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Isolation and identification of microalgae as omega-3 sources from mangrove area in Aceh Province

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ABSTRACT. Omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential fatty acids with numerous health benefits. The main sources of these fatty acids are fish and fish oils. However, fish supply is limited and the availability is uncertain. There are two main problems with regard to fish supply: overfishing and mercury contamination. In addition, food enrichment with fish oil is apparently challenging due to sensitivity of fish oil to oxidation, and fishy smell which make them less attractive to consumers. Based on above problems, alternative sources of EPA and DHA must be found. An alternative for omega-3 fatty acids are microalgae. This research aims to isolate, identify, and find microalgae biodiversity that are able to produce omega-3 fatty acids in their cells. Microalgae were isolated from fallen, senescent leaves of mangrove tree (*Kandelia candel*) in two locations in Aceh. The colonies were identified morphologically using light and stereo microscopes with 1000x magnifications. The results were compared with those in literatures. This research has isolated and identified some genus of microalgae that were predicted belong to order Labyrinthulida and family Thraustochytriidae. Morphological observations have also confirm identification of one fungi, genus *Mortierella.* All identified microalgae and fungi are omega-3 fatty acids producers, mainly EPA and DHA.
Key words: microalgae, omega-3 fatty acids, EPA, DHA, Labyrinthulid, Thraustochytrium.

Introduction

Research in the field of polyunstaurated fatty acids (PUFA) produces enormous outcomes in regards to the beneficial effects of PUFA to human health. The most important claims are: PUFA is effective in lowering cholesterol, decreasing the risk of arrhythmia, lowering the blood pressure, preventing diabetes in pregnancy, and relief of joints in arthritis (McMurray, 2007). Both omega-3 and omega-6 PUFA are precursors of hormone-like compounds, which are involved in many important biological processes in human body (Trautwein, 2001).

In early 1900s, there was a term called "Vitamin F" for omega-3 fatty acids which attracted research attention (Cannon, 2009). Incorporation of PUFA into food products is dominated by omega-3 fatty acids (α-linolenic acid (ALA) C18:3n-3, eicosapentaenoic acid (EPA) C20:5n-3, docosahexaenoic acid (DHA) C22:6n-3) (Augustin and Sanguansri, 2003). The main sources of omega-3 fatty acids are fish and seafood, particularly cold-water fish. However fish sustainability as the main source of essential fatty acids is now uncertain because of mercury contamination issue and overfishing (Cannon, 2009).

Above-mentioned problems have encouraged researchers to find alternative sources for omega-3 fatty acids. It is well known that fish does not produce fatty acids instead they are coming from the microalgae as the fish feed. Microalgae are microorganisms that produce fatty acids in their cells. Singh et al., (2011) reported that *Botryococcus braunii, Crypthecodinium cohnii, Schizochytrium* sp. (thraustochytrids) are able to produce 25-75%, 20%, and 50-77% oil (dry weight), respectively.

Exploration of microalgae as omega-3 fatty acids producers is a relatively new field and research on this topic has increased considerably in the last few years. Isolation and identification of microalgae were carried out worldwide and the results sometimes specific according to the region and climate where they were isolated. In Indonesia, a study of

PUFAs production from microalgae using glycerol had been conducted in 2010. This research found seven strains of microalgae that produce DHA. The amount of biomass resulted was in the range of 2.49-9.14 g/L (Basuki et al., 2010). In Aceh Province, isolation and identification of microalgae from mangrove area have never been done. Base on the fact that research on microalgae is growing significantly elsewhere, in Indonesia it seems that this field is not fully explore yet. A number of studies on biodiesel from microorganisms are quite many however researches on microalgae as sources of omega-3 fatty acids are scarce.

Microalgae contain bioactive components that have significant benefit to human health such as carotenoid, polysaccharides, antioxidants, and essential fatty acids. Some microorganisms reported to produce edible oils that contains omega-3 fatty acids are from genus *Schizochytrium*, *Thraustochytrium* and *Ulkenia.* The species of microalgae that are able to produce EPA including *Nitzschia spp., Nannochloropsis spp., Navicula spp., Phaeodactylum spp., Porphyridium spp.,* and *Isochrysis galbana*. Other species found with high amount of DHA in heterotrophic conditions are *Crypthecodinium cohnii* dan *Schizochytrium spp.* (Ward and Singh, 2005).

Material and Methods

Materials and equipment

Research materials used were natural sea water, distilled water, yeast extract peptone (YEP) media (consist of glucose (Difco), yeast extract (Merck), mycological peptone (Sigma), bacto agar (Difco), antibiotics (penicillin and streptomycin), lugol staining, and LCB (*Lactophenol Cotton Blue*).

The equipment and apparatus to support isolation and identification process were *light microscope* (Olympus Model BX41TF) equipped with camera (Olympus DP12), *light microscope* (Nikon Eclipse 50i), *inverted* or *stereo microscope* (Nikon), *autoclave*, incubator, *master refractometer* (Atago-Japan), pH meter, analytical scale, micropipette (Socorex-Swiss), paraffin, beaker glass, petri dish and other microbiological glassware.

Isolation and identification process

Samples were collected from two locations of mangrove area in Aceh Besar District: Hutan Kota BNI and in Neuheun-Ujung Batee, Aceh Besar. Three types of leaves brought to the laboratory; dark brown, brown, and yellow-brown colour leaves. Upon arrival in the lab, they were cut into small pieces and grown into media which consisted of yeast extract peptone as describe by Fan et al., (2002). Isolation process and microbiological experiments were carried out at Microbiology Laboratory, Dept. of Agricultural Product Technology, Syiah Kuala University. Identification process using microscopes were done at Laboratory of Pests and Plant Diseases, Faculty of Agriculture and Pathology Laboratory-Faculty of Veterinary, Syiah Kuala University in Banda Aceh-Indonesia.

The petri dishes were incubated for 2-3 days at 25°C and the colonies growth were observed daily using light microscope. At particular, the colonies' morphology (shape and colour) were monitored by stereo microscope and each different colony found must be sub-cultured immediately into the new media. The process was repeated until axenic cultures were obtained.

If the colonies were not axenic after sub-culturing, then they must be followed by a serial dilution process. An axenic culture was characterized by cells that appears in the same shape, colour, and there was no contamination at any part. These morphological characteristics were closely monitored using *light microscope* (Olympus Model BX41TF) equipped with camera (Olympus DP12) with 1000X magnification. The results were compared to literature according to the morphological point of view.

Results and Discusion

Morphological identification

Microalgae known as omega-3 fatty acids producers are classified into phylum Heterokontophyta (Stramenopiles), order Labyrinthulida, and family Thraustochytriidae. There are 12 genus in family Thraustochytriidae but genus which significantly produce EPA and DHA are Aplanochytrium, Labyrinthuloides, Schizochytrium, Thraustochytrium, dan Ulkenia (Ward and Singh, 2005).

The first group that firstly identified in this research using stereo microscope was colonies with core or pellet surrounding by white filament as can be seen in Fig 1. Figure 1 shows identification steps of Mortierella: (a) white colonies growth on solid Yeast Extract Peptone media, (b) observation using stereo microscope, and (c) the cells' morphology under light microscope with 1000x magnification. Using stereo microscope, the colonies were seen in dark green colour particularly the cores (1b). If one single touch of the colony is transferred into a glass object followed by lugol staining, the cells are as in Fig. 1c.

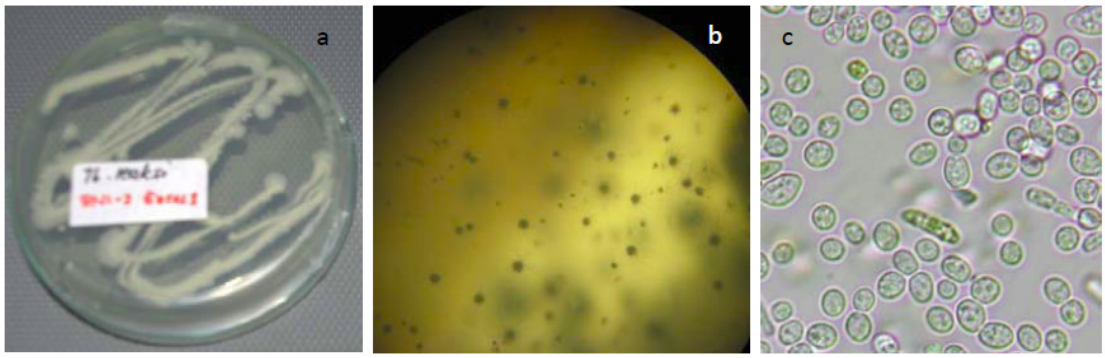

Figure 1. Morphological observation of Mortierella

According to the literatures, this group is known as filamentous microorganisms which can be categorized into fungi (genus Mortierella). The fungi is morphologically characterized by 'hairy pellet' or 'filamentous' mycelia (Park et al., 2001). Figure 2 shows the mycelia of *Mortierella alpina*. Mortierella is one of microorganism that are able to produce γ-linolenic acid (Ward and Singh, 2005), and arachidonic acid (Aki et al., 2001).

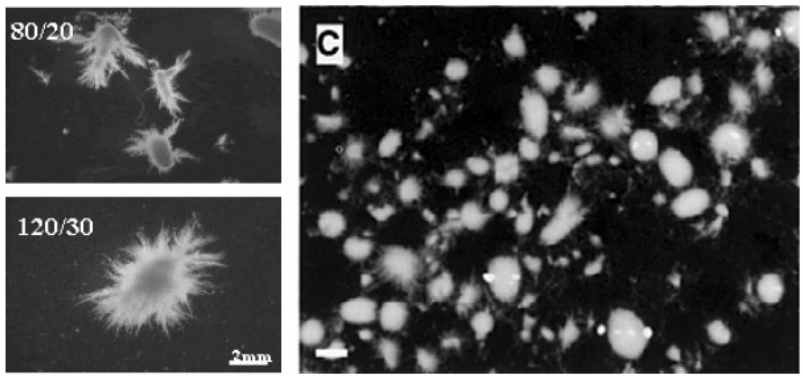

Figure 2. Photographs of mycelia morphology of *Mortierella alpina* using stereoscopic microscope as reported by Park et al., (2001)

The most important genus of microalgae reported in literature as omega-3 fatty acid source is *Schizochytrium*. This genus has been commercially grown and EPA and DHA extracted have widely applied in various food products. The cells of *Schizochytrium* are mostly spherical but some reported to be elongate amorphous cells. Based on morphological examination, *Schizochytrium* is probably also among those isolated from fallen-mangrove leaves in Aceh Province.

Colonies that commonly appeared in solid Yeast-Extract-Peptone (YEP) media after 2-3 days of incubation are the ones with flat-spherical shape, visually white in colour, and having glossy surface. Under stereo microscope these colonies are actually having dark green colour with distinct edges. Identification according to the literature confirms that the morphology of these colonies were similar with those reported by Zhou et al., (2010). They found new strain that has been identified as *Thraustochytriidae* sp. Z105. The strain is able to produce high amount of DHA (32%) from the total lipid. Comparison of colony morphology isolated from this research with results reported by Zhou et al., (2010) is can be seen in Figure 3.

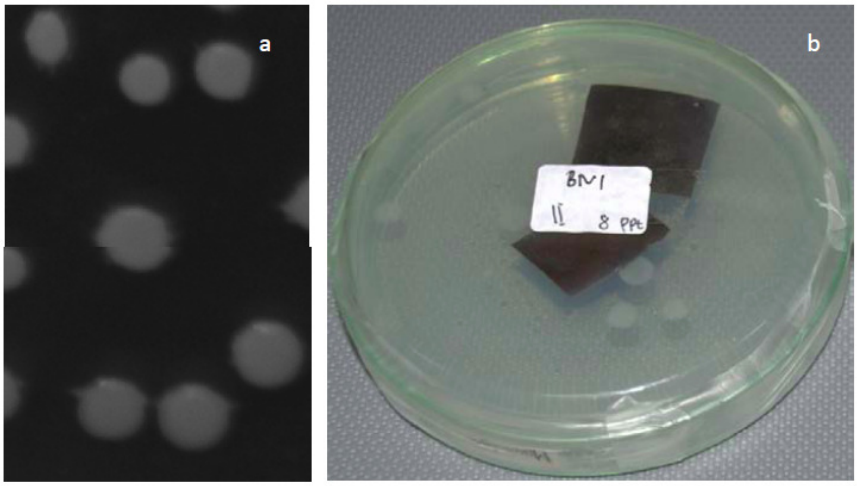

Figure 3. The colonies that are classified as Thraustochytriidae (b) and

(a) Identification results according to (Zhou et al., 2010)

Conclusion

This study has isolated and identified microalgae morphologically that are potential for omega-3 producers. According to literatures these microalgae belong to order Labyrinthulida and family Thraustochytriidae. Filamentous microorganism which can be classified as genus Mortierella was also identified.

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