forests

MDPI

Article

Secondary Metabolites from *Streptomyces araujoniae* S-03 Show Biocontrol Potential against Rhododendron Root Rot Caused by *Phytophthora cinnamomi*

Zhimin Sun [1,2], Qiuqin Wang [1,3], Yulong Li [1,3], Zizhu Shen [1,3], Xingshan Han [1,2], Peng Chen [1,2], Lin Shao [1,2], Ben Fan [1,3] and Yinjuan Zhao [1,3,*]

1 Collaborative Innovation Center of Sustainable Forestry in Southern China, Nanjing 210037, China; sunzhiminonlyone@163.com (Z.S.); qiuqinwang123@163.com (Q.W.); yulongli@njfu.edu.cn (Y.L.); 13255163465@163.com (Z.S.); hanxingshan3601@163.com (X.H.); 17388010303@163.com (P.C.); 15305160292@163.com (L.S.); fanben@njfu.edu.cn (B.F.)
2 College of Biology and Environment, Nanjing Forestry University, Nanjing 210037, China
3 College of Forestry, Institute of Forestry, Nanjing Forestry University, Nanjing 210037, China
* Correspondence: zhaoyinjuan@njfu.edu.cn

Abstract: *Phytophthora cinnamomi* is a harmful microorganism that can infect *Cinnamomum* plants and cause the rotting of plant roots. It has been reported as infecting nearly 5000 types of plants worldwide, such as avocado trees, macadamia trees, and oak and chestnut trees, and is listed among the 10 most destructive oomycetes and the 100 most severely invasive species globally. A number of chemical agents have been applied in the control of phytophthora rot diseases because of their easy application and rapid effects. However, with the extensive use of chemical agents, *P. cinnamomi* has developed resistance. More importantly, it has damaged the ecological environment and affected human health. Given that biological control should be safe and effective, the screening of biocontrol strains with highly effective antagonistic effects is regarded as the primary means to control *P. cinnamomi*. In this study, a bacterial strain was isolated from the soil of healthy rhododendron, located near to diseased rhododendron plants, and identified as *Streptomyces araujoniae*, named S-03, which inhibited *P. cinnamomi*. The plate antagonism assay showed that S-03 could effectively bend the hyphae, reduce the number of branches, and even break them, destroying the integrity of the hyphal cell membrane to inhibit the growth of *P. cinnamomi*. Moreover, strain S-03 also could inhibit the activity of *P. cinnamomi* cell wall-degrading enzymes. To analyze the inhibitory mechanism of S-03, the effects of cell wall-degrading enzymes, secondary metabolites, and volatile substances produced by S-03 on *P. cinnamomi* were assessed. It was found that the fermentation broth of S-03 had a strong inhibitory effect on *P. cinnamomi* which means metabolites play a part in inhibition. The active substance of S-03 was initially separated and purified by ethyl acetate extraction and silica gel column chromatography and had the properties of small molecules, low polarity, and solubility in methanol. The biocontrol effect was detected in the host plants and indicated that S-03 could effectively protect rhododendron from *P. cinnamomi* infection. Overall, the present study findings provide compelling evidence that *Streptomyces* S-03 could be a biocontrol agent against plant diseases caused by *P. cinnamomi*.

Keywords: *Streptomyces araujoniae*; secondary metabolites; biological control; *Phytophthora cinnamomi*

Citation: Sun, Z.; Wang, Q.; Li, Y.; Shen, Z.; Han, X.; Chen, P.; Shao, L.; Fan, B.; Zhao, Y. Secondary Metabolites from *Streptomyces araujoniae* S-03 Show Biocontrol Potential against Rhododendron Root Rot Caused by *Phytophthora cinnamomi*. *Forests* **2023**, *14*, 2054. https://doi.org/10.3390/f14102054

Academic Editor: Fred O. Asiegbu

Received: 5 September 2023
Revised: 4 October 2023
Accepted: 6 October 2023
Published: 13 October 2023

1. Introduction

Phytophthora cinnamomi, which belongs to the phylum Oomycota, is one of the most destructive plant pathogens and is responsible for substantial agro-economic losses worldwide [1]. In 2017, the global avocado production was approximately 604.8 million tons, of which Mexico accounted for a significant portion, with an annual output of approximately 583.4 million tons [2]. Current evidence suggests that the production of avocado plantations is declining due to *P. cinnamomi* [3]. In the natural ecosystem of Western Australia,

P. cinnamomi infects and kills tens of thousands of plant species, which are replaced by plant species resistant to *P. cinnamomi*, such as reeds and sedges, which severely damage the forest environment and biodiversity [4]. In China, it is widely acknowledged that cedar is susceptible to infection with *P. cinnamomi* under high temperature and high humidity conditions, causing root rot, leading to plant growth decline, yellowing and shedding of needles, and whole plant withering in severe cases. Importantly, the number of host plants of *P. cinnamomi* has increased from more than 900 in the 1980s to more than 5000 in 2020 [5]. Indeed, in recent years, there has been a burgeoning interest in refining current prevention and control strategies against *P. cinnamomi*.

Before, applying large amounts of chemical fungicides and synthetic fertilizers was the most common method to prevent rot caused by *P. cinnamomi* [6]. The most effective of these is phosphite; nonetheless, its effect is well-recognized to gradually decrease over time [7]. However, with the potential threats of chemical fungicides to the land environment and human health, there is increasing interest in the capabilities of natural active compounds and biological control agents to control *Phytophthora* [8]. Currently, the biocontrol bacteria for *P. cinnamomi* root rot are mainly *Streptomyces*, *Bacillus*, and *Pseudomonas* [9]. The active antifungal substances they produce can effectively inhibit phytopathogenic fungi and the development of symptoms after infection. It has been reported that antagonistic active substances can be divided into competitive antagonists, antibiotics and host resistance inducers, and fungal cell wall-degrading enzymes [10].

Streptomyces luridiscabiei U05 secretes chitinase, which catalyzes the cleavage of β-1,4 glycosidic bonds in the cell wall of pathogenic fungi, thereby inhibiting the growth of phytopathogenic fungi [11]. Moreover, *Streptomyces hygroscopicus* S11, with phosphite, can improve the soybean defense response to *Phytophthora sojae* [12]. Some *Streptomyces* can help avoid plant infection by pathogenic fungi and promote plant growth, which is beneficial to soils that lack nutrients. *Streptomyces* AzR-051 is an endophytic actinomycete found in the sterilized root tissue of the rice surface that can produce 13.73 μmol/mL indole acetic acid to promote plant growth while preventing tomato plant fusarium [13]. The secondary metabolites produced by *Streptomyces* are the largest source of natural products from microorganisms and are widely used in food, medicine, industry, agriculture, and other fields. Daptomycin is a cyclic lipopeptide antibiotic produced by *Streptomyces* with potent activity against Gram-positive bacteria. It has a calcium-dependent mechanism of action, which can destroy bacterial membranes. Given that it is effective against other antibiotic-resistant microorganisms, including vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin-resistant *Staphylococcus aureus* (MRSA), daptomycin is in great demand and is widely used nowadays [14]. So far, more than 2000 actinomycetes have been documented in the literature, and about 600 are from the Streptomyces genus. To our knowledge, few studies have assessed the antagonistic effect of *Streptomyces araujoniae* on *P. cinnamomi*. Indeed, there are still many undiscovered actinomycetes and potentially effective antagonistic substances. Therefore, it is of great significance to screen highly effective antagonistic strains for the prevention and control of *P. cinnamomi*.

In this study, an actinomycete strain with a robust antagonistic effect against *P. cinnamomi* was isolated from the soil. After observing cultivation, physiological and biochemical characteristics, and the 16S rDNA molecular comparison, the strains were classified and identified. Then, the anti-oomycete activity of *Phytophthora* was assessed. Analysis of the strain's cell wall-degrading enzyme activity, antagonistic activity of fermentation metabolites, and antagonistic detection of volatile substances showed that *Phytophthora* could produce secondary metabolites with antagonistic activity. Preliminary separation and purification of the active substance in the fermentation broth of S-03 showed that the substance was a small molecule substance with low polarity. Further studies are required to explore its material structure and antagonistic mechanism, providing a foothold for better prevention and control of forestry diseases caused by *Phytophthora*.

2. Materials and Methods

2.1. Microorganisms

S-03 bacteria were isolated from uninfected rhododendron soil in the diseased rhododendron cluster at Nanjing Forestry University (Nanjing, China) by using the plate dilution method, as follows: take a 10 g soil sample and add it to 90 mL sterile water. The vortex meter is sufficient. Shake, absorb 1 mL of suspension in 9 mL of sterile water to make 10% soil suspension, and dilute it with sterile water into gradient solutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Coat it on Gause's No. 1 culture medium (soluble starch(Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) 20 g, KNO_3 (Xilong Chemical Industry Co., Ltd., Shantou, China) 1.0 g, K_2HPO_4 (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) 0.5 g, $MgSO_4$ (National Medicine Group Chemical Reagent Co., Ltd., Shanghai, China) 0.5 g, NaCl (National Medicine Group Chemical Reagent Co., Ltd., Shanghai, China) 0.5 g, $FeSO_4 \cdot 7H_2O$ (National Medicine Group Chemical Reagent Co., Ltd., Shanghai, China) 0.01 g, Agar (Shanghai Generay Biotech Co., Ltd., Shanghai, China) 20.0 g, distilled water 1000 mL, pH 7.2–7.4), invert it at 28 °C, pick out the mycelium at the edge of colony after 2 days to obtain purified mold, and store it at 4 °C for later use. A *Streptomyces* strain with an inhibitory effect on *P. cinnamomi* was isolated. *P. cinnamomi* was generously provided by the Forest Protection Department of Nanjing Forestry University and was cultured on 10% (v/v) V8 agar medium [15].

2.2. Bacteria Identification

Based on the bacteriostatic experiment results, strain S-03 was isolated from the healthy soil sample next to a rhododendron bush infected by *P. cinnamomi* in Nanjing. The CTAB method was used to extract the total genomic DNA of strain S-03. The primers szm01-F (5′-AGAGTTTGATCCTGGCTCAG-3′) and szm04-R (5′-CTACGGCTACCTTGTTACGA-3′) were used to amplify the 16S rDNA. The PCR product was gel purified with a gel purification kit (Shanghai Generay Biotechnology Company, Shanghai, China). The obtained 16S rDNA sequence was analyzed by the NCBI database using the blast algorithm on the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/BLAST, accessed on 28 May 2021, MZ310452). Evolutionary history was inferred using the neighbor-joining method. Evolutionary analyses were conducted in MEGA7.

2.3. Efficacy of Inhibiting Phytophthora cinnamomi Growth on Agar

P. cinnamomi was cultured in a 10% (v/v) V8 solid medium for five days and used for the confrontation culture of *S. araujoniae* S-03. *P. cinnamomi* discs were obtained with a 6 mm cork borer placed in the center of the culture medium. The S-03 single colonies, cultured in a Gause's No. 1 agar medium at 25 °C for 5 days, were obtained with a 6 mm cork borer and inoculated around the *P. cinnamomi* disc. The culture dish was placed in a 25 °C incubator and cultured for three days. The dishes of *P. cinnamomi*, inoculated by the above method, without the inoculation of S-03, were used as the control. The hyphae of *P. cinnamomi* at the edge of the inhibition zone were picked.

Next, the following process was undertaken: cut small square samples (3 pieces each) with a size of 1.0 cm × 1.0 cm at the edge of the mycelium. Then, soak the mycelium block with 2.5% glutaraldehyde solution and fix the sample at 4 °C overnight; pour off the stationary solution and bleach it with 0.1 M phosphate buffer with a pH of 7.0.

Wash 3 times, 15 min each time; fix in 0.1% osmium acid solution for 1–2 h; pour out the stationary solution, and rinse with 0.1 M phosphate buffer with a pH of 7.0 for 3 times, each time for 15 min; dehydrate the samples with different concentrations of ethanol solutions (50%, 70%, 80%, 90%, and 95%) for 15 min at each concentration, and then treat them with 100% ethanol twice for 20 min each time. The sample was treated with the mixed solution of ethanol and isoamyl acetate (v/v = 1:1) for 30 min, and the sample was treated with pure isoamyl acetate for 1–2 h. The critical point drying process was as follows: put the processed sample into the sample cage, mark it, and dry it with the HCP-2 critical dryer. The sample coating process was as follows: take the dried samples out of the

sample cage, put them on the sample table and mark them, and coat them with the IB-5 ion sputtering instrument.

To perform a scanning electron microscopy (SEM) observation: take out the samples coated with the film, observe the hyphae with the environmental scanning electron microscope FEI Quanta 2000, take pictures, and then save them for subsequent experimental analysis. The individually cultured hyphae of *P. cinnamomi* were used as the control. Strain S-03 was cultured with Gao's No. 1 liquid at 28 °C and shaken at 160 rpm for 11 days and then centrifuged at 12,000× *g* for 10 min in a high-speed centrifuge. The supernatant was filtered with a bacterial filter (0.22 μm) and used to detect antagonistic activity to *P. cinnamomi*.

2.4. Effects of S-03 on Cell Membrane Permeability and Cell Wall-Degrading Enzyme Activities of Phytophthora cinnamomi

The 6 mm Phytophthora agar disc was placed in a 50 mL liquid PDA medium and cultured for 3 d at 25 °C and 160 rpm. 5 mL of sterile fermentation broth was added to the treatment group and 5 mL of sterile water to the control group. After culturing for 3 days, the *P. cinnamomi* mycelium was washed with sterile distilled water for use.

The remaining mycelium in the above steps was repeatedly washed with sterile ultrapure water, dried with sterile filter paper, weighed, and recorded. Then, the mycelia were ground with a cold Tris-HCl buffer (0.05 mol/L, pH 7.5, 4 °C) at a ratio of 1 to 5 (*v*/*w*). The homogenate was centrifuged at 10,000× *g* for 15 min at 4 °C. The obtained supernatant was used to determine MDA (malondialdehyde) content, β-GC enzyme activity, and polygalactosidase activity.

2.5. Detection of the Antagonistic Capacity of S-03

The S-03 strain was inoculated into the following media, a: (KH_2PO_4 (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) 0.5 g/L, $MgSO_4$ (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 0.25 g/L, gelatin (National Medicine Group Chemical Reagent Co., Ltd., Shanghai, China) 2 g/L, CMC-Na (Nanjing Oddfoni Biological Technology Co., Ltd., Nanjing, China) 2 g/L, Congo red (Shenyang Jintianyuan Chemical Co., Ltd., Shenyang, China) 0.2 g/L), b: (skim milk 1.5%, 2.5% agar), c: (colloidal chitin (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) 5.0 g, K_2HPO_4 (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) 0.7 g/L, KH_2PO_4 (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) 0.3 g/L, $MgSO_4$ (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 0.5 g/L, $FeSO_4$ (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 0.001 g/L, $ZnSO_4$ (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 0.01 g/L), and the activity of the cell wall-degrading enzyme produced by S-03 was assessed by observing the size of the transparent circle produced. Strain S-03 was inoculated into a fermentation medium (soymeal powder (Beijing Hong run Bao Shun Technology Co., Ltd., Beijing, China) 20 g/L, glucose (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 20 g/L, soluble starch (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) 5 g/L, yeast powder (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) 2 g/L, NaCl (National Medicine Group Chemical Reagent Co., Ltd., Shanghai, China) 4 g/L, K_2HPO_4 (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) 0.5 g/L, $MgSO_4$ (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 0.5 g/L, $CaCO_3$ (Nanjing Chemical Reagent Co., Ltd., Shanghai, China) 2 g/L) at 28 °C in a shaking incubator at 170 rpm and cultured for 7 days to detect the antagonistic activity of the supernatant. Strain S-03 and *P. cinnamomi* were inoculated in a V8 Petri dish with a partition to detect whether volatile antagonistic substances were produced.

2.6. Extraction and Purification of Secondary Metabolites from S-03 Culture

Strain S-03 was inoculated into the fermented seed liquid and cultured for 3 days at 28 °C and 170 rpm. The 1% seed liquid inoculum was inoculated into the fermentation medium and cultured at 28 °C and 170 rpm for 7 days. The culture broth was centrifuged at 6000× *g* for 20 min, a Büchner funnel was used to remove floating impurities, and a rotary

evaporator was used for concentration. Five times the volume of absolute ethanol was used to precipitate the impurities. The secondary metabolites were extracted by n-butanol extraction. The extracted n-butanol was evaporated to dryness in the evaporator to produce a crude extract dissolved in methanol. Then, the sample was separated and purified by silica gel column chromatography (100–140 mesh chromatography silica gel). The silica gel in the experiment was packed in a wet column and washed with chloroform. The sample was added to a small amount of silica gel powder and added to the upper layer of the chromatography column after the solvent evaporated to dryness. Trichloromethane and methanol (v/v = 24:1) were used as eluents, the flow rate was controlled at 1 drop every 3~5 s, and a tube of the sample was collected every 20 min. After the eluent in each sample was volatilized, methanol was added as the sample to be tested. *P. cinnamomi* disk was inoculated on a V8 medium with methanol on the left side of *P. cinnamomi* disk as the control and samples on the right side to detect antagonistic activity. The culture dish was placed in a 25 °C incubator and cultured for 3 days.

2.7. Biocontrol Effect of S-03 on Rhododendron Root Rot

Rhododendron with a healthy plant height of 20~30 cm was used as the host plant, and the S-03 fermentation liquid was evenly sprayed onto the rhododendron root. Then, 100 μL *P. cinnamomi* strain suspension, as suspended in sterile water and diluted to 1×10^8 CFU/mL, was sprayed onto the rhododendron root. Sterile water, S-03 fermentation liquid, and *P. cinnamomi* strain suspension were, respectively, sprayed alone on three rhododendron roots, to be used as three controls. There were 3 seedlings per treatment. Later, the growth of plants and roots was observed. The rhododendron roots were cut, dried, and weighed, and the differences among groups were compared.

3. Results

3.1. Molecular Biological Identification of Isolate S-03

The phylogenetic tree of the isolate S-03 was constructed using the 16S rDNA gene sequence (1416 bp, MZ310452). As shown in Figure 1A, the nr/nt database on the NCBI website was used to identify the length of genes with a higher homology. Cluster analysis of MEGE 7 software showed that the isolate S-03 and *S. araujoniae* were in the same category.

3.2. Inhibition of Phytophthora cinnamomi by S-03

The inhibitory effect of strain S-03 on *P. cinnamomi* was tested on the V8 plate. After five days of cultivation, the control group of *P. cinnamomi* could grow over the entire surface of the plate (Figure 1B), while the growth of *P. cinnamomi* was restricted after the addition of strain S-03 around *P. cinnamomi*, expanding in a quadrilateral shape as the hyphae could not continue to grow outwards (Figure 1B). The SEM results showed that the mycelium of the *P. cinnamomi* hyphae was deformed (Figure 2A) or broken (Figure 2B) following treatment with S-03, compared with normal hyphae. This finding indicates that strain S-03 can effectively inhibit the growth of *P. cinnamomi*. Antagonistic effects in the fermentation broth of strain S-03 were produced on day five and peaked on day seven, subsequently maintaining highly stable production levels (Figure 1D).

3.3. Effects of S-03 on Cell Membrane Permeability and Cell Wall-Degrading Enzyme Activities of Phytophthora cinnamomi

We found that the MDA (malondialdehyde) content in the hyphae of *P. cinnamomi* treated with S-03 was higher than the control group (Figure 3A). The accumulation of MDA disrupted the structure and function of the cell membrane, indicating that S-03 could disrupt *P. cinnamomi* cell membrane permeability.

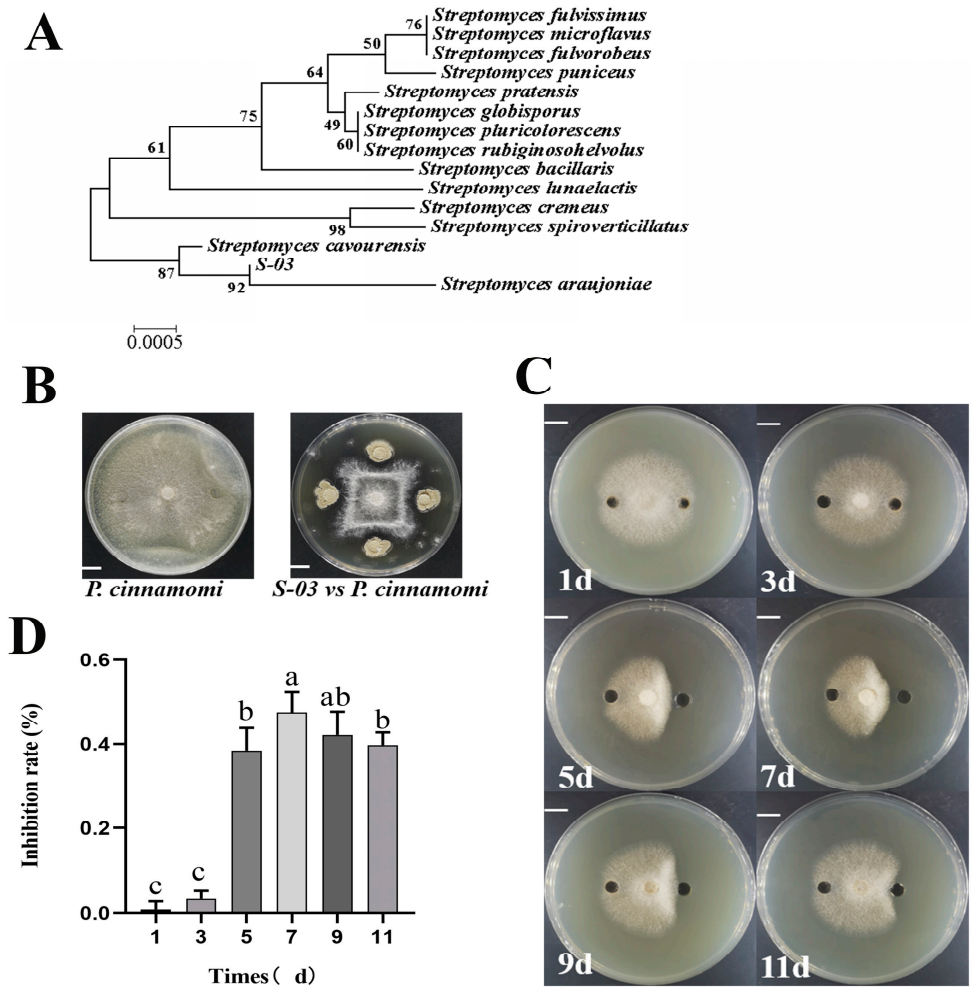

Figure 1. *Streptomyces araujoniae* inhibits *Phytophthora cinnamomi* growth. (**A**) Neighbor-joining tree based on 16S rDNA gene sequences showing the relation of strain S-03 and closely related species. The value on the left side of the node is the bootstrap support rate (>60%) of the 1000 repeated-sample dataset based on the neighbor-joining method, Bar: 0.0005 substitutions per nucleotide position. (**B**) Dual culture in vitro bacterial Phytophthora assays. The *S. araujoniae* S-03 was inoculated around the *P. cinnamomi* disc to assess the inhibitory ability of S-03 on the growth of *P cinnamomi*. Scale bar, 1 cm. (**C**) Inhibitory effect of fermentation broth from strain S-03 cultured at different times on *P. cinnamomi* within 11 days. Scale bar, 1 cm. (**D**) Inhibitory rate of strain S-03 against *P. cinnamomi* within 11 days. Different letters indicate that different treatments have been tested by Duncan's new multiple range method, and the differences are significant at the $p < 0.05$ level.

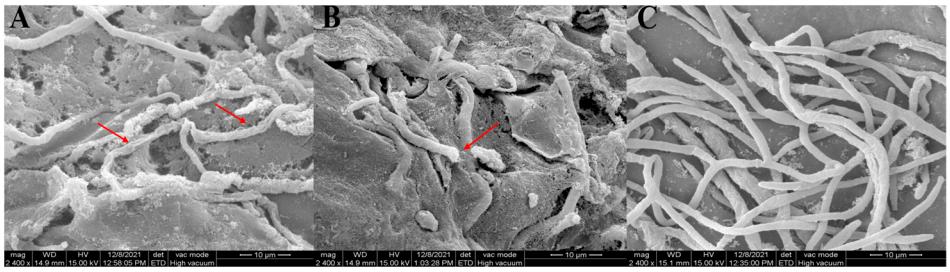

Figure 2. Effects of S-03 on *Phytophthora cinnamomi* hyphae. (**A**,**B**). Mycelium affected by S-03. Bar 10 μm, red arrows indicate shriveled and fractured mycelium. (**C**) Normally growing mycelium.

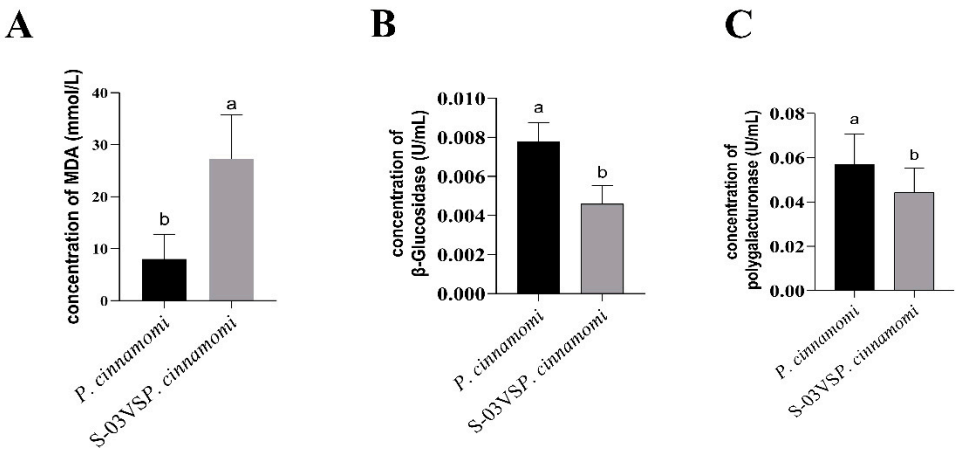

Figure 3. S-03 disrupts cell membrane permeability and reduces cell wall-degrading enzyme activity. (**A**) The effect of S-03 on MDA content of *Phytophthora cinnamomi* mycelium. (**B**) The effect of S-03 on the enzymatic content of *P. cinnamomi* mycelium β-galactosidase. (**C**) The effect of S-03 on the polygalacturonase content of *P. cinnamomi* mycelium. Different letters indicate that different treatments have been tested by Duncan's new multiple range method, and the differences are significant at the $p < 0.05$ level.

Phytophthora can infect plants via β-galactosidase and polygalacturonase. Here we found that the galacturonidase activity of *P. cinnamomi* decreased when it was inhibited by S-03 (Figure 3B,C).

3.4. Detection of the Antifungal Capacity of S-03

The ability of the cell wall-degrading enzymes produced by strain S-03 was assessed. Even though S-03 could produce cellulase and protease, their activity was not high, and it could not produce chitinase (Figure 4A). The fermentation filtrate of strain S-03 effectively inhibited the growth of *P. cinnamomi* (Figure 4A). The growth of the hyphae of S-03 and *P. cinnamomi* in a three-cell culture dish was comparable to *P. cinnamomi* cultured separately, indicating that S-03 could not produce volatile metabolites to inhibit *P. cinnamomi* (Figure 4A). Therefore, strain S-03 could inhibit the growth of *P. cinnamomi* by releasing secondary metabolites into the medium.

3.5. Extraction and Purification of Secondary Metabolites from S-03 Culture

After pretreatment, the fermentation filtrate was extracted with n-butanol. The extracted organic phase exhibited antagonistic activity (Figure 4B), unlike the extracted aqueous phase (Figure 4B). The results showed that the active antagonistic substance was a small molecule substance, which was soluble in alcohols and had low polarity. The silica gel column chromatography yielded 25 components. Bacteriostatic testing was performed on each component. It was found that the active components were mainly concentrated in three components: 8, 9, and 10 (Figure 4C).

3.6. Biocontrol Effect of S-03 Fermentation Broth on Rhododendron Root Rot

To further explore the control effect of S-03 strain under natural conditions, biocontrol experiments were carried out on rhododendron, the host plant of *cinnamomi*. The results showed that rhododendron plants treated with water and S-03 fermentation broth alone were not diseased, indicating that the S-03 strain did not affect plant growth. Plants inoculated with *Phytophthora* spores were diseased as their leaves became dry and wilted (Figure 5A) with decreased fibrous roots (Figure 5B). The dry weight of the root was also significantly lower than the other groups, suggestive of root rot (Figure 5C). However, the rhododendron did not develop any disease when initially treated with strain S-03 then with *P. cinnamomi* spore fluid (Figure 5A). These findings suggest that strain S-03 can yield a good control effect on *P. cinnamomi* under natural conditions.

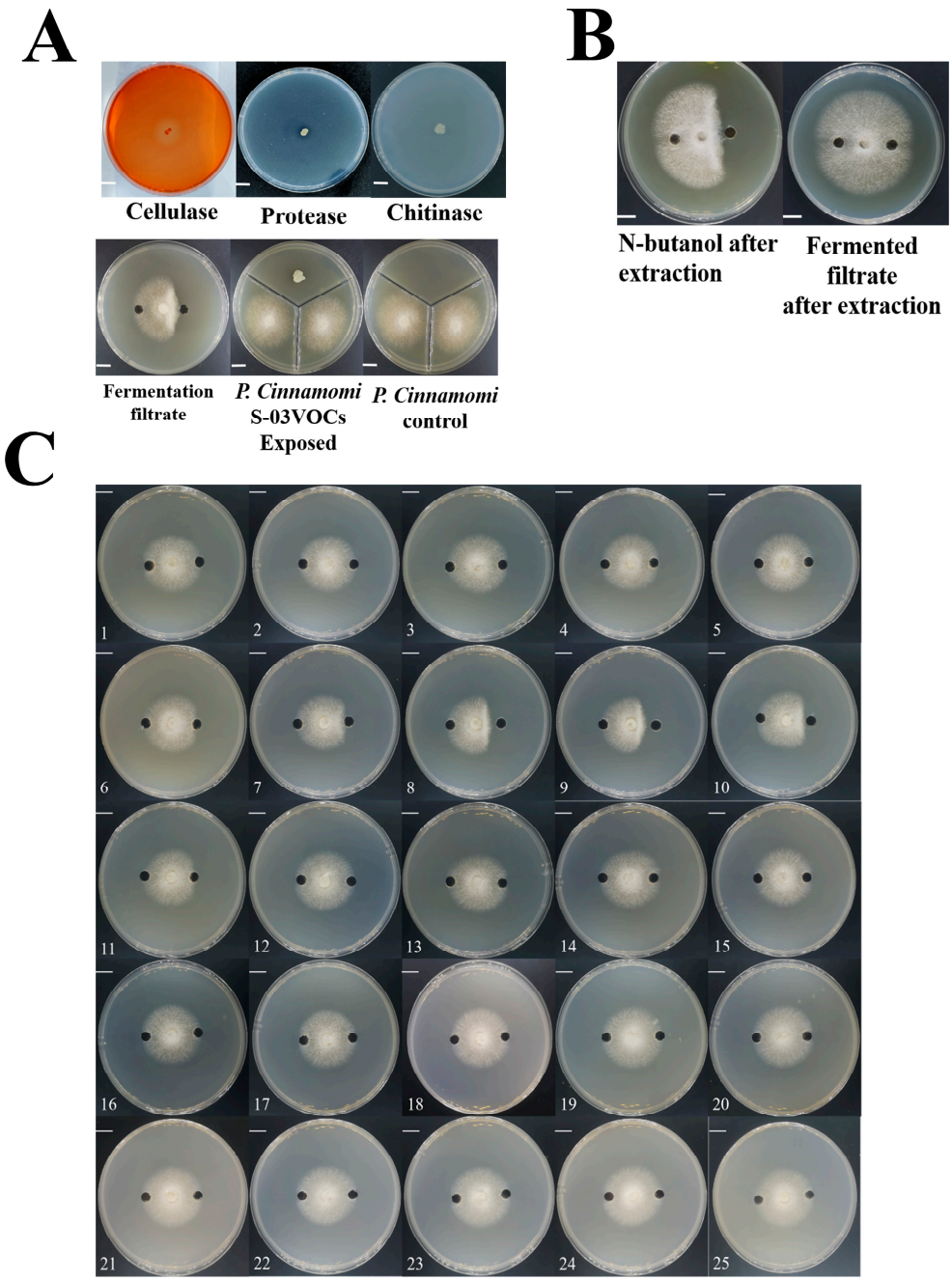

Figure 4. Detection of the antagonistic capacity of S-03. (**A**) Cellulase production identification medium; protease production identification medium; chitinase production identification medium. The medium was added on the left as a control, and the fermentation broth of S-03 was on the right to detect the S-03 volatile antagonistic substance; no S-03 was added to the control. Scale bar, 1 cm. (**B**) Antagonistic activity detection in the organic and aqueous phases after extraction, in 3 plates each. Scale bar, 1 cm. (**C**) Antagonistic detection of column chromatography components, with methanol added on the left side of the *Phytophthora cinnamomi* disc as the control, and samples added on the right side. Scale bar, 1 cm.

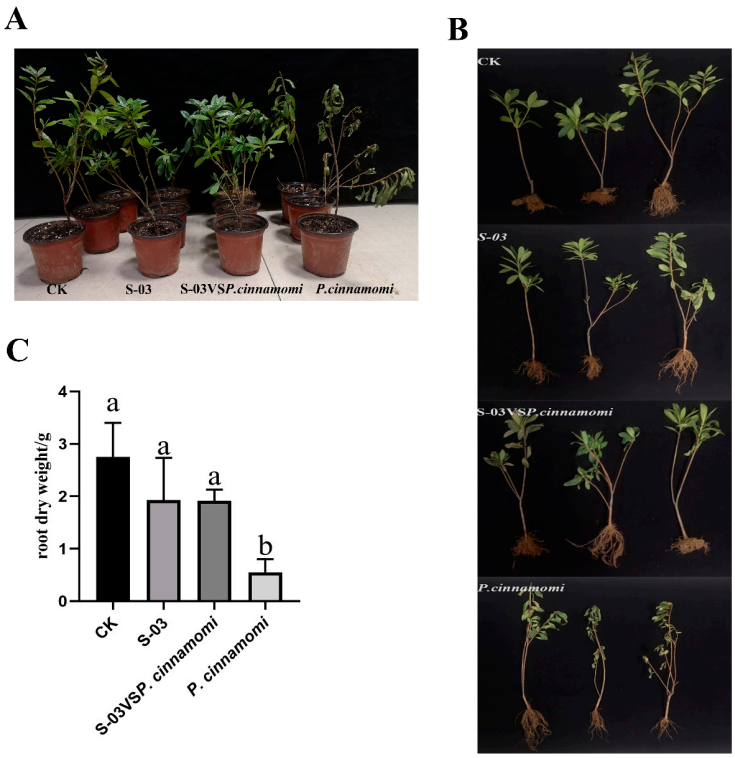

Figure 5. S-03 improves plant resistance to *Phytophthora cinnamomi*. (**A**) S-03 improves the resistance of rhododendron plants to *P. cinnamomi*. (**B**) S-03 protects rhododendron roots from *P. cinnamomi*. (**C**) Dry weight of rhododendron roots under different treatments. Different letters indicate that different treatments have been tested by Duncan's new multiple range method, and the differences are significant at the $p < 0.05$ level.

4. Discussion

During the entire growth process, plants are inevitably attacked by many soilborne pathogens [16]. Using natural biological control microorganisms to inhibit plant pathogens is an effective means to maintain the sustainable development of the ecological environment [17]. *Actinomycetes*, especially *Streptomyces*, are well-established as an important source of natural secondary metabolites [18]. Ample evidence substantiates that secondary metabolites produced by *Streptomyces* have antagonistic effects on a variety of plant pathogens [19].

P. cinnamomi is a soilborne pathogen widely distributed around the world. A global distribution map of *P. cinnamomi* from a 2020 report showed that only a small amount had been detected in the eastern coastal areas of China [7]. Accordingly, in order to prevent the further spread of *P. cinnamomi*, there is an urgent need to find economical and effective control measures to prevent the spread of *P. cinnamomi*. It is well-established that pathogen control is essential for *P. cinnamomi* due to the impact of pathogens on economic diseases and biodiversity. However, currently available methods are limited and expensive, and the primary purpose is to make plants more resistant to infection [20]. The current most effective approach to control and eliminate *Phytophthora* root rot is to treat plants with phosphite, but repeated applications are required since its effectiveness declines over time [21]. Notwithstanding that few studies have explored the long-term effects of treatments with phosphites and their impact on the ecosystem, it is widely believed that in diseased habitats, phosphite treatment significantly reduces the loss of shrub cover and bare ground and sedge cover [22]. It has been reported that phosphites do not adversely or negatively affect species composition and structure [23]. Other pathogen control options include the use of the fungicide fosetyl-aluminum [24], a treatment with copper salts to improve host resistance [25], and perilla extract [26].

Herein, an isolate of *S. araujoniae*, characterized as S. araujoniae S-03, could effectively inhibit *P. cinnamomi*. *S. araujoniae* represents a new species of *Streptomyces*, first isolated from potato roots by Brazilian scientists [27]. Few studies have assessed the biocontrol efficacy of *S. araujoniae*. It has been shown that *S. araujoniae* could inhibit *Botrytis cinerea* by producing valinomycin and macrolide antibiotics [28]. Moreover, overwhelming evidence suggests that valinomycin can slow down the movement of zoospores of *Phytophthora* species by adjusting potassium ion permeability [29]. In a study where *S. araujoniae* from the Qinghai-Tibet Plateau of China was isolated, the fermented liquid of actinomycetes could effectively inhibit fungal hyphal growth, conidia germination, and the formation of attachments [30]. To the best of our knowledge, this is the first report that shows *S. araujoniae* exerting an inhibitory effect against *P. cinnamomi*, which broadens the antagonistic spectrum of *S. araujoniae*. Moreover, we preliminarily explored the functions of this relatively new species to provide a basis for future studies.

5. Conclusions

In this study, we found that strain S-03 can effectively inhibit the growth of *P. cinnamomi* and destroy the mycelium morphology. The fermentation filtrate will destroy the integrity of the mycelium cell membrane and affect the activity of cell wall-degrading enzymes. The analysis of the fermentation filtrate of the extracted strain shows that the substance is a low-polarity small molecular substance, which is easily soluble in alcohol. The analysis of the plant biocontrol effect showed that strain S-03 could inhibit the damage of *P. cinnamomi* to plant roots, reduce root rot and effectively maintain the normal growth of plants. Therefore, it may be used to evaluate the control of *P. cinnamomi* in other hosts.

Although our research results show that S-03 has a great prospective application as a biological control agent, further research is needed to evaluate its forestry application, such as field experiments. In addition, the antagonistic mechanism of S-03 on *cinnamomi* cassia is not discussed in this study, which needs further study. Importantly, we found that S-03 can secrete small molecular secondary metabolites to inhibit cinnamon bark. Therefore, it is necessary to purify the antifungal metabolites produced by S-03 and further identify its chemical structure, so as to better study its antagonistic activity and mechanism against the forestry pathogen *cinnamomi* cassia bark.

Author Contributions: Methodology, Z.S. (Zhimin Sun); Software, Q.W.; Formal analysis, Y.Z.; Resources, Z.S. (Zizhu Shen), P.C., L.S. and B.F.; Data curation, Y.L.; Visualization, X.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors thank Tingting Dai for supply of *Phytophthora cinnamomi* strains.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dai, T.; Wang, A.; Yang, X.; Yu, X.; Tian, W.; Xu, Y.; Hu, T. PHYCI_587572: An RxLR Effector Gene and New Biomarker in A Recombinase Polymerase Amplification Assay for Rapid Detection of *Phytophthora cinnamomi*. *Forests* **2020**, *11*, 306. [CrossRef]
2. Andrade-Hoyos, P.; Victoria, H.; Romero-Arenas, O. Endophytic Trichoderma Species Isolated from *Persea americana* and *Cinnamomum verum* Roots Reduce Symptoms Caused by *Phytophthora cinnamomi* in *Avocado*. *Plants* **2020**, *9*, 1220. [CrossRef] [PubMed]
3. Silva, S.; Cantuarias-Avils, T.; Neto, H.B.; Filho, F.D.A.A.; Medina, R.B. Management of root rot in avocado trees. *Rev. Bras. Frutic.* **2016**, *38*, 175. [CrossRef]
4. Hardham, A.R. Pathogen profile *Phytophthora cinnamomi*. *Zhonghua Yi Xue Za Zhi* **2005**, *86*, 3431–3434. [CrossRef]
5. Ego, O.S.J.; Drenth, A.; Topp, B.; Henderson, J.; Akinsanmi, O.A. Prevalence of *Phytophthora* species in macadamia orchards in Australia and their ability to cause stem canker. *Plant Pathol.* **2020**, *69*, 1270–1280. [CrossRef]
6. Reeksting, B.J.; Olivier, N.A.; Van Den Berg, N. Transcriptome responses of an ungrafted *Phytophthora* root rot tolerant avocado (*Persea americana*) rootstock to flooding and *Phytophthora cinnamomi*. *BMC Plant Biol.* **2016**, *16*, 205. [CrossRef] [PubMed]
7. Loureno, D.; Branco, I.; Choupina, A. *Phytopathogenic oomycetes*: A review focusing on *Phytophthora cinnamomi* and biotechnological approaches. *Mol. Biol. Rep.* **2020**, *47*, 9179–9188. [CrossRef]

8. Martínez-Medina, A.; Alguacil, M.; Pascual, J.A.; Wees, S. *Phytohormone* Profiles Induced by *Trichoderma* Isolates Correspond with Their Biocontrol and Plant Growth-Promoting Activity on Melon Plants. *J. Chem. Ecol.* **2014**, *40*, 804–815. [CrossRef]
9. Aksoy, H.M.; Kaya, Y.; Ozturk, M.; Secgin, Z.; Onder, H.; Okumus, A. Pseudomonas putida—Induced Response in Phenolic Profile of Tomato Seedlings (*Solanum lycopersicum* L.) Infected by Clavibacter michiganensis subsp. michiganensis. *Biol. Control* **2016**, *105*, 6–12. [CrossRef]
10. Srikhong, P.; Lertmongkonthum, K.; Sowanpreecha, R.; Rerngsamran, P. *Bacillus* sp. strain M10 as a potential biocontrol agent protecting chili pepper and tomato fruits from anthracnose disease caused by *Colletotrichum capsici*. *Biocontrol* **2018**, *63*, 833–842. [CrossRef]
11. Brzezinska, M.S.; Jankiewicz, U.; Kalwasińska, A.; Witczak, J.; Ero, K. Characterization of chitinase from *Streptomyces luridiscabiei* U05 and its antagonist potential against fungal plant pathogens. *J. Phytopathol.* **2019**, *167*, 404–412. [CrossRef]
12. Arfaoui, A.; Hadrami, A.E.; Adam, L.R.; Daayf, F. Combining *Streptomyces hygroscopicus* and phosphite boosts soybean's defense responses to *Phytophthora sojae*. *Biocontrol* **2020**, *65*, 363–375. [CrossRef]
13. Verma, V.C.; Singh, S.K.; Prakash, S. Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *J. Basic Microb.* **2011**, *51*, 550–556. [CrossRef]
14. Rubio, S.; Martínez-Cámara, S.; Fuente, J.; Rodríguez-Sáiz, M.; Barredo, J.L. *Strain Improvement Program of Streptomyces roseosporus for Daptomycin Production*; Humana: New York, NY, USA, 2021; Volume 2296, pp. 351–363. [CrossRef]
15. Zhou, Z.; Yang, X.; Wu, C.; Chen, Z.; Dai, T. Whole-Genome Sequence Resource of *Phytophthora pini*, the Causal Pathogen of Foliage Blight and Shoot Dieback of *Rhododendron pulchrum*. *Mol. Plant-Microbe Interact.* **2022**, *35*, 944–948. [CrossRef]
16. Xu, J.; Yang, X.; Wu, C.; Chen, Z.; Dai, T. Recombinase Polymerase Amplification–Lateral Flow Dipstick Assay for Rapid Detection of *Fusarium circinatum* Based on a Newly Identified Unique Target Gene. *Plant Dis.* **2023**, *107*, 1067–1074. [CrossRef]
17. Zhang, Y.; Kong, W.-L.; Wu, X.-Q.; Li, P.-S. Inhibitory Effects of Phenazine Compounds and Volatile Organic Compounds Produced by *Pseudomonas aurantiaca* ST-TJ4 Against *Phytophthora cinnamomi*. *Phytopathology* **2022**, *112*, 1867–1876. [CrossRef]
18. Boukaew, S.; Plubrukam, A.; Prasertsan, P. Effect of volatile substances from *Streptomyces philanthi* RM-1-138 on growth of Rhizoctonia solani on rice leaf. *Biocontrol* **2013**, *58*, 471–482. [CrossRef]
19. Chen, J.; Xue, Q.H.; McErlean, C.S.P.; Zhi, J.H.; Ma, Y.Q.; Jia, X.T.; Zhang, M.; Ye, X.X. Biocontrol potential of the antagonistic microorganism *Streptomyces enissocaesilis* against *Orobanche cumana*. *Biocontrol* **2016**, *61*, 781–791. [CrossRef]
20. Sena, K.; Crocker, E.; Vincelli, P.; Barton, C. *Phytophthora cinnamomi* as a driver of forest change: Implications for conservation and management. *For. Ecol. Manag.* **2018**, *409*, 799–807. [CrossRef]
21. Dunstan, W.A.; Rudman, T.; Shearer, B.L.; Moore, N.A.; Paap, T.; Calver, M.C.; Dell, B.; Hardy, G. Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems. *Biol. Invasions* **2010**, *12*, 913–925. [CrossRef]
22. Romero, M.A.; González, M.; Serrano, M.S.; Sánchez, M.E. Trunk injection of fosetyl luminium controls the root disease caused by *Phytophthora cinnamomi* on *Quercus ilex* woodlands. *Ann. Appl. Biol.* **2019**, *174*, 313–318. [CrossRef]
23. Barrett, S.R.; Rathbone, D. Long-term phosphite application maintains species assemblages, richness and structure of plant communities invaded by *Phytophthora cinnamomi*. *Aust. Ecol.* **2018**, *43*, 360–374. [CrossRef]
24. González, M.; Caetano, P.; Sánchez, M.E. Testing systemic fungicides for control of *Phytophthora oak* root disease. *For. Pathol.* **2017**, *47*, e12343. [CrossRef]
25. Keast, D.; Tonkin, C.; San Fe Lieu, L. Effects of Copper Salts on Growth and Survival of *Phytophthora cinnamomi* in vitro and on the Antifungal Activity of Actinomycete Populations from the Roots of *Eucalyptus marginata* and *Banksia grandis*. *Aust. J. Bot.* **1985**, *33*, 115–129. [CrossRef]
26. Neves, D.; Caetano, P.; Oliveira, J.; Maia, C.; Horta, M.; Sousa, N.; Salgado, M.; Dionísio, L.; Magan, N.; Cravador, A. Anti-*Phytophthora cinnamomi* activity of *Phlomis purpurea* plant and root extracts. *Eur. J. Plant Pathol.* **2014**, *138*, 835–846. [CrossRef]
27. Silva, L.J.; Taketani, R.G.A.; Melo, I.S.; Goodfellow, M.; Zucchi, T.D. *Streptomyces araujoniae* sp. nov.: An actinomycete isolated from a potato tubercle. *Antonie Van Leeuwenhoek* **2013**, *103*, 1235–1244. [CrossRef]
28. Silva, L.J.; Crevelin, E.J.; Souza, W.R.; Moraes, L.A.; Zucchi, T.D. *Streptomyces araujoniae* Produces a Multiantibiotic Complex with Ionophoric Properties to Control *Botrytis cinerea*. *Phytopathology* **2014**, *104*, 1298–1305. [CrossRef]
29. Appiah, A.A.; West, P.V.; Osborne, M.C.; Gow, N. Potassium homeostasis influences the locomotion and encystment of zoospores of plant pathogenic oomycetes. *Fungal Genet. Biol.* **2005**, *42*, 213–223. [CrossRef]
30. Liu, L.; Zhao, Y.; Lu, J.; Liang, M.; Huang, S. Identification and Evaluation of UV-Tolerant *Streptomyces araujoniae* Strain Isolated from Tibet Plateau Soil as Biocontrol Agent Against Rice Blast. *Rice Sci.* **2020**, *27*, 359–362. [CrossRef]