

Article

Characterization and Evaluation of *Bacillus altitudinis* WR7 as a Biocontrol Agent for Rubber Tree Anthracnose

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Abstract

Anthracnose, caused by *Colletotrichum siamense*, is a major limiting factor for global natural rubber production. To develop sustainable control strategies, seven bacterial strains with antagonistic activity against *C. siamense* were isolated from healthy rubber tree leaves, with strain WR7 demonstrating the most significant antifungal effect, exhibiting an inhibition rate of 82.36%. Pot experiments revealed that WR7 achieved a disease control efficacy of 71.65% against *C. siamense*-induced anthracnose. Genomic analysis identified WR7 as *Bacillus altitudinis*. This strain inhibits pathogen growth through multiple mechanisms, including disruption of the pathogen's cell wall and membrane integrity, induction of reactive oxygen species accumulation in hyphae, and secretion of cellulase, glucanase, protease, and siderophores. Gene cluster analysis further confirmed the potential of WR7 to synthesize antagonistic secondary metabolites such as lichenysin, fengycin, and bacilysin, while its sterile filtrate and volatile compounds also exhibited significant antifungal activity. Moreover, treatment with WR7 activated defense-related enzymes, including catalase and superoxide dismutase in rubber tree leaves, thereby enhancing the plant's defense responses. This study is the first to report that *Bacillus altitudinis* WR7 has potential as a biocontrol agent for managing rubber tree anthracnose, offering a novel resource for sustainable disease management in rubber production.

Keywords: endophytic bacteria; anthracnose; rubber tree; *Colletotrichum siamense*; biocontrol mechanisms



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1. Introduction

Rubber tree (*Hevea brasiliensis* Muell. Arg.), a key economic crop in tropical regions, plays a vital role in the economies of tropical countries due to its ability to produce natural rubber (cis-1,4-polyisoprene), an essential industrial raw material [1,2]. However, the growth of rubber trees is often hindered by various diseases [3–5]. Anthracnose, a typical foliar fungal disease, primarily infects tender leaves, mature leaves, and branches, causing symptoms such as necrotic lesions, perforations, wilting, and even defoliation. These effects significantly weaken tree vigor and reduce latex productivity. In severe cases, anthracnose can lead to substantial yield losses across rubber plantations, making it a major factor in the decline of latex yield [6–8]. Current research suggests that the taxonomy of pathogens causing rubber tree anthracnose is complex, with notable geographical variations in dominant

pathogenic species [9,10]. In the rubber-growing regions of Hainan Province, China, the disease is mainly caused by *Colletotrichum siamense* [8,11]. The diversity of pathogenic species and their spatial distribution heterogeneity significantly complicate disease management. As a result, developing effective and sustainable management strategies remains a critical research priority. Presently, control of rubber tree anthracnose primarily depends on chemical pesticides, which, while effective in the short term, contribute to increased pathogen resistance, environmental degradation, and pesticide residue issues with long-term use [9]. Therefore, the exploration of environmentally friendly and sustainable biological control resources, along with the establishment of ecologically regulated, integrated management systems, has become both a key research focus and an urgent priority in the field of rubber tree disease control.

Plant endophytes are microbial communities that reside within plant tissues, intercellular spaces, or internal organs, establishing a stable symbiotic relationship with the host plant without causing phenotypic abnormalities or significant pathological symptoms [12,13]. Endophytes play a pivotal role in promoting plant growth and enhancing disease resistance. As a result, studies on their diversity, biological functions, and practical applications have become a prominent area of focus in plant protection research [14]. Plant endophytes enhance nitrogen absorption, trace element utilization, and disease resistance through mechanisms such as nitrogen fixation, phosphorus solubilization, potassium mobilization, secretion of growth hormones, and production of siderophores. Additionally, endophytes improve plant stress tolerance and regulate growth and development by synthesizing secondary metabolites with antimicrobial and antioxidant properties, offering significant potential for new drug development and environmental protection [14,15].

Bacillus species, known for their ability to produce various antimicrobial compounds and their broad environmental adaptability, are widely applied in the biological control of plant diseases [16,17]. Among these, *Bacillus altitudinis* has shown antagonistic effects against several crop pathogens and also promotes host growth, demonstrating strong potential for biocontrol applications [18,19]. For example, *B. altitudinis* effectively controls soybean seed rot caused by *Calonectria ilicicola* [20]. Additionally, *B. altitudinis* BRHS/S-73 promotes growth and disease control by activating soil phosphorus, secreting growth-promoting substances, inhibiting *Thanatephorus cucumeris*, and enhancing plant resistance against mung bean root rot [21]. The *B. altitudinis* KPB25 strain combats apple fire blight by reshaping the leaf microbial community, enriching beneficial bacteria that produce organic acids or antibiotics, and creating a microenvironment that inhibits *Erwinia amylovora* colonization [22].

Endophytic *Bacillus* species have been extensively studied for their potential in biological disease control. However, no reports have been made on the use of leaf-endophytic *B. altitudinis* for managing anthracnose in rubber trees. Therefore, this study isolates *B. altitudinis* from rubber tree leaves to evaluate its efficacy against rubber tree anthracnose and to preliminarily explore its biocontrol mechanisms. The findings aim to provide novel microbial resources and theoretical support for the sustainable management of rubber tree anthracnose.

2. Results

2.1. Isolation of Endophytic Bacteria from Rubber Tree Leaves and Evaluation of Their Antagonistic Activity Against *C. siamense*

A total of 42 bacterial strains were successfully isolated from rubber tree leaves using the dilution spread plate method. Dual-culture experiments revealed that seven of these strains exhibited antagonistic activity against *C. siamense*. Among them, WR7 demonstrated the strongest antifungal effect, with an inhibition rate of $82.36\% \pm 6.21\%$ against *C. siamense*.

The inhibition rates of the remaining six strains ranged from 30% to 70%. Consequently, WR7 was selected as the candidate strain for subsequent experiments (Figure 1; Table S1).

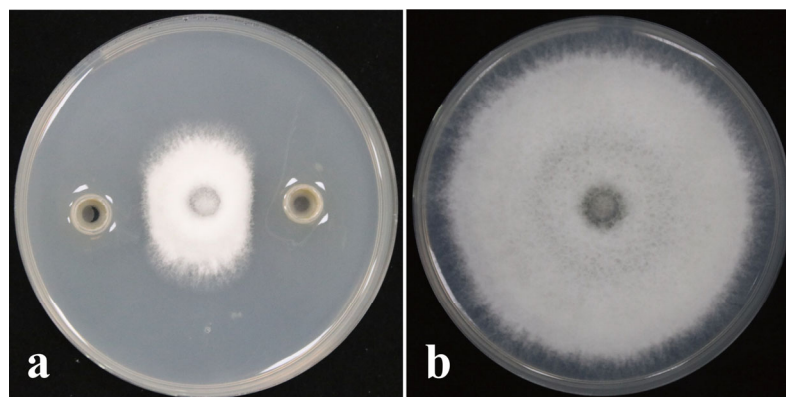


Figure 1. Antifungal activity of WR7 against *C. siamense*. A 5 mm diameter plug of *C. siamense* was inoculated at the center of each PDA plate. Bacterial culture (a) or LB medium (b) was symmetrically inoculated at two spots on the plate. Fungal colony diameters were measured.

2.2. Control Efficacy of WR7 Against Rubber Tree Anthracnose Under Greenhouse Conditions

The control efficacy of WR7 against rubber tree anthracnose was assessed using detached leaf assays and greenhouse pot experiments. In the detached leaf assay, WR7 and mancozeb achieved control efficacies of 70.07% and 73.72%, respectively, with no statistically significant difference in efficacy ($p < 0.05$) (Figure 2A,C). In the greenhouse pot experiment, WR7 and mancozeb demonstrated control efficacies of 71.65% and 67.72%, respectively, with no statistically significant difference between them ($p < 0.05$) (Figure 2B,D). Rubber tree leaves inoculated with WR7 exhibited no disease symptoms, confirming the safety of WR7 for the plants (Figure 2A,B).

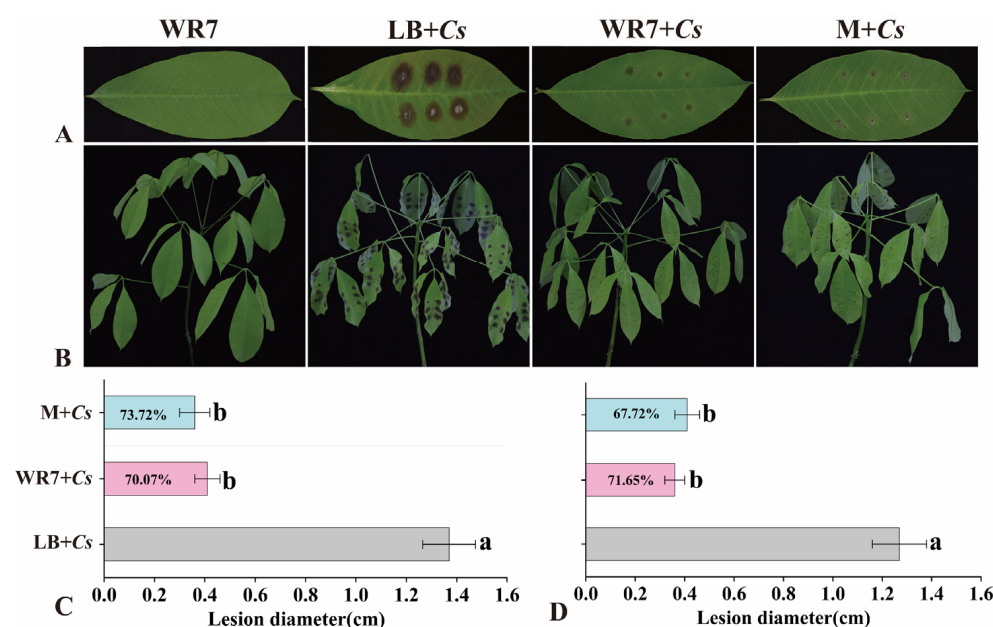


Figure 2. In vitro leaf control efficacy of strain WR7 against rubber tree anthracnose. (A) Impact of various inoculation techniques on detached rubber tree leaves. (B) Effect of different treatments on potted rubber trees in the greenhouse. (C) Control efficacy of WR7 against anthracnose caused by *C. siamense* on detached rubber tree leaves. (D) Control efficacy of WR7 against anthracnose caused by *C. siamense* in potted rubber trees. M: mancozeb; Cs: *C. siamense*; LB: Negative control. Error bars represent the standard error of the mean. According to Tukey's HSD test ($p < 0.05$), the same lowercase letters indicate no significant difference between treatment groups.

2.3. Genome Assembly and Functional Annotation of Strain WR7

Whole-genome sequencing results revealed that the genome of strain WR7 is a circular DNA molecule with no plasmids, a total length of 3,697,828 bp, and a GC content of 41.43% (Figure S1). The genome encodes 81 tRNA genes, 8 copies each of 5S rRNA, 16S rRNA, and 23S rRNA genes, and 83 copies of ncRNA (Table S2). WR7 exhibited average nucleotide identity (ANI) values of 97.77% and 97.80%, and DNA-DNA hybridization (DDH) values of 98.37% and 79.7%, respectively, when compared to *B. altitudinis* 41KF2b. In contrast, ANI and DDH values relative to other *Bacillus* species were both below the thresholds of 96% and 70%, respectively. These findings confirm that WR7 is a strain of *B. altitudinis* (Table S3).

A total of 3597 genes were annotated in the COG database and classified into 21 categories. Apart from genes of unknown function, relatively high proportions of genes were associated with transcription, amino acid transport and metabolism, and carbohydrate transport and metabolism (Figure S2). Gene Ontology (GO) annotation of the WR7 genome categorized it into biological process, cellular component, and molecular function, covering 72 functional groups. A total of 8353 genes were annotated, with the largest proportions related to molecular function, biological process, and biosynthetic processes (Figure S3). Comparative analysis of the WR7 genome with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database led to the annotation of 3924 genes across 48 metabolic pathways. Notably, a larger number of genes were involved in genetic information processing, signaling and cellular processes, and carbohydrate metabolism, with counts of 573, 552, and 376, respectively (Figure S4).

Genome prediction using antiSMASH identified multiple biosynthetic gene clusters (BGCs) in WR7, exhibiting high similarity to known secondary metabolite synthesis gene clusters. These clusters encode sporulation killing factor, lichenysin, schizokinen, fengycin, and bacilysin (Figure 3; Table S4).

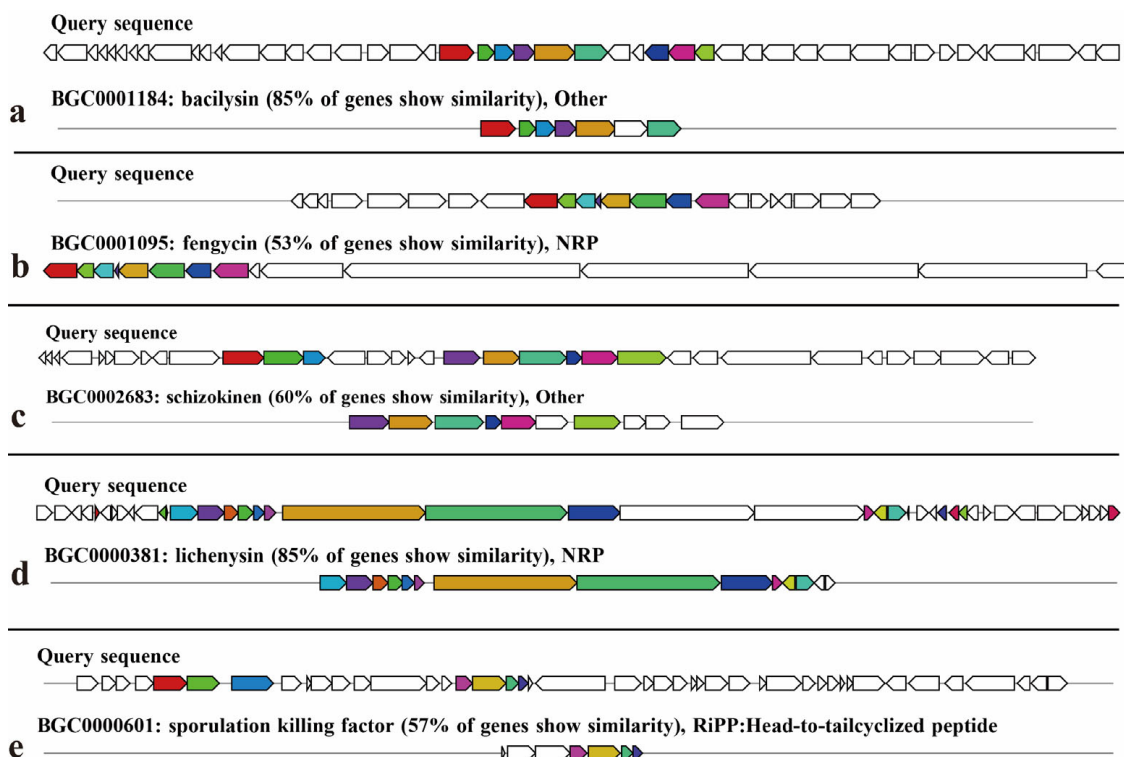


Figure 3. Prediction of Secondary Metabolite Biosynthesis Gene Clusters (BGCs) in Strain WR7. (a) Bacilysin. (b) Fengycin. (c) Schizokinen. (d) Lichenysin. (e) Sporulation killing factor.

2.4. Antifungal Effects of WR7 Volatile Compounds and BCF Against *C. siamense*

The antifungal activity of WR7 volatile compounds against *C. siamense* showed that bacterial culture spreading volumes of 25, 50, and 100 μL effectively inhibited mycelial growth, with inhibition rates of 20.70%, 33.97%, and 55.68%, respectively (Figure 4A,B). These results indicate that WR7's volatile substances significantly antagonize *C. siamense* ($p < 0.05$). Additionally, the antifungal activity of WR7 bacterial culture filtrate (BCF) against *C. siamense* demonstrated that sterile filtrates at three concentrations (1:2.5, 1:5, and 1:10) effectively inhibited mycelial growth, with inhibition rates of 30.49%, 48.03%, and 59.15%, respectively (Figure 5c,d). These results indicate a concentration-dependent enhancement in the inhibition rate as the BCF concentration increases ($p < 0.05$).

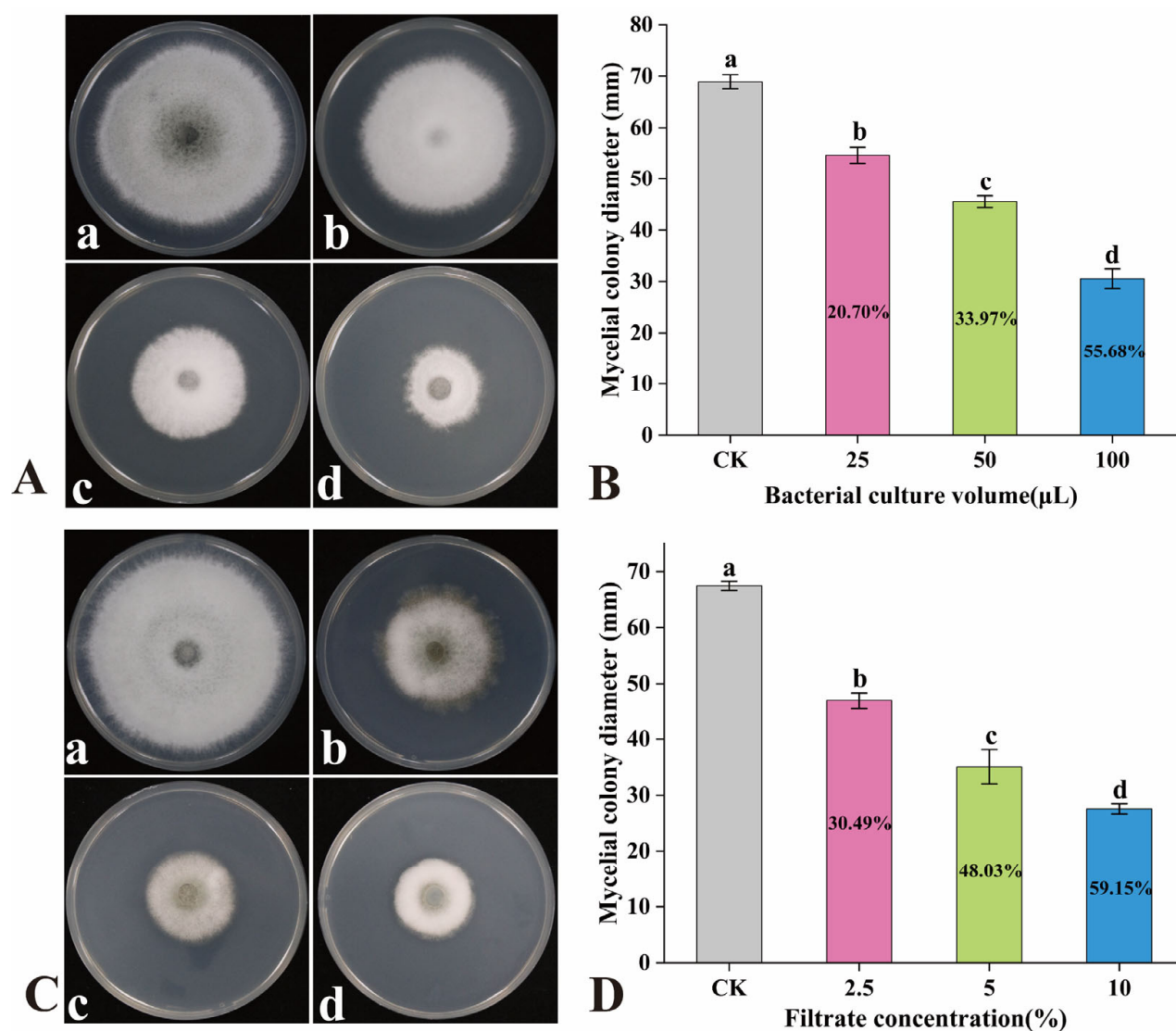


Figure 4. (A,B) Inhibitory effect of volatile organic compounds from strain WR7 on the mycelial growth of *C. siamense*. Concentrations of the bacterial cell suspension in (A): (a) 0, (b) 25 μL , (c) 50 μL , (d) 100 μL . (C,D) Inhibitory effect of the BCF of WR7 on the mycelial growth of *C. siamense*. (C) Effect at different BCF concentrations: (a) 0 (LB control), (b) 2.5%, (c) 5%, (d) 10%. Error bars represent the standard error of the mean. According to Tukey's HSD test ($p < 0.05$), the same lowercase letters indicate no significant difference between treatment groups.

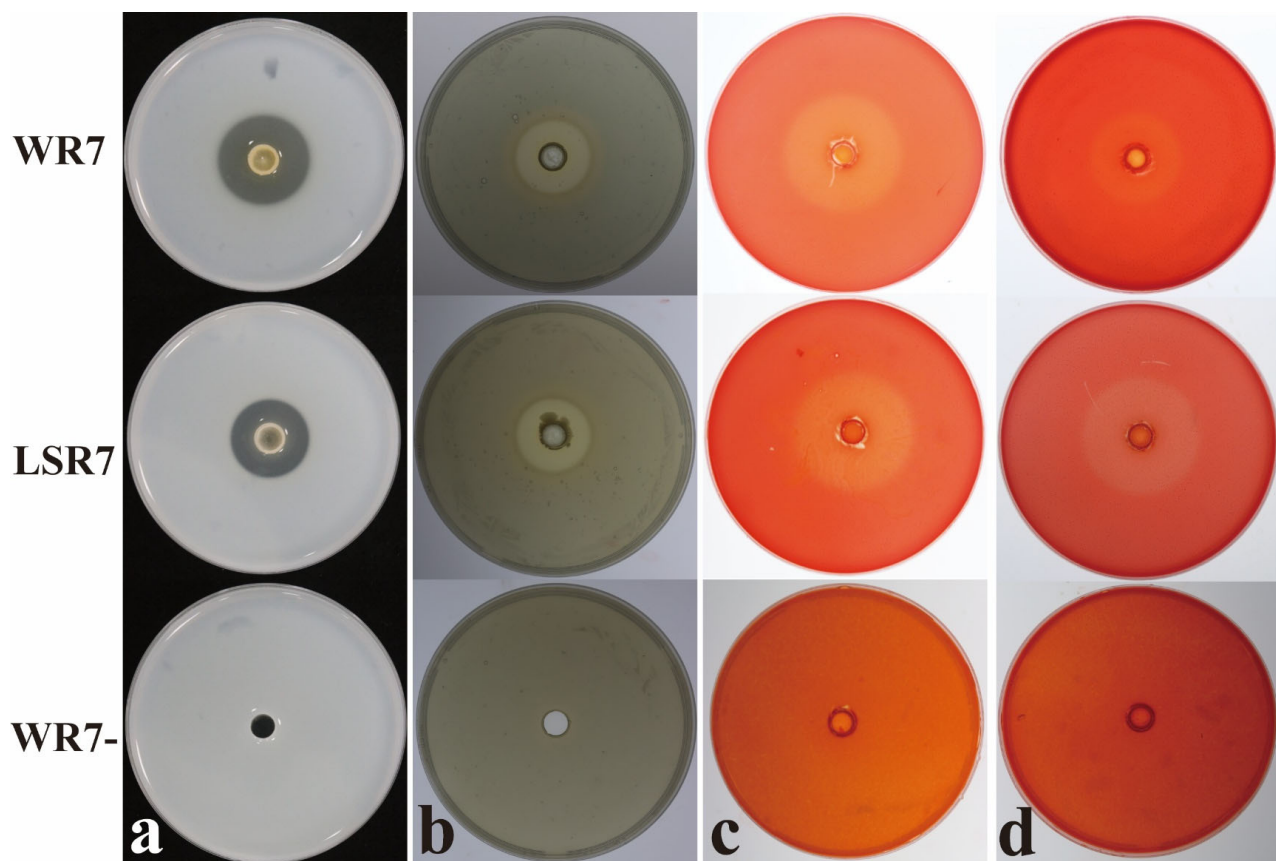


Figure 5. The ability of strain WR7 to produce extracellular enzymes and siderophores. (a) Protease production. (b) Cellulase production. (c) Pectinase production. (d) Siderophore production. LSR7, used as the positive control, is a *Bacillus* strain that has been previously demonstrated to possess the ability to produce the aforementioned extracellular enzymes and siderophores. WR7- refers to the heat-inactivated cells of strain WR7.

2.5. Experimental Confirmation of Extracellular Enzyme and Siderophore Production in Strain WR7

Culture medium-based assays confirmed that strain WR7 exhibits positive extracellular enzyme and siderophore production activities comparable to those of the positive control *B. velezensis* LSR7. In both the protease and siderophore assays, clear hydrolysis zones were observed around the colonies, confirming the secretion of these compounds. In the cellulase and glucanase assays, yellow halos formed around the colonies after Congo red staining and sodium chloride decolorization, indicating active degradation of cellulose and glucan substrates. No such activity zones were detected on LB medium control plates (Figure 5). These results demonstrate that WR7 has the functional ability to produce siderophores, proteases, cellulases, and glucanases under the tested conditions.

2.6. Effects of WR7 BCF on Membrane Integrity and ROS Levels in *C. siamense* Mycelia

After treatment with different ratios of WR7 BCF, the mycelia of *C. siamense* exhibited distinct red fluorescence following PI staining. Quantitative analysis using ImageJ showed that, compared to the control group (untreated with BCF), the mean optical density (MOD) of mycelia in all treatment groups significantly increased ($p < 0.05$), with a dose-dependent enhancement as the BCF ratio increased (1:10, 1:5, 1:2.5). These results indicate that WR7 BCF effectively disrupts the cell membrane integrity of *C. siamense*, leading to increased membrane permeability (Figure 6A,B). DCHF-DA staining results indicated a significant increase in reactive oxygen species (ROS) levels in the mycelia of *C. siamense* following treat-

ment with WR7 BCF. Fluorescence microscopy revealed intense green fluorescence in the treated mycelia, while the control group exhibited weak fluorescence signals. Quantitative analysis using ImageJ further confirmed that the ROS-associated fluorescence intensity in all treatment groups was significantly higher than in the control group ($p < 0.05$), showing a dose-dependent effect. These results suggest that WR7 BCF can induce oxidative stress in the mycelia of *C. siamense* (Figure 6A,C).

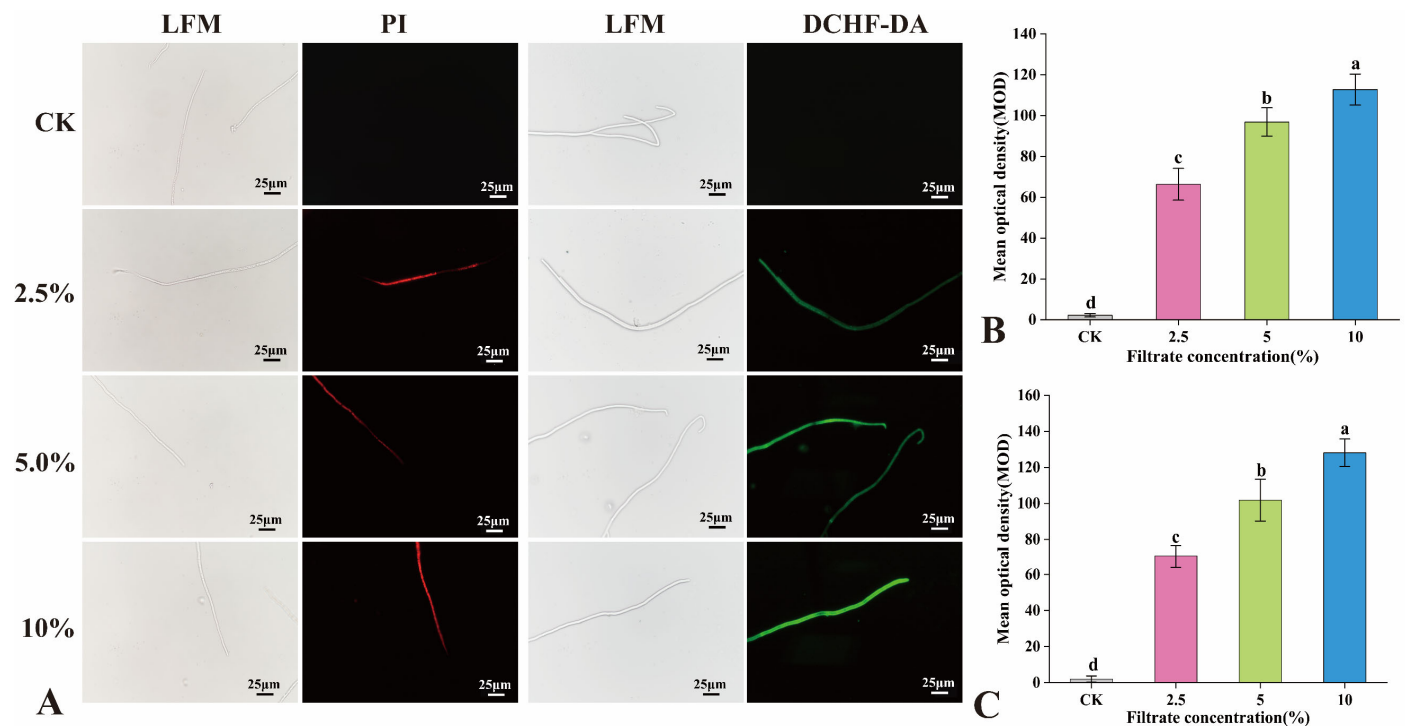


Figure 6. Effect of strain WR7 on cell membrane integrity and ROS in *C. siamense*. (A) PI staining and DCHF-DA staining of *C. siamense* mycelia. Quantitative analysis of the average fluorescence intensity of *C. siamense* mycelia stained with PI (B) and DCHF-DA (C) at different treatment times based on ImageJ. LFM, light-field microscopy. Bar = 50 μ m. Error bars represent the standard error of the mean. Columns sharing the same lowercase letter are not significantly different according to Tukey's HSD test at $p < 0.05$.

2.7. Induction of Defense Responses by WR7 in Rubber Trees

To further investigate the induction of defense responses in rubber tree leaves by WR7, the activities of defense-related enzymes, including PAL, CAT, POD, PPO, and SOD, were measured in leaves from four treatment groups at different time points: 0, 12, 24, 48, 72, and 96 h. In the control group (LB), enzyme activities remained relatively stable over time. In contrast, all three treatment groups exhibited significant peaks in enzyme activities within specific time frames. Among them, the leaves pretreated with WR7 and subsequently inoculated with *C. siamense* showed the most pronounced increase in enzyme activities. The activities of CAT, PAL, and POD peaked at 48 h, while SOD and PPO activities reached their highest levels at 24 h (Figure 7). These results suggest that pre-application of WR7 enhances plant defense mechanisms against pathogen infection by activating cellular defense responses.

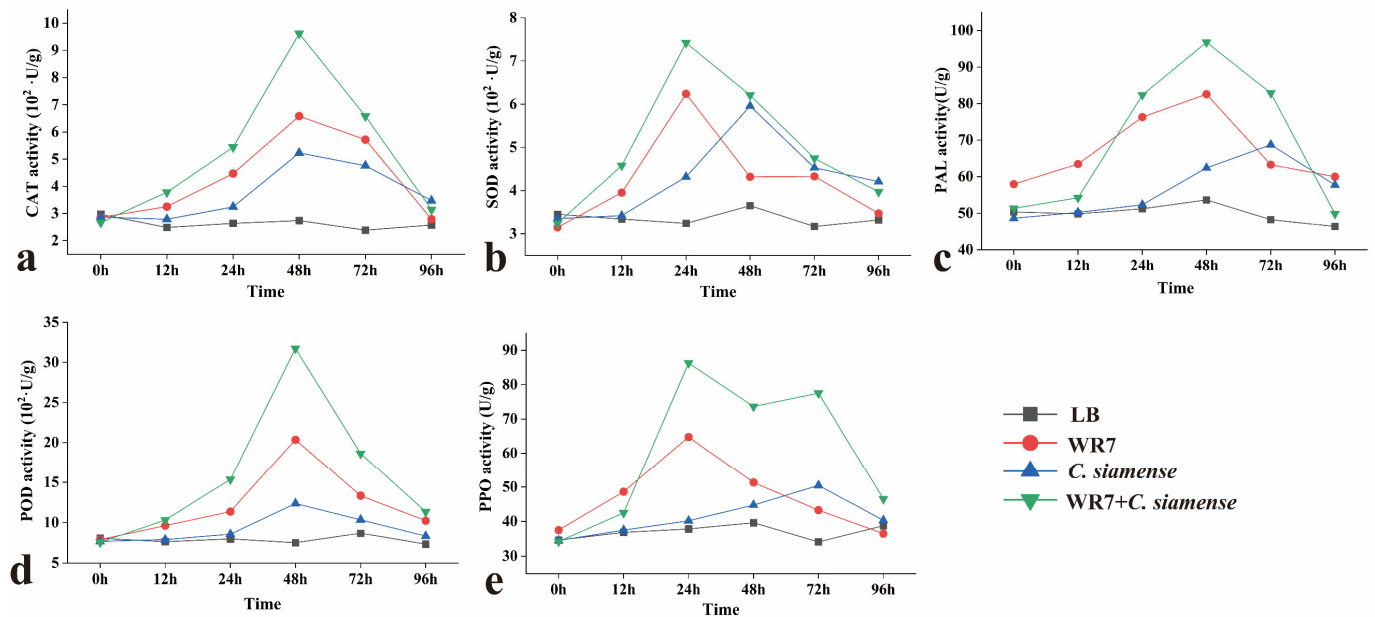


Figure 7. Changes in the activity of defense-related enzymes in rubber tree leaves under different treatments. (a–e) Impact of different inoculations on the activities of catalase (CAT), superoxide dismutase (SOD), phenylalanine ammonia lyase (PAL), peroxidase (POD), and polyphenol oxidase (PPO).

3. Discussion

Research findings highlight the potential of beneficial microorganisms as a safe, efficient, and sustainable approach for controlling rubber tree anthracnose. Previous studies have shown that *Streptomyces deccanensis* QY-3 and *Bacillus velezensis* SF334 are effective in controlling rubber tree anthracnose caused by *C. siamense* [8,23]. However, the use of endophytic *B. altitudinis* for managing this disease has yet to be explored. This study successfully isolated an endophytic bacterial strain, WR7, from healthy leaves, which exhibited significant antagonistic activity against *C. siamense*, the primary pathogen responsible for leaf anthracnose in rubber trees. The strain demonstrated a plate inhibition rate of 82.36% and a pot control efficacy of 71.65%. With its multiple disease-suppression mechanisms, *B. altitudinis* WR7 holds substantial promise as a biocontrol agent for rubber tree anthracnose. To the best of our knowledge, this is the first report on the application of an endophytic *B. altitudinis* strain for controlling rubber tree anthracnose.

Strain WR7 displayed robust direct antifungal activity against *C. siamense*, likely due to its secretion of cell wall-degrading enzymes such as cellulase, glucanase, and protease [24,25]. These enzymes effectively compromise the integrity of the fungal cell wall and membrane. Similar findings have been reported in other studies, such as the one on *B. velezensis* D61-A, which inhibits the growth of the rice sheath blight pathogen *Rhizoctonia solani* by producing protease and cellulase [26]. The resulting damage to the cell wall and membrane of *C. siamense* causes an imbalance in intracellular osmotic pressure and electrolyte leakage, disrupting mitochondrial membrane potential (MMP). This disruption triggers mitochondrial dysfunction and ATP depletion, leading to excessive endogenous ROS production via the mitochondrial respiratory chain [27]. Concurrently, membrane damage allows the entry of extracellular ROS into the cell, culminating in oxidative stress-induced cell death or growth arrest [28,29]. Similarly, *S. griseoaurantiacus* XQ-29 inhibits the growth of *Sclerotium rolfsii* by accumulating ROS in its mycelia, thereby controlling pepper southern blight [30]. The synergistic effects of these mechanisms likely explain the high in vitro antifungal efficacy of WR7.

Genomic analysis confirmed that WR7 possesses the genetic capability to synthesize lipopeptide and polyketide antibiotics, such as lichenysin, bacilysin, and fengycin. These secondary metabolites are key contributors to the antagonistic activity of *Bacillus* species against pathogenic microorganisms [31–33]. *Bacillus licheniformis* effectively inhibits the growth of *C. gloeosporioides* by producing lichenysin, which induces elongation and malformation of the pathogen's hyphae [34]. Similarly, *B. velezensis* strain GX0002980 secretes antimicrobial substances, including surfactin, bacilysin, and butirosin A. Its volatile organic compounds and sterile fermentation filtrate also exhibit significant antagonistic activity against *C. gloeosporioides* [16]. Fengycin, produced by *Bacillus subtilis*, induces apoptosis in *Magnaporthe oryzae* by triggering ROS accumulation, chromatin condensation, and collapse of the MMP [31]. Therefore, *B. altitudinis* WR7 likely inhibits the growth of *C. siamense* by producing these antagonistic substances. Additionally, WR7's ability to synthesize siderophores helps it compete for iron ions in the rhizosphere, suppressing pathogen growth and potentially promoting plant growth [35].

B. altitudinis WR7 not only acts directly against pathogenic fungi but also enhances defense-related enzyme activity in rubber tree leaves to resist pathogen invasion. This study found that WR7 treatment significantly increased the activities of PAL, PPO, CAT, SOD, and POD in rubber tree leaves. These findings align with results from other beneficial microorganisms, such as *Burkholderia vietnamiensis* C12, which enhances defense enzyme activity in rice, enabling the plant to resist invasion by *R. solani* [36]. These enzymes are critical components of the plant's phenylpropanoid metabolism pathway and antioxidant system [37,38]. Their activation promotes the synthesis of disease-resistant substances, such as phytoalexins and lignin, while also enhancing cellular antioxidant capacity. This dual action significantly improves the rubber tree's resistance to anthracnose pathogens [39–41]. Through both direct antagonism of pathogenic fungi and the induction of plant disease resistance, WR7 achieved stable control efficacy in pot experiments.

4. Materials and Methods

4.1. Isolation of Endophytic Bacteria from Rubber Tree Leaves

Healthy rubber tree leaves were collected from anthracnose-infected rubber trees at the Experimental Plantation of the Chinese Academy of Tropical Agricultural Sciences in Danzhou City, Hainan Province. Upon collection, the leaves were first rinsed with sterile water and then subjected to surface sterilization using the following procedure: immersion in 70% ethanol for 2 min, followed by treatment with 2.5% sodium hypochlorite solution for 2 min, and finally rinsing three times with sterile distilled water. To evaluate the effectiveness of the surface sterilization, the final rinse water was spread onto lysogeny broth (LB) agar plates to check for contamination. No bacterial growth on the plates indicated that the surface sterilization was successful.

The sterilized leaf tissue was then ground in a sterile mortar. A 100 µL aliquot of the tissue homogenate was subjected to tenfold serial dilutions, establishing eight dilution gradients (10^{-1} to 10^{-8}). From each dilution, 50 µL was spread onto LB agar (LA) plates. After incubation at 28 °C for 3 days, morphologically distinct single colonies were selected and streaked onto fresh LA plates for purification. The purified strains were preserved in 15% glycerol and stored at −80 °C for long-term storage [42].

4.2. Evaluation of Antifungal Activity of Endophytic Bacteria Against *C. siamense*

The dual culture method was used to assess the antagonistic activity of the isolated strains against *C. siamense*. The *C. siamense*, obtained from the Rubber Research Institute of the Chinese Academy of Tropical Agricultural Sciences, was pre-cultured on PDA plates for 3 days. A 5 mm mycelial plug was then taken and placed at the center of a fresh PDA

plate. Using a sterile cork borer, two symmetrical 5 mm diameter holes were created on the plate, 25 mm away from the central plug.

The isolated endophytic bacteria were inoculated into 50 mL of LB medium and cultured at 28 °C with shaking at 130 rpm for 48 h. Subsequently, 70 µL of bacterial culture was added to each culture well, with an equal volume of LB medium used as the negative control. All culture plates were incubated at 28 °C until the mycelium of the control group covered the edge of the plates, at which point the inhibition rate of the bacteria against the pathogenic fungi was calculated. Each treatment consisted of nine samples, and the experiment was repeated three times. The inhibition rate was calculated using the following formula: Inhibition rate (%) = [(Colony diameter of the control group – Colony diameter of the treatment group)/(Colony diameter of the control group – Initial colony diameter of the fungal plug)] × 100% [26].

4.3. Genomic Sequencing, Assembly, and Annotation of WR7

Following the methodology outlined in Section 4.2, strain WR7 was identified as exhibiting strong antagonistic activity against *C. siamense*. A single colony of WR7 was inoculated into LB medium and incubated at 28 °C with shaking at 200 rpm until the bacterial culture reached the logarithmic growth phase ($OD_{600} = 0.6$). The WR7 culture was then centrifuged at 4 °C and 5000 rpm for 15 min to harvest the bacterial cells. After discarding the supernatant, the pellet was washed twice with $1 \times$ PBS buffer. The resulting bacterial precipitate was flash-frozen in liquid nitrogen for 15 min, stored on dry ice, and sent to Personalbio (Shanghai, China). This project employed the Whole Genome Shotgun (WGS) sequencing strategy, with libraries constructed using varying insert fragment lengths. Sequencing was carried out using Next-Generation Sequencing (NGS) and third-generation single-molecule sequencing technologies.

Raw sequencing data were processed using fastp software (v0.23.1) with the following quality control parameters: paired-end reads were discarded if the average quality score of either read fell below 20, ensuring high-quality data for subsequent analysis. The third-generation sequencing data were assembled using Unicycler (v0.5.0), Flye (v2.9.1-b1781), Hifiasm (v0.18.5-r500), and Necat (v0.0.1). The assembly results were polished with Pilon software (version 1.24) using high-quality second-generation sequencing data. tRNA genes in the whole genome were predicted using tRNAscan-SE, while rRNA genes were predicted using Barrnap (version 0.9). Other non-coding RNAs were primarily predicted through comparison with the Rfam database (<https://rfam.org/>; accessed on 28 October 2025). The assembled sequences were functionally analyzed and annotated across multiple databases, including GO, KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), Swiss-Prot, NR (Non-Redundant Protein Database), TCDB (Transporter Classification Database), CAZy (Carbohydrate-Active enZymes Database), and CARD (Comprehensive Antibiotic Resistance Database). Circular genome maps were constructed using the R package (version 0.4.15) circlize to visualize genomic features. For comparative genomic analysis, the ANI was computed using MUMmer (ANIm) with the JSpeciesWS (version: 5.0.3) online tool (<https://jspecies.ribohost.com/jspeciesws/#home>; accessed on 29 October 2025), and genome-to-genome distances were determined via the GGDC web server (<https://ggdc.dsmz.de/>; accessed on 29 October 2025). Additionally, secondary metabolite BGCs were identified and analyzed with the antiSMASH online tool (<https://antismash.secondarymetabolites.org/#!/start> accessed on 30 October 2025).

4.4. Control Effects of WR7 on Anthracnose Infection in Rubber Tree Leaves

Leaves (clone ‘Reyan 73397’) of similar size, morphology, and nodal position were selected from the National Rubber Germplasm Repository in Danzhou City, Hainan Province,

China, to evaluate the control efficacy of WR7 against *C. siamense*. Following surface sterilization as described in Section 4.1, the leaves were placed in humidity chambers and uniformly sprayed with WR7 culture broth. The broth was prepared according to the method in Section 4.2, diluted with LB medium to an OD₆₀₀ of 1.0, and sprayed onto the leaf surfaces until visible droplets formed. Leaves in the control group were sprayed with an equal volume of LB medium. Twelve hours after spraying the WR7 culture broth, the leaves were punctured with sterile syringes, and a 5 mm mycelial plug (taken from a 2-day-old PDA culture) was placed onto each puncture wound. Each treatment was replicated three times independently. All leaves were incubated in a greenhouse at 28 °C under a 16 h light/8 h dark photoperiod for 72 h. After incubation, lesion diameters were measured, and the inhibition rate was calculated. Each treatment consisted of 15 leaves, and the experiment was independently repeated three times. The fungal inhibition rate was calculated using the following formula: Inhibition rate = $(1 - \text{lesion diameter of treatment group} / \text{lesion diameter of control group}) \times 100\%$ [8].

4.5. Evaluation of the Control Efficacy of WR7 Against Rubber Tree Anthracnose Under Greenhouse Conditions

The biocontrol efficacy of WR7 against rubber tree anthracnose was also evaluated in a pot experiment conducted in the greenhouse. Three-month-old rubber tree seedlings with uniform growth, at the phenological stage of light green leaves, were selected and transplanted into the greenhouse. The experiment included a positive control group treated with mancozeb (a product from Syngenta, Basel, Switzerland, active ingredient content 80%). The inoculation method for WR7 followed the procedure described in Section 4.4. Pathogen inoculation was performed using a spore suspension method, where a suspension with a concentration of 1×10^6 spores/mL was prepared and sprayed onto the leaves. The leaves were pricked as described in Section 4.4. Each treatment was independently repeated three times. After inoculation, the rubber tree seedlings were cultivated in the greenhouse under a light–dark cycle of 16:8, with air humidity maintained at 90% using an ultrasonic humidifier. Each treatment consisted of 12 plants, and the experiment was independently repeated three times. Seventy-two hours post-inoculation, the infection rate was calculated as outlined in Section 4.4.

4.6. Determination of In Vitro Antifungal Activity of WR7 Bacterial Culture Filtrate Against *C. siamense*

The WR7 bacterial culture broth was prepared as outlined in Section 4.1. After culturing the strain for 2 days, the broth was centrifuged at 10,000 rpm for 15 min to collect the supernatant, which was then filtered through a 0.22 µm microporous membrane to obtain the BCF. The filtrate was mixed with PDA medium (at 55 °C) in ratios of 1:10, 1:5, and 1:2.5 to prepare the plates. A 5 mm *C. siamense* agar plug was inoculated at the center of each plate. Each treatment was replicated three times, with an equal volume of LB added as the blank control (CK). All plates were incubated at a constant temperature of 28 °C. Once the mycelium of the control pathogen had fully covered the Petri dish, the colony diameter in the treatment groups was measured, and the inhibition rate was calculated as described in Section 4.2.

4.7. Assessment of Antifungal Activity of WR7 Volatiles Against *C. siamense*

The inhibitory effect of volatile compounds produced by WR7 against *C. siamense* was assessed using the dual-plate confrontation assay. The biocontrol strain was cultured as described in Section 4.2. Aliquots of 25, 50, and 100 µL of the bacterial suspension were spread onto LA plates. A 5 mm agar plug of *C. siamense* was obtained using a cork borer (Wuhan Heli Chemical & Instrument Co., Ltd., Wuhan, China) and inoculated at the center

of a PDA plate. The LA plate inoculated with WR7 was inverted and placed face-to-face with the PDA plate inoculated with the pathogen (with *C. siamense* on top and WR7 below), sealed with parafilm, and incubated. An LA plate supplemented with an equal volume of LB served as the control. The experiment was conducted in triplicate. All plates were incubated at a constant temperature of 28 °C. When the mycelium of the control pathogen had fully covered the Petri dish, the colony diameter in the treatment groups was measured, and the inhibition rate was calculated as described in Section 4.2.

4.8. Determination of the Enzyme and Siderophore Production Abilities of WR7

The extracellular enzyme and siderophore production abilities of WR7 were evaluated using standard culture-based methods. The strain's ability to secrete siderophores (Blue chrome azurol S medium) [43], proteases [44], cellulases [45], and glucanases [24] was assessed through specific culture medium assays. A central well was created on each plate using a 5 mm sterile punch, and WR7 bacterial culture broth (prepared as described in Section 4.2) was added to the well, with an equal volume of LB medium used as the control. After inoculation, the plates were incubated at 28 °C. In this experiment, *B. velezensis* LSR7 was used as the positive control, as this strain has been previously verified to possess the ability to produce the relevant extracellular enzymes and siderophores [46]. Meanwhile, WR7 was subjected to heat treatment at 121 °C for 20 min to obtain WR7-, which was used as the negative control. During the incubation period, the size of the clear zones around the colonies on each medium plate was observed and recorded daily, and the plates were photographed for preservation on the 3rd day of incubation. For protease and siderophore detection, enzymatic activity was indicated by the appearance of clear zones surrounding the colonies. For cellulase and glucanase assays, the corresponding media were stained with Congo red solution (1.5 mg/mL) for 20 min, followed by destaining with NaCl solution (1 mol/L) for 10 min. The appearance of yellowish halos around the colonies indicated positive enzyme activity.

4.9. Effects of WR7 Bacterial Culture Filtrate on Membrane Permeability and ROS Accumulation in *C. siamense* Hyphae

The *C. siamense* strain, cultured on PDA medium for 2 days, was transferred to PDB medium and incubated at 28 °C with shaking at 150 rpm for 48 h to obtain abundant free mycelia. The BCF of WR7 was prepared according to the method described in Section 4.6 and mixed with PDB medium containing the mycelia at ratios of 1:10, 1:5, and 1:2.5. The mixtures were incubated at 28 °C with shaking at 150 rpm for 2 h. After co-cultivation, the mycelia were collected by filtration through a sterile filter mesh (pore size 5 µm) and washed three times with 0.1% phosphate-buffered saline (PBS) for subsequent use. The experiment was repeated three times.

To evaluate membrane permeability, the treated mycelia were stained with propidium iodide (PI, Solarbio) for 30 min [47]. The stained mycelia were then observed, and images were captured using a fluorescence microscope (Nikon, Tokyo, Japan). The MOD of the stained mycelia was quantified using ImageJ software (version 1.54) to assess the effects of WR7 on the cell membrane permeability of *C. siamense*. Similarly, the accumulation of ROS in the mycelia was evaluated by staining with DCHF-DA (Solarbio), following the same method described in a previous study [30].

4.10. Assessment of the Activities of Defense-Related Enzymes in Plants

During the pot experiments assessing the biocontrol efficacy of WR7 against *C. siamense*-induced anthracnose, the effects of WR7 on the activity of defense-related enzymes in rubber tree leaves under *C. siamense* infection were also monitored. Experimental grouping, treatment methods, cultivation conditions, and sample sizes followed the descriptions

in Section 4.4 (excluding pesticide treatment groups). The activities of defense enzymes, including peroxidase (POD), phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), catalase (CAT), and superoxide dismutase (SOD), were measured at 0, 24, 48, 72, and 96 h after WR7 inoculation. The experiment was conducted with three biological replicates. Enzyme extraction and activity assays were performed strictly according to the manufacturer's instructions using commercial kits from Solarbio (Beijing, China), with the corresponding catalog numbers as follows: POD (BC0090), PAL (BC0210), PPO (BC0195), CAT (BC0200), and SOD (BC0170).

4.11. Data Analysis

Experimental results are expressed as the mean values of three independent replicates. Statistical analysis was performed using SPSS 23.0 software. Significant differences between groups were determined by analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons. The level of statistical significance was set at $p < 0.05$.

5. Conclusions

B. altitudinis WR7 exhibits potent antagonistic activity against the rubber tree anthracnose pathogen, *C. siamense*. This strain suppresses pathogen growth through multiple mechanisms, including disruption of cellular integrity, induction of ROS accumulation, and secretion of various hydrolases and siderophores. Genomic analysis further reveals the strain's potential to produce antimicrobial secondary metabolites such as lichenysin, fengycin, and bacilysin. Pot experiments confirmed a control efficacy of 71.65% and demonstrated the strain's ability to activate the defense enzyme system in rubber tree leaves, thereby enhancing resistance to pathogen invasion. This study provides the first report on the biocontrol potential of *B. altitudinis* against rubber tree anthracnose. Its multi-mechanistic synergy, derived from its endophytic habitat, offers novel strain resources and mechanistic insights for the environmentally sustainable management of rubber tree diseases.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants15050786/s1>. Figure S1: WR7 genome circlize diagram; Figure S2: COG annotation of the WR7 genome; Figure S3: GO annotation of the WR7 genome; Figure S4: KEGG pathway annotation of the WR7 genome; Table S1: Inhibition percentage of seven bacterial strains against *C. siamense*; Table S2: Genome feature of strain WR7; Table S3: The ANI and DDH values of WR7 with 11 *Bacillus* strains; Table S4: Prediction information of BCGs in strain WSR1 genome by antiSMASH.

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