

## SCREENING AND IDENTIFICATION OF MANGROVE PLANT *Sonneratia alba* Sm. ENDOPHYTIC BACTERIA FROM ENGGANO ISLAND, BENGKULU PROVINCE

Fatimatuzzahra\*, Risky Hadi Wibowo\*\*, Sipriyadi\*\*\*, Putri Hezekiel Claracia Simanjuntak\*\*\*,  
Yar Johan\*\*\*\*

\*Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Kandang Limun, Bengkulu, Indonesia, fatimatuzzahra@unib.ac.id

\*\*Biology Master Program, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Kandang Limun, Bengkulu, Indonesia, rhwibowo@unib.ac.id, sipriyadi@unib.ac.id

\*\*\*Undergraduate Student, Biology Department, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Kandang Limun, Bengkulu, Indonesia, putrihcsimanjuntak@gmail.com

\*\*\*\*Marine Science, Faculty of Agriculture, University of Bengkulu, Kandang Limun, Bengkulu, yarjohan@unib.ac.id

Email Correspondence: rhwibowo@unib.ac.id

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**Abstract:** The mangrove plants from Enggano Island are found in a well-preserved ecosystem. Mangroves contain secondary metabolite compounds that result from the interaction between plants and endophytic bacteria. This study aims to obtain endophytic bacterial isolates from the mangrove plant *Sonneratia alba* Sm. from Enggano Island and to assess bacterial diversity. Endophytic bacteria were isolated using grinding and then diluted with serial dilutions of  $10^{-1}$ ,  $10^{-3}$ , and  $10^{-5}$ . The growing bacteria were purified using the quadrant streak method. They were then identified by observing colony morphology, Gram staining, and biochemical tests including catalase, motility, citrate, urea, and sugar fermentation tests. Bacterial identification was carried out using Bergey's Manual of Determinative Bacteriology. A total of 22 endophytic bacterial isolates were collected from the mangrove plant *Sonneratia alba* from Enggano Island, consisting of 4 genera: *Bacillus*, *Marinococcus*, *Micrococcus*, and *Pseudomonas*. There were 17 isolates closely related to the genus of *Bacillus*, 1 isolate closely related to the *Marinococcus*, 2 isolates closely related to the *Micrococcus*, and 2 isolates closely related to the *Pseudomonas*.

**Keywords:** endophytic bacteria; Enggano island; bacterial screening; *Sonneratia alba* Sm

**Abstrak:** Tumbuhan mangrove asal Pulau Enggano berada pada ekosistem yang masih terjaga. Mangrove memiliki kandungan senyawa metabolit sekunder yang merupakan hasil interaksi antara tumbuhan dan bakteri endofit. Penelitian ini bertujuan untuk memperoleh isolat bakteri endofit tumbuhan mangrove *Sonneratia alba* Sm. asal Pulau Enggano dan mendapatkan keanekaragaman bakteri. Isolasi bakteri endofit dilakukan dengan metode gerus, kemudian diencerkan dengan pengenceran berseri dari  $10^{-1}$ ,  $10^{-3}$ , dan  $10^{-5}$ . Bakteri yang tumbuh dimurnikan dengan metode gores kuadran. Kemudian diidentifikasi dengan mengamati morfologi koloni, pewarnaan Gram, dan uji biokimia meliputi uji katalase, uji motilitas, uji sitrat, uji urea, uji gula-gula. Kemudian dilakukan identifikasi bakteri dengan buku *Bergey's Manual of Determinative Bacteriology*. Total

isolat bakteri endofit yang berhasil dikoleksi dari tumbuhan mangrove *Sonneratia alba* asal Pulau Enggano adalah sebanyak 22 isolat dengan keanekaragaman bakteri endofit yang terdiri dari 4 genus yaitu *Bacillus*, *Marinococcus*, *Micrococcus*, dan *Pseudomonas*. Isolat yang memiliki kedekatan dengan genus *Bacillus* adalah sebanyak 17, yang memiliki kedekatan dengan genus *Marinococcus* hanya 1 isolat, yang memiliki kedekatan dengan genus *Micrococcus* adalah 2 isolat, dan 2 isolat memiliki kedekatan dengan genus *Pseudomonas*.

**Kata kunci:** bakteri endofit; skrining bakteri; *Sonneratia alba* Sm.; Pulau Enggano

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## Introduction

Enggano Island is located in the waters of the Indian Ocean, off the western coast of Sumatra. Enggano has significant coastal and marine resources, including well-preserved mangrove forests (Kurniadi and Koeslulat 2020). Mangroves have evolutionarily adapted to the conditions of the tropical intertidal ecosystem and have been defined as "true extremophiles" because they can thrive under high salinity, hypoxic substrates, and strong tidal flows that are unsuitable for most terrestrial plants (Fatimah et al. 2022). In addition, mangroves have become a subject of conservation studies due to their many beneficial natural products and ability to host various types of microbes. As stated by Jiang et al. (2018), mangroves are a productive source of endophytic bacteria.

Endophytic bacteria are microbes that live inside plant tissues and can form colonies within plant tissues without causing negative effects on the host (Kasi et al. 2015). Endophytic microbes can be a source of biological and active natural products that can secrete various substances such as cytokinins, phytohormones, and other plant growth-promoting compounds that can affect plant growth.

Several studies have found the presence of endophytic bacteria in mangrove plants, including research by Deivanai et al. (2014) and Yulma et al. (2020), who reported endophytic bacteria belonging to the genus *Bacillus*. Tam et al. (2018) found bacteria from the genera *Pseudomonas* and *Bacillus* in the mangrove *Avicennia alba*. In a study by Jose and Cristy (2013), bacteria from the genera *Serratia*, *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Enterobacter* were isolated from the mangrove *Rhizophora mucronata* and showed potential as antimicrobials. Screening endophytic bacteria from the mangrove *Sonneratia alba* originating from Enggano Island is expected to provide scientific data on the diversity of endophytic bacteria for future research. These endophytic bacteria could become candidates for raw materials in drug development due to their antimicrobial potential. This study aims to obtain endophytic bacterial isolates from the mangrove plant *Sonneratia alba* Sm. from Enggano Island and to determine the bacterial diversity.

## Material and Methods

### Tools

The tools used in this study are Petri dishes (Sterilplan), Beaker glasses (pyrex), test tubes (Iwaki), test tube racks, analytical balance (Sartorius), measuring pipettes, measuring cups (pyrex), ose needles, autoclaves (ALP KT-S04), incubators (Jeio tech), laminar airflow (Nuair), spray bottle, oven (memmert), hot plate (basic series), tube clamp, binocular microscope (Leica), refrigerator, spiritus lamp, bulb, knife cutter, vortex mixer (Jeio tech), glass slide, and preparation cover.

### Materials

The materials used are root, stem, and leaf organs of *Sonneratia alba* plants, Nutrient Agar (NA), safranin, crystal violet, lugol, and sodium hypochlorite 5.25%, spirits, 70% alcohol, 96% alcohol, NA semi-solid, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), glucose media, lactose media, maltose media, sucrose media, distilled water, SCA (Simon Citrate Agar) agar media, urea media, cotton, cling wrap, rubber, plastic, immersion oil, and pure agar.

### Sample Collection

Samples of the mangrove plant *Sonneratia alba* Sm. were taken from the Kaana Sub District mangrove area of Enggano Island (Figure 1). The parts of the plant samples included leaves, stems, and roots. These samples were then cleaned with sterile water to remove any dirt and dried with sterile tissue paper.



Figure 1. Map of Enggano Island (photo source, Jarulis, 2021).

### Plant Verification

Plant verification is done to ensure that the plants collected and used as objects of research are real plants that will be studied and by existing literature. Verification of *Sonneratia alba* Sm. Mangrove plants were carried out at the Plant Systematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences,

University of Bengkulu.

### **Purification of Endophytic Bacteria**

The method used to purify bacterial isolates is the quadrant scratch method using NA media. NA media is a medium that supports the growth of various bacteria because it contains many nutrients needed by bacteria, making it suitable for growing endophytic bacteria (Pathmanathan, 2016). Then the endophytic bacterial isolates were incubated at 25 - 30 °C for 48 hours. Purified colonies were used for further observation. After the pure culture was obtained, the endophytic bacteria were stored in NA-tilted media to be used for further testing (Cappuccino and Sherman, 2013).

### **Identification of Colony Morphology and Gram Staining and Biochemical Characters of Endophytic Bacteria**

The pure bacterial isolates were then identified by macroscopic observation of colony morphology including colony shape, surface, colony color, appearance, edges, and elevation of bacterial colonies. Gram staining was performed and observed under a microscope with a magnification of 10 x 100. The observation of Gram-positive bacteria will be marked with purple color and Gram-negative bacteria will be marked with red color (Lay, 1994).

In addition, the isolates were tested with biochemical tests, namely citrate test, urease test, motility test, catalase test, sugar fermentation test (maltose, glucose, lactose, and sucrose), and starch hydrolysis test. The test of catalase was conducted by transferring a colony onto a clean glass slide and adding a drop of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). A positive result was indicated by the formation of bubbles (Cappuccino and Sherman, 2013). In the urease test, urea was utilized as a medium in a tube. To perform the test, the isolates were inoculated and streaked in the slant, then incubated for 24 hours at 30°C. Color change into pink in the medium showed a positive result. To perform the citrate test, Simon's Citrate Agar (SCA) medium was used in which the isolates were inoculated, followed by 24-hour incubation at 30 °C. The positive result was shown by a color change from green to blue along the slant of the medium. Furthermore, the motility test used a semi-solid NA medium in a tube. After a well-isolated colony was picked using a sterile straight needle, the medium was straight stabbed. The culture was incubated at 30°C for 24 hours. The positive result can be characterized when the colony spreads out into the medium from the inoculation site.

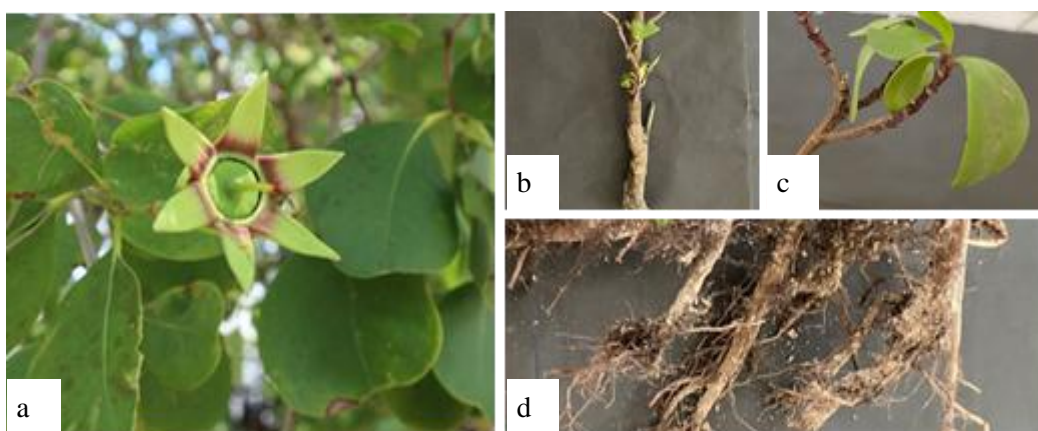
To test for the ability to ferment sugar, glucose (I), sucrose (II), lactose (III), and maltose (IV) broth media were used. Each medium was put into a tube with a Durham tube submerged in the media. One full loop of each isolate was inoculated and then incubated for 24 hours at 30°C. The observation was in the color change and trapped air bubbles in the Durham tube. In addition, data obtained from biochemical tests can determine the genus of an isolate by referring to Bergey's Manual of Systematic Bacteriology 2nd Edition Volume 3rd and Bergey's Manual

of Determinative Bacteriology 9th Edition, and high funding is also a consideration for researchers to identify using phenotypic methods compared to molecular methods.

## Results and Discussion

### *Sonneratia alba* mangrove plant verification results

Verification of *S. alba* mangrove plants was conducted at the Plant Systematics Laboratory, Department of Biology, Basic Science Building, Faculty of Mathematics and Natural Sciences, Bengkulu University, with a certificate numbered 277/UN30.12.LAB.BIOLOGI/PM/2023. The verified morphology of *S. alba* plants can be seen in Figure 2.



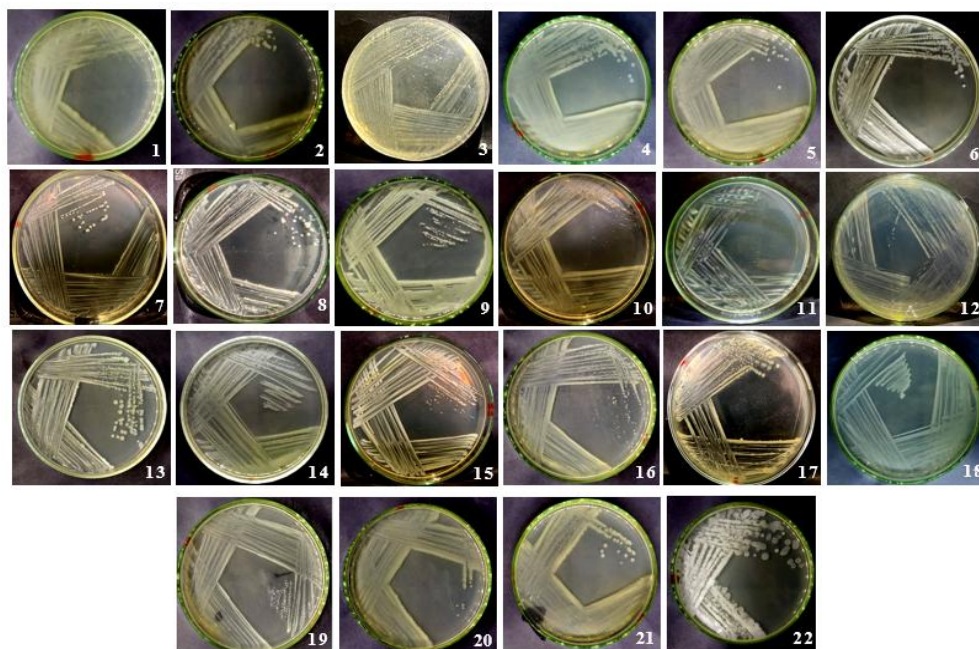
**Figure 2.** Plant morphology *Sonneratia alba* Enggano a= fruit, b= stem, c= leaf, d= root (photo source: Simanjuntak, 2023).

The isolate code of *Sonneratia alba* Enggano endophytic bacteria from the root plant organ is referred to as A, the stem is referred to as code B, and the leaf is referred to as code D.

### Isolation of Endophytic Bacteria of Mangrove Plants *Sonneratia alba* Sm.

Endophytic bacterial isolates obtained from the isolation results were 22 endophytic bacterial isolates from *S. alba*, then the endophytic bacterial colonies were rejuvenated, purified using the quadrant streak method, and incubated for 2 x 24 hours. Purification of 22 endophytic bacterial isolates from mangrove *S. alba* isolation results can be seen in Figure 3.





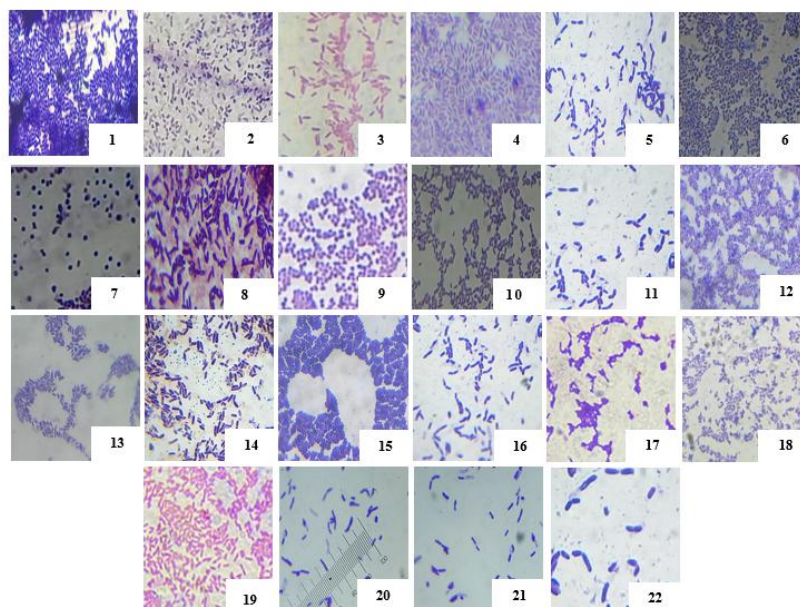
**Figure 3.** Purified *Sonneratia alba* mangrove endophytic bacterial isolates on NA medium and incubated at 30 °C for 48 hours ASAE: 1-8, BSAE: 9-15, DSAE: 16-18. DSAE: 19-22

### **Morphological Identification of Endophytic Bacteria Mangrove *Sonneratia alba* Sm.**

Endophytic bacterial isolates that grow are then observed for colony morphological characters based on appearance, surface, elevation, edges, and colony color. The results of isolation using the grinding method obtained 22 isolates of mangrove endophytic bacteria *S. alba* each have different morphological characteristics. The colony morphology of mangrove endophytic bacteria is dominated by circular shapes, but there are some irregular ones. The colony surface is mostly smooth; some isolates have concentric and wrinkled surfaces. Most elevation types are convex, but some are raised and flat. Colony edges of mangrove endophytic bacteria are mostly entire, but some are undulate, serrated, filamentous, and lobate. The colony colors of mangrove endophytic bacteria are yellow, white, cream, and greenish pigmented, but most colonies of mangrove endophytic bacteria obtained are cream.

### Gram Staining of Mangrove Endophytic Bacteria *Sonneratia alba* Sm.

The results of Gram staining of 22 *S. alba* endophytic isolates can be seen in Figure 4.



**Figure 4.** Gram staining of mangrove endophytic bacterial isolates of *Sonneratia alba* from Enggano Island, ASAE: 1-8, BSAE: 9-15, DSAE: 16-22 that was 48 hours old was observed under a binocular light microscope with a magnification of 10 x 100.

Based on the results of Gram staining that has been done, from 22 isolates of *S. alba*, isolates code of BSAE 9, ASAE 7, and BSAE 15, have a coccus shape with staphylo and strepto, diplo, and mono arrangements. Isolates ASAE 1-6, ASAE 8, BSAE 10-14, and DSAE 16-22 have a bacillus shape with mono, diplo, and strepto arrangements. The results of Gram staining showed that 22 isolates of *Sonneratia alba* were divided into 2 groups of Gram positive and negative bacteria, isolates ASAE 1, 2, 4-8, BSAE 9-15, and DSAE 16, 17, 18, 20-22 were Gram positive bacteria, while isolates ASAE 3 and DSAE 19, were Gram negative bacteria. This grouping is based on the color seen during observation under a light microscope after the bacteria are stained with the Gram staining method, caused by differences in the components that make up the cell wall. Salton and Kim (1996) claimed that Gram-positive bacteria have a thick cell wall layer (about 20 nm – 80 nm), while in Gram-negative bacteria, the peptidoglycan layer in the cell wall is thinner at 5 – 10 nm.

### Biochemical Test of Mangrove Endophytic Bacteria *Sonneratia alba* Sm.

The bacterial metabolism in biochemical tests can be seen from the ability of bacteria to use certain compounds as carbon sources and energy sources. Biochemical tests are carried out to determine the physiological characteristics of bacteria and determine the closeness of bacteria at the genus level. The results of the biochemical test can be seen in Table 1.

**Table 1.** Results of biochemical tests of mangrove endophytic bacteria *S. alba* Sm.

Isolates Code	Sugar Test				Catalase	Urea	Motility	Citrate
	Glucose	Lactose	Sucrose	Maltose				
ASAE 1	+	-	-	-	+	+	+	+
ASAE 2	+	+	-	-	+	-	+	-
ASAE 3	+	-	-	-	+	+	+	+
ASAE 4	+	-	-	-	+	+	+	+
ASAE 5	+	-	-	-	+	+	+	-
ASAE 6	+	+	+	+	+	-	+	+
ASAE 7	-	-	-	-	+	-	+	-
ASAE 8	+	+	-	+	+	+	+	+
BSAE 9	+	+	+	+	+	+	+	+
BSAE 10	-	-	-	-	+	+	+	+
BSAE 11	+	-	-	-	+	-	+	+
BSAE 12	+	-	-	-	+	-	+	+
BSAE 13	+	+	+	+	+	-	+	-
BSAE 14	+	+	+	+	+	-	+	-
BSAE 15	+	+	+	-	+	-	+	+
DSAE 16	+	-	-	-	+	+	+	+
DSAE 17	+	-	-	-	+	-	+	+
DSAE 18	+	-	-	-	+	-	+	+
DSAE 19	+	-	-	-	+	+	+	+
DSAE 20	+	+	+	+	+	-	+	+
DSAE 21	+	-	-	-	+	-	+	+
DSAE 22	+	+	+	+	+	-	+	-

Remarks: ASAE : Akar *Sonneratia alba* Enggano, BSAE : Batang *Sonneratia alba* Enggano, DSAE : Daun *Sonneratia alba* Enggano, (+) = positive, (-) = negative

The metabolic properties of bacteria in biochemical tests can be recognized by the ability of bacteria to use certain compounds as carbon sources and energy sources. The observation of the motility test in all isolates, ASAE, BSAE, and DSAE showed that these isolates are motile bacteria characterized by the movement of bacteria in the media that has been stabbed 1 ose of bacterial isolates. The ability of bacteria to move indicates that bacteria have a means of movement, namely flagella (Panjaitan et al., 2020).

The results of the catalase test showed that all isolates of mangrove endophytic bacteria ASAE, BSAE, and DSAE showed positive results characterized by the formation of gas bubbles when the isolates were tested with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) reagent. The catalase test aims to see the ability of bacteria to degrade H<sub>2</sub>O<sub>2</sub>. The formation of bubbles on the glass object indicates an enzymatic reaction from bacteria that produce the enzyme catalase. Negative catalase test results are characterized by the absence of bubbles in bacteria that are



dripped with H<sub>2</sub>O<sub>2</sub> (Harley and Prescott, 2002). Bacteria produce catalase to defend themselves against hydrogen peroxide attack (Iwase et al., 2013).

In the citrate test, the color change in the media is due to the change to sodium carbonate, resulting in an alkaline pH change and conversion of the pH indicator, bromothymol blue, from green (neutral) to blue (alkaline) (Van et al., 2016). According to Cappucino and Welsh (2018), bacteria have a citrate permease enzyme that can break down citrate into oxaloacetate and acetic acids, so bacteria that can carry citrate into cells are bacteria that can utilize citrate as one of their carbon sources.

The urea test was conducted to determine the ability of bacteria to produce urease enzymes. The occurrence of yellow color changes in the urea media is caused by the media containing a pH indicator in the form of phenol red. Bacteria that can produce the enzyme urease can decompose urea into ammonium, and CO<sub>2</sub> can use urea as the only carbon source. The accumulation of ammonium can raise the pH to alkaline so that the media changes to a pink color (Fallo and Sine, 2016).

The carbohydrate fermentation test showed the positive test of sugars is marked when there is acid formation (yellow color) with gas or without gas in the tube. The gas bubbles contained in the Durham tube are due to the carbohydrate fermentation reaction. Bacteria will ferment glucose when simple carbon sources are not available, so bacteria will ferment more complex carbon sources (Panjaitan et al., 2020).

The sucrose test results of *S. alba* endophytic bacteria in several isolates produced a positive test. The fermentation reaction is seen by the change in the color of the media when acidic products are formed. Bacteria can utilize peptone in the media which produces alkaline by-products; the pH only changes if there is excess acid. The acid produced is the result of sucrose fermentation; bacteria capable of fermenting sucrose can utilize sucrose as a carbon supply (Reiner, 2016).

The lactose test results of endophytic bacteria *S. alba* showed a positive test, which was indicated by a change in color from red to yellow. The changes that occur indicate that bacteria form acid from lactose fermentation. This is because the lactose medium contains sugar, water, peptone, and phenol red so that bacteria can break down the lactose medium, which is indicated by a change in color in the medium, from red to yellow. This bacterium has the enzyme  $\beta$ -galactosidase, which can break down lactose as its carbon supply (Lay, 1994).

Maltose test results of *S. alba* bacteria showed a positive test, which indicated the change from red to yellow color medium. Maltose is a reducing sugar; this bacterium has the enzyme  $\beta$ -amylase, which plays a role in maltose metabolism. The changes that occur indicate that bacteria can ferment maltose and use it as a carbon source (Crow et al., 2013).

### **Identification of Mangrove Endophytic Bacteria**

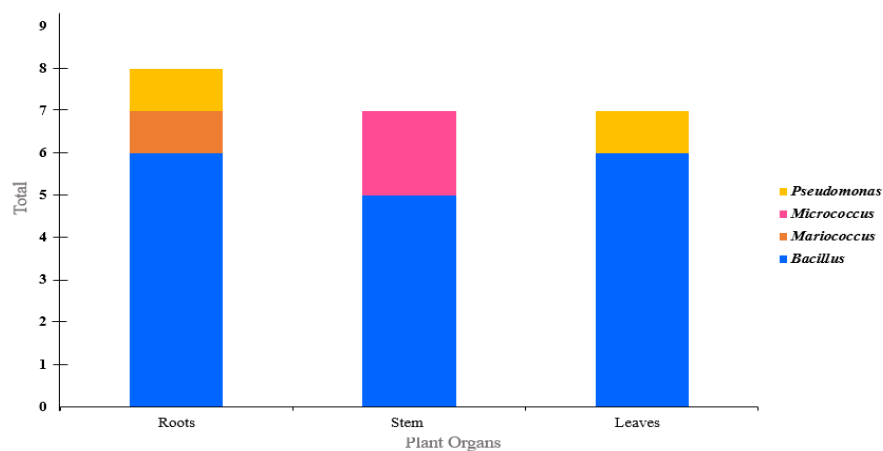
Based on the results of macroscopic morphological observations, Gram

staining, and biochemical tests, the genus of bacteria can be identified using Bergey's Manual of Determinative Bacteriology 9th Edition. The genus that has been obtained can be seen in Table 3.

**Table 3.** The identification results of bacteria by biochemical tests of *S. alba*.

Isolates Code	Origin of Plant parts	Genus
ASAE 1	Roots	<i>Bacillus</i> sp 1.
ASAE 2	Roots	<i>Bacillus</i> sp 2.
ASAE 3	Roots	<i>Pseudomonas</i> sp 1.
ASAE 4	Roots	<i>Bacillus</i> sp 3.
ASAE 5	Roots	<i>Bacillus</i> sp 4.
ASAE 6	Roots	<i>Bacillus</i> sp 5.
ASAE 7	Roots	<i>Marinococcus</i> sp 1.
ASAE 8	Roots	<i>Bacillus</i> sp 6.
BSAE 9	Stem	<i>Micrococcus</i> sp 1.
BSAE 10	Stem	<i>Bacillus</i> sp 7.
BSAE 11	Stem	<i>Bacillus</i> sp 8.
BSAE 12	Stem	<i>Bacillus</i> sp 9.
BSAE 13	Stem	<i>Bacillus</i> sp 10.
BSAE 14	Stem	<i>Bacillus</i> sp 11.
BSAE 15	Stem	<i>Micrococcus</i> sp 2.
DSAE 16	Leaves	<i>Bacillus</i> sp 12.
DSAE 17	Leaves	<i>Bacillus</i> sp 13.
DSAE 18	Leaves	<i>Bacillus</i> sp 14.
DSAE 19	Leaves	<i>Pseudomonas</i> sp 2.
DSAE 20	Leaves	<i>Bacillus</i> sp 15.
DSAE 21	Leaves	<i>Bacillus</i> sp 16.
DSAE 22	Leaves	<i>Bacillus</i> sp 17.

The results of the identification of 22 isolates of mangrove endophytic bacteria *S. alba* from Enggano Island, showed a close relationship with several genus, namely *Bacillus*, *Marinococcus*, *Micrococcus*, and *Pseudomonas*. Isolates that have similarities with the genus *Bacillus* as many as 17, 1 isolate has similarities with the genus *Marinococcus*, 2 isolates have similarities with the genus *Micrococcus*, and 2 isolates have similarities with the genus *Pseudomonas*. The diagram of endophytic bacterial diversity can be seen in Figure 5.



**Figure 5.** The diversity of endophytic bacterial genus in mangrove plants *S. alba* from Enggano Island.

The genus *Bacillus* is a group of rod-shaped, Gram-positive bacteria that produce oval or cylindrical endospores, are aerobic or facultatively anaerobic, chemoorganotrophic, and catalase-positive (Holt et al., 1994). The isolates show similarities with the genus *Bacillus*, specifically ASAE 1, 2, 4, 5, 6, 8, BSAE 10, 11, 12, 13, 14, DSAE 16, 17, 18, 20, 21, and DSAE 22. These bacteria are characterized by their rod shape, ability to ferment lactose, glucose, sucrose, and maltose, Gram-positive reaction, positive catalase reaction, motility, and ability to utilize citrate as the sole carbon source. However, there are differences among these species, such as the presence of endospores; only the ASAE 6 and ASAE 8 bacteria possess endospores. *Bacillus* is a genus of endophytic bacteria commonly found in plants. This is supported by research findings indicating that *Bacillus* is an endophyte present in the mangroves *Rhizophora apiculata* Blume. (Deivanai et al., 2014) and *Rhizophora mucronata* (Maulani et al., 2019). In a study by Castro et al. (2017), the genus *Bacillus* was found in the mangroves *Rhizophora mangle*, *Laguncularia racemosa*, and *Avicennia* sp., as well as in *Excoecaria agallocha* L. (Durai et al., 2010). *Bacillus* is known for its high survival ability due to the presence of endospores. The endospores within the cells function to protect the bacteria from unfavorable environmental conditions, including the saline water of the sea (Nicholson et al., 2000).

The genus *Micrococcus* includes a group of round-shaped, Gram-positive, aerobic bacteria, arranged in staphylococci, tetrads, and diplococci, that are catalase-positive, grow at temperatures of 25-37°C, and are usually found on mammalian skin, soil, food products, and air (Holt et al., 1994). The isolates with the codes BSAE 9 and BSAE 15 show similarities to the genus *Micrococcus*. Bacteria from the genus *Micrococcus* have been reported to be found in the mangrove *Avicennia marina* (Soldan et al., 2019). In the study by Jiang et al. (2018), it was also reported that the genus *Micrococcus* was present in the mangrove *Avicennia marina* in China. In that study, *Micrococcus* was found to inhibit the growth of pathogens *Pseudomonas aeruginosa*, *E. faecalis*, *Acinetobacter*

*baumannii*, and *Escherichia coli*.

The genus *Pseudomonas* is characterized by rod-shaped, Gram-negative cells, aerobic, motile, unable to grow in acidic conditions, catalase-positive, and can be found in humans, animals, and plants (Holt et al., 1994). The isolates with the codes ASAE 3 and DSAE 19 show similarities to the genus *Pseudomonas*. They are characterized by their rod shape, lack of spores, motility, positive catalase reaction, ability to produce the enzyme urease, and use of citrate as the sole carbon source, but they cannot ferment sugars. The genus *Pseudomonas* has been found in the mangroves *Avicennia alba* (Yahya et al., 2014) and *Rhizophora mangle* (Castro et al., 2017). Ranjan et al. (2012) stated that bacteria of the genus *Pseudomonas* dominate the mangrove ecosystems in the Bhitarkanika waters of India. The mangrove *S. alba* grows in an open zone, specifically on the coast inundated by seawater. The environmental conditions of *S. alba* are extreme, as seawater has saline conditions. This aligns with the statement by Elabed et al. (2019) that *P. aeruginosa* bacteria have a tolerance to salinity. In the study by Zhou et al. (2015), it was reported that the genus *Pseudomonas* in the plant *Atractylodes lancea* (Thunb.) can be used as a free radical scavenger, and thus has the potential as an antioxidant.

The genus *Marinococcus* is characterized by round-shaped cells, arranged as mono, diplo, or tetrads, Gram-positive, motile, non-spore-forming, catalase-positive, and grows at temperatures of 30-37°C. The isolate ASAE 7 is closely related to the genus *Marinococcus* and is therefore referred to as *Marinococcus* sp. 1. The genus *Marinococcus* is rarely found in plants and is often found in the sea with high salinity. The mangrove *S. alba* is found growing in an open zone, specifically on the coast inundated by seawater, which makes it possible for bacteria of the genus *Marinococcus* to be present in this mangrove as an endophytic bacterium. This is consistent with Noor et al. (2012), who stated that the mangrove *S. alba* is located in an open zone, specifically on the coast inundated by seawater. In the study by Tapia-Garcia et al. (2020), the genus *Marinococcus* was found in the plant *Phaseolus vulgaris*. In Tapia-Garcia et al.'s (2020) research, *Marinococcus* was found to be capable of solubilizing phosphate, indicating that this isolate could be used as a phosphate solubilizer.

## Conclusion

From the research that has been made, we concluded that the total isolates of endophytic bacteria that were successfully collected were 22 isolates from mangrove plants *Sonneratia alba* from Enggano Island. Of the 22 isolates of mangrove endophytic bacteria *Sonneratia alba* from Enggano Island, obtained bacterial diversity consisting of 4 genus, namely the genus *Bacillus*, *Marinococcus*, *Micrococcus*, and *Pseudomonas*. 17 isolates have closeness to the *Bacillus* genus, 1 has the closeness of the *Marinococcus* genus, 2 isolates have closeness to the *Micrococcus* genus, and 2 isolates have closeness to the *Pseudomonas* genus.

### Conflict of interest

The authors declare that they have no conflicts of interest.

Role of the author:

Author\* (Fatimatuzzahra): Conceptualization, methodology, and editing. Author\*, \*\* /corresponding author (Risky Hadi Wibowo). Author\*, \*\* (Sipriyadi): Methodology, data curation, checking, reviewing, evaluating, and journal submitting. Author \*\*\* (Putri Hezekiel Claracia Simanjuntak) and Author \*\*\*\* (Yar Johan): validation, evaluating, writing, editing.

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