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Isolation and Macroscopic Characterization of Molase and Alginate Bacteria from Mangrove Sediments in Shrimp Ponds

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Abstract

Bacteria are microscopic organisms capable of thriving in various environments that support their growth, including mangrove sediment. Bacterial characteristics in mangrove sediment were examined using two different media: molasses and alginate. This process included preparing solid media, diluting sediment samples, isolating bacteria, and characterizing isolates. On molasses media (both Molasses Media I and Molasses Media II), bacteria generally displayed entire, undulate, and lobate margins, with diverse shapes (round, irregular, punctiform, rhizoid), colors (opaque, clear), and elevations (convex, flat, umbonate). On alginate media (Alginat Media I and Alginat Media II), after repetition, bacteria typically had entire and lobate margins, varied shapes (round, irregular, punctiform, rhizoid), colors (opaque, clear), and elevations (convex, flat, umbonate). Bacterial isolation was carried out using the spread plate technique, inoculating microbial cultures onto solid agar media. The results indicated that molasses media supported higher bacterial colony growth compared to alginate media due to its richer nutrient profile. Colony counts were determined through serial dilution. The bacteria displayed diverse macroscopic characteristics, which were assessed using a comparison table considering colony margin, color, elevation, texture, and shape.

1. Introduction

Bacteria possess a unique ability to live, each species having its own characteristics, despite lacking a cell nucleus membrane. This is because bacteria have genetic components located within a single DNA molecule found in the cytoplasm (Hasyimuddin *et al.*, 2016). Bacteria are often considered harmful organisms for both humans and other living creatures. However, the benefits provided by bacteria to living organisms are also numerous. The utilization of bacteria in various fields, most notably in healthcare, is widespread. This is due to bacteria obtaining bioactive compounds from their hosts (Pratiwi, 2019). Bacteria are microscopic organisms capable of thriving in various environments that support their growth, including mangrove sediment.

According to Sembiring *et al.* (2014), sediment is defined as material transported in river currents due to erosion carried by the flow of water. Sediments carried by water currents can lead to the process of sedimentation, resulting in shallowing of water bodies. This shallowing can occur because the water flow in the sedimentation process is deposited at the mouth of the sea or in lakes. One of the locations where sedimentation is possible is in mangrove areas, hence it is commonly referred to as mangrove sediment. Generally, various bacteria with different characteristics are found in mangrove sediment. The diversity of bacterial characteristics in mangrove sediment is influenced by the conditions or the surrounding area of the mangrove itself, as it relates to the energy source and temperature in the vicinity of the mangrove (Lingga *et al.*, 2020).

Molasses is a thick liquid derived from sugar or sugarcane waste after the crystallization process, resulting in a sugar content of about 50% to 60%, along with various minerals and amino acids (Sutowo and Adelina, 2016). An example of molasses utilization is as a mosquito attractant. Alginate is a substance typically derived from the cell walls of brown seaweed. Alginate is commonly used as a stabilizer in mixtures and emulsions related to viscosity. An example of alginate utilization is in ice cream production (Mushollaeni and Rusdiana, 2011). Bacterial characteristics in mangrove sediment can be observed by first performing isolation using two different media: molase media and alginate media.

2. Material and methods

2.1 Preparation of Solid Media and Agar Slants

The sediment bacteria were isolated from mangrove sediment samples near shrimp ponds using the dilution technique on solid and slant media. Both types of media were divided into Molasses Media I, Molasses Media II, Alginat Media I, and Alginat Media II.

Aseptically, sample preparation was carried out by mixing 0.5 grams of molasses and 2.5 grams of NaCl, then homogenizing it and naming it Erlenmeyer 1. Erlenmeyer 2 was prepared by replacing 0.5 grams of molasses with 0.5 grams of alginate. Each Erlenmeyer flask was then filled with 100 ml of distilled water and homogenized. After homogenization, each Erlenmeyer flask was tightly sealed with cotton and placed in an autoclave. The Erlenmeyers were cooled, and then each Erlenmeyer was filled with mangrove sediment samples. The Erlenmeyers with sediment samples were then homogenized with a magnetic stirrer without heat for 30 minutes. Each Erlenmeyer was named Pure Molasses Sample and Pure Alginate Sample.

2.2 Dilution of Mangrove Sediment Samples for Bacteria

The dilution of mangrove sediment bacteria was performed through six consecutive dilutions, namely 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, in six separate reaction tubes. The dilution process was carried out by adding 1000 µL of pure molasses and pure alginate samples, respectively, to the first tube and then homogenizing it. The homogenized solution was taken as 1000 µL and added to the other reaction tubes, resulting in a 10^{-2} dilution. This process was repeated until a 10^-6 dilution was achieved.

2.3 Isolation of Mangrove Sediment Bacteria

After dilution, the samples were plated using the pour plate technique on solid media that had been prepared.

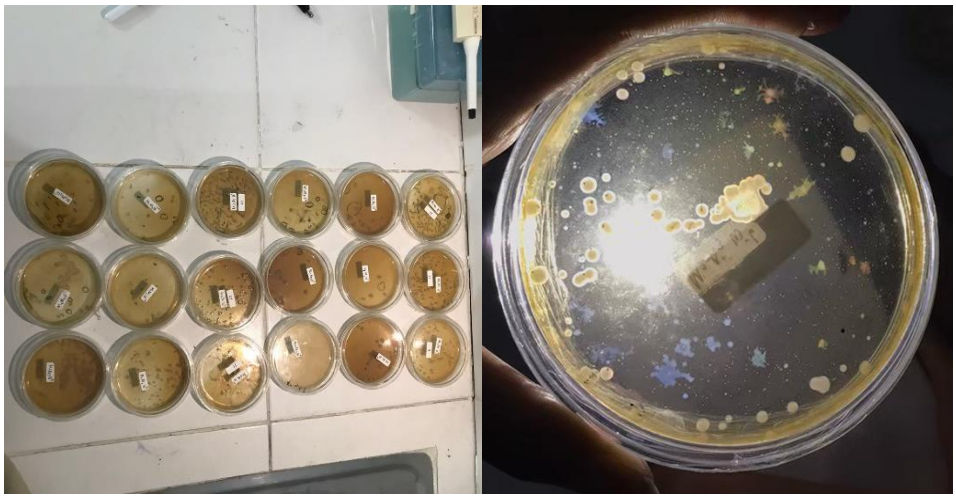

Figure 1. Bacteria Grown on a Petri Dish

They were then incubated at room temperature for 72 hours. The solid media consisted of 0.3 grams of Alginate, 1.5 grams of Agar, 0.001 grams of MgSO4, 3 grams of NaCl, 0.005 grams of FeSO4 (5000 µL), 0.7 grams of KH2PO4.3H2O, 0.2 grams of (NH4)2SO4, and 0.3 grams of KH2PO4. All these components were mixed in one Erlenmeyer flask, homogenized with 100 mL of distilled water. The result of the bacterial isolation was bacterial colonies that grew, which were then collected using an inoculating loop and streaked back onto plates to obtain purified cultures using the streak method. Purification was done on the prepared media and incubated again at room temperature for 72 hours. This purification process was repeated until distinct bacterial colonies were formed. Each isolated sediment bacterium was given a code for identification to avoid any mix-up. Isolates that showed bacterial growth were further identified and cultured on molasses and alginate slant media. Isolates that did not show bacterial growth were subjected to isolation again until bacterial growth was observed.

Table 1. Macroscopic Characteristics of Bacteria on Molasses Media

No.	Isolate Code	Macroscopic Morphology of Bacteria				Colony Count			Total ($\times 10^5$ CFU mL^{-1})
		Edge	Shape	Color	Elevation	10^{-4}	10^{-5}	10^{-6}	
1.	1MP1M1	Entire	Round	Opaque	Convex	10	11		12
2.	2MP1M1	Undulate	Irregular	Opaque	Convex		1		1
3.	3MP1M1	Lobate	Rhizoid	Bening	Flat	8			0.8
4.	4MP1M1	Entire	Punctiform	Opaque	Convex	2			0.2
5.	5MP1M2	Entire	Round	Opaque	Convex	9	10	57	580.9
6.	6MP1M2	Lobate	Rhizoid	Bening	Umbonate	1			0.1
7.	7MP1M2	Lobate	Irregular	Opaque	Umbonate	2			0.2
8.	8MP1M2	Lobate	Irregular	Opaque	Flat	12	5		6.2
9.	9MP1M2	Lobate	Irregular	Bening	Flat	0	0	0	0

2.4 Characterization of Mangrove Sediment Bacterial Isolates

The Identification Process of Mangrove Sediment Bacterial Isolates is conducted to determine the characteristics of the growing bacteria. Isolates used in this identification are pure cultures obtained from mangrove sediment samples near the shrimp ponds. Macroscopic observations are made on colony morphology. Morphological identification of bacterial shape is conducted on a macroscopic level, including the colony edge, bacterial colony, colony color, and colony elevation.

3. Results

3.1 Macroscopic Characteristics of Bacteria on Molasses Media. The Bacteria That Grew Following Incubation Were Characterized on a Petri Dish as Presented in Figure 1. The Macroscopic Characterization Results of Bacteria on Molasses Media Are Presented in Table 1.

Table 2. Table of Macroscopic Characteristics of Bacteria on Alginat Media

No.	Isolate Code	Macroscopic Morphology of Bacteria				Colony Count			Total
		Edge	Shape	Color	Elevation	10^{-3}	10^{-4}	10^{-5}	(x 10^4 CFU mL^{-1})
1.	1AP2M1	Entire	Round	Opaque	Flat	1			0.1
2.	2AP2M1	Lobate	Irregular	Bening	Flat	2			0.2
3.	3AP2M1	Lobate	Rhizoid	Bening	Flat		1		1
4.	4AP2M1	Entire	Round	Opaque	Convex		3		3
5.	5AP2M1	Entire	Punctiform	Opaque	Convex			>30	>30
6.	6AP2M2	Lobate	Rhizoid	Bening	Flat	4	6	6	66.4
7.	7AP2M2	Entire	Round	Opaque	Umbonate	1	1	1	11.1

The results of the observation of macroscopic characteristics of bacteria on molasses media, both Molasses Media I and Molasses Media II, indicate that the bacteria growing on molasses media generally have bacterial edges that are entire, undulate, and lobate, varied shapes including round, irregular, punctiform, and rhizoid, colors that are opaque and clear, and bacterial elevations that are convex, flat, and umbonate.

3.2 Macroscopic Characteristics of Bacteria on Alginat Media

The results of macroscopic characterization of bacteria on alginate media are presented in Table 2. The results of the observation of macroscopic characteristics of bacteria on alginate media after repetition, both Alginat Media I and Alginat Media II, indicate that the bacteria growing on alginate media after repetition generally have bacterial edges that are entire and lobate, varied shapes including round, irregular, punctiform, and rhizoid, colors that are opaque and clear, and bacterial elevations that are convex, flat, and umbonate.

4. Discussion

Based on the bacterial isolation results, various bacteria with different quantities, shapes, and characteristics were observed. The macroscopic characteristics include the margin (outer shape of the bacteria), color, elevation, texture, and the shape of the bacteria and bacterial colonies. Colony counts were determined through serial dilution. Serial dilution was performed to facilitate the counting of bacterial colonies. The bacterial colonies observed in Table 1 are based on the observation of macroscopic characteristics of bacteria on Molasses Media, while Table 2 is based on the observation of macroscopic characteristics of bacteria on Alginat Media. Table 2 shows the results of observations on the macroscopic characteristics of bacteria on Alginat Media with repetitions. The bacterial colony results observed in Tables 1 were obtained from dilutions of 10^{-4}, 10^{-5}, and 10^{-6}, while Table 2 used dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. Based on the macroscopic bacterial tables, both Tables 1 and 2 show that bacteria are more abundant in the highest dilution. Such results may indicate errors, such as the presence of contaminating bacteria that contaminated the sample or the media used, leading to results that are not as expected. Obtaining incongruent results is similar to the findings of a study conducted by Arifin *et al.* (2016), and in their research, they explained that errors in observations and incongruent results can be caused by contaminating bacteria originating from various sources, including the laboratory personnel or the laboratory environment, which do not meet biosafety standards based on the American Society for Microbiology (ASM) and the Clinical Laboratory Standards Institute (CLSI).

The ideal outcome that should have been obtained is a higher quantity of bacteria in lower dilutions, followed by a decrease in higher dilutions. This aligns with the statement by Seniati *et al.* (2019), which suggests that the bacterial density results will show lower numbers in larger dilution series. A favorable observation of bacterial colony counts indicates that as the dilution level increases, bacterial growth decreases. This statement is supported by Ariyanti *et al.* (2016), who state that higher dilution levels result in fewer microbes. This is based on the principles of serial dilution, which aim to reduce or decrease the quantity of bacteria/microbes in the sample.

Based on the bacterial colony counts in Table 1, the number of bacterial colonies obtained on molasses media is higher compared to the number of bacterial colonies obtained on alginate media. The bacterial colony count on alginate media appears to be lower or even absent compared to the colony count on molasses media. This also led to the repetition of bacterial isolation, the results of which are shown in Table 2. This indicates that bacteria can grow better on molasses media compared to alginate media. The content or nutrients present in molasses media can support bacterial growth more effectively compared to alginate media. This is because alginate has a long polymer structure, a chemical structure that is not easily degraded, and is rigid and brittle. This statement is supported by research conducted by Anhari *et al.* (2016), which found that bacteria can grow well on molasses waste because molasses waste contains a readily available carbon source in the form of glucose, which bacteria can easily digest. With glucose, bacteria can convert carbon into biohydrogen. This aligns with the findings of Afni *et al.* (2017), stating that alginate has a long polymer structure, which takes a considerable amount of time in the polymer degradation process for bacteria to digest. Moreover, it is also

influenced by the chemical structure of alginate, which consists of mannuronate and guluronate with different properties. Guluronate is more rigid and brittle compared to mannuronate, which is softer and more elastic, making mannuronate easier to degrade.

Bacterial growth in a specific isolation is influenced by various factors. Factors affecting bacterial growth include temperature, pH, water, oxygen, nutrients/food substances, and compounds that can inhibit bacterial growth (Adinur *et al.*, 2012). Additionally, the bacteria that grow are also influenced by the origin of the sample. This is because sediments from different regions will affect the bacteria living within them, and these bacteria will grow well, adapting to their habitat. Bacteria that grow in one sediment may differ from those that grow in another sediment. The environmental conditions in shrimp pond sediments generally deteriorate as shrimp production continues continuously in the pond. This leads to the accumulation of ammonia compounds in sediments near the shrimp ponds. Therefore, bacteria that can grow in sediments near shrimp ponds are typically bacteria capable of degrading TAN (Total Ammonia Nitrogen). These bacteria are chemoautotrophic autotrophic bacteria, which can utilize chemicals to produce food as their source of nutrition (Susanti *et al.*, 2014).

5. Conclusions

Bacterial isolation was carried out using the spread plate technique, inoculating microbial cultures onto solid agar media. The results indicated that molasses media supported higher bacterial colony growth compared to alginate media due to its richer nutrient profile. Colony counts were determined through serial dilution. The bacteria displayed diverse macroscopic characteristics, which were assessed using a comparison table considering colony margin, color, elevation, texture, and shape.

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

Inda Hawa Al Falah : Conceptualization, methodology, validation, formal analysis, investigation, resources, writing original draft preparation, writing review and editing, visualization, supervision, project administration, funding acquisition. The 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

Referrences

Adinur, C., Yoyok Budi, P., and Bhakti Etza, S. 2012. Efek Pemanfaatan Karaginan Sebagai Edible Coating Terhadap Ph, Total Mikroba Dan H2s Pada Bakso Selama Penyimpanan 16 Jam. *Animal Agriculture Journal*. 1(2): 286-292.

Afni, F., Purwaningsih, S., Nurilmala, M., and Peranginangin, R. 2017. Production of Alginate Oligosaccharides (AOS) as Prebiotic Ingredients through by Alginate lyase enzyme. *Jurnal Pengolahan Hasil Perikanan Indonesia*. 20: 109. 10.17844/jphpi.v20i1.16498

Anhari, S., Bintari, S. H., Mubarok, I., and Susilaningsih, D. 2016. Produksi Biohidrogen dari Limbah Organik Cair Molase dan Vinasse Menggunakan Bakteri *Rhodobium marinum*. *Life Sciences*. 5(2): 108-117.

Arifin, A., Hayati, Z., and Jamil, K. F. 2016. Isolasi dan identifikasi bakteri di lingkungan laboratorium mikrobiologi klinik RSUDZA Banda Aceh. *Jurnal Ilmiah Mahasiswa Kedokteran Komunitas*. 1(4).

Ariyanti, V. N., Supriharyono, S., and Widyorini, N. 2016. Hubungan Kerapatan Lamun Dengan Kelimpahan Bakteri Heterotrof Di Perairan Pantai Kartini Kabupaten Jepara. *Management of Aquatic Resources Journal*. 8. https://doi.org/10.14710/marj.v5i3.14401

Hasyimuddin, A., Djide, M. N., and Samawi, M. F. 2016. Isolasi Bakteri Pendegradasi Minyak Solar Dari Perairan Teluk Pare-Pare. *Biogenesis: Jurnal Ilmiah Biologi*. 4(1): 41-46. https://doi.org/10.24252/bio.v4i1.1119

Lingga, R., Afriyansyah, B., Septiani, R., and Miranti, I. 2020. Kepadatan Bakteri Sedimen Laut Terdampak Aktivitas Tambang Timah di Pantai Sampur Bangka Tengah. *Herbal Medicine Journal*. 3(1): 19-25.

Mushollaeni, W., and Rusdiana, E. 2011. Characterization of Sodium Alginate from *Sargassum* sp., *Turbinaria* sp. and *Padina* sp. *Jurnal Teknologi dan Industri Pangan*. 22(1): 26.

Pratiwi, R. 2019. Peranan Mikroorganisme Endofit Dalam Dunia Kesehatan: Kajian Pustaka. *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam*. 16: 21. https://doi.org/10.31851/sainmatika.v16i1.2695

Sembiring, A. E., Mananoma, T., Halim, F., and Wuisan, E. M. 2014. Analisis Sedimentasi Di Muara Sungai Panasen. *Jurnal Sipil Statik*. 2(3).

Seniati, S., Marbiah, M., and Irham, A. 2019. Pengukuran kepadatan bakteri *Vibrio harveyi* secara cepat dengan menggunakan spectrofotometer. *Agrokompleks*. 19(2): 12-19.

Susanti, E., Harpeni, E., Setyawan, A., and Putri, B. 2014. Penapisan bakteri pendegradasi total ammonia nitrogen dari sedimen tambak tradisional udang windu (*Penaeus monodon*). *Aquasains*. 2(2): 145-148.

Sutowo, I., and Adelina, T. 2016. Kualitas nutrisi silase limbah pisang (batang dan bonggol) dan level molases yang berbeda sebagai pakan alternatif ternak ruminansia. *Jurnal Peternakan*. 2: 41-47. http://dx.doi.org/10.24014/jupet.v13i2.2417