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Biological Control Potential of Bacterial Isolates Against *Fusarium proliferatum* Causing Basal Rot of Onion (*Allium cepa* L.)

Soğan (*Allium cepa* L.) Dip Çürüklüğü Etmeni *Fusarium proliferatum*'a Karşı Bazı Bakteriyel İzolatların Biyolojik Mücadele Potansiyeli

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BIOLOGICAL CONTROL POTENTIAL OF BACTERIAL ISOLATES AGAINST *FUSARIUM PROLIFERATUM* CAUSING BASAL ROT OF ONION (*ALLIUM CEPA* L.)[1]

ABSTRACT

Onion (*Allium cepa* L.) represents a crop of considerable significance in terms of both domestic consumption and international trade, while its production is continuously challenged by a range of biotic and abiotic stress factors, most notably during postharvest storage. Among the diseases affecting onion, basal rot incited by *Fusarium proliferatum* is frequently observed and is responsible for substantial yield and quality losses. Current disease management practices involve integrated approaches, including the cultivation of resistant varieties, implementation of crop rotation schemes, soil solarization, sanitation measures, and the application of chemical and biological control strategies. Nevertheless, the progressive limitations imposed on chemical fungicides, together with increasing environmental and health-related concerns, have strengthened the emphasis on biological control alternatives. In this context, the present study was designed to assess the antagonistic capacity of different bacterial isolates against *F. proliferatum*. Accordingly, twenty-two bacterial isolates were evaluated under controlled *in vitro* conditions using the dual culture assay. The obtained results demonstrated that the inhibition of mycelial growth varied between 18.85% and 66.67% among the tested isolates. Notably, isolates PM-18, TV-17C, and A-16 exhibited pronounced antagonistic effects against the pathogen. The findings of this study indicate that these bacterial isolates may serve as promising candidates for environmentally compatible and sustainable biological control strategies targeting onion basal rot.

Keywords: Biological Control, Dual Culture Assay, Soil-Borne Pathogen, Antagonistic Activity.

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SOĞAN (*ALLİUM CEPA* L.) DİP ÇÜRÜKLÜĞÜ ETMENİ *FUSARİUM PROLİFERATUM*'A KARŞI BAZI BAKTERİYEL İZOLATLARIN BİYOLOJİK MÜCADELE POTANSİYELİ

ÖZ

Soğan (*Allium cepa* L.), hem iç tüketim hem de uluslararası ticaret açısından önemli bir tarımsal ürün olup, üretimi özellikle hasat sonrası depolama sürecinde çeşitli biyotik ve abiyotik stres faktörlerinden etkilenmektedir. Soğanda yaygın olarak karşılaşılan hastalıklar arasında yer alan ve *Fusarium proliferatum* tarafından oluşturulan dip çürüklüğü, önemli düzeyde verim ve kalite kayıplarına neden olmaktadır. Hastalığın yönetiminde dayanıklı çeşitlerin kullanımı, ekim nöbeti, toprak solarizasyonu, sanitasyon uygulamaları ile kimyasal ve biyolojik mücadele stratejileri birlikte değerlendirilmektedir. Bununla birlikte, kimyasal fungisit kullanımına yönelik artan kısıtlamalar ile çevresel ve sağlık temelli kaygılar, biyolojik mücadele yöntemlerine olan ilgiyi giderek artırmıştır. Bu kapsamda, mevcut çalışma *F. proliferatum*'a karşı farklı bakteri izolatlarının antagonistik potansiyelinin belirlenmesi amacıyla yürütülmüştür. Bu doğrultuda, toplam yirmi iki bakteri izolatı ikili kültür yöntemi kullanılarak kontrollü *in vitro* koşullarda değerlendirilmiştir. Elde edilen sonuçlar, test edilen izolatların miseliyal gelişimi baskılama oranlarının %18.85 ile %66.67 arasında değiştiğini göstermiştir. Özellikle PM-18, TV-17C ve A-16 kodlu izolatlar belirgin düzeyde antagonistik etki sergilemiştir. Bulgular, söz konusu bakteri izolatlarının soğanda dip çürüklüğü hastalığının çevre dostu ve sürdürülebilir yönetimi açısından potansiyel biyolojik mücadele ajanları olabileceğine işaret etmektedir.

Anahtar Kelimeler: Biyolojik Mücadele, İkili Kültür Yöntemi, Toprak Kaynaklı Patojen, Antagonistik Aktivite.

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1. INTRODUCTION

Onion (*Allium cepa* L.), a member of the Amaryllidaceae family, is widely cultivated worldwide and represents a crop of substantial economic importance for Türkiye. With an annual production of 2,252,139 tons, Türkiye ranks fifth among onion-producing countries worldwide, underscoring the strategic importance of onion cultivation for national agriculture and export markets (FAO, 2024). In addition to its economic value, onion has attracted increasing scientific attention in recent years because of its nutritional and health-promoting properties. In particular, its high phenolic content and associated bioactive compounds have been recognized for their antioxidant activity and potential protective effects on human health (Karaman, 2008).

Despite its economic and nutritional importance, onion production remains highly vulnerable to numerous fungal and bacterial diseases arising during cultivation and, more prominently, during postharvest storage. Onion bulbs are susceptible to several fungal pathogens, including *Fusarium proliferatum*, *F. oxysporum* f. sp. *cepae*, and *F. solani* (Klokočar-Šmit et al., 2008), as well as *Alternaria alternata*, *A. porri*, *Aspergillus niger*, *A. ochraceus*, *Botrytis allii*, *Fusarium equiseti*, *F. verticillioides*, *Gliocladium roseum*, *Paecilomyces* sp., and *Penicillium* spp. (Çakır and Maden, 2016). In addition, bacterial pathogens such as *Pectobacterium* spp., *Dickeya* spp., *Enterobacter* spp., and *Pseudomonas* spp. have also been associated with onion bulb rot during storage (Kleman, 2023). These disease agents may cause considerable quantitative and qualitative losses, reaching 35–40% in some production systems (Kumar et al., 2015).

Among onion diseases, basal rot is considered one of the most destructive and is primarily caused by *F. oxysporum* f. sp. *cepae* and *F. proliferatum*. This disease is economically important because it may result in yield losses of up to 50% both in the field and during storage and is characterized by dark brown to pink root discoloration, tissue necrosis, and leaf wilting (Stanković et al., 2007; Haapalainen et al., 2016; Gálvez and Palmero, 2022).

Various conventional strategies have been employed for basal rot management, including the use of resistant cultivars, crop rotation, soil solarization, sanitation measures, and chemical fungicides. However, these approaches often provide inconsistent disease suppression because of economic constraints, environmental concerns, and risks to human health. In addition, increasing regulatory restrictions on chemical fungicides have intensified the search for alternative and sustainable disease management options (Abbo et al., 2014).

Biological control has consequently emerged as an important component of environmentally compatible plant disease management strategies. Bacterial biological control agents, in particular, have attracted substantial interest owing to their rapid proliferation, efficient rhizosphere colonization, adaptability to variable environmental conditions, and ability to exert multiple antagonistic effects simultaneously. Previous studies have shown that bacterial antagonists can suppress soil-borne pathogens through diverse mechanisms, including the production of antimicrobial and volatile metabolites, competition for nutrients and ecological niches, secretion of cell wall-degrading enzymes, and activation of systemic resistance in host plants (Villavicencio-Vásquez et al., 2025). In this context, species of *Pseudomonas*, *Bacillus*, and *Pantoea* have been reported as promising biocontrol agents against *Fusarium* spp. and other phytopathogenic fungi (Liu et al., 2014; Islam et al., 2018). In addition, several bacterial strains, including *Pantoea agglomerans*, *Bacillus subtilis*, *B. pumilus*, and *B. megaterium*, were previously found to be effective against *F. oxysporum* under *in vitro, in vivo*, and storage conditions,

with *P. agglomerans* BRTB showing particularly strong biocontrol potential (Çakar and Tozlu, 2022).

Recent studies conducted in Türkiye have demonstrated that *F. proliferatum* and other fungal pathogens are widely distributed in onion production areas and are responsible for significant yield and quality losses. In addition to studies focusing on pathogen identification and genetic diversity, increasing attention has been directed toward biological control using antagonistic bacteria, as well as alternative approaches such as wood vinegar, which has shown promising results in suppressing the pathogen under *in vitro* conditions (Bayraktar and Dolar, 2011; Kara and Soylu, 2023; Kara et al., 2023; Kara et al., 2024).

Taken together, these results highlight the suitability of bacterial agents for the long-term management of persistent soil-borne pathogens such as *Fusarium* spp. Accordingly, the aim of the present study was to evaluate the *in vitro* antagonistic activity of various bacterial isolates against *Fusarium proliferatum* and to identify potential candidates for biological control.

2. MATERIALS AND METHODS

2.1. Isolation, Purification, and Identification of the Pathogen

Onion bulbs showing characteristic basal rot symptoms, including root discoloration, basal plate rot, tissue softening, and necrosis, were obtained from local commercial markets in Erzurum, Türkiye. The symptomatic onion bulbs were thoroughly washed under running tap water to remove adhering soil particles and surface contaminants. Subsequently, small tissue pieces approximately 1-2 cm in length were excised from the margins between healthy and symptomatic tissues. The excised fragments were surface sterilized in 1% sodium hypochlorite (NaOCl), rinsed three times with sterile distilled water, and gently dried on sterile filter paper, and then aseptically transferred onto Potato Dextrose Agar (PDA) plates. All plates were incubated at 20–25°C until fungal growth was observed.

Colonies emerging on PDA that displayed stable and homogeneous morphological traits were selected for purification. These colonies were repeatedly transferred onto fresh PDA medium until pure cultures were obtained. The resulting isolates were stored at +4°C for subsequent analyses. Among the obtained pathogen isolates, three were identified as *F. proliferatum*. To determine pathogenic capacity, all isolates were subjected to pathogenicity testing based on symptom development, lesion expansion, and tissue necrosis observed after inoculation. Among the tested isolates, ET 122 was selected for subsequent analyses because it showed the most consistent pathogenicity and the most pronounced symptom development within 7–10 days after inoculation.

For pathogenicity assessment, mycelial plugs measuring 4 mm in diameter were excised from the actively growing margins of PDA cultures and inoculated into shallow artificial wounds created on healthy onion bulbs. Prior to inoculation, the bulbs were surface-disinfected and visually inspected to confirm the absence of physical damage or disease symptoms. Control bulbs received sterile PDA plugs without fungal material. After inoculation, bulbs were transferred into plastic containers (30 × 45 × 30 cm) lined with sterile moist paper towels to maintain high humidity and incubated at 25°C. Symptom development was monitored after inoculation, and pathogenicity was verified through re-isolation of the fungus from symptomatic tissues in accordance with Koch's postulates.

For molecular characterization, the selected isolate (ET 122) was propagated in Potato Dextrose Broth (PDB) at 25°C for 48 h prior to genomic DNA extraction. The internal transcribed spacer (ITS) region was amplified by PCR using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), as described by White et al. (1990). The amplified PCR products were sequenced, and the resulting sequences were compared with reference sequences available in public databases by BLASTN analysis (Zhang et al., 2000) for preliminary molecular identification. Because the ITS region alone may provide limited resolution for species-level discrimination within *Fusarium*, molecular identification was interpreted together with morphological characterization.

2.2. Morphological Characterization of the Pathogen

Macroscopic and microscopic characteristics of the selected fungal isolate were evaluated based on colony color, texture, aerial mycelium development, pigmentation on PDA, and the morphology of conidiophores, phialides, and conidia. To visually support these observations, a representative image reported by Shina et al. (2023) was included to illustrate the microscopic structures of *F. proliferatum*.

2.3. *In vitro* Experiments

A total of twenty-two bacterial isolates previously described or considered to exhibit potential biocontrol properties were retrieved from the Microbial Culture Collection of the Department of Plant Protection, Faculty of Agriculture, Atatürk University (Table 1).

Table 1. Bacterial strains used in the study

Çizelge 1. *Çalışmada kullanılan bakteriyel strainler*

Strain	Bacterial species	Accession number*	Reference
PM-18	*Pseudomonas chlororaphis*	-	Mohammadi, 2018
TV-17C	*Bacillus amyloliquefaciens*	MW751910	Mohammadi et al., 2017
A-16	*Agrobacterium radiobacter*	MN967438	Mohammadi et al., 2017
FDG-37	*Pseudomonas fluorescens*	MW740242	Çamlıca and Tozlu, 2019
TV-6F	*Bacillus subtilis*	MW751905	Tekiner et al., 2018
QST-3	*Bacillus subtilis*	-	Albastawisi, 2023
CP-1	*Bacillus subtilis*	-	Göktürk et al., 2018
TV-53D	*Brevibacillus choshinensis*	-	Gökçe and Kotan, 2016
R2/2	*Paenibacillus polymyxa*	-	Albastawisi, 2023
TV-91C	*Bacillus megaterium*	MW751922	Karagöz et al., 2014
TV-6D	*Bacillus megaterium*	MW751904	Gökçe and Kotan, 2016
CP-2	*Bacillus subtilis*	-	Göktürk et al., 2018
TV-49A	*Bacillus megaterium*	-	Tekiner et al., 2019
QST-2	*Bacillus subtilis*	-	Albastawisi, 2023
M-3	*Bacillus megaterium*	-	Kınık and Çelikel, 2017
TV-12E	*Paenibacillus polymyxa*	-	Erman et al., 2010
TV-3D	*Bacillus pumilus*	MW751902	Ekinci et al., 2014
QST-1	*Bacillus subtilis*	-	Albastawisi, 2023
TV-60D	*Bacillus megaterium*	-	Kınık and Çelikel, 2017
RK-92	*Pantoea agglomerans*	-	Karagöz et al., 2014
RK-79	*Pantoea agglomerans*	MW751900	Karabıçak and Kotan, 2014
Ca B	*Bacillus megaterium*	-	Albastawisi, 2023

Dual culture assays were performed in sterile 90-mm Petri dishes containing Potato Dextrose Agar (PDA). Mycelial plugs (6 mm in diameter) of the fungal isolate *F. proliferatum* ET 122, taken from actively growing colony margins, were aseptically placed at the center of each plate. Bacterial isolates were first grown on Nutrient Agar (NA) at 26°C for 24 h and then inoculated by streaking at equidistant positions around the fungal plug. The experiment was conducted in a randomized complete block design with three replicates, and each bacterial isolate was tested in three independent Petri dishes. Plates were incubated in darkness at 25°C, and antagonistic activity was evaluated on the 7th day of incubation, when the control plates were fully colonized by the pathogen. In control treatments, where the pathogen was grown in the absence of bacterial isolates, fungal development was allowed to continue until complete colonization of the agar surface was achieved. Antagonistic effects were determined by comparing radial mycelial expansion in bacterial treatments with that observed in the control plates. The inhibitory capacity of each bacterial isolate was expressed as percentage mycelial growth inhibition and calculated using the following formula (Wang et al., 2012):

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

where **C** is the radial growth of the fungal colony in the control,
T is the radial growth of the fungal colony in the bacterial treatment, and
M is the diameter of the initial mycelial plug.

2.4. Data Analysis

Statistical analyses were performed using JMP IN statistical software (SAS Institute, Cary, NC, USA). The effects of bacterial treatments on the inhibition of fungal growth were evaluated by analysis of variance (ANOVA). Whenever significant treatment effects were detected, mean separations were performed using the Least Squares Means (LSMeans) Student's t-test. All statistical analyses were conducted at a confidence level of $P < 0.01$. This stringent significance level was adopted to minimize the likelihood of type I error and to strengthen the reliability of the statistical inferences.

3. RESULTS AND DISCUSSION

In the present study, pathogenicity tests were conducted for all obtained isolates, and among them, ET 122 showed the highest virulence, with more rapid symptom development and more extensive tissue maceration than the remaining isolates. Therefore, ET 122 was selected as the target pathogen for subsequent experiments. The use of a single, highly virulent isolate provided a stable experimental framework, allowing consistent and reliable comparisons of antagonistic performance among the tested bacterial isolates under standardized *in vitro* conditions.

When cultured on PDA medium, the ET 122 isolate formed cottony to velvety colonies after 7 days of incubation at 25–28°C. Colony coloration varied from white to pinkish–lilac, and colony diameter reached approximately 6–7 cm (Figure 1). Microscopic examination revealed characteristic morphological features of *F. proliferatum*, including abundant microconidia produced in chains, the presence of mono- and polyphialidic conidiophores, and micro- and macroconidia. These observations were consistent with previously published descriptions of *F. proliferatum*. Representative microscopic structures are presented in Figure 2, reproduced from Shina et al. (2023). In addition, molecular identification based on ITS region sequencing confirmed the isolate as *F. proliferatum*, showing 99% sequence similarity with the GenBank reference sequence under accession number (PZ205440).

Figure 1. Colony development of *Fusarium proliferatum* ET 122 isolate on PDA medium
Şekil 1. *Fusarium proliferatum* ET 122 izolatının PDA besiyerinde koloni gelişimi

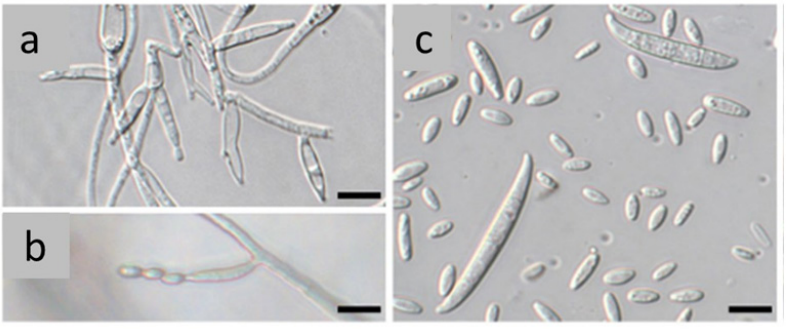

Figure 2. Representative morphological features of *Fusarium proliferatum*, including (a) mono- and polyphialides, (b) microconidial chains, and (c) micro- and macroconidia (scale bars = 10 µm) (Shina et al., 2023).
Şekil 2. *Fusarium proliferatum*'un temsili morfolojik özellikleri: (a) mono- ve polifiyalid yapılar, (b) mikrokonidi zincirleri ve (c) mikro- ve makrokonidiler (ölçek çubukları = 10 µm) (Shina et al., 2023).

A total of twenty-two bacterial isolates were screened for their *in vitro* antagonistic activity against *F. proliferatum* ET 122 using dual culture assays (Table 2).

Table 2. Efficacy of potential bioagent bacterial isolates tested against *Fusarium proliferatum* ET 122 isolate *in vitro* Petri dish assays

Çizelge 2. *Fusarium proliferatum* ET 122 izolatına karşı *in vitro* Petri kabı denemelerinde test edilen potansiyel biyolojik ajan bakteri izolatlarının etkinliği

Strain	Inhibition rate (%)		Strain	Inhibition rate (%)	
PM-18	66.67	A	CP-2	42.90	D
TV-17C	66.53	A	TV-49A	42.48	DE
A-16	65.26	AB	QST-2	34.18	EF
FDG-37	64.70	AB	M-3	33.61	F
TV-6F	63.29	AB	TV-12E	33.19	F
QST-3	57.67	BC	TV-3D	32.07	FG
CP-1	49.37	CD	QST-1	31.22	FGH
TV-53D	48.18	D	TV-60D	30.10	FGH
R2/2	47.54	D	RK-92	24.33	GHI
TV-91C	45.99	D	RK-79	23.21	HI
TV-6D	44.02	D	Ca B	18.85	I
CONTROL	0.00	J			
LSD			**8.38**		
CV			**12.07**		
F value			**29.6781**		
p value			**< 0.01**		

*Means followed by the same letter in the same column are not significantly different according to the LS Means Differences Student's t-test at p<0.01.

The bacterial isolates inhibited radial mycelial growth of the pathogen at rates ranging from 18.85% to 66.67%, with an overall mean inhibition value of 42.33%. Analysis of variance showed that the differences among treatments were statistically significant (ANOVA, $p < 0.01$). The highest levels of antifungal activity were observed for isolates PM-18 (66.67%) and TV-17C (66.53%), which were statistically grouped together and exhibited the strongest suppressive effects on fungal growth. Notably, isolates A-16 (65.26%), FDG-37 (64.70%), and TV-6F (63.29%) also produced substantial inhibition and were ranked among the most effective treatments. Within this framework, isolates PM-18, TV-17C, A-16, FDG-37, and TV-6F were identified as the most effective treatments, showing significantly higher inhibition rates than the lower-performing isolates ($p < 0.01$). In contrast, several isolates exhibited limited inhibitory activity under the experimental conditions, with Ca B (18.85%), RK-79 (23.21%), and RK-92 (24.33%) showing the lowest inhibition values.

The statistical parameters obtained in the experiment further support the biological relevance of these results. The LSD value enabled clear discrimination among treatments, and the coefficient of variation remained within acceptable limits, indicating satisfactory experimental precision. Moreover, the significant F-test result confirmed that antagonistic activity differed significantly among isolates, thereby highlighting the isolate-dependent nature of the responses observed.

Overall, the results clearly demonstrate that certain bacterial isolates possess strong antagonistic effects against *F. proliferatum* ET 122. In particular, isolates PM-18 and TV-17C consistently exhibited the highest suppression of mycelial growth, as evidenced both quantitatively and visually in dual culture assays (Figure 3), underscoring their potential as promising biological control agents. The full Petri dish image of the control treatment is also shown in Figure 3 for comparison.

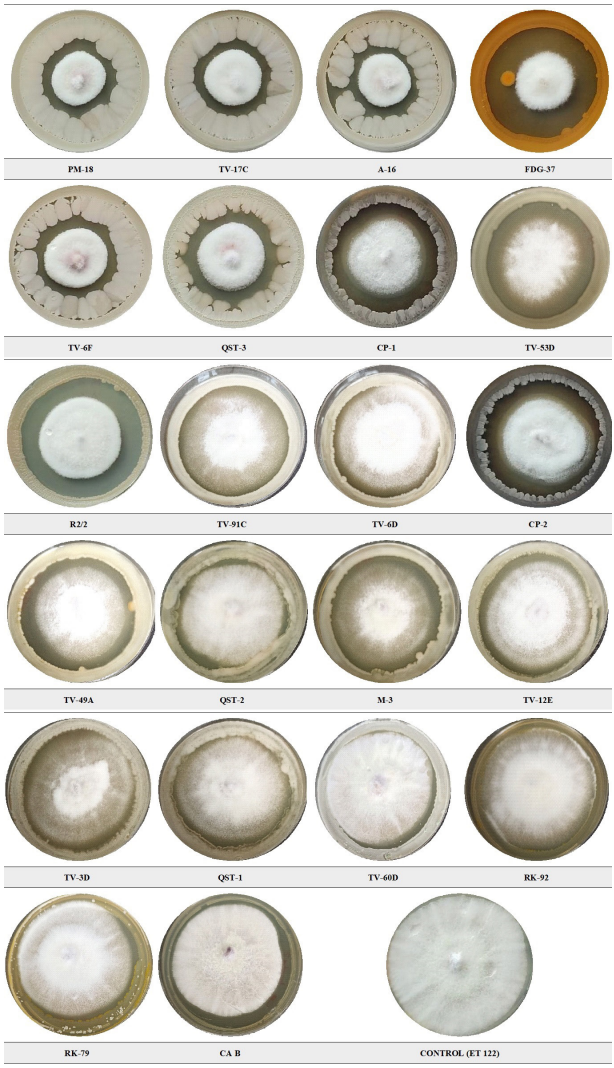

Figure 3. Efficacy of potential bacterial bioagent isolates against *Fusarium proliferatum* ET 122 *in vitro* dual culture Petri dish assays.

Şekil 3. Potansiyel bakteriyel biyolojik ajan izolatlarının *Fusarium proliferatum* ET 122'ye karşı *in vitro* dual kültür Petri kabı denemelerindeki etkinliği.

Biological control, which relies on the use of beneficial microorganisms or their metabolites to suppress plant pathogens, is widely recognized as a sustainable and environmentally friendly alternative to chemical disease management strategies (Ongena and Jacques, 2008; Tozlu et al., 2018; Tekiner et al., 2019; Tekiner et al., 2020; Lahlali et al., 2022; Tekiner et al., 2023; Şahinoğlu and Tozlu, 2023). Over several decades, extensive research has established that microorganisms including *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma*, *Pichia*, and *Candida* can effectively suppress plant pathogens through diverse mechanisms, such as antibiosis, nutrient and space competition, hyperparasitism, and induction of systemic resistance in host plants (Köhl et al., 2019; Sellitto et al., 2021; Fenta et al., 2023).

In the present study, several bacterial isolates, particularly PM-18, TV-17C, A-16, FDG-37, and TV-6F exhibited strong *in vitro* antagonism against *F. proliferatum* ET 122, as reflected by inhibition rates exceeding 60%. Such elevated inhibition levels are commonly associated with the production of antimicrobial metabolites and enhanced competitive capabilities of effective biocontrol bacteria.

The isolate PM-18, identified as *Pseudomonas chlororaphis*, has previously been reported to produce a diverse array of antifungal metabolites, which may explain its pronounced antagonistic performance (Mohammadi, 2018). *P. chlororaphis* is well recognized for its broad-spectrum activity against *Fusarium* species, mediated by mechanisms such as biofilm formation, siderophore production, and antibiotic synthesis (Compant et al., 2005). Collectively, these traits enhance rhizosphere competence and contribute to the suppression of pathogen development under competitive conditions.

Similarly, the high inhibition recorded for isolate A-16 (*Agrobacterium radiobacter*) may be linked to effective siderophore production and successful colonization ability, thereby restricting iron availability to the pathogen and promoting competitive exclusion. The antagonistic activity observed for FDG-37 (*Pseudomonas fluorescens*) can likewise be attributed to the synthesis of secondary metabolites that enhance rhizosphere competitiveness and suppress fungal growth (Güneş et al., 2015; Mohammadi et al., 2017).

Isolates exhibiting moderate antagonistic activity (42–57%) were predominantly associated with the genus *Bacillus*. Variability in inhibition levels among *Bacillus* isolates likely reflects strain-dependent differences in the production of bioactive compounds, as well as differential responses to environmental conditions (Tozlu et al., 2018; Albastawisi, 2023). Numerous studies have shown that *Bacillus* spp. suppress fungal pathogens through cyclic lipopeptides, including surfactins, iturins, and fengycins, which disrupt fungal cell membranes and lead to cellular leakage and death (Rabbee et al., 2019; Saiyam et al., 2024). Beyond their direct antifungal effects, these metabolites also contribute to biofilm formation and the induction of systemic resistance in plants (Ansari et al., 2024).

The results of the present study are consistent with those reported by Mondani et al. (2021), who demonstrated that *Bacillus subtilis* and *Streptomyces griseoviridis* inhibited *F. proliferatum* growth by more than 60%. The antagonistic performance observed for *B. subtilis* strains TV-17C and TV-6F may therefore be associated with the synthesis of antibiotic-like compounds, such as iturin and surfactin (Çakmakcı et al., 2010).

In contrast, isolates exhibiting low inhibition rates (<20%), including RK-92 and RK-79 (*P. agglomerans*), showed limited suppressive effects against *F. proliferatum*. Although *P. agglomerans* has been reported to display biocontrol potential against certain pathogens, the reduced efficacy observed here further supports the concept that antagonistic performance is strongly strain- and context-dependent (Karagöz et al., 2010; Karakurt et al., 2010).

In conclusion, the results indicate that bacterial isolates belonging primarily to the genera *Pseudomonas* and *Bacillus* represent promising candidates for the biological management of *F. proliferatum*. The high inhibition rates observed support the potential of biological control as a sustainable alternative to chemical fungicides. Nevertheless, given that the present experiments were conducted exclusively under *in vitro* conditions, extrapolation of these findings should be approached with caution. Further investigations under greenhouse and field conditions are required to validate strain performance. Moreover, considering the mycotoxin-producing capability of *F. proliferatum* (Stanković et al., 2007), future research should also address the potential of these bacterial isolates to mitigate mycotoxin accumulation.

4. CONCLUSION(S)

The results of the present study indicate that biological control constitutes a viable and environmentally sustainable approach for the management of onion basal rot caused by *F. proliferatum*. Evidence generated from the *in vitro* assays demonstrated that several bacterial isolates, particularly *Pseudomonas chlororaphis* and *Bacillus subtilis*, exhibited strong antagonistic activity against the pathogen, thereby highlighting their potential relevance as biological control agents.

The inhibitory responses observed in the dual culture assays suggest that these bacterial strains may suppress the development of *F. proliferatum* through multiple, strain-dependent mechanisms commonly associated with bacterial antagonism, including competition for resources, antibiosis, and the production of bioactive metabolites. Although the findings reported here are derived exclusively from controlled *in vitro* conditions, they nevertheless provide a scientifically robust foundation for subsequent investigations.

Future studies should therefore focus on evaluating the efficacy and ecological stability of these promising bacterial strains under greenhouse and field conditions. In addition, further research is required to elucidate their potential contributions to reducing disease severity and mitigating mycotoxin-associated risks linked to *F. proliferatum* in onion production systems

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) solely for the purpose of improving English language expression and readability. Following the use of this tool, the authors carefully reviewed and edited the content as necessary. The authors accept full responsibility for the scientific content of the publication.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

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