Original Paper

Application of Endophytic Bacteria from Tomato Stems to Control Bacterial Wilt Disease in Tomato and Enhance Plant Growth

Quang Trung Do[1]*, Danh Quang Do[1], Van Nha Pham[1], Thi Ngan Nguyen[1], Thi Thuy Linh Ngo[1], Thi Hong Van Pham[1], Trong Tri Nguyen[2], Mai Van Dinh[3]

[1]*Dai Nam University, Faculty of Biotechnology, Xom, Phu Lam, Ha Dong, Ha Noi, Vietnam*
[2]*Vietnam National University of Forestry, Xuan Mai, Ha Noi, Vietnam*
[3]*Hanoi University of Science, Vietnam National University Hanoi, 334 Nguyen Trai, Thanh Xuan, Ha Noi, Vietnam*

Article Details: Received: 2024-04-23 | Accepted: 2024-05-10 | Available online: 2024-06-30

https://doi.org/10.15414/afz.2024.27.02.179-186

Licensed under a Creative Commons Attribution 4.0 International License

SCREENED BY iThenticate® Professional Plagiarism Prevention

Bacterial green wilt (BGW) disease, caused by *Ralstonia solanacearum* bacterium, is a devastating bacterial disease of tomatoes occurring in tropical zones, resulting in substantial yield losses in production fields. Biological control agents (BCAs), particularly plant endophytes such as bacteria, are becoming increasingly popular in microbial technology for cropping systems. In this study, we assessed the potential of endophytic bacteria from tomato plants to suppress the BGW caused by *R. solanacearum* in tomato plants. The research study revealed that out of the 9 endophytic strains isolated, *Bacillus amyloliquefaciens* TO3 demonstrated a strong antagonistic ability towards *R. solanacearum*, with the zone of inhibition approximately 16.58 ±0.19 mm. This strain also produced cell wall-degrading enzymes (amylase, protease, and cellulase), and indole acetic acid (IAA). In addition, the greenhouse experiments showed that applying strain TO3 before the infection of *R. solanacearum* led to a high protective effect against the BGW and a significant increase in plant height compared to the non-inoculated ones. In field conditions, inoculating tomato seedlings with strain TO3 resulted in a disease reduction of over 55% against the BGW compared to the non-treated control. Moreover, treatment with strain TO3 increased the yield of tomato fruits by up to 47.93% compared to non-treated control plants. These findings indicate the potential use of *B. amyloliquefaciens* TO3 in preventing the BGW disease caused by *R. solanacearum* on tomato plants.

Keywords: Antagonistic ability, Bacterial wilt, Bacillus spp., Endophytic bacteria, *Ralstonia solanacearum*

1 Introduction

Bacterial green wilt (BGW) disease caused by the *R. solanacearum* is a significant threat to crop production. It was reported as a main factor causing yield losses of tomatoes up to 90% (Fanhong, 2013). Currently, preventing the BGW disease is still a challenging task and different strategies have been taken, such as using disease-resistant plant varieties, crop rotation and rootstock cultivation methods, chemical pesticides, metal nanoparticles (such as nano silver and nano copper), and biological control with microbial strains antagonistic to *R. solanacearum* (Agarwal et al., 2020). However, the effectiveness of these measures is still limited due to the complexity of the *R. solanacearum* classification system, its ability to mutate quickly, and its long-term survival in different conditions with a wide host range (Mohamed et al., 2020). Moreover, the use of chemical pesticides can have negative effects on the ecological environment, product quality, and public health (Fahime & Gholam, 2018). As a result, crop production, especially tomatoes, still faces many difficulties in various regions.

In recent years, there have been numerous studies on the use of endophytic bacteria to control diseases in many crops (Eid et al., 2021; Trung et al., 2023). According to Amaresan et al. (2012), endophytic bacteria that are effective against *R. solanacearum* belong to various genera such as *Bacillus*, *Proteus*, and *Pseudomonas*. Bahmani et al. (2021) have isolated eight endophytic bacterial strains, including *Pseudomonas brassicacearum*,

***Corresponding Author:** Quang Trung Do, Dai Nam University, Faculty of Biotechnology, 01 Xom, Phu Lam, Ha Dong, Ha Noi, 100000 Vietnam, trungdq@dainam.edu.vn; trungcnsinh@gmail.com
https://orcid.org/0000-0001-5661-4181

Bacillus licheniformis, *P. putida*, *Paenibacillus peoriae*, and *B. pumilus*, which can control the BGW disease under both *in vitro* and *in vivo* conditions. Under greenhouse conditions, the endophytic bacterium *B. subtilis* can protect mulberry plants effectively against *R. solanacearum* (Ji et al., 2008). Endophytic bacteria isolated from *Gnetum gnemon* plants are not only used to protect seedlings but also to enhance growth in tomatoes (Agarwal et al., 2020). *B. cereus* limited the manifestations of green wilt disease and reduced the disease rate by 80.0% (Achari et al., 2018).

In Vietnam, the bacterial wilt disease was reported as a factor contributing to the reduction of tomato production areas by about 25–45% (Doan & Nguyen, 2006). Various Solanaceae plants have been found to contain certain types of gram-positive bacteria belonging to the genus *Bacillus*, and gram-negative bacteria belonging to the genera *Agrobacterium* and *Pseudomonas*. These bacteria have been shown to have high antagonistic activity against *R. solanacearum* in *in vitro* conditions (Nguyen et al., 2006). However, the effectiveness of these strains in practical applications is still limited and may differ depending on the specific strain of pathogenic bacteria. Therefore, this study was conducted to isolate a new endophytic bacterial strain with high resistance to the *R. solanacearum* strain in Nam Dinh.

2 Material and methods

2.1 Isolation of endophytic bacteria

Tomato stem samples were collected in November 2021 at Nam Dinh, Vietnam. 05 stem samples of 20–30 cm in length were randomly collected from disease-free tomato plants at the growth stage about 60 days after sowing. Samples were placed in zip bags, sealed, marked, and recorded the location, and sample collection time, and finally transferred to the laboratory within 24 hours.

Plant stem samples were washed under running water for 15 minutes to remove dirt. Then the samples were cut into small pieces of 2–3 cm and disinfected on the surface with 70% ethanol for 3 minutes, continued using sodium hypochloride (NaOCl) 0.5% for 3 minutes and 70% ethanol for 30 seconds. Finally, the samples were rinsed with sterile distilled water 5 times (Agarwal et al., 2020). 0.1 ml of the final sample washing water was pipetted and inoculated on a petri plate containing Luria-Bertani (LB) medium (10 $g.l^{-1}$ peptone; 10 g $NaCl.l^{-1}$; yeast extract 5 $g.l^{-1}$; agar 18 $g.l^{-1}$). The inoculated petri dishes were incubated at 30 °C for 48 hours. If there is no growth of bacteria and fungi in these plates, it proves that sterilization has been satisfactory.

Stem samples were cut into small pieces and then ground with 5 ml of distilled water. 50 µl of the culture suspension were pipetted, spread on a petri dish containing LB medium, and cultured at 30 °C for 48–96 hours. The colonies that appeared were selected and streaked on a new LB medium several times before being used to observe colony morphology.

2.2 Antagonistic activity of isolates against R. solanacearum bacteria

The *R. solanacearum* DZ5 bacterial strain was isolated from tomato plants with bacterial wilt disease in Nam Dinh, tested for pathogenicity on the tomato, and identified by molecular method. This strain was stored in the Faculty of Biotechnology, Dai Nam University, Ha Noi, Viet Nam.

The method to evaluate the antagonistic activity of endophytic bacteria is carried out using the agar pore diffusion method (Trung et al., 2023). Briefly, 0.1 ml of *R. solanacearum* bacterial culture was spread into a petri dish containing Sucrose Peptone Agar (SPA) medium (sucrose 20 $g.l^{-1}$, peptone 5 $g.l^{-1}$, K_2HPO_4 0.25 $g.l^{-1}$, $MgSO_4 \cdot 7H_2O$ 0.25 $g.l^{-1}$, agar 15 $g.l^{-1}$). Then, a 1 cm diameter agar well was prepared by an inverted 1 ml tip in the center of the inoculated Petri dish. Next, 0.1 ml of each overnight-grown bacterial isolate was pipetted into the prepared wells. For the control, autoclaved distilled water was used. All inoculated plates were kept at 30 °C for 3 days. Each experiment was repeated 3 times.

The antagonistic activity of isolates against *R. solanacearum* bacteria was expressed through the size of the antagonistic ring, which was calculated according to the formula:

$$\textit{Antagonistic ring size}\ (\text{mm}) = D - d$$

where: D – the diameter of the resistant ring (mm);
d – the diameter of the well (mm)

2.3 Determining cellulase, amylase, and protease activities of isolates

The activity of cellulase, amylase, and protease enzymes was determined based on the ability to form lytic rings stained with Lugol solution on agar plates containing the corresponding substrates Carboxymethyl cellulose (CMC), starch, and casein at a concentration of 0.2% (Williams, 1983).

2.4 Determining the phosphorus solubilization of isolates

Phosphorus solubilizing activity was determined based on the formation of clear rings around colonies of isolates on the Pikovaskya medium (Puente et al., 2004).

2.5 Determining the IAA production of isolates

Bacterial strains were grown in nutrient agar (NA) medium supplemented with 100 mg.l^{-1} tryptophan and the produced IAA was quantified using Salkowski reagent (Trung et al., 2023). The reaction mixture included 1 ml of supernatant and 2 ml of Salkowski reagent was mixed well and incubated in the dark for 20 minutes. Then the OD of solution was measured on a spectrophotometer at a wavelength of 530 nm. The OD value of the sample was compared with the standard graph to calculate the IAA content in the culture fluid in units of µm IAA/ml.

2.6 Molecular identification of selected endophytic bacteria

The isolates were grown overnight and the bacterial DNAs were extracted by using PureLink™ Genomic DNA Mini Kit (Thermo Fisher). The extracted DNA was applied for 20 µl PCR reaction including 12.5 µl PCR buffer 2X, forward primer (27F, 5'-AGAGTTTGATCMTGGCTCAG-3–) 1pmol, reverse primer (1492R, 5'-GGTTACCTTGTTACGACTT-3') 1 pmol, DNA 50 ng (Li and de Boer 1995). Thermal cycling of the PCR reaction included: Initial denaturation at 95 °C for 1 minute; The 30 cycles included: denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 30 seconds; and finished at 72 °C for 10 minutes. PCR products were electrophoresed on a 1% agarose gel in 1X TAE buffer, at a voltage of 100V for 30 minutes. The purified PCR product (about 1.350 kb) was sequenced by First BASE company – Malaysia. The obtained nucleotide sequences were compared to sequences of genebank on NCBI (National Center for Biotechnology Information) using BLASTN (Nucleotide Basic Local Alignment Search Tool).

2.7 Evaluating the ability of isolates to stimulate growth and control bacterial green wilt disease in a greenhouse

The bacterial strain *R. solanacearum* DZ5 isolated from tomato plants with green wilt disease in Nam Dinh was provided by the Faculty of Biotechnology, Dai Nam University, Ha Noi, Vietnam.

The tomato seeds (*Solanum lycopersicum* var. TN-323) were used in testing the resistance ability of endophytic bacteria to *R. solanacearum* bacteria under greenhouse conditions at the Vietnam National University of Forestry in the Spring-Summer 2021 crop and cared for according to routine procedures.

Tomato seeds were surface sterilized and soaked for 4 hours in sterilized water. The sterilized seeds were incubated in agar plates for 6 days in the growth room set up at 28 °C, 75% relative humidity, and a 12 h photoperiod. After 6 days of germination, 3 seedlings were transferred into one pot containing sterilized soil.

The planted pots were arranged with different experiments including (T1): The seedlings were artificially infected with *R. solanacearum* and did not inoculate antagonistic bacteria; (T2): The seedlings were not artificially infected with *R. solanacearum* and were not treated with antagonistic bacteria; (T3): The seedlings were not artificially infected with *R. solanacearum* and were treated with antagonistic bacteria; (T4): The seedlings were artificially infected with *R. solanacearum* and were treated with antagonistic bacteria.

The bacteria were grown in a liquid nutrient broth medium for 36 hours before treatment (OD600 =1.0). Seedlings were treated with isolated bacteria the first time by dipping their roots in the bacterial culture for 1 hour before transferring to the soil and the second time by watering (108 cfu.ml^{-1}, 3 ml.plant^{-1}) 3 days before artificial infection with pathogenic bacteria (10^8 cfu.ml^{-1}, 15 ml^{-3} plants.pot^{-1}).

All experiments were done in a completely randomized block design with 3 replicates. Monitoring criteria included plant height and disease level at 7, 14, and 21 days after *R. solanacearum* infection (DAI) when the seedlings showed symptoms of green wilt disease based on the assessment scale of Seleim et al. (2011).

The disease index was recorded using the following formula (Seleim et al., 2011):

$$\textit{disease index}\ (\%) = \left[\frac{\sum(ni \times vi)}{(V \times N)}\right] \times 100$$

where: *ni* – number of plants with respective disease ratings; *vi* – disease rating; *V* – the highest disease rating; *N* – the number of plants observed

Disease rating was calculated on the following scale: 1 – no symptoms, 2 – one leaf wilted, 3 – two to three leaves wilted, 4 – four or more leaves wilted, and 5 – whole plant wilted.

2.8 Evaluating the effective control of green wilt disease by isolates in field conditions

The field experiment was carried out in Giao Yen commune, Giao Thuy district, Nam Dinh province in the Spring-summer crop of 2021. The field experiment was conducted on tomato seeds (*Solanum lycopersicum* var. TN-323) and arranged in a completely randomized block design with 4 replicates.

For each treatment, 20-day-old tomato seedlings were transplanted into 2 rows, the distance between rows was

50 × 40 cm, 10 plants.plot^{-1}. The lime powder was spread in line (0.3m wide) to separate plots.

For the treated experiments (C1), the antagonistic bacteria (10^8 cfu.ml^{-1}) were applied the first time by soaking with the seeds before sowing, and the second time by watering at 6 days after planting (15 ml.plant^{-1}). After 12 days from the second application of antagonistic bacteria, the culture of *R. solanacearum* bacterium (10^8 cfu.ml^{-1}) was watered at the base of plants (15 ml. plant^{-1}).

For the control, the tomato seeds were treated with sterilized water and the seedlings were applied only *R. solanacearum* strain (C2) or no antagonistic bacteria and *R. solanacearum* strains (C3).

The criteria of plant growth such as plant height and fresh weight of shoots and roots) were monitored. The development of green wilt disease and the disease index were measured as methods described in the greenhouse experiment.

To calculate the fruit yield for each plot, data obtained from all the pickings from that plot was pooled and presented as ton ha^{-1}.

2.9 Data analysis

Experimental data were statistically processed using Excel and IRRISTAT 5.0 software. Tukey's honestly significant difference (HSD) method in Statistical Package for the Social Sciences (SPSS) (version 20) was used to compare the means in all experiments.

3 Results and discussion

3.1 Characterization of isolated endophytic bacteria

From uninfected plant samples, 8 endophytic bacterial strains were isolated (Table 1). As can be seen, the isolated colonies presented different morphology indicating that they could be different species.

All of them were investigated for their resistance ability against *R. solanacearum* bacterium, a causal of wilt disease in tomato plants. The results showed that among 8 isolated endophytic bacterial strains, only 2 bacterial strains TO1 and TO3 were able to antagonize *R. solanacearum* bacterium (Table 2).

Table 2 Antagonistic ability of isolates against *R. solanacearum* under *in vitro* conditions

Isolates	Diameter of antagonistic halo-zone (mm)	
	48 hrs	72 hrs
TO1	10.21 ±0.15a*	11.93 ±0.14a
TO2	0	0
TO3	15.02 ±0.17b	16.58 ±0.19b
TO4	0	0
TO5	0	0
TO6	0	0
TO7	0	0
TO8	0	0
Control	0	0

* values are mean ± SD (n = 3); values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test ($P < 0.05$)

The data in Table 2 demonstrated that strain TO3 presented a higher antagonistic ability than strain TO1 did during the experiment, which was about 5 mm after 48 hrs and 72 hrs of incubation. The results also indicated that the resistance of two isolates, TO1 and TO3, gradually increased from 48 to 72 hours.

Furthermore, the results of experiments for the ability to solubilize phosphorus compounds showed that strain TO1 produced a phosphorus solubilization ring with dissolution diameters of about 3 mm (Table 3). The results also demonstrated that the two strains investigated in this study were all capable of producing IAA when supplemented with Tryptophan at a concentration of 0.1%, the measured amount of IAA was 5.32

Table 1 Colony characteristics of endophytic bacterial strains isolated from tomato plants

Isolates	Colony characteristics
TO1	the colony is large and round, has a rough, dry surface, and has many milky white wrinkles on the outside
TO2	the colony has a light pink color, a small round surface, and is smooth
TO3	the colony is milky white, rough, dry, and round, with a dry wrinkled surface and a rough convex center
TO4	the colony has a dry, rough, milky-white surface
TO5	the colony is dry and flat, with a dark, opaque white center with outer edges
TO6	the colony is round, the surface is wrinkled, dry, rough, milky white
TO7	the colony is large, dry, and round; the surface is wrinkled
TO8	colonies are creamy white, wrinkled, and dry

Table 3 Biological characteristics of endophytic bacteria strains, TO1 and TO3, under *in vitro* conditions

Isolates	Diameter of Phosphorus solubilization ring (mm)	IAA production (μg.ml^{-1})	Diameter of hydrolytic ring (mm)		
			CMC	Starch	Casein
TO1	3	5.32 ±0.11a	12.17 ±0.22a	-	17.21 ±0.17a
TO3	-	107.81 ±0.24b	16.58 ±0.19b	15.02 ±0.17b	30.25 ±0.23b

* values are mean ± SD (n = 3); values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test (P < 0.05)

and 107.81 μg.ml^{-1} respectively for strain TO1 and TO3 (Table 3).

In addition, the results also showed that two tested strains, TO1 and TO3, produced cellulase and protease with different degradation abilities, which were illustrated by ring diameters from 12–30 mm (Table 3). The data also indicated that only strain TO3 could produce amylase with ring diameters of about 15 mm (Table 3).

The results showed that among selected isolates, strain TO3 presented better biological characteristics than strain TO1 (Table 3). Hence, the strain of TO3 was chosen for further studies. Based on the comparison results with the 16S sequences available on NBCI's GenBank using BLAST software, strains TO3 were close to *Bacillus amyloliquefaciens* BC1 (the similarity percentage is 99.30%). The sequences were deposited on the GenBank with the accession number OR807327.

Many mechanisms have been proposed to explain the inhibition of pathogens. According to Latha et al. (2019), this mechanism is based on the protective action in the rhizosphere against plant pathogenic bacteria through the biosynthesis of metabolites such as biosurfactants – biological surfactants, (hydrogen cyanide), cleavage enzymes such as β-1,3 glucanase and proteases, plant hormones and plant growth stimulants such as auxins, IAA and siderophores. In this study, 2 strains that create inhibitory rings with pathogenic bacteria were TO1 and TO3, which produced hydrolytic enzymes (cellulase, protease, and amylase), and were also capable of producing IAA. The biosynthesis of secondary metabolites may have an impact on antagonistic activity by functioning as a signal mediator between endophytic bacteria and the host plant (Graner et al., 2003). In addition, in many previous publications, endophytic bacterial strains belonging to the species *B. amyloliquefaciens* have been isolated and demonstrated their high resistance to *R. solanacearum* bacteria (Agarwal et al., 2020; Singh et al., 2022).

3.2 Effective control of bacterial green wilt disease by strain TO3 under greenhouse conditions

The results of the pot experiments under greenhouse conditions showed that there was a significant difference in the wilting rate of seedlings among the treatments (Table 4). In addition, between observation times, different wilting rates of seedlings were also recorded (Table 4).

As can be seen from Table 4, in the T1 treatment without treating seeds with endophytic bacteria, wilting symptoms began to be observed 5 days after infection with *R. solanacearum* bacteria. After 7 days of infection, the disease rate reached very high, up to 52.12%. Meanwhile, in other treatments (T2, T3, and T4), no wilting symptoms in seedlings were observed. After 21 days, the seedlings in the T1 treatment were completely withered, with a disease rate of 100%. For the treatment with seeds treated with endophytic bacteria TO3 (T4), the disease rate (5.13%) was much lower than

Table 4 Antagonistic ability of endophytic bacteria TO3 against *R. solanacearum* on tomato seedlings under greenhouse conditions

Treatment	Disease index (%)		
	7 DAI*	14 DAI	21 DAI
T1**	52.12 ±0.13b***	85.35 ±0.31b	100 ±0.00b
T2	0 ±0.00a	0 ±0.00a	0 ±0.00a
T3	0 ±0.00a	0 ±0.00a	0 ±0.00a
T4	1.76 ±0.25a	3.91 ±0.32a	5.13 ±0.15a

* DAI – days after infection with *R. solanacearum*; ** T1 – treated without strain TO3 and with *R. solanacearum*; T2 – treated without strain TO3 and *R. solanacearum*; T3 – treated with strain TO3 and without *R. solanacearum*; T4 – treated with strain TO3 and *R. solanacearum*; the percentage data is converted to arcsin $\left(\sqrt{(x100)}\right)$ when analyzing statistically, where x is the disease index; *** values are mean ±SD (n = 3); values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test (P <0.05)

Table 5 Effect of endophytic bacteria TO3 on the development of tomato seedlings under greenhouse conditions

Treatment	Plant height (cm)			
	0 DAI*	7 DAI	14 DAI	21 DAI
T1**	22.12 ±0.24a***	25.29 ±0.33a	27.41 ±0.27a	34.32 ±0.15a
T2	22.41 ±0.12a	26.23 ±0.22a	32.51 ±0.42a	43.38 ±0.69b
T3	22.09 ±0.28a	35.09 ±0.31b	46.85 ±0.79b	57.24 ±0.51c
T4	21.78 ±0.34a	36.72 ±1.08b	46.12 ±0.93b	58.79 ±0.74c

* DAI – days after infection with *R. solanacearum;* ** T1 – treated without strain TO3 and with *R. solanacearum*; T2 – treated without strain TO3 and *R. solanacearum*; T3 – treated with strain TO3 and without *R. solanacearum*; T4 – treated with strain TO3 and *R. solanacearum;* the percentage data is converted to arcsin $\left(\sqrt{(x100)}\right)$ when analyzing statistically, where *x* is the disease index; *** values are mean ±SD ($n = 3$); values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test ($P < 0.05$)

the positive control group (Table 3). At the same time, in the T2 and T3 treatments, no green wilting symptoms were recorded in the seedlings. These results indicated that *R. solanacearum* bacteria are the causative agent of green wilt symptoms in experimental treatments and that endophytic bacterial strains TO3 had the potential to prevent green wilt disease in tomato plants. Regarding the ability to stimulate growth, the data in Table 5 indicated that strain TO3 helped inoculated seedlings grow better than non-inoculated ones did (T3), and also helped seedlings withstand and grow better when infected with *R. solanacearum* (T4).

Endophytic bacteria were reported to potentially be bacterial control agents against phytopathogen and stimulate the development of plants, which were artificially infected with the phytopathogenic agent (Trung et al., 2023). According to Vessey (2003), inoculated bacteria stimulated plant growth not only acts as microbial organic fertilizer but also stimulates plant growth by helping plants fight pathogens. In this study, the results demonstrated that the endophytic bacteria *Bacillus amyloliquefaciens* TO3 presented the highest antagonistic activity under *in vitro* and greenhouse conditions. The stimulation of disease resistance of inoculated bacteria was reported through the ability to slow down the disease manifestation process and reduce the incidence and toxicity of the disease compared to plants that were not inoculated with antagonistic bacteria (Eid et al., 2021). Collectively, the results of this study supported the potential application of endophytic bacteria *B. amyloliquefaciens* TO3 as a biocontrol agent against green wilt disease on tomatoes.

3.3 Effective control of bacterial green wilt disease by strain TO3 in field conditions

The experiment was carried out under average temperature conditions of 26–35 °C and humidity of 60–90%. The weather had little rain and lime was spread between the experimental plots, so the isolation between the experimental plots was very good.

The results of disease-control experiments under field conditions are presented in Table 6. As can be seen, the disease index of the experiments treated with strain TO3 (C1) was lower and statistically different from the one of the experiments only infected with *R. solanacearum* (C2). As we expected, there was no symptom of green wilt disease observed in the experiments treated with only sterilized water (C3).

Regarding to plant growth stimulation in the treatments, the fruit yield of the plots treated with strain TO3 was significantly higher than the ones of non-treated plots (control). The inoculation of strain TO3 influenced the development of tomato plants such as plant height, the weight of the shoot and root, and the fruit yield (Table 7). As presented in Table 7, all the investigated

Table 6 Effectiveness of green wilt disease control by strain TO3 under field conditions

Treatment	Disease index (%)				
	5 DAI*	10 DAI	20 DAI	30 DAI	35 DAI
C1**	1.21 ±0.13b***	1.72 ±0.22b	2.29 ±0.33b	2.85 ±0.21b	3.25 ±0.13b
C2	5.14 ±0.32c	6.18 ±0.31c	6.73 ±0.25c	7.15 ±0.15c	7.89 ±0.24c
C3	0 ±0.00a	0 ±0.00a	0 ±0.00a	0 ±0.00a	0 ±0.00a

* DAI – days after infection with *R. solanacearum;* ** C1 – treated with strain TO3 and *R. solanacearum*; C2 – treated without strain TO3 and with *R. solanacearum*; C3 – treated without strain TO3 and *R. solanacearum;* the percentage data is converted to $\left(\sqrt{(x)}\right)$ when analyzing statistically, where *x* is the disease index; *** values are mean ±SD ($n = 3$); values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test ($P < 0.05$)

Table 7 Effect of strain TO3 on the development of tomato plants under field conditions

Treatment	Plant height (cm)	Fresh shoot weight (kg.plant^{-1})	Fresh root weight (kg.plant^{-1})	Fruit yield (t.ha^{-1})
C1*	95.35 ±0.23b**	0.352 ±011c	0.047 ±0.17c	60.52 ±0.11c
C2	83.652 ±0.12a	0.181 ±0.41a	0.025 ±0.25a	28.57 ±0.15a
C3	89.72 ±0.42ab	0.276 ±0.27b	0.039 ±0.12b	33.97 ±0.23b

* C1 – treated with strain TO3 and *R. solanacearum*; C2 – treated without strain TO3 and with *R. solanacearum*; C3 – treated without strain TO3 and *R. solanacearum;* the percentage data is converted to $\left(\sqrt{(x)}\right)$ when analyzing statistically, where *x* is the disease index; ** values are mean ±SD (n = 3); values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test ($P < 0.05$)

criteria of plants in the experimental treatment (C1) were higher than the ones in the control (C2 and C3 treatments).

The results of field experiments could be explained by the inoculation of tomato seeds with strain TO3 generated protection for the tomato seedlings against green wilt disease at the early growth stage and this protection was strengthened by the second application of strain TO3 at the later growth stage. Moreover, strain TO3 also produced IAA, hydrolytic enzymes, and could solubilize phosphorus compounds, subsequently enhancing the growth of tomato plants under field conditions. Results of field experiments by Doan and Nguyen (2006) showed that treatments with growth-promoting bacterial strains increased productivity from 7.78 to 10.56% compared to the control. In this experiment, productivity increased from 43.86% to 47.93% compared to the control. The difference may be because the experiment of Doan & Nguyen (2006) only used bacteria to coat the seeds, but this experiment also coats the seeds and inoculates the soil with bacteria one more time later. These data indicated that strain TO3 could potentially be applied as a biocontrol agent and/or biofertilizer in organic agriculture.

4 Conclusions

In the research results, 8 strains of endophytic bacteria were isolated from tomato stem samples. In particular, an endophytic bacterial strain *Bacillus amyloliquefaciens* TO3 has been identified and had the highest resistance to *R. solanacearum* under either *in vitro* or *in vivo* conditions. The data also presented that the infection of *R. solanacearum* significantly increased in plant height of strain TO3-inoculated tomato seedlings compared to the non-treated seedlings under greenhouse conditions. Under field conditions, inoculation with strain TO3 of tomato seedlings resulted in a mean level of disease reduction of more than 55.0% against green wilt disease compared to non-treated control. In addition, treatment with strain TO3 also increased the yield of tomato fruits by up to 47.93% than water-treated control plants. These results suggest the potential application of *B. amyloliquefaciens* TO3 strain in preventing green wilt disease caused by *R. solanacearum* on tomato plants.

Acknowledgments

This research has been done under the support of Dai Nam University. The Dai Nam University provided funds for collecting samples and buying chemicals for experiments and supported experimental facilities to carry out experiments.

References

Agarwal, H. et al. (2020). Endophytes from *Gnetum gnemon* L. can protect seedlings against the infection of phytopathogenic bacterium *Ralstonia solanacearum* as well as promote plant growth in tomato. *Microbiological Research*, 238, 126503. https://doi.org/10.1016/j.micres.2020.126503

Amaresan, N. et al. (2012). Endophytic bacteria from tomato and chili, their diversity and antagonistic potential against *Ralstonia solanacearum*. *Archives of Phytopathology and Plant Protection*, 45(3), 344–355. https://doi.org/10.1080/03235408.2011.587273

Achari, G.A., & Ramesh, R. (2018). Colonization of eggplant by endophytic bacteria antagonistic to *Ralstonia solanacearum*, the bacterial wilt pathogen. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 89, 585–593. https://doi.org/10.1007/s40011-018-0972-2

Bahmani, K. et al. (2021). Isolation and identification of endophytic bacteria from potato tissues and their effects as biological control agents against bacterial wilt. *Physiological and Molecular Plant Pathology*, 116, 101692. https://doi.org/10.1016/j.pmpp.2021.101692

Doan, T.T., & Nguyen, T.H. (2006). Status of research on biological control of tomato and groundnut bacterial wilt in Vietnam. *Mitt Biol Bundesanst Land- Forstwirtsch*, 408, 105–111.

Eid, A.M. et al. (2021). Harnessing bacterial endophytes for promotion of plant growth and biotechnological applications: An overview. *Plants*, 10(5), 935. https://doi.org/10.3390/plants10050935

Fahime, S., & Gholam, K. (2018). Endophytic bacteria suppress bacterial wilt of tomato caused by *Ralstonia solanacearum* and activate defence-related metabolites. *Biological Journal of Microorganisms*, 6(24), 39–52.

Fanhong, M. (2013). *Ralstonia solanacearum* species complex and bacterial wilt disease. *Journal of Bacteriology and Parasitology*, 4, 2. https://doi.org/10.4172/2155-9597.1000e119

Granér, G. et al. (2003). Screening of oxylipins for control of oilseed rape (*Brassica napus*) fungal pathogens. *Phytochemistry*, 63(1), 89–95. https://doi.org/10.1016/s0031-9422(02)00724-0

Ji, X. et al. (2008). Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. *FEMS Microbiology Ecology*, 65(3), 565–573. https://doi.org/10.1111/j.1574-6941.2008.00543.x

Li, X., & de Boer, S.H. (1995). Selection of polymerase chain reaction primers from an RNA intergenic spacer region for specific detection of *Clavibacter michiganensis* subsp. *spedonicus*. *Phytopathology*, 85(8), 837–842.

Latha, P. et al. (2019). Endophytic bacteria: Prospects and applications for the plant disease management. In Ansari R., Mahmood, I. (eds) *Plant health under biotic stress.* Springer, Singapore.

Mohamed, A.F. et al. (2020). Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* using *Pseudomonas* species isolated from the rhizosphere of tomato plants. *Archives of Phytopathology and Plant Protection*, 53(1–2), 1–16. https://doi.org/10.1080/03235408.2020.1715756

Nguyen, T.H.H. et al. (2006). Bacterial endophyte: biological charateristics and application potential in biological control of bacterial wilt caused by *Ralstonia solanacearum*. *Science and Technology Journal of Agriculture and Rural Development*, 18(2), 77–79 (in Vietnamese).

Puente, M.E. et al. (2004). Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. *Plant Biology*, 6, 629–642. https://doi.org/10.1055/s-2004-821100

Seleim, M. et al. (2011). Biological control of bacterial wilt of tomato by plant growth promoting rhizobacteria. *Journal of Plant Pathology*, 10, 146–153. https://doi.org/10.3923/ppj.2011.146.153

Singh, D. et al. (2022). Suppression of tomato bacterial wilt incited by *Ralstonia pseudosolanacearum* using polyketide antibiotic-producing *Bacillus* spp. isolated from rhizospheric soil. *Agriculture*, 12, 2009. https://doi.org/10.3390/agriculture12122009

Trung, D.Q. et al. (2023). Application of endophytic bacterium *Bacillus velezensis* BTR11 to control bacterial leaf blight disease and promote rice growth. *Egyptian Journal of Biological Pest Control*, 33, 97. https://doi.org/10.1186/s41938-023-00740-w

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255, 571–586. https://doi.org/10.1023/A:1026037216893

William, S. (1983). Staining reaction for detection of hemicellulose degrading. *FEMS Microbiology Letters*, 20, 253–258.