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Research Article

The effectiveness of application of phosphorous and potassium solubilizing multifunctional microbes (*Aspergillus costaricaensis* and *Staphylococcus pasteuri* mutants) on maize growth

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Abstract: The use of phosphorus and potassium-solubilizing microbes as biofertilizers is an alternative method to increase the availability of phosphorus and potassium in soils. This study aimed to explore the effectiveness of phosphorus (P) and potassium (K)-solubilizing multifunctional microbes (*Aspergillus costaricaensis* and *Staphylococcus pasteuri* mutants) on maize growth. The stages of this study consisted of viability test of P and K solubilizing *A. costaricaensis* and *S. pasteuri* mutants in peat and effectiveness test of P and K solubilizing *A. costaricaensis* and *S. pasteuri* mutants on maize growth. The results showed that peat carriers could keep the fungi population stable until 18 weeks of storage times. While the bacteria at 6 and 8 weeks storage times showed a slight decrease and stable in the 10 to 12 weeks storage time. The addition of P and K-solubilizing multifunctional microbes could reduce the use of fertilizer up to 50% in the treatment with a combination of easily soluble P or K sources with not-easily soluble P or K sources, as well as a combination of treatments of not-easily soluble P and K sources. This tended to occur in the treatment with the addition of *A. costaricaensis* mutant.

Keywords: *biofertilizer, microbes, phosphorus, potassium, viability*

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Introduction

Agricultural development in Indonesia has an impact on changes in the quality of land resources. Intensive agriculture generally has high phosphorus (P) content in the soil as a result of the continuous use of P fertilizer. However, most P is not available for absorption by plants (Khan et al., 2007). Muliana et al. (2018) reported that intensive agricultural practices that routinely provide inorganic fertilizers with high doses and intensity in shallot cultivation in Brebes lead to the accumulation of P and potassium (K). Most of the P and K elements are not available, so P and K cannot be utilized by plants. In acid soils, P fixation can occur, which causes P to become unavailable for plants. If soil P availability is low due to high soil fixation ability, fertilizer application is not effective (Sanyal and de Datta,1991; Achal et al., 2007). Similarly with K, based on the soil type approach to 90-98% of the total K was found to be in the unavailable form. Maize plant is a crop that is responsive to nutrient deficiency. The availability of P is one of the limiting factors for agricultural production in Indonesia (Leiwakabessy, 1998). Soils in the tropics are dominated by acid soils which generally have high P fixation capacity. This fixation causes the availability of P for plants to be very low (Khan et al., 2007). The efficiency of P fertilization ranges from 10% -25% (Syers, 2008). Apart from the P nutrient, another limiting factor for plant growth is the availability of K. The K content in the soil varies from low to high depending on soil type and natural processes that determine K input and output to the soil (Subandi et al., 2013). A study conducted by Sofyan et al. (2000) showed that the K nutrient status of 11.66% of Indonesia's agricultural land is low. The fulfilment of K nutrient in agricultural land in Indonesia is generally through the addition of inorganic fertilizers such as potassium chloride. Potassium chloride fertilizer used in Indonesia is imported from various countries (Hadi et al., 2007; Subandi, 2013).

Microbes play important roles in increasing the availability of macronutrients such as P and K. Results of previous studies indicated that P-solubilizing microbes could solubilize P from insoluble P sources (by invitro) (Premono, 1994; Elfiati, 2004; Chen et al., 2006; Bojinova et al., 2008; Puspitawati et al., 2013). The use of P-solubilizing microbes has also been reported to reduce the use of SP-36 fertilizer until 25% in the SRI method (Puspitawati et al., 2013), and increase the efficiency of rock phosphate used in maize and wheat cultivation (Kaur and Reddy,2014). Other studies also reported that several microbes could solubilize K, such as *Bacillus mucilaginosus* (Han and Lee, 2005), *Enterobacter hormaechei* (Prajapati and Modi, 2012), *Microbacterium hominis, Flectobacillus* sp., *Agrobacterium tumefasciens*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus megaterium* (Diep and Hieu, 2013), and K-solubilizing fungi of *Aspergillus terreus* (Prajapati et al., 2013). K-solubilizing microbes can solubilize not-easily soluble K sources such as feldspar and mica (Diep and Hieu, 2013).

Indonesia is a country that is rich in natural sources of P and K that can be used as fertilizers. Indonesia has large reserves of rock phosphate deposits, which are around 7 to 8 million tons (Moersidi, 1999). Apart from P, Indonesia also has potential K sources such as feldspar and mica. However, the use of P and K sources faces constraints because they require a long time for the solubilizing process. The use of rock P as a source P fertilizer is not always effective because most rock P has the low solubility or reactivity category (Vassilev et al., 2001). The main minerals, such as apatite (Ca-P), veriscite (Al-P), and strengit (Fe-P) cause most of the low natural P reactivity (Zapata and Roy, 2004). Rock potassium also requires a long weathering process to release available K, which can be absorbed by plants. Therefore, the use of P and K solubilizing microbes is one method that can accelerate the weathering process of these rock P and K. Functional microbes such as *Aspergillus costaricaensis* and *Staphyloccocus pasteuri* are generally used as biological agents in biofertilizer as these two isolates have the multifunctional ability to solubilize P and K (Sukmadewi et al., 2019).

This study aimed to explore the effectiveness of P and K solubilizing multifunctional microbes (*Aspergillus costaricaensis* and *Staphyloccocus pasteuri* mutants) on maize growth.

Materials and Methods

The study was conducted in the Soil Biotechnology Laboratory, Chemistry and Soil Fertility Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University. *Aspergillus costaricaensis* and *Staphyloccocus pasteuri* mutants were used in this study. The sterilization of the carrier material was conducted in the Isotope and Radiation Application Center, National Nuclear Energy Agency of Indonesia, Pasar Jumat, South Jakarta. The field trial was conducted in the Cikarawang rice fields, Dramaga Bogor.

Viability test of A. costaricaensis and *S. pasteuri* mutants *in peat*

Viability is an important requirement in the quality of biofertilizer because it is related to the minimum population of microbes living in inoculants which can affect plant growth (Simanungkalit et al., 2006). One of the materials that be used as a carrier material is peat. Peat has suitable physical properties for microbes, contains a moderate microbial population, and its presence can be suppressed through the sterilization process (Mishra and Dadhich, 2010). The peat was packed in 40 g of aluminium foil bags. Sterilization was carried out using gamma irradiation at a dose of 50 kGy (Putri et al., 2010). *A. costaricaensis* and *S. pasteuri* mutants were used in this study. Microbes population calculation to determine its viability on a carrier was carried out every 2 weeks until the microbe population has decreased. The total microbes population was calculated by the Total Plate Count (TPC) method on specific media (Pikovskaya and Alexandrov).

Effectiveness test of A. costaricaensis and S. pasteuri mutants on maize growth

Testing the effect of P and K multifunctional solubilizing microbes on maize growth was conducted in the Cikarawang rice fields. The type of soil used is Inceptisol. Ten treatments (Table 1) were arranged in a single factor randomized block design with four replications. The data obtained were analyzed using Anova or variance test with a significance of 5%. Treatments that had a significantly different were further tested with the Duncan Multiple Range Test at the 5% level. The treatment plot was made with a size of 2 m x 2 m. The number of plants in one plot was 12 plants. Planting was done by dropping maize seeds as deep as 5 cm with a spacing of 75 x 40 cm (Suliasih and Widawati, 2015). Before planting, the seeds were immersed in a bacterial and fungal suspension for 30 minutes. Fertilizer was applied by burying it around the planting hole.

Table 1. Treatments for testing the effect of P and K solubilizing multifunctional microbes on the growth of maize in the field.

Code	Sources of P		Sources of K		Microbes
	Rock Phosphate	SP-36	Feldspar	KCl	
P1	100%	-	100 %		-
P2	-	100%	100%	-	-
P3	100%	-	-	100%	-
P4	-	100%	-	100%	-
P5	50%	-	50%	-	*S. pasteuri* mutant
P6	50%	-	50%	-	*A. costaricaensis* mutant
P7	-	50%	50%	-	*S. pasteuri* mutant
P8	-	50%	50%	-	*A. costaricaensis* mutant
P9	50%	-	-	50%	*S. pasteuri* mutant
P10	50%	-	-	50%	*A. costaricaensis* mutant

Note: The use of urea fertilizer for all treatments 100%.

The fertilizer dose given in this study was based on the plant population approach. The fertilizers given were urea fertilizer (200 kg / ha), KCl (50 kg / ha), SP-36 (130 kg / ha), feldspar (600 kg / ha) and rock phosphate (200 kg / ha). The urea and KCl fertilizers were given twice. SP-36, feldspar, rock phosphate, and biofertilizer were given once. The biofertilizers used were *A. costaricaensis* and *S. pasteuri* mutants. Plant height was measured every two weeks until the maximum vegetative phase. Soil and other plant parameters observed were the height of plant, root, and shoot dry weight, available P in soil, exchangeable K in the soil. Measurement of available soil P used the Bray-I method and the absorbance of the sample was measured by UV-VIS 1280 Shimadzu spectrophotometry. Extraction on soil exchangeable K measurements used ammonium acetate 1 M pH 7.0 and sample emissions were measured using a Corning 405 Flame photometer (Santari et al., 2019).

Results and Discussion

Viability of P and K solubilizing A. costaricaensis **and** *S. pasteuri mutants in peat*

The data obtained in Figure 1 shows that the peat carrier material can maintain a stable fungal population up to 18 weeks. *A. costaricaensis* mutant shows the population log 6.85 (7.13 x 10^6 CFU/g) on Pikovskaya medium and log 6.83 (6.80 x 10^6 CFU/g) on Alexandrov's medium. Population decline occurred from week 20 on both Pikovskaya and Alexandrov mediums. Although there was a decrease in the population of *A. costaricaensis* mutant, it was still in the population range of 10^6 CFU/g. Peat as a good carrier material, presumably because it can adapt to changes in pH and water content, has a high water-holding capacity, non-toxic and has a strong buffering capacity (Mishra and Dadhich, 2010; Kaljeet et al., 2011; Herman and Lesuer, 2013). The peat carrier material can provide a suitable environment for microbes during storage before the inoculant is used. The data in Figure 2 show the bacterial population for 14 weeks. The population of *S. pasteuri* mutant on Pikovskaya medium as much as log 8.08 (1.22 x 10^8 CFU/g) and log 8.04 (1.08 x 10^8 CFU/g) on the Alexandrov medium.

At 6 and 8 weeks of storage time, there is a slight decrease in population. The population of bacteria stabilized again at 10 to 12 weeks of storage time. Meanwhile, at 14 weeks of storage time, the population had decreased. At 10 weeks until 14 weeks storage time, the morphology of the bacteria changes in the margin of the colony, which is round and smooth throughout to undulate. Changes that occur in bacteria are a way of adapting bacteria to the environment of the carrier material. The conditions of carrier material begin to be incompatible with bacteria to maintain their population. The results obtained are in line with the research of Feng et al. (2002) that some rhizobia species showed changes in morphology when inoculated from liquid culture into peat carrier materials. This is thought to be one of the rhizobia's efforts to increase the adaptability of the peat carrier material. Observations using transmission electron microscopy showed changes in the cell wall and the occurrence of blockage of the periplasmic space with electron-dense material. This was observed in *Rhizobium* sp cell strains of SU343 and B*radyrhizobium lupini* WU425 after 7 days and 14 days were inoculated on peat. Low nutrition and lack of oxygen also trigger this change. In the observations, it was also seen that there was the mobilization of polyhydroxybutyrate reserves after rhizobia were inoculated on peat. The increased expression of iron-manganese superoxide dismutase was also seen in the observation of rhizobia cells when inoculated into peat. All forms of adaptation carried out by microbes are thought to be able to survive and be resistant to various types of stress in the environment.

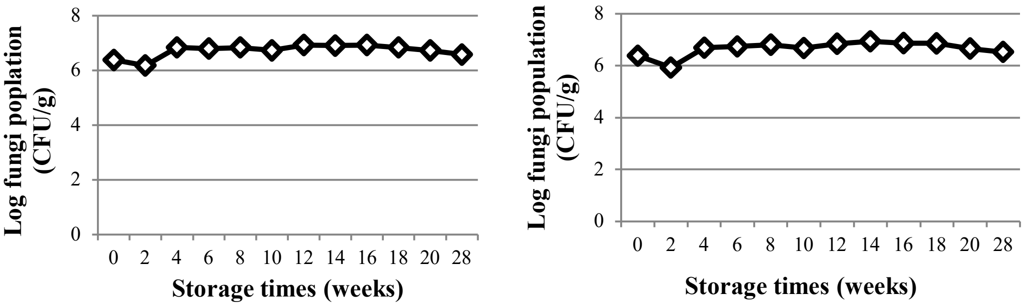

Figure 1. The population of fungi on peat carrier material for 28 weeks of storage. Total plate count on Pikovskaya medium (a) and Alexandrov medium (b).

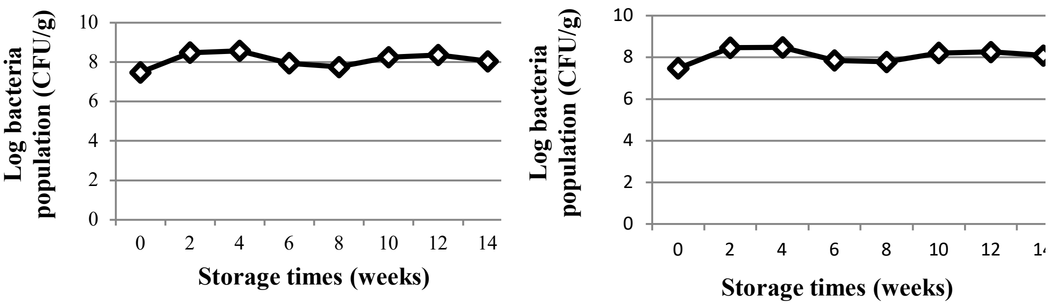

Figure 2. The population of bacteria on peat carrier material for 14 weeks of storage. Total plate count on Pikovskaya medium (a) and Alexandrov medium (b).

The effectiveness of P and K solubilizing A. costaricaensis and S. pasteuri mutants on the maize growth

Plant height, shoot dry weight, root dry weight of maize

The data presented in Table 2 show that the plant height parameter showed higher results in the treatments with the addition of *A. costaricaensis and S. pasteuri* than control. This result obtained in treatments using not-easily soluble P and K sources as well as in treatment with a combination of not-easily soluble P or K sources with easily soluble P or K sources. The shoot dry weight parameter (Table 2) also showed a tendency for higher results than control in the treatment with the addition of mutant isolates. The treatment with the addition of the *A. costaricaensis* mutant showed higher results than the control. Overall, the treatment showed that the highest shoot dry weight was the P10 treatment which was a combination of not-easily soluble P sources with easily soluble K sources (50% dose) which get the addition of *A. costaricaensis* mutant. P10 treatment was able to increase shoot dry weight 30.52%, root dry weight to 71.21% compared to control (P3) that using not-easily soluble P sources with easily soluble K sources (100% dose).The height of the plant and shoot dry weight parameters were influenced by the root dry weight parameters. This is because the higher root dry weight indicates, the wider spatial of the plant roots can reach and absorb nutrients (Tisdale et al., 1985). The nutrients absorbed will affect plant growth. The data in Table 2 show that the highest root dry weight was shown in control P4 treated with a combination of easily soluble P and K nutrient sources (dose 100%). However, the results obtained were not significantly different from treatment P5, P6, P8, and P10. The results indicated that the addition of the *A. costaricaensis* either in combination with both not-easily soluble P and K sources (50% dose) or a combination of not-easily soluble P or K sources with easily soluble P or K sources (50% dose) tended to keep up with the control treatment P4. The results indicated that the microbes applied had a positive effect, presumably because the microbes can colonize the roots to increase the availability and absorption of nutrients for plant growth. Based on the parameters of plant height, root dry weight and shoot dry weight, the addition of mutant microbes reduced the use of easily soluble P or K fertilizers with not-easily soluble P or K as well as not-easily soluble P and K combinations by up to 50%. The results appeared in the treatment with the addition of *A. costaricaensis* isolates. The results obtained were able to compensate for the control treatment using soluble P and K at a dose of 100%. The results obtained in this study are in line with the research of Viruel et al. (2014), who reported that P solubilizing bacteria could increase plant growth. This was indicated by a significant increase in plant height (45%), plant dry weight (40%) in the treatment of *Pseudomonas tolaasii* IEX.

Table 2. Effect of P and K solubilizing multifunctional microbe inoculation with different P and K sources on plant height, root dry weight and shoot dry weight at of maize (49 days after planting).

Treatments	**Plant height^a (cm)**	**Shoot dry weight^a (g)**	**Root dry weight^a (g)**
P1 (100% Rock Phosphate + Feldspar)^b	113.98 abc	15.63 a	3.21 a
P2 (100 % SP-36 + 100% Feldspar)^c	110.56 ab	32.25 bc	9.28 abc
P3 (100% Rock Phosphate + 100% KCl)^c	122.36 bc	29.19 bc	4.48 a
P4 (100% SP-36 + 100% KCl)^d	131.66 bc	31.21 bc	13.50 c
P5 (50% Rock Phosphate + 50% Feldspar + *S. pasteuri* mutant)	135.58 c	31.53 bc	8.45 bc
P6 (50% Rock Phosphate + 50% Feldspar + *A. costaricaensis* mutant)	96.67 a	34.77 c	6.12 abc
P7 (50% SP-36 + 50% Feldspar + *S. pasteuri* mutant)	134.03 bc	19.91 ab	5.87 a
P8 (50% SP-36 + 50% Feldspar + *A. costaricaensis* mutant)	138.27 c	31.73 bc	6.68 abc
P9 (50% Rock Phosphate + 50% KCl + *S. pasteuri* mutant)	134.46 bc	18.74 ab	3.90 a
P10 (50% Rock Phosphate + 50% KCl + *A. costaricaensis*)	126.11 bc	38.10 c	7.67 abc

^{a} Numbers followed by the same letters in the same column show not significantly different based on the DMRT test at the 5% level; ^{b} P1 is a control that used sources of not-easily soluble P and K; ^{c} P2 and P3 are controls that used a combination of not-easily soluble P or K with easily soluble P or K, and ^{d} P4 is a control with soluble P and K sources.

Based on field tests, the inoculation of isolates from the 1Exb strain combined with the addition of TSP fertilizer stimulated the total dry biomass of maize by 32%. This growth induced effect can be attributed to the potential for strains to increase nutrient availability such as P, siderophores, and phytohormone production (Viruel et al., 2011) as well as the capacity to colonize root systems and positive interactions with plants. Based on the research of Patil et al. (2012) also reported that inoculation of seeds with P solubilizing fungi with different P_2O_5 concentrations significantly affected plant height, number of leaves per plant, and dry weight production. Previous research related to the effect of the application of K solubilizing microbes on plant growth also showed results that were consistent with this study. Several studies have shown that inoculation of seeds and seedlings with K-solubilizing bacteria generally shows increased plant growth under greenhouse and field conditions (Awasthi et al., 2011; Lynn et al., 2013; Zhang et al., 2013; Zhang and Kong, 2014; Meena et al., 2014; Subhashini and Kumar, 2014; Meena et al., 2015; Anjanadevi et al., 2016).

Available P and exchangeable K in soil

The ability of mutant microbes to increase plant height, root dry weight, and shoot dry weight was influenced by the ability of these microbes to solubilize P and K. It makes nutrients became available for plants. The ability of mutant microbes to solubilize P and K nutrients when applied in the field can be seen through the available P in soil and exchangeable K in the soil. The available P indicates the soil P fraction that can be used by plants. The data obtained in Table 3 shows that the P5 and P10 treatments showed a higher P available in soil compared to the control. P5 treatment is a treatment that uses both sources of not-easily soluble P and K, which get the addition of *S. pasteuri* mutant. This P5 treatment shows that the added microbes can solubilize the not-easily soluble P and K sources (50% dose) into available in the soil. The highest result was shown by treatment P10. Treatment P10 is a treatment that uses a combination of not-easily soluble P with easily soluble K (50% dose). P10 treatment was able to increase P available soil 54.57% compared to control (P3).

The results obtained are in line with the measurement data for height parameters, shoot dry weight, and root dry weight in Table 2 which are classified as high compared to other treatments. Previous studies have also reported that the use of P solubilizing microbes can increase the availability of P in the soil, thereby increasing the uptake of P by plants which increases the growth and yield of various types of plants (Buntan, 1992; Premono et al., 1994; Elfianti, 2004; Singh and Reddy, 2011; Yu et al., 2012; Yadav et al., 2013). The data obtained on exchangeable K in soil (Table 3) shows the highest results in the P3 and P4 treatments. This is presumably because P3 and P4 treatments are controls that use easily soluble K source (KCl) at a dose of 100%. Other treatments approaching this result were P9 and P10 treatment which were combination treatments of not-easily soluble P with easily soluble K at a dose of 50%. A higher result was observed in P10 treatment. This is in line with the results obtained for the previously measured parameters, such as the height of the plant, shoot dry weight and root dry weight, which shows higher results than other treatments. Exchangeable K has a role in maintaining K levels in soil solution (Leiwakabessy, 2003). Exchangeable K can be a measure of K availability in the soil. Kirkman et al. (1994) reported that the application of K fertilization could be predicted based on the level of soil exchangeable K content. Therefore, the higher the exchangeable K content of the soil, the less amount of fertilizer that needs to be added. This shows that the P8 and P9 treatments have the potential to reduce the use of fertilizers, both rock phosphate and KCl.

Table 3. Effect of P and K solubilizing multifunctional microbes inoculation with different P and K sources on soil available P and exchangeable K.

Treatments	**Available P in soil (ppm)**	**Exchangeable K in soil (ppm)**
P1 (100% Rock Phosphate + Feldspar)[a]	6.50	58.5
P2 (100 % SP-36 + 100% Feldspar)[b]	6.30	50.7
P3 (100% Rock Phosphate + 100% KCl)[b]	8.96	120.9
P4 (100% SP-36 + 100% KCl)[c]	6.11	101.4
P5 (50% Rock Phosphate + 50% Feldspar + *S. pasteuri* mutant)	9.34	58.5
P6 (50% Rock Phosphate + 50% Feldspar + *A. costaricaensis* mutant)	4.92	42.9
P7 (50% SP-36 + 50% Feldspar + *S. pasteuri* mutant)	5.53	54.6
P8 (50% SP-36 + 50% Feldspar + *A. costaricaensis* mutant)	6.23	50.7
P9 (50% Rock Phosphate + 50% KCl + *S. pasteuri* mutant)	5.43	81.9
P10 (50% Rock Phosphate + 50% KCl + *A. costaricaensis* mutant)	13.85	89.7

a P1 is a control that uses both sources of not-easily soluble P and K.

b P2 and P3 are controls that use a combination of not-easily soluble P or K with easily soluble P or K .

c P4 is a control with easily soluble P and K sources.

The same microbial mutant gave different responses when combined with different P and K fertilizer sources. If the P and K sources are different, the P and K dissolution mechanism that occurs will also be different. Microbes will produce different organic acids depending on the P or K source used. The combination of not-easily soluble P and K sources or a combination of not-easily soluble P or K sources with easily soluble P or K sources is thought to influence this. When both P and K not-easily soluble used, the microbes have to work harder in dissolving P and K. In the combination of not-easily soluble P or K with easily soluble P or K sources, microbes can be more optimal in solubility increasing the P or K sources that are not-easily soluble. Therefore, in the measured parameters, the highest yield tends to be obtained in the treatment with a combination of not-easily soluble P sources with easily soluble K sources.

Conclusion

Peat carriers can keep the fungi population stable until 18 weeks of storage times. While the bacteria at 6 and 8 weeks storage times showed a slight decrease and stabilized in the 10 to 12 weeks storage time. The application of multifunctional microbes mutant P and K on the growth of maize in the field showed a positive effect. The addition of mutant microbes can reduce the use of fertilizer up to 50% in the treatment with a combination of easily soluble P or K sources with not-easily soluble P or K sources, as well as a combination of treatments of not-easily soluble P and K sources. This tended to occur in the treatment with the addition of *A. costaricaensis*. The application of P and K solubilizing multifunctional microbes can compensate for the control treatments that using easily soluble P and K sources (100% dose).

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