Synergistic Test and Bioassay of Phosphate Solubilizing Bacteria for Improving Growth of Rice Seedling

Betty Natalie Fitriatin[1], Dahlia Florencia Manurung[2], Emma Trinurani Sofyan[3], Mieke Rochimi Setiawati[4]
{betty.natalie@unpad.ac.id}

Department of Soil Sciences and Land Resouces Management, Agriculture Faculty, Universitas Padjadjaran – Jatinangor 45363-West Java – Indonesia[134], Agrotechnology, Agriculture Faculty, Universitas Padjadjaran–Jatinangor 45363-West Java – Indonesia[2]

Abstract. The increasing of conventional productivity still depends on inorganic fertilizers. The use of inorganic fertilizers continuously decreases the soil quality. One of the problems encountered is the low availability of soil P. One of the efforts to increase the availability of P in the soil is by using Phosphate Solubilizing Bacteria (PSB). This research aims to study the synergistic test between PSB and determine PSB isolates which can increase the rice growth in bioassay experiment. This experiment consisted of 2 stages, the synergistic test which was carried out in the Laboratory of Soil Biology and bioassay experiment in the Greenhouse, Department of Soil Science and Land Resources, Faculty of Agriculture, Universitas Padjadjaran from August to September 2020. The bioassay experimental design that we use was randomized block design (RBD) with 6 treatments and 5 replications. Each type of bacterial treatment was control, Burkholderia sp., Pseudomonas mallei, Bacillus substilis, Bacillus megatherium and mixed isolates. The data obtained showed that each PSB isolates were compatible with each other. The result showed that mixed PSB treatment improved rice growth.

Keywords: Compatible; Dissolve P; Phosphate solubilizing

1 Introduction

Phosphate plays an important role for plant growth and soil fertility. Phosphorus (P) fertilizer has an important role for increasing rice production. According to Doberman and Fairhurst (2000), the phosphorus fertilizer for rice plants is required as much as 2.6 kg P ha-1 per tonne of grain. High P fertilizer application is not in line with availability of P in soil because most of P is bound by Al, Fe and Ca so they are not available to plants. This causes inefficient use of P fertilizer. To overcome the problem of soil P availability by utilizing phosphate solubilizing bacteria (PSB). Phosphate solubilizing bacteria can dissolve insoluble soil P become too available for plants. The mechanism of soil P dissolution through organic acids produced by P solubilizing bacteria is through a decrease in soil pH (Kumar et al., 2018).

Organic acids are able to reduce the amount of phosphate that is fixed by Fe and Al through a chelating mechanism so that P can be available to plants (Barker and Pilbeam, 2007). The use of phosphate solubilizing bacteria can consist of more than one microbe as well as the use of biofertilizers, which can be done through the use of a single inoculation or a consortium. However, it is necessary to have compatibility between two or more PSB that will be used to increase the effectiveness of the inoculant. Phosphate solubilizing bacteria consortium inoculation was able to promote better plant growth and P uptake than single inoculation. The

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use of indigenous isolates Pseudomonas fluorescens and Burkholderia cepacia was able to increase growth, yield and nutrient uptake in wheat (Minaxi et al., 2013). According to Lacobazzi (2009), the inoculum of the PSB consortium produced more organic acids which were more effective in dissolving P. Fitriatin and Nurmala (2019) showed the interaction between of Peudomonas mallei, P. cepaceae, Aspergillus niger, and Penicillium sp. were synergistic, some isolates can be said to be synergistic when no inhibition zone is found at the junction area of the two isolates.

Phosphate solubilizing bacteria as biofertilizers and their ability to dissolve phosphate and the production of phosphatase enzymes are known and are able to produce phytohormones (Fitriatin et al., 2020). Further studies have shown the ability of PSB to synthesize metabolites to chelate metals (Vassileva et al., 2010). Walpola and Yoon (2013) reported that phosphate solubilizing bacteria (Pantoea agglomerans and Burkholderia anthina) can produce IAA, ammonia, hydrogen cyanide (HCN), and siderophore and are able to increase plant growth and P uptake and resistance to disease. Based on this background, an experiment is needed to test compatibility, where the activity between isolates will be seen compared to the single isolate used as a treatment, and a bioassay experiment with phosphate solubilizing bacteria which can increase the solubility of P and rice growth (Oryza sativa L.).

2 Materials and Methods

PSB isolates used in the compatibility and bioassay test experiments were Burkholderia sp., Pseudomonas mallei, Bacillus substilis, and Bacillus megatherium. Nutrient agar (NA) media was used for the synergistic test. Pikovskaya media was used for culturing isolates. Bioassay test using modified Murphy media (CaSO4, H2O, Ca3PO4.2H2O, MgSO4.7H2O, NaCl, KCl, ZnCl2, CuSO4 .5H2O, FeSO4).

The synergistic test was conducted qualitatively with the streak method. Each isolate was scratched in contact with each other using the streak method. After 72 hours of incubation, compatible isolates if there is no inhibition zone in the area where the two isolates meet, and incompatible if there is an inhibition zone in the area where the two isolates intersect.

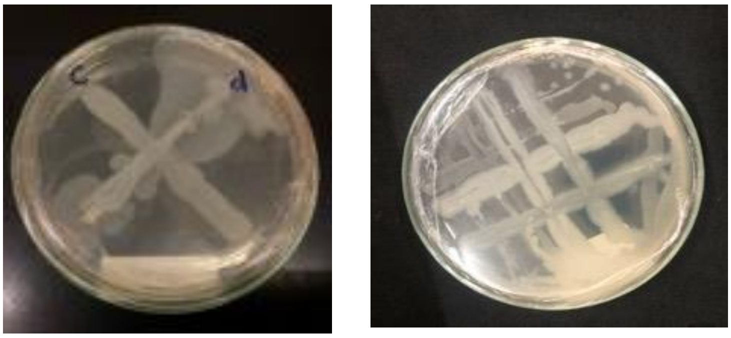
For the bioassay test, the germinated rice was planted in a 100 mL test tube filled with 95 mL of liquid Murphy media and treated with 5 mL of PSB isolates suspension (density 107 CFU mL-1). Furthermore, rice plants were maintained and observed in a greenhouse for 4 weeks. The experimental design for the bioassay was a randomized block design (RBD) with 6 treatments and repeated 5 times. The treatments were PSB isolates which consisted of (A) control, (B) Burkholderia sp.; (C) Pseudomonas mallei; (D) Bacillus substilis; (E) Bacillus megatherium; (F) mixed isolates.

3 Result and Discussion

3.1 The synergistic test

The results of the synergistic test between the phosphate solubilizing bacterial isolates, each isolate did not show any inhibition (Fig.1). There were no inhibition zones between the isolates, both isolate colonies grew well when incubated in the same place. These results showed that the four PSB isolates are compatible with each other.

When two or more species of bacteria are placed in the same petridish, there are several possible interactions that occur, namely between bacteria that are synergistic, neutral or antagonistic to one another. In general, antagonistic bacteria in the activity of one organism with another, will compete with each other for place, air, water and food materials (nutrients). These bacteria do not compete with each other but interact and synergize, and share the same source of nutrition and behave cooperatively between bacteria in their habitat, it can be said that the interactions that occur are synergistic interactions (Deng and Wang, 2016).



(a) (b)

Fig 1. The synergistic test of PSB isolates
(a) Pseudomonas mallei x Bacillus substilis; (b) mixed PSB isolates

The compatibility or synergy of two or more inoculated bacteria is a very important factor so that these bacteria can work together properly. Bacteria with the same genus or species can interact and synergize, and share the same source of nutrition. This shows the cooperative behavior between bacteria in a habitat in the form of a consortium (Bauer, et al. 2018) A form of consortium will produce products that can be used together, so that they can mutually support the growth of single isolates and others (Gosh, et al., 2016).

3.2 The Bioassay

The dry weight obtained based on the data from Table 1 showed that the application of PSB isolates has a significant effect on root dry weight, dry weight and shoot root ratio. On root dry weight, the treatment of Bacillus substilis isolate had a significant effect with a value of 0.0064 g. However, root weight does not really reflect good plant nutrient absorption when compared to root length (Griffiths and York, 2020). The absorption of P element which is immobile will increase if the contact distance between P and the root was shortened. The close contact distance with the P source in the soil can be overcome by root extension (Mohsin and Alfonzo, 1990).

Table 1. Growth of rice (at 4 weeks) as affected of PSB isolates

Treatments	**Root dry weight (g)**	**Shoot dry weight (g)**	**Shoot root ratio**
Comtrol	0,0024 a	0,0094 a	4,70 ab
Burkholderia sp.	0,0054 bc	0,018 b	3,68 a
Pseudomonas mallei	0,0038 ab	0,0168 b	4,47 ab
Bacillus substilis	0,0064 c	0,019 b	2,98 a
Bacillus megatherium	0,0048 bc	0,0174 b	4,82 ab

Mixed isolates*	0,0034 ab	0,0202 b	6,33 b

\} Mixture of Burkholderia sp., Pseudomonas mallei, Bacillus subtilis, and Bacillus megatherium isolates the average score followed by the same letter is not significantly different according to the Duncan Test at the 5% level

The application of PSB isolates increased the yield of plant dry weight. The increasing of plant dry weight was due to the activity of PSB which plays a role in providing nutrients and as a growth promoter. When the plants are in the germination period, plant growth is still supported by nutrients from the seed food reserves and hormones from the plant itself, such as auxins and gibberellins. However, the application of PSB can stimulate to help growth (Kalayu, 2019).

The root shoot ratio value which had a higher potency was owned by the treatment of mixed isolates with a value of 6.33 while the lowest shoot root ratio was owned by the treatment of Bacillus substiles with a value of 2.98. The mixed isolates showed an increase in shoot root drop ratio by 34.6%. In this case, the shoot root ratio reflected the share of the photosynthate in the growth of the plant under test. A root shoot ratio value that is more than one indicates that the growth tends to be eliminated, whereas if the shoot root ratio value is less than one, it indicates that the growth tends towards the root (Minaxi et al., 2011)

4 Conclusion

The phosphate solubilizing bacteria Burkholderia sp., Pseudomonas mallei, Bacillus substiles, and Bacillus megatherium were compatible with each other, so they can be used in mixed culture of biofertilizers. The bioassay of phosphate solubilizing bacteria isolates on rice plant showed that PSB increased plant dry weight and shoot root ratio. The application of mixed isolates had a better effect on rice plant growth than single isolates.

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