EFFECT OF PHOSPHATE-SOLUBILIZING BACTERIA ISOLATES IN ACID SOIL ON SOYBEAN (*GLYCINE MAX* L.) SEED YIELD

WIDODO, Y.[1*] – PURWANINGRAHAYU, R. D.[1] – HARNOWO, D.[1] – HARSONO, A.[1] – KRISDIANA, R.[2] – KUNTYASTUTI, H.[1] – ROZI, F.[3] – MEJAYA, M. J.[1*] – ARSANA, I. G. K. D.[1] – ASNAWI, R.[2] – ARIEF, R. W.[4]

[1]*Research Center for Food Crops – Research Organization for Agriculture and Food – National Research and Innovation Agency (BRIN), Jalan Raya Jakarta-Bogor KM. 46, Bogor, West Java 16911, Indonesia*

[2]*Research Center for Behavioral and Circular Economics – Research Organization for Governance, Economy and Community Welfare – National Research and Innovation Agency (BRIN), Jalan Gatot Subroto No. 10 South Jakarta, Jakarta 12710, Indonesia*

[3]*Research Center for Macro Economics anf Finance – Research Organization for Governance, Economy and Community Welfare – National Research and Innovation Agency (BRIN), Jalan Gatot Subroto No. 10 South Jakarta – Jakarta 12710, Indonesia*

[4]*Research Center for Agroindustry – Research Organization for Agriculture and Food – National Research and Innovation Agency (BRIN), Jl. Kawasan Puspitek, South Tangerang City, Banten 15314, Indonesia*

[*]*Corresponding authors*
e-mail: yudi_atas@yahoo.com; mmejaya@yahoo.com

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Abstract. Acidic soil is generally poor in macro nutrients, especially phosphorus (P) which is the second major nutrient after nitrogen needed for crop production. The objective of this study was to identify efficient isolates of phosphate solubilizing bacteria (PSB) in acid soils to improve soybean productivity. Two experiments were carried out: one in the greenhouse of ILETRI and the other, a field experiment, in the acid soil of Tanah Laut Two experiments were carried out namely in the greenhouse of Indonesian Legumes and Tuber Crops Research Institute (ILETRI) and the field experiment in acid soil of Tanah Laut, South Kalimantan, Indonesia. The treatments were arranged in a split-plot design with three replications. The main plots were the equivalent dose of lime in the form of dolomite: 0; 400; and 800 kg ha^{-1} dolomite. Sub plots consisted of two factors: factor 1 was P fertilizer equivalent to 0; 100; and 200 kg SP36 ha^{-1}, and factor 2 was inoculation of PSB isolates i.e. without inoculation, isolate Lampung-1, isolate Lampung-2 and isolate Lampung-3. The result of the greenhouse study revealed that, isolate Lampung-2 increased seed yield by 18%, and when combined with dolomite equivalent to 800 kg ha^{-1} it increased yield by 40% compared to control (without dolomite and P-solubilizing bacteria). Application of Lampung-2 + 200 kg ha^{-1} SP36 gave the highest yield and yield increase of 45% relative to 0 kg ha^{-1} SP36 un-inoculated as controls. The increase in yield was supported by an increase in plant P uptake, which was highest in Lampung-2 isolates. The result of the field experiment showed that at fertilization dose of 100 kg ha^{-1} SP36, Lampung-2 was able to increase yield by 27% (equivalent to seed yield obtained from 200 kg ha^{-1} SP36), followed by Lampung-1 with 18.5%, over the control treatment. The present study suggests Lampung-2 biological fertilizer combined with phosphorus fertilizer and dolomite could be recommended for enhancing growth and yield of soybean in acid soils in Indonesia.
Keywords: *biological, dolomite, fertilizer, Pseudomonas sp, P uptake*

Introduction

The expansion of soybean planting areas in Indonesia is programmed to acid dry land outside of Java Island to increase national soybean production towards self-sufficiency. The total area of dry land in Indonesia is around 148 million ha, 102.80 million ha (69.46%) of which is acid soils, which are dominated by Ultisol and Oxisol and are mostly found in Sumatra, Kalimantan and Papua islands (Mulyani et al., 2004).

Acidic soil is generally poor in macro nutrients, especially phosphorus (P). Phosphorus is the second major nutrient after nitrogen needed for crop production. Phosphorus helps in flower initiation and seed and fruit development. Phosphorus deficiency decreases photosynthate supply to the legumes roots which in turn inhibits root nodule formation and activity (Chaudhary et al., 2008; Yakubu et al., 2010).

Phosphorus availability to plant is highly dependent on soil pH with the optimum pH range of 6.5-7.5 (Mitchell, 2000). On acid soils with high levels of Al, Fe, and Mn, the P fixation occurs at the form of Al-P, Fe-P, and Mn-P, which is poorly soluble and causes P unavailable to plants. When the low soil P availability is due to a high soil phosphorus fixation, then fertilizer alone would not be effective in improving soil P availability because more than 80% of the P fertilizer may be strongly absorbed or precipitated by the soil and not be immediately available to the crop (Zhang and Zou, 2008; Syers et al., 2008).

Phosphate solubilizing bacteria (PSB) by microbe is animportant process in natural ecosystems, especially on sustainable agriculture. These microorganisms, including various bacteria, fungi and actinomycetes, have been reported to be active in the conversion of insoluble phosphates into usable forms (Whitelaw, 2000: Sharma et al., 2013). There are several microbes in acid soil that solubilize P, but they are less beneficial because the amount is not high enough to compete with other microbes that also located around the plant roots (Igual et al., 2001; Hibbing et al., 2010). Therefore, the use of effective P solubilizing as inoculants would be expected to overcome the problems of P fixation in acid soil.

The use of P-solubilizing biofertilizer in maize plants was able to increase the grain yield by 44% and P content by 56% compared to the uninoculated control (Viruel et al., 2014). The application of PSB significantly increased the content of soil phosphatase, invertase, and urease respectively by 1.18, 1.31 and 2.32 times higher than those in the control, and reduced Ca-P bonds in the soil so that P becomes available for plant uptake (Shi et al., 2017). In soybean, wheat and Chinese cabbage the application of PSB, *Bacillus cereus* YL6 strain, increased soil available phosphorus by 120.16%, 62.47% and 7.21%, respectively (Ku et al., 2018).

Some researchers reported that, certain bacteria are more active than other types of microbes in the conversion of P (Igual et al., 2001; Khan et al., 2009). Bacterial strains of *Pseudomonas*, E*scherichia*, *Micrococcus* and *Staphylococcus* genera were able to improve number of pods, and grain weight of mungbean up to 65, and 17.15% respectively, over control (Thakuria et al., 2004; Ali et al., 2010). In tomato, application PSB converted approximately 20% of less available phosphorus into labile forms (Turan et al., 2007). The objective of this study was to identify efficient isolates of phosphate solubilizing bacteria in acid soils to improve soybean productivity.

Methodology

Green house experiment

Greenhouse study was conducted from February to May 2015. The soil used for the study was an upland acid Ultisol and was taken from Gunung Makmur village, Tanah Laut district, South Kalimantan province, Indonesia.

Before planting, the soil was analyzed to obtain the data of pH, organic C, N, P, K, Ca, Mg, Al, and a natural population of P-solubilizing microbes. Soil analysis was done at Plant

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and Soil laboratory of ILETRI. The *Pseudomonas* sp isolates (Lampung-1, Lampung-2, and Lampung-3) used in the present study were originally isolated from Ultisols in Lampung Province and was selected for its phosphate solubilizing capacity under laboratory conditions (Suryantini, 2011). The isolates were maintained in nutrient agar at 4 ^{o}C in the Soil Microbiology laboratory of ILETRI until needed for experiments.

The treatments were arranged in a split-plot design with three replications. The main plots were the equivalent dose of lime in the form of dolomite: 0; 400; and 800 kg ha^{-1} dolomite. Sub plots consisted of two factors: factor 1 was P fertilizer equivalent to 0; 100; and 200 kg SP36 ha^{-1}, and factor 2 was inoculation of PSB isolates i.e. without inoculation, isolate Lampung-1, isolate Lampung-2 and isolate Lampung-3. Pots were filled with 5 kg of soil after air drying and sieving through a 10 mesh size. The soil was air-dried and pounded, then put into each pot, at amount 5 kg pot^{-1}. Lime was applied three days before planting by mixing it with the soil. The isolated were applied in a solution of nutrient broth at planting by pouring into the planting hole. Each isolate was applied at a dosage of 10 ml pot^{-1} containing 10^9 CFU (colony forming units) ml^{-1} solution of nutrient broth. P fertilizer and base-fertilizer equivalent to 50 kg. ha^{-1} Urea + 100 kg ha^{-1} KCl, were applied at planting close to the planting hole.

Four seeds of Anjasmoro soybean variety were planted per pot and were thinned to two plants per pot after ten days. Watering was done regularly to keep the soil moisture at field capacity. Sampling was done at the late flowering and setting for shoot dry weight, shoot height and shoot P content. When the plants were completely matured harvesting was done, then pod and grain weights were recorded.

Field experiment

The field experiment was conducted during the rainy season of 2015 in Gunung Makmur village, Tanah Laut district, South Kalimantan province, Indonesia. Treatment was arranged in a split-plot design with three replications. The main plots were P fertilizer consisted of no fertilized, 100 kg ha^{-1} SP36, and 200 kg ha^{-1} SP36.The subplots were inoculation with P-solubilizing isolates (the two best isolates from the greenhouse experiment) consisted of without inoculation, Lampung-1, Lampung-2, and a commercial inoculant, at a dose of 5 g inoculants kg^{-1} seed. Treatment plot size was 4 m x 5 m, plant spacing of 40 cm (between row distance) x 15 cm (within row distance), two plants per hill. For inoculant preparation, the carrier material (peat) was mixed with 0.3% $CaCO_3$ to neutralize the pH before it was sterilized in an autoclave at 121°C for one hour. Each isolate of *Pseudomonas* sp grown in 100 ml nutrient broth on a shaker at room temperature (28 ± 2°C) for 5 days and containing 4.5 x 10^9 CFU ml^{-1} was mixed with 250 g of sterilized peat aseptically. The inoculant was then packed in polythene bag and stored at room temperature for one week. The viable cell count in the carrier-based inoculum was enumerated by serial dilution plate count technique using Pikovskaya's agar. The viable cell count was 2 x 10^9 CFU g^{-1} inoculants.

Data was collected at the vegetative phase (50 DAP) from samples of 10 plants $plot^{-1}$ for plant height and plant dry weight. At harvest, 10 plants per plot were for the number of pods per plant, while the harvested plots of 3 m x 4 m were for grain yield. Data collected were subjected to statistical analysis of variance (ANOVA) by using a computer program MSTATC. Duncan's Multiple Range Test (DMRT) at 5% probability level was applied to compare the differences among treatments means. The correlation analysis was conducted between P uptake and seed yield per plant of soybean.

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Results and Discussion

Soil analysis of samples from research sites in Gunung Makmur village of Tanah Laut district, South Kalimantan province indicated soil fertility; the soil is acid (pH ≤4), very low organic matter content (<2%), low of N, P and K (*Table 1*). Population of PSB is low, below the effective number. According to Sabarudin et al. (2011), PSB was able to increase the availability of P in the soil when the population reached 1 x 10^9 CFU g^{-1} soil. Wang et al. (2015) reported that PSB inoculation studies have shown both increased phosphorus uptake and improved plant yield both in pot experiments and under field conditions. In a pot experiment where Aspergillus niger was used as a biofertilizer (using wheat husks with 20% perlite as carrier material) the soil colonization rate was 5.6 × 10^6 spores g^{-1} soil.

Table 1. *Initial chemical properties of soils from the study site*

P-solubilizing bacteria (CFU/g soil)	pH H_2O	C-org (%)	N (%)	P_2O_5 (ppm)	K	Ca	Mg	Al
					(me/100 g)			
73 x 10^5	4.00	1.20	0.80	8.88	0.76	1.71	0.56	0.94

Note: Plant and Soil laboratory of ILETRI in Malang

On acid soil, soybean tolerance of Al toxicity is at the saturation Al of ≤ 20% (Arya, 1990). In this study to reduce the Al saturation to 20% requires an addition of dolomite as 4 g pot^{-1} or equivalent to 800 kg ha^{-1} as dolomite dose recommendation.

Interaction between PSB isolates dolomite on grain yield

There was an interaction between PSB isolate the rate of dolomite for grain yield of soybean. Application of dolomite up to 800 kg ha^{-1} did not increase grain yield. PSB isolates Lampung-2 and Lampung-3 without dolomite increased grain yield by 17.7% and 5%, respectively over control treatment, whereas Lampung-1 isolates did not affect grain yield. Some PSB can improve the pH of their growth environment so that they can survive and shows effectiveness in acidic conditions. It was found that P solubilizing bacteria was able to increase the pH of the nutrient solution from 4.00 (the initial solution pH) to 7.00 after 24 h of inoculation, which impact on better plant performance (Panhwar et al., 2014). However, at dose of 400 kg dolomite per hectare isolate Lampung-1 was more effective than other isolates and was able to increase grain yield by 25%, followed by isolates Lampung-2 and Lampung-3 with 20% and 16%, respectively. At a dose of 800 kg ha^{-1} dolomite Lampung-2 isolates showed the highest yield increase (40%), whereas two other isolates showed yield decreased. This result suggests that isolates Lampung-2 had an advantage and was effective in acid soil either limed or not, compared to other isolates. Increases in grain yield showed a similar pattern with the increase in number of pods plant-1 and was influenced by the interaction between PSB isolates dolomite (*Table 2*).

Interaction between PSB isolates with SP36 on grain yield

Without P-fertilizer, isolates Lapung-1, Lampung-2 and Lampung-3 demonstrated their effectiveness by improving grain yield 4.6%, 18.7% and 5%, respectively over the control treatment. At fertilization of 100 kg ha^{-1} SP36, all isolates improved yield higher than that obtained from fertilized P alone. These are in line with those reported by (Devi

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et al., 2012) that on acid soils with low P levels, inoculation with P-solubilizing bacteria were able to increase soybean yield but the highest yield was obtained when it combined with fertilizer P. The soybean yields treated with Lampung-2 isolate were higher than fertilized with 100 kg SP36, and the results was equivalent to those fertilized with 200 kg SP36 (*Table 3*).

Table 2. *Interactive effects of dolomite and P-solubilizing isolates on filled pods number and grain yield of soybean*

Treatment		Filled pods number plant^{-1}	Grain yield (g plant^{-1})
Dolomite (kg ha^{-1})	**Isolate**		
0 (control)	Un-inoculated	36.27 fg	7.20 ef
	Lampung-1	35.10 g	7.09 f
	Lampung-2	40.95 d	8.48 c
	Lampung-3	38.61 e	7.55 d
400	Un-inoculated	36.27 fg	7.43 de
	Lampung-1	45.63 b	9.00 b
	Lampung-2	43.29 c	8.65 c
	Lampung-3	40.95 d	8.37 c
800	Un-inoculated	37.44 ef	7.61 d
	Lampung-1	40.95 d	7.55 d
	Lampung-2	47.97 a	10.12 a
	Lampung-3	37.44 ef	7.72 d
CV (%)		5.87	6.64

Note: The numbers in the same column followed by the same letter are not significantly different (P = .05); CV = Coefficient of Variation

Table 3. *Effect of phosphorus fertilizer (SP36) and PSB isolates on filled pods number and grain yield of soybean*

Treatment		Filled pods number plant^{-1}	Grain yield (g plant^{-1})
SP 36 (kg ha^{-1})	**Isolate**		
0 (control)	Un-inoculated	35.10 e	6.90 f
	Lampung-1	37.62 d	7.22 e
	Lampung-2	39.78 cd	8.19 c
	Lampung-3	38.61 d	7.25 de
100	Un-inoculated	35.10 e	7.55 d
	Lampung-1	42.12 bc	8.83 b
	Lampung-2	43.80 ab	9.13 b
	Lampung-3	39.78 cd	8.22 c
200	Un-inoculated	42.12 bc	8.15 c
	Lampung-1	43.80 ab	8.20 c
	Lampung-2	46.29 a	10.00 a
	Lampung-3	37.44 de	8.19 c
CV (%)		7.66	8.04

Note: The numbers in the same column followed by the same letter are not significantly different (P = .05); CV = Coefficient of Variation

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A more efficient use of P fertilizer can be done by combining a fertilizer dose of 100 kg ha^{-1} SP36 with Lampung-1 or Lampung-2 isolates. Both combinations were able to increase the yield of seeds by 27.9% and 32.3%, respectively, compared to the control. This result was also higher than the fertilization of 200 kg SP36 which was increase the soybean yield 18.8% compared to the control (*Table 3*). This increase in soybean yield showed the same pattern as the increase in the number of pods per plant and was influenced by the interaction between PSB isolates with a dose of SP36. (*Table 3*).

Increased pods number and grain yield in case of PSB application might be attributed to the complementary effect of enhanced phosphate availability. The application of PSB to the soil was able to increase Olsen P-soil from 14.7 mg to 23.4 mg kg^{-1} of soil after 14 days of incubation (Wang et al., 2014). This is evident from the highest plant P uptake in the Lampung-2 treatment, followed by Lampung-1, Lampung-3, and uninoculated control (*Figure 1a*). Significant interaction between PSB isolates and with P fertilization grain yield is closely related to plant P uptake, indicated by a significant positive correlation (R = 0.69) between seed yield per plant and P uptake (*Figure 1b*). Phosphate solubilization of PSB potential has been attributed to the isolates' ability to produce organic acids, such as gluconic acid, oxalic acid, and citric acid that can directly solubilize mineral phosphate as a result of anion exchange or indirectly chelate both Fe and Al ions associated with phosphate (Panhwar et al., 2014; Pande et al., 2017). This leads to increased P availability, which ultimately increases plant P uptake.

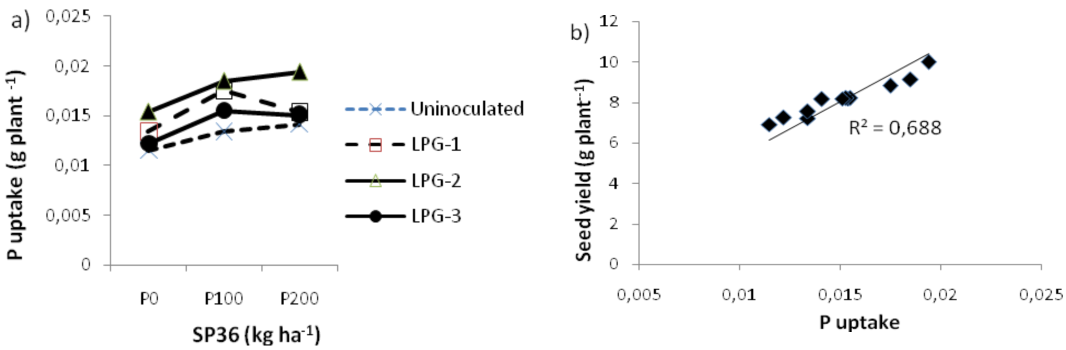

Figure 1. *Effect of inoculation with PSB on P uptake in soybean at different P fertilizer levels (a) and a positive correlation between P uptake and seed yield per plant of soybean (b); LPG = Lampung*

Best isolates obtained in green house experiment was further tested on the field in South Kalimantan. Lime was applied at half (400 kg ha^{-1} dolomite) based on the best results in the greenhouse. In the treatment without P-fertilizer, Lampung-2 isolate was able to increase grain yield by 13% compared to controls. Highest grain yield was obtained with combination treatments 100 kg ha^{-1} SP 36 + Lampung-1 and 100 kg ha^{-1} SP3 + Lampung-2 which increased yield by 18.5 and 27%, respectively, compared to the controls. Fertilizer alone at a dose of 100 kg SP36 ha^{-1} did not improve yield. This result is equivalent to grain yield at 200 kg ha^{-1} SP36 fertilization. On the other hand, commercial inoculants could not increase soybean yield. The use of P fertilizer increased the number of pods per plant with increasing dose applied, but the highest

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number of pods was obtained at 100 kg ha^{-1} SP36 combined with LPG-2 isolate, followed by combination with Lampung-1 isolate (*Table 4*).

Table 4. *Effect of SP 36 fertilizer dosage and PSB on grain yield and, number of filled pods of soybean*

Treatment		Filled pods number plant^{-1}	Grain yield (t ha^{-1})
SP36 (kg ha^{-1})	**P-solubilizing inoculant**		
0 (control)	Un-inoculated	32.29 g	1.36 d
	Lampung-1	35.00 ef	1.51 cd
	Lampung-2	36.97 def	1.54 bc
	Commercial	35.69 def	1.45 cd
100	Uninoculated	34.05 fg	1.46 cd
	Lampung-1	43.01 b	1.67 ab
	Lampung-2	47.74 a	1.73 a
	Commercial	37.21 de	1.42 cd
200	Uninoculated	43.06 b	1.70 a
	Lampung-1	40.48 bc	1.42 cd
	Lampung-2	43.29 b	1.73 a
	Commercial	38.61 cd	1.49 cd
CV (%)		5.87	12.07

Note: The numbers in the same column followed by the same letter are not significantly different (Duncan Multiple Range Test) (P = .05); CV = Coefficient of Variation

Effective P solubilization from organic and inorganic insoluble forms of P compounds to soluble P form that are easily absorbed by plants led to increased P uptake and grain yield. Although there was no observation of P in the soil after harvest, however Sandeep et al. (2008) as well as Fageria et al. (2016) reported that increasing crop productivity is also due to the role of P nutrients in root growth and increasing levels of P then the more extensive root uptake of the element so there than P also increased.

Conclusion

PSB isolated from soils of South Kalimantan and tested under greenhouse conditions were tolerant to acid and slightly acidic soil and were effective in increasing P uptake and grain yield of soybean. The effectiveness of the PSB isolates was in the order LPG-2 > LPG-1 > LPG-3. These results suggest that by using effective PSB and P fertilization can increase P availability in acidic soils.

Data Availability Statement. Data are available on request through a data access committee of the Indonesian Legumes and Tuber Crops Research Institute (ILETRI).

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Conflicts of Interests. The authors declare no conflict of interests.

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