Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Pseudomonas putida has potential for biological control of bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*

Gizem ERYİGİT[1], Hatice OZAKTAN[1]*, Utku SANVER[2]

[1] *Ege University Faculty of Agriculture Department of Plant Protection, İzmir, Türkiye*
[2] *Siirt University Faculty of Agriculture Department of Plant Protection, Siirt, Türkiye*
*Corresponding author. Email: hatice.ozaktan@ege.edu.tr

Summary. Bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*, is a serious disease that causes yield and quality losses. There has been increased focus on biological control agents as alternatives to chemical pesticides in plant disease management. In this study, 313 endophyte and epiphyte bacterial isolates, from tomato plants sampled from different locations in Turkey, were assessed for their potential for plant growth promotion and biocontrol efficacy against *X. euvesicatoria*. Results obtained from *in vitro* assays were evaluated using the weighted ranking method, and 15 isolates were selected for *in planta* biocontrol evaluation against *X. euvesicatoria*. In efficacy tests, bacteria were introduced into tomato plants by biopriming of seeds or by spraying whole plants. The two most effective isolates reduced bacterial spot by 40–45% after seed biopriming, and 30–41% from shoot application, compared to the non-treated experimental controls. Sequence analysis using 16S rRNA primers identified one representative isolate (coded KD 91/1) as *Pseudomonas putida*. Tomato plants bioprimed with KD 91/1 through seed treatment had greatest biomass compared to that for the other tested bacteria. The population of *P. putida* KD 91/1 in tomato tissues after pathogen inoculation was approx. 7.2×10^4 cfu g^{-1} in shoots and 1×10^5 cfu g^{-1} in roots. This study indicates that antagonistic *P. putida* isolates are promising candidates for biological control of *X. euvesicatoria*.

Keywords. Plant growth promotion, antagonistic bacteria, endophytes, biocontrol agents.

Citation: Eryiğit, G., Ozaktan, H., & Sanver, U. (2025). *Pseudomonas putida* has potential for biological control of bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*. *Phytopathologia Mediterranea* 64(2): 271-284. doi: 10.36253/phyto-15347

Accepted: August 18, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Ali Siah, UMR 1158 BioEcoAgro, Yncréa HdF, Lille, France.

ORCID:
GE: 0000-0001-6161-5285
HO: 0000-0001-9971-6508
US: 0000-0001-5373-2924

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important edible vegetable, with a 42.3% share of vegetable market and production of 13.2 million tons in Turkey, (FAO, 2022). Tomato production is affected by biotic factors that cause yield and quality losses, and bacterial diseases are important contributors to economic losses. Tomato bacterial spot, caused by *Xanthomonas* spp. (*X. perforans*, *X. gardneri, X. euvesicatoria* and *X. vesicatoria*), is a serious disease that impacts tomato production in many countries (Sharma and Bhattarai, 2019). This disease can cause up to 50% crop losses in

tomatoes, and prevents marketability of fresh tomatoes (Balogh *et al*., 2003).

Symptoms of bacterial spot can be observed on tomato plant leaves, stems, and fruits. The lesions are initially green then turn brown, and are irregular necrotic spots often surrounded by large chlorotic areas. Combined large lesions appear as leaf blight, and later, defoliation may occur. Necrotic lesions and cracks can occur along stems. Fruit lesions are first small with scalded appearance in water, then enlarge and become brown and scab-like rough spots. Fruit lesions can facilitate secondary fruit infections caused by entry of fungi and bacteria (Ritchie, 2000).

Conventional methods for controlling bacterial spot of tomato include application of copper-based pesticides and use of resistant varieties (Sahin and Miller, 1998). However, tomato breeding efforts involving wild lines and varieties resistant to bacterial spot have largely been unsuccessful, and there are no available tomato varieties with resistance to this disease (Sharma and Bhattarai, 2019). Efficacy of copper preparations used against the disease is rapidly decreasing due to pathogen evolution (Martin *et al*., 2004; Abbasi *et al*., 2015), and use of chemical pesticides for disease control has posed environmental hazards and caused accumulation of potentially toxic substances in human food chains.

Therefore, use of chemical pesticides for crop disease management should be minimized, and to achieve this, plant protection alternatives, such as biocontrol, must be identified (Pertot *et al*., 2016). Beneficial bacteria can directly enhance of plant growth, mitigate biotic and abiotic stresses (Khan *et al*., 2016; Tiwari *et al*., 2016), provide nutrients to agricultural crops, and stimulate plant growth by producing phytohormones, improving soil structure, bioaccumulation of inorganic compounds, and bioremediation (Mahmood *et al*., 2016). Beneficial bacteria are used in agricultural crops for biocontrol of plant pathogens through various mechanisms. Microbial biological control agents interact with plants through direct mechanisms, such as competing with targeted pathogens for ecological niches or substrates, or by producing antimicrobial metabolites that inhibit pathogen development. These agents may also exert indirect effects by inducing resistance in host plants against a broad spectrum of pathogens (Compant *et al*., 2005; Köhl *et al*., 2019).

El-Hendawy *et al*. (2005) demonstrated that tomato leaf, root, soil, and seed treatments with *Rahnella aquatilis* reduced severity of bacterial spot caused by *X. campestris* pv. *vesicatoria*. (Mirik *et al*., 2008) also showed that three *Bacillus* sp. solates (designated M1-3, M3-1 and H8-8) from rhizospheres of greenhouse and field-grown pepper plants, reduced disease caused by *X. axanopodis* pv. *vesicatoria* by 11 to 62% in greenhouse-grown pepper plants, and by 38 to 67% in field-grown plants. In addition (Shrestha *et al*., 2014) reported that lactic acid bacteria reduced bacterial spot of pepper by 57-73% under greenhouse conditions and 70–94% in the field. (Pajčin *et al*., 2020) showed that *Bacillus velezensis* IP22, isolated from fresh cheese and grown in a laboratory-scale bioreactor, suppressed pepper plant symptoms caused by *X. euvesicatoria*.

The present study aimed to assess endophyte and epiphyte bacteria from healthy tomato plants for biocontrol potential against *X. euvesicatoria* spot of tomato. bacteria associated with healthy tomato plants were isolated and characterized, and isolates were assessed for disease reduction and plant growth promotion potential. Selected isolates that showed were then assessed for tomato growth enhancement and biocontrol potential *in planta*. Identification of one of these isolates was determined using gene barcoding analysis.

MATERIALS AND METHODS

Plant material, and isolations of bacteria

Epiphytic and endophytic bacteria were isolated from root, stem, and leaf samples from 127 healty tomato plants collected from 40 different locations in the Izmir, Manisa, and Aydın provinces of Turkey.

Endophytic bacteria were isolated from internal tissues of roots, leaves, and stems of the healthy plants, following tissue surface sterilization with sequential immersion in 70% ethanol for 5 min, then 5% sodium hypochlorite solution for 10 min, followed by three rinses with sterile distilled water. Bacterial isolates were obtained using two techniques: either triturating leaves or imprinting stem and root tissues onto tryptic soy agar (TSA). A tissue surface sterility test was carried out on each sample to verify the elimination of surface microorganisms. In cases where no bacterial growth occurred in the sterility test, the isolated bacteria were classified as endophytes (Nejad and Johnson, 2000).

Epiphytic bacteria were isolated from the surfaces of roots, leaves, and stems of healthy tomato plants, and were then each suspended in 100 mL of phosphate buffer. Following extraction on a rotary shaker for 30 min at 120 rpm, ten-fold serial dilutions (10^{-1} to 10^{-3}) were prepared, and 0.1 mL of each dilution was spread onto triplicate TSA plates. The plates were then incubated at 24°C for 48 h (Akköprü and Ozaktan, 2018). The isolates obtained were then preserved in nutrient broth plus 20% glycerol at at -86°C.

A highly aggressive strain of *Xanthomonas euvesicatoria* strain183 (*X. euvesicatoria*) was obtained from the Bacteriology Laboratory of the Department of Plant Protection at Ege University, İzmir, Türkiye. This strain is known to cause bacterial leaf spot on tomatoes.

Characterization of bacterial isolates

Hydrogen cyanide (HCN) production from glycine was assessed by culturing bacteria on 10% tryptic soy agar (TSA) supplemented with glycine (4.4 g L^{-1}). Cyanogenesis was detected using 0.5% picric acid and 2% sodium carbonate (Na_2CO_3), (Bakker and Schippers, 1987). Filter paper impregnated with the reagents was affixed to the underside of Petri dish lids, and results were evaluated after 5 d incubation at 24±2°C. Colour change in the filter paper from yellow to orange-brown indicated the production of HCN. Indole acetic acid (IAA) production was quantified as described by Bric *et al.* (1991). Individual isolates were cultivated in their respective media supplemented with 100 µg mL^{-1} of L-tryptophan at 30°C for 48 h. Following cultivation, the cultures were centrifuged at 8000 rpm for 10 min. Supernatant (2 ml) from each culture was combined with 4 mL of Salkowski reagent (composed of 150 mL of concentrated H_2SO_4, 7.5 mL of 0.5 M $FeCl_3 \cdot 6H_2O$, and 250 mL of distilled water). The development of a pink colour indicated IAA production. Siderophore production was assessed using Chrome Azurol S (CAS) blue supplemented agar in Petri plates, as described by Klement *et al.* (1990). Isolates were cultivated on TSA medium for 24 h at 24±2 °C, then suspended in sterile water to achieve an optical density (OD_{600}) of 0.1. A 2 µL aliquot of each culture was inoculated onto CAS-blue agar plates. The plates were incubated for 48 h at 24±2 °C and observed daily for formation of yellow-orange haloes around colonies, with presence of a yellow-orange halo indicating siderophore production, and halo diameter was measured. Activities of 1-aminocyclopropane-1-carboxylate (ACC) deaminase for bacterial isolates were screened on sterile minimal DF salt media, utilizing ACC as the sole nitrogen source. DF salt media were prepared as described by Saravanakumar and Samiyappan (2007). A 2 µL aliquot of each culture was inoculated into the DF media, the plates were incubated for 72 h at 24±2 °C, and growth of bacterial colonies was observed. Growth on DF medium was interpreted as indicative of ACC deaminase positivity. Isolates were also screened on NBRIP agar plates for capacity to solubilize inorganic phosphate (Nautiyal, 1999). Bacterial cultures were inoculated at the centres of agar plates, which were then incubated for 3 d at 30°C. Presence of clear zones (halo zones) surrounding colonies was recorded, indicating phosphate solubilization ability. Antibiosis activity against *X. euvesicatoria* was assessed as outlined by Jetiyanon and Kloepper (2002).

In vitro *activity of isolates against* Xanthomonas euvesicatoria

In vitro activity was assessed on King's B medium (KBM). A suspension of *X. euvesicatoria* (10^8 cfu mL^{-1}; OD_{600} = 0.1) was prepared and was streak inoculated with a sterile swab into Petri dishes containing KBM. After a 30 min incubation, the plates were dot inoculated with candidate bacteria, and then incubated at 24°C for 48 h. Diameters of *X. euvesicatoria* inhibition zones formed around dot inoculation points were then measured. A modified weighted ranking method was used for assessments of bacterial isolate inhibition of *X. euvesicatoria*. This facilitated numerical classification of bacterial strains by weighting each of several traits based on relative importance, and integrating these weightings with other weighted characteristics. Data obtained were subjected to variance analysis, which enabled quantitative comparisons among all items within the test (Michelson *et al.*, 1958; Akbaba and Ozaktan, 2018). The modified "weighted-rankit" method was applied to 313 bacterial isolates obtained from tomato plant tissues. This approach was adapted to evaluate the diverse bacteria for; ability to produce siderophores, exhibit ACC deaminase activity, produce indole 3-acetic acid (IAA) and hydrogen cyanide (HCN), solubilize phosphates, and inhibit *X. euvesicatoria* growth, with different levels of significance for each factor. Fifteen isolates that were characterized through *in vitro* tests (above) were selected for testing against *X. euvesicatoria*, based on their weighted-average rankings (Table 1). These isolates were also preserved at -80°C in liquid nutrient broth plus 20% glycerol, for long-term storage.

In planta *assessments of antagonistic bacteria against* Xanthomonas euvesicatoria

An *in planta* experiment with 15 selected bacterial isolates was carried out by applying seed coating and plant leaf sprays, applied to tomato variety SC2121. This variety is well-regarded due to its early maturation, good adaption for field cultivation, appropriateness for direct human consumption, and its round, red, and thin skinned fruit (Turfan and Düzel, 2023).

The experiment was conducted in a growth chamber, set to a daily regime of 16 h light at 25°C and 8 h dark at 22°C, and lasted for 45 d.

Tomato seeds were sterilized in a solution of 1% sodium hypochlorite for 1 min, and then rinsed three times with sterile distilled water. Antagonistic bacteria and *X. euvesicatoria* were incubated in KBM medium at 24°C for 24 to 48 h (Schaad *et al*., 2001). For seed bacterization treatments, bacterial colonies were suspended in carboxymethyl cellulose (CMC) at 1% v/v, and inoculum was adjusted to OD_{600} = 0.1 using a spectrophotometer (PG Instruments T60 UV/VIS). The seeds were then soaked for 30 min in inoculum solutions, each containing an antagonistic bacterium suspension amended with carboxymethyl cellulose. The experimental control treatment applied 1% CMC to seeds. The seeds were then allowed to dry on sterile blotting paper for 24 h in a microbial-free cabinet before planting. After completing bacterization, the seeds inoculated with the candidate bacteria were individually planted at approx. 1 cm depth into 10 cm^2/500 cm^3 plastic pots containing sterile peat (TS1 Klasmann-Deilman GmbH, Germany). This substrate had electrical conductivity of 35 mS m^{-1}, pH of 6.5, nutrient composition of 14:10:18 (N:P:K), and density of 1.0 kg m^3 (Bolat *et al*., 2022).

The pots were then placed in the growth chamber. When resulting plants reached true leaf stage second applications of respective antagonistic bacteria were applied by spraying onto the plant surfaces. Isolates of antagonistic bacteria were incubated in KBM at 24°C for 24 to 48 h. Bacterial suspensions were then prepared in sterile distilled water and adjusted to OD_{600} = 0.1 (1×10^8 cfu mL^{-1}) using the UV-visible spectrophotometer (above). The suspensions were then sprayed onto tomato plants (at 3rd true leaf stage) using a hand nozzle sprayer. One to two days later the plants were inoculated with *X. euvesicatoria* (Lwin and Ranamukhaarachchi, 2006), and inoculation contol plants were treated with distilled water.

For *X. euvesicatoria* inoculum, colonies of the pathogen were grown on KB medium at 25°C for 48 h. Bacterial suspension was prepared as suspension in sterile distilled water, and with concentration adjusted to approx. 10^8 cfu mL^{-1} (OD_{600} = 0.35) using the UV-visible spectrophotometer (above) (Klement *et al*., 1990). The 3rd-leaf tomato seedlings were sprayed with *X. euvesicatoria* suspension applied to the undersides of their leaves, because *X. euvesicatoria* infiltrates through leaf stomata which are at high densities on the underside leaf surfaces. The seedlings were then placed in a controlled environment cabinet at 24°C and of 95-100% relative humidity for 2 to 3 d. They were then transferred to a transparent cabinet to preserve high relative humidity (95–100%). After inoculation, the plants were placed in a growth chamber for 2 weeks to observe any disease symptoms that may develop. Tomato plants that were not treated with bacteria and were inoculated with the pathogen were used as experimental controls. Tomato plants that were not treated with antagonistic bacteria or *X. euvesicatoria* were used as experimental controls. The experiment was structured in a completely randomized design with ten replicates, with one plant of each treatment assigned to each replicate. Two weeks after *X. euvesicatoria* inoculation, disease severity was evaluated using a 0 to 4 scale, where 0 = no symptoms, 1 = 1 to 5 lesions on leaves, 2 = multiple lesions and merged lesions on leaves, 3 = merged lesions and necrotic leaves, and 4 = dead leaves, as a modification of the scale described by Al-Dahmani *et al*. (2003). Disease indices (DI) were determined using a formula based on that of Townsend and Heuberger (1943), as DI = [Σ (number of plants in the rating × rating number)/(total number of plants × highest rating)] × 100. This experiment was conducted twice.

Colonization of tomato by antagonistic bacteria and Xanthomonas euvesicatoria

Based on the *in vitro* and *in planta* experiments, and on availability of plant resources and growth chamber capacity, two bacterial strains were chosen that gave significant antagonism against *X. euvesicatoria*. The strain population densities in roots and shoots of tomato plants growing under conditions described above and for 30 d after planting. The two selected bacterial isolates were made resistant to rifampicin (200 ppm), labelled, and tomato seeds were treated with the respective modified strains (Kloepper *et al*., 1980). Colonies were selected that had resistance to rifampicin and typical bacterial colony size and appearance to bacteria. Rifampicin resistance was checked by subject the modified isolates to 10, 50, 100 or 200 ppm concentrations of rifampicin. Isolate purification was achieved using KBM supplemented with 200 ppm of rifampicin.

Time-dependent colonization of tomato plants with beneficial bacteria in the presence and absence of *X. euvesicatoria* was carried out at sampling intervals, including seedling cotyledon stage, first true leaf stage, second true leaf stage, as well as at 24 h, 48 h , 7 d, and 14 d post- *X. euvesicatoria* inoculation. Commencing at the seed bacterization stage, 1 g of plant material (root or shoots) was taken from each assessed plant at each sampling time, and this was rinsed in 100 mL of sterile water for 15 min. A dilution series was prepared from the washing water for each seedling, and was subsamples were inoculated onto KBM supplemented with 200 ppm of rifampicin), Resulting colonies were counted, and the bacterial populations in 1 g of plant tissue (cfu g^{-1} of plant tissue) were determined. The experiment was of randomized plot design

with three replicates, and with one tomato seedling in each replicate for each sampling period.

Identification of two antagonistic bacteria through sequencing of the bacterial 16S rRNA gene

To extract DNA, antagonistic bacterial isolates KD15/1 and KD91/1 were grown on KBM for 24 to 48 h at 24°C. DNA was obtained from resulting bacterial suspensions using boiling lysis. Sterile distilled water (OD_{600} = 0.1) was used to prepare the bacterial suspensions, and the suspensions were centrifuged at 15,000 g for 10 min. The resulting pellets were each suspended in 40 µL of ultrapure water and heated at 100°C for 10 min. The suspensions were then cooled on ice and centrifuged at 15,000 g for 10 s. These resulting the pellets were stored at -20°C. The extracted DNA was used as templates for PCR amplification (Omar *et al*., 2014) . To amplify approx. 1460 base pairs of 16S rDNA, the universal primers 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) were used in the PCR (Hodkinson and Lutzoni, 2009). Each final PCR mixture included 100 ng of DNA extract, 10× Taq KCl reaction buffer, 1 mM of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 1 unit of Taq DNA polymerase (recombinant, 5 U μL^{-1}). The following steps were used in a thermocycler for amplification: 35 cycles, each with an initial denaturation at 95°C for 3 min, followed by denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min using a thermal cycler. The PCR products were then analysed by electrophoresis on a 1.5% agarose gel in 0.5× TAE buffer containing 50× Tris-acetate-EDTA, and were stained with nucleic acid staining solution at 20,000× concentration. The 80 V setting was applied to the gel for 90 min, and the resulting DNA bands plus a 1 kb DNA ladder were visualized under UV light. The amplified products were then purified using a QIAquick Gel Extraction Kit (QIAGEN). Sequencing was performed by MedSanTek Company (Turkey). Sequence editing was carried out using MEGA v10. DNA sequences were analysed using BLASTn software (http://blast.ncbi.nlm.nih.gov/) and were compared with GenBank sequences. The 16S rRNA sequences of the antagonistic bacteria used in this study were submitted to the GenBank database with relevant assigned accession numbers.

Statistical analyses

The antagonistic bacterial population data were first transformed using log root and shoot CFU g^{-1} values, before averaging. All data were analysed using the "agricolae" package in the R statistical programming language. The data obtained were subjected to analysis of variance (ANOVA), with the population data transformed to log (CFU g^{-1}). Means were compared using Duncan's multiple range test at $P < 0.05$, and standard deviations of means were calculated.

RESULTS

In vitro *PGPR and biocontrol assessments of antagonistic bacterial isolates against* Xanthomonas euvesicatoria

A total of 313 endophyte or epiphyte bacterial isolates were obtained from tomato leaf or stem tissues, and were assessed *in vitro* for their antagonistic activity against *X. euvesicatoria*, and for aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, siderophore, indole 3-acetic acid (IAA) and hydrogen cyanide (HCN) production, and to solubilize phosphates (Supplementary Table 1). From these assessments, the 15 isolates were selected for further bioassays (Table 1).

In planta *biocontrol efficacy of bacterial isolates against* Xanthomonas euvesicatoria

Of the 15 potential antagonistic bacterial isolates assessed against *X. euvesicatoria*, isolates isolates KD 4/5 and KD 15/2 were the most disease suppressive producing overall mean disease severities of, respectively, 33 and 34% (Table 2).

In the seed treatment assays, isolate KD 15/2 was the most disease suppressive (Figures 2 and 3). From seed bacterization and spray applications, isolates KD 15/2, KD 84/3, and KD 91/1 gave the greatest pathogen disease suppression (Table 3)

Colonization of tomato plants by antagonistic bacteria and Xanthomonas euvesicatoria

Isolates KD 15/2 and KD 91/1, labelled with rifampicin resistance (200 ppm) and affected systemic transport and colonization at different stages of tomato plant depending on time. At 24 h after applications of the labelled isolates KD 15/2 and KD 91/1 to tomato seeds, the beneficial bacteria were detected at populations of 7×10^9 cfu g^{-1} for KD 15/2 and 8.6×10^8 cfu g^{-1} for KD 91/1. Time-dependent changes in the population densities of the beneficial bacteria in roots and shoots of tomato plants treated with KD 15/2, KD 91/1, KD 15/2 + *X. euvesicatoria*, or KD 91/1 + *X. euvesicatoria* are

Table 1. Characteristics (including mean parameters) of 15 bacterial isolates selected for further *in planta* evaluation in this study.

Isolate code	Location obtained	Growth habit	HCN [a]	IAA [b]	SID [c]	ACC [d]	PS [e]	XE-In [f]
KD4/5	Eğlenhoca/Karaburun/İZMİR	Endophyte	-	125	4.25	+	1.00	10.00
KD7/1	Eğlenhoca/Karaburun/İZMİR	Epiphyte	-	137	3.25	+	10.25	7.25
KD7/2	Mordoğan/Karaburun/İZMİR	Epiphyte	-	114	3.25	+	4.75	10.00
KD10/3	Mordoğan/Karaburun/İZMİR	Endophyte	-	132	5.00	+	3.50	8.25
KD15/1	Ildır/Çeşme/İZMİR	Epiphyte	-	353	2.75	+	4.00	8.25
KD15/2	Ildır/Çeşme/İZMİR	Epiphyte	-	275	2.50	+	3.50	8.25
KD17/2	Ildır/Çeşme/İZMİR	Epiphyte	+	75	12.25	+	1.50	9.00
KD25/2	Nohutalan/Urla/İZMİR	Epiphyte	+	217	4.50	+	3.50	6.00
KD63/2	Dalama/Efeler/AYDIN	Epiphyte	-	256	8.50	+	0.00	6.00
KD80/2	Kınık Ovası/Kınık/İZMİR	Endophyte	-	484	15.00	+	7.75	2.75
KD84/3	Bölcek/Bergama/İZMİR	Endophyte	-	343	10.50	+	5.50	4.00
KD88/1	Yanıkköy/Menemen/İZMİR	Epiphyte	-	167	6.25	+	2.00	8.50
KD88/2	Yanıkköy/Menemen/İZMİR	Endophyte	-	311	4.00	+	1.00	3.00
KD91/1	UTEAM/Menemen/İZMİR	Endophyte	-	191	8.75	+	4.00	8.25
KD97/1	Musahoca/Kırkağaç/MANİSA	Endophyte	-	278	4.00	+	5.00	8.25

[a] Hydrogen cyanide production. [b] Indole acetic acid production (ppm)., [c] Siderophore production (mm), [d] ACC (1-Aminocyclopropane-1-carboxylic acid) deamilase activity, [e] Phosphate solubilization (mm), [f] *Xanthomonas euvesicatoria* inhibition zone (mm). All tests were set up in three replicates and repeated twice.

Table 2. Mean disease severities (DS) caused by *Xanthomonas euvesicatoria* on tomato plants inoculated with different bacterial isolates. Means accompanied by the same letters are not significantly different ($P< 0.05$).

Isolate Code	1. Trial DS (%)	2. Trial DS (%)	Mean DS (%)
Untreated Control	61.66 ± 12.07 a	56.45 ± 7.82 a	59.06 ± 10.12 a
KD 4/5	35.62 ± 5.50 cd	30.62 ± 6.80 de	33.12 ± 6.54 de
KD 7/1	56.95 ± 10.82 a	34.16 ± 3.82 bcde	45.56 ± 14.11 b
KD7/2	35.12 ± 7.64 cd	30.83 ± 6.03 cde	32.97 ± 7.05 de
KD10/3	43.25 ± 9.33 bc	35.83 ± 4.89 bcde	39.54 ± 8.19 bcd
KD15/1	34.87 ± 9.21 cd	34.87 ± 10.34 bcde	34.87 ± 9.53 cde
KD15/2	31.62 ± 12.22 d	37.12 ± 5.56 bcd	34.37 ± 9.66 cde
KD17/2	51.00 ± 19.59 ab	39.78 ± 4.65 b	45.39 ± 15.01 b
KD25/2	36.12 ± 10.23 cd	38.75 ± 4.41 b	37.43 ± 7.78 cde
KD63/2	58.12 ± 10.77 a	53.33 ± 8.95 a	55.72 ± 9.95 a
KD80/2	53.12 ± 10.31 ab	36.45 ± 5.57 bcde	44.79 ± 11.75 b
KD84/3	30.50 ± 7.95 d	30.00 ± 11.33 e	30.25 ± 9.53 e
KD88/1	44.79 ± 9.27 bc	36.25 ± 3.95 bcde	40.52 ± 8.20 bc
KD88/2	53.75 ± 9.86 ab	37.87 ± 4.50 bc	45.81 ±11.04 b
KD91/1	51.25 ± 10.85 ab	31.25 ± 6.58 cde	41.25 ± 13.47 bc
KD97/1	37.50 ± 8.83 cd	39.79 ± 7.76 b	38.64 ± 8.18 bcd

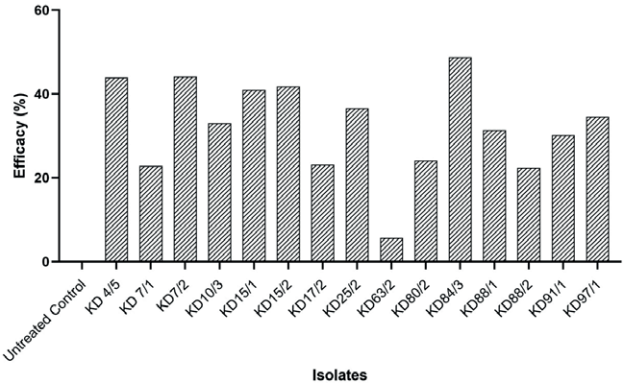

Figure 1. Efficacy percentages for 15 bacterial isolates against *Xanthomonas euvesicatoria*, after applications to surfaces of tomato plants.

shown in Figure 4. At the seedling cotyledon stage, bacterial populations of $6.2 \times 10^{4\text{-}5}$ cfu g^{-1} of internal shoot tissue were detected, and $3.2 \times 10^{5\text{-}6}$ cfu g^{-1} roots. This indicates that isolates KD 15/2 and KD 91/1 colonized plant tissues from seeds to the developing plants. At the second true leaf stage, the bacterial isolates maintained populations of 2.9×10^4 to 2.5×10^6 cfu g^{-1} in shoots and root tissues. For the shoot inoculations, at 24 h after *X. euvesicatoria* inoculation, isolate KD 15/2 was detected at 4×10^3 cfu g^{-1} in plant shoots and 1.1×10^4 cfu g^{-1} in roots. At 72 h after *X. euvesicatoria* inoculation, isolate KD 91/1 maintained a population of 7.2×10^4 cfu g^{-1} plant in the plant shoots and 1×10^5 cfu g in roots. In general, the bacterial isolates successfully colonized tomato roots and shoots in the presence and absence of *X. euvesicatoria*.

Table 3. Mean disease severities (DS) caused by *Xanthomonas euvesicatoria* on tomato plants after seed bacterization with different bacterial isolates. Means accompanied by the same letters are not significantly different ($P < 0.05$).

Isolate Code	Trial 1 DS (%)	Trial 2 DS (%)	Mean DS (%)
Untreated Control	61.67 ± 12.07 a	56.45 ± 7.82 ab	59.06 ± 10.12 a
KD 4/5	46.37 ± 17.54 cde	50.00 ± 4.16 bcde	48.18 ± 12.55 cd
KD 7/1	53.12 ± 15.09 abc	58.12 ± 4.85 a	55.62 ± 11.21 ab
KD7/2	52.29 ± 14.94 abc	51.70 ± 9.90 abcd	52.00 ± 12.34 bc
KD10/3	52.41 ± 5.48 abc	53.12 ± 7.93 abc	52.77 ± 6.64 abc
KD15/1	49.83 ± 17.41 bcd	44.37 ± 7.73 e	47.10 ± 13.41 cd
KD15/2	30.62 ± 9.72 f	34.16 ± 4.19 f	32.39 ± 7.51 e
KD17/2	46.04 ± 8.12 cde	49.58 ± 6.93 bcde	47.81 ± 7.57 cd
KD25/2	50.20 ± 9.28 abcd	55.50 ± 4.60 ab	52.85 ± 7.63 abc
KD63/2	60.62 ± 6.62 ab	56.12 ± 5.11 ab	58.37 ± 6.20 ab
KD80/2	51.25 ± 12.07 abcd	44.75 ± 7.26 de	48.00 ± 10.25 cd
KD84/3	40.20 ± 8.21 def	46.25 ± 11.85 cde	43.22 ± 10.40 d
KD88/1	54.29 ± 9.42 abc	37.50 ± 5.10 f	45.89 ± 11.34 cd
KD88/2	59.04 ± 11.87 ab	53.62 ± 10.67 ab	56.33 ± 11.33 ab
KD91/1	36.50 ± 6.36 ef	34.25 ± 4.04 f	35.37 ± 5.32 e
KD97/1	44.37 ± 3.54 cde	52.08 ± 7.41 abc	48.22 ± 6.90 cd

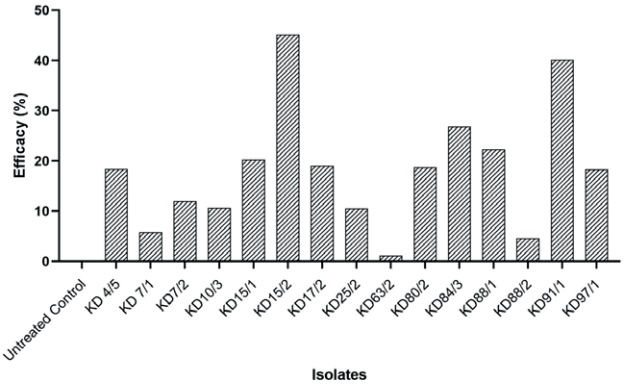

Figure 2. Efficacy percentages from 15 different bacterial isolates applied as seed bacterization treatments against *Xanthomonas euvesicatoria.*

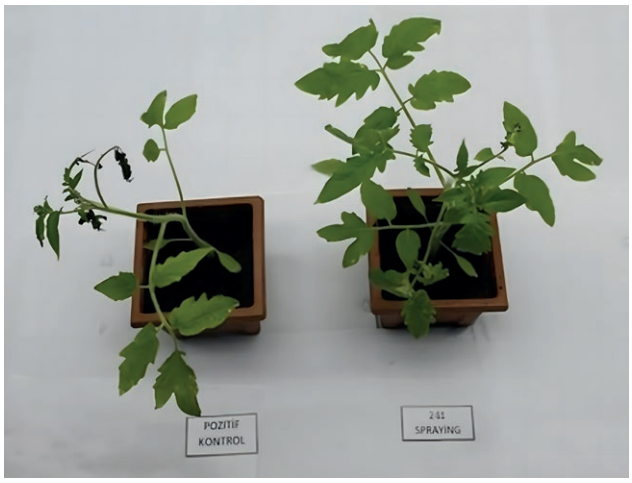

Figure 3. Tomato plants inoculated with *Xanthomonas euvesicatoria* which were either untreated (experimental control, A) or treated with bacterial isolate KD 91/1 (B).

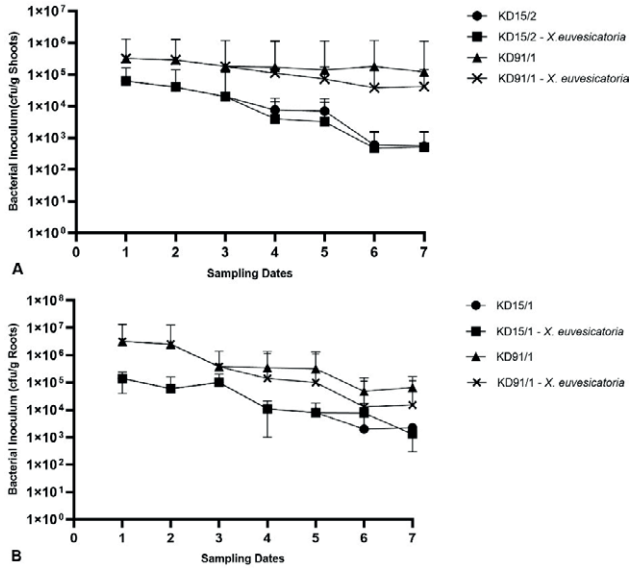

Figure 4. Time-dependent variations of mean populations of different bacteria applied to tomato plants as shoot inoculum (A) or root inoculum (B). Error bars indicate standard errors. Different letters denote significant differences at $P < 0.05$.

Molecular identification of bacteria that reduced Xanthomonas euvesicatoria *infections*

Molecular identifications of six bacteria that were most inhibitory to *X. euvesicatoria* in *in planta* tests are shown in Figure 5, the BLAST analyses of the sequence results in the NCBI database are outlined in Table 4. According to the sequence results, three isolates were identified as *Pseudomonas putida* (KD 4/5, KD 7/2, KD 91/1), one isolate as *Enterobacter aerogenes* (KD 15/1), one isolate as *Enterobacter cloacae* (KD 15/2), and one isolate as *Pantoea* spp. (KD 84/3).

DISCUSSION

Xanthomonas euvesicatoria causes significant yield losses in most tomato-growing regions (Potnis *et al.*, 2015). Important bacterial plant pathogens have devel-

Figure 5. Gel image at 1460 bp after amplification with primer pairs 27F/1492R from six isolates of bacteria that inhibited *Xanthomonas euvesicatoria* infections of tomato plants.

Table 4. Diagnosis and NCBI reference numbers for six bacterial isolates that reduced *Xanthomonas euvesicatoria* infections of tomato plants.

Isolate Code	Bacteria species	Reference Similarity	Reference Access No.	NCBI Access No.
KD 4/5	*Pseudomonas putida*	99.1	MH488985.1	OM218731
KD 7/2	*Pseudomonas putida*	93.0	KF478210.1	OM219206
KD 15/1	*Enterobacter aerogenes*	96.7	LN623608.1	OM219003
KD 15/2	*Enterobacter cloacae*	95.4	MG274270.1	OM219024
KD 84/3	*Pantoea ananatis*	93.0	MN641907.1	PP824970
KD 91/1	*Pseudomonas putida*	94.0	MF683391.1	OM219060

oped resistance to long-used chemical controls (Lucas, 2011), so management of economically important diseases has become increasingly challenging, particularly because of scarcity of effective compounds (Bailey, 2010). Therefore, biological control using microbial antagonists as alternatives to chemicals has been proposed as a positive alternative, to reduces risks chemical contamination in ecosystems and human food chains. Biological control is less affected by pest resistance development than conventional chemicals, and has advantages of minimal or zero residual toxicity and environmental pollution (Gardener and Fravel, 2002; Lazarovits *et al*., 2014; Stenberg *et al*., 2021). In recent years, research on identification and characterization of potential biocontrol agents, such as PGPRs and microbial endophyte antagonists against plant pathogens, has increased considerably.

Approximately 2–5% of rhizosphere bacteria promote plant growth (Glick, 1995). Most plant growth promoting rhizobacteria (PGPRs) belong to *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Frankia*, *Serratia*, *Thiobacillus*, *Pseudomonas*, and *Bacillus* (Goswami *et al*., 2016). The most common bacterial endophytes belong to *Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea*, and *Microbacterium* (Santoyo *et al*., 2016).

The present study assessed isolates of endophytic and epiphytic bacteria obtained from 127 different tomato plant samples from 40 different locations. Among the 313 bacterial isolates obtained, three of the six bacterial isolates shown to be most effective against *X. euvesicatoria in planta* belonged to *Pseudomonas*, two to *Enterobacter*, and one to *Pantoea*, and these results reflect previous reports. The 15 most inhibitory endophyte and epiphyte bacterial isolates were evaluated for effects on disease incidence by evaluating the *in vitro* biocontrol and plant growth-promoting parameters using weighted grading. The chosen isolates were introduced into the pathosystem by host plant seed biopriming or shoot spraying. Among selected isolates, isolates KD 91/1 and KD 15/2 reduced the severity of bacterial spot caused by *X. euvesicatoria* on tomato plants after both treatment types. *In vitro*, isolates KD 91/1 and KD 15/2 gave greatest inhibition of *X. euvesicatoria*. Inhibition zones result from production of volatile antimicrobial compounds (Raza *et al*., 2016) and is a primary antagonistic mechanism (Talibi *et al*., 2014). The 15 selected potential biocontrol agents exhibited biocontrol activity and plant growth-promoting properties, including production of IAA, siderophores, and phosphate solubility. IAA promotes plant growth, whereas siderophore and phosphate solubility help nutrient accumulation in plants, creating nutrient scarcity in soil for pathogens (Wahab *et al*., 2024). These properties of bacterial endophytes indirectly inhibit pathogen growth and promote host plant growth (Kashyap *et al.,* 2019). Of the two isolates, KD 91/1 was the most active *in vitro*, especially for siderophore production, by forming the largest yellow-orange inhibition zones in CAS blue agar.

Bacteria can enhance plant growth and stimulate production of plant growth-promoting substances, including cytokinins and indole acetic acid (Arkhipova *et al*., 2005; Sandhya *et al*., 2017). Bacterial endophytes may produce ACC deaminase, which produces ammonia and α-ketobutyrate from the ethylene precursor ACC, which can promote plant growth under nitrogen-limiting conditions (Afzal *et al*., 2019). The ability of isolate KD 91/1 to produce ACC deaminase and IAA at a high

rate (191 ppm) was probably for greater host plant biomass compared to the other 14 bacterial isolates and the untreated negative controls.

When the 15 potential biocontrol bacteria selected were evaluated for *in planta* biocontrol and plant growth promotion, the greatest effects were detected from isolate KD 91/1. Tomato seed and plant foliar applications gave, respectively, 30 and 40% increases, respectively. Isolate KD 91/1 was identified as a *Pseudomonas* sp. by molecular diagnosis. Plant phyllospheres are predominantly inhabited by *Pseudomonas* spp. (Delmotte *et al*., 2009; Maignien *et al*., 2014) because of their abilities to use effectors to leak water from cells to apoplasts (Xin *et al*., 2016), and to synthesize biosurfactants to increase water availability on leaf surfaces (Hernandez and Lindow, 2019).

Pseudomonas putida is widely recognized as non-hazardous to human and environmental health. Strain KT2440 of *P. putida* is classified by the FDA as HV1 certified, signifying its safety for use inP1 or ML1 environments (Kampers *et al*., 2019), which indicates low-risk for human exposure. The environmental compatibility and efficacy of *P. putida* in bioremediation have been demonstrated in previous studies (Xue *et al*., 2022; Tasleem *et al*., 2023). However, close examination reveals contradictions and important facts. Although *P. putida* is generally considered safe, clinical isolates of this species have been reported, albeit infrequently. These clinical strains possess genes associated with survival under oxidative stress, resistance to biocides, and toxin/antitoxin systems, potentially enhancing capacity to colonize and persist within human tissues (Molina Delgado *et al*., 2016). Furthermore, while *P. putida* shares 85% of its coding regions with the opportunistic pathogen *P. aeruginosa*, it lacks virulence factors such as exotoxins and type III secretion systems (Udaondo *et al*., 2016).

Pseudomonas isolates have been observed to directly inhibit growth of various pathogens such as *X. campestris* pv. *vesicatoria* and *P. syringae* pv. *glycinea* in laboratory and field experiments (Völksch and May, 2001; Abo-Elyousr and El-Hendawy, 2008). In the present study, no significant reduction in disease severity caused by *X. euvesicatoria* was found when bacterial isolates KD 4/5 and KD 7/2 were applied to tomato seeds by biopriming, whereas they reduced the disease severity by an average of 43% when applied by spraying on plant leaves. Molecular identifications of isolates KD 4/5 and KD 7/2 showed them to be *Pseudomonas*. Isolate KD 91/1 (*P. putida*) was an endophyte isolated from tomato roots. Compared to non-endophytic bacteria, endopytes enter and colonize their original host more readily than non-endophytes, as they are adapted to hosts without stimulating defense mechanisms. Endophytes also compete with pathogens that infect plants and survive in plant tissues, endophyte colonization prevents pathogenic organisms from entering plant tissues (Martinez-Klimova *et al*., 2017). For example, *P. putida* WCS358 effectively mitigated Fusarium wilt in radish by competing for iron through siderophore production, whereas *P. putida* RE8 induced systemic resistance against the disease (de Boer *et al*., 2003). Additionally, *P. putida* strains have demonstrated capacity to employ contact-dependent mechanisms, including the type IVB secretion system, to eliminate competing bacterial species and safeguard plants from pathogens, such as *Ralstonia solanacearum* (Purtschert-Montenegro *et al*., 2022).

Pantoea ananatis, while predominantly identified as a plant pathogen, also has PGPR characteristics. This bacterium has diverse ecological functions, including plant growth enhancement, and has potential as a biological control agent. Some strains of this bacterium have been shown facilitate plant growth in *Solanum tuberosum* (potato) and *Capsicum annuum* (pepper) (Coutinho and Venter, 2009). Additionally, *Pa. ananatis* can infect monocotyledonous and dicotyledonous plants, causing leaf blotches and die-back. The bacterium is responsible for diseasesmany economically important crop plants including *Zea mays* (maize), *Oryza sativa* (rice), *Allium cepa* (onion), and *Citrullus lanatus* (melon). symptoms of these diseases vary depending on host plant, leading to diverse agricultural challenges. For example, *Pa. ananatis* causes center rot in onions, which can result in substantial yield reductions and postharvest losses (De Maayer *et al*., 2010). This pathogen also has toxicity towards specific human cell lines, including glioblastoma cells, underscoring its relevance (Polidore *et al*., 2021).

Enterobacter cloacae, a known PGPR, exerts several effects that can be beneficial or detrimental. As a PGPR, *E. cloacae* facilitates plant growth by enhancing nutrient uptake, improving stress resistance, and promoting overall growth, thereby contributing to sustainable agriculture. However, similar to many non-native rhizobacteria, when this bacterium is introduced into ecosystems unintended consequences can result, including alteration of local microbiomes and disruption of ecosystem functions (dos Santos *et al*., 2020; Moore *et al*., 2022). Conversely, *E. cloacae* is acknowledged as a significant opportunistic pathogen in humans. It can have multidrug resistance, frequently attributed to the production of chromosomally-encoded AmpC β-lactamase, which complicates therapeutic interventions (Davin-Regli and Pagès, 2015). This organism has been associated with hospital-acquired infections, including those of urinary tracts and respira-

tory systems, and can be disseminated throughout hospital settings, often leading to considerable healthcare challenges including outbreaks in intensive care units (John *et al*., 1982; Moradigaravand *et al*., 2016).

Enterobacter aerogenes can be PGPR and an opportunistic pathogen with considerable implications. This bacterium functions as a PGPR by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces plant ethylene levels, thereby enhancing plant growth under stress conditions such as in saline alkali environments. The bacterium can also synthesize indole-3-acetic acid (IAA), which facilitates root elongation and branching, which are important for plant survival under drought stress (Jochum *et al*., 2019). Furthermore, *E. aerogenes* has ability to solubilize phosphate and produce siderophores, which contribute to improved plant nutrient availability and uptake, ultimately leading to enhanced plant productivity and yields (Liu *et al*., 2019). Conversely, *E. aerogenes* is recognized as an opportunistic pathogen associated with hospital-acquired infections, particularly in immunocompromised patients. It has been implicated in various clinical conditions, including pneumonia, bacteremia, and urinary tract infections. The bacterium can have multidrug resistance, which complicates clinical treatment options (Davin-Regli and Pagès, 2015).

For these reasons, *P. anantis*, *E. cloacae* and *E. aerogenes* are not recommended as biological control agents, despite their efficacy in controlling *X. euvesicatoria* and their beneficial impacts on plant growth.

In general, bacterial populations in plant rhizoplanes are in the range of 10^5 to 10^7 cfu g^{-1} root fresh weight (Goel *et al*., 2017). Populations of endophytes can vary depending on bacterial species, plant genotypes, plant tissue, and environmental conditions (Rosenblueth and Martínez-Romero, 2006). In the present study, bacterial population of 8.6×10^8 cfu g^{-1} seed was determined after coating *P. putida* KD 91/1 bacterial isolates onto tomato seeds. Average bacterial population of $3 \times 10^{4\text{-}5}$ to $2.5 \times 10^{4\text{-}6}$ cfu g^{-1} plant tissue was determined within tissues of tomato shoots and roots during the cotyledon and second true leaf stages. At 72 h after pathogen inoculation, the isolate KD 91/1 maintained a population of 7.2×10^4 cfu g^{-1} in shoots and 1×10^5 cfu g^{-1} in roots. Overall, *P. putida* KD 91/1 successfully colonized tomato roots and shoots in the presence and absence of *X. euvesicatoria*. Root colonization ability is a prerequisite and determinant for biocontrol agent activity and efficacy (Cavaglieri *et al*., 2005). The enhanced colonization ability of *P. putida* KD 91/1 may have contributed to biocontrol efficacy. *Pseudomonas rhodesiae* and *Pantoea ananatis*, selected for their consistent colonization of pepper stems, have been shown to mitigate disease severity caused by *Xanthomonas axonopodis* pv. *vesicatoria* and to induce systemic host resistance (Kang et al., 2007).

CONCLUSIONS

Previous research has suggested that particular antagonistic isolates may be suitable candidates for biological control of *X. euvesicatoria*. However, further research is required to gain understanding of the interactions between this pathogen, host plants, and antagonistic bacteria. Effectiveness of biocontrol against *X. euvesicatoria* should also be confirmed through molecular gene expression experiments and detection of genes involved in plant resistance induction.

ACKNOWLEDGEMENTS

The authors thank Professor Ayse GUL for providing plant chamber conditions.

FUNDING

This study was supported by national grants from the Ege University Office of Scientific Research Projects (BAP) (FDK-2019-20622).

AUTHOR CONTRIBUTIONS

GE carried out the growth chamber and colonization studies. HO participated in the study design and performed the statistical analysis. HO conceived the study and participated in its design and coordination; US performed the statistical analysis, sequence alignment, and drafted the manuscript. All authors have read and approved the final manuscript.

LITERATURE CITED

Abbasi P.A., Khabbaz S.E., Weselowski B., Zhang L., 2015. Occurrence of copper-resistant strains and a shift in *Xanthomonas* spp. causing tomato bacterial spot in Ontario. *Canadian Journal of Microbiology* 61: 753–761.

Abo-Elyousr K.A.M., El-Hendawy H.H., 2008. Integration of *Pseudomonas fluorescens* and acibenzolar-S-methyl to control bacterial spot disease of tomato. *Crop Protection* 27: 1118–1124.

Afzal I., Shinwari Z.K., Sikandar S., Shahzad S., 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiological Research* 221: 36–49.

Akbaba M., Ozaktan H., 2018. Biocontrol of angular leaf spot disease and colonization of cucumber (*Cucumis sativus* L.) by endophytic bacteria. *Egyptian Journal of Biological Pest Control* 28: 1–10.

Akköprü A., Ozaktan H., 2018. Identification of rhizobacteria that increase yield and plant tolerance to angular leaf spot disease in cucumber . *Plant Protection Science* 54(2): 67–73.

Al-Dahmani J.H., Abbasi P.A., Miller S.A., Hoitink H.A.J., 2003. Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. *Plant Disease* 87: 913–919.

Arkhipova T.N., Veselov S.U., Melentiev A.I., Martynenko E. V, Kudoyarova G.R., 2005. Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. *Plant and Soil* 272: 201–209.

Bailey K.L., 2010. Canadian innovations in microbial biopesticides. *Canadian Journal of Plant Pathology* 32: 113–121.

Bakker A.W., Schippers B.O.B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. *Soil Biology and Biochemistry* 19: 451–457.

Balogh B., Jones J.B., Momol M.T., Olson S.M., Obradovic A., … Jackson L.E., 2003. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Disease* 87: 949–954.

Bolat I., Bakır A.G., Korkmaz K., Gutiérrez-Gamboa G., Kaya O., 2022. Silicon and nitric oxide applications allow mitigation of water stress in Myrobalan 29C rootstocks (*Prunus cerasifera* Ehrh.). *Agriculture* 12: 1273–1283.

Bric J.M., Bostock R.M., Silverstone S.E., 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology* 57: 535–538.

Cavaglieri L., Orlando J., Rodriguez M.I., Chulze S., Etcheverry M., 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. *Research in Microbiology* 156: 748–754.

Compant S., Duffy B., Nowak J., Clément C., Barka E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* 71: 4951–4959.

Coutinho T.A., Venter S.N., 2009. *Pantoea ananatis*: An unconventional plant pathogen. *Molecular Plant Pathology* 10: 325–335. https://doi.org/10.1111/j.1364-3703.2009.00542.x.

Davin-Regli A., Pagès J.M., 2015. *Enterobacter aerogenes* and *Enterobacter cloacae*; Versatile bacterial pathogens confronting antibiotic treatment. *Frontiers in Microbiology* 6: 392–402.

De Boer M., Bom P., Kindt F., Keurentjes J.J.B., van der Sluis I., … Bakker P.A.H.M., 2003. Control of Fusarium wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms. *Phytopathology* 93: 626–632.

De Maayer P., Chan W.Y., Venter S.N., Toth I.K., Birch P.R.J., … Coutinho T.A., 2010. Genome sequence of *Pantoea ananatis* LMG20103, the causative agent of Eucalyptus blight and dieback. *Journal of Bacteriology* 192: 2936–2937. https://doi.org/10.1128/JB.00060-10.

Delmotte N., Knief C., Chaffron S., Innerebner G., Roschitzki B., … Vorholt J.A., 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences* 106: 16428–16433.

Dos Santos R.M., Diaz P.A.E., Lobo L.L.B., Rigobelo E.C., 2020. Use of plant growth-promoting rhizobacteria in maize and sugarcane: characteristics and applications. *Frontiers in Sustainable Food Systems* 4: 136–151.

El-Hendawy H.H., Osman M.E., Sorour N.M., 2005. Biological control of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* by *Rahnella aquatilis*. *Microbiological Research* 160: 343–352.

FAO, 2022. *Statistical Yearbook*. Available at: https://www.fao.org/faostat/en/#home.

Gardener B.B.M., Fravel D.R., 2002. Biological control of plant pathogens: research, commercialization, and application in the USA. *Plant Health Progress* 3: 17–32.

Glick B.R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41: 109–117.

Goel R., Kumar V., Suyal D.K., Dash B., Kumar P., Soni R., 2017. Root-associated bacteria: rhizoplane and endosphere. In: *Plant-Microbe Interactions in Agro-Ecological Perspectives: Volume 1: Fundamental Mechanisms, Methods and Functions*, Springer, 161–176 pp.

Goswami D., Thakker J.N., Dhandhukia P.C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food & Agriculture* 2: 1127500–1127514.

Hernandez M.N., Lindow S.E., 2019. *Pseudomonas syringae* increases water availability in leaf microenvironments via production of hygroscopic syringafactin. *Applied and Environmental Microbiology* 85: 14-19.

Hodkinson B.P., Lutzoni F., 2009. A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49: 163–180.

Jetiyanon K., Kloepper J.W., 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biological Control* 24: 285–291.

Jochum M.D., McWilliams K.L., Borrego E.J., Kolomiets M. V., Niu G., … Jo Y.K., 2019. Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. *Frontiers in Microbiology* 10.

John J.F., Sharbaugh R.J., Bannister E.R., 1982. *Enterobacter cloacae*: bacteremia, epidemiology, and antibiotic resistance. *Reviews of Infectious Diseases* 4: 13–28. https://doi.org/10.1093/clinids/4.1.13.

Kampers L.F.C., Volkers R.J.M., Martins dos Santos V.A.P., 2019. *Pseudomonas putida* KT 2440 is HV 1 certified, not GRAS. *Microbial Biotechnology* 12: 845–848.

Kang S., Cho H.S., Cheong H., Ryu C.M., Kim J.F., Park S.H., 2007. Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). *Journal of Microbiology and Biotechnology* 17(1): 96–103.

Kashyap B.K., Solanki M.K., Pandey A.K., Prabha S., Kumar P., Kumari B., 2019. *Bacillus* as plant growth promoting rhizobacteria (PGPR): a promising green agriculture technology. In: *Plant Health Under Biotic Stress*: Volume 2: Microbial Interactions, Springer, 219–236 pp.

Khan A., Zhao X.Q., Javed M.T., Khan K.S., Bano A., … Masood S., 2016. *Bacillus pumilus* enhances tolerance in rice (*Oryza sativa* L.) to combined stresses of NaCl and high boron due to limited uptake of Na+. *Environmental and Experimental Botany* 124: 120–129.

Klement Z., Mavridis A., Rudolph K., Vidaver A., Perombelon M.C.M., … Rudolph K., 1990. Inoculation of plant tissues. In: *Methods in Phytobacteriology*, Akadémiai Kiadó, Budapest, 94–124 pp.

Kloepper J.W., Schroth M.N., Miller T.D., 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology* 70: 1078–1082.

Köhl J., Kolnaar R., Ravensberg W.J., 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Frontiers in Plant Science* 10: 845–864.

Lazarovits G., Turnbull A., Johnston-Monje D., 2014. Plant Health Management: Biological Control of Plant Pathogens. In: *Encyclopedia of Agriculture and Food Systems*; Elsevier Ltd.: London, UK, 2014; pp. 388–399.

Liu J., Tang L., Gao H., Zhang M., Guo C., 2019. Enhancement of alfalfa yield and quality by plant growth-promoting rhizobacteria under saline-alkali conditions. *Journal of the Science of Food and Agriculture* 99: 281–289. https://doi.org/10.1002/jsfa.9185.

Lucas J.A., 2011. Advances in plant disease and pest management. *The Journal of Agricultural Science* 149: 91–114.

Lwin M., Ranamukhaarachchi S.L., 2006. Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. *International Journal of Agriculture and Biology* 8: 657–660.

Mahmood A., Turgay O.C., Farooq M., Hayat R., 2016. Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology* 92(8): 112–126.

Maignien L., DeForce E.A., Chafee M.E., Eren A.M., Simmons S.L., 2014. Ecological succession and stochastic variation in the assembly of *Arabidopsis thaliana* phyllosphere communities. *mBio* 5: 10–1128.

Martin H.L., Hamilton V.A., Kopittke R.A., 2004. Copper tolerance in Australian populations of *Xanthomonas campestris* pv. *vesicatoria* contributes to poor field control of bacterial spot of pepper. *Plant Disease* 88: 921–924.

Martinez-Klimova E., Rodríguez-Peña K., Sánchez S., 2017. Endophytes as sources of antibiotics. *Biochemical Pharmacology* 134: 1–17.

Michelson I.F., Lachman W.H., Allen D.D., 1958. The use of the "Weighted-Rankit" method in variety trials. *ASHS Proceedings* 71: 334–338.

Mirik M., Aysan Y., Cinar O., 2008. Biological control of bacterial spot disease of pepper with *Bacillus* strains. *Turkish Journal of Agriculture and Forestry* 32: 381–390.

Molina Delgado L., Udaondo Z., Duque E., Fernández M., Bernal Guzmán P., … Ramos J.L., 2016. Specific Gene Loci of Clinical *Pseudomonas putida* Isolates. *Plos ONE* 11(1): 147–171.

Moore J.A.M., Abraham P.E., Michener J.K., Muchero W., Cregger M.A., 2022. Ecosystem consequences of introducing plant growth promoting rhizobacteria to managed systems and potential legacy effects. *New Phytologist* 234(6): 1914–1918.

Moradigaravand D., Reuter S., Martin V., Peacock S.J., Parkhill J., 2016. The dissemination of multidrug-

resistant *Enterobacter cloacae* throughout the UK and Ireland. *Nature Microbiology* https://doi.org/10.1038/nmicrobiol.2016.173.

Nautiyal C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters* 170: 265–270.

Nejad P., Johnson P.A., 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. *Biological Control* 18: 208–215.

Omar B.A., Atif H.A., Mogahid M.E., 2014. Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. *African Journal of Microbiology Research* 8: 598–602.

Pajčin I., Vlajkov V., Frohme M., Grebinyk S., Grahovac M., … Grahovac J., 2020. Pepper bacterial spot control by *Bacillus velezensis*: bioprocess solution. *Microorganisms* 8: 1463.

Pertot I., Puopolo G., Giovannini O., Angeli D., Sicher C., Perazzolli M., 2016. Advantages and limitations involved in the use of microbial biofungicides for the control of root and foliar phytopathogens of fruit crops. *Italus Hortus* 23: 3–12.

Polidore A.L.A., Furiassi L., Hergenrother P.J., Metcalf W.W., 2021. A phosphonate natural product made by *Pantoea ananatis* is necessary and sufficient for the hallmark lesions of onion center rot. *mBio* 12: 1–18. DOI: 10.1128/mBio.03402-20.

Potnis N., Timilsina S., Strayer A., Shantharaj D., Barak J.D., … Jones J.B., 2015. Bacterial spot of tomato and pepper: Diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology* 16: 907–920.

Purtschert-Montenegro G., Cárcamo-Oyarce G., Pinto-Carbó M., Agnoli K., Bailly A., Eberl L., 2022. *Pseudomonas putida* mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system. *Nature Microbiology* 7: 1547–1557.

Raza W., Ling N., Yang L., Huang Q., Shen Q., 2016. Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. *Scientific Reports* 6: 24856.

Ritchie, 2000. Bacterial spot of pepper and tomato. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2000-1027-01.

Rosenblueth M., Martínez-Romero E., 2006. Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions* 19: 827–837.

Sahin F., Miller S.A., 1998. Resistance in *Capsicum pubescens* to *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. *Plant Disease* 82: 794–799.

Sandhya V., Shrivastava M., Ali S.Z., Sai Shiva Krishna Prasad V., 2017. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russian Agricultural Sciences* 43: 22–34.

Santoyo G., Moreno-Hagelsieb G., del Carmen Orozco-Mosqueda M., Glick B.R., 2016. Plant growth-promoting bacterial endophytes. *Microbiological Research* 183: 92–99.

Saravanakumar D., Samiyappan R., 2007. ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. *Journal of Applied Microbiology* 102: 1283–1292.

Schaad N.W., Jones J.B., Chun W., 2001. *Laboratory Guide for the Identification of Plant Pathogenic Bacteria*. American Phytopathological Society (APS Press). USA, 373pp.

Sharma S., Bhattarai K., 2019. Progress in developing bacterial spot resistance in tomato. *Agronomy* 9: 26.

Shrestha A., Kim B.S., Park D.H., 2014. Biological control of bacterial spot disease and plant growth-promoting effects of lactic acid bacteria on pepper. *Biocontrol Science and Technology* 24: 763–779.

Stenberg J.A., Sundh I., Becher P.G., Björkman C., Dubey M., … Jonsson M., 2021. When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science* 94: 665–676.

Talibi I., Boubaker H., Boudyach E.H., Ait Ben Aoumar A., 2014. Alternative methods for the control of postharvest citrus diseases. *Journal of Applied Microbiology* 117: 1–17.

Tasleem M., El-Sayed A.-A.A.A., Hussein W.M., Alrehaily A., 2023. *Pseudomonas putida* metallothionein: structural analysis and implications of sustainable heavy metal detoxification in Madinah. *Toxics* 11: 864.

Tiwari S., Lata C., Chauhan P.S., Nautiyal C.S., 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiology and Biochemistry* 99: 108–117.

Townsend G., Heuberger J., 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter* 27: 340–343.

Turfan N., Düzel Ö., 2023. Investigation of Foliar L-Glutamic Application on the Resistance to the Capacity of the SC2121 Tomato Variety (*Solanum lycopersicum*) L.) to Long-Term Salinity Stress. *Yuzuncu Yıl University Journal of Agricultural Sciences* 33: 327–337.

Udaondo Z., Molina L., Segura A., Duque E., Ramos J.L., 2016. Analysis of the core genome and pangenome of *Pseudomonas putida*. *Environmental Microbiology* 18: 3268–3283.

Völksch B., May R., 2001. Biological control of *Pseudomonas syringae* pv. *glycinea* by epiphytic bacteria under field conditions. *Microbial Ecology* 41: 132–139.

Wahab A., Bibi H., Batool F., Muhammad M., Ullah S., ... Abdi G., 2024. Plant growth-promoting rhizobacteria biochemical pathways and their environmental impact: a review of sustainable farming practices. *Plant Growth Regulation* 104: 637–662.

Xin X.-F., Nomura K., Aung K., Velásquez A.C., Yao J., ... He S.Y., 2016. Bacteria establish an aqueous living space in plants crucial for virulence. *Nature* 539: 524–529.

Xue Y., Qiu T., Sun Z., Liu F., Yu B., 2022. Mercury bioremediation by engineered *Pseudomonas putida* KT2440 with adaptationally optimized biosecurity circuit. *Environmental Microbiology* 24: 3022–3036.