In vitro test and bioassay of selected Phosphate Solubilizing Bacteria (PSB) by using maize seedlings

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Abstract. Phosphate Solubilizing Bacteria (PSB) plays a significant role to improve phosphate availability, in order to support the growth of maize in acid soil. The experiment aimed to investigate the capability of PSB isolates in solubilizing phosphate and their compatibility with maize seedlings which were conducted in the soil biology laboratory and greenhouse at Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. In vitro test using three isolates of PSB was done to understand the synergistic character by using streak method with sodium agar medium. Subsequently, those three isolates were used for bioassay. The bioassay experiment set up as randomized factorial design, consisted of 16 treatments (control, single and consortia of three PSB isolates in two concentrations) and provided by 3 replications. The solubilized phosphate, P uptake and plant dry weight was measured at 14 days after planting. The results revealed that three PSB isolates were synergistic and did not antagonistic to each other. The isolates either in single or consortia application were able to solubilize phosphate up to 23,23 ppm and increase P uptake up to 42%. The consortia of PSB isolates has the highest capability of increasing the growth of maize seedlings.

1. Introduction
Utilization of Phosphate Solubilizing Bacteria (PSB) was carried out to increase the P nutrient to be available to plants especially in acid soil. Soil acidity is one of the most predispose limitations for agricultural practices [1]. Acid soils can cause colloidal exchange sites to be dominated by H⁺ and Al³⁺ where the dominance of those elements is harmful for plants [2][3]. There are three main land orders, most of which are classified as potential acid soils for agriculture, namely Ultisols, Inceptisols and Oxisols [4].

Ultisols are soil with a further level of soil development. In general, this soil has the potential for Al toxicity and poor organic matter content with acid to very acidic soil reaction (pH 5−3.10) [5]. This soil is also poor in nutrients, especially P and exchangeable cations such as Ca, Mg, Na, and K. Ultisols have high Al content, low cation exchange capacity, and sensitivity to erosion [6]. At pH less than 5.5, elements P will be bound by Al and Fe so that P is unavailable for plant to uptake.

Element P is needed by maize plants to store and transport photosynthetic energy to all parts of the plant which is then used for cell division, formation of maize seeds and to strengthen roots and stems [7]. P deficiency can inhibit plant growth to become stunted because Al reacts strongly to P resistance which is difficult to release in Ultisols [8]. In addition, P element deficiency causes plant growth to be
slow and weak, young leaves become dark green in color and old leaves experience purple pigmentation [9].

The use of PSB is expected to escalate the availability of P in Ultisols. PSB is a bacteria that plays a role in the process of mineralization of organic P compounds into inorganic P so that it can increase phosphate availability in the soil [10]. In line with the increase in P solubility, plant uptake can also be increased and finally can increase plant growth which is characterized by plant biomass or plant dry weight. The use of PSB can be done through a single inoculation or consortium. Inoculation of PSB consortium can improve plant growth and better P uptake compared to single inoculation [11]. Synergistic test between two or more PSB isolates is needed to increase its effectiveness. Therefore, it is necessary to investigate the synergistic effect between PSB isolates in vitro, PSB isolate compatibility with maize plants in increasing the availability of P in the soil represented by P solubility and P uptake, and the ability of PSB isolates to support the growth of maize by examining biomass/plant dry weight.

2. Materials and methods

2.1. In Vitro Test for Synergistic Effect

In Vitro test for synergistic that effect of several PSB isolates, namely J1M, J3T and J5H, were carried out in vitro on NA medium using the streak method [12] in the soil biology laboratory of Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. Pure cultures of each isolate are prepared on a sloping agar medium. To see the synergy between bacterial isolates, each isolate from sloping agar medium was scratched to each other on NA medium in petridish so that the isolates would meet. 24 hours incubation and observations were carried out for three, five and seven days after inoculation by investigating clear zones which characterizes as inhibition zones around the isolate intersection. Isolate is said to be synergistic if there is no inhibition zone in the intersection area of the two isolates, and is said to be antagonistic if there is an inhibition zone in the area where the two isolates intersect. After knowing the synergistic effect of each isolate, it was continued by testing the compatibility of PSB isolates with maize plants using a bioassay in Murphy's liquid medium.

Figure 1. Synergistic test between three isolates using the streak method

2.2. Bioassay of selected Phosphate Solubilizing Bacteria (PSB) using Maize Seedlings

The experiment was arranged as randomized factorial block design, consisted of 16 treatments (one control and seven combination of singular and consortia PSB isolates with two concentrations) provided by 3 replications. Isolates were obtained and screened from maize rhizosphere in the District of Majalengka (J1M), maize rhizosphere in the District of Tasikmalaya (J3T) and natural forest in the District of Garut (J5H), West Java. All the PSB isolates were inoculated in Pikovskaya medium and incubated in 150 rpm rotary shaker for 72 h at 28°C to obtain the population density about 10^8 CFUs mL^-1.

Before treatment, maize seeds (BISI 2) were sterilized by washing it 4 times with sterilized water then air dried. Afterwards, maize seeds were planted in a paper towel that has been moistened using
aquadest then covered with other paper towel and rolled it. Kept in dark condition for four days to grow roots the upon maize seedlings is optimum for bioassay test.

The planting of maize seedlings was carried out on sterilized test tube of 20 mm x 300 mm in size. For 5% PSB concentration, added 5 ml of bacterial suspension and 90 ml Murphy liquid medium. For 10% PSB concentration, added 10 ml of bacterial suspension and 85 ml Murphy liquid medium. Subsequently, soaking the roots of maize seedlings into Murphy liquid planting media and the top of the plant is supported by sterilized plastic and floksok pipe. Then arranged in test tube rack and stored in greenhouse of Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran.

The solubility of P analysis was carried out using Bray methods, P uptake analysis was carried out using wet destruction method and the growth of maize plants was reflected through the value of plant dry weight by measuring it quantitatively.

Figure 2. Bioassay test using maize seedlings in Murphy liquid medium

2.3. Statistical analysis
Statistical analyses were processed by SPSS 16.0 software (Information Technology Service, Universitas Padjadjaran). Data were analyzed using one-way analysis of variance (ANOVA) and the difference between treatments was tested by Duncan’s multiple range test at p ≤ 0.05.

3. Results and Discussion
3.1. Synergistic effect from In Vitro test
The results of synergistic effect from in vitro test between PSB isolates showed that all three isolates (J1M, J3T, and J5H) were synergistic and showed no antagonistic signs. Inoculation of the phosphate solvent bacterial consortium has the potential to increase the availability of phosphate needed by plants compared to one type/ singular bacteria [13]. Addition of microbial consortiums need to get special attention because this has the possibility of the growth inhibition of one of the inoculated microbes [14]. The bacterial isolates used for biological testing were tested for compatibility to ensure the absence of antagonistic properties between bacteria. If there is a zone of inhibition between the scratched isolates, the isolate has antagonistic properties.

Table 1. Synergistic effect of combination selected Phosphate Solubilizing Bacteria isolate

| Isolate combination | 3 days after inoculation | 5 days after inoculation | 7 days after inoculation |
|---------------------|--------------------------|--------------------------|--------------------------|
| J1M                 | +                        | +                        | +                        |
| J3T                 | +                        | +                        | +                        |
| J5H                 | +                        | +                        | +                        |
(+) Grow
(+se) Grow synergistically
# all the results were noted with triplicate experiments.

![Image]

**Figure 3.** The synergy of J1M + J3T + J5H isolates using the streak method on NA medium

### 3.2. Solubility of P in the culture medium

Solubility of P in each PSB isolate both single and consortium and at concentrations of 5% and 10% was observed. The results of statistical analysis showed that the two treatment factors did not give a significant effect on phosphate solubility in Murphy's medium as the planting medium, but the average solubility value of P on PSB consortium treatment was greater than the control (without PSB) and single PSB isolates. The use of 10% PSB isolates also showed an average value of P solubility that was greater than the concentration of the use of 5% PSB isolates because the population in 10% concentration is more than in 5% concentration. The condition of medium culture nutrition influences bacterial growth and solubility of P [15]. Research conducted by Bras and Nahas [16] showed the results of similar studies that the use of microbial consortiums, *Burkholderia cepacea* and *Aspergillus niger* provide more phosphate in the medium culture.

### Table 2. Solubility of P in Bioassay Test due to Giving Various PSB Isolates and Concentrations

| Treatments | Solubility of P (ppm) |
|------------|-----------------------|
| b0 = control | 20,33 |
| b1 = J1M | 22,47 |
| b2 = J3T | 21,00 |
| b3 = J5H | 22,02 |
| b4 = J1M + J3T | 23,23 |
| b5 = J1M + J5H | 21,51 |
| b6 = J3T + J5H | 22,82 |
| b7 = J1M + J3T + J5H | 21,22 |
| k1 = 5% | 21,49 |
| k2 = 10% | 22,15 |

Figures followed by the same notation are not significantly different based on Duncan Multiple Range Test at 5% real level.
3.3. P uptake and growth promotion of maize seedlings

The treatment of PSB isolates which were applied singular or consortium with PSB concentration showed the effect of interaction on P uptake of maize seedlings. The treatment of single and consortium gave different responses to P uptake. Isolate J1M at 10% concentration gave the highest P uptake value of maize seedlings compared to other treatments. It was seen that there was a tendency to increase P uptake capacity of plants in treatments that experienced an increase in PSB concentration. This condition can be occur due to a higher PSB population so that P uptake is also more than at lower PSB concentrations.

PSB increases P availability through various methods including acidification of tricalcium phosphate to $H_2PO_4^-$ and $HPO_4^{2-}$, then by chelation and exchange of ligand phosphate by anionic organic acids against Al, Fe, and Ca, and fills up the soil colloidal uptake by organic acids and P-organic mineralization by phosphatase, and phytase produces phosphate [17]. In the roots of soybean plants the condition of P deficiency produces oxalate, malate and citrate to absorb P from the soil [18]. The results of similar studies conducted by Zapata and Roy [19] also showed that PSB inoculation increased P uptake of maize plants by 70-75 mg P-1 plants, P levels to 0.28%, and increased RAE to 145-150% (height > 90).

Table 3. P uptake of Bioassay Test due to the Giving of Various PSB Isolates and Concentrations

| Phosphate Solubilizing Bacteria | Concentration of PSB | 5%          | 10%          |
|---------------------------------|----------------------|-------------|--------------|
| Control                         |                      | 3.76 h      | 2.45 a       |
|                                 |                      | A           | B            |
| J1M                             |                      | 2.66 b      | 3.49 h       |
|                                 |                      | A           | B            |
| J3T                             |                      | 2.67 c      | 3.14 e       |
|                                 |                      | A           | B            |
| J5H                             |                      | 3.46 g      | 3.18 f       |
|                                 |                      | B           | A            |
| J1M + J3T                       |                      | 3.13 e      | 2.76 c       |
|                                 |                      | B           | A            |
| J1M + J5H                       |                      | 3.28 f      | 3.33 g       |
|                                 |                      | A           | A            |
| J3T + J5H                       |                      | 2.80 d      | 2.94 d       |
|                                 |                      | A           | B            |
| J1M + J3T + J5H                 |                      | 2.36 a      | 2.56 b       |
|                                 |                      | A           | B            |

Figures followed by the same notation are not significantly different based on Duncan Multiple Range Test at 5% real level. Small letters are read vertically, uppercase letters are read horizontally.

Nutrients that were absorbed by plants contribute to an increase in plant dry weight [20]. Based on the results of the analysis of variance, showed that there was an interaction effect between single PSB isolates and the consortium with PSB concentrations on plant dry weight (Table 4.). The use of a single PSB isolate and a consortium at 10% PSB concentration did not show a significant difference in plant dry weight, this is indicated by the same Duncan test notation results in each treatment, but at 5% PSB concentration it can be seen that the use of single PSB isolates is not significantly different compared to controls (without PSB administration) while the use of consortium PSB isolates (J1M + J5H and J3T + J5H) showed higher dry weight than the control and treatment J1M + J3T showed the highest dry weight compared to other treatments. This means that the use of consortium PSB isolates can increase plant dry weight compared to the use of single PSB isolates and controls (without PSB administration). In the
treatment of consortium PSB (J1M + J3T) 5% showed a significantly higher dry weight of plants compared to the use of 10% PSB (J1M + J3T) consortium.

Increased dry weight of plants due to an increase in the rate of germination due to the activity of two or more microbial strains that can work as plant growth boosters [21]. Although the nutrients obtained at the germination stage come from seed food reserves and plant hormones themselves such as auxin and giberelin, rhizobacteria can work as stimulants. This significant dry weight increase is due to a positive interaction between two or more rhizobacteria.

**Table 4. Plant Dry Weight of Bioassay Test due to Giving Various PSB Isolates and Concentrations**

| Phosphate Solubilizing Bacteria | Concentration of PSB | 5%  | 10% |
|----------------------------------|----------------------|-----|-----|
| Control                          |                      | 0.15 a | 0.15 a |
| J1M                              |                      | 0.14 a | 0.17 a |
| J3T                              |                      | 0.16 a | 0.15 a |
| J5H                              |                      | 0.18 a | 0.17 a |
| J1M + J3T                        |                      | 3.28 c | 0.14 a |
| J1M + J5H                        |                      | 1.70 b | 0.19 a |
| J3T + J5H                        |                      | 1.67 b | 0.16 a |
| J1M + J3T + J5H                  |                      | 0.11 a | 0.16 a |

Figures followed by the same notation are not significantly different based on Duncan Multiple Range Test at 5% real level. Small letters are read vertically, uppercase letters are read horizontally.

4. Conclusion

Three PSB isolates (J1M, J3T and J5H) were synergistic to each other and did not show any antagonistic responses. The isolates either in singular or consortia application were able to solubilize phosphate up to 23.23 ppm and to increase P uptake up to 42%. The consortia of three isolates application has the highest ability to increase the growth of maize seedlings reflected in higher plant dry weight compared to singular application.

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