Microalgae Isolation found in Kedonganan beach, Badung Bali, Indonesia

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Abstract. Microalgae are autotrophic organisms that are very abundant in waters. The purpose of this study was to isolate marine microalgae found in Kedonganan Beach, Badung, Bali. In this study, microalgae were isolated by taking phytoplankton samples vertically with a depth of 50 cm at the study site, filtering using a plankton net. The obtained microalgae were observed under a microscope and identified. The results showed that several genera were isolated, namely Nitzschia, Botryococcus, Nannochloropsis, Ceratium, Caetoceros, Skeletonema, Closterium, and Cyclotella. Based on research, Nannochloropsis is a stable genus with a high growth rate.

The results of the proximate analysis of biomass showed that the microalgae Nannochloropsis sp. contained 40.30±0.37% carbohydrates, 39.07±0.08% protein, 12.44±0.14% fat, 4.77±0.79% ash and water 3.41±0.37%. Nannochloropsis sp. microalgae isolated from Kedonganan Beach has the potential as a source of protein.

1. Introduction

The Indonesian Sea, which is very wide, 5.8 million km² (including 2.7 million km² of exclusive economic zone (EEZ) waters), has a variety of very abundant biological resources. One of the natural wealth in Indonesian waters is microalgae. Microalgae are autotrophic microorganisms that can live in both fresh and marine water. Microalgae are phytoplankton that have the potential to produce proteins, fats, and bioactive compounds such as alkaloids, phenols, chlorophyll, carotenoids, tocopherols, and vitamin C.

The nutritional content contained in microalgae is related to the potential of the microalgae. At present, the potential for the use of microalgae is very diverse and wide so that it becomes an interesting research topic with various objectives in various countries. The potentials of microalgae are as follows: biofuels [1] bioethanol, dyes, food supplements [2], sources of bioactive compounds for medicinal components [3] [4] [5], and biomass [6].

As a source of nutrients, the advantages of microalgae compared to other organisms are 1) it does not require a large area of land, 2) it grows quickly, 3) harvests in 4 to 10 days, 4) productivity 30 times more than land plants, 5) environmentally friendly, 6) can reduce CO₂ emissions in the air, 7) is easily genetically modified, and 8) is generally recognized as safe (GRAS) [7][8]. The purpose of this study was to isolate microalgae in Kedonganan Beach, Badung, Bali.

2. Materials and methods

2.1. Materials

The equipment used in the field are: Global Positioning System (GPS), plankton net no.50, van dorn, 30 mL sample bottle for phytoplankton samples, 10 L plastic buckets, bottles for 500 mL water samples, pH paper, hand refractometer, thermometer, ice box, and stationery. The equipment in the laboratory
used is a microscope, object glass, dropper pipette, cover glass, Erlenmeyer and phytoplankton identification books, Soxhlet equipment, oven, a set of destruction tools, Kjeldahl tool set auto distillation, furnace, and analytical balance. The materials used include Kjeldahl tablet, hexane, distilled water and 5% Lugol's solution for preservative of phytoplankton samples.

2.2. Phytoplankton Sampling Procedure
Phytoplankton sampling was carried out at 5 points, with a distance of every 100 m point in the Intertidal area. Phytoplankton sampling was carried out vertically with a depth of 50 cm using a 10 L Vannorn for each point at each research location. Phytoplankton water samples were filtered using a plankton net with a mesh size of 50 m as much as 5 L, after that the filtered water was put into a sample bottle with a volume of 30 mL and preserved using 5% Lugol's liquid until it was yellowish like tea. After that, the sample bottle is put into a cool box that has been given ice cubes to be brought to the Bioindustry and Environmental Laboratory of Udayana University, Bali to be identified. Phytoplankton analysis was carried out by observing 1 mL of water sample in the counting chamber using a binocular microscope with a magnification of 10x10. The phytoplankton that were found afterwards were identified using an identification book. The nutrition content of Nannochloropsis sp. was analysed with proximate analysis.

2.3. Morphological identification
Microalgae dripped on the object glass then covered with a cover glass and observed under the light microscope Olympus BX5 connected to the camera Olympus DP26 and personal computer with the CellSens Standard app starts from small magnification to 1000 magnification time. The shape or architecture of the cell is observed by observing the morphological characters, namely round, elongated, irregular, colonized or single, and slimy or not. Morphological characters of each isolated microalgae were then analyzed descriptive [1].

3. Results and discussion
The results showed that several types of microalgae have been isolated from Kedonganan Beach, Badung, Bali, including Botryococcus sp., Chaetoceros sp., Ceratium sp., Closterium sp., Cyclotella sp., Nitzschia sp., Skeletonema sp., and Nannochloropsis sp.

3.1. Botryococcus sp.
Morphological analysis showed that the microalgae Botryococcus sp. has been isolated at Kedonganan Beach, Badung, Bali (Figure 1), belonging to the class Chlorophyta, has a toxic effect on several types of aquatic organisms. B. braunii is rich in free fatty acids, mainly oleic and α-linolenic acids [9]. Botryococcus sp. can be used as biofuel. Botryococcus sp. was also found in Liyu Lake, Taiwan, not Labuange Bay, Barru Regency South Sulawesi Province [9] [10].

Figure 1. Botryococcus sp.
3.2. *Chaetoceros* sp.
The morphological analysis also showed that the microalgae *Chaetoceros* sp. was successfully isolated at Kedonganan Beach, Badung, Bali (Figure 2). *Chaetoceros* sp. is a microalgae belonging to the Bacillariophyta (Diatom) family. *Chaetoceros* sp. Some are round with sizes ranging from 4 – 6 μm, and some are rectangular, measuring 8 – 12 x 7 – 18 microns. Like diatoms in general, *Chaetoceros* sp. have cell walls made of silica [11].

![Figure 2. Chaetoceros sp.](image)

The optimal temperature for growth of *Chaetoceros* sp. is 25 – 30°C. The optimal salinity for growth is 17 – 25 ppt. The optimal light intensity for growth is 500 – 10,000 lux, and if the light intensity exceeds 10,000 lux, the growth will decrease [11]. Optimal growth will be achieved by using white light [12]. *Chaetoceros* sp. has good nutritional content. *C. calcitrans* which was cultivated on f/2 medium for 6 days had chlorophyll a, protein, carbohydrate, and fat levels of 0.461, 220.8, 260.8, and 262.1 picograms per cell respectively [13]. The use of agricultural media in the cultivation of *C. calcitrans* produced biomass with the highest protein content, which was 41.92% compared to the use of NPSI media, Walne media, Gillard media, and Na media [14]. The highest biomass concentration (4.1 x 10⁷ cells/mL) was produced when using NPSI media added with 36 M ferric chloride (FeCl₃) with a protein content of 25.41% [15].

Currently *Chaetoceros* sp. widely used as natural food for fish and shrimp larvae [16], as antibacterial [17], potential as antioxidants [18]. *C. calcitrans* is one of the microalgae that can absorb metal ions, such as Cd²⁺ and Cu²⁺ [19][20].

3.3. *Ceratium* sp.
*Ceratium* sp. (Figure 3) also successfully isolated from Kedonganan Beach, Badung, Bali is a marine microalgae belonging to the class Dinophyceae. This species has a straight body with a length ranging from 70 – 300 microns and a width of 15 – 50 microns. *C. furca* is a heterotroph organism that can migrate vertically from the surface down in the waters and is cosmopolitan [21][22]. This species has flagella as a tool for swimming. *C. furca* is often found in abundance and dominates other species. This species does not produce toxins, but it is often reported that blooms occur, resulting in the mass death of other marine organisms. *Ceratium* sp. are mixotrophs, obtain food both by photosynthesis and phagocytosis [23].
Ceratium sp. is contains lots of carotenoid pigments, so they are often red, brown, or gold in color, rather than green. Pyrrophyta or Dinoflagellates mostly have non-contractile vacuoles, chloroplasts, and have chlorophyll a and b. *Ceratium* sp. found in Mexican waters, namely the Gulf of Mexico [24] and also in Labuange Bay, Barru Regency South Sulawesi Province [10]. *Ceratium* microalgae can be used as an indicator of organic matter pollution in waters [10][25][26].

3.4. *Closterium* sp.

Based on morphological analysis, *Closterium* sp. (Figure 4) was also isolated from Kedonganan Beach, Badung, Bali. This microalgae is called ornamental algae because of its various shapes. The results of this study are in line with the research of Fauziah and Laily [27], which states that *Closterium* cells are in the shape of a crescent moon or in the middle of the curve to have a shape like a biscuit or a star, so that the cell consists of 2 symmetrical or symmetrical parts in each. Each part is a large chloroplast with an intricate arrangement, having one or several pyrenoids. In the center of the cell is a nucleus. Some types can crawl by means of threads of mucus that are released through holes in their cell walls.

Closterium sp. belongs to the division Chlorophyta. Chlorophyta are the main producers in aquatic ecosystems because most of the phytoplankton (single-celled and motile) is a member of the Chlorophyta which has chlorophyll pigment so that it is effective for photosynthesis. *Closterium* sp. not found in Labuange Bay, Barru Regency South Sulawesi Province, but found in Sumber Air Jaya Reservoir, Bululawang District, Malang Regency [10][27].

3.5. *Cyclotella* sp.

Based on morphology, *Cyclotella* sp. also found on the beach Kedonganan, Badung, Bali. *Cyclotella* sp. is a type of diatom microalgae, included in the class Bacillariophyceae. *Cyclotella* is a type of diatom in the form of a small disc. The center is flat. Around the edges is a wide band. *Cyclotella* cells are rectangular. Each cell contains many discoid-shaped chloroplasts, cells with a diameter of 5 – 30 microns [28].
Cyclotella sp. used as an indicator of water pollution [28]. This genus is a planktonic diatom that is commonly found throughout the world and is widely distributed in lakes, rivers, seas and brackish water. *Cyclotella* sp. was also found in the Padang Galak Estuary, Denpasar, Bali, in Lake Aur, Musi Rawas Regency but was not found in the Padang Galak Sea in Denpasar, Bali [28] [29].

3.6. *Nannochloropsis* sp.

The results of morphological analysis show that Figure 6 is a *Nannochloropsis* sp. microalgae, a type of Chlorophyta. *Nannochloropsis* sp. has green cells, does not have flagella, cell size is 2 – 4 microns, has chloroplasts and a cell nucleus covered by a membrane. Cell wall of *Nannochloropsis* sp. composed of cellulose. The optimum salinity for the growth of *Nannochloropsis* sp. is 20 – 25 ppt and the optimum temperature for its growth is 25 – 30 °C [11].

*Nannochloropsis* sp. has a relatively high nutritional content. The results showed that *Nannochloropsis* sp. isolated from Kedonganan beach, Bali contained 40.30±0.37% carbohydrates, 39.07±0.08% protein, 12.44±0.14% fat, 4.77±0.79% ash and 3.41±0.37% water. The results of this study indicate that the protein content produced is higher and the fat content is lower than that stated by Isnanstyo and Kurniastuti at 27.64% and 16%, respectively [11]. *Nannochloropsis* sp. used as natural feed for rotifers and fish larvae, biofuel, source of vitamin E, antioxidant, anticancer, anti-inflammatory, microcapsules of active compounds with a certain expiration period. The high content of carbohydrates and lipids makes it possible to utilize alternative renewable energy sources such as bioethanol [30][31][32][33][34]. *Nannochloropsis* sp. is also found in Florida, United States [35]. This microalgae was not found in Unda River, Badung River, Beratan Lake, Padang Galak Estuary, Galak Sea, Bali, nor was it found in Labuaneg Bay, Barru Regency South Sulawesi Province [10] [29].

3.7. *Nitzschia* sp.

Morphological analysis showed that *Nitzschia* sp. also successfully isolated from Kedonganan Beach, Badung, Bali. *Nitzschia* sp. included in the class Bacillariophyceae, are microalgae that have yellow-
brown chromatophores [36]. The hallmark of the class Bacillariophyceae lies in its clear shell, made of silica. The silica framework consists of two valves [37]. *Nitzschia* sp. including the Order Centrales which has radial or round symmetry cell shape characteristics [36]. Besides being found on Kedonganan Beach, Bali, it was also found in the Padang Galak Estuary, Denpasar, Bali [29].

![Figure 7. Nitzschia sp.](image)

3.8. *Skeletonema* sp.

The results of morphological analysis showed that the microalgae *Skeletonema* sp. also found on Kedonganan beach, Badung Bali (Figure 8). This microalga is a type of diatom, belonging to the class Bacillariophyceae. The color of the cells is brownish green and each cell has a frustula that produces an external skeleton. Carotenoids and diatomins are the dominant pigments in this species. *Skeletonema* sp. can grow at a temperature of 3 – 30 °C, salinity 25 – 35 ppt [11].

![Figure 8. Skeletonema sp.](image)

*Skeletonema costatum* has good nutritional content, especially protein content. The nutritional content is as follows: protein of 37%, fat of 7%, carbohydrates of 21%, crude fiber content of 0.56 – 1.41% and water content ranging from 7.78 – 12.68% [39]. *Skeletonema* sp. commonly used to stimulate the growth of fish and shrimp larvae because it has a high protein content. *Skeletonema costatum* (Greville) as a phytoremediation of heavy metal lead (Pb) in batik waste [38].

4. Conclusion

Based on the description above, it can be concluded that there are eight types of microalgae that have been isolated from Kedonganan Beach, Badung, Bali. Most of these microalgae are diatom species, namely *Chaetoceros* sp., *Ceratium* sp., *Cyclotella* sp., *Nitzschia* sp., *Skeletonema* sp. Other microalgae are *Botryococcus* sp., *Closterium* sp., and *Nannochloropsis* sp. *Nannochloropsis* sp. microalgae isolated from Kedonganan Beach has the potential as a protein source.

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