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Research Article

Properties of *Bacillus* sp. Strains with Antifungal and Plant Growth Promoting Activities

Huyen Nguyen Thanh, Canh Xuan Nguyen, Huyen Dang Khanh Nguyen,
Phuong Nguyen Thu Phan and Tam Thi Thanh Dang

Faculty of Biotechnology, Vietnam National University of Agriculture, Hanoi, Vietnam

Abstract

Background and Objective: Onion (*Allium cepa* L.) is one of the most important commercial vegetable crops worldwide with high economic value. However, fungal diseases seriously threaten onion production by reducing yield and bulb quality. For sustainable disease management, the use of biological control agents has emerged as an environmentally friendly and effective alternative to chemical fungicides. This study aimed to select potential antifungal bacteria that can promote plant growth in onion. **Materials and Methods:** Bacterial isolates were isolated from rhizosphere soil samples, screened and characterized for antifungal activities. Strong antagonistic bacterial strains were selected. These bacterial strains were identified and characterized by their biochemical features. In addition, three selected strains were evaluated for their growth-promoting capacity by pot assay. All experiments were conducted in triplicate; data were expressed as mean±SD and analyzed using one-way ANOVA followed by Tukey's test ($p<0.05$) in GraphPad Prism 9.3.1. **Results:** Three bacterial. strains exhibited strong antagonistic activity against *Sclerotium rolfsii* and *Curvularia lunata* through the production of diffusible compounds and volatile organic compounds (VOCs). In addition, physiological and biochemical characterization revealed that all strains belonged to the genus *Bacillus.* Moreover, soil amendment with the culture of *Bacillus* sp. T3, T33 and T36 significantly enhanced onion growth. **Conclusion:** These findings suggest that the selected *Bacillus* sp. strains represent promising candidates for the development of biocontrol agents against fungal pathogens.

Key words: Antagonism, *Bacillus* sp., plant growth-promoting bacteria, siderophore

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Corresponding Author: Tam Thi Thanh Dang, Faculty of Biotechnology, Vietnam National University of Agriculture, Hanoi, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant fungal pathogens are considered a major factor causing significant reduction in both crop yield and product quality[1]. These pathogens infect roots, stems, leaves, flowers and fruits, leading to visible disease symptoms such as blights, wilts and rots that decrease photosynthetic capacity and stunt plant growth, ultimately lowering yields[2]. Studies have shown that fungal pathogens accounts for a large proportion of global crop loses[3]. In addition to yield loss, crop quality is compromised through deformed seeds, lower weights, fruit rot and premature plant death, which diminish market value and nutritional content[4]. In onion (*Allium cepa* L.), fungal diseases negatively affect early plant growth, reduce bulb development and lower both yield and market quality[5,6]. One of the most damaging soil-borne pathogens in onions is *Sclerotium rolfsii*, which causes stem and bulb rot characterized by basal stem collapse, wilting[7,8]. This fungal pathogen can form the persistent sclerotia in the soil that can survive for years and complicate disease management[9]. In addition, species within the genus *Curvularia* are well-recognized phytopathogens associated with leaf spots, seed and seedling infections in various crops, including *Allium* species[10]. Infection by *Curvularia* sp. can reduce photosynthetic leaf area and weaken plant vigor, ultimately affecting bulb yield and quality[11,12]. The combined impact of *S. rolfsii*, *Curvularia* sp. species and others fungal pathogens on onions makes the challenge of fungal disease management in *Allium* production systems[13]. Up to date, chemical fungicides have been widely used to control fungal diseases in onion production. However, its repeated and intensive use has led to concerns regarding environmental contamination, fungicide resistance in pathogen populations and chemical residues on onion bulbs[14,15]. Therefore, application of biological control agents (BCAs) has been increasing attention as a sustainable approach for managing fungal diseases in onion cultivation[16,17]. Most of efficient BCAs are beneficial fungi and bacteria that can suppress fungal pathogens through multiple mechanisms such as antibiosis, competition, mycoparasitism and the induction of plant defense responses[18,19]. In onions, these microorganisms have been reported to reduce the incidence of soil-borne and foliar fungal diseases while simultaneously promoting plant growth and improving bulb yield and quality[20,21]. In the present study, three bacterial strains were isolated from rhizosphere soil samples and exhibited strong antifungal activity against fungal pathogens. The study's objectives were to evaluate the *in vitro* antagonistic effect of selected bacterial strains on *S. rolfsii* and *C. lunata* along with classifying and determining their capabilities in promoting onion growth.

MATERIALS AND METHODS

Study area: This study was conducted in the Plant Biotechnology Laboratory in the Department of Plant Biotechnology at Faculty of Biotechnology, Vietnam National University of Agriculture from August 2024 to October 2025.

Plant-pathogenic fungi: For testing the antifungal activity of bacterial strains, two fungal isolates of *S. rolfsii* and *C. lunata* were used. These fungal pathogens were grown in potato dextrose agar (PDA) medium at 28°C in the dark. The *S. rolfsii* was cultured for 3-5 days and the *C. lunata* was cultured for 7-10 days, respectively.

Screening of antifungal bacterial strains: Forty-eight bacterial strains were isolated from rhizosphere soil samples of vegetable crops in Haiphong and Hanoi, Vietnam, using the serial dilution method on Nutrient agar medium (at 35°C, 4 days). To select trains with strong antifungal activities, the collected isolates were screened with *S. rolfsii* and *C. lunata* by the dual-culture method (Fig. 1). The tested bacteria were streaked along two straight lines, each 4.5 cm long, symmetrically positioned at equal distances (3 cm) from the center of the plate (A). The fungal plug was placed at the center of the PDA plate (F). The plates were kept in the dark, at 28°C for 3-5 days. The control plate was inoculated with only the pathogenic fungus. Antifungal activity was assessed by measuring the mycelial growth diameter of the pathogens on both control (R1) and treatment plates (R2) using ImageJ software.

The percentage of growth inhibition (PIRG) was calculated as:

$$\text{PIRG (\%)} = \frac{\text{R1} - \text{R2}}{\text{R1}} \times 100$$

Testing antifungal activity of bacterial volatile organic compounds (VOCs): The antifungal activities of bacterial volatile compounds from three selected strains (T3, T33 and T36) against *S. rolfsii* and *C. lunata* were evaluated using the double Petri-dish method[22]. A petri dish containing LB was inoculated by spreading 100 μL of 24 hrs bacterial culture from a single colony of each strain. In another petri dish, a 7 mm diameter mycelial plug of *S. rolfsii* or *C. lunata* from a growing culture plate was placed in the center of the PDA dish. Then, the plate with bacteria was inverted and placed over the fungi plate. The two plates were sealed with parafilm. Plates of a dish of mycelial plug of *S. rolfsii* or *C. lunata* covered with LB dish were the controls. All plates were incubated in the dark at 28°C. Treatment plates with *S. rolfsii* were incubated for 3 days and treatment plates with *C. lunata* were incubated for 5 days. The experiments were triplicated. The inhibition rate was calculated using the formula:

$$\text{Inhibition rate (\%)} = \frac{\text{R1} - \text{R2}}{\text{R1}} \times 100$$

Where:

R1 = Diameter of the fungi in the control
R2 = Diameter of the fungi in the treatment plate. The diameter was measured by using ImageJ software

Morphological and biochemical analysis of bacterial strains: The selected bacterial strains were examined for colony morphology, Gram staining, physiological and biochemical traits, including tests for catalase, amylase, cellulase and IAA production[23,24]. For siderophore production, three bacterial strains were streaked on Chrome Azurol S (CAS) medium. Then, the testing plates were kept at 35°C in the dark for 2 days and the change of medium color was observed[25].

Identification of selected bacterial strains: To identify the bacterial strains exhibiting strong antagonistic activities against the pathogenic fungi, 16S rRNA gene sequencing was performed. Single colony of each selected bacterial strain was cultured in 5 mL LB medium in shaker (180 rpm) at 30°C for 2 days. Then, the cell pellet was collected by centrifuge. The genomic DNA of each selected bacterial strain was extracted following the method described by Masoomi-Aladizgeh *et al.*[26]. The 16S rRNA genes of bacterial strains were amplified by PCR using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') with an annealing temperature of 53°C. The PCR product (1465 bp) was purified and sequenced at 1st BASE (Singapore). The 16S rRNA sequences of selected bacterial were aligned and compared with reference sequences using the GenBank database using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Pot assay for testing the growth-promoting ability of selected bacteria: To evaluate the effects of selected bacterial strains on onion plant growth, bacterial suspensions were applied to the planting soil and then growth parameters were subsequently assessed. The sterilized soil was used for the experiment and weighed at 1.5 kg per pot (30 cm in diameter). For bacterial culture, 50 µL of bacterial suspensions from three selected strains that were preserved in glycerol were reactivated in 5 mL of LB medium. The cultures were incubated at 37°C with shaking at 180 rpm for 24 hrs. The cultures were then scaled up to 100 mL and incubated under the same conditions for an additional 48 hrs. After incubation, the bacterial cultures were centrifuged to collect cell pellets. Then, cell pellets were washed 2 times with sterile water and diluted in water to obtain a final optical density of $OD_{600} = 0.5$. The experiment consisted of four treatments, each comprising six pots (five single onion bulbs per pot). The control treatment was not inoculated with bacteria, while the experimental treatments were inoculated with each strain, T3, T33, or T36 (50 mL of bacterial suspension per pot). In this experiment, bacterial suspensions were prepared and applied to the soil three times (at weeks 1, 4 and 8) in 10 weeks. All treatments were irrigated daily with equal volumes of water. Growth parameters were evaluated, including fresh weight of plant (g), fresh weight of roots per plant (g) and dry weight of roots per plant (g).

Statistical analysis: All experiments were repeated three times and the data were expressed as the Mean±Standard Deviation (SD). Statistical analysis was performed using One-way analysis of variance (ANOVA) by GraphPad Prism (version 9.3.1). The comparisons of mean values were carried out through Tukey's multiple comparisons test with $p<0.05$.

RESULTS

Antifungal activity of selected bacterial strains in *in vitro* conditions: From collected samples, three out of forty-eight bacterial isolates with significant antagonistic activities against *S. rolfsii* and *C. lunata* were obtained. The antifungal activities of three isolates (strains) were evaluated using *in vitro* dual culture assays (Fig. 1a-b). The antagonistic activities of all three bacterial strains against both fungal strains were more than 60% compared with the control. Interestingly, the T3 strain exhibited the highest antagonistic activity, with an inhibition rate of 78.46% against *S. rolfsii* after three days of co-culture (Fig. 1a) and 72.65% against *C. lunata* after 5 days of co-culture (Fig. 1b). The antifungal activities of the T33 strain against *S. rolfsii* and *C. lunata* reached 75.81 and 63.57%, respectively. Strain T36 exhibited the lowest antagonistic activity among the three isolates.

***In vitro* antifungal activity of bacterial volatile organic compounds:** To understand the antifungal mechanism of selected bacterial strains, the effects of the VOCs produced by these strains were investigated by double petri-dish assay. As

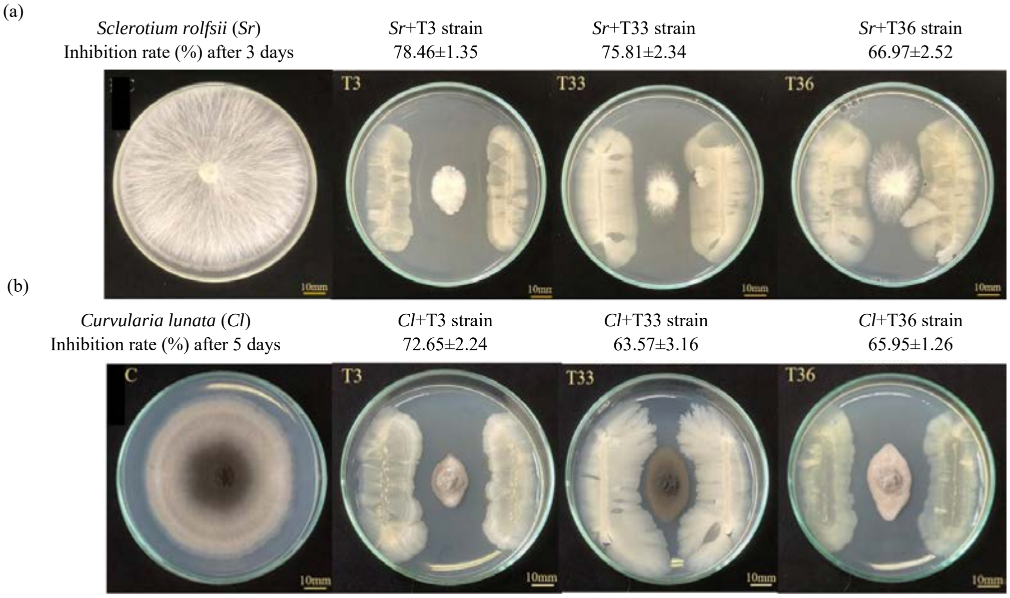


Fig. 1(a-b): Antagonistic activities of three selected bacterial strains against fungal pathogens on PDA plates, (a) The growth of *Sclerotium rolfsii* (*Sr*) on the control plate and the treatment plates (dual culture three selected bacterial strains with *R. rolfsii*) after 3 days (b) The growth of *Curvularia lunata* (*Cl*) on the control plate and the treatment plates (dual culture three selected bacterial strains with *C. lunata*) after 5 days

Inhibition rates were presented with standard deviation (SD)

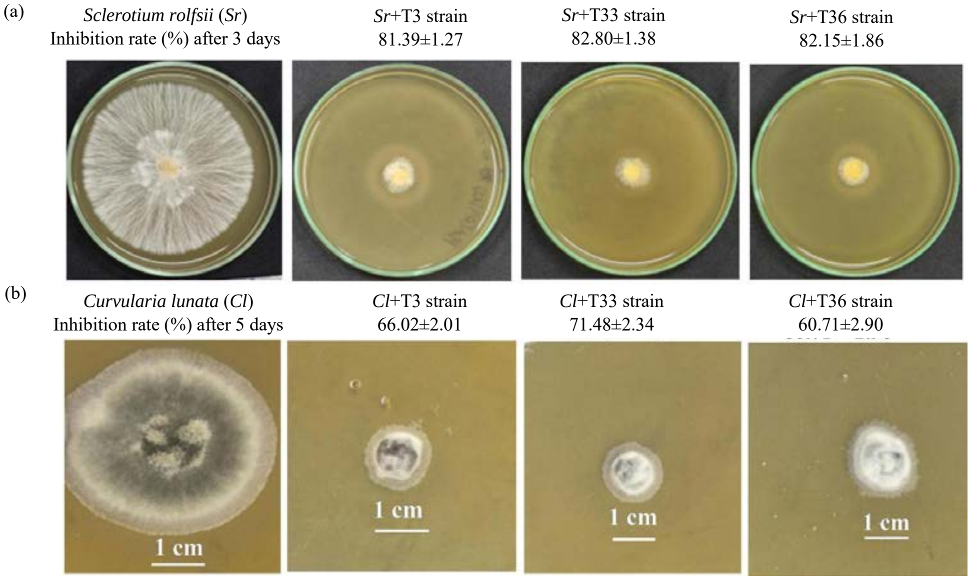
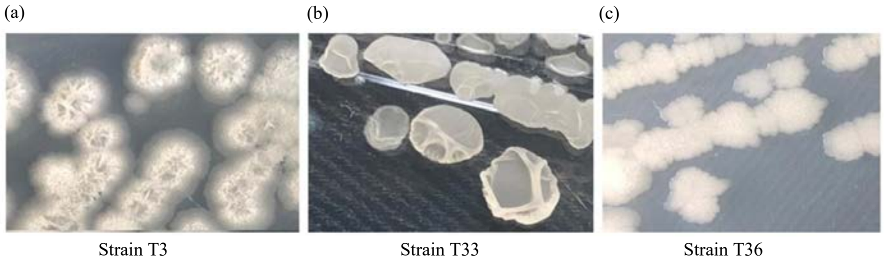


Fig. 2(a-b): Double Petri-dish assay for antifungal activity of VOCs produced by three selected bacterial strains, (a) The growth of *Sclerotium rolfsii* (*Sr*) on the control plate and the treatment plates after 3 days of treatment and (b) The growth of *Curvularia lunata* (*Cl*) on the control plate and the treatment plates after 5 days of treatment

Inhibition rates were presented with standard deviation (SD)

shown in Fig. 2(a-b), the VOCs released by all three strains had strong inhibitory effects on the growth of both fungal pathogens, *S. rolfsii* and *C. lunata*. In detail, all three T3, T33 and T36 strains exhibited stronger growth inhibition against *S. rolfsii* (81.15-81.39% on PDA medium after 3 days) (Fig. 2a). In contrast, the growth inhibitions of three strains



Strain T3 Strain T33 Strain T36

Fig. 3(a-c): Colony morphology of selected bacterial strains on LB agar plates, (a) T3 colony is milk-white, rough and dry surface, (b) T33 colony is milk-white, irregular edges, wrinkled surface and (c) T36 colony is milk-white, irregular edges, opaque surface

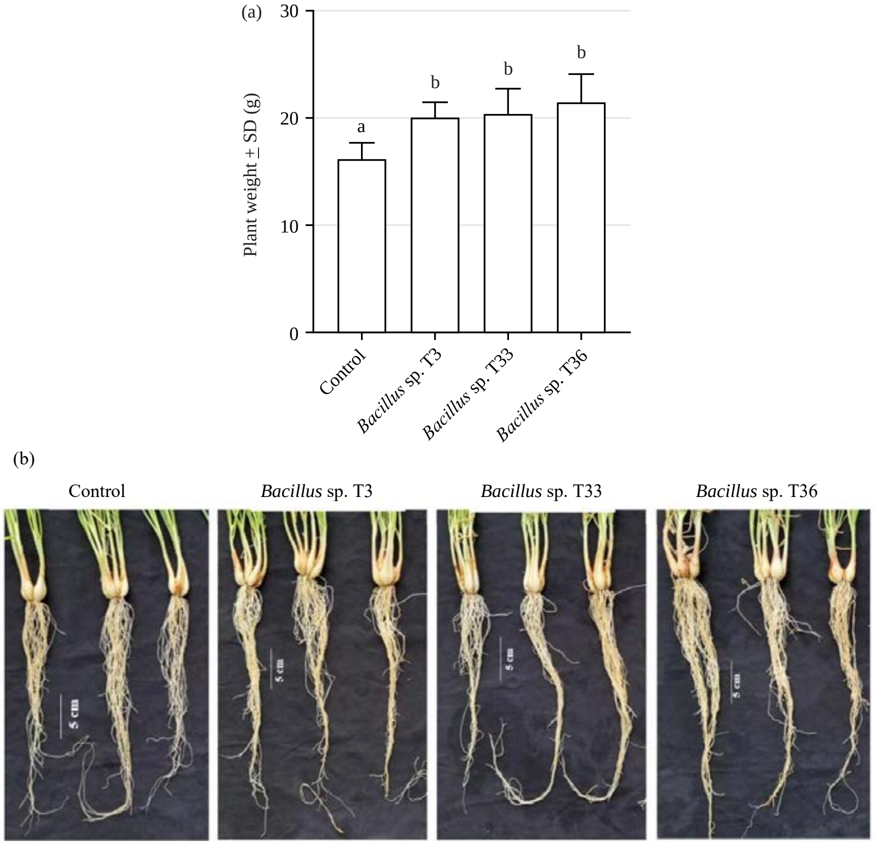


Fig. 4(a-b): Effects of *Bacillus* sp. strains on fresh plant biomass (after 10 weeks of treatment), (a) Biomass of onion plants in control and treatments; Bars marked with different letters were significantly different from one another with $p<0.05$ and (b) Photograph of onion plants in control and treatments

Table 1: Physiological, biochemical and genetic characteristic of selected bacterial strains

Characteristics	Strain T3	Strain T33	Strain T36
Gram staining	+	+	+
Cell shape	Rod-shaped	Rod-shaped	Rod-shaped
Catalase test	+	+	+
IAA production (3 days of culture)	-	+ (6.0 μg/mL)	-
Siderophore production	+	+	+
Cellulase	+	+	+
Amylase	+	+	+

-: Negative, not detected and +: Positive reaction

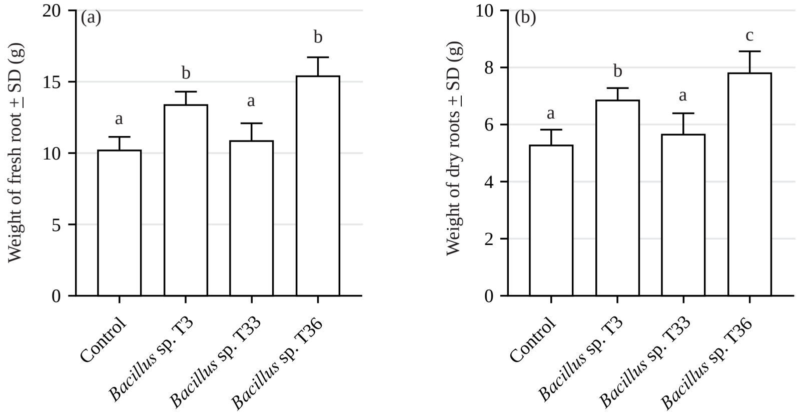


Fig. 5(a-b): Effects of *Bacillus* sp. strains on root development (a) Fresh root weight and (b) Dry root weight
Bars marked with different letters were significantly different from one another with $p<0.05$

Table 2: Molecular identification of selected bacterial strains by analyzing 16S rRNA sequence

Strains	Highest homology strain	Accession on GenBank NCBI	Percentage identity (%)	Named
T3	*Bacillus velezensis* ES1-02	PQ107481.1	98.30	*Bacillus* sp. T3
T33	*Bacillus* sp. strain Par3	OR690892.1	98.07	*Bacillus* sp. T33
T36	*Bacillus subtilis* strain Tr21H	MH210866.1	99.97	*Bacillus* sp. T36

against *C. lunata* were reduced (60.71-71.48% after 5 days of treatment) (Fig. 2b). Among three strains, T33 strains exhibited the strongest inhibitory effects against testes fungal pathogens.

Characterization and identification of selected bacterial strains: To extend knowledge about the three selected bacterial strains, the physiological, biochemical characteristic and 16S rRNA sequences were analyzed (Table 1, Fig. 3). The data revealed that all three bacterial strains are gram positive with rod-shaped. On LB medium, three strain-colonies exhibited milk-white color with distinct colony morphology (Fig. 3a-c). All three selected strains were capable of producing catalase, cellulase and amylase. Notably, all strains also generated siderophores on CAS medium, with strain T36 exhibiting stronger activity than the other two strains. However, only strain T33 was able to produce IAA and at a relatively low concentration of 6.0 µg/mL. Analysis sequences of the 16S rRNA genes showed that all three strains shared high similarity (>98 %) with members of the genus *Bacillus* (Table 2). Therefore, based on the cellular characteristics, biochemical properties and 16S rRNA gene sequences, the selected s trains were identified as belonging to the genus *Bacillus*. Consequently, three strains were named as *Bacillus* sp. T3, *Bacillus* sp. T33 and *Bacillus* sp. T36.

Effect of *Bacillus* sp. strains on onion growth: To evaluate the capacity of selected *Bacillus* sp. strains to promote plant growth, the onion buds were grown in pots and treated with bacterial culture during the growth process. As shown in Fig. 4a-b, treatments with three *Bacillus* sp. strains, T3, T33 and T36, significantly increased the plant weight of onion ($p<0.05$) compared with the control after 10 weeks. However, no significant differences were observed in the fresh plant weight of onions between treatments. In addition, analysis of the fresh and dry weight of roots showed different effects of *Bacillus* sp. strains. Data shown in Fig. 5a-b exhibited that only *Bacillus* sp. T3 and *Bacillus* sp. T36 promoted both fresh and dry weight of onion roots compared with the control. Interestingly, compared with the *Bacillus* sp. T3 strain, *Bacillus* sp. T36 exhibited the greatest increase in dry root biomass of onion. Treatment with *Bacillus* sp. T33 strain showed no difference in weight of roots compared with the control. Overall, three *Bacillus* sp. strains exhibited a growth-promoting effect on onion growth (Fig. 4).

DISCUSSION

Pathogenic fungi are common agents of plant diseases that significantly impacts crop yield and quality. Recently, biocontrol agents have become an essential component of sustainable plant disease management due to their ability to suppress fungal pathogens while reducing reliance on chemical fungicides. Among these agents, *Bacillus* species are particularly important because of their strong antagonistic activities, ability to colonize the rhizosphere and capacity to produce multiple antifungal metabolites[27]. In addition, their ability to produce stable endospores gives them high survival under harsh environmental conditions, making them more reliable in field applications than many other microbial biocontrol agents[28]. As concerns over fungicide resistance and environmental contamination increase, the use of *Bacillus* species as biocontrol agents represents a promising and necessary strategy for sustainable agriculture. In this study, we characterized three *Bacillus* strains exhibiting strong antifungal activity against plant pathogenic fungi. These *Bacillus* strains were isolated and identified based on their cellular morphology, biochemical properties and 16S rRNA gene sequences. The antifungal activities of the strains were demonstrated by their inhibitory effects on the mycelial growth of *S. rolfsii* and *C. lunata* on co-culture medium. Three *Bacillus* strains T3, T33 and T36 exhibited strong fungal inhibition (>60%) compared with the control (Fig. 1). The data indicated that these bacterial strains could secrete antifungal compounds into the agar medium that prevent the elongation of fungal hyphae. In addition, the results indicated that VOCs produced by three *Bacillus* strains suppressed the mycelial growth of fungal pathogens. Biochemical properties also indicated that all three *Bacillus* strains could produce extracellular cellulase (Table 1). These results suggested that the antagonistic activities of these *Bacillus* strains may due to their antifungal metabolites and cell wall degrading enzymes. Normally, hydrolytic enzymes could be produced together in several bacterial biocontrol agents and be a mechanism for these bacteria to fight fungal pathogens[29]. Cellulase is reported to be involved in fungal cell wall degradation by enhancing the activity of chitinases and glucanases as well as degrading cellulose or cellulose-like polysaccharides[30,31]. Interestingly, all three strains, T3, T33 and T36, also exhibited plant growth–promoting activity, resulting in increased plant biomass (Fig. 3). Biochemical assays confirmed that all strains produced siderophores (Table 1). This could be the advantageous property of these strains, explaining their antifungal mechanism. In *Bacillus*, siderophores act as high-affinity iron-chelating compounds that restrict iron availability to fungal pathogens while increasing iron uptake by plants, thereby providing a dual function in biocontrol and plant growth promotion[32,33]. Numerous *Bacillus* species with capacity of synthesizing siderophores which contributes to their effectiveness as biocontrol agents against phytopathogens[34].

Recently, many studies have discovered several potential antifungal *Bacillus* species against *S. rolfsii* or *C. lunata* fungi with diverse mechanisms. The main antagonistic mechanisms of *Bacillus* species are the productions of antagonistic compounds, volatile compounds, lipopeptides, lytic enzymes and siderophores[19-35]. For instance, endophytic *Bacillus subtilis* DZSY21 produced volatile organic compounds that significantly suppressed *C. lunata* mycelial growth, conidial germination, reduced virulence gene expression and decreased disease incidence on maize leaves[36]. *Bacillus velezensis* LHSB1 was demonstrated with strong suppression of *S. rolfsii* (up to ~93.8% radial growth inhibition) and observed effects on sclerotia formation, hyphal membrane integrity and disease severity reduction in pot trials. Its antagonistic activity might be mediated by antifungal lipopeptides such as bacillomycin A, fengycin A and surfactin A[37]. Taken together, our data indicated that *Bacillus* strains T3, T33 and T36 are potential candidates for developing biocontrol agents to manage *S. rolfsii* and *C. lunata* fungi. In addition, these strains with plant growth promotion make them attractive candidates for sustainable, integrated disease management.

CONCLUSION

In this study, three antifungal *Bacillus* sp. strains were isolated from soil samples and characterized. The strains showed strong antagonistic activities against *S. rolfsii* and *C. lunata* by diffusible compounds and VOCs. In addition, the physiological and biochemical characteristics were examined. All three strains were determined to belong to the *Bacillus* species with the capability of producing extracellular enzymes. Interestingly, adding culture medium of *Bacillus* sp. T3, T33 and T36 strains to the soil increased onion growth. Overall, our data indicated that these *Bacillus* sp. strains are promising candidates for future study in developing biocontrol agents. However, more study on these *Bacillus* strains is needed, including culture conditions, other biochemical properties and antagonistic activities in *in vivo* conditions.

SIGNIFICANCE STATEMENT

This study highlights the potential of three *Bacillus* sp. strains as promising biocontrol agents against the pathogenic fungi *S. rolfsii* and *C. lunata*. Under *in vitro* conditions, all three strains significantly inhibited fungal growth by more than 60%. In addition to their antagonistic activity, these strains promoted onion growth by enhancing plant biomass. Notably, *Bacillus* sp. T3 and *Bacillus* sp. T36 increased both the fresh and dry root weights of onion plants. Overall, these findings indicate that the selected *Bacillus* sp. strains have strong potential for managing fungal diseases in onion cultivation. However, their effects against onion fungal pathogens under *in vivo* conditions require further investigation.

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REFERENCES

1. Strange, R.N. and P.R. Scott, 2005. Plant disease: A threat to global food security. Annu. Rev. Phytopathol., 43: 83-116.
2. Sharma, I., 2021. Phytopathogenic Fungi and Their Biocontrol Applications. In: Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-Technology: Volume 1: Fungal Diversity of Sustainable Agriculture, Sharma, V.K., M.P. Shah, S. Parmar and A. Kumar (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-12-821394-0, pp: 155-188.
3. Savary, S., L. Willocquet, S.J. Pethybridge, P. Esker, N. McRoberts and A. Nelson, 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol., 3: 430-439.
4. Zeilinger, S., V.K. Gupta, T.E.S. Dahms, R.N. Silva and H.B. Singh *et al*., 2016. Friends or foes? Emerging insights from fungal interactions with plants. FEMS Microbiol. Rev., 40: 182-207.
5. Dutta, R., K. Jayalakshmi, S.M. Nadig, D.C. Manjunathagowda, V.S. Gurav and M. Singh, 2022. Anthracnose of onion (*Allium cepa* L.): A twister disease. Pathogens, Vol. 11. 10.3390/pathogens11080884.
6. Diabankana, R.G.C., M. Frolov, B. Islamov, E. Shulga, M.N. Filimonova, D.M. Afordoanyi and S. Validov, 2024. Identification and aggressiveness of *Fusarium* species associated with onion bulb (*Allium cepa* L.) during storage. J. Fungi, Vol. 10. 10.3390/jof10020161.
7. Kwon, J.H., H.D. Kim, O.H. Choi, Y.S. Kwak, Y.H. Lee and H.S. Shim, 2011. Sclerotium rot of onion caused by *Sclerotium rolfsii*. Res. Plant Dis., 17: 222-224.
8. Kwon, J.H., O. Choi, H.D. Kim, J.Y. Oh and J. Kim, 2015. The occurrence of Southern blight on *Allium hookeri* caused by *Sclerotium rolfsii* in Korea. Can. J. Plant Pathol., 37: 519-522.
9. Mullen, J., 2001. Southern blight, Southern stem blight, white mold. Plant Health Instr., Vol. 1. 10.1094/PHI-I-2001-0104-01.
10. Wang, S., Z. Lu, B. Lang, X. Wang, Y. Li and J. Chen, 2022. *Curvularia lunata* and curvularia leaf spot of maize in China. ACS Omega, 7: 47462-47470.
11. Dabire, T.G., S. Bonzi, I. Somda and A. Legreve, 2016. Identification of seed-borne fungi of onion (*Allium cepa* L.) in Burkina Faso. Int. J. Innovation Sci. Res., 25: 562-571.
12. Lahmood, W.Y. and L.F. Manher, 2017. Isolation and identification of fungi associated of growth of onion with it types white,yellow,green and red that grassed in regions surrounded of Al-Qadisia. Al-Qadisiyah J. Pure Sci., 22: 149-156.
13. Etana, M.B., M.C. Aga and B.O. Fufa, 2019. Major onion (*Allium cepa* L.) production challenges in Ethiopia: A review. J. Biol. Agric. Healthcare, 9: 42-47.
14. Aktar, W., D. Sengupta and A. Chowdhury, 2009. Impact of pesticides use in agriculture: Their benefits and hazards. Interdiscip. Toxicol., 2: 1-12.
15. Lucas, J.A., N.J. Hawkins and B.A. Fraaije, 2015. The Evolution of Fungicide Resistance. In: Advances in Applied Microbiology, Sariaslani, S. and G.M. Gadd (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-12-802275-7, pp: 29-92.
16. Shin, J.H., H.K. Lee, S.C. Lee and Y.K. Han, 2023. Biological control of *Fusarium oxysporum*, the causal agent of Fusarium basal rot in onion by *Bacillus* spp. Plant Pathol. J., 39: 600-613.
17. Waris, M., M.A. Abro, J.U.D. Hajano and A.A. Gilal, 2025. Biological control of blue mold rot of onion using *Trichoderma* species in Pakistan. Egypt. J. Biol. Pest Control, Vol. 35. 10.1186/s41938-025-00875-y.
18. El-Mougy, N.S. and M.M. Abdel-Kader, 2019. Biocontrol measures against onion basal rot incidence under natural field conditions. J. Plant Pathol., 101: 579-586.
19. Villavicencio-Vásquez, M., F. Espinoza-Lozano, L. Espinoza-Lozano and J. Coronel-León, 2025. Biological control agents: Mechanisms of action, selection, formulation and challenges in agriculture. Front. Agron., Vol. 7. 10.3389/fagro.2025.1578915.
20. Payghami, E., S. Massiha, B. Ahary, M. Valizadeh and A. Motallebi, 2001. Enhancement of growth of onion (*Allium cepa* L.) by biological control agent *Trichoderma* spp. Acta Agron. Hung., 49: 393-395.
21. Osman, H.E.M., Y. Nehela, A.A. Elzaawely, M.H. El-Morsy and A. El-Nagar, 2023. Two bacterial bioagents boost onion response to *Stromatinia cepivora* and promote growth and yield via enhancing the antioxidant defense system and auxin production. Horticulturae, Vol. 9. 10.3390/horticulturae9070780.
22. Gao, Y., H. Ren, S. He, S. Duan, S. Xing, X. Li and Q. Huang, 2022. Antifungal activity of the volatile organic compounds produced by *Ceratocystis fimbriata* strains WSJK-1 and Mby. Front. Microbiol., Vol. 13. 10.3389/fmicb.2022.1034939.
23. Lynd, L.R., P.J. Weimer, W.H. van Zyl and I.S. Pretorius, 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol. Mol. Biol. Rev., 66: 506-577.
24. Herlina, L., K.K. Pukan and D. Mustikaningtyas, 2017. The endophytic bacteria producing IAA (Indole Acetic Acid) in *Arachis hypogaea*. Cell Biol. Dev., 1: 31-35.
25. Louden, B.C., D. Haarmann and A.M. Lynne, 2011. Use of blue agar CAS assay for siderophore detection. J. Microbiol. Biol. Educ., 12: 51-53.
26. Masoomi-Aladizgeh, F., L. Jabbari, R.K. Nekouei and A. Aalami, 2016. A simple and rapid system for DNA and RNA isolation from diverse plants using handmade kit. Protoc. Exch., Vol. 2020. 10.21203/rs.2.1347/v2.

27. Etesami, H., B.R. Jeong and B.R. Glick, 2023. Potential use of *Bacillus* spp. as an effective biostimulant against abiotic stresses in crops-A review. Curr. Res. Biotechnol., Vol. 5. 10.1016/j.crbiot.2023.100128.
28. Pirttijarvi, T.S.M., M.A. Andersson and M.S. Salkinoja-Salonen, 2000. Properties of *Bacillus cereus* and other bacilli contaminating biomaterial-based industrial processes. Int. J. Food Microbiol., 60: 231-239.
29. Ajuna, H.B., H.I. Lim, J.H. Moon, S.J. Won and V. Choub *et al*., 2023. The prospect of hydrolytic enzymes from *Bacillus* species in the biological control of pests and diseases in forest and fruit tree production. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms242316889.
30. Ghasemi, S., N. Safaie, S. Shahbazi, M. Shams-Bakhsh and H. Askari, 2020. The role of cell wall degrading enzymes in antagonistic traits of *Trichoderma virens* against *Rhizoctonia solani*. Iran. J. Biotechnol., 18: 18-28.
31. Dobrzyński, J., Z. Jakubowska, I. Kulkova, P. Kowalczyk and K. Kramkowski, 2023. Biocontrol of fungal phytopathogens by *Bacillus pumilus*. Front. Microbiol., Vol. 14. 10.3389/fmicb.2023.1194606.
32. Miethke, M. and M.A. Marahiel, 2007. Siderophore-based iron acquisition and pathogen control. Microbiol. Mol. Biol. Rev., 71: 413-451.
33. Yu, X., C. Ai, L. Xin and G. Zhou, 2011. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect o n Fusarium wilt and promotes the growth of pepper. Eur. J. Soil Biol., 47: 138-145.
34. Andrić, S., T. Meyer and M. Ongena, 2020. *Bacillus* responses to plant-associated fungal and bacterial communities. Front. Microbiol., Vol. 11. 10.3389/fmicb.2020.01350.
35. Dimkić, I., T. Janakiev, M. Petrović, G. Degrassi and D. Fira, 2022. Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms-A review. Microbiol. Mol. Biol. Rev., Vol. 117. 10.1016/j.pmpp.2021.101754.
36. Xie, S., J. Liu, S. Gu, X. Chen, H. Jiang and T. Ding, 2020. Antifungal activity of volatile compounds produced by endophytic *Bacillus subtilis* DZSY21 against *Curvularia lunata*. Ann. Microbiol., Vol. 70. 10.1186/s13213-020-01553-0.
37. Chen, L., Y.D. Wu, X.Y. Chong, Q.H. Xin, D.X. Wang and K. Bian, 2020. Seed-borne endophytic *Bacillus velezensis* LHSB1 mediate the biocontrol of peanut stem rot caused by *Sclerotium rolfsii*. J. Appl. Microbiol., 128: 803-813.