Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2024) 41(3) 201-208
doi:10.55507/gopzfd.1529947

Identification of Beneficial Bacteria in Rosemary Rhizospheres and Determination of Plant Growth Promoting (PGP) Potential

Murat GÜLER[1*]

[1]Ministry of National Education, Kırşehir
***Corresponding author's email:** volvox2015@gmail.com

Alındığı tarih (Received): 07.08.2024 Kabul tarihi (Accepted): 20.11.2024

Abstract: Plant growth-promoting rhizobacteria (PGPR), which colonize the rhizosphere, are eco-friendly and beneficial bacteria that directly or indirectly promote plant growth. In this study, 13 isolates from the rhizosphere of *Rosmarinus officinalis* L. (Rosemary) were identified using MALDI-TOF-MS to assess morphology, biochemistry, and plant growth-promoting traits and to evaluate their antagonistic effects against *Fusarium oxysporum*. Among all isolates, 9 isolates fixed nitrogen, 8 isolates dissolved inorganic phosphate, 8 isolates produced siderophores, 7 isolates produced IAA (Indole-3-Acetic Acid), and 6 isolates produced HCN. Isolate BBR-6 showed the highest antifungal activity against *Fusarium oxysporum*, with an inhibition rate of 61.54 %. The isolate BBR-10 (19.40 %) showed the weakest effect against *F. oxysporum*. Although research on PGPRs has increased recently, research on rosemary is still limited. This study aimed to identify the local bacterial community in the rhizosphere of rosemary and assess its growth-promoting properties and antifungal activity against disease-causing *F. oxysporum*, potentially acting as a microbial fertilizer and biocontrol agentents.

Key words: Rosemary, *Fusarium oxysporum*, MALDI TOF MS, microbial fertilizer, PGPR

Biberiye Rizosferindeki Yararlı Rizobakterilerin Tanımlanması ve Bitki Gelişimini Teşvik Edici Özelliklerinin Belirlenmesi

Öz: Bitki büyümesini teşvik eden rizobakteriler (PGPR), rizosferde kolonize olan, doğrudan ya da dolaylı olarak bitki büyümesini destekleyen çevre dostu faydalı bakterilerdir. Bu çalışmada, *Rosmarinus officinalis* L. (Biberiye) rizosferinden on üç izolat MALDI-TOF-MS methodu ile tanımlanarak morfolojik, biyokimyasal, bitki büyümesini teşvik edici özellikleri ile *Fusarium oxysporum*'a karşı antagonistik özellikleri değerlendirildi. Tüm izolatlar arasında 9 izolatın azot fikse ettiği, 8 izolatın norganik fosfatı çözdüğü, 8 izolatın siderofor ürettiği, 7 izolatın IAA (indole-3-acetic acid) ürettiği ve 6 izolatın HCN ürettiği belirlendi. BBR-6 izolatı, *Fusarium oxysporum*'a karşı % 61.54'lük bir inhibisyon oranıyla en yüksek antifungal aktiviteyi gösterdi. BBR-10 izolatı ise % 19.40 ile *F. oxysporum*'a karşı en zayıf etkiyi gösterdi. PGPR'ler üzerindeki araştırmalar son zamanlarda artsa da biberiye üzerine yapılan araştırmalar hala sınırlıdır. Bu çalışma, biberiye rizosferindeki yerel bakteri topluluğunu tanımlamayı, bitki büyümesini teşvik edici özelliklerini, mikrobiyal gübre ve biyokontrol ajan potansiyeli ile biberiye bitkisinde kök hastalığa neden olan *F. oxysporum*'a karşı antifungal aktivitesini değerlendirmeyi amaçlamaktadır.

Anahtar Kelimeler: Biberiye, *Fusarium oxysporum,* MALDI TOF MS, mikrobiyal gübre, PGPR

1. Introduction

Rosmarinus officinalis L. (Rosemary), belonging to the Lamiaceae family, is an important medicinal and aromatic plant that can grow up to 3 meters tall. *Rosmarinus officinalis* L. which grows naturally in many Mediterranean countries, has been used for various medical and culinary purposes since ancient times. The essential oils in rosemary contain different chemical compounds such as alcohol, hydrocarbons, phenols, aldehydes, esters, and ketones. It has been indicated that the essential oils obtained from rosemary flowers and leaves are widely used to treat conditions such as asthma, cataracts, rashes, headaches, indigestion, and renal colic (Hammer & Junghanns, 2020). Chemical fertilizers are harmful to soil and ecosystems, particularly human health. Excessive use of chemical fertilizers causes significant soil pollution and decreases crop yield. Therefore, using eco-friendly natural fertilizers instead of chemical ones in growing rosemary is crucial for sustainable farming. PGPR are bacteria colonizing the plant's rhizosphere and directly or indirectly supporting plant growth. PGPRs utilize soil nutrients for plant growth, produce many regulators, protect plants from phytopathogens, improve soil structure, and reduce harmful compounds like pesticides. Therefore, rhizobacteria are crucial microorganisms for soil fertility and sustainable crop production (Rochlani et al., 2022). Although research on PGPRs has increased recently, it is still limited. Recent studies suggest that developing microbial formulations with local isolates showing activity in different ecosystems and plant species should be increased. Therefore, developing microbial fertilizers that protect the environment and comply with sustainable agriculture can significantly reduce chemical fertilizer use. This study aimed to isolate, identify, and determine the plant growth-promoting properties of rhizobacteria associated with *Rosmarinus officinalis* used in many fields, especially in medicine and pharmacy.

2. Material and Method

2.1. Sample collection

Rhizospheric soil samples were collected in May 2023 from *R. officinalis* in the Medicinal Plants Garden of the Department of Field Crops of Ankara University Faculty of Agriculture (39°57'44.2"N, 32°51'36.7"E).

2.2. Isolation of rhizobacteria

Rhizospheric bacteria were isolated from 1 g of dried soil samples by serial dilution method. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6}. These dilutions were spread on nutrient agar (NA) solidified Petri dishes and incubated at 28 °C. After incubation, different colonies were selected and planted in Petri dishes containing NA medium until a pure colony was obtained.

2.3. Identification of bacterial Isolates

Biochemical and morphological characterization of isolates

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

2.3.1. Identification of isolates

MALDI-TOF MS was used for bacterial identification by analyzing unique molecular fingerprints with the MALDI Biotyper System (Sivri & Öksüz, 2019).

2.4. Plant growth promoting (PGP) properties

2.3.1. Determination of nitrogen fixation ability

Determination of the nitrogen fixation abilities of the isolates was done according to the protocol specified by Wilson and Knight (1952) and Park et al. (2005). The nitrogen fixation activity was assigned three time intervals (+++: development after 6 hours, ++: development after 12 hours, and +: development after 24 hours).

2.3.2. Evaluation of siderophore production

Chrome Azurol S (CAS) agar media was used to identify the production of siderophores. (Schwyn & Neilands, 1987). The siderophore activity was assigned three time intervals (+++: color change after 12 h, ++: color change after 24 h, +: color change after 36 h).

2.3.3. Determination of HCN-producing isolates

The HCN production assay was carried out according to Bakker and Schippers (1987). The HCN activity was assigned three time intervals (+++: color change after 6 h, ++: color change after 12 h, +: color change after 24 h).

2.3.4. Assessment of indole-3-acetic acid (IAA) production

The Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce IAA. The IAA activity was assigned three time intervals (+++: color change after 1 h, ++: color change after 6 h, and +: color change after 12 h).

2.3.5. Determination of inorganic phosphate dissolving capacity

The inorganic phosphate dissolving capacities of the isolates were determined according to the protocol described by Mehta and Nautiyal (2001).

2.5. Assessment of antifungal activity

The antifungal activity of the isolates was tested against *Fusarium oxysporum* using the dual culture method (Landa et. al., 1997). The mycelial growth diameter of each phytopathogen was measured according to the method described by Royse and Ries (1978) to determine the percentage of fungal inhibition (FI). The experiments were conducted in triplicates.

FI (%) = (B-M) / B x 100

Where B is the average diameter of mycelial development without bacterial isolate.

M is the mean diameter of mycelial growth in the presence of the bacterial isolate.

2.6. Data analysis

Data for antifungal activity and IAA production were analyzed using JMP Pro 17.0 statistical software, based on three replicates. Dependant variables with normal distribution were shown as mean ± standard deviation (SD).

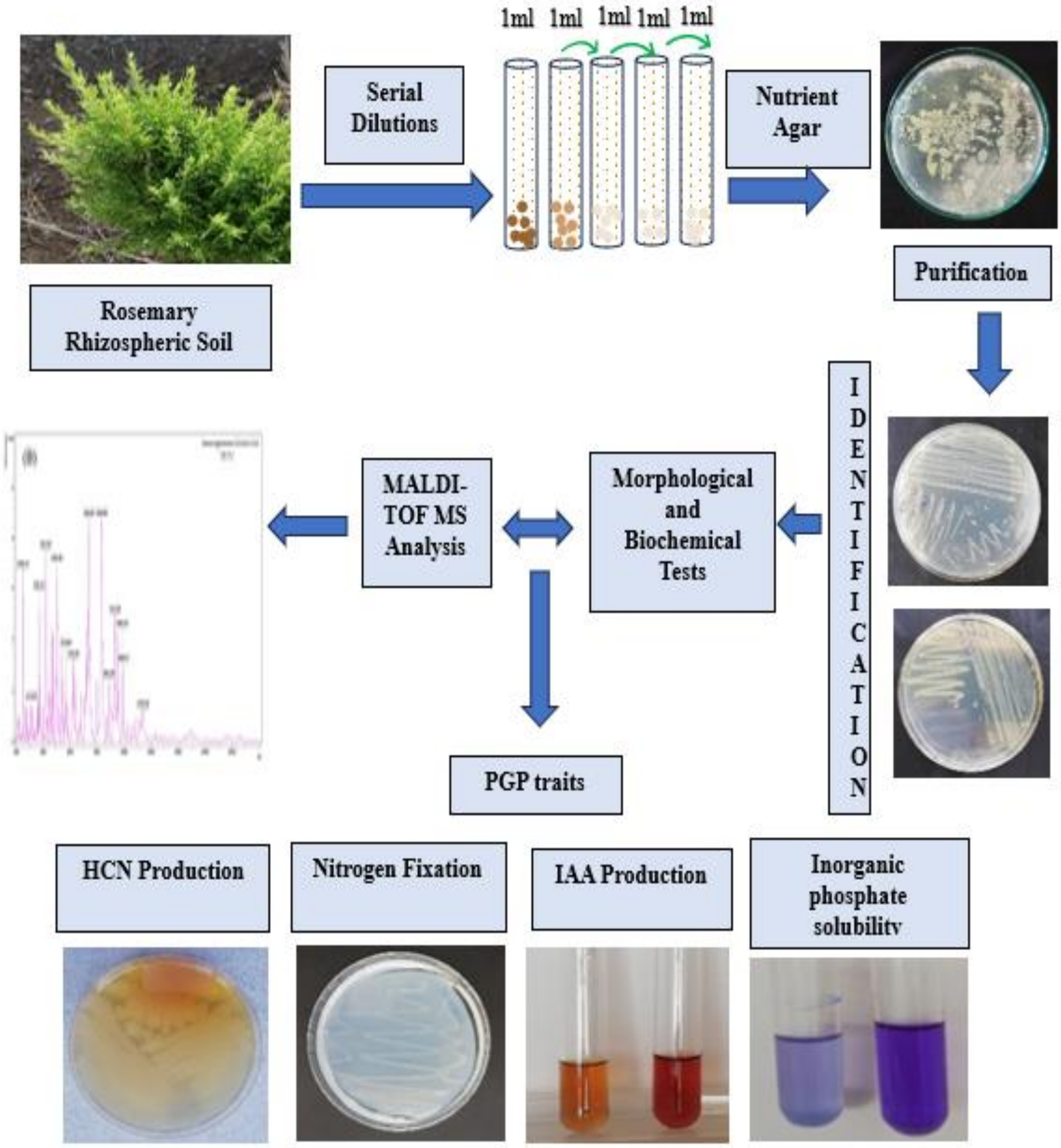

Figure 1. Flow chart for identification of isolates in Rosemary rhizospheric soil samples
Şekil 1. *Biberiye rizosferik toprak örneklerinde izolatların tanımlanmasına yönelik akış şeması*

3. Results and Discussion

3.1. Identification of isolates

In this study, 13 isolates were identified from Rosemary's rhizospheric soil samples using the MALDI-TOF MS method; these include 6 Bacillus (BBR-1, BBR-2, BBR-3, BBR-6, BBR-9, BBR-13), 3 Pseudomonas (BBR-7, BBR-8, BBR-12), 2 Lactobacillus (BBR-5, BBR-11), 1 Pantoea (BBR-4) and 1 *Pseudarthrobacter* (BBR-10). Some morphological and biochemical characteristics of rhizobacterial isolates are given in Table 1.

MALDI-TOF MS is an extremely useful tool for identifying bacteria at the genus, species, and strain levels. In previous studies, many researchers used the MALDI-TOF MS method to identify bacteria (Nazir et al., 2020; Tamura, 2023). Recently, this method has gained popularity due to its high accuracy and rapid results. Martínez-Hidalgo et al. (2021) identified endophytic bacteria from canola roots using the MALDI TOF MS method. Similarly, Öksel et al. (2022) used the MALDI-TOF MS method to identify bacteria in wheat rhizosphere. The findings of this study revealed that *Bacillus* (46%) *Pseudomonas* (23%) and were the most common bacterial genera in the Rosemary rhizosphere.

Table 1. Morphological and biochemical traits of rhizobacterial isolates
Çizelge 1. *Rizobakteriyel izolatların morfolojik ve biyokimyasal özellikleri*

Isolates No	MALDI-TOFMS results	Gram Stain Test	Motility	Colony color	Biochemical Characteristics		
					Catalase	Oxidase	KOH %3
BBR-1	*Bacillus thuringiensis*	+*	+	whitish	+	-	-
BBR-2	*Bacillus pumilus*	+	+	cream	+	**	-
BBR-3	*Bacillus megaterium*	+	+	white	+	-	-
BBR-4	*Pantoea agglomerans*	-	+	light yellow	+	-	+
BBR-5	*Lactobacillus paracasei*	+	-	white	-	-	-
BBR-6	*Bacillus mojavensis*	+	+	white	+	+	-
BBR-7	*Pseudomonas libanensis*	-	+	light yellow	+	+	+
BBR-8	*Pseudomonas chlororaphis*	-	+	white	+	+	+
BBR-9	*Bacillus cereus*	+	+	white	+	-	-
BBR-10	*Pseudarthrobacter scleromae*	+	-	white	+	-	-
BBR-11	*Lactobacillus oligofermentans*	+	-	white	-	-	-
BBR-12	*Pseudomonas fluorescens*	-	+	white	+	+	+
BBR-13	*Bacillus simplex*	+	+	cream	+	-	-

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

3.2. Plant growth-promoting properties of isolates

In the current study, 13 isolates were screened for siderophores, phosphate solubility, nitrogen fixation, IAA, and HCN production abilities. Among all isolates, 69% (BBR-2, BBR-3, BBR-4, BBR-7, BBR-8, BBR-9, BBR-10, BBR-12, BBR-13) fixed nitrogen, 46% (BBR-1, BBR-5, BBR-6, BBR-9, BBR-11, BBR-12) produced HCN, 61% (BBR-2, BBR-3, BBR-4, BBR-6, BBR-7, BBR-8, BBR-12, BBR-13) dissolved inorganic phosphate, 61% (BBR-2, BBR-4, BBR-6, BBR-8, BBR-9, BBR-10, BBR-12, BBR-13) produced siderophores, and 54% (BBR-1, BBR-4, BBR-6, BBR-7, BBR-9, BBR-10, BBR-12) produced IAA. Siderophores are low-molecular-weight, iron-chelating organic substances produced by many rhizospheric bacteria. The majority of Pseudomonas and Bacillus strains in the rhizosphere can produce siderophores (Joseph et al., 2007). Rudakova et al. (2023) reported that *Bacillus pumilus* strain 3-19, which has plant growth-promoting properties, produces siderophores. In the present study, *B. pumilus* BBR-2, *B. mojavensis* BBR-6, and *B. cereus* BBR-9 produced siderophore. Many studies are showing that *Pseudomonas* sp. produces siderophores (Saranraj et al., 2023; Clericuzio et al., 2024). Subramanium & Sundaram (2020) reported that *P. fluorescens* PSF02 isolated from agricultural soils also produced siderophores. In the current study, *P. fluorescens* BBR-12 produced siderophore. Wei et al. (2023) reported that rhizospheric *Pseudomonas chlororaphis* IRHB3 produced siderophore. Interestingly, In the current study, *P. chlororaphis* BBR-8 produced siderophore. Previous studies have shown that *Pantoeae agglomerans* also produced siderophores (Hynes et al., 2008; Shariati et al., 2017). Similarly, In the present study, *P. agglomerans* BBR-4 produced siderophore. Nitrogen is a vital element used in many structural and functional processes in the cell. There are a lot of studies that prove bacteria in the roots of plants like rosemary (Stamenov et al. 2021) and oregano (Loera-Muro et al. 2021) can fix nitrogen. *Bacillus* spp., about which a great deal of research has been conducted, are among the most abundant bacteria in soil. According to the available literature, *B. pumilus* (Agake et al. 2021), and *B. subtilis* (Sharma et al. 2021) strains were identified to exhibit nitrogen fixation. In the present study, *B. pumilus* BBR-2 *and B. megaterium* BBR-3 fixed nitrogen. Singh et al., (2023) determined that *P. koreensis* CY4 isolated from sugarcane rhizosphere fixed nitrogen. Shi et al., (2023) reported that *Pseudomonas* sp. in the rhizosphere of *A. mongolicus*, a Chinese medicinal plant, fixed nitrogen. Similarly, In the current study, *P. libanensis* BBR-7 *and P. chlororaphis* BBR-8 fixed nitrogen. According to Sharma et al. (2021), 80% of bacteria isolated from the rhizosphere can produce IAA. According to spectrophotometric analysis, the highest IAA production was obtained in *B. mojavensis* BBR-6 with 18.37 $\mu g/mL^{-1}$ in line with the present study. This was followed by *B. cereus* BBR-9 with 17.89 $\mu g/mL^{-1}$ and *P. agglomerans* BBR-4 with 17.66 $\mu g/mL^{-1}$, respectively. Stamenov et al. (2021) reported that *Pseudomonas* sp. P42 strain in rosemary rhizosphere produced IAA. Similarly, in this study, *P. fluorescens* BBR-12, and *P. libanensis* BBR-7 produced IAA. In a recent study, Patel et al. (2024) reported that rhizospheric *P. chlororaphis* did not produce IAA. Likewise, in this study, *P. chlororaphis* BBR-8 did not produce IAA. Khatami et al. (2023) reported that rhizospheric *Bacillus* sp. synthesized high amounts of IAA. Similarly, In the current study, *B. thuringiensis* BBR-1, *B. mojavensis* BBR-6, and *B. cereus* BBR-9

Table 2. Plant growth promoting traits of the rhizobacterial isolates
Çizelge 2. *Rizobakteriyel izolatların bitki gelişimini teşvik edici özellikleri*

Isolates	PGP traits					OD at 530 nm Mean ± SD
	Inorganic phosphate solubization	Nitrogen fixation	Siderophore production	HCN production	IAA production	
Bacillus thuringiensis BBR-1	-	-	-	+++*	+	8.42 ± 0.07^{d}
Bacillus pumilus BBR-2	+	++**	+	-	-	$2.33\pm0.22^{\text{hı}}$
Bacillus megaterium BBR-3	+	+++	-	-	-	3.81 ± 0.37^{f}
Pantoea agglomerans BBR-4	+	+++	+++***	-	+++****	17.66 ± 0.67^{a}
Lactobacillus paracasei BBR-5	-	-	-	+	-	$3.06\pm0.19f^{gh}$
Bacillus mojavensis BBR-6	+	-	+	+	+++	18.37 ± 0.08^{a}
Pseudomonas libanensis BBR-7	+	++	-	-	+	6.56 ± 0.29^{e}
Pseudomonas chlororaphis BBR-8	+	++	+	-	-	$2.18\pm0.05^{\text{ı}}$
Bacillus cereus BBR-9	-	+	+	+	+++	17.89 ± 0.09^{a}
Pseudarthrobacter scleromae BBR-10	-	+	+	-	++	11.55 ± 0.33^{b}
Lactobacillus oligofermentans BBR-11	-	-	-	+	-	3.27 ± 0.26^{fg}
Pseudomonas fluorescens BBR-12	+	++	+++	++	++	10.51 ± 0.12^{c}
Bacillus simplex BBR-13	++	+	+	-	-	$2.79\pm0.14^{\text{ghı}}$

* The color changes for HCN activity are as follows: +++: after 6 hours, ++: after 12 hours, +: after 24 hours.**Nitrogen fixation activity (+++: development after 6 h, ++: development after 12 h, +: development after 24 h).*** The color changes for siderophore activity are as follows: +++: color change after 12h, ++: color change after 24h., +: color change after 36h.**** The color changes for IAA activity are as follows: +++: colour change after 1h, ++: colour change after 6h, and +: colour change after 12h. For OD at 530 nm: p<0,001; statistically significant level. a-ı: The difference between the means shown by different letters in the same column is statistically significant. (Mean ± SD: Mean±Standard Deviation)

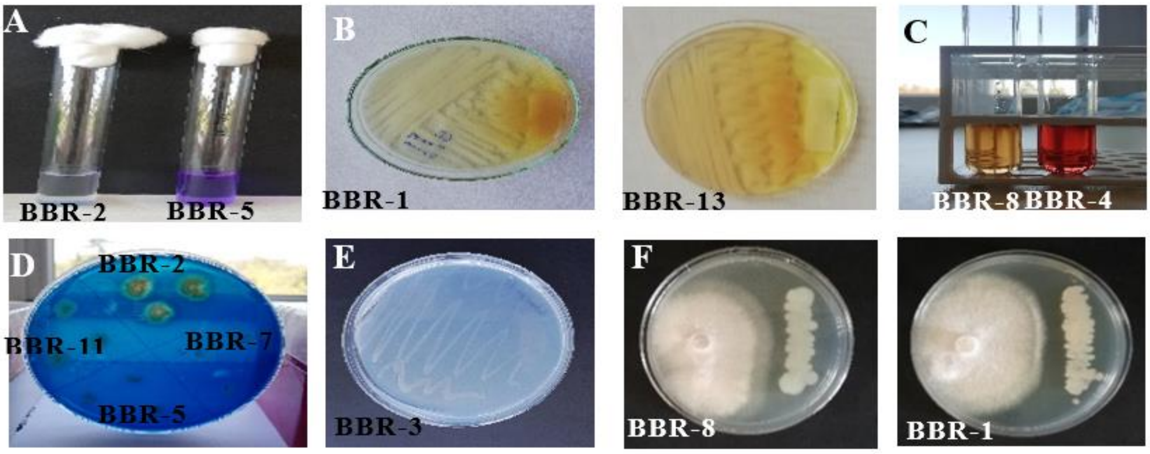

Figure 2. PGPR and antifungal activity test images of rhizobacterial isolates (A: Inorganic phosphate solubization B: HCN production C: IAA production D: Siderophore production E: Nitrogen fixation F: Antifungal test of isolates against *F. oxysporum*)
Şekil 2. *Rizobakteriyel izolatların PGPR ve antifungal aktivite testi görüntüleri (A: İnorganik fosfat çözünümü B: HCN üretimi C: IAA üretimi D: Siderophore üretimi E: Azot fiksasyonu F: İzolatların F. oxysporum'a karşı antifungal testi)*

produced IAA. According to Anderson and Kim (2018), HCN produced by *Pseudomonas* and *Bacillus* strains is an important factor that protects the plant against phytopathogens. Ahmad et al. (2008) determined that among rhizospheric bacteria, 50% of *Bacillus* isolates and 80% of *Pseudomonas* isolates were positive for HCN production. Singh et al. (2019) determined that *B. thuringiensis* SF 23, *P. aeruginosa* SF 44, *B. subtilis* SF 48, and *B. subtilis* SF 90 isolate produced HCN. Stamenov et al. (2021) determined that *Pseudomonas* and *Bacillus* obtained from rosemary rhizosphere produced HCN. Kumar et al. (2021) reported that *Bacillus pumilus* strain JPVS11 in the rice rhizosphere produced HCN. Halimursyadah et al. (2023) reported that *P. fluorescens* produced HCN among the 37 isolates from the patchouli rhizosphere. Similarly, In the current study, *P. fluorescens* BBR-12 also produced HCN. Bacteria like *Pseudomonas* and *Bacillus* in the rhizosphere employ various mechanisms to dissolve phosphate and release it into the soil. According to Rawat et al. (2021), the most prevalent inorganic phosphate-solubilizing bacteria in the rhizosphere are *Bacillus*, *Enterobacter*, and *Pseudomonas*. Similarly, in the current study, *B. pumilus* BBR-2, *B. megaterium* BBR-3, *B. mojavensis* BBR-6, *B. simplex* BBR-13 dissolved inorganic phosphate. Sharma et al. (2021) reported that *Pseudomonas libanensis* HB4N3 strain has high inorganic phosphate solubilization ability. Similarly, In the current study, *P. libanensis* BBR-7 dissolved inorganic phosphate. Amri et al. (2023) determined that *Pseudomonas fluorescens* dissolved inorganic phosphate at high density (618.57 µg mL^{-1}). In the current study, *P. fluorescens* BBR-12 dissolved inorganic phosphate. Our findings are consistent with other studies. Table 2 and Figure 2 presents the plant growth-promoting properties of the isolates.

3.3. Antifungal activity

In the current study, the Antifungal activity of isolates obtained from *R. officinalis* rhizosphere was tested against *Fusarium oxysporum* and the inhibition percentages varied between 19.40% and 61.54%. Among the isolates, isolate BBR-6 demonstrated the strongest antagonism against *F. oxysporum* with a high percentage inhibition value (61.54%), followed by isolate BBR-9 (55.35%). Isolate BBR-10 (19.40%) showed the weakest effect against the pathogen (Table 3). *F. oxysporum* is a major soil-borne plant pathogen that causes economically significant diseases in agricultural production worldwide. *R. officinalis* wilt caused by *F. oxysporum* causes yield losses. *Bacillus* spp. is regarded as a successful bacteria capable of synthesizing a diverse range of useful compounds. The production of antifungal metabolites by PGPRs such as *Bacillus* is a well-documented biocontrol agent against phytopathogens (Chowhan, et al., 2023). In the current study, *B. mojavensis* BBR-6 showed a maximum inhibition rate of 61.54%. Similar results were obtained by Diabankana et al. (2021) and Abdelkefi et al. (2024). It has been shown in many studies that *B. mojavensis* and *B. cereus* produce fungal wall-degrading enzymes (Ramírez et al., 2022; Chowhan, et al., 2023). In this study, we can suggest that the high antifungal activity of *B. mojavensis* BBR-6 and *B. cereus* BBR-9 against *F. oxysporum* can be attributed to these enzymes. Bautista et al. (2010) reported that *Bacillus megaterium* B14 inhibited the mycelium development of *F. oxysporum* by 40%. In the current study, *B. megaterium* BBR-3 showed a high inhibition rate of 30.35% against *F. oxysporum*. Numerous studies have shown that *Pseudomonas* sp., which is commonly found in soil and rhizosphere, prevents the growth of plant diseases by secreting various compounds (Wang et al., 2020). Rathore et al. (2020) determined that *P. fluorescens* Pf-5 showed 82.51% growth inhibition against *Fusarium* sp. In the current study, *P. fluorescens* BBR-12 showed a high inhibition rate of 54.04% against *F. oxysporum*. In a recent study, Yang et al. (2024) determined that *P. libanensis* P3P4 showed 78.17 % growth inhibition against *F. oxysporum*. In the current study, *P. libanensis* BBR-7 showed a high inhibition rate of 39.52% against *F. oxysporum* (Table 3).

Table 3. Antifungal activity test results of rhizobacterial isolates against *F. oxysporum*
Çizelge 3. *Rizobakteriyel izolatların F. oxysporum'a karşı antifungal aktivite test sonuçları*

Isolates	Colony diameter of *F. oxysporum* (mm) Mean ± SD	Inhibition percentage (%) of *F. oxysporum*
Bacillus thuringiensis BBR-1	51.7 ± 0.80^{g}	45.59
Bacillus pumilus BBR-2	62.1 ± 1.05^{cd}	33.21
Bacillus megaterium BBR-3	64.5 ± 0.75^{bc}	30.35
Pantoea agglomerans BBR-4	47.2 ± 1.41^{h}	50.95
Lactobacillus paracasei BBR-5	67.6 ± 3.22^{b}	26.66
Bacillus mojavensis BBR-6	38.3 ± 1.05^{ı}	61.54
Pseudomonas libanensis BBR-7	56.8 ± 1.70^{ef}	39.52
Pseudomonas chlororaphis BBR-8	53.9 ± 0.52^{fg}	42.97
Bacillus cereus BBR-9	43.5 ± 0.60^{h}	55.35
Pseudarthrobacter scleromae BBR-10	73.7 ± 0.87^{a}	19.40
Lactobacillus oligofermentans BBR-11	66.4 ± 0.91^{b}	28.09
Pseudomonas fluorescens BBR-12	44.6 ± 0.26^{h}	54.04
Bacillus simplex BBR-13	60.2 ± 1.60^{de}	35.47

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

4. Conclusion

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

References

Abdelkefi, N., Louati, I., Mechichi, H. Z., Sayahi, N., El-Sayed, W. S., El Nayal, A., & Mechichi, T. (2024). Enhanced salt stress tolerance in tomato plants following inoculation with newly isolated plant growth-promoting rhizobacteria. *Scientia Horticulturae*, 328, 112-921. https://doi.org/10.1016/j.scienta.2024.112921

Agake, S. I., Artigas Ramirez, M. D., Kojima, K., Ookawa, T., Ohkama-Ohtsu, N., & Yokoyama, T. (2021). Seed coating by biofertilizer containing spores of Bacillus pumilus TUAT1 strain enhanced initial growth of *Oryza sativa* L. *Agronomy Journal*, 113(4), 3708-3717. https://doi.org/10.1002/agj2.20747

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

Amri, M., Rjeibi, M. R., Gatrouni, M., Mateus, D. M., Asses, N., Pinho, H. J., & Abbes, C. (2023). Isolation, identification, and characterization of phosphate-solubilizing bacteria from Tunisian soils. *Microorganisms*, 11(3), 783. https://doi.org/10.3390/microorganisms11030783

Anderson, A.J., & Kim, Y.C. (2018). Biopesticides produced by plant-probiotic plant bacteria interaction Pseudomonas chlororaphis isolates. *Crop Protection*, 105, 62-69. https://doi.org/10.1016/j.cropro.2017.11.009

Bakker, AW., & Schippers, B. (1987). Microbial cyanides 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.

Bautista, D., Corrales Ramírez MSC, L.C., Cuervo Andrade, J.L., González, L., Guevara, M., & Sánchez Leal M.S.C, L.C. (2010). Evaluation of biocontrol effect of *Bacillus* spp. vs. *Fusarium* spp. under greenhouse conditions in *Rosmarinus officinalis* L. *Biomedical Sciences Journal*, 8(13), 63-75. doi.org/10.1016/0038-0717(87)90037

Chowhan, L.B., Mir, M. I., Sabra, M. A., El-Habbab, A.A., & Kumar, B.K. (2023). Plant growth promoting and antagonistic traits of bacteria isolated from forest soil samples. *Iranian Journal of Microbiology*, 15(2), 278. https://doi.org/10.18502/ijm. v15i2.1248

Clericuzio, M., Novello, G., Bivona, M., Gamalero, E., & Medana, C. (2024). A Study of metabolites from basidiomycota and their activities against *Pseudomonas aeruginosa*. *Antibiotics*, 13(4), 326. https://doi.org/10.3390/antibiotics13040326

Diabankana, R.G.C., Afordoanyi, D.M., Safin, R. I., Nizamov, R. M., Karimova, L.Z., & Validov, S.Z. (2021). Antifungal properties, abiotic stress resistance, and biocontrol ability of *Bacillus mojavensis* PS17. *Current Microbiology*. 78:3124-3132.

Halimursyadah, H., Syafruddin, S., Syamsuddin, S., & Sriwati, R. (2023). Screening of indigenous rhizobacteria isolates from patchouli rhizosphere producing HCN, siderophores and chitinolytic enzymes. In *IOP Conference Series: Earth and Environmental Science,* 1183(1), 012096. doi.org/10.1088/1755-1315/1183/1/012096

Hammer, M., & Junghanns, W. (2020). *Rosmarinus officinalis* L.: Rosemary. *Medicinal, Aromatic and Stimulant Plants*, 501-521. https://doi.org/10.1007/978-3-030-38792-11

Hynes, R.K., Leung, G.C., Hirkala, DL., & Nelson, L.M. (2008). Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada. *Canadian Journal of Microbiology*, 54(4), 248-258.

Joseph, B., Patra, R.R., & Lawrence, R. (2007). Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *International Journal of Plant Production,*1(2), 141-152.https://doi.org/10.9734/bmrj/2015/14496

Khatami, S.A., Kasraie, P., Oveysi, M., Tohidi Moghadam, H.R., & Ghooshchi, F. (2023). Impacts of plant growth-promoting bacteria, compost and biodynamic compost preparations for alleviating the harmful effects of salinity on essential oil characteristics of lavender. *Chemical and Biological Technologies in Agriculture*, 10(1), 110. https://doi.org/10.1186/s40538-023-00485-6

Kumar, A., Singh, S., Mukherjee, A., Rastogi, R. P., & Verma, J. P. (2021). Salt-tolerant plant growth-promoting *Bacillus pumilus* strain JPVS11 to enhance plant growth attributes of rice and improve soil health under salinity stress. *Microbiological Research*, 242, 126616. doi.org/10.1016/j.micres.2020.126616

Landa, B. B., Hervás, A., Bettiol, W., & Jiménez-Díaz, R. M. (1997). Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusarium oxysporum* f. sp. ciceris. *Phytoparasitica*, 25, 305-318. https://doi.org/10.1007/bf02981094

Loera-Muro, A., Caamal-Chan, M. G., Castellanos, T., Luna-Camargo, A., Aguilar-Díaz, T., & Barraza, A. (2021). Growth effects in oregano plants (*Origanum vulgare* L.) assessment through inoculation of bacteria isolated from crop fields located on desert soils. *Canadian Journal of Microbiology*, 67(5), 381-395. https://doi.org 79/cbdm.541

Martínez-Hidalgo, P., Flores-Félix, J. D., Sánchez-Juanes, F., Rivas, R., Mateos, P. F., Santa Regina, I., & Velázquez, E. (2021). Identification of canola roots endophytic bacteria and analysis of their potential as biofertilizers for canola crops with special emphasisonsporulatingbacteria. *Agronomy*, 11,9 doi.org/10.3390/agronomy1109179,

Mehta, S., & Nautiyal, C.S. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Current Microbiology* 43, 51-56.

Nazir, R., Ganai, B. A., Rahi, P., Rehman, S., Farooq, S., & AbduAllah, E.F. (2020). MALDI-TOF-MS and 16S rRNA characterization of lead tolerant metallophile bacteria isolated from saffron soils of Kashmir for their sequestration potential. *Saudi Journal of Biological Sciences*, 27(8), 2047-2053. doi.org/10.1016/j.sjbs.2020.04.021

Öksel, C., Balkan, A., Bilgin, O., Mirik, M., & Başer, İ. (2022). Investigation of the effect of PGPR on yield and some yield components in winter wheat (*Triticum aestivum* L.). *Turkish Journal of Field Crops*, 27(1), 127-133. https://doi.org/10.17557/tjfc.1019

Palleroni, N.J., Krieg, N.R., & Holt, J.G., (1984). Bergey's manual of systematic bacteriology. *Baltimore: The Willian and Wilkins. Co*, 141-219.

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.

Patel, S.K., Singh, S., Benjamin, J.C., Singh, V.R., Bisht, D., & Lal, R.K. (2024). Plant growth-promoting activities of *Serratia marcescens* and *Pseudomonas fluorescens* on *Capsicum annuum* L. plants. *Ecological Frontiers*, 44(4), 654-663

Ramírez, V., Martínez, J., Bustillos-Cristales, M.D.R., Catañeda-Antonio, D., Munive, J. A., & Baez, A. (2022). *Bacillus cereus* MH778713 elicits tomato plant protection against *Fusarium oxysporum*. *Journal of Applied Microbiology*, 132(1), 470-482.

Rathore, R., Vakharia, D.N., & Rathore, D.S. (2020). In vitro screening of different *Pseudomonas fluorescens* isolates to study lytic enzyme production and growth inhibition during antagonism of *Fusarium oxysporum* f. sp. cumini, wilt causing pathogen of cumin. *Egyptian Journal of Biological Pest Control*, 30(1), 57.

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(1), 49-68.

Rochlani, A., Dalwani, A., Shaikh, N., Shaikh, N., Sharma, S., & Saraf, M. (2022). Plant growth promoting rhizobacteria as biofertilizers: application in agricultural sustainability. *Acta Scientific Microbiology* 5(4), 2581-3226

Royse, D., & Ries, S.M. (1978). The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cincta*. Phytopathology, 68:6-37

Rudakova, N.L., Khilyas, I.V., Danilova, I.V., Pudova, D.S., & Sharipova, M.R. (2023). Evaluating of the potential of *Bacillus pumilus* 3-19 as a plant growth-promoting Strain. *Russian Journal of Plant Physiology*, 70(8),197. doi.org/10.1134/s1021443723

Saranraj, P., Sayyed, R. Z., Kokila, M., Salomi, V., Sivasakthivelan, P., Manigandan, M., & Mawar, R. (2023). Evolving concepts of biocontrol of phytopathogens by endophytic *Pseudomonas fluorescence*. *Current Microbiology* 12(7), 304

Sarwar, M., & Kremer, R.J. (1995). Determination of bacterially derived auxins using a microplate method. *Letters in Applied Microbiology,* 20(5), 282-285

Schwyn, B., & Neilands, J. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical biochemistry*, 160(1), 47-56.

Shariati J.V., Malboobi, M.A., Tabrizi, Z., Tavakol, E., Owlia, P., & Safari, M. (2017). Comprehensive genomic analysis of a plant growth-promoting rhizobacterium *Pantoea agglomerans* strain P5. *Scientific Reports*, 7(1), 15610.

Sharma, A., Dev, K., Sourirajan, A., & Choudhary, M. (2021). Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology*, 19(1), 99. https://doi.org/10.1186/s43141-021-00186-3

Singh, T., Tiwari, Y., & Awasthi, G. (2019). Understanding the impact of *Bacillus thuringiensis* proteins on non-target organisms. *International Journal of Scientific Research in Biological Sciences,* 6(2), 169-176 doi.org/10.26438/ijsrbs/v6i2.169176

Singh, P., Singh, R.K., Li, H.B., Guo, D.J., Sharma, A., Verma, K. K., & Li, Y.R., (2023). Nitrogen fixation and phytohormone stimulation of sugarcane plant through plant growth promoting diazotrophic *Pseudomonas*. *Biotechnology and Genetic Engineering Reviews*, 1-21. https://doi.org/10.1080/02648725.2023.2177814

Sivri, G.T., & Öksüz, Ö. (2019). Identification of *Propionibacterium* spp. isolated from mihaliç cheeses by MALDI-TOF MS. *Tekirdağ Ziraat Fakültesi Dergisi*, 16(2), 244-250. https://doi.org/10.33462/jotaf.526431

Subramanium, N., & Sundaram, L. (2020). Siderophore producing *Pseudomonas* spp. isolated from rhizospheric soil and enhancing iron content in *Arachis hypogaea* L. plant. *International Journal of Agricultural Technology*, 16(2), 429-442

Stamenov, D., Đurić, S., & Jafari, T. H. (2021). Biostimulatory potential of microorganisms from rosemary (l.) rhizospheric soil. *Contemporary Agriculture*, 70(4), 108-115. https://doi.org/10.2478/contagri-2021-0016

Shi, Z., Guo, X., Lei, Z., Wang, Y., Yang, Z., Niu, J., & Liang, J. (2023). Screening of high-efficiency nitrogen-fixing bacteria from the traditional Chinese medicine plant *Astragalus mongolicus* and its effect on plant growth promotion and bacterial communities in the rhizosphere. *BMC Microbiology*, 23(1), 292.

Tamura, H. (2023). A MALDI-TOF MS Proteotyping Approach for Environmental, Agricultural and Food Microbiology. Microbiological identification using maldı-tof and tandem mass spectrometry: *Industrial and Environmental Applications*, 147-182. https://doi.org/10.1002/9781119814085.ch6

Wang, S., Huang, Z., Wan, Q., Feng, S., Xie, X., Zhang, R., & Zhang, Z. (2020). Comparative genomic and metabolomic analyses of two *Pseudomonas aeruginosa* strains with different antifungal activities. *Frontiers in Microbiology*, 11, 1841.

Wei, D., Zhu, D., Zhang, Y., Yang, Z., Wu, X., Shang, J., & Chang, X. (2023). Characterization of rhizosphere *Pseudomonas chlororaphis* IRHB3 in the reduction of Fusarium root rot and promotion of soybean growth. *Biological Control*, 186, 105-349. https://doi.org/10.2139/ssrn.4514461

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

Yang, D., Zhang, X., Li, Z., Chu, R., Shah, S., & Zhang, X. (2024). Antagonistic effect of *Bacillus* and *Pseudomonas* combinations against *Fusarium oxysporum* and their effect on disease resistance and growth promotion in watermelon. *Journal of Applied Microbiology*, 135(5), 74. https://doi.org/10.1093/jambio/lxae074