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Bioprocess of Astaxanthin Production as Functional Food from Aurantiochytrium Microalgae: A Review

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ABSTRACT

The Covid-19 pandemic has increased human needs for nutrition including functional food and nutraceutical products in line with the need to boost immunity to resist viral infection, maintain a healthy life, and limit fatalities. In this regard, the choice to use functional food and nutraceuticals seems to be a promising panacea. This paper aims to examine the potential of the microalgae species Aurantiochytrium, which is commonly found in the mangrove ecosystem. This microalgae species has received a lot of attention from researchers because of its high content of lipids and other high-value-added components as well as its fast growth and resistance to environmental stress. With current bioprocess technology, Aurantiochytrium microalgae can be deployed to produce high-value-added components such as astaxanthin. During the current pandemic, the need for this product is increasing as raw material for drugs, health supplements, antioxidants, and vaccine adjuvants that are in direct contact with efforts to combat the human coronavirus and prevent the spread of viral diseases. Due to its strategic position, several large companies have positioned as pioneers in the production of these nutritional products to supply the desired products to help international programs fight the pandemic. In general, producing functional nutritional products requires stages starting from the isolation and screening of microalgae, cultivation, extraction of the desired components to product packaging. Although Aurantiochytrium microalgae are found in the mangrove ecosystem, unfortunately, studies on the production potential of functional nutrients from microalgae Aurantiochytrium native strains from Indonesia have never existed until now. From this study, it is expected that this study will become a fundamental basis for opportunities for further research in this field and increasing attention for the production of functional nutrients from microalgae isolated from mangrove forests in Indonesia. An early insight into bioprocess technology modes such as appropriate isolation techniques, cultivation, and extraction to produce astaxanthin is provided.

1. Introduction

The Covid-19 pandemic gives the lessons that human needs for nutrients to maintain healthy living are positioned as in a top priority [1]. The choice of many people to consume nutraceutical and functional nutrients are aimed at improving stamina, endurance and hence the body is resistant to avoiding viral infections [2]. Therefore, the demand for intake rich in nutraceuticals and functional nutrients in the COVID-19 pandemic is increasing [3,4]. These functional nutrients include food containing long-chain unsaturated fatty acids (PUFA), antioxidants, vitamins, and the like [5]. One source of raw materials for food intake needed by the body is microalgae that are already known biomass producers that contain high added value components (HAVC) needed for human health [6,7]. In this context, the content of HAVC contained by microalgae is helpful for sources of nutrients, pharmaceutical preparations, cosmetic ingredients, pigments, animal feed, organic fertilizers, and raw materials for industrial processes such as biofuels and biochemicals [8,9,10]. In addition, great attention is also increasing on the technology of producing single-cell algae producing lipids and fatty acids for a commercial scale [11,12].

One of the microalgae species widely studied by international researchers comes from the species Aurantiochytrium. Microalgae Aurantiochytrium is a single-cell eukaryotic microalgae (unicellular) found in mangrove ecosystems. Aurantiochytrium microalgae provide nutrients for fish and shrimp in mangrove ecosystems [13,14]. The high-value components produced by Aurantiochytrium include omega-3 unsaturated fatty acids, astaxanthin, squalene, carotenoids, and various enzymes that are beneficial for humans [15,16,17].

Therefore, the future potential of microalgae species Aurantiochytrium may be developed as a raw material for functional nutrients in the post-pandemic era. Previous studies present an increased interest in international researchers and academics to explore the omega-3 potential of Aurantiochytrium for the use of resilience physical and psychological treatment during the Covid-19 pandemic [18].

Unfortunately, although Indonesia has the largest mangrove forest globally that is the habitat of Aurantiochytrium microalgae, the study of the potential of Aurantiochytrium microalgae in Indonesia is still very minimal. The existence of microalgae Aurantiochytrium microalgae that has the potential as a raw material for the production of nutrient sources has not been widely studied in Indonesia. One of the previous studies that examined the isolation techniques and characteristics of Aurantiochytrium species from Indonesian mangrove forests was Hutari and Neuebauer [19]. A review of the potential of Aurantiochytrium microalgae from Indonesia's mangrove forests has been published previously, namely the omega-3 industry design studies [20], review of squalene production [21], and isolation from the mangrove forests of Kulon Progo Yogyakarta [22].

Therefore, this paper aims to provide a review of the potential of Aurantiochytrium as a raw material for the production of astaxanthin. The illustration of the entire production chain illustrated in Figure 1 review in this paper includes methods of isolation of microalgae from mangrove ecosystems, the potential of functional nutritional products produced along with its benefits, and a review of bioprocess technology to produce astaxanthin as a desired functional nutritional product.

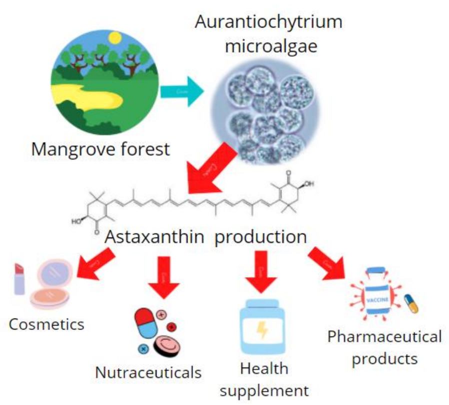

Fig. 1. Illustration of products that can be produced from Aurantiochytrium found in mangrove forests

2. Aurantiochytrium Microalgae

Aurantiochytrium microalgae are heterotrophic unicellular marine microorganisms that require organic components to grow [23]. The common habitat of Aurantiochytrium is mangrove forests and is grown by utilizing nutrients from organic matter in mangrove ecosystems, such as fallen leaves, organic matter, land, or mangrove sediments [24]. Table 1 presents taxonomic illustrations of the Aurantiochytrium species. This species is in the family Thraustocytriaceae found in the oceans from sea level to a depth of 2000 m [25]. Table 1 presents the taxonomy of the Aurantiochytrium.

Previous research has shown that the Aurantiochytrium species have advantages, including high lipid content, rapid growth, and resistance to environmental change [26]. Aurantiochytrium has long been known as oleaginous microalgae (microalgae capable of accumulating lipids in their cells) [27–29]. Because Aurantiochytrium is heterotrophic microalgae, the quantity and quality of products made from these microalgae depend on the source of nutrients, both sources of carbon, nitrogen, phosphate, and other operating conditions (temperature, pressure, pH, and salinity) [23].

Table 1. Microalgae taxonomy of Aurantiochytrium species [30]

Kingdoms	Chromista
Subkingdoms	Harosa
Division	Stramenopiles
Class	Labyrinthulomycetes
Order	Thraustochytriales
Family	Thraustochytriaceae
Genus	Aurantiochytrium

Table 2. List of some astaxanthin-producing microalgae

Microalgae	Output	Production Time	Reference
Thraustochytriidae sp. AS4-A1	±13 mg/L	5-6 days	[31]
Aurantiochytrium sp. RH-7A-7	37.9 mg/L	4 days	[32]
Chorella sp R2	0.005 mg/L	6 days	[33]
Haematococcus pluvialis	20.82 mg/L	14 days	[34]
Chlorella sp.	4.9 mg/L	4 days	[35]

3. Bioprocessing of Astaxanthin Production from Aurantiochytrium

Success story phasing out the development of processes from the upstream stage (microbiology laboratory scale) to the downstream stage (industrial scale) that uses Aurantiochytrium can be studied from preliminary publication [36,37]. The production of lipids containing long-chain unsaturated fatty acids (PUFAs) has been commercialized by the American company Omega Tech, which Martek later acquired (currently part of DSM company). The history of developing DHA-rich oil industry production from Thraustochytrids by Omega Tech has been reviewed by Barclay et al. [36].

In general, looking at the publication, the development of bioprocess technology made from microalgae Aurantiochytrium has six stages, as shown in Figure 2. The first stage is the stage of strain isolation, identification, and selection of the best strain. The second stage is developing cultivation technology to determine the design of bioreactors, optimum conditions, and nutrients used. The third stage is the determination of the technology how to obtain the desired biomass, followed by the third stage, namely the determination of extraction technology to obtain targeted components. Furthermore, the desired component is purified (fifth stage) before it is finally packaged as the final product (sixth stage). Industrial-scale application of Aurantiochytrium processing is applied by DSM and Evonik, who built this microalgae-based plant for omega-3 production [38–40].

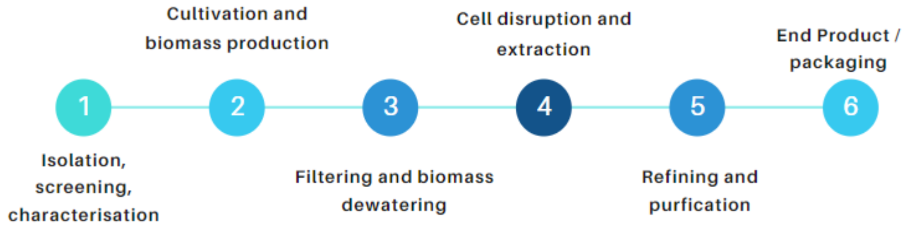

Fig. 2. The stages of production of functional nutrients from microalgae Aurantiochytrium

3.1. Aurantiochytrium Isolation Method

An important step before developing functional nutrient production technology from Aurantiochytrium is the isolation of strains to be cultivated. In general, there are two techniques of isolation of Aurantiochytrium from sea waters, namely direct planting method and polen baiting. Direct planting techniques are done by taking samples of mangrove leaves that have fallen in sediment or mangrove water. Fallen mangrove leaves are a source of carbon supply that Aurantiochytrium needs to grow. The leaves are then cut by an area of 1 cm^2 and placed on the surface of a petri dish. Some previous studies have used this technique for Aurantiocyhtrium isolation [41,42,43]. Illustration of Aurantiochytrium microalgae isolation efforts can be seen from the learning media by Suhendra [44].

After getting the desired strain isolate, the screening stage (sorting) to get the best microalgae strain that can produce products following the target process, withstand the stress condition of tool operations, product safety, and economical operating costs.

3.2. Cultivation

Nutrient components commonly used for laboratory-scale cultivation (breeding) are sugar (glucose or fructose), glycerol, agar medium, yeast extract, peptone, distillation water, NaCl, seawater (either in the form of synthetic brine water or native seawater), phosphate sources (in orthopospathic form) and urea [45,32]. In addition, antibiotics such as penicillin and streptomycin and antifungals such as nystatin are added to prevent the growth of bacterial and fungal colonies [46]. Figure 3 reveals an illustration of microalgae cultivation using Aurantiochytrium RH-7A-7 strains [32] with glucose, peptone, yeast, and antibiotic nutrients at 28 °C and a pressure of 1 atm. The yield of biomass produced from this cultivation is 37.9 mg/L with a four days-cultivation time.

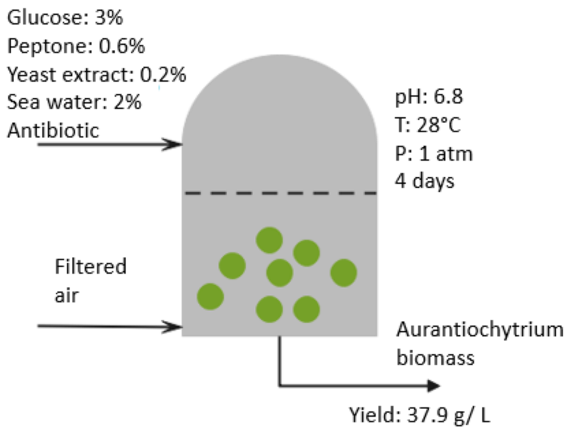

Fig. 3. Illustration of operating conditions and nutrition for the cultivation of Aurantiochytrium RH-7-A-7

Aurantiochytrium can produce unsaturated fatty acids, squalene, and carotenoids, depending on variable nutrient composition (source of carbon and nitrogen), length of fermentation and operating conditions [47,48]. The study of cultivation mode was conducted on a laboratory scale using shaken flask [49], air lift bioreactor [50] as well as technical strategies on fermenters, both in batch, fed-batch, and continuous culture [51]. Löffelholz et al. [52] study the main parameters for cultivating Aurantiochytrium so that it can be used in single-use bioreactors. Jia et al. [53] examine the balance of oxygen that needs to be supplied so that the growth of Aurantiochytrium cells is maximal.

Cultivation of some strains of Aurantiochytrium can produce high lipids, about 23% dry cells with a dry cell density above 100 g/l dry weight with cultivation time of four days [54]. To produce astaxanthin from Aurantiochytrium refers to preliminary research conducted by Aki et al. [55], which produces astaxanthin productivity per day 1.5 mg/l/day. A strain of Aurantiochytrium sp. KH105 can produce astaxanthin with a concentration of 0.30-0.45 g/l after six days of fermentation [56].

3.3. Purification and Extraction

After the cultivation stage, an important stage in producing HVAC from microalgae is the purification and extraction of intracellular metabolites. Generally, this stage greatly determines the total production cost because the investment of bioprocess technology for this stage can reach up to about 50-80% of total production, depending on the characteristics, biochemical components, types of techniques and extraction materials as well as the level of purity of the components to be obtained [49,57,58].

In general, the study of extraction techniques considered includes extraction using organic solvents, cold press, extraction using supercritical fluid [59,60] as well as enzymatic extraction [61,62]. For industrial scale, extraction using organic solvents (generally hexane) is more widely used.

The advantage of the extraction method is that biomass from microalgae is easier to obtain than the filtration method with wet-dry results because there are difficulties in breaking down the cells.

4. Conclusion

This paper has reviewed the potential of Aurantiochytrium microalgae for the production of functional nutrients from astaxanthin. Aurantiochytrium microalgae is heterotrophic microalgae hence the quantity and quality of products produced depends on the nutrients used and the conditions of his cultivation operation. Currently, research on the utilization of Aurantiochytrium microalgae from Indonesian mangrove forests for raw materials astaxanthin as functional nutrients is still very minimal. From some previous research, the industrial-scale application of Aurantiochytrium microalgae processing was addressed by DSM and Evonik by establishing a microalgae-based factory for omega-3 production. Finally, this short article review is expected to pinpoint the enormous potential of Aurantiochytrium microalgae that ubiquitous in the mangroves ecosystem. Hence, it requires further thorough studies by Indonesian researchers.

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