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Original Paper

Phosphate-solubilizing bacteria enhance the growth and lead removal of weed plants (*Echinochloa colona*)

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Heavy metal pollution of soils in being a serious problem for sustainable agriculture. A promising solution for phytoremediation of metal-contaminated soils is to use plants in combination with phosphate-solubilizing bacteria (PSB). In this study, a total of 30 soil samples were collected from different locations in Nam Dinh, Vietnam. They were used to isolate PSB from paddy soil on Pikovskaya agar media, and 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 was investigated by pot experiments. Total 07 PSB were isolated and the ND04 showed the ability in solubilizing multiple P sources ($Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and phytate) with corresponding P solubilizing levels were 530.12, 50.13, 25.02, and 3.58 mg/L PO_4^{3-}–P, respectively. Moreover, the ND04 strain was identified as *Pseudomonas putida* (accession number FJ976605.1) and produced the highest values of available P (1.67 mg/L) in $Ca_3(PO_4)_2$-incubated soil experiments. Furthermore, the ND04 inoculation significantly enhanced the growth of *E. colona* and also increased the phytoremediation efficiency of Pb from Pb-contaminated soil. These results suggest the ND04 could potentially use to construct novel constructed wetlands for phytoremediation of metal-contaminated soil.

Keywords: metal-contaminated soil, *Pseudomonas putida*, soil fertility, phytoremediation, weed

1 Introduction

In recent decades, the hectare of soil contaminated with heavy metals is increasing quickly due to industrial development, agricultural practices, and human activities as well (Aransiola et al., 2019; Xiao et al., 2021, Bortoloti & Baron, 2022). Among the metal pollutants, 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). Notably, the metal chemicals were not biodegraded leading to their accumulation in 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.

Among applying methods for metal remediation, phytoremediation is a promising one that used 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).

Echinochloa colona is a weed that has a wide distribution in an agroecosystem. *E. colona* has been reported that play a role in the uptake of heavy metals from metal-contaminated soil (Subhashini & Swamy, 2016; Noble et al., 2018). It demonstrated their efficiency in the phytoremediation of lead, nickel, zinc, cadmium, and chromium from contaminated soils (Subhashini & Swamy, 2016, Bortoloti & Baron, 2022). 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

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that phytoremediation presents some limitations such as 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).

In agricultural practices, the application of PGPR, particularly phosphate solubilizing bacteria (PSB), to improve the crop yield is becoming more and more frequent. Besides assisting plants in nutrient uptakes and disease protection, PSB also presented its ability in enhancing 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. However, the study using PSB to enhance the removal efficiency of metal pollutants from the soil by *E. colona* is not much.

Nam Dinh (19° 52′–20° 30′ N, 105° 55′–106° 35′ E) located to the south of the Red River delta is a tropical area with hot and humid rain. Agriculture has an important role in Nam Dinh province, with approximately 67% of the land used for agricultural production. However, the agricultural soil is being polluted with heavy metals that came from different sources such as overuse of chemical fertilizers in promoting crop growth or the irrigation with heavy metal-contaminated water. Therefore, the development of an environmentally friendly method to remove the contaminated heavy metal from agricultural soil plays a key role in sustainable agriculture in Nam Dinh. Hence, this study's aims were (1) to isolate PSB from Nam Dinh 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 bacteria with the phosphorus-solubilizing ability

40 alluvial soil samples were collected at 8 fields cultivating rice in Nam Dinh, Vietnam in September 2021. The sampling procedure is carried out according to TCVN 4046-1985 (TCVN 4046 – 85, 1985) as follows: soil at depths of 0–20 cm was taken according to the diagonal or zigzag rule depending on the topography of the land. Each field took 5 samples, each sample was about 0.5 kg.

About 5 g of each soil sample was carefully transferred into a sterile 50ml conical tube containing 45 ml of sterile deionized (DI) water. Then, each test tube was vortexed thoroughly and kept standing at room temperature for 5 min. After that, 1 ml of soil suspension was transferred into a tube containing 9 mL of sterile water. This mixture was shaken well and diluted (10–1 dilution), with subsequent serial 10^{-1}-fold dilution until a 10^{-6} dilution was reached. 100 µl of diluted samples were cultured on Pikovskaya (PVK) media agar plates (Pikovskaya, 1948). The plates were incubated at 30 °C for 7 days. Each treatment was done in triplicates. The bacterial colonies with clear halos in the PVK agar plate indicated solubilizing activity of the phosphate. These were sub-cultured on new PVK media agar plates (Biobasic, Canada).

The phosphate solubilization index (PSI) of bacteria grown on plates was measured as the following formula:

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

2.2 Molecular identification of isolated strains

The total DNA of isolates was extracted using a Rapid Bacteria Genomic DNA Isolation Kit (Biobasic, Canada) as per the kit instructions. The 16S rDNA fragment was amplified from the extracted DNA by PCR 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′). The PCR reaction was prepared in PCR tube with a final volume of 25.0 µL containing 0.5 µL DNA template (20–50 ng/µL), 2.5 µL 10 × Buffer (with Mg^{2+}), 1 µl dNTPs, 0.5 µL primers 27 F (10 uM), 0.5 µL primers 1492 R (10 uM), 0.5 µL Taq DNA polymerase and add ddH_2O to 25 µL. The PCR reaction was performed in a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, CA, USA) using the thermal program: 95 °C for 5 min; 30 cycles at 95 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 90 sec; and 72 °C for 7 min. The fragment of 16S rDNA sequences (about 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 PCR products were sequenced and obtained sequences were blasted on National Center for Biotechnology Information (NCBI) to identify the species. The species with high nucleotides similarity (>96%) were used for multiple cluster alignment and phylogenetic analysis on MEGA software (v.7.2).

The primary comparison of 16S rDNA sequence with ND04 strains using B*LA*ST indicated that ND04 showed 96.2% similarity with *Pseudomonas putida* (FJ976605.1). To create phylogenetic tree, some 16S rDNA sequences of previously reported phosphate solubilizing bacterial strains belonging to the genera *Pseudomonas*, which have high nucleotide similarity (>98%) with 16S rDNA sequence of ND04 strains were then aligned by CLUSTAL-W. The selected genera *Pseudomonas* including *Pseudomonas*

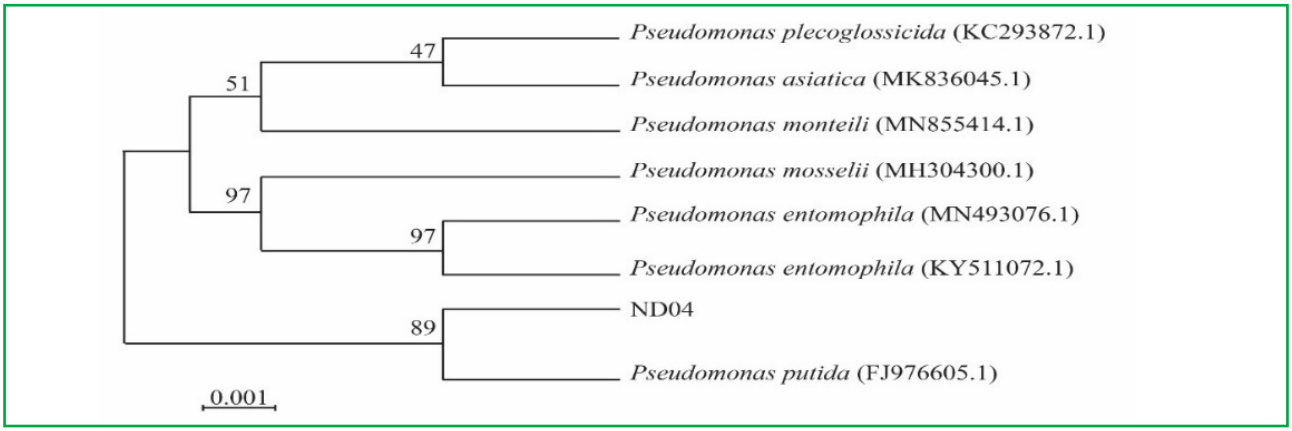

Figure 1 A neighbor-joining tree shows the phylogenetic relationships among 16S rDNA sequences of ND04 and their closely related sequences from NCBI. The scale bar indicates evolutionary distance

plecoglossicida (KC293872.1), *Pseudomonas asiatica* (MK836045.1), *Pseudomonas monteili* (MN855414.1), *Pseudomonas monteili* (MH304300.1), *Pseudomonas entomophila* (MN493076.1), and *Pseudomonas entomophila* (KY511072.1). The phylogenetic tree was shown in Figure 1.

2.3 Determine phosphate solubilizing efficiency of the isolates

Single colonies were cultured separately in Luria Broth (LB) media at 30 °C for 24 h on the shaker (150 rpm). Then, bacterial cells of each strain were collected by centrifuging and washed 5 times with sterile water and subsequently resuspended with sterile water (10^6 CFU/ml). These bacterial suspensions were designated as seed cultures and separately inoculated to PVK liquid medium containing $Ca_3(PO_4)_2$, sodium phytate, $FePO_4$, or $AlPO_4$, respectively. The culture was incubated at 30 °C for 7 days. The medium with no bacteria was used as the control.

After 7 days of incubation, the soluble P concentration in bacterial culture was measured using the molybdenum blue method as described by Waterlot (2018). Briefly, 1 ml of supernatant 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 the sample into the UV/VIS spectrophotometers (Mettler Toledo, USA) and measured. The pH measurement of the bacterial culture was carried out by using the pH meter (Mettler Toledo, USA). All measurements were performed in triplicates and LB medium was used for the blank.

In addition, the isolated PSB also characterized their P solubilizing efficacy in soil conditions as the method described by Wan et al. (2020). Soils used in the incubation experiments were collected from an uncultivated field in Hai Hau, Nam Dinh (20° 11' 19.4" N 106° 19' 20.8" E). The original pH, total carbon (TC), total nitrogen (TN), water-soluble P, Olsen P, total P, and total lead were 2.68, 0.28%, 0.62%, 0.08 mg/g, 4.20 mg/g, 0.85 mg/g, and 0.53 mg/g, respectively. Different treatments have been designed 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 Production of indole-3acetic acid (IAA) by PSB

The isolates were also screened for IAA production by using LB medium supplemented with 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. Briefly, 1 ml of bacteria strains (OD = 1) was inoculated into LB medium supplemented with 0.1% L-Tryptophan. The culture was incubated at 30 °C for 48 h. After that, bacterial cultures were centrifuged at 3,000 rpm for 30 min to collect the supernatant. Then, 2 ml of the supernatant were mixed with 100 µl of ortho-phosphoric acid and 4 ml of Solawaski's reagent (12 g/l $FeCl_3$ + 7.9 M H_2SO_4) and incubated at 37 °C for 30 min. The development of pink color indicates IAA production, which was quantified using a spectrophotometer at 535 nm. The concentrations of IAA produced by isolates were determined using a standard curve prepared from pure IAA from Sigma Company.

2.5 Evaluation of ND04 strain on the development and Pb uptake of weed plant (Echinochloa colona)

The ND04 strain with the highest efficiency of phosphate solubilization and IAA production was selected for the

pot experiment under greenhouse conditions. Pot-culture experiments were conducted with 3 replications in a greenhouse at VNU-Central Institute for Natural Resources and Environmental Studies, Ha Noi, Viet Nam.

The ND04 strain was cultured overnight, centrifugated, and washed with sterile water before being resuspended with sterile water to make a bacterial solution (OD = 1), which was used as an inoculant for *E. colona* seeds. In parallel, seeds of *E. colona* were surface sterilized by using ethanol 70% for 30 sec and sodium hypochlorite solution 1% for 5 min, respectively. Then these seeds were washed three times with sterile water and dried on autoclaved filter papers. The sterilized seeds were covered with selected PSB by soaking in the bacterial solution for 30 min before sowing. For the control, sterile water was used instead of the bacterial solution.

Soils used in these experiments were collected from an uncultivated field in Hai Hau, Nam Dinh (20° 11' 19.4" N 106° 19' 20.8" E). The soil was artificially contaminated with Pb by mixing with 600 mg/kg of $Pb(NO_3)_2$. In the beginning, 10 bacterized seeds of *E. colona* were sowed per plastic pot, which was filled up with about 1 kg of lead-contaminated soil. After plant establishment, one plant per pot was maintained. 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 weight. The plant height was measured from the aboveground to the tip of the uppermost leaf of the plant. The root was cut from the plant and washed with 1 mM $Ca(NO_3)_2 \cdot 4H_2O$ to remove Pb^{2+} bounding to its surface; after that with sterile water. The root and shoot were dried in an oven at 70 °C for 72 h and their dry weight was recorded.

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 was measured by flame atomic absorption spectrometry (Agilent, USA). All measurements were done in triplicates.

2.6 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 Statistical Package for the Social Sciences (SPSS) (version 17) was applied to compare the means in all experiments.

3 Results and discussion

3.1 Identification of isolated phosphate-solubilizing bacteria

A total of seven bacteria isolates that produced a transparent zone around colonies in Pikovskaya (PVK) medium were selected and further transferred into new PVK plates for purification (Table 1). Strains ND01, ND02, ND03, and ND04 showed white colonies, while strains ND05, ND06, and ND07 showed milky yellow colonies.

As can be seen from Table 1, after 7 days of incubation at 30 °C, these isolates showed different efficiency in solubilizing phosphorus compound that was illustrated by different values of PSI ranging from 2.13 to 302.52. The data also indicated that strain ND04 presented the highest PSI value (302.52). A further characteristic of isolates indicated their ability in IAA production, in which the highest amount of IAA (8.30 mg/L) was observed for the ND04 strain. These strains could solubilize tricalcium phosphate presenting in the media which was characterized by the dramatic increase of the amount of soluble P in the solution and pH reduction of the supernatant. These results were

Table 1 Characterization of isolated phosphate-solubilizing bacteria (PSB)

PSB isolates	Color	Phosphate solubilization index (Agar)	IAA production (mg/L)
ND01	white	54.12 ±0.21b*	2.84 ±1.34c
ND02	white	47.21 ±0.22b	2.09 ±1.03c
ND03	white	36.52 ±0.62c	3.97 ±1.12bc
ND04	white	302.52 ±0.21a	8.30 ±1.09a
ND05	yellow	2.32 ±0.31d	5.20 ±1.23b
ND06	yellow	2.13 ±0.02d	2.65 ±1.11c
ND07	yellow	2.37 ±0.01d	2.54 ±1.21c

*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)

Table 2 Similarity of 16S rDNA sequences for the isolated strains compared to those obtained from the database

Bacterial isolates	Length of 16S rDNA (Nu)	Accession number of 16S rDNA on Genbank	Blast identification	Identify similarity (%)	Accession number of sequence deposited on Genbank
ND1	1421	MK823655.1	*Bacterium strain* BS0467	99.7	MZ484514
ND2	1412	MT672505.1	*Burkholderia ubonensis*	99.8	MZ484516
ND3	1415	CP017475.1	*Enterobacter cloacae*	99.7	MZ484520
ND4	1415	FJ976605.1	*Pseudomonas putida*	96.2	MZ484521
ND5	1392	KR780381.1	*Lysinibacillus macroides*	99.5	MZ484522
ND6	1412	JX149543.1	*Bacterium* H1C	99.9	MZ484523
ND7	1426	KP851957.1	*Bacillus* sp.	99.8	ON352668

consistent with some other studies which demonstrated that PSBs produced many types of organic acids such as gluconic, oxalic, pyruvic, lactic, formic, citric, and other external metabolites that played important roles in phosphate solubilization (Kumar & Rai, 2015; Wan et al., 2020).

The identification of PSB strains based on 16S rDNA sequences was presented in Table 2. The obtained sequences were submitted to the Gene bank under accession numbers MZ484514–MZ484515, MZ484520–MZ484523, and ON352668. The results indicated that the isolates belonged to different groups such as *Bacterium*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Lysinibacillus*, and *Bacillus* with a similarity of more than 96%.

The alignment result of 16S rDNA sequence of ND04 strain with some reference *Pseudomonas* sp showed that ND04 strain was close to *Pseudomonas putida* (FJ976605.1), with the nucleotide similarity is 96.2%. The alignment result of 16S rDNA sequence was used to generate phylogenetic tree. The results were shown in Figure 1.

The *Pseudomonas putida* isolated in this study showed a significant efficiency of $Ca_3(PO_4)_2$ solubilization compared to reported *Pseudomonas* sp. (such as *Pseudomonas fluorescens* (184 mg/L) (Katiyar & Goel, 2003), *Pseudomonas putida* (247 mg/L) (Pandey et al., 2006). These differences can be explained due to the difference in isolated strains that were grown and developed under specific conditions.

3.2 Determination of phosphorus solubilizing ability

The results indicated the different capabilities in solubilizing phosphorus compounds of all isolates from different phosphate sources. The result showed that all isolated strains could solubilize multiple insoluble phosphorus compounds ($Ca_3(PO_4)_2$, $AlPO_4$, and $FePO_4$) but only ND01 and ND04 presented the phytate solubilization (Table 3). 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 (192.03–530.12 mg/L) released from this compound; and the ND04 also presented the highest efficiency. In addition to the solubilization of inorganic P, approximately 10-fold less solubilization efficiency was observed for the remaining complexed phosphate sources including $AlPO_4$ (31.63 to 78.32 mg/L) and $FePO_4$ (16.42 to 27.45 mg/L) by most of the isolates (Table 2). Furthermore, only two strains, ND01 and ND04, showed the ability in solubilizing organic phytate supplemented with a modified PVK broth medium (1.32 and 2.36 mg/L, respectively). These results indicated that ND04 could solubilize multiple P sources and might be used to reverse insoluble phosphate to soluble form in agriculture.

This observation could be 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 $AlPO_4$. Another explanation 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 enhance the phosphorus solubilization (Sánchez-Cruz, 2020; Aliyat et al., 2022). Furthermore, the results indicated the pH reduction and production of a phytase of strains ND01 and ND04 played significant roles in solubilizing inorganic phosphate. These were demonstrated by some previous research (Wan et al., 2020; Aliyat et al., 2022). 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 = 0.457), and between the pH of supernatant and the amount of soluble P released (r = 0.521). These could be related to P solubilizing mechanisms, in which the PSB produced

Table 3 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

PSB isolates	PVK with $Ca_3(PO_4)_2$		PVK with $AlPO_4$		PVK with $FePO_4$		PVK with sodium phytate	
	soluble P (mg/L)	pH	soluble P (mg/L)	pH	soluble P (mg/L)	pH	soluble P (mg/L)	pH
ND01	352.21 ±35.11b*	4.84 ±0.32b	56.12 ±5.11b	3.44 ±0.21c	13.12 ±1.22d	3.24 ±0.11d	1.32 ±0.31a	4.24 ±0.13b
ND02	202.67 ±18.20c	4.03 ±0.13c	78.32 ±2.23a	3.81 ±0.11bc	16.42 ±2.20c	3.72 ±0.13c	ND**	3.69 ±0.20c
ND03	335.23 ±15.12b	3.94 ±0.12c	45.23 ±3.62c	3.78 ±0.12bc	27.45 ±2.64a	3.75 ±0.12c	ND	3.87 ±0.11bc
ND04	530.12 ±30.32a	4.13 ±0.23c	50.13 ±5.22b	3.56 ±0.22bc	25.02 ±1.21ab	3.66 ±0.20cd	2.36 ±0.34b	3.58 ±0.33b
ND05	187.03 ±11.10c	5.12 ±0.15b	20.32 ±3.33e	4.21 ±0.13b	20.12 ±2.23b	3.91 ±0.25b	ND	3.12 ±0.25d
ND06	192.03 ±10.12c	4.65 ±0.22bc	32.03 ±4.12d	3.85 ±0.20bc	18.98 ±2.10b	3.73 ±0.21c	ND	4.04 ±0.24b
ND07	198.01 ±14.01c	4.57 ±0.20bc	31.63 ±3.10d	3.52 ±0.24c	19.87 ±2.13b	3.83 ±0.14c	ND	3.65 ±0.17c
Control media	ND	6.47 ±0.10a	ND	6.48 ±0.12a	ND	6.53 ±0.09a	ND	6.48 ±0.11a

* – 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

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.

There were some reports that demonstrated a positive correlation between the pH of culture and the solubilized amount of 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; Aliyat et al., 2022) leading to pH decrease and subsequently the increase of soluble P. Therefore, it could be said that the solubilization of phosphorus compounds simultaneously affected by pH decrease and acids production in the solution (Fankem et al., 2006).

3.3 Isolated PSB enhanced the soil fertility

The results of soil incubation showed that all isolated PSBs could solubilize the $Ca_3(PO_4)_2$ incubated in soil. The results are presented in Table 4.

The results showed that the AP content was significantly higher in all experiments than in the control after 30 days of incubation. Notably, the soil added with ND04 showed the highest amount of AP in the same soil treatments. Particularly, the AP amount in ND04-incubated soils, in T1, T2, and T3 treatments were 0.52, 0.84, and 1.67 mg/g, respectively. Additionally, the results also showed that the AP values in T3 treatment were significantly higher than those in other treatments including T1 and T2 treatment (P <0.05) (Table 3). Moreover, a significant increase in AP amount in PSB-inoculated soil when $Ca_3(PO_4)_2$ was added. These results indicated that ND04 showed a promising application in solubilizing insoluble phosphorus compounds in soil that increase soil health.

There are many studies that 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 & Reddy, 2011; Teng et al., 2019; Wan et al., 2020). In this study, the results showed that a combination of PSB and $Ca_3(PO_4)_2$

Table 4 Evaluation of available P in soil incubation (mg/L)

PSB strains	Soil + PSB	Soil + PSB + $Ca_3(PO_4)_2$	Soil + PSB + $Ca_3(PO_4)_2$ + nutrient
ND01	0.35(c)(C)*	0.57(bc)(B)	1.34(bc)(A)
ND02	0.37(bc)(C)	0.62(b)(B)	1.46(b)(A)
ND03	0.33(c)(C)	0.56(bc)(B)	1.44(b)(A)
ND04	0.55(a)(C)	0.84(a)(B)	1.67(a)(A)
ND05	0.34(c)(C)	0.53(c)(B)	1.24(c)(A)
ND06	0.38(bc)(C)	0.56(bc)(B)	1.31(bc)(A)
ND07	0.42(b)(C)	0.52(c)(B)	1.34(bc)(A)

* the presenting results are the mean value of three replicates; values with the normal letter in the same column indicate a significant difference (P <0.05); Value with the uppercase letter in the same row presents a significant difference (P <0.05)

fertilizer significantly enhanced the proportion of soluble P in treated soil. 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 efficiency of phosphorus solubilization. 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 & 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) presented in soil (Teng et al., 2019).

3.4 ND04 strain improved the development and Pb uptake of weed plant (Echinochloa colona)

The results of greenhouse experiments were illustrated in Figure 2 and shown in Table 5. As can be seen from Table 5, the ND04 strain significantly improved the plant parameters of *E. colona* compared to the control experiment. The length of ND04 inoculated plants was increased approximately 1.5 times compared to non-bacterized plants. Similarly, the increase in shoot and root dry weight was observed for the plants bacterized with ND04 with 1.5 times higher than the control.

The data in Table 5 also indicated that the Pb concentration in the shoot of bacterized *E. colona* plant was dramatically increased in the comparison with one of the non-bacterized plants. The result also showed that the inoculation of ND04 was not clearly influenced by the amount of Pb in the root. In addition, the result also presented that the ND04-treated plants contained more amount of Pb uptake in the shoot than the control did.

It was reported that plant development was inhibited when grown on heavy metal-contaminated soil (Tangahu et al., 2011; Bortoloti & Baron, 2022). However, our result showed that the ND04 enhanced the growth of *E. colona* in soil contaminated with a high amount of Pb. This might be due to the ND04 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.

Figure 2 Inoculation of ND04 strain enhances the development of weed (*Echinochloa colona*) after 30 days of planting

Lin et al. (2018) reported that the growth of *Wedelia trilobata* cultivated in Cu^{2+}-contaminated soil was significantly upregulated when inoculated with *Pseudostellariae heterophylla*, a phosphate-solubilizing bacterium. Yahaghi et al. (2018) also demonstrated the bacteria (*Brevibacterium frigoritolerans* YSP40, *Bacillus paralicheniformis* YSP151) improved the development of *Brassica juncea* that grown in a soil contaminated with heavy metals by producing IAA, siderophores, and solubilizing inorganic phosphate.

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 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; Bortoloti & Baron, 2022; Lai et al., 2022). For example, Lai et al. (2022) demonstrated that the combination of biochar and PSB (*Enterobacter* sp.)

Table 5 Enhanced effect of ND04 strain on the development and Pb uptake of *Echinochloa colona*

Phosphorus solubilizing bacteria	Plant length (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Pb concentration in the shoot (mg/kg)	Pb concentration in the root (mg/kg)	Pb uptake by shoot (µg/pot)
SDW*	51.27 ±3.21a**	17.21 ±3.17a	13.87 ±3.52a	41.15 ±2.76a	93.35 ±4.86a	70.12 ±15.48a
ND04	71.25 ±4.31b	28.05 ±4.58b	21.12 ±1.62b	72.28 ±8.65b	85.61 ±3.35a	221.23 ±32.19b

* SDW – sterile distill water; ND04 – selected phosphorus solubilizing bacteria; ** – presenting values of the mean ±standard deviation. Values with a different letter in the same column indicated a significant difference according to HSD (p <0.05)

significantly increased the acid-soluble and non-bioavailable fraction of Pb/Cd by 5/15 times and 14/5.8 times in contaminated soil. This suggests the PSB could be used as an inoculant to increase the efficiency of the phytoremediator.

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 ND04 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

To conclude, seven strains were isolated from Nam Dinh paddy soil in this study; they belong to the genus *Bacterium*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Lysinibacillus*, and *Bacillus*. They were able to produce IAA and solubilize multiple forms of P: $Ca_3(PO_4)_2$ > $FePO_4$ > $AlPO_4$ > Phytate. Especially, the isolated PSB improved the quality of Pb-contaminated soil in the presence of $Ca_3(PO_4)_3$, particularly by increasing the amount of available P. Among them, the ND04 strain identified as *Pseudomonas putida* presented the highest ability in solubilizing $Ca_3(PO_4)_3$ (530.12 mg/L), producing IAA (8.30 mg/L), and enhancing Pb-contaminated soil fertility. Additionally, the inoculation of ND04 strain into the Pb-contaminated soil not only promoted the growth-promoting traits of *E. colona* but also enhanced the Pb^{2+} uptake by the root of *E. colona*. These data suggest a potential application of isolated strain ND04 in combination with a phytoremediator for improving the phytoremediation of metal pollutants from metal-polluted soil.

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