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Nutrient Uptake, Chlorophyll Content, and Yield of Rice (*Oryza sativa*) Under the Application of PGPR Consortium

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Abstract. Indigenous paddy soil rhizobacteria are one alternative to restore biological fertility and soil health. Plant Growth Promotion Rhizobacteria (PGPR) that act as biofertilizer will help to increase the availability of nutrients and promote the plant growth. The objective of this research was to study the effect of the PGPR consortium indigenous paddy soil to nutrient uptake, chlorophyll content, and yield of rice. This research was arranged by Randomized Complete Block Design with the treatment was the combination of Plant Growth Promotion Rhizobateria isolates originated from paddy soil. The treatments consisted of control, *Rhizobium* sp. LM-5, R08 isolate, R011 isolate, R08 isolate+*Rhizobium* sp. LM-5, R011 isolate+*Rhizobium* sp. LM-5, R011 isolate+R08 isolate, R011 isolate+R08 isolate+*Rhizobium* sp. LM-5. The result showed that the the consortium of PGPR was able to increase the root growth thereby increasing nutrient uptake, chlorophyll content, and plant biomass. Application of single strain R11 isolate and the consortium of R11 isolate+*Rhizobium* sp. LM-5 were capable of giving the highest grain yield of 64.99 and 62.80 g plant^{-1} respectively. These finding were PGPR consortium between IAA-producing bacteria combined with *Rhizobium* sp. LM-5 as N_2 fixing bacteria in increasing nutrient uptake, chlorophyll contents and crop yields, it can be recommended that PGPR consortium as a biofertilizer formula in rice cultivation

Key words: *Rhizobium* sp. LM-5, rice, growth, nutrient, yield

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INTRODUCTION

Rice is the staple food for the people of Indonesia and the need for rice increases every year in line with the increasing population of Indonesians. However, the level of rice productivity in Indonesia is still stagnant at around 4-5 tons ha^{-1}, and rice production is still supported by the island of Java by 75% (Bashir & Yuliana, 2019; Arif et al., 2020). Production stagnation is mostly caused by high input technology intake, land conversion, land degradation, and decline in environmental quality (Mariyono, 2014). Rice production technology with high fertilization inputs, such as urea, which has reached 600 kg ha^{-1} on the island of Java, greater than the recommended amount of 150 kg ha^{-1}, harms nutrient balance and decreases the quality of paddy fields (Hatta & Sulakhudin, 2016). Continuous application of urea fertilizer causes a decrease in soil health through soil carbon degradation and a decrease in the structure and composition of soil biology (Bhattacharyya et al., 2017). Zhou et al. (2017) reported that the long term application of fertilizer containing nitrogen (N) had a significant effect on decreasing of the total population of bacteria from 4.28×10^9 cfu g^{-1} to 2.17×10^9 cfu g^{-1} in the wheat season and 1.79×10^{10} cfu g^{-1} to 9.35×10^9 cfu g^{-1} in the soybean season.

The application of biofertilizer containing microbes from indigenous rice fields as biological fertilizers is one alternative to restore soil biological fertility and soil health. Adedeji et al., (2020) stated that biological fertilizers consisting of useful microbes in agriculture will encourage to improve soil health and increase plant fertility through the ability to increase nutrients, as well as solubilize and mobilize the nutrients. Plant Growth Promotion Rhizobacteria (PGPR) has a positive effect on plants through the production of various secondary metabolites, growth regulators, siderophores, and organic acids, as well as helping in nitrogen fixation and solubilizing of phosphorus (P) so that plants can improve their adaptation to absorb nutrients from the soil (Tabassum et al., 2017).

The abundance of microbes and the greatest interactions occurs in the rhizosphere area. In the rhizosphere of rice plants, many useful bacteria have been found, including *Pseudomonas sp., Sphingomonas, Bacillus* sp., *Blastocatella, Thiobacillus, Bryobacter, Anaeromyxobacter, Streptococcus,* and *Staphylococcus* (Purwanto et al., 2019a; Osman et al., 2017). Santosa et al., (2018) reported that on organic rice fields the abundance of useful microbes was dominated by *Stenotrophomonas maltophilia, Acinetobacter junii, Serratia nematodiphila, Pseudomonas aeroginosa, Exiguobacterium acetylicum, Bacillus cereus, and Bacillus subtilis*. PGPR isolates from paddy soil had the capacity to produce Indole Acetic Acid (IAA) with the precursor of root exudate to increase root length, and isolate R11 used in this study had the capacity to increase the rice yield (Purwanto et al., 2019a; Purwanto et al., 2019b). Sharma et al., (2014) reported that inoculation of *Azospririllum. Lipoferum* in three rice genotypes increased the chlorophyll content caused by improving the mineral nutrition of the plant. On the other hand, the application of useful microbes such as N-fixing bacteria and P solubilizing bacteria will increase root growth so that plant N and P uptake increases, and the shoot growth rate are better when compared to the absence of bacterial inoculation (Setiawati et al., 2016; Santosa et al., 2018; Safriani et al., 2020).

Interaction and compatibility of the combination of rhizobacteria determines the effectiveness of the application of organic fertilizers on plant growth. The application of the Bacterial Consortium is expected to bring about multifaceted effects through synergies, nutrient supply, removal of inhibitory products, and interaction between bacteria through mutual stimulation by physical and biochemical activities. This can improve some beneficial aspects of the physiology of the plant. growth (Pandey et al. 2012). Simarmata et al. (2016) found that application of consortium of rhizobacteria enriched with *Trichoderma* sp. can increase significantly the grain yield of rice and reduce the sheath blight and bacterial leaf blight symptom. Tennakoon et al. (2019) reported that application of *Azospirillum* sp. strain 6 + *Rhodococcus* sp. gave a significant increase in leaf N content of tea plant at range of 3.27-3.80%. The purpose of this study was to investigate the effect of the PGPR consortium indigenous paddy soil to nutrient uptake, chlorophyll content, and yield of rice. This knowledge is very useful in developing biofertilizer technology to support sustainable rice production

METHODS

Study site and experimental design

The research was conducted from October 2018 to February 2019 at Laboratory of Agronomy and Horticulture and Experimental Station of Faculty of Agriculture, Jenderal Soedirman University. The soil type used in this research was Inceptisol soil with pH value of 5.91 (pH meter 1:5 IK5.4.c), N total of 0.24 % (Kjeldahl), K-available of 160 ppm (Morgan-Wolf), and P_2O_5 content of 6 ppm (Olsen). This study was arranged by A Randomized Complete Block Design with the treatment was the combination of PGPR isolates originated from paddy soil. The treatments consisted of 8 combinations of PGPR isolates i.e. control (K), *Rhizobium* sp. LM-5, R011 isolate, R08 isolate, R08 isolate+*Rhizobium* sp. LM-5, R011 isolate+*Rhizobium* sp. LM-5, R08 isolate + R011 isolate, R08 isolate + R011 isolate+ *Rhizobium* sp. LM-5. There were eight treatments with 3 times repetitions, so the total unit experiments were 24 units. Each unit of experimental consisted of 8 polybags and planted with 2 seedlings of rice. The rice variety used in this research was Inpago Unsoed 1 with the characteristic of rice as aromatic rice. Polybag measuring 30x30 cm was filled with 9.952 kg soil according to the treatment.

PGPR isolates preparation

PGPR isolate used was a collection of the Agronomy and Horticultural Laboratory of the Faculty of Agriculture, UNSOED. Before being used in this study, PGPR isolates were rejuvenated by transferring the isolates from the old NA medium to the new NA medium using a loop needle to improve the nutrition of the bacterial isolates. Bacterial stock cultures were prepared by dissolving 5% molasses in 1 liter of distilled water. Molasses was put in 10 Erlenmeyers with a volume of 100 ml each. Two inoculation loops of PGPR isolate were inoculated in each Erlenmeyer, then shaken at 180 rpm for 3 days. Then the total plate count (TPC) was carried out to determine the abundance of bacteria up to 10^7 cfu ml^{-1}. The stock culture was stored in a refrigerator after use. The application of the PGPR consortium was carried out by immersing the roots of rice seedlings in a 1% concentration PGPR consortium solution before planting. Additional PGPR applications were carried out at the age of 15, 30, and 45 days after planting by spraying a 1% concentration of PGPR solution.

The observed variables

The observed variable consisted of N content measured by Kjedahl method (Jiang et al. 2014), P and K content determined by HNO_3 and $HCLO_4$ reaction (Islam et al. 2016), the uptake of nutrients by rice plants calculated by multiplying the nutrient content in plants by the total plant biomass, the leaf area and plant biomass measured by Hasanah et al. (2021) methods, the chlorophyll content measured by spectrophotometric methods (Proklamasiningsih et al. 2013), the root length measured by intersection methods (Delory et al., 2017), and rice yields are harvested when the rice grains are physiologically ripe.

Data analysis

Data was analyzed by analysis of variance (ANOVA) using SAS 9.1 software. If there were significant differences in ANOVA results, we continued the analysis using DMRT α=0.05.

RESULTS AND DISCUSSION

The effect of PGPR consortiums on nutrient uptake and root growth

The results showed that inoculation of the indigenous PGPR consortium of paddy soil was able to improve the physiological characters and the level of nutrient uptake of rice plants. Plant roots are the organs that first interact directly with the soil and soil microorganisms. The results of this research showed that PGPR inoculation both single strain and consortium was able to increase rice root length by an average of 366.31% compared to control. The total root length of control group reached 301.2 cm, while longest root length was achieved in the inoculation treatment of the consortium R08 isolate + R11 isolate by 2067.70 cm, although it was not significantly different from R11 single strain isolate (1430.00 cm) and *Rhizobium* sp. LM-5 + R08 isolates (1883.10 cm) treatments (Table 1).

The ability of PGPR to increase plant root length is through the ability of plants to produce growth regulators such as the auxin group, including Indole Acetic Acid (IAA) (Paul & Sharma, 2006). The results of our previous study showed that all bacterial isolates used (R11 isolate, *Rhizobium* sp. LM-5, and R08 isolate) are indigenous rhizobacteria of paddy soil that can produce IAA and stimulate root growth of rice plants both *in vitro* and *in vivo*, and *Rhizobium* sp. LM-5 is capable of fixing N_2 from the air (Purwanto et al. 2017; Purwanto et al. 2019b). Based on the research results, it is known that the consortium application gave an increase in root length by 56.63% compared to single strain applications. The interaction between isolates showed a positive effect on rice root length. The application of a consortium of IAA-producing bacteria will increase the concentration of growth regulators to an optimum level in the rhizosphere so that it can stimulate a better growth. Oluwambe (2016) stated that the low to the optimum concentration level of IAA produced by rhizobacteria will promote root elongation.

Table 1. The effect of the PGPR consortium on rice root length.

Treatments	Roots length (cm)
Control	301.2 e
Rhizobium sp. LM-5	610.20 de
R08 isolate	1143.20 cd
R11 isolate	1430.00 abc
R08 isolate +*Rhizobium* sp. LM-5	1883.10 ab
Rhizobium sp. LM-5 + R11 isolate	1338.20 bc
R11 isolate+R08 isolate	2067.70 a
R11 isolate+ R08 isolate+*Rhizobium* sp. LM-5	1359.30 bc

Note: Numbers followed by the same letter in the same column were not significant different according to DMRT α=0.05

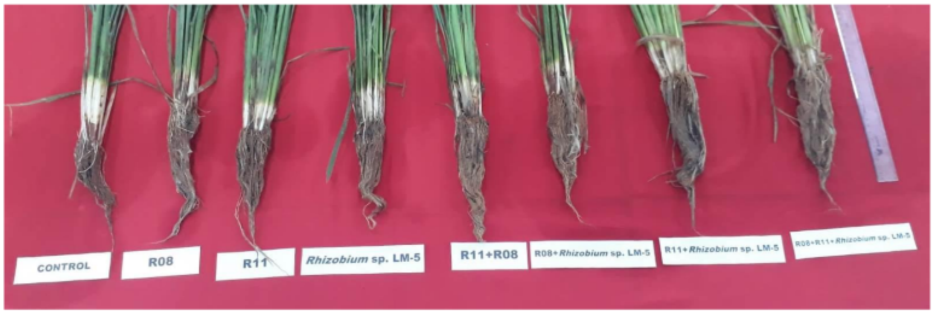

Figure 1. Roots of rice plant in different PGPR treatment.

Table 2. Impact of the PGPR Consortium on Nutrient Intake

Treatments	N ($mg\ plant^{-1}$)	P ($mg\ plant^{-1}$)	K ($mg\ plant^{-1}$)
Control	157.40 c	0.225 c	344.20 b
Rhizobium sp. LM-5	540.60 abc	0.256 b	804.40 ab
R08 isolate	779.40 ab	0.144 f	1007.20 ab
R11 isolate	350.40 c	0.169 e	648.10 ab
R08 isolate +*Rhizobium* sp. LM-5	960.70 a	0.196 d	1353.60 a
Rhizobium sp. LM-5 + R11 isolate	1067.60 a	0.324 a	1308.00 a
R11 isolate+R08 isolate	1034.30 a	0.169 e	1323.10 a
R11 isolate+ R08 isolate+*Rhizobium* sp. LM-5	1019.20 a	0.169 e	1516.10 a

Note: Numbers followed by the same letter in the same column were not significant different according to DMRT α=0.05

Increasing the rice root length will allow an increase in the area of the roots that interact directly with soil colloids, thereby enabling an increase in the process of nutrient absorption by plants through the roots. This can be seen in the nutrients absorption variables. The results showed that the PGPR application, both single strain, and consortium, were able to increase nitrogen uptake by 422.07% compared to control. The highest result was achieved in the treatment of the *Rhizobium* sp. LM-5 + R11 isolate by 1067.60 mg plant^{-1}, while the control only reached 157.40 mg plant^{-1}. In general, PGPR consortium applications have higher nitrogen uptake values than single strains with an average of 1020.45 mg plant^{-1} or an increase of 83.27%. *Rhizobium* sp. LM-5 is a nitrogen fixing bacteria indigenous paddy soil and is capable of producing IAA (Purwanto et al., 2017). Backer et al. (2018) stated that diazotrophs can provide nitrogen for various plants compared to rhizobia, and on the other hand bacteria that are unable to fix nitrogen can increase N uptake through increased nitrogen fertilization efficiency due to increased root growth.

The effect of the PGPR consortium application on P nutrient uptake shows that the *Rhizobium* sp. LM-5 + R11 isolate was a PGPR consortium that provides the highest P uptake by 0.324 mg plant^{-1}. PGPR consortium application tended to increase the P uptake by 44% compared to control, however, the increase between single strain and consortium was only 13.09%. The effect of rhizobacteria on P uptake was influenced by the ability of rhizobacteria as P solubilizing bacteria, so that the P uptake of rice plants increased both single strain or PGPR consortium.

The results of previous studies indicated that *Rhizobium* sp. LM-5, R08 isolate, and R11 rhizobacteria strain isolates did not show P solubilizing activity. Potassium (K) nutrient uptake in the PGPR application both single strain and consortium affects increasing potassium nutrient uptake. The PGPR consortium showed higher K nutrient uptake, but no significant difference from the R08 isolate+*Rhizobium* sp. LM-5, R11 isolate +*Rhizobium* sp. LM-5, R11 isolate+R08 isolate, and R08 isolate + *Rhizobium* sp. LM-5+R11 isolate treatments with 1353.60, 1308.00, 1323.10, and 1516.10 mg plant^{-1}, respectively (Table 2). The consortium application was able to increase K nutrient uptake by 67.72% compared to single strain applications with only 819.90 mg plant^{-1} in average. In general, the application of PGPR can increase nutrient uptake of nitrogen, phosphate, and potassium up to several times compared to control. Likewise, the consortium application is better in increasing nutrient uptake than the single strains. This result is in line with Jha et al. (2013) where enrichment of rice straw compost with *Pseudomonas, Azospirillum*, and *Cyanobacteria* increased nitrogen and sulfur uptake by three times, phosphate by 2 times more, and 1.3-1.9 times more of micronutrients. PGPR can increase the supply of nutrients in the rhizosphere and/or stimulate the rooted ion transport system through two mechanisms, namely phosphate solubilizing and nitrogen fixation so that nitrogen is available to plants (Vacheron et al., 2013).

The effect of PGPR consortiums on chlorophyll contents, leaf area, and plant biomass

The results showed that the PGPR application of both single strains and the PGPR consortium were able to increase the chlorophyll content. The average increase in chlorophyll a and chlorophyll b levels due to PGPR treatment was 85.71% and 83.67%, respectively. The highest chlorophyll content of rice plants in the PGPR application were achieved in the treatment of the R08 isolate+*Rhizobium* sp. LM-5 by 0.028 mg g^{-1} and 0.015 mg g^{-1}, respectively, although they did not show significant difference with the *Rhizobium* sp. LM-5 + R11 isolate, R11 isolate+R08 isolate, and R11 isolate+ R08 isolate+*Rhizobium* sp. LM-5 (Table 3). The PGPR consortium application shows a positive interaction with the increase in leaf chlorophyll levels of rice plants when compared to the control and single strain. The application of PGPR consortium was able to increase chlorophyll a and b levels by 89.28% and 101.04%, respectively.

Chlorophyll is a photosynthetic apparatus for plants so that the process of obtaining carbohydrates for energy for plant growth is available and some carbon compounds will be translocated to the storage section as plant products. Chlorophyll molecules promote the conversion of solar energy into chemical energy by collecting light energy, transferring excitation energy to the reaction center and facilitating charge separation at the reaction center. Croft & Chen (2017) stated that chloroplast contained the majority of plant nutrients and 70-80 percent of the total of leaf nitrogen content is found in chloroplastic proteins of C3 plants. The application of the PGPR consortium in rice plants can increase nitrogen nutrient uptake so that leaf chlorophyll levels increase. Nitrogen is one of the elements that make up the structure of chlorophyll. Esfahani et al. (2008) stated that leaf chlorophyll content was closely correlated with nitrogen content in leaves. Furthermore, Fiorentini et al. (2019) stated that the increasing availability of nitrogen will increase the higher chlorophyll concentration in leaf tissue which determines the increase in leaf greenness.

Table 3. The effect of PGPR consortium on leaf area, plant biomass, and chlorophyll a and chlorophyll b content.

Treatments	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Leaf area (cm^2)	Biomass (g)
Control	0.014 c	0.007 c	410.30 c	13.71 c
Rhizobium sp. LM-5	0.024 b	0.010 b	1047.00 b	42.90 ab
R08 isolate	0.026 ab	0.013 ab	1291.40 ab	45.24 ab
R11 isolate	0.026 ab	0.013 ab	973.40 b	28.15 bc
R08 isolate +*Rhizobium* sp. LM-5	0.028 a	0.015 a	1205.70 ab	43.75 ab
Rhizobium sp. LM-5 + R11 isolate	0.025 ab	0.012 ab	1492.30 ab	71.04 a
R11 isolate+R08 isolate	0.027 ab	0.013 ab	1419.50 ab	67.50 a
R11 isolate+ R08 isolate+*Rhizobium* sp. LM-5	0.026 ab	0.014 ab	1600.10 a	64.03 a

Note: Numbers followed by the same letter in the same column were not significant different according to DMRT α=0.05

The results of this research showed that the application of the PGPR consortium increased the growth of leaf organs indicated by the wider leaf area variable compared to the single strain and control groups. The widest leaf area was obtained in the R11 isolate+ R08 isolate+*Rhizobium* sp. LM-5 by 1600.10 cm^2, although it was not significantly different from the R08 isolate+*Rhizobium* sp. LM-5, Rhizobium sp. LM-5 + R11 isolate, and R11 isolate +R08 isolate with 1205.70, 1492.30, 1419.50 cm^2, respectively (Table 3). The yield of dry biomass of rice plants in the PGPR single strains and consortium treatments showed increased results compared to those without the PGPR application. The highest rice plant biomass was obtained in the R11 isolate+*Rhizobium* sp. LM-5 by 71.04 g per plant, although it was not significantly different from the R08 isolate+*Rhizobium* sp. LM-5, R11 isolate+R08 isolate, and R11 isolate+ R08 isolate+*Rhizobium* sp. LM-5 with respectively 43.75, 67.50, and 64.05 g per plant. In the single-strain treatment, especially R08 isolates and *Rhizobium* sp. LM-5, the results were not significantly different from the PGPR consortium application with 42.90 and 45.24 g per plant, respectively. Plant biomass is a plant product that is the result of the photosynthetic process. The increase in biological yield in the form of plant dry matter can be seen in the treatment of R11 *isolate+Rhizobium* sp. LM-5 as a treatment with higher leaf area, chlorophyll content, and plant nitrogen content compared to other treatments. Nitrogen supply through the ability of N_2 fixation causes an increase in leaf chlorophyll content and nitrogen content, thereby increasing leaf area and biomass of rice plants. Suharjo & Sutarno (2009) stated that an increase in plant nitrogen contents will affect photosynthesis through chlorophyll contents and photosynthetic enzymes. Chlorophyll is an indicator of leaf greenness and also indicates the adequacy of nitrogen supply in leaves, so that chlorophyll content, leaf area, and plant biomass are strongly influenced by nitrogen supply (Liu et al., 2019).

The effect of PGPR consortiums on rice yield

The results of this research showed that the PGPR treatment either single strain or consortium were able to increase the grain yield. The highest yield was achieved in the R11 isolate treatment followed by the R11 isolates+*Rhizobium* sp. LM-5 with 64.99 and 62.80 g plant^{-1}, respectively. The lowest yield was obtained in the treatment without PGPR application by 20.35 g plant^{-1} (Figure 2). The PGPR application was able to increase the grain yield by 176.97% compared to control, while the single strain application and the consortium were able to increase the yield by 176.41 and 177.38% respectively compared to the control. The grain yield is an economic yield as an assimilate partition that can be stored in the plant storage section. R11 isolates and the *Rhizobium* sp LM-5 + R11 isolate consortium were able to increase nutrient uptake, especially nitrogen, which is a macronutrient constituent of chlorophyll, resulted in increasing chlorophyll content of rice plant leaves. Chlorophyll content determines the rate of plant photosynthesis so that it can increase assimilates that can be partitioned as plant dry matter as well as economic yields in the form of grain. Furthermore, Sikuku et al. (2015) reported that the increase in leaf chlorophyll levels in plants is the fact that nitrogen is part of an enzyme related to chlorophyll synthesis, and leaf chlorophyll content reflects the relative N status and yield level. Pramanik & Bera (2013) reported that nitrogen levels have a significant effect on the grain yield of rice, and the increase in yield components is the impact of nutrient adequacy, plant growth, and increased nutrient uptake. Furthermore, Gholizadeh et al. (2017) reported that chlorophyll content has a very close correlation with grain yield due to increased levels of N in flag leaves.

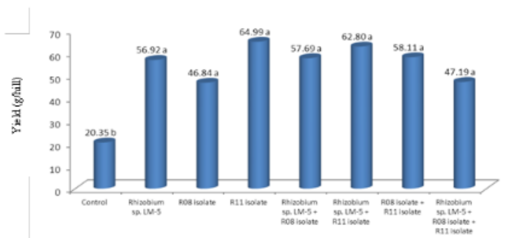

Figure 2. The effect of PGPR consortium on rice yield

Based on the results of this research, it can be seen that the PGPR consortium containing N-fixing bacteria and R11 isolate was able to increase the rice yields. This combination is a consortium that has the ability to provide nitrogen for plants and stimulate growth through the production of IAA. Rhizobium sp LM-5 is a bacterium that was successfully isolated from rice fields from the *Rhizobium* sp. family, which can stimulate the growth of rice plants (Purwanto et al., 2017). These finding were PGPR consortium between IAA-producing bacteria combined with *Rhizobium* sp. LM-5 as N_2 fixing bacteria in increasing nutrient uptake, chlorophyll contents and crop yields**.** Therefore, a consortium between N-fixing bacteria and IAA-producing bacteria can be developed for the assembly and production of biofertilizers to increase rice crop productivity.

CONCLUSION

The consortium of PGPR promotes rice root growth, which increases nutrient uptake (N, P, K), leaf chlorophyll content, plant biomass, and rice yield**.** Application of single strain R11 isolates and the consortium of R11 isolate+*Rhizobium* sp. LM-5 were the treatments capable of giving the highest grain yield of 64.99 and 62.80 g plant^{-1}, respectively. The PGPR consortium application was able to increase rice yields by 177.38% compared to control (withaout PGPR application)

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