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Identification of potential phosphate-solubilizing bacteria across different levels of humic acid application under sweet corn cultivation

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ABSTRACT: Phosphate-solubilizing bacteria (PSB) plays a crucial role in enhancing phosphorus availability to plants, thereby promoting plant growth and productivity in agricultural ecosystems. Given the inherent challenges of low soil fertility, organic matter deficiency, and phosphorus fixation in northeastern Thailand's soil, organic fertilizers such as humic acid (HA) offer a promising avenue for enhancing soil health and productivity. Therefore, further investigation into the direct effects of humic acid on PSB is considered crucial, particularly to determine the optimal application rates that can stimulate PSB efficiency in soil. This study aims to assess the influence of different concentrations of humic acid on the population of PSB and identify the potential PSB isolates from distinct HA application rates. The pot experiment was conducted using a completely randomized design with six treatments: T1 (control), T2 (chemical fertilizer), T3 (HA 0.5%), T4 (HA 1%), T5 (HA 1.5%), and T6 (HA 2%). The initial soil (O1) was also assessed. We uncovered that the highest PSB population was observed with a 1.5% HA treatment, presenting a 40% increase compared to the control. This suggests that a 1.5% HA concentration is the most favorable for increasing the PSB populations, while higher concentrations may not provide additional benefits and might potentially lead to adverse effects on the bacterial population. Soluble P content in broth culture medium was analyzed during a 9-day period with 3-day interval measurements. The five potential PSB isolates were chosen and identified as *Priestia megaterium, Bacillus subtilis, Priestia aryabhattai, Bacillus* sp., and *Mycolicibacterium* sp. The strongest solubilizing ability was observed from *Priestia megaterium* and *Bacillus* sp., with soluble P concentrations of approximately ± 607 mg L^{-1} and ± 601 mg L^{-1}, respectively, on day 6. This study provides comprehensive knowledge regarding the potential PSB community isolated from various HA treatments and suggests their potential for further biofertilizer application as plant growth-promoting rhizobacteria.

Keywords: humic acid; phosphate-solubilizing bacteria; organic amendment; bacterial population; sweet corn rhizosphere

Introduction

Phosphorus is integral to a multitude of physiological processes within plants (Khan et al., 2023). It encompasses vital functions such as energy transfer for the conversion of inorganic phosphorus to organic forms like adenosine triphosphate (ATP) and adenosine diphosphate (ADP) (Carstensen et al., 2018). This energy transfer is indispensable for photosynthesis (Khan et al., 2023; Saengwilai et al., 2023). Phosphorus facilitates the formation of essential molecules such as ribulose-1,5-bisphosphate (RuBP) (Khan et al., 2023; Saengwilai et al., 2023). It is

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also fundamental to the synthesis of nucleic acids and phospholipids (Jones et al., 2015). These components are essential for cellular structure and genetic material (Jones et al., 2015).

Ultisols soil, particularly Typic Paleustult in Northeastern Thailand, is known for their low fertility and extremely acidic soil, coupled with low cation exchange capacity (CEC) and high phosphorus fixation, which correlates with aluminium and iron oxides, posing notable challenges in providing available phosphorus in soil (Arunrat et al., 2020; Trakoonyingcharoen et al., 2005). Despite its significance, phosphorus availability in soil is often limited due to its tendency to form insoluble compounds with other elements, such as rock phosphate (RP), fluorapatite (FAP), ferric phosphate (Fe-P), and aluminum phosphate (Al-P) (Paz-Ares et al., 2022). Considering this issue, humic acid (HA) has come forward as a viable approach, as several studies have uncovered that HA offers numerous benefits for soil health, such as improving soil physicochemical attributes (Ali and Mindari, 2016; Ampong et al., 2022; Vikram et al., 2022). Humic acid enhances soil buffering capacity, thereby effectively stabilizing soil pH and increasing CEC (Xu et al., 2021), which also contributes to phosphorus availability in soil (Erdal et al., 2000; Jamal et al., 2018; Jun et al., 2017; Yuan et al., 2022).

Humic acid has been reported to stimulate microbial activity in soil, including the proliferation of phosphate-solubilizing bacteria (PSB) (Jing et al., 2022; Xiong et al., 2023). These bacteria play a crucial role in enhancing phosphate solubility by converting insoluble forms of phosphate into soluble forms, which are more readily available for plant uptake (Etesami et al., 2021; Wang et al., 2022). Humic acid creates a more favorable environment for soil bacterial growth by providing a carbon source (Tang et al., 2021; Tang et al., 2022). PSB promotes available P levels by stimulating root system growth, leading to increased root exudates; these exudates contain organic acids, such as amino acids and fatty acids that might increase available P in the rhizosphere (Pantigoso et al., 2023; Zhalnina et al., 2018). Moreover, PSB produces chelating agents like organic acids and siderophores, which effectively dissolve inorganic phosphate complexed with Ca, Fe and Al (Collavino et al., 2010; Katznelson and Bose, 1959; Prabhu et al., 2019).

Although existing studies have indicated that HA can stimulate microbial activity and promote the growth of PSB (Chaitra, 2018; Hussain et al., 2019; Jing et al., 2022; Mulyatni et al., 2018; Xiong et al., 2023), but the precise rates driving these effects remain poorly understood. Further detailed investigations are needed to elucidate the effective or optimal rates in promoting the PSB population, as existing research on these issues remains limited, especially in tropical red Ultisols soil such as Typic Paleustult. Our hypothesis proposed that increasing HA application rates will induce a higher PSB population in the rhizosphere, and the selected PSB isolates may have the ability to solubilize phosphorus, thereby exhibiting potential as effective bioinoculants for plants. Therefore, we aim to investigate the effects of humic acid application rates on the potential PSB population and solubilization activities under sweet corn cultivation. Understanding these effects is crucial for optimizing soil fertility and nutrient availability, as it provides valuable insights into the interaction between organic amendments and soil microbial efficiency.

Material and methods

Study Site Description and Experimental Design

The pot experiment was carried out at the Research Station of the Department of Soil Science and Environment, Faculty of Agriculture, Khon Kaen University, Thailand, with coordinates UTM: (48Q 266414E, 1823109N). The soil in the study site was classified as Ultisols Typic Paleustult according to Soil Taxonomy (Soil Survey Staff, 2014). The climate is characterized as tropical, with an annual rainfall of 1,705 mm/year. The sweet corn was cultivated in pots (polybags) using the Jumbo Sweet F1 corn variety. The experiment was conducted using a completely randomized design (CRD) in triplicate. Six treatments of humic acid (HA) levels and chemical fertilizer (CF) include: control without CF or HA addition (T1); CF only (T2); CF with 0.5% HA (T3); CF with 1% HA (T4); CF with 1.5% HA (T5); and CF with 2% HA (T6), and the initial soil (O1) was also included. The chemical fertilizer consisted of urea, diammonium phosphate (DAP), and muriate of Potash (MOP) as sources of nutrients for nitrogen (N), phosphorus (P), and potassium (K), respectively, following the nutrient requirements for sweet corn (N = 293.75 kg ha^{-1}; P = 58.13 kg ha^{-1}; and K = 225 kg ha^{-1}). The sweet corn was harvested manually 20 days after the corn silk had grown to a length of 2 inches.

Soil Rhizosphere Collection

Initiation soil (O1) was collected at a depth of 0–30 cm prior to planting, and the samples after harvesting were collected from the rhizosphere of sweet corn. The plants were dug out, the excess bulk soil was thoroughly mixed and removed, and the soil remaining at the roots was referred to as the soil rhizosphere. After air drying, the soil samples were completely mixed, and a portion of all samples was sieved using a 2-mm sieve. The samples were then placed in sterile zip-lock bags and immediately stored at 4 °C to maintain sample microbial biomass quality.

Isolation of Phosphate Solubilizing Bacteria

A gram of soil sample underwent serial dilution using distilled water (dH_2O), with a 100 µl aliquot spread-plated on National Botanical Research in Phosphate Medium (NBRIP) agar plates containing: 20 g; glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $MgCl_2.6H_2O$, 5 g; $MgSO_4.7H_2O$, 0.25 g; KCl, 0.2 g; and $(NH_4)_2SO_4$, 0.1 g; agar adjusted to pH 7 (Nautiyal 1999). The medium volume was adjusted to 1,000 ml with dH_2O and was dripped with bromophenol blue (0.4% ethanol), then the medium was autoclaved at 121 °C for 20 minutes. The spread medium was incubated at 28°C ±2° C for 7 days (Ndung'u-Magiroi et al. 2012; Paul and Sinha 2016). The number of colonies was the sum of all the colonies, and colony counting was carried out within 7 days. The population was interpreted as colony-forming units (CFU) (Magiroi et al., 2012) as following equation by simply counting plates containing 30–300 colonies.

$$\text{CFU g}^{-1}\text{ soil} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume of the aliquot}}$$

During a 7-day incubation period at 28°C ± 2°C, bacteria representative of the prominent morphologically characterized distinct colonies were selected by prioritizing those with halo zones, afterwards purified severally on a nutrient agar (NA) plate (peptones, 5 g l^{-1}; yeast extract, 2 g l^{-1}; beef extract, 1 g l^{-1}; agar, 15 g l^{-1}; NaCl 5 g l^{-1}; pH

7.4 was adjusted, sooner autoclaving at 121° C for 20 minutes) (Magiroi et al., 2012). The culture was maintained with 10% glycerol at -20° C (Pandey et al., 2006).

Qualitative Assay of Phosphate Solubilization

The colony selection was based on the morphological characteristics displayed on the plate medium. Specifically, representative colonies with prominent and different features, such as colony form and color, were chosen. Selected bacteria were spot inoculated using plate assay method to examine the solubilizing function. 10 µl of aliquot bacteria suspension (3×10^5 cells ml^{-1}) from NBRIP broth was dropped to sterile filter paper Grade 1 (pore size 11 µm) in duplicate on one NBRIP agar plate media with bromophenol blue 0.4% ethanol solution, then was incubated at 30 ℃ for 7 days. The diameter ratio (the halo or clear zone surrounding colonies) as well as colonies diameter calculations were used to determine the functional activity using the following formula of Edi Premono (1996) cited in Paul and Sinha (2016).

$$\text{Phosphate Solubilizing Index (SI)} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Quantitative Assay of Phosphate Solubilization

The determination of phosphate solubilizing capacity was carried out using approximately 1 ml of bacteria suspension (3×10^5 cells ml^{-1}) from purified inoculant that was stabbed using sterile toothpicks containing 100 ml of NBRIP broth into Erlenmeyer flasks (250 ml) (Pandey et al., 2006) after incubating at 28 ± 2 ℃ in a rotary shaker at 180 rpm for 9 days with a measurement interval of 3 days. The vanado-molybdate yellow color method, modified by Kitson and Mellon in 1994 and then modified by Pingale and Virkar in 2013, was used to determine soluble phosphate in supernatants using Barton's reagent, a mixture of ammonium molybdate and ammonium metavanadate in HNO_3.

The supernatant was obtained by centrifugation at 10,000 rpm for 10 minutes and then passed through a 0.45 m Millipore filter. 10 ml of the filtered supernatant was mixed with 2.5 ml of Barton's reagent, and the total volume was adjusted to 50 ml using distilled water (dH_2O). Following 10 minutes, the yellow color intensity was measured using a spectrophotometer (UV VIS Eppendorf Bio-Spectrometer) at 430 nm, and the quantity of P-solubilized was estimated from the standard curve.

Phenotypic and Genotypic Characterization of Selected Isolates

The selected isolates were observed microscopically to determine the status of Gram stain and the isolated bacteria's morphological characteristics using spore-staining according to the Hans Christian Gram procedure as described in Coico (2006). Bacterial shapes and differentially stained status as Gram-positive or negative were identified using a safranin counterstain and a crystal violet-iodine solution.

The DNA of selected isolates were extracted by following the manufacturer's protocols of the TIANamp Bacteria DNA Kit (TIANGEN®). The 16S rRNA gene products of five bacterial isolates were sequenced using the Sanger sequencing method. Following purification, the clean PCR products underwent cycle sequencing using the universal primers 518F (5'-CCAGCAGCCGCGGTAATACG-3') and 800R (5'-TACCAGGGTATCTAATCC-3'). The resulting

nucleotide sequences were then compared with the existing sequences in the GenBank database at NCBI (http://www.ncbi.nlm.nih.gov) using BLAST software.

Statistical Analysis

The significant differences among PSB populations were expressed as mean ± standard deviation and were analyzed using Tukey's post-hoc one-way analysis of variance (ANOVA) and honest significant differences (HSD). Significance was determined at a level of $P \leq 0.05$ using Statistica 8.0 software (Weiß, 2007).

Results

Potential PSB population

The potential population of phosphate-solubilizing bacteria, as depicted in **Figure 1**, was determined based on colony-forming units obtained from a 10-fold dilution in NBRIP agar. Compared to the control group, the application of HA at 1.5% and 2% concentrations resulted in a significant increase in the population of potential PSBs, with the highest number was observed with the 1.5% HA addition, which indicated a higher population number than the 2% HA addition. However, the application of HA at 0.5% and 1% concentrations did not have a significant impact on the population count. Additionally, the application of chemical fertilizer showed no significant difference compared to the control group, nor was it significantly different from the HA 1.5% and HA 2% applications.

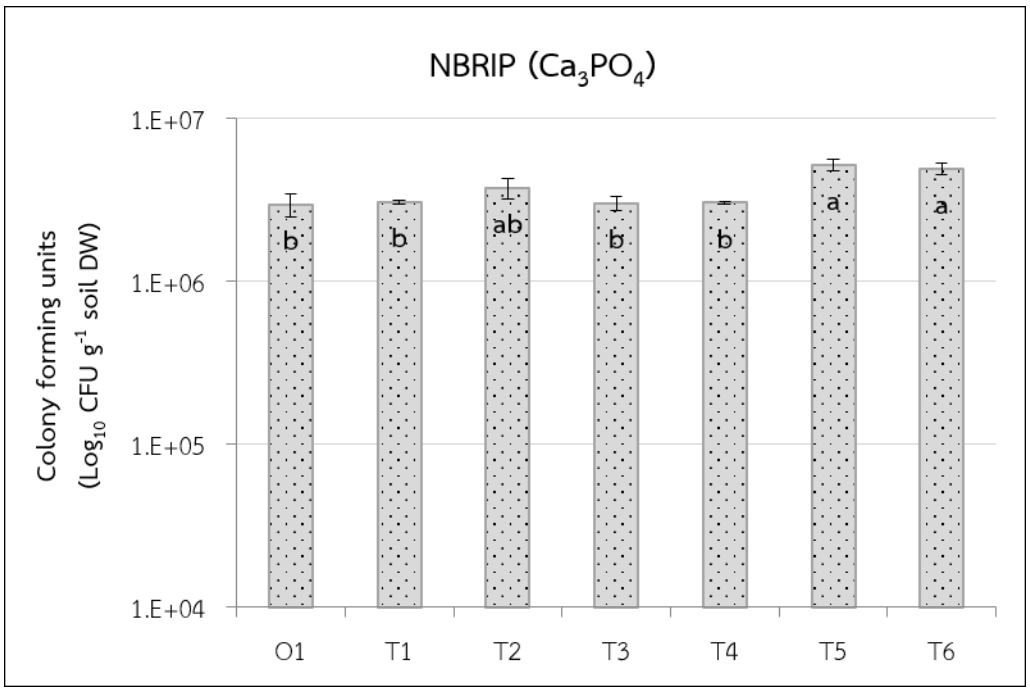

Figure 1. Potential PSB population assessing by colony forming units in different humic acid application rates, ($p < 0.05$). O1 = initial soil, T1 = control, T2 = chemical fertilizer, T3 = HA 0.5%, T4 = HA 1%, T5 = HA 1.5%, T6 = HA 2%. $^{a-b}$ the letters indicate significant differences ($p < 0.05$, Tukey's HSD).

Solubilization ability of potential PSB strain

A collective of 44 distinct phenotypic isolates was acquired throughout all treatments, encompassing 5 isolates derived from the initial soil, 6 isolates each from the control, chemical fertilizer, HA 0.5%, HA 1%, HA 1.5%, and 9 isolates from HA 2% (**Table 1**). Among the 44 isolates analysed for the qualitative solubilization assessment, approximately 17 isolates exhibited a clear zone appearance. Subsequently, these 17 isolates underwent further examination for quantitative solubilization analysis.

Table 1 Qualitative assessment of PSB isolates to solubilize phosphorus

No	Strain Code	Treatment	Solubilizing Index
1	C1F3 - 103	Control	2.1 ± 0.24
2	C1F4 - 107	Control	2.9 ± 0.10
3	C1F4 - 108	Control	2.9 ± 0.10
4	C2F4 - 111	Chemical fertilizer	3.5 ± 019
5	C3F4 - 117	HA 0.5%	2.9 ± 0.32
6	C4F3 - 122	HA 1.0%	2.9 ± 0.18
7	C4F4 -123	HA 1.0%	2.8 ± 0.12
8	C4F4 - 125	HA 1.0%	3.1 ± 0.40
9	C4F4 - 126	HA 1.0%	2.9 ± 0.10
10	C5F4 - 127	HA 1.5%	2.8 ± 0.12
11	C5F3 - 128	HA 1.5%	3.2 ± 0.0
12	C5F4 -131	HA 1.5%	2.9 ± 0.00
13	C5F4 - 132	HA 1.5%	2.5 ± 0.14
14	C6F4 - 137	HA 2.0%	3.5 ± 0.14
15	C6F4 - 138	HA 2.0%	3.3 ± 0.14
16	C6F3 - 140	HA 2.0%	3.0 ± 0.00
17	C6F3 - 141	HA 2.0%	4.1 ± 0.00

T1 = control, T2 = chemical fertilizer, T3 = HA 0.5%, T4 = HA 1%, T5 = HA 1.5%, T6 = HA 2%.

The solubilizing index was measured as indicators of the isolates' ability to solubilize insoluble phosphorus. In the control group, isolates C1F4-107 and C1F4-108 exhibited relatively large clear zones, resulting in lower solubilizing indices. The isolates from the HA 1.5% treatment, particularly C5F4-131, showed promising solubilization capabilities. The HA 2% treatment also exhibited significant solubilization potential, although the outcomes varied among individual isolates. Notably, isolates C6F4-137 and C6F3-141 displayed substantial clear zones and high solubilizing indices, indicating their ability to efficiently solubilize insoluble phosphorus. The 17 isolates resulting in the selection of 5 strains considered the strongest isolates capable of solubilizing phosphorus in insoluble Ca_3PO_4 represented in **Figure 2**.

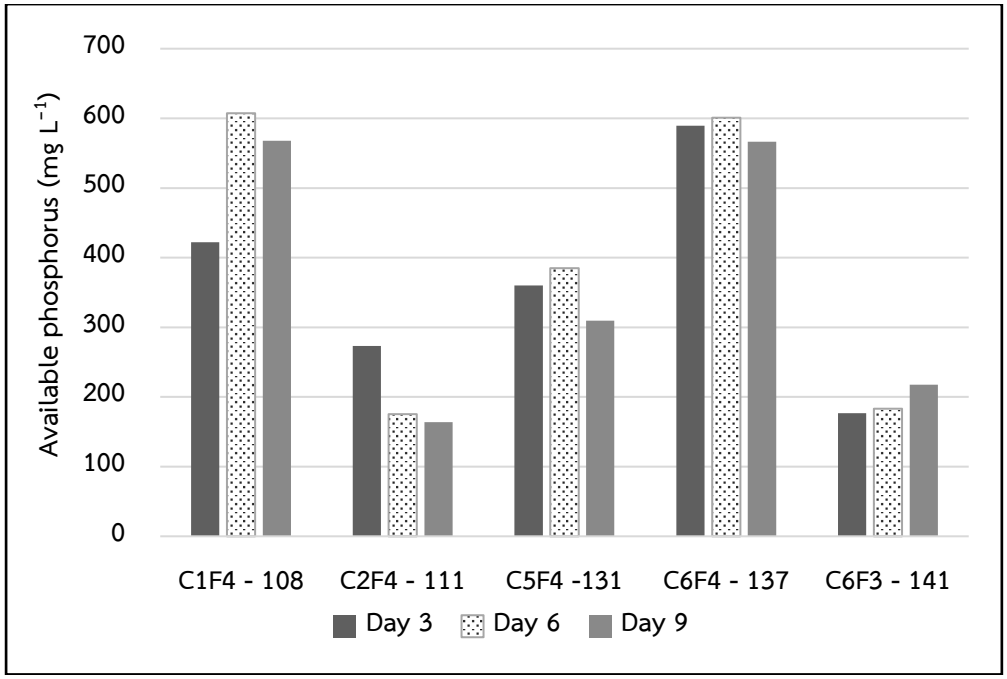

Figure 2 Solubilization capability of selected strain to solubize phosphorus

The five selected isolates were obtained from different treatments, specifically control group (C1F4-108), chemical fertilizer (C2F4-111), HA 1.5% treatment (C5F4-131), and two isolates from the 2% HA treatment (C6F4-137 and C6F3-141). The solubilization efficacy of insoluble phosphorus was analyzed over a period of 3 days, specifically on days 3, 6, and 9, by inoculating the 5 selected isolates in NBRIP broth medium. As indicated in **Table 2**, the selected isolates were identified using the 16S rRNA sequence, enabling bacterial classification. It was observed that each isolate fluctuated in solubilizing phosphorus. The isolate C1F4-108 was identified as *Priestia megaterium* and reached its solubilization peak on day 6, providing ± 607 mg L^{-1} of soluble phosphorus. However, by day 9, the soluble phosphorus had decreased to approximately 6.5%, yet it still remained higher than on day 3. C2F4-111 was classified as *Bacillus subtilis*, which exhibited the highest soluble phosphorus levels during the initial 3 days after isolation approximately ± 273 mg L^{-1} of soluble P. However, the soluble phosphorus levels in this strain decreased during day 6 and reached their lowest value on day 9, revealing that *Bacillus subtilis* efficiently solubilized P on day 3. The isolates C5F4-131 and C6F4-137, classified as *Priestia aryabhattai* and *Bacillus* sp., respectively, exhibited a similar trend. Both reached their highest soluble phosphorus levels, approximately ± 385 mg L^{-1} and ± 601 mg L^{-1} respectively, on day 6. However, by day 9, the soluble P levels decreased even further compared to day 3. Specifically, the soluble phosphorus levels on day 9 decreased by approximately 14% for *Priestia aryabhattai* and 3.9% for *Bacillus* sp. compared to day 3.

Identification of selected strain

The identification of the selected isolates indicates that all five isolates shared similar phenotype characteristics. They were identified as Gram-positive bacteria that retained the crystal violet stain and exhibited a rod-shaped cellular morphology. However, according to genotypic identification, the five isolates were

distinguished and classified as different species with an outstanding 99% similarity to species such as *Priestia megaterium, Bacillus subtilis, Priestia aryabhattai*, and *Bacillus* sp.

Table 2 Selected isolates characterization and their classification

Isolate code	Gram reaction	Cellular morphology	Relatively closest organisms			
			Species	% Homology	Query coverage (%)	16S rRNA accession no.
C1F4 - 108	(+)	Rod-shaped	*Priestia megaterium*	99%	98%	MH168997
C2F4 - 111	(+)	Rod-shaped	*Bacillus subtilis*	99%	98%	OL872188
C5F4 - 131	(+)	Rod-shaped	*Priestia aryabhattai*	99%	98%	KU598848
C6F4 - 137	(+)	Rod-shaped	*Bacillus* sp.	99%	98%	LM655314
C6F3 - 141	(+)	Rod-shaped	*Mycolicibacterium* sp.	99%	98%	AP022599

Discussion

Various studies have provided comprehensive insight into the enhanced nutrient uptake and plant growth observed with integrated humic acid and PGPR, particularly phosphate-solubilizing bacteria (Baloach et al., 2014; Cozzolino et al., 2021; Ekin, 2019; Xiong et al., 2023). However, further investigation into the direct effects of humic acid on phosphate-solubilizing bacteria (PSB) is necessary, particularly in elucidating the specific application rates that can maximize the stimulation of PSB populations in soil. Given the dynamic nature of soil bacterial communities, they are highly sensitive to environmental fluctuations (Lundquist et al., 1999). It is obvious that different types of microbes in soil are significantly impacted by agricultural inputs introduced into the soil (Hellequin et al., 2020; Lundquist et al., 1999). In the context of humic acid serving as a supplementary material, its application induces discernible modifications in soil physicochemical characteristics (Ali and Mindari, 2016; Vikram et al., 2022), consequently exerting a significant influence on soil bacterial structure (Shao et al., 2020).

In some cases, the application of humic acid significantly influences the population of phosphate-solubilizing bacteria, with notable variations across different application rates (Chaitra, 2018; De Hita et al., 2020; Mulyatni et al., 2018), which the highest bacterial population was observed when applied 20 kg ha^{-1} of humic acid (Chaitra 2018). Another study suggested that the application of humic acid at a rate of around 8 kg fed^{-1} is approximately equivalent to 19.04 kg ha^{-1}, which stimulates the bacterial population (El-Sayed and El-Sayed, 2020), also effective at 24.7 kg ha^{-1} (Hussain et al., 2019). Furthermore, it has been noted that humic acid is capable of stimulating bacterial populations even at higher doses, such as 40 kg ha^{-1}, indicating its potential efficacy across a range of application rates (Ding et al., 2021). In this research, the most substantial population of phosphate-solubilizing bacteria was found in the 1.5% humic acid treatment (5.18×10^6 cfu g^{-1}), surpassing the control by 40%. Elevating the humic acid rates to 2% led to a decrease in population by approximately 4.8% compared to the 1.5% humic acid treatment suggest that a 1.5% concentration of humic acid is optimal for promoting the population of phosphate-solubilizing bacteria. Increasing the concentration to 2% may not provide additional benefits and could potentially inhibit the proliferation of PSB. This inhibition could be attributed due to

the application of humic acid altering soil physicochemical properties, such as soil pH, total organic carbon content, and the availability of macro-nutrients for bacterial growth like nitrogen and phosphorus (Ali and Mindari, 2016; Li, Y et al., 2019; Ren et al., 2022). While soil bacteria exhibit varying preferences for pH levels, and changes in pH resulting from HA application could impact their growth and activity (Jin and Kirk, 2018; Ratzke and Gore, 2018). Additionally, the nutrient sources, such as carbon serves as the primary energy source and building block for microbial growth, while nitrogen is essential for microbial protein synthesis and cellular metabolism, and phosphorus plays a critical role in various cellular processes, including energy transfer and nucleic acid synthesis (Ali and Mindari 2016; Li, Y et al., 2019; Ren et al., 2022). Therefore, alterations in these soil parameters due to HA application may affect the availability of essential nutrients, influencing PSB proliferation.

Several previous studies have examined the solubilization activity of PSB isolates, with varying durations of measurement. For instance, Aliyat et al. (2020) assessed solubility after a 7-day incubation period, Mohamed et al. (2018) and Chen and Liu (2019) reported measurements after 5 days of incubation, although Chen et al. (2006) reported solubilization activity within 3 days after incubation. However, this variability in measurement durations poses a challenge in comparing the results across studies and may introduce inconsistencies in assessing the solubilization activity of PSB isolates. Additionally, relying on a single time point measurement, as observed in some studies, may not capture the full extent of solubilization potential over time. Therefore, measuring solubilization activity at interval day provides a more the temporal dynamics of PSB isolates' activity. This approach allows for the observation of potential changes in solubilization rates over time. In this study, *Priestia megaterium* exhibited its highest solubilization rates on day 6, *Bacillus subtilis* on day 3, *Priestia aryabhattai* on day 6, *Bacillus* sp. on day 6, and *Mycolicibacterium* sp. on day 9. These findings suggest that different bacterial species within the study exhibit varied patterns of phosphate solubilization over time.

The variation in solubilization rates among bacterial species can be attributed to several factors related to their metabolic capabilities and growth characteristics. Different bacterial species possess distinct genetic and enzymatic machinery involved in phosphate solubilization (Pan and Cai, 2023). Enzymes such as phosphatases play a crucial role in breaking down insoluble phosphate compounds into soluble forms that are accessible to the bacteria, the efficiency and expression levels of these enzymes vary among species, influencing their ability to solubilize phosphorus (Liang et al., 2020; Neal et al., 2018). Bacterial growth dynamics and metabolic activity can affect solubilization rates (Chen and Liu, 2019; Gupta et al., 2022). As reported by Gupta et al. (2022), which measured the solubilizing activity interval 3, 5, and 7 days, the bacterial isolates exhibited variability in phosphorus solubilization as the time interval increased, reaching maximum levels on day 7. A comprehensive result also reported by Pande et al. (2017), which measure the solubilizing activity of PSB isolates during the interval days 2, 4, 6, and 8, the bacterial isolates reached the highest solubilizing activity on day 6, while after 8 days they found that no further concentration of soluble P was detected. Therefore, faster-growing species may reach their solubilization peak earlier in the incubation period, as they rapidly consume available nutrients and produce metabolic byproducts that facilitate phosphate solubilization, while slower-growing species may require more time to reach optimal solubilization levels.

The potential PSB has been successful to be cultivated, which was the most frequently isolated cultivable bacterial *Bacillus*, *Rhizobium*, *Pseudomonas*, and *Burkholderia* (Jida et al., 2016; Koczorski et al., 2022;

Rodriguez and Fraga Vidal, 1999; Teng et al., 2021; Zhang et al., 2021). The dominant genus isolated in this study belonged to the genus *Bacillus*, accounting 4 out of 5 isolates (**Table 2**), consisting of species such as *Bacillus subtilis* and *Bacillus* sp., as well as *Priestia megaterium* and *Priestia aryabhattai*, formerly classified as *Bacillus megaterium* and *Bacillus aryabhattai* (Gupta et al., 2020). The capacity of this isolate to solubilize phosphorus has been thoroughly reported in previous studies, they suggest that these community were the strong phosphate solubilizer in soil, such as *Bacillus subtilis* (Mohamed et al., 2018; Sharma et al., 2013), *Bacillus* sp. (Prakash and Arora, 2019; Saeid et al., 2018), *Priestia megaterium* (Kang et al., 2014; Li, Q et al., 2022), and *Bacillus aryabhattai* (Deng et al., 2022; Koczorski et al., 2022). The promotion of *Bacillus* growth by humic acid application suggests that potential plant growth-promoting rhizobacteria (PGPR) isolated from humic acid treatments are likely to belong to the *Bacillus* genus.

Conclusion

In summary, the application of humic acid has been found to promote the growth of phosphate-solubilizing bacteria (PSB), particularly with the highest population occurring with the addition of 1.5% humic acid. In this study, PSB isolates were identified as *Priestia megaterium, Bacillus subtilis, Priestia aryabhattai, Bacillus* sp., *and Mycolicibacterium* sp., with three of these strains, including *Priestia aryabhattai, Bacillus* sp., and *Mycolicibacterium* sp., being isolated from soil treated with humic acid. Notably, *Priestia megaterium* and *Bacillus* sp. exhibited the most solubilizing capacities, demonstrating soluble P concentrations of approximately ± 607 mg L^{-1} and ± 601 mg L^{-1}, respectively, on day 6. These findings suggested the strains C1F4-108 and C6F4-137 hold great potential as bioinoculants, particularly as PGPR, that might improve both plant growth and phosphorus availability.

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