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

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**ABSTRAK**

Fosfat merupakan salah satu unsur yang berperan penting bagi pertumbuhan tanaman dan kesuburan tanah. Akan tetapi, ketersediaan unsur P terlarut yang dapat diserap oleh tanaman sangat kecil oleh 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 substilis*, *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, hasil percobaan menunjukkan bahwa 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 growth of plant However, the phosphte nutrient that can be uptake by plants is very small because it binds to cations in the soil. Effort for enhace the soil P availabilty through the phosphorus olubilizing bacteria (PSB). This study aims to test PSB isolates for increasing P solubility and rice growth in bioassay tests. The greenhouse experiment located in Jatinangor District, Sumedang Regency, West Java with Randomized Block Design (RBD) had PSB isolates with five replications. Each type of bacteria treatment was control, *Bacillus substilis*, *B. megatherium*, *Pseudomonas mallei*, *Burkholderia* sp. and mixed isolates. The results revealed that the P solubilizing isolates had varying abilities to enhace phosphatase, P solubility, and rice growth in bioassay tests. Furthermore, the mixed PSB isolates had a better effect on phosphatase activity, dissolved P and rice growth than single isolates.

*Key words : Bioassay, Dissolve P, 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 problem that is commonly encountered in agricultural soils in general (Penn & Camberato, 2019).

Effort for enhace the soil P availabilty through the application of inorganic fertilizers. However, the using of inorganic fertilizers in the long term reduce soil fertility and pollute the environment (Bhatt et al. 2019; Chandini et al. 2019). The soil that was continuously fertilized by inorganic fertilizers would reduce the C-organic content of the soil (Ozlu et al. 2019 ; Zhao et al. 2020) and damage soil aggregates so that they were 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. One example is the use of microorganisms that play a role in the transformation of P nutrients in the soil that can be used as biological fertilizers, namely phosphate solubilizing microorganisms (Kalayu, 2019).

 Phosphorus solubilizing microorganisms (PSM) are benefecial soil mcroorganims that have capable to solubilize P from the bound phosphate into soil P soluble to become available to plants (Ingle & Padole, 2017). The phosporus solubilizing microorganisms secrete organic acids that can form stable complexes with P-binding cations in the soil (Tian et al., 2021). This group of phosphate solubilizing microorganisms has many advantages in influencing the increase in 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 shows 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. The experiment is needed to study PSB isolates for increasing P solubility and bioassay of these isolates which can increase rice growth (*Oryza sativa* L.).

**MATERIALS AND METHODS**

The bioassay test was carried out in Greenhouse of Agriculture Faculty, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java. Randomized Block Design (RBD) was used for experimental with type isolates as treatments and replications five times. The PSB isolates used in this study were*, Bacillus substilis, B. megatherium, Pseudomonas mallei, Burkholderia sp.*

 Culture media for bioassays using Murphy media. The rice seeds used previously were sterilized, then germinated on straw paper for 7 days. Sprouts that have grown are 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 because the rice plants were in the vegetative phase and adjusted to the capacity of the growing medium.

**RESULTS AND DISCUSSION**

***Phosphatase Enzymes and Dissolved P***

 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 4.812 g pNP/g/h, followed by the bacterial isolate of *Burkholderia* sp. of 4.816 g pNP/g/h.

 The data in Table 1 shown that each bacterial isolate had various ability to dissolve P. The highest dissolved P value was owned by mixed isolates, 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.

Table 1. Ability of Phosphate Solubilizing Bacterial Isolates to Produce Phosphatase and P-Dissolved

|  |  |  |
| --- | --- | --- |
| Isolates | Phoshatase (µg pNP/g/h) | P-dissolved (ppm) |
| Control | 4,21 a | 870,79 a |
| *Bacillus substilis* | 4,62 b | 4994,02 c |
| *B. megatherium* | 4,56 b | 5625,44 de |
| *Pseudomonas mallei* | 4,53 b | 3909,55 b |
| *Burkholderia* sp. | 4,816 c | 5307,63 cd |
| mixed isolates | 4,812 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 isolates that had a high value of the phosphatase enzyme would produce a high value of P-dissolved as well, which was 6052.19 ppm. According to Elhaissoufi et al. (2020) that the P solubilizing from organic compounds depends on the capacity of catalytic by phosphatase produced by the P solubilizing bacteria.

The mixed isolate was equivalent to that of *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 be different. *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 influenced 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 complex compounds.

The formation of this complex compound will cause P fixation to decrease thereby increasing P-available. The results of experimental showed that the mixed treatment isolate was the best in increasing the activity of the phosphatase enzyme and the concentration of dissolved P. Mixed isolates showed an increase in the phosphatase enzyme by 14.2% by compared to the control and 3.89% by compared to single isolates. Likewise with the P content with an increase of 595% when compared to the control and 22% when compared to a single isolate. This is in line with Bradáˇcová 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 in plant height and plant root length is influenced by various factors, one of which is the availability of nutrients in the growing media. Provision of bacteria can change elements that were not previously available, become available to plants which 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 growth. 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). The isolate used in this experiment was classified as 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 |
| *Bacillus substilis* | 2,96 | 0,8 | 0,66 | 0,3 |
| *B. megatherium* | 3,6 | 1,1 | 0,48 | 0,12 |
| *Pseudomonas mallei* | 3,18 | 0,58 | 0,46 | 0,26 |
| *Burkholderia* sp*.* | 2,88 | 0,9 | 0,7 | 0,52 |
| mixed isolates | 2,9 | 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.



Table 3 shown 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 nutrients in the soil with a wide range (Asova et al., 2018). In addition, the increase in plant root length is also thought to be caused by the bacteria in the consortium that are synergistic with each other which 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, which was 0.06 cm from the previous week.

This biostimulant function is caused by the production of one of the hormones, namely 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 asIAA and GA3 (Gibberelic Acid). phytohormones that function to stimulate cell elongation at the growing point (Nenwani et al., 2010). IAA is a growth hormone of the 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 test. 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 differences in roots in the control treatment with the treatment given PSB isolates proved that PSB worked in dissolving P which was previously unavailable to become available to plants.

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

Table 3. Increasing in Root Length of Rice Plants in Bioassay of PSB

|  |  |
| --- | --- |
| **Isolates** | Increasing in Root Length **(cm)** |
| **1 WAP** | **2 WAP** | **3 WAP** | **4 WAP** |
| Control | 0,84 | 0,74 | 0,68 | 0,06 a |
| *Bacillus substilis* | 1,06 | 0,48 | 0,38 | 0,34 b |
| *B. megatherium* | 1,26 | 0,48 | 0,68 | 0,22 ab |
| *Pseudomonas mallei* | 2,22 | 0,64 | 0,96 | 0,24 ab |
| *Burkholderia* 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.

**CONCLUSION**

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

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**REFERENCES**

Asova, T.N.P., A. Jingga, M.R. Setiawati, dan T. Simarmata. 2018. Uji hayati dan karakterisasi isolate rhizobakteri fosfat dengan indikator tanaman jagung. Jurnal Penelitian Saintek, 23(1): 43–51.

Bechtaoui N., Rabiu M.K., Raklami A., Oufdou K., Hafidi M. and Jemo M. 2021. Phosphate-Dependent Regulation of Growth and Stresses Management in Plants. Front. Plant Sci. 28 Front. Plant Sci., 28 | https://doi.org/10.3389/ fpls.2021.679916

Behera, B.C., H. Yadav, S.K. Singh, R.R. Mishra, B.K. Sethi, S.K. Dutta, H.N. Thatoi. 2017. Phosphate solubilization and acid phosphatase activity of Serratia sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. Journal of Genetic Engineering and Biotechnology. Vol 15 : 169-178

Bhatt M.K., Labanya R., Joshi H.C. 2019. Influence of Long-term Chemical fertilizers and Organic Manures on Soil Fertility - A Review. Universal Journal of Agricultural Research 7(5): 177-188

Bradáˇcová K., Florea A.S., Bar-Tal A, , Minz D., Yermiyahu U., Shawahna R., Kraut-Cohen J., Zolti A., Erel R., Dietel K.,Weinmann M., Zimmermann B., Berger N. , Ludewig U., Neumann G., & Posta G. 2019. Microbial Consortia versus Single-Strain Inoculants: An Advantage in PGPM-Assisted Tomato Production. Agronomy 2019, 9, 105; doi:10.3390/agronomy9020105

Chandini, Kumar R., Kumar R., & Prakash O. 2019. The Impact of Chemical Fertilizers on Our Environment and Ecosystem. *In book*: Research Trends in Environmental Sciences (pp.69-86) Edition: 2nd Chapter: 5

Elhaissoufi W., Khourchi S., Ibnyasser A., Ghoulam C., Rchiad Z., Zeroual Y., Lyamlouli K. & Bargaz A. 2020, Phosphate Solubilizing Rhizobacteria Could Have a Stronger Influence on Wheat Root Traits and Aboveground Physiology Than Rhizosphere P Solubilization. Front. Plant Sci. 11:979. doi: 10.3389/fpls.2020.00979

Fitriatin, BN. D. Fauziah, F.N. Fitriani, D.N. Ningtyas, P.Suryatmana, R.Hindersah, M.R. Setiawati, T. Simarmata. 2020. Biochemical activity and bioassay on maize seedling of selected indigenous phosphate-solubilizing bacteria isolated from the acid soil ecosystem. Open Agriculture 5: 300–304

Ghosh, Ranjani, Soma Barman and Narayan Chandra Mandal. 2019. Phosphate defciency induced bioflm formation of Burkholderia on insoluble phosphate granules plays a pivotal role for maximum release of soluble phosphate. Science Report 9 :5477-5492

Hummel C., Boitt G., Santner J., Lehto N.J., Condron L., Wenzel W.W. 2021. Co-occurring increased phosphatase activity and labile P depletion in the rhizosphere of Lupinus angustifolius assessed with a novel, combined 2D-imaging approach. Soil Biology and Biochemistry 153 : 107963. http: //www.elsevier.com/locate/soilbio

Ingle K.P. & Padole D.A. 2017. Phosphate Solubilizing Microorganisms: An Overview. Int.J.Curr.Microbiol.App.Sci. 6(1): 844-852

Kalayu, G. 2019. Phosphate solubilizing microorganisms: Promising Approach as Biofertilizers. International Journal of Agronomy. Vol. 2019, ID 4917256: 1-7

Kim H. & Li X. 2016. Effects of Phosphorus on Shoot and Root Growth, Partitioning, and Phosphorus Utilization Efficiency in Lantana. HORTSCIENCE 51(8):1001–1009.

Kumar, M.; Giri, V.P.; Pandey, S.; Gupta, A.; Patel, M.K.; Bajpai, A.B.; Jenkins, S.; Siddique, K.H.M. 2021. Plant-Growth-Promoting Rhizobacteria Emerging as an Effective Bioinoculant to Improve the Growth, Production, and Stress Tolerance of Vegetable Crops. Int. J. Mol. Sci. 22, 12245. https:// doi.org/10.3390/ ijms222212245

Lopes MJS, Dias-Filho MB & Gurgel ESC. 2021. Successful Plant Growth-Promoting Microorganisms: Inoculation Methods and Abiotic Factors. Front. Sustain. Food Syst. 5:606454. doi: 10.3389/fsufs.2021.606454

Margalef O., Sardans J., Fernández-Martínez M., Molowny-Horas R., Janssens I. A.,Ciais P, Goll D., Richter A., M. Obersteiner M., Asensio D. & Peñuelas J. 2017. Global patterns of phosphatase activity in natural soils. Scientific Reports | 7: 1337 | DOI:10.1038/ s41598-017-01418-8

Nenwani, V., P. Doshi, T. Saha, dan S. Rajkumar. 2010. Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity. Journal of Yeast and Fungal Research 1 (1): 009- 014.

Ogunyale O.G., Fawibe O.O., Ajiboye A.A. and Agboola D.A. 2014. A Review of Plant Growth Substances: Their Forms, Structures, Synthesis and Functions. ournal of Advanced Laboratory Research in Biology. 5 (4) : 152-168

Ozlu E., Sandhu S.S, Kumar S. &Fransisco J. Arriaga. 2019. Soil health indicators impacted by long-term cattle manure and inorganic fertilizer application in a corn-soybean rotation of South Dakota. Scientific Reports : 11776

Penn,C.J. and J.J. Camberato. 2019. A Critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture. 9: 120; doi:10.3390/agriculture9060120

Pereira, N.C.M., F.S. Galindo, R.P.D.Gazola, E.Dupas, P.A.L. Rosa, E.S. Mortinho and M.C.M.T. Filho. 2020. Corn yield and phosphorus use efficiency response to phosphorus rates associated with plant growth promoting bacteria. Frontiers in Environmental Science | www.frontiersin.org. Vol 8 : 40

Rawat, P, S. Das, D. Shankhdhar & S. C. Shankhdhar. 2020. Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. Journal of Soil Science and Plant Nutrition.https://doi.org/10.1007/s42729-020-00342-7

Serna-Posso E.J.,  Sánchez-de Prager M.,

Cisneros-Rojas C,A. 2017. Organic acids production by rhizosphere microorganisms isolated from a Typic Melanudands and its effects on the inorganic phosphates solubilization. Acta Agronómica, vol. 66, no. 2, pp. 241-247, 2017

Tian, J., Ge, F., Zhang, D., Deng, S., Liu, X. 2021Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. Biology 10, 158. https://doi.org/10.3390/biology10020158

Vance S.P., Uhde-Stone C. & Allan D.L. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist. 157: 423 – 447. [www.newphytologist.com](http://www.newphytologist.com)

Wei, Y., Zhao, Y., Shi, M., Cao, Z., Lu, Q., Yang, T., Fan, Y., & Wei, Z. 2018. Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. Bioresource Technology. 247: 190 – 199.

Zhao Z., Zhang C., Li F, Songfeng Gao S., Zhang J. 2020. Effect of compost and inorganic fertilizer on organic carbon and activities of carbon cycle enzymes in aggregates of an intensively cultivated Vertisol. PLoS ONE 15(3): e0229644. <https://doi>. org/10.1371/ journal.pone.022964