Indol Acetic Acid Production of Indigenous Plant Growth Promotion Rhizobacteria from Paddy Soil

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ABSTRACT

The aim of this research was to examine the diversity of indigenous plant growth promoting rhizobacteria from paddy soil and to obtain a superior isolate that can support the growth and vigor of rice plants. This research was conducted in the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Jenderal Soedirman University from July to September 2017. The bacteria were isolated from paddy soil rhizosphere originated from rice production centers of Banyumas Regency i.e. Kebasen, Rawalo, Patikraja, Jatilawang, and Karangwangkal. Results showed that indigenous PGPR from paddy soil were able to produce Indol Acetic Acid (IAA) in the range 0.05-5.40 ppm, but did not have the ability to solubilize phosphate. Plant Growth Promoting Rhizobacteria (PGPR) inoculation in rice seedlings was able to increase seed vigor, plant height, root length, and seed germination. Based on morphological and biochemical characters, PGPR isolates were identified as Bacillus sp., Pseudomonas sp., Streptococcus sp., and Staphylococcus sp.

Keywords: PGPR, Rice, Germination, IAA, Vigor index

ABSTRAK

Penelitian ini bertujuan untuk mengkaji kelimpahan dan keragaman *Plant Growth Promoting Rhizobacteria* (PGPR) dari lahan sawah serta mendapatkan isolat unggul yang mampu mendukung pertumbuhan dan vigor bibit tanaman padi. Penelitian ini dilaksanakan di Laboratorium Agronomi dan Hortikultura Fakultas Pertanian Universitas Jenderal Soedirman dari bulan Juli sampai September 2017. Bakteria diisolasi dari rhizosfer tanaman padi di wilayah Kebasen, Rawalo, Patikraja, Jatilawang dan Karangwangkal. Hasil penelitian menunjukkan bahwa PGPR *indigenous* tanah sawah mampu menghasilkan *Indol Acetic Acid* (IAA) pada kisaran 0.05-5.40 ppm, tetapi tidak memiliki kemampuan dalam pelarutan Pospat. Inokulasi benih padi dengan PGPR mampu meningkatkan vigor bibit, tinggi tanaman, panjang akar, dan daya kecambah. Berdasarkan karakter morfologi dan biokimia, PGPR teridentifikasi sebagai *Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., and *Staphylococcus* sp.

Kata Kunci: PGPR, Padi, Perkecambahan, IAA, Vigor

INTRODUCTION

The productivity of rice plants is now showing the symptoms of falling, where the increase in production factors is not followed by an adequate increase in yield. This condition shows the carrying capacity of the soil as a growing medium of crop retreat due to excessive use of inorganic fertilizer, and without the application of organic manure so that fertilizer efficiency decreases, nutrient balance is disturbed and soil biodiversity decreases (Adiningsih, 2005; Syekhfani, 2005). Improvement of soil fertility through the application of organic materials will affect the activity and biodiversity of the soil biology. According to Aryantha et al., (2011) crop production depends not only on inorganic fertilizer, but also biological factors of both plant and soil biological activity.

The environment of rhizosphere is the root of plants that interact directly with the soil and microorganisms through the mechanism of symbiosis and free living with the utilization of root exudates so that biological activity in the area of rhizosphere is a sign of soil fertility (Hindersah and Simarmata, 2004). Biodiversity of free-living microorganisms, as well as symbiosis in the root area, opens opportunities for optimizing the function and role of microorganisms through selection and isolation of superior microbes as biological fertilizers. Some previous studies showed that some genera of the rhizospheric bacteria had been isolated such as *Azospirillum* spp., *Herbaspirillum* spp., *Burkholderia* spp., *Gluconacetobacter diazotrophicus*, dan *Pseudomonas* spp. (Muthukumarasamy et al., 2005).

The mutual interactions between. Plant Growth Promoting Rhizobacteria (PGPR) and the plant take place through the P solubilizing mechanism (Estrada et al., 2013), nitrogen fixation (Raja et al., 2006; Terakado-Tonooka et al., 2013), plant root improvement through Indol Acetic Acid (IAA) production (Ai 'shah et al., 2013; Keyeo *et al.*, 2011; Naher et al., 2009; Purwanto et al., 2017). The indirect effect of PGPR applications on plants is the ability of antagonism against pathogens through the ability to produce siderophores and induce plant resistance through Induced Systemic Resistance (ISR) mechanisms (Adesemoye & Egamberdieva, 2013; Lucas et al., 2009). Ai'shah et al. (2013) reported that *Herbaspirillum seropedicae, Microbacterium, Acetobacter,* and *Microbacterium* sp. were able to produce IAA in free environment condition and able to increase root length, fresh root weight, and protein content in palm oil seedling. In rice plants, inoculation of *Rhizobium* sp. was capable of enhancing root growth and plant biomass (Naher et al., 2009; Purwanto et al., 2017). Aiman et al. (2017) also reported that inoculation of PGPR both single and consortium inoculation significantly increased biomass production.

The abundance of beneficial microorganisms in paddy fields as well as in rhizosphere areas of rice plants opens the opportunity for their use as biofertilizers. Selection of PGPR strains is necessary to obtain an indigenous strain capable of adapting and interacting with the rooting of rice plants in wetland environment conditions. This study was aimed to examine the diversity of indigenous PGPR from paddy soil and to obtain a superior isolate that can support the growth and vigor of rice plants.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Jenderal Soedirman University from July to September 2017. The bacteria were isolated from paddy soil rhizosphere originated from rice production centers of Banyumas Regency i.e. Kebasen, Rawalo, Patikraja, Jatilawang, and Karangwangkal. The soil samples were taken by random sampling from the rhizosphere of rice plants at a depth of 10 cm. Soil sampling was analyzed according to Husen's method (2007) on rhizosphere land. Rhizosphere is a portion of the soil that is directly affected by the roots of the plant, while rhizoplane is root surface with soil strongly attached to its surface. The boundary of rhizosphere begins at the root surface to the extent that the roots no longer have a direct effect on microbial life.

Bacteria Isolation

The bacteria were isolated using NA media. A total of 10 g of soil was diluted using 90 ml of sterile distilled water, then diluted to 10 - 8 series. Dilution series 10^{-7} and 10^{-8} were taken each of 1000 µl and inoculated on NA media, then incubated for 3 days. Observed colonies were formed and single and largest colonies were isolated and purified as pure isolate bacteria for subsequent characterization of both physical and physiological.

Phosphate Solubilizing Capability Test

The Phosphate solubilizing capability of PGPR isolates was tested using Pykovkaya media. A total of 20 µL suspension cells were inoculated on Pykovkaya media and incubated for 15 days at a temperature of 30 °C. The P solubilizing capability of PGPR isolates was observed by measuring the diameter of the halo zone formed.

IAA Production Capacity Test

The IAA productivity test was performed using colorimetry method with a spectrophotometer (Susilowati et al., 2007). Bacteria were grown in NB media. Bacterial culture was incubated at 32

°C for 5 - 7 days, then centrifuged at 10,000 × g for 10 min. The supernatant was transferred to a sterile tube and treated with Salkowski reagents (20 ml FeCl 0.1 M, 400 ml H_2SO_4, 580 ml of distilled water). The supernatant was incubated for one hour, then measured its absorbance at λ = 530 nm using a spectrophotometer. The standard used was the pure IAA solution.

Inoculation of Plant Growth Promoting Rhizobacteria to Enhance Rice Seed Germination and Vigor

Plant Growth Promoting Rhizobacteria (PGPR) ability test in improving seed germination and vigor was done by a germination method in a petri dish. The PGPR isolates were prepared by culturing in Nutrient Broth (NB) media and were ready for use when the bacterial population reached 10^9cfu ml^{-1}. Fifty seeds of rice were cleaned with sterile distilled water, then soaked in a solution containing PGPR for one minute. Rice seeds were put on the wet paper in a petri dish and kept for three weeks at room temperature. After three weeks, the percentage of seed germination, plant height, and root length were observed. Vigor Index was calculated by the following formula:

Vigor Index = (shoot length + root length) x % germination.

Table 1. Morphological and Biochemical Characters of Indigenous PGPR

Isolate	Gram	Shape	Spore Forming	Catalase	Oxidase	Mannitol	Anaerob	IAA Production	P Solubilizing
J02	Positive	Bacil	+	Negative			+	+	-
J03	Positive	Bacil	+	Negative			+	+	-
J04	Positive	Bacil	+	Negative			+	+	-
J05	Positive	Bacil	+	Negative			+	+	-
J17	Positive	Bacil	+	Negative			+	+	-
K01	Positive	Coccus	-	Negative				+	-
K05	Positive	Coccus	-	Negative				+	-
P02	Positive	Coccus	+	Negative			+	+	-
P03	Positive	Coccus	-	Negative			+	+	-
P07	Positive	Bacil	+	Negative			+	+	-
P10	Positive	Bacil	+	Negative			+	+	-
R01	Positive	Bacil	+	Negative			+	+	-
R03	Positive	Short Bacil	+	Negative			+	+	-
R04	Positive	Coccus		Positive				+	-
R05	Positive	Coccus	-	Positive				+	-
R08	Positive	Coccus	-	Negative			+	+	-
R11	Positive	Bacil	+	Negative			+	+	-
R12	Positive	Bacil	+	Negative			+	+	-
R13	Positive	Short Bacil	+	Negative			+	+	-
U01	Positive	Coccus	-	Positive				+	-
U02	Negative	Coccus	+	Negative	Positive	+	-	+	-
U03	Positive	Short Bacil	+	Negative			+	+	-
U04	Positive	Coccus	-	Positive				+	-
U05	Positive	Bacil	+	Negative			+	+	-
U06	Positive	Coccus	-	Negative				+	-
U07	Positive	Coccus		Negative			+	+	-
U08	Positive	Coccus	-	Positive				+	-

Data Analysis

All data were analyzed by using Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) at the 95% confidence level.

RESULTS AND DISCUSSION

Plant Growth Promoting Rhizobacteria (PGPR) were successfully isolated from paddy fields located in the rice production center of Banyumas region that covers Karangwangkal area, Rawalo, Kebasen, Patikraja, and Jatilawang. Characteristics of PGPR isolates vary both morphologically and biochemically. Almost all isolates are gram-positive, except isolate U02 (gram negative) originating from rice fields in Karangwangkal. The shape of the cell is dominated by rod-shaped bacteria (coccus) as many as 13 isolates, followed by bacil bacteria cell form as many as 11 isolates, and short bacil form of 3 isolates. Spore forming was seen in isolated J02, J03, J04, J17, P02, P07, P10, R01, R03, R11, R12, R13, U02, U03, and U05. The catalase test showed that most of the isolates reacted negatively, except isolate R04, R05, U01, and U04 which showed a positive reaction. Isolate U02 showed a positive reaction to the oxidase and mannitol tests and showed a negative reaction to the anaerobic environment (Table 1). Based on morphological and biochemical characters, PGPR isolates were identified as *Bacillus* sp. (J02, J03, J04, J05, J17, P07, P10, R01, R03, R11, R12, R13, U03, U05), *Pseudomonas* sp. (U02), *Streptococcus* sp. (K01, P02, P03, U07), and *Staphylococcus* sp. (R04, R05, U01, U04, U08) (Table 2).

Table 2. Identification of PGPR

Genus	Isolates
Bacillus sp.	J02, J03, J04, J05, J17, P07, P10, R01, R03, R11, R12, R13, U03, U05
Pseudomonas sp.	U02
Streptococcus sp.	K01, P02, P03, U07
Staphylococcus sp.	R04, R05, U01, U04, U08

Table 3. PGPR Ability to Produce IAA

Isolates	λ (nm)	IAA (ppm)
J02	0.453	5.40
J03	0.325	3.37
J04	0.121	0.13
J05	0.352	3.79
J17	0.149	0.57
K01	0.263	2.38
K05	0.192	1.25
P02	0.231	1.87
P03	0.308	3.10
P07	0.166	0.84
P10	0.255	2.25
R01	0.150	0.59
R03	0.278	2.62
R04	0.201	1.40
R05	0.229	1.84
R08	0.251	2.19
R11	0.238	1.98
R12	0.192	1.25
R13	0.177	1.02
U01	0.332	3.48
U02	0.246	2.11
U03	0.261	2.35
U04	0.286	2.75
U05	0.247	2.13
U06	0.175	0.98
U07	0.260	2.33
U08	0.116	0.05

All isolated PGPR isolates were able to produce auxin growth regulator, namely Indol Acetic Acid (IAA). This was shown by the change of color to purple after reacting with Salkowski reagent. The results of the analysis showed that the ability to produce IAA varied between isolates. It was seen that the production of IAA varied in concentrations ranging from 0.05 to 5.40 ppm (Table 3). In average, PGPR isolates could generate IAA of 1.94 ppm, in which isolate J02 produced the highest IAA of 5.4 ppm, while isolate U08 produced the lowest IAA of 0.05 ppm.

Ability of PGPR in IAA production is the main

Table 4. The Effect of PGPR on Rice Seed Germination and Seedling Vigor

Treatments	Germination (%)	Plant height (cm)	Root length (cm)	Vigor Index
J02	95 a	8.77 bcde	56.33 abcdef	6195 abcde
J03	93 ab	9.28 bcde	52.34 abcdef	5760 abcdef
J04	85 bcde	12.77 ab	67.34 abc	6814 abcd
J05	88 abcd	6.17 de	21.84 f	2465 f
J17	91 abc	11.05 abcde	36.00 bcdef	4294 bcdef
K01	91 abc	10.14 bcde	59.67 abcde	6334 abcde
K05	92 ab	8.05 bcde	36.00 bcdef	4053 bcdef
P02	91 abc	8.69 bcde	38.34 bcdef	4236 bcdef
P03	89 abcd	6.55 cde	34.17 cdef	3623 cdef
P07	92 ab	12.15 abc	86.17 a	9044 a
P10	91 abc	11.37 abcde	65.50 abcd	7001 abc
R01	84 bcde	12.07 abc	77.50 a	7524 ab
R03	80 de	10.85 bcde	50.00 abcdef	4906 bcdef
R04	88 abcd	11.85 abcd	67.17 abc	6965 abc
R05	89 abcd	10.15 bcde	54.17 abcdef	5647 abcdef
R08	87 abcd	13.18 ab	87.50 a	8769 a
R11	91 abc	13.62 ab	62.17 abcde	6901 abc
R12	90 abc	8.72 bcde	72.34 ab	7295 abc
R13	88 abcd	16.75 a	56.34 abcdef	6474 abcd
U01	77 e	10.75 bcde	52.83 abcdef	4825 bcdef
U02	92 ab	5.78 e	33.67 cdef	3613 cdef
U03	92 ab	11.92 abcd	66.00 abcd	7168 abc
U04	96 a	8.42 bcde	21.34 f	2881 ef
U05	88 abcd	10.39 bcde	54.00 abcdef	5727 abcdef
U06	87 abcd	11.45 abcde	66.00 abcd	6721 abcd
U07	82 cde	10.55 bcde	28.34 def	3208 def
U08	90 abc	7.90 bcde	27.33 ef	3171 def

Notes: Values followed by the same letters in same column are not significantly different according to DMRT 5%.

indicator to be able to support plant growth. PGPR interacts with colonizing root plants by exploiting root exudates and influencing plant growth through the mechanism of phytohormones production, P solubilizing the mechanism, and the increase in nutrient uptake (Ashrafuzzaman et al., 2009). PGPR can synthesize IAA by using tryptophan precursors with different pathways, although the auxin synthesis pathway is generally via an independent tryptophan pathway (Bhattacharyya and Jha, 2012).

All local isolates of paddy soil can produce IAA with varying concentrations. Bacteria obtain nutrients such as tryptophan amino acids from root exudates. However, at high tryptophan concentrations, bacterial growth is disrupted so bacteria convert tryptophan to IAA (Velivelli et al., 2014). Moghaddam et al., (2012) reported that IAA production capacity in pure culture could be increased by the addition of DL-tryptophan to the media and the addition of 500 ppm tryptophan increased IAA production by 678 ppm by *Azospirillumbrasilense*.

PGPR Ability to Support Seed Germination and Vigor of Rice Seeds

The result of the analysis showed that PGPR inoculation influenced the germination of rice seed, plant height, root length, and vigor of rice seed. The effect of PGPR inoculation on vigor index varied. The P07 and R08 isolates produced the highest vigor index of 9044 and 8767, respectively, while the J05 produced the lowest vigor index of 2465 (Table 3).

The value of seed vigor index is influenced by root length and plant height. The relationship between these variables is very close. The result of correlation analysis showed that the relationship between the seed vigor index and plant height and root length were significant with r values of 0.634 and 0.984, respectively. The ability to produce IAA will improve plant growth. This is in line with the results of Purwanto et al. (2017) finding that local diazotrophic bacteria isolate of paddy soil could produce IAA and increase root length of rice seedlings, and at high IAA levels tended to suppress root growth of rice plants in in vitro cultures. Increased root growth impacts on the growth of plant shoot due to increased water supply and nutrients from plant roots.

CONCLUSION

Indigenous PGPR from paddy soil were able to produce IAA in the range of 0.05 -5.40 ppm. However, it could not demonstrate the ability to solubilize Phosphate. PGPR inoculation in rice seedlings was able to increase seed vigor, plant height, root length, and seed germination. Based on morphological and biochemical characters, PGPR isolates were identified as *Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., and *Staphylococcus* sp.

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