Fitriatin BN · Budiman MN · Suryatmana P · Kamaluddin NN · Ruswandi D

Phosphate availability, P-uptake, phosphatase, and yield of maize (*Zea mays* L.) affected by kaolin based P-solubilizer and P fertilizer in Inceptisols

Abstract. Inceptisols have problem in phosphate availability. Soil P content is very low available to plants because it is bound by soil colloids. One of the efforts to increase the P nutrient in the soil in a sustainable way by using P-Solubilizers that can dissolve phosphate in the soil so that it is available for plants. The purpose of experiment was to determine the effect of the combination dose of kaolin based P-Solubilizer and P fertilizer for improving P availability, P uptake, phosphatase, and maize yield on Inceptisols. The kaolin-based P-Solubilizer was used a consortium of phosphate solubilizing microbes (PSM) consisting of *Bacillus subtilis*, *Burkholderia cepacea, Pseudomonas mallei*, and *Trichoderma asperellum*. This experiment was conducted in the experimental field of the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, from July to December 2021. The experiment used a randomized block design (RDB) method with nine treatments and three replications, with details of 0 P-Solubilizer + 0 P-fertilizer; 100% P-fertilizer; 100% P-solubilizer; and combination 50%, 75 %, 100%, and 150% P-solubilizer with 50%, 75%, and 100% P-fertilizer. P-solubilizer 100% recommended dose 50 kg ha^{-1} and P-fertilizer recommended dose 100 kg ha^{-1}. The results showed that the dose of 100% P-Solubilizer (50 kg ha^{-1}) + 75% P (75 kg ha^{-1}) showed the best results in increased P-availability (346,93%), P-uptake (312,5%), Phosphate activity (33,5%), and maize yields (48,09%) compared to without application of P-solubilizer and P-fertilizer. This consortium isolate could be developed as a P-Solubilizer with the ability to increase the efficiency of P up to 25%.

Keywords: *Burkholderia* · Efficiency · Microbes · P-solubilizing · *Trichoderma*

Submitted: 14 November 2022, Accepted: 18 March 2023, Published: 17 April 2023
DOI: http://dx.doi.org/10.24198/kultivasi.v22i1.42847

Fitriatin BN[1] · Budiman MN[2] · Suryatmana P[1] · Kamaluddin NN[1] · Ruswandi D[3]
[1] Department of Soil Science and Land Resources Management, Faculty of Agriculture, Universitas Padjadjaran – Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia
[2] Study Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran– Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia
[3] Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran – Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia
* Corresponding author: betty.natalie@unpad.ac.id

Fitriatin BN, Budiman MN, Suryatmana P, Kamaluddin NN, Ruswandi D. 2023. Phosphate availability, P-uptake, phosphatase, and yield of maize (*Zea mays* L.) affected by kaolin based P-solubilizer and P fertilizer in Inceptisols. Jurnal Kultivasi, 22(1): 71-76

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Maize (*Zea mays* L.) is the second staple commodity after rice in Indonesia. Apart from being a food commodity, maize is an important raw material for animal fodder. The need for fodder maize has not been fully met by domestic maize production, most of it comes from imports. One of the efforts that can be made to increase maize production is by extensification. In general, maize plants can grow in almost every type of soil. Inceptisols are one type of agricultural soil in Indonesia that has the widest distribution in Indonesia. However, this land has problems in its utilization, especially its low fertility rate (Muslim et al., 2020). To overcome the problem of soil fertility, biofertilizer can be applied to inceptisols to reduce the use of chemical fertilizers.

Biofertilizers are fertilizers that contain beneficial microbes that can facilitate the availability of nutrients for plants that are environmentally friendly (Kumar et al., 2022). One of the biofertilizers that can increase the availability of soil phosphate is phosphate solubilizing microbes. Phosphate solubilizing microbes (PSM) are soil microbes that can to release P from bonds with Al, Fe, Ca, and Mg, so that it can dissolve P that was originally unavailable to plants to become available to plants (Alori et al., 2017; Tian et al., 2021). This is because the PSM secrete organic acids which can form stable complexes with P-binding cations in the soil. In this study, PSM isolates were used consisting of *Bacillus subtilis, Pseudomonas mallei, Burkholderia cepacia,* and the fungus *Trichoderma asperellum.* Recently, the genus *Burkholderia* has become important as a solvent microbe (Moreno-Conn et al., 2021).

Using the PSM consortium as a biofertilizer is expected to increase the availability of soil P to be absorbed by plants to increase fertilization efficiency while reducing the use of phosphate chemical fertilizers. Sarmah and Sarma (2022) stated that the application of phosphate-dissolving microbial biofertilizers can increase nutrient availability, plant production and be able to substitute inorganic P fertilizers. This is in line with the results of research by Timofeeva et al. (2022) that PSM can increase soil available P, soil fertility, and crop production in a sustainable way.

The effectiveness of microorganisms used as phosphate-solubilizing microbes depends on environmental factors, survival in the soil, formulation quality, and applications (Raymond et al., 2021). Bacterial and fungal cells can be immobilized in solid carriers for preservation and protection from the external environment. Carriers have an important role in maintaining the effectiveness and survival of microbes during storage (Aksani et al., 2021). According to the results of a study by Herrera-Téllez et al., (2019), kaolin as a carrier was able to maintain the viability of *Trichoderma asperellum* around 1×10^7 CFU g^{-1} soil, and this level was maintained throughout the experiment (90 days), indicating the retention and survival of *T. asperellum* which is optimal in kaolin formulations.

Based on the description above, the application of kaolin-based P-solubilizer combined with P fertilizers in this study was expected to influence P availability, P uptake, phosphatase activity, and maize yields in Inceptisols.

Materials and Methods

This experiment was conducted in the experimental field of the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, from July to December 2021. P-solubilizers were made at the Soil Biology Laboratory, Faculty of Agriculture, Padjadjaran University. Maize seeds used the Padjadjaran 1 variety. The PSB isolates used in this study were (*Bacillus subtilis, Burkholderia cepacia*, and *Pseudomonas mallei*) collection of Laboratory of Soil Biology Faculty of Agriculture, Universitas Padjadjaran, and the PSF isolates (*Trichoderma asperellum*) collection of Laboratory PT. Agritek Tani Indonesia. Carrier that P-solubilizer used is 100 g sterilized kaolin meanwhile inorganic fertilizer that used is Urea (46% N), SP-36 (36% P_2O_5), and KCl (50% K_2O), as well as manure from sheep manure 2 ton ha^{-1}.

The experiment used a randomized block design (RDB) consisting of nine treatments and three replications for each treatment. The treatments were: 0% P-solubilizer + 0% P (without P-solubilizer and without P-fertilizer); 100% P-fertilizer; 100% P-solubilizer; and combination 75 % and 100% P-fertilizer with 50%, 75%, and 100% P-solubilizer. P-solubilizer 100% recommended dose 50 kg ha^{-1} and P-fertilizer recommended dose 100 kg ha^{-1}.

P-Solubilizer Preparation. PSMs isolate were refreshed in agar medium (NA and PDA). Isolate propagation in NB (1% (v/v)) incubated for 3-5 days. Inoculation into the bulking media (mixture of molasses (5%) and potato extract 2:1) and incubated for 3 days. Inoculation into 100g sterile kaolin (carrier) (5% v/w), which was incubated for 5 days. PSMs population in biofertilizer counted by the Dilution Plate Count method.

Application of treatments. Two seeds of maize in a planting hole by 5 cm deep. P-solubilizer dissolved in water with a concentration of 5 $g.L^{-1}$; therefore P-solubilizer was applied directly into the planting hole with a dose according to the treatment. The treatment of inorganic P Super Phosphate 36 (P) was carried out at 1 WAP with a dose according to the treatment. Inorganic fertilizer such as Urea fertilizer at a dose of 350 kg ha^{-1} was applied 25% at 1 week after plant (WAP), 50% at 4 WAP, and 25% at 6 WAP. KCl fertilizer at a dose of 50 kg ha^{-1} applied at 75% at 1 WAP and the remaining 25% at 4 WAP recommended dose (Ministry of Agriculture, 2020). Observations were made every two weeks until 8 WAP.

Soil and Plant Sampling. P-availability, phosphatase activity, P uptake, and maize yield components were further conducted. Soil sampling was performed a week before planting and when plants reached their peak vegetative phase (56 days after planting). Analysis of the soil biological and chemical properties was taken from soil around the roots (rhizosphere).

Phosphatase enzyme activity was determined according to Eivazi and Tabatabai method. p-nitrophenyl was added to the substrate to form p-nitrophenol compound through enzyme activity. Then, it was consecutively stained by sodium hydroxide solution, which can be detected by 400 nm spectrophotometer.

P-Availability was determined according to Olsen and Bray I method. Soil samples from the field were air-dried, ground, sifted using a 2 mm sieve, and then put in a labeled plastic bag. Analysis of soil characteristics was carried out on soil properties that were thought to be closely related to soil P availability. The P content in the soil was estimated using 25% HCl extractor, Olsen, and Bray I.

Sampling for P uptake was carried out during the maximum vegetative period. The plant sample taken for analysis of plant P uptake was the 4^{th} leaf which is assumed to be an indicator leaf. The 4^{th} leaves were cleaned of adhering dirt, then air-dried, then cut into pieces, and dried using an oven at 87^0C. The dried leaves were then crushed using a grinder machine with a fineness of 0.5 mm and put into a film bottle, and labeled according to the treatment for further analysis in the laboratory. Plant P content was analyzed by the Kjeldahl method.

Harvesting was done when the cobs or husks were dry, the seeds were shiny, hard, and when pressed with a fingernail, it didn't leave an impression or at 99 days after planting (DAP). Furthermore, dried for 2 days and peeled, maize yield components as dry weight cob stated in dry weight/plant.

The data were analyzed by means of variance (ANOVA) using SPSS 25.0; for treatments that had a significant effect, Duncan's multiple distance test was carried out at a significance level of 5%.

Results and Discussions

Soil P-Availability. The results of laboratory analysis of the value of soil P availability showed in Table 1. The results of the statistical analysis showed that the effect of the application of kaolin biofertilizer combined with P fertilizer had a significant effect on the availability of P in the soil. The highest available P content was indicated by treatment 50% P-Solubilizer + 75% P. his treatment showed the highest available soil P among all treatments, namely 17.05 ppm P, an increase of 451.78% compared to the 0% P-solubilizer + 0% P, while the 0% P-solubilizer + 0% P treatment showed the lowest. This is consistent with the statements of Amri et al. (2022), P-solubilizers with consortium PSM can increase the availability of P to plants, thus promoting plant growth and making the use of inorganic fertilizers more efficient.

As shown in Table 1, overusing biofertilizers did not significantly affect the availability of P. Application of P-fertilizers only increases yield to an optimal point. It is believed that the application thickens the soil solution and prevents it from being taken up by plants. This was in line with Barłóg et al., (2022), who stated that even nutrients contained in fertilizers are not available to plants because over-fertilization leads to lower plant growth and

ISSN: 1412-4718, eISSN: 2581-138x

enriches the soil solution. High P content in soil interferes with the uptake of other elements in the soil and hinders plant growth.

Table 1. Effect of biofertilizers on soil P–availability and P-uptake

Treatments	P availability (ppm P)	P Uptake (g plant^{-1})
0% P-solubilizer + 0% P	3.09 a	0.80 a
100% P	7.21 bc	1.43 b
100% P-Solubilizer	9.40 cd	1.36 b
100% P-Solubilizer + 50% P	14.40 e	2.82 e
100% P-Solubilizer + 75% P	13.81 e	3.30 f
100% P-Solubilizer +100%P	10.73 de	1.90 c
50% P-Solubilizer + 75% P	17.05 f	2.45 d
75% P-Solubilizer + 75% P	12.41 de	2.51 de
150% P-Solubilizer + 75% P	12.25 de	2.82 e

Description: The same letter in the same column shows no significant difference according to the DMRT test with a level of 5%

P-Uptake. Statistical test results showed that applying a combination of P-solubilizer and P fertilizer at several dosage combinations, shown in Table 1, had a significant impact on maize's ability to uptake P nutrients. The 100% P-Solubilizer + 75% P treatment increased P-uptake by 312.5% compared to 0% P-solubilizer + 0% P treatment. This is caused by P-solubilizer containing phosphate-soluble microorganisms, whose populations are fairly high at ± 10^{12} cfu g^{-1}, and can dissolve nutrient P for plant uptake. In addition to being a phosphate-soluble microorganism used in this experiment also have properties such as plant growth-promoting rhizosphere bacteria (PGPR).

PGPR stimulates root growth through the production of phytohormones (auxin, IAA), secondary metabolites, and enzymes Chandran et al. (2021). In line with the study by Shen et al. (2018), increased plant P uptake is known to be affected by P availability, root spread, and root P uptake capacity. According to Lugli et al. (2020), phosphorus uptake is highly dependent on root contact with phosphorus in dissolved soil, and the distribution of roots in soil can be very significant to increase phosphorus uptake and plant dry weight. Phosphorus uptake by maize roots is affected by the type of root and the type of soil supplied with phosphorus (Gong et al., 2022).

Activity of Phosphatase. Based on data in Table 2 showed that the application of the P-solubilizer + P fertilizer between different doses of treatment did not show a significant difference in the phosphatase activity. However, the application of a dose of 100% P-solubilizer + 75% P and 100% P-solubilizer + 100% P showed the activity of phosphate increasing. although statistically is not significant. The application of a dose 100% P-solubilizer + 75% P and 100% P-solubilizer + 100% P by increasing 33.5% and 36% compared to 0% P-solubilizer + 0% P treatment.

The increasing phosphatase activity shows the efficiency and effectiveness of nutrient uptake (Janes-Bassett et al., 2022). Without P-solubilizer the phosphatase activity tends to be lower, while in the treatment of 150% biological fertilizer + 75% P the phosphatase activity also tended to be low, this indicates that the addition of more than 100% biological fertilizer did not increase the phosphatase activity.

Fitriatin et al. (2020) revealed that PSM isolates in various rhizospheres have the ability to dissolve P by producing organic acids, phosphatase enzymes and phytohormones. Phosphatase enzymes dissolve insoluble cation-bound P complexes, making them available for plant uptake. Secretion and phosphatase activity are ways in which some microbes and plants respond to soil acidity and P deficiency (Wu et al., 2018; Nannipieri et al., 2021)

Table 2. Effect of biofertilizers on phosphate activity

Treatments	Phosphatase (μ g^{-1} h^{-1})
0% P-solubilizer + 0% P	15.11 a
100% P	18.27 ab
100% P-Solubilizer	14.81 a
100% P-Solubilizer + 50% P	18.07 ab
100% P-Solubilizer + 75% P	20.18 b
100% P-Solubilizer + 100% P	20.56 b
50% P-Solubilizer + 75% P	16.20 ab
75% P-Solubilizer + 75% P	17.54 ab
150% P-Solubilizer + 75% P	17.45 ab

Description: The same letter in the same column shows no significant difference according to the DMRT test with a level of 5%

Yields of Maize Plants. Based on statistical test showed that the application of kaolin based biofertilizer and P fertilizer had a significant effect on the dry weight of maize (Table 3). The increase of dry weight cob per hectare reached 48,09% compared to 0% P-solubilizer + 0% P treatment and 15.8% from the average dry harvest weight of the Padjadjaran 1 variety of 11.92 ton ha^{-1}.

Table 3. Effect of biofertilizers on maize yield components

Treatment	Maize Yields Components	
	Weight of 100 Seeds (g))	Dried weight cob (ton Ha^{-1})
0% P-solubilizer + 0% P	24.20 a	8.32 a
100% P	28.61 bc	9.84 bc
100% P-Solubilizer	26.76 ab	9.21 ab
100% P-Solubilizer + 50% P	34.13 d	11.74 d
100% P-Solubilizer + 75% P	40.15 e	13.81 e
100% P-Solubilizer + 100% P	39.20 e	13.48 e
50% P-Solubilizer + 75% P	31.90 cd	10.97 c
75% P-Solubilizer + 75% P	26.53 ab	9.13 ab
150% P-Solubilizer + 75% P	31.40 cd	10.80 cd

Description: The same letter in the same column shows no significant difference according to the DMRT test with a level of 5%

Dry harvest weight maize on treatment 100% P-Solubilizer + 75% P reached 13.81 ton ha$^{-1,}$ and 100% P-Solubilizer + 100% P reached 13.48 ton ha^{-1}. From an economic point of view, providing 100% P-solubilizer + 75% P treatment has more benefits for farmers to save cost of fertilizer procurement compared to using just P-solubilizer or just P-fertilizer.

Increasing the P-availability in the soil will optimize the supply of phosphorus nutrients for plants which are useful for increasing the rate of photosynthesis and accumulation of dry matter after the flowering phase. This has a positive impact on increasing the yield of maize seeds (Zhu et al., 2012). During the generative period, especially during the seed filling phase, sufficient availability of phosphorus nutrients is required so that maximum maize yields will be obtained (Khan et al., 2014).

Conclusion

The results showed that the application of kaolin-based biofertilizer and P fertilizer (SP-36) increased the availability of P, P uptake, and components of maize yields, but had no significant effect on the soil phosphatase activity. The application of 100% P-solubilizer + 75% P gave the best yield of 13.81 tons ha^{-1} that increased up to 15.8% of the average dry harvest weight of the Padjadjaran 1 variety of 11.92 ton ha^{-1}. This consortium isolate can be developed as a P-Solubilizer with the ability to increase the efficiency of P up to 25%.

Acknowledgment

This research was funded by Directorate General of Higher Education Ministry of Research and Technology Indonesia with the applied research scheme for the fiscal year 2021 (1207/UN6.3.1/PT.00/2021). The authors are grateful to Rector, Dean of the Faculty of Agriculture, Director of Research and Community Service Universitas Padjadjaran. The authors would like to thank staff Laboratory of Soil Biology Faculty of Agriculture. Universitas Padjadjaran for their cooperation. We also thank to Mega Kartika Hermawan, SP., MP. who helped with this research.

References

Aksani D, Surono, Ginting RCB, Purwani J. 2021. The assay of carrier material and bacteria isolate formula as a P-Solubilizer on soybean in Inceptisols from West Java. IOP Conference Series: Earth and Environmental Science, 648(1).

Alori ET, Glick BR, Babalola OO. 2017. Microbial Phosphorus Solubilization and Its Potential for Use in Sustainable Agriculture. Frontiers in Microbiology, 8(971): 1-8.

Amri M, Mateus D, Gatrouni M, Rjeibi MR, Asses N, Abbes C. 2022. Co-Inoculation with Phosphate-Solubilizing Microorga-nisms of Rock Phosphate and Phosphogypsum and Their Effect on Growth Promotion and Nutrient Uptake by Ryegrass. Appl. Biosci., 1: 179–197. doi.org/10.3390/applbiosci1020012

Barłóg P, Grzebisz W, Łukowiak R. 2022. Fertilizers and fertilization strategies mitigating soil factors constraining efficiency of nitrogen in plant production. Plants, 11, 1855. doi.org/10.3390/plants11141855

Chandran H, Meena M, Swapnil P. Plant growth-promoting rhizobacteria as a green alternative for sustainable agriculture. Sustainability. 13 : 10986. doi.org/10.3390/su131910986

Fitriatin BN, Fauziah D, Fitriani FN, Ningtyas DN, Suryatmana P, Hindersah R, Setiawati MR, Simarmata T. 2020. Biochemical activity and bioassay on maize seedling of selected indigenous phosphatesolubilizing bacteria isolated from the acid soil ecosystem. Open Agriculture, 5(1): 300-304.

Gong H, Xiang Y, Wako BK, Jiao X. 2022. Complementary effects of phosphorus supply and planting density on maize growth and phosphorus use efficiency. Front. Plant Sci., 13: 983788. doi: 10.3389/fpls.2022.983788

Herrera-Téllez VI, Cruz-Olmedo AK, Plasencia J, Gavilanes-Ruíz M, Arce-Cervantes O, Hernández-León S, Saucedo-García M. 2019. The protective effect of *Trichoderma asperellum* on tomato plants against *fusarium oxysporum* and *botrytis cinerea* diseases involves inhibition of reactive oxygen species production. International Journal of Molecular Sciences, 20(8): 1-13.

Janes-Bassett V, Blackwell MSA, Blair G, Jess Davies J, Haygarth PM, Mezeli MM, Stewart G. 2022. A meta-analysis of phosphatase activity in agricultural settings in response to phosphorus deficiency. Soil Biology and Biochemistry. 165: 108537. doi.org/10.1016/j.soilbio.2021.108537

Khan MS, Zaidi A, Musarrat J. 2014. Phosphate Solubilizing Micro-Organisms: Principles and Application of Microphos Technology. Springer Cham. Springer International Publishing Switzerland.

Kumar S, Diksha, Sindhu SS, Kumar RR. 2022. P-Solubilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Current Research in Microbial Sciences, 3: 100094.

Lugli LF, Andersen KM, Aragão LEOC, Cordeiro AL, Cunha HFV, Fuchslueger L, Meir P, Mercado LM, Oblitas E, Quesada CA, Rosa JS, Schaap KJ, Valverde-Barrantes O, Hartley IP. 2020. Multiple phosphorus acquisition strategies adopted by fine roots in low-fertility soils in Central Amazonia. Plant Soil, 450: 49–6. doi.org/10.1007/ s11104-019-03963

Ministry of Agriculture. 2020. Rekomendasi Pupuk N, P, dan K Spesifik Lokasi untuk Tanaman Padi, Jagung, dan Kedelai pada Lahan Sawah (Per Kecamatan) Buku II : Jagung. Kementerian Pertanian. Jakarta.

Moreno-Conn LM, López-Casallas M, Barrera FMC. 2021. Phosphate solubilization by *Burkholderia* species isolated from Oxisols from the Colombian high plains. Ciencia y Tecnología Agropecuaria, 22(2): 1897. https://doi.org/10.21930/rcta.vol22_num2_art:1897.

Muslim RQ, Kricella P, Pratamaningsih MM, Purwanto S, Suryani E, Ritung S. 2020. Characteristics of Inceptisols derived from basaltic andesite from several locations in volcanic landform. Sains Tanah – J. of Soil Science and Agroclimatology, 17(2): 115-121.

Nannipieri P, Giagnoni L, Landi L, Renella G. 2021. Role of phosphatase enzymes in soil. In Phosphorus in Action. Springer, Berlin, Heidelberg, 215-243.

Raymond NS, Gomez-Munoz B, van der Bom FJT, Nybroe O, Jensen LS, Muller-Stover, DS, Oberson A, AE Richardson. 2021. Phosphate-solubilising microorganisms for improved crop productivity: a critical assessment. New Phytologist, 229: 1268–1277

Sarmah R, Sarma AK. 2022. Phosphate Solubilizing Microorganisms: A Review. Communications In Soil Science and Plant Analysis. https://doi.org/10.1080/00103624.2022.2142238.

Tian J, Ge F, Zhang D, Deng S, Liu X. 2021. Roles of phosphate solubilizing microbes from managing soil phosphorus deficiency to mediating biogeochemical p cycle. Biology, 10: 158. doi: 10.3390/Biology10020158

Timofeeva A, Galyamova M, Sedykh S. 2022. Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. Plants, 11: 2119. doi.org/10.3390/plants11162119

Wu L, Kobayashi Y, Wasaki J, Koyama H. 2018. Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil Science and Plant Nutrition, 64 (6): 697–704.doi.org/10.1080/00380768.2018.153709

Zhu, X-k, Li C-y, Jiang Z-q, Huang L-l, Feng C-n, Guo W-s, & Peng Y-x. 2012. Responses of Phosphorus Use Efficiency, Grain Yield, and Quality to Phosphorus Application Amount of Weak-Gluten Wheat. Journal of Integrative Agriculture, 11(7): 1103-1110.