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Isolation and Macroscopic Characterization of Molase and Bacteria from *Acanthus* sp. Mangrove Sediments

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Abstract

Acanthus sp. grows in mangrove sediments with a muddy texture that contains nutrients, allowing specific bacteria to thrive. Microbes from various sources can thrive in mangrove sediments due to the presence of nutrients as an energy source This study aims to isolate and characterize bacteria from mangrove sediment (*Acanthus* sp.) cultured in media with added molasses. Bacteria were cultivated on solid molasses media I and II. The planting process began with the sediment sample being dissolved in distilled water for 30 minutes before undergoing sixfold dilution. Subsequently, the samples were planted on molasses media I and II with different compositions. The planting was carried out using the spread plate method, where 100µL of the final three dilutions was added. The bacteria were then incubated until bacterial colonies that could use molasses as a carbon source for their metabolism grew. Molasses is a residual substance from sugar production in sugarcane plants, which still contains fermentable sugars optimally utilized by bacteria for metabolic processes. The results of bacterial cultivation revealed differences in growth diversity, with media II exhibiting greater diversity than media I. Bacterial diversity was observed using macroscopic identification methods, focusing on the colony's appearance. This suggests that the composition of media II is more suitable for bacterial growth.

1. Introduction

Mangroves are terrestrial plants commonly found in coastal areas. Indonesia has the largest mangrove coverage in the world, both in terms of area (+42,550 km2) and the number of species (+45 species) (Purnobasuki, 2004). Mangroves along the coast serve various functions, including providing habitat, a food source, spawning grounds, and acting as sediment traps. Their root systems help in trapping sediment particles that can come from various directions. Sediment transport to coastal areas is driven by marine hydrodynamics, so sediments can originate from both the land and the coastal ocean (Arifin *et al*., 2019).

Acanthus illicifolius is one of the true mangrove plants used for medicinal purposes. Traditionally, this plant has been used to treat conditions such as asthma, diabetes, diuretic, hepatitis, neuralgia, rheumatism, skin diseases, abdominal pain, infertility, and tumors (Suryati *et al*., 2018). *Acanthus ebracteatus* Vahl. (AE) is another medicinal plant used as an anti-inflammatory agent and is an ingredient in anti-cancer and anti-inflammatory formulations in traditional medicine (Wisuitiprot *et al*., 2023).

Mangrove ecosystems along the coast play a significant role in sediment deposition. Sediment deposits around mangroves include not only sand particles but also accumulated nutrients from the biological processes, such as the decomposition of mangrove ecosystems. Microbes from various sources can thrive in mangrove sediments due to the presence of nutrients as an energy source for these microorganisms (Citra *et al*., 2020).

Microbes, especially bacteria, require a medium to grow. The growth medium typically consists

of various components, with one of the essential elements being an energy source (carbohydrate). In this study, molasses, a byproduct of sugar production and currently underutilized, was used as a potential carbohydrate source for bacteria. It can serve as an alternative supplement for bacterial growth media (Ikhsan and Ariani, 2017).

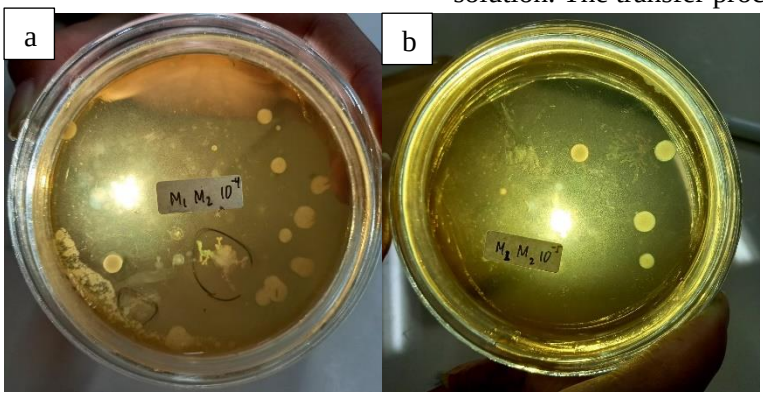

Figure 1. Pertumbuhan bakteri pada media I pengenceran10^{-4} (a) dan media II pengenceran 10^{-5} (b).

2. Material and methods

2.1. Method for the Preparation of Molasses

Solid and Slant Media Solid and slant media share the same ingredients and are divided into two types, namely media I and media II. Molasses media I is prepared by mixing 1 gram of molasses, 1.5 grams of Agar, 3 grams of NaCl, 0.1 gram of $MgSO_4$, 0.001 gram or 100 µm of $FeSO_4.7H_2O$, 0.2 gram of $K_2HPO_4.3H_2O$, 0.5 gram of $(NH_4)^2SO_4$, and 100 mL of distilled water (aquades). Meanwhile, molasses media II is prepared by mixing 1 gram of molasses, 0.3 gram of KH_2PO_4, 0.2 gram of $(NH_4)_2SO_4$, 3 grams of NaCl, 0.7 grams of $K_2HPO_4.3H2O$, 0.001 gram or 100 µm of $MgSO_4$, 0.005 gram or 500 µm of $FeSO_4$, 100 mL of distilled water (aquades), and 1.5 grams of Agar. Both media I and II are homogenized using a hot magnetic stirrer. The media are then sterilized using an autoclave for 15 minutes, counted from when the autoclave starts, and allowed to cool until the pressure inside the autoclave has subsided. Subsequently, the media are poured into petri dishes for solid media and into test tubes for slant media.

2.2. Method for the Preparation, Dilution of Bacterial Culture, Bacterial Inoculation, and Molasses Bacterial Incubation

100 mL of distilled water (aquades) is placed in an Erlenmeyer flask, and 1 gram of sediment sample is added to it. The Erlenmeyer flask is then sealed with cotton wool to make it airtight, and aluminum foil is placed on top and secured around the neck of the Erlenmeyer flask. This sample solution is stirred on a magnetic stirrer without heat for 30 minutes to ensure thorough mixing (homogenization). The preparation of this sample solution is done with the aim of transferring bacteria from the sediment sample to the liquid medium. The sediment sample solution is then diluted 6 times. Dilution is carried out aseptically by transferring 1 mL or 1000 µL of the sediment sample solution using a micropipette to test tubes containing 9 mL of 3% NaCl solution. The transfer process is repeated 6 times and is referred to as serial dilution. Bacterial inoculation is carried out using the last three dilution samples, namely 10^{-4}, 10^{-5}, and 10^{-6}. For each dilution sample, 100 µL is taken and spread evenly over the entire surface of the solid medium in petri dishes. The bacterial inoculation process is done aseptically and near a Bunsen burner flame. Petri dishes that have been inoculated with bacteria and have absorbed the water from the medium can be sealed. Subsequently, they can be incubated in a container in an inverted position (with the lid of the petri dish facing downward and the agar side facing upward) at room temperature until molasses bacteria grow.

2.3. Macroscopic Characterization of Molasses Bacteria

The solid media that have been inoculated and incubated are observed for their growth. Characterization is carried out when the majority of the media has been colonized by molasses bacteria. Bacteria are characterized based on a macroscopic bacterial table (Figures 1, 2, and 3), which observes the colony shape of the bacteria. The results of the characterization of the colony shape of bacteria are recorded. What is observed includes the color, shape, margin, and elevation of the bacterial colonies.

3. Results

The results of bacterial cultivation from *Acanthus* sp. sediment samples show that mangrove sediment bacteria can thrive in molasses media I and II with different forms of diversity (Figure 1). Details regarding the macroscopic characteristics of molasses bacteria can be observed in Table 1.

Table 1. Characterization results of *Acanthus* sediment molasses bacteria on media I and II.

Isolate Code	Macroscopic Characteristics of Bacteria	Total
7M1	*Entire, Round, Opaque, Convex*	46
8M1	*Entire, Irreguler, Opaque, Convex*	6
9M1	*Lobate, Rhizoid,* Bening, *Flat*	2

10M1	*Filamentous, Filamen, Bening, Umbonate*	1
20M2	*Entire, Round, Opaque, Convex*	86
21M2	*Lobate, Rhizoid,* Bening, *Flat*	13
22M2	*Undulate, Irregular, Opaque, Flat*	2
23M2	*Lobate, Irregular,* Bening, *Flat*	10
24M2	*Filamentous, Filamen,* Bening, *Umbonate*	2
25M2	*Lobate, Irregular, Opaque, Convex*	7
26M2	*Entire, Irregular, Opaque, Convex*	6

The results of macroscopic identification of *Acanthus* mangrove sediment molasses bacteria revealed a diversity of colony forms with different numbers. Molasses media I was populated by 4 different bacterial forms, while molasses media II was populated by 7 different bacterial forms. Based on the dominance of bacterial forms commonly found in both media, the predominant colony forms were entire, round, opaque, and convex, with 46 bacterial colonies in molasses media I and 86 bacterial colonies in molasses media II. This indicates that media II has a higher diversity of bacterial colony forms compared to the diversity of bacterial colony forms in media I.

4. Discussion

The planting process of *Acanthus* sp. mangrove sediment on the media was carried out on solid molasses media by planting 100µL of the culture that had been diluted to 10^{-4}, 10^{-5}, and 10^{-6}. This dilution is intended to make the bacterial density lower in each dilution. This is in accordance with Nuttjahyani and Shyntya (2014), who performed dilution so that the bacterial concentration had a lower density. This will facilitate the identification of the planted bacteria. Dilution was carried out 6 times with the bacterial source being a mangrove sediment sample solution made by continuous stirring for 30 minutes, aiming to mix bacteria and sediment in distilled water so that when bacteria were planted using the spread plate method, it would not damage the solid media due to sediment that had various sizes and rough textures.

The bacteria in this study were planted using two molasses media with different compositions, namely media I and media II. The difference between the two media lies in the composition of K2HPO4.3H2O and FeSO4, which are more abundant in media II, and (NH4)2SO4 and MgSO4, which have less composition in media II. In addition, KH2PO4 was added to media II but not used in media I. The composition of the two molasses media is a modification of previously existing media. Media I is derived from the research of Daboor *et al*. (2019), while media II is sourced from the research of Wang *et al*. (2013). The modification made is changing the carbon source in the media, namely alginate, into molasses. Molasses is used as a substitute for alginate because molasses is a waste product that contains simple carbon sources. This is in line with the research by Cazetta *et al*. (2007), molasses from sugarcane production waste contains fermentative sugars. These sugars are useful as a source of energy for microorganisms in metabolism.

Both molasses media were used as media for the growth of *Acanthus* sediment bacteria. The media planted with *Acanthus* sediment samples showed bacterial growth. Bacterial growth that occurred in media I and II had different characteristics. This is indicated by the domination and different colony shapes of bacteria. The results obtained from this study found 4 different colony shapes in media I, with the dominance of entire, round, convex, and opaque bacterial colony forms. Unlike media II, which has 7 different forms of bacterial colonies with the same dominance as in media I. Based on these results, it can be seen that media II has a higher diversity of bacterial colony characteristics than media I molasses.

From both media, as mentioned earlier, bacteria that grow in the media were observed using macroscopic identification methods. This macroscopic observation was carried out by directly looking at the shape of the colonies that grew on both media under study. This observation was done subjectively, focusing on the colony's shape, such as the edge shape, surface shape, elevation shape, and color of the bacterial colony. The results of macroscopic identification that were most frequently found in this study were bacteria with macroscopic characteristics of entire, round, opaque, and convex. Visually, the bacteria appeared as cloudy droplets forming a sphere. This is in accordance with the description of the division of macroscopic characteristics of bacteria by Kolwzan *et al*. (2011), entire as a non-wavy edge, round/perfectly circular, opaque as the cloudy color of the bacterial colony, and convex elevation. Macroscopic observation of bacteria was carried out to facilitate the process of bacterial identification. This is in line with Nuraini *et al*. (2020), macroscopic identification is done subjectively because it is limited to the observer's ability to observe bacterial colonies. The interpretation results in different colony shapes indicate the diversity of bacteria, which may be of different types. With the macroscopic identification, further research is needed to specifically determine the types of each bacterial colony that grew.

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5. Conclusions

Bacterial planting was carried out using the spread plate method, and macroscopic characterization was performed by observing the bacteria that grew on the media and comparing them to the bacterial macroscopic table. The composition of media that is

suitable for the growth of *Acanthus* sediment bacteria is the composition of media II molasses. This is indicated by the greater variety and abundance of macroscopic bacterial characteristics found in media II molasses compared to media I molasses.

Ethics approval
Excluding "biota."

Data availability statement
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Credit authorship contribution statement
Putri Nadhira Rachmani Kurniawan: data collection. Zaenal Arifin : Conceptualization, methodology, validation, formal analysis, investigation, resources, writing original draft preparation, writing review and editing, visualization, supervision, project administration, funding acquisition. Both authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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