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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Isolation of Beneficial Bacteria from Wheat Plant Rhizosphere and Determination of PGP (Plant Growth Promoting) Properties

Buğday Bitkisi Rizosferinden Yararlı Bakterilerin İzolasyonu ve Bitki Büyümesini Teşvik Edici Özelliklerinin Belirlenmesi

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Abstract

Wheat, an important food and industrial product, is one of the most widely grown plants in the world. Environmentally friendly alternative methods are needed to increase plant productivity. Plant growth-promoting rhizobacteria (PGPR) directly or indirectly promote plant growth and production by actively colonizing plant roots. In the current study, 49 isolates were obtained from rhizospheric soil samples of wheat fields in 6 districts of Kırşehir (Akpınar, Boztepe, Kaman, Mucur, Akçakent, Çiçekdağı) in May 2019 and the isolates were identified biochemically and by MALDI-TOF mass spectrometry. The MALDI-TOF MS results showed that 49 isolates from 14 distinct genera could be identified. Among the isolates, Bacillus, Pseudomonas, and Pseudarthrobacter ranked first through third. Plant growth promoting properties of these isolates (inorganic phosphate solubilisation, siderophore production, nitrogen fixation, HCN (Hydrogen cyanide) production, IAA (Indole-3-acetic acid) production) were screened. Among 49 isolates, 4 isolates (MH-50-6, MH-50-7, MH-60-1, and MH-60-3) dissolved inorganic phosphate, and 13 isolates (MH-28-1, MH-34-3, MH-34-8, MH-39-1, MH-39-3, MH-39-7, MH-50-8, MH-55-3, MH-55-7, MH-55-9, MH-60-1, MH-60-3, MH-60-5) nitrogen fixed, on the other hand, 9 isolates (MH-34-5, MH-34-8, MH-39-5, MH-50-6, MH-50-7, MH-50-8, MH-55-6, MH-60-1, MH-60-3) produced siderophores, 8 isolates (MH-28-1, MH-34-2, MH-34-4, MH-34-5, MH-39-1, MH-55-3, MH-60-2, MH-60-5) produced HCN, while 4 isolates (MH-34-8, MH-50-4, MH-50-8, MH-55-9) was determined to produce IAA. In conclusion, indigenous PGPR (*Pseudarthrobacter oxydans* MH-34-8, *Pseudomonas jessenii* MH-50-8, *Arthrobacter crystallopoietes* MH-60-1, *Aromatoleum evansii* MH-60-3) isolates gave positive results for at least 3 plant growth-promoting properties. The present study is the first study on growth-promoting plants isolated from wheat in Kırşehir (Turkey). This study will illuminate the preparation of effective microbial fertilizer with local bacterial species that spread in the region and the availability of local culture collections.

Keywords: Wheat, PGPR, Beneficial bacteria, Rhizosphere

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Öz

Önemli bir gıda ve sanayi ürünü olan buğday, dünyada yaygın bir şekilde yetiştirilen bitkilerden biridir. Bitki verimliliğini artırmak için çevre dostu alternatif yöntemlere ihtiyaç vardır. Bitki büyümesini teşvik eden rizobakteriler (PGPR), bitki köklerini aktif olarak kolonize ederek bitki büyümesini ve üretimini doğrudan veya dolaylı bir şekilde destekler. Bu çalışmada, Mayıs 2019'da Türkiye'de Kırşehir'in 6 ilçesinde (Akpınar, Boztepe, Kaman, Mucur, Akçakent, Çiçekdağı) buğday tarlalarının rizosferik toprak örneklerinden 49 izolat elde edilmiş ve izolatların biyokimyasal ve MALDI-TOF kütle spektrometrisi yöntemiyle teşhisleri yapılmıştır. MALDI-TOF MS sonuçlarına göre 14 farklı cinse ait 49 izolat tanımlanmıştır. 49 izolat içerisinde ilk üç sırada Bacillus, Pseudomonas ve Pseudarthrobacter cinsleri yer almaktadır. Bu izolatların bitki büyümesini teşvik edici özellikleri (inorganik fosfat çözünürlüğü, siderofor üretimi, azot fiksasyonu, HCN (Hydrogen cyanide) üretimi, IAA (Indole-3-acetic acid**)** üretimi) tarandı. 49 izolattan 4'ünün (MH-50-6, MH-50-7, MH-60-1, and MH-60-3) inorganik fosfatı çözdüğü, 13'ünün (MH-28-1, MH-34-3, MH-34-8, MH-39-1, MH-39-3, MH-39-7, MH-50-8, MH-55-3, MH-55-7, MH-55-9, MH-60-1, MH-60-3, MH-60-5) azot fiksasyonu yaptığı, 9'unun (MH-34-5, MH-34-8, MH-39-5, MH-50-6, MH-50-7, MH-50-8, MH-55-6, MH-60-1, MH-60-3) siderofor ürettiği, 8'inin (MH-28-1, MH-34-2, MH-34-4, MH-34-5, MH-39-1, MH-55-3, MH-60-2, MH-60-5) HCN ürettiği, 4'ünün (MH-34-8, MH-50-4, MH-50-8, MH-55-9) ise IAA ürettiği belirlendi. Sonuç olarak, yerli PGPR (*Pseudarthrobacter oxydans* MH-34-8, *Pseudomonas jessenii* MH-50-8, *Arthrobacter crystallopoietes* MH-60-1, *Aromatoleum evansii* MH-60-3) izolatları en az 3 bitki büyümesini teşvik edici özellikleri bakımından pozitif sonuçlar vermiştir. Bu çalışma, Kırşehir'de (Türkiye) buğdaydan izole edilen büyümeyi teşvik eden bitkiler üzerine yapılan ilk çalışmadır. Bu çalışma, bölgede yayılış gösteren yerel bakteri türleri ile etkili mikrobiyal gübre hazırlanmasına ve yerel kültür koleksiyonlarının varlığına ışık tutacaktır.

Anahtar Kelimeler: Buğday, PGPR, Yararlı bakteriler, Rizosfer

1. Introduction

With rapid population growth and industrialization, it has become necessary to differentiate between the continuation of traditional agricultural practices worldwide. Although people disregard human health in order to obtain more efficiency from the products they produce, they have not avoided trying different applications. Although agricultural production has increased at the desired rate as a result of these different applications, it has caused the destruction of nature and the environment in terms of the results it has produced and has led to the deterioration of the natural balance by increasing environmental pollution (Savci, 2012). An example of harmful applications made to obtain more yield is the use of chemical fertilizers in the soil for a long time (Pahalvi et al., 2021). Long-term, intensive and indiscriminate use of chemicals in agriculture; It has become a serious threat to human and environmental health, food security, biodiversity and agricultural sustainability (Kotan and Tozlu, 2021). Overused fertilizers cause deterioration of the effectiveness of microorganisms in the soil over time, eutrophication and nitrate accumulation in water, greenhouse gas effects, and heavy metal accumulation. In addition, gases such as N_2O and NO, which are formed as a result of the use of excessive amounts of nitrogen fertilizer, disrupt the structure of ozone in the stratosphere and cause its decomposition (Sönmez et al., 2008).

The plant root system is surrounded by millions of microorganisms in a nutrient-rich rhizosphere. The rhizosphere harbors a diverse array of living organisms, including bacteria, fungi, nematodes, protozoa, algae, and micro arthropods. Plant roots not only provide mechanical support and aid in the absorption of water and nutrients, but they also engage in the synthesis, accumulation, and secretion of diverse compounds (Walker et al., 2011; Tabassum et al., 2017). These compounds produced by plant roots have a heterogeneous structure and act as chemical attractants for actively used soil microbial communities. Some substances, such as amino acids, sugars, and aromatic acids secreted by plant roots, provide a good supply of energy and nutrients for bacteria in the rhizosphere, causing a larger microbial population to develop there than outside the region (Haas and Defago, 2005). A community of soil bacteria that competitively colonizes plant roots and stimulates growth, thus reducing the incidence of plant diseases, is called plant growth-promoting rhizobacteria (PGPR) (Kleopper, 1978). Some of the most important known features of PGPRs are their ability to dissolve organic phosphorus, produce plant hormones, produce siderophores, increase systematic durability, bind free nitrogen in the atmosphere, produce phytohormones such as IAA, Gas, kinetin and reduce the ethylene level of the plant by producing auxin. These beneficial bacteria, known as PGPR, play a crucial role in disease resistance, nutrient absorption, root and shoot formation, seed germination, and environmental stress tolerance (Hayat et al., 2010; Esertaş et al., 2023). Studies on PGPR bacteria isolated from the rhizosphere of various plants in Turkey are rapidly increasing (Oral and Kotan, 2021; Çelik, 2023). Bacteria belonging to the genera *Pseudomonas* and *Bacillus* stand out for their plant growth-promoting traits as well as their biocontrol properties. For instance, Çelikten and Bozkurt (2018) reported that bacteria of these genera, isolated from the rhizosphere of wheat fields in Hatay, exhibited plant growth-promoting characteristics.

Sustainability in agriculture requires improving the soil's physical, chemical, and biological structure. Any environmentally friendly activity that increases biological activity and soil performance in the soil is of vital importance for agricultural sustainability. Therefore, PGPR-based local microbial fertilizers need to be developed and used in agriculture (Kenneth et al., 2019). Investigating locally plant growth-promoting (PGP) bacteria and utilizing them in agricultural practices is crucial for sustainable agriculture, not only regionally but also across the entire country, particularly in areas like Kırşehir, where wheat farming is extensively practiced. The aim of the current study is to determine the roles of the local strains that we isolated from the soils in Kırşehir in supporting plant growth and to create the microbial fertilizer inventory of the region.

2. Materials and Methods

2.1. Locations of soil samples

Kırşehir, with a surface area of 6.570 km^2, is located in the Central Kızılırmak Section of the Central Anatolia Region. Ranking 53rd in Turkey in terms of surface area, Kırşehir land constitutes 0.8% of the country's land and 2.9% of the Central Anatolian Region's land (*Figure 1*).

The province, which has 6 districts (Akpınar, Boztepe, Kaman, Mucur, Akçakent, Çiçekdağı) in total, is located between 38^0 50'-39^0 50' north latitudes and 33^0 30'-34^0 50' east longitudes (*Table 1*). It has been determined that

366.073 hectares of 431.025 hectares of agricultural land exist in the province, which has an altitude of 985 m and annual precipitation of around 350-400 mm, is irrigable. An annual yield of 48.800 kg was obtained from wheat cultivated in all districts of Kırşehir, especially Boztepe and Çiçekdağı (Bayar et al., 2019).

Table 1. Locations and altitudes of wheat rhizosphere samples

No	Isolates	Location	Altıtude	Latitude	Longitude
1	*Bacillus atrophaeus* MH-28-1	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
2	*Bacillus simplex* MH-28-2	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
3	*Bacillus simplex* MH-28-3	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
4	*Bacillus simplex* MH-28-4	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
5	*Bacillus megaterium* MH-28-5	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
6	*Bacillus simplex* MH-28-6	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
7	*Bacillus simplex* MH-28-7	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
8	*Pseudomonas anguilliseptica* MH-28-8	AKPINAR/ Centre	1126	39°26'49.4"N	33°58'03.2"E
9	*Bacillus cereus* MH-34-1	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
10	*Bacillus muralis* MH-34-2	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
11	*Hafnia alvei* MH-34-3	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
12	*Bacillus mojavensis* MH-34-4	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
13	*Bacillus sp LB* MH-34-5	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
14	*Bacillus simplex* MH-34-7	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
15	*Pseudarthrobacter oxydans* MH-34-8	KAMAN/İsahocalı	1280	39°25'00.6"N	33°53'58.8"E
16	*Bacillus atrophaeus* MH-39-1	MUCUR/Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
17	*Bacillus cereus* MH-39-2	MUCUR/Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
18	*Weeksella virosa* MH-39-3	MUCUR/Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
19	*Bacillus pseudomycoides* MH-39-4	MUCUR//Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
20	*Bacillus pumilus* MH-39-5	MUCUR//Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
21	*Actinomyces viscosus* MH-39-6	MUCUR/Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
22	*Bacillus thuringiensis* MH-39-7	MUCUR//Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
23	*Bacillus cereus* MH-39-8	MUCUR/Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
24	*Bacillus simplex* MH-39-9	MUCUR/Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
25	*Bacillus simplex* MH-39-10	MUCUR//Yeşilyurt	1080	39°05'54.7"N	34°18'29.3"E
26	*Bacillus simplex* MH-50-1	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
27	*Brevibacillus brevis* MH-50-2	AKÇAKENT//Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
28	*Brevibacillus parabrevis* MH-50-3	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
29	*Staphylococcus hominis* MH-50-4	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
30	*Bacillus megaterium* MH-50-5	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
31	*Bacillus mojavensis* MH-50-6	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
32	*Bacillus subtilis* MH-50-7	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
33	*Pseudomonas jessenii* MH-50-8	AKÇAKENT/Mahsenli	1268	39°34'10.6"N	34°10'44.5"E
34	*Bacillus simplex* MH-55-1	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
35	*Bacillus simplex* MH-55-2	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
36	*Bacillus atrophaeus* MH-55-3	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
37	*Bacillus simplex* MH-55-5	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
38	*Bacillus pumilus* MH-55-6	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
39	*Glutamicibacter arilaitensis* MH-55-7	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
40	*Bacillus simplex* MH-55-8	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
41	*Pseudarthrobacter polychromogenes* MH-55-9	BOZTEPE/Külhüyük	1150	39°20'15.1"N	34°15'47.0"E
42	*Arthrobacter crystallopoietes* MH-60-1	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E
43	*Paeniclostridium sordellii* MH-60-2	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E
44	*Aromatoleum evansii* MH-60-3	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E
45	*Bacillus simplex* MH-60-4	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E
46	*Citrobacter freundii* MH-60-5	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E
47	*Paracoccus denitrificans* MH-60-6	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E
48	*Bacillus megaterium* MH-60-7	ÇİÇEKDAĞI//İbikli	1140	39°31'50.0"N	34°20'36.0"E
49	*Bacillus cereus* MH-60-8	ÇİÇEKDAĞI/İbikli	1140	39°31'50.0"N	34°20'36.0"E

Figure 1. Locations of wheat rhizospheric soil samples (Kırşehir/Turkey)

2.2. Bacteria isolation and purification from rhizosphere soil samples

To isolate the bacterial samples for use in the study, soil samples were taken at a depth of 10 cm from the rhizosphere of wheat (*Triticum aestivum*) plants in Kırşehir and its districts in 2019. Each soil sample was labelled and brought to Microbiology Laboratory of Kırşehir Ahi Evran University and studied under aseptic conditions. The soil samples were homogenized by thorough mixing and serial dilutions of 10^{-1}-10^{-6} were prepared (Naseem and Bano, 2014). Inoculation was performed using the spreading method in Petri dishes containing a nutrient agar (Merck) medium. Petri dishes were incubated for 2-4 days at 28±2ºC and after which they were examined and selected according to their distinct morphological characteristics at the end of the incubation, incubated at 28±2ºC for 48 h, and inoculated on Petri dishes containing nutrient agar by the line sowing method to obtain a pure culture (*Figure 2*). A total of 49 pure isolates (*Table 2*) obtained at the end of the incubation were transferred to a nutrient broth (Merck) medium. Purified bacteria were stored in 50% (v/v) glycerol solution at -80°C for future research.

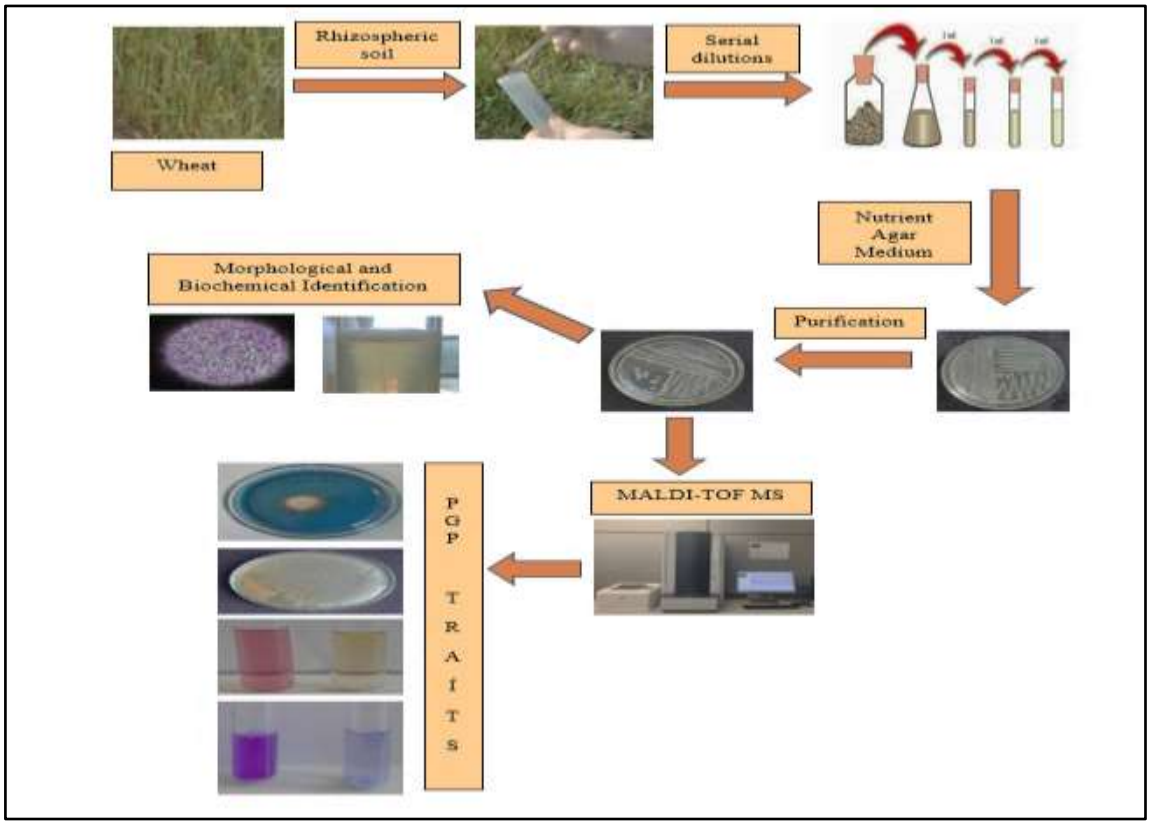

Figure 2. Flowchart representation of the process used to detect the characteristics of isolates from wheat rhizospheric soil

Table 2. Numerical distribution of the isolates on the basis of genus

Genus	Species	Number of Species	Genus	Species	Number of Species
Bacillus	*Bacillus atrophaeus*	3	Pseudarthrobacter	*Pseudarthrobacter oxydans*	1
	Bacillus simplex	14		*Pseudarthrobacter polychromogenes*	1
	Bacillus megaterium	3	Brevibacillus	*Brevibacillus brevis*	1
	Bacillus cereus	4		*Brevibacillus parabrevis*	1
	Bacillus pumilus	2	Hafnia	*Hafnia alvei*	1
	Bacillus mojavensis	2	Actinomyces	*Actinomyces viscosus*	1
	Bacillus thuringiensis	1	Staphylococcus	*Staphylococcus hominis*	1
	Bacillus muralis	1	Glutamibacter	*Glutamicibacter arilaitensi*	1
	Bacillus pseudomycoides	1	Arthrobacter	*Arthrobacter crystallopoietes*	1
	Bacillus pumilus	1	Paeniclostridium	*Paeniclostridium sordellii*	1
	Bacillus subtilis	1	Aromatoleum	*Aromatoleum evansii*	1
Pseudomanas	*Pseudomonas jessenii*	1	Paracoccus	*Paracoccus denitrificans*	1
	Pseudomonas anguilliseptica	1	Weeksella	*Weeksella virosa*	1
			Citrobacter	*Citrobacter freundii*	1

2.3. Biochemical tests and identification of isolates with MALDI-TOF MS

All isolates were grouped based on phenotypic and biochemical characteristics, such as cell form and size, Gram staining, motility, and the presence of catalase and oxidase, as described by Temiz (2010). MALDI-TOF mass spectrometer was used for bacterial identification (*Figure 2*). 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, 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. The table below displays the outcomes of the bacterial isolates identification using the MALDI-TOF MS method (*Table 3*).

2.4. Determination of plant growth promoting properties (PGP) of isolates

2.4.1. Determination of nitrogen fixing capacity

The nitrogen fixation ability of the isolates was determined according to the protocol described by Wilson and Knight (1952). The isolates were incubated at 28±2ºC for 24 h by streaking on NA (nutrient agar) medium. Each fresh culture isolate developed as a result of incubation was inoculated into petri dishes containing solid Burk's N-free medium by streaking (Wilson and Knight, 1952; Park et al., 2005). They were incubated in this medium at 28± 2 °C for four days, and the plates were checked daily and graded according to their development. Three time intervals were determined for nitrogen fixation activity (+++: development after 6 hours, ++: development after 12 hours, +: development after 24 hours).

2.4.2. Confirmation of nitrogen fixation capabilities of isolates

Bacteria that grew and fixed nitrogen in Burk's N-free medium at 30°C after 3-4 days were grown in TSA (Tryptic Soy Agar) medium at 30±2°C for 24h. It was then inoculated into sterile test tubes containing 5 ml of semi-solid JNFb medium, according to the method described by Baldani et al. (2014). The uninoculated tubes were used as the control group, and the inoculated tubes were incubated for 7 days at 30±2°C. At the end of this incubation period, while there was no change in the tubes of the control group, it was evaluated by looking at the color change of the nitrogen-fixing bacteria in the existing medium and whether they formed a membrane layer near the surface of the medium; it was observed that the nitrogen fixation tubes changed color from yellow to blue and formed a zone near the surface of the medium.

Table 3. Morphological, biochemical and MALDI TOF MS results of isolates

Location	Isolates	Gram Characteristic		Biochemical Characteristics		Motility	MALDI-TOF MS Score Value
		Gram reaction	KOH %3	Catalase	Oxidase		
AKPINAR	*Bacillus atrophaeus* MH-28-1	+*	-	+	+	+	2.188
	Bacillus simplex MH-28-2	+	-	+	-	+	1.722
	Bacillus simplex MH-28-3	+	-	+	-	+	1.775
	Bacillus simplex MH-28-4	+	-	+	-	+	1.716
	Bacillus megaterium MH-28-5	+	-	+	-	+	1.584
	Bacillus simplex MH-28-6	+	-	+	-	+	1.779
	Bacillus simplex MH-28-7	+	-	+	-	+	1.743
	Pseudomonas anguilliseptica MH-28-8	-	-	+	-	+	1.497
KAMAN	*Bacillus cereus* MH-34-1	+	-	+	+	+	1.489
	Bacillus muralis MH-34-2	+	-	+	+	-	1.892
	Hafnia alvei MH-34-3	-	-	+	-	+	1.478
	Bacillus mojavensis MH-34-4	+	-	+	+	+	1.912
	Bacillus sp. LB MH-34-5	+	-	+	-	+	1.208
	Bacillus simplex MH-34-7	+	-	+	-	+	1.750
	Pseudarthrobacter oxydans MH-34-8	+	-	+	-	+	2.086
MUCUR	*Bacillus atrophaeus* MH-39-1	+	-	+	+	+	1.891
	Bacillus cereus MH-39-2	+	-	+	+	+	1.946
	Weeksella virosa MH-39-3	-	-	+	-	-	1.394
	Bacillus pseudomycoides MH-39-4	+	-	+	+	+	1.704
	Bacillus pumilus MH-39-5	+	-	+	+	+	1.535
	Actinomyces viscosus MH-39-6	+	-	+	+	-	1.461
	Bacillus thuringiensis MH-39-7	+	-	+	-	-	1.626
	Bacillus cereus MH-39-8	+	-	+	+	+	1.747
	Bacillus simplex MH-39-9	+	-	+	-	+	1.919
	Bacillus simplex MH-39-10	+	-	+	-	+	1.849
AKÇAKENT	*Bacillus simplex* MH-50-1	+	-	+	-	+	1.758
	Brevibacillus brevis MH-50-2	+	-	+	+	-	1.663
	Brevibacillus parabrevis MH-50-3	+	-	+	+	-	1.684
	Staphylococcus hominis MH-50-4	+	+	-	+	-	1.446
	Bacillus megaterium MH-50-5	+	-	+	-	+	1.637
	Bacillus mojavensis MH-50-6	+	-	+	+	+	2.052
	Bacillus subtilis MH-50-7	+	-	+	-	+	1.430
	Pseudomonas jessenii MH-50-8	-	-	+	+	+	1.897
BOZTEPE	*Bacillus simplex* MH-55-1	+	-	+	-	+	1.735
	Bacillus simplex MH-55-2	+	-	+	-	+	1.709
	Bacillus atrophaeus MH-55-3	+	-	+	+	+	1.674
	Bacillus simplex MH-55-5	+	-	+	-	+	1.712
	Bacillus pumilus MH-55-6	+	-	+	+	+	1.831
	Glutamicibacter arilaitensis MH-55-7	+	-	+	-	+	2.309
	Bacillus simplex MH-55-8	+	-	+	-	+	1.677
	*Pseudarthrobacter polychromogenes*MH-55-9	+	-	+	-	+	1.653
ÇİÇEKDAĞI	*Arthrobacter crystallopoietes* MH-60-1	+	-	+	+	+	1.404
	Paeniclostridium sordellii MH-60-2	+	-	+	+	+	1.297
	Aromatoleum evansii MH-60-3	-	-	+	+	+	1.537
	Bacillus simplex MH-60-4	+	-	+	+	+	1.544
	Citrobacter freundii MH-60-5	-	-	+	-	+	1.437
	Paracoccus denitrificans MH-60-6	-	-	+	+	+	1.301
	Bacillus megaterium MH-60-7	+	-	+	+	+	1.603
	Bacillus cereus MH-60-8	+	-	+	+	+	1.338

Note: * +, positive; –, negative

2.4.3. Determination of inorganic phosphate dissolving capacity of isolates

The inorganic phosphate-dissolving capacities of the isolates were determined qualitatively according to the protocol described by Mehta and Nautiyal (2001). 25 microliters (approximately 0.5-1x10^9 CFU ml^{-1}) of 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 tubes with the control group were not inoculated. All tubes were incubated at 30±2°C and 180 rpm for three days. Although there was no color change

(blue-purple) in the control group tubes after incubation, it was observed that some of the inoculated tubes showed color expansion.

2.4.4. Assessment of isolates producing siderophores

To determine whether the isolates produced siderophores, Schwyn and Neilands (1987) followed the procedure and used Chrome Azurol S agar in this method. The isolates were seeded onto the medium using the spot-seeding method and incubated for 4 days at 28±2°C. At the end of the incubation, yellow-orange color formation around the bacteria was evaluated as a positive result, and the formed zone diameters (mm) were measured (Öğütcü and Avsar, 2020) (*Figure 3*). Three time intervals were determined for siderophore activity (+++: color change after 1h, ++: color change after 6h., +: color change after 24h).

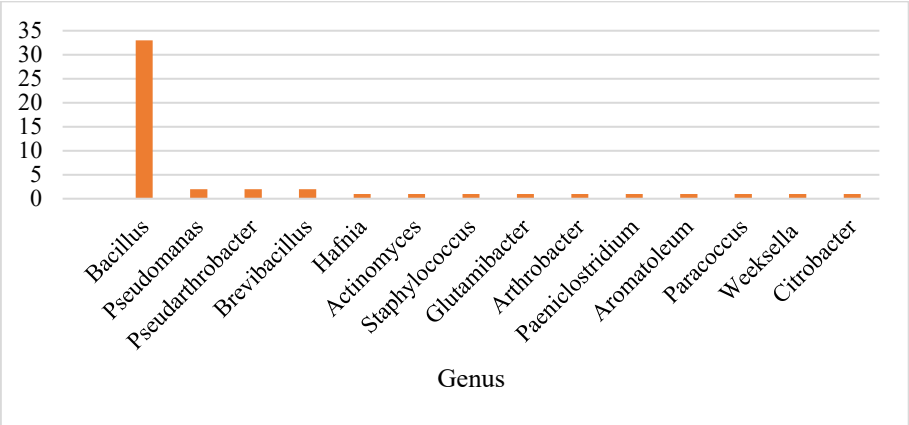

Figure 3. Number of PGPR isolates at genus level

2.4.5. Evaluation of isolates producing (Hydrogen cyanide) HCN

The method suggested by Bakker and Schippers (1987) was used to determine the HCN production by the isolates. In accordance with this purpose, 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 edge of the Petri plate without touching the medium. The mouths of the Petri dishes were tightly closed with paraffin and incubated at 28±2°C for 4 days. At the end of the incubation period, picric acid-impregnated papers turned from yellow to brown, which was considered a positive result (Temiz, 2010). Three time intervals were determined for HCN activity (+++: color change after 6h, ++: color change after 12h, +: color change after 24h).

2.4.6. Estimation of isolates producing indole-3-acetic Acid (IAA)

IAA production capabilities of the isolates were determined according to the protocol described by Sarwar and Kremer (1995). Bacteria were grown in a liquid culture for 10 min at 10,000 rpm. The cells were centrifuged, inoculated into 100 ml sterilized Luria Bertani and 0.01% g L-tryptophan medium (approximately 0.5-1x10^9 CFU ml^{-1}) in 250 ml flasks, and incubated for 3 days at 30ºC and 180 rpm in a shaker. After incubation, bacterial cultures were centrifuged at 10,000rpm for 10 minutes. 2 ml of the supernatant was put into 10 ml tubes and 3 ml of Salkowski solution (0.5 M 1 ml $FeCl_3$ and 50 ml of 98% perchloric acid) was added on it. The mixture was then left to stand for one hour in the dark at 30°C. After the waiting period, pink color change was considered positive for IAA production, and those that did not show color change were considered negative. It was evaluated as (+), (++), (+++) according to the lightening of the pink color change in the tube containing IAA.

2.5. Statistical analysis

All the statistical analysis was performed by using JMP Pro Statistics Ver. 17 software. The statistical data were expressed as the mean of three independent replications ± standard deviation (SD) of at least three replicates of each experiment and were interpreted through analysis of variance (one-way ANOVA) followed by Duncan's multiple range test at the $P \leq 0.05$ significance level (Uzun et al., 2017).

3. Results and Discussion

3.1. Identification of isolates

According to the MALDI-TOF MS results, 49 isolates belonging to 14 different genera were identified. Bacillus, Pseudomonas, and Pseudarthrobacter were in the first three ranks among the 49 isolates (*Table 2*, *Figure 3*).

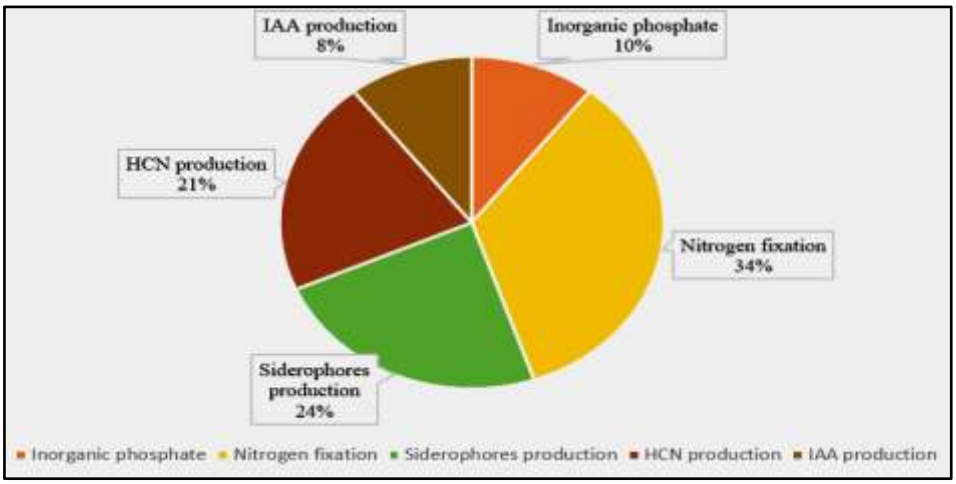

Figure 4. PGP ratios of isolates

3.2. Biochemical and morphological characterization of isolates

49 isolates were obtained from the rhizospheric soil samples of the wheat fields in 6 districts (Akpınar, Boztepe, Kaman, Mucur, Akçakent, Çiçekdağı) of Kırşehir. All isolates except seven (*Pseudomonas anguilliseptica* MH-28-8, *Hafnia alvei* MH-34-3, *Weeksella virosa* MH-39-3, *Pseudomonas jessenii* MH-50-8), (*Aromatoleum* evansii MH-60-3, *Citrobacter freundii* MH-60-5 and *Paracoccus denitrificans* MH-60-6) showed Gram (+) reactions. The catalase test was positive for all isolates except *Staphylococcus hominis* MH-50-4, whereas the oxidase test was positive for 24 isolates. However, except for 7 isolates (*Bacillus muralis* MH-34-2, *Weeksella virosa* MH-39-3, *Actinomyces viscosus* MH-39-6, *Bacillus thuringiensis* MH-39-7, *Brevibacillus brevis* MH-50-2, *Brevibacillus parabrevis* MH-50-3 and *Staphylococcus hominis* MH-50-4) other isolates were observed to be mobil (*Table 3*).

3.3. Screening for PGP characteristics of isolates

Of the 49 isolates, only 4 (MH-50-6, MH-50-7, MH-60-1, and MH-60-3) were found to dissolve inorganic phosphate. In addition, it was defined that 13 (MH-28-1, MH-34-3, MH-34-8, MH-39-1, MH-39-3, MH-39-7, MH-50-8, MH-55-3, MH-55-7, MH-55-9, MH-60-1, MH-60-3 and MH-60-5) of 49 isolates do nitrogen fixation, 9 (MH-34-5, MH-34-8, MH-39-5, MH-50-6, MH-50-7, MH-50-8, MH-55-6, MH-60-1 and MH-60-3) produce siderophores, 8 (MH-28-1, MH-34-2, MH-34-4, MH-34-5, MH-39-1, MH-55-3, MH-60-2 and MH-60-5) produce HCN, and 4 (MH-34-8, MH-50-4, MH-50-8 and MH-55-9) produce IAA (*Table 4*). Siderophore-producing isolates were analyzed by measuring zone diameter. The best siderophore-producing isolates are presented in *Table 4*. Isolates with high zone diameter are as follows in decreasing order: MH 60-1, MH- 60-3, MH-50-8, MH-55-6, MH 50-6, MH-50-7, MH 39-5, MH -34-8, MH-34-5.

Many studies have shown that different bacteria belonging to the Pseudomonas, Bacillus, Rhizobium, Acinetobacter, and Paenibacillus genera increase plant growth in the rhizosphere (Akhtar et al., 2009; Wang et al., 2013). Pseudomonas and Bacillus bacteria stand out with their plant growth-enhancing properties, as emphasized in numerous studies. Bacillus, a common soil genus, forms aerobic endospores and is considered the primary taxon cultivated from plant rhizospheres, accounting for 56.2% of soil bacteria (Sezen et al., 2016). Many research on inorganic phosphate solubility, HCN production, siderophore synthesis, nitrogen fixation, and IAA production of PGPRs in vitro have been undertaken (Baghaee and Heidarzadeh, 2014; Amara et al., 2015). Atmospheric nitrogen is converted to its (NH_4) form, which can be used by plants by microorganisms for biological nitrogen fixation (BNF). The conversion of atmospheric nitrogen to ammonium is known as biological nitrogen fixation, or diazotrophy (Deka et al., 2015). According to the findings of Zaidi and Khan (2005) the application of various

Table 4. PGP characteristics of isolates

Isolates	Inorganic phosphate solubility	Nitrogen fixation	Siderophores production	Siderophores zone diameter (mm)	HCN production	IAA production
Bacillus atrophaeus MH-28-1	-	+	-	-	+	-
Bacillus simplex MH-28-2	-	-	-	-	-	-
Bacillus simplex MH-28-3	-	-	-	-	-	-
Bacillus simplex MH-28-4	-	-	-	-	-	-
Bacillus megaterium MH-28-5	-	-	-	-	-	-
Bacillus simplex MH-28-6	-	-	-	-	-	-
Bacillus simplex MH-28-7	-	-	-	-	-	-
Pseudomonas anguilliseptica MH-28-8	-	-	-	-	-	-
Bacillus cereus MH-34-1	-	-	-	-	-	-
Bacillus muralis MH-34-2	-	-	-	-	+	-
Hafnia alvei MH-34-3	-	+++*	-	-	-	-
Bacillus mojavensis MH-34-4	-	-	-	-	+	-
Bacillus sp. MH-34-5	-	-	+	2.66±1.90e	+	-
Bacillus simplex MH-34-7	-	-	-	-	-	-
Pseudarthrobacter oxydans MH-34-8	-	+	+**	3.00±1.20de	-	++
Bacillus atrophaeus MH-39-1	-	+	-	-	+***	-
Bacillus cereus MH-39-2	-	-	-	-	-	-
Weeksella virosa MH-39-3	-	++	-	-	-	-
Bacillus pseudomycoides MH-39-4	-	-	-	-	-	-
Bacillus pumilus MH-39-5	-	-	+	4.33±1.83cde	-	-
Actinomyces viscosus MH-39-6	-	-	-	-	-	-
Bacillus thuringiensis MH-39-7	-	+	-		-	-
Bacillus cereus MH-39-8	-	-	-	-	-	-
Bacillus simplex MH-39-9	-	-	-	-	-	-
Bacillus simplex MH-39-10	-	-	-	-	-	-
Bacillus simplex MH-50-1	-	-	-	-	-	-
Brevibacillus brevis MH-50-2	-	-	-	-	-	-
Brevibacillus parabrevis MH-50-3	-	-	-	-	-	-
Staphylococcus hominis MH-50-4	-	-	-	-	-	+
Bacillus megaterium MH-50-5	-	-	-	-	-	-
Bacillus mojavensis MH-50-6	+	-	+	4.66±0.28cd	-	-
Bacillus subtilis MH-50-7	+	-	+	4.33±0.12cde	-	-
Pseudomonas jessenii MH-50-8	-	+	+	7.00±1.90b	-	+++
Bacillus simplex MH-55-1	-	-	-	-	-	-
Bacillus simplex MH-55-2	-	-	-	-	-	-
Bacillus atrophaeus MH-55-3	-	+	-	-	+	-
Bacillus simplex MH-55-5	-	-	-	-	-	-
Bacillus pumilus MH-55-6	-	-	+	5,00±1.28c	-	-
Glutamicibacter arilaitensis MH-55-7	-	+	-	-	-	-
Bacillus simplex MH-55-8	-	-	-	-	-	-
Pseudarthrobacter polychromogenes MH-55-9	-	+	-	-	-	+
Arthrobacter crystallopoietes MH-60-1	+	++	+	10.33±0.79a	-	-
Paeniclostridium sordellii MH-60-2	-	-	-	-	+	-
Aromatoleum evansii MH-60-3	+	++	+	8.33±0.77b	-	-
Bacillus simplex MH-60-4	-	-	-	-	-	-
Citrobacter freundii MH-60-5	-	+++	-	-	+	-
Paracoccus denitrificans MH-60-6	-	-	-	-	-	-
Bacillus megaterium MH-60-7	-	-	-	-	-	-
Bacillus cereus MH-60-8	-	-	-	-	-	-

*For nitrogen fixation activity (+++: development after 6 hours, ++: development after 12 hours, +: development after 24 hours).
**For siderophore 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
Data expressed as means ± standard deviation. Values with different letters differ significantly (P <0.01)

strains of PGPR resulted in a significant increase in nitrogen uptake by the wheat shoot, ranging from 27% to 94%. Rana et al. (2011) determined that two of the 10 isolates, specifically *Providencia* sp., were obtained from the wheat rhizosphere. *Providencia* sp. AW4 and *Brevundimonas diminuta* AW7, demonstrated the most significant levels of activity in the nitrogen fixation test. In a study conducted by Sezen et al. (2016) it was found that out of a total of 180 bacteria that were isolated from the rhizosphere of wheat plants, 16 of them exhibited the ability to fix nitrogen. In the present study, 13 isolates (MH-28-1, MH-34-3, MH-34-8, MH-39-1, MH-39-3, MH-39-7, MH-50-8, MH-55-3, MH-55-7, MH-55-9, MH-60-1, MH-60-3 and MH-60-5) performed nitrogen fixation (*Table 4*). MH-63-7 (*Blastomonas natatoria*) and MH-64-3 (*Aromatoleum evansii*) isolates showed high levels of nitrogen fixation (*Figure 5b*). Among all isolates, the nitrogen-fixing isolates accounted for 34% (*Figure 4*).

Many bacteria can dissolve inorganic and organic phosphorus in the soil. These are known as phosphate-solving bacteria (PSB). The presence of a significantly elevated population of phosphate solubilizing bacteria in the rhizosphere, as opposed to non-rhizospheric soil, has been well-established in previous studies (Patten and Glick, 2002). These bacteria dissolve phosphate through several mechanisms, particularly acid production. These bacteria release available phosphate into the soil by increasing the solubility of organic and inorganic P complexes. Some soil bacteria, such as Pseudomonas and Bacillus, can dissolve insoluble phosphates, which has a significant effect on plant growth-promoting activity (Mehnaz and Lazarovits, 2006). The utilization of *Bacillus* as a biofertilizer in the field of agriculture has been extensively documented. One inherent characteristic of *Bacillus* is its capacity to solubilize phosphate. *Bacillus polymyxa*, *Bacillus subtilis*, and various other species are recognized as highly proficient bacterial communities with regards to phosphate solubilization (Illmer and Schinner, 1992). Additionally, there have been reports of inorganic phosphate solubilization by *Acinetobacter* sp. (Ogut et al., 2010; Chaiharn and Lumyong, 2011). Mukhtar et al. (2017) determined that PSB5 (*Bacillus safensis*) and PSB12 (*Bacillus megaterium*) from wheat rhizosphere solubilized high phosphate levels (305.6, 217.2, and 148.1 µg ml^{-1}). In our study, isolates MH-50-6 (*Bacillus mojavensis*), MH-50-7 (*Bacillus subtilis*), MH-60-1 (*Arthrobacter crystallopoietes*) and MH-60-3 (*Aromatoleum* evansii) were shown to have inorganic phosphate solubilization ability (*Figure 5a*, *Table 4*). Among all isolates, the isolates with inorganic phosphate-dissolving ability were determined to be 10% (*Figure 4*).

Iron, which is an indispensable inorganic substance in all living organisms, is abundant in the Earth's crust in the form of ferric hydroxide in an insoluble form. Owing to the very low solubility of Fe^{+3} ions at neutral pH, they cannot be used by plants and soil bacteria. Therefore, bacteria eliminate this problem by producing siderophores. This process facilitates the accessibility of iron to plants while rendering it inaccessible to phytopathogens. Therefore, concurrently, the siderophore serves to safeguard the overall well-being of the plant (Erdem, 2013). According to Meyer (2000) various strains of Pseudomonas exhibit the capacity to synthesise significant quantities of siderophore. Rasuli et al. (2006) observed that a total of 201 strains of *Pseudomonas fluorescens*, which were obtained from the rhizosphere of wheat plants, demonstrated the ability to synthesise siderophores in their study. Recent research has shown that the use of bacteria that produce siderophores is linked to a notable rise in the amount of iron that plants absorb, which enhances the amount of chlorophyll, the area of leaves, and the rate of photosynthetic activity (Ehsan et al., 2022; Mushtaq et al., 2020). In the current investigation, siderophores were formed by the isolates MH-34-5, MH-34-8 (*Pseudarthrobacter oxydans*), MH-39-5 (*Bacillus pumilus*), MH-50-6 (*Bacillus mojavensis*), MH-50-7 (*Bacillus subtilis*), MH-50-8 (*Pseudomonas jessenii*), MH-55-6 (*Bacillus pumilus*), and MH-60-3 (*Aromatoleum evansii*) (*Figure 5c*, *Figure 6*, *Table 4*). If we compare the siderophore zone diameters of the isolates, the first three are *Arthrobacter crystallopoietes* MH-60-1, *Aromatoleum evansii* MH-60-3, *Pseudomonas jessenii* MH-50-8 isolates with zone diameters of 12mm, 9mm and 7mm, respectively (*Figure 6*). It has been demonstrated that 24% of isolates generate siderophores. Conversely, *Arthrobacter* sp. was found to produce siderophores by Sayyed et al. (2012). Likewise, we determined that the isolate of *Arthrobacter crystallopoietes* MH-60-1, generated siderophores.

Hydrogen cyanide (HCN) has been widely recognised for its significant contribution to the suppression of diseases. HCN production by soil bacteria is reported to play a role in disease suppression, as in the case of tobacco where *Pseudomonas fluorescens* helped suppression of black root rot disease (Haas and Defago, 2005). According to Siddiqui (2006), it has been reported that hydrogen cyanide (HCN) is a volatile secondary metabolic product that exhibits growth-suppressing properties against pathogens. This suppression is attributed to the inhibition of metal enzymes, particularly cytochrome c oxidases, within the electron transport system. Wani et al. (2007) reported that most rhizospheric isolates produced HCN and aided plant growth. El Habil-Addas et al. (2017) isolated RS15 (*Pantoea allii* strain BD 390) and RS 19 (*Stenotrophomonas maltophilia* strain IAM 12423) strains from wheat rhizosphere and determined that they produced HCN. When we examined the bacteria we isolated in terms of Hydrogen cyanide (HCN), it has been determined that the isolates MH-28-1 (*Bacillus atrophaeus*), MH-34-2 (*Bacillus muralis*), MH-34-4 (*Bacillus mojavensis*), MH-34-5 (*Bacillus* sp.), MH-39-1 (*Bacillus atrophaeus*), MH-55-3, MH-60-2 (*Paeniclostridium sordellii*), and MH-60-5 (*Citrobacter freundii*) possess the potential to produce HCN (*Table 4, Figure 5d*). HCN production was observed in 21% of isolates (*Figure 4*). Therefore, the use of HCN-producing bacteria as biopesticides offers an environmentally friendly approach for sustainable agriculture.

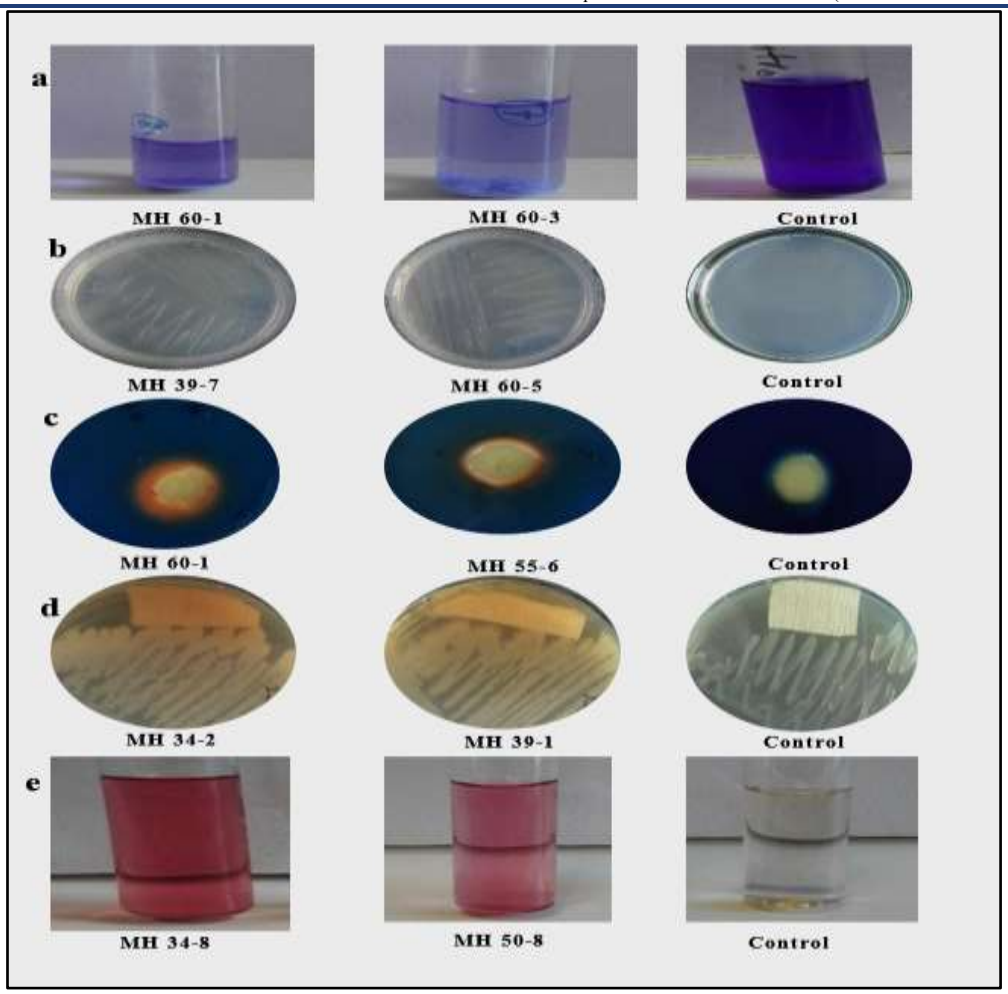

Figure 5. PGP test images of isolates (a Inorganic phosphate solubility, b Nitrogen fixation, c Siderophores production, d HCN production, e IAA production)

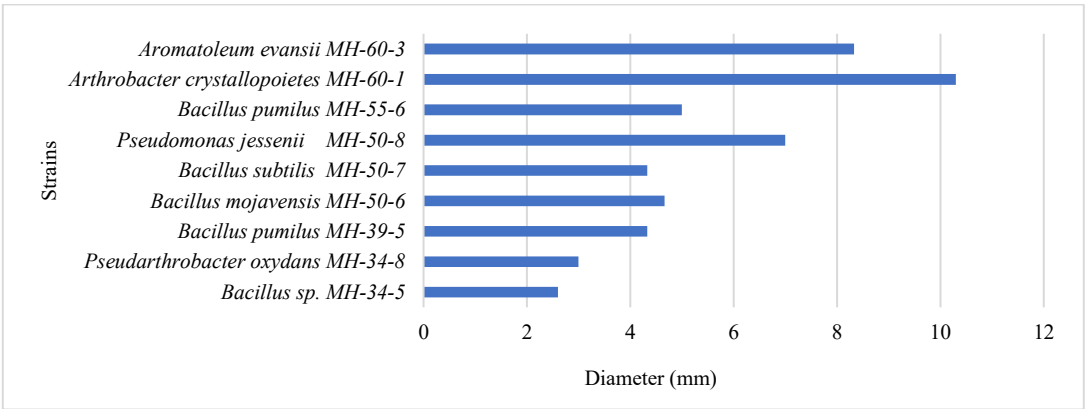

Figure 6. Siderophore production zone diameters of isolates

IAA (Indole-3-acetic acid), a member of the phytohormone group, is generally considered to be the most important natural auxin. IAA is the most active hormone involved in long-term physiological events, such as cell elongation, cell division, and differentiation in plants. It is estimated that 80% of bacteria isolated from the rhizosphere can produce IAA (Sokolova et al., 2011). Plant hormone producing microbes increase root surface area, leading to greater nutrient uptake, as reported by Bai et al. (2003). Numerous studies demonstrate the correlation between the promotion of plant growth by *Bacillus* spp. and the signalling of phytohormones (Faisal and Hasnain, 2006; López-Bucio et al., 2007; Baghaee and Heidarzadeh, 2014). In our current experiment, only four isolates MH-34-8 (*Pseudarthrobacter oxydans*), MH-50-4 (*Staphylococcus hominis*), MH-50-8

(*Pseudomonas jessenii*), and MH-55-9 (*Pseudarthrobacter polychromogenes*) were found to be IAA (*Figure 5e*). We found that it produces 8% (*Figure 4*).

4. Conclusions

Some of our isolates gave positive results for at least one PGP (Plant growth promoting) trait (IAA production, nitrogen fixation, phosphorus solubilization, siderophore, HCN production). In light of these data, 22 of 49 isolates are positive for at least one PGP feature. Therefore, it appears that 45% of the isolates we obtained as a result of our study have the potential to be used in microbial fertilizer production.

Finally, the present investigation is the first to look at plant growth-promoting bacteria isolated from the soil of a local wheat rhizosphere in Kırşehir of Turkey. Bacillus phosphate-solubilizing and siderophore-producing bacteria dominate the wheat rhizosphere soil. The recovered bacteria are expected to be useful in organic agriculture as well as biofertilizer manufacture, which will promote plant development and provide the sustainability of agriculture. Therefore, further research on PGPR is necessary to help to create more effective local rhizobacterial strains that can function in several agro-ecological environments.

Acknowledgment

This work was not supported financially by any organization.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authorship Contribution Statement

Concept: Güler, M.; Design: Öğütcü, H.; Data Collection or Processing: Güler, M.; Statistical Analyses: Numanoğlu Çevik, Y.; Literature Search: Öğütcü, H.; Writing, Review and Editing: Güler, M.

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