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by Wibowo Artikel

Submission date: 07-Oct-2022 06:34AM (UTC+0700)
Submission ID: 1918667863
File name: Wibowo_2.pdf (1.1M)
Word count: 7131
Character count: 41101

ENDOPHYTIC BACTERIA IDENTIFICATION OF RED GINGER *(ZINGIBER OFFICINALE* VAR. RUBRUM*)* FROM ENGGANO ISLAND

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Received : December 2, 2021 Accepted : August 13, 2022 Published : October 1, 2022

Abstract: Endophytic bacteria are bacteria associated with the tissues of healthy plants that are beneficial. Almost every higher plant has some endophytic bacteria, one of which is Red Ginger (*Zingiber officinale* var. Rubrum) from Enggano Island. This research aims to obtain endophytic bacteria and to identify the Red Ginger (*Zingiber officinale* var. Rubrum) endophytic bacteria collected from Enggano Island. Endophyte bacteria isolation was carried out by the paste method on medium Nutrient Agar (NA) after sterilizing the surface of plant organs using alcohol and sodium hypochlorite 5,25%. A total of 24 isolates of endophytic bacteria of Red Ginger (*Zingiber officinale* var. Rubrum) were isolated from rhizomes (stem modification), leaf sheath, and leaf sheet. Endophytic bacteria were identified based on morphological observation, biochemical tests, and Gram-staining. As a result, the diversity of bacteria consists of 7 genera, namely the genus *Bacillus, Sporosarcina, Amphibacillus, Azotobacter, Eubacterium, Pimelobacter,* and *Micrococcus*. The genus *Bacillus* consists of 4 species, the genus *Sporosarcina* consists of 6 species, the genus *Amphibacillus* consists of 1 species, the genus *Azotobacter* consists of 2 species, the genus *Eubacterium* consists of 1 species, the genus *Pimelobacter* consists of 1 species, and the genus *Micrococcus* which also consists of 1 species. *Bacillus* is the most common type of endophytic bacteria that was found in red ginger from Enggano Island.
Keywords: *Zingiber officinale* var. Rubrum; red ginger; diversity; Enggano Island

Abstrak: Bakteri endofit adalah bakteri yang berasosiasi dengan jaringan tanaman yang memiliki banyak manfaat, baik bagi manusia maupun tumbuhan itu sendiri. Hampir setiap tumbuhan tingkat tinggi memiliki beberapa bakteri endofit, salah satunya jahe merah (*Zingiber officinale* var. Rubrum) yang berasal dari Pulau Enggano. Penelitian ini bertujuan untuk mendapatkan bakteri endofit dan mengetahui keanekaragaman bakteri endofit yang diisolasi dari jahe merah (*Zingiber officinale* var. Rubrum) asal Pulau Enggano. Isolasi bakteri endofit dilakukan dengan metode tempel pada media Nutrient Agar (NA) setelah dilakukan sterilisasi permukaan organ tanaman

menggunakan alkohol dan natrium hipoklorit 5,25%. Sebanyak 24 isolat bakteri endofit jahe merah (*Zingiber officinale* var. Rubrum) diisolasi dari rimpang (modifikasi batang), pelepah daun, dan helaian daun. Bakteri endofit diidentifikasi berdasarkan pengamatan morfologi, uji biokimia, dan pewarnaan Gram. Hasilnya, keanekaragaman bakteri terdiri dari 7 genus, yaitu *Bacillus, Sporosarcina, Amphibacillus, Azotobacter, Eubacterium, Pimelobacter,* dan *Micrococcus*. Genus *Bacillus* terdiri dari 4 spesies, genus *Sporosarcina* terdiri dari 6 spesies, genus *Amphibacillus* terdiri dari 1 spesies, genus *Azotobacter* terdiri dari 2 spesies, genus *Eubacterium* terdiri dari 1 spesies, genus *Pimelobacter* terdiri dari 1 spesies, dan genus *Micrococcus* yang juga terdiri dari 1 spesies. *Bacillus* merupakan genus yang mendominasi keanekaragaman bakteri endofit yang diisolasi dari jahe merah asal Pulau Enggano.

Kata Kunci : *Zingiber officinale* var. Rubrum; jahe merah; keanekaragaman; Pulau Enggano

Recommended APA Citation :

Darwis, W., Supriyanto, A. P., Wibowo, R. H., Sipriyadi, & Supriati, R. (2022). Endophytic Bacteria Identification of Red Ginger *(Zingiber officinale* Var. Rubrum*)* From Enggano Island. *Elkawnie*, *8*(1). 119-136. https://doi.org/10.22373/ekw.v8i1.11498

Introduction

Enggano is an island located in the southwest of the island of Sumatra, which is situated in the Indian Ocean at 5° 38' south latitude and 102° 25' east longitude. This island is found in the Enggano District of the North Bengkulu Regency in the Bengkulu Province. Enggano Island is unique in terms of biogeography and evolution because it has never merged with Sumatra Island. Despite its uniqueness, Enggano Island has not been extensively explored until now. Not much is known about the local biodiversity of Enggano Island, particularly the diversity of microbes and low-level plants (Maryanto *et al*., 2017). It is believed that the geography of Enggano influences the growth and survival of local plant species. Thus, the endophytic bacteria present in the plant tissue, such as those found in the red ginger plant, are also specific to their host (Zingiber officinale var. Rubrum).

Endophytic bacteria are obtained from the surface-sterilized plant tissue that generally has no harmful effects on the plant (Santoyo et al., 2016). Various microbial communities are associated with plants. Endophytes, or microbes found in plants, are exceptional in their ability to adapt to the chemical environment of their host plants. The nature of the plant species influences the endophytic microbial community within a plant (Ding and Melcher, 2016). Endophytic bacteria are considered an untapped natural product source (Strobel, 2003; Rosenblueth and Martinez-Rodriguez, 2006). Endophytic bacteria also provide benefits to host plants because they are able to assist plants in acquiring nutrients and regulating growth hormones, allowing these plants to thrive under normal and stressful conditions (Ma et al., 2016). In response to biotic and abiotic stresses, endophytic bacteria play a role in N_2 fixation, phosphate solubilization,

phytohormone production, and inhibition of ethylene biosynthesis (Singh et al., 2017).

The Zingiberaceae family, also known as the ginger plant family, consists of rhizome-bearing plants that are frequently used in medicine due to their abundance of bioactive compounds. Different plant parts, including leaf sheaths, meristems, roots, stems, petioles, and leaves, were used to isolate endophytic bacteria affiliated with the Zingiberaceae family (Chakraborty et al., 2019). Chel et al. (2014) discovered that red ginger (Zingiber officinale Roscoe) contains a variety of endophytic bacteria, such as *Bacillus, Chryseobacterium, Pseudomonas, Flavobacterium, Agrobacterium, Serratia, Roseateles, Sphingomonas, Leclercia, Enterobacter, PaeniBacillus, Aeromonas, Acetobacter, Pantoea, Ensifer,* and *Stenotrophomonas*. Isolation of red ginger's endophytic bacteria from Enggano Island is expected to become a scientific database on the diversity of endophytic bacteria which can be used for further research. So that later these endophytic bacteria can become the candidates for raw materials for making drugs. This study aims to determine the diversity of endophytic bacteria from red ginger (Zingiber officinale var. Rubrum) on Enggano Island.

Research Method

Tools

The tools used in this study were 1 ml and 10 ml serological pipettes, rubber bulb, test tube rack, vortex mixer (Jeio tech), spritus lamp, erlenmeyer, analytical balance (Sartorius), hotplate, ose needle, object glass, Beaker glass. , autoclave, laminar air flow (Nuaire), incubator (Jeio tech), oven (Memmert), Petri dish, measuring cup, spatula, binocular microscope, colony counter (Stuart Scientific), magnetic stirrer, Durham tube, refrigerator (showcase Polytron), and test tubes.

Materials

The material used in this study was the Red Ginger plant from Enggano Island Zingiber officinale var. Rubrum), Nutrient Agar (NA), Nutrient Broth (NB), sucrose medium, glucose medium, lactose medium, maltose medium, urea medium, Simon Citrate Agar (SCA) medium, safranin, Lugol's solution, nystatin, crystal violet, Sodium hypochlorite (NaClO) 5.25%, Hydrogen peroxide (H2O2) 3% solution, distilled water, spirit, cotton, plastic wrap, aluminium foil, 70% alcohol, 96% alcohol, disposable Petri dishes, and oil immersion.

Sample Collection

Red ginger plant (*Zingiber officinale* var. Rubrum) from Enggano Island sized about 50 cm was collected by taking plant parts containing endophytic bacteria such as rhizome (modified stem), leaf midrib, and leaf sheet. Then, it is stored in a plastic bag to keep it dry so that fungus does not grow on the red ginger.

Purification of Endophytic Bacteria

The endophytic bacterial isolates were then purified by transferring the bacterial colonies to a petri dish containing NA using the scratch method (Putri et al., 2017), followed by 24-hour incubation at 27-30 ^{o}C in an incubator. Pure cultures can also be obtained by growing bacterial isolates on sloping media to multiply cultures or as supplies (Irianto, 2006).

Morphological Identification of Cell Colony and Endophytic Bacteria Staining

Endophytic bacteria growing on the media were observed for their morphological characteristics based on their shape, arrangement, colony color, surface properties, margins and elevation (Lay, 1994). One loop of an endophytic bacterial isolate was placed on an alcohol-cleaned microscope slide. A drop of distilled water was then added and a spirit light was lit. After that, crystal violet is added and allowed one minute to settle. The samples were then cleaned with distilled water, dripped with Lugol's solution, and allowed to settle for two minutes. The preparations were rinsed with 96% alcohol and safranin, waited 30 seconds, and then rinsed with distilled water again. The preparations were immersed in oil before being examined under a microscope with a 10x100 magnification. Gram-positive bacterial cells will be identified as purple, whereas Gram-negative bacterial cells will be red (Lay, 1994).

Biochemical Test

Sugar Test

The media used were sucrose, glucose, maltose and lactose media. Each medium is placed in a sterile test tube which has been given a Durham tube. The media was then sterilized using an autoclave. After cooling, pure bacterial cultures were inoculated into the media and incubated for 24-48 hours at a temperature of 27-30 ^{o}C. Positive results are indicated by a change in the color of the medium to yellow and the formation of gas in the Durham tube (Cappuccino and Sherman, 2013; Chi-Chu, 2010.

Urea Test

A sterilized urea medium is placed in a sterile test tube. The media was then inoculated with one loop of pure bacterial culture and incubated at 27-30 ^{o}C for 24-48 hours. A positive test result is shown by a change in color from orange to pink in the medium (Cappuccino and Sherman, 2013).

Catalase Test

Glass objects are cleaned using 70% alcohol. Then one loop of the bacterial isolate was placed on a slide and 3% Hydrogen Peroxide (H_2O_2) reagent was dripped on top of the bacterial isolate. Positive test results are indicated by the formation of bubbles of O_2 gas (Cappuccino and Sherman, 2013).

Citrate Test

This test uses the SCA (Simon Citrate Agar) medium. One loop of bacterial culture is inserted vertically into the medium to inoculate it. The bacteria-containing medium was cultured for 18 to 24 hours at 27-30 ^{o}C. Positive tests are indicated by a change in color from green to blue in the media (Cappuccino and Sherman, 2013).

Motility Test

The motility test utilized a semi-solid Nutrient Agar (NA) medium in an autoclave-sterilized test tube. As deep as the media, one loop of pure bacterial culture was introduced vertically. The media was incubated for 18-24 hours at 27-30 ^{o}C and then the growth of bacteria was observed. If the growth is linear, the test result is negative (not motile), whereas if it extends beyond the inoculation line, the result is positive (motile) (Fardiaz, 1992).

Data Analysis

Data is analysed qualitatively by describing the result of the identification (Sajarkani & Nasoetion, 1989).

Results and Discussions

Result of Red Ginger's Endophytic Bacterial Isolation from Enggano

24 strains of endophytic bacteria were obtained from portions of the red ginger plant (*Zingiber officinale* var. Rubrum) from Enggano Island, including 9 isolates from the rhizome (modified stem), 8 isolates from the leaf midrib, and 7 isolates from the leaf sheet. Figure 1 depicts the isolated ginger red plant components.



Figure 1. Red ginger *(Zingiber officinale* var. Rubrum) from Enggano; (a) rhizome (modified stem), (b) leaf midrib, (c) leaf sheet.

The isolate codification was made based on the name of the plant JM (Red Ginger), followed by the place where the plant was taken (E) Enggano, and ended by letter/s representing the plant organ; D (Leaves/strands), R (rhizome/stem modification), PD (leaf midrib).

The morphology observation results of endophytic bacterial isolates of Red Ginger from Enggano Island can be seen more clearly in Figure 2.

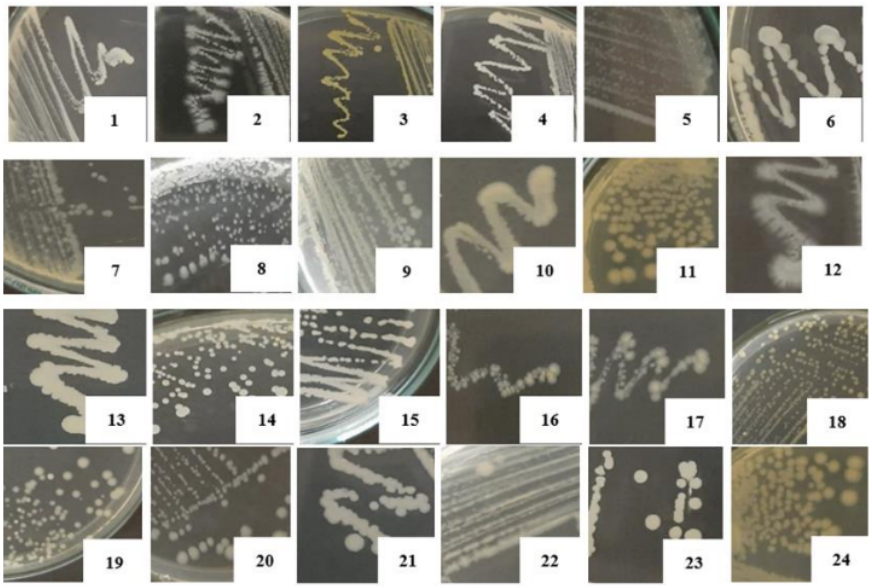


Figure 2. Morphological colony the isolates of Red ginger's endophytic bacteria 1-7 coded JMED, 8-16 coded JMER, 17-24 coded JMEB

Most of the isolates of endophytic bacteria had a circular appearance, only a small number of isolates had an irregular appearance, and one isolate had a pinpoint appearance. The surface of the colony was mostly smooth, some of the surfaces were concentric, while the others were contoured, radiated, and wrinkled. For elevation, the type of flat is more dominant, some others have a convex elevation. The edges of the colonies were mostly entire, while some other colonies had undulate, serrated, filamentous, and lobate edges. The color of endophytic bacterial colonies varies from cream, white, yellow, and yellowish white. Most endophytic bacterial colonies are white.

Red ginger's Endophytic Bacterial Identification

Bacterial identification was carried out manually by comparing data on Gram staining and biochemical tests with the characteristics of the bacterial genus contained in Bergey's Manual of Determinative Bacteriology Ninth Edition.

Gram Staining of Endophytic Bacteria

The results of Gram staining and microscopic observations of red ginger endophytic bacteria from Enggano Island are presented in Figure 3.

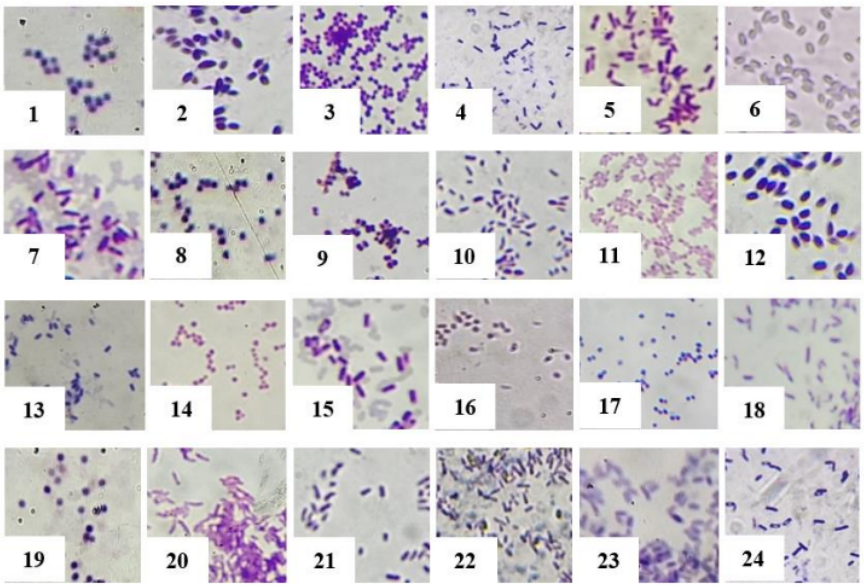


Figure 3. Gram staining of the isolates of Red ginger's endophytic bacteria 1-7 coded JMED, 8-16 coded JMER, 17-24 coded JMEB

Gram staining revealed that isolates JMED 1, JMED 3, JMER 8-9, JMER 11, JMER 14, JMER 16, JMEPD 17, and JMEPD 19 had cocci shape with mono, diplo, strepto and staphylo arrangement. isolates JMED 2, JMED 4-7, JMER 10, JMER 12-13, JMER 15, JMEPD 18, JMEPD 20-24 had bacilli forms with mono, diplo and strepto arrangements.

A total of 22 isolates of endophytic bacteria (JMED 1-7, JMER 8-10, JMER 12-13, JMER 15-17, JMEPD 18-24) were classified as Gram-positive bacteria shown as purple. Meanwhile, only 2 isolates, namely JMER 11 and JMER 14 belonged to the group of Gram-negative bacteria as indicated by the appearance of red cells on observation under a microscope. The cell wall of Gram-positive bacteria contains thick peptidoglycan so that it can retain the crystal violet-iodine complex and appear purple, whereas the cell wall of Gram-negative bacteria has a thinner peptidoglycan layer but contains a large amount of lipopolysaccharide so that it loses the crystal-iodine complex during the decolorization process with alcohol and absorbs the second dye, safranin so that the cells appear red (Lande et al., 2019).

Biochemical Test of Endophytic Bacteria

After the completion of microscopic observations of Gram staining, biochemical tests were carried out to determine the physiological characteristics of the endophytic bacterial isolates of Red Ginger from Enggano Island. The

biochemical tests included sugar tests (glucose, lactose, and sucrose), catalase test, urea test, motility test, and citrate tests. The results of biochemical tests of red ginger isolates from Enggano Island are shown in Table 1.

Table 1. Result of biochemical tests of red ginger isolates from Enggano Island

Isolate code	Sugar test			Catalase test	Urea test	Motility test	Citrate test
	Glucose	Lactose	Sucrose				
JMED 1	-	+	-	+	+	+	-
JMED 2	+	+	+	-	+	+	-
JMED 3	+	+	-	+	+	+	+
JMED 4	+	+	-	+	+	+	-
JMED 5	+	+	+	+	-	+	-
JMED 6	+	+	+	+	+	+	-
JMED 7	+	+	+	+	-	+	-
JMER 8	+	+	+	+	+	+	-
JMER 9	+	+	-	+	+	+	-
JMER 10	+	+	+	+	+	+	+
JMER 11	+	+	-	+	+	+	+
JMER 12	-	+	+	-	-	+	-
JMER 13	+	+	+	+	+	+	-
JMER 14	+	+	+	+	-	+	-
JMER 15	+	+	+	+	+	+	-
JMER 16	-	+	-	+	+	+	+
JMEPD 17	+	+	+	+	+	+	+
JMEPD 18	+	+	+	+	+	+	+
JMEPD 19	+	+	+	+	+	-	-
JMEPD 20	+	+	+	+	+	+	+
JMEPD 21	+	+	+	+	+	+	-
JMEPD 22	+	+	+	+	+	+	-
JMEPD 23	+	+	-	+	+	+	-
JMEPD 24	+	+	+	+	+	+	+

Biochemical tests were used to determine the physiological characteristics of bacterial isolates. The results of this test will greatly affect the identification of bacteria because it can be used to distinguish the species.

Three different forms of carbohydrates—glucose, lactose, and sucrose—are used in the sugar test. A change in the medium's color from orange to yellow and the presence of bubbles in the Durham tube are signs of successful results. According to Lay (1994), the fermentation of carbohydrates results in the production of acid, which lowers the pH of the media, causing the media's color to change to yellow if a phenol red indicator is present in the test medium. The gas that is created enters the Durham tube, pushing the liquid within. Only three

isolates of endophytic bacteria—JMED 1, JMER 12, and JMER 16—showed negative results in the sugar test using glucose, whilst 21 isolates of endophytic bacteria demonstrated positive results. All isolates were positive in the sugar test using lactose while seven isolates tested negative in the test using sucrose, including JMED 1, JMED 3, JMED 4, JMER 9, JMER 11, JMER 16, and JMEPD 23.

The catalase test was used to assess whether bacteria were capable of producing the catalase enzyme. According to Hadioetomo (1993), the creation of bubbles shows that the bacteria are capable of producing the enzyme catalase, which breaks down hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2). In the catalase test, 22 isolates of endophytic bacteria showed positive results or were able to manufacture catalase enzymes; just two isolates, JMED 2 and JMER 12 showed negative results

The urea test aims to determine the ability of bacteria to produce urease enzymes which are useful for breaking down urea into ammonium and carbon dioxide (CO2). A positive result is characterized by a change in the medium to pink (very pink). Color changes can occur when the enzyme urease breaks the carbon and nitrogen bonds to form ammonia. Ammonia causes the pH of the media to become alkaline so that the phenol red indicator will turn pink in the media (Cappuccino and Sherman, 2005). The urea test results showed that 20 isolates of endophytic bacteria could produce urease enzyme or positive results, while only 4 isolates, namely JMED 5, JMED 7, and JMER 12, were tested negative.

The motility test aims to see the movement of bacteria. A positive (motile) result is indicated by the presence of patches around the streak on the media which indicates that the bacteria are mobile and a negative (non-motile) result if there are no encroachments around the streak which indicates that the bacteria are immobile (Detha et al., 2019). According to the results of the motility test, only JMEPD 19 isolates were non-motile or negative. Another 23 endophytic bacteria isolates were positive or motile.

According to Lay (1994), the citrate test was used to determine the ability of bacteria to use citrate as the sole source of energy and carbon. Citrate is the product of the reaction between acetyl coenzyme A (CoA) and oxaloacetic acid (4C). A positive result is indicated by a change in the color of the medium from green to dark blue. This happens because the citrate produced by the citrase will form oxaloacetic and acetic acids which are then converted into pyruvic acid and carbon dioxide by an enzymatic process. During the reaction, the pH of the medium becomes alkaline because carbon dioxide combines with sodium (Na) and water (H_2O) to form sodium carbonate (Na_2CO_3). Sodium carbonate will change the bromothymol blue indicator in the media, so the media changes color from green to dark blue (Cappuccino & Sherman, 2005). In the citrate test, 8 isolates of endophytic bacteria showed positive results, namely isolates JMED 3,

JMER 10, JMER 11, JMER 16, JMEPD 17, JMEPD 18, JMEPD 20, and JMEPD 24.

Identification Result of Endophytic Bacteria

Using Bergey's Manual of Determinative Bacteriology, Ninth Edition, it is feasible to identify the bacterial genus based on morphological observations, Gram staining, and biochemical testing. Table 2 displays the genus acquired from morphological identification, Gram staining, and biochemical analysis.

Table 1. Identification result of red ginger's endophytic bacterial isolates from Enggano Island

Isolate code	Part of plant origin	Species name
JMED 1	Leaf sheet	*Sporosarcina* sp 1.
JMED 2	Leaf sheet	*Amphibacillus* sp 1.
JMED 3	Leaf sheet	*Sporosarcina* sp 2.
JMED 4	Leaf sheet	*Bacillus* sp 1.
JMED 5	Leaf sheet	*Bacillus* sp 2.
JMED 6	Leaf sheet	*Bacillus* sp 3.
JMED 7	Leaf sheet	*Bacillus* sp 2.
JMER 8	Rhizome (modified stem)	*Sporosarcina* sp 3.
JMER 9	Rhizome (modified stem)	*Sporosarcina* sp 4.
JMER 10	Rhizome (modified stem)	*Bacillus* sp 4.
JMER 11	Rhizome (modified stem)	*Azotobacter* sp 1.
JMER 12	Rhizome (modified stem)	*Eubacterium* sp 1.
JMER 13	Rhizome (modified stem)	*Pimelobacter* sp 1.
JMER 14	Rhizome (modified stem)	*Azotobacter* sp 2.
JMER 15	Rhizome (modified stem)	*Bacillus* sp 3.
JMER 16	Rhizome (modified stem)	*Sporosarcina* sp 5.
JMEPD 17	Leaf midrib	*Sporosarcina* sp 6.
JMEPD 18	Leaf midrib	*Bacillus* sp 4.
JMEPD 19	Leaf midrib	*Micrococcus* sp 1.
JMEPD 20	Leaf midrib	*Bacillus* sp 4.
JMEPD 21	Leaf midrib	*Bacillus* sp 3.
JMEPD 22	Leaf midrib	*Bacillus* sp 3.
JMEPD 23	Leaf midrib	*Bacillus* sp 1.
JMEPD 24	Leaf midrib	*Bacillus* sp 3.

The identification results of 24 isolates of red ginger endophytic bacteria from Enggano Island, showed close relationships with 7 genera, namely *Bacillus, Sporosarcina, Amphibacillus, Azotobacter, Eubacterium, Pimelobacter,* and *Micrococcus*. The abundance of endophytic bacteria in red ginger from Enggano Island can be seen in Figure 4

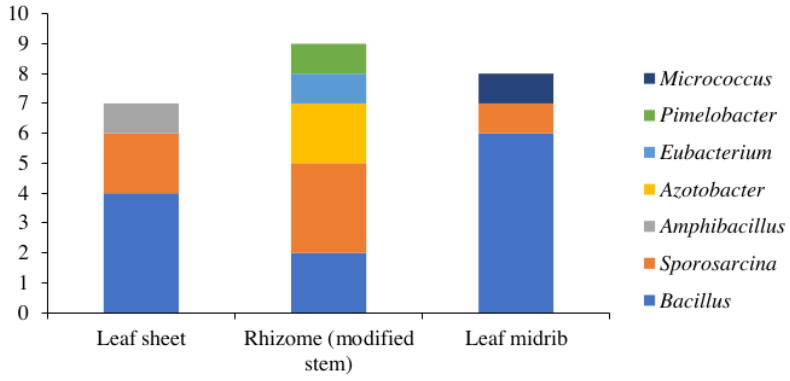


Figure 3. Abundance diagram of red ginger's endophytic bacterial isolates from Enggano Island

Twelve of the total 24 endophytic bacteria isolates belonged to the genus *Bacillus*. This is consistent with the findings of Rohini et al. (2018), who discovered that *Bacillus* is one of the most commonly isolated genera in red ginger plants. The population of endophytic bacteria in the plant's rhizome (modified stem) is greater than in the leaves. This is because the rhizome contains a high concentration of compounds. Red ginger plants store fat, protein, starch, and other nutrients in rhizomes (modified stems) that are beneficial to the plant and its endosymbionts. According to Dalal and Kulkarni (2013), the rhizome has the highest population of endophytic microbes compared to other plant parts because it is the first point of entry for microbes into the plant. Endophytic bacterial species associated with plants were influenced by the tissue taken, plant age, plant genotype, soil fertility, and the season when plant parts were explored, according to Kartikawati and Gusmaini (2018).

A *bacillus* is a group of rod-shaped bacteria, Gram-positive, producing endospores that are oval or cylindrical, aerobic or facultative-anaerobic, chemoorganotrophs, and catalase positive (Holt et al., 1994). The isolates JMED 4-7, JMER 10, JMER 15, JMEPD 18, and JMEPD 20-24 were similar to the genus *Bacillus*. which then named *Bacillus* sp 1., *Bacillus* sp 2., *Bacillus* sp 3., and *Bacillus* sp 4. *Bacillus* is a genus of endophytic bacteria that are often found. This is supported by the results of research showing that *Bacillus* is also found on the stems of *Bacopa monnieri* (Jasim et al. 2016), *Phaseolus vulgaris* leaves (Lopes et al., 2017), *Phoenix dactylifera* roots (Yaish, 2017), and *Zea mays* seeds (Rath et al., 2018).

Bacillus has been shown to produce spores that are resistant to harsh environments. *Bacillus* bacteria provide numerous benefits, including promoting

plant growth and protecting against phytopathogenic microorganisms, insects, and nematodes (Lopes et al., 2018).

The genus *Sporosarcina* is a group of spherical/coccal bacteria, sometimes arranged in diplococci or tetrads, Gram-positive, motile, having a round endosopra, chemoorganotrophic, aerobic, cream to orange colonies and growing at a temperature of 15-37 ^{o}C (Holt et al., 1994). The isolates JMED 1, JMED 3, JMER 8, JMER 9, JMER 16, and JMEPD 17 were similar to the genus *Sporosarcina*. The isolates were then referred to as *Sporosarcina sp 1., Sporosarcina sp 2., Sporosarcina sp 3., Sporosarcina sp 4., Sporosarcina sp 5.,* and *Sporosarcina sp 6*.

Sporosarcina endophytic bacteria were discovered in the leaves of Phaseolus vulgaris (Costa et al., 2012), roots of transgenic Chinese cabbage (*Brassica campestris* L.) grown in the laboratory (Haque et al., 2016), and pneumatophores of *Avicennia* marina L. *Sporosarcina* bacteria are capable of producing siderophores and IAA. Siderophores are iron-chelating ligands that help plants increase iron (Fe III) solubility (Renshaw et al., 2002). Meanwhile, IAA production promotes plant root growth by promoting cell elongation or cell division (Glick and Bashan, 1997).

The genus *Amphibacillus* is a group of rod-shaped bacteria that are Gram-positive, motile, facultative anaerobes, chemoorganotrophic cells, catalase-negative, and grow at temperatures ranging from 25 to 45 degrees Celsius (Holt et al., 1994). JMED 2 isolate resembled the genus *Amphibacillus*. The isolate was given the name *Amphibacillus* sp. 1. Endophytic bacteria of the genus *Amphibacillus* were discovered for the first time in the fern *Dicksonia sellowiana* (Barros et al., 2010). This bacterium has also been found in yellow root plant tissue from Enggano Island (*Arvangelisia flava* (L.) Merr) (Reformanda et al., 2021).

Azotobacter is an oval-shaped bacterial genus that does not produce endospores, is Gram-negative, motile or nonmotile, aerobic, chemoorganotrophic, catalase positive, lives in water and soil, and can be associated with plant roots (Holt et al., 1994). JMER 11 and JMER 14 were found to be related to the genus *Azotobacter*. These isolates were given the names *Azotobacter* sp. 1 and *Azotobacter* sp., both have distinct characteristics. *Azotobacter* endophytic bacteria were discovered in *Oryza sativa* L. var. Sabita and *O. eichingeri* (Banik et al., 2016). *Azotobacter* bacteria have the ability to bind nitrogen, promote plant growth, and dissolve phosphate (Prakash et al., 2021).

The genus *Eubacterium* is a group of irregular rod-shaped bacteria, cells arranged singly, in pairs or in chains, Gram-positive, motile, anaerobic, chemoorganotrophic, catalase negative, and can be found in feces, animals, plants, and soil (Holt et al., 1994). JMER 12 has similarities with the genus Eubacterium. The isolate was later referred to as Eubacterium sp 1. Bacteria of the genus *Eubacterium* have rarely been reported as endophytes in plants. Research

conducted by Xia et al. (2013) was the first study to report this bacterium as an endophyte on elephant grass (*Panicum virgatum* L.).

Pimelobacter is a genus of irregular rod-shaped bacteria that are Gram-positive, non-sporing, aerobic, have white or yellowish-white colonies, are catalase positive, and live in the soil (Holt et al., 1994). JMER 13 is related to the genus *Pimelobacter*. The isolate was later named *Pimelobacter* sp 1. Endophytic bacteria from the genus *Pimelobacter* have been found in the roots of banana plants (*Musa* sp.) and wheat roots (Zhai et al., 2016). (Conn and Franco, 2004).

Micrococcus is a genus of spherical, Gram-positive, aerobic, catalase-positive bacteria that grows at temperatures ranging from 25 to 37 ^{o}C and typically lives on mammalian skin, soil, food products, and air (Holt et al., 1994). Because JMEPD 19 shares characteristics with the genus *Micrococcus*, the isolate was later named *Micrococcus* sp 1. Bacteria from the genus *Micrococcus* have been found in cereal crops (Lata et al., 2019) as well as *Corchorus olitorius* (Haidar et al., 2018). According to the findings of Asghari et al. (2019), bacterial strains with the same 16S rRNA gene sequence as the genus *Micrococcus* have the best inhibitory ability against plant pathogens that cause crown gall disease, namely, *Agrobacterium tumefaciens* and *Agrobacterium vitis*. Furthermore, research has shown that endophytic bacteria from the genus *Micrococcus* can stimulate the growth of their host plants (Prakash et al., 2014).

Research conducted by Rohini et al. (2017) found 5 genera of endophytic bacteria isolated from the rhizome (modified stem) of the ginger plant (*Zingiber officinale*). The 5 genera include *Bacillus, Enterobacter, Burkholderia, Pantoea*, and *Alcaligenes*. Another study conducted by Zhang et al. (2018) discovered several genera of endophytic bacteria from ginger plants (*Zingiber officinale* Roscoe), namely *Serratia, Enterobacter, Acinetobacter, Pseudomonas, Stenotrophomonas, Bacillus, Agrobacterium*, and *Ochrobactrum*. This current study obtained 7 genera, namely *Bacillus, Sporosarcina, Amphibacillus, Azotobacter, Eubacterium, Pimelobacter*, and *Micrococcus*. This indicates that another genus has been isolated from the red ginger plant from Enggano Island (*Zingiber officinale* var. Rubrum) so that the endophytic bacteria obtained are more diverse.

Conclusion

According to the result of the current study, it can be concluded that endophytic bacterial isolation from parts of the red ginger plant (Zingiber officinale var. Rubrum) from Enggano Island succeeded in obtaining 24 isolates of endophytic bacteria. Those isolates included 9 isolates from the rhizome (modified stem), 8 isolates from the leaf midrib, and 7 of the leaves. Besides that, the study also discovered diverse bacteria from 7 genera, that includes the genus *Bacillus*, *Sporosarcina*, *Amphibacillus*, *Azotobacter*, *Eubacterium*, *Pimelobacter*,

and *Micrococcus*. These endophytic bacteria obtained are more diverse than the previous research on endophytic bacteria from ginger plants.

Acknowledgements

The author would like to thank LPPM, Universitas Bengkulu which has funded this research through the "Hibah Unggulan LPPM UNIB 2020" Research Scheme with contract number: 1985/UN30.15/PG/2020 on behalf of the chairman Drs. Welly Darwis, M.S. The authors also thank Mashudi from the Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember.ITS Keputih Campus, Sukolilo, Surabaya who help us to translate this manuscript, and Masrukhin from Biosystematics and Evolution Research, Cibinong Science Center, Cibinong who help us to identify the bacterial endophyte isolates and the people of Enggano Island, Bengkulu Province and all parties involved in this research.

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