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Effective DNA extraction method for metagenomic analysis of rhizosphere bacteria from mangrove sediments

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ABSTRACT

Mangrove, *Rhizophora mucronata*, grows in the intertidal area, which contains much organic matter and varying salinity. The organic matter content is influenced by the bacterial community that inhabits the ecosystem, but information regarding the bacterial community, especially in the mangrove root system, is not widely available. There are several challenges in completing this information, one of which is that the method used is still in a conventional form. Developments in environmental DNA analysis can support and complement this information. However, this method must be optimized because the organic matter content and salt variations affect the extraction results. Thus, this study aimed to determine the optimal approach for extracting bacterial DNA from mangrove sediments. The analysis used two methodologies: manual DNA extraction techniques based on buffer modification and DNA extraction kits. There were four different treatments, namely the soil DNA isolation plus kit (M1), the fecal / soil microbial quick-DNA miniprep kit (M2), glass powder with charcoal (M3), and glass powder with skimmed milk (M4). DNA samples were obtained from each method and assessed for concentration and purity using a nanodrop. In addition, the resulting DNA's quality was analyzed using 1.5% agarose. The results obtained were in the M2 treatment, which showed optimal results compared to the others. M2 uses a bead-based beating and spin column method to achieve optimal DNA concentration through high molecular weight. The DNA obtained was also protein-free, and several samples were contaminated with humic acid, namely KL.S1, KL.S4, and T7.S4.

Introduction

Numerous prior researchers have undertaken examinations of bacterial communities in sedimentary environments. Thus far, the data pertaining to bacterial taxa acquired remains exceedingly limited. This is due to the fact that the majority of bacterial identification sequences are still based on culture methods (Sharma and Lal, 2017). The conventional techniques of bacterial cultivation and isolation are limited in their efficacy, as only a small fraction of soil bacteria, ranging from 0.1% to 2%, can be successfully cultured through these techniques. Consequently, the extent of diversity present within soil or sedimentary bacterial communities, particularly those inhabiting marine environments, remains largely unexplored (Daniel, 2005; Sharma and Lal, 2017). Moreover, the utilization of culture techniques leads to a substantial bias rating since numerous bacteria are incapable of being cultured in laboratory conditions, and the media used is particular, allowing only a small percentage of the bacterial inhabitants from the specimen to proliferate (Wagner *et al.*, 1993)

Recombinant engineering was first introduced in the 1970s, and since then, the utilization of DNA for the examination of bacteria has experienced a significant surge. This facilitates the investigation of particular components within bacterial populations

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by scientists (Voordouw *et al.*, 1993), where process of bacterial DNA isolation is conducted in situ from the surrounding environment (Daniel, 2005; Handelsman, 2004). The obstacles are encountered during the process of bacterial DNA extraction from sedimentary or soil ecosystems. Daniel (2005) stated that soil or detritus has very complex characteristics and contains numerous compounds, such as humic acids, which can bind to DNA and inhibit enzymatic DNA modification. Fatima *et al.* (2014) added that the presence of organic compounds, specifically humic and fulvic acids, within the soil matrix can impede the activity of DNA polymerase and disrupt hybridization protocols. Humic acids have been determined to be a factor that inhibits PCR and leads to DNA contamination in soil and sediment samples (Alaeddini, 2012)

A number of methods for the isolation and purification of DNA from soil organic matter have been published or commercialized in over a decade, exhibiting varying levels of efficacy. Notwithstanding this fact, it has been reported that the utilization of chemical-based approaches, such as Sodium Dodecyl Sulfate (SDS) (Gray and Herwig, 1996) Chelex 100 (Jacobsen and Rasmussent, 1992), Mannitol (Fatima *et al.*, 2014), and physical methods including bead beating (Yeates *et al.*, 1998), sonication (Purohit and Singh, 2009), as well as the use of DNA extraction kit with diverse principles have not been universally applicable (Técher *et al.*, 2010). Alaeddini (2012) supports the notion that the selection of an appropriate method and technique for bacterial DNA isolation from sedimentary environments is contingent upon the characteristics of the substrate texture.

Soil samples from agricultural areas, plantations, lakes, peat, and deep-sea sediments have been used thus far in experiments involving the isolation of bacterial DNA. Devi *et al.* (2015) successfully isolated bacterial DNA from a soil environment abundant in organic matter, devoid of humic acid contamination, through the utilization of activated charcoal. Similarly, Kashi (2016) was able to isolate bacterial DNA from saline sediments by incorporating skim milk into the extraction buffer. The efficacy of both techniques has been experimentally verified and established for the purpose of extracting bacterial DNA from saline sediments and nutrient-dense organic matter. However, neither approach has been tested for its potential to isolate bacterial DNA from mangrove sediments with distinct features.

The sedimentary features of mangrove ecosystems exhibit marked dissimilarity from those belong to other terrestrial ecosystems. The sedimentary features of mangroves exhibit a unique combination of characteristics that are not present concurrently in sediments from terrestrial forests and deep-sea environments where these traits refer to high levels of organic matter and salinity variability. The high quantity of mangrove detritus that precipitates into sediment triggers heightened bacterial decomposition activity, leading to a surge in the production of organic acids, specifically humic acids (Madyowati and Kusyairi, 2020; Rusianti *et al.*, 2022). In addition, due to saline intrusion, mangrove habitats along the coast produce a substantial amount of salt in sediments (Kusuma, 2023). The presence of elevated levels of salt has been observed to impede the retrieval of DNA during the extraction process. Hence, there is a need for improved techniques to extract bacterial DNA from mangrove sediments that enable effective and concurrent lysis of microbial cells while minimizing interference from organic matter. The objective of this study is to ascertain the precise technique for bacterial DNA extraction from mangrove sediments through a comparative analysis of manual DNA extraction methods and DNA extraction kits.

Materials and Methods

Location and time of research

Sediment samples were obtained in August 2022 from two separate mangrove ecosystems, Kuala Langsa and Telaga Tujuh. The mangrove ecosystem in Kuala Langsa has undergone rehabilitation efforts since 2006, while Telaga Tujuh represents a pure, pristine and intact mangrove ecosystem that is thought to have lasted for more than at least two centuries ago. Hanafi *et al.* (2021) said that mangrove areas are protected forests classified as climax forests. This is evidenced by the abundance of vegetation at the tree level compared to saplings and seedlings. In terms of administrative, the study sites of Kuala Langsa and Telaga Tujuh are situated within the Langsa Barat District of Langsa City, located in the East Aceh Province of Indonesia (Table 1, Figure 1).

The laboratory analysis was conducted in November 2022 to April 2023. The sediment parameters were analyzed at the Soil Science Laboratory located at IPB University, Indonesia. The isolation of bacterial DNA was conducted at the Genomics Building of the National Research and Innovation Agency (BRIN), followed by subsequent molecular processes such as *Polymerase Chain Reaction* (PCR) and DNA electrophoresis, which were performed at PT. Ocenogen Baruga, Indonesia.

Table 1. Position of sediment sampling from mangrove ecosystem in Kuala Langsa and Telaga Tujuh, East Aceh Province

Stations	Positions
KL.S1	N 4°31'14.40 " and E 98°0'51.82 "
KL.S4	N 4°31'07.63 " and E 98°0'47.46 "
T7.S1	N 4°33'26.68 " and E 98°3'32.35 "
T7.S4	N 4°33'25.81 " and E 98°3'33.76 "

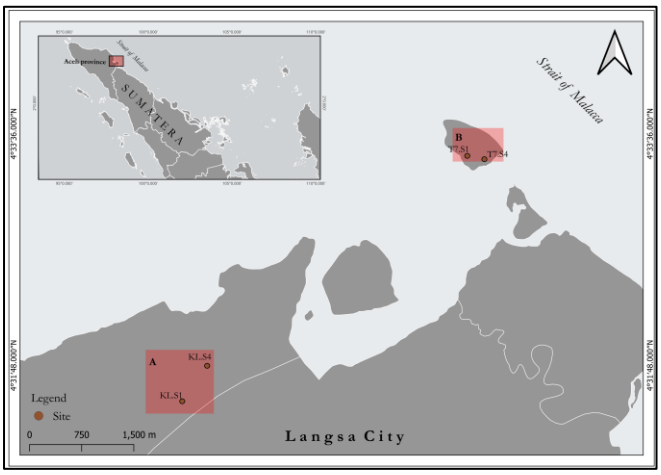

Figure 1. Maps indicating the locations (red square) of the mangrove ecosystem areas in the East Aceh Province (A) Kuala Langsa, and (B) Telaga Tujuh

The acquisition of sediment samples was conducted through purposive sampling methodology, wherein sedimentary specimens were collected from nearby areas of the *Rhizophora mucronata* mangrove's root system, specifically at a depth ranging from 1 to 10 centimeters. The collection of specimens was conducted using polyvinyl chloride (PVC) pipes in accordance with the procedures outlined by Giannopoulos *et al.* (2019). The specimens were aseptically packaged and preserved in a dry ice container prior to conveyance and stored at -20℃ until subsequent laboratory investigation.

Environmental Parameters

This study involved the analysis of various environmental parameters, including acidity (pH), reduction potential (Eh), conductivity (EC), organic matter, and substrate texture, obtained from four stations that presented in Table 1 and Figure 1. In situ pH measurements were conducted using a refractometer, whereas Eh was assessed through the use of Oxidation Reduction Potential (ORP). The measurement of sediment conductivity was taken using a specialized tool known as a conductivity meter. This particular device enables the determination of the salinity of the sediment based on the obtained value (Astuti, 2014; Fahimah *et al.*, 2021; Riyandi *et al.*, 2016). Conductivity measurements are made based on the ability of ions, such as salts and minerals, to conduct electricity. (Astuti, 2014; Fahimah *et al.*, 2021; Riyandi *et al.*, 2016). Analysis of sediment organic matter content refers to the Loss on Ignition (LOI) method by weighing the weight of the sample lost after combustion (Suryono *et al.*, 2018). Substrate texture was determined using the pipette-fraction method (Ramadhani and Muhtadi, 2016). Each fraction obtained, including dust, clay, and sand are analyzed using Millar's Triangle in Figure 2, where the substrate texture is determined based on the point of intersection among the three fractions.

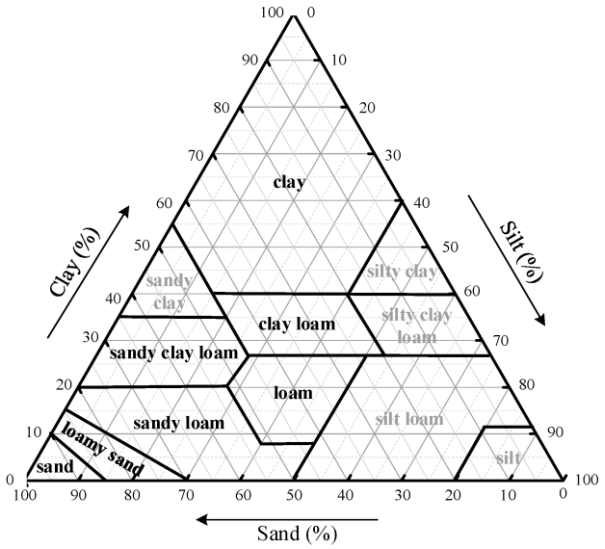

Figure 2. Millar's Triangle (USDA 2012)

DNA extraction methods testing

The process of extracting bacterial DNA from mangrove sediments was conducted through the use of a DNA extraction kit, as well as manual techniques. In this study, two types of kits were utilized, specifically the Soil DNA Isolation Plus and the Quick-DNA Fecal/Soil Microbe Miniprep. The selection of these two kits was based on their ready accessibility within the laboratory. The extraction kit utilized is a specialized kit for extracting bacterial DNA from soil or sediment environments, whereas the manual method selected alludes to the method published by Devi *et al.* (2015) and Kashi (2016). The selection of both manual methods was based on their compatibility with the properties of mangrove sediments, which are characterized by high levels of organic matter and varying salinity levels (Andriyani *et al.*, 2020; Imamsyah *et al.*, 2020). The categorization of the four extraction methods is based on their respective working principles, which can be classified

into two distinct categories: bead beating and physical grinding.

Method 1 (M1): DNA extraction using the Soil DNA Isolation Plus (Norgen Biotek, https://norgenbiotek.com/product/soil-dna-isolation-plus-kit, catalog number: 64000), 250 mg of sediment was placed into a Bead Tube and suspended with 750 µL Lysis Buffer G and 200 µL Lysis Additive A. For ten minutes, the sample was agitated. The separation of the supernatant from the sediment particle precipitate was carried out through centrifugation at a speed of approximately ~14,000 RPM for a minute The supernatant was transferred into a new tube of 1.5 mL followed by adding Binding Buffer I to the tube in a volume of 100 µL. The sample was incubated for 5 min at 4℃ then centrifuged. A total of 50 µL OSR Solution was added into the supernatant and incubated for 5 minutes at 4℃. After centrifuging the sample and transferring the supernatant to a new 1.5 mL tube, add 400 L of Lysis Buffer QP and 550 L of Ethanol, and then perform another centrifugation. The supernatant was carefully decanted into the designated spin column, followed by removing the centrifugation and liquid. The process of DNA washing entails using of Wash with Binding Buffer B and Wash with Wash Solution A, which are incorporated into the spin column. The sample was centrifuged at high speed. The binding column was transferred to a new 1.5 mL tube followed by adding 100 µL Elusion Buffer D to elute the DNA trapped on the column membrane.

Method 2 (M2): DNA extraction using the Quick-DNA Fecal/Soil Microbe Miniprep (Zymo Research, https://zymoresearch.eu/products/quick-dna-fecal-soil-microbe-dna-miniprep-kit, catalog number: D6010), 250 mg of sediment were placed in a 2 mL ZR BashingBead Lysis Tube and suspended with 750 µL BashingBead Buffer. The sample was agitated for 1 hour at ~8,000 RPM. The supernatant was separated from the sediment particle precipitate through the process of centrifugation, which was carried out at ~10,000 RCF for a minute. The supernatant was transferred to a Zymo-Spin III-F Filter and then centrifuged again for 1 minute at a speed of ~10,000 RCF for 1 minute. The filtrate contained in the collection tube was added with 1,200 µL of Genomic Buffer. The solution was transferred to the Zymo-Spin IICR Column and subjected to centrifugation for 2 minutes, with the process being repeated until the solution was fully depleted. A total of 200 µL DNA Pre-Wash Buffer was added to the Zymo-Spin IICR Column and the sample was centrifuged for 1 minute at ~10,000 RCF. After discarding the liquid in the collecting tube, the Zymo-Spin IICR Column was filled with 200 µL DNA Pre-Wash Buffer, and centrifugation was performed once again. Add 500 µL g-DNA Wash Buffer into the Column followed by centrifugation for 1 minute. Following that, the Zymo-Spin IICR Column was carefully relocated to a new 1.5 mL tube, and then 100 µL Elusion Buffer D was added into the Column to remove the DNA trapped on the membrane. On the other hand, preparation of Zymo-Spin III-HRC Filter was done by adding 600 µL Prep Solution and was centrifuged for 3 minutes. The Zymo-Spin III-HRC Filter was transferred into a new 1.5 mL tube and transferred the eluted DNA into the Zymo-Spin III- HRC Filter. Centrifuge at ~16,000 RCF for 3 minutes.

Method 3 (M3): DNA extraction using glass powder and activated charcoal (Devi *et al.*, 2015), 1 g of sediment sample and 1 g of borosilicate <0.2 cm were pulverized using a pestle and mortar for 5 minutes. The mixture was then put into a 50 mL falcon tube. 1 mL of Extraction Buffer (100 mM Tris, 100 mM EDTA, 1.5 M NaCl, pH 8.0) and 10 mg of activated charcoal were added to the sample and incubated under 65℃ for 10 minutes. The sample was centrifuged at a speed of ~12,000 RCF for 5 minutes. The supernatant was then transferred to a 2 mL tube, and subsequently, 100 µL of 3M Sodium Acetate pH 5.2 and 400 µL of PEG 8000 were added. The sample was incubated at -20℃ for 20 minutes. The sample should be centrifuged for five minutes to separate the pellet from the liquid. The pellet was suspended using 500 µL of TE Buffer (10 mM Tris, 01 mM EDTA, pH 8.0). The next step was added chloroform to the suspension to separate the organic and aqueous phases. DNA extraction was performed by taking the aqueous phase and then precipitated using 500 µL of cold Isopropanol. The sample was centrifuged for 10 minutes then the supernatant liquid was discarded. The pellet went under a triple washing process with 70% alcohol solution, followed by a final elimination step using cold absolute ethanol. Dry out the pellet and dissolved it by adding 100 µL of TE Buffer.

Method 4 (M4): DNA extraction using glass powder and skim milk (Kashi, 2016), A total of 10 g of sediment was washed using phospate buffered saline (PBS) one time. This stage, which is repeated three times, seeks to eliminate the salt content in the sediment as well as other contaminants such tiny roots that may be present in the sediment. Subsequently, 0.4 grams of purified sediment were solubilized in 100 mL of PBS 1x then solution was

then subjected to an overnight incubation at ambient temperature (21℃ to 25℃). The solutions was carefully put into a 50 milliliter falcon tube in a slow, steady process to prevent disturbances of the sediment. The sample was centrifuged for 10 minutes at ~5,000 RPM. The supernatant liquid was discarded and the pellet was washed using 1x PBS. The sample was centrifuged again for 3 minutes. A total of 5 mL of 1% SDS and 1 g of beads were added to the sample and agitated for 5 minutes. A total of 0.5 mL Lysozyme (Buffer TE 10 mg/mL) was added to the sample and incubated at 37℃ for an hour. After that, 10 mL of Lysis Buffer (4% SDS, 50 mM Tris-HCL, 100 mM EDTA, 1% CTAB, pH 8.0) and 10 mg of skim milk were added to the sample and incubated again for 1 hour at 56℃. After breaking down the bacterial cells, the bacterial DNA was purified. The upper water phase was extracted using Phenol which was added to the sample as 1x volume. The sample was centrifuged at ~10,000 RCF for 2 minutes. The supernatant was transferred into a new tube followed by the addition of chloroform by volume and then centrifuged again. Two distinct layers were obtained, the upper layer or aqueous phase containing nucleic acids and the lower organic layer containing pigments and cell debris. DNA extraction is done by taking the aqueous phase. Ethanol as much as 3 liquid volume and 2M Sodium Acetate as much as 0.1x liquid volume were added to the sample and then incubated at -20℃ overnight. The sample was centrifuged for 10 minutes and the supernatant liquid was discarded. The pellets underwent a triple washing process with 70% alcohol and were at last purified with cold absolute ethanol. Dry out the pellet and dissolved it by adding 50 µL of TE Buffer.

Concentration, Purity and Quality of DNA

The final product of the extraction process will produce genome or commonly called genomic DNA (gDNA). DNA concentration and purity were determined through nanodrop spectrophotometry method (Wilmington, USA) at wavelengths of 230, 260, and 280 nm. 260 nm is used to determine the concentration of DNA while the ratio of A260/A280 and A260/A230 is used to determine the purity of DNA. DNA requirements are said to be pure if has a ratio of A260/A280 of more than 1.7 and A260/A230 of more than 2.0. DNA purity with ratios less than 1.7 and 2.0 indicates protein and humic acid contamination (Mahmoudi *et al.*, 2011).

The quality of DNA was analyzed using a 1.5% agarose gel containing red dye (GelRed Biotium) as a substitute for EB (Ethidium Bromide) which to impart pigmentation, thereby enabling the visualization of DNA. A total of 4 µL of DNA extract was mixed with 1 µL Loading dye and subsequently loaded into agarose wells in 1x TAE buffer. Gel electrophoresis was run at 100 V voltage, 100 mA current for 30 minutes. The results of this electrophoresis were then visualized using Ultraviolet transilluminator light through the Gel Documentation machine.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) aims to multiply copies of DNA in the target region (Madduppa, 2013). This study used primer pairs, specifically bactery 16S, namely 341F (5' CCTAYGGGRBGCASCAG 3') and 806R (5' GGACTACNNGGGTATCTAAT 3') in order to selectively amplify the V3 and V4 regions, aiming to target a specific segment of DNA with a length of 478 basepairs (Lund *et al.*, 2022; Muwawa *et al.*, 2021).

Bacterial DNA amplification used a total volume of 25 µL, which consists of 12.5 µL Mytaq Hs Red Mix by Bioline Meridian Bioscience, 1.25 µL Forward and Reverse Primer, 9 µL ddH20, 2 µl DNA template. The DNA amplification process is divided into several stages, including: (1) pre- denaturation of template DNA at 95°C for 7 minutes; (2) denaturation of template DNA at 94°C for 45 seconds; (3) annealing or primer attachment to the template at 53 °C for 30 seconds; (4) extension or primer elongation at 72°C for 1 minute and (5) final extension at 72 °C for 7 minutes (Lund *et al.*, 2022; Muwawa *et al.*, 2021).

Results

Environmental Parameters

This study revealed the presentation of the physical and chemical parameters of mangrove sediments from two observation sites, Kuala Langsa and Telaga Tujuh. The parameters include pH, conductivity (ms/cm), reduction potential (mV), organic matter (%), and substrate texture, and are listed in Table 2.

Table 2. Analysis of sediment from mangrove ecosystem in Kuala Langsa and Telaga Tujuh, East Aceh Province

Characteristics	Kuala Langsa	Telaga Tujuh
pH	5.25±0.31	4.96±0.38
EC (mS/cm)	18.58±3.00	17.92±3.08
Eh (mV)	120.55 ±128.62	243.60±35.49
Organic matter (%)	21.53±4.10	25.47±2.37
Clay (%)	22.12±6.05	11.11±3.23
Silt (%)	21.34±8.81	10.65±2.32

Characteristics	Kuala Langsa	Telaga Tujuh
Sand (%)	56.53±14.87	78.23±5.56
Substrate texture	Sandy loam	Loamy sand

The pH of the mangrove sediments located in Kuala Langsa and Telaga Tujuh has been determined to be moderately acidic, with an average value of 5.25 ± 0.31 and 4.96 ± 0.38, respectively. According to the data provided in Table 3, it can be observed that the concentration of organic matter in the sediments of Telaga Tujuh mangrove is higher compared to that of Kuala Langsa. This discrepancy in organic matter concentration is likely to have an impact on the pH value. The average concentration of organic matter derived from sediments in Kuala Langsa was determined to be 21.53 ± 4.10%, while in Telaga Tujuh was found to be 25.47 ± 2.37%. As the organic matter content in the sediment increases, there is a corresponding increase in the accumulation of organic acids. Consequently, the pH of the sediment is lowered.

The pH value of the sediment is subject to alteration by various factors, including the composition of organic matter, the textural characteristics of the substrate, and the concentration of oxygen provide. Based on the substrate fraction analysis using Millar's Triangle, the substrate texture of Kuala Langsa and Telaga Tujuh mangroves has been identified as sandy loam and loamy sand. The sediment's oxygen content can be determined by analyzing the Eh value acquired. The obtained values of Eh measurements from sediments of Kuala Langsa and Telaga Tujuh were 120.55± 128.62 mV and 243.60 ± 35.49 mV, respectively. Both of these results exhibit positive Eh values, indicating their inclusion within the oxidizing state.

Mangrove ecosystems exhibit remarkable adaptability to physicochemical fluctuations, particularly in relation to salinity. In this study, sediment salinity was determined based on conductivity (EC) values. The average results of sediment conductivity measurements in Kuala Langsa and Telaga Tujuh showed values of 18.58 ± 3.00 mS / cm and 17.92 ± 3.08 mS / cm or equivalent to 9.85 ± 1.34 PSU and 9.85 ± 1.76 PSU, thus the salinity of the sediment acquired from the two ecosystems is included in the brackish water classification.

Comparison of DNA Concentration, Purity, and Quality

Table 3 gives the quantification of DNA concentration obtained by the implementation of four distinct extraction methodologies. Meanwhile, Table 4 displays the outcomes regarding DNA purity. The four extraction methods successfully obtained DNA with varying concentration values. M3 and M4, both included in the manual DNA extraction method. The DNA concentrations acquired through the implementation of M4 exhibited elevated values in comparison to the remaining three methodologies, with DNA concentrations exceeding 30 ng/µL for all sediment samples. In contrast, the results obtained through the implementation of Method M3 indicate a DNA concentration that is the lowest among the tested samples, measuring less than 6.0 ng/µL. Extraction methods using kits, namely M1 and M2 obtained DNA concentrations that were not much different ranging from 6.0 - 11.3 ng/µL.

Table 3. Concentration of DNA from Kuala Langsa and Telaga Tujh mangrove sediments using nanodrops (Wilmington, USA)

Stations	A260 (ng/µL)			
	M1	M2	M3	M4
KL.S1	7.8±0.7	6.2±0.5	4.7±0.1	50.6±0.4
KL.S4	6.0±0.4	11.3	5.2±0.6	33.0±0.6
T7.S1	6.8±0.9	5.3	4.7±0.2	66.6±0.8
T7.S4	9.9±0.5	6.6±0.2	5.9±2.1	37.6±2.8

Describe
*A*260 is the concentration of DNA DNA (ng/µL)
M1 is DNA extraction using Soil DNA Isolation Plus Kit
M2 is DNA extraction using Quick-DNA Fecal/Soil Microbe Miniprep Kit
M3 is DNA extraction using glass powder and activated charcoal
M4 is DNA extraction using glass powder and skim milk

Table 4. Purity of DNA from Kuala Langsa and Telaga Tujuh mangrove sediments using nanodrops (Wilmington, USA)

Stations	A260/A280			
	M1	M2	M3	M4
KL.S1	2.1±0.05	1.8±0.02	1.4±0.06	1.3
KL.S4	2.1±0.05	1.8±0.10	1.4±0.10	1.4±0.10
T7.S1	1.8±0.04	1.8±0.07	1.4±0.02	1.3
T7.S4	2.0±0.01	2.1±0.06	1.4±0.12	1.3±0.02
Stations	A260/A230			
	M1	M2	M3	M4
KL.S1	0.24±0.21	1.7±0.75	0.05	1.5±0.01
KL.S4	0.37±0.11	0.33±0.01	0.1±0.02	0.9±0.45
T7.S1	0.35±0.02	2.4±0.33	0.11	1.8±0.05
T7.S4	0.17	0.27±0.007	0.08±0.07	1.3±0.06

Describe
260/280 is the purity ratio of DNA to protein
260/230 is the DNA purity ratio of humic acid

The extraction method using M1 and M2 successfully obtained DNA free from protein contaminants. This was indicated by the A260/A280 ratio of all tested samples were greater than 1.7. The purity of the other two methods, M3 and M4, was below 1.7, indicating the presence of protein contamination in the DNA. Moreover,

DNA purity in the A260/A230 ratio showed values less than 2.0 for each extraction method used. M3 has the lowest ratio with the highest humic acid contamination compared to the three methods others, with a ratio below 0.2 for all sediments. The results of the experiment conducted on mangrove sediment samples, as presented in Table 3, indicate that only the M2 was successful in obtaining A260/A230 values greater than 2.0 for sediment sample T7.S1.

In addition to measuring the concentration and purity of raw DNA, DNA quality analysis was also performed using 1.5% agarose. According to the observed DNA bands presented in Table 3, the M2 had sufficient DNA concentration to be clearly visible on a agarose gel with a molecular weight >20 Kb and there was one sediment sample with a faint visible band (KL.S4). The other methods, namely M1, M3, and M4 did not show any appearance of DNA bands on a agarose gel.

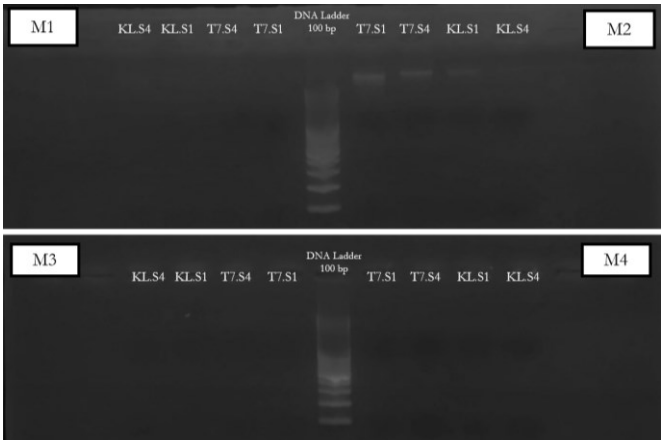

Figure 3. Visualization of DNA from extraction methods using Soil DNA Isolation Plus (M1), Quick-DNA Fecal/Soil Microbe Miniprep (M2), glass powder with charcoal (M3), and glass powder with skim milk (M4)

Polymerase Chain Reaction (PCR)

Figure 4 is the result of visualization of DNA amplicons using 2 µL of DNA template from each extraction method. The M2 was able to provide the highest quality DNA for all sediment samples, while the M1 amplification and DNA reading through a 1.5% agarose gel was only read on sample KL.S4, while the other three did not show any appearance of DNA bands. Similar to the previous condition, DNA bands from the amplicons were not visible on the electrophoresis gel for the use of M3 and M4.

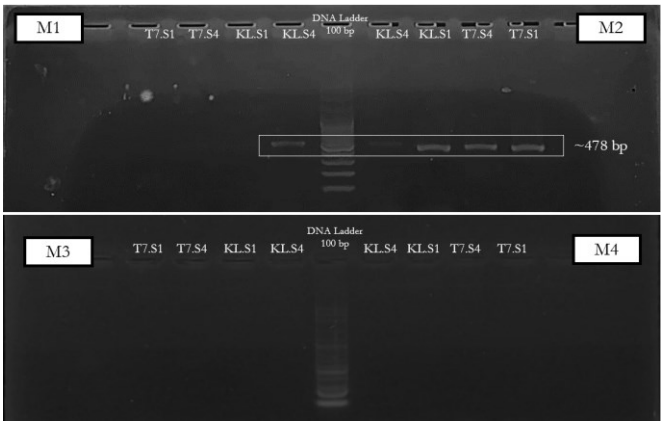

Figure 4. Visualization of DNA amplicons extraction methods using Soil DNA Isolation Plus (M1), Quick-DNA Fecal/Soil Microbe Miniprep (M2), glass powder with charcoal (M3), and glass powder with skim milk (M4)

Discussion

The mangrove areas of Telaga Tujuh exhibit a comparatively lower pH value in comparison to other mangrove ecosystems, within the range of 6 – 7 as reported by English *et al.* (1997) in Agustin *et al.* (2012). The low pH value observed in the present investigation exhibits a correlation with the concentration of organic matter present within the sedimentary, as elucidated by Rusianti *et al.* (2022). Agroklimat (2005) in Barus *et al.* (2019) separated the sediment's organic matter concentration into separate groups based on various criteria. These categories included very low concentrations (<3.5%), low concentrations (3.5-7%), moderate concentrations (7-17%), high concentrations (17-35%), and very high concentrations (>35%). The obtained measurements indicate that the sediments in Kuala Langsa and Telaga Tujuh exhibited a high concentration of organic matter. The concentration of organic matter typically observed in terrestrial species varies within the range of 2-5% (Tangketasik *et al.*, 2012). According to the findings of Rusianti *et al.* (2022), higher concentrations of organic matter have the potential to enhance the accumulation of acidic organic compounds within the sediment, thereby leading sediment to a sour and decrease in pH levels.

According to the findings presented in Table 2, it has been determined that the mangrove sediments found in Kuala Langsa and Telaga Tujuh can be classified as sandy loam and loamy sand. The present observation aligns with the conclusions drawn by Magfirah *et al.* (2014) wherein they stated that regions exhibiting the most notable accumulations of organic matter were predominantly situated in areas

characterized by clay-based substrates. Sediments characterized by diminutive particle sizes exhibit a raised potential for the absorption of organic materials due to the bonding nature interactions strongly with organic matter. Mangrove ecosystems possess the capacity to gather organic matter through the utilization of their root system as a trapping mechanism (Ulumuddin, 2019)

According to Odum (1993), sediment can be classified based on its potential reduction value and reaction chemistry. This classification involves the identification of three distinct zones. The first zone is the reduction zone, characterized by an Eh value below 0 mV. The second zone is the transition zone, which exhibits an Eh value ranging from 0 to +200 mV. Lastly, the third zone is the oxidation zone, where the Eh value exceeds 200 mV. The observed conditions at each location indicate that the sediment from Kuala Langsa occurs within the transition zone, whereas the sediment from Telaga Tujuh is found within the oxidation zone. However, it is noteworthy that both entities exhibit positive Eh values, thereby indicating their inclusion in the oxidation process. The observed phenomenon of oxidation in sediments indicates that the chemical reactions occurring in these sediments are predominantly aerobic, meaning they rely on the presence of oxygen for the decomposition of organic matter (Suwoyo *et al.*, 2015). The Telaga Tujuh mangrove area is directly adjacent to the marine environment, allowing for the acquisition of abundant oxygen through the aeration process encouraged by active seawater movements, such as currents and waves.

Mangrove ecosystems exhibit remarkable adaptability to fluctuating physicochemical conditions, particularly in relation to salinity. The salinity of sediment is assessed through the measurement of conductivity or electrical power transmission (EC). The measurement of conductivity allows for the acquisition of precise data related to the salinity of groundwater or sediment, this is achieved by evaluating the abundance of salts and minerals concentration within the sediment (Darmanto and Cahyadi, 2013; Riyandi *et al.*, 2016). According to the classification by Effendi (2012), sediment can be categorized into three distinct groups based on the concentration of salt present. The first category comprises freshwater sediments, characterized by a salinity level below 0.5 PSU. The second category consists of brackish water sediments, which exhibit salinity levels ranging from 0.5 PSU to 30 PSU. Lastly, the third category refers to seawater sediments, characterized by salinity levels exceeding 30 PSU. Based on the average conductivity measurements of sediments in Kuala Langsa and Telaga Tujuh, it can be concluded that both ecosystems are in the brackish water category.

The two primary prerequisites for extracting DNA from sediment samples are the achieving a high molecular weight and the absence of inhibitors (Yeates *et al.*, 1998). Organic matter is frequently cited by researchers as a hindering factor during the process of DNA isolation from sedimentary environments. In Table 3, it is known that mangrove sediments from Kuala Langsa and Telaga Tujuh have a high content of organic matter, where the levels of organic matter Telaga seven is greater than Kuala Langsa. The presence of organic matter is related to the amount of organic acid produced from the decomposition process of litter by bacteria (Rusianti *et al.*, 2022). As the organic matter content increases, there is a corresponding increase in the accumulation of organic acids, which can lead to a reduction in sediment pH. Organic acids, particularly humic acids, present a concern for numerous researchers engaged in the isolation of bacterial DNA from soil or sediment environments due to their potential interference with hybridization protocols (Fatima *et al.*, 2014). Humic acid has DNA like characteristics and charge, so the purity of DNA from the influence of humic acid can be measured based on the ratio of wavelengths of 260 nm and 230 nm (Devi *et al.*, 2015). Apart from the presence of organic matter, salinity parameters are also known to impact the process of DNA extraction. In particular, the concentration of salts present in the sediment can affect the stability of the extracted sediment. High salt content can reduce DNA stability which causes DNA precipitation so that extraction becomes more difficult (Kashi, 2016).

Table 4 shows that the highest concentration of DNA was achieved with M4, exceeding >30 ng/µL, whereas M3 yielded the lowest concentration of DNA, measuring less than 6.0 ng/µL. The concentration of DNA is reliant upon the extraction methodology employed. Specifically, during the DNA extraction's cell lysis stage where there are two treatment stages, namely using mechanical interference and without interference by relying on enzyme assistance. In this study, all extraction methods used mechanical disruption to lyse bacterial cells. DNA extraction using mechanical disruption can accelerate the destruction of cell walls so that the concentration of DNA obtained increases (Fujimoto *et al.*, 2004). There are two types of mechanical disturbances given to sediment samples, namely bead beating using a bead beater (M1, M2, and M4) and physical grinding using a pestle and mortar (M3).

Based on the DNA measurements obtained from each extraction method employed, it has been determined that M3 exhibits the minimum DNA concentration. The observed condition can be attributed to the manual mechanical treatment applied to the sample via pestle and mortar grinding. The efficacy of cell wall destruction is based upon the user's physical strength during the grinding process.

The working principle of bead beating has been widely applied by previous researchers to isolate DNA from sedimentary environments. This method has the advantages of being easy to apply by all people, has a fast processing time, and the costs incurred tend to be cheaper (Fujimoto *et al.*, 2004). However, the success of the extraction method with the bead beating principle is determined by the size of the beads used and the bead beating time (Devi *et al.*, 2015). In accordance with the standard procedures of the company, M1 and M2 exhibit distinct variations in their respective bead sizes. Specifically, M1 employs a bead size of 1.0 mm, whereas M2 utilizes two distinct bead sizes, which is 1.0 and 0.5 mm. Fujimoto *et al.* (2004) proved that the cell lysis process using beads with a diameter of 0.5 mm produces more DNA than beads with a size of 1.0 mm. Beads with a smaller diameter have a larger surface area per unit mass and thus provide more frequent physical interactions with bacteria in the sediment that have a diameter of about 1 µm. Furthermore, the length of bead beating time given will affect the molecular weight of the resulting DNA. According to De Lipthay *et al.* (2004), as the duration of bead beating increases, there is a reduction in the molecular weight of DNA. Therefore, bead beating for more than 1 minute is not recommended.

The extraction using M1 and M2 successfully obtained DNA free from protein contaminants. This is indicated by the A260/A280 ratio of all tested samples greater than 1.7. In this study, extraction using the spin column-based kit method successfully removed protein impurities present in sediment samples. The function of the silica membrane present in the spin column is to effectuate the isolation of DNA from extraneous particles, such as proteins and other particles based on their size. According to (Tan and Yiap, 2009) the size of DNA is larger than proteins, so the use of spin columns with small pore sizes will hold DNA while proteins and other small particles will pass through the filter membrane. DNA that is retained in the membrane is eluted using a buffer or certain solvents to produce DNA that is free from proteins and humic acids.

Overall, the purity of DNA from humic acid at the A260/A230 ratio was low for all sediment samples. In Table 4, sediments from stations KL.S1 and T7.S1 obtained higher purity values than KL.S4 and T7.S4. The level of DNA purity from humic acid contamination is influenced by the level of organic matter contained in the sediment. Sediment sampling at locations close to the sea will have low organic matter (Hakim *et al.*, 2016) because it has undergone several washing processes by tidal sea water (Siringoringo, 2013). In addition, T7.S1 has more sand substrate fraction than clay and silt. According to (Yuwono, 2009), the sand fraction has large grains with high permeability and is easily leached, making it difficult to store organic matter. Station KL.S1 is primarily characterized by a high proportion of clay particles. However, its proximity to the pond area results in a comparatively lower concentration of organic matter compared to KL.S2. The results obtained by Muwawa *et al.* (2021) support the notion that the proximity of mangroves to human-impacted areas leads to a reduction in the concentration of organic matter. At stations KL.S4 and T7.S4 have different conditions from the two stations described, where the location of these two stations are far from the pond and sea so that the accumulation of organic matter is not disturbed which resulted in increased levels of organic matter in the sediment followed by increased humic acid contamination. Therefore, DNA from KL.S4 and T7.S4 samples must be purified again to meet the predetermined purity requirements.

Voytas (2000) mentioned that the utilization of agarose gel is highly efficacious in the process of segregating, characterizing, and refining DNA fragments with dimensions ranging from 0.5 to 25 kilobases (Kb). Based on the visible DNA bands, the DNA extraction method using M2 has sufficient DNA concentration to be clearly visible on a 1.5% agarose gel with a molecular weight of >20 Kb. This result contradicts what De Lipthay *et al.* (2004) stated that the bead beating time should not exceed 1 minute because it can reduce the molecular weight of DNA. Based on the extraction protocol submitted, the bead beating time given by M2 took duration up to 1 hour. Supposedly, the DNA molecular weight from M2 is smaller than the other two methods, M1 (10 minutes) and M4 (5 minutes), but in the study conducted the results obtained are the opposite. The observed phenomenon may be attributed to the utilization of M2, which incorporates a DNA extraction technique employing a patented device. The procedures executed and buffers employed in this method have been standardized to enable facile

acquisition of high molecular weight DNA without necessitating any alterations to the current protocols. Voytas (2000) mentioned several factors that affect the DNA bands from M1, M3, and M4 are not visible including the selection of inappropriate agarose gel concentration, the voltage applied, and the length of time electrophoresis. The most frequent cause is the selection of inappropriate agarose concentration. Low percentage agarose gel is used to break up DNA fragments with high molecular weight, while high percentage gel is used to break up DNA fragments with low molecular weight. In addition, the application of voltage to facilitate the separation of DNA fragments is directly proportional to the speed at which the fragments traverse the gel matrix. Specifically, an increase in the voltage magnitude results in a corresponding increase in the rate of DNA fragment movement. Too low voltage makes the rate of DNA movement slow and lengthens the time required to separate the DNA fragments. In the context of DNA electrophoresis, short DNA fragments have a lighter molecular weight than longer DNA fragments. So it can be temporarily concluded that the cause of the non-appearance of DNA bands on the agarose gel is because the DNA from the extraction of M1 and M4 is assumed to have a large molecular weight so that a greater voltage and longer time is needed.

Evaluation of DNA extraction results from all methods used based on absorbance at 230 nm, 260 nm, and 280 nm is not sufficient to indicate reliable DNA quality (Kuhn *et al.*, 2017). The difference in DNA extraction results is clearly visible based on electrophoretic analysis on a 1.5% agarose gel between the DNA purity determined by nanodrop spectrophotometry and the original purity possessed by DNA. Gel electrophoresis is a technique that is often used for the identification and purification of DNA fragments separated by size and shape (Hanada, 2020). In this context, DNA is separated from other components, such as proteins, RNA, and humic acids present in the raw DNA based on their electrical charge (Hanada, 2020). According to Kuhn *et al.* (2017), common contaminates such as RNA, proteins, EDTA and/or phenol, as well as humic acids in raw DNA can severely interfere with spectrophotometer quantification by absorbing UV light at the same wavelength, resulting in high bias values. This uncertainty may affect the calculation of the quantity of DNA obtained. Such cases can occur at any time, including in this study. The application of M4 for DNA isolation resulted in a quantity of DNA, as evidenced by the spectrophotometer reading, which exceeded 30 ng/µL. However, the electrophoresis results showed that DNA bands did not appear during gel visualization after DNA amplification (Figure 4). On the other hand, the use of the kit method, M1, also has a similar case with M4, where the reading of the amount of DNA with a spectrophotometer gives results that are not much different between M1 and M2. However, visualization of DNA on 1.5% agarose gel only showed DNA bands from the M2 method. This is due to the contamination of the raw DNA, where the M4 method is in the A260/A280 and A260/A230 ratio and M1 is in the A260/A230 ratio, which is a result of the many contaminating factors read by the spectrophotometer.

In addition to DNA purity, DNA molecular weight is also a key requirement in isolating DNA. The M2 method provided DNA with high molecular weight quality (Figure 3) and was successful in amplifying bacterial 16S region V3-V4 for all sediment samples (Figure 4). DNA molecular weight refers to the size or length of DNA which is generally measured in base pairs (bp) or kilobases (Kb). The DNA isolation method using M2 has a molecular weight of >20 Kb. The high molecular weight of DNA is required to avoid chimera formation during the PCR process. The larger the DNA size, the less likely the formation of chimeras during PCR (Liesack *et al.*, 1991).

Based on the analysis that has been done in this study on the DNA samples obtained, both using nanodrop spectrophotometric measurements, testing the visualization of DNA on a 1.5% agarose gel, and confirming the success of DNA samples after PCR. The M2 is able to isolate bacterial DNA from the sediment environment with the characteristics presented in Table 2. The raw DNA obtained from the M2 is then subjected to the next stage, known as DNA sequencing. The commercial kit issued by the Zymoresearch company is relatively expensive compared to the other three extraction methods. Alternatively, we recommend an appropriate extraction method in isolating bacterial DNA that is based on the bead beating principle, where the recommended glass bead size is less than 0.05 mm in diameter to increase the chance of collision between the cell surface and the bead beating surface. The implementation of membrane filtration during the extraction phase, together with the addition of activated charcoal and skim milk into the extraction buffer used for sediment samples, helps minimize the presence of humic acid and protein contaminants.

Conclusion

Principle beating beads with a bead size below 0.05 mm were assessed capable increase the concentration of DNA while the addition of activated charcoal and skim milk in the extraction buffer can be minimized contamination sour humat. The application of the spin column method to the manual method can be one way to reduce risk contaminants of the protein in DNA.

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References

Agustin, Y.L., M. Muryono, H. Purnobasuki. 2012. Estimasi stok karbon pada Tegakan Pohon rhizophora stylosa di Pantai Talang Iring, Pamekasan Madura. Working paper, Biology Department, Institut Teknologi Sepuluh Nopember, Surabaya, 5 Mei.

Aksornkoae, S. 1993. Ecology and Management of Management of Mangroves. IUCN.

Alaeddini, R. 2012. Forensic implications of PCR inhibition - A review. Forensic Science International Genetic, 6: 297–305.

Andriyani, L.W., D.G. Bengen, M.S. Ismet. 2020. Relationship between gastropods (Cassidula nucleus and Cassidula vespertilionis) and mangroves (Avicennia marina and Sonneratia alba) in rehabilitated mangrove ecosystem in Pantai Indah Kapuk, Jakarta, Indonesia. AACL Bioflux, 13: 2327–2335.

Astuti, A.D. 2014. Kualitas air irigasi ditinjau dari parameter DHL, TDS, pH, pada lahan sawah Desa Bulumanis Kidul, Kecamatan Margoyoso. Jurnal Litbang, 10(1): 35–42.

Barus, B.S., R. Aryawati, W.A.E. Putri, E. Nurjuliasti, G. Diansyah, E. Sitorus. 2019. Hubungan N-total dan C-organik sedimen dengan makrozoobentos di Perairan Pulau Payung, Banyuasin, Sumatera Selatan. Jurnal Kelautan Tropis, 22(2): 147-156.

Daniel, R. 2005. The metagenomics of soil. Nature Review Microbiology, 3(6): 470–478.

Darmanto, D., A. Cahyadi. 2013. Kajian intrusi air laut melalui sungai di Pesisir Kabupaten Semak Jawa Tengah. Majalah Geografi Indonesia, 27(1): 1–10.

De Lipthay, J.R., C. Enzinger, K. Johnsen, J. Aamand, S.J. Sørensen. 2004. Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis. Soil Biology Biochemistry, 36(10): 1607–1614.

Devi, S.G., A.A. Fathima, S. Radha, R. Arunraj, W.R. Curtis, M. Ramya. 2015. A rapid and economical method for efficient DNA extraction from diverse soils suifigure for metagenomic applications. PLoS One, 10(7): 1-16.

Fahimah, N., A.D. Damayanti, V.U. Bunga, H. Mubiarto. 2021. Profil vertikal dan horizontal parameter salinitas, DHL, dan TDS berdasarkan variasi musiman di estuari Sungai Citarum. Oseana, 46(1): 1–12.

Fatima, F., N. Pathak, S.R. Verma. 2014. An improved method for soil DNA extraction to Study the microbial assortment within rhizospheric region. Molecular Biology International, 20(1): 1-6.

Fujimoto, S., Y. Nakagami, F. Kojima. 2004. Optimal bacterial dna isolation method using bead-beating technique. Medical School, 3: 33–38.

Gehlen, M., H.J. Trampisch, W. Dott. 1985. Physiological characterization of heterotrophic bacterial communities from selected aquatic environments. Microbiology Ecology, 11: 205–219.

Giannopoulos, G., D. Lee, S. Neubauer, B. Brown, B. Franklin. 2019. A simple and effective sampler to collect undisturbed cores from tidal marshes. BioRvix, 9: 1-11.

Gray, J.P., R.P. Herwig. 1996. Phylogenetic analysis of the bacterial communities in marine sediments. Applied and Enviromental Microbiology, 62(11): 4049–4059.

Hakim, M., A., N.K.T. Martuti, A. Irsadi. 2016. Estimasi stok karbon mangrove di Dukuh Tapak Keluarahan Tugurejo Kota Semarang. Life Science, 5: 88-94.

Hanada, K. 2020. Introduction and perspectives of DNA electrophoresis. Springer, Germany.

Hanafi, I., H. Basri. 2021. Analisis vegetasi mangrove (studi kasus di hutan mangrove Pulau Telaga Tujuh Kecamatan Langsa Barat). Jurnal Ilmiah Mahasiswa Pertanian, 6(4): 1-11.

Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. Applied and Enviromental Microbiologi, 68(4): 669–685.

Imamsyah, A., D.G. Bengen, M.S. Ismet. 2020. Struktur vegetasi mangrove berdasarkan kualitas lingkungan biofisik di Taman Hutan Raya Ngurah Rai Bali. Ecothrophic, 14(1): 88–99.

Jacobsen, C.S., O.F. Rasmussent. 1992. Development and application of a new method to extract bacterial DNA from soil based on separation of bacteria from soil with cation-exchange resin. Applied and Enviromental Microbiology, 58(8): 2458–2462.

Kashi, F.J. 2016. An improved procedure of the metagenomic DNA extraction from saline soil, sediment, and salt. International Letters of Natural Sciences, 60: 38–45.

Kuhn, R., J. Böllmann, K. Krahl, I.M. Bryant, M. Martienssen. 2017. Comparison of ten different DNA extraction procedures with respect to their suitability for environmental samples. Journal Microbiology Methods, 143: 78–86.

Kusuma, A.H. 2023. Produksi serasah mangrove Avicennia alba di Desa Sungai Nibung, Kecamatan Dente Teladas, Kabupaten Tulang Bawang, Provinsi Lampung. Jurnal Akuatik Lestari, 6(2): 179–186.

Liesack, W., H. Weyland, E. Stackebrandt. 1991. Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic bacteria. Microbiology Ecology, 21(3): 191–198.

Lund, M., A.J. Rasmussen, R.J. Madrigal, R. Sawers, M.T.P Gilbert, C.J. Barnes. 2022. Rhizosphere bacterial communities differ among traditional maize landraces. Environmental DNA, 4(6): 1241–1249.

Madduppa, H. 2013. Bioekologi dan biosistematika ikan terumbu, Pertama. IPB Press, Bogor.

Madyowati, S.O., A. Kusyairi. 2020. Keanekaragaman komunitas makrozobenthos pada ekosistem mangrove di Desa Banyuurip Kecamatan Ujung Pangkah Kabupaten Gresik. Journal Fisheris and Marine Research, 4(1): 116–124.

Magfirah, Emiyarti, L.O.M.Y. Haya. 2014. Characteristics of sediment and its relationship with macrozoobenthos community structure in Tahi Ite River of Rarowatu District of Bombana. Jurnal Mina Laut Indonesia, 14: 117-131.

Mahmoudi, N., G.F. Slater, R.R. Fulthorpe. 2011. Comparison of commercial DNA extraction kits for isolation and purification of bacterial and eukaryotic DNA from PAH-contaminated soils. Canadian Journal of Microbiology, 57(8): 623–628.

Muwawa, E.M., C.C. Obieze, H.M. Makonde, J.M. Jefwa, J.H.P. Kahindi, D.P. Khasa. 2021. 16S rRNA gene amplicon-based metagenomic analysis of bacterial communities in the rhizospheres of selected mangrove species from Mida Creek and Gazi Bay, Kenya. PLoS One, 16: 1-22.

Purohit, M.K., S.P. Singh. 2009. Assessment of various methods for extraction of metagenomic DNA from saline habitats of coastal

Gujarat (India) to explore molecular diversity. Microbiology, 49(3): 338–344.
Ramadhani, S.F., A. Muhtadi. 2016. Hubungan panjang bobot dan kondisi ekologi ikan gelodok di Pantai Bali Desa Mesjid Lama Kecamatan Talawii Kabupaten Batu Barat Provinsi Sumatera Utara. Working paper, Institut Pertanian Bogor, Bogor, 30 Mei.
Riyandi, N., E. Muliawan, J. Sampurno, M.I. Jumarang. 2016. Identifikasi nilai salinitas pada lahan pertanian di Daerah Jungkat berdasarkan metode daya hantar listrik (DHL). Prisma Fisika, 4(2): 69–72.
Rusianti, C.R., F.I.E. Saleh, S. Talakua, A. Alianto, S. Mangando, Y.E. Demena, E. Manalu, F.C. Eldiester, F.N.Y. Rumbino, S. Raharjo. 2022. Struktur vegetasi mangrove dan produksi serasah mangrove Sonneratia alba di Telaga Wasti Kabupaten Manokwari, Papua Barat. Jurnal Pembangunan Berkelanjutan, 4(2): 93–103.
Sharma, A., R. Lal. 2017. Survey of metagenomic approaches for understanding microbial community dynamics. Indian Journal Microbiology, 57(1): 23–38.
Siringoringo, H.H. 2013. Potensi sekuestrasi karbon organik tanah pada pembangunan hutan tanaman Acacia mangium Wills. Pusat Litbang Konservasi dan Rehabilitasi, 10(2): 193–213.
Suryono, N. Soenardjo, E. Wibowo, R. Ario, E.F. Rozy. 2018. Estimasi kandungan biomassa dan karbon di hutan Mangrove Perancak Kabupaten Jembrana, Provinsi Balik. Buletin Oceanografi Marina, 7(1): 1–8.
Suwoyo, H.S., K. Nirmala, D. Djokosetiyanto, S. Mulyaningrum. 2015. Dominan factors affecting sediment oxygen consumpsion level in intensive white shrimp. Jurnal Ilmu dan Teknologi Kelautan Tropis, 7(2): 639–654.
Tan, S.C., B.C. Yiap. 2009. DNA, RNA, and protein extraction: the past and the present. Journal Biomed Biotechnology, 2009: 1-11.
Tangketasik, A., N.M. Wikarniti, N.N. Soniari, I.W. Narka. 2012. Kadar bahan organik pada tanah sawah dan tegalan di Bali serta hubungannya dengan tekstur tanah. Agrotropikal, 2(2): 101–107.
Técher, D., C. Martinez-Chois, M. D'Innocenzo, P. Laval-Gilly, A. Bennasroune, L. Foucaud, J. Falla. 2010. Novel perspectives to purify genomic DNA from high humic acid content and contaminated soils. Sep Purif Technology, 75(1): 81–86.
Ulumuddin, Y.I. 2019. Metana: emisi gas rumah kaca dari ekosistem karbon biru, mangrove. Jurnal Ilmu Lingkungan, 17(2): 341- 359.
Voordouw, G., Y. Shen, C.S. Harrington, A.J. Telang, T.R. Jack, D.W.S. Westlake. 1993. Quantitative reverse sample genome probing of microbial communities and its application to oil field production waters. Applied and Enviromental Microbiologi, 59(12): 4101–4114.
Voytas, D. 2000. Resolution and recovery of large DNA fragment, in: Current Protocols in Molecular Biology.
Wagner, M., R. Amann, H. Lemmer, K.H. Schleifer. 1993. Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. Applied Environment Microbiologi, 59(5): 1520–1525.
Yeates, C., M.R. Gillings, A.D. Davison, N. Altavilla, D.A. Veal. 1998. Methods for microbial DNA extraction from soil for PCR amplification. Biological Procedur Online, 1(1): 41-47.
Yuwono, N.W. 2009. Membangun kesuburan tanah di lahan marginal. Jurnal Ilmu Tanah dan Lingkungan, 9(2): 137–141.

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