

BIOASSAY OF PHOSPHORUS SOLUBILIZING ISOLATES FOR ENHANCE P SOLUBILITY AND GROWTH OF RICE (*Oryza Sativa* L.)

UJI HAYATI ISOLAT PELARUT FOSFOR UNTUK MENINGKATKAN KELARUTAN P DAN PERTUMBUHAN PADI (*Oryza Sativa* L.)

Betty Natalie Fitriatin^{1*}, Dahlia Florencia Manurung², Mieke Rochimi Setiawati¹

¹⁾ Department of Soil Sciences and Land Resources Management, Faculty of Agriculture, Universitas Padjadjaran – Jatinangor 45363-West Java

²⁾ Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran–Jatinangor 45363-West Java

*Corresponding Author: betty.natalie@unpad.ac.id

Accepted: 13 April 2022 / Agreed: 13 Juli 2022

ABSTRAK

Fosfat merupakan salah satu unsur yang berperan penting bagi pertumbuhan tanaman dan kesuburan tanah. Namun, ketersediaan unsur P terlarut yang dapat diserap oleh tanaman sangat kecil karena berikatan dengan kation yang berada di dalam tanah. Salah satu upaya dalam meningkatkan P tersedia dalam tanah adalah dengan pemanfaatan agen hayati Bakteri Pelarut Fosfat (BPF). Penelitian ini bertujuan untuk menguji isolat BPF yang dapat meningkatkan kelarutan P dan pertumbuhan padi pada uji hayati. Percobaan ini dilaksanakan di Rumah Kaca Departemen Ilmu Tanah dan Sumber Daya Lahan, Fakultas Pertanian, Universitas Padjadjaran. Rancangan percobaan yang digunakan adalah Rancangan Acak Kelompok (RAK) dengan 6 perlakuan dan 5 ulangan. Masing-masing perlakuan jenis bakteri adalah kontrol, *Bacillus subtilis*, *B. megatherium*, *Pseudomonas mallei*, *Burkholderia* sp., dan isolat campuran. Hasil percobaan menunjukkan isolat BPF yang diuji memiliki kemampuan yang bervariasi dalam meningkatkan enzim fosfatase, kelarutan P, dan pertumbuhan padi pada uji hayati. Lebih lanjut, perlakuan BPF campuran memberikan pengaruh lebih baik terhadap aktivitas fosfatase, P terlarut dan pertumbuhan padi dibandingkan isolat tunggal.

Kata kunci: Fosfatase, Isolat, Pelarutan P, Uji hayati

ABSTRACT

Phosphorus is an element that important for soil fertility and plant growth. However, the phosphate nutrient can be uptaken by plants only in a small amount because it binds to cations in the soil. The effort for enhancing the soil P availability is by the phosphorus solubilizing bacteria (PSB). This study aimed to test PSB isolates for increasing P solubility and rice growth using bioassay. The experiment conducted at the greenhouse in Jatinangor District, Sumedang Regency, West Java with Randomized Block Design (RBD) for PSB isolates with five replications. Each type of bacteria treatment was control, *Bacillus subtilis*, *B. megatherium*, *Pseudomonas mallei*, *Burkholderia* sp., and mixed isolates. The results showed that the P solubilizing isolates had various abilities to enhance phosphatase, P solubility, and rice growth using bioassay. Furthermore, the mixed PSB isolates had a better effect on phosphatase activity, dissolved P and rice growth than single isolates.

Keywords: Bioassay, P Dissolved, Isolate, Phosphatase

INTRODUCTION

Plant growth requires sufficient soil macronutrients. Phosphorus is an important macro nutrient that has problems in its availability. Soil phosphorus content is high but in a state not available to plants. The presence of strong P fixation by Al and Fe hydroxides is a common problem encountered in agricultural soils (Penn & Camberato, 2019; Johan et al., 2021).

Effort for enhance the soil P availability through the application of inorganic fertilizers. However, the using of inorganic fertilizers in the long term can reduce the soil fertility and pollute the environment (Bhatt et al. 2019; Chandini et al. 2019). The soil fertilized by inorganic fertilizers continuously will reduce the C-organic content of the soil (Ozlu et al. 2019 ; Zhao et al. 2020) and damage soil aggregates then unable to support plants (Ghosh et al., 2019).

To increase plant growth and fertilization efficiency as well as soil quality, it is necessary to develop the utilization of potential biological resources to facilitate the availability of soil nutrients. An example of microorganism usage that play a role in the transformation of P nutrients in the soil and can be used as biological fertilizers is phosphate solubilizing microorganism (Kalayu, 2019; Dai et al., 2020).

Phosphorus solubilizing microorganism (PSM) is beneficial soil microorganism that can solubilize P from the bound phosphate into soluble soil P and available to plants (Ingle & Padole, 2017). The phosphorus solubilizing microorganism secretes organic acids that can form the stable complexes

with P-binding cations in the soil (Tian et al., 2021). These group of phosphate solubilizing microorganisms have many advantages in affecting the plant growth, besides being able to release fixed P, it can also produce phosphatase enzymes (Behera et al., 2017). The phosphatase enzyme can mineralize organic P into inorganic P (Margalef et al., 2017). Rawat et al. (2020) stated that soil P dissolution by PSM generally occurs through the dissolution of anorganic P and mineralization of organic P.

Several research showed the role of P solubilizing microorganisms in increasing soil nutrient availability and crop production. An increase in yield of maize and plant P uptake caused by the application of phosphate solubilizing bacteria as Plant Growth Promoting Rhizobacteria (PGPR) *Azospirillum brasilense*, *Bacillus subtilis* and *Pseudomonas fluorescens* have been reported by Pereira et al. (2020). Fitriatin et al. (2020) isolated PSB isolates from the acid soil ecosystem in various rhizosphere and characterized its ability to dissolve P by producing organic acids, phosphatase enzymes and phytohormones.

Rice requires P nutrients to increase the yield. The experiment is needed to study PSB isolates for increasing P solubility and bioassay of these isolates to the rice growth (*Oryza sativa* L.).

MATERIALS AND METHODS

The bioassay was carried out in the greenhouse of Agriculture Faculty, Universitas Padjadjaran, Jatinangor District,

Sumedang Regency, West Java. Randomized Block Design (RBD) was used for the experiment with type isolates as treatments and five time replications. The PSB isolates used in this study were *Bacillus subtilis*, *B. megatherium*, *Pseudomonas mallei*, and *Burkholderia* sp. These isolates were collected at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Padjadjaran, which were isolated from maize and rice rhizosphere that had been selected to dissolve P.

Bioassay of phosphate solubilizing bacteria was applied in liquid Murphy medium according to Murphy and Riley (1962) (CaSO₄. H₂O 0.25 g; KH₂PO₄.2H₂O 0.25 g; MgSO₄.7H₂O 0.25 g; NaCl 0.08 g; KCl 0.52 g; ZnCl₂ 0.017 g; CuSO₄.5H₂O 0.005 g; FeSO₄ 0.025 g in 1 L distilled water, pH 7).

The rice seeds used previously were sterilized and germinated on the straw paper for 7 days. Sprouts that have grown were planted in test tubes filled with 95 mL of Murphy media and treated with 5 mL of PSB isolates by 10⁷ CFU mL⁻¹ density. Observations were carried out for 4 weeks, refer to the rice vegetative phase and adjusted to the capacity of the growing medium.

Phosphatase activity, P-dissolved and rice plant growth were further conducted. Phosphatase enzyme activity was determined according to Eivazi and Tabatabai method (Margesin, 1996). p-nitrophenyl was added to the substrate to form p-nitrophenol compound through enzyme activity. Then, it was consequently stained by sodium hydroxide solution, which can be detected by 400 nm

spectrophotometer. The data were analyzed by means of variance (ANOVA) using SPSS, for treatments had a significant effect, Duncan's multiple distance test was carried out at a significance level of 5% (Gomez & Gomez, 1995).

RESULTS AND DISCUSSION

Phosphatase enzymes and P-dissolved

The phosphatase enzyme produced by each type of phosphate solubilizing bacteria was different (Table 1). The isolate of bacteria that produced the highest phosphatase enzyme was owned by the mixed isolate by 4.83 g pNP g⁻¹ h⁻¹, followed by the bacterial isolate of *Burkholderia* sp. of 4.82 g pNP g⁻¹ h⁻¹.

The data in Table 1 showed that each bacterial isolate had various ability to dissolve P. Mixed isolate dissolves P higher than other treatments, which was 6052.19 ppm. The activity of phosphatase enzyme from each isolates was one of the factors that influenced the variation in the dissolved P-value produced by each isolate. Hummel et al. (2021) reported that the activity of the phosphatase enzyme affected the solubility of P and the availability of P in the soil.

The mixed isolates that had a high value of the phosphatase enzyme would produce a high value of P-dissolved as well as 6052.19 ppm. However, P-dissolved by mixed isolates was not significant with *B. megatherium* (5625.44 ppm). According to Elhaissofi et al. (2020), the P solubilizing from organic compounds depends on the capacity of catalytic by phosphatase produced by the P solubilizing bacteria.

Table 1. Ability of phosphate solubilizing bacterial isolates to produce phosphatase and P-dissolved

Isolates	Phosphatase ($\mu\text{g pNP g}^{-1} \text{h}^{-1}$)	P-dissolved (ppm)
Control	4.21 a	870.79 a
<i>Bacillus substilis</i>	4.62 b	4994.02 c
<i>B. megatherium</i>	4.56 b	5625.44 de
<i>Pseudomonas mallei</i>	4.53 b	3909.55 b
<i>Burkholderia</i> sp.	4.82 c	5307.63 cd
mixed isolates	4.83 c	6052.19 e

Note: The average value in the same column marked with the same letter is not significantly different according to Duncan's Multiple Distance Test at 5% level.

The mixed isolate was equivalent to *Burkholderia* sp., which had the highest yield in secreting the phosphatase enzyme, while the solubility of *Bacillus megatherium* P was equivalent to that of mixed isolates. This can be presumably because the types of organic acids produced are different so that the ability of these organic acids to chelate P bonds will also differ. *Burkholderia* sp. isolate quantity in a single treatment can produce a phosphatase enzyme, which is equivalent to the quantity of *Burkholderia* sp. consortium with other isolates. Variations in the solubility of elemental P are affected by the ability of organic acids to chelate P (Serna-Posso et al. 2017). According to Wei et al. (2018), PSB can produce organic acids that can form a complex compound.

The formation of this complex compound will cause P fixation to decrease, thereby P-available will increase. The results showed that the mixed treatment isolate was the best in increasing the activity of the phosphatase enzyme and the concentration of P-dissolved. Mixed isolates showed phosphatase enzyme enhancement by 14.2% compared to the control and 3.89% compared to single isolates. Likewise, the P content with an increase of 59.5% compared to the control and

22% compared to a single isolate. This is in line with Bradáčová et al. (2019) that the use of microbial consortia tends to give better results than the use of single isolates because it is expected that the enzyme work of each type of microbe can complement each other in order to survive using available nutrient sources.

Rice plant growth

Phosphate solubilizing bacteria isolates had no significant effect on increasing of plant height (Table 2), but had an effect on root length (Table 3). The increase of plant height and plant root length was affected by various factors such as the availability of nutrients in the growing media. Provision of bacteria can change elements that were not previously available become available to plants and have an impact on increasing plant height and plant root length (Lopes et al. 2021).

The results revealed that P-solubilizing bacteria had no significant effect on plant height. Based on the data in Table 2, the treatment of *Burkholderia* sp. tended to have the highest average increase in plant height when compared to other treatments. Figure 1. showed the plant height without isolates tends to be lower than the isolate treatment. The isolate used in this experiment was classified as the PGPR (Plant Growth Promoting Rhizobacteria)

which acts as a biostimulant, bioprotectant and biofertilizer (Kumar et al., 2021).

Table 2. The increasing of plant height as affected by PSB

Isolates	Increasing of Plant Height (cm)			
	1 WAP	2 WAP	3 WAP	4 WAP
Control	2.62	0.62	0.44	0.12
<i>Bacillus substilis</i>	2.96	0.8	0.66	0.3
<i>B. megatherium</i>	3.6	1.1	0.48	0.12
<i>Pseudomonas mallei</i>	3.18	0.58	0.46	0.26
<i>Burkholderia sp.</i>	2.88	0.9	0.7	0.52
mixed isolates	2.90	0.82	0.28	0.22

Note: The average value in the same column marked with the same letter is not significantly different according to Duncan's Multiple Distance Test at 5% level.

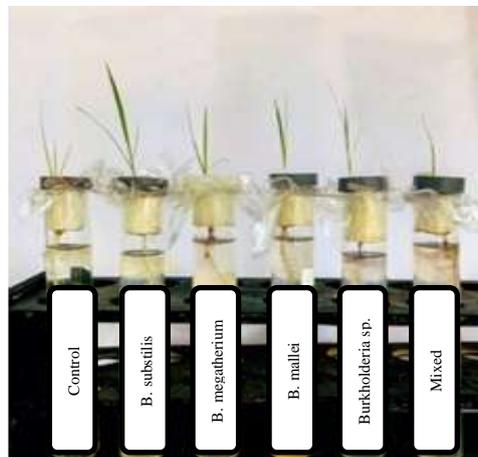


Figure 1. Bioassay of PSB in rice plant 4 WAP

Table 3 showed the effect of PSB isolates on plant root length was not significantly different at one to three weeks after planting (WAP). However, PSB isolate increased plant root length significantly. In four weeks after planting, rice plants with mixed isolate had the largest for increasing in plant root length, which was 0.4 cm from the previous week.

Phosphate solubilizing bacteria are able to produce growth hormone IAA, which functions in root extension. In addition, the activity of other growth hormones such as gibberellins made the application of PSB isolates more likely to

work on the roots. Root length tends to determine nutrient absorption more than root weight because long roots will easily absorb the nutrients in the soil with a wide range (Asova et al., 2018). In addition, the enhancement of plant root length is also assumed to be caused by the bacterium in the consortium that are synergistic with each other. This bacterium makes the inoculation effective, so that the nutrients needed by plants can be fulfilled. Meanwhile, the control treatment showed the smallest increase in plant root length of 0.06 cm from the previous week.

This biostimulant function is caused by the production of a hormone i.e IAA (Indole Acetic Acid) as a natural compound that plays a role in cell division and encourages the formation of adventitious roots. Phosphate solubilizing bacteria produce phytohormones such as IAA and GA3 (Gibberelic Acid). Phytohormones had a function to stimulate cell elongation at the growing point (Nenwani et al., 2010). IAA is a growth hormone of auxin group that functions to stimulate plant growth. Auxins play a role in increasing stem cell growth, inhibiting leaf shedding, stimulating fruit formation, and stimulating cambium growth (Ogunyale et al., 2014).

The application PSB isolates increased the length of plant roots in the bioassay. Phosphorus has a very important role in cell division and for the development of meristem tissue that can stimulate root growth, especially in seeds and young plants (Bechtaoui et al., 2021). Rice plants

treated with PSB isolates were found to have long adventitious roots and more root hairs. The roots function to absorb nutrients. This is the effect of element P because it can increase the surface area of the roots and increase the number of root hairs (Kim & Li, 2016). The finding of difference roots in the control treatment and the treatment given PSB isolates proved that PSB worked in dissolving P, which was previously unavailable to become available to plants.

The phosphate formed available to plants will be absorbed and bring out the functions of element P for better plant growth when compared to the control treatment. Phosphorus element acts as a carrier and store of energy in the form of ATP in plant metabolism so that cell division, enlargement, and root development can be obtain properly (Vance et al., 2002).

Table 3. Increasing in root length of rice plants in bioassay of PSB

Isolates	Increasing of Root Length (cm)			
	1 WAP	2 WAP	3 WAP	4 WAP
Control	0.84	0.74	0.68	0.06 a
<i>Bacillus substilis</i>	1.06	0.48	0.38	0.34 b
<i>B. megatherium</i>	1.26	0.48	0.68	0.22 ab
<i>Pseudomonas mallei</i>	2.22	0.64	0.96	0.24 ab
<i>Burkholderia</i> sp.	0.94	0.28	0.8	0.2 ab
mixed isolates	1	0.8	0.66	0.4 b

Note: The average value in the same column marked with the same letter is not significantly different according to Duncan's Multiple Distance Test at 5% level.

The inoculation of PSB had a significant effect on root dry weight, shoot dry weight and S/R ratio (Table 4). *Bacillus substilis* had a significant effect on root dry weight compared to control with a value of 6.4 mg, while the control treatment was 2.4 mg. However, root weight does not reflect the good absorption of plant nutrients when

compared to root length (Asova et al., 2018). The immobile P element will increase its absorption if the contact distance between P and roots is shortened. The close contact distance with the P source in the soil can be reached by root extension (Lucas et al., 2019). Mixed isolates affected shoot dry weight better than single isolates.

Growth rates of shoots and roots during period of vegetation continually adjust to plant growth and environmental conditions (Bláha, 2019). S/R ratio had a higher potential for the mixed isolate treatment of 5.94 while the lowest shoot root ratio showed by the *Bacillus subtilis* isolate treatment of 2.97. Mixed isolates showed an increase in S/R ratio of 51.3% when compared to the control. In this case, the S/R ratio reflects the division of the photosynthetic yield in the plant growth under the test. The S/R ratio more than one indicates that growth tends to shoot, whereas if the value of S/R ratio is less than

one, growth tends to the root (Minaxi et al., 2011). In the vegetative phase, it would be better if the roots were larger than the shoot. Wu et al. (2016) stated that the roots affect plant growth because nutrient absorption depends on the length and number of plant roots.

The results showed that inoculation of phosphate solubilizing bacteria affected root growth. This is in line with Verbon and Liberman (2016) that rhizobacteria affect cell division and differentiation leading to changes in root system architecture, which contributes to enhanced shoot growth.

Table 4. Root dry weight, shoot weight, and S/R ratio affected by PSB

Isolates	Shoot Dry Weight (mg)	Root Dry Weight (mg)	S/R
Control	9.4 a	2.4 a	3.92 ab
<i>Bacillus subtilis</i>	19.0 b	6.4 c	2.97a
<i>B. megatherium</i>	17.4 b	4.8 bc	3.63 ab
<i>Pseudomonas mallei</i>	16.8 b	3.8 ab	4.42 ab
<i>Burkholderia</i> sp.	18.0 b	5.4 bc	3.33 a
mixed isolates	20.2 b	3.4 ab	5.94 b

Note: The average value in the same column marked with the same letter is not significantly different according to Duncan's Multiple Distance Test at 5% level.

CONCLUSION

Phosphorus solubilizing bacteria *Bacillus subtilis*, *B. megatherium*, *Pseudomonas mallei* and *Burkholderia* sp. had various abilities to increase the phosphatase enzyme, P solubility and rice growth in the bioassay. The mixed PSB isolates had a better effect on phosphatase activity, dissolved P and rice growth than single isolates. These isolates of phosphorus solubilizing bacteria can be developed as biofertilizers to increase soil P availability and plant growth.

ACKNOWLEDGMENTS

The research was supported by the Directorate General of Higher Education Indonesia (Applied Research: 1827/UN6.3.1/LT/ 2020). We also thank to Yeni Wispa who helped with this research.

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