RESEARCH ARTICLE

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Isolation and characterization of plant growth promoting rhizobacteria (PGPR) from rhizosphere of *Helianthus annuus* L.

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Abstract

Plant growth-promoting rhizobacteria (PGPR) support plant growth through direct and indirect mechanisms. To investigate PGPR strains that support plant growth, 21 bacterial isolates, mostly *Bacillus* ssp. and *Pseudomonas* ssp., were isolated from different rhizospheric soils of sunflowers in Kırşehir districts in 2020. All isolates were characterized morphologically, biochemically by screening under in vitro conditions for plant growth-promoting properties such as nitrogen fixation, IAA (indoleacetic acid) production, siderophore production, HCN (hydrogen cyanide) production, inorganic phosphate solubility. It was also screened for extracellular enzyme production and antifungal activity against *Fusarium oxysporum*. Among the 21 isolates, 3 isolates (MH-35-4, MH-49-4, MH-64-3) fixed nitrogen, 2 isolates (MH-59-6, MH-64-3), produced siderophores, 8 isolates (MH-35-4, MH-35-6, MH-54-3, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produced HCN, 6 isolates (MH-35-6, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produced IAA, and 7 isolates (MH-35-4, MH-35-6, MH-59-1, MH-59-2, MH-59-4, MH-59-8, MH-64-3) solubilized inorganic phosphate. Additionally, only 2 isolates (MH-54-3, MH-54-4) were positive amylase tests, 8 isolates (MH-35-6, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-6, MH-59-7, MH-59-8) were positive citrate tests, 8 isolates (MH-35-1, MH-35-4, MH-35-7, MH-49-4, MH-54-4, MH-59-6, MH-59-7, MH-64-3) were positive protease tests, and 6 isolates (MH-35-1, MH-35-3, MH-35-7, MH-54-3, MH-54-4, MH-59-7) were positive gelatin hydrolysis tests. Among 21 isolates, 38% were determined as hydrogen cyanide producers, 10% as siderophore producers, 29% IAA producers, 33% as phosphate solubilizers and 14% as nitrogen fixers. Isolate MH-35-6 showed the highest antifungal activity against *Fusarium oxysporum* with an inhibition rate of 53.57%. This was followed by isolates MH-54-1 (51.19%), MH-54-3 (47.61%) and MH-59-2 (38.09%), respectively. Therefore, our study reveals that bacteria that promote plant growth in sunflowers can be used to increase crop yield and as a biocontrol agent.

Keywords: *Helianthus annuus* L., Plant growth-promoting rhizobacteria (PGPR), *Fusarium oxysporum*, MALDI-TOF MS

INTRODUCTION

The need for food has gradually increased due to the growing global population, which has highlighted the significance of agricultural productivity and prompted the development of sustainable agricultural practices. The cornerstone of ecological agriculture and the growth of green production policies is the use of biofertilisers, which are fertilisers that do not damage the environment or the natural world, as opposed to chemical fertilisers used in traditional agriculture (Yadav, 2020).

Food security has become a major problem worldwide due to the increasing global population, decreasing arable land resources, and climate change. Therefore, limited arable land resources need to be used more efficiently to produce more food. It is known that chemical fertilizers contributed to the continuous increase in agricultural food production in previous years. Even though agricultural production has increased at the desired rate, it has led to the destruction of nature and the environment in terms of its results, increasing environmental pollution and causing the natural balance to deteriorate. The development and use of alternative products against the use of chemical fertilizers is of great importance for sustainable agriculture and environmental protection. Therefore, in recent years, research on the development of new methods that are beneficial to the environment and human health in agriculture has gained momentum. One of these studies is the use of environmentally friendly plant growth-promoting formulations (inoculants), which have a significant effect on increasing crop yield (Jiang et al. 2021).

The soil we live on is home not only to visible creatures but also to millions of microorganisms. The rhizosphere is a habitat in which the soil is rich in nutrients, intense biological and chemical activities occur, the plant root system is surrounded, and millions of microorganisms live (Tabassum et al. 2017). Lorenz Hiltner was the first to define the term "rhizosphere" in 1904 (Shrivastava et al. 2015). Plant roots synthesise, accumulate, and secrete various compounds in addition to providing mechanical support and facilitating water and nutrient uptake (Walker et al. 2011). These heterogeneous compounds produced by plant roots act as chemical attractants for actively used soil microbial communities. Chemicals called root exudates are substances that roots release into the soil. Microorganisms found in plant roots that have many benefits for the development and growth of the plant are called Plant Growth Promoting Rhizobacteria (PGPR). Although the term PGPR was first used by Kloepper et al. (1980) for fluorescent Pseudomonas, which are used for biocontrol purposes against pathogens and contribute to the growth of the plant, its current meaning was used by Kapulnik et al. (1981) for rhizobacteria that have the feature of promoting plant growth. Today, PGPR is expressed for all bacteria in the rhizosphere that ensure the growth and development of the plant through one or more mechanisms (Haghighi et al. 2011). Some of the most important known properties of PGPRs are: phosphate dissolving, producing IAA hormone and siderophore, and nitrogen fixation.

Numerous bacteria from the genera *Pseudomonas*, *Bacillus*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Rhizobium*, *Enterobacter*, and *Phyllobacterium* have been identified as PGPR. The most extensively documented PGPRs among these are *Pseudomonas*, *Azospirillum*, and *Bacillus*. They significantly increase the growth and yield of agronomic crops (Bashan et al. 2010). Sunflower (*Helianthus annuus* L.) is an important oilseed plant with high adaptability and is cultivated in large areas around the world (Mahapatra et al. 2021). A crucial raw material for the edible oil, chemical, cosmetic, paint, motor oil, biodiesel, hydraulic oil, soap, polish, and plastic industries is the sunflower. Sunflower, whose homeland is known as North America, has the largest cultivation area and production in Türkiye. Today, more than half of the vegetable oils produced in Türkiye are obtained from sunflowers. Sunflowers cultivation areas must be expanded, and the yield obtained per unit area must be increased to close the vegetable oil deficit in our country (Abdullah et al. 2023).

The soil physico-chemical structure, climatic conditions, and microbial population distribution of each region vary. Therefore, in order to prepare effective biofertilizer formulations, the biotic and abiotic conditions of the soil in that region must be well known. Particularly local bacterial species that are widespread in their own region are of great importance in the preparation of these formulations and the creation of local culture collections, and studies are concentrated on this subject. The isolation and identification of PGPR bacteria from the soil where sunflower cultivation is carried out in Kırşehir province and its districts and their PGP (Plant Growth Promoting) properties were investigated in this study. It was aimed at obtaining the microbial fertiliser inventory in the sunflower rhizosphere cultivated in our province in this context. This study will lead to the preparation of effective microbial fertilizer with local bacterial species prevalent in the region for future studies.

MATERIALS AND METHODS

Sample Collection and Isolation of Rhizobacteria

The soil samples collected in 2020 from the rhizosphere region of the sunflower (*Helianthus annuus* L.) plant in Kırşehir (Table 1) and its districts were thoroughly mixed and homogenised, and serial dilutions of 10^{-1}-10^{-6} were prepared from each of them to isolate the bacterial samples for use in the study (Figure 1).

In petri dishes with nutrient agar media, planting was done using the spreading technique. To obtain a pure culture, petri dishes were incubated for 2-4 days at 28±2°C. All the isolates obtained at the end of the incubation were transferred to a nutrient broth medium. Each purified colony was kept in a 20% glycerol solution. Bacterial isolates were deposited in the Culture Collection at the Microbiology Laboratory of Kırşehir Ahi Evran University in Türkiye.

Identification of Bacterial Strains

MALDI-TOF mass spectrometry was used to identify the resulting rhizospheric bacteria. The MALDI Biotyper CA System is a powerful tool for rapid and accurate identification of microorganisms and uses unique molecular fingerprints.

Morphological and Biochemical Characterization

The strains were identified using Bergey's Manual of Determinative Bacteriology and characterized by Gram stain, colony color, motility test, and biochemical tests including catalase activity, oxidase activity, and KOH (3%) (Krieg &Holt, 1984).

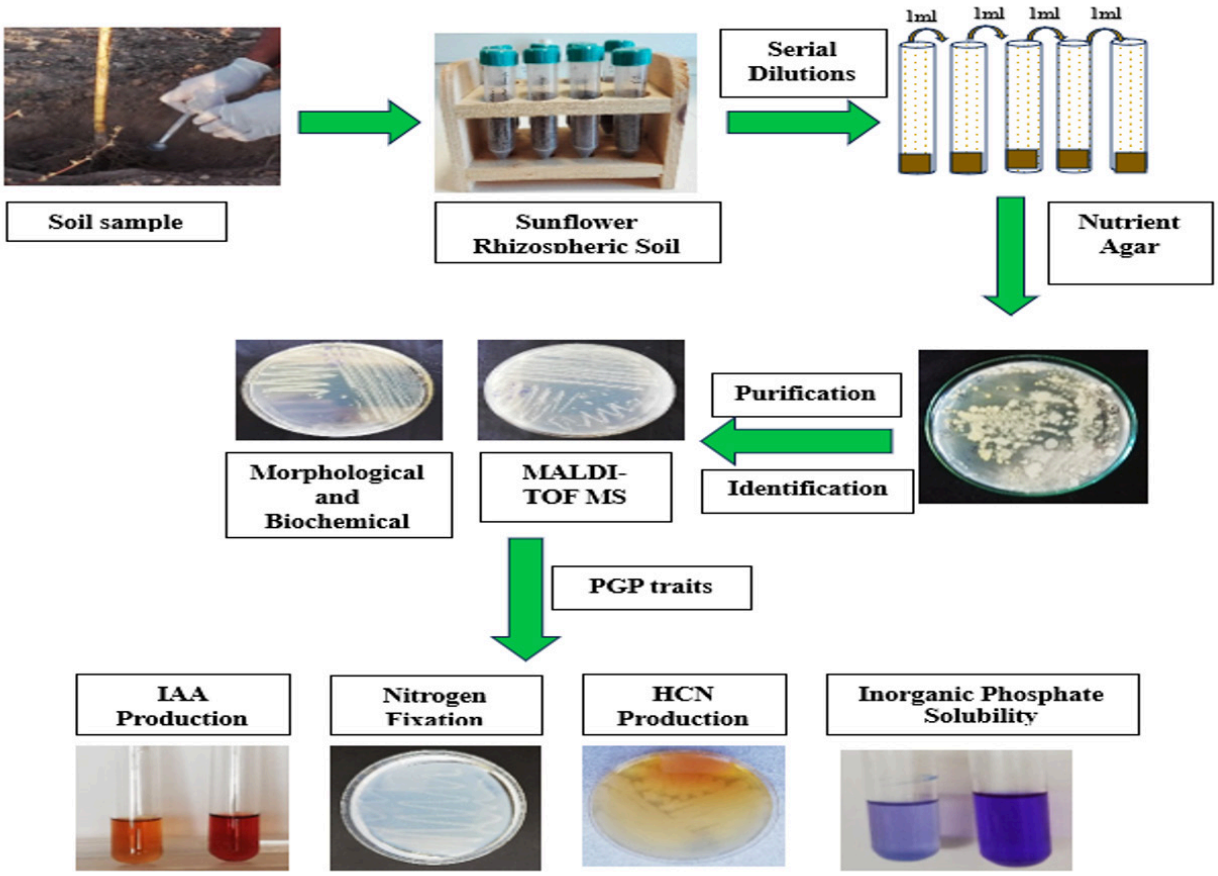

Figure 1. Flowchart Representation of the Process Used to Detect the Characteristics of Isolates From Sunflower Rhizospheric Soil

Assessment of Extracellular Enzyme Production

Screening for amylase production was done as per the methodology Smibert & Kreig (1994). Starch agar medium (yeast extract 5g, NaCl 10g, tryptone 10g, starch 5g, agar 15g, pure water 1000 ml) was prepared to determine amylase activity. After planting on starch agar medium, it was placed in an oven set at 30ºC and incubated for 1 day. At the end of the incubation, iodine solution (I_2 1g, KI 2g, 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 & Kreig (1994). Agar (skimmilk 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. For the citrate test, 10ml of slanted Simmans agar medium was prepared and the pH was adjusted to 6.9. Isolates were inoculated into 10ml tubes with a loop and incubated at 28˚C for 7 days. At the end of incubation, the color change from green to blue in the tube was evaluated as positive (Marakana et al. 2018). After preparing the required amount of gelatin agar medium, isolates taken from the 24-hour fresh culture were inoculated into 5ml tubes and then incubated at 28ºC for 7-14 days. After incubation, it was placed in the refrigerator at +4ºC. The tubes were kept in the refrigerator for 2-3 hours. Gelatin production was considered

positive if the tubes remained in liquid form when removed from the refrigerator (Nathan et al. 2011). Extracellular enzyme activity assays were conducted in triplicate.

Table 1. Locations, Altitudes, Longitude and Latitude of Sunflower Rhizosphere Samples

Isolates	Location	Altıtude	Latitude	Longitude
Bacillus pseudomycoides MH-35-1 *Bacillus megaterium* MH-35-3 *Chryseobacterium elymi* MH-35-4 *Pseudomonas koreensis* MH-35-6 *Bacillus weihenstephanensis* MH-35-7 *Bacillus simplex* MH-35-8, *Bacillus oligofermantans* MH-35-9 *Bacillus cereus* MH-49-2 *Pseudarthrobacter polychromogenes* MH-49-4 *Bacillus simplex* MH-49-8	Kaman/İsahocalı	1280	39°25'00.6"	33°53'58.8"
Bacillus megaterium MH-54-1 *Bacillus mojavensis* MH-54-3 *Stenotrophomonas sp* MH-54-4	Akçakent/Mahsenli	1268	39°34'10.6"	34°10'44.5"
Pseudomonas koreensis MH-59-1 *Pseudomonas koreensis* MH-59-2 *Pseudomonas koreensis* MH-59-4 *Acinetobacter calcoaceticus* MH-59-6 *Stenotrophomonas rhizophila* MH-59-7 *Pseudomonas koreensis* MH-59-8	Boztepe/Külhüyük	1150	39°20'15.1"	34°15'47.0"
Bacillus simplex MH-64-2 *Aromatoleum evansii* MH-64-3	Çiçekdağı/İbikli	1140	39°31'50.0"	34°20'36.0"

Evaluation of Plant Growth Promotion

Determination of Nitrogen Fixing Capacity

The isolates were tested using the nitrogen fixation protocol described by Wilson & Knight (1952). Firstly, the isolates were streaked on nutrient agar medium and incubated for 24h at 28±2ºC. Using the streaking method, each of the newly isolated cultures that had emerged from incubation was injected into petri dishes that contained solid Burk's N-free medium (Wilson & Knight, 1952; Park et al. 2005). They were incubated in this medium at 28±2°C for four days, and the plates were checked hourly and graded according to their development. Three-time intervals were determined for nitrogen fixation activity (+++: development after 6h, ++: development after 12h, +: development after 24h).

Evaluation of Siderophores-Producing Isolates

Schwyn & Neilands (1987) used Chrome Azurol S agar in this method to determine whether the isolates produced siderophores. The isolates were seeded onto the medium using the spot-seeding method and incubated at 28°C for 4 days. At the end of the incubation, the formation of yellow-orange colour around the bacteria was considered positive, and the formed zone diameters (mm) were measured (Öğütcü & Avsar, 2020). For siderophore activity, three-time intervals were determined (+++: color change after 1h, ++: color change after 6h, +: color change after 24h).

Assessment of Isolate Inorganic Phosphate Dissolving Capacity

The isolates' inorganic phosphate-dissolving capacities were determined using the protocol described by Mehta & Nautiyal (2001). Pure bacterial cultures grown on nutrient agar medium were inoculated into tubes containing 5 ml of NBRIP-BPB Medium (National Botanical Research Institute's Phosphate), and the control tubes were not inoculated. For three days, all tubes were incubated at 30±2°C and 180 rpm. Although there was no color change (blue-purple) in the control group tubes after incubation, color expansion was observed in some of the inoculated tubes.

HCN-Producing Isolates

The isolates' HCN production was determined using the method proposed by Bakker & Schippers (1987). Bacteria were inoculated on a nutrient agar medium containing 0.44% glycine, and filter papers (1.5 cm in diameter) impregnated

with picric acid (0.5% picric acid, 2% sodium carbonate) were placed on the Petri plate's edge without touching the medium.

The petri dish mouths were tightly sealed with paraffin and incubated at 28±2°C for 4 days. Picric acid-impregnated papers turned from yellow to brown at the end of the incubation period, which was considered a positive result (Temiz, 2010). For HCN activity, three-time intervals were determined (+++: color change after 6h, ++: color change after 12h, +: color change after 24h).

Identification of Isolates Producing Indole-3-Acetic Acid (IAA)

The isolates' IAA production abilities were determined using the protocol described by Sarwar & Kremer (1995). Pure bacterial cultures were grown for and 48h at 36±2°C. Fresh cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). The appearance of pink indicates the presence of IAA. For IAA activity, three time intervals were determined (+++: color change after 1h, ++: color change after 6h, +: color change after 24h).

Antifungal Activity

The fungal isolate (*Fusarium oxysporum*) used in the study was obtained from the culture collection unit of Ahi Evran 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 centre 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.

$$\% \text{ Inhibition} = (C - T) / (C - M) \times 100$$

C: Colony diameter of the pathogen in the control application

M: Diameter of micellar disc (6 mm)

T: Colony diameter of the pathogen in bacterial application

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). Analysis of variance (ANOVA) was used for antifungal activity measurements. The Tukey test was used to determine the differences between the mean levels of factors.

RESULTS AND DISCUSSIONS

Identification 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. Therefore, rhizobacteria contribute to soil fertility and sustainability. In this report, we looked at the bacterial ecology in the rhizosphere of sunflower. Soil samples were collected from 4 different locations (Kaman, Akçakent, Boztepe, *Çiçekdağı*) of Kırşehir city in T*ürkiye*. 21 isolates were obtained by serial dilution method. For bacterial identification, MALDI-TOF mass spectrometer was used. According to the MALDI-TOF MS results, 21 isolates belonging to 7 different genera were identified. Among these isolates, *Bacillus*, *Pseudomonas* and *Stenotrophomonas* were in the first three places (Table 2, Figure 2). Pramanik et al. (2018) used MALDI-TOF MS and FAME analysis to identify the bacteria they isolated from heavy metal-contaminated rice rhizospheres in India and tested them for plant growth-promoting properties. Similarly, Çevik & Ogutcu (2020) identified 51 isolates from non-agricultural soils using the MALDI TOF MS method. They determined that some bacteria (*Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., and *Paenibacillus* sp.) possessed plant growth-promoting properties.

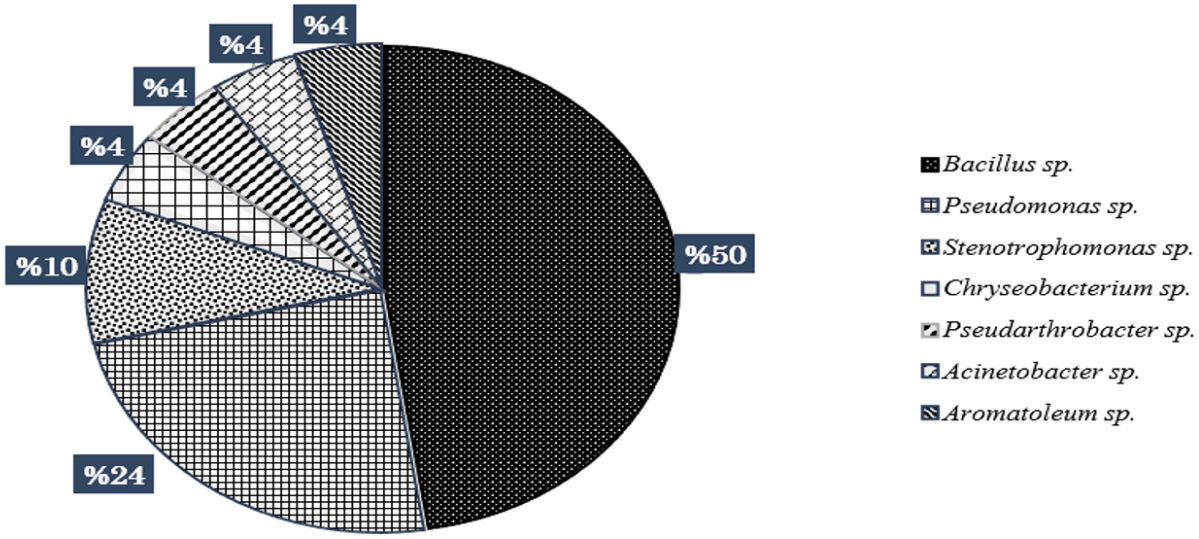

Figure 2. Percentages of Bacteria Isolated from Sunflower Rhizosphere

Biochemical and Morphological Characterization of Isolates

The biochemical tests such as catalase, and oxidase test were carried out for phenotypic identification of isolates (Krieg & Holt, 1984). It has been observed that bacterial isolates obtained from sunflowers have different biochemical and morphological characters. The majority of the isolated bacteria were Gram-positive, with a lower proportion being Gram-negative. In the current study, Among all isolates, 11 were Gram (+) and 10 were Gram (-); Except for two isolates (MH-49-4, MH-64-3), the others were catalase (+); Except for 7 isolates (MH-35-4, MH-35-6, MH-54-3, MH-59-1, MH-59-2, MH-59-4, MH-59-8), the others were oxidase (-). Details are shown in Table 2.

Production of Extracellular Enzymes

Among 21 isolates, all except for two isolates (*Bacillus mojavensis* MH-54-3, *Stenotrophomonas sp*. MH-54-4) were negative amylase test, 8 isolates were positive citrate and protease test, and 6 isolates (*Bacillus pseudomycoides* MH-35-1, *Bacillus megaterium* MH-35-3, *Bacillus weihenstephanensis* MH-35-7, *Bacillus mojavensis* MH-54-3, *Stenotrophomonas sp*. MH-54-4, *Stenotrophomonas rhizophila* MH-59-7) were positive gelatin hydrolysis test in the present study (Figure 3, Table 3). Biocontrol agents are antagonists that reduce disease intensity by acting against pathogens in plants. Antagonists show their effect against pathogens by producing hydrolytic enzymes such as protease, amylase and chitinase that damage the fungal cell wall. Similarly, PGPs in the rhizosphere produce hydrolytic enzymes such as proteases, cellulases, amylases, and so on to combat phytopathogens, which disrupt the pathogens' cell walls and cause cell death (Khalil et al. 2022). According to Petrović et al. (2024), enzymes such as protease, pectinase, and xylanase help bacteria colonise plant tissues and form symbiotic relationships with host plants. Bashir et al. (2021) reported that *Exiguobacterium auranticum*, *Paenibacillus* sp, and *Priestia koreensis* isolated from sunflower leaves produced amylase, protease, and chitinase. Fatima et al. (2022) reported that *Pseudomonas aeruginosa* IR-57, *Bacillus subtilis* IR-27, and *Serratia* sp. (IS-1) from chickpea rhizosphere produced protease, amylase, and HCN, and this resulted in an increase in the antifungal potential of the isolates. Among our isolates, some isolates that produced protease enzymes and were positive for HCN production (*Chryseobacterium elymi* MH-35-4, and *Stenotrophomonas sp*. MH-54-4) exhibited relatively high antifungal activity against the pathogen. Similarly, the protease-producing *Bacillus weihenstephanensis* MH-35-7 isolate exhibited antifungal activity (27.38%) in present study (Table 3). Our results for enzymatic activity are consistent with other researchers (Moustaine et al., 2017; Bashir et al., 2023).

Table 2. Morphological and Biochemical Characterization of Isolates

MALDI-TOF MS results	Morphological Characterization			Biochemical Characterization		
	Gram staining	Colony color	Motility test	KOH %3	Catalase activity	Oxidase activity
Bacillus pseudomycoides MH-35-1	+*	cream	-	-	+	-
Bacillus megaterium MH-35-3	+	white	+	-	+	-
Chryseobacterium elymi MH-35-4	-	pale yellow	-	+	+	+
Pseudomonas koreensis MH-35-6	-	white	+	-	+	+
Bacillus weihenstephanensis MH-35-7	+	white	+	-	+	-
Bacillus simplex MH-35-8,	+	cream	+	-	+	-
Bacillus oligofermantans MH-35-9	+	white	-	-	+	-
Bacillus cereus MH-49-2	+	whitish	+	-	+	-
Pseudarthrobacter polychromogenes MH-49-4	+	white	-	-	-	-
Bacillus simplex MH-49-8	+	cream	+	-	+	-
Bacillus megaterium MH-54-1	+	pale yellow	+	-	+	-
Bacillus mojavensis MH-54-3	+	white	+	-	+	+
Stenotrophomonas sp. MH-54-4	-	yellow	+	+	+	-
Pseudomonas koreensis MH-59-1	-	white	+	+	+	+
Pseudomonas koreensis MH-59-2	-	white	+	+	+	+
Pseudomonas koreensis MH-59-4	-	white	+	+	+	+
Acinetobacter calcoaceticus MH-59-6	-	white	-	+	+	-
Stenotrophomonas rhizophila MH-59-7	-	yellow	+	+	+	-
Pseudomonas koreensis MH-59-8	-	white	+	+	+	+
Bacillus simplex MH-64-2	+	cream	+	-	+	-
Aromatoleum evansii MH-64-3	-	white	+	+	-	-

Note: * +, positive; –, negative

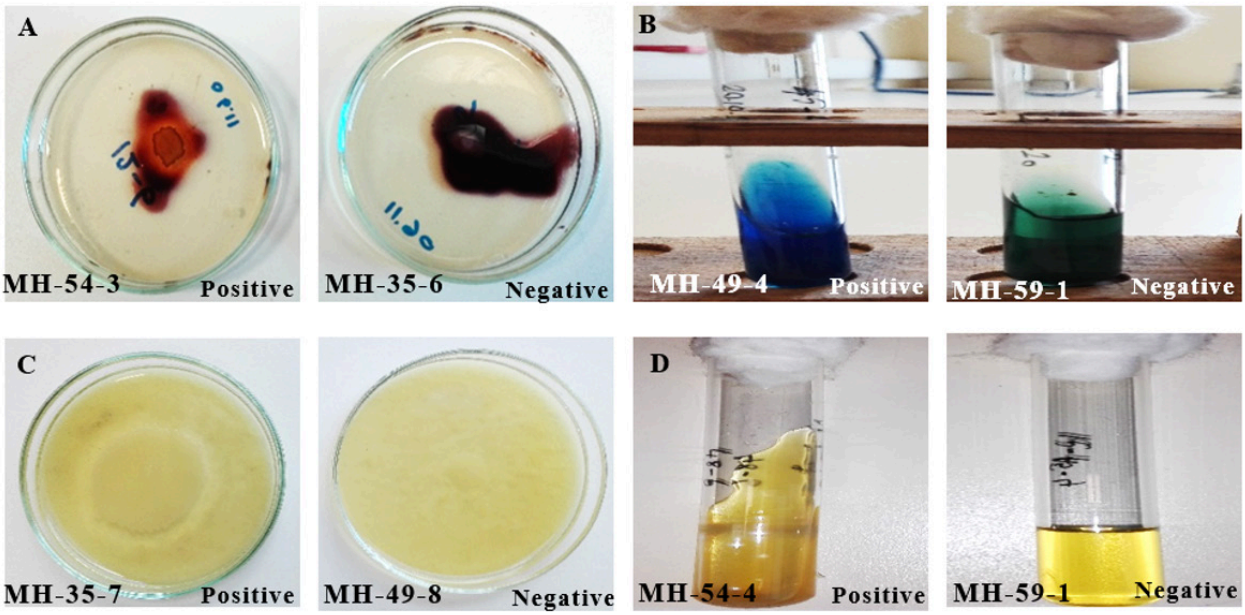

Figure 3. Extracellular Enzyme Production of Isolates (A. Amylase test B. Citrate test C. Protease test D. Gelatin hydrolysis)

Table 3. Extracellular Enzyme Production Results of Isolates

Isolates	Extracellular Enzymes			
	Amylase Test	Citrate Test	Protease Test	Gelatin Hydrolysis
Bacillus pseudomycoides MH-35-1	-	-	+	+
Bacillus megaterium MH-35-3	-	-	-	+
Chryseobacterium elymi MH-35-4	-	-	+	-
Pseudomonas koreensis MH-35-6	-	+	-	-
Bacillus weihenstephanensis MH-35-7	-	-	+	+
Bacillus simplex MH-35-8,	-	-	-	-
Bacillus oligofermantans MH-35-9	-	-	-	-
Bacillus cereus MH-49-2	-	-	-	-
Pseudarthrobacter polychromogenes MH-49-4	-	-	+	-
Bacillus simplex MH-49-8	-	-	-	-
Bacillus megaterium MH-54-1	-	-	-	-
Bacillus mojavensis MH-54-3	+	-	-	+
Stenotrophomonas sp MH-54-4	+	+	+	+
Pseudomonas koreensis MH-59-1	-	+	-	-
Pseudomonas koreensis MH-59-2	-	+	-	-
Pseudomonas koreensis MH-59-4	-	+	-	-
Acinetobacter calcoaceticus MH-59-6	-	+	+	-
Stenotrophomonas rhizophila MH-59-7	-	+	+	+
Pseudomonas koreensis MH-59-8	-	+	-	-
Bacillus simplex MH-64-2	-	-	-	-
Aromatoleum evansii MH-64-3	-	-	+	-

Note: * +, positive; –, negative

PGP Attributes of Isolates

Of the 21 isolates, only 7 (MH-35-4, MH-35-6, MH-59-1, MH-59-2, MH-59-4, MH-59-8, MH-64-3) were found to dissolve inorganic phosphate (Figure 4). This showed that it had a share of 33% among all isolates. In addition, it was defined that 3 (MH-35-4, MH-49-4, MH-64-3) of 21 isolates do nitrogen fixation (14%), 2 (MH-59-6, MH-64-3) produce siderophores (10%), 8 (MH-35-4, MH-35-6, MH-54-3, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produce HCN (38%) and 6 (MH-35-6, MH-54-4, MH-59-1, MH-59-2, MH-59-4, MH-59-8) produce IAA (29%) (Table 4).

PGPR promotes plant growth via a variety of mechanisms, including IAA production, nitrogen fixation, siderophore production, and phosphate solubilization. According to Beattie (2006), approximately 30% of the bacteria isolated from the sunflower rhizosphere consist of the genera *Stenotrophomonas*, *Agrobacterium*, *Pseudomonas* and *Rhizobium*. Recent studies are showing that *Stenotrophomonas* sp., a member of the PGPR family, supports plant growth (Ghosh et al. 2020; Singh et al. 2020; Mushtaq et al. 2021). In the current study, it was determined that *Stenotrophomonas* sp. MH-54-4 isolate produced HCN and IAA. *Stenotrophomonas* strains have been found as endophytes in rice (Sun et al. 2008), sugarcane (Morgado et al. 2015), wheat (Majeed et al. 2015) and maize (Ercole et al. 2023). Pseudomonas are among the foremost rhizospheric bacteria because they colonise aggressively and use a variety of carbon sources (Dorjey et al. 2017). Pandey et al. (2013) reported that *Pseudomonas* sp. isolate RP1 showed plant growth-promoting including phosphate solubilization, IAA production, and HCN production.

An essential macronutrient, phosphorus is required for many important plant metabolic functions, including signal transduction, membrane integrity, synthesis of energy, cell division, and photosynthesis. The bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium*, and *Erwinia* are known to be capable of phosphorus solubilization. According to reports, *Rhizobium*, *Bacillus*, and *Pseudomonas* sp. have the highest P solubilization activity (Ahmad et al. 2008; Chaiharn & Lumyong, 2011). Beneduzi et al. (2008) studied the phosphate solubility of bacteria isolated from wheat rhizosphere and found only 9 phosphate-solubilizing bacteria. Hameeda et al. (2008) isolated 207 bacteria from the maize rhizosphere and found that only 5 dissolved phosphate. Ambrosini et al. (2012) determined that among 299 isolates from the sunflower rhizosphere, 59 were phosphate-soluble (including *Burkholderia* sp. and *Achromobacter* sp.), and especially *Azospirillum* sp.Vi 22 isolate had a high rate of nitrogen fixation.

According to reports, rhizosphere soil typically contains higher concentrations of phosphate-solubilizing bacteria than non-rhizospheric soil (Verma & Shahi, 2015; Rawat et al. 2021). The eports available on *Pseudomonas* sp. isolated from different sources showed phosphate solubilization (Rosas et al. 2006; Khan et al. 2014; Paul & Sinha, 2017). Phosphate-solubilizing bacteria play a crucial role in the growth of plants by converting insoluble phosphorus into soluble phosphates that plants can use. Therefore, the use of plant growth-promoting phosphate-solubilizing bacteria in agriculture not only preserves soil fertility but also reduces costs. Liang et al. (2023) determined that among 31 isolates obtained from the rhizosphere of *Festuca arundinacea*, *Acinetobacter calcoaceticus*, *Buttiauxella* sp, and *Erwinia pyriflorinigrans* solubilized inorganic phosphate between 203.96 and 412.22 µg/mL. Soares et al. (2023), who investigated the phosphate solubilization abilities of isolates in different pH environments, determined that *P. aeruginosa* UFT01 and *B. cereus* UFT42 dissolved phosphate in all pH ranges. In present study, *Pseudomonas koreensis* MH-59-1, *Pseudomonas koreensis* MH-59-2, *Pseudomonas koreensis* MH-59-4 showed phosphate solubilization (Figure 4E).

IAA (indole-3-acetic acid), produced by bacteria, takes part in physiological events such as increasing root development, cell elongation, and cell division differentiation in plants. (Glickmann & Dessaux, 1995). IAA is the most common and best-characterized phytohormone. It is estimated that 80% of bacteria isolated from the rhizosphere can produce IAA (Sokolova et al. 2011). Several PGPR genera, including *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pantoea*, *Pseudomonas*, and *Serratia*, have been found to produce IAA. Sivasakthivelan and Stella (2012) reported that the *Azospirillum lipoferum* SA-17 strain isolated from the sunflower rhizosphere produced IAA in high amounts (89.9 µg 25 ml^{-1}), and that *Pseudomonas fluorescens* SP-10 and *Bacillus megaterium* SB-17 strains fixed nitrogen. Raval and Desai (2012) measured the IAA production of bacteria isolated from sunflower rhizosphere on a spectrophotometer (535 nm) and determined that the highest IAA production was in *Pseudomonas* M6S3 and *Bacillus* M7S3 isolates. Similarly, Pandey et al. (2013) determined that some of the bacteria they isolated from the sunflower rhizosphere produced IAA. They also determined spectrophotometrically that the highest IAA producer belonged to the *Pseudomonas stutzeri* (78 µg/ml). Adeleke et al. (2022) reported that 20 of 50 endophytic bacteria obtained from sunflower had plant growth-promoting properties among them, *S. maltophilia* JVB5 (23.36 µg/ml), *B. cereus* T4S (20.72 µg/ml) and *S. indicatrix* Bovıs40 (46.43 µg/ml) isolates produced IAA.

The findings of this study revealed that *Pseudomonas* (24%) and *Bacillus* (50%) were the most common bacterial genera in sunflower rhizospheres. Further, all five isolates identified as *Pseudomonas koreensis* (MH-35-6, MH-59-1, MH-59-2, MH-59-4, MH-59-8) were positive for IAA production and solubilizing inorganic phosphate when evaluated for PGP properties (Figure 4A, Figure 4E). *Bacillus* species, which are the most common in soil, have a high potential to become microbial fertilizers, especially due to their ability to form spores, being easy to grow and store, and having protective properties against plant pathogens (Forchetti et al. 2007). *Bacillus* species used as biofertilizers promote plant growth by synthesizing plant growth hormones, fixing nitrogen, solubilizing phosphates and producing siderophores (Borriss, 2011; Riaz et al. 2021; Mushtaq et al. 2021). PGP features were not determined in *Bacillus* isolates, except for isolate *Bacillus mojavensis* MH-54-3 in current study (Table 4).

Nitrogen, which is necessary for all living things, participates in the structure of many substances such as amino acids, vitamins and nucleotides. Although nitrogen is 78% concentrated in the atmosphere, it cannot be used directly by plants. The conversion of atmospheric nitrogen into ammonium is known as the process of biological nitrogen fixation or diazotrophy. Biological nitrogen fixation, which occurs thanks to the nitrogenase enzyme complex, occurs in two ways: symbiotic and nonsymbiotic (Deka et al. 2015). The genera *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mesorhizobium*, and *Frankia* contain symbiotic nitrogen-fixing bacteria (Hayat et al. 2012). Non-symbiotic bacterial genera are; *Azotobacter*, *Azospirillum*, *Clostridium*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Pseudomonas* are bacteria. Goes et al. (2012) showed that 3 *Bacillus* species among 57 bacteria obtained from different sunflower tissues (roots, stems, florets, and rhizosphere) fixed nitrogen. Chai et al. (2023) determined that *Acinetobacter calcoaceticus* HMAJ1, *Pseudomonas piscium* HMAJ5, and *Pseudarthrobacter psychrotolerans* NCZ1 have the nitrogenase activity necessary for nitrogen fixation. In the current study, isolates of *Chryseobacterium elymi* MH-35-4, *Pseudarthrobacter polychromogenes* MH-49-4 and *Aromatoleum evansii* MH-64-3 fixed nitrogen (Figure 4B). In previous studies, the genus *Chryseobacterium* and *Pseudarthrobacter* isolated from different plant rhizospheres was reported to fix nitrogen (Lucas Garcia et al. 2004; Nishioka et al. 2016; Dhole et al. 2017; Gopalakrishnan et al. 2017; Bushra et al. 2023). Moreover, *Chryseobacterium* can produce siderophore, protease, cellulase, amylase, xylanase, and exhibit antifungal properties (Pathma & Sakthivel, 2013).

One of the many strategies for preventing pathogenic microorganisms from growing in the rhizosphere is the production of HCN (Pandey et al. 2013). It inhibits electron transport chains and energy sources in cells, leading to pathogen death. Studies have shown that bacterial species like *Bacillus* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., and *Alcaligenes* sp. may prevent the pathogen attach in plants by stimulating the HCN production mechanism (Miljaković et al. 2020; Hamid et al. 2021; Ferioun et al. 2023). HCN production by *Bacillus* sp. and *Pseudomonas*

aeruginosa has been previously reported (Kumar et al. 2012; Sebastian et al. 2021; Devi et al. 2023). Manasa et al. (2017) reported that the *Rhizobium* RR-1 strain obtained from sunflower rhizosphere produced HCN. Singh et al. (2019) determined that *Bacillus thuringiensis* SF 23, *Pseudomonas aeruginosa* SF 44, *B. subtilis* SF 48, and *Bacillus subtilis* SF 90 isolate produced HCN. Similarly, Bashir et al. (2021) determined that *Exiguobacterium auranticum* SV10 and *Priestia koreensis* LV19 isolates, which are endophyte bacteria obtained from sunflower leaves, produced HCN. Isolates *Chryseobacterium elymi* MH-35-4, *Pseudomonas koreensis* MH-35-6, *Bacillus mojavensis* MH-54-3, *Stenotrophomonas sp.* MH-54-4, *Pseudomonas koreensis* MH-59-1, *Pseudomonas koreensis* MH-59-2, *Pseudomonas koreensis* MH-59-4 and *Pseudomonas koreensis* MH-59-8 produced HCN in present study (Figure 4C).

The amount of iron available in the rhizosphere for microbial assimilation is very limited. In this case, organisms secrete iron-binding ligands known as siderophores, which bind to the ferric ion and make it available to the host organisms so that they can survive (Gupta & Gopal, 2008). Rhizospheric bacteria produce siderophores, which enhance rhizosphere colonisation and are crucial for iron mineralization and plant supplementation. Fluorescent pseudomonads are known to produce siderophores such as pyoverdines. Khare et al. (2011) reported that *P. aeruginosa* could produce siderophores and pyoverdines at different NaCl concentrations (0-500 mM).

Ambrosini et al. (2012) reported that the bacteria they obtained from the sunflower rhizosphere mostly belonged to the *Enterobacter*, *Burkholderia* and *Klebsiella* genera, and 27 of them produced siderophores. Similarly, Sivasakthivelan and Stella (2012) reported that high amounts of siderophores were produced by strains of *Pseudomonas fluorescens* (8.26 µg ml^{-1}) and *Bacillus megaterium* (7.80 µg ml^{-1}) among the various isolates obtained from the sunflower rhizosphere. Further studies revealed that bacterial strains are siderophore producers (Zou et al. 2020; Fiodor et al. 2023). Koçak and Boyraz (2024) reported that among 5 isolates obtained from sunflower rhizosphere (*Bacillus cereus*, *Bacillus simplex*, *Brevibacterium frigoritolerans*, *Bacillus toyonensis*, *Bacillus toyonensis*), the most effective siderophore producer was *Brevibacterium frigoritolerans*. Huang et al. (2023) reported that *Acinetobacter calcoaceticus* DP25 and *Acinetobacter calcoaceticus* DP27, obtained from the rhizosphere of *Lespedeza davurica*, produced 53.13% and 86.67% of siderophores, respectively. Interestingly, In the current study, 2 isolates (*Acinetobacter calcoaceticus* MH-59-6 and *Aromatoleum evansii* MH-64-3) produced siderophores (Figure 4D).

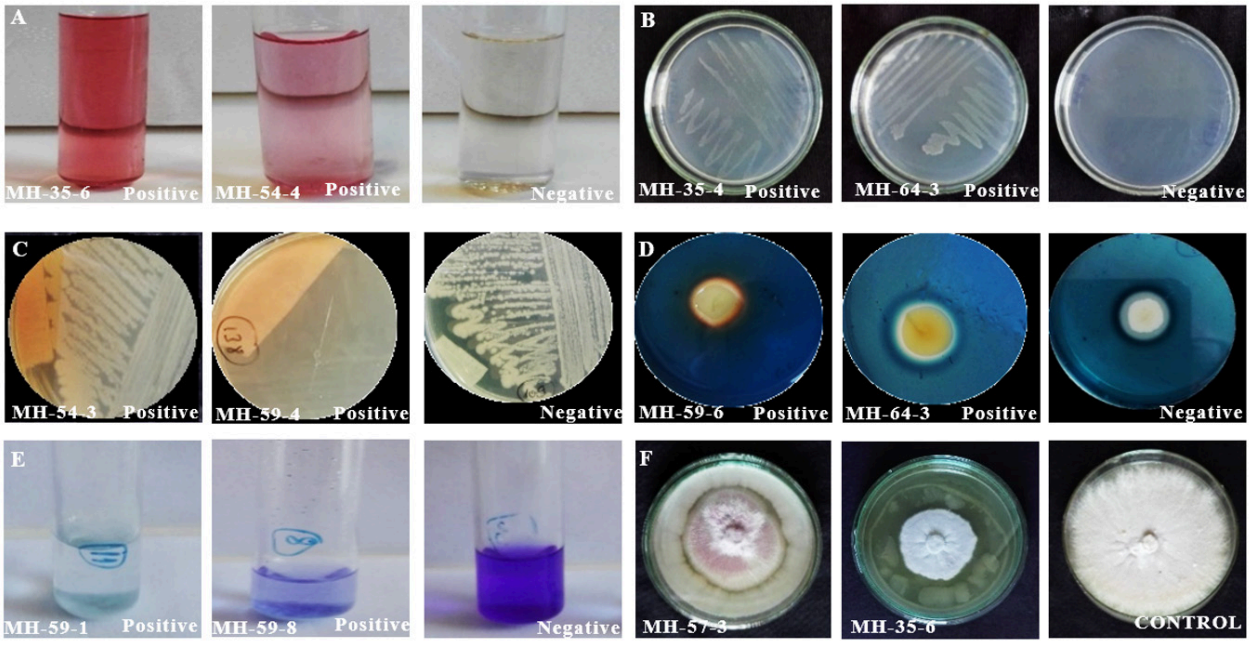

Figure 4. PGPR Test Results of Isolates (A. IAA production B. Nitrogen fixation; C. HCN production D. Siderophore production E. Inorganic phosphate solubization) and antifungal activity against *Fusarium oxysporum* (F)

Antifungal Activity

Sunflower wilt is caused by *Fusarium oxysporum*, the most frequent soil-borne pathogen. *F. oxysporum* invades the host's root system and blocks the water-conducting tissues, causing root rot and eventual plant death. Synthetic fungicides used against *Fusarium* sp. are both ineffective and harmful to environmental health (Gulya, 2016; Bashir et al. 2021). Therefore, the use of biofungicides as an alternative to chemical pesticides is gaining importance day by day. Forchetti et al. (2007) determined that endophyte bacteria (*Bacillus pumilus*, *Achromobacter xiloxidans*) in sunflowers grown under drought conditions showed antifungal activity at different rates against pathogenic fungi (*Fusarium* sp., *Sclerotinia sclerotiorum*, *Alternaria* sp.).

In this study, Antifungal activity of isolates obtained from sunflower rhizosphere was tested against *F. oxysporum* using PDA (Potato dextrose agar) medium and the inhibition percentages varied between 16.66% and 53.57%. Among the isolates, *Pseudomonas koreensis* MH-35-6 isolate showed the strongest antagonism against the pathogen with a high percentage inhibition value (53.57%), followed by *Bacillus megaterium* MH-54-1 isolate (51.19%). The *Stenotrophomonas rhizophila* MH-59-7 isolate (15.47%) showed the weakest effect against the pathogen (Table 4, Figure 5). Previous studies have shown that PGPRs improve plant growth and fungal diseases in tomatoes, wheat and sunflowers (Shittu et al. 2009; Moussa et al. 2013; Waqas et al. 2015).

Bacillus spp. is considered an effective microorganism with remarkable abilities for synthesising a wide range of beneficial substances. Production of antifungal metabolites by PGPR such as *Bacillus* is a well-documented biocontrol agent against phytopathogens (Majeed et al. 2018). Recently, several Bacillus species have been accepted as biocontrol agents against phytopathogens because of their ability to produce biosurfactant lipopeptides with antimicrobial activity. According to Koumoutsi et al. (2004), *B. amyloliquefaciens* FZB42 secretes fengycin and bacillomycin D, which have antagonistic activity against *Fusarium oxysporum*. Shobha and Kumudini, (2012) reported that seven Bacillus isolates revealed significant inhibitory effects on mycelial radial growth against *F. oxysporum* in vitro. Singh et al. (2017) determined that fifteen *B. subtilis* strains reduced *F. oxysporum* pathogen growth by varying rates ranging from 47% to 85.5%. Singh et al. (2019) determined the inhibition rates of *Bacillus* strains (*Bacillus thuringiensis* Rhizo SF23 and *Bacillus subtilis* Rhizo SF48) isolated from the sunflower rhizosphere against *Fusarium* sp. as 43.54% and 47.85%, respectively. Similarly, Mishra et al. (2023) determined that *Pseudomonas guariconensis* IIPRMKCP-9, *Bacillus amyloliquefaciens* IIPRAJCP-6, *Bacillus haynesii* IIPRMKCP-10, *Bacillus cereus* IIPRAMCP-5, *Bacillus subtilis* IIPRSHEP-6, and *Seratia macrescens* IIPRMKCP-3 isolates inhibited *F. oxysporum* mycelial growth by more than 80%. Bacillus isolates revealed varying degrees of antifungal activity against the fungal pathogen *F. oxysporum* in the present study. Among the Bacillus ssp., *Bacillus megaterium* MH-54-1 showed the maximum inhibition rate of 51.19%, followed by *Bacillus mojavensis* MH-54-3 (47.61%) and *Bacillus pseudomycoides* MH-35-1 (35.71%) in current study (Table 4). Our findings are consistent with the other works.

PGPR's antagonistic nature against plant pathogens is associated with the production of secondary metabolites that impede pathogen growth and progression. *Pseudomonas* spp. produces a number of antifungal compounds: chitinases, glucanases, proteases, siderophores, butylbenzenesulfonamide, hydroxymethyl, hydroxyphenyl, which prevent various pathogen diseases. Parveen et al. (2020) determined that *Pseudomonas aeruginosa* PGPR-11 showed antifungal activity against sunflower root pathogenic fungi (*Rhizoctonia solani*, *F. solani*, *Macrophomina phaseolina* and *F. oxysporum*). Bashir et al. (2021) examined the antifungal activities of endophytic bacteria (*Exiguobacterium auranticum* SV7, *Paenibacillus* sp. SV10 and *Priestia koreensis* LV19) obtained from sunflower against *Fusarium* sp. They determined that among the three endophytic bacteria, the most effective isolate against *Fusarium* sp. belonged to the *Priestia koreensis* LV19 isolate with an inhibition rate of 53%, and the least effective isolate belonged to the *Paenibacillus* sp. SV10 isolate with an inhibition rate of 19.2%. Thakker et al. (2023) reported that *Pseudomonas aeruginosa* OG101 inhibited *F. oxysporum* mycelial growth by 24.4%. Similarly, Chaurasiya et al. (2023) reported that *Pseudomonas* sp. PGP 18 isolate inhibited the *F. oxysporium* that causes lentil wilt disease by 67.41%. Likewise, Among the Pseudomonas ssp., *Pseudomonas koreensis* MH-35-6 isolate showed the highest antifungal activity (53.57 %), followed by *Pseudomonas koreensis* MH-59-2 (39.09%) and *Pseudomonas koreensis* MH-59-8 (36.90%) in present study (Table 4, Figure 4F). Our result coincides with Majeed et al. (2018) who showed *Pseudomonas* sp. AF-54 antifungal activity against *F. oxysporum* in Arabidopsis.

Table 4. Plant Growth Promoting Test Results and Antifungal Activity Values Against *Fusarium oxysporum* of the Isolates

Isolates	PGP Traits					Antifungal Activity	
	Inorganic phosphate solubility	Nitrogen fixation	Siderophores production	HCN production	IAA production	Colony diameter of F. oxysporum (mm) Mean ± SD	Inhibition percentage (%)
Bacillus pseudomycoides MH-35-1	-	-	-	-	-	60±1.52 ^{ij}	35.71
Bacillus megaterium MH-35-3	-	-	-	-	-	68±0.57 ^{fgh}	26.19
Chryseobacterium elymi MH-35-4	+	++*	-	+***	-	65±1.52 ^{h}	29.76
Pseudomonas koreensis MH-35-6	+	-	-	+	+++	45±1.15 ^{l}	53.57
Bacillus weihenstephanensis MH-35-7	-	-	-	-	-	67±0.57 ^{gh}	27.38
Bacillus simplex MH-35-8	-	-	-	-	-	70±1.52 ^{cde}	23.80
Bacillus oligofermantans MH-35-9	-	-	-	-	-	74±1.52 ^{bcd}	19.04
Bacillus cereus MH-49-2	-	-	-	-	-	75±1.0 ^{ab}	17.85
Pseudarthrobacter polychromogenes MH-49-4	-	+	-	-	-	68±0.57 ^{gh}	26.19
Bacillus simplex MH-49-8	-	-	-	-	-	76±0.57 ^{ab}	16.66
Bacillus megaterium MH-54-1	-	-	-	-	-	47±1.52 ^{kl}	51.19
Bacillus mojavensis MH-54-3	-	-	-	+	-	50±0.33 ^{k}	47.61
Stenotrophomonas sp. MH-54-4	-	-	-	+	+	70±1.33 ^{efg}	23.80
Pseudomonas koreensis MH-59-1	+	-	-	+	+++	60±1.0 ^{ij}	35.71
Pseudomonas koreensis MH-59-2	+	-	-	+	+++	58±0.33 ^{j}	38.09
Pseudomonas koreensis MH-59-4	+	-	-	+	+++	63±1.52 ^{i}	32.14
Acinetobacter calcoaceticus MH-59-6	-	-	+	-	-	75±0.57 ^{abc}	17.85
Stenotrophomonas rhizophila MH-59-7	-	-	-	-	-	77±1.0 ^{a}	15.47
Pseudomonas koreensis MH-59-8	+	-	-	+	+++	59±0.57 ^{ij}	36.90
Bacillus simplex MH-64-2	-	-	-	-	-	71±0.33 ^{def}	22.61
Aromatoleum evansii MH-64-3	+	++	+	-	-	76±1.0 ^{a}	16.66

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

For antifungal activity: 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)

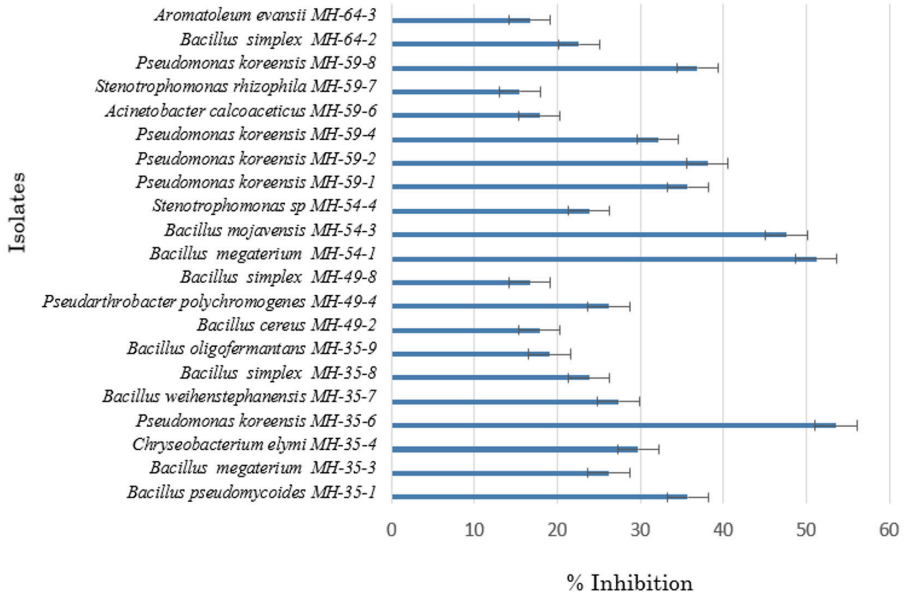

Figure 5. Percent Inhibition Rates of Isolates Against *Fusarium oxysporum*

CONCLUSION

This study reported the isolation, characterization and identification of PGPR from the rhizosphere of sunflowers grown in Kırşehir province of Türkiye. Among the PGPB properties of the obtained isolates, biological nitrogen fixation, phosphorus solubilization, siderophores production, HCN production, IAA production and antifungal activity against the fungal pathogen *Fusarium oxysporum* are the most interesting features. Numerous studies conducted in the past few decades have documented the advantages of using *Bacillus* species as biocontrol and biofertilizers, such as *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus cereus* and *Bacillus pumilus*. The predominance of bacillus species among our isolates can be considered as an advantage in terms of developing inoculant-based formulations from bacillus species. *Bacillus* ssp. and *Pseudomonas* ssp. in sunflower acts as an antipathogen because it inhibits the growth of specific pathogens. In future, PGPR is expected to eventually replace artificial growth regulators, chemical fertilizers, and pesticides, which have many harmful effects on the environment and human health.

The isolates obtained in this study are suitable candidates for the development of biotechnological tools aimed at increasing the yield of sunflower plants in environmentally friendly ways and contributing to the protection against the *Fusarium oxysporum* that causes disease in this plant. Moreover, Further study, including efficiency tests in greenhouses and fields, is needed to determine the role of PGPR-based microbial fertilizers as biofertilizers.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

Abdullah, Çil., Çil, A.N., Şahin, V., Yaşar, Y. (2023). Sunflower (*Helianthus annuus* L.) advanced breeding materials in field trials in different geographical regions of Turkey. MAS Journal of Applied Sciences, 8(2), 384-390. https://doi.org/10.5281/zenodo.8051748

Adeleke, B. S., Ayangbenro, A. S., Babalola, O.O. (2022). In vitro screening of sunflower associated endophytic bacteria with plant growth-promoting traits. Frontiers in Sustainable Food Systems, 6, 903114. https://doi.org/10.3389/fsufs.2022.903114

Ahmad, F., Ahmad, I., Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research, 163 (2), 173-181. https://doi.org/10.1016/j.micres.2006.04.001

Ambrosini, A., Beneduzi, A., Stefanski, T., Pinheiro, F. G., Vargas, L.K., Passaglia, L.M. (2012). Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). Plant and Soil, 356, 245-264. https://doi.org/10.1007/s11104-011-1079-1

Bakker, A.W. & Schippers, BOB. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. Soil Biology and Biochemistry ,19(4), 451-457. https://doi.org/10.1016/0038-0717(87)90037-x

Bashan, Y. & De-Bashan, L.E. (2010), How the plant growth-promoting bacterium *Azospirillum* promotes plant growth-a critical assessment. Advances in Agronomy, 108, 77-136. https://doi.org/10.1016/s0065-2113(10)08002-8

Bashir, S., Iqbal, A., Hasnain, S., White, J.F. (2021). Screening of sunflower associated bacteria as biocontrol agents for plant growth promotion. *Archives of Microbiology*, 203, 4901-4912. https://doi.org/10.1007/s00203-021-02463-8

Bashir, T., Iqbal, M., Javed, S., Riaz, J., Bukhari, N. T., Nisa, I., Khan, N. (2023). Isolation and characterization of plant growth promoting bacteria from rhızosphere of Okra. Journal of Population Therapeutics and Clinical Pharmacology, 30(18), 1257-1268. https://doi.org/10.53555/jptcp.v30i18.3264

Beattie, G.A. (2006) Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances.

In: Gnanamanickam SS (ed) Plant-associated bacteria. Journal of Applied Sciences, (6), 1–56. https://doi.org/10.1007/1-4020-4538-7_1

Beneduzi, A., Peres, D., Vargas, L.K., Bodanese-Zanettini, M.H., Passaglia, L.M.P. (2008). Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. Applied Soil Ecology, 39:311–320. https://doi.org/10.1016/j.apsoil.2008.01.006

Borriss, R. (2011). Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents in agriculture. Bacteria in Agrobiology*:* Plant Growth Responses, 41-76. https://doi.org/10.1007/978-3-642-20332-9_3

Bushra, R., Uzair, B., Ali, A., Manzoor, S., Abbas, S., Ahmed, I. (2023). Draft genome sequence of a halotolerant plant growth-promoting bacterium *Pseudarthrobacter oxydans* NCCP-2145 isolated from rhizospheric soil of mangrove plant *Avicennia marina*. Electronic Journal of Biotechnology, 66, 52-59. https://doi.org/10.1016/j.ejbt.2023.08.003

Chai, J., Wang, X., Liu, X., Li, C., Han, J., Yao, T. (2023). Inoculation of cold-adapted microbial consortium screened from alpine meadows promotes the growth of mixed grasses by changing soil properties and enzyme activity. Rhizosphere, 28, 100782. https://doi.org/10.1016/j.rhisph.2023.100782

Chaiharn, M. & Lumyong, S. (2011). Screening and optimization of Indole-3-Acetic production and phosphate solubilization from rhizobacteria aimed at improving plant growth. Current Microbiology. 62, 173-181. https://doi.org/10.1007/s00284-010-9674-6

Chaurasiya, D. K., Sahni, S., Prasad, B. D., Kumar, B. (2023). Biological management of Lentil (*Lens culinaris*) *Fusarium* wilt by using the potential *Pseudomonas* isolates. International Journal of Plant & Soil Science, 35(2), 7-11. https://doi.org/10.9734/ijpss/2023/v35i22752

Çevik, Y.N. & Ogutcu, H. (2020). Identification of bacteria in soil by MALDI-TOF MS and analysis of *Bacillus* spp., *Paenibacillus* spp. and *Pseudomonas* spp. with PCA. Analytical Chemistry Letters, 10(6), 784-797. https://doi.org/10.1080/22297928.2021.1877194

Deka, H., Deka, S. & Baruah, C.K. (2015). Plant growth promoting rhizobacteria for value addition: mechanism of action. In Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants, Soil Biology, (42), 305-321. https://doi.org/10.1007/978-3-319-13401-7_15

Devi, S., Sharma, S., Tiwari, A., Bhatt, A. K., Singh, N. K., Singh, M., Kumar, A. (2023). Screening for multifarious plant growth promoting and biocontrol attributes in *Bacillus* strains isolated from Indo Gangetic soil for enhancing growth of rice crops. Microorganisms, 11(4), 1085. https://doi.org/10.3390/microorganisms11041085

Dhole, A., Shelat, H., Panpatte, D. (2017). *Chryseobacterium indologenes* a novel root nodule endophyte in *Vigna radiata*. International Journal of Current Microbiology and Applied Sciences*,* 6, 836-44. https://doi.org/10.20546/ijcmas.2017.604.104

Dorjey, S., Dolkar, D. & Sharma, R. (2017). Plant growth promoting rhizobacteria *Pseudomonas*: a review. International Journal of Current Microbiology and Applied Sciences, 6(7), 1335-1344. https://doi.org/10.20546/ijcmas.2017.607.160

Ercole, T.G., Kava, V.M., Aluizio, R., Pauletti, V., Hungria, M., Galli-Terasawa, L.V. (2023). Co-inoculation of *Bacillus velezensis* and *Stenotrophomonas maltophilia* strains improves growth and salinity tolerance in maize (*Zea mays* L.). Rhizosphere, 27, 100752. https://doi.org/10.1016/j.rhisph.2023.100752

Fatima, I., Hakim, S., Imran, A., Ahmad, N., Imtiaz, M., Ali, H., Mubeen, F. (2022). Exploring biocontrol and growth-promoting potential of multifaceted PGPR isolated from natural suppressive soil against the causal agent of chickpea wilt. Microbiological Research, 260, 127015. https://doi.org/10.1016/j.micres.2022.127015

Ferioun, M., Srhiouar, N., Tirry, N., Belahcen, D., Siang, T. C., Louahlia, S., El Ghachtouli, N. (2023). Optimized drought tolerance in barley (Hordeum vulgare L.) using plant growth-promoting rhizobacteria (PGPR). Biocatalysis and Agricultural Biotechnology, 50, 102691. https://doi.org/10.1016/j.bcab.2023.102691

Fiodor, A., Ajijah, N., Dziewit, L. Pranaw, K. (2023). Biopriming of seed with plant growth-promoting bacteria for improved germination and seedling growth. Frontiers in Microbiology, 14, 1142966. https://doi.org/10.3389/fmicb.2023.1142966

Forchetti, G., Masciarelli, O., Alemano, S., Alvarez, D., Abdala, G. (2007). Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Applied Microbiology and Biotechnology, 76, 1145-1152. https://doi.org/10.1007/s00253-007-1077-7

Ghosh, R., Chatterjee, S. & Mandal, N.C. (2020). *Stenotrophomonas*. In Beneficial Microbes in Agro-Ecology. Academic Press, 427-442. https://doi.org/10.1016/b978-0-12-823414-3.00020-4

Glickmann, E. & Dessaux, Y. (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Applied Environmental. Microbiology 61, 793–796. https://doi.org/10.1128/aem.61.2.793-796.1995

Goes, K.C.G.P.D., Fisher, M.L.D.C., Cattelan, A.J., Nogueira, M.A., Carvalho, C.G.P.D., Oliveira, A.L.M.D. (2012). Biochemical and molecular characterization of high population density bacteria isolated from sunflower. Journal of Microbiology and Biotechnology, 22(4), 437-447. https://doi.org/10.4014/jmb.1109.09007

Gopalakrishnan, S., Srinivas, V. & Samineni, S. (2017). Nitrogen fixation, plant growth and yield enhancements by diazotrophic growth-promoting bacteria in two cultivars of chickpea (*Cicer arietinum* L.). Biocatalysis and Agricultural Biotechnology, 11, 116-123. https://doi.org/10.1016/j.bcab.2017.06.012

Gulya, T. J., Mathew, F., Harveson, R., Markell, S., Block, C. (2016). Diseases of sunflower. Handbook of Florists' crops diseases, handbook of plant disease management. 1-20, Springer International Publishing. https://doi.org/10.1007/978-3-319-32374-9_27-1

Gupta, A. & Gopal, M. (2008). Siderophore production by plant growth promoting rhizobacteria. Indian Journal of Agricultural Research, 42(2), 153-156.

Haghighi, B.J., Alizadeh, O. & Firoozabadi, A.H. (2011). The role of plant growth promoting rhizobacteria (PGPR) in sustainable agriculture, Advances in Environmental Biology, 5(10), 3079-3083. https://doi.org/10.1007/978-981-13-6790-8_2

Hameeda, B., Harini, G., Rupela, O.P., Wani, S.P., Reddy, G. (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Research, 163(2), 234–242. https://doi.org/10.1016/j.micres.2006.05.009

Hamid, S., Lone, R. & Mohamed, H.I. (2021). Production of antibiotics from PGPR and their role in biocontrol of plant diseases. Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management, 441-461. https://doi.org/10.1007/978-3-030-66587-6_16

Hayat, R., Ahmed, I. & Sheirdil, R.A. (2012). An overview of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. Crop Production for Agricultural Improvement, 557-579. https://doi.org/10.1007/978-94-007-4116-4_22

Huang, C., Jiang, L., Liang, Y.P., Han, L.J., Yang, K.Y., Qin, Y.M., & Zhao, X. (2023). *Acinetobacter calcoaceticus* promotes the seedling growth of *Lespedeza daurica* under saline-alkaline stress, Acta Microbiologica Sinica, 8, 3264-3278.

Jiang, Z., Zheng, H. & Xing, B. (2021). Environmental life cycle assessment of wheat production using chemical fertilizer, manure compost, and biochar-amended manure compost strategies. Science of The Total Environment, 760, 143342. https://doi.org/10.1016/j.scitotenv.2020.143342

Kapulnik, Y., Okon, Y., Kigel, J., Nur, I., Henis, Y. (1981). Effects of temperature, nitrogen fertilization, and plant age on nitrogen fixation by *Setaria italica* inoculated with *Azospirillum brasilense* (strain cd), Plant Physiology, 68(2), 340-343. https://doi.org/10.1104/pp.68.2.340

Khalil, M.S.M., Hassan, M.H.A.R., Mahmoud, A.F., Morsy, K.M.M. (2022). Involvement of secondary metabolites and extracellular lytic enzymes produced by plant growth promoting rhizobacteria in inhibiting the soilborne pathogens in Faba Bean Plants. Jurnal Hama dan Penyakit Tumbuhan Tropika, 22(2), 100-108. https://doi.org/10.23880/oajmms-16000161

Khan, M. S., Zaidi, A. & Ahmad, E. (2014). Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. Phosphate solubilizing microorganisms: Principles and Application of Microphos Technology, Springer International Publishing Switzerland, 31-62. https://doi.org/10.1007/978-3-319-08216-5_2

Khare, E., Singh, S., Maheshwari, D.K., Arora, N.K. (2011). Suppression of charcoal rot of chickpea by fluorescent Pseudomonas under saline stress condition. Current Microbiology, 62, 1548-1553. https://doi.org/10.1007/s00284-011-9895-3

Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N. (1980). Enhanced plant growth promoting rhizobacteria, Nature, 286 (5776), 883-885. https://doi.org/10.1038/286885a0

Koçak, R. & Boyraz, N. (2024). Determination of In Vitro and In Vivo Efficacy of Some Bacterial Antagonists Against *Sclerotinia sclerotiorum* (Lib.) De Bary in Sunflowers. Tekirdağ Ziraat Fakültesi Dergisi, 21(2), 362-374. https://doi.org/10.33462/jotaf.1259380

Koumoutsi, A., Chen, X. H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Borriss, R. (2004). Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. Journal of Bacteriology, 1084–1096. https://doi.org/10.1128/jb.186.4.1084-1096.2004

Kumar, P., Dubey, R. C., & Maheshwari, D. K. (2012). *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiological Research, 167(8), 493-499. https://doi.org/10.1016/j.micres.2012.05.002

Krieg, N.R. & Holt, J.G. (1984). Bergey's manual of systemic bacteriology. The William and Wilkins Co. Baltimore. (1), 964. https://doi.org/10.1099/00207713-35-3-408

Liang, Y., Xu, Z., Xu, Q., Zhao, X., Niu, S., & Yin, X. (2023). Isolation of Inorganic Phosphorus-Solubilizing Bacteria from the Rhizosphere of *Festuca arundinacea* Schreb. Geomicrobiology Journal, 40(6), 538-546.

Lucas Garcia, J.A., Probanza, A., Ramos, B., Barriuso, J., Gutierrez Manero, F.J. (2004). Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and *Sinorhizobium fredii* on biological nitrogen fixation, nodulation and

growth of *Glycine max* cv. Osumi. Plant and Soil, 267, 143-153. https://doi.org/10.1007/s11104-005-4885-5

Mahapatra, A.N.I.T.A., Gouda, B. & Ramesh, K. (2021). Productivity and profitability of summer sunflower (*Helianthus annuus* L.) with integrated nutrient management. Journal of Oilseeds Research, 38(1), 106-109. https://doi.org/10.56739/jor.v38i1.137020

Majeed, A., Abbasi, M. K., Hameed, S., Imran, A., Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology, (6), 198. https://doi.org/10.3389/fmicb.2015.00198

Majeed, A., Abbasi, M.K., Hameed, S., Imran, A., Naqqash, T., Hanif, M.K. (2018). Isolation and characterization of sunflower associated bacterial strain with broad spectrum plant growth promoting traits. International Journal of Biosciences, 13, 110-123. http://dx.doi.org/10.12692/ijb/13.2.110-123.

Majeed, A., Abbasi, M. K., Hameed, S., Yasmin, S., Hanif, M. K., Naqqash, T., & Imran, A. (2018). *Pseudomonas* sp. AF-54 containing multiple plant beneficial traits acts as growth enhancer of *Helianthus annuus* L. under reduced fertilizer input. Microbiological Research, 216, 56-69.

Manasa, K., Reddy, S. & Triveni, S. (2017). Characterization of potential PGPR and antagonistic activities of *Rhizobium* isolates from different rhizosphere soils. Journal of Pharmacognosy and Phytochemistry, 6(3), 51-54. https://doi.org/10.20546/ijcmas.2017.605.316

Marakana, T., Sharma, M. & Sangani, K. (2018). Isolation and characterization of halotolerant bacteria and it's effects on wheat plant as PGPR. The Pharma Innovation Journal, 7(7*)*, 102-110.

Mari, M., Guizzardi, M. & Pratella, G.C. (1996). Biological control of gray mold in pears by antagonistic bacteria. Biological Control, 7(1), 30-37. https://doi.org/10.1006/bcon.1996.0060

Mehta, S. & Nautiyal, CS. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. Current Microbiology, (43), 51-56. https://doi.org/10.1007/s002840010259

Miljaković, D., Marinković, J., & Balešević-Tubić, S. (2020). The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms, 8(7), 1037.

Mishra, R. K., Pandey, S., Rathore, U. S., Mishra, M., Kumar, K., Kumar, S., & Manjunatha, L. (2023). Characterization of plant growth-promoting, antifungal, and enzymatic properties of beneficial bacterial strains associated with pulses rhizosphere from Bundelkhand region of India. Brazilian Journal of Microbiology, 54(3), 2349-2360.

Moussa, T.A.A., Almaghrabi, O.A., Abdel-Moneim, T.S. (2013). Biological control of the wheat root rot caused by *Fusarium graminearum* using some PGPR strains in Saudi Arabia. Annals of Applied Biology, 163(1), 72-81. https://doi.org/10.1111/aab.12034

Moustaine, M.R. Elkahkahi, A., Benbouazza, R., Benkirane, E., Achbani. (2017). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth in tomato (*Solanum lycopersicum* L.) and characterization for direct PGP abilities in Morocco. International Journal of Environment, Agriculture and Biotechnology, 2:238708. https://doi.org/10.22161/ijeab/2.2.5

Morgado González, A., Espinosa Victoria, D., Gómez Merino, F. C. (2015). Efficiency of plant growth promoting rhizobacteria (PGPR) in sugarcane. Terra Latinoamericana, 33(4), 321-330. Retrieved in March 12, 2024, from https://www.scielo.org.mx/scielo.php?pid=S0187-57792015000400321&script=sci_arttext.

Mushtaq, Z., Faizan, S. & Hussain, A. (2021). Role of microorganisms as biofertilizers. Microbiota and Biofertilizers: A Sustainable Continuum for Plant and Soil Health, 83-98. https://doi.org/10.1007/978-3-030-48771-3_6

Nathan, P., Rathinam, X., Kasi, M., Rahman, Z.A., Subramaniam, S. (2011). A pilot study on the isolation and biochemical characterization of *Pseudomonas* from chemical intensive rice ecosystem. African Journal of Biotechnology, 10(59), 12653-12656.

Nishioka, T., Elsharkawy, M.M., Suga, H., Kageyama, K., Hyakumachi, M., Shimizu, M. (2016). Development of culture medium for the isolation of *Flavobacterium* and *Chryseobacterium* from rhizosphere soil. Microbes and Environments, 31(2), 104-110. https://doi.org/10.1264/jsme2.me15144

Öğütcü, H. & Avsar, H. (2020). Characterization and siderophores production of *Rhizobium* spp. Isolated from wild legumes, International Journal of Computational and Experimental Science and Engineering (IJCESEN), 6(3), 176-179.

Paul, D. & Sinha, S.N. (2017). Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. Annals of Agrarian Science, 15(1), 130-136. https://doi.org/10.1016/j.aasci.2016.10.001

Pandey, R., Chavan, P. N., Walokar, N. M., Sharma, N., Tripathi, V., Khetmalas, M.B. (2013). *Pseudomonas stutzeri* RP1: a versatile plant growth promoting endorhizospheric bacteria inhabiting sunflower (*Helianthus annuus*). Journal of Biotechnology, 8(7), 48-55.

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S., Sa, T. (2005). Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiological Research, 160(2), 127-133. https://doi.org/10.1016/j.micres.2004.10.003

Parveen, G., Saleem, S.M.S., Raıt, N., Rahman, A. (2020). Role of *Pseudomonas aerugınosa* in Enhancing The Growth of Sunflower and Suppressıon of Root Rottıng Fungi. International Journal of Biology Research 8 (12): 43-55.

Pathma, J. & Sakthivel, N. (2013). Molecular and functional characterization of bacteria isolated from straw and goat manure based vermicompost. Applied Soil Ecology, 70, 33-47. https://doi.org/10.1016/j.apsoil.2013.03.011

Petrović, M., Janakiev, T., Grbić, M. L., Unković, N., Stević, T., Vukićević, S., Dimkić, I. (2024). Insights into Endophytic and Rhizospheric Bacteria of Five Sugar Beet Hybrids in Terms of Their Diversity, Plant-Growth Promoting, and Biocontrol Properties. Microbial Ecology, 87(1), 1-25. https://doi.org/10.1007/s00248-023-02329-0

Pramanik, K., Mitra, S., Sarkar, A., Soren, T., Maiti, T.K. (2018). Characterization of a Cd2+-resistant plant growth promoting rhizobacterium (*Enterobacter* sp.) and its effects on rice seedling growth promotion under Cd2+-stress in vitro. Agriculture and Natural Resources, 52(3), 215-221. https://doi.org/10.1016/j.anres.2018.09.007

Raval, A.A. & Desai, P.B. (2012). Rhizobacteria from rhizosphere of sunflower (*Helianthus annuus* L.) and their effect on plant growth. Research Journal of Recent Sciences, ISSN, 2277-2502.

Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S. C. (2021). Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. Journal of Soil Science and Plant Nutrition, 21, 49-68. https://doi.org/10.1007/s42729-020-00342-7

Riaz, U., Murtaza, G., Anum, W., Samreen, T., Sarfraz, M., Nazir, M.Z. (2021). Plant Growth-Promoting Rhizobacteria (PGPR) as biofertilizers and biopesticides. Microbiota and Biofertilizers: A Sustainable Continuum for Plant and Soil Health, 181-196. ttps://doi.org/10.1007/978-3-030-48771-3_11

Rosas, S.B., Andrés, J. A., Rovera, M., Correa, N.S. (2006). Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia–legume symbiosis. Soil Biology and Biochemistry, 38(12), 3502-3505. https://doi.org/10.1016/j.soilbio.2006.05.008

Sarwar, M. & Kremer, R.J. (1995). Determination of bacterially derived auxins using a microplate method. Letters in applied microbiology, 20(5): 282-285. https://doi.org/10.1111/j.1472-765x.1995.tb00446.x

Sebastian, A. M., Umesh, M., Priyanka, K., Preethi, K. (2021). Isolation of plant growth-promoting *Bacillus cereus* from soil and its use as a microbial inoculant. Arabian Journal for Science and Engineering, 46(1), 151-161. https://doi.org/10.1007/s13369-020-04895-8

Schwyn, B. & Neilands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry, 160(1), 47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Shittu, H.O., Castroverde, D.C., Nazar, R.N., Robb, J. (2009). Plant-endophyte interplay protects tomato against a virulent *Verticillium*. Planta, 229, 415-426. https://doi.org/10.1007/s00425-008-0840-z

Shobha, G., & Kumudini, B. S. (2012). Antagonistic effect of the newly isolated PGPR Bacillus spp. on *Fusarium oxysporum*. International Journal of Applied Sciences and Engineering Research, 1(3), 463-474. https://doi.org/10.6088/ijaser.0020101047

Shrivastava, S., Egamberdieva, D. & Varma, A. (2015). Plant growth-promoting rhizobacteria (PGPR) and medicinal plants: The state of the art. Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants, 1-16. https://doi.org/10.1007/978-3-319-13401-7_1

Singh, N., Raina, S., Singh, D., Ghosh, M., Heflish, A.I.A.I. (2017). Exploitation of promising native strains of *Bacillus subtilis* with antagonistic properties against fungal pathogens and their PGPR characteristics. Journal of Plant Pathology, 27-35.

Singh, S.B., Gowtham, H.G., Murali, M., Hariprasad, P., Lakshmeesha, T.R., Murthy, K.N., Niranjana, S.R. (2019). Plant growth promoting ability of ACC deaminase producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). Biocatalysis and Agricultural Biotechnology, 18, 101089. https://doi.org/10.1016/j.bcab.2019.101089

Singh, R.K., Singh, P., Li, H. B., Guo, D.J., Song, Q.Q., Yang, L.T., Li, Y.R. (2020). Plant-PGPR interaction study of plant growth-promoting diazotrophs *Kosakonia radicincitans* BA1 and *Stenotrophomonas maltophilia* COA2 to enhance growth and stress-related gene expression in *Saccharum* spp. Journal of Plant Interactions, 15(1), 427-445. https://doi.org/10.1080/17429145.2020.1857857

Sivasakthivelan, P. & Stella, D. (2012). Studies on the phytohormone producing potential of agriculturally beneficial microbial (ABM) isolates from different rhizosphere soils of sunflower in Tamil Nadu. International Journal of Pharmaceutical and Biological Archives, 3(5), 1150-1156.

Smibert, R.M. & Kreig, N.R. (1994). "Phenotypic characterization in Gerhardt P.et al. Method for general and molecular bacteriology", American Society for Microbiology, Washington DC, 607-654.

Soares, A.S., Nascimento, V.L., De Oliveira, E.E., Jumbo, L.V., Dos Santos, G.R., Queiroz, L. L., de Souza Aguiar, R.W. (2023). *Pseudomonas aeruginosa* and *Bacillus cereus* Isolated from Brazilian Cerrado Soil Act as Phosphate-Solubilizing Bacteria. Current Microbiology, 80(5), 146. https://doi.org/10.1007/s00284-023-03260-w

Sokolova, M.G., Akimova, G.P. & Vaishlya, O.B. (2011). Effect of phytohormones synthesized by rhizosphere bacteria on plants. Applied Biochemistry and Microbiology, 47, 274-278. https://doi.org/10.1134/s0003683811030148

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., Song, W. (2008). Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots

estimated by 16S rDNA sequence analysis. Microbial Ecology, 55, 415-424. https://doi.org/10.1007/s00248-007-9287-1

Tabassum, B., Khan, A., Tariq, M., Ramzan, M., Khan, M.S.I., Shahid, N., Aaliya, K. (2017). Bottlenecks in commercialisation and future prospects of PGPR, Applied Soil Ecology, 121, 102-117. https://doi.org/10.1016/j.apsoil.2017.09.030

Temiz, A., 2010, Genel Mikrobiyoloji Uygulama Teknikleri, *Hatipoğlu Yayıncılık*, Ankara, 1- 277.

Thakker, J. N., Badrakia, J., Patel, K., Makwana, U., Parmar, K., Dhandhukia, P. (2023). Potential of a marine *Pseudomonas aeruginosa* strain OG101 to combat *Fusarium oxysporum* associated wilt in legume crops. Archives of Phytopathology and Plant Protection, 56(4), 284-294. https://doi.org/10.1080/03235408.2023.2183800

Verma, P. & Shahi, S.K. (2015). Characterization of plant growth promoting rhizobacteria associated with potato rhizosphere. International Journal of Advanced Research, (3)6, 564-572.

Waqas, M., Khan, A.L., Hamayun, M., Shahzad, R., Kim, Y.H., Choi, K.S., Lee, I.J. (2015). Endophytic infection alleviates biotic stress in sunflower through regulation of defence hormones, antioxidants and functional amino acids. European Journal of Plant Pathology, 141, 803-824. https://doi.org/10.1007/s10658-014-0581-8

Walker, V., Bertrand, C., Bellvert, F., Moënne-Loccoz, Y., Bally, R., Comte, G., (2011). Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*, New Phytologist, 189(2), 494-506. https://doi.org/10.1111/j.1469-8137.2010.03484.x

Wilson, M. & Knight, D. (1952). Methods of Plant Pathology, Ed. Tuite, J. London: Academic Press, 343

Yadav, A.N. (2020). Plant microbiomes for sustainable agriculture: current research and future challenges. Springer International Publishing, 475-482. https://doi.org/10.1007/978-3-030-38453-1_16

Zou, D., Zheng, H., Zhang, Y., Gu, Y., Cao, Y., Song, Y., Li, L. (2020). Screening of rhizosphere growth promoting bacteria and their growth promoting ability of sunflower in cold black soil area. In IOP Conference Series: Earth and Environmental Science, (526),10, 12039. https://doi.org/10.1088/1755-1315/526/1/012039.