



Potential of endophytic *Bacillus toyonensis* BAC3151 for phytopathogen control and growth promotion in common bean

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Abstract

Bean (*Phaseolus vulgaris* L.) crops in Brazil are affected by several fungal diseases such as anthracnose (*Colletotrichum lindemuthianum*), Fusarium wilt (*Fusarium oxysporum* f. sp. *phaseoli*), and white mold (*Sclerotinia sclerotiorum*). Disease management through biological control is sustainable and environmentally friendly. The purpose of the study was to investigate the potential of endophytic *Bacillus toyonensis* BAC3151, applied via seed treatment, to control phytopathogens *C. lindemuthianum*, *F. oxysporum* f. sp. *phaseoli*, and *S. sclerotiorum*, and promote common bean growth. *Bacillus toyonensis* BAC3151 reduced 95.2%, 24.2%, and 8.2% of the mycelial growth of *S. sclerotiorum*, *C. lindemuthianum*, and *F. oxysporum* f. sp. *phaseoli*, respectively. Additionally, the disease severity of white mold was reduced by 87.5% in the plants. *Bacillus toyonensis* BAC3151 showed growth-promoting characteristics, including nitrogen fixation and production of volatile organic compounds, siderophores, indole acetic acid, chitinase, protease, carboxymethyl cellulase, and amylase, as well as significantly increased root length by 31.5% and root dry mass by 33.6%. These findings indicate that *B. toyonensis* BAC3151 may be used as a biocontrol agent and growth promoter for common bean.

Keywords Bacteria · Endophyte · Enzymes · Volatile organic compounds · Biological control

Introduction

Common bean (*Phaseolus vulgaris* L.) is highly nutritious and one of the most widely consumed crops worldwide. The world common bean production was approximately 28.3 million tons in 2022 (FAO 2024). However, a critical constraint to bean cultivation is loss resulting from diseases such as anthracnose caused by *Colletotrichum lindemuthianum*, Fusarium wilt caused by *Fusarium oxysporum* f.

sp. *phaseoli* (FOP), and white mold caused by *Sclerotinia sclerotiorum* (Sartorato and Rava 1994).

Research on microorganisms that benefit the culture with little or no environmental impact, promote plant growth, and control pathogenic microorganisms and nematodes is constantly increasing to raise current productivity levels and reduce production costs. Studies have been conducted to identify microorganisms that can be safely integrated into bean cultures (Wang et al. 2010; Sabaté et al. 2020; Souza et al. 2023). Many fungi and bacteria have already shown potential to be used as biocontrol agents for important diseases in major agronomic crops. Among them, *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Pochonia* spp. are examples of microorganisms applied for crop protection against diseases caused by fungi, bacteria, and nematodes. (Ayaz et al. 2023). Moreover, adopting these beneficial microorganisms in integrated disease management, which aims to combine different strategies for crop protection, has proven to be an effective and sustainable alternative to mitigate the economic losses caused by phytopathogens (Kumar et al. 2024).

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Members of the *Bacillus* genus can act as bioactive and eco-friendly agents for disease control and plant growth promotion (Sabaté et al. 2020; Souza et al. 2023). They have various biological control mechanisms, such as the secretion of hydrolytic enzymes, the production of secondary metabolites, and the induction of systemic resistance in plants. Additionally, *Bacillus* has plant growth-promoting mechanisms, including the synthesis of hormones and volatile organic compounds (VOCs) that regulate phytohormone metabolism, the supply of nutrients through processes such as nutrient solubilization (biofertilization), inhibition of senescence, and regulation of the expression of plant genes responsive to abiotic stress (Shan et al. 2024; Bakki et al. 2024; Portieles et al. 2021; Patel et al. 2023; Patani et al. 2024).

Bacillus toyonensis is a Gram-positive, facultative anaerobic species that belongs to the *Bacillus cereus* group. This species is known for its ability to sporulate under environmental and nutritional stress (Checinska et al. 2015) and has been reported as a probiotic in animal nutrition (Jiménez et al. 2013a, 2013b). Endophytic *B. toyonensis* isolated from pine roots demonstrated nematode-control capacity under greenhouse conditions (Li et al. 2023). Moreover, the endophytic *Bacillus* strain isolated from *Arthrocaulon* sp. was also identified as *B. toyonensis* AA1EC1 and exhibited traits associated with plant growth promotion and quorum-quenching activity (Roca et al. 2024).

Previously, an endophytic bacterium (BAC3151) was isolated from common bean (*P. vulgaris*) leaves and was initially identified as *Bacillus thuringiensis* using 16S rRNA gene sequencing (Costa et al. 2012). Genome analysis of this strain revealed 99.49% identity with the *B. toyonensis* (BCT-7112 strain), which led to its reclassification. Moreover, *B. toyonensis* BAC3151 has a genetic repertoire of antimicrobials, including gene clusters of bacteriocins, non-ribosomal peptide synthetases genes, N-acyl homoserine lactones, and chitinases, representing a broader and functionally diverse set of biosynthetic genes compared with those typically found in other *Bacillus* strains (Lopes et al. 2017). This study assessed the potential of the common bean endophyte *B. toyonensis* BAC3151 for its ability to control common bean fungal diseases. Additionally, the direct and indirect mechanisms contributing to the growth promotion of common bean were analyzed.

Materials and methods

Microorganisms and growth conditions

Bacillus toyonensis BAC3151, *C. lindemuthianum* A₂ 2–3, *F. oxysporum* f. sp. *phaseoli*, and *S. sclerotiorum* were

obtained from the Microbial Molecular Genetics Laboratory/BIOAGRO at Universidade Federal de Viçosa. The bacteria were cultured in 10% tryptone-soy agar (TSA) medium (1.5 g/L tryptone, 0.5 g/L soy peptone, and 1.5 g/L NaCl; pH 7.3) at 28 °C with stirring at 150 rpm for 24 h (Lopes et al. 2017).

Colletotrichum lindemuthianum was cultivated in a potato-dextrose-agar (PDA) medium and incubated for 10 days at 22 °C under a 12-h photoperiod. The mycelial discs were transferred to sterilized bean pods and incubated under the same conditions to allow the fungus to sporulate. *F. oxysporum* f. sp. *phaseoli* and *S. sclerotiorum* were cultured in a PDA medium and incubated for 14 d at 25 °C and 7 d at 24 °C, respectively, under a 12-h photoperiod.

Characterization of antifungal potential

In vitro analysis

From a culture of *B. toyonensis* BAC3151, a 100 µL aliquot with an optical density (OD) of 0.5 was spread on cellophane discs deposited onto PDA, and the plates were incubated at 28 °C for 48 h. After incubation, the cellophane disc was removed, and a 2 mm fungal mycelium disc was placed in the center of the plate. The plates were then incubated in a BOD incubator under the appropriate conditions for each fungus. A fungal mycelium disc was inoculated onto a plate containing PDA without prior bacterial inoculation as a control. The fungal colony diameter was measured after 7 days of growth to examine the antagonistic activity of *B. toyonensis* BAC3151.

The percentage of fungal growth inhibition was calculated using the following formula:

$$IC = [(C - T) / (C)] \times 100 \%$$

Where *C* is the diameter of the fungal colony in the control (without previous inoculation of the bacteria) and *T* is the diameter of the mycelial growth in the treatment (with prior inoculation of the bacteria).

Data were subjected to analysis of variance (ANOVA), and means were compared by the Tukey's test at a 5%. Three biological and experimental replicates were analyzed for each phytopathogen.

In vivo analysis

Bean seeds from Manteigão Fosco 11 (resistant to Fusarium wilt) and BRS Estilo (susceptible to Fusarium wilt) were used to examine Fusarium wilt suppression. In contrast, seeds from the BRS Pérola (resistant to Fusarium wilt) have been used to analyze the suppression of white mold and

anthracnose diseases (Yokoyama et al. 1999; Pereira et al. 2011; Melo et al. 2010).

The seeds were superficially disinfected by immersing them in a 2.5% sodium hypochlorite solution for 5 min and subsequently washed three times with sterile distilled water by shaking (5 min each). Next, the disinfested seeds were placed in a filter paper disc, moistened with distilled water in a sealed Petri dish, and germinated in the dark at 25 °C. After 96 h, the uniformly germinated seeds were selected.

Seeds were placed for 10 min in tubes containing 1 mL of bacterial culture of *B. toyonensis* BAC3151 grown in 10% TSA medium. As a control, seeds were placed in tubes containing 1 mL of sterile distilled water for 20 min. The seeds were sown in 500 mL pots containing an unsterilized Topstrato® vegetable substrate. The experimental design was completely randomized (CRD), with one plant per pot. Three experiments with six replicates were performed. After seed development, the plants were grown in a greenhouse for 10 days with irrigation whenever necessary (Botta et al. 2013).

Assessment of anthracnose suppression

Colletotrichum lindemuthianum was cultivated in bean pods for 10 days. After that, the pods were collected and placed into 10 mL tubes, and 3 mL of distilled water was added to prepare spore suspensions. Using a Neubauer chamber, the conidia were counted, and a suspension was prepared at a concentration of 1×10^6 conidia mL⁻¹. An aqueous suspension of conidia was used to inoculate the plants with the fungus. Plants of the BRS Pérola (10-day-old) were sprayed with the spore suspension on their shoot parts. After inoculation, the plants were maintained for 12 h in an inoculation chamber without light (dark), and then transferred to the greenhouse.

Treatment: (1) Negative control (plants treated with water); (2) Positive control (plants inoculated only with the conidial suspension of the phytopathogen); (3) Seeds previously treated only with *B. toyonensis* BAC3151; and (4) Seeds previously treated with *B. toyonensis* BAC3151 and subsequently with a conidial suspension of the phytopathogen. The test was conducted in a completely randomized design (CRD), with three experiments and six replications.

Disease severity was evaluated in two leaves per plant by quantifying the injured leaf area using the Quant V.1.0 program 5 days after pathogen inoculation (Vale et al. 2001). Disease severity was expressed as a percentage of the injured area. Moreover, protection was calculated as the reduction in disease severity and expressed as a percentage relative to the control (Moraes et al. 1976).

The values were subjected to ANOVA, and the Tukey's test compared the means at a 5% probability.

Evaluation of Fusarium wilt suppression

Fusarium oxysporum f. sp. *phaseoli* (FOP) was cultured in a PDA medium and incubated for 14 days at 25 °C under a 12-h photoperiod. A conidial suspension was prepared at 1×10^6 conidia mL⁻¹, including macro and microconidia (Pastor-Corrales and Abawi 1987).

Root immersion in the conidial suspension was performed to inoculate plants with the fungus. At stage V2 (fully expanded primary leaf), the plants were removed from the pots, their roots were washed, and 1/3 of these roots were excised. The roots were immediately immersed in a suspension of macroconidia and microconidia for 5 min. The plants were then transplanted into plastic pots containing 500 mL of Topstrato® substrate and maintained in a greenhouse under irrigation (Pastor-Corrales and Abawi 1987).

Treatment: (1) Negative control (Plants treated with water); (2) Positive Control (Plants inoculated only with the conidial suspension); (3) Plants previously treated only with *B. toyonensis* BAC3151 and (4) Plants treated with *B. toyonensis* BAC3151 and a conidia suspension. The test was conducted in CRD, three experiments with six replicates were performed.

The response of plants to Fusarium wilt was evaluated using a Disease Severity Rating Scale (Pastor-Corrales and Abawi 1987), which is mainly based on shoot wilt intensity. The following scoring was used: 1=no visible symptoms, 3=1–10% symptomatic leaves (leaves with mild wilt and chlorosis), 5=11–25% symptomatic leaves (leaves with moderate wilt and chlorosis), 7=26–50% symptomatic leaves (leaves with severe wilt and chlorosis), and 9=dead or severely infected plants. Evaluations were performed 20 days after inoculation (DAI) to determine disease severity and incidence.

Using the symptom data, the disease severity index (DI) was calculated using the following formula:

DI (%) = $\frac{\sum(\text{score} \times \text{number of plants with the same score})}{(\text{maximum number of scale scores} \times \text{total number of plants evaluated})} \times 100$ (Mckinney 1923).

The area under the disease progress curve (AUDPC) was obtained according to Shaner and Finney (1977), using the following equation:

$$AUDPC = \sum_{(i=1)}^n \left[\left(\frac{Y_{i+1} + Y_i}{2} \right) (T_{(i+1)} - T_i) \right]$$

Here, Y_i is the Fusarium wilt severity in the i -th observation, T_i is the time (days after inoculation) at the i -th observation, and n is the total number of evaluations. Values were subjected to ANOVA, and means were compared by the Tukey's test at a 5% probability.

White mold suppression assessment

Mycelial discs (5 mm in diameter) containing the fungal mycelium were used to inoculate plants. The discs were inoculated and gently pressed into the primary leaves of 10-day-old bean plants, leaving them in contact with the midrib. The inoculated plants were maintained in a growth chamber for 7 d (Fagundes-Nacarath et al. 2018).

Treatment: (1) Negative control (Plants treated with water and PDA discs without mycelia); (2) Positive Control (Plants treated only with *S. sclerotiorum* mycelia on PDA discs); (3) Plants were treated with *B. toyonensis* BAC3151 and (4) Plants treated with *B. toyonensis* BAC3151 and mycelia of *S. sclerotiorum* on PDA disks. The test was conducted in CRD, three experiments with six replicates were performed.

White mold severity was assessed using the grading scale described by Tolêdo-Souza and Costa (2003), which is based mainly on the intensity and length of the lesion. In this scale, 1=no visible symptoms; 2=beginning of circular lesion development; 3=lesion up to 1.0 cm in length; 4=lesions between 1.0 and 1.5 cm; 5=lesions larger than 1.5 cm; 6=lesions on the peduncle and presence of mycelia and wilt on one of the leaves; 7=lesions on the peduncle and presence of mycelia and wilt on both leaves; 8=symptoms of general wilt; and 9=dead plants. The severity and incidence of the disease were evaluated up to 7 days after inoculation (DAI).

The DI and AUDPC were calculated using the severity data. Values were subjected to ANOVA, and means were compared by the Tukey's test at a 5% probability.

In vitro evaluation of plant growth-promoting activities

At least three replicates were performed for all plant growth-promoting activities, and the trial averages are discussed.

Nitrogen fixation

Bacillus toyonensis BAC3151 was inoculated into test tubes (picking method) containing a nitrogen-free semi-solid malate medium (Nfb). The cultivation conditions were adapted according to Lopes et al. (2015), and the cultures were incubated at 28 °C for 7 d. A change in the color of the

Nfb medium from green to blue indicated nitrogen fixation (Dobereiner 1995).

Phosphate solubilization

The ability to solubilize phosphate was evaluated by inoculating *B. toyonensis* BAC3151 into National Botanical Research Institute's phosphate growth medium (NBRIP). A halo zone around a colony was considered a positive result for phosphate solubilization (Nautiyal 1999).

Siderophore production

Bacillus toyonensis BAC3151 (10 µL) was inoculated onto chrome azurol-S agar in Petri dishes (Schwyn and Neilands 1987). The culture conditions were adapted for optimal growth of *B. toyonensis* BAC3151, and Petri dishes were incubated at 28 °C for 7 days. A halo zone around the colonies, with a color change from blue to yellow, was considered a positive result for siderophore production (Schwyn and Neilands 1987).

Indole acetic acid (IAA) production

The ability of *B. toyonensis* BAC3151 to produce indole acetic acid was evaluated by transferring a bacterial colony to Erlenmeyer flasks containing 10% TSA supplemented with 5 mM L-tryptophan (an IAA precursor). The culture was maintained under agitation at 125 rpm at 28 °C for 24 h without light.

After the growth period, 2 mL of the bacterial culture was centrifuged at $10,000 \times g$ for 10 min. A 900 µL aliquot of the supernatant was then removed and added to 400 µL of Salkowski's solution (50 mL of 35% perchloric acid and 1 mL of 0.5 M FeCl_3). The mixture was incubated at 25 °C for 30 min. Results were positive when the culture medium turned pink (Ramos 2016).

Protease production

Bacillus toyonensis BAC3151 was inoculated onto a skim milk agar medium (10 g/L skim milk powder and 15 g/L agar). Cultures were incubated at 28 °C for 2–3 days. Protease activity was detected by developing a halo around the colonies, indicating that milk proteins were hydrolyzed (Chang and Yang 2009).

Chitinase production

Bacillus toyonensis BAC3151 was inoculated onto solid minimal medium containing 1% colloidal chitin and 10 mL of the fluorescent dye Calcofluor White M2R to facilitate

halo visualization. Petri dishes were incubated at 28 °C for 2–3 days. Chitinase activity was revealed by the development of a halo around the bacterial colonies, indicating the occurrence of chitin hydrolysis (Slama et al. 2019).

Cellulase production

Bacillus toyonensis BAC3151 was inoculated onto carboxymethylcellulose (CMC) agar medium (minimum salt medium supplemented with 0.2% CMC). The plates were incubated at 28 °C for 7 d. After that, a 0.5% Congo red solution was added to the plates for 40 min, followed by 1 mol L⁻¹ NaCl washing for 20 min. Cellulase activity was detected by forming a clear zone around the colony, indicating the occurrence of cellulose hydrolysis (Chang and Yang 2009).

Amylase production

Bacillus toyonensis BAC3151 was inoculated onto a nutrient agar medium (Merck®) supplemented with 0.5% soluble starch. The plates were incubated at 28 °C for 2 days. Iodine solution (0.3 g/L of iodine and 0.6 g/L of KI) was added to the surface of the Petri dishes, and the appearance of a clear zone around the colony indicated a positive result (Slama et al. 2019).

Hydrogen cyanide (HCN) production

Four microdrops of the bacterial culture (10 µL each) were inoculated at equidistant points on a nutrient agar medium (Merck®) supplemented with 4 g/L glycine. A disk of Whatman filter paper was soaked in HCN reagent solution (1% picric acid in 10% NaCO₃) and placed on the plate lid. The plates were incubated at 28 °C for 4–5 days. After incubation, the development of a yellow to reddish-brown color indicated a positive result (Lorck 1948).

In vitro assay to evaluate the effect of VOCs on common bean plants

Seeds of the BRS Pérola were inoculated onto a sterile tissue culture dish (12 cm × 12 cm square) with Murashige and Skoog medium (Sigma®), and a 15 mL sterile round Petri dish without the lid was placed on top of the dish. *Bacillus toyonensis* BAC3151 was inoculated onto a plate containing 10% TSA. The plates were sealed with parafilm and incubated in a chamber at 25 °C with a photoperiod of 12 h light and 12 h darkness for 3 d. The control was similarly processed without bacterial inoculation (Fiers et al. 2013; Rath et al. 2018). The vigor index was calculated as follows:

Vigor index = germination percentage × seedling length (shoot length + root).

Data were subjected to ANOVA, and means were compared by the Tukey's test at 5% probability.

Evaluation of plant growth promotion

The bean seeds were immersed for 20 min in tubes containing 1 mL of *B. toyonensis* BAC3151 culture grown in 10% TSA medium (T2), 1 mL of sterile distilled water (negative control), and 1 mL of bacterial culture of *B. toyonensis* BAC3151 grown in 10% TSA medium that was killed by autoclaving (T3). The treated seeds were sown in 500 mL pots containing an unsterilized Topstrato® vegetable substrate. A complete CRD was used with six replications, a portion of one pot of each plant, and three experimental replications. At the end of the experiment (45 days after sowing), the following evaluations were performed: the number of leaves per plant, considering all live and open leaves; root length measured with a graduated ruler from the base of the stem to the root tip; and fresh and dry masses of shoots and roots. For the purpose mentioned above, the plant material was cut into smaller pieces and weighed on a precision scale (Aaker Bel Engineering; min 0.01 g, max 500 g) and then placed in paper bags, and dried in an oven at 65 °C till constant weight (5 d) (Botta et al. 2013; Santos et al. 2014). Data were subjected to ANOVA, and means were compared by the Tukey's test at 5% probability. Three replicates were analyzed.

Results

Assay for the characterization of antifungal potential

In vitro analysis

The antagonistic activity of *B. toyonensis* BAC3151 against the three phytopathogens was evaluated by analyzing the secretion of antifungals into the culture medium. The results revealed that *B. toyonensis* BAC3151 secreted compounds capable of suppressing the growth of all three phytopathogens (Fig. 1; Table 1). The inhibitory activity of the compounds against *S. sclerotiorum* was the strongest (95.2% growth inhibition); however, the compound was weaker against FOP (8.1% growth inhibition).

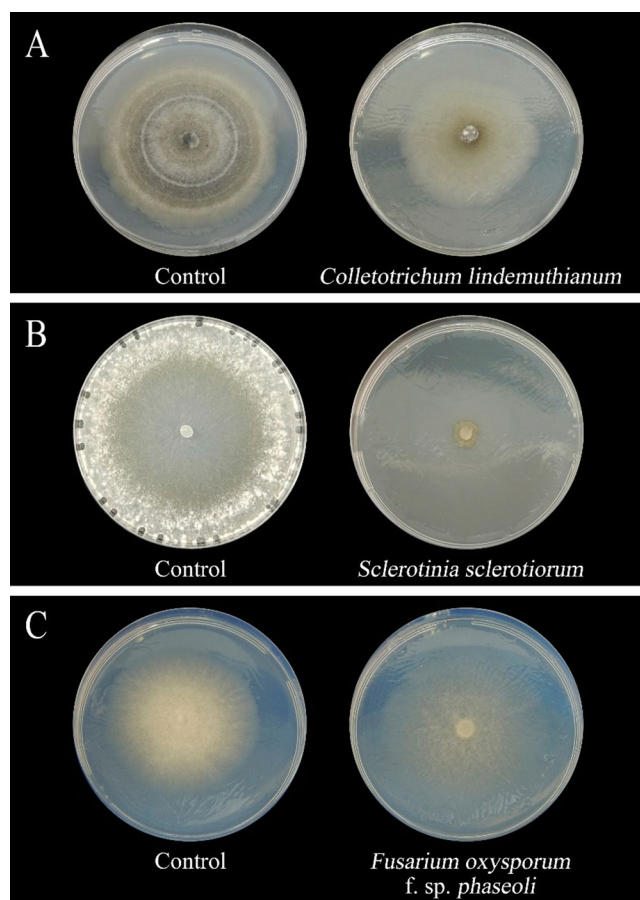


Fig. 1 Antagonistic activity of *B. toyonensis* BAC3151 against common bean pathogens. Controls are plates with a PDA culture medium, where the pathogen was inoculated without previous inoculation of *B. toyonensis* BAC3151. Treatments are plates with PDA, where the pathogen was inoculated with a previous inoculation of *B. toyonensis* BAC3151 on cellophane. Examined pathogens: **A** *C. lindemuthianum*, **B** *S. sclerotiorum*, and **C** *F. oxysporum* f. sp. *phaseoli*

Table 1 Diameter of fungal colonies cultured in the presence of compounds secreted by *B. toyonensis* BAC3151

Diameter of colonies (mm) ⁽¹⁾			
Pathogenic Fungi	Control (mm)	Treatment (mm)	Growth Reduction ⁽²⁾
<i>C. lindemuthianum</i>	65.5 a	49.7 ± 2.6 b	24.2 %
<i>F. oxysporum</i> f. sp. <i>phaseoli</i>	57.5 a	52.8 ± 1.4 b	8.1 %
<i>S. sclerotiorum</i>	77.1 a	3.7 ± 0.1 b	95.2 %

⁽¹⁾ Means ± standard error followed by equal letters on the line do not differ by Tukey test at a 5 % probability (n = 3). ⁽²⁾ Relative to control.

In vivo analysis

Assessment of anthracnose disease suppression

Five days after *C. lindemuthianum* inoculation, disease symptoms were observed in all plants not treated with *B.*

Table 2 Severity of anthracnose caused by *C. lindemuthianum* in common bean cultivated on substrate inoculated with *B. toyonensis* BAC3151⁽¹⁾

Treatments ⁽²⁾	Leaf area injured by plant	Severity reduction ⁽³⁾
<i>C. lindemuthianum</i>	7.1 ± 0.5% a	-
<i>C. lindemuthianum</i> + <i>B. toyonensis</i>	4.3 ± 0.3% b	39.3%

⁽¹⁾ Means ± standard error followed by equal letters in the column do not differ by Tukey test at a 5% probability (n = 4). ⁽²⁾ Isolate of *B. toyonensis* BAC 3151 was inoculated onto bean seed 10 days before inoculation with *C. lindemuthianum*. ⁽³⁾ Relative to the control

toyonensis BAC3151. Plants treated with the bacterium demonstrated a significant decrease in leaf lesion area (39.3%) (Table 2) compared with the leaves of untreated plants, indicating the efficiency of *B. toyonensis* BAC3151 in controlling *C. lindemuthianum* (Fig. 2A).

White mold suppression assessment

White mold symptoms were observed on the fourth day after *S. sclerotiorum* inoculation. Plants not treated with bacteria exhibited symptoms of white mold 72 h after inoculation, reaching 100% severity at the time of evaluation (dead plants). In contrast, plants inoculated with *B. toyonensis* BAC3151 demonstrated no evident mycelial growth, only slight necrosis around the disc, and only 12.5% severity (Fig. 2B). Furthermore, the treatment of plants with *B. toyonensis* BAC3151 revealed stimulatory and non-phyto-toxic effects (Fig. 2B).

Disease progression in the common bean was tracked using a white mold progress curve based on parameters from the area below the curve. Comparing *B. toyonensis* BAC3151-treated plants with untreated plants, a significant reduction in disease progression (smaller AUC) was observed, implying that the bacteria effectively suppressed the severity of white mold (Fig. 3).

Assessment of Fusarium wilt disease suppression

Disease symptoms were observed from day 10 onward. The yellowing and wilting of leaves treated with the fungus were evident. The symptoms gradually increased in all the plants inoculated with the pathogen until death, including those treated with *B. toyonensis* BAC3151.

In vitro plant growth-promoting activity

Plate-based assays revealed that *B. toyonensis* BAC3151 could fix nitrogen, produce VOCs, siderophores, and IAA, and secrete enzymes such as proteases, chitinases,

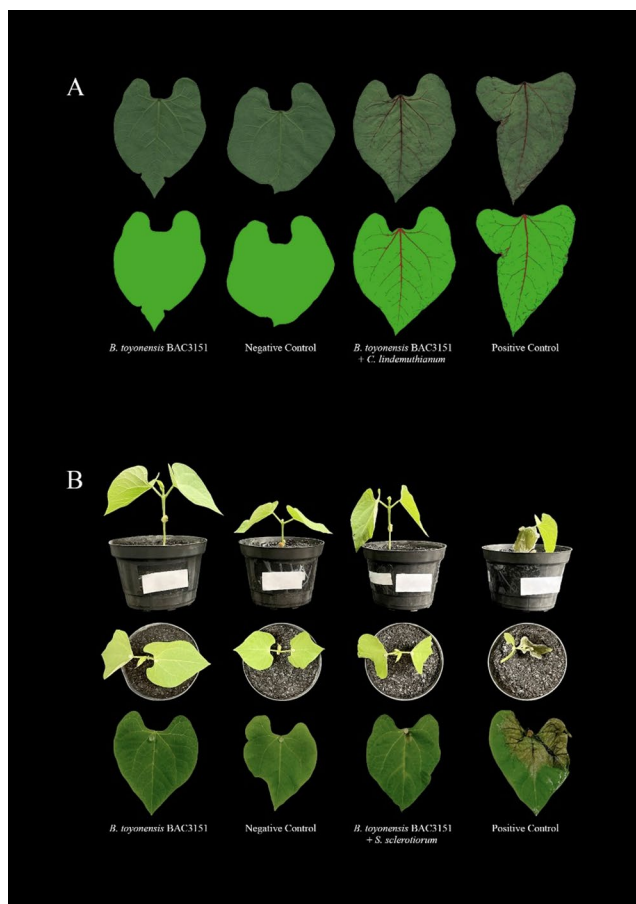


Fig. 2 Antagonistic activity of *B. toyonensis* BAC3151 against *C. lindemuthianum* and *S. sclerotiorum*. **A** Representation of real leaves showing lesions caused by the *C. lindemuthianum* and a computational representation of lesion severity, highlighting the extent of the affected area. Negative Control - plants treated with water; Positive Control - plants inoculated only with the conidial suspension of *C. lindemuthianum*; *B. toyonensis* BAC3151 - plants inoculated only with *B. toyonensis* BAC3151; *C. lindemuthianum*+*B. toyonensis* BAC3151 - plants inoculated with *B. toyonensis* BAC3151 and conidial suspension of *C. lindemuthianum*. **B** Representation of real leaves showing lesions caused by *S. sclerotiorum*. Negative Control - plants inoculated with agar disk; Positive Control - plants inoculated only with *S. sclerotiorum* mycelium disk; *B. toyonensis* BAC3151 - plants inoculated with *B. toyonensis* BAC3151 and agar disk; *B. toyonensis* BAC3151+*S. sclerotiorum* - plants inoculated with *B. toyonensis* BAC3151 and *S. sclerotiorum* mycelium disk

carboxymethyl cellulase (CMCase), and amylases (Supplementary Figure S1). However, *B. toyonensis* BAC3151 could not solubilize phosphate or produce HCN under the analyzed conditions.

The effect of VOCs produced by *B. toyonensis* BAC3151 on common bean was evaluated in plate tests. Seeds treated with *B. toyonensis* BAC3151 demonstrated a significant expansion in seedling shoot and root formation (Fig. 4) and a higher vigor index than untreated seeds (1300% control and 3250% *B. toyonensis* BAC3151).

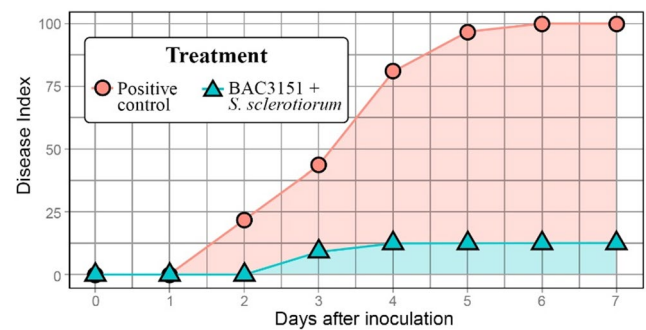


Fig. 3 White mold disease progression. The pink line represents the advancement of white mold disease during the days following the treatment of common bean leaves with *S. sclerotiorum* mycelium discs. The blue line represents the advance of white mold following inoculation with a disc of *S. sclerotiorum* mycelium in bean plants previously treated with *B. toyonensis* BAC3151

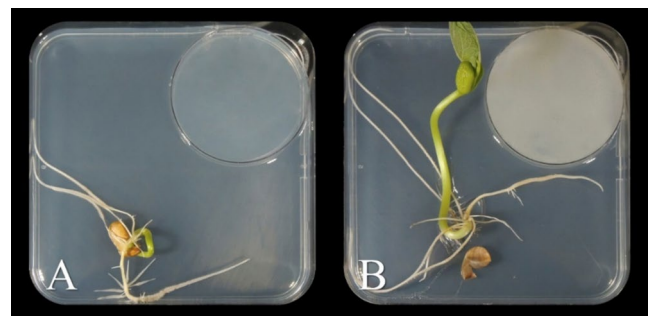


Fig. 4 Plant growth promotion assays and the effect of volatile organic compounds from *B. toyonensis* BAC3151 on bean growth and development. **A** Control seedlings (without *B. toyonensis* BAC3151 inoculation) and **B** three-day-old seedlings exposed to *B. toyonensis* BAC3151 VOC in a rectangular Petri dish

Evaluation of plant growth promotion

The length and number of leaves were evaluated in evaluating the plant shoot, and no significant difference was observed between the treatments. Assessment of the root length and fresh and dry masses of the shoot parts and roots at the experiment's end revealed significant differences between the treatments. Plants treated with *Bacillus toyonensis* BAC3151 showed an increase in root length and dry mass by 31.5% and 33.6%, respectively (Fig. 5).

Discussion

The findings of this study are significant concerning the control of fungal diseases that affect common bean crops and pose severe threats to agriculture. The diseases are initially controlled using chemical pesticides and/or resistant cultivars. However, the chemicals selected for resistant fungi can harm the environment. Additionally, acquiring durable resistance to several fungal diseases and high productivity is

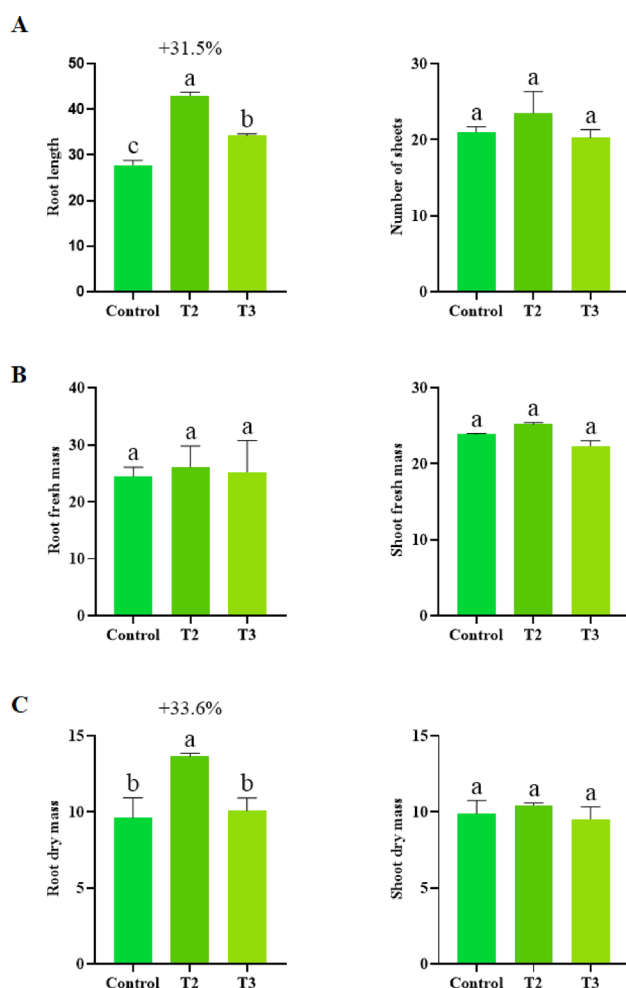


Fig. 5 Comparison of parameters promoting the growth of common bean plants. T2, plants treated with *B. toyonensis* BAC3151; T3, plants treated with autoclave-killed *B. toyonensis* BAC3151. **A** Percentage of length (left) and dry mass of roots (right); **B** Percentage of the fresh mass of the root (left) and shoot (right); **C** Percentage of the root (left) and shoot (right) dry mass. Error bars indicate the standard deviation. Equal letters do not differ by the Tukey test at a 5% probability

challenging for resistant cultivars. Owing to these disadvantages, the search for alternative biological control methods, such as antagonistic bacteria and fungi, is constantly rising, favoring integrated strategies for disease control and agricultural production (Wang et al. 2010).

This study examined the ability of the common bean endophyte *B. toyonensis* BAC3151 to control phytopathogens and promote common bean growth. We evaluated whether the compounds produced and secreted by *B. toyonensis* BAC3151 had a positive or negative effect on controlling fungal diseases and promoting the growth of common bean.

The compounds produced and secreted by *B. toyonensis* BAC3151 into the culture medium inhibited the growth of fungal colonies. Lopes et al. (2015) reported the potential production of antimicrobial compounds by *B. toyonensis*

BAC3151. They identified genes encoding several antimicrobials, such as bacteriocins and hydrolytic enzymes, in the bacterial chromosome and plasmids.

In vivo, antagonism assays revealed that *B. toyonensis* BAC3151 inhibited white mold development and reduced anthracnose severity. These findings are consistent with previous reports, reinforcing that *B. toyonensis* BAC3151 likely acts through the same mechanisms described for other *Bacillus* strains—namely, the secretion of antifungal metabolites and the induction of systemic plant defenses during colonization, or a synergistic combination of both. Likewise, other studies have demonstrated similar antifungal actions in *Bacillus* species. For instance, two strains of *B. toyonensis* (B1 and B2) produced antimicrobial compounds capable of inhibiting *S. sclerotiorum*, reducing mycelial growth by 54.4% and 50.2% in dual culture assays. In the plant, strain B2 decreased lesion size caused by *S. sclerotiorum* in sunflower by 31–32% (Koçak et al. 2024). A comparable mode of action is also observed in the chitinolytic strain *B. toyonensis* AGBE1.2.TL, which suppresses *Colletotrichum capsici*, the causal agent of anthracnose in chili (Yanti et al. 2023).

Bacillus toyonensis BAC3151 produces hydrolytic enzymes (proteases and chitinases), which may be responsible for its antifungal activity, as the enzymes degrade the fungal cell wall and are considered essential for its antifungal action (Chang et al. 2010; Wong et al. 2008; Medison et al. 2022). Other *B. toyonensis* have been reported to produce proteases and chitinases, indicating that enzyme secretion is a strategy employed by these biocontrollers (Contreras-Pérez et al. 2019; Rojas-Solis et al. 2020; Shurigin et al. 2022; Foughalia et al. 2024). Lopes et al. (2017) reported the presence of three chitinases genes in the genomes of *B. toyonensis* BAC3151.

Bacillus toyonensis BAC3151 also produced CMCase and amylases (Supplementary Figure S1). The production of hydrolytic enzymes is essential for colonizing plant roots and movement toward the interior of plants, which helps plant growth (Choubane et al. 2016). *Bacillus cereus* 0–9 has genes that code for amylase production. Mutant analysis revealed that the production of extracellular amylase was closely associated with the bacterium's ability to colonize wheat roots and with its biocontrol activity against *R. cerealis*. The extracellular amylase produced by *B. cereus* 0–9 hydrolyzes starch, releasing glucose, maltose, and other nutrients that support bacterial growth (Huang et al. 2022).

The effects of *Bacillus toyonensis* on plant growth are related to several factors, such as plant protection against pathogens, production of plant growth hormones, increased absorption, translocation of nutrients and minerals, and increased solubility and availability of various micronutrients (Alaylar 2022; Rojas-Solis et al. 2020; Andriūnaitė et al., 2021; Shurigin et al. 2022; Shin et al. 2023). Among growth-promoting factors,

VOCs are vital for increasing plant growth, phytohormonal synthesis, metabolism, and the inhibition of fungal growth. Studies have reported that many *B. toyonensis* secrete VOCs that stimulate plant development and growth and exert antimicrobial action. Contreras-Pérez et al. (2019) reported the genome of the blueberry (*Vaccinium* spp. var. Biloxi) growth-promoting endophyte *B. toyonensis* COPE52, and to validate in silico analyses, they detected the production of indole acetic acid, protease activity, and VOC as potential mechanisms of promoting plant growth. Furthermore, *B. toyonensis* COPE52 promoted the growth of biomass and chlorophyll content in blueberry plants and showed antifungal action against the phytopathogen gray mold (*Botrytis cinerea*) in vitro assays. *Bacillus toyonensis* BAC3151 stimulated seedling development through the production of indole acetic acid (IAA) and growth-promoting VOCs, resulting in higher vigor index and accelerated germination and seedling growth. Although the strain clearly enhances bean development via these volatile and hormonal signals, the specific VOCs involved still require identification and detailed characterization.

Another critical factor in promoting plant growth is the absorption and translocation of nutrients and minerals. A significant result was the ability of *B. toyonensis* BAC3151 to fix nitrogen and produce siderophores. The results obtained are supported by the analysis of the *B. toyonensis* BAC3151 genome in which biosynthetic genes of petrobacin and bacillibactin siderophores were found (Lopes et al. 2017). Nitrogen and iron are essential for plant growth and productivity. Certain *Bacillus* species can fix nitrogen and supply it to plants through two different mechanisms: symbiotic and nonsymbiotic (Maheshwari 2010; Yousuf et al. 2017). Previous studies have reported that *Bacillus* sp. eliminates iron from the environment due to the low bioavailability of iron, favoring plant growth (Ferreira et al. 2019).

As presented in Figs. 2 and 5, plants treated with *B. toyonensis* BAC3151 demonstrated a stimulatory effect compared to non-treated plants. They displayed a significant difference in dry mass and root length, increasing the nutrient and water absorption areas, which contributed positively to the growth and development of bean plants and their tolerance to stresses against phytopathogens. The results of this work are consistent with those by Es-Sahm et al. (2024) and Mohammed et al. (2023) that describe *B. toyonensis* as being able to increase significantly stem length, number of leaflets, leaf area, and root biomass.

Conclusion and perspective

The findings of this study imply that *B. toyonensis* BAC3151 is a potential biocontrol agent as it inhibits white mold and reduces the severity of anthracnose in common bean. This

could be due to the production of enzymes that degrade the fungal cell wall, antifungal metabolites, and VOCs, the induction of a systemic plant response against phytopathogens, or a combination of these factors. In addition, it promotes plant growth by increasing the dry mass and length of bean roots through growth-promoting mechanisms.

However, controlling fungal diseases, particularly white mold, is costly and challenging because it manifests in the late plant development period. Few studies have been conducted on resistant cultivars. Based on the results obtained in this study, the ease of inoculation of *B. toyonensis* BAC3151 in common bean seeds and the efficiency of white mold control, we concluded that *B. toyonensis* BAC3151 may be of great significance for common bean crops.

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Authors' contributions Marisa Vieira de Queiroz conceived the project. Mirele Lopes da Silva and Bruna Caetano de Freitas performed the data analysis and interpretation of results under the supervision of Marisa Vieira de Queiroz, Maurício Dutra Costa, and José Eustáquio de Souza Carneiro. Mirele Lopes da Silva wrote the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not required.

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References

- Botta AL, Santacecilia A, Ercole C et al (2013) *In vitro* and *in vivo* inoculation of four endophytic bacteria on *Lycopersicon esculentum*. New Biotechnol 30(6):666–674
- Alaylar B (2022) Isolation and characterization of culturable endophytic plant growth-promoting *Bacillus* species from *Mentha longifolia* L. Turkish J Agric Forestry, 46(1)
- Andriunaitė E, Tamosiunė I, Aleksandravičiūtė M et al (2021) Stimulation of *Nicotiana tabacum* L. *In vitro* shoot growth by endophytic *Bacillus cereus* group bacteria. Microorganisms 9(9):1893
- Ayaz M, Li CH, Ali Q et al (2023) Bacterial and Fungal Biocontrol Agents for Plant Disease Protection: Journey from Lab to Field, Current Status, Challenges, and Global Perspectives. Molecules, 21;28(18):6735
- Bakki M, Banane B, Marhane O et al (2024) Phosphate solubilizing *Pseudomonas* and *Bacillus* combined with rock phosphates promoting tomato growth and reducing bacterial canker disease. Front Microbiol 15:1289466
- Chang CH, Yang SS (2009) Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. Bioresour Technol 100:1648–1658
- Chang WT, Chen ML, Wang SL (2010) An antifungal chitinase produced by *Bacillus subtilis* using chitin waste as a carbon source. World J Microbiol Biotechnol 26(5):945–950
- Checinska A, Paszczynski A, Burbank M (2015) *Bacillus* and other spore-forming genera: variations in responses and mechanisms for survival. Annu Rev Food Sci Technol 6(1):351–369
- Choubane S, Cheba BA, Benourrad A (2016) Screening and phenotypic diversity of amylase producing rhizospheric bacteria from some North African plants. Procedia Technol 22:1197–1204
- Contreras-Pérez M, Hernández-Salmerón J, Rojas-Solis D et al (2019) Draft genome analysis of the endophyte, *Bacillus toyonensis* COPE52, a blueberry (*Vaccinium* spp. var. Biloxi) growth-promoting bacterium. 3Biotech, 9(371)
- Costa LEO, Queiroz MV, Borges AC et al (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). Brazilian J Microbiol 43(4):1562–1575
- Dobereiner J (1995) Fixação biológica de nitrogênio. Brasília: Associação Brasileira de Educação Agrícola Superior, 1995. 54 p. (ABEAS- Curso de Agricultura Tropical – Módulo 2. Biologia e Recuperação dos Solos Tropicais – Módulo 2.2. Fixação Biológica de Nitrogênio)
- Es-Sahm I, Esserti S, Dich J et al (2024) Common *Bacillus* mitigate tomato verticillium wilt and bacterial specks when combined with an essential oil extract. Rhizosphere, 29;100865.
- Fagundes-Nacarath IRF, Debona D, Novaes MIC et al (2018) A set of standard area diagrams to assess white mold severity on the leaflets of common beans. Crop Prot 114:60–67
- FAO – Food and Agriculture Organization Accessed 10/06/2024 in Applied Soil Ecology
- Ferreira CMH, Vilas-Boas Â, Souza CA et al (2019) Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. AMB Express 9:78
- Fiers M, Lognay G, Fauconnier ML et al (2013) Volatile compound-mediated interactions between barley and pathogenic fungi in the soil. PLoS One. <https://doi.org/10.1371/journal.pone.0066805>
- Foughalia A, Hamiroune M, Chandeysson C et al (2024) Biocontrol potential of native Algerian bacteria isolated from greenhouses against *Botrytis cinerea* and *Oidium neolycopersici* on tomato. J Plant Dis Prot 131:847–861
- Huang Q, Liu H, Zhang J et al (2022) Production of extracellular amylase contributes to the colonization of *Bacillus cereus* 0–9 in wheat roots. BMC Microbiol 22:205
- Jiménez G, Urdiain M, Cifuentes A et al (2013b) Description of *Bacillus toyonensis* sp. nov., a novel species of the *Bacillus cereus* group, and pairwise genome comparisons of the species of the group by means of ANI calculations. Syst Appl Microbiol 36(6):383–391
- Jiménez G, Blanch AR, Tamames J et al (2013a) Complete Genome Sequence of *Bacillus toyonensis* BCT-7112 T, the Active Ingredient of the Feed Additive Preparation Toyocerin. Genome Announcements ASM 1(6):13
- Koçak R, Boyraz N (2024) Determination of *In Vitro* Efficacy of Some Bacterial Antagonists Against *Sclerotinia sclerotiorum* (Lib.) De Bary in Sunflowers. J Tekirdag Agricultural Fac, 21(2)
- Kumar S, Biswas SK, Kumar A et al (2024) Effect of Integrated Disease Management (IDM) Practices on Disease Severity and Incidence of Common Scab of Potato. Potato Res 67(1):271–292
- Li D, Li Y, Wang X et al (2023) Engineered pine endophytic *Bacillus toyonensis* with nematocidal and colonization abilities for pine wilt disease control. Front Microbiol 14:1240984
- Lopes R, Costa LEO, Vanetti MCD, Araújo EF, Queiroz MV (2015) Endophytic bacteria isolated from common bean (*Phaseolus vulgaris*) exhibiting high variability showed antimicrobial activity and quorum sensing inhibition. Curr Microbiol 71:509–516
- Lopes R, Cerdeira L, Tavares GS et al (2017) Genome analysis reveals insights of the endophytic *Bacillus toyonensis* BAC3151 as a potentially novel agent for biocontrol of plant pathogens. World J Microbiol Biotechnol 33(10):185
- Lorek H (1948) Production of hydrocyanic acid by bacteria. Physiol Plant 1(2):142–146
- Maheshwari DK (ed) (2010) Plant growth and health promoting bacteria, vol 18. Springer Science and Business Media
- Mckinney H (1923) Influence of soil, temperature and moisture on infection of wheat seedlings by *Helmonthosporium sativum*. J Agric Res 26:195–217
- Medison RG, Tan L, Medison MB et al (2022) Use of beneficial bacterial endophytes: A practical strategy to achieve sustainable agriculture. AIMS Microbiol 8(4):624–643
- Melo LC, Del Peloso MJ, Pereira HS et al (2010) BRS Estilo cultivar de grão tipo comercial carioca, com arquitetura de planta ereta associado com alto potencial produtivo. EMBRAPA
- Mohammed M, Badaluddin NA, Khandaker MM et al (2023) Potential of *Bacillus toyonensis* and *Paenibacillus alvei* as Plant Growth Promoter on Melon Manis Terengganu (MMT). Basra J Agricultural Sci, 36(2)
- Moraes WBC, Martins EMF, Musumeci MR et al (1976) Induced protection to *Hemileia vastatrix* in coffee plants. Summa Phytopathologica 2:39–43
- Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170(436):265–270
- Pastor-Corrales MA, Abawi GS (1987) Reactions of selected bean germ plasms to infection by *Fusarium oxysporum* f.sp. phaseoli. Plant Dis 71:990–993
- Patani A, Patel M, Islam S et al (2024) I. Recent advances in *Bacillus*-mediated plant growth enhancement: a paradigm shift in redefining crop resilience. World Journal of Microbiology and Biotechnology, 40(2):77
- Patel M, Vurukonda SSKP, Patel A (2023) Multi-trait halotolerant plant growth-promoting bacteria mitigate induced salt stress and enhance growth of *Amaranthus viridis*. J Soil Sci Plant Nutr 23(2):1860–1883

- Pereira MJZ, Ramalho MAG, Abreu AFB (2011) Reação de linhagens de feijoeiro ao fungo *Fusarium oxysporum* f. sp. *phaseoli* em condições controladas. *Cienc Agrotecnol* 35:940–947
- Portieles R, Xu H, Yue Q et al (2021) Heat-killed endophytic bacterium induces robust plant defense responses against important pathogens. *Sci Rep* 11(1):12182
- Ramos APS (2016) Estudo de bactérias potencialmente promotoras de crescimento em *Citrus* sp. Tese (Doutorado)- Programa de Pós-Graduação em Ciência. do Solo da Universidade Federal Rural de Pernambuco, Recife
- Rath M, Mitchell TR, Gold SE (2018) Volatiles produced by *Bacillus mojavensis* RRC101 act as plant growth modulators and are strongly culture-dependent. *Microbiol Res* 208:76–84
- Roca A, Cabeo M, Enguidanos C et al (2024) Potential of the quorum-quenching and plant-growth promoting halotolerant *Bacillus toyonensis* AA1EC1 as biocontrol agent. *Microb Biotechnol* 17(3):e14420
- Rojas-Solís D, Vences-Guzmán MA, Sohlenkamp C et al (2020) *Bacillus toyonensis* COPE52 modifies lipid and fatty acid composition, exhibits antifungal activity, and stimulates growth of tomato plants under saline conditions. *Curr Microbiol* 77:2735–2744
- Sabaté DC, Petroselli G, Erra-Balsells et al (2020) Beneficial effect of *Bacillus* sp. P12 on soil biological activities and pathogen control in common bean. *Biol Control* 141:104131
- Santos JF, Sacramento BL, Mota KNAB et al (2014) Crescimento de girassol em função da inoculação de sementes com bactérias endofíticas. *Pesquisa Agropecuária Trop (Agricultural Res Tropics)* 2(1):10–15
- Sartorato A, Rava CA (1994) Principais doenças do feijoeiro comum e seu controle. EMBRAPA
- Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160:47–56
- Shan Y, Wang D, Zhao F-H et al (2024) Insights into the biocontrol and plant growth promotion functions of *Bacillus altitudinis* strain KRS010 against *Verticillium dahliae*. *BMC Biol* 22(1):116
- Shaner G, Finney RE (1997) The effect of nitrogen fixation fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67(8):1051–1056
- Shin J-H, Lee H-K, Lee S-C et al (2023) Biological control of *Fusarium oxysporum*, the causal agent of fusarium basal rot in onion by *Bacillus* spp. *Plant Pathol J* 39(6):600–613
- Shurigin V, Alimov J, Davranov K et al (2022) The diversity of bacterial endophytes from *Iris pseudacorus* L. and their plant beneficial traits. *Curr Res Microb Sci* 3:100133
- Slama HB, Cherif-Silini H, Chenari BA et al (2019) Screening for *Fusarium* antagonistic bacteria from contrasting niches designated the endophyte *Bacillus halotolerans* plant warden against fusarium. *Front Microbiol* 10:1–24
- Souza AES, Filla VA, Silva JPM et al (2023) Application of *Bacillus* spp. phosphate-solubilizing bacteria improves common bean production compared to conventional fertilization. *Plants* 12(22):3827
- Tolêdo-Souza ED, Costa JLS (2003) Métodos de inoculação de plântulas de feijoeiro para avaliação de germoplasma quanto a resistência a *Sclerotinia sclerotiorum*. *Pesqui Agropecu Trop* 33(2):57–63
- Vale FXR, Liberato JR, Maffia LA et al (2001) Quant: quantificação de doenças. Versão 1.0.1. UFV, Viçosa
- Wang N, Hatcher DW, Tyler RT et al (2010) Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Res Int* 43(2):589–594
- Wong JH, Hao J, Cao Z et al (2008) An antifungal protein from *Bacillus amyloliquefaciens*. *J Appl Microbiol* 105(6):1888–1898
- Yanti Y, Hamid H, Yaherwandi R (2023) The ability of chitinolytic bacteria to control *Colletotrichum capsici* in Chili Plants. *IOP Conference Series: Earth and Environmental Science*, 1228
- Yokoyama LP, Del Peloso MJ, Di Stefano JG et al (1999) Nível de aceitabilidade do cultivar de feijão Pérola: avaliação preliminar. Embrapa Arroz e feijão
- Yousuf J, Thajudeen J, Rahiman M et al (2017) Nitrogen fixing potential of various heterotrophic *Bacillus* strains from a tropical estuary and adjacent coastal regions. *J Basic Microbiol* 57(11):922–932

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