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Isolation and Characterization of Phosphate-Solubilizing Bacteria from Compost and Open Dump Sites: Implications for Plant Growth

K. M. P. I. Jayathilake[1,2], P. M. Manage[1] and F. S. Idroos[1]†

[1]Centre for Water Quality and Algae Research, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka

[2]Faculty of Graduate Studies, University of Sri Jayewardenepura, Sri Lanka

†Corresponding author: F. S. Idroos; sumaiyaidroos@sci.sjp.ac.lk

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ABSTRACT

Phosphorus (P) is a vital macronutrient for plant growth. The bioavailability of P in soils is limited due to the insolubility of P. This study aimed to isolate and characterize Phosphate Solubilizing Bacteria (PSB) strains and determine their efficiency of phosphate solubilization and their effect on plant growth. pH, Electrical Conductivity (EC), Total Organic Carbon (TOC), organic nitrogen, and available phosphorus in soil were analyzed. Pikovskaya's Agar (PKV) was used to isolate PSB strains and identified through biochemical tests and 16S rRNA gene sequencing. The solubilization efficiency of the isolates was assessed in PVK broth supplemented with 0.5% tricalcium phosphate (TCP), and their effect on plant growth was evaluated in pot experiments. Mung bean (*Vigna radiata*) was selected as the experimental plant. Five *Bacillus* strains were identified, and the genotypic test was confirmed as *Bacillus pumilus* strain PRE14, *Bacillus altitudinis* 41KF2b, *Bacillus cereus* ATCC 14579, *Bacillus siamensis* strain KCTC 13613, and *Bacillus subtilis* strain NCIB 3610. All isolated strains were positive for the catalase test, gram staining, motility test, starch hydrolysis test, and spore staining test. The citrate utilization test and the spot test for indole production were negative for isolated PSB strains. *B. siamensis* strain KCTC 13613 showed the highest phosphate solubilizing efficiency with the maximum phosphorus concentration of 9.17±0.07 mg/kg recorded after 6 days of incubation. Pot experiments revealed that potting media inoculated with *B. siamensis* strain KCTC 13613 exhibited the highest shoot length (mean increase of 16.77 ± 0.74 cm), root length (mean increase of 10.99 ± 0.41 cm), and wet weight (mean increase of 0.60 ± 0.08 g) compared to other isolated PSB strains. All isolated *Bacillus* spp. Strains demonstrated a significant difference (n=9, $P < 0.05$) in measured growth parameters compared to plants grown under the control potting media. Soil pH increased post-germination, with the lowest pH recorded at 7.40±0.09 for media inoculated with *B. siamensis* strain KCTC 13613, indicating organic acid production. These findings reveal the potential of *Bacillus* spp. as bio-inoculants to promote sustainable agriculture by enhancing phosphorus availability and plant growth.

INTRODUCTION

Phosphorus (P) is an essential macronutrient and plays a vital role in various physiological and biochemical processes like energy transfer, photosynthesis, and nutrient uptake of plant growth (Khan et al. 2023, Jayathilake et al. 2024). Conventional agricultural practices mainly depend on the application of chemical fertilizers to meet the phosphorus demands of plant growth (Srivastav et al. 2024). However, the excessive use of chemical fertilizers results in serious negative impacts on the natural environment: soil and water pollution, disturbance in the microbial community of the soil, nutrient runoff causing eutrophication in aquatic life, etc. (Kumar et al. 2019, Hossain et al. 2022). Therefore, researchers turned their attention to alternative ways that would not pollute the environment and thus avoid other negative factors associated with chemical fertilizers (Ajmal et al. 2018, Jayathilake et al. 2024). The bioavailability of phosphorus in many agricultural

soils is limited due to its predominance in insoluble forms like phosphate minerals and organic compounds (Phytates, Humic substances, and Phospholipids). This limitation creates a significant challenge to agricultural productivity, and plants struggle to access an adequate P supply. The forms of P that are taken up by plants from soil solution are phosphate anions (mainly $H_2PO_4^-$ and HPO_4^{2-}) (Motamedi et al. 2016). To address the issue of phosphorus availability while mitigating environmental risks, alternative approaches rooted in sustainable agricultural practices have gained prominence. Among such approaches, the use of Phosphate Solubilizing Bacteria (PSB) stands out as a very promising strategy. PSB plays a significant part in agroecosystems by converting insoluble soil phosphate into soluble forms ($H_2PO_4^-$ and HPO_4^{2-}), consequently making sufficient phosphorus available for use as an essential plant nutrient. This conversion process enhances the availability of phosphorus, a vital nutrient for plant growth and development. By solubilizing phosphate, these bacteria make it more accessible to plants, thus promoting better nutrient uptake and ultimately leading to improved crop yields. This ability of PSB highlights their significance in sustainable agriculture practices aimed at enhancing soil fertility and productivity (Shome et al. 2022, Silva et al. 2023).

PSB is a group of microorganisms with diversity that can convert insoluble phosphorus to soluble forms through various mechanisms. These include organic acid production, proton extrusion, and enzymatic activity (Patel & Goswami 2020). The identification and characterization of these bacteria from different soil environments is very promising for enhancing phosphorus uptake in plants. Previous research has been able to show the potential of PSB not only in increasing available phosphorus in soils but also in promoting overall plant growth through improved uptake of other nutrients and soil health (Yadav et al. 2023). The benefits resulting from PSB-mediated phosphate solubilization are not only limited to the availability improvement of phosphorus. Inoculation studies with PSB have also reported positive effects on soil health parameters, nutrient cycling process, and promoting overall plant growth. By promoting nutrient uptake, improving soil structure, and reducing the reliance on chemical fertilizers, PSB contributes to the sustainability and resilience of agricultural systems, aligning with the principles of agroecology and ecological farming (Arjumend et al. 2022).

The isolation and characterization of PSB from different soil environments hold significant potential for enhancing phosphorus availability in agricultural soils (Janati et al. 2022). Compost and open dump sites represent unique ecological niches rich in microbial diversity, including PSB, due to the decomposition of organic matter and the accumulation of nutrient-rich materials. Therefore, exploring these sites for the isolation of PSB offers an opportunity to identify novel strains with efficient phosphate solubilization capabilities. For their effective application as biofertilizers in sustainable agriculture, it is essential to understand the characteristics and behavior of isolated PSB strains. Biochemical tests that help evaluate metabolic activities, genotypic identification by techniques such as sequencing 16S rRNA genes, and evaluating phosphate solubilization efficiency under laboratory conditions are generally involved in efforts toward characterizing these isolates. These analyses provide valuable insights into the diversity, functionality, and potential agricultural benefits of the isolated PSB strains (Asghar et al. 2023, Dodino-Gutiérrez et al. 2024). PSB have attracted increasing attention for their role in improving phosphorus availability for legume plants and also for their wider application in other crops. Recent studies have already shown the potential of PSB in improving nutrient uptake, growth, and yield in both legume plants and nonlegume plants and fast-growing plants, illustrating their diversity and versatility in supporting various agricultural sectors (Biswas & Shivaprakash, 2021, Kumar et al. 2016, Angadi et al. 2017, Jayathilake et al. 2024) This study aims to isolate and characterize PSB strains from compost and open dump sites and determine the phosphate solubilization efficiency and their potential impact on plant growth. The research results are significant in terms of contributing to sustainable developments in agriculture by harnessing the potential benefit of soil-microbe-plant interactions to enhance nutrient cycling and agricultural production.

MATERIALS AND METHODS

Collection and Analysis of Physiochemical Properties of Soil Sample

Compost piles associated with soil and open dump soil in Karadiyana (6°48'51.8" N, 79°54' 17.0" E) were collected to isolate the phosphate-solubilizing bacteria. Soil pH, Electrical Conductivity (EC), total soil carbon, soil organic nitrogen, and soil available phosphorus were determined by standard protocols and methods (Walkley & Black 1934, AOAC 2000, Mussa et al. 2009).

Isolation of Phosphate Solubilizing Bacteria

Phosphate-solubilizing bacterial strains were isolated using Pikovskaya's Agar medium. 10 g of each soil sample was homogenized in 90 mL sterilized saline. A series of 10-fold

dilutions of the suspension were prepared for each sample. 100µL of each concentration was spread directly onto the surface of Pikovskaya Agar medium plates (Ha & Chu 2020). Pure cultures of five isolated strains were labeled as PSB-A, PSB-B, PSB-C, PSB-D, and PSB-E.

Biochemical Analysis of Bacterial Isolates

Rhizospheric microbes were characterized by different biochemical methods, catalase test, gram staining test, motility test, starch hydrolyses test, spore staining, citrate utilization test, and spot test for assessment of indole production (Hamza et al. 2017). These biochemical tests aimed to confirm the potential of isolates for nutrient cycling and plant nutrition enhancement.

Genotypic Identification of Bacterial Isolates

The DNA extraction was performed using the Wizard Genomic DNA Purification Kit. The 16srRNA gene was amplified by Polymerase Chain Reaction (PCR) and subjected to sequencing at Macrogen, Korea.

Phylogenetic Tree Construction

The phylogenetic tree was constructed using MEGA software based on 16S rRNA gene sequences. Sequence alignment was performed using the CLUSTALW algorithm, and the tree was generated using the Neighbor-Joining method with 1,000 bootstrap replicates to assess branch reliability.

Phosphate Solubilization Efficiency of Bacterial Isolates

The phosphate solubilization activity test was conducted in 150 mL conical flasks containing 100 mL of PVK broth supplemented with 0.5% TCP (pH = 7). Exactly 1 mL of bacterial inoculant (10^7 CFU/mL) was inoculated into prepared broth media, while sterile distilled water served as the control. The experimental setup was performed in triplicate. The cultures were then incubated on a rotary shaker at 28°C and 130 rpm for 8 days. Aseptic aliquots of 1.5 mL of culture supernatant were collected on the 2nd, 4th, 6th, and 8th days. Following the centrifugation at 12,000 rpm for 2 minutes to remove suspended solids and mycelial fragments, 0.1 mL of each culture supernatant was taken to estimate the phosphorus released from TCP. The available soluble phosphate in culture supernatants was assessed using the Bray extraction method at 882 nm (Hui et al. 2011, Doilom et al. 2020).

Preparation of Potting Media

The growth medium for the pot experiment contained a blend of compost, coir dust, and soil in a 1:1:1 ratio and isolated bacterial strains. The bacterial inoculant was prepared in sterilized distilled water. Subsequently, each pot was inoculated with 10 mL of bacterial inoculant (10^8 CFU/mL) from each isolate, ensuring consistent distribution of the strains throughout the sterilized media in the pots.

Determination of the Effect of PSB Strains on Mung Bean (*Vigna radiata*) Plant Growth

Plant growth parameters (shoot length and root length) were recorded to determine the effect of the selected isolates on plant growth. A control pot was treated with 10 mL of sterilized water. The pots were irrigated twice daily with 50 mL of water per day. The experiment followed a completely randomized block design with five replications per treatment. The potting media was kept sterile through aseptic techniques at the time of planting and irrigation to avoid microbial contamination from the outside environment. The physiochemical parameters of potting media (pH, electric conductivity, available phosphorus, and soil organic carbon) were determined before seed germination and after 30 days of seed germination (Walkley & Black 1934, Mussa et al. 2009, Pal et al. 2009).

Statistical Analysis

Data analysis and graphical representations were conducted using Microsoft Excel. Data from different treatments were calculated and statistically analyzed with a one-way analysis of variance (ANOVA) using Python 3.11 (VS Code).

RESULTS AND DISCUSSION

Bacterial strains were isolated from compost piles associated with soil and open dump site soil to determine the potential of phosphate-solubilizing bacteria (PSB) on plant growth. Fig. 1 represents the physicochemical properties of compost soil and open dump site soil samples. Both soil samples have shown acidic characteristics. The compost soil has a lower pH value compared to the open dump site soil. This acidic condition is optimal for the proliferation of PSB strains. Li et al. (2022) and Zhao et al. (2024) have shown that acidic conditions are more conducive to the growth of PSB strains. The collected soil samples have high concentrations of organic carbon, organic nitrogen, and soil moisture content. Previous studies have shown that organic carbon, organic nitrogen, and soil moisture content are vital for the growth of PSB and essential for their proper development (Atif et al. 2023, Wang et al. 2023).

Table 1 presents the isolated PSB strains. Five PSB strains were isolated and labeled as PSB-A, PSB-B, PSB-C, PSB-D, and PSB-E, all of which belong to the genus *Bacillus*.

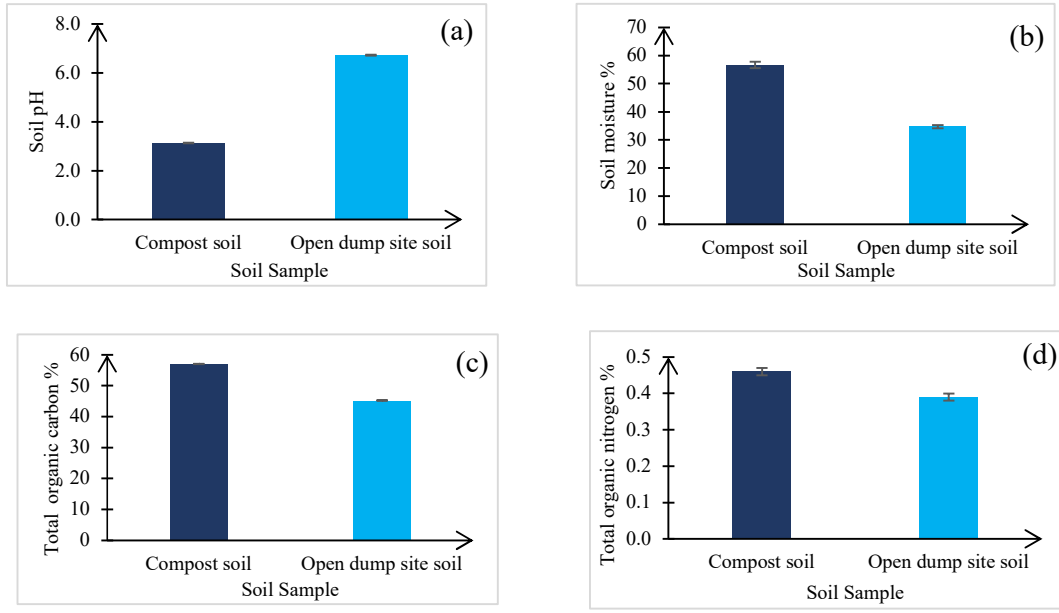


Fig. 1: Physiochemical properties of collected soil samples (a) soil pH, (b) soil moisture content, (c) total organic carbon, and (d) total organic nitrogen.

Table 1: Isolated PSB Strains.

Isolated Strains	Bacterial strains
PSB-A	*Bacillus pumilus* strain PRE14
PSB-B	*Bacillus altitudinis* 41KF2b
PSB-C	*Bacillus cereus* ATCC 14579
PSB-D	*Bacillus siamensis* strain KCTC 13613
PSB-E	*Bacillus subtilis* strain NCIB 3610

Genotypic identification confirmed that the five isolated PSB strains are *B. pumilus* strain PRE14, *B. altitudinis* 41KF2b, *B. cereus* ATCC 14579, *B. siamensis* strain KCTC 13613, and *B. subtilis* strain NCIB 3610. Table 2 illustrates the results of the biochemical analysis test for the isolated PSB strains. All isolated *Bacillus* species tested positive for the catalase test, Gram staining, motility test, starch hydrolysis test, and spore staining test, while they tested negative for the citrate utilization test and the spot test for indole production.

The catalase test indicates the presence of the enzyme catalase, which is essential for breaking down hydrogen peroxide, a toxic byproduct of aerobic metabolism (Gebicka & Krych-Madej 2019). Gram staining confirms the cell wall structure characteristic of *Bacillus* species. The motility test reveals the ability of these bacteria to move, which is crucial for colonization and nutrient acquisition (Madigan et al. 2015). Starch hydrolysis demonstrates the production of amylase, allowing the bacteria to utilize starch as a carbon source. Spore staining identifies the presence of endospores, which are vital for bacterial survival under adverse conditions. The negative citrate utilization test indicates

Table 2: Biochemical analysis of isolated strains.

Bacterial strains	Biochemical test						
	Catalase test	Gram staining test	Motility test	Starch hydrolyses test	Spore Staining	Citrate Utilization Test	Spot Test for Assessment of Indole Production
Bacillus pumilus (PRE14)	+	+	+	+	+	-	-
Bacillus altitudinis (41KF2b)	+	+	+	+	+	-	-
Bacillus cereus (ATCC 14579)	+	+	+	+	+	-	-
Bacillus siamensis (KCTC 13613)	+	+	+	+	+	-	-
Bacillus subtilis (NCIB 3610)	+	+	+	+	+	-	-

that these strains cannot use citrate as their sole carbon source, and the negative indole production test shows they lack the enzyme tryptophanase necessary for breaking down tryptophan. These biochemical characteristics are critical for identifying and understanding the metabolic capabilities of the PSB strains (Hamza et al. 2017).

The phylogenetic tree (Fig. 2) shows the evolutionary relationships among phosphate-solubilizing *Bacillus* isolates, constructed through 16S rRNA gene sequencing and MEGA software. This analysis includes strains *Bacillus pumilus* (PRE14), *Bacillus altitudinis* (41KF2b), *Bacillus siamensis* (KCTC 13613), *Bacillus subtilis* (NCIB 3610), and *Bacillus cereus* (ATCC 14579). These species have been documented for their efficient phosphate-solubilizing capabilities, beneficial for agricultural applications as biofertilizers (Tahir et al. 2013, Cherif-Silini et al. 2016, Ait-Ouakrim et al. 2023). In the phylogenetic tree, *Bacillus pumilus* (PRE14) is closely clustered with *Bacillus altitudinis* (41KF2b), suggesting they share recent common ancestry and may exhibit similar functional traits in phosphate solubilization. *Bacillus siamensis* (KCTC 13613) and *Bacillus subtilis* (NCIB 3610) form a secondary cluster, indicating moderate genetic divergence from the first cluster. *Bacillus cereus* (ATCC 14579) branches off earlier, indicating a greater evolutionary distance and potentially different ecological roles or mechanisms in phosphate solubilization. These clustering patterns reinforce previous research on *Bacillus* spp. as effective phosphate solubilizers, crucial for enhancing nutrient availability in agricultural soils. The close relationship between *B. pumilus* and *B. altitudinis* aligns with findings by Ait-Ouakrim et al. (2023) on efficient phosphate solubilization by *Bacillus* strains, while the functional diversity within this genus supports its broad application in biofertilizer development. Previous studies have shown that phylogenetic analysis of soil microbes underlines their multifunctional roles in sustainable agriculture by emphasizing that they promote plant growth through ammonia production, siderophore synthesis, and phosphorus solubilization (Bramhachar et al. 2027, Verma & Saharan 2020).

Fig. 3 illustrates the solubilized phosphorus concentrations after 2, 4, 6, and 8 days of incubation in PVK broth inoculated with PSB isolates. *B. siamensis* strain KCTC 13613 exhibited the highest phosphate solubilizing efficiency on the 6th day of incubation compared to the other isolated PSB strains. The other *Bacillus* strains showed the highest available phosphorus levels on the 2nd day of incubation, with a gradual decrease in phosphorus concentration thereafter. Fig. 4 shows the pH values of TCP-containing PVK broth inoculated with PSB isolates after 2, 4, 6, and 8 days of incubation. The lower pH value is attributed to the formation of organic acids during the incubation period. PSB strains solubilize insoluble phosphorus (PO_4^{3-}) into plant-available phosphorus ($H_2PO_4^-$, HPO_4^{2-}) through the production of organic acids during the phosphate solubilizing process (Aruna et al. 2024). This plant-available phosphorus is crucial for proper plant growth and nutrient availability, emphasizing the role of PSB in enhancing soil fertility and promoting agricultural productivity (Yadav et al. 2023).

Fig. 5 represents the variation of growth parameters in Mung bean plants. Mung beans were selected due to their numerous advantages, including ease of cultivation, short growth cycle, wide availability, adaptability to various environments, and sensitivity to nutrient levels (Hou et al. 2019). The potting media inoculated with *B. siamensis* strain KCTC 13613 exhibited the highest shoot length, root length, and wet weight of the grown plants. This indicates the significant role of *B. siamensis* strain KCTC 13613 in promoting plant growth through the solubilization of insoluble phosphorus. Additionally, potting media inoculated with *B. pumilus* strain PRE14, *B. altitudinis* 41KF2b, *B. cereus* ATCC 14579, and *B. subtilis* strain NCIB 3610 showed significant

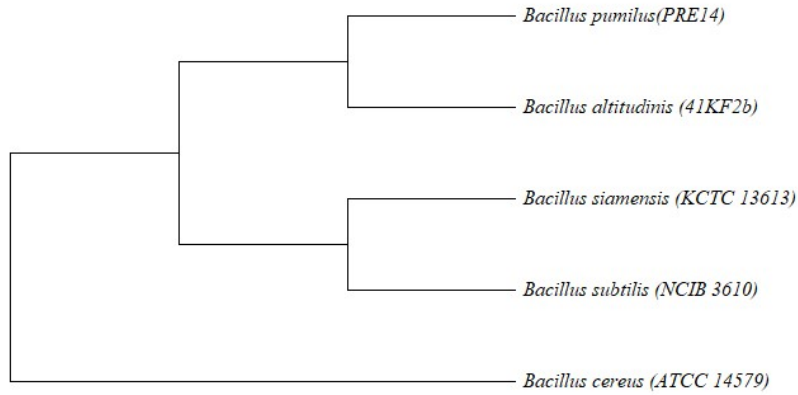
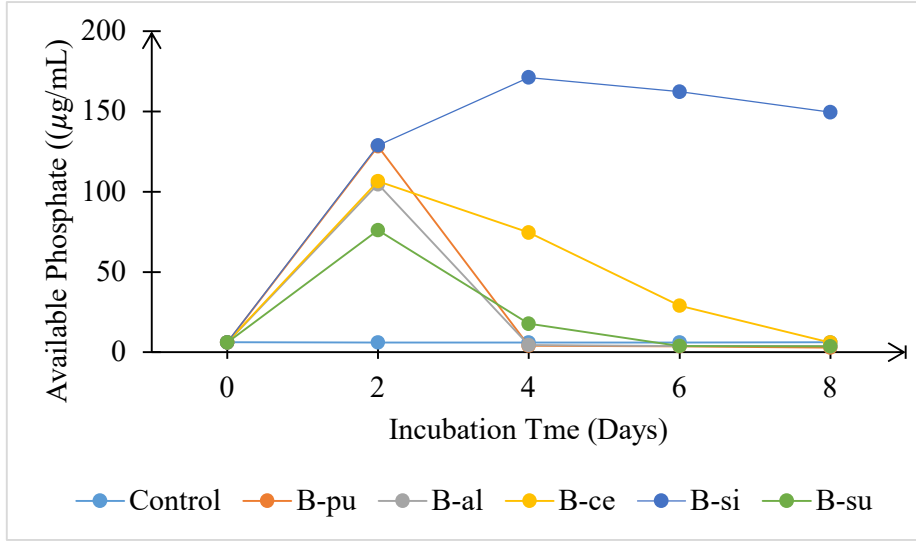
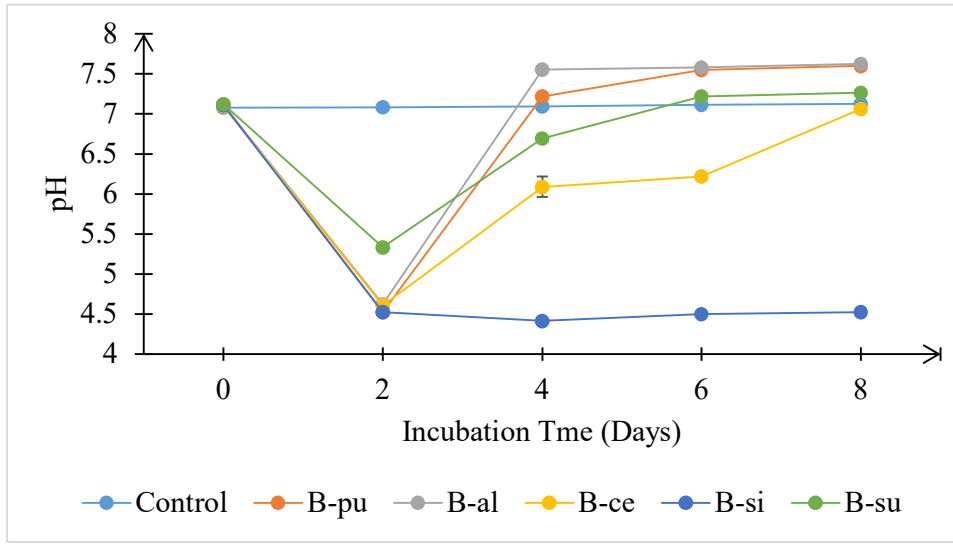


Fig. 2: The evolutionary relationships among isolated phosphate-solubilizing bacteria.



(Note: B-pu- *Bacillus pumilus* strain PRE14, B-al- *Bacillus altitudinis* 41KF2b, B-ce *Bacillus cereus* ATCC 14579, B-si - *Bacillus siamensis* strain KCTC 13613, B-su- *Bacillus subtilis* strain NCIB 3610)

Fig. 3: Solubilized P concentrations after 2, 4, 6, and 8 days of incubation in PKV broth inoculated with PSB isolates.



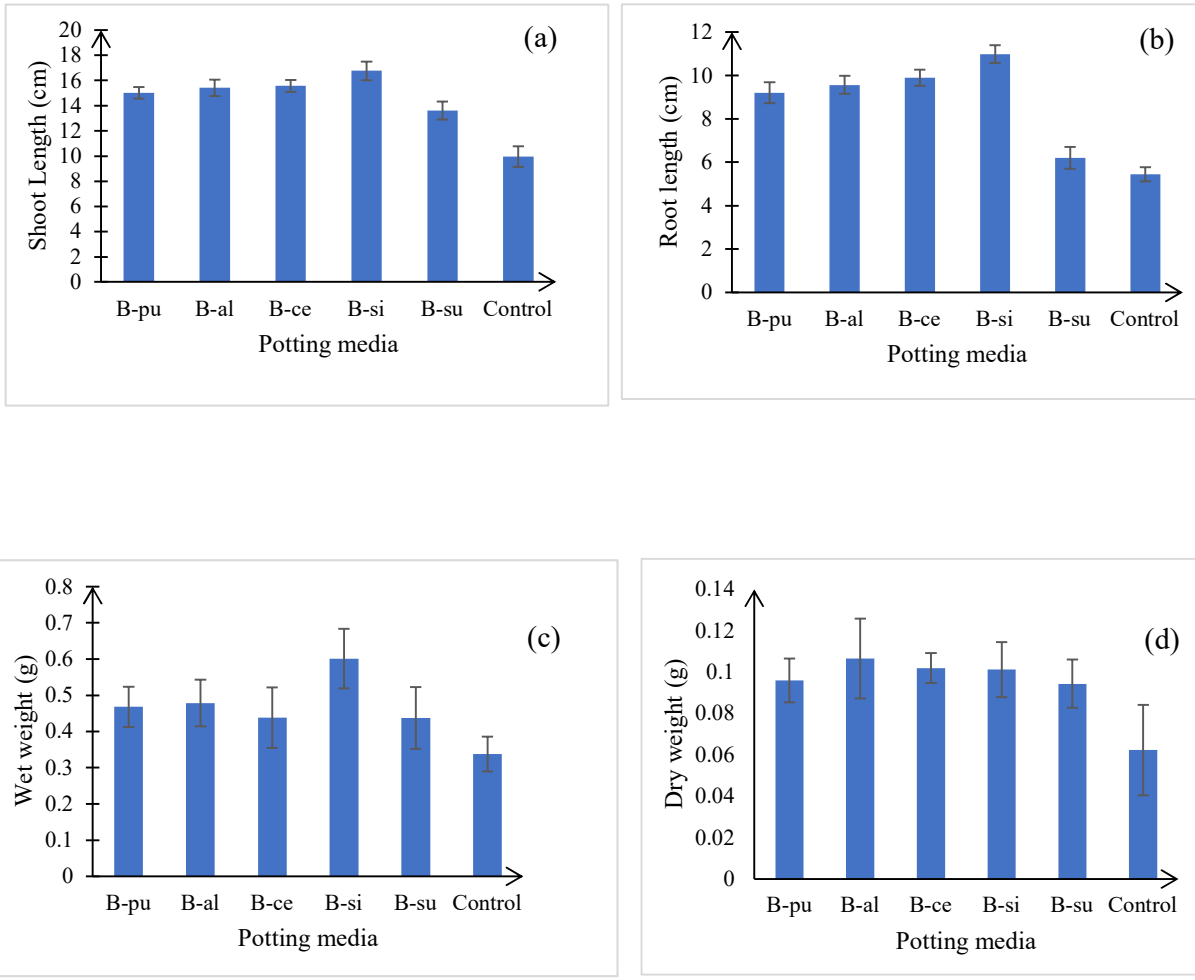
(Note: B-pu- *Bacillus pumilus* strain PRE14, B-al- *Bacillus altitudinis* 41KF2b, B-ce *Bacillus cereus* ATCC 14579, B-si - *Bacillus siamensis* strain KCTC 13613, B-su- *Bacillus subtilis* strain NCIB 3610)

Fig. 4: pH values of TCP containing PVK broth inoculated with PSB isolates after 2, 4, 6, and 8 days of incubation.

differences ($p<0.05$) in the measured growth parameters (shoot length, root length, wet weight, and dry weight of the grown plants). The involvement of these strains in enhancing plant growth can be attributed to their ability to increase nutrient availability, particularly phosphorus, which is crucial for various physiological and biochemical processes in plants.

Table 3 presents the variation in physicochemical parameters of the potting media before seed germination (initial) and after 30 days of seed germination.

The initial pH value of the potting media was acidic. After 30 days of seed germination, the pH value of the potting media inoculated with PSB strains became basic. Specifically, the potting media inoculated with *B. siamensis* strain KCTC 13613 had a lower pH value (7.40±0.09) compared to the media inoculated with other PSB strains and the control. This shift in pH suggests the metabolic activity of PSB strains, which can alter soil pH through the production of organic acids and subsequent phosphate



(Note: B-pu- *Bacillus pumilus* strain PRE14, B-al- *Bacillus altitudinis* 41KF2b, B-ce *Bacillus cereus* ATCC 14579, B-si - *Bacillus siamensis* strain KCTC 13613, B-su- *Bacillus subtilis* strain NCIB 3610)

Fig. 5: Variation of growth parameters of Mung Bean (*Vigna radiata*) plants (a) shoot length, (b) root length, (c) wet weight, and (d) dry weight.

Table 3: The variation of physiochemical parameters of potting media before seed germination (Initial) and after 30 days of seed germination.

Parameter	Before seed germination	After 30 days of seed germination					
	Initial	B-pu	B-al	B-ce	B-si	B-su	Control
pH	6.83 ± 0.02	7.62±0.07	7.52±0.05	7.66±0.11	7.40±0.09	8.00±0.06	8.23±0.08
EC [$\mu S.cm^{-1}$)]	1040.67±7.51	128.77±9.18	134.83±3.13	124.73±3.33	125.40±1.75	116.97±2.01	115.23±1.26
AP [$mg.kg^{-1}$)]	1.74±0.01	3.33±0.13	2.19±0.07	3.07±0.09	9.17±0.07	2.79±0.06	1.90±0.11
OC%	7.73±0.13	6.06±0.21	7.07±0.20	5.71±0.15	6.69±0.28	4.35±0.48	7.01±0.08

solubilization (Patel & Goswami 2020). The initial EC of the potting media was high (1040.67±7.51 $\mu S.cm^{-1}$) compared to the media after 30 days of seed germination. The EC values of the media inoculated with PSB strains were lower, indicating improved nutrient utilization by the plants. Initially, the potting media had low available phosphorus (1.74±0.01 $mg.kg^{-1}$). After 30 days, the media inoculated with *B. siamensis* strain KCTC 13613 showed the highest available phosphorus level (9.17±0.07 $mg.kg^{-1}$), highlighting the strain's efficiency in solubilizing phosphorus. Other PSB-inoculated media also showed significant differences ($p<0.05$) in available phosphorus compared to the control.

The initial organic carbon percentage of the potting media was 7.73±0.13%. Post germination, the media inoculated with PSB strains exhibited noticeable changes in organic carbon content, generally showing lower values compared to the initial media. This reduction could be attributed to microbial activity and plant uptake, indicating an active nutrient cycling process facilitated by the PSB strains.

The positive outcomes in catalase, Gram staining, motility, starch hydrolysis, and spore staining tests highlight physiological characteristics in the isolated *Bacillus* species. Catalase activity is critical for degrading hydrogen peroxide, thereby reducing oxidative stress on plants. Gram-positive staining indicates the structural integrity of bacterial cell walls, which is crucial for stability and persistence in the rhizosphere (Paray et al. 2023, Ghorpade et al. 2023). Motility facilitates bacterial movement towards plant roots, enhancing colonization and improving nutrient uptake efficiency. Starch hydrolysis capability suggests proficiency in carbohydrate metabolism, providing energy for bacterial growth and activities that support plant nutrition. Spore formation ensures survival during unfavorable conditions, enabling prolonged interactions with plants. These characteristics suggest that the isolated *Bacillus* species possess mechanisms that promote plant growth by enhancing nutrient availability, stress tolerance, and root colonization. In spite of testing negative for the citrate utilization test and the spot test for indole production, which are associated with nutrient solubilization and plant hormone modulation, respectively. The absence of citrate utilization suggests potential limitations in organic acid production, which could impact the solubilization of insoluble phosphates in soil. However, positive results for phosphate solubilization efficiency indicate that these strains can lower pH levels and increase available phosphorus, suggesting alternative pathways or microbial interactions that facilitate nutrient release beneficial for plant uptake. The lack of indole production capability implies a deficiency in auxin synthesis, which is crucial for root development and growth stimulation. However, the observed enhancement in plant growth parameters suggests that these strains may utilize other mechanisms, such as direct phosphate solubilization or synergistic interactions with soil microbes, to effectively promote plant growth. Moreover, previous studies have demonstrated that the isolated *Bacillus* spp. Possess significant potential for nitrogen fixation and nutrient solubilization that plants require (Saha et al. 2023, Novello et al. 2024). However, the absence of field trials limits the ability to evaluate scalability and applicability on-site. Moreover, the effect of PSB application could not be studied for long-term impacts on soil health as well as crop productivity. Future studies should focus on large-scale field trials, testing PSB efficacy across various types of soils and climatic conditions, and investigating problems associated with commercial production and distribution.

These findings demonstrate the potential of utilizing *Bacillus* spp. as bio-inoculants to promote sustainable agriculture through improved phosphorus availability and enhanced plant growth.

CONCLUSIONS

This study successfully isolated and identified five phosphate-solubilizing bacterial (PSB) strains from compost and open dump sites in Karadiyana, which were identified as *B. pumilus* strain PRE14, *B. altitudinis* 41KF2b, *B. cereus* ATCC 14579, *B. siamensis* strain KCTC 13613, and *B. subtilis* strain NCIB 3610. The biochemical and genotypic analyses confirmed the efficacy of these strains in solubilizing phosphate under acidic soil conditions, which are conducive to their growth and activity. The phosphate solubilization efficiency varied among the isolates, with *B. siamensis* strain KCTC 13613 exhibiting the highest phosphate solubilizing activity. This strain demonstrated the greatest ability to lower the pH of the growth medium, indicating significant organic acid production, which is essential for phosphate solubilization. Furthermore, the pot experiment with mung bean plants revealed that inoculation with PSB strains significantly enhanced plant growth parameters, including shoot length, root length, and biomass. Particularly, *B. siamensis* strain KCTC 13613 significantly improved the availability of phosphorus in the potting media, leading to better plant growth outcomes compared to other strains and the control. The study also observed a shift in soil pH towards basicity and a reduction in electrical conductivity and organic carbon content post-germination, suggesting active microbial involvement in nutrient cycling and soil fertility enhancement. The focus of future research should shift toward evaluating the efficiency of PSB on different soil types under field conditions and their compatibility with other microbial consortia for further optimization of applications under different agricultural systems. These findings highlight the potential of utilizing *Bacillus* spp. as bio-inoculants to promote sustainable agriculture through improved phosphorus availability and enhanced plant growth.

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