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Original Research Article

Isolation and identification of phytoplankton from aquatic ecosystems of Lampung Mangroves Center (LMC) as biological feed

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Abstract

In this paper we studied the isolation and identification of phytoplankton from aquatic ecosystems of Lampung Mangroves Center (LMC) as biology feed. The analysis of sea water quality parameters in the location of the study consisting of pH, salinity, brightness, water temperature, nitrate, nitrite and phosphate, revealed that all the parameters meet the quality standard, except for nitrates and nitrites. Isolation and identification of Phytoplankton taken from aquatic ecosystem of the LMC using net plankton ± 15 was carried out to obtain pure cultures in Live Feed Laboratory of the Center for Marin Culture Lampung, Indonesia. The Phytoplankton samples were isolated in the bacto agar and were added the fertilizers Conwy and Gillard to spur the growth of green and brown algae. The isolation of media was done by scratch method and cultured in a petri dish with upside down position for seven days, and observed every day for evaluating the algae growth. The results showed that there were 14 genera of phytoplankton identified. Six types of 14 genera of phytoplankton identified have potential to be developed as a biological feed: *Thalassiosira*, *Cyclotella*, *Chaetoceros*, *Tetraselmis*, *Nannochloropsis*, and *Isochrysis*, The isolates of phytoplankton produced can be stored in the refrigerator as pure stock cultures and can last for up to 6 months before being used in a medium culture or laboratory culture scale.

Keywords: Aquatic Ecosystem, Phytoplankton, Isolation, Biological Feed.

Introduction

Lampung Mangrove Center (LMC) covering an area of 700 hectares, located in the Village of Margasari, District of Labuhan Maringgai, Regency of East Lampung, Lampung Province, Indonesia, is determined by a decree No. 660/305/04/SK/2005/1546/J.26/KL/2005 dated May 10, 2005 (Lampung University, 2010). The mangrove ecosystem has multifunction which include: a) as a breakwater, thus preventing abrasion, b) as cycles of nutrients for flora and fauna, c) as a counterweight quality of the environment and neutralize pollutants, d) ecological function as a spawning grounds, hiding grounds and nursery grounds of marine organisms (Chen et al., 2009; Ponnanbalam et al., 2012; Suprakto et al., 2014). Aquatic ecosystems of Lampung Mangrove Center is very supportive for the growth of fish populations, showed by species diversity index (H = 2.35), and the condition factor (CF) of most types of fish are very healthy and growing well with the value of CF > 1

(Tugiyono and Master, 2015).

The high nutrients in the mangrove ecosystem causes high plankton productivity, especially phytoplankton has a very important role in the food chain in the mangrove ecosystem. Microalgae is unicellular or multicellular organisms from photoautotrophic water, and is a microscopic organism that require inorganic nutrients for photosynthesis (Biondi and Tredici, 2011). The phytoplankton in aquatic ecosystem has the potential to be developed as a biological feed through isolation of phytoplankton from aquatic ecosystem and then it was cultured in the laboratory. (Rocha et al., 2003). Three groups of marine aquaculture organisms using algae as feed are shrimp, bivalve mollusks (especially clams and oyster) and rotifers (De Pauw and Persoone, 1988), and algae are fed to shrimp and mollusks for only a short period of time during larval stages and rotifers are not strictly a target organisms themselves but are in turn used as feed for marine finfish larvae (Raymond and Maxey, 1994).

Based on the analysis of the stomach contents of 13 species of fish caught in the aquatic ecosystem of LMC mostly fish eat the shrimp larvae, fish larvae and larvae of aquatic insects, zooplankton and phytoplankton. Zooplankton groups found are *Thalassiosira sp*, *Chaetoceros* sp, and *Brachionus* sp, while phytoplankton groups found are *Nannochloropsis* sp, *Tetraselmis* sp, *Nitzhia* sp (Tugiyono and Master, 2016).

The availability of food is one of the factors that determine population size, growth, reproduction and population dynamics and the condition of the fish in the waters (Tugiyono and Master, 2015). Besides, as a natural feed phytoplankton, micro algae play an important role in outlining the waste, improve soil structure and increase fertility and produce methane and fuels to energy. As well as grown for animal feed including the fisheries, human food, and pharmaceutical (Sivakumar et al., 2012). The use of microalgae *Chlorela vulgaris* and *Scenedesmus obliquus* as the inoculum used in wastewater treatment, is known to eliminate nutrients such as nitrogen > 90% and phosphorus > 98% (Gauveia et al., 2016). Micro algae produce 10 to 20 times more biomass per unit area than terrestrial plants. More importantly, micro algae can be cultivated in seawater, using leftover nutrients and at the same time can reduce greenhouse gas (GHG) emissions (Sivakumar and Rajendran, 2013). Algae are capable of producing lipids and hydrocarbons quickly and their photosynthetic abilities make them a promising candidate for an alternative energy source (Sivakumar et al., 2012). Besides that, some microalgae can be used to adsorb heavy metals to save our environment (Buhani et al., 2012; 2012a; 2011; Buhani and Suharso, 2009), and remove nitrogen and phosphorus nutrient in wastewater sewage (Wong et al., 2016).

The availability of natural feed (phytoplankton and zooplankton) under normal conditions in the natural aquatic ecosystem is available in enough even abundant. This feed (phytoplankton and zooplankton) can be used as a natural feed by each trophic level efficiently in food web, especially fish that occupy the highest trophic level. Problems with feed requirements usually only appear when the organism is in a farming environment. Natural food, especially phytoplankton is needed in cultivation for commercial purposes, such as fish (larvae or adults), shrimp (stadia early larva), sea cucumbers (larval, juvenile and adult) and mussels (larval, juvenile and adult) (Fulk and Main, 1991). The quality of phytoplankton is outstanding and has been a key factor for hatchery culture success especially with delicate species (Bluebiotech, 2017). So as to meet the needs of the natural feed, use instant natural food in the form of powder produced by the factory are goods imported, so the price is very expensive, for example, the price of freeze dried *Nannochloropsis sp*. is more expensive than *Nannochloropsis sp.* concentrate or frozen paste *Nannochloropsis sp.* (Bluebiotech, 2017). Based on the important role of phytoplankton in the cultivation of aquatic organisms (fish, shrimp, sea cucumbers and other commodities) the possibility of the potential development of biological feed on phytoplankton isolated from aquatic ecosystems of LMC is essential for development.

Materials and Methods

Sampling of phytoplankton

Sampling was conducted on aquatic ecosystems phytoplankton Lampung Mangrove Center in June 2016, retrieval using a plankton net No. 15, with a concentration method: filtered water as much as 50 liters of water with plankton net to 2 liters. Samples were placed in a cool box filled with ice and taken to Live Feed Laboratory of the Center for Marine culture Lampung, Indonesia. Furthermore, the sample was poured into a glass vessel and was aerated and maintained its growth with Conwy liquid fertilizer at a dose of 1 ml/Lit per week and the samples were identified every day. On the spot measurement was conducted for water quality data, namely pH, temperature, salinity and brightness, and collected water samples for analysis of nitrate, nitrite and phosphate in laboratory.

Isolation sample of phytoplankton on bacto agar

Three grams of bacto agar put in sterile sea water with a salinity of 25 ppt (parts per thousand) and a volume of 200 mL bacto agar heated to boil and dissolve completely, the solution became clear or translucent. During heating the bacto agar solution was stirred to prevent the crust and clotting. Then agar- agar solution was cooled, after cooling the bacto agar solution fertilizer Conwy was added at a dose of 1 ml/Lit for the possibility of green algae and partly by the addition of fertilizer Gillard at a dose of 1 ml/Lit for the possibility of brown algae. Bacto agar was then poured into a petri dish with a thickness of 3 mm or less and allowed to stand until hardened.

Then etched germ samples of phytoplankton using ose needle sterilized with alcohol and heating. Bacto agar in a petri dish was streaked in zigzag pattern, the petri dish was closed, sealed and placed isolated with upside down on a rack culture and illuminated by two lamps TL 40 watts continuously, for 7 days (Andersen and Kawachi, 2005; Rusyani, 2007). The growth of phytoplankton isolates was observed every day. One drop of the culture medium was placed on the glass slide and observed under microscope for type identification. Data from the isolation and identification were analyzed using qualitative methods to compare the characteristics of observed phytoplankton with literature.

Results and Discussion

Based on identification of the isolat, it has been known 14 phytoplankton genera existed in water ecosystem of Lampung Mangrove Center (LMC) as presented in Table 1. According to the literature studies, from 14 genera identified of the phytoplankton, there are 6 genera that have potentially to be developed as a live feed such as: Thalassiosira, Cyclotella, Chaetoceros, Tetraselmis, Nannochloropsis, and Isochrysis (Tomas, 1997,Raymond and Maxey, 1994; Aquatrain, 2017,) and Photographs of the observations of the six genera of phytoplankton are presented in Figure 1.

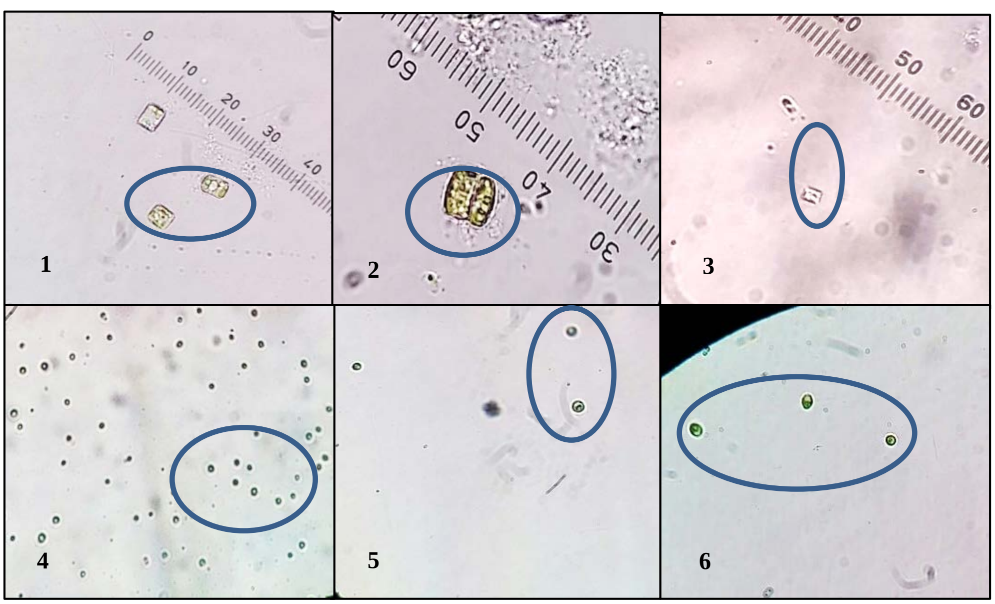

Figure 1: Photographs of the observations of the six genera of phytoplankton, at 40X magnification.
Note: 1. Thalassiosira 2. Cyclotella 3. Chaetoceros sp 4. Nannochloropsis 5. Isochrysis 6. Tetraselmis

Table 1: Identification results of phytoplankton genera isolated from the aquatic ecosystem of LMC.

Phaeophyceae	Chlorophyceae	Chyanophyceae	Chrysophyceae	Bacillariophyceae
Actinocyclus	Radiosphaera	Anabena	Isochrysis	Nitzchia
Melosira	Pseudochlorococcum	Chroococcus		
Thalassiosira	Chlorococcum	Gloeocapsa		
Cyclotella	Tetraselmis			
Chaetoceros				

Thalassiosira sp. has a characteristic cylindrical shape with a diameter ranging from 4 to 32 µm, could be found individually or in groups. *Thalassiosira* sp. cell wall composed of silicate with two valves, which epivalve larger and smaller hypovalve. The size and shape of the valve which is owned *Thalassiosira weissflogii* vary but essentially irregularly shaped ring with one labiate. Another characteristic common to *Thalassiosira weissflogii* is irregular pores. For the largest cells (valve diameter > 10 µm), the valve diameter was equal to or greater than the pervalvar length; in smaller cells the valve diameter was much less than the pervalvar length. Habitat *Thalassiosira weissflogii* is in marine, brackish and freshwater and can match Antlantik Ocean and the Pacific Ocean (Fryxell and Hasle, 1977; Dassow, et al., 2006). Based on the research results of (Panjaitan, 2014) suggest cultivating larvae of Vaname shrimp fed Phytoplankton with a different kind, namely *Thalassiosira weissflogii*, *Chaetoceros calcitrans* and a combination of both, resulting in the highest survival rate of shrimp larvae by feeding from a combination of both. Giving *Thalassiosira weissflogii* it self has a number of larvae survival rate is greater than *Chaetoceros calcitrans.*

Cyclotella sp. characterized unicellular round cylindrical shape or centric category, its diameter is 18.1-48.2 µm. Its pigment is carotene and xantofil so yellowish green and golden brown. Valve with differential ornamentation between the central and marginal area, central area flat to tangentially undulate dan single rimoportula positioned on a costa. *Cyclotella* sp can live marine and fresh waters, and it is one type of phytoplankton have the best potential for development as economical rotifer feeds (Raymond and Maxey, 1994).

Chaetoceros sp. has the characteristics of a single cell that can combine to form a chain. The main body of an elliptic cylinder such as a petri dish with a body length of 2 to 45 µm and diameter 2-85 µm. The main characteristic of *Chaetoceros* sp. is their spines protruding at the corner of the main body and yellow-brown the body colour. *Chaetoceros* sp generally utilized as feed for rotifer, shellfish, oysters, and shrimp larvae. Some species can cause physical damage to organisms' gills when present in large number (blooming) (EOS-Phytoplankton Encyclopedia Project, 2012).

Tetraselmis sp. has a characteristic elliptical oval to move actively with the four flagella measuring 0.75 to 1.2 times the length of its body (Butcher, 1959). Tetraselmis sp. also known as the green chlorophyll flagellates, sizes ranging from 7-12 µm, a single cell with a cell wall composed of cellulose and pectin (EOS-Phytoplankton Encyclopedia Project, 2012). One type of phytoplankton that have potential as biological feed because it contains high nutrients is *Tetraselmis sp* (Rocha et al., 2003).

Nannochloropsis sp. has a characteristic oval-shaped and can move on because it has two flagella. *Nannochloropsis sp* size ranges between 2-4 µm. In addition *Nannochloropsis sp* also have a stigma or eye spots, so *Nannochloropsis sp* sensitive to light. *Nannochloropsis sp* has a pigment of chlorophyll c to make them look green. Another interesting feature is that it has the composition of the cell wall in the form of cellulose (Sleigh, 1989). *Nannochloropsis sp.* is also one type of phytoplankton that have potential as biological feed feed due to its nutritional value (Rocha et al., 2003). Reducing the concentration of inorganic nitrate ($NaNO_3$) in the culture medium tends to put pressure on the growth of *Nannochloropsis sp.* (Hudaidah et al., 2013; Muhaemin et al., 2014). *Nannochloropsis sp and*

Tetraselmis sp. which maintained a 24-hour exposure in the growth media with sufficient nitrogen supplementation and at salinity of 33 gL^{-1} showed the fastest growth, the highest chlorophyll content, and the highest dry weight (Alsull and Omar, 2012).

Isochrysis sp. has a characteristic shape of an ellipse with a length of 5 to 6 μm wide and 2 to 4 μm, a stigma and two smooth flagella. Mainly benthic and typically in spherically round shape. Individual unicellular life and can move on with two flagella. Chloroplasts of *Isochrysis* sp bowl shaped with a ratio of 2/3 from the cell body (Yahya et al., 2013). The species has been known to be used as aquaculture diet and has been proposed as a source of decosahexanoic acid (DHA) for nutraceuticals. To a lesser extent too, it has been tried for lab-scale biodiesel production (Rusyani, 2010 and Flammini, 2011). *Isochrysis sp* has been used especially as a food source for variety shellfish, shrimp and marine fish larvae (De Pauw and Persoone, 1988; Jalal et al., 2013). It is known as one of the most commonly used marine unicellular algae in many marinculture system (Sukenik and Wahnon, 1991).

The analysis of sea water quality parameters in the location of the study consisting of pH, salinity, brightness, water temperature, nitrate, nitrite and phosphate can be seen in Table 2. Based on the analysis parameters of seawater quality in this research, all measurable water quality parameters are suitable for the growth of phytoplankton (Hobson et al., 1979, Andersen and Kawachi, 2005, Jalal et al., 2013, Aquatrain, 2017,).

Table 2: Measurement results of water quality parameters in the waters ecosystems LMC.

No	Parameter	Area1	Area2	Area3	Area4	Area5
1	Salinity	13	19	24	30	20
2	Brightness (cm)	24	25	43	6.5	51
3	pH	6.93	6.91	6.91	6.89	6.91
4	Temperature	32	34	34	31	34
5	Nitrite (NO_2) (mg L^{-1})	0.154	0.232	0.152	0.120	0.156
6	Nitrate (NO_3) (mg L^{-1})	2.714	3.402	3.724	7.419	10.037

Conclusion

Biological feed development of phytoplankton isolates from aquatic ecosystems of Lampung Mangrove Center there are 6 types of phytoplankton are likely to be developed. These activities will support the biological availability of feed in cultivation of marine organisms such as fish, shrimp, sea cucumbers and shellfish (larvae or adults).

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