Study on Phosphate Solubilizing Bacteria from Banana Pseudostem IMO as Biofertilizer on System of Rice Intensification

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Abstract. The two rice farmer groups namely “Asemjajar” and “Makmur” in Wonorejo District-Pasuruan, Indonesia make a banana pseudostem IMO which is used to replace synthetic P fertilizer. This study aimed to: 1. determine if the banana pseudostem IMO contains P elements that can replace synthetic phosphate fertilizers; 2. Determined whether the banana pseudostem IMO contains phosphate solubilizing bacteria and identified it 3. verified the effect of bacterial inoculant application which was found to the rice growth and production in System of Rice Intensification. The greenhouse study was a simple experiment using a completely randomized design with 4 treatments i.e. A: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer; B: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + banana pseudostem IMO; C: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + bacterial inoculants; D: 1 dose of synthetic fertilizer. The results showed that banana pseudostem IMO contains only 0.025% P2O5. Morphological and biochemical identification results showed that the phosphate solubilizing isolates which were found in banana pseudostem IMO was Serratia marcescens. The greenhouse test results showed that the application of S. marcescens inoculant had a significant effect on the number of tillers at the age of 4-6 weeks after transplanting, increased the weight of 1000 seeds, lowered the empty grains percentage and speeded up the harvest time.

Keyword: Banana pseudostem IMO, Phosphate Solubilizing Bacteria, Biofertilizer, Serratia marcescens, SRI

1. Introduction
Phosphorus, one of the most critical elements for the growth of plants, is not a renewable resource and its future use in agriculture will be impacted by declining availability and increased cost (Hameeda, Harini, Wani and Reddy, 2008). Chemical fertilizer is one of the major sources which provide phosphorus in plants, but the high costs of chemical fertilizers, and environmental problems that caused by their overuse have led the researchers seek ways to reduce chemical fertilizers (Vahed, Shahinrokhshar, & Heydarnezhad, 2012)

Rice is the most important staple food in Indonesia, and chemical fertilizer is the most important input required for intensive rice cultivation. Many serious problems about fertilization have encouraged rice farmers in Pasuruan East Java to find an alternative fertilizer that can replace synthetic fertilizers. They make a liquid fertilizer from fermented banana pseudostem waste, called banana pseudostem IMO fertilizer, and then it is used to replace synthetic phosphate fertilizer. The result of nutritional analysis shows that the phosphate content in banana pseudostem IMO fertilizers is too low to substitute the synthetic phosphate fertilizers. Thus, it is hypothesized that there are other
functions of banana pseudostem IMO fertilizer which have a significant impact on improving rice production.

A considerable number of bacterial species are able to exert a beneficial effect upon plant growth. Some of them would carry out the functions as a provider of nutrients for the plants, so they are called as biofertilizers, while others increase the resistance of plants against disease, and it is called as biopesticide. ‘Biofertilizer’ is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003; Raja, 2013; Khan, Jameel & Khan, 2018).

Cultivated crops and its parts may be considered as complex ecosystems where different niches are inhabited by a broad diversity of bacteria. When the crops have been harvested and the waste is used to make IMO fertilizer, the bacteria that live in it will be still carried away. Some of these bacteria may be able to have a role as a phosphate solubilizer. This research was conducted to reveal the role of banana pseudostem IMO fertilizers as the substitution of inorganic phosphate fertilizer.

This study aims to: 1. determine if the banana pseudostem IMO fertilizer contains P elements that can replace synthetic phosphate fertilizers; 2. determine whether the banana pseudostem IMO fertilizer contains phosphate solubilizing bacteria. 3. identify phosphate solubilizing bacteria from banana pseudostem IMO fertilizer. 4. verify the effect of bacterial inoculant application which was found on the rice growth and production in SRI. The hypothesis are: 1. The banana pseudostem IMO fertilizer contains elements of P and phosphate solubilizing bacteria; 2. The application of phosphate solubilizing bacteria inoculant gives the effect on the rice growth and production in SRI.

2. Material and Methods
2.1. Material and Location
A laboratory and pot experiment was conducted in Microbiology Laboratory and glass house of Widyagama Malang University, Malang, Indonesia. Testing is carried out on phosphate content in banana pseudostem IMO, bacteria isolation, morphology and biochemical identification. All of the experiment was conducted from December 2018 to April 2019.

2.2. Methods
One kind of banana pseudostem IMO fertilizer was made of the waste of banana pseudostem + sugar + rice waste water as a study material. A series of dilutions $10^{-1}$ to $10^{-7}$ were made of 10 ml of banana pseudostem IMO material that had been prepared. 0.2 ml of each dilution was taken with a sterile pipette and poured into a sterile petri dish, then poured with the solid medium Pikovskaya which was composed of 5g Ca$_3$(PO$_4$)$_2$, 0.5g (NH$_4$)$_2$SO$_4$, 0.2g NaCl, 0.1g MgSO$_4$.7H$_2$O, 0.2g KCl, 10g Glucose, 0.5 g of yeast extract, 20 grams agarose, a little bit of MnSO$_4$ and FeSO$_4$, and distilled water 1000 ml.

Colonies which were surrounded by halozone, showed that the bacteria in concerned can solubilize phosphate. Furthermore, the phosphate solubilizing bacteria which was found was then purified and grown in NA medium for preservation and identification.

The pot experiment was conducted to verify the effect of phosphate solubilizing bacterial inoculants applications on rice growth and production in the System of Rice Intensification. Simple experiment using a completely randomized design with 4 treatments i.e. A: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer; B: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + banana pseudostem IMO; C: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + phosphate solubilizer bacteria inoculants; D: 1 dose of synthetic fertilizers (400Kg Urea + 200Kg Phonska + 100Kg SP-36 + 100Kg ZA). Each treatment was repeated 5 times. The observation was conducted on plant height, number of tillers, number of productive tillers, shoot dry weight, root dry weight, number of grains per panicle, grain number per pot, weight of 1000 seeds and the harvest time.

2.3. Data Analysis
Analysis of Variance and T-test were used in data processing.
3. Results and Discussion

3.1. Laboratory Experiment

The laboratory analysis found that the Phosphate content in banana pseudostem IMO was 0.025% (Table 1). Based on the IMO concentrations which commonly used by the farmers is 3 L/spray tank with 5000L dose per hectare, that the total P$_2$O$_5$ received by plants is 225 grams per hectare, whereas for reached rice production 7 tons / ha required elements of P as much as 18.2 Kg (Abdulrachman &Sembiring, 2006). It can be concluded that the P element contained by banana pseudostem IMO cannot meet the needs of the P rice plants.

Table 1. Content Analysis Results of Banana Pseudostem IMO

| Analysis Component | Results       |
|--------------------|--------------|
| pH                 | 3.8          |
| C-Organic          | 1.38         |
| N-Total (%)        | 0.25         |
| C/N                | 5            |
| Organic Matter (%) | 2.39         |
| P$_2$O$_5$ (%)     | 0.025        |
| K$_2$O (%)         | 0.254        |
| NaO (%)            | 0.174        |
| CaO (%)            | 0.185        |
| MgO (%)            | 0.132        |
| S SO$_4$ (%)       | 0.033        |
| Fe (ppm)           | 180,00       |
| Zn (ppm)           | 3.00         |

One isolate of phosphate solubilizing bacteria has been found from banana pseudostem IMO fertilizer. Pathogenicity test results showed that this bacteria is not a pathogen for the rice plants or for the banana plants. So if it is proven to be able to solubilize P, then it must be able to be used as a biofertilizer agent for the rice plants. The morphological test result showed that the isolate is Gram negative bacteria and the single colony is round, slimy, convex, turns red on NA medium and turns white on Pikovskaya medium (Figure 1).

Figure 1. The single colony of *Serratia marcescens*. Cultured on Nutrient Agar medium (left), cultured on Pikovskaya medium (right). The photo was taken with the help of electron microscope.

The biochemical characterization results are shown in the table 2.

Table 2. Morphological and Biochemical Test Results of Phosphate Solubilizing Isolates

| Characterization     | Results   |
|----------------------|-----------|
| Gram (Staining & KOH 3%) | Negative  |
| Cell shape           | Rod       |
| Catalase activity    | +         |
| Glucose              | +         |
| Lactose              | -         |
| Sucrose              | +         |
Based on the test results above, the red-bacterial isolate which has the ability to solubilize phosphate, has strong similarities with the *Serratia marcescens*. An important feature of bacterial species *Serratia marcescens* is the ability to produce gelatinase enzyme, therefore almost certainly that the bacterial isolates which recovered from IMO-1 is *Serratia marcescens*.

Study by Hameeda *et al.* (2008) and by Mohamed, Farag & Youssef (2018) proved that *Serratia marcescens* has the ability to solubilize phosphate. Several soil bacteria, particularly those belonging to phosphate solubilizing bacteria (phosphobacteria/PSB), posses the ability to solubilize insoluble inorganic phosphate and make it available to plants. The solubilization effect is generally due to the production of organic acids by these organisms.

![Figure 2](image2.png)

Figure 2. The streak culture of *Serratia marcescens* on Nutrient Agar medium (left), the Pikovskaya medium before being inoculated with bacterial inoculant (middle), the Pikovskaya medium after being inoculated (right).

From Figure 1 above, it can be seen that there is a difference between the pikovskaya fresh media contains insoluble phosphate (left) with the inoculated pikovskaya medium (middle). The inoculated one becomes clear.

The phosphate solubilizing ability had been already apparent at 24 hours after inoculation, with the appearance of halozone around the colonies. The diameter of the halozone became wider with the increasing of the colony age (Figure 2).

![Figure 3](image3.png)

Figure 3. The halozone which was formed by the *Serratia marcescens* bacterial colony in 24 hours after inoculation (left) and 48 hours after inoculation (right)

Pot experiment shows that the application of bacteria pure inoculums gives significantly different results in the number of tillers at the age of 4 – 6 weeks after transplanting compared to other treatments (Table 3). Several previous studies indicate that phosphate solubilizing bacteria also have the ability to produce growth hormone.

| TREATMENTS | TOTAL OF TILLERS |
|------------|------------------|
|            | **TOTAL OF TILLERS** |
| 4 WAT      | 5 WAT  | 6 WAT  | 7 WAT  | 8 WAT  | HAR VEST |
| 4 WAT      | 5 WAT  | 6 WAT  | 7 WAT  | 8 WAT  | HAR VEST |
A: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer

6.4 a

B: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + Banana pseudostem IMO

6.6 a

C: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + PSB inoculant

8.6 c

D: 1 dose of synthetic fertilizer

7.4 b

BNT 5%

0.63

WAT refers to weeks after transplanting
ns refers to non significant effect of the treatment
IMO is the abbreviation of Indigenous Microorganisms
PSB refers to Phosphate Solubilizing Bacteria
The numbers in the same column which are marked by the same letters indicate that the treatment effect were not significantly different in the T-test 5%

The study which was conducted by Panhwar, Othman, Rahman, Meon, & Ismail (2012) showed that there was a close association between PSB strains and the rice plants. It can prove that local isolated PSB strains were able to colonize the root interior and root surface of rice at different P fertilizer rates. The highest bacterial population was found in the rhizosphere. Beneficial association of the inoculated strains produced highest plant biomass. Bacillus sp. PSB16 formed a natural association with aerobic rice leading to improved plant growth. In this case, the rhizosphere plays a major role in PSB activity due to the root exudates which supply carbon compounds as a major source of energy to the bacteria.

The highest weight of 1000 seeds is achieved by C treatment, followed by B, A and D. Actually, synthetic fertilizer which was given to treatment D is the greatest, twice that of treatments A, B and C, but because of the absence of additional organic matter which was given, the fertilization becomes ineffective. The weight of 1000 seeds of D treatment was equal to A treatment that received only half of dose, but there is the addition of organic matter such as manure fertilizer. This suggests that the application of phosphate solubilizing bacterial inoculants and manure fertilizer can meet the needs of the plant to be able to produce the most perfect grain quality compared to all other treatments (Table 4). In this case, the addition of banana pseudostem IMO also gives significant effect on the increase in the weight of 1000 seeds. Alleged that the addition of banana IMO has increased the diversity of microbes in the area of plant roots. In accordance with the opinion of Zhen, Liu, Wang, Guo, Meng, Ding, Wu, and Jiang (2014) that the application of compost tends to increase organic matter and available phosphorus. Moreover, compost directly affected microbial community in soils.

Table 4. The Effect of the Treatments towards the weight of 1000 seeds (gram), percentage of empty grains and the harvest age

| Fertilizers treatment                                             | Weight of 1000 seeds | % of empty grains | Harvest age |
|-----------------------------------------------------------------|---------------------|-------------------|-------------|
| A: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer | 26.86 a             | 3.74 b            | 108.6 c     |
| B: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + banana pseudostem IMO | 27.27 b             | 2.35 a            | 107.0 b     |
| C: ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + PSB inoculant | 27.51 c             | 2.12 a            | 106.4 a     |
| D: 1 dose of synthetic fertilizers                              | 26.81 a             | 3.99 b            | 112.6 d     |
| T-test 5%                                                        | 0.15                | 1.29              | 0.34        |

IMO is the abbreviation of Mikroorganisme Lokal that mean Indigenous Microorganisms
- PSB refers to Phosphate Solubilizing Bacteria
- The numbers in the same column which are marked by the same letters indicate that the treatment effects were not significantly different in the T-test 5%

The average age of harvest due to the treatment shown in Table 4. It can be seen that the longest harvest age is indicated by the D treatment, while the shortest harvesting is achieved by C treatment. As it is known that the elements of P can speed up the harvest time, and vice versa deficiency of these components will be delaying the maturity. Abdulrachman & Sembiring (2006) stated that the P element has the function of accelerating flowering and the ripening of grain. Therefore, based on the results of this study, the treatment of ½ dose of manure fertilizer + ½ dose of synthetic fertilizer + PSB inoculant give the best effect on the parameters of harvest age and weight of 1000 seeds in the System of Rice Intensification.

According to Hameeda et al. (2008), seed coating treatment with Serratia marcescens inoculum on maize have given the enhancement of root length, plumule length, plant weight, percentage of germination, and vigor index. The P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant P nutrition. Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PGPB is advantageous in the sustainable agricultural practices.

Actually the application of IMO fertilizer on paddy field aimed more to increase soil biodiversity rather than to add plant nutrition because of the low nutrient content in IMO fertilizer. Based on biological theory, the higher the biodiversity, the more stable the environment. And, the more stable the environment, the better quality of life will be given for all organisms in it. In addition, the indigenous organism will be more adaptable to their extreme conditions in its environment, compared to the foreign organisms. Thus, application of IMO fertilizer on the paddy field has economical and biological advantages as well as environmental safety.

Phosphorus (P) is an essential element classified as a macronutrient, because of the relatively large amounts of P required by plants. Phosphorus is one of the three nutrients generally added to soils in fertilizer. Soil may contain several hundred to several thousand pounds of phosphate per acre. However, much of the phosphate in soil is not available to growing plants. Phosphate in the soil solution P pool is immediately available but the amount is very small in comparison to the total P in soil. The active P pool is phosphorus that can be released into solution but is generally small in comparison to the fixed P (Busman Lamb, Randall, Rehm, & Schmitt, 2009)

Release of P by Phosphate Solubilizing Bacteria from insoluble and fixed / adsorbed forms is an important aspect regarding P availability in soil. There are strong evidences that soil bacteria are capable of transforming soil P to available forms to plants (Khan, Jilani, Akhtar, Naqvi, & Rasheed, 2009).

In the rhizosphere, the PSB’s number increased significantly up to 5 to 20 fold when compared with soil outside of the rhizosphere. The PSB propagates very quickly, and are able to use amino-acids and water-soluble sugars and are resistant to some antibiotics. Thus, to circumvent P deficiency, PSBs could be exploited as biofertilizer to increase the availability of accumulated phosphates in an environmentally friendly and sustainable manner.

4. Conclusion
- Banana pseudostem IMO substances is not a fertilizer, because the nutrient content in it is too low.
- 1 isolate phosphate solubilizing bacteria was found from banana pseudostem which was identified as Serratia marcescens. It has significant ability to solubilize phosphate on Pikovskaya medium.
- The application of bacterial inoculant Serratia marcescens has significant effects on System of Rice Intensification in the glass house trial. The variables which were affected are the total of tillers in 4-6 weeks after transplanting, weight of 1000 grains, the percentage of empty grain, and the length of the harvest time.
**Acknowledgement**

This research was supported/partially supported by [Name of Foundation, Grant maker, Donor]. We thank our colleagues from [Name of the supporting institution] who provided insight and expertise that greatly assisted the research, although they may not agree with all of the interpretations/conclusions of this paper.

**References**

[1] Abdulrachman, S., H. Sembiring. 2006. Penentuan Takaran Pupuk Fosfat untuk Tanaman Padi Sawah. ejurnal.litbang.pertanian.go.id: 1(1)

[2] Bushman, L., J. Lamb, G. Randall, G. Rehm and M. Schmitt. 2009. The Nature of Phosphorous in Soils. Phosphorous in Agriculture Environment. University of Minnesota

[3] Dobermann, A and T. H. Fairhurst, 2000. Rice. Nutrient Disorders & Nutrient Management. 1st Ed. Oxford Graphic Printers Pte Ltd.

[4] Hameeda, B., G. Harini, OP. Rupela, SP. Wani and G. Reddy. 2008. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Res.:163(2):234-42

[5] Khan, A. A., G. Jilani, M. S. Akhtar, S.M.S. Naqvi, m. Rasheed. 2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. J. Agric. Biol. Sci. 1(1): 48-58

[6] Khan, N.T., N. Jameel, M.J. Khan. 2018. Microbes as Biofertilizers. OAJ of Biomed Eng and Biosci

[7] Mohamed, E. A. H., A. G. Farag, S. A. Youssef, 2018. Journal of Environmental Protection 9, 266-277

[8] Panhwar, QA., R. Othman, ZA. Rahman, S. Meon, MR. Ismail. 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. African Journal of Biotechnology: 11(11)

[9] Vahed, H.S., P. Shahinroksar and F. Heydarneshad. 2012. Performance of Phosphate Solubilizing Bacteria for Improving Growth and Yield of Rice (Oryza sativa L.) in the Presence of Phosphorus Fertilizer. Intl J Agri Crop Sci. 4(17): 1228-1232

[10] Vessey, J. K., 2003. Plant Growth Promoting Rhizobacteria as Biofertilizer, Plant Soil 255:571-586

[11] Zhen, Z., H. Liu, N. Wang, L. Guo, J. Meng, N. Ding, G. Wu, G. Jiang. 2014. Effects of Manure Compost Application on Soil Microbial Community Diversity and Soil Microenvironments in a Temperate Cropland in China. Plos/On