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Endophytic bacteria isolated from a weed plant as a potential biocontrol agent against stem end rot pathogen of pitaya in Vietnam

Do Quang Trung^{1*} , Luu The Anh^{1,2}, Nguyen Thi Thuy³, Dinh Mai Van⁴ and Tran Thi Hang⁴

Abstract

Background: Stem end rot (SER) disease caused by *Alternaria alternata* is one of the main fungal diseases in pitaya and other crops in Nam Dinh, Vietnam, that leads to extensive yield and economic losses. Biocontrol of SER, using endophytic bacteria, is environmentally friendly and compatible with other control measures. Hence, it is emerging as an alternative disease management strategy in sustainable agriculture. This study aimed to screen antagonistic bacteria isolated from the weed, *Eleusine indica*, with the potential to manage SER.

Results: A total of 16 endophytes were isolated from the stems, leaves, and roots of the weed, *E. indica*. Of those, 6 strains presented antagonistic effects against *A. alternata* growth, and one isolate, EI-15, showed a significant inhibitory effect on SER. In addition, analyzing the 16S rDNA sequence indicated that EI-15 was a strain of *Bacillus amyloliquefaciens*. Moreover, the results of the antagonistic spectrum assay showed that EI-15 significantly inhibited some plant and fruit tree pathogens, especially the suppression of *A. alternata*. Notably, the culture filtrate of strain EI-15 exhibited in vitro apparent activity against *A. alternata*. Furthermore, an in vivo antagonistic experiment of EI-15 on pitaya twig showed a significant reduction of lesion on twigs than the control.

Conclusions: Overall, this study suggested the potential application of the EI-15 strain as a biological agent and needs to be further studied in the field to control SER.

Keywords: *Alternaria alternata*, *Eleusine indica*, Endophyte bacterium, 16S rDNA, *Bacillus amyloliquefaciens*

Background

Pitaya is an important fruit crop with high economic value and grown well in Nam Dinh, Vietnam. In this region, however, fungal pathogens are widely spreading, particularly from stem end rot (SER) caused by *Alternaria alternata* YZU, resulting in severe economic losses (Luu et al. 2021). Currently, control of SER is highly dependent on the application of agrochemicals resulting in many problems to human health and the environment and especially it might induct the pesticide resistance of

phytopathogenic agents (Crane et al. 2013). Moreover, the control efficiency is reduced because the SER mostly occurs during the rainy season meaning rainwater could wash away the pesticides. Thus, biocontrol of the plant disease, using microorganisms as a biological agent, is strongly required to meet the consumer demand for pesticide-free food, while environmental safety is maintained. Endophytes are microorganisms which are widely distributed in all living plants and do not directly cause any plant diseases. They were reported that they not only antagonized the infection, colonization, and development of other plant pathogens but also enhanced plant growth under both abiotic (heat, drought, salinity, heavy metal exposure, etc.) and biotic stresses, including

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stem end rot (Luu et al. 2021). For example, those authors isolated 19 endophytic bacteria from the weed, *Echinochloa colonum*, and 5 of them presented in vitro antagonistic activity against *A. alternata* on pitaya. These studies indicated that endophytes had a significant potential as a biocontrol agent against SER.

In agro-ecosystems, although weeds and crops exist parallel, weeds are well adapted to the environment and grow or reproduce aggressively in association with crops (Blanco 2016). *Eleusine indica* is a member of the *Poaceae* family (Zhang et al. 2015) and is able to survive under a wide range of environmental conditions (Nandula et al. 2005). *E. indica* has been used as a traditional medicinal plant to treat influenza, hypertension, oliguria, and urinary complaints (Balangcod et al. 2012). In addition, the same authors reported that it contained certain secondary metabolites with antibacterial activities. The endophytic bacterial associates might play a role in those abilities. While living in the weeds, endophytic bacteria could benefit their weed host in various ways such as the production of bioactive compounds or the generation of induced systemic resistance (Luu et al. 2021). Hence, exploiting these interesting bacteria in crop production is getting more interest from scientists (Khan et al. 2020). Presumably, applying weed endophytic bacteria to biocontrol plant diseases for sustainable agriculture can be a very promising tool because of their short lifecycle and high selection of antagonistic forms.

In Vietnam, discovering of endophytic bacteria from weeds and their potential usage in sustainable crop production is not much. Therefore, this study aimed to isolate endophytic bacteria from the weed (*E. indica*) and to explore the antagonistic form of the isolates on *A. alternata*, a casual of pitaya stem end rot disease in Vietnam.

Methods

Isolation of endophytic bacteria from weed

Healthy *E. indica* plants were collected from different pitaya farmland of Nam Dinh province, Vietnam. The samples (stems, roots, and leaves) were immediately washed with clean water to remove dust and soil. The samples were then disinfected by successive treatment procedures including soaking in 75% alcohol for 30 s, rinsing once in sterile water, soaking for 5 min in 1% mercury chloride, and rinsing 3 times with sterile water. Aliquots of 0.1 ml of the last wash water were inoculated onto the plates containing Luria Broth (LB) media (yeast extract 5 g/l, tryptone 10 g/l, NaCl 5 g/l, agar 15 g/l, pH 7.0–7.5) to check the effectiveness of the disinfection process. The disinfected samples were cut into fragments (5 × 5 mm) and placed onto the LB plates to isolate bacteria living in the samples. The sterile distilled

water was also inoculated on LB plates as controls. All the plates were incubated at 28 °C for 7 days and observed daily for the appearance of different types of bacterial colonies. The observed colonies were picked up and transferred to new LB plates several times by repeated streaking (quadrant streaking) till single type and isolated colonies were obtained. Obtained pure cultures were inoculated onto LB slants. After overnight incubation at 28 °C, they were stored at 4 °C till further use.

Identification of endophyte isolates

The modified cetyltrimethylammonium bromide (CTAB) method was used to extract the DNA of the endophytic bacteria isolated (Liu et al. 2019). Then, the extracted DNA was used as a template to amplify the target fragments by using polymerase chain reaction (PCR) amplification with the 16S rDNA universal primers (27F: 5'-CAGAGTTTGGATCCTGGCT-3', 1492R: 5'-AGGAGGTGATCCAGCCGCA-3'). The amplified PCR products were sequenced by Apical Scientific (1st BASE, Singapore). Obtained sequences were BLAST on the NCBI website and subsequently determining the possible species of the isolates. Closed BLAST results were downloaded to construct the phylogenetic tree using MEGA7.0 (Kumar et al. 2016).

Dual culture assays for antagonistic tests

Dual culture assays were used to test the antagonistic activity of isolated bacteria on *A. alternata* (Luu et al. 2021). For each repetition, a cylinder of a 1-cm² fungal plug (*A. alternata*) was placed in the center of a sterile potato dextrose agar (PDA, potato infusion 200 g/l; dextrose 20 g/l; agar 15 g/l; pH = 7.0–7.3) plate and the bacterial isolates were inoculated between the fungal plug and the plate edge. The plates that grew only *A. alternata* were used as controls. Then, inoculated and control plates were incubated at 28 °C in the dark for 72 h. The antimicrobial activity was determined by measuring the diameter of the inhibition zone and subsequently was statistically analyzed. The diameter of the inhibition zone was measured by the longest line that was drawn from the plate center to the edge and also overlapping the endophyte growth area. Each endophyte/pathogen combination was done with triplicated and 2 independent experiments were repeated for each strain.

To identify the bacterial spectrum, the same method was used to detect the inhibitory effects of antagonistic endophyte on other common plant pathogens, including *Fusarium moniliforme* (a cotton pathogen), *Sclerotium rolfsii* (a rice pathogen), *Alternaria alternata* (a pear fruit pathogen), *Curvularia lunata* and *Cladosporium perangustum* (two pitaya pathogens), and *Colletotrichum gloeosporioides* (an apple fruit pathogen). Each treatment included 3 repetitions.

Antifungal activity of extracellular filtrates

The overnight culture of the isolated bacterium with antifungal activity was used and filtrated to obtain culture filtrate (CF) (Shafique et al. 2019). Then, biocontrol assays (0, 15, 30, 45, 60, and 75% dilutions of stock) were made. After that, a 5-mm plug of PDA with actively growing *A. alternata* was inoculated in a potato dextrose medium amended with CF at different concentrations. The inoculated and control media were incubated at 28 °C for 7 days and observed every 12 h. After that, the biomass of *A. alternata* was collected by filtrating it through filter paper and oven-dried at 40 °C overnight. The reduction of fungal biomass was calculated by using the following formula (Luu et al. 2021): Growth inhibition (%) = [(Growth in control – Growth in treatment)/Growth in control] × 100. The fungal growth inhibition was used as an indicator for the antifungal activity of filtrate.

Disease suppression on the twigs under laboratory conditions

Antagonistic bacteria were assessed for their efficacy in suppressing the *A. alternata* YZU under laboratory conditions (modified from Mohd et al. 2013). The bacterial antagonist was cultured in LB at 28 °C for 48 h. Two milliliters of this bacterial suspension (at 10⁸ cfu ml⁻¹) was pipetted onto a pitaya twig, wounded with a sterile needle, and placed in a closed plastic container with a layer of moistened filter paper at the bottom. The treatments were designed randomly with 3 replications including (1) the bacterial suspension was sprayed onto the wounded twig immediately before the PDA plug of *A. alternata* YZU was placed onto the wounded tissue, (2) the bacterial suspension was sprayed onto the wounded twig for 24 h before inoculation with a PDA plug of *A. alternata* YZU, (3) the PDA plug of *A. alternata* YZU was placed onto the wounded twig for 24 h before spraying the bacterial suspension, (4) only the bacterial suspension was sprayed onto the wounded twig, and (5) only the PDA plug of *A. alternata* YZU was placed onto the wounded twig. Symptom of stem end rot disease was observed after 15 days inoculating the wounded pitaya with *A. alternata* YZU and accessed by measuring the length of lesions produced.

Disease suppression on the twigs in the greenhouse

The efficacy of antagonistic bacteria against *A. alternata* YZU was also assessed in a greenhouse (Mohd et al. 2013). A similar procedure described above was applied, except some modifications including 10 replications were used under the greenhouse conditions.

Statistical analysis

Data analysis was done with significance ($p < 0.05$) of treatment effects using one-way ANOVA, followed by

posthoc comparisons (Tukey's HSD). The significance of the results was determined by Duncan's tests.

Results

Isolation and identification of endophytic bacteria

In total, 16 strains of endophytic bacteria were isolated from different tissues of weed (*E. indica*) including 6 strains from the roots, 5 from stems, and 5 from leaves. The results of dual culture assays are illustrated and shown as in Fig. 1. The results indicated that there were 6 out of 16 strains that had antagonistic effects on the mycelial growth of *A. alternata* YZU. Among them, the EI-15 strain presented the best antagonistic effect with the average diameter of the inhibition zone (33.24 mm), which was significantly larger than the other 5 strains (EI-5, EI-8, EI-9, EI-10, EI-11). In addition, the molecular identification used universal 16S rDNA primers which indicated that the EI-15 was highly homologous to sequences belonging to *Bacillus* families. The phylogenetic tree showed that EI-15 was classified as *Bacillus amyloliquefaciens* (Fig. 2).

Antimicrobial spectrum of the EI-15 strain

The EI-15 strain was also investigated under *in vitro* conditions for their antagonistic spectrum against several common pathogens on pitaya and other common crops and fruit trees. The results showed that EI-15 had significant inhibitory effects on the growth of all 6 tested pathogens with a mean inhibition diameter ranging from 20.1 to 31.1 mm (Table 1). The results suggested the EI-15 strain had a broad antimicrobial spectrum. Interestingly, EI-15 also presented the strongest inhibition against *A. alternata* (a casual of black spot disease on pear), with an inhibition zone reaching 31.1 mm.

Effect of cultural filtrates of the EI-15 strain on the growth of *Alternaria alternata*

The culture filtrates of *B. amyloliquefaciens* with different concentrations showed a significant inhibition of the mycelial growth of *A. alternata* YZU grown on potato dextrose broth (Fig. 3). The results indicated that an increase in extract concentration of *B. amyloliquefaciens* resulted in a significant decrease in the biomass production of *A. alternata*. As shown in Fig. 3, the highest percentage of fungal biomass reduction was approximately 85%, recorded at the highest CF concentration (75%), while the lowest one (38.86%) was observed at the lowest CF concentration (15%).

Disease suppression test on the twigs under laboratory and greenhouse conditions

The biocontrol of stem end rot disease under laboratory and greenhouse conditions showed that spraying the twigs of pitaya with the EI-15 strain either 24 h before or

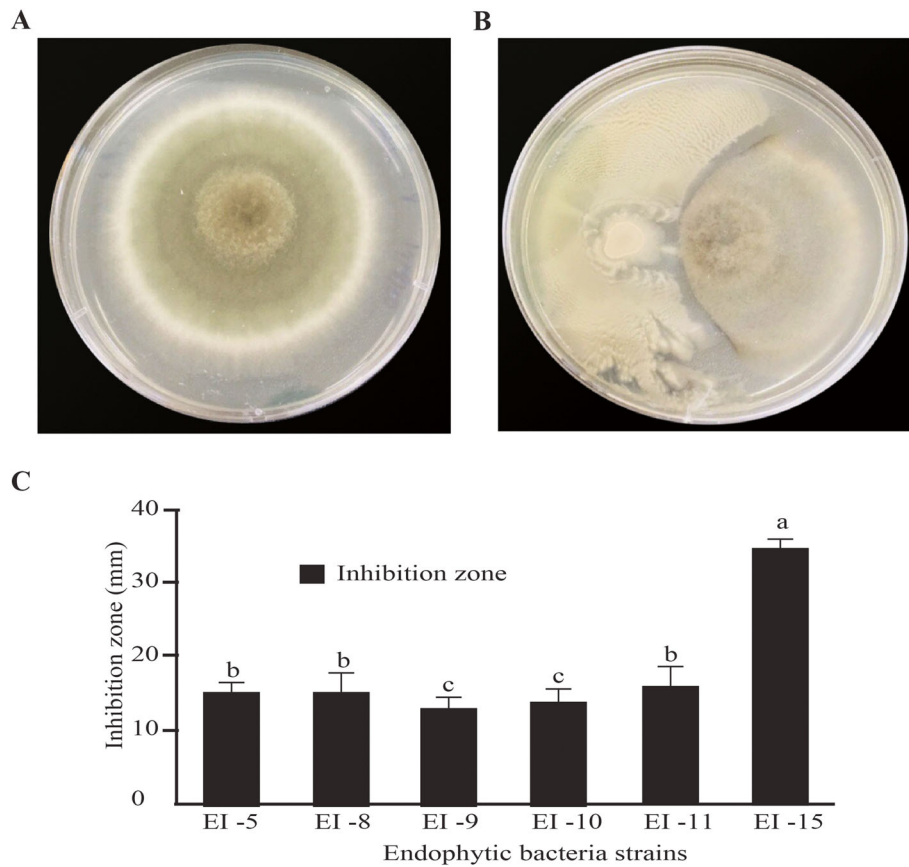


Fig. 1 Endophytic bacteria inhibited the mycelial growth of *Alternaria alternata* YZU. **A** *A. alternata* YZU growth alone. **B** The antagonistic effect of EI-15 against *A. alternata* YZU and **C** antagonistic activity of six endophyte isolates against *A. alternata* YZU. Values are means \pm SD ($n = 10$). Different letters above the bars indicate a significant difference at $p < 0.05$

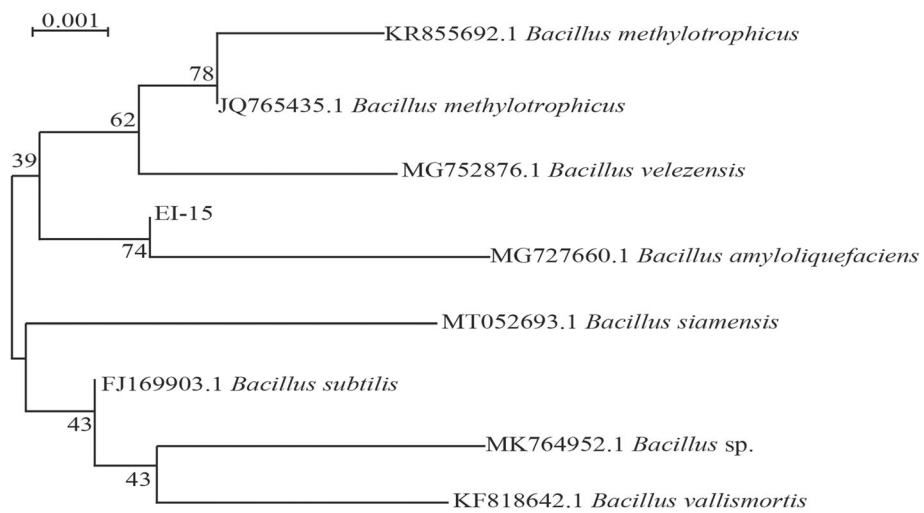


Fig. 2 A neighbor-joining tree shows the phylogenetic relationships among 16S rDNA sequences of EI-15 and their closely related sequences from NCBI. The scale bar indicates evolutionary distance

Table 1 In vitro inhibition of growth of different plant pathogens by *Bacillus amyloliquefaciens* on a PDA medium

Phytopathogen	Mean diameter of the inhibition zone (mm)
<i>Colletotrichum gloeosporioides</i>	20.1 ± 3.7 d
<i>Fusarium moniliforme</i>	23.7 ± 5.8 c
<i>Sclerotium rolfii</i>	28.4 ± 3.1 b
<i>Alternaria alternata</i>	31.1 ± 1.3 a
<i>Curvularia lunata</i>	23.2 ± 1.4 c
<i>Cladosporium perangustum</i>	22.8 ± 1.2 c

Data are means ± SD (n = 3). Values in the same column with the same letter(s) are not significantly different as determined by Duncan’s multiple range (DMR) test

immediately after fungal (*A. alternata*) inoculation was effective to suppress stem end rot disease severity than the untreated control inoculated with the pathogenic fungi only (Fig. 4). Nevertheless, spraying the twigs of dragon fruit with the EI-15 strain, 24 h before fungal inoculation, gave relatively better control efficacy. The results also indicated that the control efficacy slightly decreased under greenhouse conditions (Fig. 4). Presumably, applying the bacteria to the pitaya twigs before inoculating phytopathogenic fungi was recommended to inhibit stem end rot disease development.

Discussion

In Vietnam, the stem end rot disease on pitaya is being more and more serious. Currently, the farmer controlled the SER disease mainly by using chemical pesticides, which might lead to the increase of chemical pesticide-resistant strains resulting in losing the efficacy of many fungicides (Yin et al. 2018). Moreover, native weed has been demonstrated as a promising resource for the isolation of endophytic bacteria antagonizing the

phytopathogens (Luu et al. 2021). Therefore, the development of new strategies using weed endophytic bacteria for controlling the pitaya SER is urgently required.

In this study, endophytic bacteria strain EI-15 isolated from weed, *E. indica*, showed an inhibitory ability against not only *A. alternata* causing pitaya SER disease, but also other phytopathogens including *Fusarium moniliforme* (a cotton pathogen), *Sclerotium rolfii* (a rice pathogen), *Alternaria alternata* (a pear fruit pathogen), *Curvularia lunata* and *Cladosporium perangustum* (two pitaya pathogens), and *Colletotrichum gloeosporioides* (an apple fruit pathogen). EI-15 also presented the best antagonistic effect against another strain of *A. alternata*, a causal of pear fruit disease. Therefore, the results suggested that EI-15 generates a wide antagonistic spectrum and could potentially be used as a biocontrol agent for controlling phytopathogens, especially for *A. alternata*.

In addition, the result of morphological observation and 16S rDNA sequence analysis indicated that the antagonistic bacteria EI-15 was *B. amyloliquefaciens*, which had demonstrated antifungal and antibacterial activities

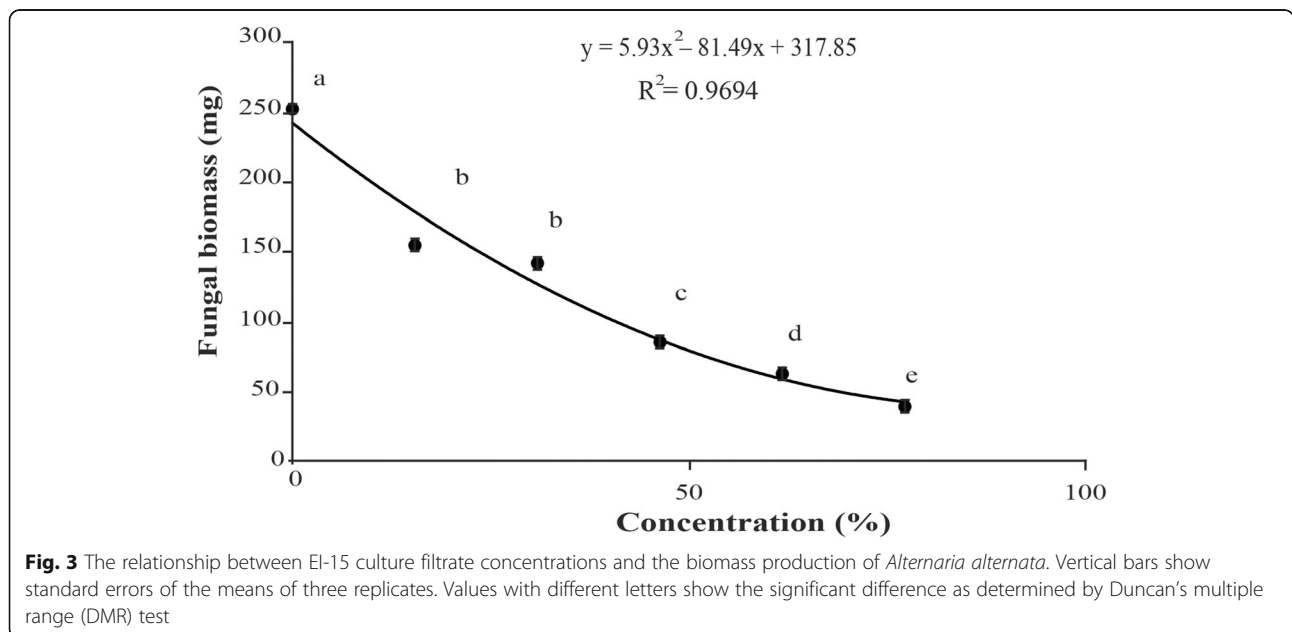
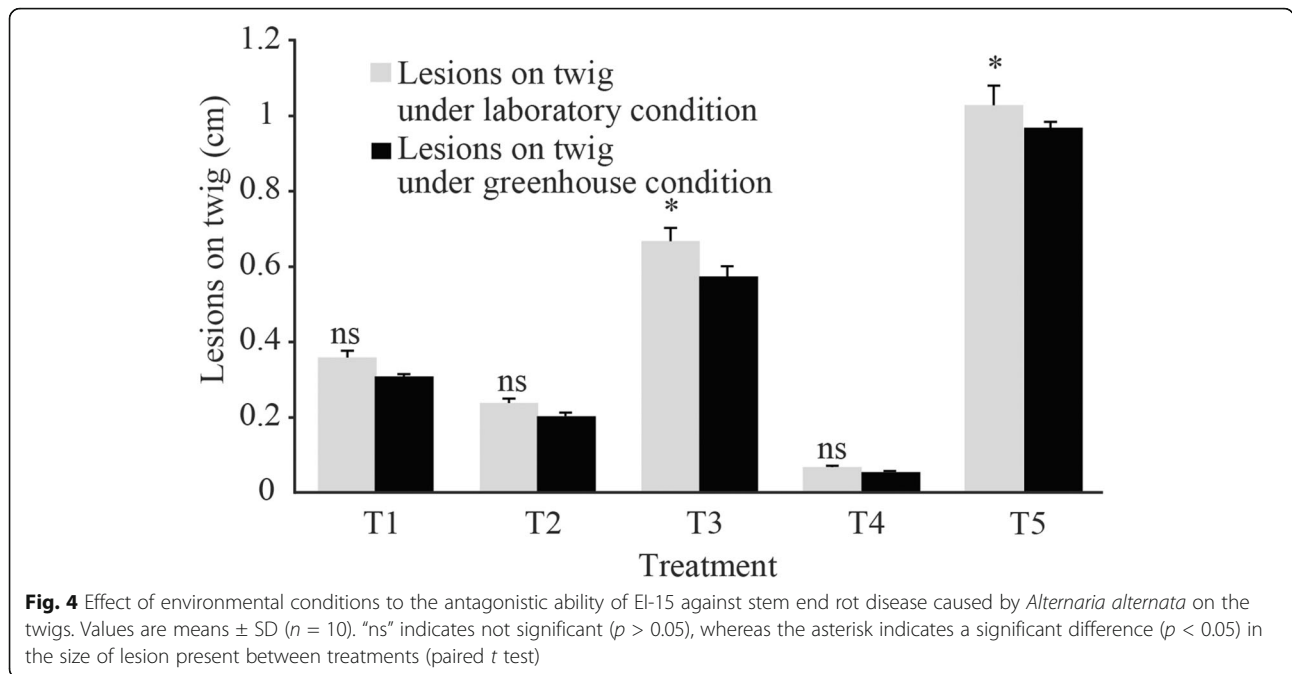


Fig. 3 The relationship between EI-15 culture filtrate concentrations and the biomass production of *Alternaria alternata*. Vertical bars show standard errors of the means of three replicates. Values with different letters show the significant difference as determined by Duncan’s multiple range (DMR) test



against many plant pathogens on many plant species (Zhang et al. 2019). For example, *B. amyloliquefaciens* DH-4 had demonstrated broad antifungal activity against several common crop and fruit tree pathogens, including several citrus *Penicillium* spp. diseases, *P. expansum* (apple blue mold), *G. citri-aurantii* (citrus sour rot), *Alternaria citri* (citrus or tomato black rot), *Phomopsis citri* (citrus stem end rot), *C. gloeosporioides* (citrus anthracnose), *B. dothidea* (kiwi fruit soft rot), and the typical food pathogen *Aspergillus niger* (Chen et al. 2018). Consistency with these studies, the present study also reported that the *B. amyloliquefaciens* had an antagonistic effect against several crop and fruit tree diseases. Moreover, the CF of EI-15 also showed an antagonistic effect on the *A. alternata*, a plant-fungal pathogen. This suggested that EI-15 produced external antibiotics into CF. This explanation was strengthened by previous reports, in which *B. amyloliquefaciens* grown on PDB produced some antimicrobial compounds such as macrolactin, bacillaene, iturins, fengycin, and surfactin exhibiting different activities against the 17 fungi (Chowdhury et al. 2015). All of these results suggested that EI-15 isolated from weed, *E. indica*, is a valuable resource for developing biocontrol agents.

The results of biocontrol of *A. alternata* under laboratory and greenhouse experiments showed that *B. amyloliquefaciens* was effective to suppress disease development when these bacteria were applied either 24 h before or followed immediately with fungal inoculation on the pitaya twigs. These results were not consistent with some studies that suggested *Bacillus* species are root-dwelling bacteria (Chowdhury et al. 2015).

However, there are some other studies that demonstrated that *B. amyloliquefaciens* isolated from wheat spikelets or mulberry leaves could inhibit the development of head blight in spikelets or the growth of several phytopathogenic fungi and bacteria (Chowdhury et al. 2015). These data indicated that *B. amyloliquefaciens* could exist in non-root tissues. Moreover, the details of plant protection by *B. amyloliquefaciens* are still unclear. Chowdhury et al. (2015) suggested that a direct effect of the numerous antimicrobial secondary metabolites in suppressing pathogens is not the key factor in protecting plants from pathogenic microorganisms. The authors also reported that *B. amyloliquefaciens* triggered pathways of induced systemic resistance (ISR) by bacterial metabolites, such as surfactin and volatiles, which is the key mechanism. Hence, the results suggested that EI-15 could be a potential biocontrol agent to control plant diseases.

Conclusions

The present study revealed that endophytic bacteria isolated from weed were able to produce in vitro antagonistic inhibition on the mycelial growth of *A. alternata*. EI-15 that identified as *B. amyloliquefaciens* was the most effective in suppressing stem end rot diseases' development on pitaya twigs under laboratory and greenhouse conditions. More work is needed to evaluate the antagonistic effects of EI-15 under field conditions.

Abbreviations

CF: Culture filtrate; CTAB: Cetyltrimethylammonium bromide; ISR: Induced systemic resistance; LB: Luria Broth; PDA: Potato dextrose agar; SDW: Sterile distilled water; SD: Standard deviation; SER: Stem end rot

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Authors' contributions

DQT was involved in the conceptualization, supervision, final interpretation of data, and editing of the manuscript. LTA and NTT regenerated Fig. 4, double checked the data analyses, and revised the manuscript. DMV and TTH regenerated Fig. 2 and revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

All data are available in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declares that they have no competing interests.

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References

- Balangcod TD, Vallejo VL, Patacsil M, Apostol O, Luran MVA, Manuel JJ, Cortez S, Gutierrez RM (2012) Phytochemical screening and antibacterial activity of selected medicinal plants of Bayabas, Sablan, Benguet province, Cordillera administrative region, Luzon, Philippines. *Indian J Tradit. Knowl.* 11:580–585
- Blanco Y (2016) Review: The role of weeds as a component of biodiversity in agro-ecosystems. *Cultivos Trop* 37(4):34–56. <https://doi.org/10.13140/RG.2.2.10964.19844>
- Chen HM, Wu HX, Yan B, Zhao HG, Liu FH, Zhang H, Sheng Q, Miao F, Liang Z (2018) Core microbiome of medicinal plant *Salvia miltiorrhiza* seed: a rich reservoir of beneficial microbes for secondary metabolism. *Int J Mol Sci.* 19(3):672. <https://doi.org/10.3390/ijms19030672>
- Chowdhury SP, Hartmann A, Gao X, Borriss R (2015) Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42—a review. *Front Microbiol.* 6:780. <https://doi.org/10.3389/fmicb.2015.00780>
- Crane JM, Gibson DM, Vaughan RH, Bergstrom GC (2013) Iturin levels on wheat spikes linked to biological control of *Fusarium* head blight by *Bacillus amyloliquefaciens*. *Phytopathology* 103(2):146–155. <https://doi.org/10.1094/PHYTO-07-12-0154-R>
- Khan M, Gao J, Chen X, Zhang M, Yang F, Du Y, Moe T, Munir I, Jing X, Zhang X (2020) Isolation and characterization of plant growth-promoting endophytic bacteria *Paenibacillus polymyxa* SK1 from *Lilium lancifolium*. *Biomed Res Int* 2020:1–17. <https://doi.org/10.1155/2020/8650957>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Boil Evol.* 33(7):1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Liu HF, Zhou J, Liao J, Yi JP, Ma DF, Deng JX (2019) Grafted twig rot on *Citrus sinensis* caused by a member of the *Fusarium solani* species complex. *Can J Plant Pathol* 1–7. <https://doi.org/10.1080/07060661.2019.1633412>

- Luu T, Phi Q, Nguyen T, Dinh V, Pham B, Do T (2021) Antagonistic activity of endophytic bacteria isolated from weed plant against stem end rot pathogen of pitaya in Vietnam. *Egypt J Biol Pest Control* 31(1):14. <https://doi.org/10.1186/s41938-021-00362-0>
- Mohd MH, Salleh B, Zakaria L (2013) Identification and molecular characterizations of *Neoscytalidium dimidiatum* causing stem canker of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. *J Phytopathol* 161(11–12):841–849. <https://doi.org/10.1111/jph.12146>
- Nandula VK, Reddy KN, Duke SO, Poston DH (2005) Glyphosate-resistant weeds: current status and future outlook. *Outlooks Pest Manag.* 16(4):183–187. <https://doi.org/10.1564/16aug11>
- Shafique S, Shafique S, Javed A, Akhtar N, Bibi S (2019) Analysis of antagonistic potential of secondary metabolites and organic fractions of *Trichoderma* species against *Alternaria Alternata*. *Biocontrol Sci.* 24(2):81–88. <https://doi.org/10.4265/bio.24.81>
- Yin JL, Zhang X, Ma DF, Lu C, He YQ, Zhu YX (2018) A study on the sensitivity and synergistic effect of prochloraz and cypermethrin on *Gibberella zeae* in wheat. *Acta Agric Univ Jiangxiensis* 40:920–924
- Zhang P, Zhu Y, Ma D, Xu W, Zhou J, Yan H, Yang L, Yin J (2019) Screening, identification, and optimization of fermentation conditions of an antagonistic endophyte to wheat head blight. *Agronomy* 9(9):476. <https://doi.org/10.3390/agronomy9090476>
- Zhang T, Li F, Tian X, Yang C, Gao J (2015) Use of chlorophyll fluorescence and P700 absorbance to rapidly detect glyphosate resistance in goose grass (*Eleusine indica*). *J Integrative Agric* 14(4):714–723. [https://doi.org/10.1016/S2095-3119\(14\)60869-8](https://doi.org/10.1016/S2095-3119(14)60869-8)

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