The ability of some microbes to solubilize the hardly soluble phosphorous and potassium from various sources in vitro

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Abstract. Utilization of bio-fertilizer is an alternative option to maintain the available phosphorus (P) and potassium (K) in the soil. To develop such a bio-fertilizer, the ability of microbes to solubilize phosphate and potassium is one of the most important things. A set of experiments was conducted to evaluate the ability of some microbes (7 bacteria and 7 fungi isolates) to solubilize the hardly soluble P (Ca₃PO₄, AlPO₄, FePO₄ and Blora rock phosphate) and K (Malang feldspar) sources in vitro. The results showed that the solubilization rate of hardly soluble P and K sources by microbes are different depending on isolates type and sources applied. The amounts of P released from Ca₃PO₄, AlPO₄, FePO₄ and rock phosphate in liquid medium were significantly increased by the isolates, respectively up to 8.8, 69.3, 928, 26 times more than the control (un-inoculated). Some of them also significantly increased the solubility of the K containing mineral (feldspar), up to 2.3 times more than the control. Acidification of the medium has been observed to be the most important mechanism for the P and K solubilization, especially for Ca₃PO₄, AlPO₄. The JK1 fungal isolate was the most promising phosphate-potassium solubilizing microbe.

1. Introduction
Phosphorus (P) and potassium (K) are the major essential nutrients which are necessary for plant growth and development [1]. These nutrients play an important role for many biochemical and physiological processes in plants such as photosynthesis, respiration, energy generation, nucleic acid biosynthesis cell, osmotic regulation and enzyme activation [2, 3]. Most of plants consist of roughly 0.5% to 1% P and 1% to 5% K in their dry weight [4]. However, the concentrations of bioavailable both P and K in soil are generally very low. Usually, only 1% of total P in the soil exists in soluble form which is available for plant [5]. Similarly, the biggest portion of K in the soil is unavailable for plants as well, only 2% to 10% of K is either fixed or exchangeable and water-soluble ion [3]. In order to meet the plant needs of these elements, application of chemical fertilizers has been commonly used by farmers. However, most of developing countries, including Indonesia are often in limited supply of these fertilizers and they are also too expensive for poor farmers. In addition, the application of chemical fertilizer has been often inefficient and added in excessive dose. As a consequence, it causes economic loss and also environmental damage [6] such as groundwater contamination and waterway eutrophication [7].

In Acidic soils, inorganic P can be adsorbed by Al/Fe oxides and hydroxides to form Al-P and Fe-P complexes, whereas in neutral-to-calcareous soils, P retention is dominated by precipitation reactions to form Ca-P complex [8]. These things cause inorganic P fertilization to be often inefficient, a large portion of soluble inorganic phosphate applied to soil can be rapidly immobilized and transformed into...
these complexes which are unavailable for plants [9, 10]. Meanwhile, in most soils only less than 100 ppm K is exchangeable or dissolved in soil water, which can be absorbed by plant, about 90% to 98% K are insoluble rocks, minerals and deposits which exist in slow release forms [3]. The most important potassium-bearing minerals in soils are alkali feldspars (30 to 20 g K kg⁻¹), muscovite (K mica, 60 to 90 g K kg⁻¹), biotite (Mg mica, 36 to 80 g K kg⁻¹), and illite (32 to 56 g K kg⁻¹), which dissolution reactions and exchange processes of these minerals is a major issue [11].

Utilization of microbes and indigenous sources of P and K are alternatives for maintaining available P and K in the soil and reducing chemical fertilizer consumption. Microbes are integral to the soil P and K cycle and therefore play an important role in mediating the availability of these elements to plants [12, 13]. Several studies have reported the ability of different microbe species to solubilize insoluble inorganic phosphate compounds, including rock phosphate as a natural P source in vitro [10, 14-16] and also to increase plant growth and yield on fields scale [17, 18]. The solubilization of P by microbes occurred because of the production of mineral dissolving compounds such as organic acids, siderophores, protons, hydroxyl ions and CO₂ [5, 19]. The excretion of these organic acids decrease pH that causes the acidification of the microbial cells and the surroundings, hence, P ions are released by substitution of H⁺ for Ca²⁺ [19]. Similarly, the K solubilization mechanism also mainly occurs because of organic acid production by microbes [20]. These microbes release organic acid, which quickly dissolves rock and chelate silicate ions, and releases K ions into the soil [21]. Many studies have recently revealed that some microbes can release K from insoluble minerals such as feldspar, mica, illite and others [1, 12, 22].

The ability of microbes to solubilize both P and K is an advantage to make a proper bio-fertilizer. Therefore, the evaluation of this ability is required and one of the most important stages. The study has been performed to test the solubilizing activity of some collection microbes (7 bacteria isolates and 7 fungi isolates) to soluble several hardly soluble P sources (Ca₃PO₄, AlPO₄, FePO₄ and Blora rock phosphate) and K source (Malang feldspar mineral) in vitro.

2. Materials and methods

The study was conducted at the Soil Biology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University, and Soil and Plant Nutrition Laboratory, Agricultural Department, Centre for Application of Isotope and Radiation, National Nuclear Energy Agency of Indonesia (BATAN) from July to December 2016.

2.1. Phosphorus and potassium sources

Sources of P in the form of Ca₃PO₄, AlPO₄, FePO₄ were used as chemical P sources. Rock phosphate (RP) taken from Blora, Central Java, Indonesia and feldspar (potassium containing mineral) taken from Malang, East Java, Indonesia were used as the local natural P and K source, respectively. Blora rock phosphate contains 26.6% total P₂O₅ and 18.1% P₂O₅ soluble in 2% citric acid and Malang Feldspar contains 1.74% K₂O, 70.8% SiO₂ and 14.3% Al₂O₃. Rock phosphate and Feldspar were pounded and passed through a 270 mesh sieve before used.

2.2. Microbial strains and inoculants preparation

Seven fungi isolates (JK1, JK2, PN1, FPF4, FPF5, FPFE1, SS10.6) and 7 bacteria isolates (F21, F22, PS1, BPF7, BPF9, SS1.2, SS19.7) were obtained from culture collection of Soil Biology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University, and from culture collection of Soil and Plant Nutrition Laboratory, Agricultural Department, Centre for Application of Isotope and Radiation, National Nuclear Energy Agency of Indonesia. These isolates were identified at preliminary characterization as phosphate solubilizing microbes (unpublished). The bacteria isolates were cultured in nutrient broth (NB) medium, and then incubated on an orbital shaker at 150 rpm for 28 hours at room temperature. The cell concentration in the suspension was determined by a total-plate-count method. The pure cells in the suspension were collected by centrifugation at 5,000 rpm for 20 minutes and washed with distilled water. The pelleted cell as centrifuged result was resuspended with distilled water to adjust cell concentration to 10⁶ cell ml⁻¹. Each fungi isolate was cultured in potato dextrose agar (PDA) medium, and then incubated on PDA medium for 168 hours at
room temperature. The spores in cultured PDA were collected by flooding the medium with distilled water. The spore density in suspension was directly measured using hemocytometer. Each spore suspension was re-suspended using distilled water to adjust spore concentration to 10⁹ spore ml⁻¹.

2.3. P and K solubilization index test

All microbial isolates were tested by an agar assay to obtain the solubilizing index value. Solubilization of P in solid medium was tested on Pikovskaya agar medium containing (g L⁻¹): glucose (10), Ca₃(PO₄)₂ (5), MgSO₄·7H₂O (0.1), KCl (0.2), (NH₄)₂SO₄ (0.5), FeSO₄·7H₂O (0.0025), MnSO₄·7H₂O (0.0025), yeast extract (0.5) and bacterial agar (15) [23]. Solubilization of K was tested on Alexandrov agar medium containing (g L⁻¹): glucose (10), Ca₃(PO₄)₂ (2), MgSO₄·7H₂O (0.5), FeCl₃ (0.005), CaCO₃ (0.1), feldspar (5), yeast extract (0.5) and bacterial agar (30) [24].

The bacterial isolates were stabbed on agar plate in triplicate using an inoculation loop. The fungal isolates were cultured by putting 0.5 x 0.5 cm plugs of 5-day-old PDA culture in the center of the agar plate in triplicate using a sterile toothpick. All cultures were incubated at room temperature for 72 hours. The ability of the microbe to solubilize insoluble P or K in solid medium was described by the solubilization index [22, 25].

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\text{Solubilization index (SI)} = \frac{\text{The total diameter colony + halo zone (cm)}}{\text{Colony diameter (cm)}} \tag{1}
\]

2.4. Quantitative measurement of released P from Ca₃PO₄, AlPO₄, FePO₄ and rock phosphate in liquid medium

All isolates were examined for their ability to release of hardly soluble P sources in broth medium. One ml of culture suspense of each isolate (containing 10⁹ ml⁻¹ bacteria cell or 10⁹ ml⁻¹ fungi spore) was inoculated on 25 ml of Pikovskaya broth containing 5 g L⁻¹ of each P source in inoculated flask. All cultures were incubated for 168 hours at room temperature. The initial pH of medium containing Ca₃PO₄, AlPO₄, FePO₄ and rock phosphate were 6.04, 5.35, 5.58, 7.39, respectively. During incubation times, only bacteria cultures were shaken on an orbital shaker at 150 rpm. Non inoculated treatments were made as controls which there are 2 types of control, shaken un-inoculated broth as bacteria control and non-shaken un-inoculated broth as fungi control. All treatments were replicated in three times. After incubation times, the broth cultures were filtered through Whatman No.1 filter paper and then centrifuged at 12,000 rpm for 20 minutes. The soluble P content in the supernatants was determined by phosphomolybdic blue color method using spectrophotometer [26]. The pH value of medium was measured using pH meter.

2.5. Quantitative measurement of released K from feldspar mineral

All isolates were also studied for their potential ability to release K from feldspar in broth medium containing (g L⁻¹): glucose (10), feldspar (5) and yeast extract (0.5). The initial pH of liquid medium containing feldspar was 4.37. The inoculation technique, incubation times, incubation temperature and control treatments were the same with the measurement of P released. All treatments were replicated in three times. After incubation times, the broth cultures were filtered through Whatman No.1 filter paper and then centrifuged at 12,000 rpm for 20 minutes. The soluble K content in the supernatant was measured using flame photometer [27]. The pH value of medium was measured using pH meter.

2.6. Statistical analysis

The raw data generated during the whole experiment were subjected to statistical analysis by following the Complete Randomized Design and the observations were analyzed with the appropriate ANOVA table. Overall significance of treatment were tested by Duncan Multiple Range Test (SPSS 16) at 5% probability level (P>0.05). The strength of a linear relationship between two parameters was tested by Pearson Correlation Coefficient at 1% (P>0.01) and 5% (P>0.05) probability level (SPSS 16).
3. Results and discussion

3.1. Activity of isolates on agar plates
The results showed that all isolates were capable to solubilize Ca₃PO₄ present in Pikovskaya agar which was indicated by halo zone formed surrounding colony (figure 1). It was seen that the colony diameter and the halo zone diameter were formed with varied size (table 1), which the highest P solubilization index was reached by F22 isolate (2.68). From all of the isolates, nine of them, 5 fungal isolates and 4 bacterial isolates were also capable to solublize feldspar present in Alexandrov agar, which the K solubilization indexes were about 1.02 to 2.11. The others did not show halo zone on Alexandrov agar; however, they were still capable to grow on the medium. The F21 isolate was found to be the greatest K solubilization index (2.11).

The solubilization index value helps to evaluate the solubilized activity of isolates, which the higher value indicated, the higher solubilized activity of the isolates tested. The activity was associated with the production of organic acids into the surrounding medium [28]. Variation of solubilization index value might be caused by the difference of isolates ability to secrete their extracellular organic acids, such as oxalic acid and tartaric acid, and also the production of polysaccharides [29].

### Table 1. Phosphate and potassium solubilization index at 72-hour incubation.

| Isolate | Diameter colony (cm) | Diameter halo zone (cm) | P solubilization index | Diameter colony (cm) | Diameter halo zone (cm) | K solubilization index |
|---------|----------------------|------------------------|------------------------|----------------------|------------------------|------------------------|
| **fungi isolates** | | | | | | |
| JK1 | 3.05<sup>bc</sup> | 3.71<sup>c</sup> | 1.22<sup>c</sup> | 3.08<sup>d</sup> | 3.40<sup>c</sup> | 1.11<sup>d</sup> |
| JK2 | 2.60<sup>b</sup> | 2.90<sup>b</sup> | 1.12<sup>abc</sup> | 2.43<sup>b</sup> | 2.54<sup>b</sup> | 1.05<sup>c</sup> |
| PN1 | 1.33<sup>a</sup> | 1.44<sup>a</sup> | 1.09<sup>ab</sup> | 1.20<sup>a</sup> | 0.00<sup>a</sup> | 0.00<sup>a</sup> |
| FPF4 | 2.71<sup>bc</sup> | 3.13<sup>b</sup> | 1.15<sup>bc</sup> | 2.51<sup>b</sup> | 2.61<sup>b</sup> | 1.04<sup>c</sup> |
| FPF5 | 3.19<sup>c</sup> | 3.70<sup>c</sup> | 1.16<sup>bc</sup> | 3.65<sup>c</sup> | 3.73<sup>d</sup> | 1.02<sup>b</sup> |
| FPFE1 | 3.13<sup>c</sup> | 3.21<sup>b</sup> | 1.03<sup>a</sup> | 2.61<sup>b</sup> | 2.66<sup>b</sup> | 1.02<sup>b</sup> |
| SS10.6 | 3.18<sup>c</sup> | 3.28<sup>b</sup> | 1.03<sup>a</sup> | 2.83<sup>a</sup> | 0.00<sup>a</sup> | 0.00<sup>a</sup> |
| **bacteria isolates** | | | | | | |
| F21 | 0.57<sup>c</sup> | 1.33<sup>c</sup> | 2.35<sup>c</sup> | 0.29<sup>ab</sup> | 0.60<sup>c</sup> | 2.11<sup>d</sup> |
| F22 | 0.42<sup>b</sup> | 1.12<sup>d</sup> | 2.68<sup>d</sup> | 0.39<sup>b</sup> | 0.77<sup>d</sup> | 1.99<sup>d</sup> |
| PS1 | 0.60<sup>cd</sup> | 0.70<sup>b</sup> | 1.17<sup>a</sup> | 0.30<sup>b</sup> | 0.00<sup>a</sup> | 0.00<sup>a</sup> |
| BPF7 | 0.30<sup>a</sup> | 0.40<sup>a</sup> | 1.33<sup>b</sup> | 0.34<sup>ab</sup> | 0.00<sup>a</sup> | 0.00<sup>a</sup> |
| BPF9 | 0.83<sup>c</sup> | 1.13<sup>d</sup> | 1.36<sup>b</sup> | 0.27<sup>a</sup> | 0.00<sup>a</sup> | 0.00<sup>a</sup> |
| SS1.2 | 0.63<sup>d</sup> | 0.87<sup>c</sup> | 1.37<sup>b</sup> | 0.50<sup>c</sup> | 0.51<sup>b</sup> | 1.02<sup>b</sup> |
| SS19.7 | 0.83<sup>c</sup> | 0.93<sup>c</sup> | 1.12<sup>a</sup> | 0.49<sup>c</sup> | 0.62<sup>c</sup> | 1.32<sup>c</sup> |

Data following same letter in superscripts in each column are not significantly different at 5% probability level as per Duncan’s multiple range test.

3.2. Quantity of released P from Ca₃PO₄, AlPO₄, FePO₄ and Rock Phosphate
Table 2 summarizes the total amounts of P released (µg P ml<sup>-1</sup>) and the percent of P released (%) from each P source in broth culture at the end of incubation times. The higher amounts of P released indicate the higher isolates ability to solubilize hardly soluble P source in broth medium. Most of the isolates tested significantly increased the solubility of Ca₃PO₄, which the amounts of P released were about 19.7 to 152.7 µg P ml<sup>-1</sup>. The JK1 fungi isolate has the highest ability to solubilize Ca₃PO₄ among the other fungal isolates, which the amount of P released by that isolate was up to 8.8 times more than the control. In bacteria isolate group, the highest P released from Ca₃PO₄ was reached by BPF7 isolate, which increased solubility of Ca₃PO₄ up to 2.9 times more than the control. The results also showed that fungal isolates as a group has the ability to solubilize Ca₃PO₄ about 184% higher than bacteria.
In the media supplemented with AlPO₄ as a sole P source, the highest amounts of P released were obtained by the FPF4 isolate in fungi group and the F22 isolate in bacteria group. These isolates increased the solubility of AlPO₄ in broth medium about 69.3 and 65.8 times more than the control, respectively. The maximum P released from AlPO₄ was about 100.5 mg ml⁻¹ or about 9.1% of total AlPO₄ in the medium. Similarly, with the Ca₃PO₄ solubilization, fungi as a group had higher ability to solubilize AlPO₄ than bacteria. The fungal isolates increased the solubility of AlPO₄ about 110% higher than bacteria.

The results also showed that the isolates tested significantly increased the solubility of P sources in the media supplemented with FePO₄ and rock phosphate. The isolates increased the solubility of FePO₄ and rock phosphate in the medium up to 928 and 29 times more than the control, respectively. The fungi isolate JK1 reached the highest solubilization rate of the Blora rock phosphate. Fungi as a group had higher ability to solubilize Blora rock phosphate about 140% than bacteria. However, different from others P sources, bacteria isolate as a group showed greater ability to solubilize FePO₄ than fungi. The bacteria isolate SS19.7 reached the highest solubilization rate of the FePO₄, which solubilized P from that source about 83.5 mg ml⁻¹ or about 12.02% of total FePO₄ in the medium.

Our research showed that the different isolate tested has different abilities to solubilize P in liquid medium. It may due to the different ability of isolates to produce and to secrete organic acid among these isolates. Organic acids such as acetic, citric, lactic, fumaric, tartaric, propionic, glycolic, oxalic, malonic, succinic, malonic, gluconic, etc. have been identified to solubilize phosphate [5, 9]. Such organic acids can either directly dissolve the mineral phosphate as a result of anion exchange of PO₄³⁻ by acid anion or chelate both iron and aluminum ions associated with phosphate [9]. In summary, our results had revealed that fungi as a group has a greater ability to solubilize P than bacteria except in solubilization of FePO₄. The most likely explanation is that the fungi may produce and secrete more acids than the bacteria [5]. Soil fungi have been reported to be able to traverse long distances within the soil more easily than bacteria and may be more important to the solubilization of inorganic phosphate in soils as they typically produce and secrete more acids, such as gluconic, citric, lactic, 2-ketoglucronic, oxalic, tartaric and acetic acid, than bacteria [5]. In general, we had been revealed that JK1 isolate was the most promising phosphate solubilizing microbe, which has the most constant ability to solubilize P from various sources in liquid medium.

Our results also showed that the solubilization rate of hardly soluble P sources in liquid medium depends on the type of the P source. In general, the amount of phosphate solubilized decreased in the order Ca₃PO₄ > AlPO₄ > FePO₄ > rock phosphate. This result may due to the different solubility of these
P sources in the liquid medium. This agrees with results obtained by previous study which tested some fungal isolates to solubilize Ca₃PO₄₂, AlPO₄, FePO₄ and rock phosphate [14]. Similar results were also found by other report who described this phenomenon is probably due to the higher solubility of the Ca phosphate in the culture solution [16].

**Table 2.** Phosphate solubilization activity in liquid medium amended with the four phosphate types recorded 168 hours after inoculation.

| Isolate | P released (µg P ml⁻¹) | P released (%) |
|---------|------------------------|----------------|
|         | Ca₃PO₄ | AlPO₄ | FePO₄ | RP | Ca₃PO₄ | AlPO₄ | FePO₄ | RP |
| fungi isolates |
| Control | 17.4a | 1.5a | 0.2a | 0.14a | 1.7a | 0.1a | 0.02a | 0.02a |
| JK1     | 152.7e | 91.5d | 8.0e | 3.72e | 15.3e | 8.3d | 1.14c | 0.64c |
| JK2     | 69.3c | 37.6b | 9.5c | 2.88d | 6.9c | 3.4ab | 1.36c | 0.50d |
| PN1     | 42.9b | 42.2b | 3.0b | 0.23a | 4.3b | 3.8b | 0.43b | 0.04a |
| FPF4    | 124.5d | 100.5d | 3.1b | 0.94b | 12.5d | 9.1d | 0.44b | 0.16b |
| FPF5    | 128.5d | 85.5cd | 2.2b | 1.89c | 12.9d | 7.7cd | 0.31ab | 0.33c |
| FPF6E1  | 134.2de | 69.7bcd | 4.8b | 1.18b | 13.9de | 6.3bcd | 0.69b | 0.20bc |
| SS10.6  | 67.9c | 51.4bc | 15.8d | 0.19a | 6.8c | 4.6bc | 2.27d | 0.03a |
| bacteria isolates |
| Control | 16.6e | 0.9a | 0.1a | 0.05a | 1.7a | 0.1a | 0.01a | 0.01a |
| F21     | 19.7b | 57.0f | 27.2d | 0.27a | 2.0b | 5.2d | 3.91d | 0.05a |
| F22     | 35.7d | 59.2e | 40.2c | 0.26a | 3.6de | 5.4e | 5.78c | 0.05a |
| PS1     | 31.8bc | 50.3d | 10.5b | 0.31ab | 3.2bcd | 4.5d | 1.50b | 0.06ab |
| BPF7    | 49.1c | 2.9a | 17.8c | 0.16a | 4.9e | 0.3a | 2.56c | 0.03a |
| BPF9    | 28.6bc | 34.5c | 29.0d | 0.66b | 2.9abc | 3.1c | 4.17d | 0.11b |
| SS12    | 44.5de | 5.7a | 15.1c | 1.42c | 4.5de | 0.5a | 2.17bc | 0.24c |
| SS19.7  | 45.9de | 18.5b | 83.5f | 1.46c | 4.6e | 1.7b | 12.02f | 0.25c |

Data following same letter in superscripts in each column are not significantly different at 5% probability level as per Duncan’s multiple range test.

3.3. **Quantity of released K from feldspar**

The amounts of K released (µg K ml⁻¹) and the percent of K released (%) from feldspar in liquid medium by the isolates tested is presented in Table 3. Five bacteria isolates (F21, F22, PS1, BPF9, SS19.7) significantly increased the solubility of feldspar as compared to the control. The maximum K released was recorded by BPF9 isolate, which released the amounts of K up to 2.3 times more than the control. In contrary, we observed that all fungi isolates did not significantly increased the solubility of feldspar compared to the control. However, a slight increase but statistically not different in the amount of K released by fungi isolates was observed. The JK1 isolate caused the biggest amount of K released among the fungi isolates.

This result showed that some isolates have the ability to release the inorganic insoluble potassium as well. However, this ability was lower than the ability to solubilize P sources. It was probably due to the very low K contained in feldspar (1.74% K₂O) which was used in this study. Previous studies showed that some microbes were able to solubilize insoluble K minerals, such as K-feldspar, Illite and waste mica [1, 12, 30]. They produced several kinds of organic acids which broke down the mica or feldspar structure to satisfy their Si⁴⁺ and K requirements, bringing them into solution consequently lowering the pH of the inoculated broth [31]. As previously documented, organic acids, such as citric, oxalic, succinic, and α-ketogluconic acids and capsular polysaccharide of microbial origin are capable of mobilizing K from various K-bearing minerals [24]. Recent studies showed that some microbes have the ability to release both P and K from insoluble sources in the liquid medium such as rock phosphate, waste muscovite and waste biotite [1, 32].
Table 3. Potassium solubilization activity in liquid medium amended with the feldspar recorded 168 hours after inoculation.

| Isolate | K released (µg P ml⁻¹) | % K released (%) |
|---------|------------------------|-----------------|
| **fungi isolates** | | |
| Control | 20.9ᵃ | 29.4ᵃ |
| JK1 | 35.7ᵃ | 50.2ᵃ |
| JK2 | 26.5ᵃ | 37.3ᵃ |
| PN1 | 24.8ᵃ | 35.0ᵃ |
| FPF4 | 25.3ᵃ | 35.6ᶜ |
| FPF5 | 21.5ᵃ | 30.3ᵃ |
| FPFE1 | 22.2ᵃ | 31.3ᵃ |
| SS10.6 | 22.2ᵃ | 31.3ᵃ |
| **bacteria isolates** | | |
| Control | 26.5ᵃ | 37.3ᵃ |
| F21 | 55.1ᵉ⁄ᶠ | 77.7ᵉ |
| F22 | 50.9ᵉ⁄ᶠ | 71.7ᵈᵉ |
| PS1 | 40.8ᵈ⁄ᵉ⁄ᶠ | 57.5ᵇᵉ⁄ᵈ |
| BPF7 | 37.5ᵇᶜ | 52.7ᵇᶜ |
| BPF9 | 59.8ᶠ | 84.2ᵉ |
| SS1.2 | 31.1ᵃ⁄ᵇ | 43.8ᵃ⁄ᵇ |
| SS19.7 | 48.8ᵈᵉ | 68.8ᵈᵉ |

Data following same letter in superscripts in each column are not significantly different at 5% probability level as per Duncan’s multiple range test.

3.4. The pH value of broth culture

The pH values of control treatment were relatively unchanged after 168-hour incubation times (compared to the initial pH before treatment). In general, the pH broth cultures of inoculation treatments after 168 hours incubation time were significantly lower than the control (table 4). The reduction in pH may due to the production and secretion of different kinds of organic and inorganic acids by the isolates tested [19]. The results showed that fungi as a group caused lower in pH medium than bacteria. It was indicated that fungi have higher ability to produce and secrete organic acid than bacteria, which was caused acidification of broth medium. However, the maximum drop in pH was recorded in broth culture containing feldspar by BPF9 isolate. This lowest pH medium caused the biggest amount of released K.

The lowest pH in liquid medium containing Ca₃(PO₄)₂ was 3.84, which was reached by JK1 isolate. The lowest drop in pH medium by this isolate was associated with the biggest amount of released P. In this study, the pH medium affected by isolate was between 3.84 and 5.80. This range was almost similar to the range found by two previous studies that studied the solubilization of Ca₃(PO₄)₂ using the *Penicillium* sp., *Talaromyces* sp. and the dark septate endophytes (DSE) [15, 16].

In the media supplemented with each AlPO₄ and FePO₄ as sole P source, the pH values range from 2.88 to 4.18 and 2.23 to 5.4, respectively. These results were almost similar to the result found by previous study [16], which reported that the pH medium containing AlPO₄ and FePO₄ was about 2.23 to 2.71 and 2.37 to 3.40, respectively. These ranges were reached lower than the range of pH medium containing Ca₃PO₄.
3.5. Correlation between Solubilization Index, pH medium with nutrients released

We observed that there is no significant correlation between the values of the P solubilization index and the abilities of isolates to solubilize Ca$_3$(PO$_4$)$_2$, AlPO$_4$, FePO$_4$ and rock phosphate (P>0.5) in liquid medium (Table 5). Similar results occurred also for the K Solubilization, which is also the presence of correlation between the values of K Solubilization index and the abilities of microbes to solubilize feldspar in liquid medium. These results indicated that the size of clearing zone produced by microbe on agar plate did not always indicate the ability of microbe to solubilize hardy soluble P and K in the liquid medium. Previous study reported that some isolates which did not produce any visible halo zone on agar plates could still solubilize various types of insoluble inorganic phosphates in the liquid medium [10]. In addition, a previous study reported that some strains which showed a well-developed ability for phosphate solubilization lost their abilities after several cycles of inoculation and cultivation [33]. However, the method can be regarded as generally reliable for isolation and preliminary characterization of phosphate-solubilizing microbes.

We also found that the amounts of P released from Ca$_3$PO$_4$ by the isolates correlated significantly with the amounts of P released from AlPO$_4$ and rock phosphate. These results can indicate that the solubilization of Ca$_3$PO$_4$, AlPO$_4$ and rock phosphate by microbe had a similar mechanism. Otherwise, we haven’t found significant correlation between the amount of P released from Ca$_3$PO$_4$, AlPO$_4$ and rock phosphate and the amount of P released from FePO$_4$. These results may due to differences of the mechanism to solubilize FePO$_4$ with other sources. Interestingly, we found significant correlation between the amount of P released from FePO$_4$ and the amount of K released from feldspar by the isolates, which was probably caused by the similarity of solubilization mechanism both these sources. Our study also showed that the solubility of Ca$_3$PO$_4$ and AlPO$_4$ are strongly influenced by the pH medium, which was the lower pH caused a higher amount of released P (Table 6). This study found a significant negative correlation between the amount of P released from Ca$_3$PO$_4$ and AlPO$_4$ with the pH medium. It indicated that P released from Ca$_3$(PO$_4$)$_2$ and AlPO$_4$ were possible by simple acidification of the medium. This concurs with the findings of several studies which reported that a reduction in pH is the most important mechanism for solubilizing pure Ca-P source [14,16,34]. However, acidification solely could not be the explanation of Fe-P, rock phosphate and feldspar solubilization. In this study, we

| Isolate | Ca$_3$(PO$_4$)$_2$ | AlPO$_4$ | FePO$_4$ | RP | Feldspar |
|---------|-----------------|-----------|-----------|----|----------|
| **fungi isolates** | | | | | |
| Control | 5.78 | 4.84 | 5.59 | 6.90 | 4.40 |
| JK1 | 3.84 | 2.88 | 2.29 | 5.22 | 2.25 |
| JK2 | 4.48 | 3.01 | 2.27 | 4.19 | 2.64 |
| PN1 | 4.76 | 3.14 | 2.34 | 4.89 | 3.13 |
| FPF4 | 3.93 | 3.59 | 2.40 | 5.75 | 2.44 |
| FPF5 | 3.92 | 3.38 | 2.28 | 4.90 | 2.41 |
| FPFE1 | 3.88 | 3.43 | 2.39 | 5.31 | 2.27 |
| SS10.6 | 4.57 | 3.89 | 2.23 | 3.43 | 2.29 |
| **bacteria isolates** | | | | | |
| Control | 6.06 | 5.58 | 5.59 | 7.47 | 3.76 |
| F21 | 5.80 | 3.16 | 3.17 | 7.09 | 2.34 |
| F22 | 5.72 | 3.33 | 3.18 | 7.00 | 2.28 |
| PS1 | 5.52 | 3.52 | 5.14 | 7.25 | 5.15 |
| BPF7 | 5.26 | 5.54 | 4.02 | 7.32 | 6.25 |
| BPF9 | 5.74 | 3.40 | 3.35 | 7.19 | 1.94 |
| SS1.2 | 5.56 | 5.53 | 3.81 | 7.30 | 4.97 |
| SS19.7 | 5.44 | 4.18 | 3.63 | 7.10 | 2.40 |

Data following same letter in superscripts in each column are not significantly different at 5% probability level as per Duncan’s multiple range test.
observed no significant correlation between the amount of nutrient released from Fe-P, rock phosphate, feldspar and pH medium. This concurs with the findings of several studies that described acidification not the only mechanism of P solubilization [10, 35].

**Table 5.** Pearson correlation between P and K Solubilization Index with P and K released from hardly soluble sources.

|                | P released | K released | solubilization Index |
|----------------|------------|------------|----------------------|
|                | Ca₃PO₄    | AlPO₄      | FePO₄    | RP          | (feldspar) | P | K |
| P solubilization Index | -0.44 | 0.01 | 0.30 | -0.32 | 0.63* | 1.00 | 0.66* |
| K solubilization Index | 0.10 | 0.29 | 0.33 | 0.26 | 0.26 | 0.66* | 1.00 |
| P released (Ca₃PO₄) | 1.00 | 0.71** | -0.46 | 0.62* | -0.63* | -0.44 | 0.10 |
| P released (AlPO₄) | 0.71** | 1.00 | -0.43 | 0.29 | -0.29 | 0.01 | 0.29 |
| P released (FePO₄) | -0.46 | -0.43 | 1.00 | -0.13 | 0.65* | 0.30 | 0.33 |
| P released (RP) | 0.62** | 0.29 | -0.13 | 1.00 | -0.25 | -0.32 | 0.26 |

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

**Table 6.** Pearson correlation between P and K released from hardly soluble sources with pH medium.

|                | pH medium (Ca₃PO₄) | pH medium (AlPO₄) | pH medium (FePO₄) | pH medium (RP) | Feldspar |
|----------------|-------------------|-------------------|-------------------|----------------|---------|
| P released (Ca₃PO₄) | -0.94** | -0.27 | -0.62** | -0.54* | -0.33 |
| P released (AlPO₄) | -0.68** | -0.70** | -0.55* | -0.43 | -0.58* |
| P released (FePO₄) | 0.57* | 0.18 | 0.34 | 0.48 | -0.16 |
| P released (RP) | -0.55* | -0.27 | -0.40 | -0.35 | -0.27 |
| K released (feldspar) | 0.79** | -0.03 | 0.53 | 0.75** | -0.06 |
| pH medium (Ca₃PO₄) | 1.00 | 0.34 | 0.73** | 0.75** | 0.33 |
| pH medium (AlPO₄) | 0.34 | 1.00 | 0.49 | 0.44 | 0.74** |
| pH medium (FePO₄) | 0.73** | 0.49 | 1.00 | 0.81** | 0.70** |
| pH medium (RP) | 0.75** | 0.44 | 0.81** | 1.00 | 0.44 |
| pH medium (Feldspar) | 0.33 | 0.74* | 0.70** | 0.44 | 1.00 |

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

4. Conclusions
The solubilization rate of hardly soluble phosphate and potassium sources by microbes are varied depending on isolates type and sources applied. The amounts of P released from Ca₃PO₄, AlPO₄, FePO₄ and rock phosphate were significantly increased by the isolates tested respectively up to 8.8, 69.3, 928, 26 times more than the control. Some of them also significantly increased the solubility of K mineral (feldspar), up to 2.3 times more than the control. Acidification of the medium has been observed to be the most important mechanism for P and K solubilization, especially for Ca₃PO₄ and AlPO₄. A significant negative correlation was observed between the amount of released P from Ca₃PO₄ and AlPO₄ and the pH medium. Fungi as a group have a greater ability to solubilize Ca₃PO₄, AlPO₄ and rock phosphate than bacteria. Fungi have dissolved Ca₃PO₄, AlPO₄ and Blora rock phosphate respectively 184%, 110% and 140% bigger than bacteria. In contrary, bacteria have a greater ability to solubilize FePO₄ and feldspar than fungi. The JK1 fungal isolate was the most promising phosphate-potassium solubilizing microbe.
Acknowledgments
The authors acknowledge the research grant from The Ministry of Research, Technology, and Higher Education, Indonesia.

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