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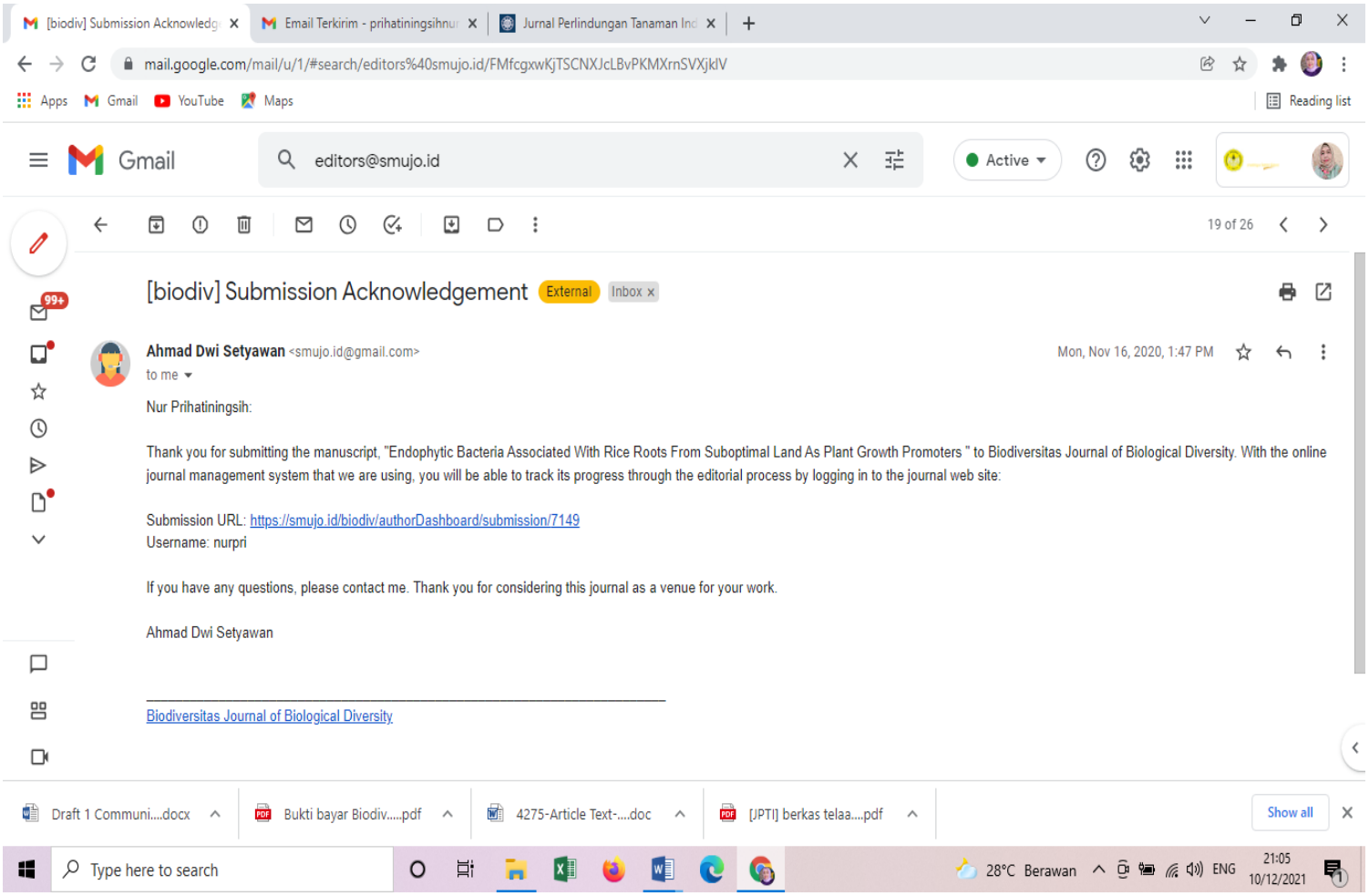

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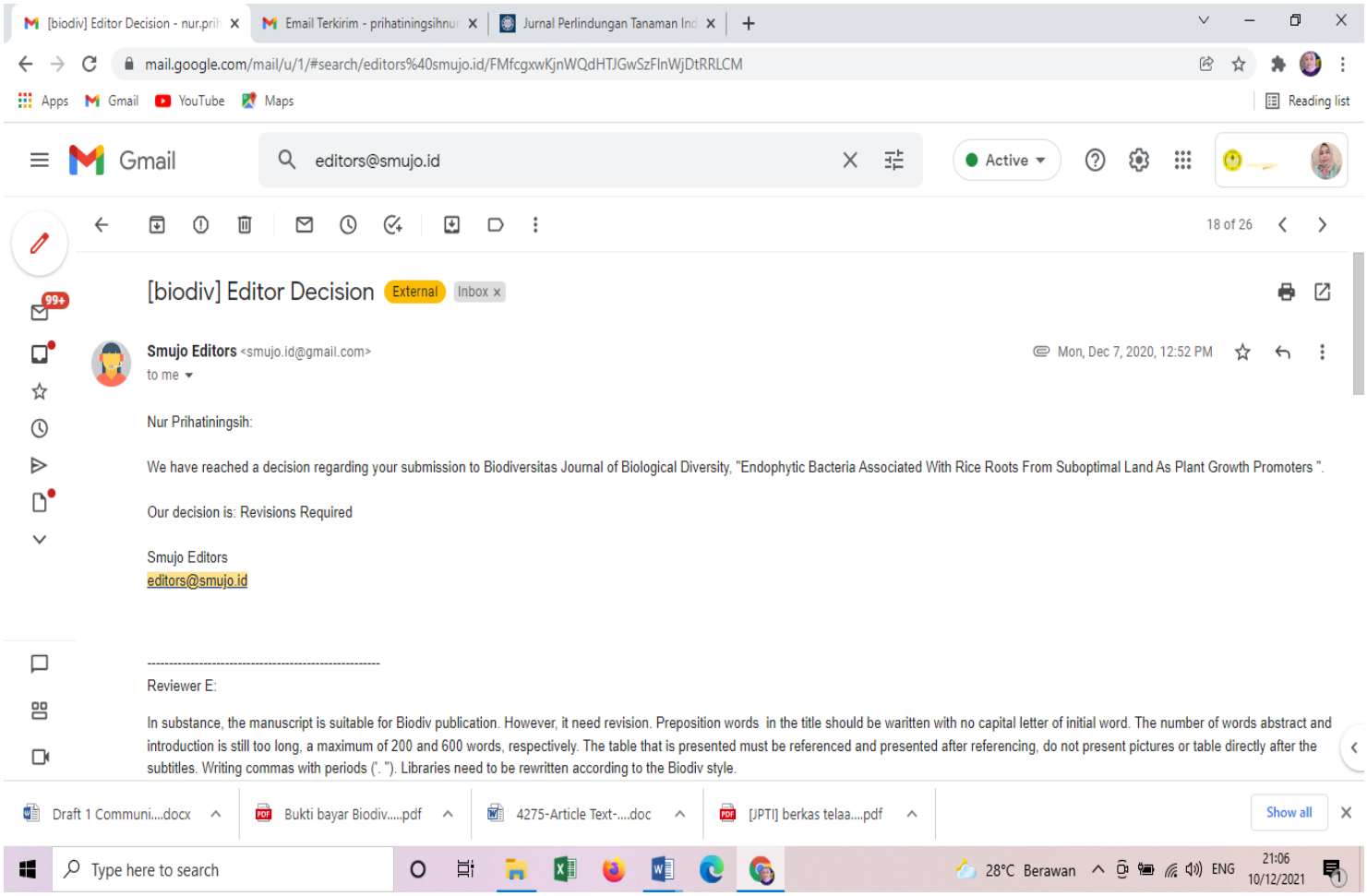

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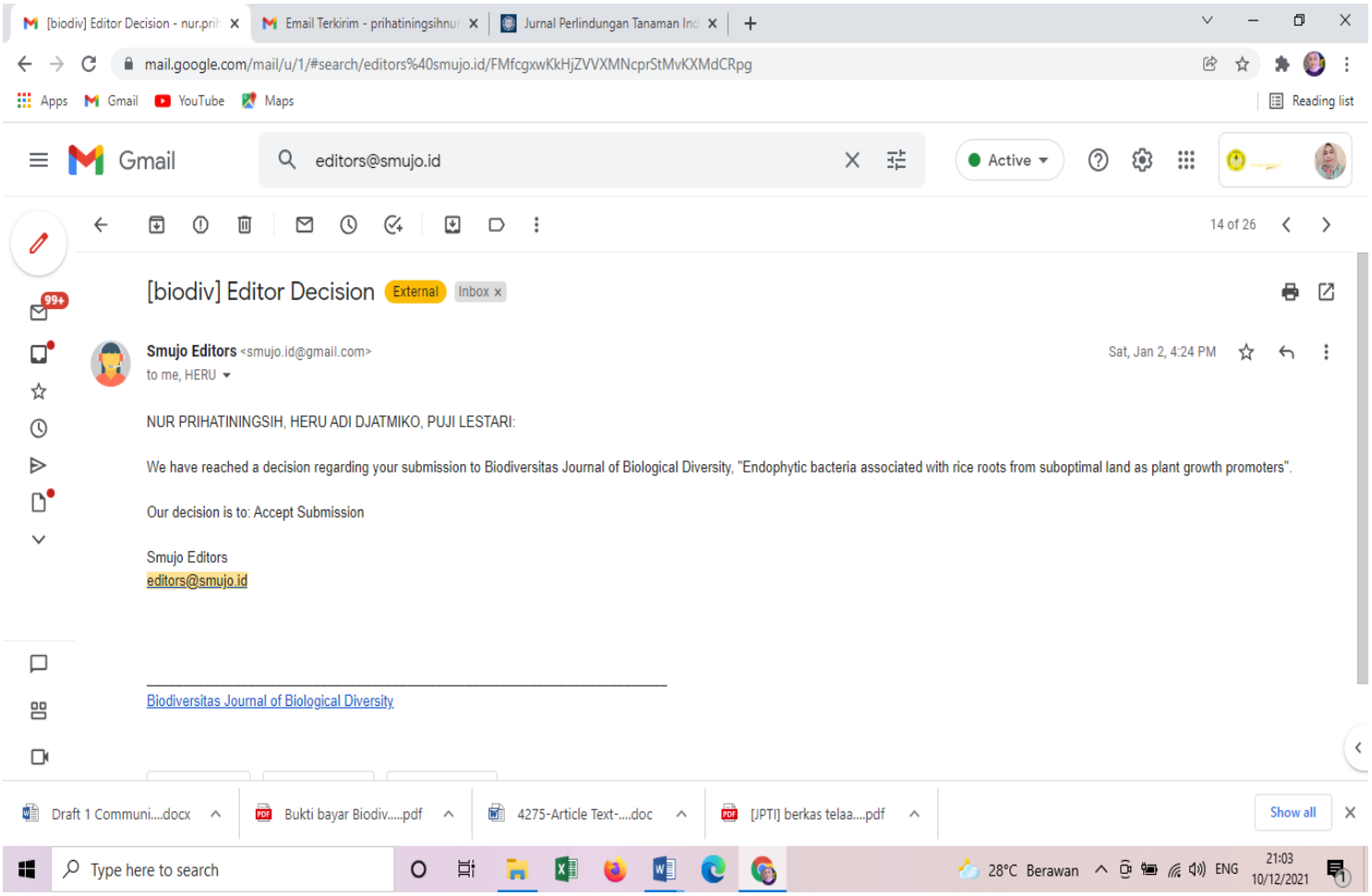

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Endophytic Bacteria Associated with Rice Roots from Suboptimal Land as Plant Growth Promoters

Author(s) name:

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Find of endophytic bacteria associated with rice roots from suboptimal land as plant growth promoters and control of bacterial leaf blight on rice

Statements:

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Place and date:

Purwokerto, 16 November 2020

Sincerely yours,

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Nur Prihatiningsih

Revisi 1

Endophytic bacteria associated with rice roots from suboptimal land as plant growth promoters

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Manuscript received: DD MM (Date of abstract/manuscript submission). Revision accepted: ..... . (8 pt)

Abstract. The role of the endophytic bacteria as plant growth promoted directly and controlled to the pathogens undirectly. This research aimed to evaluate endophytic bacteria associated with rice root and their activity to promote the plant growth and to control rice diseases. The study was conducted at the Laboratorium of Plant Protection and experimental farm Faculty of Agriculture Jenderal Soedirman University, from April to August 2020. This endophytic bacterial from suboptimal land were evaluated for promotes plant growth with soaking seed before seedling and spraying them at 10, 20 and 30 days after transplanting. The experiment was arranged with six replications and four treatments namely control (untreated endophytic bacteria) SM1 (isolated from Somagede); SB1, and SB3 (from Sumbang). *Xanthomonas oyzae* pv.*oryzae* was nature inoculation (the location is in the endemic category of bacterial leaf blight and around of rice crops). The variable observed were the plant growth components i.e plant height, number of tillers, panicles, incubation period, disease intensity, infection rate and effectivenes control. The result of this research showed that endophytic bacteria from Somagede (SM1) was the best an enchanced plant height and number of tillers, and suppressed disease intensity, delayed of incubation period.

Key words: endophytic bacteria, plant growth, rice, *Xanthomonas oyzae* pv.*oryzae*

INTRODUCTION (10 PT)

Endophytic bacteria are bacteria that live in plant tissue, are not pathogens, and do not cause damage to these plants, even benefit plants because they produce compounds that help plant growth. Endophytic bacteria that are in plant tissue reach sufficient populations and are capable of producing plant growth-promoting compounds or phytohormones such as IAA (*indole acetic acid*) and gibberellins. Endophytic bacteria are associated with plants to help the absorption of nutrients because they are able to dissolve phosphate into a form available to plants (Adesemoye et al, 2009; Dawwam et al 2013).The direct effect of endophytic bacteria on plants is to increase plant growth because endophytic bacteria can help provide nutrients for plants. Ability as a phosphate solubility, producing siderophore which is able to chelate iron into iron-siderophore bonds available to plants. Endophytic bacteria are correlated with the enhanced plant growth by the production of hormones that increase accessibility of nutrients, such as nitrogen, potassium and phosphorus (Glick, 2012). Like rhizobacteria, according to Kesaulya et al (2015), they are able to act as biostimulants (produce phytohormones), biofertilizers (to help absorb nutrients for plants), and bioprotectants (suppress disease). Endophytic bacteria indirectly affect plants as a bioprotectant or biocontrol that can control several plant diseases, both by fungi and bacteria (Muthukumar et al, 2017).

The colonize of bacterial endophytes was same as that of plant pathogens, which can act as biocontrol agents (Berg et al. 2005). The major mechanism of endophytic bacteria in plant disease control is (i) to assist nutrient availability and uptake (ii) to enhance stress tolerance and (iii) to provide disease resistance (Ryan et al. 2008; Hamilton et al. 2012). While induced disease resistance activities are allied with the abilities to produce secondary metabolites, such as antibiotics or chitinase enzyme, which can inhibit growth of plant pathogens. Hence they act as biocontrol agents (Christina et al. 2013; Wang et al. 2014). Further, endophytic bacteria have the capacity to cope with phytopathogenic fungi with induced systemic resistance (ISR) (Pieterse et al. 2014). Due to their beneficial function such as plant growth promotion and disease control, endophytes can be used in the form of bio-formulations (seed treatment, soil application and seedling dip) in agriculture.

Rice cultivation often experiences problems with plant pathogens. Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important disease because it causes damage to leaves and can cause yield losses of up to 74% in Southeast Asia (Sarra et al. 2010). The severity of the disease depends on the virulence of the bacterial isolate which causes two primary symptoms, namely blight (seed blight) and crackle (wilt phase). During the blight phase bacteria enter through wounds or water pores on the edges and tops of the leaves causing systemic symptoms, resulting in irregular

yellow wet spots, with wavy edges, irregular leaves, and spots starting from the edge near the tip of the leaf (Singh et al., 2013). Xoo. is a rod-shaped bacteria measuring 0.55-0.75 x 1.35-2.17 μm, with a single polar flagellum, not spore-forming, motile, single or paired, and sometimes chained, a Gram negative, the colony is light yellow. More than 30 races were found with sub-groups within the species (Furutani et al., 2009).

Efforts to improve plant growth and control bacterial leaf blight have been carried out by using antagonistic bacteria from the rice rhizosphere, namely *Bacillus subtilis* B1 in an organic fertilizer formula (Djatmiko et al. 2017). However, the results are not optimal because leaf blight is classified as vascular disease, so it is difficult to control only with antagonistic bacteria, it is necessary to do this with endophytic bacteria that can infect the tissue so that they will interact directly with the Xoo bacteria. The research previously showed control of bacterial leaf blight on screen house with effectiveness of more than 60% (Prihatiningsih et al., 2020).

Suboptimal land development for agriculture is generally faced with several problems, including high soil acidity and toxicity of Fe and Al as well as deficiency of nutrients N. P. K. Ca, Mg. Therefore, improvements to the condition of the soil chemistry such as the addition of organic matter (Sirappa and Titahena, 2014). One of them using the endophytic bacteria to improve the soil biology and chemistry could be properties of productivity land. The suboptimal land for rice based distribution maps unit land is estimated at about 5791.31 ha scattered in various physiographyc. Suboptimal land spread on the embankment of river, swamp the back, lower part of the river terraces, alluvial depression, plain fluvio marine and peat topogen (Sirappa and Titahena, 2014). The specification of this research is the application of endophytic bacteria technology innovation associated with rice plant roots as antagonistic endophytic bacteria in suboptimal land to improve plant health and growth by improving physical, chemical, and biological characteristics. This research aimed to evaluate endophytic bacteria associated with rice root and their activity to promote the plant growth directly and to control rice bacterial leaf blight indirectly.

MATERIALS AND METHODS

Preparations of endophytic bacterial associated of rice root (EBARr) from suboptimal land.

Endophytic bacteria from root samples of healthy rice plants from suboptimal land around Banyumas were prepared from the results of previous research isolation. Initially, 21 isolates were obtained and then selected to produce nine potential endophytic bacterial isolates. Then the nine endophytic bacterial isolates were tested in vitro for inhibition against Xoo and in the screen house, the endophytic bacteria were tested to suppress bacterial leaf blight (Prihatiningsih et al. 2020). The results of the two series of studies were 3 isolates that were able to suppress disease, namely SM1 (Somagede 1 isolate), SB1 (Sumbang 1), and SB3 (Sumbang 3). Furthermore, in this study, the three endophytic bacteria were tested in rice fields.

The evaluate of endophytic bacterial associated of rice root (EBARr) to promote plant growth with observed of promoter component

a. Hydrogen Cyanide (HCN) production test. The nine endophytic bacterial isolates were tested as HCN producers. The paper disc was immersed in a 0.5% picric acid solution in a 2% sodium carbonate solution, placed on a NB medium containing 0.44% glycine, then the paper disc was affixed to the test tube wall. Endophytic bacteria that produce HCN gas are shown by changing the color of the paper disc from yellow to brown (Reetha et al., 2014).

b. The siderophore production test was carried out with SD-CASA medium, dark blue, 2% agar w /v in order to be added as a compactor. As much as 10 ml of CAS-blue agar as the basis of the plate, After solidifying it was coated with 6 ml of nutrient medium, the plate was incubated overnight at 32^{o}C, 10 μl of supernatant endophytic bacteria was dropped on a paper disk, forming an orange or pink zone (Hu & Hu, 2011).

c. Testing IAA production, endophytic bacteria isolates were grown on slanted NA medium with and without L-Tryptophan (0.5%), after 2 days of incubation at room temperature then dropping 100 μl of Salkowski reagent A (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution). Colonies that produce IAA show a change in color to pink (Mohite, 2013).

d. The phosphate solubility activity test was assessed qualitatively using potato-dextrose yeast extract agar (PDYA)-CaP. Each bacterial culture was scratch inoculated in PDYA-CaP medium at 4 places, incubated at 28 $\pm$ 2^{o}C for 10 days. The clear zone shows that the endophytic bacteria is capable of phosphate solubility (Khan et al., 2009).

e. Resistance Test of Isolates to Antibiotics. The resistance test of isolates against antibiotics was carried out by growing endophytic bacteria on the NA medium by means of the pour plate method then dipping the sterile paper disc pieces in antibiotics after draining and then placing them on the bacterial medium, incubated for 24 hours. If a clear zone is formed,

it means that the endophytic bacteria is senisitive to these antibiotics, while those that do not form a clear zone means that they are resistant to these antibiotics (Yasmin et al, 2009).

f. Testing of endophytic bacteria to promote plant growth. Observation of the growth components of rice plants is intended as a direct effect of endophytic bacteria on plants, including plant height and a number of tillers, number of panicle. The study was conducted with a completely randomized block design with 4 treatments and 6 replications. The treatments were control (untreated endophytic bacteria), B: SM1 (endophyte bacteria isolate Somagede 1), C: SB1 (endophytic bacteria isolate Sumbang 1) and D: SB3 (endophytic bacteria isolate Sumbang 3). The treatment of endophytic bacteria was started by soaking the seeds before seeding for 1 night, followed by spraying the plants at 20, 30, and 40 days after planting. The variables observed were plant height, number of tillers, and number of panicle.

g.The activity of EBARr as biocontrol of bacterial leaf blight. Endophytic bacteria as disease control as an indirect effect is carried out in bacterial leaf blight endemic areas and uses varieties that are susceptible-tolerant to bacterial leaf blight, namely Ciherang. Treatment and design such as testing on plant growth. The endophytic bacterial density was 10^9 cfu / mL. Variables observed: components of the pathosystem (incubation period, disease intensity, infection rate, control effectiveness). Observation of disease intensity using the formula DI = Σ (n xv) / (Z xN) x 100%, DI = disease intensity, n = number of plants from each attack category, v = attack category, N = number of plants observed, Z = highest category value. Xoo's attack category is: 0 = no attack, 1 = damage scale 1–5%, 3 = damage scale 6–12%, 5 = damage scale 13–25%, 7 = damage scale 26–50%, 9 = scale 51–100% damage (Djatmiko et al, 2011). The rate of infection is calculated based on the proportion of diseased tissue obtained from the value of disease intensity with the van der Plank formula (1963), $Xt = Xo. e^{rt}$, where Xt = proportion of diseased intensity at time t and Xo = proportion of diseased intensity at initial time, e = logarithmic number, r = infection rate and t = interval of observation time. Because of the bacterial leaf blight is a simple interest disease, calculate r = 2,3 / t (log 1 / (1-xt) - log 1 / (1-Xo) unit / day.

Data analysis

Data at the laboratory experiment were analyzed by descriptive methods and then communicated and comparative to references. Data from experimental ex-farm were analyzed by anova, if significant effect, it's continued with LSD 5%

RESULTS AND DISCUSSION

Based on the characterization of endophytic bacteria in previous studies and by heating treatment in an oven at 80°C for 20 minutes of the suspension of rice root endophytic bacteria in the 1st dissolution, most of the endophytic bacteria obtained showed Gram positive, in the form of short rod shape - rod shape, forming endospores, then grouped into the genus *Bacillus* sp. (Gordon et al., 1973; Prihatiningsih et al., 2020).

Plant growth promoter component of EBARr

The component of plant growth promoter showed from nine endophytic bacteria that could produce HCN, siderophore, IAA, and phosphate solubility as qualitative test (Table 1). The various respon of four assays showed the relative of qualitative.

Table 1. Plant growth components of nine rice root endophytic bacteria

Isolate Code	HCN production	Siderophore production	IAA production	Phosphate solubility
Kr4	+	+	+	+
Kr5	+	+	+	+
Kr7	+	-	+	-
SR1	+	+	+	+
SR5	+	+	+	+
SR7	+	-	+	+
SM1	+	+	+	++
SB1	+	+	++	+
SB3	+	+	+++	++

Notes: +: produce; ++ and +++: produce with more colour (IAA) ++: more width zone of phosphate solubility test, -: unproduced

Figure 1. HCN production test of the nine endophytic bacteria
A. Before reaction and B. After reaction as HCN production

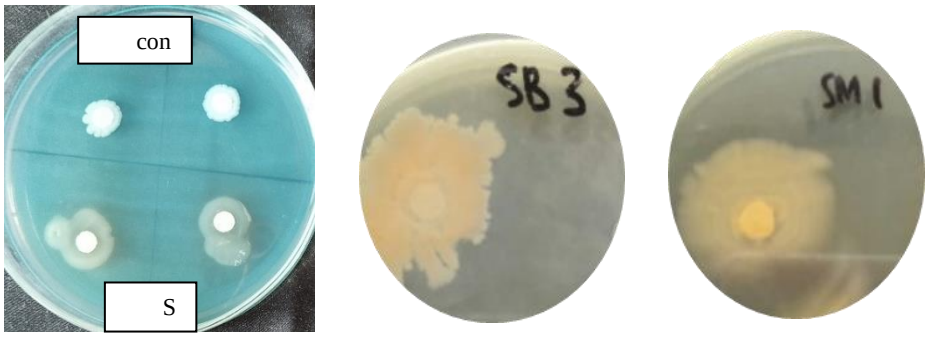
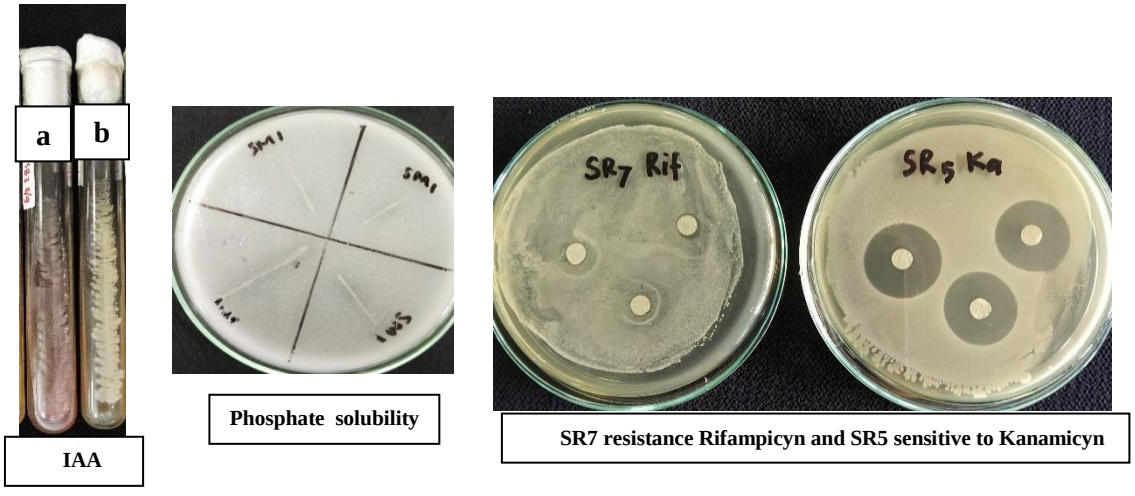


Figure 2. Siderophore production of the endophytic bacteria

Endophytic bacteria are able to stimulate plant growth by observing plant growth-promoting components, namely bacteria as a producer of HCN, siderophore, IAA, and as a phosphate solubility (Table 1). The response of plant growth promoter components varied from the nine isolates of endophytic bacteria that were tested as a result of the selection from the first year. Bacteria that are able to produce HCN show that these bacteria are capable of being biocontrol. This is because the role of HCN is to inhibit the proliferation or multiplication of pathogens, stimulate the formation of root hairs, thereby helping to increase nutrient absorption (Olanrewaju et al., 2017).

Testing the ability of endophytic bacteria to produce siderophores (Figure 2) was shown by the seven endophytic bacteria. Siderophores are low molecular weight compounds produced by bacteria and are capable of chelating iron into a form of siderophore-Fe $^{3+}$ bonds available to plants. This siderophore is also one of the biocontrol mechanisms by binding to Fe^{3+} so the pathogen is limited in the need for Fe^{3+} so that the pathogen is inhibited from reproducing to reach the rhizosphere due to iron deficiency (Glick, 2012; Prihatiningsih et al., 2017).



IAA

Phosphate solubility

SR7 resistance Rifampicyn and SR5 sensitive to Kanamicyn

Figure 3. IAA production, phosphate solubility and resistance to antibiotics of the endophytic bacteria

The nine endophytic bacteria were able to produce IAA which was marked with pink or pink color in the endophytic bacteria culture which was dripped with Salkowski reagent (Figure 3., a:produce of IAA and b:unproduce of IAA). This indicates that endophytic bacteria are capable of producing the plant hormone IAA. IAA functions for cell division and elongation stimulates seed and tuber germination increases the rate of xylem development and root development. IAA also functions as a vegetative growth controller, initiating the formation of adventitious and lateral roots, so that plant growth is better (Glick, 2012).

The ability of endophytic bacteria as a phosphate solubility is shown by a positive reaction in Table 1 and Figure 3, with the formation of a clear zone. The ability of bacteria as a phosphate solubility helps to provide nutrients available for plants because of the phosphate present in the soil in a form that is not available to plants becomes available (Olanrewaju et al., 2017).

Table 2. Intrinsic antibiotic resistance (IAR) test of rhizobacterial isolates

Isolate	Rifampicin		Kanamycin	
	Reaction	Diameter of zone (mm)	Reaction	Diameter of zone (mm)
Kr4	-	4	-	8
Kr5	-	4	-	8
Kr7	-	4	-	8
SR1	+	0	+	0
SR5	-	5	-	8
SR7	+	1	-	6
SM1	-	6	-	8
SB1	-	7	-	9
SB3	+	1	-	7

Notes: +: resistance= not zone, very small (1 mm), -: sensitive: zone formed

Not zone = antibiotic resistance (+) means that be still growing in the medium with the antibiotic.

The resistance of endophytic bacteria to antibiotics (Figure 3) is very important as a pathogen biocontrol mechanism because it is exposed to many microorganisms that produce various antibiotics in the soil. Antibiotic-resistant endophytic bacteria will be able to survive so that they are able to compete in space and become antagonistic to pathogens (Ulloa-Ogaz et al., 2015).

Isolates that are resistant to antibiotics as shown in Figure 3. SR7 isolates are resistant to Rifampicin antibiotics which are characterized by no zone formation, meaning that the growth of SR7 endophytic bacteria still occurs around the antibiotic. On the other hand, the SR5 isolate was not resistant or sensitive to the Kanamycin antibiotic which was indicated by the formation of a clear zone around the filter paper treated with the Kanamycin antibiotic, meaning that there was no growth around the antibiotic. This is in accordance with the opinion of Yasmin et al. (2009) that the IAR test is needed to identify the endophytic bacteria resistant or sensitive to antibiotics. IAR can also be used to genotypically identify bacterial species.

Endophytic bacteria to promote plant growth

Table 3. The component of plant growth with endophytic bacteria application

Isolate Code	Plant height (cm)	Number of tiller	Number of panicle/clump
A (control)	96,6b	13,3b	9,875
B (SM1)	101,3a	12,6b	13,005
C (SB1)	98,5b	15,3ab	12,125
D (SB3)	98,8b	18,3a	18,875

The treatment of endophytic bacteria in the land showed that the endophytic bacteria selected from the first year SM1 study (Somagede isolate) was able to increase plant height compared to other endophytic bacteria treatments and controls, while isolate Sumbang 3 (SB3) was increase the number of tiller. This is in accordance with the opinion of Glick (2012) that endophytic bacteria are able to stimulate plant growth because they produce phytohormones such as IAA.

Suppressing bacterial leaf blight by EBARr

Table 4. Components of plant pathosystems against bacterial leaf blight

Isolate Code	Incubation period (dat)	Disease intensity (%)	Effectiveness (%)	Infection rate (unit/hari)
A (control)	24	23,13a	0	0,0469
B (SM1)	30,2	10,17b	56,03	0,0435
C (SB1)	36,3	9,07b	60,79	0,0246
D (SB3)	34,2	9,27b	59,92	0,0454

Observation of the pathosystem components of rice bacterial leaf blight (Table 4) showed that the treatment of endophytic bacteria was different from the control. However, between endophytic bacteria was not significantly different, with control effectiveness ranging from 56.03 to 60.79%.

The opinion of Nagendran et al. (2013) stated that endophytic bacteria were able to control bacterial leaf blight on rice with a low disease intensity of 2.80% compared to without treatment of endophytic bacteria with a disease intensity of 19.82%. Four isolates, OS52, OS40, OS23 and OS53, which were identified by nucleotide sequence analysis as *Enterobacte*r sp., *B. subtilis, Bacillus* sp. and *Pseudomonas putida*, respectively, could increase plant growth and decrease Xoo infection under greenhouse conditions. Seed treatment with these antagonists caused disease to cease in over 60% of plants. These species have been found as endophytes in rice (Yousefi et al., 2018). Endophytic bacteria able to control the disease caused by bacteria and fungi because of them produced hydrolitic enzyme as well as chitinase, protease similarly with rhizospheric bacteria *Bacillus subtilis* from potato rhizosphere (Lestari et al., 2017).

CONCLUSION

The conclusion based on this research showed that three endophytic bacteria associated rice root (EBARr) as prospectives as plant growth promoter, and biocontrol agents to bacterial leaf blight. The inhibition activity of EBARr similar with in an exfarm test by suppresing the disease until 60,79% effectiveness. The rice growth enhanched by applied of EBARr with plant height and number of tiller so increase. Furthermore endophytic bacteria was prospected to develope biofertilizer and biocontrol.

ACKNOWLEDGEMENTS

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Endophytic Bacteria Associated ~~W~~with Rice Roots ~~F~~from Suboptimal Land ~~A~~as Plant Growth Promoters [1]

Abstract[2]**.** Endophytic bacteria are bacteria associated with plant growth. The role of this bacteria as plant growth promoted directly and controlled to the pathogens undirectly. This research aimed to evaluate endophytic bacteria associated with rice root and their activity to promote the plant growth directly and to control rice diseases. The study was conducted at the Laboratorium of Plant Protection and experimental farm Faculty of Agriculture Jenderal Soedirman University, from April to August 2020. The endophytic bacterial associated with rice root (EBARr) from suboptimal land were evaluated for promotes plant growth with soaking seed before seedling and spraying them at 10, 20 and 30 dat (days after transplanting). The experiment was arranged with four treatments namely control (untreated endophytic bacteria) SM1 (Endophytic bacteria isolate from Somagede); SB1 (from Sumbang 1, and SB3 (from Sumbang 3). The treatments were conducted in six replications. *Xanthomonas oyzae* pv.*oryzae* was nature inoculation because the experiment location is in the endemic category of bacterial leaf blight and around of the experiment fields were rice crops. The variable observed were the plant growth components i.e plant height, number of tillers, number of panicles, incubation period, disease intensity, infection rate and effectivenes control. The result of this research showed that endophytic bacteria from Somagede (SM1) was the best an enchanced plant height and number of tillers, and suppressed disease intensity, delayed of incubation period. Endophytic bacteria was prospected to develop in the formula as biofertilizer and biopesticide.

Key words: endophytic bacteria, plant growth, rice, *Xanthomonas oyzae* pv.*oryzae*

INTRODUCTION[3] (10 PT)

Endophytic bacteria are bacteria that live in plant tissue, are not pathogens, and do not cause damage to these plants, even benefit plants because they produce compounds that help plant growth. Endophytic bacteria that are in plant tissue reach sufficient populations and are capable of producing plant growth-promoting compounds or phytohormones such as IAA (*indole acetic acid*) and gibberellins. Endophytic bacteria are associated with plants to help the absorption of nutrients because they are able to dissolve phosphate into a form available to plants (Adesemoye et al, 2009; Dawwam et al 2013).The direct effect of endophytic bacteria on plants is to increase plant growth because endophytic bacteria can help provide nutrients for plants. Ability as a phosphate solubility, producing siderophore which is able to chelate iron into iron-siderophore bonds available to plants. Endophytic bacteria are correlated with the enhanced plant growth by the production of hormones that increase accessibility of nutrients, such as nitrogen, potassium and phosphorus (Glick, 2012). Like rhizobacteria, according to Kesaulya et al (2015), they are able to act as biostimulants (produce phytohormones), biofertilizers (to help absorb nutrients for plants), and bioprotectants (suppress disease). Endophytic bacteria indirectly affect plants as a bioprotectant or biocontrol that can control several plant diseases, both by fungi and bacteria (Muthukumar et al , 2017).

The colonize of bacterial endophytes was same as that of plant pathogens, which can act as biocontrol agents (Berg et al. 2005). The major mechanism of endophytic bacteria in plant disease control is (i) to assist nutrient availability and uptake (ii) to enhance stress tolerance and (iii) to provide disease resistance (Ryan et al. 2008; Hamilton et al. 2012). While induced disease resistance activities are allied with the abilities to produce secondary metabolites, such as antibiotics or chitinase enzyme, which can inhibit growth of plant pathogens. Hence they act as biocontrol agents (Christina et al. 2013; Wang et al. 2014). Further, endophytic bacteria have the capacity to cope with phytopathogenic fungi with induced systemic resistance (ISR) (Pieterse et al. 2014). Due to their beneficial function such as plant growth promotion and disease control, endophytes can be used in the form of bio-formulations (seed treatment, soil application and seedling dip) in agriculture.

Rice cultivation often experiences problems with plant pathogens. Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important disease because it causes damage to leaves and can cause yield losses of up to 74% in Southeast Asia (Sarra et al. 2010). The severity of the disease depends on the virulence of the bacterial isolate which causes two primary symptoms, namely blight (seed blight) and crackle (wilt phase). During the blight phase bacteria enter

through wounds or water pores on the edges and tops of the leaves causing systemic symptoms, resulting in irregular yellow wet spots, with wavy edges, irregular leaves, and spots starting from the edge near the tip of the leaf (Singh et al., 2013). Xoo. is a rod-shaped bacteria measuring 0.55-0.75 x 1.35-2.17 μm, with a single polar flagellum, not spore-forming, motile, single or paired, and sometimes chained, a Gram negative, the colony is light yellow. More than 30 races were found with sub-groups within the species (Furutani et al., 2009).

Efforts to improve plant growth and control bacterial leaf blight have been carried out by using antagonistic bacteria from the rice rhizosphere, namely *Bacillus subtilis* B1 in an organic fertilizer formula (Djatmiko et al. 2017). However, the results are not optimal because leaf blight is classified as vascular disease, so it is difficult to control only with antagonistic bacteria, it is necessary to do this with endophytic bacteria that can infect the tissue so that they will interact directly with the Xoo bacteria. The research previously showed control of bacterial leaf blight on screen house with effectiveness of more than 60% (Prihatiningsih et al., 2020).

Suboptimal land development for agriculture is generally faced with several problems, including high soil acidity and toxicity of Fe and Al as well as deficiency of nutrients N. P. K. Ca, Mg. Therefore, improvements to the condition of the soil chemistry such as the addition of organic matter (Sirappa and Titahena, 2014). One of them using the endophytic bacteria to improve the soil biology and chemistry could be properties of productivity land. The suboptimal land for rice based distribution maps unit land is estimated at about 5791.31 ha scattered in various physiographyc. Suboptimal land spread on the embankment of river, swamp the back, lower part of the river terraces, alluvial depression, plain fluvio marine and peat topogen (Sirappa and Titahena, 2014). The specification of this research is the application of endophytic bacteria technology innovation associated with rice plant roots as antagonistic endophytic bacteria in suboptimal land to improve plant health and growth by improving physical, chemical, and biological characteristics. This research aimed to evaluate endophytic bacteria associated with rice root and their activity to promote the plant growth directly and to control rice bacterial leaf blight indirectly.

MATERIALS AND METHODS

Preparations of endophytic bacterial associated of rice root (EBARr) from suboptimal land.

Endophytic bacteria from root samples of healthy rice plants from suboptimal land around Banyumas were prepared from the results of previous research isolation. Initially, 21 isolates were obtained and then selected to produce nine potential endophytic bacterial isolates. Then the nine endophytic bacterial isolates were tested in vitro for inhibition against Xoo and in the screen house, the endophytic bacteria were tested to suppress bacterial leaf blight (Prihatiningsih et al. 2020). The results of the two series of studies were 3 isolates that were able to suppress disease, namely SM1 (Somagede 1 isolate), SB1 (Sumbang 1), and SB3 (Sumbang 3). Furthermore, in this study, the three endophytic bacteria were tested in rice fields.

The evaluate of endophytic bacterial associated of rice root (EBARr) to promote plant growth with observed of promoter component

a. Hydrogen Cyanide (HCN) production test. The nine endophytic bacterial isolates were tested as HCN producers. The paper disc was immersed in a 0.5% picric acid solution in a 2% sodium carbonate solution, placed on a NB medium containing 0.44% glycine, then the paper disc was affixed to the test tube wall. Endophytic bacteria that produce HCN gas are shown by changing the color of the paper disc from yellow to brown (Reetha et al., 2014).

b. The siderophore production test was carried out with SD-CASA medium, dark blue, 2% agar w /v in order to be added as a compactor. As much as 10 ml of CAS-blue agar as the basis of the plate, After solidifying it was coated with 6 ml of nutrient medium, the plate was incubated overnight at 32^{o}C, 10 μl of supernatant endophytic bacteria was dropped on a paper disk, forming an orange or pink zone (Hu & Hu, 2011).

c. Testing IAA production, endophytic bacteria isolates were grown on slanted NA medium with and without L-Tryptophan (0.5%), after 2 days of incubation at room temperature then dropping 100 μl of Salkowski reagent A (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution). Colonies that produce IAA show a change in color to pink (Mohite, 2013).

d. The phosphate solubility activity test was assessed qualitatively using potato-dextrose yeast extract agar (PDYA)-CaP. Each bacterial culture was scratch inoculated in PDYA-CaP medium at 4 places, incubated at 28 $\pm$ 2^{o}C for 10 days. The clear zone shows that the endophytic bacteria is capable of phosphate solubility (Khan et al., 2009).

e. Resistance Test of Isolates to Antibiotics. The resistance test of isolates against antibiotics was carried out by growing endophytic bacteria on the NA medium by means of the pour plate method then dipping the sterile paper disc pieces in

antibiotics after draining and then placing them on the bacterial medium, incubated for 24 hours. If a clear zone is formed, it means that the endophytic bacteria is senisitive to these antibiotics, while those that do not form a clear zone means that they are resistant to these antibiotics (Yasmin et al, 2009).

f. Testing of endophytic bacteria to promote plant growth. Observation of the growth components of rice plants is intended as a direct effect of endophytic bacteria on plants, including plant height and a number of tillers, number of panicle. The study was conducted with a completely randomized block design with 4 treatments and 6 replications. The treatments were control (untreated endophytic bacteria), B: SM1 (endophyte bacteria isolate Somagede 1), C: SB1 (endophytic bacteria isolate Sumbang 1) and D: SB3 (endophytic bacteria isolate Sumbang 3). The treatment of endophytic bacteria was started by soaking the seeds before seeding for 1 night, followed by spraying the plants at 20, 30, and 40 days after planting. The variables observed were plant height, number of tillers, and number of panicle.

g.The activity of EBARr as biocontrol of bacterial leaf blight. Endophytic bacteria as disease control as an indirect effect is carried out in bacterial leaf blight endemic areas and uses varieties that are susceptible-tolerant to bacterial leaf blight, namely Ciherang. Treatment and design such as testing on plant growth. The endophytic bacterial density was 10^9 cfu / mL. Variables observed: components of the pathosystem (incubation period, disease intensity, infection rate, control effectiveness). Observation of disease intensity using the formula DI = Σ (n xv) / (Z xN) x 100%, DI = disease intensity, n = number of plants from each attack category, v = attack category, N = number of plants observed, Z = highest category value. Xoo's attack category is: 0 = no attack, 1 = damage scale 1–5%, 3 = damage scale 6–12%, 5 = damage scale 13–25%, 7 = damage scale 26–50%, 9 = scale 51–100% damage (Djatmiko et al, 2011). The rate of infection is calculated based on the proportion of diseased tissue obtained from the value of disease intensity with the van der Plank formula (1963), Xt = Xo. e^{rt}, where Xt = proportion of diseased intensity at time t and Xo = proportion of diseased intensity at initial time, e = logarithmic number, r = infection rate and t = interval of observation time. Because of the bacterial leaf blight is a simple interest disease, calculate r = 2.3 / t (log 1 / (1-xt) - log 1 / (1-Xo) unit / day.

Data analysis

Data at the laboratory experiment were analyzed by descriptive methods and then communicated and comparative to references. Data from experimental ex-farm were analyzed by anova, if significant effect, it's continued with LSD 5%

RESULTS AND DISCUSSION

Based on the characterization of endophytic bacteria in previous studies and by heating treatment in an oven at 80ºC for 20 minutes of the suspension of rice root endophytic bacteria in the 1st dissolution, most of the endophytic bacteria obtained showed Gram positive, in the form of short rod shape - rod shape, forming endospores, then grouped into the genus *Bacillus* sp. (Gordon et al., 1973; Prihatiningsih et al., 2020).

Plant growth promoter component of EBARr

The component of plant growth promoter showed from nine endophytic bacteria that could produce HCN, siderophore, IAA, and phosphate solubility as qualitative test (Table 1). The various respon of four assays showed the relative of qualitative.

Table 1. Plant growth components of nine rice root endophytic bacteria

Isolate Code	HCN production	Siderophore production	IAA production	Phosphate solubility
Kr4	+	+	+	+
Kr5	+	+	+	+
Kr7	+	-	+	-
SR1	+	+	+	+
SR5	+	+	+	+
SR7	+	-	+	+
SM1	+	+	+	++
SB1	+	+	++	+
SB3	+	+	+++	++

Notes: +: produce; ++ and +++: produce with more colour (IAA) ++: more width zone of phosphate solubility test, -: unproduced

Figure 1.[4] HCN production test of the nine endophytic bacteria
A. Before reaction and B. After reaction as HCN production [5]

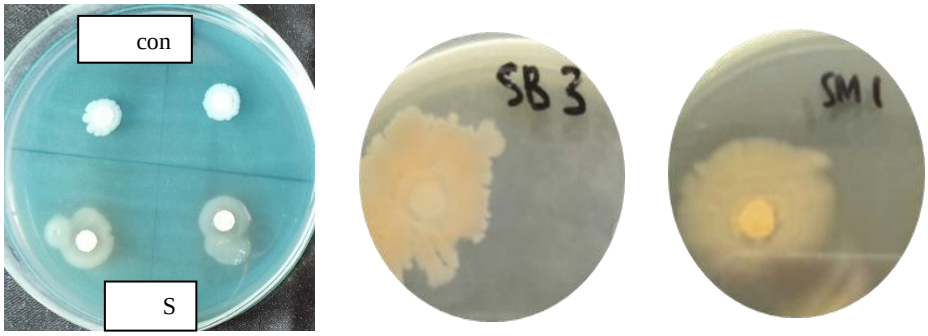
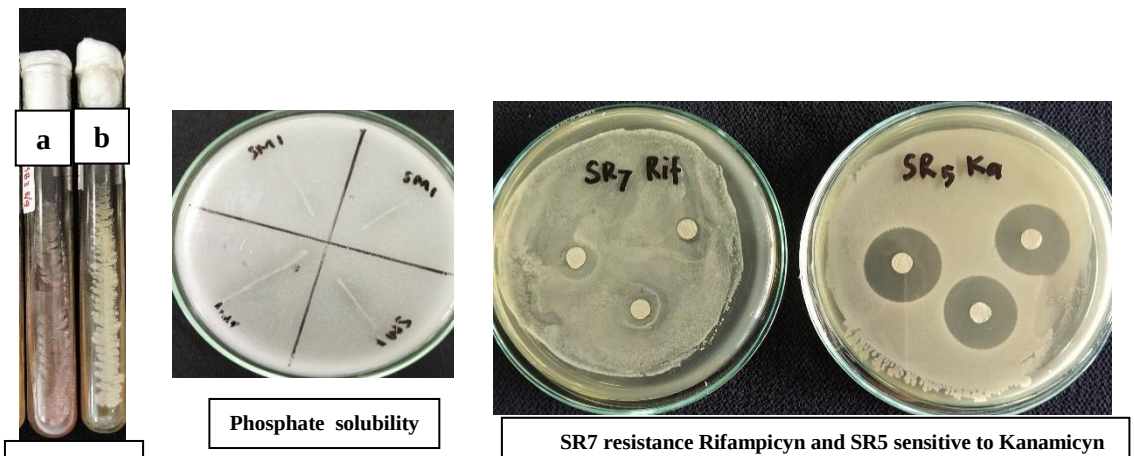


Figure 2. [6]Siderophore production of the endophytic bacteria[7]

Endophytic bacteria are able to stimulate plant growth by observing plant growth-promoting components, namely bacteria as a producer of HCN, siderophore, IAA, and as a phosphate solubility (Table 1). The response of plant growth promoter components varied from the nine isolates of endophytic bacteria that were tested as a result of the selection from the first year. Bacteria that are able to produce HCN show that these bacteria are capable of being biocontrol. This is because the role of HCN is to inhibit the proliferation or multiplication of pathogens, stimulate the formation of root hairs, thereby helping to increase nutrient absorption (Olanrewaju et al., 2017).

Testing the ability of endophytic bacteria to produce siderophores (Figure 2) was shown by the seven endophytic bacteria. Siderophores are low molecular weight compounds produced by bacteria and are capable of chelating iron into a form of siderophore-Fe $^{3+}$ bonds available to plants. This siderophore is also one of the biocontrol mechanisms by binding to Fe^{3+} so the pathogen is limited in the need for Fe^{3+} so that the pathogen is inhibited from reproducing to reach the rhizosphere due to iron deficiency (Glick, 2012; Prihatiningsih et al., 2017).



IAA

Phosphate solubility

SR7 resistance Rifampicyn and SR5 sensitive to Kanamicyn

Figure 3. IAA production, phosphate solubility and resistance to antibiotics of the endophytic bacteria

The nine endophytic bacteria were able to produce IAA which was marked with pink or pink color in the endophytic bacteria culture which was dripped with Salkowski reagent (Figure 3., a:produce of IAA and b:unproduce of IAA). This indicates that endophytic bacteria are capable of producing the plant hormone IAA. IAA functions for cell division and elongation stimulates seed and tuber germination increases the rate of xylem development and root development. IAA also functions as a vegetative growth controller, initiating the formation of adventitious and lateral roots, so that plant growth is better (Glick, 2012).

The ability of endophytic bacteria as a phosphate solubility is shown by a positive reaction in Table 1 and Figure 3, with the formation of a clear zone. The ability of bacteria as a phosphate solubility helps to provide nutrients available for plants because of the phosphate present in the soil in a form that is not available to plants becomes available (Olanrewaju et al., 2017).

Table 2. Intrinsic antibiotic resistance (IAR) test of rhizobacterial isolates

Isolate	Rifampicin		Kanamycin	
	Reaction	Diameter of zone (mm)	Reaction	Diameter of zone (mm)
Kr4	-	4	-	8
Kr5	-	4	-	8
Kr7	-	4	-	8
SR1	+	0	+	0
SR5	-	5	-	8
SR7	+	1	-	6
SM1	-	6	-	8
SB1	-	7	-	9
SB3	+	1	-	7

Notes: +: resistance= not zone, very small (1 mm), -: sensitive: zone formed

Not zone = antibiotic resistance (+) means that be still growing in the medium with the antibiotic.

The resistance of endophytic bacteria to antibiotics (Figure 3) is very important as a pathogen biocontrol mechanism because it is exposed to many microorganisms that produce various antibiotics in the soil. Antibiotic-resistant endophytic bacteria will be able to survive so that they are able to compete in space and become antagonistic to pathogens (Ulloa-Ogaz et al., 2015).

Isolates that are resistant to antibiotics as shown in Figure 3. SR7 isolates are resistant to Rifampicin antibiotics which are characterized by no zone formation, meaning that the growth of SR7 endophytic bacteria still occurs around the antibiotic. On the other hand, the SR5 isolate was not resistant or sensitive to the Kanamycin antibiotic which was indicated by the formation of a clear zone around the filter paper treated with the Kanamycin antibiotic, meaning that there was no growth around the antibiotic. This is in accordance with the opinion of Yasmin et al. (2009) that the IAR test is needed to identify the endophytic bacteria resistant or sensitive to antibiotics. IAR can also be used to genotypically identify bacterial species.

Endophytic bacteria to promote plant growth

Table 3. [8]The component of plant growth with endophytic bacteria application

Isolate Code	Plant height (cm)	Number of tiller	Number of panicle/clump
A (control)	96,6b	13,3b	9,875
B (SM1)	101,3a	12,6b	13,005
C (SB1)	98,5b	15,3ab	12,125
D (SB3)	98,8b	18,3a	18,875[9]

The treatment of endophytic bacteria in the land showed that the endophytic bacteria selected from the first year SM1 study (Somagede isolate) was able to increase plant height compared to other endophytic bacteria treatments and controls, while isolate Sumbang 3 (SB3) was increase the number of tiller. This is in accordance with the opinion of Glick (2012) that endophytic bacteria are able to stimulate plant growth because they produce phytohormones such as IAA.

Suppressing bacterial leaf blight by EBARr

Table 4.[10] Components of plant pathosystems against bacterial leaf blight

Isolate Code	Incubation period (dat)	Disease intensity (%)	Effectiveness (%)	Infection rate (unit/hari)
A (control)	24	23,13a	0[11]	0,0469
B (SM1)	30,2	10,17b	56,03	0,0435
C (SB1)	36,3	9,07b	60,79	0,0246
D (SB3)	34,2	9,27b	59,92	0,0454[12]

Observation of the pathosystem components of rice bacterial leaf blight (Table 4) showed that the treatment of endophytic bacteria was different from the control. However, between endophytic bacteria was not significantly different, with control effectiveness ranging from 56.03 to 60.79%.

The opinion of Nagendran et al. (2013) stated that endophytic bacteria were able to control bacterial leaf blight on rice with a low disease intensity of 2.80% compared to without treatment of endophytic bacteria with a disease intensity of 19.82%. Four isolates, OS52, OS40, OS23 and OS53, which were identified by nucleotide sequence analysis as *Enterobacte*r sp., *B. subtilis, Bacillus* sp. and *Pseudomonas putida*, respectively, could increase plant growth and decrease Xoo infection under greenhouse conditions. Seed treatment with these antagonists caused disease to cease in over 60% of plants. These species have been found as endophytes in rice (Yousefi et al., 2018). Endophytic bacteria able to control the disease caused by bacteria and fungi because of them produced hydrolitic enzyme as well as chitinase, protease similarly with rhizospheric bacteria *Bacillus subtilis* from potato rhizosphere (Lestari et al., 2017).

CONCLUSIONS

The conclusion based on this research showed that three endophytic bacteria associated rice root (EBARr) as prospectives as plant growth promoter, and biocontrol agents to bacterial leaf blight. The inhibition activity of EBARr similar with in an exfarm test by suppresing the disease until 60,79% effectiveness. The rice growth enhanched by applied of EBARr with plant height and number of tiller so increase. Furthermore endophytic bacteria was prospected to develope biofertilizer and biocontrol.

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Endophytic bacteria associated with rice roots from suboptimal land as plant growth promoters

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Abstract. The role of endophytic bacteria as plant growth promoted directly and controlled to the pathogens undirectly. This research aimed to evaluate endophytic bacteria associated with rice root and their activity to promote the plant growth and to control rice diseases. The study was conducted at the Laboratorium of Plant Protection and experimental farm Faculty of Agriculture Jenderal Soedirman University, from April to August 2020. The endophytic bacterial from suboptimal land were evaluated for promotes plant growth with soaking seed before seedling and spraying them at 10, 20 and 30 days after transplanting. The experiment was arranged with six replications and four treatments namely control (untreated endophytic bacteria) SM1 (endophytic bacteria isolate from Somagede); SB1 and SB3 (from Sumbang). *Xanthomonas oyzae* pv.*oryzae* was nature inoculation because the experiment location is in the endemic category of bacterial leaf blight. The variable observed were the plant growth components i.e plant height, number of tillers, number of panicles, incubation period, disease intensity, infection rate and effectivenes control. The result of this research showed that endophytic bacteria from Somagede (SM1) was the best an enchanced plant height and number of tillers, and suppressed disease intensity, delayed of incubation period.

Key words: endophytic bacteria, plant growth, rice, *Xanthomonas oyzae* pv.*oryzae*

INTRODUCTION (10 PT)

Endophytic bacteria live in plant tissue, non-pathogens, they produce compounds that help plant growth. Endophytic bacteria reach sufficient populations and capable of producing plant growth-promoting compounds such as IAA (*indole acetic acid*) and gibberellins. Endophytic bacteria associated with plants to help the absorption of nutrients because they are able to dissolve phosphate into a form available to plants (Adesemoye et al. 2009; Glick 2012; Dawwam et al. 2013). Ability producing siderophore to chelate iron into iron-siderophore bonds available to plants. Like rhizobacteria, according to Kesaulya et al. (2015), they are able to act as biostimulants (produce phytohormones), biofertilizers (to help absorb nutrients for plants), and bioprotectants (suppress disease). Endophytic bacteria indirectly affect plants as a bioprotectant or biocontrol to several plant diseases (Berg et al. 2005; Muthukumar et al. 2017).

The major mechanism of endophytic bacteria in plants are (i) to assist nutrient availability and uptake (ii) to enhance stress tolerance (iii) to provide disease resistance (Ryan et al. 2008; Hamilton et al. 2012). While induced disease resistance activities are allied with the abilities to produce secondary metabolites, such as antibiotics or chitinase (Christina et al. 2013; Pieterse et al. 2014; Wang et al. 2014). Due to their beneficial function such as plant growth promotion and disease control, endophytes can be used in the form of bio-formulations (seed treatment, soil application, and seedling dip) in agriculture.

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important disease because it causes damage to leaves and can cause yield losses of up to 74% in Southeast Asia (Sarra et al. 2010). The severity of the disease depends on the virulence of the bacterial isolate which causes two primary symptoms, namely blight (seed blight) and crackle (wilt phase). During the blight phase bacteria enter through wounds or water pores on the edges and tops of the leaves causing systemic symptoms, resulting in irregular yellow wet spots, with wavy edges, irregular leaves and spots starting from the edge near the tip of the leaf (Singh et al. 2013). Xoo. is a rod-shaped bacteria 0.55-0.75 x 1.35-2.17 µm, with a single polar flagellum, not spore-forming, motile, single or paired, and sometimes chained, a Gram-negative, the colony is light yellow. More than 30 races were found with sub-groups within the species (Furutani et al. 2009).

Efforts to improve plant growth and control bacterial leaf blight have been carried out by using antagonistic bacteria from the rice rhizosphere, namely *Bacillus subtilis* B1 in an organic fertilizer formula (Djatmiko et al. 2017). The leaf blight is classified as vascular disease, so it is difficult to control only with antagonistic bacteria, it is necessary to do this with endophytic bacteria that can infect the tissue, interact directly with the Xoo. The research previously showed control of bacterial leaf blight on-screen house with the effectiveness of more than 60% (Prihatiningsih et al. 2020a). Suboptimal land development for agriculture is generally faced with several problems, including high soil acidity and toxicity of Fe

and Al as well as deficiency of nutrients N. P. K. Ca, Mg. Therefore, improvements to the condition of soil biology and chemistry, using the endophytic bacteria. Suboptimal land spread on the embankment of the river, the lower part of the river terraces, alluvial depression, plain fluvial marine and peat (Sirappa and Titahena 2014). The specification of this research is the application of endophytic bacteria associated with rice roots as antagonistic to improve plant growth. This research aimed to evaluate endophytic bacteria associated with rice root and their activity to promote the plant growth directly and to control rice bacterial leaf blight indirectly.

MATERIALS AND METHODS

Preparations of endophytic bacterial associated of rice root (EBARr) from suboptimal land.

Endophytic bacteria from root samples of healthy rice plants from suboptimal land around Banyumas were prepared from the results of previous research isolation. Initially, 21 isolates were obtained and then selected to produce nine potential endophytic bacterial isolates. Then the nine endophytic bacterial isolates were tested in vitro for inhibition against Xoo and in the screen house, the endophytic bacteria were tested to suppress bacterial leaf blight (Prihatiningsih et al. 2020a). The results of the two series of studies were 3 isolates that were able to suppress disease, namely SM1 (Somagede 1 isolate), SB1 (Sumbang 1), and SB3 (Sumbang 3). Furthermore, in this study, the three endophytic bacteria were tested in rice fields.

The evaluate of endophytic bacterial associated of rice root (EBARr) to promote plant growth with observed of promoter component

a. Hydrogen Cyanide (HCN) production test. The nine endophytic bacterial isolates were tested as HCN producers. The paper disc was immersed in a 0.5% picric acid solution in a 2% sodium carbonate solution, placed on a NB medium containing 0.44% glycine, then the paper disc was affixed to the test tube wall. Endophytic bacteria that produce HCN gas are shown by changing the color of the paper disc from yellow to brown (Reetha et al. 2014).

b. The siderophore production test was carried out with SD-CASA medium, dark blue, 2% agar w /v in order to be added. As much as 10 ml of CAS-blue agar as the basis of the plate, After solidifying it was coated with 6 ml of YPGA (yeast extract pepton glucose agar) medium, the plate was incubated overnight at 32^{o}C, 10 µl of supernatant endophytic bacteria was dropped on a paper disk, forming an orange or pink zone (Hu and Hu 2011).

c. Testing IAA production, endophytic bacteria isolates were grown on slanted NA medium with and without L-Tryptophan (0.5%), after 2 days of incubation at room temperature then dropping 100 µl of Salkowski reagent A (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution). Colonies that produce IAA show a change in color to pink (Mohite 2013).

d. The phosphate solubility activity test was assessed qualitatively using potato-dextrose yeast extract agar (PDYA)-CaP. Each bacterial culture was scratch inoculated in PDYA-CaP medium at 4 places, incubated at 28 $\pm$ 2^{o}C for 10 days. The clear zone shows that the endophytic bacteria is capable of phosphate solubility (Khan et al. 2009).

e. Resistance Test of Isolates to Antibiotics. The resistance test of isolates against antibiotics was carried out by growing endophytic bacteria on the NA medium by means of the pour plate method then dipping the sterile paper disc pieces in antibiotics after draining and then placing them on the bacterial medium, incubated for 24 hours. If a clear zone is formed, it means that the endophytic bacteria is senisitive to these antibiotics, while those that do not form a clear zone means that they are resistant to these antibiotics (Yasmin et al. 2009).

f. Testing of endophytic bacteria to promote plant growth. Observation of the growth components of rice plants is intended as a direct effect of endophytic bacteria on plants, including plant height and a number of tillers, number of panicle. The study was conducted with a completely randomized block design with 4 treatments and 6 replications. The treatments were control (untreated endophytic bacteria), B: SM1 (endophyte bacteria Somagede 1 isolate), C: SB1 (endophytic bacteria Sumbang 1 isolate) and D: SB3 (endophytic bacteria Sumbang 3 isolate). The treatment of endophytic bacteria was started by soaking the seeds before seeding for 1 night, followed by spraying the plants at 20, 30, and 40 days after planting. The variables observed were plant height, number of tillers, and number of panicle.

g.The activity of EBARr as biocontrol of bacterial leaf blight. Endophytic bacteria as disease control as an indirect effect is carried out in bacterial leaf blight endemic areas and uses varieties that are susceptible-tolerant to bacterial leaf blight, namely Ciherang. Treatment and design such as testing on plant growth. The endophytic bacterial density was 10^9 cfu / mL. Variables observed: components of the pathosystem (incubation period, disease intensity, infection rate, control effectiveness). Observation of disease intensity using the formula DI = Σ (n xv) / (Z xN) x 100%, DI = disease intensity, n = number of plants from each scale category, v = scale category, N = number of plants observed, Z = highest category value. Xoo's attack category is: 0 = healthy, 1 = damage scale 1–5%, 3 = damage scale 6–12%, 5 = damage scale 13–25%,

7 = damage scale 26–50%, 9 = scale 51–100% damage (Djatmiko et al. 2011). The rate of infection is calculated based on the proportion of diseased tissue obtained from the value of disease intensity with the van der Plank formula (1963), Xt = Xo. e^{rt}, where Xt = proportion of diseased intensity at time t and Xo = proportion of diseased intensity at initial time, e = logarithmic number, r = infection rate and t = interval of observation time. Because of the bacterial leaf blight is a simple interest disease, calculate r = 2,3 / t (log 1 / (1-Xt) - log 1 / (1-Xo) unit / day.

Data analysis

Data at the laboratory experiment were analyzed by descriptive methods and then communicated and comparative to references. Data from experimental ex-farm were analyzed by anova, if significant effect, it's continued with LSD 5%

RESULTS AND DISCUSSION

Based on the characterization of endophytic bacteria in previous studies and by heating treatment in an oven at 80°C for 20 minutes of the suspension of rice root endophytic bacteria in the 1st dissolution, most of the endophytic bacteria obtained showed Gram positive, in the form of short rod shape - rod shape, forming endospores, then grouped into the genus *Bacillus* sp. (Gordon et al. 1973; Prihatiningsih et al. 2020a).

Plant growth promoter component of EBARr

The component of plant growth promoter showed from nine endophytic bacteria that could produce HCN, siderophore, IAA, and phosphate solubility as qualitative test (Table 1). The various respon of four assays showed the relative of qualitative. Figure 1 showed HCN production from nine endophytic bacteria before reaction to produce HCN (A) with yellow and (B) after production HCN, the paper disk become brown.

Table 1. Plant growth components of nine rice root endophytic bacteria

Isolate Code	HCN production	Siderophore production	IAA production	Phosphate solubility
Kr4	+	++	+	+
Kr5	+	+	+	+
Kr7	+	-	-K	-
SR1	+	-	+	+
SR5	+	+	+	+
SR7	+	++	+	+
SM1	+	++	+	++
SB1	+	+	++	+
SB3	+	-	+++	++

Notes: +: produce; ++ and +++: produce with more colour (IAA) ++: more strong of produce siderophore and phosphate solubility, -: unproduced; Kr: Karangwangkal, SR: Serayu, SM: Somagede, SB: Sumbang

Figure 1. HCN production test of the nine endophytic bacteria
A. Before reaction and B. After reaction as HCN production with brown colour of paper

Endophytic bacteria are able to stimulate plant growth by observing plant growth-promoting components, namely bacteria as a producer of HCN, siderophore, IAA, and as a phosphate solubility (Table 1). The response of plant growth promoter components varied from the nine isolates of endophytic bacteria that were tested as a result of the selection from the first year. Bacteria that are able to produce HCN show that these bacteria are capable of being biocontrol. This is because the role of HCN is to inhibit the proliferation or multiplication of pathogens, stimulate the formation of root hairs, thereby helping to increase nutrient absorption (Olanrewaju et al. 2017).

Figure 2 showed the six endophytic bacteria produced siderophore with orange zone exept the Kr7, SR1 dan SB3, the medium still blue. The bacterial siderophore test formed an orange zone indicating that the type of siderophore was hydroxamate (Ahmed and Holmstrom 2014; Prihatiningsih et al. 2017). Siderophores are low molecular weight compounds produced by bacteria and are capable of chelating iron into a form of siderophore-Fe $^{3+}$ bonds available to plants. This siderophore is also one of the biocontrol mechanisms by binding to Fe^{3+} so the pathogen is limited in the need for Fe^{3+} so that the pathogen is inhibited from reproducing to reach the rhizosphere due to iron deficiency (Glick 2012; Prihatiningsih et al. 2017).

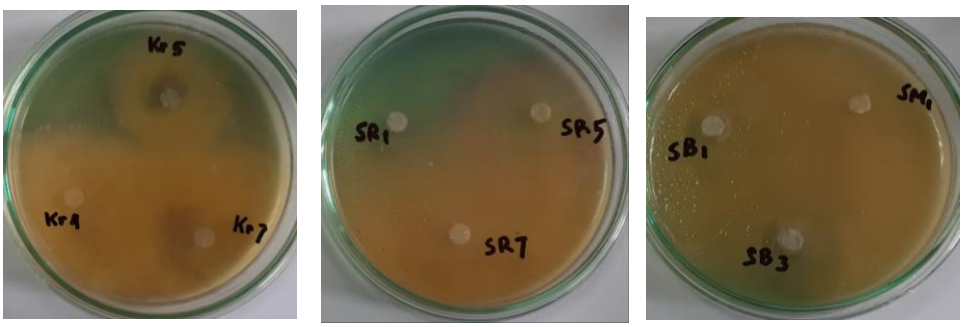

Figure 2. Siderophore production of the endophytic bacteria

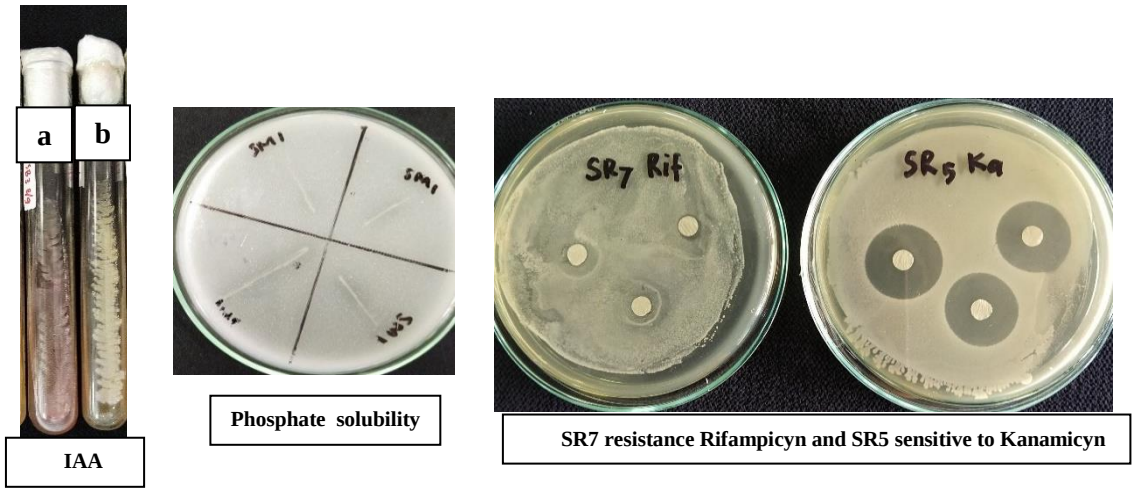

Figure 3. IAA production (a:produce of IAA and b:unproduce of IAA), phosphate solubility and resistance to antibiotics of the endophytic bacteria

The endophytic bacteria were able to produce IAA which was marked with pink or pink color in the endophytic bacteria culture which was dripped with Salkowski reagent (Figure 3). This indicates that endophytic bacteria are capable of producing the plant hormone IAA. Quantitative analysis of IAA production have conducted that SR7 and SB1 isolate were active at 79,33 and 75,88 ppm respectively (Prihatiningsih et al. 2020a). IAA functions for cell division and elongation stimulates seed and tuber germination increases the rate of xylem development and root development. IAA also functions as a vegetative growth controller, initiating the formation of adventitious and lateral roots, so that plant growth is better (Glick, 2012).

The ability of endophytic bacteria as a phosphate solubility is shown by a positive reaction in Table 1 and Figure 3, with the formation of a clear zone. Solubilization of different form of phosphate by endophytic bacteria associated with maize can be increasing its availability for plants to promote plant growth and increase the yield (Mugiastuti et al. 2020). The ability of bacteria as a phosphate solubility helps to provide nutrients available for plants because of the phosphate present in the soil in a form that is not available to plants becomes available (Olanrewaju et al. 2017).

Table 2. Intrinsic antibiotic resistance (IAR) test of rhizobacterial isolates

Isolate	Rifampicin		Kanamycin	
	Reaction	Diameter of zone (mm)	Reaction	Diameter of zone (mm)
Kr4	-	4	-	8
Kr5	-	4	-	8
Kr7	-	4	-	8
SR1	+	0	+	0
SR5	-	5	-	8
SR7	+	1	-	6
SM1	-	6	-	8
SB1	-	7	-	9
SB3	+	1	-	7

Notes: +: resistance= not zone, very small (1 mm), -: sensitive: zone formed
Not zone = antibiotic resistance (+) means that be still growing in the medium with the antibiotic.

The resistance of endophytic bacteria to antibiotics (Figure 3) is very important as a pathogen biocontrol mechanism because it is exposed to many microorganisms that produce various antibiotics in the soil. Antibiotic-resistant endophytic bacteria will be able to survive so that they are able to compete in space and become antagonistic to pathogens (Ulloa-Ogaz et al. 2015). Isolates that are resistant to antibiotics as shown in Figure 3. SR7 isolates are resistant to Rifampicin antibiotics which are characterized by no zone formation, meaning that the growth of SR7 endophytic bacteria still occurs around the antibiotic. On the other hand, the SR5 isolate was not resistant or sensitive to the Kanamycin antibiotic which was indicated by the formation of a clear zone around the filter paper treated with the Kanamycin antibiotic, meaning that there was no growth around the antibiotic. This is in accordance with the opinion of Yasmin et al. (2009) that the IAR test is needed to identify the endophytic bacteria resistant or sensitive to antibiotics. IAR can also be used to genotypically identify bacterial species.

Endophytic bacteria to promote plant growth

Based on the result of the laboratory experiment than the endophytic bacteria selected to promote plant growth in the exfarm showed in Table 3, which three endophytic bacteria can promote increase plant height, the number of tiller, and the number of panicle. The SM1 (Somagede isolate) was able to increase plant height compared to other endophytic bacteria treatments and controls, while isolate Sumbang 3 (SB3) was increase the number of tiller. This is in accordance with the opinion of Glick (2012) that endophytic bacteria are able to stimulate plant growth because they produce phytohormones such as IAA. The plant growth

Table 3. The component of plant growth with endophytic bacteria application

Isolate Code	Plant height (cm)	Number of tiller	Number of panicle/clump
A (control)	96,6b	13,3b	9,875
B (SM1)	101,3a	12,6b	13,005
C (SB1)	98,5b	15,3ab	12,125
D (SB3)	98,8b	18,3a	18,875

Suppressing bacterial leaf blight by EBARr

The observation of the pathosystem components of rice bacterial leaf blight conducted at endemic area showed that the treatment of endophytic bacteria was different from the control (Table 4). However, between endophytic bacteria was not significantly different, with control effectiveness ranging from 56.03 to 60.79%. The endophytic bacteria can delayed incubation period, and suppress the infection rate. The ability of endophytic bacteria as well as the rhizosphere bacteria to control bacterial disease with antibiosis mechanism as bacteriostatic with produce hydrolitic enzyme like amylase, protease and lipase (Prihatiningsih et al. 2020b; Mugiastuti et al. 2020)

Table 4. Components of plant pathosystems against bacterial leaf blight

Isolate Code	Incubation period (dat)	Disease intensity (%)	Effectiveness (%)	Infection rate (unit/hari)
A (control)	24	23,13a	0	0,0469
B (SM1)	30,2	10,17b	56,03	0,0435
C (SB1)	36,3	9,07b	60,79	0,0246
D (SB3)	34,2	9,27b	59,92	0,0454

The opinion of Nagendran et al. (2013) stated that endophytic bacteria were able to control bacterial leaf blight on rice with a low disease intensity of 2.80% compared to without treatment of endophytic bacteria with a disease intensity of 19.82%. Four isolates, OS52, OS40, OS23 and OS53, which were identified by nucleotide sequence analysis as *Enterobacte*r sp., *B. subtilis, Bacillus* sp. and *Pseudomonas putida*, respectively, could increase plant growth and decrease Xoo infection under greenhouse conditions. Seed treatment with these antagonists caused disease to cease in over 60% of plants. These species have been found as endophytes in rice (Yousefi et al. 2018). Endophytic bacteria able to control the disease caused by bacteria and fungi because of them produced hydrolitic enzyme as well as chitinase, protease similarly with rhizospheric bacteria *B. subtilis* from potato rhizosphere (Lestari et al., 2017).

CONCLUSION

The conclusion based on this research showed that three endophytic bacteria associated rice root (EBARr) as prospectives as plant growth promoter, and biocontrol agents to bacterial leaf blight. The inhibition activity of EBARr similar with in an exfarm test by suppresing the disease until 60,79% effectiveness. The rice growth enhanched by applied of EBARr with plant height and number of tiller so increase. Furthermore endophytic bacteria was prospected to develope biofertilizer and biocontrol.

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