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Isolation and Identification of Phosphate Solubilizing Bacteria (PSB) from the Rhizosphere of *Thymus vulgaris* L.

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Abstract
Phosphate-solubilizing bacteria dissolve insoluble form of phosphate in the soil with different mechanisms, converting them into a form that can be used, and replaced by chemical fertilizers providing beneficial use of a natural reserve. Although research on phosphate solubilizing bacteria has increased recently, research on the thyme rhizosphere is still limited. It is necessary to investigate different rhizospheric local bacteria that can solubilize phosphate and replace chemical fertilizers. 42 bacterial isolates were obtained from the rhizosphere of Thyme (*Thymus vulgaris* L.) in this study. Among these isolates, 13 phosphate-solubilizing bacterial isolates were selected which formed a transparent (halo) region around the colonies on Pikovskaya's Agar (PKA) plates. Isolates were identified using the MALDI-TOF MS method. The morphological, biochemical and IAA production of these isolates as well as quantitative measurements of phosphate solubilization of the isolates in NBRIP broth medium was evaluated. The highest efficiency was noted from *Bacillus pumilus* PCB-6 with solubilization value of 326.8 ppm. This was followed by *Acinetobacter calcoaceticus* PCB-3 with solubilization value of 313.8 ppm and *Pantoeae agglomerans* PCB-4 with solubilization value of 307.4 ppm, respectively. Among the Phosphate solubilizing bacterial isolates, P solubilization index was defined as between 1.6 and 4.2 on PKA agar medium. Additionally, the highest IAA production, at 18.43 µg ml^{-1}, was obtained from *Pantoeae agglomerans* PCB-4. This was followed by *Bacillus pumilus* PCB-6 with 17.40 µg ml^{-1} and *Acinetobacter calcoaceticus* PCB-3 with 16.71 µg ml^{-1}. This study clearly shows that selected local isolates can be used as effective phosphate-based microbial fertilizers.

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1. Introduction

Phosphorus, is a crucial element for plant growth, development, and seed formation, and is a key component of many biological molecules, including nucleic acids and enzymes. Biological oxidation plays a key role in metabolic reactions such as photosynthesis and respiration. Phosphorus is stored in plant seeds and pollen as phytate. It is the primary source of inositol in plants. Soil phosphorus deficiency causes plants to slow down root formation, limit photosynthetic activity, and decrease tolerance to phytopathogens (Aktas and Toğay, 2022). Although phosphorus is abundant in the soil in both organic (such as phytate and nucleotides) and inorganic forms (such as $Ca_3(PO_4)_2$), plants cannot use it directly because they do not dissolve. Plants can only take up phosphorus from the soil in the form of phosphate anions. Orthophosphorus (H_2PO^- and HPO_4^{-2}) is the primary inorganic form of phosphorus that plants can absorb and utilize. Inorganic phosphate fertilizers are commonly added to agricultural soils to increase available phosphorus and plant yield. The applied phosphorus is a highly reactive molecule, it forms complexes with cations like Fe^{+3}, Ca^{+2}, Al^{+3}, and Mg^{+2} in the soil. Therefore, in general only less than 30 % of the applied P fertilizers is utilized by the plants and rest of this phosphorus converts into a form that can not be used by plants and contributes to environmental pollution. To enhance P uptake efficiency, innovative and sustainable eco-friendly alternatives, such as microbial fertilizer applications, are necessary (Selçuk and Çakıcı, 2022).

Many microorganisms (like bacteria, fungi, and algae) are well-known for their ability to efficiently solubilize phosphorus and maintain soil health. The most well-known efficient phosphate-solubilizing groups are *Bacillus*, *Pseudomonas*, *Rhizobium*, *Penicillium*, *Aspergillus*, *Actinomycetes*, and Arbuscular Mycorrhizal (Haile et al., 2022). Bacteria are key microorganisms that regulate soil phosphate levels. Despite the extremely limited capacity of plants to absorb phosphorus, phosphate-solubilizing bacteria in their immediate surroundings and a small part of the rhizosphere offer plants a chance to take up phosphorus directly. Some Plant Growth-Promoting Rhizobacteria (PGPR) are particularly effective in solubilizing highly insoluble tricalcium phosphate, hydroxyapatite, and rock phosphate (Akçura and Çakmakçı, 2023; Cheng et al., 2023).

Phosphate-solubilizing bacteria play a vital role in agriculture due to their ability to enhance plant growth and improve nutrient uptake. By solubilizing phosphorus in the soil, these bacteria make this essential nutrient more readily available to plants, leading to increased growth and development. Additionally, the improved availability of phosphorus can enhance the uptake of other nutrients by plants, further supporting their nutritional requirements. Moreover, plants treated with phosphate-solubilizing bacteria Show to exhibit increased resistance to various environmental stresses, such as drought and disease. This highlights the significant impact that these bacteria can have utility in improving agricultural productivity and sustainability, and enhance play a vital role soil fertility, promoting plant growth, and improve nutrient uptake (Billah et al., 2019). The environmental and health hazards posed by chemical fertilizers, coupled with their soaring costs, have underscored the need for sustainable methods in cultivating medicinal and aromatic plants such as Thyme. Consequently, the importance of alternative, eco-friendly fertilizers has been brought to the forefront by these concerns. This study aimed to identify the local bacterial community in the thyme rhizosphere using MALDI TOF MS and evaluate its potential as a phosphate solubilising microbial fertilizer.

2. Materials and Methods

2.1. Collection of soil samples

Rhizospheric soil samples were collected on May 2023 from *T. vulgaris* in the Medicinal Plants Garden of the Department of Field Crops, Faculty of Agriculture (39°57'44.2"N, 32°51'36.7"E) Ankara University. Following aseptic procedures, the soil samples were

promptly collected from the rhizosphere soil of *Thymus vulgaris L.* at a depth of 10 cm, by collecting them in sterile plastic bags and transferred to the laboratory for further analysis. Figure 1 shows the flowchart of the process from isolating bacteria in the Thyme rhizospheric soil sample to determine their phosphate solubilizing abilities.

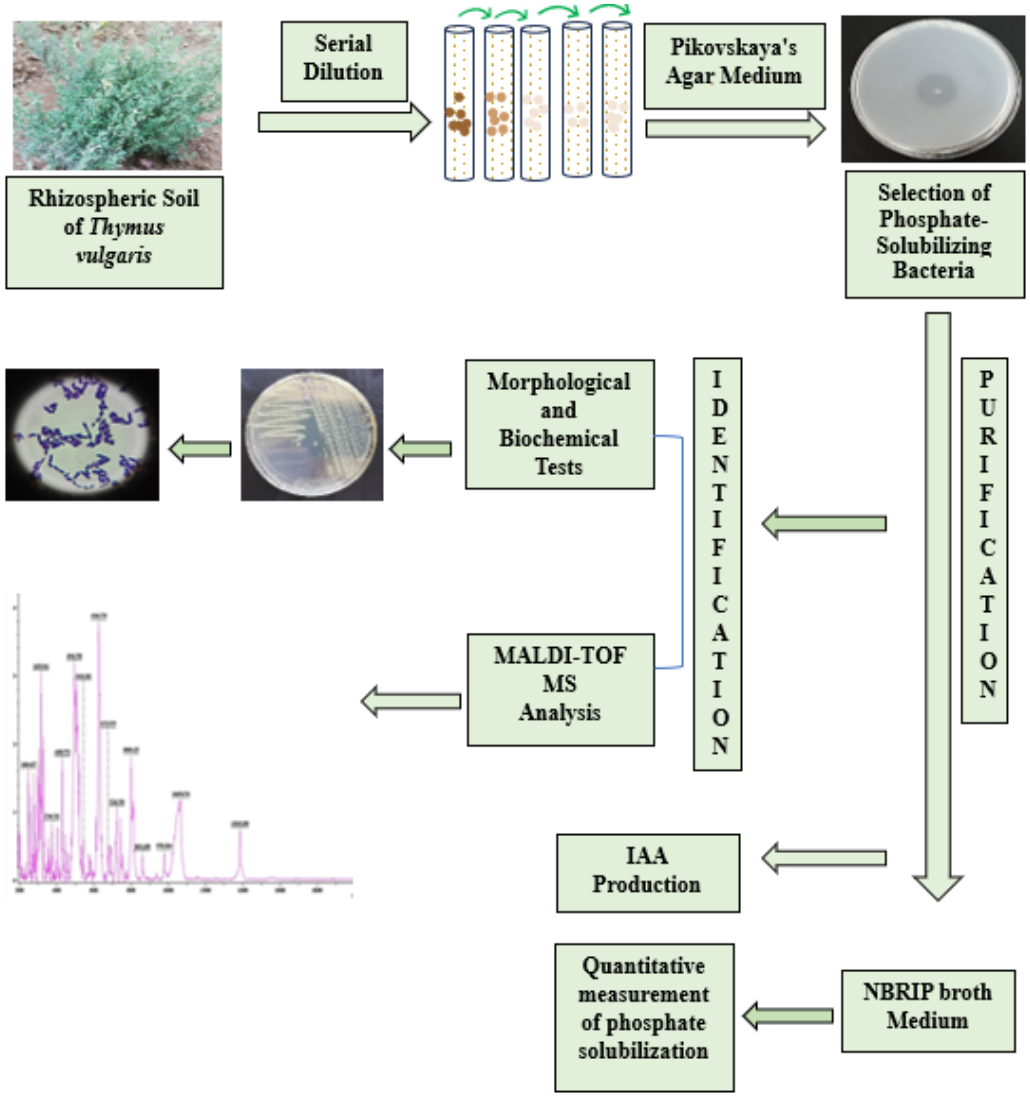

Figure 1. Flow chart for identification of isolates in Thyme rhizospheric soil samples

2.2. Isolation of phosphate solubilizing bacteria

Phosphate solubilizing bacteria were isolated from 1 g of rhizospheric soil samples using the serial dilution method. Soil samples were homogenized in 10 ml of sterile isotonic saline water. The soil samples (1 g) were mixed with 9 ml of 0.85 % saline (NaCl) sterile water and then homogenized in a shaker for 10 min. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6}. These dilutions were spread on Pikovskaya's Agar (PKA) (0.2 g L^{-1} NaCl, 10 g L^{-1} glucose, 0,2 g L^{-1} KCl, 5 g L^{-1} $Ca_3(PO_4)_2$, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.002 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.5 g L^{-1} yeast extract, 0.002 g L^{-1} $MnSO_4 \cdot H_2O$, and 1000 ml distilled water) and incubated for 5 days at 30 °C. The formation of a clear halo zone around the colonies on PKA agar plates indicated the presence of phosphate solubilizing bacteria. To obtain pure cultures, single colonies with clear zones were transferred to plates containing the same medium. Pure phosphate solubilizing bacteria

colonies were spot inoculated at the center of the Pikovskaya agar medium. After 10 days of incubation at 30 °C, the zones of phosphate solubilization around the colonies were measured. The experiments were performed in triplicate. The purified isolates were maintained on nutrient agar plates at 4 °C, and duplicates of each isolate were preserved in 40% glycerol stocks at -30 °C. The solubilization index (SI) was determined using measurements taken after seven days of growth from a point inoculation on PKA medium at 28 °C (Meena et al. 2015).

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

2.3. Quantitative measurement of phosphate solubilization

The phosphate solubilizing efficiency of 13 isolates that previously created a transparent (halo) zones on Pikovskaya Agar was assessed using the methodology developed by Barton (1948). NBRIP broth (5 g L^{-1} $Ca_3(PO_4)$, 2.5 g L^{-1} $MgCl_2.6H_2O$, 10 g L^{-1} glikoz, 2.25 g L^{-1} $MgSO_4.7H_2O$, 0,1 g L^{-1} $(NH_4)_2SO_4$, and 0.2 g L^{-1} KCl) was used to quantitatively determine the phosphate solubilizing abilities of phosphate-solubilizing isolates. For this purpose, 0.1 ml of fresh isolate (10^8 CFU ml^{-1}) was inoculated in triplicate into test tubes containing 10 ml of NBRIP growth medium and incubated at 30 °C at 180 rpm for 7 days. After incubation, the tubes were centrifuged at 5000 rpm for 10 min, and then the supernatant of each culture was analyzed for phosphate concentration in ppm. The experiments were performed in triplicate. Non inoculated medium was used as control.

2.4. Determining IAA production by phosphate-solubilizing bacteria

The Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce IAA. Bacterial cultures were cultivated for 48 hours at 30±2 °C. These freshly prepared cultures were centrifuged for 30 minutes at 3000 rpm. The supernatant (2 ml) formed after centrifugation was mixed with 4 ml of the Salkowski reagent (reagent (50 ml, 1 ml 0.5 M $FeCl_3$ solution, 35 % perchloric acid) and two drops of orthophosphoric acid. Pink appearances indicated presence of IAA. The presence of IAA in the culture supernatant was determined spectrophotometrically (SHIMADZU UVmini-1240 Spectrophotometer) at 530 nm.

2.5. Morphological and biochemical characterization of phosphate-solubilizing bacteria

The morphological characterization of 13 phosphate-solubilizing bacteria (PCB) was carried out using color, motility, and Gram staining assays, while the biochemical characterization was performed using catalase and oxidase tests. The catalase and oxidase test of the isolates was determined according to the protocol described by Clarke and Cowan (1952). For the catalase test, 2 drops of 30% hydrogen peroxide were dropped on the colonies taken with a sterile loop and the emergence of gas bubbles was observed. The observation of gas bubbles indicated a positive result. For the oxidase test, 1 % tetramethyl-p-phenylenediamine was dropped on the colonies using a sterile loop, and a change in color to blue indicated a positive result.

2.6. Identification of phosphate-solubilizing bacteria

The MALDI TOF MS method was used to identify 13 isolates with phosphate solubilizing ability. Microorganisms were identified by their unique molecular fingerprints using the MALDI Biotyper CA System. In this method, the protein profiles of microorganism biomolecules (such as proteins, peptides, sugars, and polymers) were ionized and passed through an electric and/or magnetic field. These profile spectra were compared graphically to reference microorganisms in the database of the system to accurately identify

them by genus and species (Sivri and Öksüz, 2019).

2.7. Data analysis

Data on phosphate solubilization efficiency and solubilization index (SI) were analyzed in triplicate with JMP Pro 17.0 statistical software. Dependant variables with normal distribution were presented as mean ± Standart Devision (SD) (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Morphological and biochemical characterization

13 of the 42 isolates isolated from the thyme rhizosphere were determined to form a transparent (halo) region around the colonies on Pikovskaya's Agar (PKA) medium in this study. These isolates were selected for morphological, biochemical and phosphate quantification. Among the 13 isolates, 6 were Gram positive; and 7 showed Gram-negative reactions. The catalase test was positive for all isolates except for PCB-5, whereas the oxidase test was positive for 8 isolates except for PCB-2, PCB-3, PCB-4, and PCB-10. All isolates were positive in motility test and the colony color was mostly white. Morphological and biochemical characteristics of rhizobacterial isolates are given in Table 1.

Table 1. Morphological and biochemical traits of isolates

Isolates No	MALDI-TOF MS results	Gram Stain Test	Motility	Colony color	Biochemical Characteristics	
					Catalase	Oxidase
PCB-1	*Pseudomonas koreensis*	-	+*	white	+	+
PCB-2	*Bacillus subtilis*	+	+	cream	+	-
PCB-3	*Acinetobacter calcoaceticus*	-	+	cream	+	-
PCB-4	*Pantoeae agglomerans*	-	+	light yellow	+	-
PCB-5	*Stenotrophomonas rhizophila*	-	+	white	-	+
PCB-6	*Bacillus pumilus*	+	+	cream	+	+
PCB-7	*Lactobacillus paracasei*	+	+	white	+	+
PCB-8	*Bacillus mojavensis*	+	+	white	+	+
PCB-9	*Bacillus pumilus*	+	+	cream	+	**
PCB-10	*Pantoea agglomerans*	-	+	light yellow	+	-
PCB-11	*Bacillus mojavensis*	+	+	white	+	+
PCB-12	*Pseudomonas libanensis*	-	+	light yellow	+	+
PCB-13	*Pseudomonas chlororaphis*	-	+	white	+	+

Note: * +, positive; –, negative ** Not detected

3.2. Identification of isolates by MALDI-TOF-MS method

MALDI-TOF MS analysis identified 13 isolates from soil samples in the rhizosphere, including 5 *Bacillus* (PCB-2, PCB-6, PCB-8, PCB-9, and PCB-11), 3 *Pseudomonas* (PCB-1, PCB-12, and PCB-13), 2 *Pantoeae* (PCB-4, and PCB-10), 1 *Stenotrophomonas* (PCB-5), 1 *Lactobacillus* (PCB-7) and 1 *Acinetobacter* (PCB-3) (Figure 1).

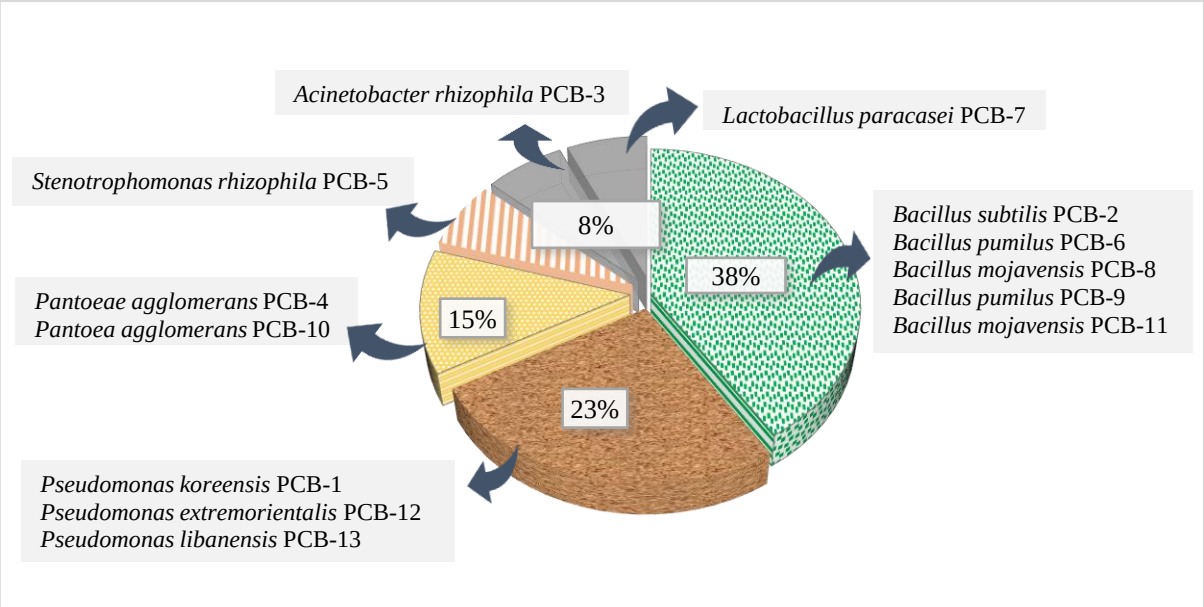

Figure 1. Percentage distribution of phosphate-solubilizing bacteria

MALDI-TOF MS is an extremely useful tool for identifying bacteria at the genus, species, and strain levels. Recently, this method has gained popularity due to its high accuracy and rapid results. In previous studies, many researchers used the MALDI-TOF MS method to identify phosphate-solubilizing bacteria (Lacava et al., 2021; Omar, 2022). Muthuri et al. (2012) identified forty-three phosphate-solubilizing endophytic bacteria strains, including *Pseudomonas*, *Bacillus*, and *Klebsiella*, in bananas using the MALDI TOF method. Çelikten and Bozkurt (2018) used the MALDI-TOF method to identify 120 bacteria they isolated from the wheat rhizosphere to investigate plant growth-promoting bacteria. Martínez-Hidalgo et al. (2021) determined that the phosphate-solubilizing bacteria in canola rhizospheres, which they identified with the MALDI TOF method, mostly belonged to the Paenibacillus and Pseudomonas genera. Similarly, Öksel et al. (2022) used the MALDI-TOF MS method to identify bacteria in wheat rhizospheres. The findings of this study revealed that *Bacillus* (38 %) *Pseudomonas* (23 %) were the most common bacterial genera in *T. vulgaris* rhizosphere. Dip et al. (2024) determined that the phosphate-solubilizing bacteria in the root zones of *Sporobolus indicus* and *Panicum coloratum*, identified using the MALDI TOF method, mainly belonged to the Enterobacter and Pseudomonas genera.

3.3. Phosphate solubilization and IAA production properties of isolates

In current study, P solubilization index of PSB strains are defined to be between 1.6 to 4.2 on PKA agar medium (Figure 2). On the other hand, in the NBRIP broth medium a rate of 84.3 to 326.8 ppm was detected. According to the phosphate solubilizing abilities of phosphate solubilizing bacteria in NBRIP broth medium, the highest value was obtained from *Bacillus pumilus* PCB-6 with 326.8 ppm. This was followed by *Acinetobacter calcoaceticus* with 313.8 ppm and *Pantoeae agglomerans* PCB-4 with 307.4 ppm, respectively. The phosphate solubilizing activities and solubilization index (SI) of the isolates were shown in Table 2.

Table 2. Phosphate solubilization activities and solubilization index (SI) of the isolates

Isolates No	Phosphate solubilizing activity (ppm)	Solubilization index (SI)
Pseudomonas koreensis PCB-1	287.6±5.92^{c}	2.4±0.26^{cd}
Bacillus subtilis PCB-2	233.5±1.93^{e}	2.8±0.26^{bc}
Acinetobacter calcoaceticus PCB-3	313.8±7.45^{b}	3.5±0.36^{ab}
Pantoeae agglomerans PCB-4	307.4±3.68^{b}	3.8±0.40^{a}
Stenotrophomonas rhizophila PCB-5	84.3±2.10^{h}	1.8±0.36^{de}
Bacillus pumilus PCB-6	326.8±5.10^{a}	4.2±0.26^{a}
Lactobacillus paracasei PCB-7	233.1±1.45^{e}	1.4±0.36^{e}
Bacillus mojavensis PCB-8	221.3±1.90^{f}	1.7±0.17^{de}
Bacillus pumilus PCB-9	197.4±1.68^{g}	1.6±0.5^{de}
Pantoea agglomerans PCB-10	242.7±2.10^{e}	1.8±0.1^{de}
Bacillus mojavensis PCB-11	206.6±1.60^{g}	1.7± 0.1^{de}
Pseudomonas libanensis PCB-12	262.1±4.16^{d}	2.3±0.26^{cd}
Pseudomonas chlororaphis PCB-13	281.5±2.47^{c}	2.1±0.17^{cde}

*For Data Analysis: p<0,001; statistically significant level. a-ı: The difference between the means shown by different letters in the same column is statistically significant. (Mean ± SD: Mean±Standard Deviation)

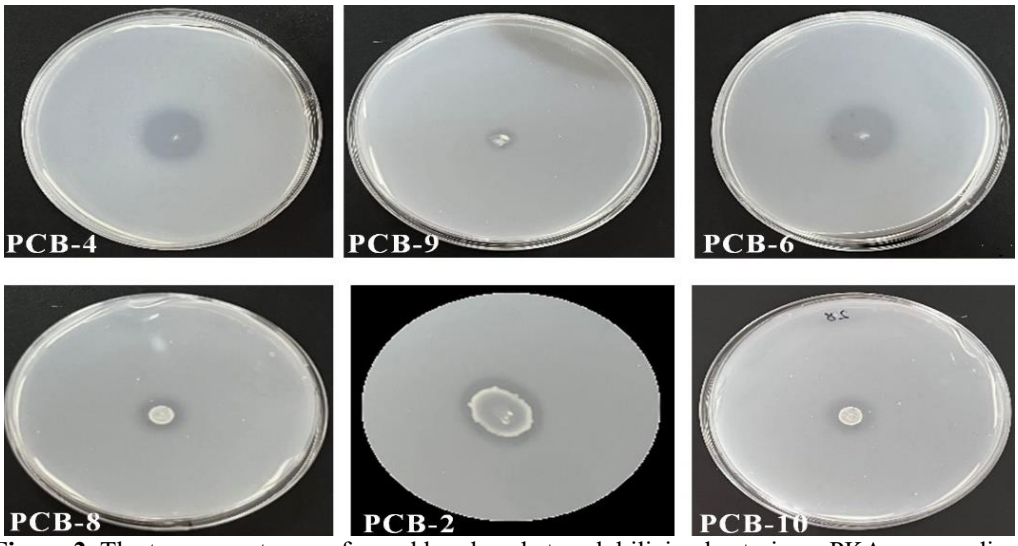

Figure 2. The transparent zones formed by phosphate solubilizing bacteria on PKA agar medium

IAA is a hormone that promotes cell elongation, division, and differentiation in plants, as well as root system development, nutrient uptake, and overall plant growth. Plant hormone producing bacteria increase root surface area, leading to greater nutrient uptake, as reported by Bai et al. (2003). Since PGPRs in the rhizosphere produce IAAs, plant roots are more influenced by these IAAs from PGPRs, which boosts root system growth and increases the root surface area in contact with the soil (Randive et al., 2024). IAA synthesis is common among soil and plant-associated bacteria. Asra et al. (2024) reported that PGPRs in the rhizosphere can support plant growth by producing IAA. In this study, it was determined that indigenous PSB strains isolated from thyme rhizome can produce IAA at different rates except *Stenotrophomonas rhizophila* PCB-5. According to the findings of the current study, the highest IAA production was obtained in *Pantoeae agglomerans* PCB-4 with 18.43 µg ml^{-1}. This was followed by *Bacillus pumilus* PCB-6 with 17.40 µg ml^{-1} and *Acinetobacter calcoaceticus* PCB-3 with 16.71 µg ml^{-1}, respectively (Figure 3A and 3B).

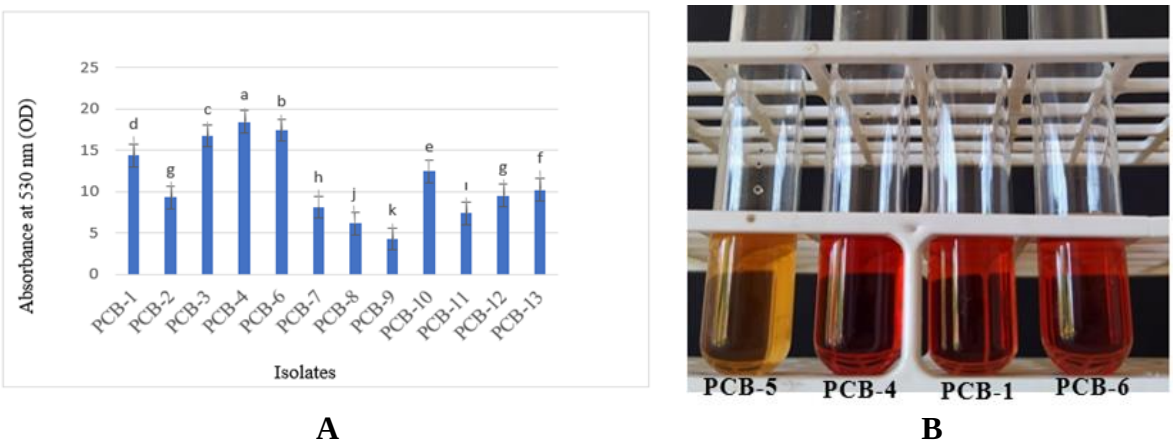

Figure 3. A. IAA production by isolates on NBRIP medium B. Pink appearances formed by some IAA producing isolates

In previous studies, researchers found similar results regarding phosphate solubilization and IAA production of strains isolated from different plant rhizospheres. Panhwar et al. (2012) determined that *Bacillus* sp. PSB9 isolated from aerobic rice in Malaysian rice fields was an effective phosphate solubilizer and IAA producer. Rfaki et al. (2020) reported that among the phosphate-solubilizing bacteria isolated from the wheat rhizosphere, the strain producing the highest amount of IAA was *Pseudomonas* sp. B26 with 21.2 mg ml^{-1}, while the strain producing the least amount of IAA was *Serratia* sp. B107 with 2.9 mg ml^{-1}. According to Sharma et al. (2021), 80 % of bacteria isolated from the rhizosphere can produce IAA. Khatami et al. (2023) reported that rhizospheric *Bacillus* sp. synthesized high amounts of IAA. On the other hand, In the current study, *Bacillus subtilis* PCB-2 (9.29 µg ml^{-1}), *Bacillus mojavensis* PCB-8 (6.16 µg ml^{-1}) and *Bacillus pumilus* PCB-9 (4.27 µg ml^{-1}) produced IAA with low potential.

Audipudi et al. (2012) tested the phosphate solubilization abilities of bacteria using solid and liquid medium and determined that their phosphate solubilization abilities ranged 80 to 100 ppm. Pramanik et al. (2017) utilized MALDI-TOF MS for the identification of the K5 strain as *Klebsiella pneumoniae*, which demonstrated phosphate solubilization at 80.25 ppm. Mei et al. (2021) documented that the strain *Pantoea agglomerans* IALR1325, isolated from flora in the Appalachian Mountains of Central Virginia, USA, exhibited a high phosphate dissolution rate of 372.8 µg ml^{-1}. Kirui et al. (2022) determined that among 71 phosphate-solubilizing bacteria from different agricultural regions in Kenya, the bacteria with the highest phosphate solubilization index of 5.883 was *Burkholderia cepacia*.

Aliyat et al. (2022) reported that bacteria taken from the phosphate mining area had the ability to dissolve different phosphate forms (tricalcium phosphate, aluminum phosphate and iron phosphate), and the highest phosphate solubilization index (SI) belonged to *Pantoea agglomerans* CB19 with 4.79. In another study, Ma et al. (2023) documented that the solubilization index (SI) of *Pantoea rhizosphaerae* MQR6T, a phosphate-solubilizing bacterium isolated from the rhizosphere of the *A. truncatum* in China, ranged from 3.20 to 3.98. It was determined that *Pantoeae agglomerans* PCB-4 had a high (307.4 ppm) phosphate solubilization ability in the current study.

The presence of a significantly elevated population of phosphate solubilizing bacteria in the rhizosphere, as opposed to non-rhizospheric soil, has been well-established in previous studies (Linu et al., 2019; Isiya, 2024). These bacteria, primarily pseudomonas bacteria, solubilize phosphate through various mechanisms, especially acid production. According to Billah et al. (2019), phosphate-solubilizing bacteria solubilize phosphate in

the soil through the release of several organic acids and enzymes and convert it into a form that is available to the plant. Amri et al. (2023) determined 28 phosphate-solubilizing bacteria from soil samples collected from different regions of Tunisia. They found that the solubilization index ratios of these bacteria ranged 2.14 to 3.51, with the highest SI ratio belonging to *Pseudomonas fluorescens*. It was determined that the phosphate solubilization index in the Pseudomonas genus was 2.1-2.4 in the present study. These results align with the findings of Roychowdhury et al. (2019), and Blanco-Vargas et al. (2020), which demonstrated that the solubilization index among various bacterial isolates, including *Pseudomonas* spp., ranged 2.56-4.50. They also showed that the formation of halo zones by these bacteria on growth plates is due to the production of organic acids, thus identifying them as effective phosphate solubilizers. Kaur et al. (2022) documented that 19 phosphate-solubilizing bacteria, isolated from the potato rhizosphere and identified using the MALDI-TOF-MS method, exhibited phosphate solubility ranging between 115 -747 µg ml^{-1}. Ben Zineb et al. (2020) reported that *Pseudomonas koreensis* LT62, which they isolated from Tunisian agricultural soil, had a phosphate solubilization ability of 325.21 µg ml^{-1} in NBRIP broth medium. Murgese et al. (2020) determined that *Pseudomonas koreensis* TFD26 isolated from the rhizosphere of *Cucumis melo* solubilized phosphate. Valli et al. (2023) reported that among 200 bacterial strains isolated from rice rhizosphere, *Pseudomonas koreensis* 69RS strain solubilized three forms of inorganic phosphate (AlPO4, $Ca_3(PO4)_2$ and Fe $(PO)_4$) in vitro. Similarly, In the present study, it was determined that *Pseudomonas koreensis* PCB-1 had a phosphate solubilization ability of 287.6 ppm in NBRIP broth medium. Aula et al. (2023) determined that 12 out of 17 isolates from Indonesian mangrove soil could solubilize phosphate, with *Pseudomonas libanensis* TL-8 emerging as a promising phosphate solubilizer with a PSI value of 2.82. Similarly, the phosphate solubilization index of *Pseudomonas libanensis* PCB-12 was determined as 3.2 in the present study. Yu et al. (2011) determined that *Pseudomonas chlororaphis* (W24) strain, among the 34 isolates they isolated from the walnut rhizosphere, dissolved tricalcium phosphate (TCP) in solid and liquid medium. Similarly, *Pseudomonas chlororaphis* PCB-13 solubilized phosphate in both liquid (281.5 ppm) and solid medium (SI: 2.1) in the current study.

Rawat et al. (2021), has mentioned that the most prevalent inorganic phosphate-solubilizing bacteria in the rhizosphere are *Bacillus*, *Enterobacter*, and *Pseudomonas*. Previous studies reported that among phosphate-solubilizing bacteria, *Bacillus* was an effective phosphate solubilizer (Swain et al., 2012; Abdelmoteleb and Gonzalez-Mendoza, 2020). Solubilization of phosphate is one of Bacillus's inherent characteristics (Li et al. 2023). Mukhtar et al. (2017) determined that *B. safensis* PSB5 and *B. megaterium* PSB12 from wheat rhizosphere solubilized high phosphate levels (305.6, 217.2, and 148.1 µg ml^{-1}). Prajakta et al. (2019) reported that *Bacillus mojavensis* PB-35 had a phosphate solubilization ability of 86.88 among 95 rhizobacterial strains isolated from soybean rhizosphere. In the current study, it was determined that *Bacillus mojavensis* PCB-8 with 221.3 ppm and *Bacillus mojavensis* PCB-11 with 206.6 ppm had a higher phosphate solubilization ability. Jiang et al. (2020) isolated 23 phosphate solubilizing bacteria, most of which belong to *Bacillus* sp., from the peanut rhizosphere and determined that it has a high potential to dissolve calcium phosphate (65- 496 mg L^{-1}).

Wang et al. (2020) reported that the *Bacillus subtilis* BPM12 strain in the corn rhizosphere dissolved phosphate with a ratio of 189.1 µg ml^{-1}. Gupta et al. (2022) determined that *B. subtilis* PS4, isolated from 3 different rice fields, was the strain with the highest phosphate solubilization efficiency with a ratio of 50.9. It was determined that *Bacillus subtilis* PCB-2 had a phosphate solubilization ability of 233.5 ppm in NBRIP broth medium in the present study. Dipta et al. (2017) found that

Bacillus pumilus MK5 in the cauliflower rhizosphere has phosphate solubilizing abilities at different tri-calcium phosphate concentrations, with the highest phosphate solubilization being at 500 ppm. Abdel-Hamid et al. (2021) measured the diameters of the clear zones formed by the endophytic isolates *Bacillus velezensis* T13 and *Bacillus licheniformis* T11 on Pikovskaya agar medium, obtained from the roots of *Thymus vulgaris*. They found that the phosphate solubilizing activity of these diameters was 7.5±0.3 mm and 8.9±0.2 mm, respectively. Sanchez-Gonzalez et al. (2022) reported that *Bacillus pumilus* A3, among 5 strains in the potato rhizosphere, had a phosphate solubilizing potential of 246 mg L^{-1}. These findings are consistent with the results of the current study. In the present study, *Bacillus pumilus* PCB-6 was determined to have a high (326.8 ppm) phosphate solubilizing potential.

4. Conclusion

Low phosphorus in agricultural soils is an urgent problem affecting agricultural systems worldwide, significantly limiting crop productivity and leading to a significant decrease in crop yields. The supplementation of chemical P fertilizers to low phosphorus deficiency in soils causes serious damage to the environment by disrupting the ecosystem. In addition, plants can absorb less than 30 % of chemical phosphorus fertilizers. Therefore, it is an inevitable fact that alternative natural fertilizers are needed to eliminate phosphorus deficiency in soils. Phosphate-solubilizing bacteria contribute to the high availability of soluble phosphates that can be assimilated by plants by dissolving insoluble phosphate in the soil and alleviate the problem of immobilization of a large part of inorganic phosphates applied to the soil by chemical fertilizers. Several studies have shown that many rhizosphere PGPR strains act as phosphate solubilizers. Screening strategies are needed to select the best local rhizobacteria strains that are environmentally friendly to prevent the long-term use of chemical phosphorus fertilizers that cause environmental and ecological problems. The use of phosphate-solubilizing local bacteria in different rhizospheres is crucial for sustainable agriculture as it reduces the need for chemical fertilizers, lowers fertilizer costs, and minimizes environmental harm. Although the isolation of bacteria from different thyme rhizospheres (*Thymus danenensis*) and their plant growth promoting properties have been investigated, there is no study on phosphate-solubilizing bacteria in the *Thymus vulgaris* rhizosphere. This is the first study on the isolation of phosphate solubilizing bacteria from the *Thymus vulgaris* rhizosphere. In this study, 13 bacterial isolates from the rhizosphere of Thymus (*Thymus vulgaris* L.) had good phosphate solubilization ability. When the phosphate solubilization abilities of these phosphate solubilizing bacteria were compared in NBRIP medium, the most effective one was *Bacillus pumilus* PCB-6 with 326.8 µg ml^{-1}. This was followed by *Acinetobacter calcoaceticus* PCB-3 with 313.8 µg ml^{-1} and *Pantoeae agglomerans* PCB-4 with 307.4 µg ml^{-1}, respectively. These selected local isolates should be evaluated in terms of competence, plant growth performance, and antifungal activity against different pathogens at greenhouse and field levels.

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