

Isolation of phosphate solubilizing bacteria from root rhizosphere to supplement biofertilizer

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Abstract: In soil, a large amount of supplemented phosphorus (P) are immediately transferred into insoluble forms and only 0.1 % of them is available for plant uptake. Therefore, exploring naturally occurring phosphate-solubilizing microorganisms is an essential activity to exploit them in reducing mineral phosphorus added to agricultural soils. In this study, we screened and isolated 7 bacteria that solubilized phosphate at different phosphate solubilization indexes, ranging from 4.2 to 226.1. Of them, the most efficient isolate is PSB31, which solubilized tri calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) at a rate of 962 mg l^{-1} and molecularly identified as *Bacillus* sp. (in: Bacteria) strain IMAU61039. This bacterial strain generated the low supernatant pH and the phosphatase, which are involved in the phosphorus solubilization mechanism. Furthermore, greenhouse experiments showed that tomato seedlings grown in PSB31-inoculated soil contained higher P amount and had much higher biomass than those plants grown in soil without PSB31 addition. These results suggest that the PSB31 strain has potential use as a biofertilizer.

Key words: phosphates-solubilizing bacteria; plant growth promoting bacteria; biofertilizer; tomato; phosphatase

Izolacija bakterij, ki sproščajo fosfat iz rizosfere kot nadomestek biognojilom

Izvleček: V tleh se velike količine dodanega fosforja hitro spremenijo v netopne oblike tako, da ostane rastlinam raspoložljivega le okrog 0,1 %. Zaradi tega je izkoriščanje v naravi prisotnih fosfat sproščajočih mikroorganizmov nepogrešljiva aktivnost, ki omogoča njihovo uporabo in zmanjšuje dodajanje mineralnega fosforja v kmetijska tla. V raziskavi smo preverili in izolirali 7 bakterij, ki sproščajo fosfat z različnimi indeksi sproščanja od 4,2 do 226,1. Med njimi je bil najučinkovitejši izolat PSB31, ki je sproščal tri kalcijev fosfat ($\text{Ca}_3(\text{PO}_4)_2$) v velikosti 962 mg l^{-1} , na osnovi molekularnih testov določen kot IMAU61039 soj bakterije iz rodu *Bacillus*. Ta soj bakterije je generaliziral nizek pH v raztopini in fosfataze, ki so vključene v mehanizem sproščanja fosforja. Nadalje je poskus v rastlinjaku pokazal, da so vsebovale sejanke paradižnika, ki so rastle v tleh inokuliranih z izolatom PSB31 večjo vsebnost fosforja in mnogo večjo biomaso kot tiste, ki so rastle v tleh brez dodatka PSB31. Izsledki nakazujejo, da bi se izolat PSB31 lahko uporabljal kot biognojilo.

Ključne besede: fosfor sproščajoče bakterije; rast rastlin vzpodbujajoče bakterije; biognojila; paradižnik; fosfataze

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1 INTRODUCTION

Phosphorus is an essential macronutrient that together with others such as C, N, plays an important role in the normal growth of a plant. In agriculture, chemical fertilizer was used to supplement the phosphorus source to promote plant development and increase crop yield. However, it is reported that only a small proportion of phosphorus in provided fertilizer is available for plant uptake, and 95 %–99 % of it were insoluble, immobilized, or precipitated under effect of environmental factors such as soil pH (Khan et al., 2009). Subsequently, the proportion of insoluble phosphates deposited in the soil are increased (Morales et al., 2011). Therefore, the best strategy in crop management is limit the addition of phosphorus into soil under chemical fertilizer form; increase the agents converting P reserved in the soil; and reclaim the chemically-bound P from insoluble form (Cordell et al., 2009). Although there are many solutions to balance the input/output ratio, identification of the least risky alternatives to traditional practices is carrying out. Among those, using phosphate-solubilizing bacteria (PSB) that can solubilize the insoluble phosphate is an emerging solution.

Phosphate-solubilizing bacteria (PSB) living in the root rhizosphere present the ability in reversing the insoluble phosphate into a soluble form easily used by plants. Chen et al. (2006) demonstrated the low molecular mass organic acids produced by PSB played role in dissolving the phosphate complexed minerals. In another study, Vyas and Gulati (2009) showed that PSB-produced low molecular mass organic acids also chelated the cations that formed complexes with P ions (PO_4^{3-}) to release P directly into the rhizosphere soil. These results demonstrated soil PSB could release P from the insoluble forms to increase soil fertility. In addition, some PSB genera (such as *Arthrobacter*, *Burkholderia*, *Beijerinckia*, *Erwinia*, *Bacillus*, *Rhizobium*, *Pseudomonas*, and *Mesorhizobium*) have been exploited as soil inoculants to promote plant growth and subsequently increase the yield (Kumar et al., 2017).

Hence, using PSB as a biofertilizer was determined as an alternative for expensive and environmentally damaging fertilizers in the future. Despite the role in solubilizing insoluble P and promoting plant development, the application of PSB as a bio-fertilizer is still needed further studies due to the composition or variation in soils and bacterial community (Barea, 2015).

Thai Binh province play important role in providing agricultural products for market in Vietnam. However, the overuse of chemical fertilizer is one of major factors

which has caused soil pollution in Thai Binh. Therefore, development of biofertilizer plays a key role for the sustainable agriculture in this province. Hence, we aimed to isolate and characterize high phosphorus-solubilizing bacteria from agricultural soil collected in Thai Binh, and also investigated their potential in developing these strains as biofertilizer.

2 MATERIAL AND METHODS

2.1 ISOLATION OF BACTERIA WITH PHOSPHORUS-SOLUBILIZING ABILITY

The soil samples were collected from the field grown maize, rice, and tomato in Thai Binh (Vietnam) on December 14, 2020. Different locations in Thai Binh province were located including Tien Hai ($20^{\circ}24'27.7''\text{N}$ $106^{\circ}31'00.1''\text{E}$) and Kien Xuong ($20^{\circ}22'54.0''\text{N}$ $106^{\circ}23'43.2''\text{E}$), which provided more than 80% agricultural products (rice, maize and tomato) for the market in Vietnam.

Sampling procedure is carried out according to TCVN 4046-1985 (TCVN 4046 – 85, 1985) as follows: soil samples were taken according to the diagonal or zig-zag rule depending on the topography of the land. Each site took from 15 to 20 samples, each sample was about 0.5 kg and the samples were mixed to be represented by the diagonal rule of about 0.5 kg.

PSB was isolated from soil samples by serially diluting up to 10^{-10} by sterilized water and inoculating in Pikovskaya's agar (PVK) agar medium by pour plate method (Cao et al., 2018). For control, only sterilized water was used. The incubation of all plates was done at 30°C for 7 days. After 7 day of incubation, the bacteria generated a clear zone around colonies were identified as strains having the phosphorus-solubilizing ability. Then, the isolated single colonies presenting a clearing zone around were transferred onto new PVK plates. The strains generated the highest clearing zones are considered as potential PSB and were selected for the next experiments.

2.2 DETERMINE PHOSPHATE SOLUBILIZING ACTIVITY OF BACTERIA ON AGAR MEDIUM

After 7 days of incubation, the clearing zones around single colonies on the reinoculated plates were measured and validated (Sharon et al., 2016):

Phosphate solubilizing index (PSI) = $[(\text{colony diameter} + \text{clearing zone}) / \text{colony diameter}] \times 100$

2.3 DETERMINE PHOSPHATE SOLUBILIZING ACTIVITY OF BACTERIA IN PVK LIQUID MEDIUM

The P solubilizing efficiency of the isolates was investigated by growing in the PVK liquid medium. 200 µl of selected isolate was cultured in 9.8 ml of PVK medium with 0.5 % $\text{Ca}_3(\text{PO}_4)_2$ (w/v) on the shaker at 30 °C. The culture was collected and centrifuged to obtain the supernatant using to determine the solubilized P by vanadomolybdate method (Pearson, 1976). Briefly, 1 ml of supernatant (distilled water for the blank) was transferred into a clean cuvette. Then, added 0.25 ml of vanadate-molybdate reagent and mixed well by pipetting up and down several times. After 10 minutes, placed the cuvette with sample into the UV/VIS spectrophotometers (METTLER TOLEDO, USA) and measured.

The efficiency of the bacteria in solubilizing the insoluble phosphorus compound was identified as the percent of the total phosphorus presenting in the medium. The culture pH was also measured by using a benchtop pH meter (Mettler Toledo, USA). All experiments were performed in triplicates.

2.4 DETERMINE PHOSPHATE SOLUBILIZING ACTIVITY OF BACTERIA IN A POTTING SAND MATRIX

The P solubilization efficacy of the isolates was also investigated in a less-nutrient environment like acid-washed and sterilized sand. The experiment was prepared in triplicates as follows: 9 g of treated sand were added into a 15 ml tube containing a 5 ml reaction. After mixing well the reaction mixture, added 1 ml of PVK media (5 % $\text{Ca}_3(\text{PO}_4)_2$) inoculating with or without PSB strain (2×10^6 cfu ml⁻¹) into the reaction. Then, the inoculated samples were kept in the incubator at 30 °C for 24 h. After incubation, adding the distilled water into the sample to make a final volume of 10 ml was done before shaking it at 200 rpm for 1 h, and followed by centrifugation at 3,500 rpm. After that, the supernatant was collected by filtrating the culture media through a 0.45 µm filter. Then, the amount of released P was identified by vanadomolybdate method (Pearson, 1976).

2.5 PHOSPHATASE ENZYMATIC ASSAY

Phosphatase was explored by using the method described by Tabatabai and Bremner (1969). Briefly, the selected strain was grown in a 250 ml conical flash containing 100 ml broth PVK medium for 80 h. In every 5

h, the culture was taken and removed the bacterial cells by centrifuging at 10,000 rpm for 10 min at 4 °C. After that, 1ml of cell-free supernatant was mixed with 4 ml of modified universal buffer (pH 6.5). Then added 1 ml of 0.025 mM disodium p-nitrophenyl phosphate (tetrahydrate) into this mixture. The solution was mixed well and incubated at 37 °C for 1 h. After 1 hour incubation, 4 ml of 0.5 M NaOH and 1 ml of 0.5 M CaCl_2 was added to stop the reaction. The solution was then filtered through Whatman No. 42 filter paper. The filtered solution was used to measure the concentration of p-nitrophenol by using the UV/VIS spectrophotometers (Mettler Toledo, USA) at 420 nm. The values were identified on the standard curve. Each measurement was done in triplicate.

The standard curve was obtained by serially diluting the standard p-nitrophenol solution. The control was also prepared as above procedure but the additions of 0.5M CaCl_2 and 0.5M NaOH was applied before the addition of 1 ml of 0.025 mM disodium p-nitrophenyl. The amount of enzyme that used to release 1 µmol of p-nitrophenol ml⁻¹ min⁻¹ from di-Na p-nitrophenyl phosphate (tetrahydrate) under the assay condition was defined as one unit (U) of phosphatase activity.

2.6 MOLECULAR IDENTIFICATION OF PSB31 STRAIN

The total DNA of strain PSB31 was extracted using a Rapid Bacteria Genomic DNA Isolation Kit (Biobasic, Canada) as per the kit instructions. The PCR amplification of 16S rDNA was done with the extracted DNA by using the universal primers 27 F (5'-AGA GTT TGA TCC TGG CTC AG-3'), and 1492 R (5'-TAC GGT TAC CTT GTT ACG ACT T-3') (Mohamed et al., 2018). The amplification was done in a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, CA, USA) using the following program: 95 °C for 5 min; 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s; and 72 °C for 7 min. The fragment of 16S rDNA sequences (1.5 kb) was obtained by running the PCR product on the 1 % agarose gel in an electrophoresis tank. Then the expected band was cut and purified by using the QIAquick PCR Purification Kit (Qiagen, USA). The purified 16S rDNA fragment was sequenced by Fisrt Base Company (Singapore). The obtained sequence was blasted on NCBI to identify the species. The sequences with high similarity (more than 99 %) were used for multiple cluster alignment and phylogenetic analysis on MEGA software (v.7.2).

The nucleotide sequence data reported in this paper deposited on GenBank with the accession numbers is OL753109.

2.7 GREENHOUSE TESTING

The ability of selected PSB strains in promoting plant growth was investigated by pot experiments under greenhouse condition (with a temperature of 30°C and constant humidity of 85-95%) at the VNU Central Institute for Natural Resources and Environmental Studies, Ha Noi, Vietnam.

The sand matrix was pretreated by washing with 0.1 M hydrochloric acid (HCl). Then, the acid-washed sand was submerged in the 0.1 M HCl for another 24 h. After that, the submerged sand was drained and washed three times with DI water. The pH of the sand was adjusted to 7-7.5. Finally, this sand was autoclave at 121 °C for 15 min. The sterilized sand was used as the potting medium. In this pot experiment, the insoluble form of P used is $\text{Ca}_3(\text{PO}_4)_2$, which was added into the potting matrix as a P source.

Tomato seeds (*Lycopersicon esculentum* ‘Thuan Dien’) were used as an indicator and were surface-sterilized by using alcohol and Javen solution as described by Li et al. (2017). After that, the sterilized seeds were germinated on agar plates, which were covered by the aluminum foil for 3 days at room temperature. Homogenous seedlings were chosen for further experiments, some of which were covered with selected bacterial strain by dipping their roots for 30 min in bacterial culture (OD = 1). Then, four bacterized seedlings were planted in each plastic pot, which was kept in a greenhouse with long-day condition. Each treatment was repeated in triplicate.

The experimental treatments were: (T1) tomato seedlings inoculated with selected bacteria; (T2) non-inoculated tomato seedlings were considered as a negative control; and (T3) the positive control is the seedlings that were not bacterized but were regularly watered with the solution added 0.25 mM KH_2PO_4 . All pots were provided the macro and micronutrients by watering them with 30 ml of the nutrient solution only or added KH_2PO_4 , where applicable.

The irrigated nutrient solution was referenced from Li et al. (2017) and consists of 0.65 mM MgSO_4 , 2 mM NH_4NO_3 , 2 mM CaCl_2 , 0.75 mM K_2SO_4 , 0.1 mM KCl, 0.25 mM KH_2PO_4 , 0.2 mM Fe-EDTA, 1×10^{-3} mM MnSO_4 , 1×10^{-3} mM ZnSO_4 , 1×10^{-4} mM CuSO_4 , and 5×10^{-6} mM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 1×10^{-3} mM H_3BO_3 .

The seedlings were grown in three weeks until harvested. The root samples were removed from the adhering soil by washing with sterile water. The measurement of shoot and root length was carried out for three plants. After that, the plant samples were dried in the oven for 30 min at 105 °C to inactivate the enzyme, then reduced the temperature to 65 °C and kept at that temperature until

the plant weight is constant. The weight of the dried plant was recorded and analyzed.

Finally, the oven-dried samples were powdered and then digested by an H_2SO_4 - H_2O_2 mixture at 370 °C. The vanadomolybdate method (Hanson, 1950) was applied to identify the P amount in the solution.

2.8 STATISTICAL ANALYSIS

All experiments were repeated three times, the results were presented as mean values with \pm SD. Tukey's honestly significant difference (HSD) method in SPSS (version 17) was applied to compare the means in all experiments.

3 RESULTS AND DISCUSSION

3.1 ISOLATION OF PHOSPHATE-SOLUBILIZING BACTERIA

The overuse of chemical fertilizer caused the increase of insoluble P in the soil leading to many problems for humans and other living creatures. Hence, soil-isolated microorganisms having the ability in solubilizing phosphorus is emerging as an alternative to chemical fertilizer because of their environment-friendly nature.

The results showed that a total of 7 colonies grown and generated a circular clearing zone on PVK medium was obtained. Among obtained colonies, five of them (PSB11 to PSB51) were from soil grown maize while only one colony was observed for soil grown rice (PSB61) and tomato (PSB71). The results were presented in Table 1 and illustrated in Figure 1.



Figure 1: Insoluble phosphate solubilization studies on Pikovskaya's agar (PVK): PSB31 show efficient phosphate-solubilizing isolate (large haloes), whereas three others show weak (PSB41 and PSB21) or no activity (PSB62, no visible halo)

Table 1: Biochemical properties of isolated PSB strains

PSB isolates	Phosphate solubilizing index (Agar) ^a	Final pH of PVK Liq. Med.	Color of colonies	Soluble P (mg l ⁻¹) ^b
PVK medium only	0	6.5	N/A	11.12 ± 4.5 ^a
PSB11	4.2	5.5	Yellow	53 ± 7.5 ^b
PSB21	13.8	5	Yellow	63 ± 9.2 ^c
PSB31	226.1	4.5	Yellow	962 ± 11.3 ^f
PSB41	78.3	4.5	Yellow	303 ± 6.3 ^d
PSB51	67.5	4.5	White	313 ± 7.2 ^e
PSB61	73.4	4.5	White	301 ± 8.2 ^d
PSB71	91.1	4.5	White	310 ± 9.1 ^e

^a All solubilization rates were measured from cultures grown for 24 h in a liquid medium

^b Data are means ± SE of three independent biological replicates. Bearing different letters in the same row are significantly different from each other according to the least significant difference (LSD) test ($p < 0.05$)

As can be seen from Table 1, the PSB31 strain produced the largest clearing zone, with a PSI of 226.1. On the other side, the lowest index value was 4.2 produced by PSB11 isolate. The results also present the un-correlation between the PSI and the insoluble phosphate solubilization ability of the other isolates including PSB51, PSB61, and PSB71, those were isolated from soil grown maize, rice, and tomato, respectively. These results were demonstrated by a study of Sharon et al. (2016), who reported the solubilization of $\text{Ca}_3(\text{PO}_4)_2$ of microbial communities living in the rhizosphere of potato roots is higher than the one produced by microbes in the rhizosphere of tomato roots. In addition, the results showed that four of the seven isolates (PSB11, PSB21, PSB31, PSB41) produced colonies that were opaque and bright yellow while the colonies formed by the other three isolates (PSB51, PSB61, PSB71) were opaque and white (Table 1). These results suggest the variation in phosphate solubilization of the isolates could be due to the differences of microbial communities that were strongly affected by soil properties and plant species (Sharon et al., 2016). The explain was strengthened by the discovery of Grayston et al. (1998), in which the microbial diversity in the rhizosphere was highly affected by metabolites exuded by different plant species into the rhizosphere such as amino acids, carbohydrates, and carboxylic acids.

3.2 IDENTIFICATION OF BACTERIAL STRAIN PSB31

The 16S rRNA gene sequences of the PSB31 strain was blasted against the one of the other microorganisms on the NCBI (Figure 2). As can be seen from Figure 2, the PSB31 strain closed to the *Bacillus* sp. strain IMAU61039

(Accession number: MF803700.1). The *Bacillus* sp. have been reported as phosphorus solubilizers (Kumar et al., 2017; Mohamed et al., 2018).

3.3 FACTORS AFFECTED TO PHOSPHATE SOLUBILIZATION OF PSB31

3.3.1 pH

The results also showed that the highest amount

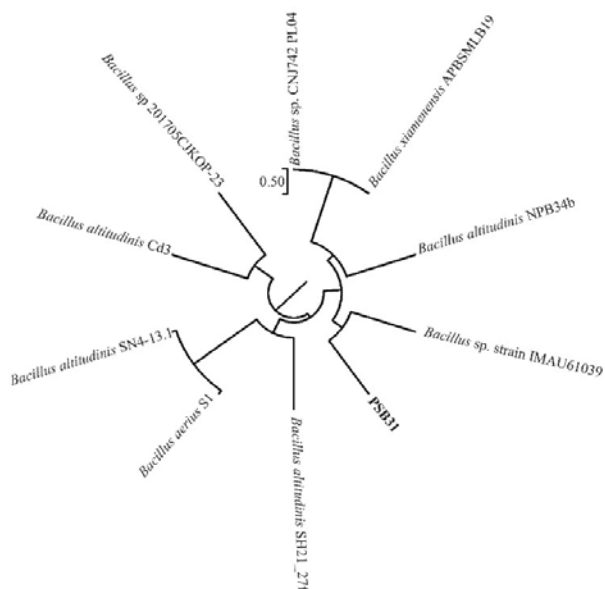


Figure 2: A neighbor-joining tree shows the phylogenetic relationships among 16S rDNA sequences of PSB31 and their closely related sequences from NCBI. The scale bar indicates evolutionary distance

of soluble P ($962 \pm 11.3 \text{ mg L}^{-1}$) was calculated for the PSB31 culture, while the lowest one ($53 \pm 7.5 \text{ mg L}^{-1}$) was observed in PSB11 culture (Table 1). Furthermore, the results also indicated that the decrease of pH of final filtrate from isolates compared to the control (Table 1). This phenomenon was reported by some previous studies which demonstrated that the organic acid production of PSB reduced the medium pH facilitating phosphate solubilization (Sharon et al., 2016; Mohamed et al., 2018). Moreover, our results also showed the supernatant pH was uncorrelated with soluble P presented in the culture of PSB31. This could be due to the calcium ion freed from the linkage with PO_4^{3-} by PSB31 strain neutralized the produced acids during the experiment (Nelofer et al., 2015). These results suggest the mechanism of P solubilization by PSB was not only produced low molecular acids but also generated others factors such as hydrolytic enzymes.

3.3.2 Phosphatase activity

The result of the phosphatase experiment showed that the insoluble $\text{Ca}_3(\text{PO}_4)_2$ was completely solubilized in the solution containing 1 ml of supernatant from PSB31 strain after 48 h of incubation was indicated by the color change of culture from milky to transparent. The result suggested that the PSB31 strain produced phosphatase to degrade the $\text{Ca}_3(\text{PO}_4)_2$ in the medium (Figure 3). The enzymatic activities rapidly increased from 0 to 18 UI after about 25 hours of incubation. After that, the enzymatic activities were slightly increased and reached 20.2 UI after 50 hours of incubation before dropped out to 17.3 UI after 55 hours. Then the enzymatic activities were slightly decreased to 16.1 UI at the end of the experiment.

The current study also showed the presence of phos-

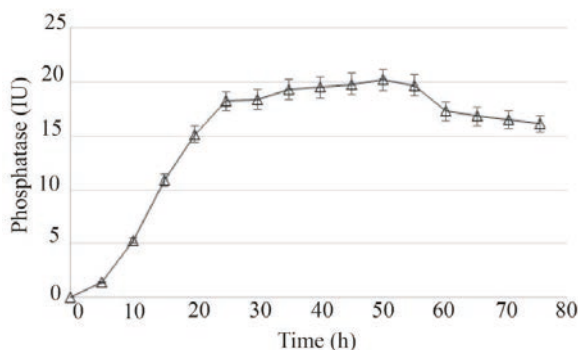


Figure 3: Phosphatase profile of phosphorus-solubilizing bacteria PSB31 for 72 h of incubation and $5 \text{ g l}^{-1} \text{Ca}_3(\text{PO}_4)_2$ in the medium

phatase in the supernatant of PSB31 with amounts that were higher than the results of previous studies (Mendoza-Arroyo et al., 2020). All in all, the result suggested the PSB31 strain is a very promising agent that could be used to solubilize the phosphorus compound in the soil to increase the P availability for crops.

3.4 PSB31 ENHANCED THE GROWTH OF TOMATO SEEDLINGS

The results of greenhouse experiments clearly showed that PSB31 was able to promote tomato growth under the stress of nutrient conditions. The results were illustrated in Figure 4A and presented in Figure 4B. As can be seen from Figure 4B, tomato seedlings supplemented with both PSB31 and $\text{Ca}_3(\text{PO}_4)_2$ (T2) had a greater increase of root and shoot length than those with no bacteria + $\text{Ca}_3(\text{PO}_4)_2$ + KH_2PO_4 (T3) and with no bacteria + $\text{Ca}_3(\text{PO}_4)_2$ (T1).

The result also presented a maximum quantity of shoot length for the seedlings that were not bacterized but were regularly watered with the P solution (positive control). Furthermore, the results also showed the tomato seedlings received both insoluble $\text{Ca}_3(\text{PO}_4)_2$ and PSB31 strain had the fresh and dry mass moderately higher than the negative control but slightly lower than the positive control (Table 2). It was consistent with reports in which PSB when applied into soil could enhance significantly the development and phosphate uptake in many crop species (Kumar et al., 2017; Mendoza-Arroyo et al., 2020).

Additionally, the results of the experiment for potting medium indicated that PSB31 could solubilize $\text{Ca}_3(\text{PO}_4)_2$ in sand increasing the soluble P in the sand matrix to $106.7 \pm 3.5 \text{ mg l}^{-1}$. This could be PSB31 strain generated phosphatase or low molecular organic acids to converted the phosphate from insoluble to soluble form that provided a P balance for plant development resulting in plant growth promotion (Mendoza-Arroyo et al., 2020). These results suggest the potential application of PSB31 as a biofertilizer for sustainable agriculture.

As can be seen from Table 2, tomato seedling inoculated with PSB31 strain had the fresh and dry shoot weights 2 times higher than the one that grew in sand mixed with the $\text{Ca}_3(\text{PO}_4)_2$ but lower than the one supported with soluble P. These results were similar to the one of Lee et al. (2020), in which the *Arabidopsis thaliana* (L.) Heynh. seedlings bacterized with *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 strain L1 via the roots had a considerable increase in plant mass. These results suggested a possible role of PSB31 strain in the process of assimilation by pho-

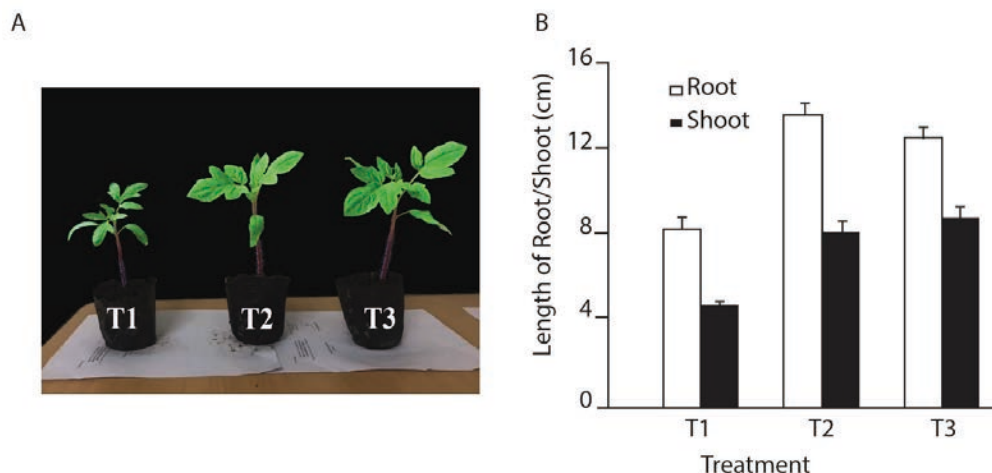


Figure 4: Plant growth promotion activity by phosphate-solubilizing bacteria. A. Illustration for plant growth. B. Length of root and shoot of plant under different conditions. T1: no bacteria + $\text{Ca}_3(\text{PO}_4)_2$, T2: with PSB31 + $\text{Ca}_3(\text{PO}_4)_2$, T3: no bacteria + $\text{Ca}_3(\text{PO}_4)_2$ + KH_2PO_4

Table 2: Effect bacterial isolates on plant biomass production

Treatment	Root mass (g)		Shoot mass (g)		Total P (%)
	Fresh	Dry	Fresh	Dry	
No bacteria + $\text{Ca}_3(\text{PO}_4)_2$	0.153 ± 0.057^a	0.022 ± 0.002^a	0.378 ± 0.006^a	0.044 ± 0.003^a	0.15
PSB31 + $\text{Ca}_3(\text{PO}_4)_2$	0.425 ± 0.223^a	0.025 ± 0.007^a	0.608 ± 0.027^b	0.089 ± 0.013^b	0.31
No bacteria + $\text{Ca}_3(\text{PO}_4)_2$ + KH_2PO_4	0.492 ± 0.257^a	0.26 ± 0.004^a	0.697 ± 0.034^b	0.091 ± 0.032^b	0.35

Data are means \pm SE of three independent biological replicates. Bearing different letters in the same row are significantly different from each other according to the least significant difference (LSD) test ($p < 0.05$)

tosynthesis, subsequently, the plant mass improvement (Wu et al., 2019).

Moreover, the higher P amount measured in bacterized seedlings (0.31 %) compared to the one grown in the sand with only $\text{Ca}_3(\text{PO}_4)_2$ (0.15 %) indicated that the PSB31 strain functioned in releasing the soluble P from $\text{Ca}_3(\text{PO}_4)_2$ enhancing P uptake of seedlings. The results also presented the P amount in seedling grown in pot watered with nutrient containing P was the highest among treatments with 0.35 % (Fig. 4B). These results suggested strain PSB31 could enhance P uptake in the bacterized seedlings led to the high amount of water in plant contributing to biomass formation. These results endorsed that the optimistic result of PSB31 on crop yield due to the increase of nutrients uptake (predominantly phosphorus).

4 CONCLUSIONS

The soil microorganisms capable of converting insoluble P to soluble P are being explored as an environ-

mentally friendly agent to promote plant development and subsequently increasing yields. The results showed that among 7 isolates, strain PSB31 has good phosphate solubilization activity and promoted the growth of tomato seedlings under phosphate limiting conditions. This PSB31 strain had been identified belonging to *Bacillus* sp. (in: Bacteria) strain IMAU61039. All of these results suggested the PSB31 strain could be potentially used as a microbial biofertilizer candidate for commercial applications in the future.

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