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Identification of Plant Growth Promoting Rhizobacteria (PGPR) Isolated from Lavender (*Lavandula angustifolia* L.) and Determination of Their Antifungal Activity against *Fusarium oxysporum*

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Abstract
Plant growth promoting rhizobacteria (PGPR) have a variety of beneficial activities, including improved nutrient absorption, phytohormone regulation, and biological control of phytopathogens. Sixteen isolates from the *Lavandula angustifolia* L. rhizosphere were screened for their morphological, biochemical, and plant growth promoting properties in this study. Isolates were identified using the MALDI-TOF MS method. Additionally, its antagonistic properties against the phytopathogen *Fusarium oxysporum* were evaluated. Among all isolates, 10 isolates (QLN-1, QLN-2, QLN-5, QLN-6, QLN-7, QLN-8, QLN-11, QLN-13, QLN-14, QLN-15) fixed nitrogen, 11 isolates (QLN-2, QLN-4, QLN-5, QLN-6, QLN-7, QLN-8, QLN-12, QLN-13, QLN-14, QLN-15, QLN-16) dissolved inorganic phosphate, 8 isolates (QLN-1, QLN-2, QLN-6, QLN-7, QLN-9, QLN-12, QLN-14, QLN-16) produced siderophore, 9 isolates (QLN-2, QLN-3, QLN-4, QLN-6, QLN -7, QLN-8, QLN-12, QLN-15, QLN-16) produced IAA and 6 isolates (QLN-5, QLN-8, QLN-9, QLN-14, QLN-15, QLN-16) produced HCN. Isolate QLN-4 showed the highest antifungal activity against *Fusarium oxysporum* with an inhibition rate of 57.14 %. Isolate QLN-8 isolate (24.76 %) showed the weakest effect against *F. oxysporum*. Although research on PGPRs has increased recently, research on lavender is still limited. Our study reveals that local bacterial isolates obtained from lavender rhizosphere can be used as microbial fertilizers and biocontrol agents.

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1. Introduction

Lavender, meaning to wash in Latin, is a shrub belonging to the Lamiaceae family. Lavender oil, which has antibacterial and antioxidant properties, contains linalool, terpinen-4-ol, linalyl acetate, ocimene, acetate lavandulol, and cineole. The content of lavender essential oil varies depending on the type of lavender, the climate and soil structure of the region where it grows, and its productive and morphological characteristics (Denner, 2009). Lavender is grown all over the world, and the aromatic oils obtained from its flowers are frequently used in aromatherapy, baked goods, candles, cosmetics, perfumes, shampoos, and soaps (Çakar et al., 2021). It is thought that lavender extract prevents dementia, slows the growth of cancer cells, and is good for skin diseases. Additionally, various studies have revealed that lavender exhibits multiple pharmacological effects, such as sedative, anticonvulsant, analgesic, and local anesthetic roles (Rehman et al., 2020; Kozuharova et al., 2023). The most commonly used type of lavender is English lavender (*Lavandula angustifolia*). *L. angustifolia*, which has high commercial value and various applications, especially in the fields of medicine and pharmacy, needs to be grown naturally (Batiha et al., 2023).

Chemical fertilizers causing harm to the environment and human health, along with the excessive price increases, have necessitated the development of sustainable approaches in the cultivation of medicinal and aromatic plants like lavender. Thus, the significance of alternative environmentally friendly fertilizers is once again highlighted by this circumstance. PGPR are bacteria that colonize the plant's rhizosphere and support plant growth directly or indirectly. They promote plant growth through various mechanisms, such as nitrogen fixation, phosphate solubilization, ACC deaminase production, facilitating the uptake of N, P, K elements, and phytohormones like IAA. Indirectly, PGPRs induce systemic resistance (ISR), parasitism, antibiosis, and the production of metabolites (such as hydrogen cyanide) and antimicrobial metabolites (Akçura and Çakmakçı, 2023). PGPRs utilize soil nutrients for plant growth, produce many regulators, protect plants from phytopathogens, improve soil structure, and reduce harmful compounds like pesticides. Therefore, rhizobacteria are crucial microorganisms for soil fertility and sustainable crop production (Riaz et al., 2021).

Although research on PGPRs has increased recently, it is still limited. Recent studies suggest that developing microbial formulations with local isolates showing activity in different ecosystems and plant species should be increased. Therefore, developing microbial fertilizers that protect the environment and comply with sustainable agriculture can significantly reduce chemical fertilizer use. This study aimed to identify bacteria in the *Lavandula angustifolia* rhizosphere using MALDI TOF MS and to determine their plant growth-promoting properties and antifungal activities against *Fusarium oxysporum*.

2. Materials and Methods

2.1. Sample collection

Rhizospheric soil samples were collected in June 2023 from *L. angustifolia* 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. The sample was taken from the plant rhizosphere at 3 different locations at a depth of 10 cm. Each soil sample was labeled and brought to the bacteriological Laboratory and studied under aseptic conditions.

2.2. Isolation of rhizobacteria

Rhizospheric bacteria were isolated from 1 g of dried soil samples by serial dilution method. Soil samples were homogenized in 10 ml of sterile isotonic saline water. Each 1 g of soil sample was mixed in 9 ml of 0.85 % saline (NaCl) sterile water and then homogenized in a shaker for 1 hour. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6}. These dilutions were spread on nutrient agar (NA) solidified Petri dishes and incubated at 28 °C. After incubation, different colonies were

selected and planted in Petri dishes containing NA medium until a pure colony was obtained.

A total of 16 pure isolates were obtained after incubation in Luria-Bertani Broth (LB) and stored in stock solution at -85 °C for use in research. Figure 1 shows the flowchart of the process from isolating bacteria in the rhizospheric soil sample to determine their plant growth-promoting properties.

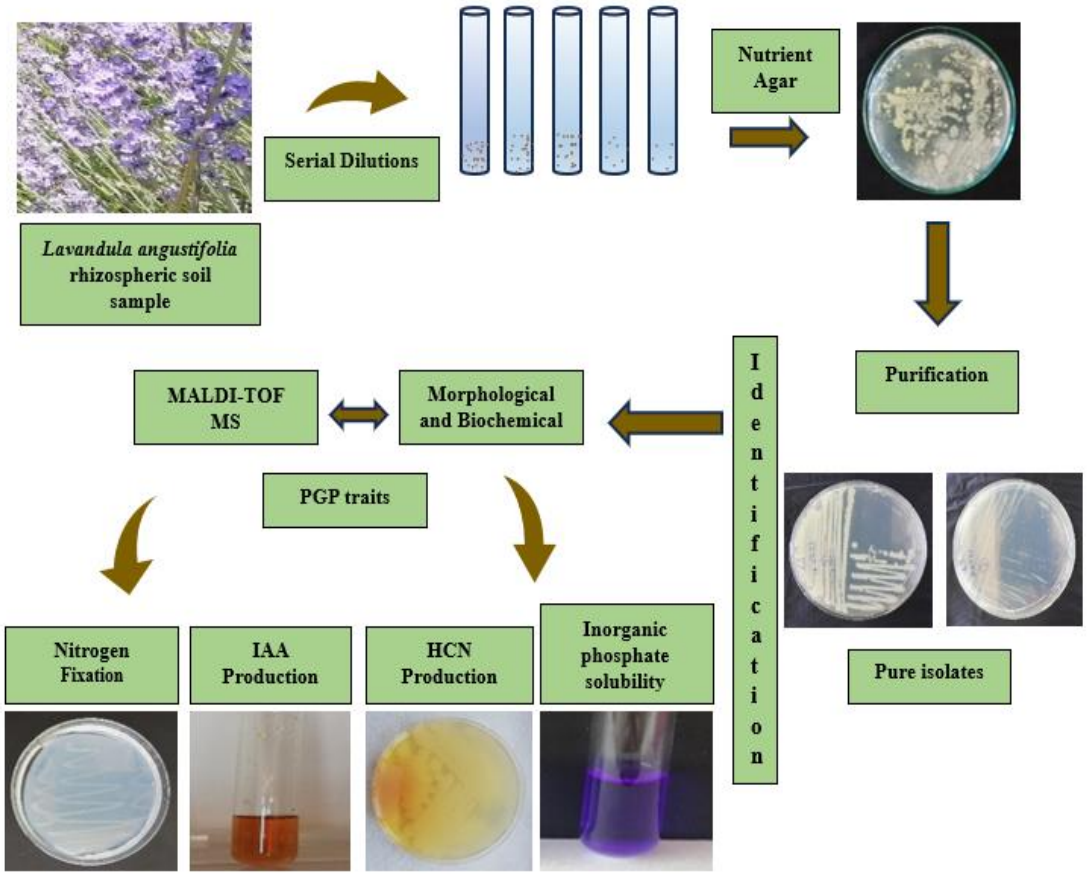

Figure 1. Flowchart representation of the process used to detect the characteristics of isolates from *L. angustifolia* rhizospheric soil

2.3. Identification of bacterial isolates

2.3.1. Biochemical and morphological characterization of isolates

Physiological, biochemical tests and Gram staining of the bacterial isolates were examined using methods described by Palleroni et al. (1984).

2.3.2. Identification of isolates with MALDI-TOF MS

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

2.4. Screening of the isolates for plant growth promoting (PGP) properties

2.4.1. Determination of nitrogen fixing capacity

Determination of the nitrogen fixation abilities of the isolates was done according to the protocol specified by Wilson and Knight

(1952) and Park et al. (2005). The isolates were streaked on NA medium and incubated at 28 ºC for 24 hours. Thereafter incubation, the fresh isolates were incubated for 4 days at 28±2 ºC by inoculating the Petri dishes with solid Burk's N-free medium (0.05 g L^{-1} Na_2SO_4, 10 g L^{-1} glucose, 0.2 g L^{-1} $CaCl_2$, 0.41 g L^{-1} KH_2PO_4, 0.52 g L^{-1} K_2HPO_4, 0.0025 g L^{-1} Na_2MoO_4. $2H_2O$, 0.1 g L^{-1} $MgSO_4$ $7H_2O$, 0.005 g L^{-1} $FeSO_4$ $7H_2O$, and 1.8 g L^{-1} agar 1 L dsH_2O) by line sowing method. After adjusting the pH of the medium to 7±0.1, it was autoclaved at 121 ºC for 15 minutes. Three time intervals were determined for nitrogen fixation activity (+++: development after 6 h, ++: development after 12 h, +: development after 24 h).

2.4.2. Determination of siderophores-producing isolates

Chrome Azurol S (CAS) agar media was used to identify the production of siderophores (Schwyn and Neilands, 1987). The isolates grown in 24-hour fresh culture were inoculated on CAS agar plates and incubated for 24-48 hours at 30±2 °C. Following the incubation period, the production of siderophores was confirmed to be positive by the appearance of an orange halo surrounding the colony on CAS agar plates. For siderophore activity, Three time intervals were determined (+++: color change after 12h, ++: color change after 24h, +: color change after 36h).

2.4.3. Determination of HCN-producing isolates

The HCN production assay was carried out according to Bakker and Schippers (1987). Bacteria were inoculated on NA medium and filter papers impregnated with picric acid (0.5 % picric acid, 2 % sodium carbonate), which were placed on the edge of the Petri dishes. The Petri dishes were tightly closed with paraffin and incubated at 28±2 °C for 4 days. After incubation, yellow-colored picric acid-impregnated papers turned brown, which was considered a positive result. For HCN activity, three time intervals were determined (+++: color change after 6h, ++: color change after 12h, +: color change after 24h).

2.4.4. Determination of isolates producing indole-3-acetic Acid (IAA)

The Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce IAA. Bacterial cultures were grown for 48 hours at 36±2 °C. These fresh cultures were centrifuged for 30 min at 3000 rpm. The supernatant (2 ml) was combined with 4 ml of the Salkowski reagent (50 ml, 35 % perchloric acid, 1 ml 0.5 M $FeCl_3$ solution) and two drops of orthophosphoric acid. Pink appearances indicate that IAA is present. The presence of IAA in the culture supernatant was determined spectrophotometrically (Multiskan SkyHigh Microplate Spectrophotometer) at 530 nm. Three time intervals were determined for IAA activity: (+++: color change after 1 h, ++: color change after 6 h, and +: color change after 12 h).

2.4.5. Determination of isolates' inorganic phosphate dissolving capacity

The inorganic phosphate dissolving capacities of the isolates were determined qualitatively according to the protocol described by Mehta and Nautiyal (2001). 25 microliters of fresh pure bacterial culture were inoculated in tubes containing 5 ml of NBRIP-BPB medium (Brom phenol supplemented National Botanical Research Institues's phosphate growth medium) (0.2 g L^{-1} KCl, 10 g L^{-1} glucose, 5 g L^{-1} $Ca_3(PO_4)$, 2.25 g L^{-1} $MgSO_4$. $7H_2O$, 2.5 g L^{-1} $MgCl_2.6H_2O$, 0.1 g L^{-1} $(NH_4)_2SO_4$ and 0.025 g L^{-1} BPB). It was incubated at 180 rpm at 30±2 °C for 3 days. The discoloration of the blue color of the NBRIP-BPB medium after incubation indicated that the isolates were phosphate solubilizers.

2.5. Assessment of extracellular enzyme production

Screening for amylase production was done as per the methodology of Smibert and Kreig (1994). Starch agar medium (yeast extract 5 g, NaCl 10 g, tryptone 10 g, starch 5 g, agar 15 g, pure water 1000 ml) was prepared to determine amylase activity. After planting on starch agar medium, it was placed in an oven set at 30 ºC and incubated for 1 day. At the end of the

incubation, iodine solution (I_2 1 g, KI 2 g, pure water 300 ml) was dropped onto the Petri dishes and waited for 5 minutes to examine the zone formation. Zone formation around the colony indicates the presence of amylase. The protease production of the isolates was determined according to the protocol described by Smibert and Kreig (1994). Agar (skim milk agar) medium containing milk was prepared for the protease production test, bacteria were cultivated using the streak seeding method and incubated at 37 °C for three days. Zones around the colony formed after incubation showed the presence of protease. The pectinase activity of the isolates was determined according to the protocol described by Geetha et al. (2014). Pectinase enzymes were identified in an M9 medium supplemented with 4 grams of pectin per liter. The plates were incubated for two days at 28±2 °C. The appearance of a clear halo surrounding colonies is indicative of pectinase production. Colloidal chitin was created using the Rodriguez-Kabana (1983) method. The isolates were cultured for four days on a chitin medium at 28±2 °C. There were clear zones around the bacterial colonies, indicating the utilization of colloidal chitin.

2.6. Assessment of antifungal activity

The fungal isolate (*Fusarium oxysporum*) used in the study was obtained from the culture collection unit of Ankara University, Faculty of Agriculture, Department of Plant Protection. Using a potato dextrose agar (PDA) medium, all the isolates were tested for antifungal activity against *F. oxysporum*. Bacterial isolates were grown on a nutrient agar medium at 25 °C for 24h to obtain fresh cultures. A 6 mm mycelial disc of fungi, *F. oxysporum* was placed in the center of the plates and incubated at 28°C for 7 days. Bacterial isolates were drawn on the edge of the Petri dish with a swab and incubated in the dark at 25 °C for one week. As a control, only the pathogenic fungus isolate was placed in the middle of the Petri dish containing PDA, and the evaluation was made when the pathogen fungus isolates covered the control Petri dish. The diameter of the fungus in the application Petri dishes was measured in mm. The percent inhibition rate of bacteria and fungus colony development was determined by Mari et al. (1996), it was calculated using the percentage of inhibition of radial development formula. For each isolate, the experiments were conducted with three replicates.

% Inhibition =(A−B) / (A−M) × 100

A: Colony diameter of the pathogen in the control application

M: Diameter of micellar disc (6 mm)

B: Colony diameter of the pathogen in bacterial application

2.7. Data analysis

Data for antifungal activity were analyzed in three replicates for using JMP Pro 17.0 statistical software. Dependant variables with normal distribution were presented as mean ± Standart Devision (SD). The antifungal activity was measured using analysis of variance (ANOVA). The differences in the mean amounts of variables were determined using the Tukey test (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Identification of isolates

In the present study, 16 isolates were obtained from the rhizospheric soil samples of *L. angustifolia* in Ankara, Turkey. MALDI-TOF MS analysis identified 16 isolates from soil samples in the rhizosphere, including 7 Bacillus (*B. endophyticus* QLN-1, *B. cereus* QLN-3, *B. subtilis* QLN-4, *B. mycoides* QLN-9, *B. pumilus* QLN-12, *B. megaterium* QLN-13, *B. mojavensis* QLN-16), 4 Pseudomonas (*P. koreensis* QLN-2, *P. putida* QLN-5, *P. fluorescens* QLN-8, *P. extremorientalis* QLN-15), 1 Stenotrophomonas (*S. rhizophila* QLN-7), 1 Pantoea (*P. agglomerans* QLN-6), 1 Enterococcus (*E. faecium* QLN-10) and 2 Lactobacillus (*L. plantarum* QLN-11, *L. paracasei* QLN-14). Among all isolates, 10 showed Gram (+) reaction. The catalase test was positive for all isolates except for *E. faecium* QLN-10 and *L. plantarum* QLN-11, whereas the oxidase test was positive for 12 isolates except for *B. subtilis* QLN-4, *P. agglomerans* QLN-6, *B. mycoides* QLN-9, and *E. faecium* QLN-10. Except *P. agglomerans*

QLN-9, *E. faecium* QLN-10 and *L. plantarum* QLN-11, motility tests all others samples were positive. MALDI-TOF MS is an extremely useful tool for identifying bacteria at the genus, species, and strain levels. In previous studies, many researchers used the MALDI-TOF MS method to identify bacteria (Pierce et al., 2007; Stîngu et al., 2008; Vega-Castano et al., 2012). Recently, this method has gained popularity due to its high accuracy and rapid results. 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. Ünlü et al. (2023) used MALDI-TOF MS to identify bacterial strains isolated from the alfalfa rhizosphere as belonging to the genera *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus*, *Acinetobacter*, and *Enterobacter*. 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* (44 %) *Pseudomonas* (25 %) and were the most common bacterial genera in *L. angustifolia* rhizosphere. The percentage distributions of bacteria obtained from the rhizosphere of *L. angustifolia* are shown in Figure 2 and morphological, biochemical results and MALDI TOF MS score values of the isolates are presented in Table 1.

Table 1. Morphological and biochemical traits of isolates

Isolates	Gram Characteristic	Biochemical Characteristics				MALDI-TOF MS Score Value
	Gram reaction	KOH 3%	Catalase	Oxidase	Motility	
Bacillus endophyticus QLN-1	+	-	+	+	+	2.043
Pseudomonas koreensis QLN-2	-	+	+	+	+	1.915
Bacillus cereus QLN-3	+	-	+	+	+	2.148
Bacillus subtilis QLN-4	+	-	+	-	+	1.745
Pseudomonas putida QLN-5	-	+	+	+	+	1.894
Pantoeae agglomerans QLN-6	-	+	+	-	+	1.907
Stenotrophomonas rhizophila QLN-7	-	+	+	+	+	1.935
Pseudomonas fluorescens QLN-8	-	+	+	+	+	2.077
Bacillus mycoides QLN-9	+	+	+	-	-	1.874
Enterococcus faecium QLN-10	+	-	-	-	-	1.931
Lactobacillus plantarum QLN-11	+	-	-	+	-	1.924
Bacillus pumilus QLN-12	+	-	+	+	+	1.965
Bacillus megaterium QLN-13	+	-	+	+	+	2.103
Lactobacillus paracasei QLN-14	+	-	+	+	+	1.842
Pseudomonas extremorientalis QLN-15	-	+	+	+	+	1.904
Bacillus mojavensis QLN-16	+	-	+	+	+	2.105

Note: * +, positive; –, negative

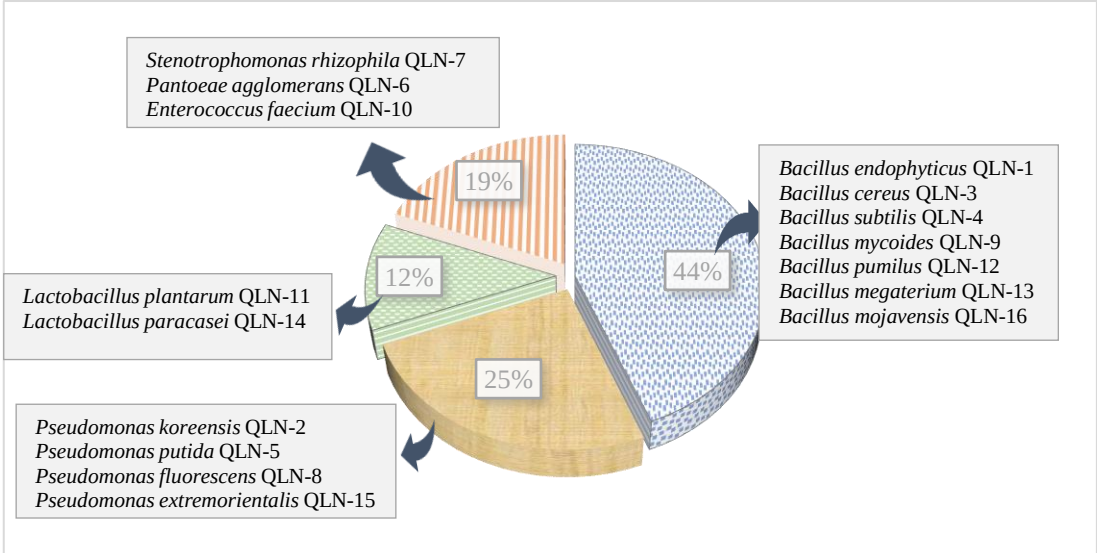

Figure 2. Percentages of bacteria isolated from *L. angustifolia* rhizosphere

3.2. Plant growth-promoting properties of isolates

PGPR supports plant growth directly or indirectly by colonizing plant roots and reducing the population of harmful microorganisms. The rhizosphere is the ecological niche with a rich nutrient presence between plant roots and soil microorganisms. These bacteria contribute to soil fertility and sustainability by producing IAA and siderophore for plants, solubilizing free phosphate, and fixing nitrogen. 16 isolates were tested for siderophores, phosphate solubility, nitrogen fixation, IAA, and HCN production abilities in vitro in the current study. Among all isolates, 62 % (QLN-1, QLN-2, QLN-5, QLN-6, QLN-7, QLN-8, QLN-11, QLN-13, QLN-14, QLN-15) fixed nitrogen, 37 % (QLN-5, QLN-8, QLN-9, QLN-14, QLN-15, QLN-16) produced HCN, 68 % (QLN-2, QLN-4, QLN-5, QLN-6, QLN-7, QLN-8, QLN-12, QLN-13, QLN-14, QLN-15, QLN-16) dissolved inorganic phosphate, 50 % (QLN-1, QLN-2, QLN-6, QLN-7, QLN-9, QLN-12, QLN-14, QLN-16) produced siderophores, and 56 % (QLN-2, QLN-3, QLN-4, QLN-6, QLN -7, QLN-8, QLN-12, QLN-15, QLN-16) produced IAA.

Nitrogen is one of the most important building blocks for plant growth and development. It is found in the structure of nucleic acids, amino acids, vitamins, and many organic molecules of living beings. Plants cannot access N_2, but N-fixing microorganisms can turn N_2 into ammonia, which plants can use right away, which reduces the use of N chemical fertilizer use and production costs (Khatoon et al., 2020). According to the available literature, *B. pumilus* (Cruz-Martín et al., 2015), *B. simplex* (Navid et al., 2023), and *B. subtilis* (Sharma et al., 2024) strains were identified to exhibit nitrogen fixation. In the present study, *Bacillus subtilis* QLN-4 fixed nitrogen. On the other hand, Zhao et al. (2011) reported that the *Bacillus cereus* MQ23 strain isolated from *Sophora alopecuroides* fixed nitrogen. In contrast, In the current study, *Bacillus cereus* QLN-3 did not fix nitrogen. Sezen et al. (2016) determined that 16 of the 180 bacteria isolated from the rhizosphere of wheat plants could fix nitrogen. Alnefai et al. (2020) determined that 8 isolates identified using MALDI-TOF MS showed strong nitrogen-fixing ability. Singh et al. (2023) determined that *P. koreensis* CY4 and *P. entomophila* CN11 isolated from sugarcane rhizosphere fixed nitrogen. Similarly, In the current study, *P. koreensis* QLN-2 fixed nitrogen (Table 2).

Many soil bacteria naturally synthesize the volatile secondary metabolite hydrogen cyanide (HCN). HCN disrupts cellular respiration in eukaryotic pathogens by

blocking the enzyme cytochrome c oxidase located in mitochondria. Additionally, it binds to and deactivates various other metalloenzymes, obstructing the mitochondrial electron transport chain and consequently stopping aerobic respiration. There are studies on the use of HCN-producing rhizospheric bacteria as biocontrol agents (Sehrawat et al., 2022; Thakur and Thakur, 2023). Bacteria produce hydrogen cyanide through bacterial cyanogenesis. The bacterial production of HCN has been linked to the suppression of growth and the killing of other organisms. Many bacterial species, especially *Pseudomonas* spp., and *Bacillus* spp., produce cyanide as a secondary metabolite. Ahmad et al. (2008) determined that among rhizospheric bacteria, 50 % of *Bacillus* isolates and 80 % of *Pseudomonas* isolates were positive for HCN production. Quesssaoui et al. (2017) determined that *P. fluorescens* Q110B and *P. fluorescens* Q036B, obtained from tomato rhizosphere, produced HCN. Likewise, Halimursyadah et al. (2023) found that *P. fluorescens* produced HCN among the 37 isolates from the patchouli rhizosphere. In the present study, *P. fluorescens* QLN-8 also produced HCN. Singh et al. (2019) determined that *B. thuringiensis* SF 23, *P. aeruginosa* SF 44, *B. subtilis* SF 48, and *B. subtilis* SF 90 isolate produced HCN. Panchami et al. (2020) determined that *P. putida* Td4 obtained from cardamom rhizosphere produced high amounts (82.5 ± 2.38 µg ml^{-1}) of HCN. Similarly, In the current study, *P. putida* QLN-5 also produced HCN (Figure 3).

Siderophores are low molecular weight organic substances produced by many bacteria. Bacillus species produce a wide array of siderophores such as bacillibactin, pyoverdine, pyochelin, and petrobactin, which are crucial for their survival. The production of siderophores by Bacillus species leads to competition with pathogens for the limited iron in the environment, thereby inhibiting the growth of phytopathogens. The majority of *Pseudomonas* and *Bacillus* strains in the rhizosphere can produce siderophores (Joseph et al., 2007). González-Sánchez et al. (2010) demonstrated that *B. cereus* and *B. subtilis* obtained from avocado rhizosphere are capable of producing siderophores to promote the protection of avocado trees against *Rosellinia necatrix*. Pereira et al. (2016) reported that the endophytic *B. cereus* LR 1-11 strain isolated from *Lavandula dentata* produces siderophores. Guerrero-Barajas et al. (2020) determined that *B. mycoides* (A1, A2) isolated from avocado rhizospheric soil produced siderophore. In the present study, *B. endophyticus* QLN-1, *B. mycoides* QLN-9, *Bacillus pumilus* QLN-12, and *B. mojavensis* QLN-16 produced siderophore. Siderophores are iron chelating compounds synthesized by many *Pseudomonas* sp. Many studies are showing that *Pseudomonas* sp. produces siderophores (Saranraj et al., 2023; Qessaouı et al., 2024). Gu et al. (2020) determined that *P. koreensis* strain S150 isolated from tobacco rhizosphere produced siderophores. Interestingly, In the current study, *P. koreensis* QLN-2 produced siderophore. Subramanium and Sundaram (2020) reported that *P. fluorescens* PSF02 isolated from agricultural soils also produced siderophores. Previous studies have shown that *Pantoeae agglomerans* also produced siderophores (Hynes et al., 2008; Shariati et al., 2017). Similarly, In the present study, *P. agglomerans* QLN-6 produced siderophore.

IAA promotes the growth of longer roots with more root laterals, which are involved in nutrient uptake. Numerous reports have indicated that IAA produces PGPR species, which are present in the rhizosphere of numerous plants (Dashti et al., 2021; Arzoo et al., 2024). According to Sokolova et al. (2011), 80 % of bacteria isolated from the rhizosphere can produce IAA. IAA production has been determined in several PGPR genera, including *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pantoea*, and *Pseudomonas*. Devanathan et al. (2021) reported that *Bacillus* sp. in *Capsicum annum* L. rhizosphere synthesized high amounts of IAA. Khatami et al. (2023) determined that *Bacillus sp., Azotobacter* sp., and *Azospirillum* sp. produced IAA in lavender rhizospheres grown under salt stress. Similarly, In the current study, *B. cereus* QLN-3, *B. subtilis*

QLN-4, *B. pumilus* QLN-12, and *B. mojavensis* QLN-16 produced IAA. In recent study, Patel et al. (2024) reported that *P. fluorescens* isolated from the *Capsicum annuum* L. rhizosphere did not produce IAA. On the contrary, In the present study, *P. fluorescens* QLN-8 produced IAA (Figure 3).

Some bacteria known as phosphate solubilizing bacteria (PSB) can dissolve inorganic and organic phosphorus in the soil. These bacteria dissolve phosphate using various mechanisms, primarily producing acid. They release phosphorus into the soil through their organic and inorganic P solubility (Selçuk and Çakıcı, 2022). Some soil bacteria like *Pseudomonas* and *Bacillus* can dissolve insoluble phosphates, which has a significant impact on promoting plant growth. 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}). According to Rawat et al. (2021), the most prevalent phosphate-solubilizing bacteria in the rhizosphere are *Bacillus*, *Enterobacter*, and *Pseudomonas*. Similarly, in the current study, *B. pumilus* QLN-12, *B. subtilis* QLN-4, *B. megaterium* QLN-13, *B. mojavensis* QLN-16, *P. koreensis* QLN-2, *P. putida* QLN-5, *P. fluorescens* QLN-8 and *P. extremorientalis* QLN-15 dissolved inorganic phosphate. Soares et al. (2023) demonstrated that *P. aeruginosa* UFT01 and *B. cereus* UFT42 dissolved inorganic phosphate but on the other hand, *B. cereus* QLN-3 did not dissolve inorganic phosphate in current study. Table 2 and Figure 3 presents the plant growth-promoting properties of the isolates.

Table 2. Plant growth promoting traits of the isolates

Isolates	Inorganic phosphate solubization	Nitrogen fixation	Siderophore production	HCN production	IAA production
Bacillus endophyticus QLN-1	-	+*	+**	-	-
Pseudomonas koreensis QLN-2	+	+++	+++	-	+***
Bacillus cereus QLN-3	-	-	-	-	+
Bacillus subtilis QLN-4	+	-	-	-	+
Pseudomonas putida QLN-5	+	++	-	++*	-
Pantoeae agglomerans QLN-6	+	+++	+	-	+++
Stenotrophomonas rhizophila QLN-7	+	+	+	-	+
Pseudomonas fluorescens QLN-8	+	++	-	+	++
Bacillus mycoides QLN-9	-	-	+	+	-
Enterococcus faecium QLN-10	-	-	-	-	-
Lactobacillus plantarum QLN-11	-	+	-	-	-
Bacillus pumilus QLN-12	+	-	+	-	+
Bacillus megaterium QLN-13	+	+++	-	-	-
Lactobacillus paracasei QLN-14	+	+	+	+	-
Pseudomonas extremorientalis QLN-15	+	++	-	+	+
Bacillus mojavensis QLN-16	+	-	+	+	+

*For nitrogen fixation and HCN activity (+++: development after 6 hours, ++: development after 12 hours, +: development after 24 hours). **For siderophore activity: (+++: color change after 12h, ++: color change after 24h., +: color change after 36h). *** For IAA activity (+++: development after 1h, ++: development after 6 h, +: development after 12 h.

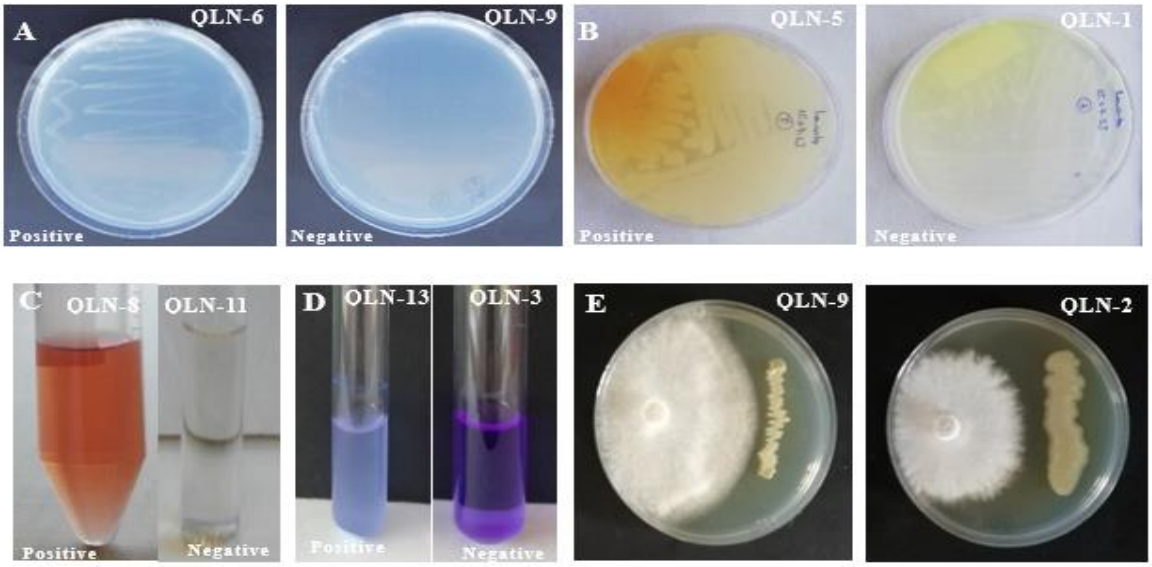

Figure 3. PGPR and antifungal activity test images of isolates (A: Nitrogen fixation B: HCN production C: IAA production D: Inorganic phosphate solubilization E: Antifungal test of isolates against *F. oxysporum*)

3.3. Antifungal activity

In the current study, it was determined that out of 16 isolates; 75 % have amylase; 56 % have protease; 62 % have pectinase; and 37 % have chitinase activity. *B. cereus* QLN-3, *B. subtilis* QLN-4, *P. fluorescens* QLN-8, and *B. pumilus* QLN-12 gave positive results for all extracellular enzyme tests. Plant growth-promoting rhizobacteria (PGPR) not only promote plant growth but also protect the plant against fungal pathogens by producing various extracellular enzymes such as chitinase, protease, and pectinase. Fungal cell walls contain chitin, glucan, cellulose, lipids, and proteins. Therefore, bacterial cellulases, lipases, and proteases play a crucial role in breaking down cell walls. These enzymes disrupt the structure of the fungal cell wall and eventually lead to the disintegration of the fungal cell. According to Dukare et al. (2020), extracellular enzymes stop the growth and development of the pathogen by preventing the germination of fungal spores and germ tube extension. Many studies show that bacteria of the genus *Bacillus* and *Pseudomonas* produce these enzymes and kill different pathogens (Nagrale et al., 2023). Shin et al. (2023) reported that enzymes such as protease, pectinase, and xylanase help bacteria colonize plant tissues, establish symbiotic relationships with host plants, and thus show antifungal activity by inhibiting the growth of pathogenic fungi. Fathalla Mohammed (2020) reported that *B. subtilis* SBMP4, which has plant growth-promoting properties, produced high amounts of chitinase. In the current study, *B. subtilis* QLN-4 produced chitinase. According to Rathore et al. (2020), lytic enzymes such as β-1,3 glucanase and chitinase produced by *P. fluorescens* are an antagonistic feature of this strain against *Fusarium* sp. Our findings support this study. In the present study, *P. fluorescens* QLN-8 produced chitinase. Table 3 presents the extracellular enzyme production of isolates.

Table 3. Extracellular enzyme production of isolates

Isolates	Extracellular enzymes			
	Amylase test	Protease test	Pectinase test	Chitinase test
Bacillus endophyticus QLN-1	+	+	-	+
Pseudomonas koreensis QLN-2	-	-	+	-
Bacillus cereus QLN-3	+	+	+	+
Bacillus subtilis QLN-4	+	+	+	+
Pseudomonas putida QLN-5	-	+	+	+
Pantoeae agglomerans QLN-6	+	-	+	-
Stenotrophomonas rhizophila QLN-7	-	+	-	-
Pseudomonas fluorescens QLN-8	+	+	+	+
Bacillus mycoides QLN-9	+	-	+	-
Enterococcus faecium QLN-10	-	+	-	-
Lactobacillus plantarum QLN-11	+	-	-	-
Bacillus pumilus QLN-12	+	+	+	+
Bacillus megaterium QLN-13	+	+	-	-
Lactobacillus paracasei QLN-14	+	-	-	-
Pseudomonas extremorientalis QLN-15	+	-	+	-
Bacillus mojavensis QLN-16	+	-	+	-

Note: * +, positive; –, negative

The cultivation of medicinal and aromatic plants has significantly increased in recent years because of the high global demand for plant-based medicines and aromatic compounds. Medicinal and aromatic plants are significantly impacted by root rot, wilt, anthracnose, and dieback resulting from fungal and bacterial infections. These plants often have problems with various fungal diseases like leaf spots, blight, rust, powdery mildew, root rot, and dieback. Lavender wilt is caused by the fungus *F. oxysporum* (Özer et al., 2021). There have been many reports worldwide of *Fusarium* sp. infections in *Lavender* sp. (Vasileva, 2015; Soylu and Incekara, 2017; Li et al., 2024). In current study, Antifungal activity of isolates obtained from *L. angustifolia* rhizosphere was tested against *F. oxysporum* and the inhibition percentages varied between 24.76 % and 57.14 %. Among the isolates, *B. simplex* QLN-4 isolate showed the strongest antagonism against the pathogen with a high percentage inhibition value (57.14 %), followed by *Pseudomonas koreensis* QLN-2 isolate (52.38 %). The *P. fluorescens* QLN-8 isolate (24.76 %) showed the weakest effect against the pathogen (Table 4).

Bacillus spp. is regarded as a successful bacteria capable of synthesizing a diverse range of useful compounds. The production of antifungal metabolites by PGPRs such as *Bacillus* is a well-documented biocontrol agent against phytopathogens (Majeed et al., 2018). Gajbhiye et al. (2010) reported that *B. subtilis* in the cotton rhizosphere inhibited mycelial growth by 50 % against *F. oxysporum*. Singh et al. (2017) determined that fifteen *B. subtilis* strains reduced *F. oxysporum* pathogen growth by varying rates ranging from 47 % to 85.5 %. In a recent study, Koumoutsi et al. (2004) reported that *B. amyloliquefaciens* FZB42 secretes fengycin and bacillomycin D, which have antagonist activity against *F. oxysporum*. Our findings are consistent with the other works. In the current study, *Bacillus subtilis* QLN-4 showed the maximum inhibition rate of 57.14 % (Table 4). According to Snook et al. (2009), *Bacillus mojavensis* promotes plant growth by colonizing the rhizosphere and destroys plant pathogens by stimulating the immune response. Moreover, *B. mojavensis* produces antifungal compounds such as fengicin and iturin. Diabankana et al. (2021) determined that *B. mojavensis* PS17, which can produce chitinase, protease, cellulase, and β-glucanase, inhibited the development of *F. oxysporum*. Galitskaya et al. (2022) reported that lipopeptides produced by *B. mojavensis* P1709 inhibited the growth of *F. oxysporum* by 93 %. In the current study, *Bacillus mojavensis* QLN-16 showed an inhibition rate of 30.23 % against *F. oxysporum.*

Numerous studies have shown that *Pseudomonas*, which are commonly found in

soil and rhizosphere, prevent the growth of plant diseases by secreting various compounds (Koche et al., 2012; Wang et al., 2020; Yue et al., 2023). Rafikova et al. (2016) determined that *P. koreensis* IB-4 showed high antagonist activity against *F. oxysporum.* Similarly, in the current study, *P. koreensis* QLN-2 showed an high inhibition rate of 52.38 % against *F. oxysporum*. Priya et al. (2019) reported that *Pseudomonas* sp. inhibited *F. oxysporum* mycelial growth with an inhibition rate of 57.77 %. Moreover, Ali et al. (2020) determined that *P. putida* MS6, which has protease activity in the sunflower rhizosphere, showed a 79 % antagonist effect against *F. oxysporum*. Similarly, in the current study, *P. putida* QLN-5 showed an inhibition rate of 36.54 % against *F. oxysporum*. Rathore et al. (2020) determined that *P. fluorescens* Pf-5 showed 82.51 % growth inhibition against *Fusarium* sp. On the other hand, In the current study, *P. fluorescens* QLN-8 showed a low inhibition rate of 24.76 % against *F. oxysporum*. The plant growth-promoting properties of *Stenotrophomonas* spp. as well as their antagonistic behavior against soil-borne plant pathogens are well documented (Ryan et al. 2009). Schmidt et al. (2012) reported that *S. rhizophila* DSM14405T significantly reduced disease severity against *Fusarium* spp. Aktas et al. (2023) determined that *S. maltophilia*, which can produce chitinase, inhibits mycelial development in *F. oxysporum*. In present study, *S. rhizophila* QLN-7 showed an high inhibition rate of 45.95 % against *F. oxysporum.*

Previous studies have shown that Enterococcus species not only have antifungal activities, but also increase shoot and root length, as well as fresh weight of plants like rice and melon by producing indole acetic acid and gibberellic acid (Lee et al., 2015). Mussa et al. (2018) determined that *E. casseliflavus* and *E. gallinarum* bacteria in rhizospheric soil samples inhibited *F. oxysporum* by 83 % and 78 %, respectively. Morales et al. (2020) reported that *E. mundtii* and *E. faecium* exhibited antifungal and antimicrobial activity by producing different compounds. Naik et al. (2023) reported that *E. faecium* MYSBC14 showed an inhibition percentage of 71.70 % against *Fusarium* sp. In present study, *E. faecium* QLN-10 showed a high inhibition rate of 45.23 % against *F. oxysporum*. Table 4 shows the percentage inhibition of the isolates against *F. oxysporum.*

Table 4. Antifungal activity test results of isolates against *F. oxysporum*

Isolates	**Colony diameter of *F. oxysporum* (mm) Mean ± SD**	**Inhibition percentage (%) of *F. oxysporum***
Bacillus endophyticus QLN-1	58±1.0^{c}	38.09
Pseudomonas koreensis QLN-2	46±1.0^{de}	52.38
Bacillus cereus QLN-3	62.7±2.33^{ab}	32.50
Bacillus subtilis QLN-4	42±1.0^{e}	57.14
Pseudomonas putida QLN-5	59.3±2.33^{bc}	36.54
Pantoeae agglomerans QLN-6	65.6±1.33^{a}	29.04
Stenotrophomonas rhizophila QLN-7	51.4±4.33^{d}	45.95
Pseudomonas fluorescens QLN-8	69.2±1.0^{a}	24.76
Bacillus mycoides QLN-9	49.5±1.0^{de}	48.21
Enterococcus faecium QLN-10	52.0±2.3^{d}	45.23
Lactobacillus plantarum QLN-11	67.2±1.33^{a}	27.14
Bacillus pumilus QLN-12	52.8±1.33^{dc}	44.28
Bacillus megaterium QLN-13	66.7±2.33^{a}	27.73
Lactobacillus paracasei QLN-14	65.3±1.33^{a}	29.40
Pseudomonas extremorientalis QLN-15	58.7±2.33^{bc}	37.26
Bacillus mojavensis QLN-16	64.6±1.33^{a}	30.23

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

4. Conclusion

To our knowledge, the current study is the first study in Turkey to isolate PGPR from *L. angustifolia* rhizosphere. Over the last two decades, multiple studies have indicated PGPR strains in many plant rhizospheres, aid plant growth and development. PGPR plays roles in producing phytohormones, increasing nutrient availability, and protecting the plant against many pathogens. Research is scarce on determining the ecology of PGPR. There is a need to screen strategies for selecting the best local rhizobacterial strains for use as environmentally friendly biofertilizers to prevent long-term use of fungicides that cause environmental and ecological problems. Rhizobacteria isolated from *L. angustifolia* exhibit significant plant growth-promoting properties and antifungal activities. These isolates can serve as effective microbial fertilizers, offering an environmentally friendly alternative to chemical fertilizers and contributing to sustainable agriculture. Therefore, further research on PGPR is necessary to help create more effective local rhizobacterial strains that can function in several agroecological environments.

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