

Research Article

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Biochemical activity and bioassay on maize seedling of selected indigenous phosphate-solubilizing bacteria isolated from the acid soil ecosystem

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Abstract: Phosphorus availability is the major constraint for plant growth in the acid soil ecosystem, due to high fixation by Al and Fe. Microbial fertilizers such as phosphate-solubilizing bacteria (PSB) can increase P availability in soils for root uptake. The objective of the research was to verify the ability of four isolates of PSB isolated from acid soil to solubilize unavailable inorganic phosphate, produce phosphatase, malic acid and indole acetic acid (IAA), as well as increase plant height of maize seedling. The bioassay by growing maize seedling in liquid nutrients has been performed to study the response of seedling to PSB inoculation. The experimental design of bioassay was a randomized block design with five replications. The results showed that the isolates RR 1 and SPR 4 had a relatively high solubilizing index. Moreover, all the PSB isolates had the ability to produce phosphatase and IAA and dissolve P. The performance of PSB-inoculated seedling was better visually and the root length was increased by 66.7–74.5% compared to the control. This result concludes that the species of four isolates needs to be identified by a biomolecular method and formulated as biofertilizers for increasing the maize productivity in the acid soil ecosystem.

Keywords: biochemical activity, bioassay, PSB, indigenous, acid soils, Ultisols

1 Introduction

The major growth-limiting factor in tropical soil associated with acid soil infertility includes toxicity of aluminum (Al) and manganese (Mn) (Bojorquez et al. 2017). This condition indicates that the soil has a low pH. The pH value indicates the amount of hydrogen ion concentration (H^+) in the soil. The higher the levels of hydrogen ions in the soil, the lower the pH value of the soil and the more acidic the soil. Most of the agricultural soils of Indonesia belong to the acid soil ecosystem and face serious problems (Sarno et al. 2004). Generally, soils are acidic with an average pH value of 4.0–5.5. Soil acidity is often the main reason of the decline in productivity of various types of plants. The soil pH greatly affects the growth and reproductive investment of plants (Gentili et al. 2018).

Soil microbes play an important role in enzymatic phosphorus transformation as an integral component of the P cycle in the soil. The release of phosphate from inorganic and organic P through the mechanism of dissolution and mineralization has been reported (Sharma et al. 2013). The phosphate-solubilizing bacteria (PSB) are plant growth-promoting rhizobacteria (PGPR) that can solubilize insoluble P and release phosphate (PO_4^-) into the soil solution where they were absorbed by plant roots (Kalayu 2019). The various isolates of indigenous PSB are present in the soil and associated with the rhizosphere in various types of plants (Arfarita et al. 2017). Each isolate has the ability to dissolve P and different enzyme activities (Fitriatin et al. 2014). Consequently, selection of PSB isolates is needed to obtain the superior biochemical traits in solubilizing the fixed P, mineralize the organic P and produce plant growth-promoting substances, such as indole acetic acid (IAA), gibberellin and other organic acids (Bhatt and Vyas 2014).

The effective isolates that have certain biochemical characteristics of PGPR are needed to increase the availability of P. The ability of each isolate to dissolve P and produce

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phosphatase can be determined by conducting tests on the Pikovskaya medium and evaluating their effect on plant growth by a bioassay (Pathak et al. 2017). The research objective was to characterize the indigenous PSB isolated from acid soils (Ultisols) and investigate their ability to produce biochemically beneficial metabolite for enhancing the seedling growth in the acid soil ecosystem

2 Materials and methods

2.1 Isolation and characterization of PSB

The four isolates of PSB were isolated from acid soil (Ultisols soil order), in Kentrong Village, Lebak Regency, Banten Province, Indonesia. The physiochemical properties of the area are as follows: pH 4.5; texture clay; Al 50.65%; P_2O_5 3.45 ppm, N 0.17%; and cation exchange capacity (CEC) 11.34 cmol kg⁻¹. PSB were isolated from several ecosystems including rice rhizosphere (RR), peanut rhizosphere (PR) and sweet potato rhizosphere (SPR).

The composite soil samples were taken from 20 cm depth using rhizosphere probe sample. Isolation of PSB was carried out by a serial dilution plate method using Pikovskaya media. Furthermore, isolates were observed to determine the clear zone of colony diameter and the solubilizing index (SI) was calculated by the formula discussed by Premono et al. (2007):

$$SI = \frac{\text{halozone diameter} + \text{colony diameter}}{\text{colony diameter}}.$$

The isolates that were superior in this testing stage were analyzed for the activity of the enzyme phosphatase that was produced, and the isolates with the highest activity in the liquid medium were selected. Four superior rhizobacteria isolates with best phosphate solubilizing activities were selected and characterized through biochemical identification and phosphatase activity with methods as described by Eiviaz and Tabatabai in Margesin (1996), and malic acid production was measured by high-performance liquid chromatography (Photodiode Array Detector, Singapore Product Waters 2998). Dissolved phosphate and phytohormone production (IAA) were determined based on the colorimetric method of Gordon and Weber (Sarker and Al-Rashid, 2013).

2.2 Bioassay of PSB on maize seedling

Bioassay test aims to obtain the superior of PSB isolates that are capable to increase the growth of maize seedlings.

The experimental design was a randomized block design, which consisted of five treatments (control and four PSB isolates) with five replications.

The bioassay of PSB isolates was carried out using Murphy's liquid media (0.25 g $CaSO_4 \cdot H_2O$, 0.25 g $KH_2PO_4 \cdot 2H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.08 g NaCl, 0.52 g KCl, 0.017 g $ZnCl_2$, 0.005 $CuSO_4 \cdot 5H_2O$, 0.025 $FeSO_4$ and 1 L aquadest) in accordance to Murphy and Riley (1962). Before treatment, maize seeds were sterilized with 0.2% $HgCl_2$ and 70% ethanol. Maize seeds were germinated at 30°C for 72 h. The maize seedlings were grown in a 100 mL reaction tube filled with 95 mL of liquid Murphy medium. The plant height (cm), root length (cm) and dry weight (g) were measured at 5, 10 and 15 days after planting.

2.3 Statistical analysis

Data of the bioassay test were collected by analysis of variance, and the mean values of treatment were compared using Duncan's multiple range test (DMRT) at $p < 0.05$.

3 Results

3.1 Characteristics of PSB

The PSB isolates were selected from agricultural soils and natural forest, resulting in four potential PSB isolates from rice rhizosphere (RR 1 and RR 2), peanut rhizosphere (PR 1) and sweet potato rhizosphere (SPR 4) as indicated by the largest halozones and SI of PSB on the Pikovskaya agar (Table 1).

The ability of four PSB isolates to dissolve P from fixed P can be observed from the phosphatase activity, organic acid production and the concentration of P dissolved in the Pikovskaya broth. Table 2 shows that the ability of

Table 1: Selected four PSB isolates from rice rhizosphere (RR 1 and RR 2), peanut rhizosphere (PR 1) and sweet potato rhizosphere (SPR 1)

No.	Isolates	Colony diameter + halozone diameter a (cm)	Colony diameter b (cm)	SI (a/b)
1	RR 1	1.63	0.67	2.43
2	RR 2	1.13	0.63	1.79
3	PR 1	0.43	0.27	1.59
4	SPR 4	1.43	0.63	2.27

Table 2: Biochemical activity of selected PSB to produce phosphatase enzyme, malic acid, P-dissolve and IAA

No.	PSB isolates	Phosphatase ($\mu\text{g pNP g}^{-1} \text{h}^{-1}$)	Malic acid ($\mu\text{g g}^{-1}$)	P-dissolve (ppm)	IAA (ppm)
1	RR 1	58.72	0.33	20.07	15.3
2	RR 2	61.2	0.36	30.48	15.83
3	PR 1	21.2	0.66	23.24	15.26
4	SPR 4	76.59	1.13	37.69	15.64

bacteria to produce phosphatase, malic acid as well as the ability to dissolve phosphate varied. The SPR 4 isolates had the highest ability to produce phosphatase up to $76.59 \mu\text{g pNP g}^{-1} \text{h}^{-1}$, followed by isolates RR 2, RR1 and PR 1 that showed the phosphatase activity of 61.20, 58.72 and $21.20 \mu\text{g pNP g}^{-1} \text{h}^{-1}$, respectively.

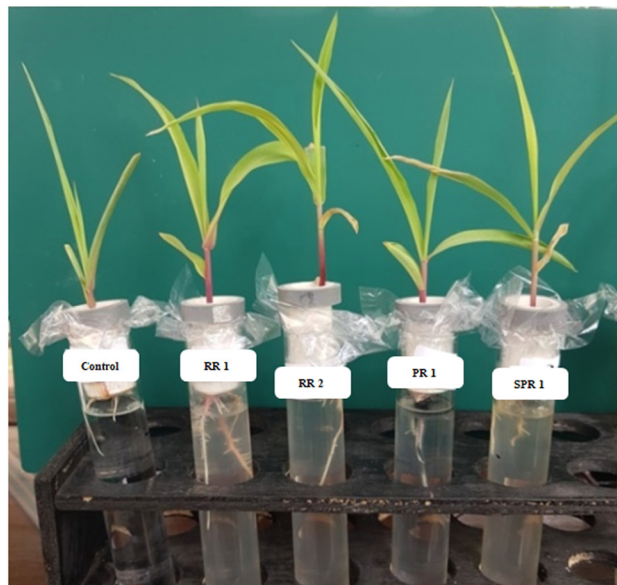
The organic acids produced by PSB from acid soil are dominated by organic malic acid. Highest to lowest concentrations of malic organic acids (malic acid) in Pikovskaya broth are produced by isolates SPR 4, PR 1, RR 2 and RR 1.

The ability of PSB to dissolve fixed P might be influenced by the ability of bacterial isolates to produce phosphatase and organic acids that affect the amount of dissolved P in the Pikovskaya broth. The highest to lowest amount of dissolved P produced by bacterial isolates was SPR 4 isolate of 37.69 ppm, RR 2 of 30.48 ppm, PR 1 of 23.24 ppm and the smallest RR 1 of 20.07 ppm.

3.2 Bioassay of PSB

The effect of PSB isolated from acid soil on maize height on days 5, 10 and 15 after inoculation is determined using Bioassay test and is shown in Table 3.

Although PSB inoculation did not increase the height of maize seedlings at days 5, 10 and 15 significantly by the

**Figure 1:** The visual growth performance of maize seedling due to the inoculation with PSB isolates.

PSB inoculation, the growth performance (vigor and leaf color) of the PS-inoculated seedling improved compared to that of the control (Figure 1). The difference in growth of maize seedlings between inoculated one and the control can be seen on days 10 and 15. The ability of PSB isolates to increase plant height from the largest and smallest was RR 1, RR 2, SPR 4 and PR 1.

The effect of PSB was significant on the root length (Table 4 and Figure 2). The ability of PSB isolates increased the root extension of the largest and smallest plants produced by the treatment of isolates RR 2, SPR 4, PR 1 and RR 1 on day 15 after inoculation. The shoot to root (S/R) ratio of each treatment varied. The S/R of the controls was 0.33, while the S/R of maize seedling treated with RR 1, RR 2, PR 1 and SPR 4 was 0.54, 0.43, 0.44 and 0.38, respectively (Table 5).

Table 3: Effect of PSB on plant height of maize

Treatments	Plant height (cm)		
	Day 5	Day 10	Day 15
Control	$8.96^a \pm 1.50$	$12.38^a \pm 2.10$	$14.72^a \pm 2.15$
RR 1	$10.42^a \pm 1.99$	$15.08^a \pm 3.62$	$19.66^a \pm 3.39$
RR 2	$10.02^a \pm 3.61$	$14.92^a \pm 3.68$	$17.66^a \pm 5.19$
PR 1	$10.20^a \pm 1.50$	$12.70^a \pm 1.95$	$17.06^a \pm 2.91$
SPR 4	$8.98^a \pm 2.66$	$13.60^a \pm 5.03$	$17.20^a \pm 4.89$

Values followed by similar letters under the same column are not significantly different at $p = 0.05$ according to DMRT.

4 Discussion

The ability of four PSB isolates has been tested in terms of the production of enzyme phosphatase, P-dissolved, IAA growth hormone, organic acids and bioassay (bioassay) in maize plants. The SI relates to the ability of PSB to solubilize phosphate qualitatively, which can be seen from the ability of bacteria to form halozones around the colony on Pikovskaya agar (Rodríguez and Fraga, 1999). Each isolate has the ability to form halozones that vary in time and diameter. The halozone indicates the magnitude of the

Table 4: Effect of PSB on root length of maize seedling

Treatments	Day 5 (cm)	Day 10 (cm)	Day 15 (cm)
Control	4.60 ^a ± 0.55	5.10 ^a ± 0.65	6.26 ^a ± 1.01
RR 1	7.50 ^b ± 1.46	8.56 ^b ± 1.29	10.10 ^b ± 1.68
RR 2	7.86 ^b ± 1.51	8.90 ^b ± 1.64	11.06 ^b ± 0.72
PR 1	7.94 ^b ± 2.00	8.70 ^b ± 1.99	10.66 ^b ± 1.70
SPR 4	7.40 ^b ± 2.82	8.50 ^b ± 3.22	10.82 ^b ± 2.81

Values followed by similar letters under the same column are not significantly different at $p = 0.05$ according to DMRT.

ability of bacteria to solubilize phosphate. The mechanism of P solubilization in Pikovskaya media is that the organic acids produced by bacteria chelate the calcium in $\text{Ca}_3(\text{PO}_4)_2$, so that P dissolves and halozone forms in Pikovskaya (Atekan et al. 2014).

The PSB isolated from Ultisols had a lower SI compared to the SI value of PSB isolates from other regions. The PSB from the rhizosphere of potato and tomato grown in California, USA, had a phosphate solubility index between 9.1 and 15.1 (Sharon et al. 2016).

The phosphatase is an enzyme that catalyzed organic P mineralization by PSB to produce PO_4^- , which in turn increases the availability of P for plants. The production of high levels of phosphatase and organic acid will help the process of organic P mineralization (Margalef et al. 2017). This is in line with the ability to produce the highest phosphatase and organic acid compared to other isolates, so they have the highest dissolved P.

Each isolate of PSB from acid soil produces IAA phytohormone. The high concentration of IAA produced by bacterial isolates indicated that the bacterial isolates

Table 5: Effect of PSB on dry weight of maize and shoot root ratio

Treatments	Root dry weight (g)	Shoot dry weight (g)	Shoot root ratio
Control	0.130 ^a ± 0.02	0.044 ^a ± 0.02	0.33
RR 1	0.152 ^a ± 0.01	0.052 ^a ± 0.02	0.54
RR 2	0.144 ^a ± 0.02	0.064 ^a ± 0.03	0.43
PR 1	0.132 ^a ± 0.02	0.058 ^a ± 0.01	0.44
SPR 4	0.152 ^a ± 0.03	0.056 ^a ± 0.03	0.38

Values followed by similar letters under the same column are not significantly different at $p = 0.05$ according to DMRT.

have a higher ability to synthesize IAA. The average concentration of IAA produced by the PSB isolates from Ultisols was 15.26–15.83 ppm.

The PSB effect towards root elongation was greatly influenced by IAA production. Dawwama et al. (2013) reported that PSB isolated from potato produced IAA up to 3.06–10.73 $\mu\text{g/mL}$ and were able to increase root length significantly. The S/R ratio reflects the photosynthate partitioning in the plant. The R/S more than one indicates higher growth of shoot, whereas if the S/R was less than one the root growth was dominant (Reham and Abogadallah 2016).

The PSB have the potency to increase the shoot and dry weight of maize seedlings (Table 5) and increase in plant dry weight was due to the role of PSB to provide nutrients mainly P and growth-promoting substances (Minaxi et al. 2011). It has also been reported that consortia of PSB increased growth of maize seedling reflected in higher plant dry weight (Khumairah et al. 2018). Simarmata et al. (2017) reported that the application of biofertilizer and bioameliorant increased the rice grain yield by increasing to 42.3%.

**Figure 2:** The performance and the root length of PSB-inoculated maize seedling.

5 Conclusion

Four isolates RR1, RR2, PR1 and SPR 4 that have been isolated from acid soil enabled to dissolve P as well as to produce phosphatase malic acid and IAA. The visual growth performance of maize seedling was improved remarkably and the root length was increased by 67.7–74.5% compared to the control. This finding concluded that the four PSB isolates are potential microbes to be developed as biofertilizers for increasing the P availability and maize growth in the acid soil ecosystem. Further molecular identification of selected PSB as well as green house and field trials are needed to obtain the comprehensive information about the effect of PSB on the fertilizer efficiency and crop productivity.

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