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Enhance the phytoremediation efficiency of *Echinochloa colona* (L.) Link for Pb-contaminated soil by phosphorus solubilizing bacteria

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Abstract: A promising solution for phytoremediation of metal-contaminated soils is to use plants in combination with phosphate-solubilizing bacteria (PSB). In this study, we subjected to isolate PSB from paddy soil and investigate their ability in improving the phytoremediation of lead (Pb^{2+}) by a weed plant (*Echinochloa colona* (L.) Link) as well as in promoting the growth of *E. colona* under Pb stress condition. Total 06 PSB (labeled from TB01 to TB06) were isolated and the TB04 showed the strongest phosphate-solubilizing activity with the highest values of phosphorus solubilization index (PSI = 7.13) obtained from $Ca_3(PO_4)_2$. Especially, the phosphorus solubilizing ability of the TB04 strain was not affected by the high Pb^{2+}concentration. The TB04 strain was identified as *Pseudomonas putida* Trevisan, 1889 (accession number FJ976601.1). Furthermore, *E. colona* inoculated with TB04 strain significantly increased the phytoremediation efficiency of Pb from Pb-contaminated soil and the growth was enhanced clearly. These results suggest that the TB04 strain could potentially use as an inoculant in combination with *E. colona* to construct novel constructed wetlands for phytoremediation of metal-contaminated soil.

Key words: lead immobilization; *Pseudomonas putida*; soil fertility; phytoremediation; metal-contaminated soil

Povečanje fitoremediacijske učinkovitosti vrste *Echinochloa colona* (L.) Link z bakterijami, ki sproščajo fosfor v tleh, onesnaženih s svincem

Izvleček: Obetajoča rešitev za fitoremediacijo s kovinami onesnaženih tal je uporaba rastlin v kombinaciji z bakterijami, ki sproščajo fosfor (PSB). V raziskavi so bili preučevani izolati teh bakterij iz riževih polj in njihova sposobnost izboljšanja fitoremediacije svinca (Pb^{2+}) s plevelno vrsto kostrebe (*Echinochloa colona* (L.) Link) kot tudi izboljšanje rasti te rastline v razmerah kovinskega stresa zaradi onesnaženja s svincem. Celokupno je bilo izoliranih 6 izolatov PSB (označenih kot TB01 do TB06), pri čemer je imel izolat TB04 največjo sposobnost sproščanja fosforja (z indeksom PSI = 7,13) iz kalcijevega fosfata ($Ca_3(PO_4)_2$). Na sposobnost sproščanja fosforja pri sevu TB04 niso vplivale velike koncentracije Pb^{2+}. Sev TB04 je bil identificiran kot vrsta bakterije *Pseudomonas putida* Trevisan, 1889 (številka akcesije FJ976601.1). Inokulacija kostrebe s sevom TB04 je značilno povečala njeno fitoremediacijsko učinkovitost za svinec v s svincem onesnaženih tleh, pri čemer je bila njena rast značilno pvečana. Rezultati nakazujejo, da bi sev TB04 lahko potencialno uporabili kot inokulant kostrebe kot nov način fitoremediacije s kovinami onesnaženih močvirnih tal.

Ključne besede: imobilizacija svinca; *Pseudomonas putida*; rodovitnost tal; fitoremediacija; s kovinami onesnažena tla

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1 INTRODUCTION

It is a fact that industrial development, agricultural practices, and human activities caused a quick increase in areas of soil contaminated with heavy metals (Xiao et al., 2021). Importantly, pollution with lead (Pb) was the most concern because it has no function in biology or physiology for the living cells, but was determined as a toxic chemical for living cells (Yahaghi et al., 2018; Aransiola et al., 2019). Especially, the metal chemicals were not biodegraded leading to their accumulation in the soil, which can increase the risk of these metals entering the food chain by uptake activity of crops (Noble et al., 2018; Xiao et al., 2021). Hence, the removal of metal pollutants from the soil is very important and necessary. Although there are several methods have been applied to remediate the metal pollution in soil, phytoremediation of heavy metals is a promising one that uses plants to uptake the metal pollutants from soil accumulating them in the above-ground part of the plant for disposal. Hence, phytoremediation is environmentally friendly, low-cost, and easy to set up (Noble et al., 2018; Xiao et al., 2021).

In agricultural practices, the application of PGPR, particularly phosphate solubilizing bacteria (PSB), to improve crop yield is becoming more and more frequent. Besides assisting plants in nutrient uptakes and disease protection, PSB also presented its ability to enhance plant growth in harsh conditions caused by contaminants in the soil such as metal pollutants (Noble et al., 2018; Adhikari et al., 2020). Therefore, the inoculation of PSB in the phytoremediation of metal pollutants from the soil is very potential. It was reported that a weed named *Echinochloa colona* (L.) Link that has a wide distribution in an agroecosystem and has played role in the uptake of heavy metals from metal-contaminated soil (Subhashini and Swamy, 2016; Noble et al., 2018). It demonstrated their efficiency in the phytoremediation of lead, nickel, zinc, cadmium, and chromium from contaminated soils (Subhashini and Swamy, 2016). In addition, Noble et al. (2018) reported that with the assistance of plantain peels the phytoremediation of Pd and Cd in soil by *E. colona* was significantly enhanced. Therefore, the application of *E. colona* for phytoremediation of metal-contaminated soils is very promising. However, it is a fact that phytoremediation presents some limitations such as being time-consuming, and the removal efficiency of metals depends strongly on the plants vegetated in that system.

Interestingly, the combination of plants and plant growth-promoting rhizobacteria (PGPR) could improve the phytoremediation efficiency (Noble et al., 2018; Xiao et al., 2021). However, the study using PSB to enhance the removal efficiency of metal pollutants from the soil by *E. colona* are scarce. Hence, this study's aims were (1) to isolate PSB from Thai Binh paddy soil and (2) to investigate their ability in improving the phytoremediation of lead (Pb^{2+}) by a weed plant (*Echinochloa colona*) as well as in promoting the growth of *E. colona* under Pb stress condition.

2 MATERIAL AND METHODS

2.1 ISOLATION OF PHOSPHORUS-SOLUBILIZING BACTERIA

Samples of soil were collected from different locations at agricultural fields in Thai Binh Province, Vietnam for isolation of PSB. About 2 g of each soil sample adhered to the rice roots was collected and carefully transferred into sterile tubes containing sterile deionized (DI) water (about 2 ml). Then, each test tube was vortexed thoroughly and let set for 5 minutes at room temperature. The 10-fold dilutions in the same buffer were applied. After that, it took 100 µl of diluted samples to plate onto Pikovskaya (PVK) media agar plates (Pikovskaya, 1948). The bacterial colonies with clear halos in the PVK agar plate indicated solubilizing activity of the phosphate. These were sub-cultured on PVK (Biobasic, Canada).

Similar methods were applied to screen for microorganisms that could solubilize aluminum phosphate ($AlPO_4$) and iron phosphate ($FePO_4$). In this experiment, the medium was modified from the PVK medium, in which the $Ca_3(PO_4)_2$ was altered by either 5 g l^{-1} of $AlPO_4$ or 5 g l^{-1} of $FePO_4$.

The PVK medium used in study include (g l^{-1}): glucose, 10; $(NH_4)_2SO_4$, 0.5; $MgSO_4.7H_2O$, 0.1; yeast extract, 0.5; KCl, 0.2; NaCl, 0.2; $FeSO_4.7H_2O$, 0.002; $MnSO_4.7H_2O$, 0.002; $Ca_3(PO_4)_2$, 5; pH 6.5 (for agar plate, 15 g of agar was added). The plate incubation was carried out at 30 °C for 7 days. All media and glassware used were sterilized in an autoclave before use.

2.2 MOLECULAR IDENTIFICATION OF TB04 STRAIN

The total DNA of strain TB04 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 27F and 1492R. The sequence of 16S rDNA sequences obtained was blasted on NCBI to identify the species. The sequences with high similarity were used for multiple cluster alignment and phylogenetic analysis on MEGA software (v.7.2).

2.3 DETERMINE PHOSPHATE SOLUBILIZING EFFICIENCY OF THE ISOLATES

Single colonies were cultured separately in liquid LB media at 30 °C for 24 h on the shaker (150 rpm). Then, bacterial cells of each strain were collected by applying a described procedure. The bacterial suspension of isolates (10^6 CFU ml^{-1}) was determined for their ability to solubilize different insoluble phosphorus compounds ($Ca_3(PO_4)_2$, sodium phytate, $FePO_4$, or $AlPO_4$) on either solid or liquid PVK medium. The condition for plate incubation was at 30 °C for seven days. The medium with no bacteria was used as the control.

After seven days of incubation, the determination of soluble P concentration in bacterial culture was done using the molybdenum blue method (Waterlot, 2018), and the phosphate solubilization index (PSI) of bacteria grown on plates was measured as the method described by Liu et al. (2015). The pH measurement of the bacterial culture was carried out by using the pH meter.

In addition, the isolated PSB were also characterized their P solubilizing efficacy in soil conditions by a method adapted from Wan et al. (2020). Different treatments have been done in triplicates: (T1) 100 g sterilized soil + 10 ml bacterial solution; (T2) 95 g sterilized soil + 10 ml bacterial solution + 5 g $Ca_3(PO_4)_2$, (T3) 95 g sterilized soil + 10 ml bacterial cultures + 5 g $Ca_3(PO_4)_2$ + 10 ml nutrient solution (PVK liquid medium removed $Ca_3(PO_4)_2$). Soil moisture in the experiments was adjusted to 80 % by sterile water and kept for 30 days at 25 °C. After that, the amount of available P (AP) in treated soils was determined by the molybdenum blue method (Waterlot, 2018).

2.4 INDOLE-3ACETIC ACID (IAA) PRODUCTION OF PSB

The isolates were also screened for IAA production by using LB medium supplemented 0.1 % L-tryptophan. The colorimetric method using ferric chloride-perchloric acid reagent ($FeCl_3$–$HClO_4$) as described by Luu et al. (2021) was applied to measure the amount of IAA produced.

2.5 PHOSPHORUS SOLUBILIZATION ABILITY OF TB04 ISOLATE UNDER LEAD STRESS

The isolates were then investigated for the solubilization of $Ca_3(PO_4)_2$ under Pb^{2+} stress. The Pb-contaminated soil was artificially produced by mixing the sterilized soil with $Pb(NO_3)_2$ (0, 200, 400, 800, 1600, or 2400 mg Pb/ kg soil) and $Ca_3(PO_4)_2$ (as the P source). Then, 100 ml of the culture of isolated PSB were added to the prepared soil and were kept for four months at room temperature. For control experiments, soil with only $Ca_3(PO_4)_2$. The moisture in all experiments was kept at 80 % by watering with sterile water every five days. After four months of incubation, the soil sample was collected, air-dried, ground, sieved through a 0.2-mm sieve, and subsequently extracted at room temperature for 30 min by a mixed solution of 0.025M HCl and 0.03 M NH_4F (1:10 soil:water ratios). The amount of the available P in the treated soil was determined by the molybdenum blue method (Waterlot, 2018).

2.6 EFFECT OF TB04 STRAIN INOCULATION TO THE DEVELOPMENT AND PB UPTAKE OF WEED PLANT (*Echinochloa colona*)

The pot experiments were prepared as the method described in Luu et al. (2021). Briefly, seeds of *E. colona* were sterilized on their surface by using ethanol 70 % for 30 s and sodium hypochlorite solution 2 % for 5 minutes. Then these seeds were washed three times with sterile water and dried on autoclaved filter papers. The TB04 strain with the highest efficiency of Pb uptake and IAA production was overnight grown, centrifugated, and washed with sterile water before being resuspended with sterile water to make a bacterial solution with OD = 1. The sterilized seeds were covered with selected PSB by soaking in the bacterial solution for 30 minutes before sowing. For the control, sterile water was used instead of the bacterial solution.

The treatment was done in triplicates by sowing ten bacterized seeds of *E. colona* per plastic pot filled with about 1 kg of lead-contaminated soil (600 mg kg^{-1} of $Pb(NO_3)_2$). After plant establishment, one plant per pot was done. The pots were kept in the nursery garden and soil moisture was held at 60 % of water holding capacity during the experiment by adding a specific amount of sterile water as the method described by Steadman et al. (2004). After one month, 100 ml of the bacterial culture (OD = 1) were added to the treated pot as biofertilizer while sterile water was used for the control.

The experiments were carried out in 3 months. The measured parameters for plant growth were plant height, shoot and root dry mass. The plant height was measured from the aboveground to the tip of the upper-most leaf of the plant. The root was cut from the plant and removed Pb ions bounding to its surface by washing with 1 mM $Ca(NO_3)_2.4H_2O$ and sterile water. The dry mass of root and shoot were determined after dried in an oven at 70 ˚C for 72 h. The Pb in the oven-dried shoot and root

was extracted by using a solution of HNO_3-HCl (70 %) and H_2O_2 (30 %) (Jones et al., 1990) and were measured by FAAS. All measurements were done in triplicates.

2.7 DATA ANALYSIS

All experiments were repeated three times the results were presented as mean values with ± 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 AND CHARACTERIZATION OF PHOSPHATE-SOLUBILIZING BACTERIA

Bacteria isolates that produced a transparent zone around colonies in the Pikovskaya (PVK) medium were determined as phosphate-solubilizing bacteria and were selected. There were six single colonies were observed and further transferred into new PVK plates for purification (Table 1).

All isolates showed different efficiency in solubilizing phosphorus after 7 days of incubation at 30 °C, which was illustrated by different values of PSI ranging from 1.53 to 7.13 (Table 1). A further characteristic of isolates indicated their ability in IAA production, in which the highest amount of IAA (7.86 mg l^{-1}) was observed for the TB04 strain.

Furthermore, the results also presented the different capabilities in solubilizing phosphorus compounds of all isolates from different phosphate sources. All isolated strains could solubilize multiple insoluble phosphorus compounds ($Ca_3(PO_4)_2$, $AlPO_4$, and $FePO_4$) but only TB03 and TB04 presented the phytate solubilization (Table 2). For inorganic P, the results indicated that $Ca_3(PO_4)_2$ was the most favorable compound for all strains demonstrated by the highest amount of soluble P (173.11-572.13 mg l^{-1}) released from this compound; and the TB04 also presented the highest efficiency. In addition, approximately 10-fold less of solubilization efficiency was observed for the remaining complexed phosphate sources including $AlPO_4$ (21.17 to 72.13 mg l^{-1}) and $FePO_4$ (10.51 to 29.73 mg l^{-1}) by most of the isolates (Table 2). Furthermore, only two strains, TB01 and TB04, showed the ability in solubilizing organic phytate supplemented with a modified PVK broth medium (1.53 and 3.61 mg l^{-1}, respectively). These results indicated that TB04 could solubilize multiple P sources and might be used to reverse insoluble phosphate to soluble form in agriculture.

Table 1: Characteristics of isolated phosphate-solubilizing bacteria (PSB)

PSB isolates	Phosphate solubilization index (Agar)	IAA production (mg l^{-1})
TB01	4.13 ± 0.11^{b}	1.87 ± 1.21^{c}
TB02	4.12 ± 0.12^{b}	2.02 ± 1.02^{c}
TB03	3.37 ± 0.31^{bc}	3.25 ± 1.01^{bc}
TB04	7.13 ± 0.15^{a}	7.86 ± 1.01^{a}
TB05	1.53 ± 0.23^{c}	4.52 ± 1.12^{b}
TB06	1.67 ± 0.12^{c}	1.91 ± 1.12^{c}

Data are means ± SE of three independent biological replicates. Data with the same letters in the same column are not significantly different from each other according to the honestly significant difference (HSD) test ($p < 0.05$)

It was a fact that the solubilization of $AlPO_4$ and $FePO_4$ was lower than the one of $(Ca_3(PO_4)_2$. It can be explained by two possible reasons. Firstly, it was reported that the interaction of aluminum (Al^{3+}) and iron (Fe^{3+}) with phosphate ion (PO_4^{3-}) is a reversible reaction. Hence, it could be that the acids produced by PSB during the solubilization might force the reversible reaction of aluminum (Al^{3+}) and iron (Fe^{3+}) with phosphate ion (PO_4^{3-}) to form insoluble complexes (Sánchez-Cruz, 2020) leading to an inefficient in solubilizing $FePO_4$ and $AlPO_4$. Secondly, it could be differences in affinity among cations and anions in the solution, in which the anions generated by PSB such as carboxylic and hydroxylic groups preferred calcium (Ca^{2+}) to aluminum (Al^{3+}) and iron (Fe^{3+}) and subsequently enhanced the phosphorus solubilization (Sánchez-Cruz, 2020). Moreover, the results indicated the pH reduction and production of a phytase of strains TB01 and TB04 played significant roles in solubilizing inorganic phosphate. These were demonstrated by some previous research (Kumar and Rai., 2015; Wan et al., 2020). All of these suggest the organic acids and/or phosphate solubilizing enzymes produced by PSBs play important roles in mineralizing phosphorus compounds (Walpola et al., 2013).

Moreover, the correlation analysis showed a low correlation between the values of PSI and the amount of soluble P released (r = 0.442), and between pH of supernatant and the amount of soluble P released (r = 0.501). These could be related to P solubilizing mechanisms, in which the PSB produced external metabolites such as hydrolytic enzymes, and/or organic acids that enhanced the solubilization of mineral phosphates and could reduce the pH of bacteria culture.

Some reports demonstrated a positive correlation between the pH of culture and the solubilized amount of

Table 2: Determination of phosphate solubilization ability in PVK broth medium with $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and sodium phytate by isolated PSB

	PVK with $Ca_3(PO_4)_2$		PVK with $AlPO_4$		PVK with $FePO_4$		PVK with sodium phytate	
PSB isolates	Soluble P (mg l^{-1})	pH	Soluble P (mg l^{-1})	pH	Soluble P (mg l^{-1})	pH	Soluble P (mg l^{-1})	pH
TB01	251.15 ± 10.71^{b}	4.95 ± 0.21^{b}	54.31 ± 4.71^{b}	3.55 ± 0.12^{c}	10.51 ± 1.53^{d}	3.47 ± 0.23^{d}	ND	4.35 ± 0.21^{b}
TB02	176.15 ± 7.12^{c}	3.93 ± 0.11^{c}	72.13 ± 2.35^{a}	3.30 ± 0.17^{bc}	13.25 ± 1.31^{c}	3.64 ± 0.17^{c}	ND	3.71 ± 0.18^{c}
TB03	248.12 ± 12.72^{b}	3.78 ± 0.14^{c}	44.16 ± 2.31^{c}	3.25 ± 0.31^{bc}	29.73 ± 2.42^{a}	3.82 ± 0.16^{c}	1.53 ± 0.19^{b}	3.92 ± 0.13^{bc}
TB04	572.13 ± 12.41^{a}	4.22 ± 0.15^{c}	51.34 ± 3.17^{b}	3.27 ± 0.12^{bc}	23.15 ± 1.27^{ab}	3.53 ± 0.12^{cd}	3.61 ± 0.71^{a}	3.53 ± 0.51^{b}
TB05	182.13 ± 11.10^{c}	5.21 ± 0.13^{b}	21.17 ± 2.73^{e}	4.31 ± 0.15^{b}	20.53 ± 1.17^{b}	4.05 ± 0.13^{b}	ND	3.37 ± 0.33^{d}
TB06	173.11 ± 9.13^{c}	4.57 ± 0.21^{bc}	33.17 ± 2.23^{d}	3.78 ± 0.17^{bc}	19.56 ± 1.15^{b}	3.77 ± 0.32^{c}	ND	4.17 ± 0.27^{b}
Control media	ND	6.51 ± 0.11^{a}	ND	6.52 ± 0.13^{a}	ND	6.47 ± 0.15^{a}	ND	6.53 ± 0.31^{a}

Data are means ± SE of three independent biological replicates. Value with the same letter in the same row is not significantly different from each other according to the honestly significant difference (HSD) test ($p < 0.05$). ND: not detected

phosphorus complexes ($Ca_3(PO_4)_2$) (Marra et al., 2019). However, the results showed an uncorrelation between the soluble P release and pH reduction. This might be chelation between metal cations (Ca^{2+}, Al^{3+}, Fe^{3+}) and anion groups of produced organic acids (Stevenson, 2005) leading to pH decrease and subsequently the increase of soluble P. Therefore, it could be said that the solubilization of phosphorus compounds is simultaneously affected by pH decrease and acid production in the solution (Fankem et al., 2006).

3.2 MOLECULAR IDENTIFICATION OF STRAIN TBO4

The molecular identification of TB04 indicated that this strain was *Pseudomonas putida* (accession number FJ976601.1) (Figure 1). The sequence of 16S rDNA of TB04 was deposited in GenBank with an accession number OP141766. This strain showed a significant efficiency of $Ca_3(PO_4)_2$ solubilization compared to reported *Pseudomonas* sp. (such as *Pseudomonas fluorescens* (Flügge 1886) Migula, 1895 (184 mg l^{-1}) (Katiyar and Goel, 2003), *Pseudomonas putida* (247 mg l^{-1}) (Pandey et al., 2006). These differences can be explained due to the difference in isolated strains that were grown and developed under specific conditions.

3.3 EFFECT OF PSB AND TRICALCIUM PHOSPHATE IN UNCULTIVATED SOIL

Next steps, we investigated the phosphate solubilizing ability of the isolates in $Ca_3(PO_4)_2$ -rich soil conditions. As we expected, all isolated PSB could solubilize the $Ca_3(PO_4)_2$ incubated in soil (Figure 2).

After 30 days of incubation, the AP content in soil supplemented with TB04 was significantly higher in all experiments than the one in the control. Notably, the soil added with TB04 showed the highest amount of AP in the same soil treatments. Particularly, the AP amount in TB04-incubated soils, in T1 (soil + PSB), T2 (Soil + PSB + $Ca_3(PO_4)_2$), and T3 (Soil + PSB + $Ca_3(PO_4)_2$ + Nutrient) treatments were 0.55, 0.87, and 1.72 mg g^{-1}, respectively. Especially, the significantly higher values of AP in T3 treatment compared to those in T1 and T2 treatments ($p < 0.05$) were observed. This might be because of the results of the addition of sufficient nutrients for bacterial growth (Figure 2). As can be seen from Figure 2, the positive correlation between the AP amount in PSB-inoculated soil and added amount of $Ca_3(PO_4)_2$ in the presence of TB04 strain. These results were consistent with previous reports, which demonstrated the potential application of PSB in improving soil quality, particularly by increasing the amount of available P that directly influences the plant development and plant uptake and subsequently the yield (Himani and Reddy, 2011; Teng et al., 2019; Wan et al., 2020). These improvements might be due to the inoculated PSB in treatment solubilized the $Ca_3(PO_4)_2$ fertilizer to release soluble P that was partially used for the development of PSB, subsequently enhancing the phosphorus's efficiency. These explanations were demonstrated by studies that reported a positive correlation between the change in the amount of soil organic carbon and the change in bacterial development in soil (Nakhro and Dkhar, 2010; Wan et al., 2020). In addition, another contributor to the improvement of soluble P amount in treated soil might be the difference in hydrolytic enzymes (such as phosphatase, and phytase)

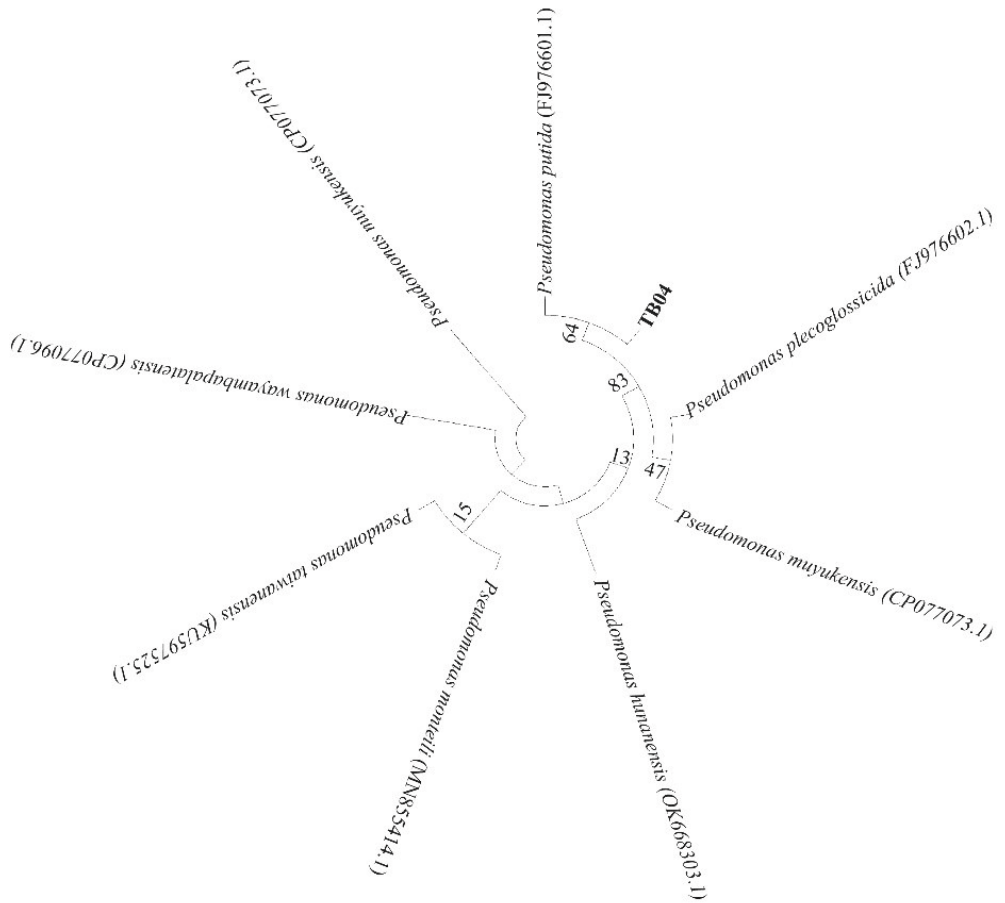

Figure 1: A neighbor-joining tree shows the phylogenetic relationships among 16S rDNA sequences of TB04 and their closely related sequences from NCBI

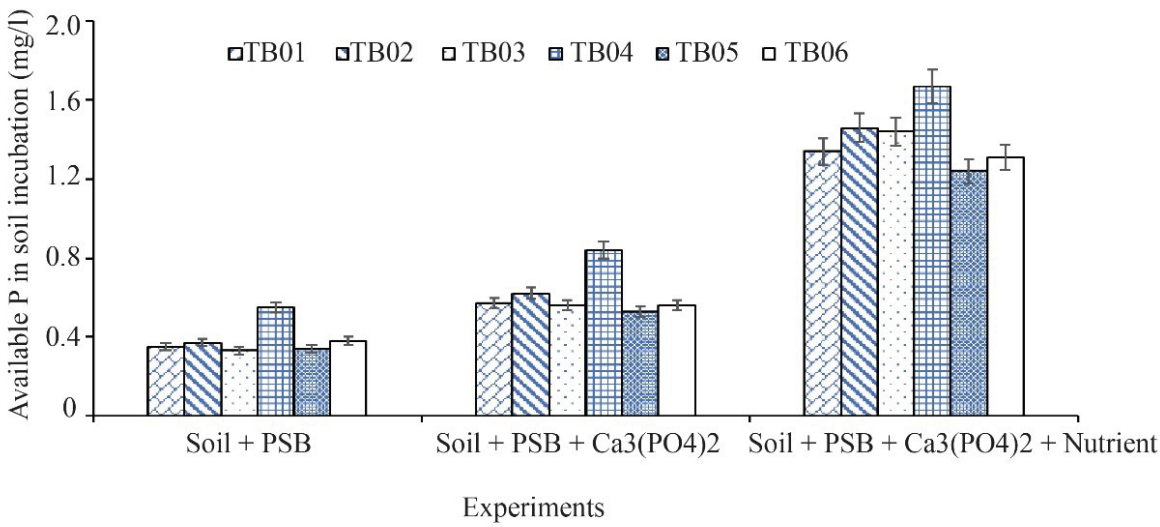

Figure 2: Evaluation of available P in soil incubation (mg l^{-1}). The presenting results are the mean value of three replicates. PSB: phosphorus solubilizing bacteria; TB01 to TB06 are phosphorus solubilizing bacteria

presented in soil (Teng et al., 2019). Presumably, the results indicated that TB04 showed a promising application in solubilizing insoluble phosphorus compounds in soil that increase soil health.

3.4 EVALUATION OF THE PHOSPHORUS SOLUBILIZATION ABILITY OF TB04 ISOLATES UNDER LEAD STRESS

In fact, the agricultural soil was contaminated with metals caused by the overuse of chemical fertilizer. Hence, we investigated the ability of strain TB04 to solubilize phosphorus compounds in the presence of lead with different concentrations. As shown in Figure 3, the amount of available P in treated soil was higher than that of initiated soil (about 0.19 mg g^{-1}). These results indicated that the TB04 strain could solubilize phosphorus compounds in soil and this ability was not affected by an increasing amount of Pb concentration.

3.5 INOCULATION OF TB04 STRAIN IMPROVES THE DEVELOPMENT AND PB UPTAKE OF WEED PLANT (*ECHINOCHLOA COLONA*)

The effect of TB04 inoculation on the growth properties of weed (*Echinochloa colona*) under greenhouse conditions was studied. The obtained results of greenhouse experiments were shown in Table 3. As can be seen, the TB04 strain significantly improved the plant growth parameters of *E. colona* compared to the control experiment, which used sterile water instead. Particularly, the length of TB04 inoculated plants was increased approximately 1.5 times compared to non-bacterized plants. Similarly, the increase in shoot and root dry mass observed for the plants bacterized with TB04 with 1.5 times higher than the control.

These data were not in agreement with some previous studies, which reported that plant development was inhibited when grown on heavy metal-contaminated soil (Tangahu et al., 2011). This might be due to the TB04 strain produced IAA (a plant up-regulator) and solubilized phosphorus compounds increasing the amount of available P in soil, and subsequently enhancing the plant development. Lin et al. (2018) demonstrated the growth of *Wedelia trilobata* (L.) H.Rob. & Cuatrec. cultivated in Cu^{2+}-contaminated soil was significantly upregulated when inoculated with *Paenibacillus polymyxa* (Prazmowski, 1880) Ash et al., 1994, a phosphate-solubilizing bacterium. Another example is the study of Yahaghi et al. (2018) who showed the inoculation of a bacterial mixture (*Brevibacterium frigoritolerans* Delaporte and Sasson, 1967 YSP40 and *Bacillus paralicheniformis* sp. nov. YSP151) improved the development of *Brassica juncea* (L.) Czern that grown in a soil contaminated with heavy metal by producing IAA, siderophores, and solubilizing inorganic phosphate.

The data in Table 3 also indicated that the Pb concentration in the shoot of bacterized *E. colona* plant was dramatically increased in the comparison with one of non-bacterized plants. The result also showed that the inoculation of TB04 was not clearly influenced by the amount of Pb in the root. In addition, the result also presented that the TB04-treated plants contained more amount of Pb uptake in the shoot than the control did.

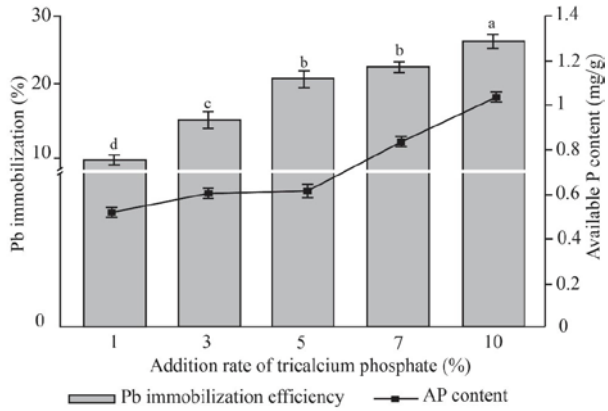

Table 3: Enhanced effect of TB04 strain on the development and Pb uptake of *Echinochloa colona*

Table 3: Enhanced effect of TB04 strain on the development and Pb uptake of Echinochloa colona

Phosphorus solubilizing bacteria	Plant length (cm)	Shoot dry mass (g/plant)	Root dry mass (g/plant)	Pb concentration in shoot (mg kg^{-1})	Pb concentration in root (mg kg^{-1})	Pb uptake by shoot (μg/pot)
SDW[a]	52.37 ± 1.79 a[b]	18.32 ± 2.73 a	14.05 ± 2.27 a	40.27 ± 3.02 a	94.17 ± 2.79 a	71.28 ± 11.53 a
TB04	73.51 ± 3.73 b	29.21 ± 3.72 b	22.07 ± 1.17 b	73.17 ± 5.27 b	84.92 ± 2.76 a	223.72 ± 18.74 b

[a] TB04: selected phosphorus solubilizing bacteria; SDW: Sterile distill water

[b] Presenting values the mean ± standard deviation. Values with a different letter in the same column indicated a significant difference according to HSD ($p < 0.05$)

The data showed that the bacterial inoculation increased the Pb concentration in the shoot of bacterized *E. colona* plant and was not clearly influenced by the amount of Pb in the root. The increase in Pb^{2+} absorption could be due to the inoculated PSB produced metabolites (such as organic acids) that enhanced the bioavailability of Pb^{2+} in the root rhizosphere, and subsequently improved the Pb^{2+} absorption of root (Aransiola et al., 2019; Xiao et al., 2021). In addition, the result also indicated a higher amount of Pb uptake in the shoot than the control did. This might be the result of the improvement in shoot biomass and the Pb^{2+} translocation caused by the TB04 inoculation. Yahaghi et al. (2018) reported that the Pb^{2+} uptake in the shoot of *B. juncea* inoculated with *Brevibacterium frigoritolerans* YSP40 and *Bacillus paralicheniformis* YSP151 strains was increased 3 and 4 times, respectively.

4 CONCLUSIONS

This study demonstrated the potential application of PSB isolated from paddy soil collected from Thai Binh province for enhancing the removal efficiency of Pb^{2+} pollutants from metal-contaminated soil by *E. colona*. The inoculation of PSB isolated into the Pb-contaminated soil not only promoted the plant growth of *E. colona* but also enhanced the Pb^{2+} uptake by the root of *E. colona*. These data suggest a potential application of isolated PSB combined with a phytoremediator for improving the phytoremediation of metal pollutants from metal-polluted soil.

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