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Isolation of phosphate solubilizing bacteria from different medicinal aromatic plants and identification using MALDI TOF MS

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Abstract

Phosphate-solubilizing bacteria, which are among the plant growth-promoting bacteria, dissolve insoluble phosphate in the soil by several pathways and promote plant growth. Therefore, it offers an alternative option instead of applying chemical fertilizers that disrupt soil chemistry and ecological balance. Although research on phosphate solubilizing bacteria has increased recently, the research on the peppermint and fennel rhizosphere is still limited. Investigating different rhizospheric local bacteria that can solubilize phosphate and replace chemical fertilizers is necessary. It was determined that 15 of the 53 bacterial isolates obtained from peppermint (*Mentha piperita* L.) and fennel (*Foeniculum vulgare* L.) rhizospheres formed a transparent (halo) region around the colonies on Pikovskaya Agar (PKA) medium using the MALDI-TOF MS method. The morphological, biochemical and IAA production of these isolates as well as quantitative measurements of phosphate solubilization by the isolates in NBRIP broth medium was evaluated. The highest efficiency was noted from *Bacillus subtilis* MMS-7 with solubilization value of 281.6 mg L^{-1}. This was followed by *Pseudomonas fluorescens* MMS-11 with solubilization value of 263.4 mg L^{-1} and *Bacillus thuringiensis* MMS-3 with solubilization value of 172.1 mg L^{-1}, respectively. Among the Phosphate solubilizing bacterial isolates, P solubilization index ranged 1.2-3.7 on PKA agar medium. Additionally, the highest IAA production was noted at 23.38 µg ml^{-1}, using *Bacillus subtilis* MMS-7. This was followed by *Pseudomonas fluorescens* MMS-11 with value of 19.72 µg ml^{-1} and *Bacillus thuringiensis using* MMS-3 with value of 18.98 µg ml^{-1}. This study demonstrated that selected local isolates can be used as effective phosphate-based microbial fertilizers.

Keywords: *Mentha piperita*, *Foeniculum vulgare*, phosphate solubilizing bacteria, MALDI TOF MS

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INTRODUCTION

Phosphorus (P) is one of the primary macronutrients that limits plant growth after nitrogen. Phosphorus, found in biomolecules like nucleic acids, enzymes, coenzymes, nucleotides, and phospholipids, play a role in many cellular processes, especially photosynthesis and cell division. It is essential for biochemical and physiological processes such as root development, flower and seed formation, and nitrogen fixation. Moreover, phosphorus is an important element that plant gain resistance against diseases. Phosphorus makes about 0.2 % - 0.8 % of a plant's dry weight. The healthy growth of a plant depend on the amount of phosphorus in the soil, along with other elements (Li et al., 2023). The abundance of phosphorus in the soil does not mean that the plant can use it. Most soils contain significant amounts of P, but a large proportion of it is bound to soil components. Phosphorus, a reactive element, is not found in elemental form in the soil, but is found as insoluble inorganic phosphorus and organic phosphorus. Plants can't use this insoluble phosphorus directly. Plant cells absorb and utilize a significant amount of the phosphorus in the soil in the form of phosphate ions. Chemical phosphorus fertilizers applied to agricultural land are immobilized by cations like Ca^{+2}, Al^{+3}, and Fe^{+3}, turning them into insoluble forms that

plants can't use. However, some microorganisms in the soil can change this situation in favor of plants (Pan & Cai, 2023). Microorganisms play a significant role in the phosphorus cycle. They enrich the soil with phosphorus by hydrolyzing insoluble compounds into organic and inorganic phosphorus compounds. Therefore, they naturally operate as a phosphorus reserve without harming the environment. Among the phosphorus-solubilizing microorganisms (*Bacillus* spp., *Pseudomonas* spp., and *Rhizobium* spp.), fungal genera (Penicillium and Aspergillus), actinomycetes, and arbuscular mycorrhizal (AM) fungi are very important (Thampi et al., 2023).

Phosphate-solubilizing bacteria in agriculture enhance plant growth and improve nutrient uptake. These bacteria solubilize phosphorus in the soil and make it available to plants, to facilitate their increased growth and development. Furthermore, the developing plants gain increased resistance to various abiotic stresses (drought) and biotic stresses (diseases). Numerous studies carried under in vitro conditions confirm that the phytochemicals and secondary metabolites found in peppermint (*Mentha piperita*) and fennel (*Foeniculum vulgare*) have antibacterial and antiviral characteristics (Patil et al., 2023; Rafieian et al., 2024). Despite their importance in medicine, pharmacy and perfumery, studies on the phosphate solubilizing bacteria are limited and their impact on agricultural productivity is not reaped in a meaningful way (Cheng et al., 2023). The environmental and health hazards posed by chemical fertilizers, coupled with their soaring costs, underline the need for use of sustainable methods during cultivation of medicinal and aromatic plants like peppermint and fennel. Consequently, the importance of alternative, eco-friendly fertilizers has been brought to the forefront by these concerns.

Bacterial identification techniques rely on culture media and tests assessing morphology, physiology, and biochemistry, typically requiring 3–5 days and expert skills. Additionally, nucleic acid-based methods like DNA-DNA hybridization, 16s rRNA, G+C ratio, RT-PCR, and fluorescent in-situ hybridization have been developed. Nonetheless, these methods are expensive, slow, and require significant expertise. Thus, there is a need for rapid and accurate identification methods to address bacteria and help isolate novel environmental strains. MALDI TOF MS offers advantages over traditional microbiological methods, including reliability, speed, simplicity, cost-effectiveness, and ease of use (Ashfaq et al. 2022). This study aimed to identify the local bacterial strains in the fennel and peppermint rhizosphere using MALDI TOF MS protocol and evaluate their potential as a phosphate solubilising microbial fertilizer.

MATERIALS AND METHODS

Collection of Soil Samples

Rhizospheric soil samples were collected in May 2024 from 2 different medicinal plants peppermint (*Mentha piperita* L.) and fennel (*Foeniculum vulgare* L.) 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, Türkiye. The soil samples were promptly collected from the rhizospheric soil of these plants at a depth of 10 cm, and stored in sterile plastic bags, which were transferred to the laboratory for further analysis. Figure 1 shows the flowchart of the process from isolating bacteria in the medicinal plants' rhizospheric soil sample to determine their phosphate solubilizing abilities.

Isolation of Phosphate Solubilizing Bacteria

The bacterial isolates were tested for their ability to solubilize phosphate according to the methods of Pikovskaya (1948) using PKA medium. Phosphate solubilizing bacteria were isolated from 1 g of rhizospheric soil samples using the serial dilution method. The 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. Single colonies with clear zones were subcultured to obtain pure cultures. Pure phosphate solubilizing bacterial 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 the duplicates of each isolate were preserved in 40% glycerol stocks at -80 °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).

$$\mathrm{SI} = \frac{\text{Colony diameter} + \text{Halo zone diameter.}}{\text{Colony diameter}}$$

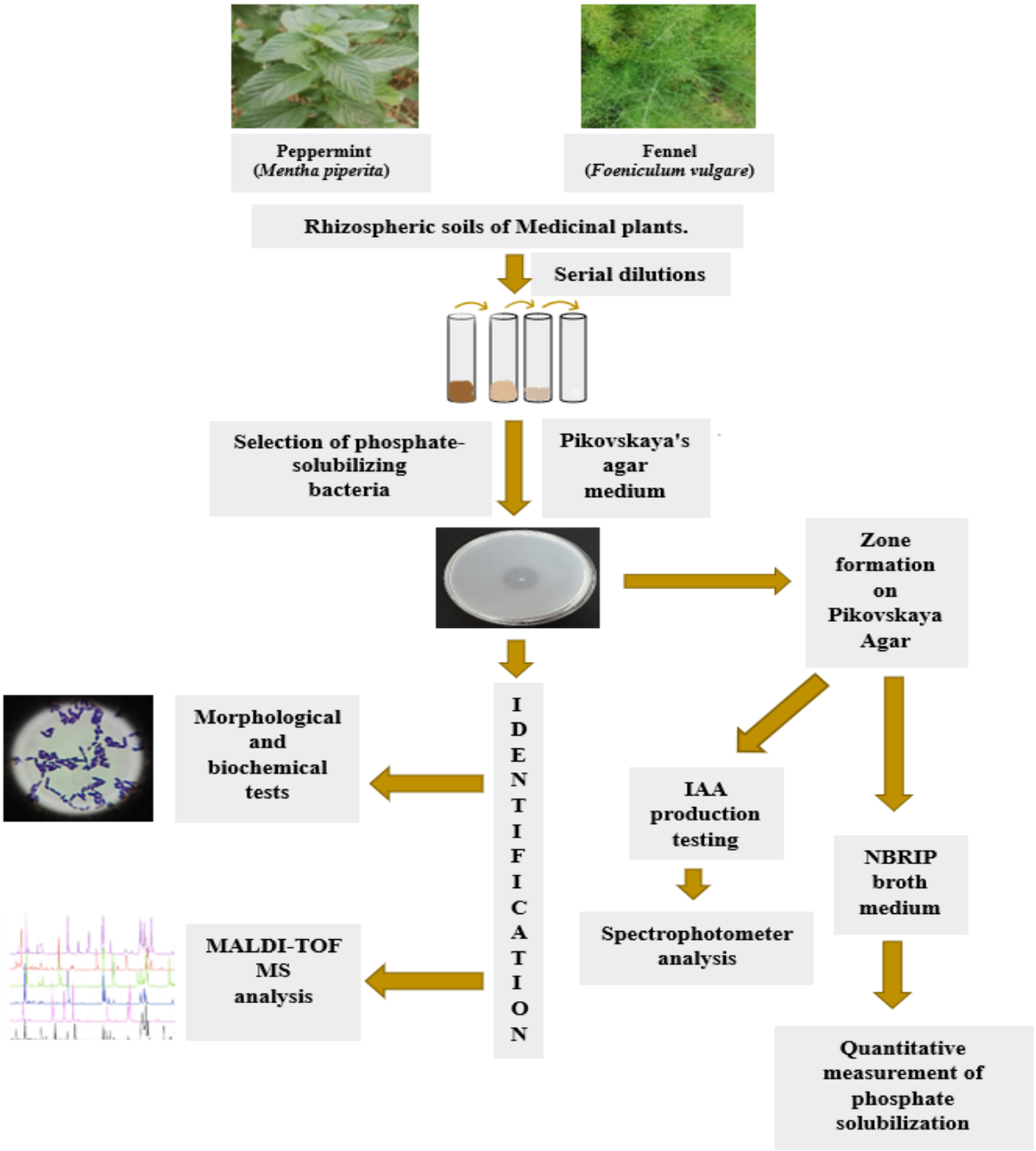


Figure 1. Process flowchart for identifying isolates in rhizospheric soil samples

Quantitative Measurement of Phosphate Solubilization

The phosphate solubilizing efficiency of 15 phosphate solubilizing bacteria 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} glucose, 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 quantify the phosphate solubilizing ability 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 mg L^{-1}. The experiments were performed in triplicate. Non inoculated medium was used as control.

Morphological and Biochemical Characterization of Bacterial Strains

The morphological characterization of phosphate solubilizing bacteria was carried out using color, motility, and Gram staining tests. 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). Two drops of 30% hydrogen peroxide were dropped on the colonies taken with a sterile loop and the

emergence of gas bubbles was observed after the catalase test. 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.

Identification of Phosphate Solubilizing Bacteria

MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) was used for bacterial identification. Microorganisms were identified by their unique molecular fingerprints by the MALDI Biotyper CA System. Protein profiles of microorganisms' biomolecules (such as protein, peptide, sugar, and polymer) are ionized and then passed through an electric and/or magnetic field in this method. The profile spectra are compared graphically with reference microorganisms in the system's database to accurately identify them at genus and species level (Sivri & Öksüz, 2019).

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 were centrifuged for 30 minutes at 3000 rpm. The supernatant (2 ml) formed after centrifuge was mixed with 4 ml of the Salkowski reagent (50 ml, 1 ml 0.5 M $FeCl_3$ solution, 35% perchloric acid) and two drops of orthophosphoric acid. Pink appearances indicated the presence of IAA. The presence of IAA in the culture supernatant was determined spectrophotometrically (SHIMADZU UVmini-1240 Spectrophotometer) at 530 nm.

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 & Soysal, 2018).

RESULTS AND DISCUSSION

Identification of Isolates

Out of the 53 isolates taken from the rhizospheres of 2 different medicinal plants, it was determined that 15 of them created a clear zone around the colonies including 5 Bacillus (*B. thuringiensis* MMS-3, *B. subtilis* MMS-7, *B. megaterium* MMS-8, *B. simplex* MMS-10, *B. simplex* MMS-12), 3 Pseudomonas (*P. lutea* MMS-4, *P. fluorescens* MMS-11, *P. boreopolis* MMS-13), 1 *Pantoea* (*P. agglomerans* MMS-15), 1 Flavobacterium (*F. hydatis* MMS-1), 2 Stenotrophomonas (*S. rhizophila* MMS-9, *S. rhizophila* MMS-14), 1 Acinetobacter (*A. baumanni* MMS-6), 1 Enterobacter (*E. cloacae* MMS-5), and 1 Rhizobium (*R. radiobacter* MMS-2) on Pikovskaya Agar (PKA) medium in this study. These isolates were selected for morphological, biochemical and phosphate quantification. The distribution of bacterial species in plants according to MALDI-TOF MS results is given in Table 1. Percentage distribution of phosphate-solubilizing bacteria in peppermint and fennel rhizosphere is given in Figure 2.

Table 1. Bacterial species distribution according to MALDI-TOF MS result in plants

Plant	Number of species	Phosphate solubilizing bacteria identified by MALDI TOF -MS	NCBI No
Foeniculum vulgare L.	6	*Flavobacterium hydatis* MMS-1	991
		Rhizobium radiobacter MMS-2	362
		Bacillus thuringiensis MMS-3	1340496
		Pseudomonas lutea MMS-4	243924
		Enterobacter cloacae MMS-5	1328422
		Acinetobacter baumanni MMS-6	470
Mentha piperita L.	9	*Bacillus subtilis* MMS-7	1423
		Bacillus megaterium MMS-8	1404
		Stenotrophomonas rhizophila MMS-9	216778
		Bacillus simplex MMS-10	1478
		Pseudomonas fluorescens MMS-11	294
		Bacillus simplex MMS-12	1478
		Pseudomonas boreopolis MMS-13	86183
		Stenotrophomonas rhizophila MMS-14	216778
		Pantoea agglomerans MMS-15	549

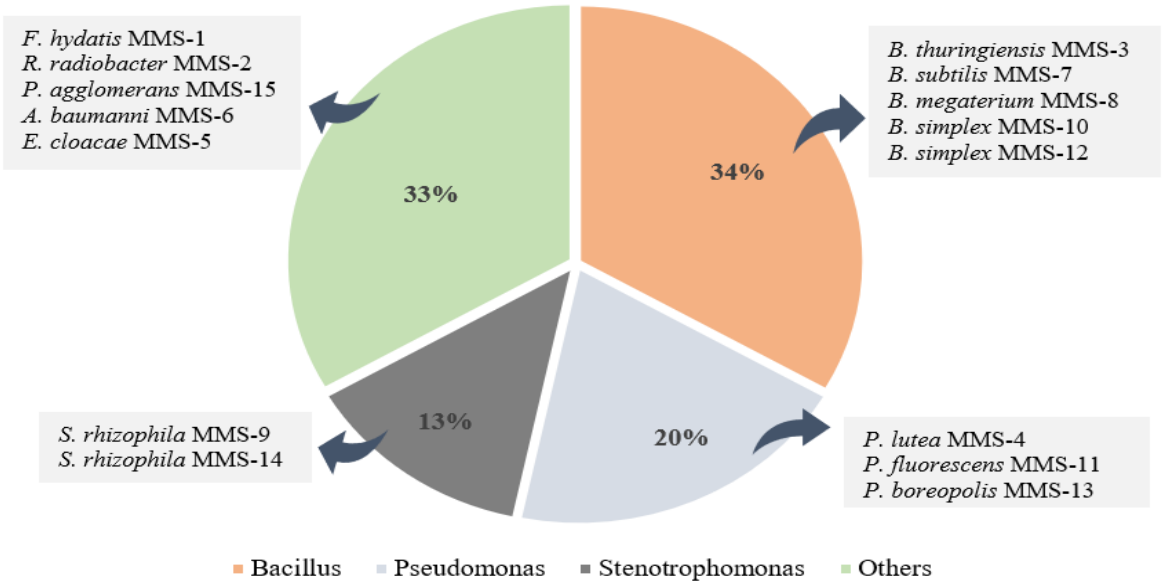


Figure 2. Percentage distribution of phosphate solubilizing bacteria by species

MALDI-TOF MS has become an invaluable tool for identifying bacteria down to the genus, species, and strain levels. Its recent rise in popularity can be attributed to its high precision and the speed at which it delivers results by MALDI-TOF MS to identify phosphate-solubilizing bacteria (Muthuri et al., 2012; Nazir et al., 2020). Çelikten & Bozkurt (2018) identified 120 bacteria from the wheat rhizosphere using MALDI-TOF to study plant growth-promoting bacteria. Bektaş (2021) used the MALDI-TOF MS method to identify 15 phosphate solubilizing bacteria isolated from 35 locations. Similarly, Öksel et al. (2022) identified bacteria in wheat rhizospheres using MALDI-TOF MS. The findings of this study revealed that Bacillus (34 %) pseudomonas (20 %) were the most common bacterial genera in *Mentha piperita* and *Foeniculum vulgare* rhizosphere. In a recent study, Dip et al. (2024) determined that the phosphate-solubilizing bacteria in the rhizosphere of *Sporobolus indicus* and *Panicum coloratum*, which were identified through the MALDI TOF method, predominantly belonged to the genera Enterobacter and Pseudomonas.

Morphological and Biochemical Characterization of Isolates

Five (5) out of the 15 isolates showed Gram (+) reaction in this study. The catalase test was positive for all isolates except for *F. hydatis* MMS-1, *B. subtilis* MMS-7, *S. rhizophila* MMS-9, and *S. rhizophila* MMS-14; whereas the oxidase test was positive for 6 isolates. Except *B. subtilis* MMS-7, motility tests all others samples were positive. Morphological and biochemical characteristics of phosphate-solubilizing isolates are given in Table 2.

IAA Production and Phosphate Solubilization Properties of Isolates

It was determined that a total of 6 isolates (MMS-7, MMS-11, MMS-3, MMS-9, MMS-14, MMS-15) from peppermint and fennel rhizospheres produced IAA in different amounts in this study. *Bacillus, Stenotrophomonas, Pseudomonas*, and *Pantoea* are among the identified genera of the IAA producing bacteria investigated in this study. The maximum IAA production was obtained in *Bacillus subtilis* MMS-7 with 23.38 µg ml^{-1} according to the findings of the current study. This was followed by *Pseudomonas fluorescens* MMS-11 with 19.72 µg ml^{-1} and *Bacillus thuringiensis* MMS-3 with 18.98 µg ml^{-1} (Figure 3A).

Indole-3-acetic acid (IAA), a primary auxin, is crucial for various plant processes from germination to maturity. It participates in cell division and expansion, leaf development, and the initiation and growth of roots, lateral roots, and root hairs. This phytohormone plays a vital role in communication between bacteria and their host plants. Bacteria producing IAA increase auxin levels, thereby boosting plant health and productivity (Ratnaningsih et al., 2023). According to Alemneh et al. (2021), 80% of bacteria isolated from the rhizosphere can produce IAA. IAA production has been determined in several PGPR genera, including *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, and *Pantoea*. Ahmed et al. (2014) reported that among 112 isolates isolated from different medicinal plant rhizospheres, 36 of them produced IAA and among them, *Pseudomonas fluorescens* Th98 and *Bacillus thuringiensis* Th100 strains produced IAA at the rate of 0.1 to 17 µg/100 ml. In the present study, *Pseudomonas fluorescens* MMS-11 (19.72 µg ml^{-1}) and *Bacillus thuringiensis* MMS-3 (18.98 µg ml^{-1}) produced IAA. Hassan et al. (2017) determined that endophytic *Bacillus cereus* Tp.1B isolated from the root of *Teucrium polium* L, a medicinal plant, produced IAA at a rate of 23.4 µg ml^{-1}. Shakeela et al. (2017) reported that *Bacillus subtilis* PkR34 isolated from the rhizosphere of a medicinal plant *Picrorhiza kurroa* produced IAA

at high levels (30.00 µg ml^{-1}). Similarly, the highest IAA producer (23.38 µg ml^{-1}) was *Bacillus subtilis* MMS-7 in the current study.

Table 2. Morphological and biochemical traits of phosphate-solubilizing bacteria

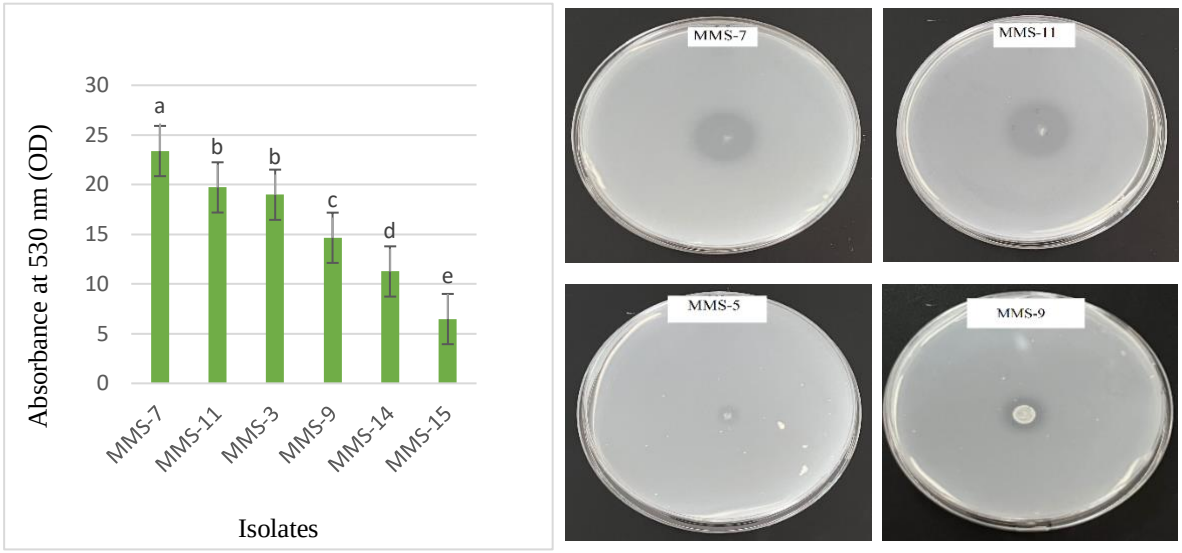
Isolates No	Gram Stain Test	Motility	Colony color	Biochemical Characteristics		
				Catalase	Oxidase	KOH 3%
Flavobacterium hydatis MMS-1	-	+	White	-	-	+
Rhizobium radiobacter MMS-2	-	+	White	+	+	+
Bacillus thuringiensis MMS-3	+	+	Cream	+	-	-
Pseudomonas lutea MMS-4	-	+	White	+	+	+
Enterobacter cloacae MMS-5	-	+	White	+	-	+
Acinetobacter baumanni MMS-6	-	+	Cream	+	-	+
Bacillus subtilis MMS-7	+	-	White	-	-	-
Bacillus megaterium MMS-8	+	+	White	+	-	-
Stenotrophomonas rhizophila MMS-9	-	+	White	-	+	+
Bacillus simplex MMS-10	+	+	Whitish	+	-	-
Pseudomonas fluorescens MMS-11	-	+	Cream	+	+	+
Bacillus simplex MMS-12	+	+	Cream	+	-	-
Pseudomonas boreopolis MMS-13	-	+	White	+	+	+
Stenotrophomonas rhizophila MMS-14	-	+	White	-	+	+
Pantoea agglomerans MMS-15	-	+	Cream	+	-	+

Note: * +, positive; –, negative

In previous studies, the researchers found similar results regarding IAA production from strains isolated from the rhizospheres of different medicinal plants. Liu et al. (2020) determined that *Bacillus subtilis* B9 strain isolated from sugarcane roots produced IAA and that B9 application increased sugarcane root development, root length, root weight and height in greenhouse experiments. Khan et al. (2020) reported that endophytic *Bacillus velezensis* Lle-9 isolated from *Lilium leucanthum* bulbs produced IAA at a rate of 23.2 µg ml^{-1}. Alaylar (2022) reported that endophytic Bacillus strains (ML-12, ML-36, ML-43, ML-46, ML-49, ML-55, ML-ML-59, ML-61, and ML-63) isolated from *Mentha longifolia* L. produced IAA.

Many studies have shown that *Pseudomonas* sp. obtained from the rhizospheres of different medicinal plants produces IAA (Thakur et al., 2017; Chandra et al., 2020). Al-Habib (2021) reported that *Pseudomonas putida* in the rhizosphere of *Eruca sativa*, which has medicinal properties, produced IAA and that the application of *P. putida* in greenhouse experiments increased rooting and germination. Wu et al. (2022) reported that *Pseudomonas fluorescens* BsEB-1 isolated from *Bletilla striata* root is an effective IAA producer. Similarly, it was determined that *Pseudomonas fluorescens* MMS-11 produced high concentrations of IAA in the current study. Marfungah et al. (2023) determined that the strain *Pseudomonas aeruginosa* RE81 produced the highest concentration of IAA among the PGPR strains isolated from the *Eucalyptus pellita* rhizosphere. Meneguzzi et al. (2024) determined that among 22 bacteria isolated from the rhizosphere of *Minthostachys verticillata*, a medicinal and aromatic plant, *Pseudomonas* sp. SM8 and *Pseudomonas putida* SM 21 produced high levels (556.7 ng/mL ve 717.8 ng/mL) of IAA in a recent study.

Shankar et al. (2011) stated that *Enterobacter* spp. and *Pantoea* spp. were among the plant growth-promoting bacteria predominantly found in the rhizosphere. Bose et al. (2016) reported that *Enterobacter cloacae* SN19 in the rizosphere of *Teramnus labialis* produced a high amount (382.23 µg ml^{-1}) of IAA. Abd el-Megeed and Youseif (2018) reported that *Pantoea* sp. NGB-W48 and *Pantoea* sp. NGB-W53 in the rhizosphere of the medicinal plant *Zygophyllum album* produced 164 and 127 µg ml^{-1} IAA, respectively. Panigrahi et al. (2020) reported that *Enterobacter cloacae* MG00145, isolated from the roots and stems of the medicinal plant *Ocimum sanctum*, produced IAA at a concentration of 17.807 µg ml^{-1}. On the other hand, *Enterobacter cloacae* MMS-5 did not produced IAA in the current study. According to Chiellini et al. (2023), Many *Stenotrophomonas* spp. are plant-probiotic bacteria that promote plant growth through different mechanisms. Vijayalakshmi and Senthilkumar (2023) determined that *Stenotrophomonas rhizophila*_SS11 was the most effective IAA producer among 86 isolates obtained from the rhizosphere of the medicinal plant *Tephrosia purpurea*. Similarly, *Stenotrophomonas rhizophila* MMS-9 and *Stenotrophomonas rhizophila* MMS-14 were determined to be effective IAA producers in the present study (Figure 3A).



A **B**

Figure 3. A. IAA production by isolates on NBRIP medium **B**. The transparent zones formed by phosphate solubilizing bacteria on PKA agar medium

Some bacteria, referred to as phosphate solubilizing bacteria (PSB), have the ability to dissolve both inorganic and organic forms of phosphorus in the soil. These bacteria employ various mechanisms to solubilize phosphate, predominantly through acid production. Consequently, they release phosphorus into the soil by means of their capacity to solubilize organic and inorganic phosphorus. Previous research has shown that the rhizosphere contains a substantially higher population of phosphate solubilizing bacteria than non-rhizospheric soil (Linu et al., 2019; Ibáñez et al., 2021). Moreover, According to Anand et al. (2016), phosphorus solubilizing bacteria (PSB) are more efficient than fungal species in the solubilization of phosphorus, and make up 1–50% of the total microbial population in soil. Siddique et al. (2021) mentioned that phosphate-solubilizing bacteria render phosphate available to plants by releasing various organic acids and enzymes, which solubilize the phosphate in the soil. P solubilization index of PSB strains are ranged 1.2 to 3.7 on PKA agar medium (Figure 3B). On the other hand, a rate of 67.8 to 281.6 mg L^{-1} was detected in the NBRIP broth medium. The highest value was obtained from *Bacillus subtilis* MMS-7 with 281.6 mg L^{-1} according to the phosphate solubilizing abilities of phosphate solubilizing bacteria in NBRIP broth medium. This was followed by *Pseudomonas fluorescens* MMS-11 with 263.4 mg L^{-1} and *Bacillus thuringiensis* MMS-3 with 172.1 mg L^{-1}, respectively. The phosphate solubilizing activities and solubilization index (SI) of the isolates are shown in Table 3.

Table 3. Phosphate solubilization activities and solubilization index (SI) of the isolates

Isolates No	**Phosphate solubilizing activity (mg L^{-1})**	**Solubilization index (SI) Mean ± SD**
Flavobacterium hydatis MMS-1	67.8± 2.28^{i}	1.2 ± 0,26^{f}
Rhizobium radiobacter MMS-2	135.9± 3.60^{e}	2.1±0,32^{cde}
Bacillus thuringiensis MMS-3	172.1± 3.96^{c}	3.1±0,26^{ab}
Pseudomonas lutea MMS-4	122.5± 3.55^{fg}	1.5±0,17^{def}
Enterobacter cloacae MMS-5	98.54± 3.53^{ijk}	2.2±0,60^{bcd}
Acinetobacter baumanni MMS-6	91.2± 1.47 ^{k}	1.3±0,2^{ef}
Bacillus subtilis MMS-7	281.6± 6.94^{a}	3.7±0,55^{a}
Bacillus megaterium MMS-8	107.8± 4.81^{hı}	2.3±0.1^{bcd}
Stenotrophomonas rhizophila MMS-9	158.7± 4.45^{d}	2.9±0,26^{abc}
Bacillus simplex MMS-10	95.9± 1.47^{jk}	1.6±0,26^{def}
Pseudomonas fluorescens MMS-11	263.4± 1.35^{b}	3.5±0,26^{a}
Bacillus simplex MMS-12	102.3± 2.69^{ij}	1.5±0,43^{def}
Pseudomonas boreopolis MMS-13	117.6± 2.48^{gh}	1.4±0,17^{def}
Stenotrophomonas rhizophila MMS-14	137.1± 1.04^{e}	2.4±0,2^{bcd}
Pantoea agglomerans MMS-15	131.9± 1.77^{ef}	1.9±0,36^{def}

*p<0,01; statistically significant level. a-l: The difference between the means shown by different letters in the same column is statistically significant. (Mean ± SD: Mean±Standard Deviation)

Han et al. (2020) mentioned that *Bacillus*, *Enterobacter*, and *Pseudomonas* were the most common inorganic phosphate-solubilizing bacteria in the rhizosphere. Previous research indicated that Bacillus was an excellent phosphate solubilizer (Azaroual et al., 2020; Zhong et al., 2021). Mishra et al. (2016) reported that *Bacillus subtilis* NRCSS-II isolated from the rhizosphere of *Foeniculum vulgare* had phosphate solubilizing ability and when applied to the seed of this plant, provided yield increase up to 1744.35 kg ha^{-1}. In another study, 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 of 50.9%. It was determined that *Bacillus subtilis* MMS-7 had a phosphate solubilization ability of 281.6 mg L^{-1} in NBRIP broth medium in the present study. ALKahtani et al. (2020) determined that *Bacillus thuringiensis* had a phosphate solubilization efficiency between 7.6 and 9.6 among 13 isolates they isolated from the rhizosphere of medicinal plants *Fagonia mollis* and *Achillea fragrantissima*. In the present study, the phosphate solubilizing index of *Bacillus thuringiensis* MMS-3 was determined as 3.1. Similar findings for *Bacillus thuringiensis* were reported by Ambreen et al. (2020) and Pantigoso et al. (2022).

It has been mentioned by Paul & Sinha, (2017); and Adhikari et al. (2021) that *Pseudomonas* spp. found in the rhizosphere are effective phosphate solubilizers and support plant growth. Amri et al. (2023) identified 28 phosphate-solubilizing bacteria in soil samples from various regions of Tunisia. They discovered that the solubilization index (SI) percentages of these bacteria ranged 2.14 to 3.51, with *Pseudomonas fluorescens* exhibiting the highest SI percentage. It was determined that the phosphate solubilization index in the Pseudomonas genus (*Pseudomonas boreopolis* MMS-13 and *Pseudomonas fluorescens* MMS-11) was 1.4-3.5 in the present study (Figure 3B). These results are in agreement 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}.

CONCLUSION

Phosphorus is crucial for plant metabolism and its non availability can significantly decrease crop yields. Around 67 % of agricultural soils are deficient in P, making it essential to address this issue. Chemical phosphorus fertilizers can disrupt soil fertility and cause eutrophication. Phosphate-solubilizing bacteria (PSB) can help eliminate phosphorus deficiency in soils. A study on 53 bacterial isolates from *Mentha piperita* and *Foeniculum vulgare* rhizospheres found such that *Bacillus subtilis* MMS-7 and *Pseudomonas fluorescens* MMS-11 were the most effective in phosphate solubilization. These bacteria can be used as cost-effective phosphate-solubilizing microbial fertilizers, contributing to sustainable agriculture. Further research is needed to assess their competence, plant growth performance, and antifungal effectiveness against various pathogens.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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