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Use of phosphorus- and potassium-solubilizing multifunctional microbes to support maize growth and yield

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ARTICLE INFO	ABSTRACT
Keywords:	Intensive chemical fertilizer use has led to environmental problems, ecological impacts,
Nutrient uptake	and dependence on chemical fertilizers. Microbial inoculants (biofertilizers) combined with
Biofertilizer	mineral fertilizers can be used to establish an environmentally friendly and sustainable
Viability	agricultural practice. This study aimed to observe the effectiveness of multifunctional
Plant support	microbes (S. pasteuri and A. costaricaensis) in their wild-type and mutant forms. The
Maize ear	microbes can simultaneously solubilize phosphorus and potassium from minerals (rock P
	and feldspar) to support maize growth and yield. Microbial viability in the zeolite carrier
Article history	was tested, and the treatment was applied to the field to determine the effect on maize
Submitted: 2021-12-24	growth and yield. The results showed that zeolite could maintain the microbe population
Accepted: 2022-04-17	at an average of 10 ⁸ CFU g ⁻¹ during 4 months of storage. A field test revealed that all
Available online: 2022-06-10	microbes treatments combined with minerals without the addition of chemical fertilizers
Published regularly: June 2022	could support maize growth and yield by producing maize ear. In particular, mutant A.
	costaricaensis can support dry stalk weight and maize ear length as effective as chemical
* Corresponding Author	fertilizers due to its ability to increase available P and exchangeable K in the soil. Overall,
Email address:	microbes could provide P but not K from the minerals and soil for plant uptake.
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1. INTRODUCTION

Since the Green Revolution in the late 1960s, the agricultural system in Indonesia has been intensively using chemical fertilizers to increase food yield and meet the needs of a growing population. However, this action has caused environmental problems and several ecological impacts, such as decreased soil fertility, nutrient deficiencies, and soil and water pollution (Shiva, 1991). BPS (2018) recorded that around 14 million hectares of land have entered a critical state until 2018. Given that critical land has an impact on decreasing crops, farmers became dependent on chemical fertilizers to improve their harvests.

Phosphorus (P) and potassium (K) are needed in large amounts after nitrogen but are minimally present in the soil. Tropical soils, such as those in Indonesia, are predominantly acidic and often extremely phosphorus deficient with high phosphorus sorption (fixation) capacities (Roy et al., 2017). Similar to potassium, the majority of K in the soil is present in a fixed form (non-available to plant indirectly) depending on the soil type, thus leading to K depletion in the soil system (Etesami et al., 2017). The addition of chemical fertilizers, especially SP-36 (source of P) and KCI (source of K), helped the plant grow but did not improve the soil properties. Furthermore, almost 75–90% of added phosphatic fertilizer is precipitated by the metal cation complexes present in the soils (Stevenson & Cole, 1986). Most soil types have P availability not exceeding 0.01% of the total P (Ginting et al., 2006). Similarly, the total K content is generally low at around 0.2–3.0% (El-Ramady et al., 2014).

Sustainable agriculture is a new approach that requires agricultural practices that are friendly to the environment and could maintain a long-term soil ecosystem. In this context, the use of microbial inoculants (biofertilizers), including phosphate- (PSM) and potassium-solubilizing microbes (KSM) combined with mineral fertilizers represents an environmentfriendly alternative. As a source of mineral fertilizers, rock P and feldspar can be the best choice in Indonesia because they are abundant in this country's soil. According to the Kemenesdm (2020), Indonesia has around 4 million tons of P rock reserves and 465 million tons of feldspar reserves. However, these minerals need a long dissolution period to be absorbed by plants. Hence, microbes are needed as an agent to speed up this process and maximize the absorption of chemical fertilizers to reduce environmental pollution and soil damage. PSM addition could reduce the use of SP-36 fertilizer by 25% in lowland rice using the SRI method (Puspitawati et al., 2014). Meena et al. (2014) confirmed that KSM could dissolve insoluble K sources such as feldspar and mica. Staphylococcus pasteuri and Aspergillus costariecaensis can simultaneously solubilize P and K and thus were named as multifunctional microbes (Sukmadewi et al., 2021).

This study aimed to explore the effectiveness of P- and Ksolubilizing multifunctional microbes (*A. costaricaensis* and *S. pasteuri*) from the previous study by Sukmadewi et al. (2021). Both microbe mutants used in the present work were irradiated with gamma rays at a dose of 2.5 kGy. In the previous study, the effectiveness was observed only in the vegetative phase. By contrast, the present work observed the effectiveness of the microbes in solubilize rock P and feldspar until the generative phase of maize.

2. MATERIAL AND METHODS

2.1. Test of microbe viability in zeolite

A viability test was conducted in Soil Biotechnology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University. Wild-type and mutant (gamma ray dose of 2.5 kGy) S. pasteuri and A. costaricaencis were obtained from the previous study by Sukmadewi et al. (2021). Zeolite was used as a microbe carrier, a component generally included in biofertilizer formulation to keep microbes alive and stable during storage. In brief 150 g of zeolite (water content 5.02%) was crushed to pass a 2 mm sieve, placed in a 250 ml Erlenmeyer flask, and sterilized using an autoclave at 121°C and 1 atm for 1 hour twice within 2 days. Afterward, 15 ml of several media each containing 2.4 × 10^8 CFU g⁻¹ wild-type *S. pasteuri*, 6.7×10^7 CFU g⁻¹ mutant *S.* pasteuri, 9.5×10^7 CFU g⁻¹ wild-type A. costaricaencis, and 5.0 \times 10⁷ CFU g⁻¹ mutant *A. costaricaencis* were individually inoculated to the sterilized zeolite. Microbes were counted at 7, 14, 21, 28, 64, 92, and 120 days after inoculation using total plate count method on nutrient agar and potato dextrose agar media.

Table 1. T	reatment	combinations	used in	ו the	study
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Code	Source P	Source K	Microbes
P1M0	SP-36	KCI	No microbes
P2M0	Rock P	Feldspar	No microbes
P2M1	Rock P	Feldspar	wild-type S. pasteuri
P2M2	Rock P	Feldspar	mutant S. pasteuri
P2M3	Rock P	Feldspar	wild-type A. costaricaencis
P2M4	Rock P	Feldspar	mutant A. costaricaencis

Remark: All treatments use the same dose of urea (N fertilizer)

2.2. Test of microbe effect on maize growth and yield

The effectiveness test was conducted in Cikabayan experimental field, IPB University. The soil was identified as latosol with average pH of 4.4. Treatment was arranged in six combinations (Table 1) in a single-factor randomized block design with four replications. Each plot had 30 plants in 2 m x 5 m size. The fertilizer dosages were 150 kg ha⁻¹ for KCl, 130 kg ha⁻¹ for SP-36, 1400 kg ha⁻¹ for feldspar, and 200 kg ha⁻¹ for rock P. Urea (N fertilizers) was given at the same dose of 300 kg ha⁻¹ for all treatments. Urea and KCl fertilizers were given twice at 7 days after planting (DAP) and 30 DAP. SP-36 fertilizer, feldspar, rock P, and biofertilizer (microbes in zeolite) were applied once at planting. Biofertilizer was given at 2 g per planting hole.

The plants were observed from the vegetative to the generative phase, and the soil was observed after harvesting. The variable parameters were plant height (cm) every 2 weeks until the maximum vegetative phase, root, and stalk dry weight (g), average maize ear weight after peeling (g), maize ear length (cm), maize ear diameter (cm), average 1000 dry seed weight (g), and P and K uptake (dry-ashing method using HClO₄ HNO₃). Soil parameters were total P, total K (Walkley and Black method), available P, available K (Bray I), and exchangeable K (N ammonium acetate pH 7). All P variables were measured using UV-vis 1280 Shimadzu spectrophotometry, and all K variables were measured using a Cornong 405 Flame photometer (Eviati & Sulaeman, 2009). Observation data were analyzed statistically with SAS software version 9.1 using ANOVA to determine the effect of single or combination of treatments on the measured parameters. A significant difference between treatments was determined. Further tests were carried out using Duncan Multiple Range Test at a level of 5%.

3. RESULTS

3.1. Viability of *S. pasteuri* and *A. costaricaensis* in zeolite carrier

During 4 months of storage, the zeolite carrier maintained all microbe populations at an average of 10^8 CFU g⁻¹. The final total microbe population in Fig. 1a revealed that the zeolite carrier could maintain the population of wild-type *S. pasteuri* up to log 8.9 (7.1 × 10^8 CFU g⁻¹) and mutant *S. pasteuri* up to log 8.6 (3.8×10^8 CFU g⁻¹). Fig. 1b also shows that the zeolite carrier could maintain the population of wild-type *A. costaricaensis* up to log 9.1 (1.2×10^9 CFU g⁻¹) and mutant *A. costaricaensis* up to log 8.7 (5.0×10^8 CFU g⁻¹).

3.2. Effect of *S. pasteuri* and *A. costaricaensis* on maize growth and yield

3.2.1. Plant height, stalk dry weight, and root dry weight of maize

Maize plant height, stalk dry weight, and root dry weight data showed significant difference under different fertilizer treatments (P < 0.01) (Table 2). Table 3 shows that all the plant parameters in the treatment with microbes were generally higher than those in the control negative group (not easily soluble P and K source without microbes) but not compared with those in the control positive group (easily soluble P and K source).



Figure 1. Population of S. pasteuri (a) and A. costaricaensis (b) in zeolite carrier during storage

Table 2. Effec	ct of S. pa	steuri and A.	costaricaensis	on all	parameters
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Treatment	Significance test <i>p</i> value	Treatment	Significance test <i>p</i> value
Height	<.0001**	population of K-solubilizing microbes	0.1051 ^{ns}
Stalk dry weight	0.0026**	P uptake	0.0108*
Root dry weigh	0.0052**	K uptake	0.0066**
Maize ear weight after peeling	<.0001**	P-total (P ₂ O ₅)	0.7208 ^{ns}
Maize ear length	0.0002**	P-available	0.0005**
Maize ear diameter	0.0015**	K-total (K ₂ O)	0.0695 ^{ns}
1000 dry seed weight	<.0001**	K-available	0.4125 ^{ns}
Population of P- solubilizing microbes	0.2155 ^{ns}	K- exchangeable	0.6037 ^{ns}

Remarks: ** Significant at the 0.01 level, * Significant at the 0.05 level, ^{ns} Not significant

Among the plants treated with microbe addition, those treated with mutant *A. costaricaensis* showed higher plant height than those treated with *S. pasteuri*. Meanwhile, the plants treated with *S. pasteuri* showed no significant difference from the control negative group.

The stalk dry weight of plants treated with mutant *A. costaricaensis* was not significantly different from that of the control positive group (Table 3). The control positive group had significantly higher root dry weight than all the other plant groups. Among the plants treated with microbe addition, those treated with mutant *S. pasteuri* tended to have high root dry weight.

3.2.2. Maize ear weight after peeling, maize ear diameter, maize ear length, and 1000 dry seed weight

Maize ear weight after peeling, maize ear diameter, maize ear length, and 1000 dry seed weight exhibited significant difference under different fertilizer treatments (P<0.01) (Table 2). Table 4 shows that the control positive group had the highest values of all the yield variables, which is in contrast to the control negative group that did not produce maize ears at all. The different types of maize ear vigor are presented in Fig. 2. The maize ear length of the plant treated with mutant *A. costaricaensis* was not significantly different from that of the control positive group. Among the plants treated with microbe addition, those treated with wild-type *A. costaricaensis* showed higher average values of maize ear weight, maize ear diameter, and 1000 dry seed weight than those treated with the other microbes.

3.3. P and K nutrient uptake by plant

Nutrient uptake data showed the amount of nutrients absorbed by plant biomass obtain by multiplying the total nutrient content with the total plant dry weight. P nutrient uptake significantly varied at the 0.05 level under different fertilizer treatments, and K nutrient uptake significantly varied at the 0.01 level under different fertilizer treatments (Table 2). The highest P and K nutrient uptake values were observed in the control positive group, and the lowest uptake values were found in the control negative group (Table 5). However, the P nutrient uptake of control positive group was not significantly different from those of other plant treatments with microbe addition, except for those added with wild-type *S. pasteuri*.

3.4. Effect of *S. pasteuri* and *A. costaricaensis* on the soil

Total PSM and KSM population showed no significant difference under various treatments (P > 0.05) (Table 2). As illustrated in Fig. 3, the addition of mutant *S. pasteuri* produced the highest value, and the addition of either mutant or wild-type *A. costaricaensis* generated the lowest average value. The PSM population appeared to be more dominant than the KSM population in the soil.

Analysis of soil P and K nutrients revealed that the treatments significantly affected the soil P-available (P < 0.01) but not the soil P-total (P > 0.05), K-total (P > 0.05), K-available (P > 0.05), and K-exchangeable (P > 0.05) as shown in Table 2. Mutant *S. pasteuri* treatment produced the significantly highest P-available (Figure 4a), which was in line with the data of total PSM and KSM population (Fig. 3).

Table 3 Effect of S	nasteuri and A	costaricaensis on	nlant hoight	stalk dry	woight	and root dry	woight
Table 5. Effect of 5.	pusteun and A.	costancaensis on	plant neight	, SLAIK ULY	' weight,	and root dry	/ weight

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Treatments	Height	(cm)ª	Stalk dry v ° (g)	veight	Root dry	weight ^a
			\6/		\6	
P1M0 (SP-36 + KCl) ^b	173.10	а	124.87	a	13,57	а
P2M0 (Rock P + Feldspar) ^c	65.42	с	10.46	C	2,00	b
P2M1 (Rock P + Feldspar + wild-type <i>S. pasteuri</i>)	89.59	bc	37.64	с	2,14	b
P2M2 (Rock P + Feldspar + mutant <i>S. pasteuri</i>)	91.51	bc	49.83	с	6,11	b
P2M3 (Rock P + Feldspar + wild-type A. costaricaens	is) 113.27	b	65.17	bc	4,94	b
P2M4 (Rock P + Feldspar + mutant A. costaricaensis)	112.09	b	106.24	ab	5,02	b

Remarks: ^aNumbers followed by the same letter in the same column show results that are not significantly different based on the DMRT test at a 5% significance level; ^b P1MO: control with easily soluble P (SP-36) and K (KCl) sources; ^c P2MO: control with not easily soluble P (rock P) and K (feldspar) sources



Note: P1M0 (SP-36 + KCl); P2M0 (Rock P + Feldspar); P2M1 (Rock P + Feldspar + wild-type *S. pasteuri*); P2M2 (Rock P + Feldspar + mutant *S. pasteuri*); P2M3 (Rock P + Feldspar + wild-type *A. costaricaensis*); P2M4 (Rock P + Feldspar + mutant *A. costaricaensis*)

Figure 2. Effect of S. pasteuri and A. costaricaensis on maize-cob production

The plants treated with the addition of wild-type *A. costaricaensis* had the highest average values of K-total, K-available (Fig. 4b), and K-exchangeable (Fig. 4c), which were in line with their high average values of plant height (Table 3), maize ear weight, maize ear diameter, 1000 dry seed weight (Table 4), and nutrient K uptake (Table 5).

4. DISCUSSION

The addition of A. costaricaensis, either mutant or wildtype, could better support maize growth than S. pasteuri or only mineral treatments (control negative). Although the treatment with chemical fertilizer (control positive) produced the highest values of all variables, microbe treatment supplemented only by mineral fertilizers generated almost equal results. Stalk dry weight and ear length in mutant A. costaricaensis treatment were not significantly different from those in chemical fertilizer treatment (Table 3). The average ear weight after peeling, ear diameter, and 1000 dry seed weight in wild-type A. costaricaensis treatment were the closest to those in chemical fertilizer treatment (Fig. 2). Treatment with only mineral fertilizers did not generate maize ears (Table 4), indicating that S. pasteuri treatments also could support maize ear production. The effectiveness of microbe inoculation can be evaluated on the basis of plant dry weight and yield. High values indicate the high ability of microbes to provide nutrients for plants. Microbes could colonize roots and are positively associated with other root-area microbes (Viruel et al., 2011).



- Notes: P1M0 (SP-36 + KCl); P2M0 (Rock P + Feldspar); P2M1 (Rock P + Feldspar + wild-type *S. pasteuri*); P2M2 (Rock P + Feldspar + mutant *S. pasteuri*); P2M3 (Rock P + Feldspar + wild-type *A. costaricaensis*); P2M4 (Rock P + Feldspar + mutant *A. costaricaensis*)
- Figure 3. Effect of *S. pasteuri* and *A. costaricaensis* on the total population of P- and K-solubilizing microbes in rhizosphere area



- Notes: P1M0 (SP-36 + KCl); P2M0 (Rock P + Feldspar); P2M1 (Rock P + Feldspar + wild-type *S. pasteuri*); P2M2 (Rock P + Feldspar + mutant *S. pasteuri*); P2M3 (Rock P + Feldspar + wild-type *A. costaricaensis*); P2M4 (Rock P + Feldspar + mutant *A. costaricaensis*)
- Figure 4. Effectiveness of *S. pasteuri* and *A. costaricaensis* on nutrient P (P-total (P₂O₅) and P-available) (a), nutrient K (K-total (K₂O) and K-available) (b) and K- exchangeable (c) of soil in rhizosphere area
- **Table 4.** Effect of *S. pasteuri* and *A. costaricaensis* on maize ear weight after peeling, maize ear diameter, maize ear length, and 1000 dry seed weight

	Maize ear	Maize ear	Maize ear	1000 Dry
Treatments	weight after	length	diameter	seed
	peeling (g) ^a	(cm) ^a	(cm)ª	weight (g) ^a
P1M0 (SP-36 + KCl) ^b	204.00 a	23.56 a	47.00 a	353.00 a
P2M0 (Rock P + Feldspar) ^c	0 e	0 с	0 d	0 d
P2M1 (Rock P + Feldspar + wild-type S. pasteuri)	30.00 de	15.15 b	20.80 c	88.75 c
P2M2 (Rock P + Feldspar + mutant S. pasteuri)	51.25 cd	14.73 b	26.50 bc	160.42 b
P2M3 (Rock P + Feldspar + wild-type A. costaricaensis)	131.25 b	16.25 b	34.40 b	204.67 b
P2M4 (Rock P + Feldspar + mutant A. costaricaensis)	72.50 c	25.83 a	25.83 bc	199.77 b

Remarks: ^aNumbers followed by the same letter in the same column show results that are not significantly different based on the DMRT test at a 5% significance level; ^b P1M0:control with easily soluble P (SP-36) and K (KCl) sources; ^c P2M0: control with not easily soluble P (rock P) and K (feldspar) sources

Stalk dry weight in mutant *A. costaricaensis* treatment was not significantly different from that in chemical fertilizer treatment, indicating that mutant *A. costaricaensis* could provide nutrients as much as chemical fertilizer to support stalk dry weight. A similar result was also reported by Sukmadewi et al. (2021), who found that treatment with mutant *A. costaricaensis* combined with rock P and KCl (50% dose) could produce the highest stalk dry weight. Stalk dry weight is the weight of stalk tissue that is not affected by plant water content. Although mutant *A. costaricaensis* can support the stalk dry weight as good as chemical fertilizer, it cannot support the plant height. Mutant *A. costaricaensis* could provide the nutrients responsible for building plant tissues but not those responsible for maintaining plant water content. Gebreslassie (2016) explained that K is needed by plants in water regulation. In plant growth, K will increase the turgor of the plant body, and plants will become resistant to stress. This turgor plant body is influenced by good water regulation in the plant body. Mutant *A. costaricaensis* can provide sufficient nutrients for plant growth except K. Sukmadewi et al. (2021) also reported that mutant *A. costaricaensis* tended to show the same effects on plant height, stalk, and root dry weight as the easily soluble P and K source control (chemical fertilizer) treatment. According to nutrient uptake data (Table 5), the P nutrient uptake showed no significant difference under mutant *A. costaricaensis* and chemical fertilizer treatments. *A. costaricaensis* could support the plant's P uptake from the soil as good as chemical fertilizer. Meanwhile, microbe treatments could not compete with chemical fertilizer treatments on providing K for plants.

Treatments	P uptake ^a (mg)	K uptake ^a (mg)
P1M0 (SP-36 + KCl) ^b	196.80 a	1756.47 a
P2M0 (Rock P + Feldspar) ^c	17.16 c	26.95 b
P2M1 (Rock P + Feldspar + wild-type S. pasteuri)	98.90 bc	133.88 b
P2M2 (Rock P + Feldspar + mutant S. pasteuri)	138.08 ab	204.44 b
P2M3 (Rock P + Feldspar + wild-type A. costaricaensis)	114.57 ab	326.45 b
P2M4 (Rock P + Feldspar + mutant A. costaricaensis)	178.31 ab	301.24 b

Table 5. Effect of S. pasteuri and A. costaricaensis on P and K nutrient uptake

Remarks: ^aNumbers followed by the same letter in the same column show results that are not significantly different based on the DMRT test at a 5% significance level; ^b P1M0:control with easily soluble P (SP-36) and K (KCl) sources; ^c P2M0: control with not easily soluble P (rock P) and K (feldspar) sources

The results above were correlated with the P and K properties in the soil data (Fig. 4). Soil analysis on P and K showed that P properties (P-total and P-available) (Figure 4a) were higher than K properties (K-total, K-available, and Kexchangeable) (Fig. 4b and Fig. 4c). Mutant S. pasteuri treatment generated significantly higher P-available than chemical fertilizer or other treatments. This result was in line with the total PSM data, which was also high. However, mutant S. pasteuri could not support maize growth and yield as good as A. costaricaencis treatment. The K content of the soil in the mutant S. pasteuri treatment was not as high as its P content, indicating nutrient imbalance. This phenomenon will not have a positive effect on plant development. Liebig's Law of Minimums states that plant growth is limited by the least amount of nutrients and also explains why plants could not use the N given in the same dose in all treatments. Plants cannot absorb N when P and K are insufficient. Similarly, plants cannot properly use N and P when K is insufficient. This phenomenon is reflected in the data on maize growth and yield. Previous (Sukmadewi et al., 2021) and present results confirmed that either A. costaricaensis or S. pasteuri could not provide available K from feldspar for plant uptake and in soil properties.

Only the negative controls did not produce maize ear (Table 4). This finding implied that the addition of multifunctional microbes could support maize yield by producing maize ears, but the effect was not as good as that of chemical fertilizer. Treatment with mutant *A. costaricaensis* also generated a good yield and almost the same maize ear length as that with chemical fertilizer. Other variables such as maize ear weight, maize ear diameter, and 1000 dry seed weight were the highest in wild-type *A. costaricaensis* treatment among the treatments with microbe addition.

The most desirable part of maize is the seeds. Table 4 shows that treatment with *A. costaricaensis* gave the highest 1000 dry seed weight, although the value was not as good as that from chemical fertilizer treatment. These results showed that even without the addition of chemical fertilizer, treatment with *A. costaricaensis* still showed satisfactory results. Therefore, the minimal addition of chemical elements (especially K) will give maximum results. A previous study also reported that according to their measured vegetative parameters, the highest yield was obtained from the treatment with a combination of rock P sources with chemical K fertilizers (Sukmadewi et al., 2021).

On the basis of the above data, *A. costaricaensis* (fungi) was better than *S. pasteuri* (bacteria) in supporting maize ear production. However, according to soil analysis data on total PSM and KSM population in Fig. 3, *A. costaricaensis* had lower population than *S. pasteuri* either in total PSM or KSM. Despite its lower population in the soil, the fungus was a better agent in solubilizing and providing P and K for plants than the bacteria. Dandessa and Bacha (2018) also reported that soil fungi are better PSM agents than bacteria. Among all the types of PSMs that have been reported, fungi have a higher ability to dissolve insoluble P than bacteria (Klaic et al., 2017). Especially in acid soils, the activity of microorganisms is dominated by fungi and decreases with the increase in pH (Flatian et al., 2020; Ginting et al., 2006).

The multifunctional microbes A. costaricaensis and S. pasteuri support plant growth through two mechanisms. First, these microbes act as solvents for minerals (rock P and feldspar). P and K become available and then absorbed by plants to be used as materials for photosynthesis. Microbesecreted organic acids, such as gluconic acid, oxalic acid, and citric acid, dissolve and release P, which is previously bound to metal compounds (Ingle & Padole, 2017). Similarly, the K dissolution mechanism occurs through the production of organic and inorganic acids and protons (acidolysis mechanism). The organic acids that can effectively dissolve K include oxalic acid, tartaric acid, and gluconic acid (Etesami et al., 2017). Second, PSM and KSM produce phytohormones that stimulate cell elongation at the growing point. According to Astriani et al. (2020), PSM produces indole acetic acid, which functions to stimulate cell elongation at the growing point. Viruel et al. (2011) also argued that P-solubilizing bacteria have the potential to produce phytohormones and siderophores.

As effective biofertilizers, the microbes in this study, especially *A. costaricaensis*, needed a suitable carrier to maintain their population. The zeolite used as carrier material showed a good performance in maintaining total microbe viability (Fig. 1) at an average of 10⁸ CFU/cell. Several studies also reported that zeolite was better at maintaining microbe viability than husk charcoal, and limestone (Ishaq et al., 2021).

Microbe population of 10⁸ CFU/cell is categorized as a high population range and sufficient to be applied in the field according to Kementan (2019). The zeolite used in this study has a pH of 8.5, which is considered ideal for most microbes to live, and therefore could maintain the microbe population on a high average. Zeolite is easy to sterilize, free from toxic materials, economical, and environmentally friendly characteristics that are ideal for a biofertilizer carrier (Malusá et al., 2012).

5. CONCLUSION

Multifunctional microbes combined with mineral fertilizers could support maize growth and yield under field conditions. In particular, mutant *A. costaricaensis* yielded similar results on dry stalk weight and ear length to chemical fertilizer treatment. Zeolite is an ideal biofertilizer carrier that is economical and environmentally friendly.

Multifunctional microbes could not provide sufficient K nutrients for plants and thus could not produce maize ear as good as that under chemical fertilizer treatments. Further research is needed for these microbes with the addition of K fertilizer (chemical and organic) under field conditions.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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