be of importance in the near future.

TABLE 1. Examples of *Aurantiochytrium* strains for DHA production.

Strain name	DHA content (gL-1)	Cultivation time (h)	DHA productivity (mg/Lh-1)	Reference
Aurantiochytrium limacinum SR21	13.3	96	138	[12]
Aurantiochytrium mangrovei Sk-02	5.5	48	115	[13]
Schizochytrium sp. G13/2S	6.1	49	125	[14]
Aurantiochytrium sp. HX-308	17.7	160	111	[15]
Aurantiochytrium sp. SD116	17.4	110	158.4	[16]
Aurantiochytrium sp. BUCHHT 093	20.7	96	215.6	[17]

With more than 3 million hectares of mangrove forests, Indonesia is recognized as the richest mangrove country worldwide [18]. Fallen mangrove leaves might be colonized by a plentiful *Aurantiochytrium* microalgae, as *Aurantiochytrium* feed on the leaves resulting in their decay [19]. In addition, *Aurantiochytrium* microalgae inhabit on the above ground roots, free living on the mud and pelagic water column.

Despite the widest mangrove forest as abundant resources of *Aurantiochytrium* ecosystem, scientific publications concerning experimental work of isolation, characterization, screening and utilization of Indonesian *Aurantiochytrium* microalgae have been rarely observed and published. Hutari et.al [20] isolated, characterized and did some cultivation work of *Aurantiochytrium sp*. from Lampung mangrove forest. There are two review journals on the application of Aurantiochytrium microalgae [21], [22]. However, there is a lack of experimental studies in isolating, screening and cultivating of Aurantiochytrium sp. from Indonesian mangrove.

Therefore, this paper presents our biodiscovery experience in isolating microalgae from a mangrove forest in Kulonprogo, Yogyakarta. This research is expected to promote the potentials of Aurantiochytrium microalgae as sources of valuable products. Following on this the modified method in the isolation of Aurantiochytrium microalgae shall be useful for further work in this field.

RESEARCH METHODOLOGY

Sampling Location

Targeting microalgae are isolated from a mangrove area in Kulonprogo, Yogyakarta. Isolation work was done on July 1st, 2019. The chosen mangrove areas are located close to the Hindia Ocean. The samples were cultured in media in the Laboratory of Pharmacy, Ahmad Dahlan University. Their morphological characteristics will be investigated afterward.

Isolation of Microalgae

Isolation of microalgae was carried out with direct plating method [23]. Fallen mangrove leaves of yellow and brown-black color were collected from the selected mangrove area. The illustration of isolation work was visualized in our education video [24]. Each leaf was cut into pieces of app. 1 x 1 cm size and placed directly on By+ agar medium and then incubated at 23°C for two days. The medium contained 50% (v/v) seawater, 1% (w/v) agar, 0.5%

(w/v) glucose, 0.1% (w/v) peptone, and 0.1% (w/v) yeast extract. The addition of streptomycin and penicillin is excluded in this preliminary research.

After incubation, colonies were picked and transferred into 50 mL flasks containing 20 mL liquid By+ broth medium (without agar) and incubated for 3 days at 23°C. Ten microliters of these liquid cultures were spread on By+ agar medium and incubated for 3 days. Streak plate technique was applied for obtaining pure isolates of each colony without contaminants.

Morphological Characterization

Morphological characterization was carried out during isolation of pure isolates to distinguish *Aurantiochytrium* colonies from other microorganisms. The selected isolates were observed under a light microscope and the figures were captured by digital camera.

RESULTS AND DISCUSSIONS

Figure 1 represents an insight of the recent research project. Figure 1A shows the sampling work located in a mangrove area of Kulonprogo, Yogyakarta, whereas Figure 1B shows some isolates inoculated from the collected samples. Different colonies were identified by their morphology as the microbes grew. Spherical, uneven, and slimy producing colonies were taken in cultivating media, as shown in Figure 1C, for further analysis to obtain pure colonies.

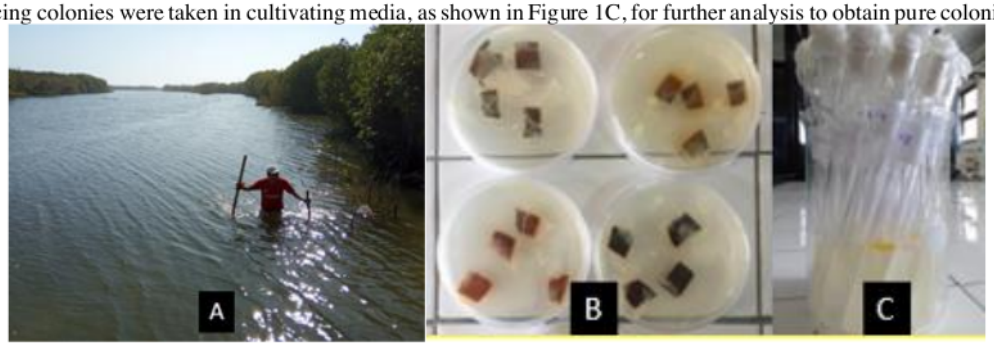

FIGURE 1: An overview of isolation steps of targeting microalgae from a mangrove forest in Kulonprogo, Yogyakarta, Indonesia. A. situation of the mangrove area, B. isolates inoculated from the collected samples, and C. colonies were taken in agar media

Figure 2 depicted the micrograph of isolated microalgae from Kulonprogo mangrove forest. The figure shows typical morphological characteristics of *Aurantiocythrium´*s cell as compared with *Aurantiochytium* LR 52 [21].

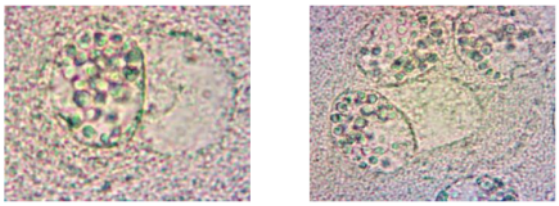

FIGURE. 2: Micrograph of discovered microalgae from Kulonprogo mangrove forest, Yogyakarta, Indonesia.

DISCUSSION

The work presented in this paper observed that *Aurantiochytrium* microalgae has been successfully isolated from fallen leaves of Kulonprogo mangrove forest. It is suggested that direct plating method presented in this paper shall be suitable for experimental work in isolating *Aurantiochytrium sp*. from other Indonesian mangrove forests. This method was also applicable with the results obtained from previous results [20], [25]–[27].

The genus Aurantiochytrium is attributed to a DHA content of about 80% of the polyunsaturated fatty acids [28]–[30]. For example ty acid profile of Aurantiochytrium R52 reveals a simple fatty acid profile with DHA content of more than 80% of PUFA and other fatty acids such as eicosapentaenoic acid (EPA; 20:5, n-3) and arachidonic acid (ARA; 20:4 n-6) [31].

As mentioned previously, the DHA from *Aurantiochytrium* microalgae becomes an emerging important sustainable resource to replace fish oil as a sole omega-3 resource. Recent industrial application also convinces that strains of *Aurantiochytrium* species are the most productive microbial omega-3 producers [32].

Other main interest the usage of *Aurantiochytrium* microalgae is in the field of nutritional, pharmaceutical and biochemical roducts (e.g., proximate nutrients, amino acids, and fatty acid composition). Among others, fatty acid profiles of palmitic acid (C16:0), pentadecylic acid (C15:0), heptadecanoic acid (C17:0 n-6) has been of interest to be observed in previous publications [33]–[35].

With the continuous emerging observation of promising benefits from *Aurantiocytrium* as an alternative omega-3 resource, however, there have been so far very few biodiscovery and isolation work of valuable strains of *Aurantiocytrium* from Indonesia, as the country with the world's largest mangrove area. Therefore, this research describing *Aurantiochytrium* isolation work from one selected mangrove areas in Indonesia is relevant and important for future research field of biochemicals production.

CONCLUSION

Experimental work in this paper shows that the isolation of *Aurantiochytrium* microalgae has been successfully done using direct plating method. The results indicate that the investigating microalgae can be cultured in the media prepared in this experiment. Further work in in this field shall explore characterization of indigenous Indonesian *Aurantiochytrium* in terms of their fatty-acid profiles and biochemical compositions. In addition, cultivation shall be conducted to analyze growth rate and high added value components from isolated *Aurantiochytrium* species. Following this successfully isolation work, it is expected that this result can contribute to further thorough observation in the field of producing valuable nutritional compounds from *Aurantiochytrium* microalgae isolated in other Indonesian mangrove forests.

Further study is required to demonstrate the important applicability of Indonesian *Aurantiochytrium* strains in strategic industrial sectors, including pharmaceuticals, nutraceuticals and biofuel companies.

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