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RESEARCH ARTICLE

STIMULATE THE GROWTH OF RICE USING ENDOPHYTIC BACTERIA FROM LOWLAND RICE PLANT TISSUE

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ABSTRACT

Exploration and selection of endophytic bacteria from healthy food crops grown in lowland ecosystem is important to be conducted in order to get growth-stimulating endophytic bacteria at soil with low fertility level so that capable to optimize initial growth of food crops and subsequently can increase productivity level of lowland soil.The research objective was to isolate and to test the IAA-producing endophytic bacteria isolate in stimulating the rice crop growth at lowland area. Endophytic bacteria are isolated from tissues of rice, corn and peanut crops which grown at shallow swamp land in OganIlir and OganKomeringIlir Districts, South Sumatra, Indonesia. There was nine isolates of nitrogen-fixer endophytic bacteria that capable to contribute IAA phytohormone into their growth media. The P31 isolate from rice crop tisssue of 2 months old produce the best rice sprouts than other isolates. This isolate can contribute of about 10 mg kg^{-1} IAA to its growth medium and increase the crownsdry weight and the roots dry weight respectively with magnitudes of 133% and 225% compared to control treatment. Concentration and absorbtion of N for rice crops innoculated with P31 isolates had increased by 169% and 400%, recpectively. The P31 isolates had been identified as ***Burkholderiapseudomallei*** (also known as *Pseudomonas pseudomallei)*.

Keywords : IAA phytohormone, endophytic bacteria, lowland soil

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INTRODUCTION

The role of swamp land is becoming more important in effort to maintain rice self-sufficiency and to attain self sufficiency of other food crops due to limited fertile land as results of functional shift for settlement areas and other non-agricultural needs. One of swamp land that has potential to be utilized for agricultural enterprise is lowland. Potential area of lowland in Indonesia was 13.28 million hectares consisting of shallow lowland with area of 4,166,000 ha, medium lowland with area of 6,076,000 ha and deep lowland with area of 3,039,000 ha. Part of this lowland area is found at South Sumatra with area of 650,000 ha and only 190,000 ha had been utilized for agricultural enterprise (Gofar 2015). Lowland area development for agriculture on the other hand has some constraints, for instance nutrients availability which is usualy very low (Tjimpolo and Kesumaningwati 2009). Constraint of N nutrient availability needs improvement efforts such as fertilizing and resource utilization of N-fixer endophytic bacteria which are found within plant tissues.

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Endophytic bacteria are the one that associates with tisssue or cells of high level plants which produce no harmful effect on plants (Tarabily *et al*. 2003). Endophytic bacteria are defined as those bacteria that can be isolated from surface-disinfected plant tissues or extracted from within the plants and that are not observed to harm the host plants. Those bacteria have a multitude of applications that enhance agricultural production, they enhance wheat growth through production of phytohormones, increase rice production by increasing mineral availability, fix nitrogen in rice and wheat (Sun *et al*. 2007). Gofar*et al*. (2004) showed that IAA phytohormone contribution from consortium of leave growth-stimulating microbe which is applied on seeds can stimulate the growth of corn crops through increase of roots dry weight and increase of crop P content. It was reported by Gofar*et al*. (2008) that endophytic bacteria of *Pseudomonas aeruginosa* and *Bacillus cereus* isolated from red chilly crop tissue grown at lowlaand soil had produced IAA with magnitudes of 4.16 $mgkg^{-1}$ and 3.93 mg kg^{-1}, respectively. Morever, Gofar *et al.* (2009) showed that innoculation with endophytyic bacteriafor lowland swamp rice crop resulted in rice yield increase compared to rice crop without innoculation.

Rice (*Oryza sativa* L.) is the most important cereal crop in the world, feeing more than 50% of the world's population (Gyaneshwar*et al*. 2001), and its yield must be increased to match the rise in consumption. The application of chemical fertilizer for long time may adverse soil environment (Sudadi *et al*. 2014). This must be achieved without the mass use of chemical fertilizers and pesticides, which may cause environmental pollution and negatively influence human health. The application of endophytic bacteria having beneficial characteristics to the cultivation of rice as well as other plant is crucial(Mano and Morisaki 2008).

Exploration and selection of endophytic bacteria from healthy food crops grown in lowland ecosystem is important to be conducted in order to get growth-stimulating endophytic bacteria at soil with low fertility level so that capable to optimize initial growth of food crops and subsequently can increase productivity level of lowland soil. This study objective is to isolate and to test the capability of IAA-producing endophytic bacteria isolates from several food crops tissue which are grown at lowland in stimulating the rice crop growth at soil of lowland area.

MATERIALS AND METHODS

Sampling of plants and field site description

The method used to get endophytic bacteria isolate that stimulate crop growth was conducted by using field survey and isolation within laboratory. The healthy leaves of food crops (rice, corn and peanut) which received no application of inorganic fertilizers or synthetic pesticides are collected from several locations of lowland at Ogan Ilir and Ogan Komering Ilir, South Sumatra, Indonesia. The physico-chemical characteristics of the field soil were as follows: pH 4.3, 46% clay, 1.3 g kg^{-1} C-organic, 0.22 g kg^{-1} N-total, and 12 mg kg^{-1} P_2O_5.

Leaves tissue as endophytic bacteria source are immediately cleaned in laboratory to remove dirts that attached on the leaves surface and subsequently are strelized by soaking within 70% alcohol solution for 3 minutes and then recleaned with steril aquadest. These leaves tissue are cut with dimension of 1 cm. One gram of leave cut is poured with 10 mL steril physiological solution and grinded until achieving the suspension form. One mL of suspension is diluted with steril physiological solution up to 1000 times dilution. Suspensionfrom each isolate is grown on solid JNFb (0.5 g malic acid/100 ml; 0.06 g K_2HPO_4/100 ml; 0.18 g KH_2PO_4/100 ml; 0.02 g $MgSO_4.7H_2O$/100 ml; 0.01 g NaCl/100 ml; 0.002 g $CaCl_2.2H_2O$/100 ml; 0.2 mL nutrients solution/100 ml; 0.2 mL bromothymol blue/100 ml; 0.4 mL Fe EDTA/100 ml; 0.l mL vitamin solution/100 ml; 0.45 g KOH/100 ml) medium for 4 days. The growing colonies were subsequently tested for their ability to produce IAA at liquid medium. Isolates that capable to produce IAA were then purified and the test was conducted in relation to their capability to stimulate plant growth.

Assay for detection of plant growth promoting ability

IAA production by the bacterial isolates was measured by the method of Wohler (1997). The bacteria were grown overnight on nutrient broth and then collected by centrifugation at 7000 g for 5 min. The bacterial pellet was incubated at 37^0C for 24h with 3 mL of phosphate buffer (pH 7.5) with glucose (1%) and tryptophan (1%). After incubation, 2 mL of 5% trichloroacetic acid and 1 mL of 0.5 M $CaCl_2$ were added. The solution was filtered (Whatman no. 2 of pore size) and to 3 mL of the filtrate 2 mL of salper solution (2 mL 0.5 M $FeCl_3$ and 98 mL 35% perchloric acid) were added. This mixture was incubated fot 30 min at 250C in the dark. The absorbance of the resulting solution was measured at 535 nm with a Shimadzu UV-1603 spectrophotometer.

Paddy growth-experimental design

The effect of endophytic bacteria from food crops tissue which are grown at lowland area in stimulating plant growth as a treatment was conducted at greenhouse level by using the method of Completely Randomized Design (CRD). Each treatment was conducted with three replications. Rice seed was soaked in suspension of endophytic bacteria isolate (10^8 CFU mL^{-1}) that had been proved capable to produce IAA and then were planted on sand medium containing nutrients solution of Johnson minus N. Composition of Johnson nutrients medium per litre was as follows : [$CaHP0_4$ 1 g ; K_2HP0_4 0.2 g ; $MgS0_4.7H_20$ 0.2 g ; NaCl 0.2 g ; $FeCl_3$ 0.1 g ; and micro nutrients (0.5% B; 0.05% Mn; 0.005% Zn; 0.005% Mo; 0.002% Cu) with magnitude of 1 mL]. The observed parameters at this greenhouse level study were crop height, crop biomass, and N absorption by ricecrop grown on free-nitrogen sand media at 3 weeks after planting.

Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS Inc., IL Version 15.0). The data were analysis of variance (ANOVA). To detect the statistical significance of differences ($P<0.05$) between means, the HSD (Honestly Significance Difference) test was performed.

RESULTS AND DISCUSSION

Some endophytic bacteria isolates originated from leaves tissue of food crops (rice, corn, peanut and cowpea) had been found at several lowland locations in South Sumatra. Endophytic bacteria have been considered to originate from the outside environment and either the plant througt stomata, lenticels, wounth, areas of emergence of lateral roots and germinating radicles, as mentioned above (Gyaneshwar*et al*. 2001).

Testing of colonies which are grown at solid JNFb medium and their abilities to produce IAA at liquid JNFb medium showed that there were 9 endophytic bacteria isolates which capable to contribute IAA on their

Table 1. Capability of growth-stimulator endophytic bacteria isolates in producing IAA (mg kg^{-1})

No	Location	Isolate code	Isolate source crops	IAA content (mg kg^{-1})
1	Pemulutan Village, OganIlir District	P11	IR 64 rice variety	7.0
2	PulauGemantung 1 Village, OKI District	P21	IR 64 rice variety	9.0
3	PulauGemantung 1 Village, OKI District	P31	IR 64 rice variety	10.0
4	PulauGemantung 1 Village, OKI District	P41	Local rice variety	6.0
5	KelurahanTimbangan, OganIlir District	J11	Corn	10.0
6	LubukDalam Village, OKI District	J21	Sweet Corn	8.0
7	LubukDalam Village, OKI District	J31	Sweet Corn	9.0
8	Indralaya Village, OganIlir District	KP1	Cowpea	10.0
9	Indralaya Village, OganIlir District	KT1	Peanut	6.0

Table 2. The effect of several endophytic bacteria isolates in stimulating rice crop growth

Iso-late code	Average Data of Rice Crop							
	Crop height (cm) at the weeks of				Crown wet weight (g)	Crown dry weight (g)	Root wet weight (g)	Root dry weight (g)
	2	3	4	5				
0	4.03 a	8.75 a	18.93 a	25.17 a	0.19 a	0.06 a	0.05 a	0.04
P11	5.13 ab	14.75 b	24.83 b	29.50 b	0.36 c	0.12 b-e	0.12 abc	0.05
P21	5.87 bcd	17.25 c	25.75 bc	34.17 d	0.34 bc	0.09 a-e	0.12 ab	0.05
P31	7.07 e	21.25 d	28.43 d	34.50 e	0.49 d	0.14 e	0.30 e	0.13
P41	5.23 bc	18.25 bc	27.07 cd	33.17 cd	0.48 d	0.12 cde	0.15 bcd	0.07
J11	6.33 cde	15.75 bc	23.33 b	29.37 b	0.26 ab	0.07 a	0.10 ab	0.05
J21	6.57 de	21.00 d	28.00 d	31.83 bd	0.46 d	0.13 e	0.23 d	0.07
J31	6.93 de	20.50 d	25.03 bc	31.50 bc	0.31 bc	0.09 a-e	0.11 ab	0.06
KP1	6.97 de	20.15 d	24.67 b	33.67 cd	0.45 d	0.13 de	0.18 cd	0.07
KT1	6.53 de	15.70 bc	23.50 bc	29.50 b	0.25 ab	0.06 a-d	0.11 ab	0.05
LSD0.05	1.12	1.48	2.56	2.52	0.10	0.04	0.07	ns

Remarks : ns (not significant)
0 : without innoculation
P11 : Isolate from rice crop tissue at Pemulutan having age of 1.5 months
P21 : Isolate from rice crop tissue at PulauGemantung having age of 2.5 months
P31 : Isolate from rice crop tissue at PulauGemantung having age of 2 months
P41 : Isolate from rice crop tissue at PulauGemantung having age of 2 months, local variety
J11 : Isolate from corn crop at Indralaya having age of 2 months
J21 : Isolate from corn crop tissue at LubukDalam having age of 1 month
J31 : Isolate from corn crop tissue at LubukDalam having age of 1 month
KP1 : Isolate from cowpea crop tissue at Indralaya having age of 45 days
KT1 : Isolate from peanut crop tissue at Indralaya having age of 1 month

growth media. The IAA content produced by these endophytic bacteria was presented in Table 1. The ability of growth-stimulator endophytic bacteria in stimulating crop growth is affected by their capability to produce phytohormone, e.g. IAA. Nine of endophytic bacteria isolates had proved capable to produce IAA in the range of 6-10 mg kg^{-1}.

Table 1 showed that IAA-producer endophytic bacteria can be isolated from several food crops which were grown at lowland soil at several locations and different crop ages. IAA hormone is key factor for some aspects of crop growth and development so that its synthesis by certain bacteria is one of reason for the increase of crop growth (Aryantha*et al*. 2004).

Table 2 showed height, wet weight (WW) and dry weight (DW) of crowns and roots of rice crop which was innoculated by several endophytic bacteria produced from tissue isolation from some crop types and ages that capable to contribute IAA on their growth media.

Rice seeds response to innoculation of endophytic bacteria isolates suspension consisted of two types: not responsive as indicated by insignificant growth compared to control treatment and other was responsive or stimulate crop growth as indicated by insignificant growth compared to control treatment. These results were different than the previous study results which produced 3 groups of crop growth response toward endophytic bacteria isolates : stimulate the crop growth, without significant effect on the crop growth, and resist the crop growth (Gofar*et al*. 2004; Gofar *et al*., 2009). The absence of isolates which resist the crop growth was due to isolates that had been previously selected, especially the one that had capability to produce IAA within optimum range in stimulating the crop growth.

Table 2 showed that rice crop growth innoculated with endophytic bacteria isolates every week had higher crop height, wet weight and dry weight of crowns and roots of 5 weeks old crop than control treatment which was not innoculated by endophytic bacteria. Isolate with P31 code had the best effect on rice crop growth. This isolate was obtained from leaves tissue of rice crop having 2 months of age which was grown without application of inorganic fertilizers and synthetic pesticides. Some endophytic bacteria in rice plants have been reported to promote host growth. When *Herbaspirillum-seropedicae* Z 67 (James *et al*.2002), *Herbaspirillum* sp. B501 (Zakria*et al*. (2007), and some strains of *Herbaspirillum-seropedicae* and *Burkholderia* spp. (Baldani*et al*. 2000) are inoculated on rice seedlings, the inoculated plants show a significant increase in weight compared to the controls. A significant increase in biomass and grain yiel has also been recorded in greenhouse grown rice plants inoculated with *Rhizobium leguminosarum*bv. Phaseoli (Singh *et al*. 2006). *Pantoeaagglomerans* YS19 shows nitrogen fixing activity in N-free medium, produces four categories of phytohormones (IAA, abscisic acid, cytokinin and gibberelic acid) in Luria-Bertani medium and can enhance the biomass of the host rice seedlings (Feng*et al*. 2006).

Test results showed that rice sprout growth innoculated with IAA-producer endophytic bacteria were better than control treatment. This was estimated due to positive effect of IAA-producer endophytic bacteria on rice seeds so that rice sprouts had higher capability for IAA secretion and more sensitive in converting their IAA. IAA produced by endophytic bacteria isolate gave impact on root morphology such as increasing the density, length and surface area of roots. These roots development results in increase of nutrients absorption area so that produced more biomass of crowns and roots (Lestari *et al*. 2007).

Table 2 showed that P31 isolate produced the best growth than other isolates. This isolate was capable to contribute 10 mg kg^{-1} IAA into its growth medium and increase crown dry weight and root dry weight with magnitude of 133% and 225% respectively compared to control treatment.

Table 3.Nitrogen content and adsorption on rice and corn crops tissue having 5 months of age due to innoculation of several endophytic bacteria isolates

No	Isolate Code	Rice crop tissue	
		N content (%)	N Absorption (mg/crop)
1	Without innoculation	1.343 a	1.00 a
2	P11 (Isolate from rice crop tissue at Pemulutan having age of 1.5 months)	3.773 b	3.00 abc
3	P21 (Isolate from rice crop tissue at PulauGemantung having age of 2.5 months)	3.881 b	3.00 abc
4	P31 (Isolate from rice crop tissue at PulauGemantung having age of 2 months)	3.615 b	5.00 c
5	P41 (Isolate from rice crop tissue at PulauGemantung having age of 2 months, local variety)	3.200 b	4.00 bc
6	J11 (Isolate from corn crop tissue at Indralaya having age of 2 months)	3.521 b	2.00 ab
7	J21 (Isolate from corn crop tissue at LubukDalam having age of 1 month)	3.468 b	4.50 bc
8	J31 (Isolate from corn crop tissue at LubukDalam having age of 1 month)	3.308 b	2.50 abc
9	KP1 (Isolate from cowpea crop tissue at Indralaya having age of 45 days)	3.600 b	4.50 c
10	KT1 (Isolate from peanut crop tissue at Indralaya having age of 1 month)	3.618 b	2.00 ab
	$HSD_{0,05}$	0.418	2.63

Based on Table 2, all endophytic bacteria isolates were capable to increase the growth of rice and corn crops compared to treatment without innoculation. These endophytic bacteria isolates were capable to increase crop biomass because they can produce IAA phytohormone as the stimulating growth hormone on crops. Some bacteria isolates of diazotrophendophytic and growth stimulating were found on rice and corn crops that produce different IAA phytohormones (Gofar*et al*. 2004; Gofar*et al*. 2009). Root is one of the most sensitive crop organ to IAA quantity. Crop gives response to IAA through the main root elongation mechanism, development of lateral roots and adventive roots (Leveau and Lindow 2005).

Endophytic bacteria ability to fix nitrogen is tested by analyzing nitrogen content and adsorption by rice crop (Table 3). Increase of nitrogen content and adsorption was occurred on tissue of rice crop having 5 months of age. Table 3 showed that rice crops treated with endophytic bacteria isolates were capable to increase N absorption. N content in rice crop tissue is categorized as sufficient in the range of 2.6-3.2%. Rice crop is considered experience N defficiency if N content is less than 2.4%. Table 3 also showed that rice crop having no innoculation with endophytic bacteria is categorized as experience nitrogen defficiency, whereas rice crop innoculated with endophytic bacteria isolates had capability to increase N content until sufficient criteria for crop requirement.

The highest N absorption was found on rice crop innoculated with isolate from tissue of local rice variety having 2 months of age (P31). N content and adsorption for rice crop innoculated with P31 isolate had increased respectively by 169% and 400% compared to control treatment. High value of N adsorption by crop innoculated with endophytic bacteria was due to ability of these bacteria in N_2 fixing which contribute to crops. Moreover, it is estimated that IAA phytohormone produced by endophytic bacteria can stimulate development of root's hair which results in better nutrients uptake. It was evidence that endophytic bacteria which produce IAA phytohormone and fix N_2 isolated from forest plants of Air Hitam Ecosystem, Central Kalimantan and food crops from tidal swamp land of South Sumatra that were innoculated on rice and corn seeds can increase N content of these crops tissue (Gofar*et al*. 2008; Gofar*et al*. 2009). Environmentally, the use of specific endophytic bacteria may be preferable to the use of nonspecific chemical fertilizers and pesticides because of cost, time effectiveness, and contributions to sustainable agricultural system.

P31 isolate had been identified as *Burkholderiapseudomallei* P31 (also known as *Pseudomonas pseudomallei*) is a Gram-negative, bipolar, aerobic, motile rod-shaped bacterium. It is capable of infecting plants. This isolate may promote rice growth in Yoshida's medium. Application of biofertilizer containing *Pseudomonas pseudomallei* at 300-400 kg ha^{-1} dose combined with inorganic fertilizer at 75% of crop requirement dose was the best combination in increasing NPK nutrient uptake for rice crop and weight of milled dry rice (Marlina *et al*. 2014). Application combination of azolla, phosphate rock and rice hull ash increase soil organic matter content, cation exchange capacity, available-P and exchangeable-K as well as rice yield (Sudadi *et al*. 2014).

CONCLUSION

P31 isolate from rice crop tissue having 2 months of age had produced the best growth of rice sprouts at lowland soil area compared to other isolates. This isolate was capable to contribute 10 $mg.kg^{-1}$ of IAA into its growth medium and increase dry weight of crowns and roots with magnitude of 133% and 225% respectively compared to control treatment. N content and adsorption of rice crop innoculated with P31 isolate had increase by 169% and 400%. P31 isolate had been identified as ***B. pseudomallei*** (also known as *Pseudomonas pseudomallei)*.

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