

Identification of Navicula Genus Isolated from Pari Island, Seribu Island Regency, Indonesia

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Abstract: Indonesia is an archipelagic country with a water area of up to 5.9 million km², where this water area is a habitat for marine diversity. One such diversity consists of macroalgae and microalgae. Diatoms are a group of microalgae with high diversity. Diatoms are one of a group of microscopic algae that can live in various habitats and are abundant in various ecosystems. Diatoms can be used as bioindicators of aquatic environmental quality. Navicula genus is an isolate that was successfully isolated from the waters of Pari Island, Kepulauan Seribu Regency. The total protein was analyzed by the Lowry-Follin method. The total fat content was determined by the soxhletation method. This genus Navicula contains a total protein content of 30,944 mg/L and a lipid content of 3.17%.

Keywords: Navicula; Pari Island; Protein; SEM; Total Fat

Introduction

Indonesia is one of the archipelagic countries that has been recognized internationally and is contained in Law No. 17 of 1985. Indonesia's total sea area is 5.9 million km² consisting of 3.2 million km² of territorial waters and 2.7 km² of exclusive economic zone waters; the area does not include other subdivisions (Lasabuda, 2013). In addition, Indonesia has a total of 17,504 islands spread from Sabang to Merauke. Therefore, the picture of abundant diversity in the seas and coasts of Indonesia can be further explored (Pursetyo et al., 2015).

As is well known, Indonesia is one of the archipelagic countries with a water area of up to 2/3 of the land, where this water area is the habitat of marine diversity (Zakiyah et al., 2020), one of which consists of macroalgae (Risjani et al., 2020) and microalgae, including diatoms with high diversity (Risjani et al., 2021). Diatoms are a group of microscopic algae that can live in various habitats and abound in various ecosystems. Diatoms can be used as bioindicators of the quality of the aquatic environment (Koc et al., 2016).

Diatoms in the Bacillariophyceae class can respond to water quality conditions by changing their composition in quantity, abundance, and other taxa (Pouladi et al., 2017; Prasertsin et al., 2021).

The genus Navicula was described in 1822 by Bory de St. Vincent to accommodate isopolar biraphid species with spindle-shaped, uniseriate striae composed of slit-like areoles, a valve outline, two plate-like segments, and a rather simple raphe system with internal accessory ribs and plastids (Vidakovic et al., 2014). The order Naviculales has a brownish color, is oblong in shape, elongated like a boat, and has a cell wall consisting of silica (Chudaev, 2019). Diatoms are one of the most widely untapped aquatic microorganisms, and over the decades, they have attracted the attention of researchers to make further use of them. This study aims to explore diatoms of the genus Navicula by isolating them from the waters of Pari Island and analyzing their protein content and total fat content.

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Method

Location and Time of Research

This research was conducted in July – October 2022. The sampling location was on Pari Island, Seribu Islands Regency, Indonesia, with coordinates 5°51'37.67"S and 106°37'4.91"E. Sample isolation was carried out in the hydrobiology laboratory of the fish resources division of Universitas Brawijaya, protein content testing was carried out at the Chemistry Laboratory of the State University of Malang, and testing of total fat content was carried out at Airlangga University.



Figure 1. Sampling sites of this research in Pari Island, Indonesia

Sampling

Microalgae sampling can be conducted in various ways: substrate/sediment, rattling on rocks, and water columns. Sampling was carried out in this study by modification according to Nasution et al. (2019) vertically and horizontally in the water column of Rahmah et al. (2022), which can be done using a plankton net mesh size 20 μm on the water column.

Isolation

Isolation was carried out in this study using the pipetting method (Alalayah et al., 2014). The prepared sample is then poured ± 5 ml into a petri dish. Prepared equipment such as an Olympus IX 53 inverted microscope with a minimum magnification of 40x and used a glass pipette to carry out insulation. The capillary pipette is heated with Bunsen and then pulled with tweezers to form a small diameter.

Culture

A pure culture is carried out after success at the isolation stage. Sterilization is an initial activity that must be carried out to sterilize tools and containers from

unwanted microorganisms (contaminants) used for microalgae cultivation. The equipment used, such as test tubes, Erlenmeyer, and aeration hoses, are immersed in a chlorine solution at a dosage of 1 mL per 1L of water. Then, it waited for 24 hours. An aeration hose is inserted in a carboy containing a chlorine solution for sterilization. Then, it is rinsed with clean water and dried (Buwono et al., 2018). In addition, the prepared seawater must also be sterilized with chlorine and neutralized with a solution of sodium thiosulfate. The medium used in this study was seawater. This medium is used starting from the isolation stage to maintain the condition of the culture environment so that it remains stable. Treatment by the normal method or pure breeding using F/2 Walne (Tan et al., 2021), fertilizer, silica, and vitamin B12.

Total Protein Content Test

According to Slocombe et al. (2013), the protein was tested using the Lowry-Follin method with several reagents. Determination of protein on the sample dissolved 0.25 g of material in aquades to a volume of 100 ml, then filtered. Next, I put a 1 ml snippet into a test tube, added 1 ml of the reagent that had been made, immediately shook it until it was homogeneous, and incubated it at room temperature for 15 minutes.

Added another 3 ml of reagent to the trailer tube, and it should be shaken as soon as possible, then incubated at room temperature for 45 minutes. Immediately measure the absorbance at 550 nm. The blue color formed remains stable after 45–80 minutes of incubation. The application results are plotted against the regression equation of the standard solution that has been made so that the protein concentration is known.

Total Fat Test

The analysis of lipid content used the Soxhletation method, according to Kavadikeri et al. (2020) and Sanjay et al. (2013) with a procedure in which the sample is weighed at up to 2 g and then put into cotton-treated filter paper. The filter paper containing the sample was corked with cotton and dried in the oven at a temperature of no more than 80 °C for 1 hour. Next, the filter paper containing the dried sample was fed to the soxhlet device. The Soxhlet tool was connected to a fat gourd containing boiling stones that had been dried, and the sample was extracted with petroleum ether solvent for six hours 23. Petroleum ether was oven-dried at 105°C, cooled, and weighed to a fixed weight. The result obtained is calculated using the following formula (1):

$$\% \text{ Fat} = \frac{(C - A)}{B} \times 100\% \quad (1)$$

Description:

A = Fat pumpkin weight blanks (gr)

B = Sample weight (gr)

C = Fat pumpkin weight and excretory fat yield (gr)

Scanning Electron Microscope (SEM)

The sample to be analyzed was placed on a holder measuring ± 10 mm. samples were used for testing using Au-Pd coating to make the sample more conductive. The sample was inserted into the SEM Chamber, then pumped, and after it was completely vacuumed, the SEM machine was ready to use (Beam On).

Result and Discussion

Scanning Electron Microscope (SEM)

The results of the SEM test show that the successfully isolated isolates can be seen morphologically, indicating the genus *Navicula* can be seen in Figure 2.

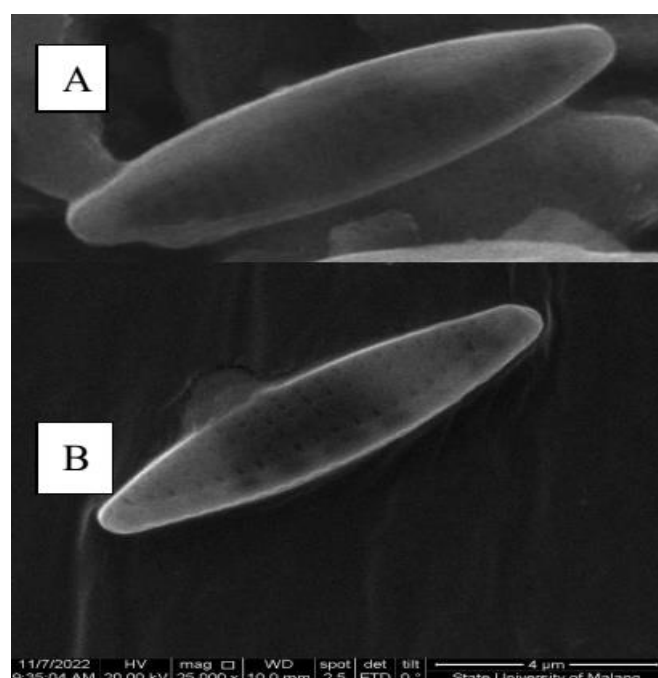


Figure 2. *Navicula* genus was isolated from Pari Island

Morphologically the genus *Navicula* belongs to the symmetric category where this diatom is present. A raphe on each valve is termed biraphid. Genus *Navicula* has an areola, one of the pores in a row that forms a stria on a valve. The plural is areolae. The type of the areola can be important in diatom taxonomy. Some examples of different types of areolae include lineolate (*Navicula*), loculate (*Diploneis*), and punctate (*Gomphoneis*). An areola may be covered partially or completely by one or two thin, porous layers of silica, known as an occlusion. Electron microscopy is usually needed to resolve occlusions. According to Fimbres-Olivarría et al. (2016), *Navicula* has an oblong shape, brownish in colour. In

some species, the navicula has central nodules, polar nodules, and stalking at the ends. Further, the longitudinal elements formed by the aligned genus *Navicula* are often thickened, so externally, the valve appears covered by longitudinal silica ribs. Klein et al. (2014) stated that diatom cells were grown on an orbital shaker and then freeze-dried to have diatoms with their initial mucilage only. Eandar et al. (2012), the formation of the diatom cell wall serves as resistance to the environment.

The research location was Pari Island, administratively in the Seribu Islands Regency, DKI Jakarta Province. According to the Ministry of Marine Affairs and Fisheries, the status of Pari Island is a "tourist area, research area, and mangrove habitat with a white sandy beach type." Pari Island is directly adjacent to the high seas, so water conditions can be affected by currents and waves that occur in the region. The existence of diatoms related to habitat or nature with limiting factors such as currents can affect the distribution of nutrients and the spread of diatomaceous microalgae (Sidabutar et al., 2016). The study's results by Rizqina et al. (2018) confirmed 14 genera from the Bacillariophyceae class, one genus from the Chlorophyceae class, and one genus each from the Cyanophyceae class and the Dinophyceae class. The abundance of microalgae obtained from the Bacillariophyceae class is influenced by the ability of these microorganisms to adapt to the surrounding environment and can multiply quickly compared to other classes.

Total Protein Content

Based on the results of the study, the protein content of the *Navicula* genus isolate was obtained at 30,944 mg/L. Total protein testing in this study was carried out on pure isolate cultures without treatment. The nitrogen content influenced protein levels in diatom culture media. Nitrogen is needed as a macronutrient to support the growth and density of microalgae (Fakhri et al., 2020; Kafouris et al., 2019). Research has shown that differences in nutrient composition can affect the composition of fats, proteins, and carbohydrates. Water microorganisms will form proteins when the nutrients in the cultivation medium are sufficient, and measurements are usually taken when entering the exponential phase. Srivastava et al. (2022), if there is a lack of macronutrients, microalgae cells will experience a decrease in protein content followed by degradation of other cell components related to protein synthesis. Pinandoyo et al. (2021) also stated that the nutritional content that can be utilized optimally would increase growth.

The results of the research of Kurnia et al. (2020) stated that the protein content of *Navicula* was 16.9%.

Navicula yang diisolasi dari perairan Korea Marine Microalgae Culture Center dengan medium F/2 Walne diperoleh kandungan protein sebanyak 6-52% (Kang et al., 2011). Navicula isolates in the study were obtained from the National Fisheries Research and Development Institute (NFRDI), Jeju Island, South Korea. The results of the research of Khatoon et al. (2010) also obtained protein content in *Amphora sp.*, *Navicula sp.*, and *Cyambella* by 16.9%-36.3%.

Total Fat Content

Based on the study's results, the results of isolates of the genus Navicula contained a total fat of 3.17%. Total fat testing in this study was carried out on pure isolate cultures without treatment. The administration of nutrients F/2 Walne, silica, and vitamin B12 was given at a dose of 1 ml/L. Fat content in microalgae is influenced by culture conditions such as engineering on the light intensity, carbon dioxide, temperature, pH, and nutrients (Zullaikah et al., 2019). Extraction methods used for application involve the separation of composition active portions by using selective solvents (Tiwari et al., 2011).

Nitrogen is an important macronutrient for fat growth and metabolism (Fakhry et al., 2015). Furthermore, the total lipid content in Navicula obtained by Kurnia et al. (2020) is 27.2% in pure isolate culture. In general, the lipid content in microalgae is usually in the form of glycerol and fatty acids with a chain length of C14 to C22 that can be found in saturated and unsaturated forms. The highest fatty acid obtained during the cultivation of the microalga *Navicula salinicola* was palmitic acid (C16:0), with percent fatty acids in the medium with a nitrogen concentration of 2.5, 5, and 7.5%, respectively, at 64.04%, 65.03%, and 53.55%.

Conclusion

Based on the results of isolation from sampling that has been carried out, isolates of the genus Navicula were obtained on Pari Island, Seribu Islands Regency. This genus Navicula contains a total protein content of 30,944 mg/L and a lipid content of 3.17%.

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