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RESEARCH

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Using certain endophytic bacteria as biocontrol agents against bacterial speck disease of tomato

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Abstract

Background Bacterial speck, caused by *Pseudomonas syringae* pv. *tomato* (Pst), is a major constraint to tomato production worldwide and the limited efficacy of chemical control indicates that there are sustainable alternatives. This study aims to evaluate the antagonistic potential of endophytic bacteria isolated from healthy tomato tissues as biocontrol agents against Pst in order to identify effective candidates for sustainable disease management.

Results In vitro screening of 15 isolates revealed that strains KAU7 and KAU11 exhibited the strongest antibacterial activity, producing inhibition zones of approximately 1.33 and 1.23 cm, comparable to streptomycin. Biochemical characterization showed that both isolates produced siderophores, solubilized phosphate and synthesized indole-3-acetic acid (IAA), indicating their ability to promote plant growth and suppress pathogens. Molecular identification confirmed that KAU7 and KAU11 were *Bacillus halotolerans* and *Bacillus paralicheniformis*, respectively. In greenhouse trials, both strains significantly reduced disease severity and *B. paralicheniformis* achieved the highest reduction (32–45%), depending on application method. Treatments also enhanced plant growth and increased defense-related enzyme activity, specifically peroxidase and polyphenol oxidase.

Conclusion The present study demonstrates that *B. halotolerans* and *B. paralicheniformis* effectively suppress bacterial speck disease in tomato plant growth and inducing defense responses. These strains represent promising alternatives to chemical bactericides in integrated disease management strategies.

Keywords *Pseudomonas syringae* pv. *tomato*, Endophytic *Bacillus*, Biological control, Induced systemic resistance, Defense-related enzymes, Tomato diseases, Plant–microbe interactions

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Background

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops worldwide and represents a major component of protected and open-field production systems. Among the bacterial diseases of tomato, bacterial speck, incited by *Pseudomonas syringae* pv. *tomato* (Pst), is a major foliar diseases of tomato. Symptoms vary, but common signs include small necrotic spots on leaves, ranging in color from white to black. Stems are often surrounded by a chlorotic halo, which may coalesce and lead to premature leaf senescence under conductive conditions (Akila et al. 2024). Disease symptoms on the fruit include superficial black specks and scab-like lesions, which may reduce market quality and consumer acceptance, even under conditions of moderate yield loss (Soliman 2023). Disease progress is promoted by cool to mild temperatures and high humidity or prolonged leaf wetness, which are widely distributed in greenhouse and irrigated cultivated systems (McManus et al. 2002). As a result, bacterial speck can lead to substantial economic losses due to fruit downgrading in the field, post-harvest rejection, and increased management costs (Giovanard et al., 2025).

Managing bacterial speck disease is particularly difficult. Chemical control, including copper-based compounds and antibiotics, are often inconsistent and largely ineffective as Pst rapidly develops resistance while maintaining high level of habitat fitness (Bonaterra et al. 2022). Furthermore, mounting restrictions on the use of antibiotics in agricultural practice along with increasing public demand for residue-free produce has intensified the requirement for sustainable interventions suitable for integrated disease management (Tyagi et al. 2024). These problems have increased interest in BCAs, especially plant-associated bacteria, as environmentally friendly alternatives to traditional bactericides (Brown et al. 2023). Within biocontrol agents (BCAs), *Bacillus* species are of particular interest because they can form spores, survive under harsh ecological conditions, and secrete a wide variety of antimicrobial metabolites. Numerous studies have shown that *Bacillus* spp. can suppress *P. syringae* pv. *tomato* via antibiosis, competition for nutrients such as iron, and triggering of induced systemic resistance in tomato plants (Köhl et al. 2019). However, significant previous work has focused mainly on well-defined rhizospheric or epiphytic species such as *B. subtilis*, *B. amyloliquefaciens*, and *B. velezensis* (reporting disease reduction without extensive strain comparisons or evaluation of the host physiology) (Giovanard et al.,Giovanardi et al. 2025). Accordingly, the strain-specific basis of effective disease suppression has not been well elucidated.

Endophytic bacteria are an especially attractive but understudied source of BCAs. These microbes reside within internal plant tissues without causing apparent disease and may form enduring rhizoplane and endophytic associations that enhance plant growth, nutrient absorption and defense mechanisms against pathogens (Eid et al. 2021). Endophytic *Bacillus* spp., in particular, has been reported to synthesize siderophores, phytohormones and lytic enzymes, and to induce host defense responses during pathogen infection (Abo-Elyousr et al. 2021). However, few studies have focused endophytic *Bacillus* spp. in relation to bacterial speck disease and few comparative studies have systematically evaluated the relationship between their antagonistic potential, plant growth promotion, and the induction of defense-related enzymes (Zhang et al. 2023). Furthermore, the biocontrol capabilities of endophytes such as *Bacillus halotolerans* and *B. paralicheniformis* against Pst have never been systematically reported.

The current study fills these gaps by examining the potential of endophytic *Bacillus* strains obtained from healthy tomato plants to suppress bacterial speck disease. In particular, this study determines (i) in vitro antagonism of selected endophytic isolates against *P. syringae* pv. *tomato* (ii) Identification of the selected isolates through molecular methods and analyses of plant growth promotion, (iii) their capacity to reduce disease severity and induce defense enzymes under the greenhouse conditions, after being applied by two delivery methods. Linked to pathogen suppression was an overall response involving biochemical and host defense reactions, indicating a potentially encouraging role for endophytic *B. halotolerans* and *B. paralicheniformis* in controlling bacterial speck disease in tomato plants as sustainable biocontrol agents, especially when used following organic standard production.

Methods

Source of the pathogen

A highly virulent strain of *Pseudomonas syringae* pv. *tomato*, previously isolated by Abd El-Fatah et al. (2023) was obtained from culture collection at Assiut University, Assiut, Egypt.

Plant material

The common tomato variety "Super Strain B" seedlings were cultivated in 30 cm pots filled with peat moss (1:3). After three to four leaf stages, the plants were moved to new pots and placed in a greenhouse. Ten days after transplanting, 10 ml/pots NPK fertilizer (12:4:6) was given to the soil (Abo-Elyousr and El-Hendawy 2008). Irrigation of the plants was done when needed.

Preparation of inoculum

Inoculum pathogen *P. syringae* pv. *tomato* was prepared. In brief, 50 ml of sterile conical flasks containing

25 ml of nutrient broth were placed on a rotary shaker at 28 ± 2 °C, and a loopful of 48-hour-old bacterial culture was suspended in liquid nutrient broth (prepared as NA). The 72-hour-old culture was then centrifuged at 4 °C for 20 min at 6000 rpm. The supernatant was transferred to a separate tube, and the pellet was discarded. A spectrophotometer was used to adjust the concentration of bacterial cells in suspension for each isolate to an OD610. The cell density (cells ml^{-1}) was maintained, and the suspension was further adjusted by diluting it with sterile distilled water (Abd El-Fatah et al. 2023).

Source of endophytic bacteria and identification

Fifteen bacterial isolates were used as candidate antagonists, collected from various healthy tomato plants and obtained from the Laboratory of Phytopathology's culture collection (King Abdulaziz University, Faculty of Environmental Sciences, Department of Agriculture, Saudi Arabia). 16 S RNA was used to identify the potential antagonist bacterial isolates used in the experiment.

In vitro antibacterial effect of antagonists

Using the dual culture test described by Abo-Elyousr et al. (2020), the antibacterial effects of possible antagonist bacterial isolates were evaluated for the ability to inhibit the growth of *P. syringae* pv. *tomato*. After getting speck applied as a three-point inoculation on Tryptic Soy Agar (TSA) media, putative antagonist bacterial isolates were cultured for 48 h at 25 °C. For each strain, three replicates were performed (Horuz et al. 2018). The inhibitory zones that developed in the Petri dishes were assessed following a 48-hour incubation period at 25 °C in an incubator.

Effects of antagonists against bacterial speck disease in in vivo conditions

Tomato seedlings (cv Super Strain B) were used in pot testing in a greenhouse. Three different methods were used to apply the antagonist bacterial isolates to tomato plants: first, 10 ml of the bacterial suspension (10^8 CFU/ml) was sprayed on the leaves of the plants using a handheld sprayer; second, the seeds were soaked in the bacterial suspension for 10 min prior to sowing; and third, 10 ml of the bacterial suspension was soaked in the soil for each pot. The seedlings were treated with sterile water as a negative control, pathogen suspension as positive control, and streptomycin (0.02%) as an antibiotic (Bashan and de-Bashan 2002). For each treatment, five replicates were used, with four plants per replicate. To initiate bacterial infection, plants were covered with polythene bags for 48 h. Plants were monitored daily, and after 14 days of inoculation, symptoms began to appear. According to literature, a 1–3 disease rating scale, (0 = no spots; 1 = 1–10 spots per leaflet; 2 = 11–20 spots per leaflet; 3 = more than 20 spots per leaflet), was used to determine the severity was recorded (Abd El-Fatah et al. 2023). The experiment was repeated twice.

Determination of the indoleacetic acid (IAA), siderophore and phosphate-solubilizing activities of antagonist bacterial isolates

The ability of putative antagonist bacterial isolates to produce IAA, siderophores, and phosphate-solubilizing activities was evaluated. The Salkowski method was used to assess the capacity of candidate bacterial strains to produce IAA in the presence of L-tryptophan.

In Petri dishes with Pikovskaya agar (PVK) medium containing tricalcium phosphate, the prospective bacterial strains' phosphate dissolving abilities were assessed. Seven days following inoculation, the development of a clear zone surrounding the pathogen colony proved positive (Filiz and Bozkurt 2022).

On Blue-CAS agar, antagonist bacteria have been identified to produce siderophores (Schwyn and Neilands 1987). All antagonist strains were cultivated on a rotary shaker (120 rpm) in Nutrient Broth medium for 48 h at 30 °C. After that, 0.05 ml of the pathogen suspension was triplicate-plated on CAS agar plates and cultured for 7 days at 30 °C. The pathogen colony zone and the widths of the yellow-orange zones produced by the antagonist bacteria were measured after a 7-day incubation at 25 ± 1 °C.

Defense-related enzymes activity

Defense-related enzyme activity was measured 15 days after treatment using leaves from the various tomato plants. Among the defense-related enzymes were polyphenol oxidase (PPO) and peroxidase (POD). Both healthy and diseased plants were assessed for POD and PPO activities. The crude enzyme was extracted using the methodology described by Elshahawy et al. (2022). Polyphenol oxidase activity was evaluated spectrophotometrically at 420 nm using the methodology of Duckworth and Coleman (1970), and peroxidase activity was tested using the method of Maehly and Chance (1954).

Statically analysis

All experimental data were analyzed using MSTATC statistical software. Analysis of variance (ANOVA) was performed; means were compared by LSD multiple range test when P values indicated a significant difference ($p \leq 0.05$).

Results

In vitro antibacterial activity of antagonistic bacterial isolates

Fifteen bacterial isolates were evaluated for antagonism against *Pseudomonas syringae* pv. *tomato* (Pst) in vitro. The isolates showed a broad range of antibacterial activity; inhibition zones ranged from 0.17 to 1.33 cm (Table 1). Significant differences were found in the treatments ($p \leq 0.05$), thereby demonstrating diversity in the antagonistic capacity of the studied strains. Isolate KAU7 produced the strongest inhibition, with an inhibition zone of 1.33 ± 0.02 cm, which was not significantly different from that of the streptomycin control (1.20 ± 0.08 cm). Isolates KAU11 and KAU15 were also showed significant inhibition, with inhibition zones measuring 1.23 ± 0.19 cm and 1.18 ± 0.06 cm, respectively. A second group of isolates were KAU12, KAU33, KAU4, and KAU5 exhibited intermediate antibacterial activity (0.80–0.92 cm) (Table 1). The remaining isolates showed lower activity, with zones of inhibition less than 0.40 cm (data not shown) for KAU1, KAU8, KAU9, and KAU10. Based on these findings, KAU7 and KAU11 were selected as the most promising isolates for further mechanistic studies and molecular identification using 16 S rRNA analysis, as well as for evaluation under greenhouse conditions.

Mechanisms of antagonism

Biochemical comparisons between the two most effective isolates showed several antagonistic characteristics. Phosphorus-solubilizing activities were observed in both the strains, where KAU11 showed very strong (+++) and KAU7 showed moderate (++) activity. KAU7 exhibited high siderophore production, whereas it was moderate for KAU11. Both isolates further produced indole-3-acetic acid (IAA) and generation was higher in KAU11 (data not show).

Table 1 In vitro antibacterial effect of fifteen isolates antagonists against *Pseudomonas syringae* pv. *tomato* (Pst)

	Isolates	Inhibition zone (cm)
1	Streptomycin	$1.20^{a} \pm 0.08$
2	KAU 1	$0.17^{b} \pm 0.06$
3	KAU 2	$0.67^{c} \pm 0.03$
4	KAU33	$0.9^{b} \pm 0.09$
5	KAU 4	$0.8^{c} \pm 0.03$
6	KAU 5	$0.8^{c} \pm 0.02$
7	KAU 6	$0.6^{d} \pm 0.07$
8	KAU 7	$1.33^{a} \pm 0.02$
9	KAU 8	$0.25^{g} \pm 0.06$
10	KAU 9	$0.37^{f} \pm 0.02$
11	KAU 10	$0.28^{g} \pm 0.03$
12	KAU 11	$1.23^{a} \pm 0.19$
13	KAU 12	$0.92^{b} \pm 0.15$
14	KAU 13	$0.58^{d} \pm 0.06$
15	KAU 14	$0.48^{e} \pm 0.06$
16	KAU 15	$1.18^{b} \pm 0.06$

* Means in a column followed by the same letter are not significantly different at $P = 0.05$ considering the results of Duncan's multiple range tests

Molecular identification of the bioagents

The two isolates were identified using molecular approaches based on the 16 S rRNA gene sequence. Phylogenic analysis showed that the isolates fell into *Bacillus* spp. and were closely clustered with reference strains of *Bacillus halotolerans (*PX601583) and *B. paralicheniformis* (PX601584) of NCBI (Fig. 1). The sequence similarity analysis identified these two isolates to be similar (≥ 98%) with other *Bacillus* species, confirming their identity.

Greenhouse evaluation of antagonists against bacterial speck disease

Significantly less incidence of bacterial speck was present in all treatments under greenhouse conditions as compared to the infected untreated control ($p \leq \leq 0.05$). The untreated inoculated plants showed 90–92% disease severity, indicating successful establishment of the pathogen (Table 2). Treatments with the two *Bacillus* spp., on the other hand, caused significant decreases. *B. paralicheniformis* was the least affected bioagent, with disease severity of 32, 45, and 39% from foliar spray, soil drench and seed treatments, respectively. *B. halotolerans* also significantly reduced disease severity to 45–63%, depending on the application method. The streptomycin treatment produced the greatest reduction, maintaining disease severity at 15–20%.

Effect of treatments on plant growth

The growth promotion of the *Bacillus* treatments was considerably higher than that of the infected control (Table 3). Pst infection caused a dramatic reduction of plant vigor, regardless of the method applied, with the lowest fresh (2.4–3.0 g) and dry (0.9–1.2 g) weight recorded in inoculated control plants compared to mock-inoculation ones. The distilled water (DW) of healthy control plants was nearly attained (6.2–7.2 g) by *B. halotolerans*-treated ones. Similarly, streptomycin treatment produced biomass levels comparable to those of *B. halotolerans*. *B. paralicheniformis* also enhanced plant growth but produced slightly lower fresh and dry weights than *B. halotolerans*.

Effect of *Bacillus halotolerans*, *B. paralicheniformis* and Streptomycin defense-related enzymes activity after 15 days from treatment

Peroxidase activity was significantly induced in all treated plants and in both healthy and infected controls compared to untreated controls on the fifteenth day after application (Table 4). *B. paralicheniformis* showed high peroxidase levels (up to 3.23 μ/mg protein), especially through foliar spray and seed treatment. The peroxidase reaction with *B. paralicheniformis* was higher than with

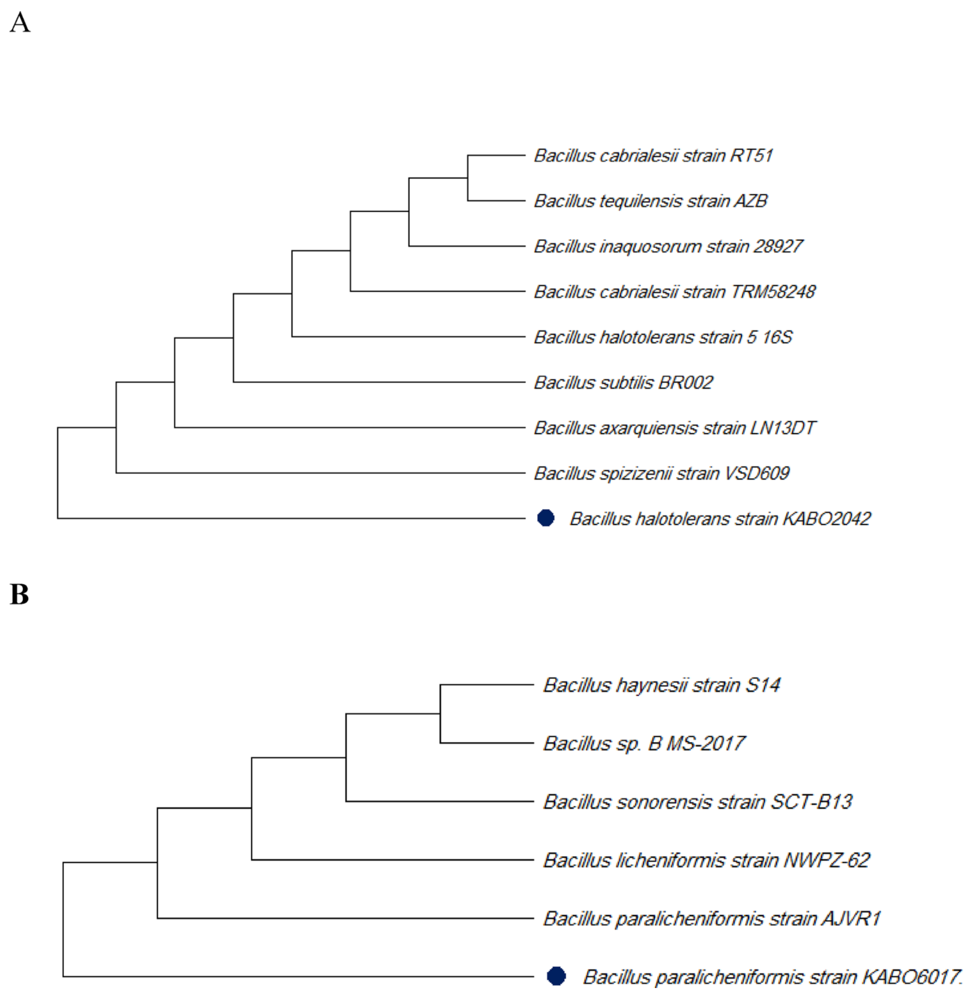

Fig. 1 Semistrict concurrence of most-parsimonious trees based on retrieved bacteria sequences data from NCBI public domain (**A**) Bacillus halotoerans PX601583 and (**B**) Bacillus paralicheniformis PX601584

Table 2 Effect of *Bacillus halotolerans*, *B. paralicheniformis* and Streptomycin on disease severity percentage under greenhouse conditions

Treatments	Methods of application		
	Foliar spray	Soil drench	Seed treatment
Bacillus halotolerans	45 b	63 b	60 b
B. paralicheniformis	32 c	45 c	39 c
Streptomycin	16 d	15 d	20 d
Infected control	90 a	92 a	90 a
Healthy control	0.0 e	0.0 e	0.0 e

Means in a column followed by the same letter are not significantly different at $P=0.05$ considering the results of Duncan's multiple range tests

B. halotolerans and streptomycin; however, it was lower than the *B. paralicheniformis* treated.

Polyphenol oxidase activity

The same tendency was apparent for the activity of polyphenol oxidase (PPO) (Table 5). *B. halotolerans* presented the highest PPO induction (1.5–1.7 μ/mg protein), and *B. paralicheniformis* also significantly increased PPO with respect to all controls (both healthy and infected treatments). The enhancement of PPO activity was observed at a moderate level under the streptomycin treatments.

Discussion

Results revealed that endophytic *B. halotolerans* and *B. paralicheniformis* effectively suppress tomato bacterial speck disease under greenhouse conditions, accompanied by enhanced plant growth and the induction of host defense responses. Rather than simply presenting numerical results, these findings are interpreted in light of the current literature and discuss their biological significance and limitations.

The in vitro antagonistic activity of both *Bacillus* against *Pseudomonas syringae* pv. *tomato* is in accordance with previous studies on potent antibacterial activity of *Bacillus* spp. against phytopathogenic bacteria (Karačić et al., 2024). Such differences in studies might be attributed to strain origins, endophytic or

Table 3 Effect of *Bacillus halotolerans*, *B. paralicheniformis* and Streptomycin on fresh and dry weights (g/plant) of tomato plants under greenhouse conditions

Treatments	Methods of application					
	Foliar spray		Soil drench		Seed treatment	
	Fresh weight (g/plant)	Dry weight (g/plant)	Fresh weight (g/plant)	Dry weight (g/plant)	Fresh weight (g/plant)	Dry weight (g/plant)
Bacillus halotolerans	6.8 a ± 0.18	2.8 a ± 0.12	7.2 b ± 0.12	3.1 b ± 0.15	6.2 b ± 0.2	1.8 a ± 0.14
B. paralicheniformis	4.7 b ± 0.12	1.8 b ± 0.10	5.9 c ± 0.14	2.3 b ± 0.18	4.1 c ± 0.18	1.5 b ± 0.18
Streptomycin	6.4 a ± 0.16	2.4 a ± 0.12	7.3 b ± 0.16	3.4 a ± 0.18	4.3 c ± 0.22	1.4 b ± 0.23
Infected control	2.4 c ± 0.13	1.2 c ± 0.12	2.4 d ± 0.19	1.0 c ± 0.19	3.0 d ± 0.23	0.9 c ± 0.31
Healthy control	6.8 a ± 0.12	2.4 a ± 0.13	8.8 a ± 0.17	3.5 a ± 0.15	7.8 a ± 0.34	1.4 b ± 0.23

Means in a column followed by the same letter are not significantly different at $P = 0.05$ considering the results of Duncan's multiple range tests

Table 4 Effect of *Bacillus halotolerans*, *B. paralicheniformis* and Streptomycin peroxidase activity (μ/ mg protein) of tomato plants under greenhouse conditions

Treatments	Methods of application		
	Foliar spray	Soil drench	Seed treatment
Bacillus halotolerans	2.45 c	2.9 a	2.1 c
B. paralicheniformis	3.23 a	2.6 b	3.2 a
Streptomycin	3.15 b	2.1 c	2.9 b
Infected control	2.15 d	1.9 d	2.0 c
Healthy control	1.94 e	1.5 e	1.7 d

Means in a column followed by the same letter are not significantly different at $P = 0.05$ considering the results of Duncan's multiple range tests

Table 5 Effect of *Bacillus halotolerans*, *B. paralicheniformis* and Streptomycin Polyphenolactivity (μ/ mg protein) of tomato plants under greenhouse conditions

Treatments	Methods of application		
	Foliar spray	Soil drench	Seed treatment
Bacillus halotolerans	1. 7 a	1. 3 a	1. 5 a
B. paralicheniformis	1. 4 c	1. 2 b	1. 4 b
Streptomycin	1. 5 b	1. 2 b	1. 4 b
Infected control	1. 2 d	1. 3 a	1. 3 c
Healthy control	0. 9 e	0. 96 c	1 0 d

Means in a column followed by the same letter are not significantly different at $P = 0.05$ considering the results of Duncan's multiple range tests

epiphytic organisms and experimental conditions such as nutrient media and incubation time (Bonaterra et al. 2022). The endophytic origin of strains tested in this study may provide a benefit, since internal colonization will enable closer association with host tissues and likely more persistent antagonism than surface colonizers.

In addition to direct antibiosis, the observed biochemical characteristics such as siderophore production, phosphate solubilization and indole-3-acetic acid (IAA) synthesis indicate that disease suppression is probably multicomponent. Competition for iron by growth limitation as mediated by siderophores is a well-established mechanism limiting pathogen growth, especially for the iron-requirement bacteria such as *Pseudomonas* spp. (Zhang et al. 2023). The increased siderophore production in *B. halotolerans* is, though intermediate between the two standards, partly responsible for its strong antagonistic activity; phosphate solubilization and IAA production should predominantly support plant vigor and indirect disease suppression by *B. paralicheniformis*. Such mechanism sharing between *Bacillus* strains has also been reported (Giovanard et al., 2025) and highlights that disease curative efficacy is not due to a unique mechanism.

In the greenhouse, both strains significantly suppressed disease severity, but they were less effective than streptomycin. Although those BCAs that are currently marketed for biocontrol in Europe provide only partial and rarely complete protection (Marchand 2023), these results support earlier findings that agents of biological control generally provide a symptomatic rather than curative effect (Bonaterra et al. 2022). It should be noted, however, that statements regarding field applications must be made with caution. Preliminary comparisons in the greenhouse or pot between biocontrol and fungicide materials to reduce these sources of variability are not possible. Therefore, despite promising results,, extrapolation to open fields requires validation through multi-location field testing and formulation studies as also noted by Ayaz et al. (2023).

The activation of defense-related enzymes, especially peroxidase (POD) and polyphenol oxidase (PPO), provides insight into the biological significance of the observed disease suppression. These enzymes are essential to defend plants, being involved in lignification and oxidative crosslinking of the cell wall proteins as well as in synthesis of antimicrobial phenolic compounds (Choudhary et al. 2007). The greatest induction of POD by *B. paralicheniformis* indicates a strong elicitation of oxidative defense responses, which might contribute to more effective containment of pathogen spread in host tissues. Likewise, high PPO activity in *B. halotolerans* would lead to an increased production of harmful quinones, and thus killing off the pathogen (Elshahawy et al. 2022). Similar patterns of enzyme induction were observed for tomato plants treated with *Bacillus* spp. and inoculated with bacterial pathogens, providing evidence that ISR is a complementary mechanism to direct antagonism of strains (Abbas et al. 2024).

However, enzyme induction is also not a definite assurance of resistance. The strength and timing of defense induction as well as cross-talk between salicylic acid (SA), jasmonic acid (JA) and ethylene signaling pathways influence the efficacy of ISR (Hönig et al. 2023). Although the present analysis clearly shows enhanced enzyme activity at specific time points, further studies investigating the timing of defense response(s) and gene-specific markers are needed to dissect this pathway more precisely. Furthermore, over activation or prolonged activation of oxidative defenses may induce metabolic costs on the plant, an aspect rarely considered in bio-control studies warranting more research.

Conclusion

It was concluded that the biocontrol efficacy likely reflects antagonism, nutrient competition, plant growth promotion and subprime priming of host defense. Utilization of endophytic *B. halotolerans* and *B. paralicheniformis* further broadens the spectrum of *Bacillus* species known as potential biocontrol agents for bacterial speck disease. Nonetheless, the greenhouse experiments represent a preliminary step toward field application, rather than proof of parasite agronomic efficacy. Additional studies on formulation, persistence, field efficacy and interaction with indigenous microbiota are required prior to practical deployment in commercial tomato production.

Abbreviations

PPO	Polyphenol oxidase
PO	Peroxidase
BCAs	Biocontrol agents
PST	*Pseudomonas syringae* pv. *tomato*
IAA	Indol acetic acid
CFU	Colony forming units
CRD	Completely randomized design

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Author contributions

NMA contributed to conceptualization, validation, formal analysis, writing—review and editing, and draft preparation. MA contributed to methodology, data curation, writing—original, funding acquisition, and software. All authors have read and agreed to the published version of the manuscript.

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Data availability

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

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants or animals requiring ethical approval. All experimental procedures were conducted in accordance with institutional guidelines for plant and microbial researchA.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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