Research Article

International Journal of Agriculture and Wildlife Science

Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi

2024, 10*(1)*: 96 – 116, *doi: 10.24180/ijaws.1386741*

Determination of Antagonistic Activities of Endophytic Bacteria Isolated from Different Wheat Genotypes Against *Fusarium culmorum**

Farklı Buğday Genotiplerinden İzole Edilen Endofitik Bakterilerin *Fusarium culmorum*'a Karşı Antagonistik Aktivitelerinin Belirlenmesi

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Geliş Tarihi (*Received*): 06.11.2023 **Kabul Tarihi (*Accepted*):** 08.01.2024 **Yayın Tarihi (*Published*):** 29.04.2024

Abstract: This study aimed to evaluate the physiological and biochemical properties and enzyme activities of endophytic bacteria obtained from different wheat genotypes, as well as their effectiveness against *Fusarium culmorum*, which causes root and crown rot in wheat. The results obtained from double culture tests of isolates against *F. culmorum* showed that the inhibition rate varied between 80.56% and 13.90%. The inhibition rate against *F. culmorum* was 80.59% for *Bacillus subtilis* (MM11), 69.41% for *Stenotrophomonas maltophilia* (EY5), and 61.10% for *Enterobacter* sp. (MY3) under *in vitro* conditions, the most effective isolates. *Pseudomonas putida* (EM9) and *Pseudomonas orientalis* (MM21) isolates gave positive results in all tests in the production of amylase, cellulase, phosphatase, ACC deaminase, and siderophore. To identify six promising isolates, 16S rRNA gene-based sequence analysis was utilized. The efficacy of bacterial strains against *F. culmorum*, pot experiments were conducted in a growth room (*in vivo*). The results demonstrated that the combination of *S. maltophilia*, *Enterobacter* sp., and *B. subtilis* (MY3+EY5+MM11) yielded the most favorable outcomes in terms of disease severity, plant height, wet weight, dry weight, root wet weight, and root dry weight. The combination of *Stenotrophomonas rhizophila*, *P. putida*, and *P. orientalis* (EY1+EM9+MM21) exhibited promising results. Utilizing effective bacterial strains is anticipated to reduce the dependence on and costs associated with chemical fertilizers and pesticides while minimizing their environmental impact. Furthermore, these strains show potential for commercial applications pending further validation procedures. The findings from this study significantly contribute to the field of biological control strategies against *F. culmorum* by leveraging the diverse capabilities of endophytic bacteria.
Keywords: Endophyte, *Fusarium culmorum*, biological control, wheat

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Öz: Bu araştırmada, farklı buğday genotiplerinden elde edilen endofitik bakterilerin, fizyolojik ve biyokimyasal özelliklerinin ile enzim aktivitelerinin yanısıra buğdayda kök ve kökboğazı çürüklüğüne neden olan *Fusarium culmorum*'a karşı etkinliğinin değerlendirilmesi amaçlanmıştır. İzolatların *F. culmorum*'a karşı ikili kültür testlerinden elde edilen sonuçlara göre inhibisyon oranı %80.56- %13.90 arasında değişiklik göstermiştir. *In vitro* koşullarda *Fusarium culmorum*'a karşı inhibisyon oranı %80.59 oranıyla *Bacillus subtilis* (MM11), %69.41 oranıyla *Stenotrophomonas maltophilia* (EY5) ve %61.10 oranıyla *Enterobacter* sp. (MY3) en etkili izolatlar olmuştur. Amilaz, selülaz, fosfataz, ACC deaminaz ve siderofor üretiminde *Pseudomonas putida* (EM9) ve *Pseudomonas orientalis* (MM21) izolatları tüm testte pozitif sonuç vermiştir. Umut vadeden altı izolat 16S rRNA geninin sekans analizi kullanılarak tanımlanmıştır. Bakteri suşlarının *F. culmorum*'a karşı etkinliğini değerlendirmek için iklim odası koşullarında (*in vivo*) saksı denemeleri kurulmuştur. Sonuçlar, *Enterobacter* sp., *S. maltophilia* ve *B. subtilis* (MY3+EY5+MM11) kombinasyonunun hastalık şiddeti, bitki boyu, yaş ağırlık, kuru ağırlık, kök yaş ağırlığı ve kök kuru ağırlığı açısından en uygun sonuçları verdiğini göstermiştir. *Stenotrophomonas rhizophila*, *P. putida* ve *P. orientalis* (EY1+EM9+MM21) kombinasyonu da oldukça etkili olmuştur. Etkili bakteri suşlarının kullanılmasının, çevresel etkilerini en aza indirirken, kimyasal gübre ve pestisitlere olan bağımlılığı ve bunlarla ilişkili maliyetleri azaltması beklenmektedir. Ek doğrulama prosedürlerinin ardından bu suşların, ticari uygulamalar için potansiyelinin olduğu düşünülmektedir. Bulgular, *F. culmorum*'a karşı çeşitli endofitik bakterilerin kullanıldığı biyolojik kontrol çalışmalarına katkıda bulunacaktır.
Anahtar Kelimeler: Endofit, *Fusarium culmorum*, biyolojik mücadele, buğday

Cite as: Güler Güney, İ., Derviş, S., Özer, G., Aktaş, H. & Keske, M. A. (2024). Determination of antagonistic activities of endophytic bacteria isolated from different wheat genotypes Against *Fusarium culmorum*. International Journal of Agriculture and Wildlife Science, 10(1) 96-116 doi: 10.24180/ijaws.1386741

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INTRODUCTION

In Turkey, wheat cultivation occupies a preeminent position within the cereals sector, boasting an expansive span of 66.3 million hectares of cultivated land and a notable aggregate production volume of 19.8 million tons, as per the Turkish Statistical Institute (TURKSTAT, 2023) data. This production landscape is characterized by the allocation of 54.2 million hectares for the cultivation of bread wheat (*Triticum aestivum* L.), yielding 16 million tons, and 12.05 million hectares dedicated to durum wheat (*Triticum durum* Desf.), which yields 3.8 million tons. Remarkably, the province of Mardin accounts for 11.4% of the nation's total wheat production. However, the wheat cultivation sector faces considerable challenges, primarily stemming from the deleterious impact of pests and diseases on crop yields. These issues are particularly pervasive given the nationwide prevalence of soil-borne pathogens, which significantly contribute to disease incidence and yield losses throughout wheat-growing regions (Wildermuth and McNamara, 1994).

At the forefront of these soil-borne afflictions lies *Fusarium* root rot, a pathology recognized for its paramount importance in engendering substantial yield diminishment. This phenomenon is especially pronounced in arid regions, both within the global context and, more acutely, within Turkey (Akinsanmi et al., 2004; Cook, 2010; Shikur Gebremariam, et al., 2018; Özer et al., 2020, 2023; Bozoğlu et al., 2022).

Among the pantheon of pathogens inflicting root and root collar ailments in wheat, the *Fusarium* genus commands notable attention. Notable members include *F. culmorum*, *F. graminearum*, *F. nivale*, *F. poae*, *F. avenaceum*, in addition to *Bipolaris sorokiniana*, *Rhizoctonia* spp. (comprising *R. solani*, *R. oryzae*, and *R. cerealis*), *Pythium* spp., *Gaeumannomyces graminis* var. *tritici*, and *Alternaria* spp. It is pertinent to underscore that *Fusarium* spp. stands out prominently as a pivotal contributor to root and root collar rot in Turkish wheat production, accentuating its significance (Tunalı et al., 2008; Shikur Gebremariam et al., 2018).

Of these pathogenic agents, *F. culmorum* takes precedence as the predominant and consequential *Fusarium* species, imposing its deleterious influence across a spectrum of cereals, with wheat and barley occupying a central role. This insidious pathogen has registered its adverse impact on global and Turkish agriculture, underscoring its prominence (Aktaş et al., 1997; Demirci, 2003; Arıcı et al., 2013; Kosiak et al., 2003; Goswami and Kistler, 2004; Wang et al., 2006; Wagacha and Muthomi, 2007; Özer et al., 2020, 2023; Alkan et al., 2021). Notably, *F. culmorum*'s presence in wheat-growingh regions has been observed to engender severe reductions in both yield and quality, thus exacerbating the challenges faced by the wheat production sector (Cook, 2001; Gökçe and Kotan, 2016; Xu et al., 2018; Bozoğlu et al., 2022).

Endophytic bacteria are recognized for their effectiveness in mitigating the impact of plant pathogens, either by preventing their harmful effects or diminishing their severity (Berg and Hallman, 2006). These microorganisms also exhibit the ability to enhance a plant's resilience to biotic stressors and confer systemic resistance against pathogenic invaders. Within the realm of biological control, endophytes engage in competitive interactions with microbial pathogens and demonstrate antagonistic activities, thereby exerting a significant influence on disease reduction (Kloepper et al., 1992; Chen et al., 1995; Pleban et al., 1995; Soylu et al., 2016).

Endophytic microorganisms, virtually ubiquitous in nearly all plant species (Owen and Hundley, 2004; Ryan et al., 2008; Khan et al., 2015), establish residence within the living tissues of their host plants without causing any discernible harm (Reinhold-Hurek and Hurek, 2011; Brader et al., 2014; Türkölmez et al., 2023). These endophytic bacteria possess a diverse array of functional traits, encompassing the capacity for phosphate solubilization (Wakelin et al., 2004), phytohormone production (Shi et al., 2009), hydrolytic enzymatic activities, including lytic hydrolases (Chernin and Chet, 2002), glucanases (Singh et al., 1999), chitinases (Frankowski et al., 2001), antibiotic synthesis (Demain, 1981; Ezra et al., 2004), siderophore secretion (Lodewyckx et al., 2002), and nitrogen fixation (Watanabe et al., 1979). These multifaceted abilities contribute significantly to nutrient acquisition by the plant and serve to augment overall plant growth. Such endophytic bacteria, endowed with growth-promoting mechanisms, are favored choices in the context of eco-friendly plant production and the pursuit of sustainable agricultural practices. Endophytes' desirability extends beyond agriculture and finds application in diverse domains, ranging from medicine

to industry, thanks to their microbial biosynthetic capabilities and distinctive chemical profiles, which confer protective attributes (Jasim et al., 2013).

The present study is dedicated to probing the efficacy of endophytic microorganisms that stimulate plant growth and fortify plant defense mechanisms. These microorganisms are also prolific producers of various hydrolytic enzymes and antibiotic compounds. The primary objective is to assess their effectiveness against the notorious *F. culmorum* pathogen, which poses a substantial threat to wheat cultivation. Identifying and promoting endophytic bacteria that exhibit antagonist effects against *F. culmorum* holds the promise of reducing the losses incurred due to pathogen-induced diseases, thereby facilitating more productive agricultural practices. Furthermore, these endophytes hold potential as sustainable alternatives to conventional fungicides, facilitating the production of agricultural products with reduced chemical residues and promoting environmentally responsible farming practices.

MATERIAL AND METHOD

Isolation of Endophytic Bacteria

The isolation of endophytic bacteria involved the collection of wheat samples from fields located in the geographical coordinates of Küçükköy Mahallesi, Artuklu district, Mardin province, Turkey (37°07'47.9"N, 40°51'11.2"E). Subsequently, wheat plant segments, carefully measuring 1-2 centimeters in length, were precisely excised from the root and root collar regions of visibly healthy bread wheat and durum wheat genotypes. These plant segments served as the primary source for isolating the endophytic bacteria employed in the study. To ensure surface sterilization, these plant segments underwent a rigorous decontamination process. Initially, they were immersed in a 70% ethanol solution for a duration of 2 minutes, followed by a subsequent immersion in a 1% NaClO solution for 1 minute. Following this, the samples were thoroughly rinsed on five separate occasions using sterile distilled water.

Concurrently, a portion of the final rinse water was subjected to direct inoculation onto nutrient agar (NA) medium (Merck, Darmstadt, Germany). This procedure served as a critical assessment of the efficacy of the surface decontamination process. The absence of any colony development on the NA agar affirmed the successful sterilization of the plant samples.

Subsequently, Petri dishes containing NA agar were placed in an incubation chamber and maintained at a temperature of 24°C for a period spanning from 24 to 72 hours. Colonies that exhibited discernibly different growth characteristics, including variances in morphology, color, and edge attributes, were systematically categorized, purified, and subsequently preserved for subsequent analysis (Zvyagintsev, 1991).

Obtaining and Preservation of Pathogen Isolates

The *F. culmorum* isolate denoted as Fc22, utilized in the present investigation, was originally procured from a diseased wheat specimen acquired during a preceding research endeavor (Alkan et al., 2019). Fc22 has previously demonstrated marked pathogenicity, instigating severe disease symptoms in the crown and root tissues of wheat plants belonging to the Seri 82 cultivar (*T. aestivum*). The quantification of disease severity, assessed on a graduated scale from 1 to 4, revealed a mean score of 3.80, signifying the considerable virulence inherent in this specific isolate. The deleterious consequences of Fc22 were conspicuously observable, resulting in substantial morbidity among the subjected plants and, in certain instances, culminating in the demise of entire specimens within the confines of the experimental pots (Özer et al., 2020). The origin of the isolate can be traced to the Culture Collection housed within the Department of Plant Protection at the Faculty of Agriculture, Bolu Abant Izzet Baysal University, Bolu, Turkey.

To ensure the viability of all bacterial isolates acquired during this study, a fresh culture was derived from a 24-48 hour incubation period. Subsequently, these cultures were inoculated onto slant agar medium enriched with NA (Nutrient Agar). The isolates were then subjected to an incubation period of 24 to 72 hours at 24°C to facilitate observable growth. Once successfully established, the isolates were conserved at a temperature of 4°C. For prolonged conservation and to facilitate their utilization in both *in vitro* and *in vivo* experiments conducted under controlled conditions, all representative isolates were subjected to a

secondary purification process on NA agar medium. Following this, they were meticulously preserved in a solution of 30% glycerol and stored at a temperature of -80°C.

Morphological Characteristics of Bacterial Isolates

The investigation of the isolated bacterial strains encompassed Gram staining procedures (Demirbağ and Demir, 2005). In the catalase test, fresh cultures of the isolates were developed in Nutrient Broth (NB, Merck, Darmstadt, Germany) at 28°C for 48 hours. Subsequently, a 3% H_2O_2 solution was introduced to the isolates, and their reactions, characterized by the presence or absence of foaming, were assessed (Holt et al., 1994). As for the oxidase test, samples were collected from bacterial isolates and applied to filter paper. Following this, oxidase reagent (Fluka, N,N-dimethyl-p-phnylenediamine oxalate, α-naphthol) was administered, and any alterations in color were meticulously evaluated to determine the oxidase activity of the isolates (Holt et al., 1994).

Enzymatic Activity

For all enzymatic experiments, cultures of the isolates were prepared to match a McFarland standard of 5. All enzymatic assays were conducted with triplicate repetitions.

Amylase Activity (Hydrolysis of Starch)

Isolates were subjected to streaking onto Petri dishes containing starch agar, followed by a 2-day incubation period at 28°C. The colonies that emerged on the agar were subsequently evaluated for their amylase activity. A positive indication of starch hydrolysis was characterized by the development of a distinct bright color upon the addition of Lugol's solution, while a blue-black coloration was interpreted as a negative outcome (Egamberdieva et al., 2008).

Phosphatase Activity

The NBRIP (National Botanical Research Institute's Phosphate) medium, formulated with glucose (10 g l^{-1}), $Ca_3(PO_4)_2$ (5 g l^{-1}), $MgCl_2.6H_2O$ (5 g l^{-1}), $MgSO_4.7H_2O$ (0.25 g l^{-1}), KCl (0.2 g l^{-1}), $(NH_4)_2SO_4$ (0.1 g l^{-1}), and agar (15 g l^{-1}), was carefully prepared and adjusted to a pH of 7.0 before undergoing sterilization. Subsequently, 10 µl of cultures were inoculated onto Petri dishes containing this medium. Incubation at 28°C for a period of 2-4 days resulted in the development of a clear zone surrounding the colonies, which was considered a positive indicator of phosphatase activity (Nautiyal, 1999). For control purposes, isolates were also cultured in media devoid of $Ca_3(PO_4)_2$ supplementation.

Cellulase Activity

To evaluate cellulase activity, sterile solutions B and D were prepared. Solution B comprised 1M $MgSO_4.7H_2O$, while Solution D consisted of a 7.5% $CaCl_2$ solution. One milliliter from each of these solutions was extracted and incorporated into a sterile mixture of Solutions A and C. Solution A consisted of NaCl (0.25 g), carboxymethylcellulose (2.5 g), K_2HPO_4 (1.5 g), and distilled water (400 ml). Meanwhile, Solution C contained Na_2HPO_4 (3 g), yeast extract (0.5 g), glycerol (2.5 g), NH_4Cl (0.5 g), agar (6.5 g), and distilled water (100 ml).

Isolates were subjected to streaking onto this composite medium and were subsequently incubated at 28°C for a duration of 96 hours. Following incubation, a 0.1% Congo red solution was applied to the colonies and allowed to stand for 20 minutes. Subsequent to this, the colonies underwent a washing procedure utilizing a 1 M NaCl solution. Colonies displaying a discernible clear zone around them were regarded as exhibiting positive cellulase activity (Egamberdieva, 2005).

ACC Deaminase Production

To assess ACC (1-Aminocyclopropane-1-carboxylate) deaminase production, the DF salt minimal medium method, originally outlined by Dworkin and Foster in 1958, was employed. Isolates that exhibited growth during incubation were categorized as positive for ACC deaminase production.

Siderophore Production

To assess siderophore production, the Blue agar medium, originally developed by Schwyn and Neilands in 1987, was employed. The evaluation of siderophore production was based on the observation of color changes within the Blue agar medium.

Physiological and Biochemical Characteristics of Isolates

To assess the tolerance of endophytic bacteria to different temperature levels, the growth of isolates was monitored at temperatures of 4°C, 15°C, 27°C, 37°C, and 41°C for 7 days (Gardner et al., 1984).To evaluate the tolerance of isolates to varying salt concentrations, NB medium was prepared with NaCl concentrations of 0%, 1%, 2%, 3%, and 4%, and the isolates were incubated at 28°C for 7 days to assess their growth under these conditions (Cappuccino and Sherman, 1992).

Carbohydrate Tests

To assess carbohydrate utilization, individual carbohydrates, including glucose, maltose, fructose, xylose, mannitol, tryptophan, and 0.1 M inositol, were separately filtered through a 0.45 µm filter and added to a sterile mineral salt medium. The growth of isolates was monitored for 3, 7, and 14 days at 28°C, following the methodology established by Ji and Wilson in 2002.

Pectolytic Activity Test

For the purpose of sterilization, unblemished and freshly harvested potato tubers were immersed in a 5% NaOCl solution for a duration of 10 minutes. Subsequently, these tubers were sliced into approximately 5 mm thick sections and carefully positioned within Petri dishes containing sterile, moist paper.A volume of 1 ml from 24-hour-old endophytic bacterial cultures was aseptically withdrawn and injected into the potato slices. The inoculated potato slices were then subjected to incubation at 28°C. The assessment of softening in the potato slices was conducted between 24 to 72 hours, with the presence of softening being regarded as a positive outcome. Sterile distilled water served as the control medium.

Tobacco Hypersensitivity (HR) Test

Endophytic bacteria cultures, aged 24 hours, were streaked onto NA agar plates and subsequently incubated. These bacterial cultures were then transferred into sterile distilled water and thoroughly mixed using a vortex device. The resulting mixture's concentration was standardized to 10^8 colony-forming units per milliliter (cfu ml^{-1}).Through the use of an injector, this prepared solution was precisely introduced into the leaf veins of tobacco plants (*Nicotina tabacum* L. cv. Samsun). Following the inoculation, the treated plants were placed in controlled plant growth chambers for a duration of 2 days. This period allowed for the observation of any necrotic tissue formation in the regions where the inoculation had been administered. For experimental validation, a positive control employed *Pseudomonas syringae* pv. *syringae* isolate, while sterile distilled water served as the negative control. Those displaying HR (+) indicated the presence of necrotic tissue formation, whereas those without such tissue formation were classified as HR (-) negative.

Antagonistic Activity

To assess the antagonistic activity of endophytic bacteria against the pathogen, the following formula was applied:

$$\%RI = R - r / R \times 100 \quad (1)$$

Where:

%RI represents the inhibition rate.

R represents the growth of the pathogenic fungus in the absence of bacteria (measured in millimeters).

r represents the growth of the pathogenic fungus towards the presence of bacteria (measured in millimeters).

Molecular Identification of Endophytic Bacteria

Before progressing to the climate chamber experiments, we conducted species-level identification of the top six isolates exhibiting the highest antagonist activity, which were isolated from the sampled areas. To

achieve this, we performed nucleic acid isolation from fresh cultures using the DNeasy Blood and Tissue kit (Cat No. ID; 69504, Qiagen, Hilden, Germany) following the manufacturer's instructions. The resulting DNA quantity was quantified using a DS-11 FX+ spectrophotometer (Denovix Inc., Wilmington, DE, USA), and the DNA was diluted to a concentration of 10 ng μl^{-1} with sterile ultrapure water.

The 16S rRNA regions of these isolates were amplified using specific primers, namely (Amp-F) 5′-GAG AGT TTG ATY CTG GCT CAG-3' and (Amp-R) 5′- AAG GAG GTG ATC CAR CCG CA-3', as described by Baker et al. (2003). The PCR amplification process was carried out in a total volume of 50 µl, consisting of 1 µl DNA, 5 µl of 10× PCR buffer, 0.5 µl of 2 mM dNTPs, 1 µl of 10 µM primers, 0.25 µl of 5 U µl Dream Taq DNA polymerase (Thermo Fischer Scientific, Waltham, MA, USA), and sterile ddH_2O. The PCR reaction followed a specific program: initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 60 seconds, and extension at 72 °C for 90 seconds, with a final extension step at 72 °C for 10 minutes. Subsequently, 10 µl of the resulting PCR products were loaded onto a 1.5% (w v^{-1}) agarose gel and electrophoresed in 1× TAE buffer for 2 hours. Afterward, the gel was stained with ethidium bromide and visualized using the G:BOX F3 gel documentation system (Syngene, Cambridge, UK).

The sequences obtained from this process underwent bidirectional sequence analysis, and species-level identification was established by comparing them with sequences of known species in the GenBank database through BLAST analysis. Furthermore, the sequences corresponding to the isolates were deposited in the GenBank database, and unique accession numbers were obtained.

In Vivo Studies

Climate Chamber Studies

In this research, we utilized sterilized seeds of the vulnerable wheat variety, known as Pehlivan. Before sowing, meticulous surface disinfection of these seeds was diligently performed. The process involved a 5-minute rinse with sterile distilled water, followed by a 30-second immersion in 75% ethanol, and a subsequent 1-minute exposure to a 0.5% NaOCl solution for thorough surface disinfection. Following this rigorous procedure, the seeds underwent two additional rounds of rinsing with sterile distilled water and were carefully dried under sterile conditions. They were then planted in vials. After a span of 10 days, the seedlings were meticulously transplanted into individual pots. Notably, the pots were first inoculated with bacteria, and subsequently, after a 2-hour interval, the introduction of the pathogen took place.

Bacterial Inoculation

In this study, we employed various combinations of endophytic bacterial isolates carefully chosen based on their specific characteristics. These combinations included MY3+EY5+MM11 isolates, EY1+EM9+MM21 isolates, MM21 isolate on its own, MM21+MY3+EY5 isolates, and MY3+EY5+MM11+EM9+MM21 isolates. Each of these isolate mixtures was suspended in a 250 ml bacterial solution with a concentration of 10^7 cfu ml^{-1}, and a 1% Carboxy Methyl Cellulose (CMC) adhesive was added to enhance adhesion.

Wheat seedlings underwent a 2-hour immersion of their roots in these bacterial suspensions before being transplanted into individual pots. Soil application of the endophytic bacterial suspension (10^7 cfu ml^{-1}) was carried out for each pot after transplantation, with an additional application applied 30 days post-inoculation. As a control group, sterile distilled water was utilized.

Pathogen Inoculation

In preparation for the pathogenicity test, an inoculum of the Pehlivan wheat variety was meticulously created. This process involved soaking Pehlivan wheat seeds in distilled water overnight, followed by thorough drainage. The hydrated seeds were then carefully arranged within 500 ml Erlenmeyer flasks. To ensure the sterilization of the seeds, approximately one-third of the Erlenmeyer flask volume was filled with seeds, which subsequently underwent a rigorous autoclaving procedure spanning three consecutive days.

Subsequently, 5 mm discs were meticulously excised from 7-day-old *F. culmorum* cultures that had been cultivated on Potato Dextrose Agar (PDA) plates. These discs were judiciously employed to inoculate the hydrated wheat seeds within the Erlenmeyer flasks. Daily agitation was maintained, and the flasks were

subjected to a four-week incubation period at a temperature of 25°C. This duration allowed for the complete colonization of *F. culmorum* within the wheat grains. Upon the conclusion of the inoculation process, the wheat grains underwent a 24-hour drying period at 30°C. Subsequently, they were transformed into flour via mechanical blending techniques employing a blender, following the methodology outlined by Wallwork et al. in 2004.

Each individual pot was then endowed with 0.5 g of the prepared wheat inoculum. These pots were methodically irrigated, hermetically sealed using sterile polyethylene bags, and afforded a one-week period to facilitate the pervasive distribution of the fungus within the soil.

For the potted plant experiment, a mixture of sterilized sand, peat, and field soil in a 1:1:1 ratio was filled to one-third of the volume in sterile plastic pots with a 20 cm diameter. Wheat seedlings, previously grown in vials, were transplanted into each pot, with four wheat plants in each. The following treatments were applied:

1. *S. maltophilia* + *Enterobacter* sp. + *B. subtilis* (MY3+EY5+MM11) + *F. culmorum*
2. *S. maltophilia* + *Enterobacter* sp. + *B. subtilis* (MY3+EY5+MM11)
3. *P. putida* + *S. rhizophila* + *P. orientalis* (EY1+EM9+MM21) + *F. culmorum*
4. *P. putida* + *S. rhizophila* + *P. orientalis* (EY1+EM9+MM21)
5. *P. orientalis* (MM21) + *F. culmorum*
6. *P. orientalis* (MM21)
7. *P. orientalis* + *Enterobacter* sp. + *S. maltophilia* (MM21+MY3+EY5) + *F. culmorum*
8. *P. orientalis* + *Enterobacter* sp. + *S. maltophilia* (MM21+MY3+EY5)
9. *Enterobacter* sp. + *S. maltophilia* + *B. subtilis* + *S. rhizophila* + *P. orientalis* (MY3+EY5+MM11+EM9+MM21) + *F. culmorum*
10. *Enterobacter* sp. + *S. maltophilia* + *B. subtilis* + *S. rhizophila* + *P. orientalis* (MY3+EY5+MM11+EM9+MM21)

Additionally, there were control groups consisting of an uninoculated negative control and a positive control with only *F. culmorum* inoculum.

The potted plants were cultivated within a controlled environment inside a growth chamber, maintaining specific conditions such as 50-65% humidity, a 14-10 hour light dark photoperiod, and temperatures held within the range of 25 20±2°C. This cultivation period extended over 60 days, aligning with the protocol established by Ekmekçi and Terzioglu in 1998.

To assess their influence on plant development, wheat plants were subjected to distinct bacterial treatments. The impact on key parameters including plant height, plant fresh weight, plant dry weight, root fresh weight, and root dry weight was scrutinized.

Subsequent to the 60-day post-inoculation period, the wheat plants underwent careful removal from their respective pots, ensuring minimal disruption to the root system. A gentle wash was administered using tap water to remove the surrounding soil. The study evaluated the effects of endophytic bacterial isolates on plant growth and their efficacy in combatting the *F. culmorum* pathogen.

Each isolate underwent triplicate pot experiments, with every pot housing four wheat plants, resulting in a total of 24 plants for each isolate treatment and a cumulative total of 144 plants. The research investigation focused on various parameters, encompassing plant height, fresh weight, total plant dry weight, total root fresh weight, and total root dry weight for the wheat seeds.

Disease Assessments

For disease assessments, the seeds of bread wheat variety Pehlivan were germinated in vials, and later, pots with four wheat seedlings were established for each treatment, including both individual and combined isolates, with one control group and three replicates, totaling four replicates. An evaluation scale ranging from 0 to 5, following the guidelines of Wildermuth and McNamara (1994), was employed to assess disease severity levels based on lesion and necrosis development:

0: Healthy or symptomless plant 1: Plant affected less than 25% 2: Plant affected between 25-50% 3: Plant affected between 51-75% 4: Plant affected more than 75% 5: Dead plant

To ensure disease inoculation, the pathogen was introduced into the soil, and the experiment was set up with four replicates. After four weeks of inoculation, the potted plants were uprooted, evaluated based on the 0-5 scale, and disease index values were calculated. Statistical analyses of the data collected from the pot experiments were conducted using the JMP program and Tukey's Test method. Following observations, Koch's postulates were applied to each sample for reisolation. Disease severity was calculated using the Townsend-Heuberger formula:

$$Disease\ Severity\ (\%) = \frac{\sum(Scale\ Value\ x\ Number\ of\ Plants\ at\ that\ Scale\ Value)^{100}}{Total\ Number\ of\ Plants\ x\ Highest\ Scale\ Value} \qquad (2)$$

Statistical analysis of the study data involved one-way analysis of variance (ANOVA), and differences between means were determined using the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

Isolation of Endophytic Bacteria

A total of 69 endophytic bacterial isolates were obtained from the roots and root collars of wheat plants, including 9 from local bread wheat genotypes, 23 from modern bread wheat genotypes, 13 from local durum wheat genotypes, and 24 from modern durum wheat genotypes (Table 1).

Morphological characteristics of Bacterial Isolates

The phenotypic test results of the bacterial isolates selected for *in vivo* pot experiments based on *in vitro* Petri dish trials are presented in Table 1.

Table 1. Phenotypic test results of isolates.
Çizelge 1. İzolatların fenotipik test sonuçları.

Bacteria[#]	Gr Staining	Catalase	Oxidase	Amylase	Cellulase	Phosphatase	ACC Deaminase	Siderophore
EY1	-	+	+	+	-	+	+	-
EY2	-	+	+	+	-	+	+	W
EY3	-	+	+	+	-	+	+	W
EY4	-	+	+	+	+	+	+	-
EY5	-	+	+	+	+	-	+	-
EY6	-	+	+	-	-	+	+	W
EY7	-	+	+	-	-	+	+	W
EY8	-	+	+	-	+	+	+	+
EY9	+	+	+	+	-	+	+	+
EM1	-	+	+	-	W	-	+	-
EM2	-	+	+	-	W	-	+	-
EM3	-	+	-	-	-	+	-	-

Table 1. Continued.
Çizelge 1. Devamı.

Bacteria#	Gr Staining	Catalase	Oxidase	Amylase	Cellulase	Phosphatese	ACC Deaminase	Siderophore
EM4	-	+	+	W	-	+	-	-
EM5	-	+	+	+	-	+	+	+
EM6	-	+	+	-	-	-	-	+
EM7	-	-	+	W	-	-	-	+
EM8	-	+	+	-	-	-	-	+
EM9	-	+	+	+	+	+	+	-
EM10	-	W	+	+	+	-	+	-
EM11	-	+	+	W	-	+	+	-
EM12	-	+	+	W	-	+	+	-
EM13	-	W	+	W	-	+	+	W
EM14	-	-	-	+	-	+	-	-
EM15	-	+	+	-	-	W	W	-
EM16	+	+	+	W	-	W	+	+
EM17	-	+	+	-	-	+	-	-
EM18	-	+	+	-	-	+	+	-
EM19	-	+	+	-	-	+	+	-
EM20	-	+	+	W	-	-	-	-
EM21	-	+	+	-	-	+	+	-
EM22	-	-	-	W	-	+	-	-
EM23	-	+	+	W	-	+	+	-
MY1	-	+	+	+	-	W	+	-
MY2	-	+	+	+	-	+	+	-
MY3	-	+	-	+	-	W	+	W
MY4	-	+	+	+	-	W	+	-
MY5	-	+	-	+	-	+	W	-
MY6	-	+	+	+	-	-	+	+
MY7	-	+	-	+	-	+	+	W
MY8	-	+	+	W	-	-	W	+
MY9	-	W	+	W	-	W	-	W
MY10	-	+	-	-	-	-	+	+
MY11	-	+	+	+	-	-	+	-
MY12	-	+	+	W	-	-	+	-
MY13	-	+	+	-	-	-	+	-
MM1	-	+	+	-	-	+	+	-
MM2	-	+	+	-	-	+	+	-
MM3	-	+	+	-	+	-	+	+
MM4	-	+	+	-	+	-	-	W
MM5	-	+	+	-	+	+	+	+
MM6	-	+	+	+	-	+	+	+
MM7	-	W	+	-	-	+	-	+
MM8	-	+	+	W	-	+	+	+
MM9	-	+	+	-	-	+	+	-
MM10	-	+	-	W	-	+	-	-
MM11	+	+	+	+	-	-	+	W
MM12	-	+	+	W	-	+	+	+
MM13	-	+	+	-	+	+	+	+
MM14	-	+	+	-	+	+	+	+
MM15	-	+	+	+	+	+	+	-
MM17	-	+	+	-	+	+	+	-
MM18	-	+	+	-	+	+	+	-
MM19	-	+	+	W	-	-	+	W
MM20	-	W	+	-	+	+	+	+
MM21	-	+	+	+	+	+	+	W
MM22	-	+	+	-	+	-	+	+
MM23	-	W	+	W	-	-	W	+
MM24	-	W	+	-	-	-	-	+
MM25	-	+	+	-	-	W	+	-

#EY: Local Bread Wheat; EM: Modern Bread Wheat; MY: Local Durum Wheat; MM: Modern Durum Wheat. Assessments in columns: Weak (W), Negative (-), Positive (+).

Enzymatic Activity

The enzymatic activities of amylase, cellulase, phosphatase, and ACC deaminase were examined in 69 endophytic bacterial isolates. Isolates displaying observable growth were categorized as positive, while those without growth were deemed negative, as summarized in Table 1. In the case of amylase activity, 29 isolates yielded negative results, while all other isolates exhibited positive outcomes (Table 1) (Figure 1a). Regarding phosphatase activity, 19 isolates demonstrated negativity, while the remaining isolates displayed positive results (Table 1) (Figure 1b). For cellulase activity, 51 isolates displayed cellulase positivity, whereas the rest were negative (Table 1) (Figure 1c). In the ACC deaminase test, 14 of the endophytic isolates were found to be negative, while the rest exhibited positive reactions (Table 1). Concerning siderophore production, 35 endophytic isolates tested negative, while all other isolates exhibited positive siderophore production (Table 1) (Figure 1d).

In a study conducted by Pang et al. (2022), the isolation of endophytic bacteria from the roots, stems, leaves, and seeds of three winter wheat varieties was documented. These isolated bacteria were subjected to various investigations, including their capacity to produce indole-3-acetic acid (IAA), solubilize potassium and phosphate, and thrive in a nitrogen-depleted environment. Additionally, their impact on plant growth parameters, such as dry root weight, above-ground dry weight, and plant height, was assessed through pot experiments. A total of 127 strains were thoughtfully selected from a pool of 610 isolated endophytic bacterial cultures, representing 10 different genera and 22 species. Remarkably, *Bacillus aryabhattai*, *B. stratosphericus*, *Pseudomonas oryzihabitans*, and *Leclercia adecarboxylata* were identified for the first time as endophytes within wheat plants using 16S rRNA gene sequencing.

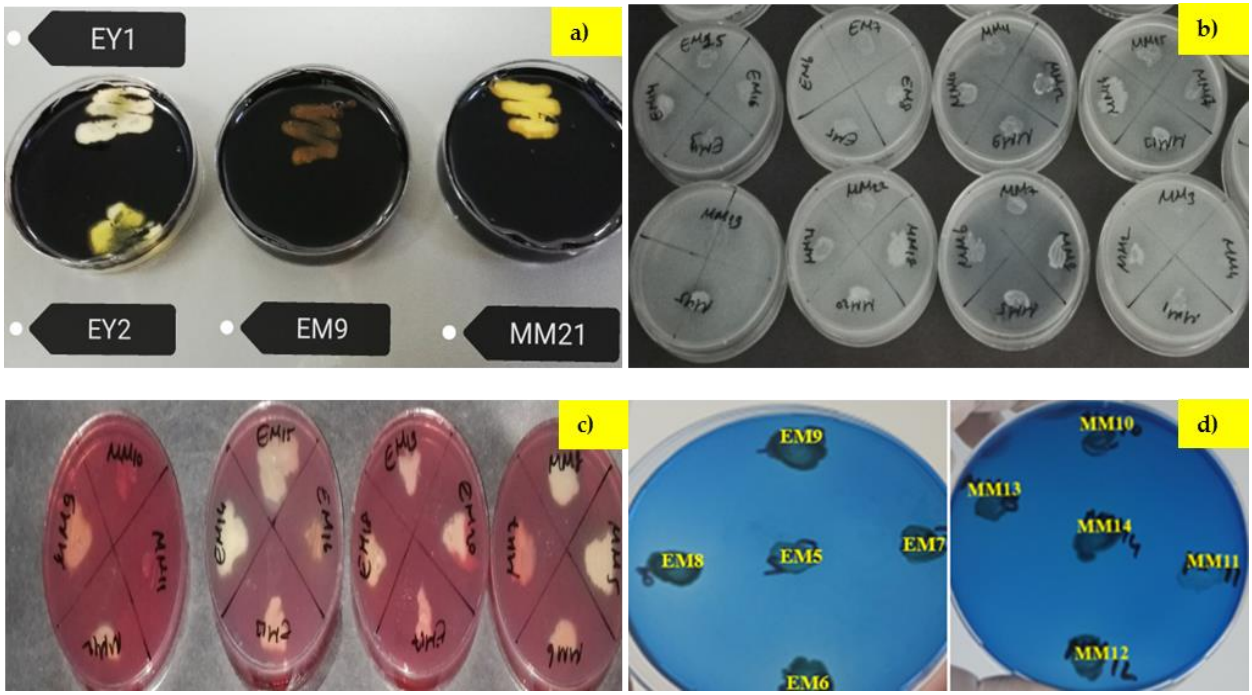

Figure 1. a) Amylase activity of endophytic bacteria; b) Phosphatase activity of endophytic bacteria; c) Cellulase activity of endophytic bacteria; d) Siderophore production of endophytic bacteria.

Şekil 1. a) Endofitik bakterilerin amilaz aktivitesi; b) Endofitik bakterilerin fosfataz aktivitesi; c) Endofitik bakterilerin selülaz aktivitesi; d) Endofitik bakterilerin siderofor üretimi.

The study unveiled noteworthy findings, with 45% of the 127 strains exhibiting IAA production, 29% displaying inorganic phosphate solubility, 37% demonstrating organic phosphate solubility, 2.4% showcasing potassium solubility, and 37.8% thriving in a nitrogen-deprived environment. In pot experiments, each bacterial strain displayed distinctive effects on wheat plants, influenced by the evaluated growth parameters. *Bacillus* emerged as the predominant bacterial taxon isolated from winter wheat plants. These findings underscored the pivotal role of wheat roots as the primary reservoir for

screening endophytic bacteria with biofertilizer potential. Furthermore, our studies revealed that 69 isolates exhibited notably effective results, with 58% displaying amylase activity, 72% phosphatase activity, 74% cellulase activity, 80% ACC deaminase enzyme activity, and 49% demonstrating siderophore production.

Physiological and Biochemical Characteristics of Isolates

A statistical analysis was conducted to evaluate variations in temperature tolerance among isolates at different concentrations. Notably, a significant difference was observed solely at 4°C ($p < 0.01$), while no statistically significant disparities were detected at other temperature levels. Similarly, when assessing tolerance to varying salt concentrations among isolates, no statistically significant differences emerged.

Carbohydrate Tests

In the context of carbohydrate utilization tests, it was observed that specific isolates displayed varying responses in different carbon sources. In a mineral medium supplemented with glucose, eight isolates failed to exhibit growth, while all other isolates demonstrated robust growth. A similar pattern was observed when fructose was incorporated into the mineral medium, with six isolates showing no growth, and all other isolates thriving. Mannitol supplementation in the mineral medium resulted in growth inhibition for 21 isolates, while the remaining isolates displayed growth. In the case of tryptophan-supplemented mineral medium, 21 isolates did not grow, while the others thrived. For maltose-supplemented mineral medium, growth was inhibited in eight isolates, with the rest showing robust growth. When xylitol was introduced to the mineral medium, nine isolates did not grow, while others flourished. Lastly, in the presence of myo-inositol, 15 isolates failed to grow (Table 2).

Endophytic bacteria with a diverse carbon source utilization profile are speculated to contribute to disease severity reduction. Their capacity to utilize carbon sources could position them as promising biological control agents. The carbohydrate test serves as a valuable tool to ascertain the presence of carbon similarity among bacterial strains. Therefore, considering the *in vitro* results alongside carbon source utilization profiles, siderophore production, and enzyme activities, it can be inferred that endophytic bacteria hold promise as effective biological control agents (Ji and Wilson, 2002).

Table 2. Carbohydrate test results of endophytic bacteria.
Çizelge 2. Endofitik bakterilerin karbonhidrat test sonuçları.

Bacteria[#]	Glucose	Fructose	Mannitol	Tryptophan	Maltose	Xylose	M-Inositol
EY1	+	+	W	+	+	+	+
EY2	+	+	-	+	+	+	+
EY3	+	+	-	+	+	+	+
EY4	+	+	W	+	+	+	+
EY5	+	-	W	-	+	-	-
EY6	-	-	-	+	+	+	-
EY7	+	+	+	+	+	-	+
EY8	+	+	+	+	+	+	+
EY9	W	-	W	+	+	-	W
EM1	+	+	W	-	-	+	-
EM2	+	+	+	W	+	+	+
EM3	+	+	+	W	+	+	+
EM4	+	+	W	W	+	+	+
EM5	+	+	W	+	+	+	+
EM6	-	-	-	-	-	+	-
EM7	+	+	W	+	+	+	+
EM8	+	+	W	+	+	+	+
EM9	-	+	-	-	+	W	W
EM10	-	W	-	-	+	+	-
EM11	+	+	+	+	+	+	+
EM12	+	+	+	+	+	+	+
EM13	+	+	+	+	+	+	+
EM14	+	+	W	+	+	+	+
EM15	+	+	W	W	-	+	-
EM16	+	+	W	W	+	+	+

Table 2. Continued.

Çizelge 2. Devamı.

Bacteria[#]	Glucose	Fructose	Mannitol	Tryptophan	Maltose	Xylose	M-Inositol
EM17	W	+	W	W	+	+	-
EM18	+	+	+	+	+	+	+
EM19	+	+	+	+	+	+	+
EM20	+	+	+	+	+	+	+
EM21	+	+	W	+	+	W	+
EM22	+	+	+	-	+	+	+
EM23	+	+	+	+	+	+	+
MY1	+	+	W	+	+	+	W
MY2	+	+	W	-	+	+	+
MY3	+	+	W	+	+	+	+
MY4	+	+	+	+	+	+	+
MY5	W	+	-	-	W	-	W
MY6	W	+	-	+	W	-	W
MY7	W	+	-	-	W	W	-
MY8	W	+	-	+	W	-	W
MY9	+	+	W	-	W	+	W
MY10	+	+	W	-	W	+	W
MY11	+	+	W	-	-	+	W
MY12	+	+	W	+	-	+	W
MY13	+	+	W	+	-	+	W
MM1	+	W	-	+	+	+	+
MM2	+	W	-	+	+	+	+
MM3	+	+	+	-	-	W	-
MM4	+	W	-	-	+	+	-
MM5	+	+	-	+	+	+	+
MM6	+	+	-	+	+	+	+
MM7	+	+	+	-	+	+	+
MM8	+	+	+	-	+	+	+
MM9	-	+	-	+	+	+	+
MM10	+	+	+	+	+	+	+
MM11	+	+	W	-	+	+	-
MM12	+	+	-	+	+	+	+
MM13	+	+	W	+	+	W	+
MM14	+	+	+	+	+	W	+
MM15	+	W	W	+	+	W	+
MM17	W	W	W	+	+	+	W
MM18	+	W	+	+	+	W	+
MM19	+	W	W	+	+	+	-
MM20	W	+	W	+	+	+	+
MM21	-	-	W	+	-	-	-
MM22	W	W	-	-	W	W	-
MM23	-	W	-	-	W	W	-
MM24	-	-	-	-	+	-	-
MM25	+	W	-	-	W	-	+

[#]EY: Local Bread Wheat; EM: Modern Bread Wheat; MY: Local Durum Wheat; MM: Modern Durum Wheat. Assessments in columns: Weak (W), Negative (-), Positive (+).

Pectolytic Activity Test

The results of the pectolytic activity test indicated that all isolates exhibited a negative response.

Tobacco Hypersensitive Reaction (HR) Test

In the HR test, all endophytic bacteria were evaluated as negative (HR–) since they did not induce the formation of necrotic tissue.

Antagonistic Activity

In the evaluation of antagonistic activity exhibited by endophytic bacteria, a spectrum of inhibition rates was observed, spanning from a minimum of 13.90% to a maximum of 80.56% (Table 3). Notably, the MM11 isolate demonstrated the highest inhibition rate at 80.59%, followed by the EY5 isolate at 69.41%, and the

MY3 isolate at 61.10%. Conversely, the lowest inhibition rates were identified in the MY7 and MY10 isolates, both registering at 13.90%.

The endophytic bacteria exhibited notable antagonistic activities against F. culmorum, as depicted in Figure 2. A statistical analysis using one-way analysis of variance (ANOVA), as detailed in Table 4, revealed significant differences ($p < 0.001$) among the mean effects displayed by these bacterial strains.

Table 3. Antagonistic activity of isolates against *Fusarium culmorum.*
Çizelge 3. İzolatların Fusarium culmorum'a karşı antogonistik etkinlikleri.

Bacterium[#]	Effect (%)	Bacterium	Effect (%)	Bacterium	Effect (%)	Bacterium	Effect (%)
EY1	58.31$^{d##}$	EM10	41.69^{j}	MY5	30.56^{n}	MM10	41.69^{j}
EY2	30.56^{n}	EM11	27.79^{o}	MY6	30.59^{n}	MM11	80.59^{a}
EY3	41.69^{j}	EM12	30.59^{n}	MY7	13.90^{r}	MM12	33.41^{m}
EY4	41.69^{j}	EM13	36.10^{l}	MY8	25.00^{p}	MM13	33.41^{m}
EY5	69.41^{b}	EM14	36.10^{l}	MY9	30.59^{n}	MM14	25.00^{p}
EY6	36.10^{l}	EM15	44.41^{i}	MY10	13.90^{r}	MM15	50.00^{g}
EY7	44.41^{i}	EM16	30.59^{n}	MY11	25.00^{p}	MM17	52.79^{f}
EY8	30.59^{n}	EM17	44.41^{i}	MY12	30.59^{n}	MM18	50.00^{g}
EY9	22.21^{q}	EM18	36.10^{l}	MY13	50.00^{g}	MM19	36.10^{l}
EM1	36.10^{l}	EM19	33.31^{m}	MM1	41.69^{j}	MM20	47.21^{h}
EM2	52.79^{f}	EM20	30.59^{n}	MM2	30.59^{n}	MM21	61.10^{c}
EM3	55.59^{e}	EM21	30.59^{n}	MM3	36.10^{l}	MM22	38.90^{k}
EM4	52.79^{f}	EM22	33.31^{m}	MM4	33.31^{m}	MM23	36.10^{l}
EM5	44.41^{i}	EM23	22.21^{q}	MM5	30.59^{n}	MM24	25.00^{p}
EM6	38.90^{k}	MY1	41.69^{j}	MM6	30.59^{n}	MM25	44.41^{i}
EM7	44.41^{i}	MY2	50.00^{g}	MM7	44.51^{i}		
EM8	38.90^{k}	MY3	61.10^{c}	MM8	52.79^{f}		
EM9	55.59^{e}	MY4	50.00^{g}	MM9	41.69^{j}		

[#]EY: Local Bread Wheat; EM: Modern Bread Wheat; MY: Local Durum Wheat; MM: Modern Durum Wheat.
[##]Means sharing the same letter within the same column do not exhibit statistically significant differences.

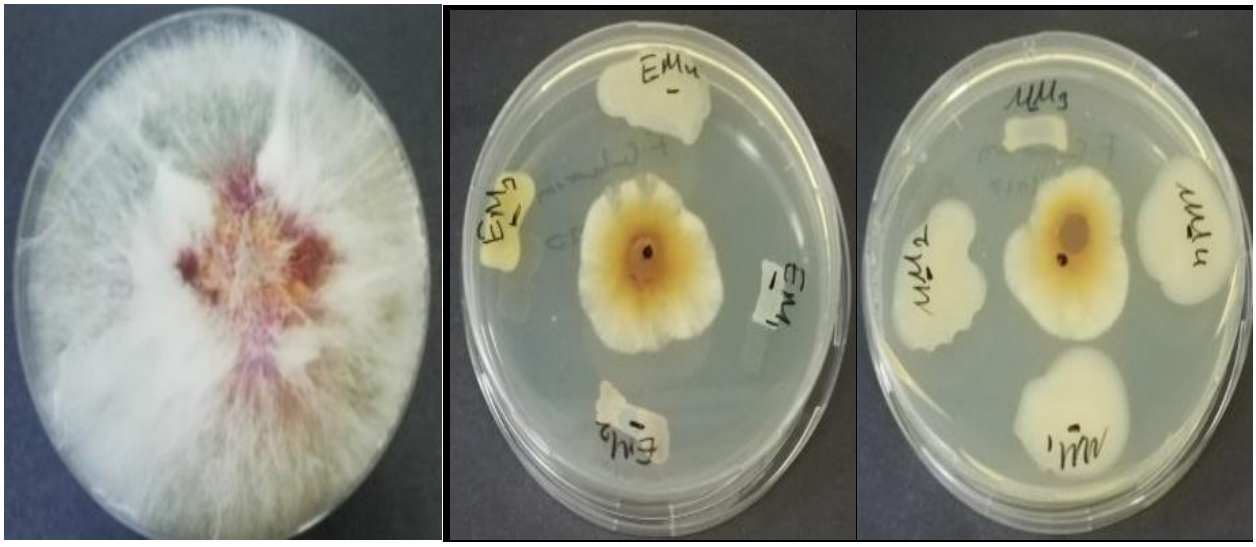

Figure 2. The antagonistic activities of endophytic bacteria EM1, EM2, EM3, EM4, MM1, MM2, MM3, and MM4 in comparison to the control.
Şekil 2. Kontrole göre EM1, EM2, EM3, EM4, MM1, MM2, MM3 ve MM4 endofitik bakterilerin antagonistik aktiviteleri.

Table 4. Analysis of variance for antagonistic activities of isolates.
Çizelge 4. İzolatların antagonistik aktivitelerinin varyans analiz tablosu .

Sources of Variation	DF#	SS#	MS#	*F* Value	Prob>*F*
Isolate	68	30337.94	446.15	6606.83	0.0001***
Error	138	9.32	0.07		
General	206	30347.26			

#DF: Degrees of Freedom SS: Sum of Squares MS: Mean Squares.
*** Significant at $p \leq 0.001$ level.

In a biological control investigation, *Bacillus amyloliquefaciens* subsp. *plantarum* xh-9 exhibited the potential to serve as a biocontrol agent against *F. oxysporum* and various other phytopathogens when applied to a locally cultivated wheat field, as documented in the study by Wang et al. in 2018. Another study conducted by Jankiewicz et al. in 2012 highlighted the inhibitory effects of the bacterium *Stenotrophomonas maltophilia* on the growth of fungal phytopathogens from the *Fusarium*, *Rhizoctonia*, and *Alternaria* genera through its enzymatic activity. Our current research similarly suggests the biocontrol potential of certain endophytic bacteria isolated from wheat, including *S. rhizophila* (EM9), *P. putida* (EY1), *Stenotrophomonas maltophilia* (EY5), *Enterobacter* sp. (MY3), *Bacillus subtilis* (MM11), and *P. orientalis* (MM21), against *F. culmorum*.

Molecular Identification of Endophytic Bacteria

The isolates were visualized using a 16S rRNA Agarose gel (Figure 3). PCR amplification yielded DNA fragments of approximately 1500 base pairs corresponding to the 16S rRNA region of the rDNA. BLASTn queries, based on the 16S rRNA region, revealed that all isolates exhibited a remarkable similarity, ranging from 99% to 100%, with species documented in the GenBank database. All sequences obtained in our study have been registered in GenBank, each assigned a unique accession number, as detailed in Table 3.

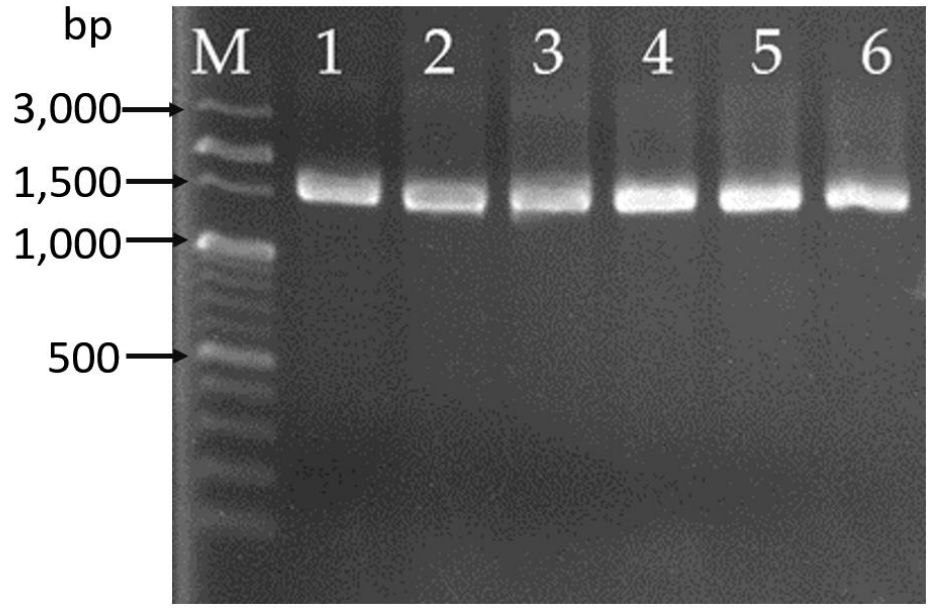

Figure 3. 16S rRNA agarose gel image of bacterial isolates obtained as part of the study. M: 100 bp DNA Ladder (Solis BioDyne, Tartu, Estonia).
Şekil 3. Çalışmadaki elde edilen bakteriyel izolatlara ait 16S rRNA agaroz jel görüntüsü. M: 100 bp DNA Ladder (Solis BioDyne, Tartu, Estonya).

In a prior investigation focused on bacteria that stimulate plant growth within the wheat rhizosphere, 16 bacterial isolates were obtained, considering various traits such as ammonium production, siderophore production, phosphate solubilization, nitrification, and IAA (indole acetic acid) production. Taxonomic classification of these isolates was determined through 16S rDNA sequencing, placing them within the genera *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, and *Lysinibacillus* (Di Benedetto et al., 2019).

In a similar vein, our current study selected six isolates based on a comprehensive set of criteria, including morphological, physiological, and biochemical characteristics, carbohydrate utilization patterns, enzyme activities, siderophore production, and antagonistic activities. These isolates were subsequently identified through 16S rRNA gene sequencing as *Stenotrophomonas rhizophila* (EM9), *Pseudomonas putida* (EY1), *Stenotrophomonas maltophilia* (EY5), *Enterobacter* sp. (MY3), *Bacillus subtilis* (MM11), and *Pseudomonas orientalis* (MM21), as summarized in Table 5.

Table 5. Isolates in the antagonistic efficiency assays and their GenBank accession numbers.
Çizelge 5. Antogonistik etkinlik denemelerinde kullanılan izolatlar ve GenBank erişim numaraları.

Species	Isolate code	GenBank accession number
Stenotrophomonas rhizophila	EM9	OP811184
Pseudomonas putida	EY1	OP811185
Stenotrophomonas maltophilia	EY5	OP811186
Enterobacter sp.	MY3	OP811187
Bacillus subtilis	MM11	OP811188
Pseudomonas orientalis	MM21	OP811189

Climate Chamber Studies

The findings from the pot experiment clearly indicate that the endophytic bacterial isolates exhibited significant disease control efficacy when the plants were inoculated with *F. culmorum*. This efficacy was particularly notable when compared to the positive control group, which was solely inoculated with *F. culmorum* and lacked bacterial treatment. The positive control group exhibited an average disease incidence of 91.7% on the 0-5 scale, along with a disease severity of 79.1%. In contrast, when considering the mixtures of endophytic bacterial isolates with *F. culmorum*+MM21 displayed the highest disease incidence at 46.5% and a disease severity of 41.3%. On the other hand, the mixture of MY3+EY5+MM11 with *F. culmorum* showed the lowest average disease incidence at 35.0%, accompanied by a disease severity of 29.1%. In a similar vein, the combination of EY1, EM9, and MM21 yielded the second lowest average disease incidence, standing at 38.2%, while concurrently demonstrating a disease severity of 32.7% (Table 6).

Table 6. Efficacy of isolates against the disease.
Çizelge 6. *İzolatların hastalığa etkileri.*

Application#	Average disease incidence##	Average disease severity##
Control –	0	0
Control+*F.c*	91.7 ± 0.08 ^{a}	79.1 ± 0.85 ^{a}
MY3+EY5+MM11+*F.c*	35.0 ± 0.19 ^{f}	29.1 ± 1.00 ^{f}
EY1+EM9+MM21+*F.c*	38.2 ± 0.21 ^{e}	32.7 ± 0.20 ^{e}
MM21+*F.c*	46.5 ± 0.38 ^{b}	41.3 ± 1.00 ^{b}
MM21+MY3+EY5+*F.c*	44.4 ± 1.11 ^{c}	38.3 ± 1.14 ^{c}
MY3+EY5+MM11+EM9+MM21+*F.c*	41.1 ± 0.85 ^{d}	35.3 ± 0.43 ^{d}
F value	3627.2***	1423.6***

Application: **Control –** (no pathogen, no endophytic bacteria); ***F.c*: *Fusarium culmorum*; **MY3:** *Enterobacter* sp.**; EY5:** *Stenotrophomonas maltophilia***; MM11:** *Bacillus subtilis*; **EY1:** *Pseudomonas putida***; EM9:** *Stenotrophomonas rhizophila***; MM21:** *Pseudomonas orientalis*.
Mean values indicated by the same letter within the same column are not significantly different, as determined by the Tukey multiple comparison test.
***: Significance level $p < 0.001$.

Among wheat seedlings that were not inoculated with *F. culmorum*, those exposed to the combination of *Enterobacter* sp., S. *maltophilia*, and *B. subtilis* (MY3+EY5+MM11) exhibited the most favorable results, with a plant height of 67.7 cm, a plant fresh weight of 1.29 g, a total dry weight of 0.18 g, a root fresh weight of

0.082 g, and a root dry weight of 0.014 g (Table 7). Conversely, among wheat seedlings exposed to both endophytic bacteria and *F. culmorum*, the MY3+EY5+MM11+*F*.c mixture demonstrated superior outcomes compared to the control, with a plant height of 59 cm, a plant fresh weight of 0.947 g, a total dry weight of 0.163 g, a root fresh weight of 0.058 g, and a root dry weight of 0.009 g (Table 7).

Table 7. Impact of effective isolates on selected plant parameters in the pot experiment.
Çizelge 7. Saksı denemesinde etkili bulunan izolatların bazı bitki parametrelerine etkisi.

Application#	BB##	YA##	TKGA##	TK YA##	TK KA##
Control	59.7^{bcd***}	0.975^{bc}	0.170^{a}	0.059^{cd}	0.011^{abc}
Control+*F.c*	47.0^{f}	0.190^{e}	0.087^{b}	0.020^{g}	0.006^{c}
MY3+EY5+MM11+*F.c*	59.0^{bcd}	0.947^{bc}	0.163^{a}	0.058^{cd}	0.009^{abc}
EY1+EM9+MM21+*F.c*	55.3^{cde}	0.653^{d}	0.143^{a}	0.054^{de}	0.009^{bc}
MM21+*F.c*	50.0^{ef}	0.563^{d}	0.133^{ab}	0.031^{fg}	0.006^{c}
MM21+MY3+EY5+*F.c*	59.0^{bcd}	0.860^{c}	0.152^{a}	0.058^{cd}	0.009^{bc}
MY3+EY5+MM11+EM9+MM21+*F.c*	52.0^{def}	0.523^{d}	0.143^{ab}	0.040^{ef}	0.009^{bc}
MY3+EY5+MM11	67.7^{a}	1.287^{a}	0.177^{a}	0.082^{a}	0.014^{a}
EY1+EM9+MM21	63.3^{ab}	1.250^{a}	0.173^{a}	0.075^{ab}	0.012^{ab}
MM21	60.0^{abc}	1.017^{b}	0.170^{a}	0.074^{ab}	0.010^{abc}
MM21+MY3+EY5	62.3^{abc}	0.970^{bc}	0.165^{a}	0.063^{bcd}	0.010^{abc}
MY3+EY5+MM11+EM9+MM21	62.3^{abc}	1.083^{b}	0.172^{a}	0.070^{abc}	0.010^{abc}
Mean	58.1^{b-d}	0.860^{c}	0.154^{a}	0.057^{cd}	0.010^{abc}
***F* value**	14.245***	122.93***	5.612**	47.915***	5.417***

Application: **Control –** (no pathogen, no endophytic bacteria); ***F.c*: *Fusarium culmorum*; **MY3:** *Enterobacter* sp.**; EY5:** *Stenotrophomonas maltophilia***; MM11:** *Bacillus subtilis*; **EY1:** *Pseudomonas putida***; EM9:** *Stenotrophomonas rhizophila***; MM21:** *Pseudomonas orientalis.*
##PH: Plant height (cm); FW: Fresh weight (g plant); TDW: Total dry shoot weight (g); TRW: Total root fresh weight (g); TKW: Total dry root weight (g).
***: Significantly different at $p < 0.001$. Means sharing the same letter within the same column, according to Tukey's multiple comparison test, are not significantly different.

In their 2017 study, Mnasri et al. investigated the potential of biocontrol agents against *F. culmorum* root infections in durum wheat. They found that endophytic bacteria exhibited antagonistic effects on the *in vitro* growth and sporulation of this pathogen. These bacteria, mainly belonging to the *Bacillus* genus and residing in cereal roots, demonstrated significant antagonistic activities against *F. culmorum*. Some of these antagonistic bacteria could even inhibit key aspects of *F. culmorum*'s life cycle, such as mycelial growth, conidial germination, and sporulation. This inhibition was attributed to the production of volatile antifungal compounds by these bacteria, highlighting their biocontrol potential for cereal root diseases. Importantly, the effectiveness of *F. culmorum*'s biocontrol, especially in greenhouse conditions, depended not only on the pathogen's virulence but also on the specific interactions between bacterial and fungal strains. Our research aligns with these findings, indicating that endophytic bacteria from genera such as *Stenotrophomonas*, *Pseudomonas*, *Enterobacter*, and *Bacillus* possess significant potential for antifungal activity in cereal root biocontrol. Moreover, our greenhouse experiments yielded results consistent with the efficacy of *F. culmorum* biocontrol observed in previous studies.

In a study by Quartana et al. (2022), the potential of five bacterial endophytic isolates derived from durum wheat seeds was investigated for their ability to inhibit *F. culmorum* growth, both *in vitro* and *in vivo*. These isolates were identified as belonging to the *Pantoea* genus (A1, F7, F15, and G1 isolates) and the *Paenibacillus* genus (isolate B). The findings revealed that the combination of F7 + F15 isolates significantly increased shoot height by 94% and improved root length by 47%, along with an 81% enhancement in the vitality index. Moreover, the combination of A1 + B isolates notably reduced *Fusarium* rot incidence by 21% and its severity by 30%. Isolate A1, when used alone, also contributed to a 15% reduction in disease incidence and a 16% reduction in disease severity. These results underscore the potential of the identified bacterial

seed endophytes as promising candidates for the sustainable management of *Fusarium* root rot in durum wheat.

In a separate investigation, *B. subtilis* strains 53 and 71, *P. fluorescens* strain biov1 32, and *Streptomyces* sp. Strain 3 were evaluated as potential biological agents for controlling *Fusarium* head blight. These bacterial antagonists demonstrated a remarkable capacity to inhibit *F. graminearum* pathogen growth, reducing its mycelial growth by 37-97% through the production of volatile metabolites. Notably, *Streptomyces* sp. Strain 3 effectively reduced disease severity after 21 days and resulted in significantly higher wheat yields compared to control groups. Furthermore, it was observed that *Streptomyces* sp. alone led to increased wheat yield compared to uninoculated controls (Nourozian et al., 2006).

Similarly, in our study, we found that endophytic bacteria exhibited significant efficacy against the *F. culmorum* pathogen in both *in vitro* and *in vivo* experiments. These bacteria demonstrated superior performance in various aspects, including plant height, fresh weight, dry weight, root fresh weight, and root dry weight, when compared to the control group.

CONCLUSION

In this study, we explored the antagonistic activity of endophytic bacteria isolated from wheat plants in the Southeastern Anatolia Region of Turkey against *F. culmorum*, the pathogenic agent responsible for inducing root and crown rot in wheat crops. The study involved the isolation of endophytic bacteria from both the root and root crown regions of wheat plants in Mardin province. Subsequently, we conducted comprehensive assessments of the physiological and biochemical activities of these microorganisms, including enzyme assays. Furthermore, we examined their antagonistic potential against *Fusarium culmorum* under controlled laboratory conditions (*in vitro*). To gain deeper insights, we performed molecular analyses on the six most promising isolates that exhibited remarkable efficacy in laboratory settings. Subsequently, we transitioned to greenhouse conditions (*in vivo*) to evaluate the effectiveness of these bacterial strains through pot experiments. The results were particularly noteworthy for the combination of *Enterobacter* sp., S. *maltophilia*, and *B. subtilis* (MY3+EY5+MM11), which demonstrated superior performance across various parameters, including plant height, fresh weight, dry weight, root fresh weight, and root dry weight. Following closely, the combination of *P. putida*, S. *rhizophila*, and *P. orientalis* (EY1+EM9+MM21) also exhibited commendable outcomes.

In summary, the remarkable efficacy of *Enterobacter* sp., S. *maltophilia*, and *B. subtilis* (MY3+EY5+MM11) endophytic bacterial strains against *F. culmorum*, a prominent wheat pathogen, underscores their potential to mitigate the economic and environmental impacts associated with pesticide and chemical fertilizer use in wheat cultivation. Future investigations will further validate this potential and explore its practical applications for commercial use.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

İG, MAK, and GÖ: Executed laboratory experiments. İG, MAK, SD, and HA collected samples and established the experimental framework. İG, SD, and GÖ: Designed the study, performed statistical analysis, and wrote the manuscript.

ACKNOWLEDGMENT

I acknowledge the support and funding provided by Mardin Artuklu University for Project Number MAÜ.BAP.20.KMY.018.

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