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Biological Diversity of Seawater Microalgae Isolated from Ujung Genteng Sukabumi and Their Novel Genomic DNA Isolation Technique

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Abstract.

The Ujung Genteng Beach, located in Sukabumi, is renowned for its extensive seagrass beds and functions as an intertidal area. However, there has been limited scientific investigation into the diversity of microalgae in this ecosystem. This research aimed to identify the diversity of microalgae sourced from the seawater of Ujung Genteng Beach based on morphological characteristics and optimize DNA isolation and amplification techniques. Sampling was conducted three times at predetermined research locations. Morphological identification was performed using a binocular microscope, while DNA isolation and amplification were performed using CTAB methods with modification. The number of species found at Ujung Genteng Beach, Sukabumi is 47 species, 12 of which are often found at the three research locations both morning and afternoon, namely Navicula sp., Frustulia sp., Diploneis parma, Nitzschia sp., Achnanthidium sp., Amphora sp., Oscillatoria teneuis, Achanthes sp., Planothidium sp., Uronema sp., Zygnema sp., and Flagillaria sp.Based on the gel image, genomic DNA of seawater microalgae was successfully isolated, despite the relatively low purity and concentration. Analysis of diversity index and species diversity revealed variations in species abundance and diversity among different locations, particularly in seawater microalgae found within distinct zones: 0-2 meters from the low tide line (location I), seawater within seagrass areas located 2-5 meters away (location II), and seawater near the open sea, situated 5-10 meters away (location III).Therefore, the diversity of microalgae at all sampling locations was in the medium category and the uniformity index between species was low.

Keywords: DNA Isolation, Genomic, Microalgae, Seawater, Ujung Genteng Sukabumi

1. Introduction

Indonesia has an area of around 7.81 million km2, of which 5.9 million km2 is sea with a coastline of 108,920 km. This fact makes Indonesia one of the largest marine mega biodiversity areas in the world, one of which is microalgae (Setiawan 2018). Microalgae are microscopic microscopic plants (between 3-30 µm in diameter) which are included in the algae class and live as colonies or single cells in all fresh and marine waters (Amini and Susilowati 2010; Fendiyanto et al. 2023). The habitat of microalgae is that it occupies damp places such as rocks, tree trunks, cliffs, and is able to live in hot springs (Gusrianto 2012). Microalgae have an important role as primary producers of oxygen produced from the photosynthesis process (Waluyo 2004). Economically, microalgae are widely used as raw materials for the production of medicines, cosmetics, bioremediation and bioenergy (Purbani et al. 2019; Pratami et al. 2022).

Microalgae identification is divided into two ways, namely morphological identification which is used to determine the type of microalgae species, and molecular identification which is used to support the morphological identification. Microalgae are a very heterogeneous group of organisms with different characteristics, including differences in cell tissue type, cell size, cell morphology, and cell color (Mercer and Armenta 2011; Fendiyanto et al. 2023). Based on the research results of Suharno and Lantang (2012) who have observed the morphology of the microalgae found in Manokwari and Sorong Sea Waters, West Papua, it is dominated by the types Diatoma sp., Euglena sp., Lyngbya sp., Navicula sp., Proboschia sp., Spirogyra sp., Nitzschia sp., Bacteriastrum sp., Peridinium sp., and Rhisosolenia sp. Other morphological identification results were shown by the identification of 21 genera of microalgae in Tanah Merah Bay, Jayapura (Sujarta et al. 2011).

Molecular identification is needed to strengthen morphological data which has limited characters and tends to be influenced by the environment. Identification based on molecular characters is obtained from DNA sequences taken from nucleus, chloroplasts and mitochondria (Suparman 2012; Pratami et al. 2023). Molecular identification in plants including microalgae is also widely

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studied based on RNA through gene expression analysis (Satrio et al. 2019; Fendiyanto et al. 2021; Fendiyanto et al. 2023) and/or molecular dynamics i.e., metabolomic approaches (Fendiyanto et al. 2020; Fendiyanto et al. 2021). Based on the research results of Purbani et al. (2019) showed that 14 isolates of marine microalgae from Tambrauw, West Papua which were traced using the NCBI website generally had a homology percentage of 98-99% to the closest type strain. A similarity value of 95-100% can be stated as the same species (Henry et al. 2000). Another study revealed that a total of 1,293 representative sequences of seawater microalgae from Kuwaiti waters could be classified into different eukaryotic taxa. The results showed that the microalgae communities in Kuwait waters were diverse and varied significantly between different ones during winter and summer (Kumar et al. 2021).

Ujung Genteng is a coastal area on the southern coast of West Java which is included in the Pangumbahan Beach area, Ciracap District, Sukabumi Regency (Ruswandi 2014). Ujung Genteng is one of the beaches in Sukabumi with the highest seagrass cover, namely around 10-15%. The results of the identification of the Directorate General of Aquaculture in collaboration with the Mina Bisnis Science and Technology Clinic Ujung Genteng Sukabumi Regency show the diversity of seagrass from the type Enhalus sp. and Thalasia sp. generally grows on coral flats at Ujung Genteng Beach. Seagrass beds are one of the energy sources in food webs in aquatic ecosystems (Paramasivam et al. 2015). Apart from that, Ujung Genteng also includes an intertidal zone which is a tidal zone where there are various types of living biota, one of which is microalgae (Widiansyah et al. 2016). Therefore, this research aimed to identify the diversity of microalgae originating from sea water based on morphological characteristics and Bulk DNA isolation optimation of seawater-microalgae.

2. Materials and Methods

2.1. Sample collection

Microalgae sampling and aquatic environmental parameters were determined based on the conditions of the research area as a whole (Evita et al. 2021).

Figure 1 The research location was at Ujung Genteng Beach, Sukabumi. The research location is marked with a red location symbol (https://www.google.com/maps/)

The sampling location was divided into three locations, namely the first location with a distance of 0-<2 m from the low tide line, the second location (seagrass area) with a distance of 2-<5 m, and the third location with a distance of 5-10 m which is connected to the open sea. Sampling was carried out at low tide (Rachmawan et al. 2021). Sample analysis was carried out at the Plant Physiology and Molecular Biology Laboratory, Department of Biology IPB Dramaga Bogor. The study area of this research was Ujung Genteng Beach, Sukabumi, Indonesia (7°17'42.1"S 106°25'32.7"E [-7.295038, 106.425741]) (Figure 1). The sampling was conducted from January to June 2022. Sampling was performed using a purposive sampling method (Fendiyanto et al. 2023) at low tide at Ujung Genteng Beach, Sukabumi for one day at intervals, namely in the morning (09.00 to 10.00 West Indonesian Time/WIT) and in the afternoon (12.00 to 13.00 WIT) (Rizqina et al. 2017).

Sampling was carried out vertically by filtering 10 liters of water using a plankton net, then the net that had been sunk to a depth of 1 m was pulled from above the surface and left for 5 minutes at the three predetermined sampling locations. Water samples were taken and put into 35 ml sample bottles and labeled with the name, location and date of sampling (Fachrul 2012, Evita et al. 2021). The sampling point was created by drawing a straight line from the lowest low tide point (05.00 to 11.00 WIT), with a distance of 10 m towards the sea and divided into three sampling locations. Sampling was carried out 3 times at each location (Erlangga et al. 2022; Fendiyanto et al. 2023). A total of 18 microalgae samples were obtained, then 1.5 ml of 4% formalin and 2 to 4 drops of betadine were added to keep the chlorophyll from being damaged. Next, the samples were taken to the Plant Physiology and Molecular Biology Laboratory, Department of Biology IPB. Apart from seawater sampling, environmental data collection was also carried out once in the morning and afternoon, namely pH, light intensity, water temperature, air humidity and wind speed. This data measurement is to describe the physico-chemical environmental conditions that support the growth of microalgae.

2.2. Morphological characterization

Identification of microalgae morphology was carried out by observation using a binocular microscope. Observation of microalgae was carried out by dripping water samples on an object glass, then covering it with a cover glass and observing under a binocular microscope connected to an Olympus CX33 camera and personal computer with the IndomicroView application starting from 4x objective lens magnification to 100x magnification (100x magnification using immersion oil). Observation of each sample using a microscope was carried out one repetition. Microalgae that are found and often appear on the microscope are documented and given the name and sampling location to facilitate identification of microalgae from the three sampling locations. The shape or architecture of the cells is observed by observing the morphological characters, namely round, elongated, irregular, in colonies or single, and slimy or not (Fendiyanto et al. 2023). Identification was carried out by referring to the identification book entitled "The Freshwater Algae" (Prescott 1978) and the book

"Introduction to the Algae Structure and Reproduction second edition" (Bold and Wynne 1985).

2.3. Biological data analysis

The morphological characteristic data obtained was analyzed descriptively to find out a complete description of each type. Quantitative data was processed using the R program version 4.0.4. Diversity parameter values include index values for species richness, abundance, diversity and evenness (Fendiyanto et al. 2023). the Menhinick index (MeI), the Margalef index (MaI), Rarefaction (Ra), the Shannon-Wiener Index (H'), Simpson Index (D), and Pilou evenness (J) were performed in this study (Magurran 1988; Boontawee et al. 1995; Odum 1996; Fendiyanto et al. 2023).

$$\text{MeI} = \frac{n}{\sqrt{N}} \qquad \text{MaI} = \frac{n-1}{lnN}$$

where n is the number of species and N is the number of individuals:

$$Ra = \sum 1 - \left[\frac{(\frac{\mathbf{N-Ni}}{\mathbf{n}})}{(\frac{\mathbf{N}}{\mathbf{n}})}\right]$$

where N is the total number of individuals in the new rarefied taxa, and Ni is the total number of individuals in each original

$$\text{H'} = -\sum\left(\frac{ni}{N}\, x\, ln\frac{ni}{N}\right) \qquad \text{D} = \frac{N(N-1)}{\sum ni(ni-1)}$$

where ni is the number of individuals of each species i and N is the total number of individuals

$$\text{J} = \frac{H'}{\ln S}$$

where H' is the true diversity value (Shannon-Wiener Index) and S is the number of species

2.4. DNA isolation and amplification

DNA isolation was carried out using the method of Sambrook et al. (1989) which has been modified following Fendiyanto et al. (2019a), namely by preparing a water sample then placing it in a 15 ml tube, then centrifuging at a speed of 4,000 rpm, temperature 4 ℃, for 5 minutes. After that, the supernatant was discarded and repeated 3 times so that the volume of each sample became 45 ml. The initial stage was the destruction of cell membranes and walls using liquid nitrogen. The powder obtained was added with 3 ml of CTAB buffer, stirred and added 60 µl of β Mercaptoethanol; after that it was vortexed until evenly mixed (Fendiyanto et al. 2019b). The mixture was incubated in a water bath at a temperature of 65℃ for 30 minutes, which served to optimize the work of the extraction buffer added to the sample (Cheng et al. 2003), then turned over every 10 minutes to make it homogeneous.

In the next stage, 30 µl of polyvilpyrrolidone was added and incubated again for 30 minutes, turned over until homogeneous every 10 minutes, then incubated at -20 ℃ for 5 minutes. A total of 3 ml of Chloroform Isoamylalcohol was added and turned over until homogeneous for 10 minutes. The mixture was centrifuged at 3600 rpm, temperature 4 ℃, for 20 minutes. The supernatant was transferred to a 15 ml tube and labeled, 3 ml of isopropanol was added, stirred back and forth, incubated at -20 ℃ for 24 hours. Precipitation is carried out with the aim of precipitating DNA (Syafaruddin and Santoso 2011). After 24 hours, the mixture was centrifuged at 4,000 rpm, temperature 10 ℃, for 30 minutes. The supernatant was discarded, and the pellet was dried in an incubator for 60 -120 minutes. The pellet was added with 20 µl free nucleic water and then vortexed and spundown. The results of the genomic DNA are viewed using a spectrophotometer or electrophoresis to determine the purity and concentration of the genomic DNA. A total of 1 µl of DNA was diluted with 199 µl of ddH_2O, then read on a spectrophotometer with a wavelength of 260 nm and 280 nm for proteins. Amplification of DNA fragments was carried out using 4 pairs of primers (18_KWT_F and 18_KWT_R, 18_ss5_F and 18_ss3_R, 16_Stiller_PS_F and 16_Stiller_U_R, 16_Stiller_PS_R and 16_Stiller_U_F) on a thermocycler machine (Stiller 2005; Table 1). PCR analysis was carried out with a total of 15 µl reactions containing 1 ng template DNA, DreamTaq Green PCR Master Mix (2x), 10 pmol forward primer, 10 pmol reserve primer, ddH_2O. The mixture is homogenized then put into a thermocycler machine.

The PCR reaction goes through the following stages, namely initial denaturation at 95ºC for 3 minutes, then denaturation at 95ºC for 30 seconds, annealing at 51ºC for 30 seconds, and existence at 72ºC for 1 minute followed by final existence at 72ºC for 10 minutes and cooling at 4ºC for 3 minutes. The amplification results were visualized using horizontal electrophoresis with a 1% (w/v) agarose gel in 1x TAE buffer. A total of 5 µl of loading dye and 10 µl of genomic DNA were inserted into the wells of a 1% agarose gel. Electrophoresis was carried out for 30 minutes at 100 Volts with 1X TAE buffer as running buffer. Gel soaked in a solution of Ethidium Bromide (EtBr). The gel can be visualized by placing the gel on a UV illuminator to see whether there are bands of genomic DNA. Fragment size is determined by comparing it to the 1 kb Ladder standard.Genetic diversity in PCR products is determined based on whether DNA bands appear or not. DNA bands resulting from PCR are measured based on the ladder used, namely 1 kb DNA ladder. In addition, this research will be continued by future researchers with metagenomic analysis using 18S rDNA primers.

Table 1. Primers used in DNA amplification of seawater microalgae at Ujung Genteng Beach (Stiller 2005; Khaw et al. 2020; Kumar et al. 2021)

No	Primer	Sekuen (5'-3')
1	18_KWT_F	GCG GTA ATT CCA GCT CCA A
	18_KWT_R	AAT CCR AGA ATT TCA CCT CT
2	18_ss5_F	GGT GAT CCT GCC AGT AGT CAT
	18_ss3_R	ATG CTT G GAT CCT TCC GCA
3	16_Stiller_PS_F	GGT TCA CCT ACG GAA ACC
	16_ Stiller_U_R	GGG ATT AGA TAC CCC WGT AGT CCT
4	16_ Stiller_U_F	ACG GYT ACC TTG TTA CGA CTT
	16_ Stiller_PS_R	GAG AGT TTG ATC CTG GTC AG
		CCC TAA TCT ATG GGG WCA TCA GGA

3. Results

3.1. Aquatic Environmental Parameters

Ujung Genteng Beach is at coordinates 07°22'55.7"S and 106°24'24.0"E. The results of absolute measurements of aquatic environmental parameters are presented (Table 2).

Table 2. Water environmental parameters at Ujung Genteng Beach, Sukabumi at the time of sampling

Abiotic Parameters	Condition	
	Morning	Midday
Temperature (ºC)	29.2	30.0
Humidity (%)	77.0	73.6
Wind velocity (m/s)	1.2	1.1
Light intensity (lux)	10,430.0	11,370.0
Water pH	7.7	7.9

The water temperature in the morning and afternoon in Ujung Genteng is 29,2-30,0 ºC. This shows that it is still suitable for the growth of microalgae because the optimum water temperature for microalgae growth is around 20-30 ºC (Nybakken 1982; Barten et al. 2020). The high and low water temperature is influenced by the intensity of incoming light and the depth of the water, and can directly influence and control the rate of metabolic processes in microalgae cells (Rianto et al. 2008).

The light intensity at Ujung Genteng Beach ranges from 10.430-11.370 lux. Light greatly influences photosynthesis in microalgae. High light intensity can make the rate of photosynthesis in algae also high and vice versa. Microalgae can be distributed based on the influence of light intensity and temperature. The higher the light intensity (range 2,500-8,000 lux) and water temperature (25-32 ºC), the higher the photosynthesis process of microalgae so that the types of microalgae can be diverse (Novasaraseta et al. 2018). Light intensity that is too high (>11,700 lux) will affect the increase in water temperature so that microalgae have problems in growth (Prasetyo et al. 2018). Based on this phenomenon, high light intensity (10,430-11,370 lux) at Ujung Genteng Beach can affect microalgae cell population density. Each type of microalgae has a different tolerance to variations in light intensity (Huang et al. 2011).The presence of seagrass and microalgae in an ecosystem can also cause competition, because they require the same nutrients to live, which can reduce the diversity (number of species) of microalgae on Ujung Genteng Beach (Roem et al. 2017).

The results of measuring the pH of sea water at Ujung Genteng Beach in the morning and afternoon were 7.7-7.9. This pH value is included in the optimum pH for microalgae growth which ranges from 7.0 to 8.5 (Pescod 1971; Filali et al. 2021). The pH value is a description of the intensity of acidity and alkalinity of water which can influence water biochemical processes, such as nitrification. Water with a pH value of less than 4 is very acidic water. This condition can cause death to aquatic organisms. Meanwhile, waters with a pH value of more than 9.5 are very alkaline waters and can reduce the productivity of aquatic organisms because they are toxic, causing an increase in ammonia content (Siregar 2009).

3.2. Diversity of seawater microalgae

The results of morphological identification of all seawater samples from Ujung Genteng Beach, Sukabumi showed 47 species of microalgae covering six classes, namely the Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae, Chrysophyceae, and Euglenida classes (Figure 2). Location II in the research results graph shows that the diversity of species found is less than in locations I and III, in fact some species found in locations I and III were not found in location II. The presence of microalgae often becomes a competitor for seagrass that lives in the same ecosystem, causing several species not to be found at location II due to competition in obtaining nutrients and placement of growth locations (Riniatsih et al. 2017).

Species abundance of classes Bacillariophyceae in all locations is relatively high among other classes. Species abundance of class Bacillariophyceae in Location III is higher than location I and II. Location I had high species number of class Chlorophyceae while Location II had the lowest number of species in class Chlorophyceae, Cyanophyceae, Dinophyceae, Chrysophyceae, and Euglenida. In addition, Location III has highest species abundance in class Bacillariophyceae, Cyanophyceae, Dinophyceae, and Euglenida (Figure 2).

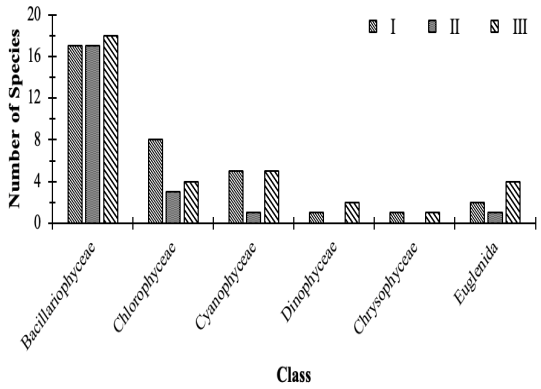

Figure 2. Diversity of microalgae at Ujung Genteng Beach, Sukabumi

The most common species found from the three sampling locations in both morning and afternoon conditions were from the Bacillariophyceae Class with 12 species, namely *Navicula* sp., *Frustulia* sp., *Diploneis parma*, *Nitzschia* sp., *Achnanthidium* sp., *Amphora* sp., *Oscillatoria teneuis*, *Achanthes* sp., *Planothidium* sp., *Uronema* sp., *Zygnema* sp., and *Flagillaria* sp. (Figure 3). According to Arinardi et al. (1997), the Bacillariophyceae class includes microalgae that are widely distributed and are able to adapt to the environment and have high tolerance. This is in accordance with the statement that the microalgae found in marine waters are generally of the diatom type (Bacillariophyceae) followed by the Dinophyceae class and blue algae (Cyanophyceae) (Nontji 1984). Diatoms will dominate waters when light intensity is high and temperatures are low (Welch 1980).

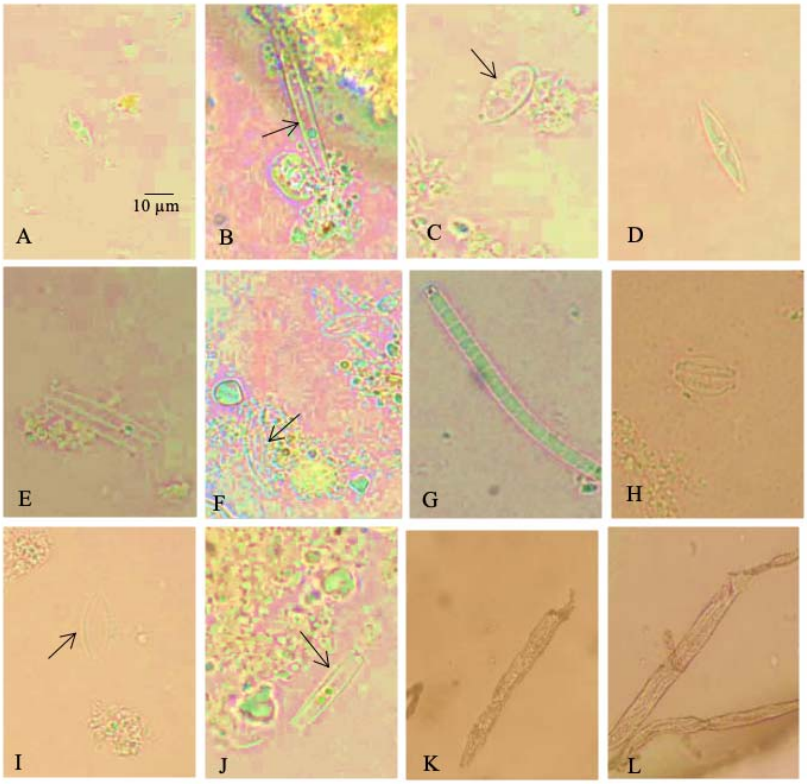

Figure 3. Microgae species that are often found on Ujung Genteng Beach, Sukabumi: A. *Navicula* sp.; B. *Frustulia* sp.; C. *Diploneis parma*; D. *Nitzschia* sp.; E. *Achnanthidium* sp.; F. *Amphora* sp.; G. *Oscillatoria teneuis*; H. *Achanthes* sp.; I. *Planothidium* sp.; J. *Flagillaria* sp.; K. *Uronema* sp.; and L. *Zygnema* sp.. The scale lines in image A apply to images A-L with 100x magnification.

Location I, both in the morning and afternoon, found microalgae consisting of the classes Bacillariophyceae (17 species), Chlorophyceae (8 species), Cyanophyceae (5 species), Euglenida (2 species), while only 1 species was found in the Dinophyceae and Chrysophyceae classes. In location II, during the morning and afternoon, the microalgae found were in the classes Bacillariophyceae (17 species), Chlorophyceae (3 species), Cyanophyceae and Euglenida (1 species), while Dinophyceae and Chrysophyceae did not find any type of microalgae. The microalgae found at location III in both morning and afternoon conditions included the Bacillariophyceae class (18 species), Cyanophyceae (5 species), Chlorophyceae and Euglenida (4 species), Dinophyceae (2 species), while for the Chrysophyceae class only 1 species was found. The diversity of microalgae at Ujung Genteng Beach depicted in the graph shows that the eight classes of microalgae that were identified can be found from the three research locations (Table 3, Figure 4).

Table 3. Types of microalgae found at Ujung Genteng Beach, Sukabumi

No.	Microalgae	Location					
		I		II		III	
		M	D	M	D	M	D
	Class Bacillariophyceae						
1.	*Naviculla* sp.	+	+	-	+	+	+
2.	*Pinnularia gibba*	-	+	-	-	+	-
3.	*Pinnularia braunii*	+	-	-	+	-	-
4.	*Pinnularia legume*	-	-	+	-	-	+
5.	*Frustulia* sp.	+	+	-	+	-	+
6.	*Diploneis parma*	+	-	-	+	+	+
7.	*Nitzschia* sp.	+	+	+	+	+	+
8.	*Cymbella turgida*	-	-	+	-	-	-
9.	*Cymbella tumida*	-	-	-	+	-	-
10.	*Epithemia alpestris*	-	+	+	+	-	+
11.	*Rhoicospenia* sp.	-	-	+	-	+	+
12.	*Flagillaria* sp.	-	+	-	+	+	-
13.	*Fragillaria vaucheriae*	+	-	-	-	+	+
14.	*Synedra ulna*	-	+	-	-	+	-
15.	*Achnanthidium* sp.	+	-	-	+	+	+
16.	*Brachysira* sp.	+	-	+	+	+	+
17.	*Rhopalodia gibberula*	-	-	+	-	-	-
18.	*Amphora* sp.	+	+	-	+	+	-
19.	*Melosira varians*	+	-	-	-	-	-
20.	*Planothidium* sp.	-	+	-	+	+	+
21.	*Achanthes* sp.	-	+	+	-	+	+
22.	*Craticula* sp.	-	-	-	-	+	+
23.	*Humidophila* sp.	-	+	-	-	+	-
	ClassChlorophyceae						
24.	*Tetrahedron obesum*	+	-	-	-	-	-
25.	*Tetrahedron minimum*	+	-	-	-	-	-
26.	*Sachroederia setigera*	-	+	+	-	+	-
27.	*Uronema* sp.	-	+	+	+	-	+
28.	*Zygnema* sp.	-	+	-	+	-	+
29.	*Pleurotaenium* sp.	+	-	-	-	-	+
30.	*Ulothrix* sp.	-	+	-	-	-	-
31.	*Protococcus viridis*	-	+	-	-	-	-
	ClassCyanophyceae						
32.	*Chroococcus linnclius*	+	-	-	-	-	-
33.	*Chroococcus disperses*	-	-	-	-	+	-
34.	*Oscillatoria teneuis*	+	+	+	-	+	+
35.	*Rhabdoderma lineare*	-	+	-	-	+	-
36.	*Aphanocapsa gravillei*	-	+	-	-	-	-
37.	*Schizothrix tinctoria*	-	+	-	-	-	+
38.	*Microcystis* sp.	-	-	-	-	-	+
	Class Dinophyceae						
39.	*Glenodinium palustre*	-	-	-	-	-	+
40.	*Gymnodium coudata*	-	+	-	-	-	-
41.	*Ceratium hirundinella*	-	-	-	-	+	-
	ClassEuglenida						
42.	*Trachelomonas superba*	-	+	-	-	-	+
43.	*Trachelomonas rotunda*	-	-	-	-	+	+
44.	*Trachelomonas abrupta*	-	-	-	-	-	+
45.	*Trachelomonas kelloggiii*	-	-	+	-	-	+
46.	*Trachelomonas volvocina*	+	-	-	-	-	-
	ClassChrysophyceae						
47.	*Dinobryon tabellaria*	+	+	-	-	+	-
	Total	17	23	12	14	22	24

+ = found; - = not found

Location I = sea water which is 0- <2 m from the low tide line, location II = sea water in the seagrass area which is 2- < 5 m away, location III = sea water which is close to the open sea, 5-10 m away m. M = conditions in the morning; D = conditions during the day.

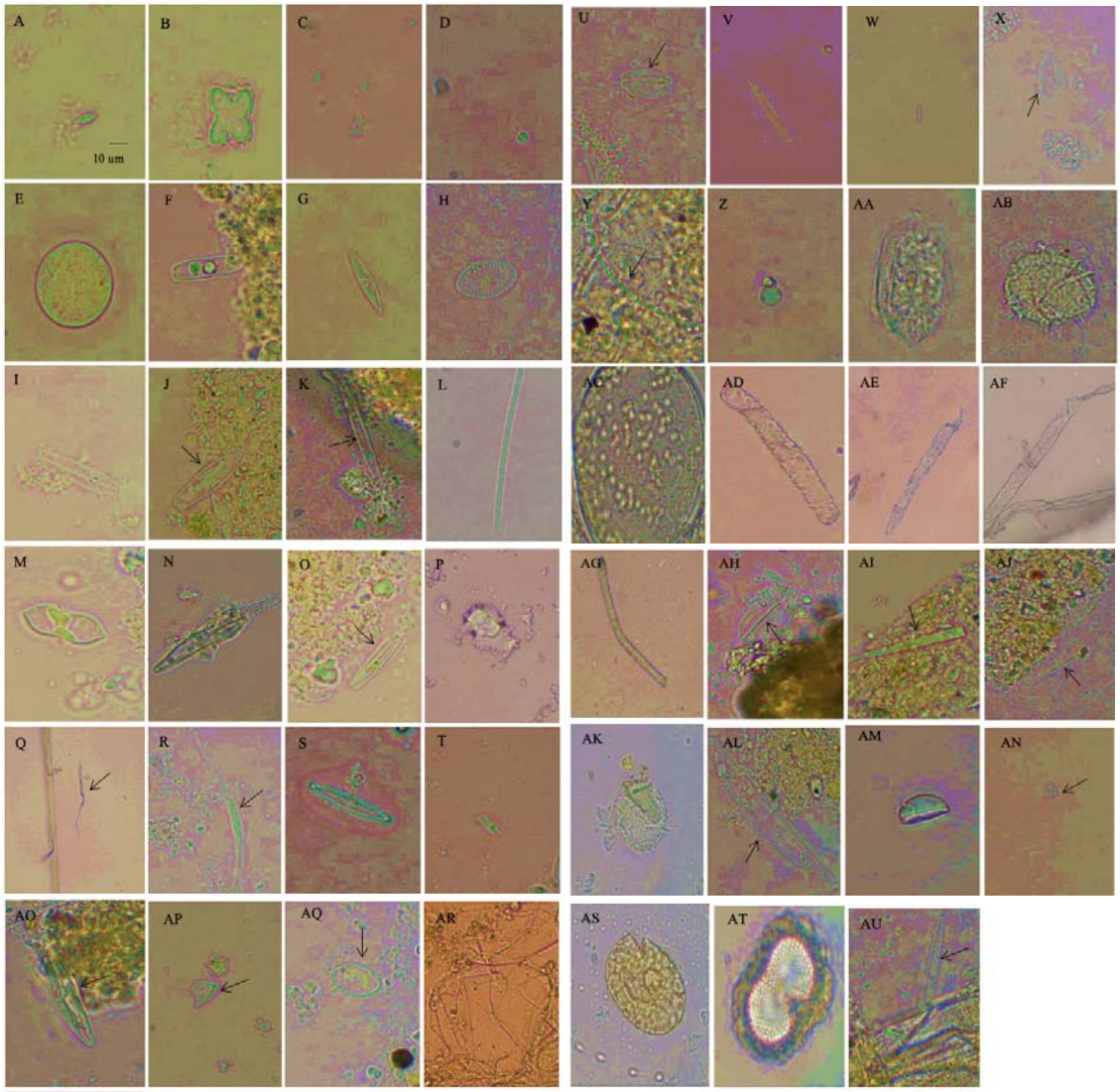

Figure 4. Microalgae species found from the three research locations on Ujung Genteng Beach during the morning and day: A. *Tetrahedron obesum*; B. *Minimum tetrahedron*; C. *Dinobryon Tabellaria*; D. *Chroococcus linnchius*; E. *Trachelomonas volvocina*; F. *Navicula* sp.; G. *Nitzschia* sp.; H. *Diploneis parma*; I. *Achnanthidium* sp. J. *Brachysira* sp.; K. *Frustulia* sp.; L. *Oscillatoria teneuis*; M. *Pinnularia braunii*; N. *Amphora* sp.; O. *Flagillaria* sp.; P. *Melosira variance*; Q. *Pleurotaenium* sp.; R. *Sachroederia setigera*; S. *Pinnularia gibba*; T. *Humidophila* sp.; U. *Achanthes* sp.; V. *Synedra ulna*; W. *Rhabdoderma lineare*; X. *Planothidium* sp.; Y. *Schizothrix tinctoria*; Z. *Protococcus viridis*; AA. *Gymnodium coudata*; AB. T*rachelomonas superba*; AC. *Aphanocapsa gravillii*; AD. *Epithemia alpestris*; A.E. *Uronema* sp.; AF. *Zygnema* sp.; AG. *Ulothrix* sp.; AH. *Rhopalodia gibberula*; A.I. *Rhoicospenia* sp.; A.J. *Pinnularia legume*; AK. *Trachelomonas kelloggii*; AL. *Cymbella turgida*; A.M. *Cymbella tumida;* AN. *Chroococcus disperses*; AO. *Craticula* sp.; AP. *Ceratium hirundinella*; I. *Trachelomonasrotunda*; AR. *Glenodinium palustre*; US. *Trachelomonas abrupta*; AT. *Microcystis* sp.; AU. *Flagillaria vaucheriae*. The scale lines in image A apply to image A-AU at 100x magnification.

3.3. Microalgae Diversity Index

Analysis of microalgae diversity parameters is based on the values of species richness, abundance, diversity and uniformity. Species richness and abundance can be tested using the rarefaction method, Menhinick index (MeI) and Margalef index (MaI), which indicates the richness and abundance of Ujung Genteng seawater microalgae species. The highest Ra value is found at location II in the morning conditions at 2.96, which means that the species at that location are unique and the majority can only be found at that location. Locations I and III have high wealth values, while the lowest wealth is in location II during the day. The greater the value of the Menhinick index (MeI) and Margalef index (MaI), the greater the value of microalgae species richness (Boontawee et al. 1995).

Microalgae diversity can also be tested with two types of indices, namely the Shannon-Wiener Index (H') and the Simpson Index (D). Based on testing these two types of index, the microalgae community as a whole has a diversity index (H') value of around 1.0<H'<3.3 (Table 4). This diversity index value shows that the abundance of microalgae types on Ujung Genteng Beach is moderate and indicates that the waters have a stable, balanced ecosystem. This refers to the diversity index criteria: H'<1: Low community stability, 1.0<H'<3.3: Medium community stability, H'>3: High community stability (Odum 1998).

Table 4. Parameter values for diversity and uniformity of microalgae from three research locations on Ujung Genteng Beach

Location	SR	MeI	MaI	SA	Ra	H'	D	J	E_{a-b}
Conditions in the morning									
Location I	17	3,57	4,75	29	2,80	2,60	0,90	0,32	0,14
Location II	12	3,33	4,29	13	2,96	2,46	0,91	0,37	0,21
Location III	22	3,28	5,51	45	2,87	2,90	0,93	0,30	0,12
Conditions during the day									
Location I	23	3,35	5,71	47	2,83	2,85	0,92	0,29	0,11
Location II	14	2,60	3,86	29	2,82	2,50	0,90	0,34	0,18
Location III	24	3,27	5,77	54	2,87	2,97	0,94	0,30	0,11

Note: SR: *Species richness*; MeI: Menhinick's index; MaI: Margalef's index; SA: *Species abundance*; Ra: *Rarefaction*; H': Shannon-Wiener Index; D: Simpson's Index; J: *Pilou evenness*; $E_{a:b}$: *Hill's ratios*.

The species uniformity value shows the inverse value of the diversity index value (both in the Shannon-Wiener Index and Simpson Index). In this study, two types of uniformity index were used, namely Pilou (J) and Hill ratio (Ea:b). Locations I and III during the day have low Pilou and Hill's ratio uniformity values, while location II in the morning has high Pilou and Hill's ratio uniformity values. The uniformity index value (J') can describe the distribution and number of microalgae in Ujung Genteng which has a low uniformity value, namely 0.29 -0.37, which means that the distribution of individuals for each type is not the same, with a tendency to be dominated by certain types (Odum 1993). The uniformity value category ranges from 0 to 1 (Odum 1993). This is reinforced by the statement that if the uniformity is close to zero, it means that the uniformity between species in the community is low and, conversely, if the uniformity is close to one, it can be said that the uniformity between species is even or the same (Pirzan et al. 2008).

3.4. Genomic DNA Isolation and DNA Quantity Measurement

The DNA isolation results showed poor results because the average of the seven samples showed low DNA concentration values. Likewise, from the DNA purity value, none of the samples showed good quality purity because the absorbance value was below 1.8 or above 2.0. Good quality DNA purity will have an A260/A280 ratio of between 1.8-2.0 (Maftuchah et al. 2014). The range of values in the results of this study indicates that the amount of DNA in the sample is less than the amount of protein, or indicates that the DNA isolation results are still contaminated with RNA and protein (Murtiyaningsih 2017).

The results of analysis of the quality of genomic DNA in 7 samples of seawater microalgae using PCR and electrophoresis methods using four pairs of primers (18_KWT_F and 18_KWT_R, 18_ss5_F and 18_ss3_R, 16_Stiller_PS_F and 16_Stiller_U_R, 16_Stiller_PS_R and 16_Stiller _U_F) are presented (Figure 5, Figure 6) . It can be seen that the DNA bands amplified by the KWT primer and SS primer have a size of 250 bp (Figure 5). Samples I1-6 of genomic DNA in each primer pair did not show any bands. The shape of the band pattern that appears indicates the presence of primary dimers. Primary dimers are potential byproducts in polymerase chain reactions consisting of two primary molecules that stick together due to complementary base sequences in the primer. The reason the 6 seawater microalgae samples did not contain bands was because the DNA concentration in the isolated samples was very low and the DNA purity was not good. However, for sample I7 of seawater microalgae (Figure 6) with a low concentration and DNA purity with a ratio of A260/A280 nm, namely 1.40-1.75, the PCR process was successful in proving the appearance of a band measuring > 250 bp by amplification using the KWT primer. Whether or not bands appear in DNA PCR electrophoresis can be influenced by differences in the primers used as well as the concentration and purity ratio of the DNA (Dzikrina et al. 2022).

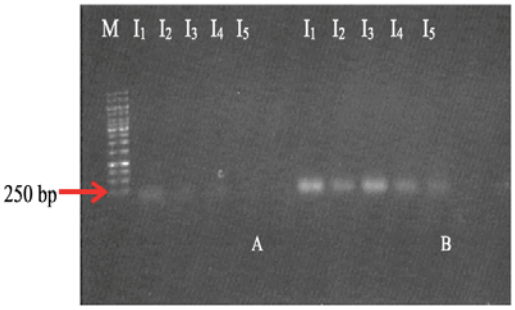

Figure 5. Gel image of Microalgae genomic DNA amplification in Ujung Genteng, M= marker 1 kb; I1-5= Genomic DNA of Ujung Genteng seawater microalgae; A= KWT primer; B= SS primer.

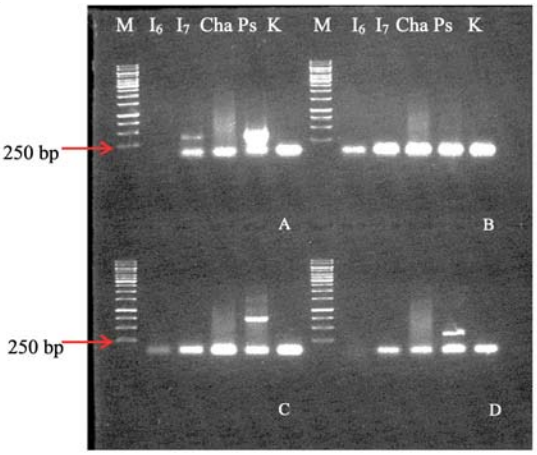

Figure 6. Gel image of amplification of genome DNA electrophoresis of seawater microalgae and microalgae culture, M= marker 1 kb; I6-7= Genomic DNA of Ujung Genteng seawater microalgae; A= KWT primer; B= SS primer, C= PS_F,U_R primer; D= PS_R,U_F primer; Cha, Ps= microalgae culture, and K= positive control.

This research also conducted experiments on microalgae culture, with the results obtained available in Figure 6. Culture of the microalgae Cha (*Chaetosceros* sp.) and Ps (*Porphyridium* sp.) on primer KWT, primer PS_F,U_R, and primer PS_R,U_F showed quite good results because thick DNA bands appeared with band sizes ranging from 250-1000 bp. Seawater microalgae samples showed that many genomic DNA samples could not be amplified properly (Figure 5, Figure 6). Poor amplification results can be caused by mismatched primers, efficiency and optimization of the PCR process. Primers that are not specific or appropriate can cause amplification of other

regions in the genome that are not targeted or, conversely, no regions of the genome are amplified (Azizah 2009).

Primers PS_F,U_R and PS_R,U_F are primers specifically designed for phytoplankton. 18S rDNA primers can target all phototrophic eukaryotes with coverage that can span the entire diversity (Stiller 2005). The KWT_R primer and KWT_F primer were used in several studies to see the abundance of Chlorophyta and other types of algae (Kumar et al. 2021). Likewise, the primer pair ss5 and ss3, these primers showed the highest universality among 18S rDNA primer sets in sequencing microalgae. However, these primers are group specific and are not suitable for determining precise identity and phylogenetic alimony because the resulting DNA sequences are short (Moro et al. 2009).

PCR optimization needs to be carried out to produce the desired characteristics which are related to the DNA denaturation and annealing temperature in the PCR machine. A low denaturation temperature can cause the double-stranded DNA to not open so that primer attachment does not occur. The process of attaching a primer to an open DNA strand requires an optimum temperature because a temperature that is too high can cause amplification to not occur because the primer does not stick or, conversely, a temperature that is too low causes the primer to stick to the other side of the genome which is not the homologous side. This causes the amplification of many non-specific regions in the genome. The annealing temperature is determined based on the primer. There are several factors that greatly influence the success of the electrophoresis process. These factors include the size of the DNA molecule, agarose gel concentration, voltage, the presence of DNA dye, and the composition of the electrophoresis buffer (Sinaga et al. 2017).

4. Discussion

Several types of microalgae found at Ujung Genteng Beach such as *Navicula* sp. and *Oscillatoria* sp. can be an alternative source of lipid production as renewable biodiesel raw material. The average lipid content of microalgae cells varies between 1-70% but can reach 90% dry weight under certain conditions (Spolaore et al. 2006). Microalgae have the potential to be used as biodiesel as an alternative renewable energy because they contain lipids that are suitable for esterification or transesterification (Umdu et al. 2009). *Oscillatoria* sp. can produce lipid extraction, namely 18.16%, while *Navicula* sp. (a type of diatom) can produce up to 60% of the cellular mass as triglycerides (TAG), which will be converted easily into biodiesel through a transesterification reaction (Lebeau and Robert 2003).

At all sampling locations, Bacillariophyceae class microalgae were found. This is in accordance with the statement that the phytoplankton found in marine waters are generally of the diatom type (Bacillariophyceae) followed by Dinophyceae and blue algae (Cyanophyceae) (Nontji 1984). The Bacillariophyceae class of microalgae is a type of microalgae that is widely distributed and is able to adapt to environmental conditions and utilize nutrients optimally for its growth. The form of adaptation of the Bacillariohyceae class is by utilizing the frustule structure on its body to float on the surface of the water. This aims to get sunlight to carry out photosynthesis (Sachlan 1982; Duxbury et al. 2002). The Chlorophyceae class was also found in all three research locations. The Chlorophyceae class is microalgae that has a high abundance in both marine and fresh waters and lives in solitary or colony form.

Types of microalgae from the Dinophyceae class were only found in location I and location III. The Dinophyceae class has chlorophyll a and chlorophyll c in their bodies (Bold and Wyne 1985), and reproduces by self-division. The difference between this type of microalgae and diatoms is that their bodies have flagella. The Cyanophyceae class is a type of microalgae that is prokaryotic and has the form of single cells, colonies or filaments. The process of nitrogen fixation can occur in microalgae in the form of colonies or filaments, so it can cause an explosion in microalgae populations in both marine and fresh water (Sachlan 1982). According to sampling results at location II, very few types of this class were found, namely only 1 type (*Oscillatoria teneuis*). The Chrysophyceae class is often known as golden algae because the carotene and xanthophyll pigments contained in their chloroplasts are greater in quantity than the chlorophyll pigments. Based on sampling results at Ujung Genteng Beach, Sukabumi, microalgae of the Chrysophyceae class were found in locations I and III. This shows that microalgae belonging to the Cyanophyceae and Chrysophyceae classes can be found in all waters including the sea (Luttge 1976).

Another type of microalgae found at Ujung Genteng Beach is the Euglenida class. This class is a type of microalgae that is very rarely found in the three research locations. The number of microalgae species on Ujung Genteng Beach during the day is greater than in the morning. According to the sampling results of the three research locations, the microalgae species found during the day are location I (23 species), location II (14 species), and location III (24 species); the microalgae species found in the morning are location I (17 species), location II (12 species), and location III (22 species).

5. Conclusion

The number of species found at Ujung Genteng Beach, Sukabumi is 47 species, 12 of which are often found at the three research locations both morning and afternoon, namely *Navicula* sp., *Frustulia* sp., *Diploneis parma*, *Nitzschia* sp., *Achnanthidium* sp., *Amphora* sp., *Oscillatoria teneuis*, *Achanthes* sp., *Planothidium* sp., *Uronema* sp., *Zygnema* sp., and *Flagillaria* sp. The diversity of microalgae at all sampling locations was in the medium category and the uniformity index between species was low. Examination of diversity indices and species diversity indicated differences in species abundance and diversity across various sites, specifically in seawater microalgae inhabiting distinct zones: within the ranges of 0-2 meters from the low tide line (location I), in seawater within seagrass areas situated 2-5 meters away (location II), and in seawater close to the open sea, approximately 5-10 meters away (location III). All DNA sample of the Ujung Genteng seawater microalgae genome that was successfully well amplified as evidenced by the appearance of a thick band pattern measuring 250 bp using KWT and SS specific primers.

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