The role of phosphate solubilizing bacteria from Rhizosphere of upland rice in the growth and yield of upland rice on ultisol soil

S N Hadi*, Fatichin², A Fauzi¹, I Widiyawati¹ and Y R Ahadiyat¹

¹Agroecology Laboratory, Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University Jl. Dr. Soeparno No. 61 Purwokerto, Indonesia 53123, Telp/Fax: (0281) 638791
²Plant Breeding and Biotechnology Laboratory, Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University Jl. Dr. Soeparno No. 61 Purwokerto, Indonesia 53123, Telp/Fax: (0281) 638791

Corresponding authors: sapto.hadi@unsoed.ac.id

Abstract. The aim of this study was to determine the role of B. proteolyticus GT2, B. paramycoides SR1, and A. delafieldii PA1 in the growth and yield of upland rice grown on ultisol soil, determine the best bacteria that showed the best upland rice growth and yield, and determine the interactions between upland rice varieties and bacterial type. The research was conducted in a greenhouse, Pasir Lor Village, Karanglewas District, Banyumas Regency, Central Java, Indonesia from December 2019 to June 2020. The research used a factorial randomized block design consisting of two factors: the upland rice varieties type (INPAGO UNSOED 1 (V1), INPAGO UNSOED PARIMAS (V2), and INPAGO 8 (V3)) and the bacteria type (control (B), B. proteolyticus GT2 (B1), B. paramycoides SR1 (B2), and A. delafieldii PA1 (B3)). Each combination was repeated three times. The results showed that PSB played a role in increasing the number of productive tillers, root volume, and root dry weight. A. delafieldii PA1 was the best bacteria in increasing the number of productive tillers, while B. paramycoides SR1 was the best bacteria in increasing root volume and dry weight. The results showed that there was no interaction between upland rice varieties and bacterial type.

1. Introduction
Phosphorus (P) is a macro nutrient which is essential for plant growth. Plants absorb P from the soil in the form of phosphate ions, especially H₂PO₄⁻ that abundant in acid soils and HPO₄²⁻ that dominant in alkaline soils. Soil bacteria that play a role in providing nutrients are phosphate solubilizing bacteria (PSB). These bacteria will release P from clay minerals and provide it to plants [1].

Phosphate solubilizing bacteria (PSB) are able to dissolve P ions bound to soil cations in the form of Al, Fe, Ca, and Mg, then convert them into available form for plants to absorb naturally [2]. PSB are able to convert insoluble phosphate by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric, and succinic acids [3]. Groups of soil bacteria that are able to dissolve P include: Bacillus, Pseudomonas [4-6], Acinetobacter, Massilia, Arthrobacter, Stenotrophomonas, Ochrobactrum, Cupriavidus [6], and Pantoea [4].
The existence of PSB play a role in the increasing level of available nutrient content, especially P in marginal soils such as ultisols. Ultisol soil is classified as marginal land, which is one type of land in Indonesia, covering about 25% of Indonesia's total land area. Utilization of this land for agricultural cultivation activities faces constraints on soil characteristics that can inhibit plant growth, especially low macro nutrient content like P, K, Ca and Mg as an addition to minimal organic matter content.

Previous study obtained a number of indigenous soil bacterial isolates from the rhizosphere of rice plants from several marginal land locations in Banyumas Regency [7]. This soil bacteria has characteristics as PSB. The biochemical and molecular identification showed that these bacterial isolates were Bacillus proteolyticus, B. paramycoides, and Acidovorax delafiedi species.

Bacillus is a group of soil bacteria that is often found in plant rhizosphere. This bacteria is very tolerant to unfavorable environmental conditions such as extreme environmental temperatures and low nutrients due to their ability to form endospores. The ability of Bacillus to form endospores allows this bacteria to adapt to agrochemicals compound applied in agricultural soils [8,9]. The application of Bacillus can effectively increase plant growth [10]. Plant growth can be increased through root colonization by Bacillus and Paenibacillus [11]. Meanwhile Acidovorax is bacteria that come from the environment (soil and water), which potential as a plant growth promoting rhizobacteria (PGPR) [12].

The aim of this study was to determine the role of B. proteolyticus GT2, B. paramycoides SR1, and A. delafiedi PA1 in the growth and yield of upland rice grown on ultisol soil, determine the best bacteria that showed the best upland rice growth and yield, and determine the interactions between upland rice varieties and bacterial type.

2. Materials and methods

2.1. Observations

The research was conducted in a greenhouse, Pasir Lor Village, Karanglewas District, Banyumas Regency, Central Java from December 2019 to June 2020. This research used a factorial randomized block design consisting of two factors. The first was the upland rice varieties type consist of INPAGO UNSOED 1 (V1), INPAGO UNSOED PARIMAS (V2), and INPAGO 8 (V3). The second was the bacteria type consisting of control (K), B. proteolyticus GT2 (B1), B. paramycoides SR1 (B2), and A. delafiedi PA1 (B3). Each combination was repeated three times.

Observation variables were plant height (cm), number of tillers (stem), number of productive tillers (stem), total length of the panicles (cm), number of filled grains per plant (seeds), dry grain weight per plant (g), weight of 100 unhulled seeds (g), root dry weight (g), and root volume (ml).

The data from the observations were analyzed using analysis of variance (ANOVA) with the f test at 5% level and if there was a real difference, it was continued with the least significant difference test (LSD) at the 5% level.

2.2. Application of bacteria in upland rice cultivation in ultisol soil

Rice seeds were immersed in sterile distilled water containing bacteria (density 10⁹ cfu / mL) for 48 hours. Rice seeds were cultivated into seedling medium for 12 days then planted into ultisol soil which has been added with manure (180.2 grams/polybags or equivalent to 20 tonnes/ha) in polybags (30 cm in diameter). The number of rice that planted was one plant per polybag. The plant were fertilized by NPK phonska. Fertilization was carried out three times (14 days after planting (dap), 24 dap, and 35 dap) [13]. When the rice was 30 dap and in primordial phase, the addition of 100 ml bacteria/polybag (density 10⁹ cfu / mL) was carried out. Rice harvesting were carried out based on plant age and characteristics such as rice panicles bowing because they support pithy grains, grain feels hard when pressed, when peeled, the grain looks white and hard when bitten, and the grain has a moisture content of 22-25%.
3. Results and discussion

The analysis result of the various treatment on upland rice varieties type, bacterial type, and the interaction between upland rice varieties and bacterial type on growth and yield of upland rice are presented in Table 1.

| No | Variable                             | Variety (V) | Bacteria (B) | (V x B) |
|----|--------------------------------------|-------------|--------------|---------|
| 1  | Plant height (cm)                     | ns          | ns           | ns      |
| 2  | Number of tillers (stem)              | *           | ns           | ns      |
| 3  | Number of productive tillers (stem)   | ns          | *            | ns      |
| 4  | The total length of the panicles (cm) | ns          | ns           | ns      |
| 5  | The number of filled grains per plant (seed) | ns | ns | ns |
| 6  | Dry grain weight per plant (g)        | ns          | ns           | ns      |
| 7  | Weight of 100 unhulled seeds (g)      | ns          | ns           | ns      |
| 8  | Root volume (ml)                      | ns          | *            | ns      |
| 9  | Root dry weight (g)                   | ns          | *            | ns      |

Remarks: V = Upland rice varieties type, B = Bacteria type, ns = not significantly different, * = significantly different at level p 0.05

Based on Table 1, the treatment of upland rice type had a very significant effect on growth parameters, namely the number of tillers. Meanwhile, the treatment of bacterial type had a very significant effect on the growth and yield components, namely the number of productive tillers, root volume, and root dry weight. There was no interaction between the upland rice varieties type and the bacterial type. The average effect of upland rice varieties type, bacterial type, and interactions between upland rice varieties type and bacteria type on the observed variables was presented in Table 2.

Table 2. The average values and analysis result of growth and yield variables of upland rice in treatment of upland rice varieties and bacteria type

| Application | Variables                  | PH (cm) | NT (stem) | NPT (stem) | TLP (cm) | NFG | DGW (g) | WUS (g) | RV (ml) | RDW (g) |
|-------------|---------------------------|---------|-----------|------------|----------|-----|---------|---------|---------|---------|
| V1          | V3                        | 72.27 a | 22.58 a   | 1.2 a      | 28.05 a  | 410.87 a | 8.27 a  | 1.91 a  | 34.16 a | 4.7 a   |
| V2          | V3                        | 76.00 a | 22.81 b   | 1.2 a      | 27.74 a  | 551.66 a | 12.17 a | 2.14 a  | 32.50 a | 4.42 a  |
| V3          | V3                        | 72.15 a | 13.32 b   | 0.91 a     | 26.50 a  | 413.87 a | 9.11 a  | 2.23 a  | 33.12 a | 4.61 a  |
| K           | V3                        | 71.73 a | 18.06 a   | 0.33 a     | 26.15 a  | 498.94 a | 10.43 a | 2.01 a  | 25.55 a | 3.07 a  |
| B1          | V3                        | 73.86 a | 20.40 a   | 1.05 ab    | 27.18 a  | 450.88 a | 9.73 a  | 2.07 a  | 35.27 b | 4.9 b   |
| B2          | V3                        | 74.58 a | 21.01 a   | 0.66 a     | 27.84 a  | 362.77 a | 8.43 a  | 2.07 a  | 42.5 c  | 6.43 c  |
| B3          | V3                        | 73.74 a | 18.80 a   | 2.38 b     | 28.55 a  | 522.61 a | 10.81 a | 2.22 a  | 29.72 a | 3.9 a   |
| C.V. (%)    |                           | 4.92    | 17.79     | 2.16       | 10.49    | 53.79 | 53.94   | 14.84   | 8.16    | 12.49   |

Remark: The number followed by the same letter in the column and the same treatment is not significantly different according to the Least Significant Difference (LSD) at the 5% error level, V1= Inpago Unsoed 1, V2= Inpago Unsoed Parimas, V3= Inpago 8, K = kontrol, B1= B. proteolyticus GT2, B2= B. paramycoides SR1, B3= A. delafiedii PA1, PH= Plant height, NT= Number of tillers, NPT= Number of productive tillers, TL= Total length of the panicles, NFG = The number of filled grains per plant, DGW= Dry grain weight per plant, BSB= Weight of 100 unhulled seeds, RV= Root volume, RDW= Root dry weight.

3.1. The role of bacteria in the number of productive tillers

According to LSD at the 5% error level, the bacteria type has a significant effect on the number of productive tillers of upland rice plants.

Based on Figure 1, the application of A. delafiedii PA1 (B3) gave the highest average number of productive tillers, 2.38 compared to the application of B. proteolyticus GT2 (B1) of 1.05 and B. paramycoides SR1 (B2) of 0.66, and control treatment (K) equal to 0.33. The role of PSB is thought to support the root formation and development which causes increasing of P uptake of plants that supports
the formation of productive tillers numbers. Uptake increased due to phosphorus was needed by plants in the cell division process [14]. One of the functions of P is to stimulate the roots and stems of rice plants and increase the formation of productive tillers, it also plays a role in the process of photosynthesis, respiration, energy transfer and storage, cell division and cell enlargement, and other processes in plants [15]. Nutrients such as P which are sufficiently available in the soil can stimulate the formation of productive tillers [16].

![Figure 1. The number of productive tillers histogram in bacterial type treatment. K= control, B1= B. proteolyticus GT2, B2= B. paramycoides SR1, B3= A. delafieldii PA1](image)

3.2. The role of bacteria in root volume
According to LSD at the 5% error level, several types of rhizosphere bacteria had a significant effect on the root volume of upland rice plants.

![Figure 2. Histogram of root volume in bacterial application. K=kontrol, B1= B. proteolyticus GT2, B2= B. paramycoides SR1, B3= A. delafieldii PA1](image)

Based on Figure 2, the use of B. paramycoides SR1 (B2) gave an average root volume of 42.5 g followed by B. proteolyticus GT2 (B1) with an average root volume of 35.27 g. The two bacteria had the best average root volume compared to the use of A. delafieldii PA1 (B3) and control treatment (K) which gave an average root volume of 29.72 and 25.55, respectively. These bacteria are thought to be
able to encourage growth and development of plant roots through their ability to dissolve phosphate. P elements can stimulate the growth and development of plant roots because they play a role in cell metabolism and as an activator of several enzymes [13].

An increase in P uptake increases root weight and volume because phosphorus is needed by plants for metabolic and physiological process as well as root growth and development [14; 17]. Phosphorus plays a role in the development of roots, especially lateral roots and fine roots [18]. An increase in root volume followed by an increase in the availability of water and nutrients will increase the amount of water and nutrients in plant absorption, further, it will stimulate physiological and metabolic processes in plants [17].

3.3. The role of bacteria in root dry weight
According to LSD at the 5% error level, several types of rhizosphere bacteria had a significant effect on the dry weight of upland rice roots.

![Figure 3. Histogram of root dry weight on bacterial type treatment.](image)

**Figure 3.** Histogram of root dry weight on bacterial type treatment. K=control, B1= *B. proteolyticus* GT2, B2= *B. paramycoides* SR1, B3= *A. delafielii* PA1

Figure 3 showed that the use of *B. paramycoides* SR1 (B2) provides a better average root dry weight, 6.43 g compared to the use of *B. proteolyticus* GT2 (B1), *A. delafielii* PA1 (B3) and control treatment (K). The average root volume was 4.9 g, 3.9 g, and 3.07 g, respectively. The role of P possessed by rhizosphere bacterial is thought to be very important in supporting root growth and development so that the resulting root dry weight is also better. The role of P is important for the formation of root hairs so that it can increase root weight up to 10 times [18]. Phosphorus element is absorbed and used to modify the root epidermis to form hairs so that the density of the root hairs is high [19]. Figure 3 also indicates that a given rhizosphere bacteria is very compatible so that it contributes to plant growth.

4. Conclusion
The results showed that PSB played a role in increasing the number of productive tillers, root volume, and root dry weight. *A. delafielii* PA1 was the best bacteria in increasing the number of productive tillers, while *B. paramycoides* SR1 was the best bacteria in increasing root volume and dry weight. The results showed that there was no interaction between upland rice varieties type and bacterial type.

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