

Screening of bacteria to control *Sclerotinia sclerotiorum*, the common-bean white-mold causal agent

Seleção de bactérias para o controle de *Sclerotinia sclerotiorum* agente causal do mofo-branco em feijão

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ABSTRACT - *Sclerotinia sclerotiorum*, a fungus that causes white-mold, is an important hard-to-control phytopathogen of several commercially valuable crops. It is necessary to search for sustainable tools for the management of this pathogen with the objective of achieving sustainability in the field. So, the research objective was to prospect bacteria for the biocontrol of this disease. A strain of *S. sclerotiorum* (VPB015) was used as phytopathogen. In total, 26 soil bacterial strains obtained from different collections were used, and 23 strains showed antagonistic action against fungus; of these, six produced thermostable metabolites with inhibitory action against the pathogen. Production of volatile compounds by the bacteria was not observed. The GN01 strain, identified as *B. velezensis*, decreased the disease severity in common-bean seedlings in soil infested with *S. sclerotiorum*. Five strains decreased disease severity in detached leaves. *B. velezensis* GN01 showed potential in disease control by seed treatment.

RESUMO - *Sclerotinia sclerotiorum*, fungo causador do mofo-branco, é um importante fitopatógeno de difícil controle em muitas culturas de valor comercial. É necessária a busca por ferramentas sustentáveis para o manejo desse patógeno com objetivo de atingir a sustentabilidade no campo. Assim, o objetivo da pesquisa foi prospectar bactérias para o biocontrole da doença. Foi utilizado um isolado de *S. sclerotiorum* (VPB015) como fitopatógeno. Portanto, foram utilizadas 26 cepas bacterianas obtidas de diferentes coleções, e 23 apresentaram ação antagonista contra fungo. Destas, seis produziram metabólitos termooestáveis com ação inibitória ao patógeno. Não foi constatada a produção de compostos voláteis pelas bactérias. O isolado GN01, identificado como *B. velezensis* diminuiu a incidência da doença em plântulas de feijão em solo infestado com *S. sclerotiorum*. Cinco cepas diminuíram a severidade da doença em folhas destacadas. O *B. velezensis* GN01 apresentou potencial no controle da doença pelo tratamento de semente de feijoeiro.

Keywords: Antagonism. *Bacillus*. Metabolites. *Paenibacillus*. Phytopathogen.

Palavras-chave: Antagonismo. *Bacillus*. Metabólitos. *Paenibacillus*. Fitopatógeno.

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INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of white-mold in several crops, is a phytopathogenic fungus that infects more 500 plants species in 75 families. It has agricultural importance for causing disease in commercially valuable crops, such as soybean, bean, cotton, and some vegetables, such as Cucurbitaceae, Brassicaceae and Solanaceae species (SAHARAN; MEHTA, 2008). This pathogen has in its life cycle a resistance structure called sclerotium, which can survive in soil for many years (SAHARAN; MEHTA, 2008). The ability to produce resistant sclerotia and to infect different plant species makes this pathogen difficult to manage.

One of the main management practices of white-mold continues to be chemical control (MEYER et al., 2025). Although there are advantages, the excessive use of agrochemicals can bring on harm such as harmful effects on human health (GARCIA; LARA, 2020) and environmental impacts (PEREIRA; COSTA; LIMA, 2019). Furthermore, there are reports on efficiency loss of some molecules, resulting from the selection of resistant individuals (CORKLEY; FRAAIJE; HAWKINS, 2022). In the study presented by Meyer et al. (2025), chemical products achieved between 47% and 66% of efficiency in white-mold management. Despite this efficiency, other practices must be adopted (MEYER et al., 2025).

Another alternative to minimize the harmful effects of diseases is biological control, which is essential for sustainable agriculture, preserving ecosystem diversity and health (LEE et al., 2023). In addition, the biocontrol agents are sustainable and efficient in the management of several phytopathogens (FATOUROS et al., 2018; MELO; SERRA, 2021; VILLARREAL-DELGADO et al., 2021).

Of the biocontrol agents, two genera stand out in *S. sclerotiorum* management, *Bacillus* and *Paenibacillus*. Among *Bacillus*, it is possible to

mention species such as *B. subtilis*, *B. amyloliquefaciens* and others (AYAZ et al., 2024, SANTOS et al., 2023). *Paenibacillus alvei* has been reported in the control of *S. sclerotiorum* and other soil fungi, such as *Pythium* sp. and *Rhizoctonia* sp. (FATOUROS et al., 2018). These bacteria can produce antimicrobial metabolites that inhibit fungal growth (SANTOS et al., 2023) and compete for space and nutrients (THAMPIBAN-UDOM et al., 2018). However, more studies are necessary, as according to Lee et al. (2023) more research and development of biocontrol agents are essential for the future of sustainable agriculture.

The study objective was to prospect and to screen bacterial strains in *S. sclerotiorum* biocontrol *in vitro* and to evaluate the effects of their application on the control of the pathogen in detached leaf and seedlings of common-beans.

MATERIAL AND METHODS

Obtaining strains

The *Sclerotinia sclerotiorum* strain (VPB015) was

Table 1. Origin of the bacterial strains used in the experiments.

Strain	Region	Institution	Strain	Region	Institution
GA155	South of Minas	VIVA	B05A	Alto Paranaíba	LAMAM
GA156	South of Minas	VIVA	17AN673	Alto Paranaíba	LAMAM
GA157	South of Minas	VIVA	GN01	Alto Paranaíba	GENEB
GA158	South of Minas	VIVA	GN16	Alto Paranaíba	GENEB
GA159	South of Minas	VIVA	GN29	Alto Paranaíba	GENEB
GA160	South of Minas	VIVA	GN109	Alto Paranaíba	GENEB
GA161	South of Minas	VIVA	GN110	Alto Paranaíba	GENEB
GA162	South of Minas	VIVA	GN120	Alto Paranaíba	GENEB
GA163	South of Minas	VIVA	GN130	Alto Paranaíba	GENEB
BC04	Alto Paranaíba	LAMAM	GN149	Alto Paranaíba	GENEB
B04	Alto Paranaíba	LAMAM	GN151	Alto Paranaíba	GENEB
AN672 B04	Alto Paranaíba	LAMAM	GN154	Alto Paranaíba	GENEB
B05	Alto Paranaíba	LAMAM	GN164	Alto Paranaíba	GENEB

Bacteria screening for Dual Culture Assay

This research step was performed in the R&D Laboratory of VIVA. Antagonistic bacteria were screened using the Dual Culture assay, adapting the methodology described by Sabaté et al. (2018). Petri plates with PDA medium + Nutrient Agar (NA) in the proportion of 1:1 [Medium composition: PDA 39 g; NA (20 g of Agar KASVI, 3 g of beef extract, 5 g of peptone, 9 g of sodium chloride L⁻¹)] were used. One mycelial plug (5 mm) of of seven days old fungus was deposited at 10 mm from the plate edge. On the opposite side, three streaks containing bacteria with seven days of cultivation were made at 10 mm from the edge. In the control treatment, only the fungus was placed on the plate. Two additional treatments were included as positive controls, consisting of products registered with the Brazilian Ministry of Agriculture and Livestock (MAPA). Biological product I contained *B. subtilis*, *B. velezensis*, and *B. pumilus* (4.6×10^8 viable spores mL⁻¹),

obtained by the collection of sclerotia in soybean crop (*Glycine max*) in the municipality of Carrancas, MG, in January 2024. The sclerotia were taken to the laboratory [Research and Development (R&D), Viva Soluções Biológicas LTDA (VIVA), Machado, MG] to obtain mycelium. Initially, the sclerotia were disinfected for one minute in a hypochlorite solution (1%), then kept for one minute in alcohol (70%), washed in distilled water, autoclaved, and dried on sterilized filter paper. After disinfection, the sclerotia were placed in Petri dishes (90 mm) with Potato Dextrose Agar medium [39 g L⁻¹ (PDA, KASVI)], taken to an incubator (ALFAMARE AM 5010) and kept for seven days with 25 °C and 12 hours of photoperiod.

In total, 26 bacterial strains were used as antagonists in the study, provided by three institutions: the VIVA company, Laboratory of Genetics and Biotechnology (GENEB) from the University Center of Patos de Minas (UNIPAM); and Minas Agricultural and Microbial Analysis Laboratory Ltda. (LAMAM). The strains were isolated from soil, in the Alto Paranaíba and South of Minas regions, in the State of Minas Gerais, Brazil, according to Table 1.

and Biological product II contained *Bacillus velezensis* (3×10^9 CFU mL⁻¹), according to the product labels. The plates were incubated (ALFAMARE AM 5010) at 25 °C for seven days. Evaluations were based on inhibition halo size and fungal colony diameter, measured in millimeters. Growth inhibition percentage (I%) was calculated using the formula: $I = [(90 - CS)/90] \times 100$, where 90 represents the plate diameter (mm) and CS represents colony size. The experiment consisted of 29 treatments with three replicates arranged in a completely randomized design. Each experimental unit consisted of one Petri dish.

Multiplication and Sporulation Assay

For the multiplication and sporulation assay, the 19 strains that showed the greatest fungal inhibition in the previous assay were selected. In 250-mL Erlenmeyer flasks, 75 mL of Luria Bertani medium (LB, KASVI) at 25 g L⁻¹ were added, corresponding to 30% of flask capacity, and

autoclaved at 121 °C for 30 minutes. After cooling, bacterial colonies grown on solid NA medium were inoculated into the flasks. After inoculation, the flasks were sealed and incubated in an orbital shaker at 190 rpm for 72 h at 28 °C. Subsequently, fermented cultures were evaluated for endospore formation using a microscope (OLYMPUS CX43RF). Sporulating strains were selected for subsequent assays.

Thermostable Metabolite Assay

For the thermostable metabolite (TSMs) assay, 2 mL of each of the 16 bacterial fermentation broths were autoclaved at 121 °C for 30 min. On PDA medium, a mycelial plug of fungal strain VPB015 was placed at 10 mm from the edge of the Petri dish, and 100 µL of the autoclaved fermentation broth was added to the opposite edge. The control treatment consisted only of the fungal plug, and an additional treatment containing only autoclaved uninoculated liquid medium was included to verify the presence of any antifungal components in the culture medium. Three replicates were prepared and incubated in a growth chamber (INCB, ALFAMARE) at 25 °C for 17 days. Mycelial growth inhibition was assessed qualitatively. As a selection criterion for subsequent assays, only bacterial strains that consistently inhibited fungal growth in all replicates were selected.

To quantify the mycelial growth inhibition caused by thermostable metabolites (TMs), six bacterial strains that showed consistent inhibition in the previous assay were selected. PDA medium amended with the fermentation broth of each strain at concentrations of 10% and 20% (v/v) was prepared. For the 10% concentration, 39 g of PDA (KASVI) were dissolved in 900 mL of distilled water and supplemented with 100 mL of fermentation broth. For the 20% concentration, 39 g of PDA (KASVI) were dissolved in 800 mL of distilled water and supplemented with 200 mL of fermentation broth. The media were homogenized, autoclaved at 121 °C for 30 min, and poured into 90-mm-diameter Petri dishes. A 5-mm-diameter mycelial plug taken from a seven days old culture of the fungal pathogen was placed at the center of each plate. Control plates consisted of PDA medium without fermentation broth. The plates were sealed and incubated in a growth chamber (ALFAMARE AM 5010) at 25 °C for 20 days. After the incubation period, colony diameters were measured and the percentage of inhibition was calculated using the formula: $I\ (%) = [(C - T)/C] \times 100$, where I represents the percentage of inhibition, C is the colony diameter in the control treatment, and T is the colony diameter in the treatment. The experiment was conducted in a 6 × 2 factorial arrangement, consisting of six bacterial strains and two fermentation broth concentrations (10% and 20%), in a completely randomized design. Each experimental unit consisted of one Petri dish, with five replicates per treatment. The experiment was performed twice.

Volatile Organic Compounds (VOCs) Assay

The assay for volatile organic compounds (VOCs) was performed following an adapted methodology described by Rahman et al. (2016). The six bacterial strains selected in the previous assay were used. Bipartite Petri dishes containing PDA medium on one side and nutrient agar (NA) medium on the other were prepared. A 5-mm-diameter mycelial plug of

the fungal pathogen was placed at the center of the PDA compartment, while 50 µL of bacterial suspension was deposited at the center of the NA compartment. In the control treatment, only the fungal plug was placed on PDA medium. The plates were sealed and incubated in a growth chamber (ALFAMARE AM 5010) at 25 °C for 7 days. After the incubation period, fungal growth inhibition was assessed qualitatively. Each experimental unit consisted of one Petri dish, with five replicates per treatment. The assay was repeated three times.

Sclerotial Germination Inhibition Assay

To determine the inhibition of sclerotial germination, the methodology described by Rodrigues and Cunha (2021) was adapted. Surface-disinfected sclerotia were submerged in solutions containing the respective treatments. A total of 10 treatments were evaluated, including a control, three commercial products registered with the Brazilian Ministry of Agriculture and Livestock (MAPA) (two biological products and one chemical fungicide), and six bacterial strains. The treatments consisted of: T1, control (sterile distilled water); T2, Biological Product I (*Bacillus subtilis*, *Bacillus velezensis*, and *Bacillus pumilus*; 4.6×10^8 viable spores mL⁻¹); T3, chemical fungicide (thiophanate-methyl 37.5% + fluazinam 37.5%; 10 g L⁻¹); T4, strain 17AN673; T5, strain GN01; T6, strain GN130; T7, strain GA155; T8, strain GA157; T9, strain GA163; and T10, Biological Product II (*Trichoderma harzianum* IB19/17; 1×10^{10} CFU g⁻¹, applied at 2 g L⁻¹). All bacterial fermentation broths were adjusted to a concentration of 1.0×10^8 CFU mL⁻¹. Five sclerotia were placed on PDA medium in each Petri dish. The plates were sealed with plastic film and incubated in a growth chamber (ALFAMARE AM 5010) at 25 °C. After 20 days, the number of germinated sclerotia was recorded. The experiment was conducted in a completely randomized design with four replicates, with each Petri dish representing one experimental unit.

A second assay was conducted to assess the viability of non-germinated sclerotia obtained from the previous experiment to determine their viability. Seven treatments containing bacterial strains from the previous assay were evaluated: T1, Biological Product I (*Bacillus subtilis*, *Bacillus velezensis*, and *Bacillus pumilus*; 4.6×10^8 viable spores mL⁻¹); T2, strain 17AN673; T3, strain GN01; T4, strain GN130; T5, strain GA155; T6, strain GA157; and T7, strain GA163. The sclerotia were surface-disinfected by immersion in sodium hypochlorite solution (5%), followed by ethanol (70%), and subsequently rinsed with sterile distilled water. After drying, the sclerotia were transferred to PDA plates following the same procedure described in the previous assay. After 7 days of incubation, the number of germinated sclerotia was recorded. Each Petri dish containing the sclerotia represented one experimental unit.

Taxonomic Identification

The six bacterial strains that showed positive results in the previous assays were subjected to phylogenetic characterization for species identification. The strains were subcultured on nutrient agar (NA) plates and sent to a specialized laboratory for molecular analysis. Partial sequencing of the 16S rRNA gene was performed for taxonomic identification. The obtained sequences were

compared using the BLAST (Basic Local Alignment Search Tool) platform. Only type-strain sequences were considered for comparison to ensure greater accuracy in taxonomic identification.

Sequence alignments were performed using the ClustalW software (THOMPSON et al., 1994). Phylogenetic trees were constructed using three different methods: Neighbor-Joining (SAITOU; NEI, 1987), Maximum Parsimony, and UPGMA (Unweighted Pair Group Method with Arithmetic Mean). The Jukes–Cantor evolutionary model (JUKES; CANTOR, 1969) was applied in all analyses. The robustness of the phylogenetic trees was assessed through bootstrap analysis with 1,000 replicates, providing a statistical estimate of the reliability of the resulting clusters. The obtained results enabled the partial identification of the bacterial strains based on sequence similarity and their phylogenetic positioning relative to type-strain sequences available in public databases.

Biocontrol of white-mold in common-bean under infested soil conditions

Pathogen inoculum production and soil infestation were performed following an adaptation of the methodology described by Sabaté et al. (2018). Briefly, 200 g of parboiled rice moistened to 30% moisture content with distilled water were placed in autoclavable plastic bags and sterilized at 121 °C for 30 min. After cooling, ten 5-mm-diameter PDA plugs containing actively growing fungal mycelium were transferred to each bag. The cultures were incubated at 22–28 °C for 10 days, with the rice substrate manually shaken every two days to ensure uniform colonization. The soil used in the experiment was collected from a cereal production area in the municipality of Lavras, Minas Gerais, Brazil, and classified as a Red-Yellow Oxisol. The soil was sterilized at 121 °C for 30 min. Colonized rice was incorporated into the soil at a rate of 0.5% (w/w) and manually homogenized. After soil infestation, 100 seeds were sown per tray, arranged into four blocks of 25 seeds each. Seeds of Pérola Dama-type common-bean were used. The seeds received the following treatments: T1, negative control; T2, chemical treatment (carboxin 20% + thiram 20%); T3, biological product (*Bacillus subtilis*, *Bacillus velezensis*, and *Bacillus pumilus*; 4.6×10^8 viable spores mL⁻¹); T4, strain 17AN673 (9.46×10^8 CFU mL⁻¹); T5, strain GN01 (9.6×10^8 CFU mL⁻¹); T6, strain GN120 (8.7×10^8 CFU mL⁻¹); T7, strain GA155 (1.3×10^8 CFU mL⁻¹); T8, strain GA157 (6.7×10^8 CFU mL⁻¹); T9, strain GA163 (9.1×10^8 CFU mL⁻¹), all applied at a rate of 300 mL per 100 kg of seeds; and T10, positive control, without pathogen infestation or seed treatment. The trays were maintained under laboratory conditions at Viva, with temperatures ranging from 22 to 28 °C.

Evaluations were conducted daily until 15 days after sowing. The number of emerged plants, symptomatic plants, dead plants, non-emerged seedlings, and seeds colonized by the pathogen were recorded. Disease incidence was calculated as a percentage using the following formula: $I = S \times 100 / N$, where I is the disease incidence (%), S is the number of dead plants, symptomatic plants, non-emerged seedlings, and seeds showing pathogen colonization, and N is the total number of seeds sown, adapted from Sabaté et al. (2018). The experiment was arranged in a completely randomized design (CRD) with ten treatments and four replicates. Each

experimental unit consisted of 25 seeds.

S. sclerotiorum biocontrol in detached leaves

To determine the efficiency of bacterial strains in controlling lesions on common-bean leaves, the methodology described by Ayaz et al. (2024) was adapted. The first trifoliate leaves were collected from 24-day-old common-bean plants grown in plastic pots. The leaves were washed and allowed to dry on three sheets of paper towel. In 90-mm-diameter Petri dishes, two sheets of Germitest paper were placed and moistened with 1 mL of sterile distilled water. The leaves were positioned on the paper with a small piece of cotton attached to the petiole and subsequently maintained moist according to the moisture level of the Germitest paper.

Treatments were applied using micropipettes, with 50 µL per leaf, and spread uniformly with a Drigalski spatula. The treatments consisted of: T1, distilled water; T2, chemical control (Thiophanate-methyl 37.5% + Fluazinam 37.5%, 10 g L⁻¹); T3, biological control (*Bacillus subtilis*, *Bacillus velezensis*, and *Bacillus pumilus*, 4.6×10^8 viable spores mL⁻¹); T4, strain 17AN673; T5, strain GN01; T6, strain GN120; T7, strain GA155; T8, strain GA157; T9, strain GA163; and T10, non-inoculated control. Bacterial suspensions were applied at the same concentrations used in the previous assay. Treatments 2 and 3 are registered with the Brazilian Ministry of Agriculture and Livestock (MAPA) for disease control.

Following treatment application, the pathogen was inoculated by placing a 5-mm mycelial plug at the center of each leaf. Petri dishes were sealed with plastic film and incubated in a growth chamber (ALFAMARE AM 5010) at 25 °C. Evaluations were performed five days after pathogen inoculation (DAI) by assessing disease severity through digital photographs analyzed using the Pliman package in RStudio Team version 4.4.1 (2025). Images were processed to identify lesion areas, healthy tissue, and background. The software quantified the lesion area and expressed the results as the percentage of diseased leaf area relative to the total leaf area.

The experiment was conducted in a completely randomized design with nine treatments and five replicates, with each individual leaf considered an experimental unit.

Statistical analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using the F-test ($P \leq 0.01$), except for the biocontrol assay in infested soil, which was analyzed at a significance level of $P \leq 0.05$. Residual normality was assessed using the Shapiro–Wilk test, and data were transformed when necessary to meet the assumptions of the analysis. Treatment means were compared using the Scott–Knott clustering test. All statistical analyses were performed using RStudio Team version 4.4.1 (2025).

RESULTS AND DISCUSSION

In the dual culture assay, 11 of the 26 bacterial strains evaluated (25 *Bacillus* spp. and one *Paenibacillus* sp.) GN130, GN109, GA159, GA155, GA158, GN01, GA156, B05A, GA157, GN120, and GA163 showed performance

similar to that of the two positive controls (Biological Products I and II), significantly inhibiting the pathogen and achieving inhibition rates ranging from 26.77% to 34.85% (Table 2). Among these 11 strains, ten belonged to the genus *Bacillus* and one to the genus *Paenibacillus*.

An additional 12 strains (AN672B04, GN151, GN110, GN29, GN16, GA162, GA161, GA154, BC04, GN164, 17AN673, and GA160) showed intermediate performance, resulting in significantly greater inhibition than the negative control, with inhibition rates ranging from 20.29% to 26.33% (Table 2). Although these strains were less effective than the commercial products in this assay, some demonstrated promising results in other experiments.

These findings are consistent with those reported by Ribeiro et al. (2021), who evaluated 28 bacterial strains, including 24 *Bacillus* spp. and four *Paenibacillus* spp., of which only five *Bacillus* strains exhibited antifungal activity against *Sclerotinia sclerotiorum*. This demonstrates that,

despite the diversity of *Bacillus* species investigated as antagonists, not all possess antifungal properties. *Paenibacillus alvei* has also been reported to inhibit the in-vitro growth of *S. sclerotiorum* (FATOUROS et al., 2018). It is possible that these strains harbor gene clusters associated with the production of antimicrobial metabolites. Ribeiro et al. (2021) reported that *Bacillus* strains associated with the control of *S. sclerotiorum* contained gene clusters involved in the biosynthesis of bacilysin and lichenysin, the latter being related to surfactin production. Inhibition of mycelial growth may be associated with the production of secondary metabolites such as fengycin, iturin, surfactin, and bacillomycin (SANTOS et al., 2023). Further investigation of strains such as these is necessary to improve our understanding of their mechanisms of action, metabolite production, and other characteristics related to biological control.

Table 2. Effect of bacterial strains on mycelial growth inhibition (%) and inhibition zone diameter (mm) of *Sclerotinia sclerotiorum* in a dual culture assay on Petri dishes.

Treatment	Mycelial Growth Inhibition (%)	Inhibition Zone Diameter (mm)
Control	0.00 d*	0.00 d
Biological I**	27.70 a	9.39 c
Biological II***	34.85 a	11.17 a
17AN673	26.08 b	12.18 b
AN672B04	20.29c	11.67b
B04	0.00 d	0.00 d
B05	0.00 d	0.00 d
B05A	29.91 a	13.69 a
BC04	25.20 b	11.60 c
GA155	28.38 a	14.09 a
GA156	29.73 a	13.77 a
GA157	29.99 a	14.33 a
GA158	28.92 a	13.98 a
GA159	28.37 a	11.19 a
GA160	26.33 b	10.33 c
GA161	24.08 b	8.76 c
GA162	24.04 b	9.11 c
GA163	31.67 a	14.59 a
GN01	28.95 a	14.20 a
GN16	23.86 b	10.07 c
GN29	22.40 c	9.12 c
GN109	26.94 a	9.34 c
GN110	21.94 c	9.08 c
GN120	30.62 a	14.99 a
GN130	26.77 a	11.90 b
GN149	0.00 d	0.00 d
GN151	21.98 c	6.57 c
GN154	24.84 b	10.54 c
GN164	25.30 b	8.93 c

*Means followed by the same letter within a column do not differ significantly according to the Scott-Knott test at $P \leq 0.01$. Data were transformed using the arcsine square-root transformation [$\arcsin\sqrt{(x + 0.5)/100}$].

**Commercial product registered with MAPA for disease control (mixture of three *Bacillus* spp., 4.6×10^8 viable spores mL⁻¹).

***Commercial product registered with MAPA for disease control (*Bacillus velezensis*, 3×10^9 CFU mL⁻¹).

Regarding the inhibition halo diameter (mm), thirteen strains (17AN673, AN672B04, BC04, B05A, GN01, GN120, GN130, GA155, GA156, GA157, GA158, GA159, and GA163) produced the largest inhibition zones, ranging from 11.60 to 14.99 mm, and differed significantly from all other treatments, including the commercial biological control products. Biological Products I and II showed intermediate results, together with 11 strains, and were significantly different from the negative control according to the Scott–Knott test ($P \leq 0.01$). The negative control and three strains did not produce inhibition halos (Table 2).

The inhibition halo represents a “clear zone” in which no microbial growth is observed. According to Thampiban-Udom et al. (2018), the formation of this zone is associated with the production of antimicrobial substances that inhibit fungal mycelial growth, thereby indicating an antibiosis mechanism. Although some strains exhibited high levels of pathogen growth inhibition, they produced relatively small inhibition halos compared with other strains (GA160, GA161, GA162, GN16, GN109, GN154, and GN164). In these cases, pathogen suppression may be more closely associated with competition, particularly for space, rather than antibiosis. Similar results were reported by Onaran and Yanar (2011),

who observed inhibition halos ranging from 16 to 20 mm for *Bacillus* sp. and *Paenibacillus* sp. strains in dual culture assays against *Sclerotinia sclerotiorum*.

Regarding the multiplication and sporulation assay, 16 of the 19 strains evaluated were able to produce endospores in Luria-Bertani (LB) broth (Table 3). Elisashvili, Kachlishvili, and Chikindas (2019) suggested that sporulation is influenced by both common and species-specific characteristics. The production of spores at high concentrations is a complex process, and individual strain development as well as bioprocess optimization are required for each specific strain (BIERMANN; BEUTEL, 2023).

A qualitative assessment revealed that 14 autoclaved bacterial fermentates (17AN673, B04, B05, GA155, GA157, GA159, GA160, GA161, GA162, GA163, GN01, GN16, GN19, and GN120) inhibited the mycelial growth of *Sclerotinia sclerotiorum* in at least one replicate (Table 3). However, only strains that consistently inhibited pathogen growth in all replicates were selected for subsequent assays (17AN673, GN01, GA157, GN120, GA163, and GA155). These strains were identified as *Bacillus subtilis*, *Bacillus velezensis*, *Bacillus amyloliquefaciens*, two *Bacillus siamensis* strains, and one *Paenibacillus polymyxa* strain, respectively.

Table 3. Sporulation of bacterial strains in Luria–Bertani (LB) broth and their ability to inhibit *Sclerotinia sclerotiorum* through the production of thermostable metabolites (TSMs).

Strains	Sporulation	TSMs Inhibition
Control		-
17AN673	+	+++**
B05A	+	++-
BC04	+	+--
GA155	+	+++
GA156	+	---
GA157	+	+++
GA158	-	
GA159	+	---
GA160	+	+--
GA161	+	+--
GA162	+	+--
GA163	+	+++
GN01	+	+++
GN109	+	+--
GN120	+	+++
GN130	-	
GN154	-	
GN16	+	+--
GN164	+	---

*The symbol (+) indicates sporulation, whereas the symbol (–) indicates no sporulation.

**The symbol (---) indicates that thermostable metabolites (TSMs) did not inhibit pathogen growth; (+--) indicates inhibition without the formation of an inhibition zone; (++-) indicates inhibition with an inhibition zone up to 5 mm; and (+++) indicates inhibition with an inhibition zone greater than 5 mm.

In the second experiment, which evaluated the effect of TSMs concentrations on fungal mycelial growth, all treatments achieved inhibition levels above 87%, demonstrating that the metabolites retained their antifungal activity after autoclaving and were therefore thermostable (Figure 1). No significant interaction was observed between the factors concentration and bacterial strain. For the

concentration factor, no significant differences were detected among treatments. However, significant differences were observed among bacterial strains. Strains GA155 and GA163 were significantly more effective than the others, exhibiting mycelial growth inhibition of 91.8% and 90.9%, respectively (Table 4).

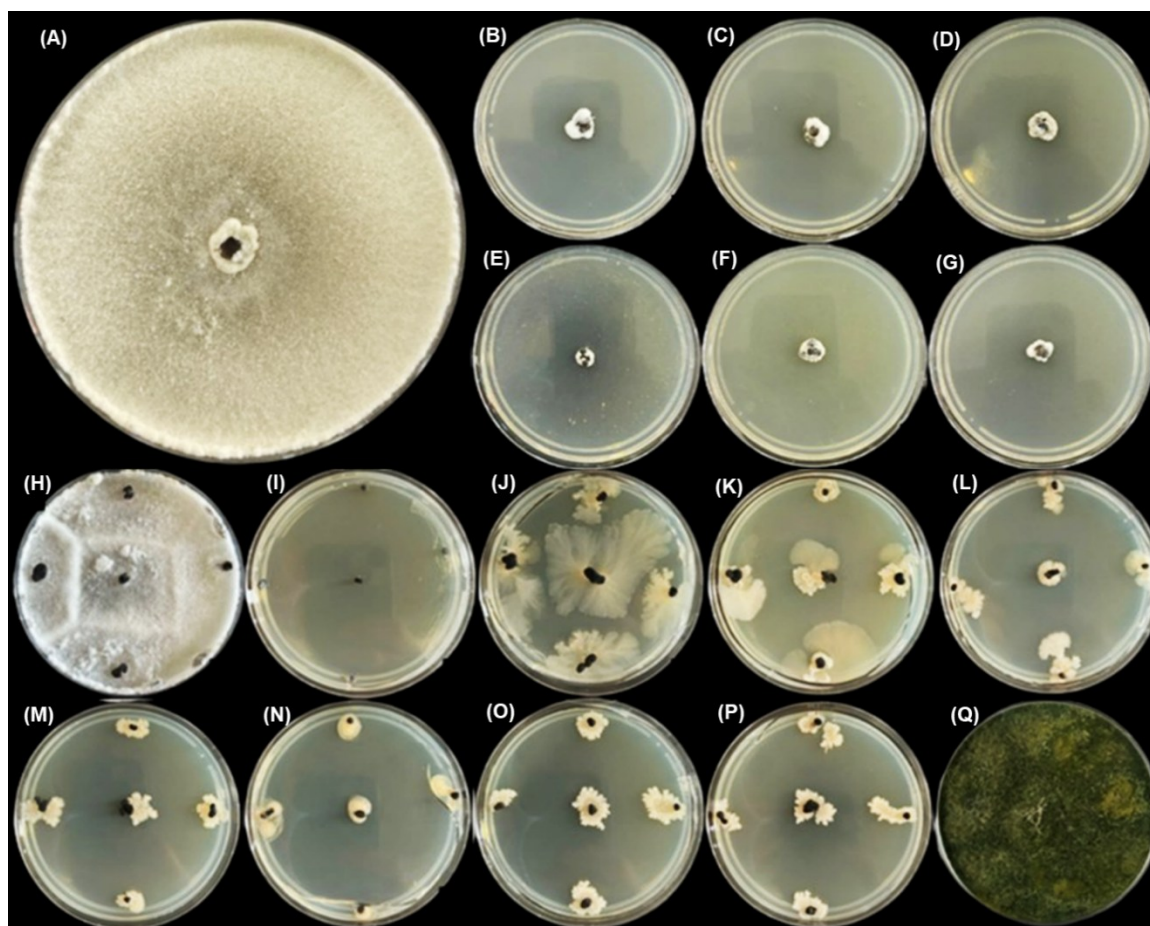


Figure 1. (A-G) *Sclerotinia sclerotiorum* colonies on PDA medium amended with bacterial fermentates. (A) Control; (B) 17AN673 (C) GN01; (D) GN120; (E) GA155; (F) GA157; (G) GA163. (H-Q) *Sclerotinia sclerotiorum* on Petri dishes containing potato dextrose agar (PDA), subjected to different treatments. (H) Control; (I) Thiophanate-methyl + Fluazinam; (J) Mixture of *Bacillus* sp.; (K) 17AN673; (L) GN01; (M) GN130; (N) GA155; (O) GA157; (P) GA163; (Q) *Trichoderma harzianum*.

Table 4. Mycelial growth inhibition of *Sclerotinia sclerotiorum* on Petri dishes containing potato dextrose agar (PDA) amended with autoclaved fermentates of different bacterial strains at two concentrations (10% and 20%) after 20 days of incubation.

Treatment	Inhibition (%)
GN120	87.8b*
17AN673	88.2b
GN01	89.4b
GA157	89.4b
GA163	90.9a
GA155	91.8a
CV (%)	2.0

*Means followed by the same letter within a column do not differ significantly according to the Scott-Knott test at $P \leq 0.01$.

The inhibitory activity promoted by thermostable metabolites produced by bacteria has been reported recently by Mian et al. (2024), who demonstrated that autoclaved metabolites from *Bacillus velezensis* Ag109 inhibited pathogen growth by 55.9%. Likewise, culture filtrates of *Paenibacillus elgii* were able to suppress the growth of ten phytopathogens, including *Sclerotinia homoeocarpa* (KIM et al., 2020). The control of *Sclerotinia sclerotiorum* through bacterial metabolites has also been previously reported (CAO et al., 2023). Metabolites such as fengycin, iturin, surfactin, and bacillomycin are commonly associated with antifungal activity (SANTOS et al., 2023); however, it is not possible to confirm the presence of these compounds in the fermentates used in the present study.

None of the strains exhibited inhibitory activity against mycelial growth through the production of volatile organic compounds (VOCs). After seven days of incubation, all Petri dishes were completely colonized by the fungus, similarly to the control treatment. Santos et al. (2023) demonstrated that different *Bacillus* strains may or may not produce the same volatile compounds and that these compounds may or may not be associated with pathogen suppression. Rahman et al. (2016) reported variable levels of suppression of *S. sclerotiorum* growth by VOCs produced by eight *Bacillus* spp. strains. Huang et al. (2018) further demonstrated that both the amount of volatile compounds produced by *Bacillus mycoides* and its antagonistic activity against pathogens may vary depending on the culture medium used. Therefore, the production of volatile compounds and their biological effects are strain-dependent characteristics and are strongly influenced by the growth medium.

All treatments differed significantly from the control and completely inhibited sclerotial germination (100% inhibition), with no statistical differences among treatments, as shown in Figure 1 (H–Q). However, in the assay conducted after surface disinfection, all sclerotia germinated (100% germination). These results indicate that sclerotia are unable to germinate only in the presence of the bacterial strains. Due to rapid bacterial growth, sclerotial germination is likely inhibited through competition mechanisms. Strains of *Bacillus* spp. and *Paenibacillus* spp. have previously been reported to inhibit sclerotial germination (KAMAL et al., 2015; ONARAN; YANAR, 2011); however, some strains evaluated in those studies were unable to completely suppress sclerotial viability. Rodrigues et al. (2025) reported that a *Bacillus* strain exerted a fungistatic effect on the sclerotia of *S. sclerotiorum*, preventing germination while the bacterium was present.

According to the phylogenetic analysis based on 16S rRNA gene sequences, the selected strains belonged to two genera: *Bacillus* and *Paenibacillus*. In the phylogenetic trees, 17AN673 was the only strain that clustered in two distinct clades, being associated with both *Bacillus subtilis* and *Bacillus amyloliquefaciens* subsp. *plantarum*. Strain GN01 clustered with a *Bacillus methylotrophicus* strain, a species currently classified as *Bacillus velezensis*. The remaining strains, GN120, GA155, GA157, and GA163, clustered with *Bacillus siamensis*, *Paenibacillus polymyxa*, *Bacillus amyloliquefaciens*, and *Bacillus siamensis*, respectively (Figure 2). Bootstrap values exceeded 60% for all

phylogenetic methods applied.

The conflicting classification of strain 17AN673 may be related to the molecular marker employed, as this strain was positioned within clades corresponding to two different species, *B. subtilis* and *B. amyloliquefaciens*. According to Maughan and Auwera (2011), taxonomy based solely on 16S rRNA gene sequences has important limitations, as it does not account for ecological characteristics and relies on species designations and sequence similarity thresholds that are essentially arbitrary.

Regarding the percentage of emerged seedlings of Pérola Dama-type common-bean in infested soil, treatments 2 and 5 (Carboxin + Thiram and *Bacillus velezensis* GN01, respectively) showed superior performance compared with the other treatments, although both were inferior to the absolute control (T10), which was not inoculated with the pathogen. Seeds treated with the chemical product and *B. velezensis* GN01 exhibited increases of 36% and 30%, respectively, in seedling emergence compared with untreated seeds grown in soil infested with *Sclerotinia sclerotiorum* (Figure 3).

Seedlings or seeds showing symptoms of infection caused by the pathogen were considered diseased. The chemical treatment and *B. velezensis* GN01 resulted in the lowest disease incidence, with values of 72% and 77%, respectively. These two treatments differed significantly from the remaining treatments according to the Scott–Knott test ($P \leq 0.05$). Compared with the untreated control, treatments 2 and 5 reduced disease incidence by 21% and 16%, respectively (Figure 3). The remaining treatments did not show positive effects on reducing disease incidence.

These results are consistent with those reported by Sabaté et al. (2018), who demonstrated that *Bacillus* spp. used as a seed treatment in white bean were able to increase seed germination and reduce disease incidence in infested soil. Villarreal-Delgado et al. (2021) showed that a *Bacillus* strain was capable of inhibiting the growth and infection of *Sclerotinia sclerotiorum* in common-bean seedlings when seeds were inoculated with both microorganisms. In the same study, Villarreal-Delgado et al. (2021) reported that the strain was able to produce cell wall-degrading enzymes, such as proteases and cellulases, which may contribute to pathogen suppression. The increased seedling emergence and reduced disease incidence observed in the present study are likely associated with the protection of seeds and seedlings provided by seed treatment. As previously reported, some *Bacillus* spp. are capable of producing antimicrobial metabolites and compounds (SANTOS et al., 2023; VILLARREAL-DELGADO et al., 2021), which contribute to the protection of seeds and seedlings against pathogen attack.

In the detached leaf biocontrol assay, all treatments, except *Paenibacillus polymyxa* and the mixture of three bacterial species (*Bacillus subtilis*, *Bacillus velezensis*, and *Bacillus pumilus*), differed significantly from the inoculated control treatment, with disease severity ranging from 0 to 13.94% (Table 5). The remaining strains (17AN673, GN01, GN120, GA157, and GA163) were effective in suppressing fungal growth and partially preventing infection. These results indicate that five of the six selected strains have potential as protective agents when applied before pathogen inoculation.

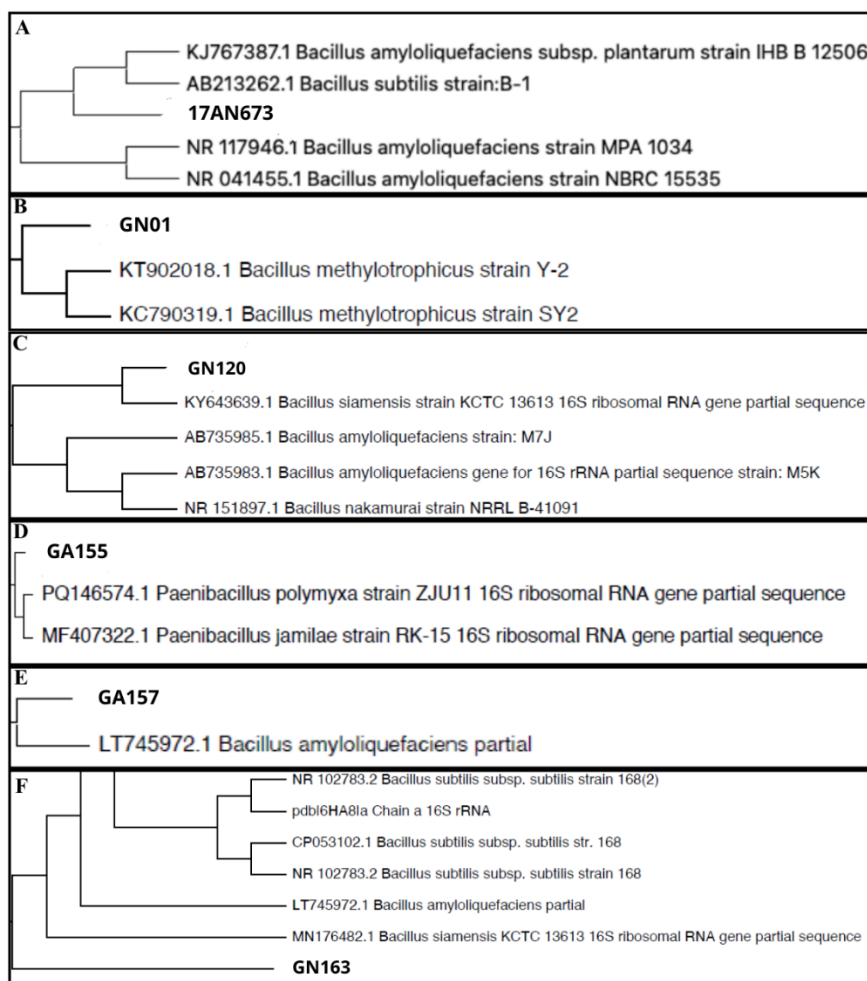
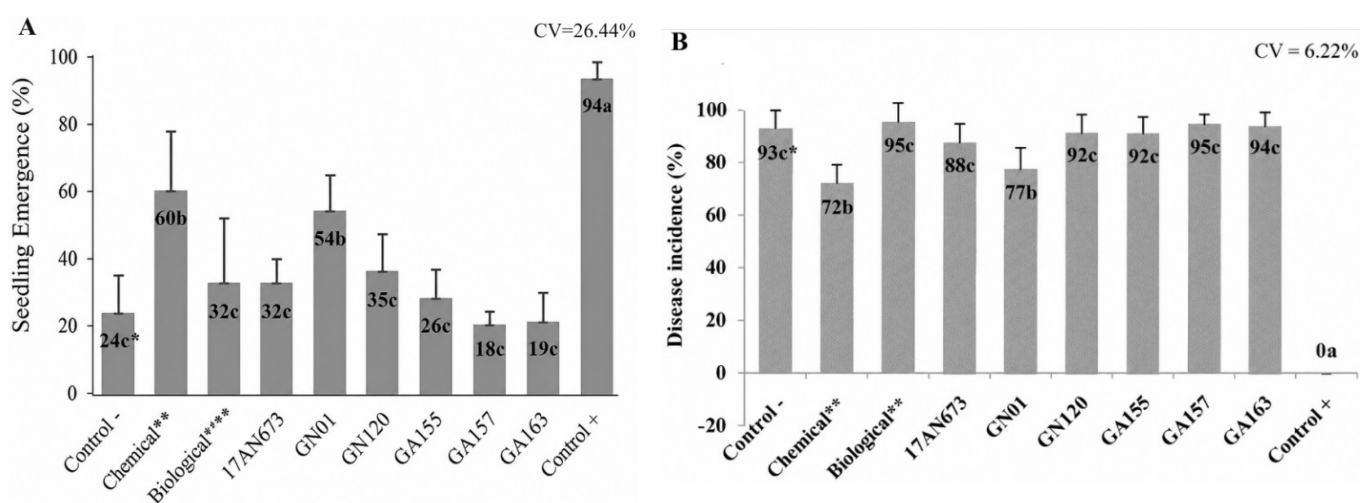


Figure 2. Phylogenetic trees of the bacterial strains evaluated in this study based on 16S rRNA gene sequences: (A) 17AN673, (B) GN01, (C) GN120, (D) GA155, (E) GA157, and (F) GA163. Trees were constructed using the Neighbor-Joining (SAITOU; NEI, 1987), Maximum Parsimony, and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) methods.



*Means followed by different letter differ significantly according to the Scott-Knott test at $P \leq 0.05$. Data were transformed to $x + 0.5/100$.

** Commercial product registered with MAPA for disease control (Carboxin +Thiram).

*** Commercial product registered with MAPA for disease control (mixture of three *Bacillus* spp., 4.6×10^8 viable spores mL^{-1}).

Figure 3. Seedling emergence of Pérola Dama-type common-bean (A) and disease incidence of white-mold caused by *Sclerotinia sclerotiorum* (B).

Table 5. Disease severity (%) caused by *Sclerotinia sclerotiorum* on detached common-bean leaves subjected to different treatments.

Treatment	Disease Severity (%)
Control -	55.7a
<i>Bacillus</i> sp. 17AN673	9.4b
<i>Bacillus velezensis</i> GN01	0.2b
<i>Bacillus siamensis</i> GN120	13.9b
<i>Paenibacillus polymyxa</i> GA155	38.5a
<i>Bacillus amyloliquefaciens</i> GA157	0.2b
<i>Bacillus siamensis</i> GA163	7.2b
Mixture of 3 Bacteria**.	48.2a
Thiophanate-Methyl + Fluazinam	0.0b
Control +	0.0b
Coefficient of Variation CV (%)	51.8

*Means followed by the same letter do not differ significantly from each other according to the Scott-Knott test at 1% significance level. Data were transformed using $\sqrt{(x + 0.5)/100}$.

**Commercial product registered with MAPA for disease control (mixture of three *Bacillus* spp., 4.6×10^8 viable spores mL⁻¹).

Similar findings were reported by Ayaz et al. (2024), who observed that a *Bacillus subtilis* strain reduced lesion diameter on detached leaves by 50%. These results are also in agreement with those of Rahman et al. (2016), who demonstrated that the application of *Bacillus* spp. on detached mustard leaves reduced disease severity caused by *S. sclerotiorum*. Application of bacterial suspensions to detached leaves can delay pathogen infection for a certain period of time (RAHMAN et al., 2016). In the present study, the application of bacterial fermentates to detached leaves likely promoted pathogen suppression through the presence of living bacterial cells competing for space and through the production of antifungal substances, thereby inhibiting pathogen growth and infection.

CONCLUSION

The strains 17AN673, GN01, GN120, GA155, GA157, and GA163 were effective in the control of *Sclerotinia sclerotiorum* *in vitro* through antibiosis and the production of thermostable compounds. *Bacillus velezensis* GN01, when applied as a seed treatment, protected seedlings against disease in pathogen-infested soil and increased seedling emergence. *Bacillus* sp. 17AN673, *B. velezensis* GN01, *B. amyloliquefaciens* GA157, and *B. siamensis* GN120 and GA163 reduced infection and white-mold severity in detached leaf assays. These strains show potential for disease control; however, further level studies and a better understanding of the metabolites involved are still needed.

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