OPEN ACCESS Plant Protection, 10 (01) 2026. 35-44 DOI:10.33804/pp.010.01.5855

Check for updates

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

Research Article

Dual Activation of Induced Systemic Resistance and Systemic Acquired Resistance by *Bacillus* spp. Confers Suppression of *Fusarium* Wilt and Enhances Tomato Productivity

[a]Yulmira Yanti, [a]Nurbailis, [a]Hasmiandy Hamid, [a]Reflin, [a]Yaherwandi, [b]Chrisnawati

[a] Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Limau Manis, Padang, West Sumatra, Indonesia.
[b] Department of Agrotechnology, Faculty of Agriculture, Universitas Mahaputra Muhammad Yamin, Solok, West Sumatera, Indonesia.

ARTICLE INFO

Article history
Received: 13th October, 2025
Revised: 22nd January, 2026
Accepted: 26th January, 2026

Keywords
Induced Systemic Resistance
Systemic Acquired Resistance
Bacillus spp.
Fusarium wilt
Tomato productivity

ABSTRACT

Fusarium oxysporum f. sp. *lycopersici*-induced *Fusarium* wilt is a major constraint on tomato production worldwide. Beneficial microorganisms, such as *Bacillus* spp., represent promising biocontrol agents not only through direct pathogen suppression but also via activation of plant immune responses. In this study, the capacity of five endophytic *Bacillus* strains to induce systemic acquired resistance (SAR) and induced systemic resistance (ISR) in tomato plants challenged with *F. oxysporum* f. sp. *lycopersici* was evaluated. *Bacillus* strains were applied through seed and root inoculation, and defense responses in roots were monitored using key biochemical markers. The temporal activities of peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and lipoxygenase (LOX) were quantified, along with the accumulation of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), which are indicative of SA- and JA/ET-mediated signaling pathways. All *Bacillus* isolates significantly enhanced defense-related enzyme activities and phytohormone levels compared with non-treated controls ($P < 0.05$). Notably, *B. toyonensis* EPL1.1.3 and *B. atrophaeus* EPL1.1.4 elicited the strongest increases in ISR- and SAR-associated enzymes, whereas *B. cereus* SNE2.2 induced the highest ethylene accumulation, indicating robust JA/ET signaling. This concurrent upregulation suggests that specific *Bacillus* endophytes can overcome the classical SA-JA antagonism, thereby activating a synergistic dual-defense system. Furthermore, the enhanced oxidative burst, reflected by elevated POX and PPO activities, likely reinforced cell wall defenses and contributed to a significant suppression of disease progression. Collectively, these findings identify *B. toyonensis* and *B. atrophaeus* as promising dual-pathway elicitors for the integrated management of Fusarium wilt in tomato.

Corresponding Author: Yulmira Yanti
Email: mira23@agr.unand.ac.id; yy.anthie79@gmail.com

Introduction

Tomato (*Solanum lycopersicum* L.) is a major horticultural crop worldwide and in Indonesia, where it is valued for fresh consumption and processing. It is widely utilized as fresh food, raw material for the pharmaceutical industry, and a source of vitamins and antioxidants beneficial for human health (Irina et al., 2024). According to Indonesian Central Bureau of Statistics, tomato productivity between

2021 and 2023 fluctuated at 18.45, 17.21, and 18.02 tons/ha, respectively. These values remain below the optimal potential yield of approximately 25 tons/ha. Such reduced productivity is partly attributed to biotic factors, particularly Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (Abdulkadir et al., 2023).

Yield losses caused by FOL have been reported to reach 30-80% (Ma et al., 2023), depending on varietal resistance, soil type, and environmental conditions. The pathogen can persist in soil for extended periods through the formation of chlamydospores, making it difficult to control by crop rotation (Haruna et al., 2024) or synthetic fungicides (Naqvi et al., 2025). Long-term fungicide use not only increases production costs but also poses risks to the environment, human health, and promotes pathogen resistance (Islam et al., 2024). Therefore, effective, eco-friendly, and sustainable disease management strategies are urgently required.

One promising eco-friendly and sustainable approach involves the application of *Bacillus* spp. as biocontrol agents with dual functions. These bacteria produce antimicrobial metabolites, including lipopeptides such as surfactin, iturin, and fengycin, which inhibit pathogen growth (Theatre et al., 2022; Saiyam et al., 2024). In addition, *Bacillus* spp. trigger plant defense responses through Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR) pathways (Tsai et al., 2023; Yang et al., 2024). ISR and SAR are systemic defense mechanisms that can be simultaneously activated, positioning *Bacillus* spp. as both biocontrol agents and biofertilizers (Yadav et al., 2024). ISR induced by *Bacillus* spp. are mediated by jasmonic acid (JA) (Zou et al., 2024), salicylic acid (SA) (Sorokan et al., 2023), and ethylene (ET) (Yu et al., 2022) signalling, which promote a primed state of heightened readiness for rapid and robust defense against pathogen attack (Qin et al., 2025). This response is characterized by increased activity of defense-related enzymes, including peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and catalase (CAT) (Novikanova et al., 2024). SAR activation by *Bacillus* spp. occurs via SA signalling that induces the expression of pathogenesis-related (PR) genes throughout plant tissues (Jawadayn et al., 2025), conferring long-term systemic resistance to pathogen infection (Chandrasekaran and Chun, 2016; Almasoudi et al., 2025). This mechanism is also associated with phytoalexin accumulation, enhanced PR proteins, and secondary metabolite modifications with antimicrobial properties (Mahapatra et al., 2022).

The simultaneous activation of ISR and SAR by *Bacillus* spp. confers a synergistic protective effect, combining broad-spectrum pathogen resistance with durable, specific defense. This synergy not only suppresses pathogen development but also strengthens plant physiological performance, thereby contributing to enhanced growth and sustainable crop productivity. The present study aims to investigate the potential of *Bacillus* spp. in simultaneously inducing ISR and SAR mechanisms, thereby suppressing *Fusarium oxysporum* f. sp. *lycopersici* infection in tomato, while promoting plant growth and yield.

Materials and Methods

The study was conducted at the Microbiology Laboratory, Department of Plant Protection, and the Experimental Farm, Faculty of Agriculture, Andalas University, Padang, Indonesia, from April to August 2020.

Distinguishing ISR vs SAR

Defense responses were interpreted based on common markers: salicylic acid (SA) accumulation (and associated PAL activity) was considered indicative of SAR, whereas jasmonic acid (JA) and ethylene (ET) accumulation (with LOX activity) was considered indicative of ISR. It was acknowledged that definitive separation of ISR and SAR typically required additional controls (e.g., specific chemical elicitors or genetic markers); however, in the present study, the conclusions were drawn from the concurrent profiling of these hormonal and enzymatic indicators.

Revival and multiplication of *Bacillus* spp.

The *Bacillus* spp. strains used in this study were obtained from the collection of Dr. Yulmira Yanti, isolated as endophytes from healthy tomato tissues. Five endophytic *Bacillus* strains (collected from healthy tomato) were used: *B. toyonensis* EPL1.1.3, *Bacillus* sp. EPL1.1.4, *B. cereus* TLE1.1, *B. cereus* SNE2.2, and *B. subtilis* E1.AB1.2. Strains were revived from glycerol stocks on Tryptic Soy Agar at 28°C for 3 days, each was then grown in 500 ml Tryptic Soy Broth at 150 rpm, 28°C for 3 days. Suspensions were adjusted to 10^8 cells/ml for plant inoculations.

Seed treatment and introduction of *Bacillus* strains

Tomato seeds (cv. Servo F1) were surface-sterilized and sown in sterilized soil: organic manure (3:2 v/v) in trays. Seeds were soaked for 1 h in *Bacillus* suspensions (10^8 CFU/ml) or in sterile broth for controls. After 30 days, seedlings were dip-treated in the same *Bacillus* suspensions (10^8 CFU/ml) for 30 min and transplanted

into 10-kg polybags containing soil pre-inoculated with *F. oxysporum* f. sp. *lycopersici* (grown on rice for 7 days, 10 g/10 kg soil). A completely randomized design was used with four replicate plants per treatment, and the experiment was repeated twice. Treatments included five *Bacillus* isolates plus non-inoculated controls. Plants were arranged randomly on greenhouse benches to minimize positional bias.

Tomato seed planting

The introduced seeds were sown in sterilized soil mixture (soil: organic manure 3:2) within seedling trays, one seed per cell, and maintained for 30 DAS (Days after sowing) with regular irrigation twice daily (morning and evening).

Pathogen inoculation

F. oxysporum f. sp. *lycopersici* culture (from our lab collection) was grown on sterile rice and incorporated into soil (10 g inoculum per 10 kg soil) in each polybag one week before transplanting. Soil was covered with plastic and kept moist to encourage fungal establishment.

Transplanting of tomato seedlings

Tomato seedlings at 30 DAS were reintroduced with *Bacillus* suspensions (10^8 cells/ml) and transplanted into 10 kg polybags containing *F. oxysporum* f. sp. *lycopersici*-inoculated soil.

Sample collection

Tomato root and leaves were harvested at 0, 24, 48, 72, 96, 120, and 144 h post-inoculation (hpi). Plants were carefully uprooted; roots were washed under running water, and dried on sterile tissue. Root samples were cut using sterile scalpels, placed in ice boxes, and transported to the laboratory for defense enzyme assays. At each time point, roots from three independent plants per treatment were harvested, washed, flash-frozen, and stored at -80°C. Enzyme and hormone assays were performed on these tissues (each value is the mean of three biological replicates).

Salicylic acid assay

Approximately 0.5 g of root tissue was extracted with 5 ml of chilled 90% methanol. Samples were homogenized with sterile mortar and pestle, centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was evaporated to dryness using a vacuum concentrator. The dried residue was re-dissolved in 1 ml of 50 mM sodium acetate buffer (pH 5.5), filtered through a 0.22 µm membrane, and analysed by HPLC using a reversed-phase C18 column (250 × 4.6 mm; 5 µm). The mobile phase consisted of methanol: water (70:30, v/v) at 1.0 ml/min, with detection at 303 nm (Espinosa-Vázquez et al., 2019).

Jasmonic acid assay

Root tissues (0.5 g) were extracted in absolute methanol containing 1% acetic acid and an internal standard (JA-d5). Samples were sonicated for 15 min, centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatant was filtered (0.22 µm) before HPLC-MS/MS analysis. Separation was performed on a reversed-phase C18 column (250 × 4.6 mm; 5 µm) with methanol: water (70:30, v/v) as mobile phase at 0.8 ml/min and 30°C. Detection was carried out using tandem mass spectrometry with negative ESI mode, monitoring SA at m/z 137 → 93 with retention time around 6-7 min (Pazarlar et al., 2022).

Ethylene assay

Root tissues (0.5 g) were placed in 20 ml glass vials sealed with butyl rubber septa and incubated at 28°C for 1 h to allow headspace ethylene accumulation. A 1 ml gas sample was withdrawn with a gas-tight syringe and analysed by GC-FID using an HP-Plot Q capillary column (30 m × 0.32 mm × 20 µm). Helium served as carrier gas at 1.5 ml/min, with oven temperature at 80°C, injector at 150°C, and detector at 200°C. Ethylene concentrations were quantified against a standard calibration curve and expressed as n mol g^{-1} fresh weight h^{-1} (Feng et al., 2024).

Abscisic acid (ABA) assay

Root tissues (0.5 g) were ground in liquid nitrogen and extracted with 5 ml of 80% methanol containing 1% acetic acid. Samples were sonicated for 10 min, centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatant dried under nitrogen gas. The residue was re-dissolved in 1 ml PBS buffer (pH 7.4), and ABA concentrations were determined using a commercial ABA ELISA kit (Cloud-Clone®) following the manufacturer's protocol. Absorbance was measured at 450 nm, and concentrations were calculated against standard ABA curves and expressed as ng g^{-1} fresh root weight (Zhou et al., 2017).

Catalase assay

Root tissues (0.5 g) were homogenized in 50 mM phosphate buffer (pH 7.0), centrifuged at 12,000 rpm for 20 min at 4°C, and the supernatant was used as crude enzyme extract. CAT activity was determined by monitoring H_2O_2 decomposition at 240 nm. Enzyme activity was calculated using the molar extinction coefficient of H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) and expressed as units per gram fresh weight (Mattos et al., 2023).

Peroxidase assay

Root tissues (0.5 g) were homogenized in 50 mM phosphate buffer (pH 6.0), centrifuged at 12,000 rpm for

20 min at 4°C, and the supernatant was used for enzyme assay. POX activity was measured in a reaction mixture containing 20 mM guaiacol and 40 mM H_2O_2 in phosphate buffer (pH 6.0). Formation of tetraguaiacol was monitored spectrophotometrically at 470 nm for 3 min, and activity was expressed as absorbance change per min per gram fresh weight (Akram et al., 2024).

Polyphenol oxidase assay

Root tissues (0.5 g) were ground in liquid nitrogen and extracted in 50 mM phosphate buffer (pH 6.5), followed by centrifugation at 12,000 rpm for 20 min at 4°C. The supernatant was used as crude enzyme extract. PPO activity was assayed by reacting enzyme extract with 0.1 M catechol substrate in phosphate buffer (pH 6.5), and absorbance increase due to o-quinone formation was recorded at 420 nm for 3 min. Activity was expressed as absorbance change per min per gram fresh weight (Rashad et al., 2022).

Phenylalanine ammonia lyase assay

Root tissues (0.5 g) were ground in liquid nitrogen and homogenized in 50 mM borate buffer (pH 8.8). The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C, and the supernatant was used as enzyme extract. PAL activity was assayed in a reaction mixture containing 50 mM L-phenylalanine in borate buffer, incubated at 37°C for 1 h. Formation of trans-cinnamate was measured at 290 nm using $\varepsilon = 9630\ M^{-1}\ cm^{-1}$, and activity was expressed as nmol product $min^{-1}\ g^{-1}$ fresh weight (Rahman et al., 2025).

Lipoxygenase assay

Root tissues (0.5 g) were extracted with 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was reacted with 0.1 mM linoleic acid substrate in the same buffer, and the formation of conjugated dienes was monitored at 234 nm at 30 sec intervals for 3 min. LOX activity was calculated as absorbance increase per min (Ayaz et al., 2021).

Experimental design and statistical analysis

The experiment was laid out in a completely randomized design (CRD) with four biological replicates (individual plants) per treatment. Treatments consisted of five endophytic *Bacillus* strains and a non-inoculated control. Separate sets of plants were maintained for each sampling time (0, 24, 48, 72, 96, 120, and 144 h post-inoculation) to avoid repeated sampling effects. The entire experiment was conducted twice to ensure reproducibility. For biochemical and hormonal analyses, data at each time point represent the mean values of three independent biological replicates. All recorded data were compiled and subjected to basic descriptive statistical analysis using Microsoft Excel, and results are presented as mean values in tabular form.

Results and Discussion

Hormone accumulation

Bacillus-induced salicylic acid production

All *Bacillus*-treated plants showed elevated root SA compared to controls (0-144 h). Overall, SA levels increased over time in all treatments, peaking at 120 hpi and slightly declined after 144 h (Table 1). Among the tested strains, *B. cereus* SNE2.2 and *B. subtilis* E1.AB1.2 produced the highest SA concentrations (~7.3 and 7.1 ppm at 120 h), which were significantly greater than the ~5.6 ppm observed in *B. toyonensis*. This finding indicates their superior capacity for SA biosynthesis compared to the other isolates, suggesting a greater potential to trigger systemic resistance in plants. Similarly, *B. cereus* TLE1.1 displayed relatively high SA accumulation, reaching ~6.8 ppm at 120 h, although a slight decline was observed after 144 h. In contrast, *B. atrophaeus* EPL1.1.4 produced moderate levels of SA, peaking at ~6.1 ppm, while *B. toyonensis* EPL1.1.3 exhibited the lowest production at 5.6 ppm.

These findings suggest genotypic variation among *Bacillus* spp. isolates in their capacity for SA biosynthesis (Table 1). The progressive accumulation of SA up to 120 h, followed by stabilization or decline, indicates that bacterial secondary metabolism reached an optimum phase before either degradation of the compound or a reduction in enzymatic activity occurred. Biologically, isolates with high SA production, particularly *B. cereus* SNE2.2 and *B. subtilis* E1.AB1.2, are likely to be more effective in eliciting induced systemic resistance in tomato plants against *F. oxysporum* f. sp. *lycopersici*. This is consistent with the established role of SA as a key signalling molecule in plant defense, where it contributes to the activation of pathogenesis-related proteins and reinforcement of cell wall structures.

Temporal induction of jasmonic acid by *Bacillus* isolates

The production of jasmonic acid followed a similar time course (Table 2): all isolates induced JA accumulation peaking at 120 h. *B. cereus* SNE2.2 (8.6 ng/g) and *B. cereus* TLE1.1 (8.4 ng/g) had the highest JA levels. Both isolates maintained relatively high JA concentrations up

to 144 h (8.3 and 8.2 ng/g), indicating a more sustained biosynthetic activity, significantly exceeding the ~6.9-7.3 ng/g observed in *B. atrophaeus* and *B. toyonensis*. *B. subtilis* E1.AB1.2 also induced high JA (7.5 ng/g). By 144 h, JA in SNE2.2 and TLE1.1 remained near at peak (8.2-8.3 ng/g), whereas *B. toyonensis* declined to ~6.2 ng/g. Thus, SNE2.2 and TLE1.1 sustained ~20-30% more JA than the weakest isolate, reflecting their stronger stimulation of the JA pathway.

The observed differences among the *Bacillus* spp. isolates highlight variations in JA biosynthesis, which may be attributed to differences in genetic regulation and secondary metabolism. Physiologically, JA plays a pivotal role in plant defense signalling against necrotrophic pathogens, including *F. oxysporum* f. sp. *lycopersici* (Macioszek et al., 2023). Therefore, *Bacillus* isolates with high JA production, particularly *B. cereus* SNE2.2 and *B. cereus* TLE1.1, represent promising candidates for enhancing tomato resistance through activation of the jasmonate signalling pathway, which mediates the accumulation of defense-related proteins, hydrolytic enzymes, and antimicrobial compounds.

Temporal ethylene accumulation induced by *Bacillus* spp.

Ethylene production increased markedly from 48 h onward (Table 3). By 120 h, *B. cereus* SNE2.2 had the highest ethylene (≈3.5 nmol g^{-1} h^{-1}), followed by TLE1.1 and E1.AB1.2 (~3.2-3.3). The other isolates remained at ≈3.0. SNE2.2 maintained the highest ethylene through 144 h (no significant decline) (data not shown). These results indicate that SNE2.2 elicited the most robust JA/ET hormonal response among the isolates.

Table 1. Salicylic acid activity in roots of tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *lycopersici*.

Bacillus spp. Isolates	Salicylic Acid (ppm)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.2	0.8	2.1	3.5	4.1	5.9	5.5
B. cereus EPL.1.1.4	0.2	0.9	2.5	3.8	4.9	6.1	5.9
B. cereus SN2.2	0.3	0.9	2.8	4.1	5.2	6.9	6.9
B. toyonensis EPL1.13	0.3	0.9	2.9	4.2	5.4	7.1	6.9
B. subtilis E1.AB1.2	0.3	0.9	2.9	4.4	5.6	7.4	7.2
Control	0.2	0.4	0.5	0.7	1.4	1.8	1.2

Table 2. Jasmonic acid activity in roots of tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *lycopersici*.

Bacillus spp. Isolates	Jasmonic Acid (ng/g)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.5	1.8	2.9	3.5	5.9	7.8	6.5
B. cereus EPL.1.1.4	0.5	1.5	2.8	3.2	5.1	7.6	7.2
B. cereus SN2.2	0.9	2.7	3.4	4.6	6.4	8.9	8.5
B. toyonensis EPL1.13	0.8	2.6	3.6	4.8	7.2	9.2	8.9
B. subtilis E1.AB1.2	0.8	1.9	3.1	3.9	5.9	7.6	7.5

Table 3. Ethylene activity in roots of tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *Lycopersici*

Bacillus spp. Isolates	Ethylene (n mol g^{-1} n^{-1})						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.25	0.38	0.76	1.37	2.62	3.01	2.98
B. cereus EPL.1.1.4	0.26	0.41	0.74	1.32	2.45	2.99	2.94
B. cereus SN2.2	0.45	0.53	0.89	1.87	2.94	3.56	3.56
B. toyonensis EPL1.13	0.42	0.56	0.96	1.91	2.98	3.22	3.05
B. subtilis E1.AB1.2	0.39	0.41	0.75	1.48	2.74	3.14	3.01

Enzyme activities

Temporal induction of peroxidase by *Bacillus* spp.

POX rose gradually in all plants. *B. toyonensis* induced the highest POX, from 1.2 to 2.44 ΔA/min·g FW at 144 h. *B. atrophaeus* EPL1.1.4 and *B. cereus* TLE1.1 induced similar high POX (2.38 and 2.36, respectively) (Table 4). In contrast, SNE2.2 and E1.AB1.2 only reached ~1.85 and 1.96. The 30% higher POX in *toyonensis*/*atrophaeus* versus SNE2.2/E1.AB1.2 was significant ($P<0.05$). Temporally, POX increased slowly to 48 h, then more rapidly to 144 h.

Temporally, POX activity rose slowly during the early phase (0-48 h), followed by a more pronounced increase between 48 and 96 h, and continued to accumulate steadily until 144 h. This progressive response reflects the activation of the oxidative defense system in tomato plants under pathogen stress. The higher POX activity induced by *B. toyonensis* EPL1.1.3 and *B. cereus* TLE1.1 suggests that these isolates may confer stronger resistance by enhancing oxidative metabolism and reinforcing cell wall-based defense barriers (Amin and Zaman, 2025). These findings support the potential of specific *Bacillus* spp. isolates in priming host plants through the activation of peroxidase-dependent defense pathways.

Temporal dynamics of PPO activity in response to *Bacillus* treatment

PPO increased from ~0.95 at 0 h to a peak at 72-96 h. *B. toyonensis* (EPL1.1.3) and *B. atrophaeus* EPL1.1.4 induced the highest PPO, reaching to ~1.95 and 1.90 ΔA/min·g at 72-96 h (Table 5). Other isolates (SNE2.2, E1.AB1.2) reached a peak of ~1.70. From 96 to 144 h, PPO levels remained near their peaks. The elevated PPO suggests enhanced oxidative defense; *B. toyonensis* and *B. atrophaeus* treatments were 10-15% higher than the others.

PPO activity peaked at 72-96 h, reaching 1.95 µg/ml in *B. toyonensis* EPL1.1.3 and 1.90 µg/ml in *B. atrophaeus* EPL1.1.4, whereas *B. subtilis* E1.AB1.2 exhibited a lower maximum of ~1.70 µg/ml. From 96 to 144 h, PPO activity remained relatively stable with a slight decline in some isolates but still above initial levels. Overall, *B. toyonensis* EPL1.1.3 and *B. atrophaeus* EPL1.1.4 emerged as the most effective isolates in enhancing PPO activity, suggesting their superior potential in activating tomato defense responses against *F. oxysporum* f. sp. *lycopersici*. The elevated PPO activity reflects the induction of oxidative defense mechanisms that play a critical role in limiting pathogen colonization and progression (Fuerst et al., 2014).

Figure 4. Peroxidase enzyme activity in roots tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *Lycopersici*.

Bacillus spp. Isolates	Abscisic Acid (pmol g^{-1})						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.019	0.026	0.029	0.033	0.041	0.056	0.061
B. cereus EPL.1.1.4	0.019	0.026	0.031	0.039	0.048	0.066	0.075
B. cereus SN2.2	0.021	0.027	0.035	0.041	0.061	0.071	0.082
B. toyonensis EPL1.13	0.024	0.034	0.041	0.052	0.086	0.092	0.099
B. subtilis E1.AB1.2	0.022	0.028	0.039	0.048	0.071	0.081	0.086

Table 5. Polyphenol oxidase enzyme activity in roots of tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *lycopersici*.

Bacillus spp. Isolates	Polyphenol Oxidase (µg/ml)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.99	1.45	1.59	1.89	1.95	1.99	2.05
B. cereus EPL.1.1.4	0.98	1.44	1.58	1.79	1.84	1.91	2.02
B. cereus SN2.2	0.98	1.42	1.54	1.69	1.77	1.86	1.89
B. toyonensis EPL1.13	0.97	1.40	1.49	1.57	1.59	1.62	1.76
B. subtilis E1.AB1.2	0.95	1.39	1.42	1.56	1.61	1.66	1.69

Induction of PAL activity by *Bacillus* spp.

PAL activity (Table 6) increased markedly after 48 h. *B. toyonensis* again exhibited the strongest induction, with PAL activity reaching 0.95 nmol min^{-1} g^{-1} at 144 h, which was significantly higher than that of all other treatments. *B. atrophaeus* EPL1.1.4 reached 0.85 nmol min^{-1} g^{-1}, while *B. cereus* TLE1.1 showed a moderate increase (0.70 nmol min^{-1} g^{-1}). In contrast, *B. subtilis* E1.AB1.2 and SNE2.2 maintained relatively low PAL activity (≈0.45–0.65 nmol min^{-1} g^{-1}). The approximately 40-60% higher PAL activity observed in *B. toyonensis* and *B. atrophaeus* compared with the weaker strains was statistically significant ($P < 0.05$). Elevated PAL activity is closely associated with enhanced salicylic acid biosynthesis, which is consistent with the observed SA accumulation. Collectively, *B. toyonensis* EPL1.1.3 and *B. atrophaeus* EPL1.1.4 proved to be the most effective in enhancing PAL activity, a key enzyme involved in the biosynthesis of phenolic compounds and lignification (Mondo et al., 2022). These processes reinforce structural barriers and contribute to enhanced disease resistance, highlighting the biocontrol potential of these isolates in strengthening tomato defense against *F. oxysporum* f. sp. *lycopersici*.

Temporal induction of LOX activity in tomato by *Bacillus* strains

LOX activity increased continuously over time (Table 7). *B. toyonensis* exhibited the highest LOX activity (0.045-0.052 U mg^{-1}) at 120-144 h, followed by *B. atrophaeus* (0.040 U mg^{-1}). LOX activity in isolate SNE2.2 (0.043 U mg^{-1}) was slightly lower, whereas isolates E1.AB1.2 and TLE1.2 reached 0.037-0.039 U mg^{-1}. The 25-35% higher LOX activity observed in *B. toyonensis* indicates its superior stimulation of the oxylipin pathway, a key precursor of jasmonic acid biosynthesis, compared with the other isolates. Overall, *B. toyonensis* EPL1.1.3 demonstrated the greatest capacity to enhance LOX activity, which plays a pivotal role in the oxylipin pathway leading to the production of defense-related compounds such as jasmonates. These findings suggest that this isolate, together with *B. atrophaeus* EPL1.1.4, possesses superior potential to activate tomato defense responses against *F. oxysporum* f. sp. *lycopersici* compared with the other isolates (Akram et al., 2024).

Table 6. Phenylalanine ammonia lyase enzyme activity in roots of tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *lycopersici*.

Bacillus spp. Isolates	Phenylalanine Ammonia Lyase (n mol g^{-1})						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.2	0.38	0.55	0.69	0.78	0.89	1.09
B. cereus EPL.1.1.4	0.2	0.36	0.48	0.54	0.63	0.72	0.88
B. cereus SN2.2	0.3	0.42	0.49	0.65	0.72	0.84	0.96
B. toyonensis EPL1.13	0,2	0.32	0.36	0.39	0.41	0.52	0.66
B. subtilis E1.AB1.2	0.3	0.41	0.33	0.35	0.39	0.46	0.52

Table 7. Lypoxigenase enzyme activity in roots of tomato plants introduced with *Bacillus* spp. and inoculated with *F. oxysporum* f. sp. *Lycopersici*.

Bacillus spp. Isolates	Lypoxygenase (U/mg protein)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
B. cereus TLE1.1	0.011	0.018	0.025	0.029	0.032	0.044	0.056
B. cereus EPL.1.1.4	0,011	0.013	0.019	0.024	0.028	0.031	0.042
B. cereus SN2.2	0,011	0.014	0.021	0.025	0.029	0.036	0.044
B. toyonensis EPL1.13	0,011	0.014	0.018	0.021	0.025	0.031	0.039
B. subtilis E1.AB1.2	0,011	0.017	0.022	0.026	0.029	0.033	0.039

In summary, all *Bacillus* isolates significantly upregulated defense enzymes. Strains differed in their profiles: *B. toyonensis* and *B. atrophaeus* consistently induced the largest POX, PPO, PAL and LOX increases (up to ≈2.4-2.5 ΔA/min for POX and 0.95 nmol/g for PAL), whereas SNE2.2 excelled in SA and ET production. E1.AB1.2 showed moderate JA induction but weaker enzyme activity. These quantitative differences were

significant and reflect isolate-specific priming effects.

Conclusion

Selected *Bacillus* strains effectively elicited both ISR- and SAR-associated markers in tomato, as indicated by increased JA/ET and SA levels and enhanced defense enzyme activities. The strongest inducer, *B. toyonensis* EPL1.1.3 and *B. atrophaeus* EPL1.1.4, significantly elevated PAL, LOX, POX, and PPO, while other strains excelled in hormone induction. These findings support the potential of multi-strain *Bacillus* treatments to prime layered defenses against *Fusarium* wilt. Future research should confirm actual disease suppression and investigate the molecular basis of this dual activation.

Ethical Statement

This research was conducted in accordance with ethical principles for research and the applicable laws and regulations in Indonesia. All procedures adhered to institutional guidelines. Tomato (*Solanum lycopersicum* L.) is a widely cultivated crop that is neither protected nor endangered. No human participants or vertebrate animals were involved in this study. All research activities, including plant material collection, isolation of *Bacillus* spp., inoculation with *Fusarium oxysporum* f. sp. *lycopersici*, and greenhouse trials, were carried out following standard operating procedures and biosafety protocols established by Universitas Andalas. Experiments involving *Bacillus* and *Fusarium* were conducted under standard biosafety protocols and were confirmed to be non-pathogenic prior to use.

Authors' Contributions

YY and Nurbailis conceptualized and designed the study. YY conducted the experimental work, acquired funding, managed project administration and resources, supervised the study, collected and curated the data, performed formal analyses, validated and visualized the results, and prepared the original draft of the manuscript. Nurbailis and Reflin contributed to the investigation, methodology development, formal data analysis, supervision, validation, and drafting of the manuscript. HH and Yaherwandi contributed to formal analysis, methodology, supervision, validation, and both writing and critical revision of the manuscript. Chrisnawati assisted with data curation, investigation, formal analysis, visualization, and preparation of the original draft. All authors critically reviewed the manuscript and approved the final version.

Research Funding

This research was funded by the Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM) of Universitas Andalas, Indonesia, under the Competitive Research Grant Scheme (T/1/UN.16.17/PP.Pangan-PTUKRP2GB/LPPM/2020).

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

References

Abdulkadir, H.K., Ekefan, E.J., Gwa, V.I., 2023. Pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) isolates in causing tomato wilt disease on two tomato (*Solanum lycopersicum* L.) varieties. Bio Science Research Bulletin-Biological Sciences 39(2).

Akram, W., Sharif, S., Rehman, A., Anjum, T., Ali, B., Aftab, Z.A., Shafqat, A., Afzal, L., Munie, B., Rizwana, H., Li, G., 2024. Exploring the potential of *Bacillus subtilis* IS1 and *B. amyloliquificiens* IS6 to manage salinity stress and *Fusarium* wilt disease in tomato plants by induced physiological responses. Microorganisms 12, 2092.

Almasoudi, N.M., Sallam, N.M., Ali, E.F., Alqurashi, A.S., Issa, A.A., Althobaiti, F., Abo-Elyousr, K.A., 2025. Development of *Bacillus* spp. for controlling wilt disease and improving the growth of tomato. European Journal of Plant Pathology 172, 355-366.

Amin, A., Zaman, W., 2025. Integrative perspectives on multi-level mechanisms in plant-pathogen interactions: from molecular defense to ecological resilience. Phyton-International Journal of Experimental Botany 94, 1973-1996.

Ayaz, M., Ali, Q., Farzand, A., Khan, A.R., Ling, H., Gao, X., 2021. Nematicidal volatiles from *Bacillus atrophaeus* GBSC56 promote growth and stimulate induced systemic resistance in tomato against *Meloidogyne incognita*. International Journal of Molecular Sciences 22, 5049.

Chandrasekaran, M., Chun, S.C., 2016. Expression of PR-protein genes and induction of defense-related enzymes by *Bacillus subtilis* CBR05 in tomato

(*Solanum lycopersicum* L.) plants challenged with *Erwinia carotovora* subsp. *carotovora*. Bioscience, Biotechnology, and Biochemistry 80, 2277-2283.

Espinosa-Vázquez, M.Á., Espinoza-Medinilla, E.E., Orantes-García, C., Garrido-Ramírez, E., Rioja-Paradela, T.M., 2019. Salicylic acid and *Bacillus subtilis* as control of early blight (*Alternaria solani*) in tomato plants (*Solanum lycopersicum* L.). Revista de la Facultad de Ciencias Agrarias UNCuyo 51, 161-171.

Feng, L., Li, Q., Zhou, D., Jia, M., Liu, Z., Hou, Z., Yu, J., 2024. *B. subtilis* CNBG-PGPR-1 induces methionine to regulate ethylene pathway and ROS scavenging for improving salt tolerance of tomato. The Plant Journal 117, 193-211.

Fuerst, E.P., Okubara, P.A., Anderson, J.V., Morris, C.F., 2014. Poplyphenol oxidase as a biochemical seed defense mechanism. Frontiers in Plant Science 5, 689.

Haruna, S.G., Yahuza, L., Tijjani, I., 2024. Management of Fusarium wilt of tomato (*Fusarium oxysporum* f. sp. *lycopersici*) and related soil-borne diseases using eco-friendly methods: a review. Asian Journal of Research in Crop Science 9, 154-168.

Irina, Z., Irina, P., Dmitriy, E., Inessa, P., Alla, C., Alena, P., Natalya, H., 2024. Assessment of vitamin-and mineral-content stability of tomato fruits as a potential raw material to produce functional food. Functional Foods in Health and Disease 14, 14-32.

Islam, T., Danishuddin, Tamanna, N.T., Matin, M.N., Barai, H.R., Haque, M.A., 2024. Resistance mechanisms of plant pathogenic fungi to fungicide, environmental impacts of fungicides, and sustainable solutions. Plants 13, 2737.

Jawadayn, A., Alkooranee, N., Alobedi, S., 2025. Induced Systemic Resistance in Plant. Dijlah Journal of Agricultural Sciences 4, 1-10.

Ma, M., Taylor, P.W., Chen, D., Vaghefi, N., He, J.Z., 2023. Major soilborne pathogens of field processing tomatoes and management strategies. Microorganisms 11, 263.

Macioszek, V.K., Jecz, T., Ciereszko, I., Kononowicz, A.K., 2023. Jasmonic acid as a mediator in plant response to necrotrophic fungi. Cells 12, 1027.

Mahapatra, S., Chakraborty, S., Samanta, M., Das, S., Islam, T., 2022. Current understanding and future directions of biocontrol of plant diseases by *Bacillus* spp., with special reference to induced systemic resistance. In: Bacilli in Agrobiotechnology: Plant Stress Tolerance, Bioremediation, and Bioprospecting. Springer International Publishing, Cham, pp. 127-150.

Mattos, A.D.P., Rissato, B.B., Itako, A.T., Tolentino, J.B., Estrada, K.R.F.S., 2023. *Bacillus amyloliquefaciens* PKM16 acts as an antagonist of white mold and an inducer of defense enzymes in tomato plants. Acta Scientiarum Agronomy 45, e59586.

Mondo, A.D., Sansone, C., Brunet, C., 2022. Insights into the biosynthesis pathway of phenolic compounds in microalgae. Computational and Structural Biotechnology Journal 20, 1901-1913.

Naqvi, S.A.H., Farhan, M., Ahmad, M., Kiran, R., Shahbaz, M., Abbas, A., Sathiya Seelan, J.S., 2025. Fungicide resistance in *Fusarium* species: exploring environmental impacts and sustainable management strategies. Archives of Microbiology 207, 31.

Novikova, I.I., Popova, E.V., Kovalenko, N.M., Krasnobaeva, I.L., 2024. The Effect of *Bacillus subtilis* in combination with chitosan salicylate on peroxidase and catalase activity in *B. sorokiniana* infected wheat. Applied Biochemistry and Microbiology 60, 241-250.

Pazarlar, S., Madriz-Ordeñana, K., Thordal-Christensen, H., 2022. *Bacillus cereus* EC9 protects tomato against *Fusarium* wilt through JA/ET-activated immunity. Frontiers in Plant Science 13, 1090947.

Qin, X., Xiao, Y., Xiong, Q., Kong, W.L., Borriss, R., Gao, Z., Fan, B., 2025. Four antimicrobial compounds and ISR induction are involved in biocontrol of crown gall disease by the plant beneficial rhizobacterium *Bacillus velezensis* FZB42. Plant Disease (ja).

Rahman, M.M., Almasoudi, N.M., Asiry, K.A., Abo-Elyousr, K.A., 2025. Rhizobacteria-mediated biocontrol of tomato gray mold: mechanisms of induced systemic resistance and enzymatic activity. European Journal of Plant Pathology 1-15.

Rashad, Y.M., Abdalla, S.A., Sleem, M.M., 2022. Endophytic *Bacillus subtilis* SR22 triggers defense responses in tomato against rhizoctonia root rot. Plants 11, 2051.

Saiyam, D., Dubey, A., Malla, M.A., Kumar, A., 2024. Lipopeptides from *Bacillus*: Unveiling biotechnological prospects-Sources, properties, and diverse applications. Brazilian Journal of

Microbiology 55, 281-295.

Sorokan, A., Burkhanova, G., Gordeev, A., Maksimov, I., 2023. Exploring the role of salicylic acid in regulating the colonization ability of *Bacillus subtilis* 26D in potato plants and defense against *Phytophthora infestans*. International Journal of Plant Biology 14, 242-253.

Théatre, A., Hoste, A.C.R., Rigolet, A., Benneceur, I., Bechet, M., Ongena, M., Jacques, P., 2022. *Bacillus* sp.: A remarkable source of bioactive lipopeptides. In: Biosurfactants for the Biobased Economy. Springer International Publishing, Cham, pp. 123-179.

Tsai, S.H., Hsiao, Y.C., Chang, P.E., Kuo, C.E., Lai, M.C., Chuang, H.W., 2023. Exploring the biologically active metabolites produced by *Bacillus cereus* for plant growth promotion, heat stress tolerance, and resistance to bacterial soft rot in *Arabidopsis*. Metabolites 13, 676.

Yadav, U., Anand, V., Kumar, S., Verma, I., Anshu, A., Pandey, I.A., Singh, P.C., 2024. *Bacillus subtilis* NBRI-W9 simultaneously activates SAR and ISR against *Fusarium chlamydosporum* NBRI-FOL7 to increase wilt resistance in tomato. Journal of Applied Microbiology 135, lxae013.

Yang, Q., Niu, A., Li, S., Liu, J., Zhou, G., 2024. Unveiling Metabolic Crosstalk: *Bacillus*-Mediated Defense Priming in Pine Needles Against Pathogen Infection. Metabolites 14, 646.

Yu, Y.Y., Si, F.J., Wang, N., Wang, T., Jin, Y., Zheng, Y., Jiang, C.H., 2022. *Bacillus*-secreted oxalic acid induces tomato resistance against gray mold disease caused by *Botrytis cinerea* by activating the JA/ET pathway. Molecular Plant-Microbe Interactions 35, 659-671.

Zhou, C., Zhu, L., Xie, Y., Li, F., Xiao, X., Ma, Z., Wang, J., 2017. *Bacillus licheniformis* SA03 confers increased saline-alkaline tolerance in *Chrysanthemum* plants by induction of abscisic acid accumulation. Frontiers in Plant Science 8, 1143.

Zou, X., Ning, J., Zhao, X., Lv, H., Qin, N., Yin, H., Ren, L., 2024. *Bacillus velezensis* LY7 promotes pepper growth and induces resistance to *Colletotrichum scovillei*. Biological Control 192, 105480.