Phosphate-solubilizing bacterial activity and their effect to rice growth in acid sulphate soil

Y Lestari1 and W Annisa

Indonesian Swampland Agriculture Research Institute (ISARI), Banjarbaru, Indonesia

1Email: yulibalitra70@yahoo.com

Abstract. Efficiency of P fertilization in acid sulphate soil for rice cultivation can be improved by phosphate solubilizing bacteria (PSB) application. This study aims to observe the capability of PSB dissolved P insoluble and their effects on rice growth in acid sulphate soils at different level of P-fertilizer. PSB isolates namely P17.4.1, P17.4.2, P21.4.1 were observed their ability to dissolve tricalcium phosphate, aluminum phosphate and rock phosphate in liquid pikovskaya medium. The three PSB isolates were also examined for their effects on rice growth at different level of P-fertilizer (without fertilizer, 30 kg.ha\(^{-1}\), 45 kg.ha\(^{-1}\) and 60 kg.ha\(^{-1}\) P\(_2\)O\(_5\)) in pot experiment. The result showed that PSB were observed able to dissolve tricalcium phosphate and rock phosphate but were unable to dissolve aluminium phosphate. The pot experiment showed that application of PSB and their interaction with P-fertilizer is not significantly influenced rice plant growth. Rice plant height and shoot dry weight are significantly influenced by application of P-fertilizer. The average of plant height in application P-fertilizer 30 kg.ha\(^{-1}\), 45 kg.ha\(^{-1}\) and 60 kg.ha\(^{-1}\) P\(_2\)O\(_5\) increased 4.19 cm; 3.63 cm and 3.39 cm respectively compared with control. The shoot dry weight given 60 kg.ha\(^{-1}\) P\(_2\)O\(_5\) increased by 52.13%.

1. Introduction

Acid sulphate soils are widespread in the world including Indonesia. In Indonesia, the area of acid sulphate soil is 6.1 Mha (1), spread over 4 major islands, namely Sumatra, Kalimantan, Sulawesi and Papua (2). The soils are characteristic by the presence of pyrite layer or jarosite (3).

Utilization of acid sulphate soil is one of the strategies to increase rice production to maintain food security. However, this soil does not produce optimum until it has ameliorant material. The main characteristic of acid sulphate soil causing low rice production is high soil acidity from pyrite oxidation. High soil acidity results in stunted growth of rice crops, increased availability of micro elements such as Fe and Al and decreased macro nutrients such as phosphorus. The highest of P availability at pH 6.5. At low pH, soil-P and P fertilizer will be fixed by iron oxide and Al, then precipitated as Al-P or Fe-P. P deficiency causes the cell division to be disrupted so that plant growth is reduced (4).

The effort that can be done to increase the solubility of P in the soil and the efficiency of fertilizing P is application of phosphate solubilizing microorganisms. The use of P solubilizing microorganisms biofertilizers has many advantages, including energy saving, environment friendly, increase P-soluble P that is absorbed, prevents the absorption of P fertilizer by absorbent elements and reduces the toxicity of Al\(^{3+}\), Fe\(^{3+}\), and Mn\(^{2+}\) to plants on acid soils (5). These bacteria also increase soil pH due to the production of polysaccharides resulting in precipitated Al (6). Therefore, using of phosphate
solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield (7). Increasing of plant growth due to application of PSB as biofertilizer is also caused by the fitohormone produced. The aim of these study were to: (i) Determine the ability of PSB to dissolve P insoluble, (ii) Observe the effect of PSB application on rice growth in acid sulphate soil at different P fertilizer level.

2. Materials and Methods

2.1. Determination of PSB’s ability to dissolve insoluble-P in liquid medium
The ability of PSB to dissolve P-insoluble was carried out at the Soil and Plant Laboratory, ISARI, Banjarbaru in May to July 2017. PSB is a collection of ISARI. The insoluble-P source used for examining are tricansium phosphate (Ca-P), aluminum phosphate (Al-P), and rock phosphate. The experiment were arranged by complete randomized design with 3 replications. The treatment was inoculation of PSB isolate (without PSB, P17.4.1, P17.4.2, P21.4.1). The prior isolate was cultured in Pikovskaya liquid medium containing calcium phosphate (Ca-P), Aluminum phosphate (Al-P), and rock phosphate on a 75 rpm speed shaker for 14 days at room temperature. The observed parameters are soluble-P and medium pH. The soluble-P and medium pH were measured by P-Bray methode and glass electrode pH meter respectively. The solubility of P by PSB determined based on the increase in P solubility of the inoculated treatment.

2.2. The effect of PSB application on rice growth at acid sulphate soil at different level of P-fertilizer
A pot experiment to determine the effect of PSB applications on rice growth in acid sulphate soils at different P levels was carried out in the greenhouse of the Swamp Land Agricultural Research Institute, Banjarbaru in July to November 2017. The experiment used potential acid sulphate soil was taken from the Belandean experimental station, Alalak Village, Mandastana District, Barito Kuala Regency, South Kalimantan on S 03’16’90,5◦; E114’60’39,5◦. This experiment used a completely randomized design arranged in factorial with 3 replications. First factor is the PSB isolate, namely (i) without PSB, (ii) P17.4.1, (iii) P17.4.2, (iv) P21.4.1. Second factor is the level of P fertilizer (TSP): (a) without P fertilizer, (b) 30 kg.ha⁻¹ P2O5, (c) 45 kg.ha⁻¹ P2O5 and (d) 60 kg.ha⁻¹ P2O5.

The acid sulphate soil as much as 15 kg was put in a pot experiment and given of lime at a dose of 2 t.ha⁻¹. PSB application and planting of Inpara 2 rice seedlings are conducted 1 week and 2 weeks after liming respectively. Fertilizing of P according to treatment is done at the time of planting. Basic fertilizers such as urea 226 kg.ha⁻¹ and KCl 150 kg.ha⁻¹ were applied when the plant was 1 week and 4 weeks old. Maintenance included watering using local well water and intensive eradication of pests and diseases. Plants were maintained until the maximum vegetative phase, harvested at 8 weeks after planting (WAP). Characteristics of lime, urea, TSP, and KCl are presented in Table 1.

| Chemical properties | Dolomit | Urea | KCl | TSP |
|---------------------|---------|------|-----|-----|
| Ca (%) | 22.98 | | | |
| CaO (%) | 32.17 | | | |
| N (%) | | 39.76 | | |
| K2O (%) | | | 43.94 | |
| P2O5 | | | | 33.61 |
| Mg (%) | | | | 8.69 |

Observations of acid sulphate soils were made at the time before planting including: pH (H2O), C-organic, N-total, P-available, P-total, K-available, Al-available and Fe. Observation on plant growth at
maximum vegetative phase (end of the experiment) in terms of height, number of tiller and shoot dry weight.

Analysis of pH used a glass electrode pH meter HORIBA Model. Analysis of N-total by the Kjeldahl method used H2SO4 wet ashing and then titrated. Analysis of organic-C by ashing. Available-P was analyzed by P-bray methods using Spectrophotometer Model Spectronic 20 (λ = 889 nm). Analysis Al3+ by titration used KCl 1M extractor. Iron was extracted using NH4OAc pH7 and analyzed using AAS (Atomic Absorption Spectrometry ) GBC Model 933 plus.

The data obtained in this experiment were analyzed statistically using SAS software Portable 9.1.3. Analysis of varian was done to determine the treatment difference. The duncan multiple range test (DMRT) was used to compare treatment means at P<0.01 or P<0.05.

3. Result

3.1 Ability of PSB to dissolve insoluble-P in liquid medium

Three isolates of PSB were examined on Pikovskaya medium, 17P4.1, 17P4.2 and 22P4.1 capable of dissolving Ca3PO4 and rock phosphate. Ability of PSB to dissolve insoluble phosphates such as Ca3PO4, AlPO4 and rock phosphate was showed in table 2. Table 2 showed that the inoculation of phosphate solubilizing bacteria has a positive effect on Ca3PO4 and rock phosphate solubility. Concentrations of available-P on Pikovskaya medium with Ca3PO4 and rock phosphate inoculated by PSB were higher than controls. The highest of P-solubility pada Pikovskaya with Ca3PO4 was 70,60 ppm PO4 due to inoculate by isolates 17P4.2. It is not significantly different compared to isolate 17P4.1, otherwise significantly different to isolate 21P4.1. According to (8), the ability of phosphate solubilizing microorganisms depending on the type of microbes.

Rock phosphate (natural phosphate) is one fertilizer that contains high P and Ca that is slow release, dissolves in acidic conditions and is suitable for acid soils (9). Application of PSB can increase rock-phosphate solubility (Table 2). Based on the statistical analysis shows that 21P4.1 isolates have the highest ability to dissolve rock-phosphate which is significantly different from isolate 17P4.2 but not different from isolate 17P4.1. According to (10), the process of dissolving natural phosphate on land is as follows:

\[
\text{Ca}_{10}\text{a-hNaaMgb} (\text{PO}_4) \text{6-xF}_2 + 0.4x + 12\text{H}^+ + 10\text{Ca}^{2+} + (6-x) \text{H}_2\text{PO}_4^- + x\text{CO}_2 + (2 + 0.4x) \text{F} + x\text{H}_2\text{O}
\]

The above equation shows that to dissolve 1 mole of natural phosphate requires 12 H^+.

Table 2 showed that the inoculation of PSB to Pikovskaya medium containing AlPO4 had an effect on decreasing soluble-P concentration compared to control. This means that the PSB isolates examined were unable to dissolve AlPO4. The decrease in soluble-P concentration is caused by phosphate solubilizing bacterial isolates utilizing soluble-P.

| Isolate type | Ca3PO4 | AlPO4 | Rock phosphate |
|--------------|---------|-------|----------------|
| Control      | 7,646 c | 6,67 a | 2,83 b         |
| 17P4.1       | 67,26 ab| 2,70 b | 38,09 a        |
| 17P4.2       | 70,60 a | 2,02 c | 12,07 b        |
| 21P4.1       | 55,62 b | 1,85 c | 44,46 a        |

Description: **Numbers in 1 column followed by the same letters do not differ according to Duncan's test with a confidence level of 99%
3.2. Changes of the medium pH due to inoculation of PSB

According to (11), that PSB isolates in their metabolism are able to excrete a number of organic acids which can affect the pH of the medium. The increase in organic acids is followed by a decrease in pH. This pH change plays an important role in phosphate solubility. The results of this study indicate that the PSB isolates examined were able to dissolve tricalcium phosphate, but did not decrease the pH of the medium (Table 3) showed that phosphate solubility phosphate by isolates examined not because of organic acid activity, but due to the activity of the enzyme phosphatase excreted by PSB

Table 2 showed that inoculation of PSB isolates to the Pikovskaya medium with AlPO₄ caused significantly decreased the medium pH, but there is a decrease in dissolved PO₄. It might be that the amount of dissolved PO₄ produced is utilized by the PSB growth.

Table 3. Effect of PSB inoculation to Pikovskaya medium pH with insoluble phosphate Ca₃PO₄, AlPO₄ and rock phosphate.

| Isolate type | Ca₃PO₄ | AlPO₄ | Rock fosfat** |
|--------------|--------|-------|--------------|
| Control      | 5.66 a | 5.12 a| 7.27 a       |
| 17P4.1       | 5.67 a | 2.91 b| 7.37 a       |
| 17P4.2       | 5.81 a | 2.74 b| 4.92 c       |
| 21P4.1       | 5.70 a | 2.96 b| 6.09 b       |

Description: ** Numbers in 1 column followed by the same letters do not differ according to Duncan's test with a confidence level of 99%

Inoculation of PSB on Pikovskaya medium containing rock phosphate caused very significant effect on the medium pH. The pH of the medium inoculated with PSB 17P4.1 isolates was not significantly different compared to the control, whereas those inoculated 17P4.2 and 21P4.1 were significantly different. If observer further, showed that the pH decrease value is not related to the ability to dissolve rock phosphate. According to (12), the dissolution of phosphate by PSB is due to the activity of organic acids which are excreted by microbes (the more organic acids are excreted the pH decreases and the dissolved P increases) also due to enzyme phosphatase activity. Thus it can be said that the PSB isolates examined were able to secrete H⁺ into Pikovskaya medium containing rock phosphate. The results of the study by (12), show that the dissolution of rock phosphate by the fungus of Aspergillus niger phosphate solubilization is related to proton activity which causes a decrease in pH and production of organic acids.

3.3. Soil chemical properties of acid sulphate soil before experiment

The acid sulphate soil used in this study is classified as very acidic (pH H₂O = 4.16) (table 4). It was classified as potential acid sulphate soil according to (13). According to (13), one of the characteristics of potential acid sulphate soil is to show a very acid-rather acidic soil reaction (pH> 4.0).

C-organic content of acid sulphate soil used in this study was 3.699% and classified as high. This is related to the condition of acid sulphate soil where the sampling is hit by tidal runoff, so that the organic decomposed is slow. The total N content of potential acid sulphate used in this study was 0.248% and classified as moderate.

Concentration of available-P in acid sulphate soil used in this study were 10.596 µ.g-1 and relatively low. Factors that influence available-P in soil are the supply of P, soil pH, type of clay, temperature and organic matter (14), amount of clay, application time, aeration, compaction, moisture, temperature and other nutrients (15). In this study the available-P in the soil related with pH, exchangeable-Al and soluble-Fe. Phosphate is widely available at pH 5.5-7 (15) and maximum at pH 6.5 (3). In acid soil, phosphate was fixed by Fe and Al (16; 15) in the form of AlPO₄ and FePO₄ (4).
Table 4. Initial characteristics of acid sulphate soil before experiment.

| Chemistry properties            | Value  | Classified |
|---------------------------------|--------|------------|
| pH H₂O                          | 4.16   | Very acid  |
| Organic-C (%)                   | 3.699  | High       |
| Total-N (%)                     | 0.248  | Moderate   |
| Available-P (ppm)               | 10.596 | Low        |
| Exchangeable-Al (c mol.kg⁻¹)    | 9.774  | High       |
| Fe (µg.g⁻¹)                     | 269,997| High       |

3.4. The effect of PSB on rice growth

The application of P fertilizer significantly affected the height of rice plants at 8 WAP, but the application of PSB had no effect (table 5). Table 5 showed that there is no interaction between the application of PSB and P-fertilizer to plant height. The average rice plant height that was treated with P fertilizer was higher than the control (without P fertilizer). According to (17), that phosphor play a role in cell formation and division. It is suspected that the application of P fertilizer can increase cell formation and division so that growth is increased which is reflected in plant height.

Based on statistical analysis, showed that the effect of application 30, 45 and 60 kg.ha⁻¹ P₂O₅ to rice plant height not different. This might be related with nutrients balance absorbed by plants. According to (18), that plant growth and development is strongly influenced by the application of fertilizers and nutrient availability in the soil. Nutrients that are in a minimum concentration will limit plant growth (Liebig Minimum Law). Thus the lowest nutrient status will control plant growth. Possibility in this study there were nutrients which were at minimal conditions so that an increase in the dose of P fertilizer did not increase plant growth (plant height).

Table 5. Effect of PSB inoculations and P-fertilizer application to the height of the Inpara 2 variety of rice at 8 WAP.

| Type of PSB          | Level of P-fertilizer ( P₂O₅) | Average |
|----------------------|--------------------------------|---------|
|                      | Without P                      | 30 kg.ha⁻¹ | 45 kg.ha⁻¹ | 60 kg.ha⁻¹ | Average** |
| Without inoculation  | 86.50                          | 86.17    | 88.33      | 84.83      | 86.46      |
| 17P4.1               | 83.00                          | 87.67    | 89.67      | 88.83      | 87.68      |
| 17P4.2               | 83.75                          | 91.00    | 87.00      | 92.33      | 89.15      |
| 21P4.1               | 83.50                          | 88.67    | 86.00      | 84.33      | 85.82      |
| Average**            | 84.19 B                        | 88.38 A  | 87.82 A    | 87.58 A    |

** Description: Numbers in 1 row followed by the same capital letters not different according to Duncan's test with a confidence level of 99%.

The number of tillers at 8 WAP was not influenced by application of PSB, P-fertilizer and the interaction of both (Table 6). The average number of tillers Inpara 2 variety at 8 MST was 22.48 per clump. According to (19), that in addition to being affected by nutrient P, the formation of paddy seedlings is also influenced by nutrient N. Because the dosage of N (urea) fertilizer applied is the same ie 90 kg N.ha⁻¹ (226 kg urea.ha⁻¹) the number of tillers formed does not differ between treatments.
Table 6. Effect of PSB and P-fertilizer application to the number of tillers Inpara 2 variety at 8 WAP.

| Type of PSB | Without P | 30 kg.ha$^{-1}$ | 45 kg.ha$^{-1}$ | 60 kg.ha$^{-1}$ | Average |
|-------------|------------|-----------------|-----------------|-----------------|---------|
| Without PSB | 21,50      | 20,50           | 21,83           | 21,83           | 21,42   |
| 17P4.1      | 21,25      | 22,00           | 23,00           | 23,33           | 22,50   |
| 17P4.2      | 27,00      | 23,00           | 21,75           | 19,83           | 22,60   |
| 21P4.1      | 20,75      | 24,17           | 22,83           | 25,33           | 23,50   |
| Average     | 22,50      | 22,42           | 22,41           | 22,58           |         |

Application of P fertilizer had a very significant effect on the shoot dry weight of rice at 8 WAP, but the inoculation of PSB had no effect (Table 7). Table 7 showed no interaction between PSB and P fertilizer application to shoot dry weight. Average of shoot dry weight treated with 60 kg .ha$^{-1}$ P$_2$O$_5$ application was higher than control (without P-fertilizer). Compared to control, the average of shoot dry weight given P-fertilizer 30, 45 and 60 kg .ha$^{-1}$ P$_2$O$_5$ were not significantly different. According to (17), that phosphorus play a role in the photosynthesis process. The maximum photosynthesis process will occur, if the inorganic P concentration in the chloroplasts achieve 2.0-2.5 mM. Inorganic P concentrations in the chloroplast are less than 1.5-1.0 mM, caused photosynthesis process is inhibited. Furthermore, according to (20), one of the factors that influences P uptake is the availability of P in the soil. This might be caused P- fertilizer can increase available -P in the soil so that P-uptake increases. Increasing P in plant tissues including chloroplasts speed up rate of photosynthesis and increasing assimilates are translocated to the shoot. Furthermore (21), stated that shoot dry weight is the result of assimilates accumulation which are translocated to the shoot. The accelerate of assimilate translocated to the shoot caused high shoot dry weight.

Table 7. Effect of PSB and P-fertilizer application to shoot dry weight Inpara 2 varieties on 8 WAP.

| Type of PSB | Without P | 30 kg.ha$^{-1}$ | 45 kg.ha$^{-1}$ | 60 kg.ha$^{-1}$ | Average |
|-------------|------------|-----------------|-----------------|-----------------|---------|
| Without inoculation | 24,80      | 31,10           | 27,87           | 38,85           | 29,91   |
| 17P4.1      | 22,37      | 24,20           | 31,03           | 42,63           | 30,59   |
| 17P4.2      | 29,85      | 30,70           | 27,87           | 36,33           | 31,31   |
| 21P4.1      | 24,37      | 29,97           | 29,83           | 35,18           | 30,25   |
| Average**   | 24,94 B    | 29,43 B         | 29,15 B         | 37,94 A         |         |

** Description: Numbers in 1 row followed by the same capital letters not different according to Duncan’s test with a confidence level of 99%.

4. Conclusion

Three isolates of PSB were examined on Pikovskaya medium, P17.4.1, P17.4.2 and P22.4.1 capable of dissolving Ca3PO4 and rock phosphate. Concentration of pikovskaya medium contained tricalcium phosphate inoculated by P17.4.1, P17.4.2 and P22.4.1 were 67.26 ppm PO$_4^{2-}$, 70.60 ppm PO$_4^{2-}$ and 55.62 ppm PO$_4^{2-}$ respectively. Isolates P21.4.1 has highest ability rock phosphate dissolve as much as 44.46 ppm PO$_4^{2-}$ follow by P17.4.1 (38.09 ppm PO$_4^{2-}$) and P17.4.2 (12.07 ppm PO$_4^{2-}$). Application of PSB does not improve plant growth. Application of P fertilizer increase the rice plant height. Shoot dry weight increased by 52.13% due to application of P fertilizer as much as 60 kg. ha$^{-1}$ P$_2$O$_5$.
References

[1] Tim Sintesis Kebijakan 2008 Utilization of acid sulfate soil with environmental insight to support the increase of national rice production Pengembangan Inovasi Pertanian 1(2)129-131

[2] Mulyani A, Rachman A and Dairah A 2012 Distribution of Acid Land: Its Potential and Availability for Agricultural Development balittanah.litbang.deptan.go.id/dokumentasi buku/fosfat alam/anny_mulyani.pdf. accessed 12 December 2012

[3] Havlin J L, Tsidale S L, Beaton J D and Nelson W L 2012 Soil Fertility and Fertilizers. An Introduction to Nutrient Management: Eight Edition Pearson Prentice Hall New Jersey

[4] Shamshuddin J, Jamaludin A F, Panhwar Q and Shazana R S 2017 Formation and utilization of acid sulfate soil in Southeast Asia for sustainable rice cultivation. Pertanika J. Trop. Agric. Sci. 40(2):225-46

[5] Dewanti A, Pratiwi W E and Nuraini Y 2017 Viabilitas dan aktivitas enzim fosfatase serta produksi asam organic bakteri pelarut fosfat pada beberapa suhu simpan. J.Tanah dan Sumberdaya Lahan 3(1) 311-18

[6] Panwar Q A, Naher U A, Radziah O, Shamshuddin J and Razi I M 2015 Eliminating Aluminium Toxicity in an Acid Sulfate Soil for Rice Cultivation Using Plant Growth Promoting Bacteria. Molecules. 20(3) 3628-46

[7] Rodriguez, H. and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Av. 17(4-5)

[8] Ivanova R, Bojinova D and Nadialkova K 2006 Rock phosphate solubilization by soil bacteria J.of the university of Chemical Technology and Mineralogy. 41(3)297-302

[9] Kasno A, Setyorini D and Dwiningsih S 2008 Solubility of rock phosphate fertilizers and their effects on growth and yield of maize in ultisol soils. agris.fao.org/agris-search/search.do?record ID.DJ2012055959

[10] Rahayu F, Mastur and B Santoso 2014 Potential of several phosphate solvent bacteria isolates from sugar cane fields in East Java based on the activity of the enzyme phosphatase. Bulletin Tanaman Tembakau, Serat dan Minyak Industri 6(1) 23-31

[11] Santi L P, Goenadi D H, Siswanto I Sailah and Isroi. 2000. Solubilization of insoluble phosphates by Aspergillus niger Menara Perkebunan 68(2)

[12] Subagyo, H 2006 Klasifikasi dan penyebaran lahan rawa in D A Suriadi-karta, U Kurnia, H S Mamat, W Hartatik and D Setyorini (eds.) Swamp land characteristics and management. Center for Research and Development of Agricultural Land Resources. Agricultural Research and Development Agency. Agriculture Department

[13] Novriani 2010. Management alternative of P (phosphorus) nutrients in maize cultivation. Agronobis, Jakarta

[14] Winarso S 2005 Soil Fertility: Basic Health and Soil Quality Gaya Media. Yogyakarta

[15] Rosmarkam, A and Yuwono N W 2002 Soil Fertility: Science Kanisius, Yogyakarta

[16] Marschner P 2005 Mineral Nutrition of Higher Plants: Elsevier. USA.

[17] Pahan I 2008 Panduan Lengkap Kelapa Sawit: Management Agribisnis dari Hulu hingga hilir: Penebar Swadaya Jakarta

[18] Dobermann A and Fairhurst T 2000 Rice: Nutrient Disorders and Nutrient Management: IRRI. Philippines

[19] Hasibuan S Y, Damanik M M B, and Sitanggang G 2014 Aplikasi pupuk SP-36 dan pupuk kandang ayam terhadap ketersediaan dan serpan fosfor serta pertumbuhan tanaman jagung pada ultisol Kwala Bekala. Journal Online Agroekoteknologi 2(3)1118-25

[20] Dewi E S 2012 Effect of combination nitrogen (N) sources from cow manure and urea fertilizer on growth and yield of corn seeds. Thesis. Graduate Program. Faculty of Agriculture. Gadjah Mada University