fortune Journals

Research Article

ARCHIVES OF MICROBIOLOGY & IMMUNOLOGY

ISSN: 2572-9365

Bacillus Bombysepticus JAB01 Unleashes Antifungal Defense Against *Sclerotinia Sclerotiorum* White Mold Disease

Paula Klotz Brandão Rodrigues*, Luis Angel Chicoma Rojas, Max Hervot de Mattos Vaz and Eliana Gertrudes de Macedo Lemos*

Abstract

White mold disease, caused by the pathogenic fungus *Sclerotinia sclerotiorum*, affects over 600 plant species globally, including key crops such as soybeans, beans, cotton, and tomatoes. This fungus leads to significant reductions in yield and quality, posing a substantial threat to grain production. Biological control offers an environmentally safe and effective alternative against *S. sclerotiorum*, with the genus Bacillus emerging as a promising tool. In this study, the strain JAB01 was identified as *Bacillus bombysepticus* through morphological examinations and confirmed by whole genome sequencing. In vitro assays demonstrated that *B. bombysepticus* JAB01 produced diffusible substances and volatile organic compounds that effectively suppressed *S. sclerotiorum* growth by 80% and inhibited sclerotia germination by 100%, significantly reducing disease infection on seeds and leaves. These findings suggest that *B. bombysepticus* JAB01 could serve as a promising biological agent against white mold disease. This research has the potential to significantly impact agriculture and the phytopathogen control industry by contributing to sustainable agricultural practices in the cultivation of soybeans and other host plants. By reducing the reliance on fungicides for white mold disease control, this study offers benefits to farmers, consumers, and the environment, promoting more responsible and effective agricultural practices.

Keywords: Antifungal activity; *Bacillus*; Biological control agents; phytopathogenic fungus; VOCs

Introduction

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, which belongs to the Phylum Ascomycota, Class Leotiomycetes, Order Helotiales, and Family Sclerotiniaceae is a necrotrophic and pathogenic fungus that infects more than 600 plant species worldwide, including important crops such as beans, soybeans, cotton and tomato (**1**). In Brazil, *S. sclerotiorum* was reported for the first time in 1921 in São Paulo, on the potato crop, and since then it has been identified in several other plant species like soybean, beans, cotton, tomato, sunflower, and sun hemp (**2**). On soybean in the country, it is one of the most important diseases and can lead to significant reductions in yield and quality in grain production, as the infection can lead to necrosis, girdling, and possibly death of the plant (**3**). The fungus has the ability to synthesize oxalic acid (OA) and lytic enzymes that act on host plant cell wall causing the formation of soft watery areas, resulting in the rots of roots, stems, and other plant organs (**4**). Yield reductions can be atributted to a decrease in

Affiliation:

[1]Biochemistry of Microorganisms and Plants Laboratory, Agricultural, Livestock and Environmental Biotechnology Department, São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, São Paulo 14884-9, Brazil

***Corresponding author:**

Paula Klotz Brandão Rodrigues, Biochemistry of Microorganisms and Plants Laboratory, Agricultural, Livestock and Environmental Biotechnology Department, São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, São Paulo 14884-9, Brazil.

Eliana Gertrudes de Macedo Lemos, Biochemistry of Microorganisms and Plants Laboratory, Agricultural, Livestock and Environmental Biotechnology Department, São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, São Paulo 14884-9, Brazil.

Citation: Paula Klotz Brandão Rodrigues, Luis Angel Chicoma Rojas, Max Hervot de Mattos Vaz and Eliana Gertrudes de Macedo Lemos. *Bacillus Bombysepticus* JAB01 Unleashes Antifungal Defense Against *Sclerotinia Sclerotiorum* White Mold Disease. Archives of Microbiology and Immunology. 9 (2025): 07-20.

Received: December 28, 2024
Accepted: January 06, 2025
Published: January 28, 2025

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

both seed number and weight that are consquences of stems girdling which disrupts the xylem and phloem (**5**).

This soil pathogen, with a high destructive potential, can manifest on any part of the plant, characterized by the abundant formation of white cottony mycelium and sclerotia—structures that ensure its survival in the soil for extended periods. The longevity of sclerotia varies significantly according to different authors, ranging from at least two years to up to eleven years, with one of the factors influencing their persistence being the depth at which they are buried in the soil (**6**). The spread of the pathogen occurs primarily through seeds infected with the mycelium or contaminated by sclerotia (**7**). Pathogenic factors associated with this fungus include mycelial growth, production of sclerotia (**8**), and the secretion of oxalic acid (**9**, **10**). The most commonly used control method is chemical using fungicides. However, the use of chemicals can lead to the development of resistant populations of the fungus (**11**, **12**, **13**). In addition, it can cause damage to the environment acting on non-target organisms, including humans. Therefore, it is necessary to search for alternatives with fewer adverse effects. Faced with this demand, biological control of plant diseases is important, as it is an ecologically sustainable and environmentally safe alternative to chemical control for managing various pests and pathogens (**14**). In recent years, progress has been made in the biological control of *Sclerotinia* stem rot, among different biological approaches, the use of microbial antagonists, like yeasts (**15**) , fungi (**16**, **17**), and bacteria (**18**, **19**, **20**) have been reported as efficient against *S. sclerotiorum*. According to several studies *in vivo* and *in vitro* with bacteria with antagonistic activity against *S. sclerotiorum* the species of *Bacillus* genus are in evidence (**21**, **22**, **19**). This fact can be attributed to the ability of *Bacillus* to generate several biologically active compounds with antimicrobial properties like hidrolytic enzymes, toxins, antibiotics, siderophores, volatile organic compounds (VOCs), and others (**23**, **24**). To date, there is no information in the literature about the effects of *Bacillus bombysepticus* on the soil pathogen *Sclerotinia Sclerotiorum*.

Considering the importance of controlling diseases caused by *Sclerotinia sclerotiorum* while minimizing the use of chemical fungicides, this investigation aimed to evaluate the antagonistic activity of the bacterial strain *Bacillus bombysepticus* JAB01 against *S. sclerotiorum* in vitro. The results of the study revealed promising potential for this bacterial strain as a biological control agent. These findings not only contribute to advancements in managing diseases caused by this fungal phytopathogen but also pave the way for the development of commercial biological products for farmers, reducing reliance on chemical inputs. Consequently, this research holds significant potential to benefit farmers, consumers, and the environment by fostering more sustainable and responsible agricultural practices.

Materials and Methods

Isolates and culture conditions

In a preliminary bioprospecting study with fifty-one bacterial isolates of the internal collection of genomes and metagenomes of the LBMP (Biochemistry of Microorganisms and Plants Laboratory), one isolate (JAB01) was identified as a promising against the fungus *Sclerotinia sclerotiorum*. The bacterial isolates were randomly isolated from the rhizospheric soil samples of various plants collected from different places in São Paulo State University (UNESP), Jaboticabal- São Paulo, Brazil, and are stored in -80ºC freezers of the LBMP. For preliminary tests, they have been cultivated in Luria-Bertani (LB) medium at 30ºC and 150 rpm for 24 h. The microorganism used here, *Bacillus bombysepticus* JAB01, was isolated from mimosa soil, and after carrying out its bacterial growth curve, it was cultivated in LB medium at 30ºC and 150 rpm for 4 h. The fungus *S. sclerotiorum* was obtained from the Jco Biofertilizer industry and was grown on Potato Dextrose Agar (PDA) for 4 days at 25°C and maintained at glycerol stock.

Partial sequencing of the 16S rRNA region

For the isolate that showed antagonist activity, partial sequencing of the 16S ribosomal RNA was performed in order to confirm the identification of the genus and obtain an approximation of the species. Total DNA extraction was performed using the Insta Gene matrix Bio-Rad Kit according to the manufacturer's instructions. Subsequently, DNA amplification was performed by Polymerase Chain Reaction (PCR) with primers specific for the 16S rRNA gene, fD1 (5'-CCG AAT TCG TCG ACA ACA GAG TTT GAT CCT GGC TCA G - 3') and rD1 (5'-CCC GGG ATC CAA GCT TAA GGA GGT GAT CCA GCC - 3') (**25**), the amplified products were sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing kit on the capillary sequencer model ABI 3130 - Perkin Elmer. The sequences obtained were submitted to a nucleotide similarity query in the GenBank database accessed through the NCBI website ("National Center for Biotechnology Information"), through the local BLAST tool - "Basic Local Alignment Search Tools".

Whole genome sequencing of *Bacillus bombysepticus* JAB01

The *Bacillus bombysepticus* JAB01 genome was sequenced using Ilumina Novaseq6000 and Oxford Nanopore (MinION) technologies. For genome assembly, the Novaseq6000 pair-end fastq files were trimmed to adapters, and potential contaminating human sequences were mapped using Bowtie2 using the human reference genome. Additionally, the fastq files from the MinION run (SQK-LSK110 kit, Oxford Nanopore) were trimmed for low-quality reads with NanoFilt and adapters were trimmed

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

using Porechop (v0.2.4, https://github.com/rrwick/Porechop). All trimmed reads from Illumina and Nanopore were then used for hybrid assembly using the MaSuRCa (**26**) assembler according to the developer's instructions. The filtered reads were combined to create a single contig with no gaps and the Fasta file contains a final assembly. The genome map was constructed and visualized using Proksee (**27**).

Phylogenomic analysis and Average Nucleotide Identity (ANI)

To reconstruct and position *Bacillus bombysepticus* JAB01 within the *Bacillus* genus, we conducted a phylogenetic analysis using GToTree v1.1.10 (**28**). The phylogenetic analysis was performed using 1050 *Bacillus* genomes available in GenBank. We utilized the hidden Markov model (HMM) single-copy gene set and employed the maximum likelihood (ML) method for tree reconstruction, generated from 1000 bootstrap replicates. The phylogenomic tree was edited and annotated with iTol (Interactive Tree Of Life) (v. 6.7.5) (**29**). In addition, the Orthologous Average Nucleotide Identity Tool software (OAU) (**30**) was used to determine the ANI values among biocontrol *Bacillus* genomes.

Phase Contrast Microscopy (PCM)

For morphological identification, samples were analyzed with a PCM. The JAB01 isolate was cultured in liquid EMBRAPA (**31**) medium for 72 h at 150 rpm and 30°C. After culture, a drop of the bacterium was placed on a slide with a sterile pipette and covered with a coverslip, a drop of immersion oil was added to the coverslip, and visualization of the samples was performed on a Zeiss Z2 Axionvsion microscope at 1000x magnification.

Bioassay of antagonist activity *in vitro* by diffusible substances

The antagonist activity of the *Bacillus bombysepticus* JAB01 was tested against the phytopathogen by dual-culture plate technique. Discs of *S. sclerotiorum* mycelium (≈ 7 mm Ø) cultured on solid PDA medium were cut using a sterilized punch and placed in the center of PDA Petri dishes. At the end of the plate (≈ 2.5 cm from the center) 10 µl ($1.13x10^8$ CFU/ml) of the bacterial isolate JAB01, previously incubated for 4 h at 30°C in liquid LB medium, was inoculated. The plates were incubated at 25°C in a B.O.D (Bio-Oxygen Demand) for 5 days. Inhibition zones were measured from the edge of the JAB01 colony to the end of fungal mycelial growth. PDA plates inoculated with the fungus disc and 10 µl of sterile LB medium were used as controls. Each treatment was applied to three replicate plates and repeated at least three times.

Germination and viability of sclerotia and oxalic acid production

For the evaluation of sclerotia germination, an aliquot of 100 µL ($1.13x10^8$ CFU/ml) of bacterial suspension was spread with a Drigalsky loop sterilized in a Petri dish with PDA medium, then two sclerotia were placed at the ends of the plates and incubated at 25°C. Germination was assessed after 7 days. Additionally, sclerotia were submerged for 1 min in a spore suspension ($1.13x10^8$ CFU/ml) of the antagonist, then collected and transferred to a Petri dish containing PDA. After the germination, the sclerotia were collected and the surface was sterilized with 3 washes with sodium hypochlorite (5%) for 1 minute interspersed with sterile water to eliminate the JAB01 inoculum. After disinfection, sclerotia were collected and transferred to Petri dishes containing PDA medium supplemented with 50 mg/L bromophenol blue to assess myceliogenic germination viability and oxalic acid production. Bromophenol blue is an acid-base indicator, which emits a blue color at pH ≥ 4.6 and a yellow color at pH ≤ 4.6 (**32**). These experiments had three repetitions and for the control treatments plates with sclerotia non-inoculated with JAB01 was used.

Seed bacterization

Susceptible soybean seeds of BMX Valente, upon surface disinfection, were immersed in bacterial culture ($1.13x10^8$ CFU/ml) JAB01 for 30 min. The method established by (**33**) with modifications was used to assess seed infection. Two mycelial plugs (≈ 7 mm Ø) of *S. sclerotiorum* were placed on both sides of a PDA plate, subsequently, five bacterial seeds were placed forming a row between the mycelial plugs. After incubation for 3 and 7 days at 25 °C, the number of germinated healthy seeds and infected seedlings was recorded. Untreated soybean seeds were used as a control and the experiment was performed in five replicates.

Pathogenicity on detached leaves

Fully expanded leaves of selected soybean plants were detached, rinsed with sterile distilled water, and air dried. The leaves were placed on plastic Petri plates (8 by 8 cm) on damp paper towels to avoid direct contact with water. The leaves that were inoculated with the JAB01 isolate were immersed in a bacterial solution grown under the conditions described above. Plugs of *S. sclerotiorum* fungal hyphae that were prepared as described above were placed on the upper part of the mid rib of the leafs, while their petioles were wrapped with damp cotton to prevent desiccation. For the control treatments, there has been no inoculation of the leaves with JAB01. All treatments were incubated at 25°C and fungal pathogenicity was assessed from 72 h to 144 h post-inoculation (hpi) by the presence of disease symptoms. The experiment was replicated three times.

Evaluation of the inhibitory activity of VOCs produced by *Bacillus* JAB01 *in vitro* against *S. sclerotiorum*

In addition, the double-plate assay was used to study

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

the antagonistic activity of volatile compounds released by JAB01 against *S. sclerotiorum*. Two Petri dishes were placed opposite each other. The lower Petri dish contained LB agar, which was inoculated with 100 µl of JAB01 ($1.13x10^8$ CFU/ml). The upper Petri dish contained PDA, where a ≈ 7 mm diameter disk of actively growing *S. sclerotiorum* was placed. The upper and lower Petri dishes were sealed with parafilm to prevent the loss of volatiles and incubated at 25°C for four days. Control plates were also prepared in the same way, except that an uninoculated LB plate was used in place of the JAB01 plate.

To further evaluate the effects of *Bacillus* VOCs on *S. sclerotiorum*, ≈ 7 mm diameter fungal plugs were taken from an *S. sclerotiorum* mycelial mat previously exposed to *Bacillus* VOC for 96 h. The plugs were then placed at the center of PDA Petri dishes (7 mm Ø), sealed, and incubated at 25°C. The Petri dishes containing VOC-untreated fungal mycelial plugs served as a control. Growth and development of fungal mycelia were monitored for 96 h by recording mycelial growth (in millimeters) at intervals of 24 h. The *in vitro* mycelial growth inhibition rate (R) was calculated by the equation:

$$\mathbf{R\ (\%) = D1 - D2/D1 - D0 \times 100}$$

where R is the percentage of inhibition of mycelial extension; D1 is the mycelial diameter (mm) of the negative control set; D2 is the mycelial diameter of the treated plate, including the size of the fungal agar plug (mm); and D0 is the original mycelium diameter (7 mm Ø) of the fungal agar plug (**34**). The experiment had three replicates and was replicated at least three times.

Effects of VOCs on sclerotia germination

To evaluate the effects of JAB01 VOCs on *S. sclerotiorum* sclerotia germination, sclerotia were taken from an *S. sclerotiorum* plate and placed on one side of a split Petri dish. On the other side of the plate, 50 µl of the bacterial solution, grown under the conditions described above, was spread with a Drigalsky loop and the plates were sealed with parafilm and kept in BOD at 25°C. After 5 days, the mycelial germination of the resistance structure of the fungus and antagonistic activity was considered when there was an inhibition zone. Each treatment had five replicates.

Effects of VOCs on detached leaves

To evaluate the effects of JAB01 VOCs on soybean leaves inoculated with *S. sclerotiorum*, fully expanded V3 leaves of the BMX Valencia line grown in a greenhouse were cut, washed in sterile distilled water, and a single leaflet was carefully placed on wet paper towels on one side of a 9 cm diameter bipartite Petri dish. Plugs of ≈ 7 mm in diameter were taken from an *S. sclerotiorum* mycelial mat and placed on the upper part of the leaf, while its petioles were wrapped with damp cotton. On the other side of the plate, 60 µl of the bacterial solution, grown under the conditions described above, was spread with a Drigalsky loop and the plates were sealed with parafilm and kept in BOD at 25°C for 9 days. After 9 days, the disease incidence was evaluated, and the antagonist activity was considered according to the presence of disease symptoms. The experiment had five replicates for each treatment.

Scanning Electron Microscopy (SEM)

The influence of VOCs emitted by *Bacillus* JAB01 on the morphology of *S. sclerotiorum* hyphae was assessed by SEM. Fungal mycelia were extracted from VOC-treated and untreated Petri dishes. Samples were fixed in 2.5% glutaraldehyde (Solarbio, Co. Ltd.) in 0.1M sodium cacodylate buffer for 18 h and postfixed at 4°C for 6 h with the same buffer in 1% osmium tetroxide, followed by dehydration using a number (30 to 100%) ethanol gradient (vol/vol). Samples were dried in a critical point dryer and then metalized in gold and visualized in a Zeiss scanning electron microscope, Evo MA 10, at 10KV.

Greenhouse experiment

To evaluate the antagonistic potential *in vivo* of *B. bombysepticus* JAB01 against *S. sclerotiorum* in greenhouse conditions, four treatments were used, these being: T1= soybean seedlings inoculated with S. sclerotiorum (referred to as S-Ss), T2= soybean seedlings inoculated with *B. bombysepticus* (S-Bb), T3= mixture of *S. sclerotiorum* and *B. bombysepticus* (S -Bb-Ss), and soybean seedlings as a control treatment (S). The experimental design was completely randomized (DIC), with five replicates in each treatment. Commercial soybean seeds of the cultivar "BMX VALENTE" were used. The seeds had Standak top technology with fungicidal action without spectrum to control *S. sclerotiorum*.

Pots of 7 liters of capacity were previously prepared, containing a mixture composed of 4 parts of soil, 1 part of sand, 1 part of substrate, and 1 part of tanned manure. These pots were then placed in the greenhouse. Subsequently, each pot was sown with 5 soybean seeds of the cultivar BMX Valente. After a germination period of 10 days, 2 plants were removed from each pot, thus ensuring the maintenance of 3 viable plants in each designated experimental plot. The fungus was cultivated in a 500 ml Erlenmeyer with 250 ml of liquid PDA medium, to which 30 actively growing mycelium discs were added. The centrifuged mycelial mass was then resuspended with previously sterilized distilled water. The inoculated vial was kept under agitation of 200 rpm at 28 ºC for 5 days. The mycelial masses from the discs were collected and centrifuged in Falcon tubes for 15 minutes at 10000 rpm and 4 ºC and, at the end, the supernatant was removed. The tubes were centrifuged again for another 10 minutes under the same conditions. The supernatant was again removed. The

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

centrifuged mycelial mass was resuspended with distilled water previously sterilized and then ready for inoculation.

The inoculation of the microorganisms was done when the plants were in the V3/V4 stage. This was performed according to the mycelium drop methodology described by **35** with modifications. A wound of approximately 2 cm was made on the main stem of the plants with a scalpel, between the 2nd and 3rd trefoil. The homogenized mycelium suspension was dripped with a sterile pipette into the wound, at a dose of approximately 1 ml/plant. The plants comprising the treatment with the antagonist (S-Bb and S-Bb-Ss) were inoculated with 60µl of the bacterial solution which had been cultivated in accordance with the previously described method. All wounds were sealed with parafilm and after 3 days the first obvious symptoms of the disease appeared on S-Ss treatment.

Data analysis

Data were examined with one-way ANOVA and Tukey´s test was applied when one-way ANOVA revealed significant differences ($p \leq 0.05$). All statistical analyses were performed with the SISVAR ver. 5.8 statistical software (**31**).

Results

The molecular identification of strain JAB01, by sequencing of the 16S rRNA gene, showed high similarity (99,93%) and coverage (99%) with *Bacillus sp.* (Supplementary table S1). In addition, after sequencing and genome assembly, the 6,094,706 bp long JBA01 genome was found to consist of a 5,374,157 bp circular chromosome (Figure 1) and four circular plasmids (121,263 bp, 60,442 bp, 220,629 bp, and 315,703 bp) with an average GC content of 35.3%. NCBI Genome Annotation shows that the chromosome contains a total of 6,215 genes, of which 6,061 encode CDS, and 154 RNA genes. According to the annotation results, 5,833 CDS were attributed to putative biological functions, whereas 228 CDS were characterized as hypothetical proteins (Supplementary table S2). According to the results of the phylogenomic tree (Figure 2) JAB01 is positioned in the same clade as *Bacillus bombysepticus* Wang indicating high similarity between the two strains, this result was confirmed by the ANI test (Figure 3), in which the average nucleotide identity between the two strains was 99.04% and therefore we confirm that JAB01 is a *Bacillus bombysepticus*. It was also possible to infer that JAB01 has similarity with strains of *Bacillus thuringiensis* and *Bacillus cereus*. In addition, using phase contrast microscopy and SEM, it was confirmed that isolate JAB02 has a bacillary morphology, also presents endospores, and lacks crystals (Supplementary figure S1).

The results showed that, in in vitro plate assays, *Bacillus*

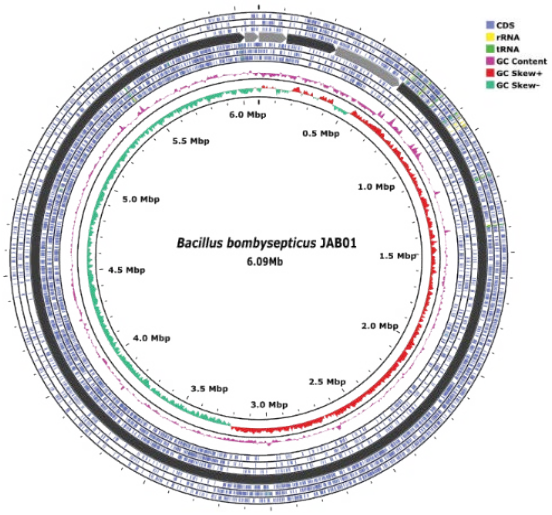

Figure 1: Circular representation of the JAB01 genome. In blue, coding regions are distributed as a function of the open reading frame. From outside to inside, rRNA (yellow), tRNA (light green), GC content (pink), positive GC bias (red), negative GC bias (matte green), and DNA coordinates (black).

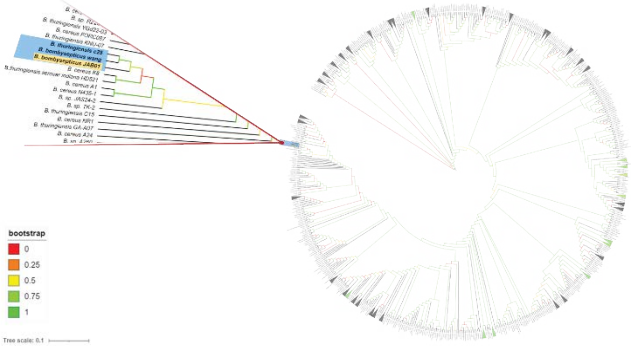

Figure 2: Phylogenomic tree constructed from 1050 genomes of the genus *Bacillus* stored in GenBank. The maximum likelihood (ML) method was used to create the tree. The bootstrap values for each branch are shown in different colors: red for 0, orange for 0.25, yellow for 0.5, lime green for 0.75 and light green for 1. The location of *Bacillus bombysepticus* JAB01 within the tree is highlighted in the expanded view.

JAB01 exhibited significant antagonistic activity through the production of diffusible secondary metabolites, as evidenced by the formation of an inhibition zone between the microorganisms. In dual culture assays, JAB01 reduced the mycelial growth of *Sclerotinia sclerotiorum* by 67% compared to the control (Figure 4D). Furthermore, when the bacterial isolate was applied directly to the mycelial plugs of the fungus, complete inhibition of the disease was observed. These inhibitory effects on the mycelial growth of *S. sclerotiorum* warrant further investigation into whether the metabolites produced by *Bacillus* JAB01 can also affect the germination and viability of sclerotia. The diffusible secondary metabolites produced by *Bacillus* also exhibited

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

fungistatic effects against sclerotia of *S. sclerotiorum*, as evidenced by the complete inhibition of sclerotia germination when the resistance structures were inoculated with *Bacillus* on Petri plates, unlike the control plates where the sclerotia germinated normally (Figure 4 B). Additionally, the absence of yellow coloration in plates PDA supplemented with bromophenol blue containing treated sclerotia indicated that there was no production of oxalic acid (Figure 4 C).

Bacillus JAB01 demonstrated significant antifungal activity through the production of volatile organic compounds (VOCs), which inhibited *Sclerotinia sclerotiorum* mycelial growth by 78.43% compared to the control (Figure 5B-D). Furthermore, in split-plate assays, sclerotia exposed to VOCs produced by JAB01 exhibited halos of mycelial inhibition, indicating that these volatile compounds were also effective in suppressing the mycelial growth of the fungal resistance structures. In detached leaf assays, untreated fungal hyphal plugs caused brownish lesions on host plant leaves, while fungal plugs treated with JAB01 VOCs showed significantly reduced lesion development. To investigate the effects of the VOCs emitted by *Bacillus* JAB01 on *S. sclerotiorum* hyphae, scanning electron microscopy (SEM) analyses were conducted. The scanning electron images captured from the VOC-untreated control exhibited healthy, dense, and cylindrical hyphae (Figure 5 C). However, the fungal hyphae treated with the VOCs of *Bacillus* displayed signs of dryness and an increase in the number and size of pores in certain parts. These observations revealed that the VOCs emitted by B. JAB01 have a discernible impact on the morphology and structure of *S. sclerotiorum* hyphae, leading to notable changes compared to the untreated control.

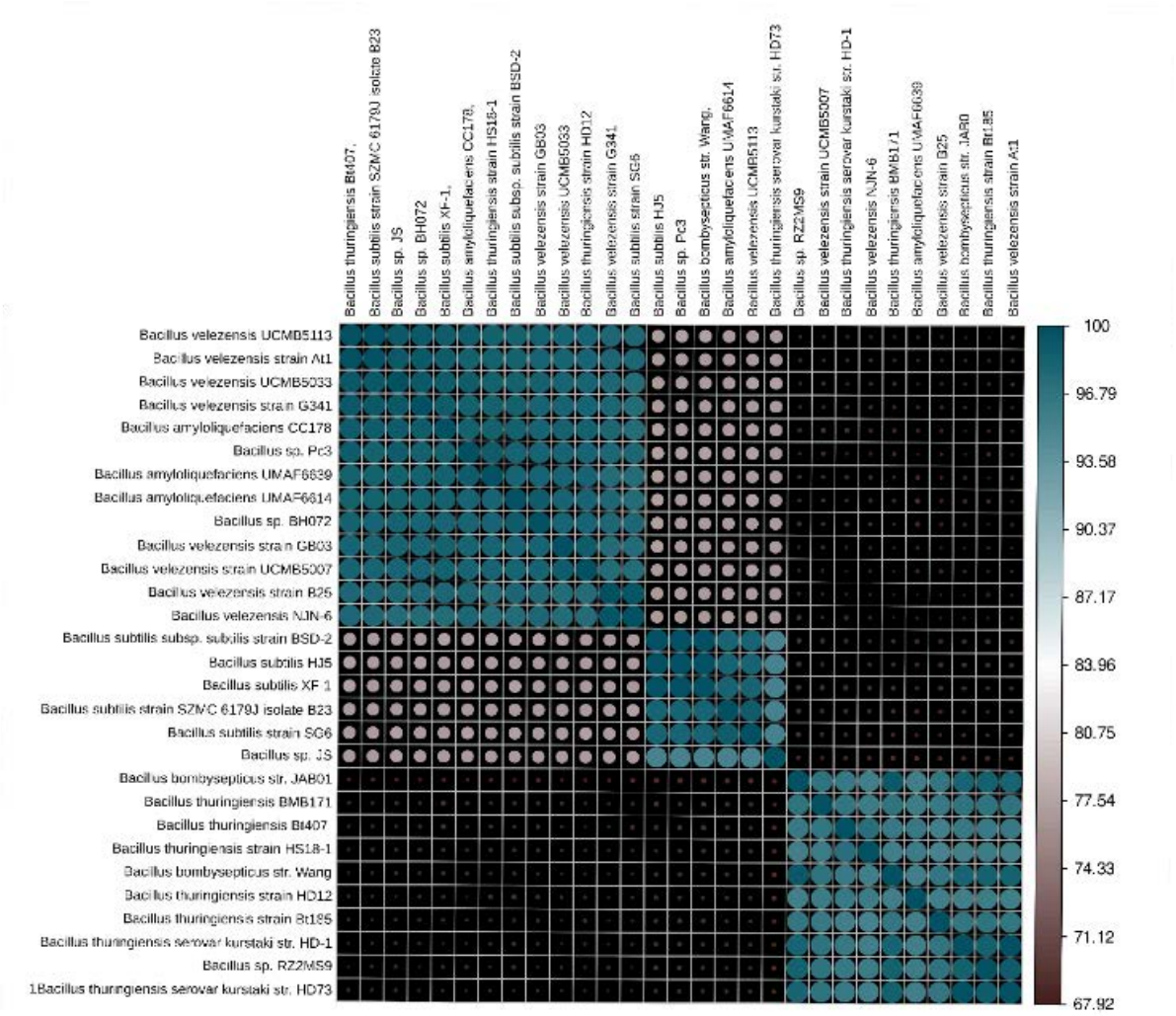

Figure 3: Heat map of genomic Average Nucleotide Identity (ANI) values for pairwise comparisons between 29 *Bacillus* genomes using the Orthologous Average Nucleotide Identity Tool. Percentage identity is shown on the color gradient side ladder. ANI values >95% between genomes indicate that they belong to the same species.

Citation: Paula Klotz Brandão Rodrigues, Luis Angel Chicoma Rojas, Max Hervot de Mattos Vaz and Eliana Gertrudes de Macedo Lemos. Bacillus Bombysepticus JAB01 Unleashes Antifungal Defense Against Sclerotinia Sclerotiorum White Mold Disease. Archives of Microbiology and Immunology. 9 (2025): 07-20

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

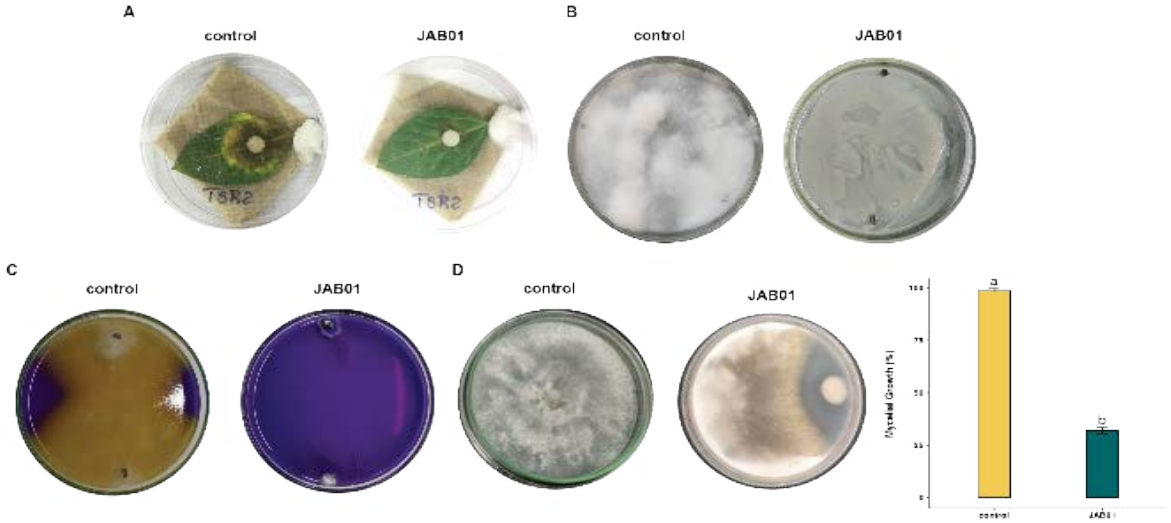

Figure 4: Antagonistic effects of diffusible substances produced by bacterial isolate JAB01 on mycelial growth of *Sclerotinia sclerotiorum*. (A) Effect of JAB01 for protection of soybean leaves against *Sclerotinia sclerotiorum*. **(B)** Myceliogenic germination of sclerotia on PDA plates. **(C)** Myceliogenic germination of sclerotia and oxalic acid production on PDA suplemmented with bromopheenol blue. **(D)** Mycelial growth in PDA medium of *Sclerotinia sclerotiorum* on control and JAB01-incultured plates.

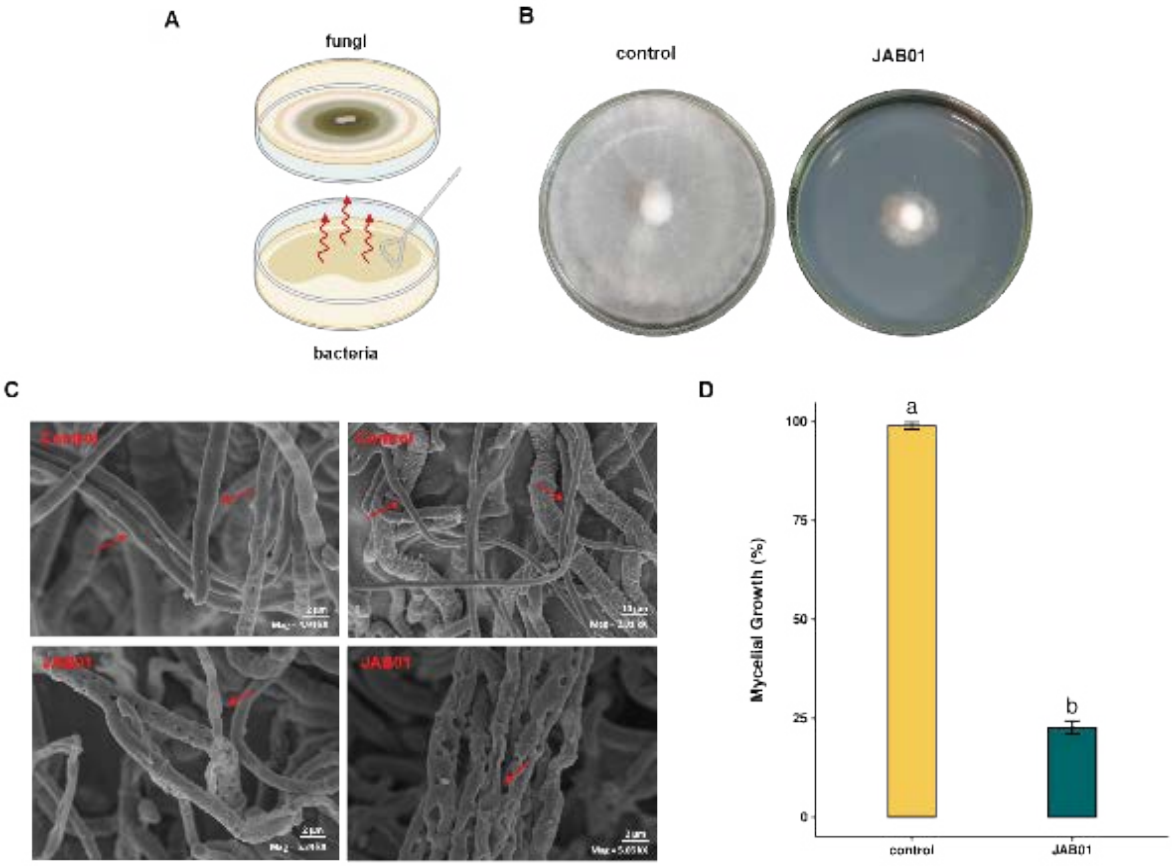

Figure 5: (**A**) Schematization of plates (sadwich models) for testing of volatile compounds. (**B**) Effects of VOCs of JAB01 on mycelial growth of *Sclerotinia sclerotiorum* on PDA. **(C)** Scanning electron microscopy of the mycelium of *Sclerotinia sclerotiorum* exposed to VOCs. (**D**) Growth rate (percentage) of *Sclerotinia sclerotiorum*.

Citation: Paula Klotz Brandão Rodrigues, Luis Angel Chicoma Rojas, Max Hervot de Mattos Vaz and Eliana Gertrudes de Macedo Lemos. Bacillus Bombysepticus JAB01 Unleashes Antifungal Defense Against Sclerotinia Sclerotiorum White Mold Disease. Archives of Microbiology and Immunology. 9 (2025): 07-20

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

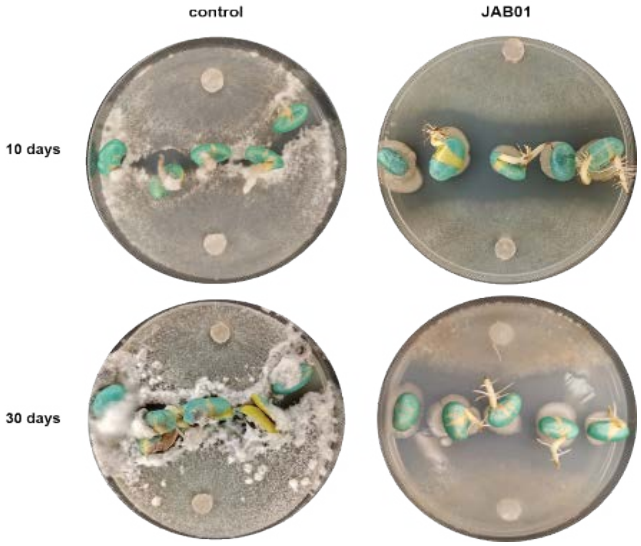

Figure 6: Protective effect of JAB01 on soybean seeds against *Sclerotinia sclerotiorum*. Soybean seeds inoculated by immersion with JAB01 were germinated in Petri dishes with solid PDA medium in the presence of *Sclerotinia sclerotiorum* at the ends of the plate. Evaluation conducted at 10 and 30 days.

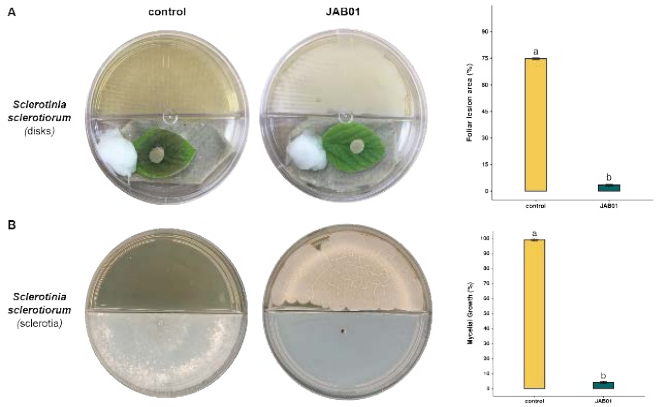

Figure 7: In vitro effects of VOCs on the growth of *Sclerotinia sclerotiorum*. (**A**) Soybean leaves infested with PDA discs of *Sclerotinia sclerotiorum*, one end of the Petri dish, and bacterial isolate JAB01 on solid LB medium, the opposite end of the dish. Leaves treated with JAB01 showed 3% damaged leaf area compared to 75% of the control. (**B**) Sclerotia was grown on PDA medium and JAB01 was inoculated on a solid LB medium at the opposite end of the Petri dish. For the controls, JAB01 was not inoculated. Plates inoculated with JAB02 inhibited 96.23% of sclerotia growth.

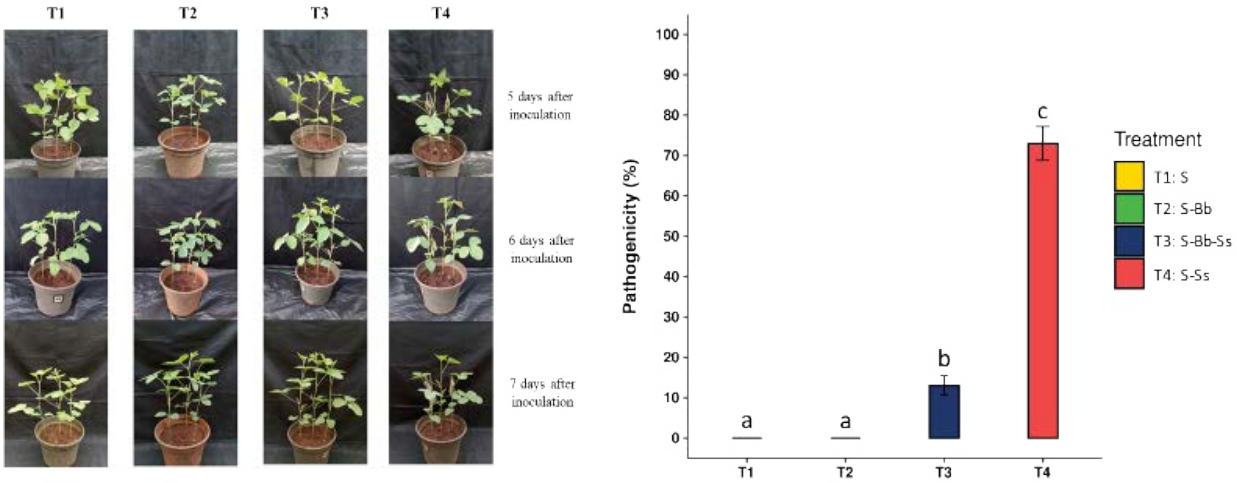

Figure 8: In-house study of antagonistic activity of *Bacillus bombysepticus* JAB01 (60µl/plant) on soybean plants (cultivar "BMX Valente") infested with *Sclerotinia sclerotiorum* (1 ml/plant). Treatment: **T1**= soybean seedlings as control treatment (S), **T2** = soybean seedlings inoculated with *Bacillus bombysepticus* JAB01 (S-Bb), **T3** = mixture of *Sclerotinia sclerotiorum* and *Bacillus bombysepticus* JAB01 (S -Bb-Ss), and **T4** = soybean seedlings inoculated with *Sclerotinia sclerotiorum* (named S-Ss). Evaluations made at 5, 6 and 7 days after inoculation. Incidence of white mold at 7 days after inoculation was 73% and 13% in T4 and T3, respectively, and 0% for T2 and T1.

The results of seed bacterization show that seed inoculation with *Bacillus* JAB01 provided complete protection against seed rot caused by *S. sclerotiorum* infection. When the seeds were treated with JAB01, at least 80% of the resulting seedlings remained healthy. In contrast, the control treatment without bacterial inoculation resulted in seedlings being infected by the fungus within 10 days of incubation (Figure 6). The metabolites produced by JAB01 effectively decreased both the size and incidence of lesions, with limited or no lesion formation observed on all tested host plant leaves 72 hours post-infection, compared to the untreated control (Figure 7). Similarly, pathogenicity assays using JAB01 VOCs on detached soybean leaves demonstrated a comparable reduction in lesion size and disease progression, further highlighting the potential of JAB01 as a biocontrol agent. After 4 days of inoculation by liquid culture of mycelium,

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

the plants that made up the positive control treatments (S+Ss) already had the symptom of wilting, indicating that there was infection and consequently dryness of the affected parts (Figure 8). On the other hand, in the plants submitted to the treatment of interaction between the fungus and the bacterial antagonist (S+ Bb + Ss), no visible symptoms of the disease were observed, and the plants developed normally. These results lead us to believe that Bb JAB01 was able to inhibit the development of the fungus in soybean plants in greenhouse.

Discussion

In this study, the 16S rRNA partial gene sequencing of the isolate that showed antagonistic performance against *S. sclerotiorum* confirmed that the isolate belongs to the *Bacillus* genus. Since sequencing 16S rRNA gene sequencing isn't reliable at the species level and *Bacillus* is a bacterial genus with great genomic diversity, its taxonomic affiliation is complex. Therefore, to certify the specie of strain JAB01, the whole genome sequencing and phylogenomic analysis was done, and the isolate was identified as *Bacillus bombysepticus* JAB01. To date, there are no reports of *B. bombysepticus* antagonizing *S. sclerotiorum*.

Based on microscopic observations, *Bacillus bombysepticus* JAB01 exhibits typical morphological characteristics of the *Bacillus* genus. Although the literature reports the presence of parasporal crystals in *B. bombysepticus* (**36**), our examination using contrast microscopy did not reveal any evidence of such structures in *B. bombysepticus* JAB01. The genus *Bacillus* is widely recognized for its importance in biological control due to its ability to produce a diverse range of biologically active molecules (**36**). These microorganisms offer several advantages over other biocontrol agents, including their capacity to form endospores, which enables them to tolerate extreme pH, temperature, and osmotic conditions (**37**). In this study, we focused on the antifungal activity of *B. bombysepticus* JAB01 isolated from mimosa soil and its potential as a biocontrol agent against white mold disease caused by *Sclerotinia sclerotiorum*. This strain demonstrated significant antifungal properties by producing diffusible substances and volatile organic compounds (VOCs), which suppressed the mycelial growth of *S. sclerotiorum*, inhibited sclerotia germination, and slowed disease progression in detached leaves and soybean plants under greenhouse conditions. These findings align with the results of **19**, who reported that *Bacillus velezensis* VM11 could produce diffusible compounds and VOCs that effectively inhibited the mycelial growth of *S. sclerotiorum*.

The zone of inhibition formed in the dual culture bioassay plates indicated the presence of biologically active metabolites that diffused in an agar medium. Our findings are in agree with other studies that revealed the antagonistic activity exerted by *Bacillus* spp (**33**). In the present study, the growth suppression during dual culture assay was less than the volatile inhibition; these results are like the findings of (**38**). The results about germination and production of oxalic acid indicate that JAB01 has genetic mechanisms to suppress the myceliogenic germination of the resistance structure and the oxalic acid secretion. At the moment of infecting plants, *S. sclerotiorum* secrets oxalic acid to acidify the surrounding ambient creating the ideal conditions for cell wall degrading enzyme activity. According to **39** , the secretion of oxalic acid and a battery of acidic lytic enzymes kill cells ahead of the advancing mycelium and causes the death of cells at the injection sites. So oxalic acid is important to sclerotia production and pathogenesis. (**40**) showed that under neutral or alkaline pH sclerotial formation is inhibited.

The sclerotia are the resistance structure of the fungus, which can withstand extremely adverse conditions and persist in the soil for many years until favorable conditions appear, making it difficult to control the disease (**41**). Sclerotia can germinate either myceliogenically or carpogenically with favorable environmental conditions. Myceliogenic germination produces infective hyphae (**42**). The observed inhibition of sclerotia germination may turn to be a very useful and valuable factor in the development of biological methods of plant protection. Our results align with the findings of **43** and **44**, suggesting a promising control approach given the critical role of sclerotia in the persistence of *Sclerotinia sclerotiorum*. Additionally, the absence of oxalic acid secretion in this study deserves attention, as this compound significantly influences the virulence and pathogenicity of the fungus. In fact, previous reports have highlighted the development of resistant transgenic plants based on oxalic acid degradation (**45**). Further tests are necessary to fully understand the mechanisms underlying this suppression.

The observations from our seed bacterization experiments (Figure 6) are consistent with other studies reporting the positive effects of seed treatment with *Bacillus* isolates in suppressing seedling diseases caused by various phytopathogens (**46**; **37**; **47**). Similarly, the results of the detached leaf assays agree with previous findings showing that *Bacillus* isolates effectively reduce disease progression on the leaves of different host plants (**21**; **48**). These findings can be attributed to the extensive arsenal of antimicrobial substances produced by *Bacillus* species, which are known to inhibit phytopathogens. These substances include secondary metabolites such as lipopeptides, volatile compounds (**34**), and hydrolytic enzymes (**49**). Together, these mechanisms underline the potential of *Bacillus* isolates as effective biocontrol agents against plant diseases. The fungal cell wall is rich in cellulose and chitin. The activity of cell wall-degrading enzymes is considered an important mechanism of

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

microbial antifungal action (**50**). Thus, hydrolytic enzymes produced by biocontrol agents, such as chitinases, glucanases, and proteases, are important mechanisms involved in the biocontrol of fungal plant pathogens. So, the mechanism involved in the *in vitro* disease suppression might involve the production of these degradation enzymes.

The data obtained in the present study corroborate with previous studies in which species of the genus *Bacillus* were able to control fungal pathogens *in vitro* by the volatile organic compounds (**51**, **52**, **53**, **54**). For example, (**21**) related the antagonistic effect of VOCs produced by *Bacillus amyloliquefaciens* strain NJZJSB3 that adversely affected the growth of *S. sclerotiorum* due to its antifungal activity. The volatile organic compounds (VOCs) produced by *Bacillus* species have demonstrated significant antifungal activity against various phytopathogens. For instance, **52** reported that VOCs emitted by *Bacillus velezensis* inhibited the mycelial growth of several phytopathogens. Moreover, according to **55**, VOCs can function as biofumigants, offering advantages over traditional biological and chemical fungicides, such as the absence of residue and pollution, as they do not require direct spraying.

In this study, we hypothesized that the VOCs produced by *Bacillus bombysepticus* JAB01 might exhibit fungicidal effects against *Sclerotinia sclerotiorum* hyphae. While the VOCs effectively inhibited hyphal development, the hyphae regained their growth momentum upon transfer to fresh PDA medium, suggesting that the inhibitory effects of the VOCs are not long-lasting or permanent. However, it is noteworthy that the VOCs emitted by JAB01 significantly impacted sclerotial germination, as healthy sclerotia co-cultured with the antagonist lost viability in vitro. These results align with findings reported by **18** and **19**, emphasizing the potential of VOCs in disrupting fungal resistance structures. Furthermore, scanning electron microscopy (SEM) observations (Figure 3B) revealed damage to the fungal hyphae following exposure to JAB01 VOCs. This damage likely weakens the hyphae, reducing their ability to infect and colonize host plants. Such antagonistic activity could explain the smaller lesions observed on soybean leaves treated with JAB01 VOCs in detached leaf assays. These results underscore the potential of *Bacillus bombysepticus* JAB01 VOCs as effective biocontrol agents by targeting both the mycelial growth and sclerotia viability of *S. sclerotiorum*, thereby limiting its pathogenicity.

The results of this study suggest that *Bacillus bombysepticus* JAB01 has significant potential as a biocontrol agent against white mold disease in host plants. This hypothesis was supported by its demonstrated effectiveness in controlling the disease in soybean plants grown under greenhouse conditions. These findings pave the way for the development of innovative biological methods to manage white mold, either through the identification of bioactive molecules, the creation of biocontrol products, or the utilization of *B. bombysepticus* JAB01 as a source of genes for further applications. Despite these promising results, it is crucial to emphasize the importance of conducting field studies to assess the in vivo potential of *Bacillus* JAB01 as a disease control agent under real agricultural conditions. Field trials are essential to determine the efficacy of the antifungal compounds produced by JAB01 in controlling *Sclerotinia sclerotiorum*, especially considering the influence of various abiotic factors such as temperature, humidity, and competition with other soil microorganisms, which may affect its performance.

Such studies would not only validate the practical application of this isolate but also provide critical insights into its mechanisms of action. Future research should focus on understanding how *B. bombysepticus* JAB01 suppresses *S. sclerotiorum* infection in host plants and the potential pathways involved in its antifungal activity. These investigations will further strengthen the case for its adoption in agricultural settings. However, it is also important to consider the broader ecological implications of introducing microorganisms into the soil at high concentrations as biocontrol agents. While their use offers substantial advantages, little is currently known about the potential consequences for native soil microbial communities. Addressing these concerns through comprehensive ecological studies will ensure the sustainable and responsible use of *Bacillus* JAB01 in agricultural practices.

Conclusion

In conclusion, our study demonstrates that *Bacillus bombysepticus* JAB01 effectively suppressed *S. sclerotiorum* growth and sclerotia germination in *in vitro* assays. Besides that, the *Bacillus* significantly reduced the disease infection on seeds, so it has the potential to be used as a seed treatment biocontrol agent. Notably, it has proven effective in inhibiting fungal development within soybean plants under controlled greenhouse conditions.

Looking ahead, future investigations should focus on evaluating the performance of *B. bombysepticus* JAB01 in field trials conducted under diverse environmental conditions. This step is critical for assessing the practical applicability and real-world effectiveness of this promising biocontrol agent.

References

1. Liang X & Rollins JA. Mechanisms of broad host range necrotrophic pathogenesis in sclerotinia sclerotiorum. Phytopathology 108 (2018): 1128–1140

2. Sousa Melo B, Voltan AR, Arruda W, Cardoso Lopes FA, Georg RC, et al. Morphological and molecular aspects

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

of sclerotial development in the phytopathogenic fungus Sclerotinia sclerotiorum. Microbiological Research 229 (2019): 126326.

3. McDonald MR & Boland GJ. Forecasting diseases caused by Sclerotinia spp. in eastern Canada: fact or fiction? 26 (2010): 480–488.
4. Yaderets VV, Karpova NV, Glagoleva EV, Ovchinnikov AI, Petrova KS, & et al. Inhibition of the growth and development of sclerotinia sclerotiorum (Lib.) de bary by combining azoxystrobin, penicillium chrysogenum vkm f-4876d, and bacillus strains. Agronomy 11 (2021): 2520.
5. Danielson GA, Nelson BD & Helms TC. Effect of Sclerotinia Stem Rot on Yield of Soybean Inoculated at Different Growth Stages 88 (2007): 297–300.
6. Brustolin R, Reis EM & Pedron L. Longevity of Sclerotinia sclerotiorum sclerotia on the soil surface under field conditions. Summa Phytopathologica 42 (2016).
7. Bolton MD, Thomma BPHJ & Nelson BD. Sclerotinia sclerotiorum (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Molecular Plant Pathology 7 (2006): 1–16.
8. Bloomfield BJ & Alexander M. Melanins and Resistance of Fungi to Lysis. Journal of Bacteriology 93 (1967): 1276–1280.
9. Kyoung SK, Min JY & Dickman MB. Oxalic Acid Is an Elicitor of Plant Programmed Cell Death during Sclerotinia sclerotiorum Disease Development 21 (2008): 605–612.
10. Xu L, Li G, Jiang D & Chen W. Sclerotinia sclerotiorum: An Evaluation of Virulence Theories 56 (2018): 311–338.
11. Brent KJ & Hollomon DW. Fungicide resistance in crop pathogens: how can it be managed? 2nd revised edition (2007).
12. Kuang J, Wang J & Zhou M. Monitoring carbendazim and dimethachlon resistance of Sclerotinia sclerotiorum obtained from the blight stems of rape in Jiangsu Province. Chin. Agric. Sci. Bull 27 (2011): 285-291.
13. Firoz MJ, Xiao X, Zhu FX, Fu YP, Jiang DH, Schnabel G, et al. Exploring mechanisms of resistance to dimethachlone in Sclerotinia sclerotiorum. Pest Management Science 72 (2016): 770–779.
14. M Mari, F Neri and P Bertolini. Novel approaches to prevent and control postharvest diseases of fruits. Stewart Postharvest Review 3 (2008): 1–7.
15. Cavalcanti VP, Araújo NAF, Machado NB, Costa Júnior PSP, Pasqual M, Alves E, et al. Yeasts and Bacillus spp. as potential biocontrol agents of Sclerotinia sclerotiorum in garlic. Scientia Horticulturae 261 (2020): 108931.
16. You-you G, Xiao-yu L, Gui-ming D & Yu-chao M. Antimicrobial Activities and Efficacy of Endophytic Streptomyces sp. SSD49 in Plant Disease Control and Plant-growth-promoting. Biotechnology Bulletin 33 (2017): 121.
17. de Rezende LC, de Andrade Carvalho AL, Costa LB, de Almeida Halfeld-Vieira B, Silva LG, et al. Optimizing mass production of Trichoderma asperelloides by submerged liquid fermentation and its antagonism against Sclerotinia sclerotiorum. World Journal of Microbiology and Biotechnology 36 (2020): 1–14.
18. Fernando WGD, Nakkeeran S, Zhang Y & Savchuk S. Biological control of Sclerotinia sclerotiorum (Lib.) de Bary by Pseudomonas and Bacillus species on canola petals. Crop Protection 26 (2007): 100–107.
19. Massawe VC, Hanif A, Farzand A, Mburu DK, Ochola SO, et al. Volatile compounds of endophytic Bacillus spp. have biocontrol activity against Sclerotinia sclerotiorum. Phytopathology 108 (2018): 1373–1385.
20. Liu J, Hu X, He H, Zhang X, Guo J, Bai J & Cheng Y. Digital gene expression profiling of the transcriptional response to Sclerotinia sclerotiorum and its antagonistic bacterium Bacillus amyloliquefaciens in soybean. Frontiers in Microbiology 13 (2022): 4404.
21. Wu Y, Yuan J, Raza W, Shen Q & Huang Q. Biocontrol Traits and Antagonistic Potential of Bacillus amyloliquefaciens Strain NJZJSB3 Against Sclerotinia sclerotiorum, a Causal Agent of Canola Stem Rot. J. Microbiol. Biotechnol 24 (2014): 1327–1336.
22. Ansary WR, Prince FRK, Haque E, Sultana F, West HM, Rahman M, et al. Endophytic Bacillus spp. from medicinal plants inhibit mycelial growth of Sclerotinia sclerotiorum and promote plant growth. Zeitschrift Fur Naturforschung - Section C Journal of Biosciences 73 (2018): 247–256.
23. Legein M, Smets W, Vandenheuvel D, Eilers T, Muyshondt B, et al. Modes of Action of Microbial Biocontrol in the Phyllosphere. Frontiers in Microbiology 11 (2020): 1619.
24. Weisburg WG, Barns SM, Pelletier DA & Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173 (1991): 697–703.
25. Benchlih S, Esmaeel Q, Aberkani K, Tahiri A, Belabess Z, et al. Modes of Action of Biocontrol Agents and Elicitors for sustainable Protection against Bacterial Canker of Tomato. Microorganisms 11 (2023): 726.
26. Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, et al. The MaSuRCA genome assembler. Bioinformatics 29 (2013).

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

27. Grant JR, Enns E, Marinier E, Mandal A, Herman EK, et al. Proksee: In-depth characterization and visualization of bacterial genomes. Nucleic Acids Research 51 (2023).

28. Lee MD. GToTree: A user-friendly workflow for phylogenomics. Bioinformatics 35 (2019).

29. Letunic I & Bork P. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Research (2024): 1–5.

30. Lee I, Kim YO, Park SC & Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. International Journal of Systematic and Evolutionary Microbiology 66 (2016): 1100–1103.

31. Monnerat R, Montalvão SCL, Martins ES, Queiroz PR, Silva EYY, et al. Manual de produção e controle de qualidade de produtos biológicos à base de bactérias do gênero Bacillus para uso na agricultura. - Portal Embrapa (2020).

32. Kreft S & Kreft M. Quantification of dichromatism: a characteristic of color in transparent materials. Journal of the Optical Society of America. A, Optics, Image Science, and Vision 26 (2009): 1576.

33. Abdullah MT, Ali NY & Suleman P. Biological control of Sclerotinia sclerotiorum (Lib.) de Bary with Trichoderma harzianum and Bacillus amyloliquefaciens. Crop Protection 27 (2008): 1354–1359.

34. Gao H, Li P, Xu X, Zeng Q & Guan W. Research on Volatile Organic Compounds from Bacillus subtilis CF-3: Biocontrol Effects on Fruit Fungal Pathogens and Dynamic Changes During Fermentation. Frontiers in Microbiology 9 (2018).

35. Chen Y & Wang D. Two Convenient Methods to Evaluate Soybean for Resistance to Sclerotinia sclerotiorum 89 (2007): 1268–1272.

36. Tserkovniak LS & Kurdish IK. Phosphate-mobilizing bacteria Bacillus subtilis as phenolic producers. Applied Biochemistry and Microbiology 45 (2009): 279–284.

37. Ashwini N & Srividya S. Potentiality of Bacillus subtilis as biocontrol agent for management of anthracnose disease of chilli caused by Colletotrichum gloeosporioides OGC1. 3 Biotech 2013 4:2, 4 (2013): 127–136.

38. Chaurasia B, Pandey A, Palni LMS, Trivedi P, Kumar B, et al. Diffusible and volatile compounds produced by an antagonistic Bacillus subtilis strain cause structural deformations in pathogenic fungi in vitro. Microbiological Research 160 (2005): 75–81.

39. Kamal MM, Savocchia S, Lindbeck KD & Ash GJ. Biology and biocontrol of Sclerotinia sclerotiorum (Lib.) de Bary in oilseed Brassicas. Australasian Plant Pathology 45 (2015): 1.

40. Rollins JA & Dickman MB. pH signaling in Sclerotinia sclerotiorum: Identification of a pacC/RIM1 homolog. Applied and Environmental Microbiology 67 (2001): 75–81.

41. Giorgio A, De Stradis A, Lo Cantore P & Iacobellis NS. Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on Sclerotinia sclerotiorum. Frontiers in Microbiology 6 (2015).

42. Willetts HJ & Wong AL. Ontogenetic diversity of sclerotia of Sclerotinia sclerotiorum and related species. Brit Mycol Soc Trans (1971).

43. Tyagi S, Lee KJ, Shukla P & Chae JC. Dimethyl disulfide exerts antifungal activity against Sclerotinia minor by damaging its membrane and induces systemic resistance in host plants. Scientific Reports 10 (2020): 1–12.

44. Haddad PE, Leite LG, Lucon CMM & Harakava R. Selection of Trichoderma spp. strains for the control of Sclerotinia sclerotiorum in soybean. Pesquisa Agropecuária Brasileira 52 (2017): 1140–1148.

45. Yang X, Yang J, Wang Y, He H, Niu L, Guo D, et al. Enhanced resistance to sclerotinia stem rot in transgenic soybean that overexpresses a wheat oxalate oxidase. Transgenic Research 28 (2019): 103–114.

46. Moustafa SA, Kamil Z, Rizk M, Saleh M & Moustafa S. Isolation and Identification of Rhizosphere Soil Chitinolytic Bacteria and their Potential in Antifungal Biocontrol. Global Journal of Molecular Sciences 2 (2007): 57–66.

47. Abbas A, Khan SU, Khan WU, Saleh TA, Khan MHU, et al. Antagonist effects of strains of Bacillus spp. against Rhizoctonia solani for their protection against several plant diseases: Alternatives to chemical pesticides. Comptes Rendus Biologies 342 (2019): 124–135.

48. Rahman MME, Hossain DM, Suzuki K, Shiiya A, Suzuki K, et al. Suppressive effects of Bacillus spp. on mycelia, apothecia and sclerotia formation of Sclerotinia sclerotiorum and potential as biological control of white mold on mustard. Australasian Plant Pathology 45 (2016): 103–117.

49. Oztopuz O, Sarigul N, Liaqat F, Park RD & Eltem R. Chitinolytic Bacillus subtilis Ege-B-1.19 as a biocontrol agent against mycotoxigenic and phytopathogenic fungi. Turkish Journal of Biochemistry 44 (2019): 323–331.

50. Sadeghy B & Hatamy N. Isolation and investigation of extracellular metabolites of Streptomyces sp. for biological control of Fusarium subglutinans, the causal

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

agent of wilting cucumber greenhouse 47 (2014): 171–177.

51. Yuan J, Raza W, Shen Q & Huang Q. Antifungal activity of Bacillus amyloliquefaciens NJN-6 volatile compounds against Fusarium oxysporum f. sp. cubense. Applied and Environmental Microbiology 78 (2012): 5942–5944.

52. Gao Z, Zhang B, Liu H, Han J, & Zhang Y. Identification of endophytic Bacillus velezensis ZSY-1 strain and antifungal activity of its volatile compounds against Alternaria solani and Botrytis cinerea. Biological Control 105 (2017): 27–39.

53. Tahir HAS, Gu Q, Wu H, Niu Y, Huo R & Gao X. Bacillus volatiles adversely affect the physiology and ultra-structure of Ralstonia solanacearum and induce systemic resistance in tobacco against bacterial wilt. Scientific Reports 7 (2017): 1.

54. Myo EM, Liu B, Ma J, Shi L, Jiang M, Zhang K & Ge B. Evaluation of Bacillus velezensis NKG-2 for bio-control activities against fungal diseases and potential plant growth promotion. Biological Control 134 (2019): 23–31.

55. Li Q, Wu L, Hao J, Luo L, Cao Y & Li J. Biofumigation on Post-Harvest Diseases of Fruits Using a New Volatile-Producing Fungus of Ceratocystis fimbriata. PLOS ONE 10 (2015): e0132009.

Rodrigues PKB, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500206

Supplementary figure S1: – Microscopic visualization of the morphology of the bacterial isolate JAB01.

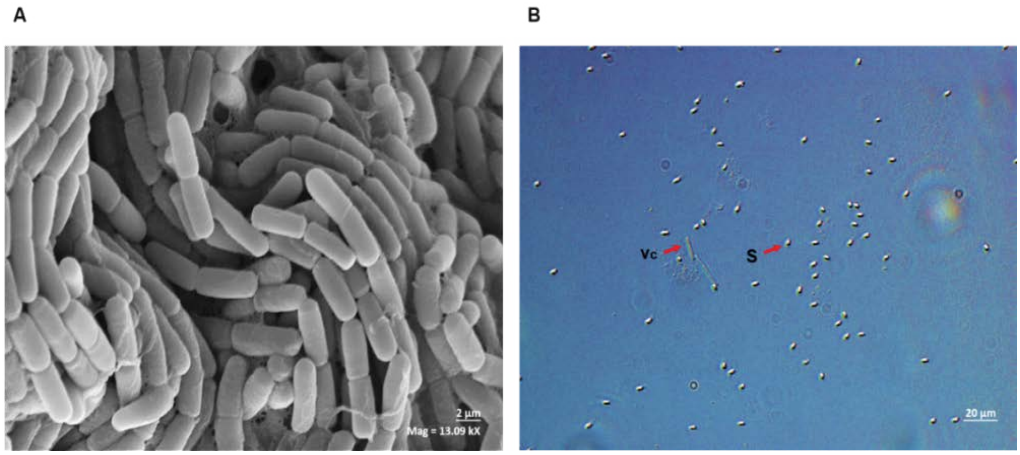

Figure 1: Morphological characteristics of *Bacillus* JAB01. (**A**) JAB01 cells under scanning electron microscopy. (**B**) Spores (S) and vegetative cells (Vc) of JAB01 under phase contrast microscopy at 1000x magnification.