The Potency of Plant Growth Promoting Rhizobacteria (PGPR) of Coastal Poaceae (*Phragmites karka*) to Stimulating of Paddy (*Oryza sativa* L.) Growth

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Abstract: The aim of this research was to obtain and to observe the activity of plant growth promoting rhizobacteria (PGPR) isolated from coastal Poaceae, *Phragmites karka,* on stimulating paddy's growth. The bacteria were qualitatively on three different selective media: Pikovskaya, JNFb, and tryptophan containing LB media to observe the ability of bacteria to solubilize phosphate, to fix nitrogen, and to produce indol acetic acid (IAA). The selected potential bacteria from each test were studied further as PGPR candidates on stimulating paddy growth in sterilized soil medium. The results showed that there were 6 isolates able to solubilizing phosphate, 6 isolates capable to fixing nitrogen and 8 isolates able to producing IAA. The synergy test resulted in three selected isolates (PP03, RG08 and PI05) were synergistic each other. The application of three isolates as single culture and their consortium stimulated of paddy growth significantly comparing to control (without bacteria) especially in the plant height and root length parameter. The best performance of paddy growth was achieved from consortium of 3 bacterial isolates treatment.

1 INTRODUCTION

Indonesia is one of the most populous countries in the world. Rapid population growth leads to increase in food demand. Society and government use various measures to maintain food availability. Food production is needed to improve of food demand fulfillment. Efforts that have been made to increase food production still need to be improved. One effort that can be done is extended farmlands by utilized marginal land such as coastal area. Marginal land is a nutrient-poor land with environmental conditions do not support the plant growth. Coastal area have sandy soils and high salinity content. Only tolerance organism can grow in this area. Indonesian Centre for Rice Research have breeding program to improve and find a salinity tolerance of rice (Hairmansis *et al.,* 2017). Beside the plant tolerant to salinity, other efforts to make marginal land become usable are attempted the organism plant growth promoting tolerance to salinity.

Plant Growth Promoting Rhizobacteria (PGPR) was a numerous soil bacteria which occupy roots or rhizosphere area, stimulating plant growth in various mechanism and also induced plant resistance (Vessey,2003). Investigation of PGPR microorganism from many sources is the purpose to making it as biofertilizers. In association of plant and PGPR, microorganism given some benefit action to stimulating plant growth specifically by fixing dinitrogen from atmosphere, solubilizing unsoluble phosphate, producing growth hormone of Indole-3-acetic acid (IAA), antimicrobial compound to reduced plant pathogen and induced plant resistance (Vessey, 2003), (Sandilya *et al.,* 2016). Bacteria *Burkholderia cepaciai* is one of species PGPR bacteria that potential as stimulated growth of maize and also have biocontrol activity to *Fusarium* spp. (Bevivino *et al.,* 1998).

Many PGPRs are diazotrophs (symbiosis or non symbiosis nitrogen fixing bacteria). Many diazotrophs bacteria are phosphate solubilizing and IAA producer (Vessey, 2003). In lower conditions of N or P level in soil, diazotroph microorganism adapt in the rhizosphere but harmless to their host. Population and activity of microbial in rhizosphere of sandy soil land is more higher than in rhizosphere of humus soil, and it's call as rhizosphere effect

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(Dotaniya and Meena, 2015). Low land moisture such as sandy soil affect microorganism to move toward the plant root zone. Availability nutrient in rhizosfer influence the rhizosphere population microorganism (Dotaniya and Meena, 2015).

In this research will be found a new candidate the plant growth promoting rhizobacteria of *Phragmites karka* which collected from coastal area.

2 MATERIAL AND METHODS

2.1 Material Research

The soil from *Phragmites karka* rhizosphere were collected as much as 100 g from Coastal location in Percut Sei Tuan area. The soil was collected from 3 samples point and stored on sterile of sealed plastics sample before transfer to laboratory. Seed of paddy was obtained from commercial seed seller. Several medium Pikovkayas, JNFb (medium free nitrogen source) and Luria Bertani (LB)+ Tryptophan medium were used for cultivation and selection of PGPR activity.

2.2 Isolation of PGPR Bacteria

The amount of 1 g of soil sample was diluted into 9 ml of sterile distilled water and than homogenized. Serial dilution was conducted to obtain a 1x 10^{-5} dilution. A total of 0.1 ml of soil suspension from the last dilution was spread to the surface of Pikovskaya, JNFb and LB + tryptophan medium aseptically. The culture medium was incubated for 2-3 days at room temperature. Activity of phosphate solubilizing bacteria were detected qualitatively by clear zone formation around colony of bacterial on pikovskaya medium. The positive test of nitrogen-fixing bacteria were characterized by the presence of colonies bacteria on JNFb medium. The medium of JNFb was free nitrogen source. Presence of bacteria in JNFb medium indicated the bacteria can fix bacteria from atmosphere. Bacteria produced IAA in medium LB + tryptophan broth medium were checked by added Salkowski's reagent (2% 0.5M $FeCl_3$ in 35% $HClO_4$ solution). The color medium become purple if there was indol compound in the medium. The positive sign bacteria growth in selective medium were purified and further characterized.

2.3 Selection of Phosphate Solubilizing Bacteria

The purified bacteria isolates were further tested for their ability to dissolve the phosphate to determine its solubility index, fixing nitrogen and produce IAA. Phosphate solubility index (PSI) score was obtained from the comparison of the diameter of clear zone around the bacterial colony and diameter of colony bacteria (Dotaniya and Meena, 2015).

2.4 Selection of Nitrogen Fixing Bacteria

Nitrogen fixation from purified bacteria were obtained from pellicle size formed of bacteria when cultured on semi solid JNFb medium. The white pellicle formed will be visible in the surface medium on tube and measured after 10 days incubation at room temperature.

2.5 Selection of IAA Producing Bacteria

The IAA produced by each bacteria was measured with colorimetric technique using the Salkowski's method (Ehmann, 1977). Bacterial suspension (3 ml) with cell density in Optical Density (OD) 600 was 0.5 (≈ 10^8 Colony Forming Unit, CFU / ml) was introduced into 27 ml of liquid LB + tryptophan broth and incubated at 28°C for 6 days and shaken at 100 rpm. Every 2 days as much as 10 ml of culture fluid is taken and then centrifuged at 5500 rpm for 10 minutes. The supernatant was transferred to a new sterile tube and then added Salkowski reagent with 4 : 1 ratio (supernatant : salkowski) and incubated for 20 minutes at room temperature. Colorimetrically the color change formed was measured by a spectrophotometer at a wavelength of 535 nm. The concentration of IAA from the sample was calibrated from linear regression equation of pure IAA.

2.6 Application of Selected Bacteria to Improved Paddy Growth

Paddy seeds were cultivated on 1 kg of sterile humus-sandy soil with composition humus: sandy soil (3:1) for 1 week. The potential bacteria were checked their synergistic each other by cross inoculated onto agar medium. When two bacteria was in synergism condition, they can live together with no inhibited each other. The selected bacteria was cultivated on Nutrient Broth for 24 hours. Ten milliliter of bacterial

culture broth OD 600 (0,5) or in consortium form were poured into rhizosphere of paddy seedling on soil medium. Each treatment was 3 replications. Parameters of paddy growth were analyzed as plant height, root length, biomass of plant and how much the dead plant. Observation of rice plant growth was done at 30 days after planting.

3 RESULTS AND DISCUSSION

3.1 Isolation Results

A total of 20 bacteria were isolated from *Phragmites karka* rhizosphere. Six isolates were each growth on Pikovkaya and JNFB medium and also 8 isolates on LB+Tryptophan medium. bacteria and 8 isolates are IAA producing bacteria. Based on the colony morphology observations showed that the 20 isolates had various characteristics in shape, edge, elevation and color. The colony shapes were circular, irregular and rhizoid. Almost all isolates were had white color and several cream. Only one isolate had orange color (Table 1).

3.2 Phosphate Solubilizing, Nitrogen Fixing and IAA Producing Bacteria

From 20 isolates bacterial tested were only 7 isolates potential to give positive results to all tree test. Results for phosphate solubilizing activity showed that 14 isolates bacterial were able to dissolve phosphate and one isolate with the highest ability was PP03. In the nitrogen fixing test was indicated by pellicle size showed that only 9 isolates produced the pellicle when growth on semisolid medium of JNFb and seven isolates produced thick pellicle with the size 5-9 mm. All isolates tested were IAA producer, and only two isolates produced IAA in concentration rage 28-40 ppm i.e. PP03 and RG08. Seven bacterial isolates were only dissolved phosphate and produced IAA and two isolates were only fixed nitrogen and produced IAA. From all tested bacteria, four isolates had one ability that only as IAA producer (Table 2). Three isolates bacteria which have best perform to all selected criteria were collected i.e. PP03, RG05 and PI05) and then used in paddy growth application. The synergistic test results showed that tree bacteria synergistically (Table 1).

Three selected bacteria in this research were potential as biofertilizer candidate. Existence of microorganisms as biofertilizer increases the growth of plants by enrichment of soil nutrients (nitrogen fixation) or making nutrient like phosphate more available to plant or increasing number of roots to be increasing nutrient absorption from soil or producing growth hormone to stimulated plant growth (Vessey, 2003). PGPR may

Table 1: Morphological character of colony bacteria from *Phragmites karka*, cultivated on 3 different agar medium

No	Code	Agar medium	Shape	Edge	Elevation	Color
1	PP01	Pikovskaya Agar	Irregular	Entire	Flat	White
2	PP02	Pikovskaya Agar	Irregular	Undulate	Flat	White
3	PP03	Pikovskaya Agar	Circular	Filamentous	Raised	White
4	PP04	Pikovskaya Agar	Circular	Undulate	Raised	White
5	PP05	Pikovskaya Agar	Circular	Entire	Flat	White
6	PP06	Pikovskaya Agar	Rhizoid	Filamentous	Flat	White
7	RG01	JNFB	Circular	Entire	Flat	White
8	RG02	JNFB	Circular	Undulate	Flat	White
9	RG05	JNFB	Rhizoid	Filamentous	Flat	White
10	RG06	JNFB	Circular	Undulate	Raised	Orange
11	RG07	JNFB	Circular	Undulate	Raised	White
12	RG08	JNFB	Rhizoid	Filamentous	Flat	White
13	PI01	LB	Irregular	Filamentous	Flat	White
14	PI02	LB	Irregular	Entire	Raised	Cream
15	PI03	LB	Circular	Entire	Flat	White
16	PI04	LB	Circular	Entire	Raised	Cream
17	PI05	LB	Irregular	Filamentous	Raised	Cream
18	PI06	LB	Irregular	Entire	Flat	White
19	PI07	LB	Irregular	Filamentous	Flat	White
20	PI08	LB	Rhizoid	Filamentous	Flat	White

colonize plant on rhizosphere or endophytes. Not any soil bacteria can colonize in that area. Association between rhizobacteria and plant were symbiotically give benefit to plant. Non-symbiotic diazotroph bacteria like Azospirillum, Azotobacter, *Gluconacetobacter diazotrophicus, Beijerinckia* sp. can live in rhizospher and promoting plant growth (Vessey,2003).

3.3 Analyzed of Paddy Growth

Application of 3 isolates bacteria to paddy seed were observed to several plant growth parameter . Inoculated of 3 bacteria and their consortium treatment statistically were not give significant results for plant biomass measured but had significantly results than control (P0/no bacterial treatment) to stimulated plant height and root length.

Table 2: Combination ability of phosphate solubilizing bacteria,nitrogen fixing bacteria and IAA producing bacteria

NO	Bacterial Isolate	Phosphate solubilizing index	Pellicle size	Consentration of IAA
1	PP01	+	-	+
2	PP02	+	-	++
3	PP03	+++	+++	+++
4	PP04	+	-	++
5	PP05	++	-	+
6	PP06	+	-	+
7	RG01	+	+++	+
8	RG02	+	+++	+
9	RG05	+	+++	+
10	RG06	+	-	++
11	RG07	+	+++	+
12	RG08	+	-	+++
13	PI01	-	+	+
14	PI02	-	-	+
15	PI03	-	-	+
16	PI04	-	-	+
17	PI05	++	+++	++
18	PI06	+	++	+
19	PI07	-	-	++
20	PI08	-	+++	+

Noted. Phosphate solubilizing index: - (0), + (1-2), + + (2-3), + + + (3-4); Pellicle Size (mm): - (0), + (1-3), + + (3-5), + + + (5-9); IAA Consentration (ppm): - : 0, + (1-14), ++ (15-27), + + + (28-40)

Table 3: Average paddy seedling growth parameter after 30 days planting

Treatment	Average seedling Growth Parameters					
	Plant height (cm)	Root length (cm)	Wet weight canopy (g)	Weight of wet root (g)	Dry weight of canopies	Dry weight of root(g)
P0 (without bacteria)	35,04^{a}	4,71^{a}	0,75^{a}	0,20^{a}	0,13^{a}	0,07^{a}
P1 (PP03)	39,99^{b}	8,67^{bc}	0,71^{a}	0,36^{a}	0,11^{a}	0,09^{a}
P2 (RG08)	39,10^{b}	10,50^{c}	0,61^{a}	0,35^{a}	0,08^{a}	0,09^{a}
P3 (PI05)	42,09^{bc}	5,08^{a}	0,73^{a}	0,42^{a}	0,10^{a}	0,10^{a}
P4 (PP03+RG08)	41,90^{bc}	9,43^{bc}	0,74^{a}	0,37^{a}	0,11^{a}	0,10^{a}
P5 (PP03+PI05)	41,31^{bc}	8,10^{bc}	0,48^{a}	0,19^{a}	0,08^{a}	0,06^{a}
P6 (RG08+PI05)	42,55^{bc}	8,44^{bc}	0,67^{a}	0,43^{a}	0,09^{a}	0,09^{a}
P7 (PP03+RG08+PI05)	44,84^{c}	7,81^{b}	0,80^{a}	0,47^{a}	0,13^{a}	0,14^{a}

Description: The average value that has the same letter in the same column shows no significant difference according to the DMRT test at the real level of 5%

The best results for plant height was from P7 treatment and for length of roots was P2 treatment (isolate of RG08) (Table 3). All treatment of PGPR bacterial and their consortium provided best performance to paddy growth than control, even though from growth parameter P7 treatment was the best results (Figure 1). The research about application of biofertilizer to Sugarcane soil showed that biofertilizer will substitute 25%-50% of NPK fertilizer in soil (Mulyani *et al.,* 2017). Inoculation of PGPR bacteria to maize plant increased plant performance in chlorophyll content, root and shoot length (Ullah *et al.,* 2017).

Figure 1: Performance of paddy seedlings after treated with rhizobacteria of *Phragmites karka* and observed on 30 days planting. Noted: P0 (without bacteria), P1-P7 were treated by P1 (PP03 isolate), P2 (RG08 isolate), P3 (PI05 isolate), P4 (PP03 and RG08 isolates), P5 (PP03 and PI05 isolates), P6 (RG08 and PI05 isolates) and P7 (PP03, RG08 and PI07 isolates).

4 CONCLUSION

Selective isolation of rhizobacteria from *Phragmites karka* were obtain 20 isolates and were selected three isolates as PGPR candidate. Only seven isolates were able to fixing nitrogen, solubilizing phosphate and producing IAA. Three selected isolates had the best activity for all test. Application of three selected isolates and their consortium to paddy growth resulted best performance to paddy growth especially for plant height and length of root than control (without bacterial treatment).

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