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SCREENING OF COMPETENT RICE ROOT ENDOPHYTIC BACTERIA TO PROMOTE RICE GROWTH AND BACTERIAL LEAF BLIGHT DISEASE CONTROL

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ABSTRACT

Screening of competent rice root endophytic bacteria to promote rice growth and bacterial leaf blight disease control. This study was aimed to collect isolate endophytic bacterial of rice roots which able to produce IAA, determine the effect of endophytic bacteria application in stimulating rice plant growth, and evaluate the potential of rice root endophytic bacteria for controlling bacterial leaf blight. This reasearch was carried out at the Screen House, Plant Protection Laboratory, and Agrohorti Laboratory of the Agriculture Faculty, Jenderal Soedirman University. Isolation of rice root endophytic bacteria was carried out by purposive random sampling from several marginal lands. The results showed that 8 isolates of rice root endophytic bacteria were able to produce IAA, ranging from 57.56 to 79.33 ppm and B07 isolate from Serayu produced the highest amount of IAA. The B04 and B07 isolates were contributed to increase the rice plant growth. The application of rice root endophytic bacteria was effective in controlling bacterial leaf blight.

Key words: bacterial leaf blight, endophytic bacteria, IAA, plant growth, rice

INTRODUCTION

Rice is a staple food for almost all Indonesian. The increase in population causes an increasing in food needs. Therefore, efforts are needed to increase national rice productivity. The need for rice in 2017 reached 111.58 kg/capita/year, it was equivalent to 29.13 million tons of rice (BPS, 2019). Thus, improvement in technology is needed to increase the productivity of rice plants that are environmentally friendly and sustainable (Kasno, 2010). The continuous use of chemical fertilizers will cause damage to the physical properties of the soil, the soil becomes denser and phosphate accumulation occurs. Efforts to overcome this problem is using alternative fertilizers that can increase productivity and maintain soil fertility (Havlin *et al.,* 2005).

The application of PGPR (Plant growth-promoting rhizobacteria) is one of the efforts to increase rice productivity, one of which is the utilization of beneficial root endophytic bacteria for plant growth. Endophytic bacteria are bacteria that live and associated with plant tissues without causing any disease to the plant (Spaepen *et al.,* 2007). The presence of endophytic bacteria in plant tissues was not only play a role in improving plant growth but also producing growth promoters, fixing nitrogen, solubilizing phosphate, and playing role in plant health (Wozniak *et al.*, 2019). The N-fixing endophytic bacteria can increase nitrogen fixation from the air. Increasing the concentration of N-fixing endophytic bacteria in biological fertilizer tends to significantly increase plant N uptake (Setiawati *et al.,* 2002; Mieke, 2008). The superiority of endophytic bacteria as biological control agents, which increase the availability of nutrients, produce growth hormones, control plant diseases, and can induce plant resistance (Kloepper *et al.,* 1992; Hallmann, 2001).

Plants can experience a decrease in productivity caused by endogenous hormones, for example, auxin reduction can cause abnormal growth of plant roots. One of the important auxin group hormone for rice plants is indole acetic acid (IAA). The IAA functions is controlling many important physiological processes including cell enlargement and division, tissue differentiation and response to light and gravity (Khalid *et al.,* 2004). One of the efforts to increase IAA in the paddy fields is through the application of PGPR (Cahyaty *et al.,* 2017). IAA is an endogenous auxin hormone that synthesized in various parts of the plant and generally associated with parts of the plants that actively growing and developing such as in all meristem

tissues of shoot tips, root tips, and cambium. IAA also stimulates cell extension, regulates apical dominance, and the formation of lateral and adventitious roots (Patil, 2011).

Endophytic bacteria could serve as plant growth promoter and pathogen biocontrol. One important disease in rice plants is bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). This disease causes yield losses of more than 40% (Swamy *et al.,* 2006). *Bacillus methylotrophicus* and *B. subtilis* were reported to be able to suppress Xoo by 50.29 and 57.86% (El-shakh *et al.,* 2015). Isolation of endophytic bacteria as control of targeted plant diseases *Bacillus* sp. carried out by heating the suspension of endophytic bacteria for 30 min at a 80 °C. *B. subtilis* var. *amyloloquefaciens* (FZB 24) can inhibit Xoo in vitro and endophytic EPB 18, EPB 11, FZB 24 can stimulate rice plant growth, and significantly reduce the intensity of rice leaf blight disease up to 85–87% (Nagendran *et al.,* 2013).

The aim of this research was to collect the rice root endophytic bacterial isolates (RREB) which able to produce IAA, understand the effect of RREB application in stimulating rice plant growth and suppress bacterial leaf blight disease, and evaluate the potential of rice root endophytic bacteria for controlling bacterial leaf blight disease.

MATERIALS AND METHODS

Research Site. This research was conducted from March to September 2019 at the Screen House, Plant Protection Laboratory, and Agrohorti Laboratory of the Agriculture Faculty, Jenderal Soedirman University. The endophytic bacteria were isolated from Karangwangkal, Sumbang, Serayu, and Somagede Banyumas Regency, Central Java Province.

Isolation of Endophytic Bacteria. The experimental design used in the isolation and identification of endophytic bacteria was descriptive, starting with plants root sampling by purposive random sampling. The isolation was done by choose two healthy rice plants, took their roots, cleaned in running water, and weighed 100 g each. Rice root endophytic bacteria were isolated by sterilizing the root surface with sodium hypochlorite, then rinsed with sterile water 3 times, macerated with sterile porcelain mortal, and added 100 mL of sterile water. The 1 mL of suspension then added 9 mL of sterile water and incubated at 80 °C for 30 min for *Bacillus* sp. target (Singh *et al.,* 2016). Five times serial dillution then carried out, the fourth and fifth dilutions were cultured on the NA (nutrient agar) medium with spread plate method. The growing colonies then observed the macroscopic and microscopic morphology.

Quantitative and Qualitative IAA Production Test. Each RREB isolate was grown on LB (Luria Bertani) medium with 40 g/mL L-Tryptophan added and incubated in a shaker in room temperature 29 ± 1 °C at 150 rpm for 48 h. After that, it was centrifuged at 3000 rpm for 15 min. Subsequently, 1 mL of filtrate was mixed with 1 mL of Salkowski reagent (1.5 mL of $FeCl_3$. $6H_2O$ 0.5 M in 80 mL H_2SO_4 60% solution), incubated at room temperature 29 ± 1 °C for 30 min. Qualitatively, IAA production was indicated by pink color, then IAA concentrations were measured quantitatively by a spectrophotometer with a wavelength of 550 nm, compared to a predetermined IAA standard curve (Rana *et al.,* 2011).

Experimental Design of RREB Application to the Growth of Rice Plants. The study was conducted using a randomized complete block design (RCBD) with treatments consisting of 8 RREB isolates and control (no RREB). Each treatment was repeated 3 times and each experimental unit contained 3 polybags. The rice used in this study was Ciherang variety. The treatments were B01 (Karangwangkal 7), B02 (Karangwangkal 5), B03 (Karangwangkal 8), B04 (Sumbang 1), B05 (Sumbang 2), B06 (Serayu 5), B07 (Serayu 7) and B08 (Somagede 1) and control (no isolate). The observed variables were plant height, leaf size, number of tillers, root length, root volume, fresh root weight, dry plant weight, and dry root weight.

Application of RREB to Suppress Bacterial Leaf Blight. The RREB was applied by soaking the seeds before seeding and spraying at 30 days after planting with RREB suspension (10^8 CFU/mL) and sterile water for control. Three plants were prepared for each treatment group and each plant was inoculated with Xoo suspension (10^8 CFU/mL) on the two leaves by leaf-clipping method using a sterile scissors that was dipped earlier in Xoo suspension (Singh *et al.,* 2013). The disease intensity was calculated with formula (Suganda *et al*., 2016):

$$DI = \frac{\sum (n \times v)}{Z \times N} \times 100\%$$

DI : disease intensity
n : number of infected plants for each score
v : infection score
N : number of observed plants
Z : maximum disease score

The disease infection scores were: 0= no infection, 1= 1–5 squares (4–20 mm), 2= 6–10 squares (24–40 mm), 3= 11–15 squares (40–60 mm), 4= 16–20 squares (64–80 mm), 5= 21–25 squares (84–100 mm).

The collected data were analyzed using analyses of variance (ANOVA and followed by DMRT (Duncan Multiple Range Test) on 5% significance level.

RESULTS AND DISCUSSION

Isolation of Endophytic Bacteria. The macroscopic and microscopic characteristics of endophytic bacteria was presented in Table 1. All collected isolates were gram-positive bacteria with dominant rod shape (bacilli) cell and had endospore with malachite green staining. The colony were yellowish, white and creamy. The colony elevation is convex and flat (Figure 1). This result was in accordance with Nadeem *et al*. (2012) that bacterial colonies on the surface of the medium were vary in shapes, such as circular (round), irregular (round irregular) and also varies in elevation, such as flat, raised and convex.

Quantitative and Qualitative IAA Production Test. Qualitative test results was characterized by a change in the color of each endophytic bacterial suspension to pink reacted to Salkowski's reagent (Figure 2). This is in line with the opinion of Rahman *et al*. (2010), the pink discoloration of isolates after Salkowski's reagent drops was due to the reaction between Salkowski's reagent with IAA or with IAA-forming compounds. The observation result showed that isolate has the highest IAA production was B07 isolate, while the lowest production was B03 isolate (Table 2). The lowest IAA production isolates were originated from Karangwangkal area, it has more fertile land compared to marginal land, so that the potential of bacteria to be able to promote plant growth is lower. One of marginal land character was acidity due to the high level of total phosphate, however, some bacteria could solubilize phosphate and make it available for the plant. On marginal land, the limited nutrient content causing bacteria to be more active in producing IAA as a secondary metabolite that produced in extreme conditions. The bacteria as plant growth promoter capability produce IAA, the phosphate solubilizing ability, and siderophore secretion that chelates iron (Yu *et al*., 2011; Lwin *et al.*, 2012). Kholida & Zulaika (2015) stated IAA is a major member of the auxin group which controls many important physiological processes including cell enlargement and division. IAA

Table 1. The morphological characteristics of RREB

Isolates	Microscopic morphology		Macroscopic morphology			
	Size (µm)	Gram test	Shape	Elevation	Margin	Color
B01	2–3 × 6–7	+	Rod	Flat	Undulate	White
B02	2–3 × 6–7	+	Rod	Convex	Undulate	Yellow
B03	2–3 × 6–7	+	Rod	Flat	Undulate	White
B04	2–3 × 4–5	+	Rod	Flat	Undulate	White
B05	2–3 × 6–7	+	Rod	Flat	Undulate	White
B06	2–3 × 6–7	+	Rod	Convex	Entire	Yellow
B07	2–3 × 6–7	+	Rod	Convex	Entire	White
B08	2–3 × 6–7	+	Rod	Convex	Entire	Yellow

B01 (Karangwangkal 7), B02 (Karangwangkal 5), B03 (Karangwangkal 8), B04 (Sumbang 1), B05 (Sumbang 2), B06 (Serayu 5), B07 (Serayu 7), B08 (Somagede 1).

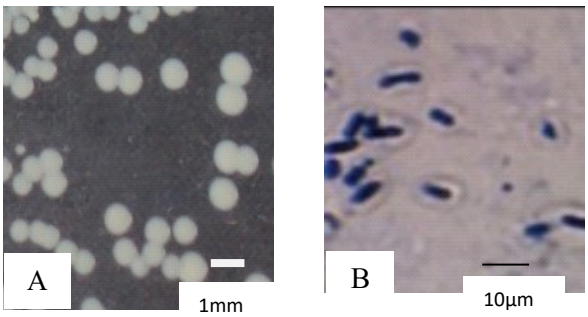

Figure 1. Rice root endophytes bacteria. (A) Colony, (B) Cells.

stimulate plants to produce more lateral roots, root hairs and root hair branches (Rahman *et al.,* 2010).

Effect of RREB Application to the Rice Plants Growth. The application of RREB significantly affected root length, root fresh weight, root volume, and root dry weight (Table 3). The application of bacterial isolates B04 and B07 showed an increase in root length of 39.96% and 36.53%, root volume of 82.72%, root fresh weight of 79.37% and root dry weight with an increase of 52 , 13% (Table 3). This was presumably due to the IAA production as a growth hormone which causes faster root development and wider root surface so that nutrient uptake is increased followed by cell enlargement and root fresh weight will increase. Increased root fresh weight is mainly due to increased uptake of water. IAA produced by bacteria will be utilized by plants and will follow the metabolic processes in the plant so that it

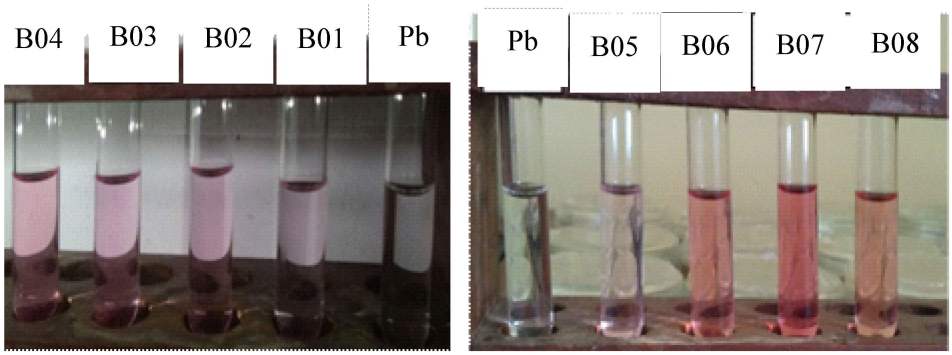

Figure 2. The IAA production test. Pb: control, B01-B08: endophytic bacteria isolates.

Table 2. IAA producing activity, quantitatively and qualitatively

Isolates	Qualitative	Quantitative (ppm)
B01	+	67.64
B02	+	75.12
B03	+	57.56
B04	+	75.88
B05	+	66.63
B06	+	69.91
B07	+	79.33
B08	+	73.63

B01 (Karangwangkal 7), B02 (Karangwangkal 5), B03 (Karangwangkal 8), B04 (Sumbang 1), B05 (Sumbang 2), B06 (Serayu 5), B07 (Serayu 7), B08 (Somagede 1).

Table 3. Application of RREB towards root length, root fresh weight, root volume, and root dry weight of ciherang paddy at 6 weeks after planting

Isolates	Root length (cm)	Root volume (mL)	Root fresh weight (g)	Root dry weight (g)
Control	3753.10 c	44.33 e	38.98 e	5.16 cd
B01	3636.55 d	74.67 abc	62.01 b	4.58 d
B02	3750.48 c	69.67 cd	59.57 bc	5.75 c
B03	3654.88 d	67.33 d	55.15 cd	3.83 e
B04	3876.19 a	81.00 a	69.29 a	7.85 a
B05	3255.48 f	73.00 bcd	59.34 bc	5.77 c
B06	2781.43 g	71.67 bcd	52.87 d	6.69 b
B07	3797.62 b	75.00 abc	52.75 d	4.66 d
B08	3496.43 e	78.00 ab	68.50 ab	4.92 d

Number followed by same letter in the same coloumn were not signifficantly different based on DMRT 5%. B01 (Karangwangkal 7), B02 (Karangwangkal 5), B03 (Karangwangkal 8), B04 (Sumbang 1), B05 (Sumbang 2), B06 (Serayu 5), B07 (Serayu 7), B08 (Somagede 1).

helps in the process of adding height, root volume, root length, and root fresh weight (Spaepen *et al.,* 2007).

The analysis results from the observation of the number of tillers, plant height and leaf size was shown in Table 4. The application of B04 isolate showed the highest number of tillers (12.21 tillers) with an increase at 6.88 tillers or 129% relatives to control. The increase occurred due to the application of endophytic bacteria, so that nutrient needs were met in the process of photosynthesis at the age of 4 and 6 weeks after planting which included in the vegetative phase to form more rice tillers. According to Yoshida (1981), the number of tillers is strongly influenced by the availability of nitrogen and phosphorus in the soil. If enough nitrogen is found in the soil, plants can produce a large number of tillers, although not all of the growing tillers produce panicles. Based on the bi-weekly growth report above, the B04 treatment was the best in producing the number of tillers with the highest increase compared to the control treatment at 6 weeks after planting.

The B07 treatment was the best in increasing plant height with the highest increase at 10.28 cm or 14.39% relative to control. This showed that the association between endophytic bacterial isolates and their host plants was not only able to stimulate the formation of roots, but also can stimulate the height of rice plants.

The best treatment for increasing leaf size was B04 isolate with an increase in total leaf size at 1069.31 cm^2 or 122.92% relative to control. Plant growth was increased by endophytic bacteria in the vegetative phase during cell division because it can stimulate plant growth by increasing leaf size. Ningrum *et al.* (2017) explained that, the higher consentration of PGPR, promote leaf surface area and vice versa. So that it can be in line with the application of endophytic bacteria.

Effect of RREB Application to Suppress Bacterial Leaf Blight. The bacterial leaf blight disease could be suppressed by application of RREB (Table 5). The best

Table 4. Application of RREB towards number of tillers, plant height and leaf size at 5 wap

Isolates	Number of tillers	Plant height (cm)	Leaf size (cm^2)
Control	5.33 d	71.39 c	869.91 g
B01	10.58 b	80.67 ab	1831.38 b
B02	10.24 b	80.67 ab	1750.73 cd
B03	10.57 b	80.44 ab	1809.24 bc
B04	12.21 a	80.33 ab	1939.22 a
B05	9.11 c	79.17 b	1724.26 d
B06	9.00 c	81.11 a	1684.63 e
B07	10.56 b	81.67 a	1514.03 e
B08	10.67 b	81.22 a	1260.45 f

Number followed by same letter in the same coloumn were not signifficantly different based on DMRT 5%. B01 (Karangwangkal 7), B02 (Karangwangkal 5), B03 (Karangwangkal 8), B04 (Sumbang 1), B05 (Sumbang 2), B06 (Serayu 5), B07 (Serayu 7), B08 (Somagede 1).

Table 5. Phatosystem component of bacterial leaf blight disease on the application of RREB

Isolates	Incubation period (dai)	Disease intensity (%)	Effectiveness (%)
Control	4.2	36.44 c	-
B01	5.8	18.67 ab	48.76
B02	4.8	20.22 b	44.51
B03	5.7	13.78 ab	62.18
B04	6.1	11.56 a	68.27
B05	5.8	15.78 ab	56.69
B06	5.8	14.22 ab	60.98
B07	5.6	13.11 ab	64.02
B08	6.1	21.11 b	42.06

Number followed by same letter in the same coloumn were not signifficantly different based on DMRT 5%. dai = day after inoculation. B01 (Karangwangkal 7), B02 (Karangwangkal 5), B03 (Karangwangkal 8), B04 (Sumbang 1), B05 (Sumbang 2), B06 (Serayu 5), B07 (Serayu 7), B08 (Somagede 1).

was isolates collected from Sumbang, with an effective disease suppression of 68.27% compared to control. This was due to the isolates collected from non-suboptimal or marginal land with adequate water conditions, and obtained from healthy plants among diseased plants. These conditions indicate the suitability for the growth of antagonistic bacteria because of adequate nutrition, so that it has the potential as a biological control agent and promoter of plant growth. Moustaine *et al.* (2017) suggested that, the rhizosphere and endophytes bacteria associated with the roots, are able to benefit plants which are shown by their ability to produce plant hormones such as auxin, N2 fixation, and phosphate solubilizer. In addition, it was also stated that endophytic bacteria and rhizosphere as antagonists to pathogens by producing antibiotics, siderophore, chitinase and other nutrients that are able to effectively colonize the roots as a trigger for plant growth.

CONCLUSION

In total, 8 isolates of root endophyte bacteria was collected from paddy roots. The isolates could produce IAA up to 79.33 ppm, among others the B07 isolate produce highest amount of IAA. The B04 isolate from Sumbang and B07 from Serayu was able to promote the paddy growth. The application of endophytic bacteria could effectively control the bacterial leaf blight.

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