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ENDOPHY BACTERIAL ISOLATE TEST FOR INHIBITORS OF LEAF DROPPING DISEASE (Pestalotiopsis sp) IN THE LABORATORY

Fenty Maimunah Simbolon[1], Syamsafitri[2], Mindalisma[3] and Ramayanti A[4]
[1234]Faculty of Agriculture, Islamic University of North Sumatra , Indonesia
Email: fenty.simbolon@gmail.com

Abstract
This research was conducted at the Sungai Putih Research Center, Rubber Research Center, Kec. Galang, Kab. Deli Serdang North Sumatra, North Sumatra Province. This research was conducted from December 2020 to April 2021. The aim of this study was to test the ability of Endofid bacterial isolates to inhibit the development of leaf fall disease (Pestalotiopsis sp) in the laboratory. The study used a non-factorial completely randomized design (RAl) with endophytic bacteria treatment with 6 levels of treatment, namely control (SPE0), isolate 1 from Sungai Putih (SPE1), isolate 2 from Sungai Putih (SPE2), isolate 3 from Bandar Betsi (BBE3).), isolate 4 from Bandar Betsi (BBE4), isolate 5 from Bandar Betsi (BBE5). The parameters observed were the identification of the fungus Pestalotiopsis sp and indophytic bacteria, the percentage of broad inhibition of the fungus Pestalotiopsis sp, the Antagonist Test of Endophytic Bacteria against Pestalotiopsis sp by In Vitro, the Pathogenicity Test of Chili Against Selected Endophytic Bacteria, the Growth Test of Chili Plants against Selected Endophytic Bacteria. The results of statistical analysis showed that the endophytic bacterial isolate had a significant effect on the percentage of inhibition of the area of Pestalotiopsis sp. The growth of chili seed sprouts by immersing the endophytic bacteria solution resulted in a smaller percentage than the control, but it could increase the growth of plant height and root length of chili plants.

Keywords: Endofit Bacteria, Pestalotiopsis sp.

1. Introduction

Rubber plant (Havea brasilliensis) is one of the important agricultural commodities for Indonesia and internationally. In Indonesia, rubber is one of the agricultural products that support the country's economy a lot. The foreign exchange earnings obtained from rubber are quite large, even Indonesia has once controlled world rubber production by surpassing the results from other countries and the country of origin of the rubber plant itself, namely in the plains of South America. Indonesia's position as the number one rubber producer in the world was finally pushed by two neighboring countries, Malaysia and Thailand. At first, Malaysia shifted Indonesia's position to number two, but unexpectedly Thailand poked Malaysia and is now the largest rubber producer in the world, while Indonesia is still in second place. The third position was occupied by Malaysia, which was thrown from the number one and second positions. The area of rubber land owned by Indonesia reaches 3-3.5 million hectares. This is the largest rubber plantation in the world. The productivity of rubber land in Indonesia is low on average and the quality of the rubber produced is also unsatisfactory (Anonymous, 2010).

Pestalotiopsis sp, as one of the causes of leaf spot disease, has recently been reported to be one of the fungi that causes rubber leaf fall disease (GDK). The attack on GDK disease caused by Pestalotiopsis sp, is almost the same as GDK disease caused by Fusicoccum sp because they both have concentric lines on the affected leaves. Which is one of the factors that causes difficulties in identifying the cause of the disease that attacks if you only look at the external symptoms.

This disease was initially found to attack rubber in North Sumatra in 2016, then spread to South Sumatra in 2017 to 2018 (Samsi, 2019).

The first outbreak of Pestalotiopsis sp. in rubber plantations in Malaysia was in 1987 and 2003. Meanwhile, in Indonesia, the first outbreak of leaf fall was in 2016 and spread from rubber plantations in North Sumatra to South Sumatra. Until mid-2018, this disease has spread to Lampung, Central Java, East Java, South Kalimantan and Central Sulawesi. The total area of this disease is more than 22,000 ha, causing canopy loss > 25% and latex production loss > 50 (Cahyo, 2018).

Some of the controls that help suppress the leaf fall disease of Pestalotiopsis sp are Preventive by misting sulfur at night. Sulfur fumigation can be given at a dose of 5-7 kg/ha, 1 week intervals with 4 applications and exhalation is done at 02.00 -

05.00. The blowing agent used is the active ingredient Thiophanate methyl with a dose of 2 ml/1 on the leaves above the soil surface to control the spores of Pestalotiopsis sp. Application of fungicides with the active ingredients of propicon azole, hexaconazole at a dose of 5 m/1 on the plant crown 3 times with 1 week intervals.

Endophytic bacteria are bacteria that naturally live in plant tissues without causing negative effects. Several types of endophytic bacteria have been reported to have antibacterial, antimalarial, and antifungal activities (Purwanto et al., 2014). Currently, many studies have been carried out on endophytic bacteria for the search for new bioactive compounds. The advantages of utilizing this endophyte are (Kharwar et al., 2008). Extracts from this plant are also known to have antimicrobial activity.

Utilization of endophytic bacteria is a biological control agent that is currently being developed for the control of various plant diseases. Endophytic bacteria are reported to produce antibiotics and degrading enzymes that can inhibit the growth of pathogens in vitro, increasing plant resistance to pathogens by inducing plant resistance reactions (Haliman, 2005).

Biological control using antagonistic microorganisms is an alternative that is currently being studied and widely used as plant disease control. Biological control is the protection of plants from pathogens, including the spread of antagonistic microorganisms after or when a pathogen infection occurs (Agrios, 2011).

2. Methods

2.1 Place and Time of Research

The research was carried out at the Sungai Putih Research Center, Karet Research Center, Kec. Galang, Kab. Deli Serdang, North Sumatra. at an altitude of ± 25 meters above sea level with a flat topography. This research was conducted in December 2020 to April 2021.

2.2 Research Methods

a. Exploration method: To obtain or isolate endophytic bacteria from rubber leaves of clone Pb630 from Sungai Putih and Bandar Betsi gardens.
b. This research will be carried out using a non-factorial completely randomized design (CRD) consisting of 6 treatments and 1 control using 3 replications. The treatments tested consist of:
   SPE0 = No Treatment/Control
   SPE1 = Use of Isolated Endophytic Bacteria 01
   SPE2 = Use of Isolated Endophytic Bacteria 02
   BBE3 = Use of Isolated Endophytic Bacteria 03
   BBE4 = Use of Isolated Endophytic Bacteria 04
   BBE5 = Use of Isolated Endophytic Bacteria 05.

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3. Results and Discussion

3.1 Results of Isolation of Endophytic Bacteria from Rubber Leaves

From the results of this exploration of endophytic bacteria, 5 isolates were obtained from the leaves of healthy rubber plants taken from the rubber plantations of Bandar Betsi and Sei Putih.

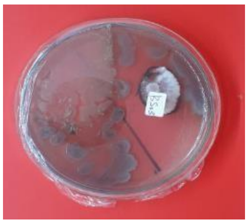
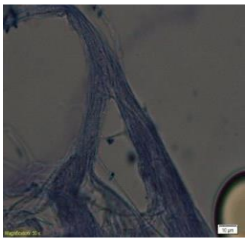
Table 1. Results of Isolation of Endophytic Bacteria from Rubber Leaves

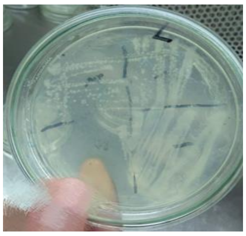
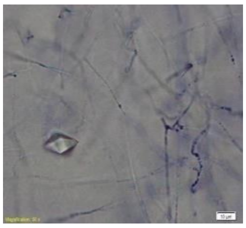
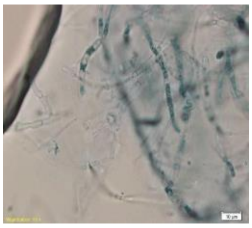
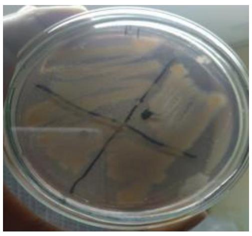
Isolation Code	Origin of Isolation	Healthy Leaf Rubber Clone	Treatment Code	Gram
SPE1	B P. Sungai Putih	Pb630	Isolat 1	+
SPE2	B P. Sungai Putih	Pb630	Isolat 2	+
BBE3	B P. Bandar Betsi	Pb630	Isolat 3	-
BBE4	B P. Bandar Betsi	Pb630	Isolat 4	+
BBE5	B P. Bandar Betsi	Pb630	Isolat 5	-

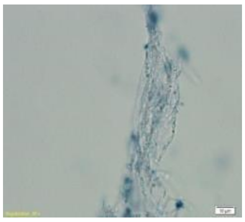
The results of macroscopic and microscopic identification showed that the isolation of endophytic bacteria had different characteristics and characteristics on NA (Nutrient Agar) media. The 5 isolates of endophytic bacteria can be seen in Table 2.

3.2 Macroscopic Characteristics of Endophytic Bacteria Shape, Elevation, Edge, Gram The endophytic bacteria that were isolated were from Sungai Putih and Bandar Betsi.

Table 2. Macroscopic Characteristics of Endophytic Bacteria Shape, Elevation, Edge, Gram

No	Isolation Code	Bacteria Shape	Elevation	Edge	Gram
1.	 SPE 1 (Macroscopic)	Bund ar (Bulat)	Flat	Flat	Gram Positif
	 Microscopic zoom in 100 x 10	Streptococc us (hand in chain)	Flat	Flat	Gram Positif

2.	 SPE 2 (Macroscopic)	Bundar(Bulat)	Flat	Flat	Gram Positif
	 Microscopic zoom in 100 x 10	Streptococc us, (hand in chain)	Flat	Flat	Gram Positif
3.	 BBE 3 (Macroscopic)	Bundar (Bulat)	Flat	Flat	Gram Negatif
	 Microscopic zoom in 100 x 10	Streptococc us, (hand in chain)	Flat	Flat	Gram Negatif
4.	 BBE 4 (Macroscopic)	Bundar(Bulat)	Flat	Flat	Gram Positif

	 Microscopic zoom in 100 x 10	Cocci shape	Flat	Flat	Gram Positif
5.	 BBE 5 (Macroscopic)	Bundar(Round)	Flat	Flat	Gram Negatif
	 Microscopic zoom in 100 x 10	Staphylacoc cus (bunch of ol)	Flat	Flat	Gram Negatif

Source: Research Collection

Endophytic bacteria that have been isolated have obtained several isolates, based on the morphological characteristics of these isolates showing different characters from one isolate to another. The morphology of the obtained endophytic bacterial colonies have a round and irregular shape. The edge of the colony is dominated by flat type and the rest is split and jagged. The type of elevation varies from flat, raised, and convex. The results of Gram staining are known from the bacterial isolates that belong to the group of Gram positive bacteria.

The results of the characterization of isolated endophytic bacteria varied greatly from one isolate to another in terms of their morphological characteristics, thus indicating the possibility that the isolated bacterial isolates were of different species. Based on these morphological differences, it can be assumed that there is more than one type of bacteria found in one plant tissue.

The diversity of endophytic bacteria that can be affected can also be influenced by plant tissues and environmental conditions. Balosi et al. (2014) stated that the population of endophytic bacteria was more abundant in roots and decreased in stems and leaves. Munif (2012), stated that the presence of endophytic bacteria in plant tissues is influenced by biotic and abiotic factors. Biotic factors are plant tissue, plant genotype and plant age used for isolation. Meanwhile, abiotic factors that influence are environmental factors such as organic matter in the soil, fertilization, pesticide application and soil properties. The number of endophytic bacteria in plants cannot be determined with certainty, but can be isolated using agar media. Purwanto et al, (2014) stated that endophytic bacteria that can be isolated are bacteria that can adapt to new environments.

3.3 Percentage of Inhibition of Bacteria (%)

The results of analysis of variance showed that the use of endophytic bacteria had a significant effect on the inhibition of the broad development of the fungus Pestalotiopsis sp. The results of the average percentage of bacterial inhibition are presented in Table 3.

Table 3. Percentage of Bacterial Inhibition of 8 HIS (%)

Treatment	Endophytic Bacteria Inhibitory	
SPE0 (kontrol)	0,00 (0,71)	e
SPE1 (Isolat 1, Sei Putih)	35,68 (6,01)	d
SPE2 (Isolat 2, Sei Putih)	43,37 (6,62)	c
BBE3 (Isolat 3 Bandar Betsi)	77,16 (8,81)	a
BBE4 (Isolat 4 Bandar Betsi)	56,12 (7,50)	b
BBE5 (Isolat 5 Bandar Betsi)	54,40 (7,40)	b

Note: Numbers followed by letters that are not the same in the same treatment column and row are significantly different at the 5% level based on the DMRT . test

From Table 3 it can be seen that the largest percentage of bacterial inhibition was found in the BBE3 treatment (77.16%) which was significantly different from other treatments, the BBE4 treatment (56.12%) was not significantly different from the BBE5 treatment (54.40%), and both the treatment was significantly different from the treatment of SPE2 (43.37%), SPE1 (35.68%) and SPE0 (0.00%). The difference in the percentage of bacterial inhibition that occurred was due to the different bacterial isolates tested, resulting in different inhibition and activity. The graph of the inhibition of endophytic bacteria on the widespread development of the fungus Pestalotiopsis sp can be seen in Figure 1.

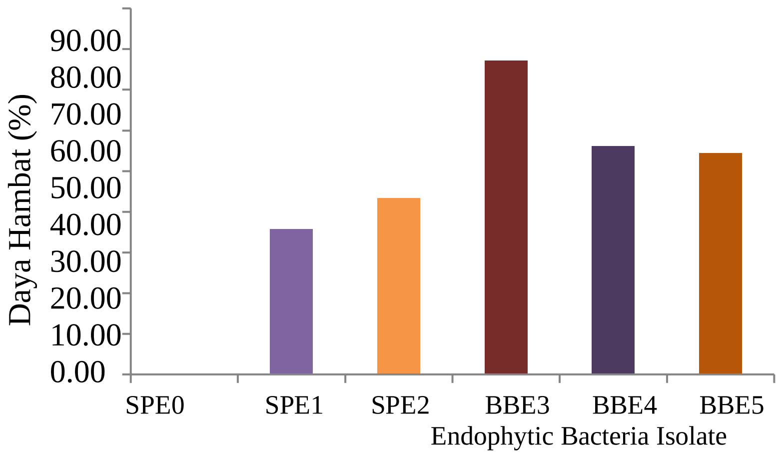


Figure 1. Graph of Inhibitory Power of Endophytic Bacterial Isolates on Broad Development of Pestalotiopsis sp

From Figure 1 it can be seen that the percentage of inhibition of the broad development of the fungus Pestalotiopsis sp. showed that endophytic bacteria from Bandar Betsi isolate in isolate 3 were more able to inhibit the growth of the pathogen Pestalotiopsis sp. Lingga (2010), said that the advantage of endophytic fungi as biological control agents is that they are able to increase the availability of nutrients and produce growth hormones in their host plants.

The control treatment showed that it did not have the potential to inhibit or had no potential as a biological control agent against Pestalotiopsis sp because it was unable to inhibit Pestalotiopsis sp. Even the controls experienced rapid growth of the Pestalotiopsis sp.

3.4 Antagonist Test of Endophytic Bacteria Against Pestalotiopsis sp.

The results of the average difference test for the antagonist of endophytic bacteria to the inhibition of the Pestalotiopsis sp fungus in each observation are presented in Table 4.

Table 4. Average Data of Endophytic Bacteria Antagonist Tests on Fungus Inhibition Pestalotiopsis sp.

Treatment	Percentage of Inhibitory Power							
	2 HSI		4 HSI		6 HSI		8 HSI	
SPE0 (control)	0,00 (0,71	b	0,00 (0,71)	c	0,00 (0,71)	c	0,00 (0,71)	c
SPE1 (Isolat 1, Sei Putih)	40,51 (6,39)	a	44,53 (6,64)	b	42,77 (6,54)	b	38,64 (6,25)	b
SPE2 (Isolat 2, Sei Putih)	43,87 (6,65)	a	54,02 (7,36)	b	75,23 (8,70)	a	72,34 (8,53)	a
BBE3 (Isolat 3 Bdr. Betsi)	45,19 (6,73)	a	71,62 (8,48)	a	83,38 (9,16)	a	76,60 (8,78)	a
BBE4 (Isolat 4 Bdr. Betsi)	46,46 (6,84)	a	67,80 (8,25)	a	82,26 (9,10)	a	75,76 (8,73)	a
BBE5 (Isolat 5 Bdr. Betsi)	48,41 (6,99)	a	74,17 (8,64)	a	79,54 (8,95)	a	72,02 (8,51)	a

Note: Numbers followed by letters that are not the same in the same treatment column and row are significantly different at the 5% level based on the DMRT . test

From Table 4. it is known that at 2 DAI all endophytic bacterial isolates had a significant effect on the control, while at 4 to 8 DAI, differences began to be seen between endophytic bacterial isolates from Sungai Putih and Bandar Betsi, and based on the results of observations at 4 to 8 DAI the percentage was obtained. inhibition of endophytic bacterial isolates that were able to inhibit the growth of Pestalotiopsis sp with a value of more than 70% found in isolates SPE2, BBE3, BBE4, and BBE5. The high and low percentage of inhibition can be seen from the size of the inhibition zone produced by bacteria. Dharmawan, (2009) stated that there was a large variation in the inhibition zone obtained, presumably due to differences in the characteristics of the test bacteria used both morphologically and physiologically. In addition, it was also caused by each isolate having a different chemical structure, composition and content/concentration.

Increased inhibition of all types of isolates at 2 to 6 DAI, but decreased inhibition at 8 DAI. Based on the percentage of inhibition of the mycelium of the Pestalotiopsis sp fungus, it showed that there were several endophytic bacteria that had the potential to inhibit the growth of the Pestalotiopsis sp. The inhibition of mycelium growth of the Pestalotiopsis sp fungus can occur due to the antagonism of endophytic bacteria which involves the influence of antifungal compounds or secondary metabolites produced by bacteria and are toxic to the Pestalotiopsis sp fungus or also due to nutritional competition, according to the statement of Mukerji & Garg (1988).) Microbes can suppress the development of pathogens or diseases by means of competition for nutrients or space, antibiosis (producing antibiosis), and parasitism.

One form of response shown by bacteria is to form secondary metabolites as a form of defense against other microbial attacks (Nofiani et al. 2009). For example, the bacterium Aeromonas hydrophila produces the enzyme chitinase which is anti-fungal against the fungal pathogen A. flavus and

3.5 Oxysporum with destruction of pathogenic cell walls (Halder et al., 2013).

The ability of endophytic bacteria to inhibit the growth of Pestalotiopsis sp can be seen from the growth of fungal mycelium which is shorter than the control. The inhibition carried out by each endophytic bacteria has differences, this is in accordance with the statement of Hallmann and Berg (2006) that the form of bacterial antagonism against pathogens occurs through a mechanism that can be in the form of lysis of cell components of cell metabolism so that the pathogen will be disrupted and cause the pathogen to die. It is suspected that the obstacles that occur are the result of competition for nutrients and space, this is in accordance with the statement of Schulz et al. (2006) in addition to the formation of the inhibition zone, competition is considered a very important factor in controlling pathogenic fungi by endophytic bacteria, the inhibition zone competition occurs when both organisms are in the same place and use the same nutrients.

3.6 Pathogenicity Test of Selected Endophytic Bacteria Against Chili Seed

Pathogenicity test was carried out on selected endophytic bacterial isolates, the test results showed that the total bacterial isolates used affected the growth of chili sprouts. The bacteria found were dominated by pathogenic and potentially pathogenic bacteria. Pathogenic bacteria cause seeds to not germinate, while potentially pathogenic bacteria can still cause seeds to germinate. According to Kartika (2013),

What is meant by normal growth sprouts are sprouts with good/perfect development of the root system, hypocotyl, plumule, and cotyledon without any damage or abnormalities in the tissues.

Table 5. Percentage of Chili Seed Growth Due to Soaking Endophytic Bacteria

Treatment	Repeat			Avarage
	I	II	III	
E0	34,07 (5,88)	21,07 (4,64)	55,52 (7,48)	36,88 (6,00) b
E1	86,60 (9,33)	63,30 (7,99)	93,30 (9,69)	81,07 (9,00) a
E2	80,00 (8,97)	73,30 (8,59)	100,00 (10,02)	84,43 (9,20) a
E3	70,00 (8,40)	60,00 (7,78)	96,60 (9,85)	75,53 (9,20) a

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E4	76,60 (8,78)	46,60 (6,86)	66,60 (8,19)	63,27 (8,68) a
E5	73,30 (8,59)	63,30 (7,99)	86,60 (9,33)	74,40 (8,68) a
Avarage	70,09 (8,33)	54,59 (7,31)	83,10 (9,10)	69,26 (8,24)

The results showed that some isolates of endophytic bacteria were able to increase the growth of chili seedlings. Chili seed growth which was given a liquid NA solution that was shaken for 24 hours, endophytic bacteria had better growth than the control. Several previous studies also showed that endophytic bacteria have the ability to promote plant growth. Endophytic bacteria are known to produce various growth regulators and hormones that are important for plant growth (Munif et al. 2012).

Provision of endophytic bacteria on chili seed sprouts was able to increase plant height 17.55 cm - 22.89 cm and root length 9.85 cm - 12.20 cm, while the control only had a plant height of 15.88 cm and a root length of 8.26. cm.

The presence of endophytic bacteria associated with chili plants has been reported to increase crown height by 33% and root length by 48% of rice seedlings compared to controls (Vasudevan et al. 2002). The results of other studies also reported that endophytic bacteria can associate and stimulate the growth of several types of plants, including potato (Sturz and Nowak, 2000), cucumber (Hallmann et al. 1997), and chili (Sundaramoorthye et al. 2012). Research conducted by Wibowo (2013) showed that endophytic bacteria derived from forestry plants were able to act as a growth promoter of tomato plants. The mechanism of endophytic bacteria in increasing plant growth is by producing IAA which plays an important role in plant growth (Miliūtė et al. 2011). Some endophytic bacteria were also reported to inhibit plant germination, but were able to increase the speed of plant growth during the generative period of the plant (Munif et al. 2012).

The mechanism of action of endophytic bacteria as biological agents is to produce antimicrobial compounds to fight pathogens, produce growth regulators, fix nitrogen, and mobilize phosphate which plays a role in stimulating and strengthening the growth of plant resistance (Ikeda et al. 2010). Endophytic bacteria are also known to be able to suppress disease-causing pathogens as reported by Munif et al. (2012) that endophytic bacteria from tomato roots can suppress pathogens F. oysporum f. sp. radicus-lyocopersici and F. oysporum f. sp. lyocopersici.

4. Conclusion

1. Endophytic bacteria were significantly able to inhibit the widespread development of the fungus Pestalotiopsis sp. Isolate 3 endophytic bacteria from Bandar Betsi (BBE3) with an inhibitory power of 77.16% resulted in better inhibition than other isolates.
2. Chili seeds soaked in a solution of endophytic bacteria resulted in a higher growth percentage than the control.
3. Isolated endophytic bacteria were able to increase plant height by 77.55 cm while the control resulted in plant height of 15.88 cm and plant root length of 8.26 cm.

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