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Biological Control Effect of Antagonistic Bacteria on Potato Black Scurf Disease Caused by *Rhizoctonia solani*

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Abstract: Potato black scurf, caused by *Rhizoctonia solani*, is a severe soil-borne disease that affects the quality and production of potatoes worldwide. In our study, we used *Paenibacillus polymyxa* YF and *Bacillus amyloliquefaciens* HT to determine the antagonistic ability of *R. solani*, with a particular focus on the antagonistic action of *P. polymyxa* YF to *R. solani* and its biocontrol effect on potato black scurf. In fermentation, filtrate assay, 50% filtrate of *P. polymyxa* YF and *B. amyloliquefaciens* HT inhibited the growth of *R. solani* by 85.55% and 82.86%, respectively. Microscopic observations showed notable morphological changes with mycelial collapse, atrophy, and deformation following treatment with the antagonistic filtrates. Moreover, cell membrane permeability results showed increased conductivity in bacteria-treated samples compared to the control. *P. polymyxa* YF exhibited stable colonization on potato plants and secreted various extracellular enzymes (protease, amylase, and cellulase), along with the synthetic substances with growth-promoting effects, such as siderophores and Indole-3-acetic acid (IAA). Whether it is in the excised tissue inoculation or potted experiment, the negative control showed the highest rank of disease symptoms. In the pot experiment, after YF treatment, physiological parameters showed remarkable changes in plant height, root length, stem thickness, and dry and fresh weight. Compared to blank control, the activities of the four resistant enzymes increased significantly in the *P. polymyxa* YF treatment group. The upregulation in the *P. polymyxa* YF group was 4.04, 0.54, 0.46, and 3.10 times, respectively. PCR analysis identified genes in both bacterial strains coding for antimicrobial lipopeptides, including fenB, ituC, and srfAA, which are associated with fengycin, iturin, and surfactin synthesis. These findings demonstrated that *P. polymyxa* YF has a prominent antagonistic effect on *R. solani*, suggesting its potential as an effective biological control agent for controlling potato black scurf.

Keywords: antagonistic effect; biocontrol bacteria; growth promotion; potato black scurf; *Rhizoctonia solani*

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1. Introduction

Potato black scurf, caused by *Rhizoctonia solani*, is a destructive soil-borne disease that significantly threatens potato cultivation worldwide [1]. *R. solani*, known for extensive damage to various agricultural and horticultural crops, has become a significant concern in potato-cultivating countries, including China [2]. For instance, in Dingxi City, Gansu province, the incidence rate in severely affected fields has reached 75%, with a general incidence rate of 10–20% in ordinary fields. Furthermore, the incidence of diseases in plots continuously cultivated for 7 years is as high as 96.71% [3,4].

R. solani, belonging to the genus *Rhizoctonia* [5], is characterized by colorless, branched mycelium forming right or near-right angles. Studies have shown that *R. solani* is one species that has many strains. Most countries have proved that the main strain causing potato black scurf is AG-3. *R. solani* AG-3 TB causes tobacco target spot disease, that can seriously damage the quality and output of tobacco. *R. solani* AG-3 mainly infects the underground parts of plants and causes ulcers of roots, buds, stems, and stolons through mycelia and sclerotia that survive on soil and seed tubers [6–8]. When *R. solani* encounters a potato plant, it begins to attach to the plant and secrete cell-wall-degrading enzymes, and the hyphae begin to infect plant cells, eventually causing the entire cell structure to fall apart and the cells to die [9]. The disease transmission and damage mainly depend on diseased seed potatoes, which facilitate the disease in low-temperature and high-humidity soil [10]. Currently, the challenge in controlling *R. solani* is attributable to the stability of its sclerotium, which can survive in the soil for a long time.

Current methods for managing potato black scurf include chemical fumigation [11], breeding resistant varieties [12], crop rotation [13–15], and so on. However, the chemical control of *R. solani* is not very effective as most chemicals cause hazardous effects on non-targeted organisms and the environment [16], and disease-resistant varieties may have problems such as low product yield, poor processing quality, and low output [17]. Crop rotation is not suitable for the large-scale cultivation of potato agriculture [17]. Therefore, it is necessary to explore efficient and environmentally friendly methods for managing potato black scurf, such as biological control. Currently, microorganisms have attracted people's attention as biological control agents for plant diseases and have thus become a research focus [18,19].

A large number of studies have proved that biocontrol bacteria, such as *Bacillus velezensis*, *Brevibacillus parabrevis*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Trichoderma harzianum*, *Trichoderma reesei*, and *Serratia plymuthica* B4, can inhibit the infection of pathogens and can also promote plant growth [20–27]. The *Bacillus amyloliquefaciens* SB14 significantly controlled damping-off disease caused by *R. solani* AG-4 by 58% and that caused by *R. solani* AG-2-2 by 52.5; *Bacillus subtilis* V26 induced significant suppression of root canker and black scurf tuber colonization in incidence disease of 63% and 81%, respectively [28,29]. Furthermore, there are substances that promote plant growth, such as iron carriers and IAA [30,31]. However, there are few reports on using *B. polymyxa* YF as a biological control agent for potato black scurf. This study investigates the inhibitory effects of *Paenibacillus polymyxa* YF and *Bacillus amyloliquefaciens* HT on *R. solani*, through plate, pot, and in vitro inoculation experiments. Additionally, it seeks to elucidate the potential disease resistance and growth-promoting effects of these antagonistic bacteria. These findings lay a theoretical foundation for developing *P. polymyxa* YF as a feasible biological control agent with the potential to control potato black scurf and promote potato growth.

2. Materials and Methods

2.1. Pathogens Strain and Antagonistic Bacteria

The fungal pathogen *Rhizoctonia solani* (GenBank Accession OR824714) strain was provided by the Gansu Academy of Agricultural Sciences (Lanzhou, China). *R. solani* was maintained under in vitro conditions on potato dextrose agar (PDA) slants with regular sub-culturing after every 15–20 d [32].

P. polymyxa YF (GenBank ID: 16S rRNA, MW205750; gyrA, OP888972; RpoB, OP888973) and *B. amyloliquefaciens* HT (GenBank ID: 16SrRNA, MW776428; gyrA, OP888971; RpoB, OP888976) were used in this study. They were isolated from sheep manure compost or the rhizosphere soil of healthy vegetables, and stored on Lysog-enic Agar (10 g/L (Basebio, Hangzhou, China), yeast extract 5 g/L (Macklin, Shanghai, China), NaCl 10 g/L (Guangnuo, Shanghai, China), and agar (BioFroxx, Guangzhou, China) 20 g/L) at 4 °C. *P. polymyxa* YF were conserved at the China General Microbiological Culture Collection Center (CGMCC No.21517) [33]. The strains of microorganisms used in the study have been preserved in the Microbiology Laboratory of Lanzhou Jiaotong University.

2.2. Antifungal Activity Assay of *P. polymyxa* YF and *B. amyloliquefaciens* HT against *R. solani*

The antagonistic ability of biocontrol bacteria was measured in vitro on PDA plates in a confrontation assay [34]. The 6 mm diameter cakes from *R. solani* cultured at 28 °C for 7 d were inoculated in the center of all plates. Then, the bacteria isolates were transferred in three symmetric spots 2 cm from the *R. solani*. In the control, sterile distilled water was used instead of the antagonistic bacteria. Plates were incubated for 7 d at 28 °C in the dark, and the growth of the fungus towards the bacterial and control treatments was measured on day 7 [35]. The percentage of mycelium growth inhibition was calculated by using the following formula: inhibition rate (%) = $[(R - r)/R] \times 100$. Here, R is the radius of fungal growth from the center of the plate towards the control treatment and r is the radius of fungal growth towards the bacterial treatment.

The poisoned food technique investigated the antifungal activity of extracellular metabolites from *P. polymyxa* YF and *B. amyloliquefaciens* HT [36]. Two bottles of LB broth (100 mL) were inoculated with 200 µL *P. polymyxa* YF and *B. amyloliquefaciens* HT (OD 600 = 1), respectively, and incubated simultaneously at 28 °C for 48 h. The culture was removed by centrifugation for 10 min at a speed of 3640 g, and the supernatant was filtered aseptically by a disk membrane with a diameter of 0.22 µm. The obtained cell-free culture filtrate (CF) was mixed with the molten PDA medium (45 ± 5 °C) so that the final medium volume ratio was 10%, 20%, and 50% (*v*/*v*). The media amended with different concentrations of CF were poured into Petri plates and inoculated with *R. solani* plug in the center. PDA medium amended with LB broth in the same concentrations was used as the control treatment. Inoculated plates were incubated for 7 d (the average time for *R. solani* to cover the plate surface in the control treatment). Plates were photographed, and pathogenic hyphae were measured. Inhibition rate (%) = $[(D1 - D2)/D1] \times 100$; here, 'D1' is the colony diameter of *R. solani* in the control and 'D2' is the colony diameter of *R. solani* in the treatment plates.

2.3. Determination of the Plant-Growth-Promotion Properties of *P. polymyxa* YF and *B. amyloliquefaciens* HT

2.3.1. Test of Siderophore Production

Siderophore production was analyzed by Modified CAS Agar Medium Kit (Hopebio, Qingdao, China, CAS: chrome azurol sulphonate 10.87 g, 1 L distilled water) according to the methods outlined previously [37]. *P. polymyxa* YF and *B. amyloliquefaciens* HT were inoculated onto Lysogenic Agar plates and incubated at 30 °C for 24 h. The strain was inoculated on CAS medium and the plate was incubated at 30 °C for 3 days. The clear and transparent circle around the colony is believed to indicate the production of iron carriers.

2.3.2. Detection of Extracellular Enzymes

P. polymyxa YF and *B. amyloliquefaciens* HT can produce extracellular enzymes such as protease, cellulase, and amylase [38]. Biocontrol antagonists were inoculated into corresponding chromogenic mediums. (Amylase detection medium; cellulase detection medium; protease detection medium. The specific formula of the medium can be found in Supplement I.) All experiments were carried out at 28 °C for 3 d and observed conformational changes; these were recorded via photos.

2.3.3. IAA Production

The generation of IAA by *P. polymyxa* YF and *B. amyloliquefaciens* HT strains was qualitatively and quantitatively determined using the colorimetric method, as detailed in previous studies [30,31]. Briefly, the cultures of *P. polymyxa* YF and *B. amyloliquefaciens* HT were placed in King's medium with and without L-tryptophan (Macklin, Shanghai, China) for 2 d at 30 °C and 180 rpm. After centrifugation at 5000× *g* rpm for 5 min, the supernatant was mixed with Salkowski's reagent (150 mL concentrated H_2SO_4 (Nanjing Reagent, Nanjing, China), 250 mL dH_2O, 7.5 mL 0.5 M $FeCl_3 \cdot 6H_2O$ (FuChen, Tianjin, China)) in a 1:1 ratio using brief vortexing. After incubation at 30 °C in the dark for 30 min,

the absorbance of the mixture was measured at 530 nm using a spectrophotometer. The IAA concentration was quantified by comparing these readings against a standard IAA solution (Sigma Aldrich, Burlington, MA, USA).

2.4. Effect of P. polymyxa YF and B. amyloliquefaciens HT on Mycelial Morphology of R. solani

Five mm diameter plugs from *R. solani* cultures were inoculated into 100 mL Potato Dextrose Broth medium (composed of 200 g of potato, 20 g of glucose (Xilong, Guangzhou, China), and 1 L of distilled water). These cultures were incubated at 25 °C, agitated at 180 r/min for 4 d, and then mixed with 10% *P. polymyxa* YF or strain HT bacteria-free filtrates that had been previously incubated for 24 h. The non-inoculated medium filtrate was added to the control treatment. After maintaining for 4 d, scanning electron microscopy was used to observe the morphological changes of *R. solani* hyphae in each treatment group (SEM, ZEISS Gemini 500, Oberkochen, Germany). This experiment is in triplicate.

2.5. Effects of P. polymyxa YF and B. amyloliquefaciens HT on the Membrane Permeability of R. solani

The effect of the bacterial culture supernatant on the permeability of the *R. solani* cell membrane was studied by measuring electrical conductivity [39]. The hyphae of *R. solani* were cultured in 100 mL of PDB at 25 °C for 7 d. The obtained *R. solani* hyphae were centrifuged at 8000× *g* for 2 min and washed thrice with sterile distilled water. After that, 10% bacterial culture supernatant was added to the culture. Eventually, the supernatant was analyzed by a conductivity meter (INESA, Shanghai, China) within 24 h. The control was performed without the bacterial supernatant; this experiment was performed in triplicate.

2.6. Observation on Colonization of P. polymyxa YF Strain in Potato Plants

GFP-labeled *P. polymyxa* YF were constructed using GFP-marked vector pGFP4412 [40]. The colonization of *P. polymyxa* YF-GFP in the potato stems and roots was performed with minor modifications in the methods described [41]. *P. polymyxa YF-GFP* was incubated overnight in 100 mL LB liquid medium containing 100 μg/mL Amp (Rhawn, China) at 30 °C. The surfaces of the well-grown 5-week-old potato stems and roots were disinfected; then, they were transplanted into pots with sterilized soil. Next, they were treated with 100 mL *P. polymyxa* YF-GFP culture (10^8 CFU/mL) and provided with 100 mL of water as a blank control for watering. Then, the plants were kept in a greenhouse with a temperature cycle of 25 °C/15 °C (day and night). To visualize the colonization of *P. polymyxa* YF-GFP in potato stems and roots, the potato stem and root tissue samples were randomly collected at 5, 10, 15, 20, and 25 d after inoculation with bacteria. The collected stems and roots were washed with water and cut into thin slices of about 1 mm for observation using confocal laser scanning microscopy (CLSM, Olympus FV3000, Olympus, Tokyo, Japan) with an excitation wavelength of 488 nm. To further determine the colonization rate of potato roots and stems, a portion of each stem and root sample (0.1 g) was ground in a mortar containing 0.9 mL of sterile distilled water, until a fine homogenate was obtained. After diluting the suspension, it was placed on a modified LB medium (100 μg/mL, Amp) and incubated at 30 °C for 24 h, followed by CFU counting [42].

2.7. Control Effect on R. solani and Growth Promotion for Potato of P. polymyxa YF in the Greenhouse

The Atlantic variety of potatoes from Ronghui market, Anning district, Lanzhou city, Gansu province, was selected. Fifteen healthy and plump potato tubers were used for sprouting potato seedlings. Wash the potato tubers, disinfect them by soaking in ethanol (70%) for 1 min and NaClO (5%) for 2 min, rinse them three times with excess sterile distilled water, and finally cut them into pieces with a sterile scalpel. Each potato tuber was placed individually in a plastic pot containing sterile soil and cultured for 5 weeks under natural conditions. The experimental design included 5 treatment groups: (1) drenching plant roots with 50 mL suspension of *R. solani* conidia (10^5 conidia/mL); (2) applying 50 mL suspension of both *R. solani* conidia (10^5 conidia/mL) and *P. polymyxa* YF suspension (10^8 CFU/mL) to

the roots; (3) drenched the roots of each plant with 50 mL of *P. polymyxa* YF suspension and, 48 h later, drenched them with 50 mL of *R. solani* conidial suspension (10^5 conidia/mL); (4) a sole application of 50 mL *P. polymyxa* YF suspension; (5) drenched the roots of each plant with 50 mL of sterile water as a control. Two weeks post-treatment, we observed and recorded the symptoms and severity of disease manifestation in the *R. solani*-treated plants. In addition, the physiological parameters were also measured, including plant height (cm), root length (cm), stem thickness (mm), fresh weight (g), and dry weight (g). The disease incidence in each treatment group was quantified as a percentage of infected plants out of the total. Two weeks after inoculation, the phenotypes such as stems and leaves and browning were observed. These come as a result of the occurrence of the disease in the upper ground tissue; the extent to which the underground tissue is affected is assessed based on the characteristics of the potato black scurf after the seedlings are removed from the pot: rotten, the dark brown discoloration of the vascular tissue. Moreover, another result of the disease is that the plants die; in this case, the occurrence of the disease can be determined. To assess the pathogenic data obtained from the pathogenic test, we used one-way analysis of variance (ANOVA) and then Tukey's HSD test (honest and significant differences) to determine the presence of any significant differences at the $p < 0.05$ level (SPSS Version 27.0) [43].

The severity of the disease affecting the potato seedlings was evaluated after treatment. According to the nonparametric marginal effect analysis of disease severity grade, based on 95% CI of $\hat{p}$ij, the pathogenicity of all treatments differed from that of the control plants without inoculation. Disease severity was evaluated using a numerical rating scale from 0 to 9 [44]. Two weeks after inoculation, the disease severity of the underground part of the plant was classified and counted, and the following scores were assigned: 0 = no disease spots on leaves; 1 = disease spot area < 5% of total leaf area; 3 = disease spot area 6~10% of total leaf area; 5 = disease spot area 11~20% of total leaf area; 7 = disease spot area 21–50% of total leaf area; 9 = disease spot area > 50% of total leaf area [45]. The disease severity was calculated as a percentage of the total number of experimental plants for the underground plant part. Data were analyzed in IBM SPSS Statistics 27.0.

2.8. Potato Excised Tissues Experiment

Material varieties and sources are consistent with potted experiments. Specifically, healthy potatoes were selected, ensuring similarity in shape. The potato tubers were cut into fragments with an average size of 6.39 cm × 5.93 cm and a thickness of 1.5 cm. The potato tubers were washed and then disinfected by soaking in ethanol (70%) for 1 min and NaClO (5%) for 2 min; then, they were rinsed three times with excess sterile distilled water. Finally, they were cut into pieces with a sterile scalpel [46]. The slices were subjected to five treatments: (1) only sprayed with 100 µL of *R. solani* conidial suspension (10^5 conidia/mL) on the surface of the tuber; (2) sprayed with 100 µL of *R. solani* conidial suspension (10^5 conidia/mL) and 100 µL of *P. polymyxa* YF suspension (10^8 CFU/mL) on the surface of the tuber; (3) sprayed with 100 µL for 48 h *P. polymyxa* YF suspension (10^8 CFU/mL) and then sprayed with *R. solani* conidial suspension (10^5 conidia/mL) on the surface of the tuber; (4) only sprayed with 100 µL of biocontrol bacteria (OD600 = 0.4) on the surface of the tuber; (5) sprayed with sterile dH_2O (control) on the surface of the tuber. The treated slices were placed in a Petri dish (90 mm) lined with sterile moist filter paper; then, they were incubated at 25 °C for 4 days. Lesion diameter was measured and calculated at 7 d post-inoculation. Disease incidence and severity were calculated using the method described in Section 2.7.

2.9. Production of Resistant Enzymes in Plants Induced by P. polymyxa YF

Enzymes involved in plat resistance, such as peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT) and phenylalanine ammonia-lyase (PAL) were examined. The fermentation broth of *R. solani* and the cultures of *P. polymyxa* YF were prepared. Five treatment groups were established according to the descriptions provided in Section 2.7.

After the potato plant reached the development stage of 7–8 compound leaves, a random sample of 0.5 g of root tissue from each treatment group was selected for analysis. The root tissues were then ground for the assays assessing the enzymatic activities of POD, PAL, CAT, and PPO; these were analyzed using commercial assay kits (AIDISHENG, Yancheng, China), following the manufacturer's instructions [47,48]. The quantification of enzyme activities was achieved by recording the absorbance values using a spectrophotometer (UA-2800A, UNIC, Shanghai, China).

2.10. Detection of Synthesis Genes of Antimicrobial Lipopeptides of P. polymyxa YF and B. amyloliquefaciens HT

Using the primers designed in Table S1, the genes for iutirin, fengycin, suractin, polyketide synthase, and nonribosomal peptide synthesis were detected in the *P. polymyxa* YF and *B. amyloliquefaciens* HT. The primer sequences used in this study are described by Zhang et al. [33] (Table S1). The reaction system of the 25 μL PCR included 1.5 μL of the bacterial suspension (10^8 CFU/mL), 1 μL of primer (10 μM), 12.5 μL 2× master mix with standard buffer (Sangon Biotech in Shanghai), and 10.5 μL nuclease-free water. The primer sequence was provided by Sangon Biotech in Shanghai. The reaction conditions were set: pre-denaturation at 94 °C for 7 min, denaturation at 94 °C for 45 s, annealing at 56 °C for 45 s, and extension at 72 °C for 120 s. This cycle was repeated 35 times. After the cycles, there was a final extension at 72 °C for 10 min and an infinite cycle at 4 °C. The PCR amplicons were analyzed by electrophoresis in 1% (*w*/*v*) agarose gel, and the sizes of the bands were determined using the 2 kb marker.

2.11. Identification of P. polymyxa YF Surfactin and Polymyxin B Sulfate Components by HPLC

P. polymyxa YF was inoculated into LB of 100 mL and cultured for 72 h; next, the bacterial suspension was centrifuged at 12,000× *g* rpm for 15 min. The collected supernatant was used for liquid phase analysis. The supernatant was filtered with a sterile filter membrane (0.22 μm); then, 50 mL of macroporous resin particles were added to every 100 mL supernatant; finally, the flask was shaken at room temperature for 4 h. The obtained mixture was shaken with a separatory funnel and then allowed to stand and separate. The separation was concentrated in a rotary evaporator with a condition of 40 °C and 60 rpm.

Samples and standard (10 μL) were injected into the HPLC, SHIMANZU Essentia LC-16 series device, consisting of an SPD-16 absorption and a C18 reversed-phase (ShimNex CS C18, 250 × 4.6 mm, 5 μm) column. For the crude extract of *P. polymyxa* YF and the standard sample of surfactin (purity ≥ 98%, Macklin-Aldrich Trading Co., Ltd., Shanghai, China), the mobile phase consisted of an acetonitrile–methanol solution (Damao, Tianjin, China) containing 0.1% TFA (Macklin, Shanghai, China) and water (90:10, *v*/*v*). The flow rate was maintained at 1 mL min^{-1}, and the chromatograms were detected at 205 nm. For the crude extract of *P. polymyxa* YF and polymyxin B sulfate (purity ≥ 96%, Med Chem Express Trading Co., Shanghai, China), the mobile phase was composed of acetonitrile and water (50:50, *v*/*v*), and the flow rate was 1 mL min^{-1}; the chromatograms were detected at 215 nm. The column temperature was 40 °C.

Accuracy was evaluated within and between the day data of the triple injections of each concentration and expressed as a relative error. The precision of the HPLC method was evaluated in terms of the relative standard deviation of the retention time, measured concentration, and the peak area of the studied solution within and between days. In this experiment, a 3-fold S/N ratio was used as the detection limit of the method. A polymyxin B sulfate and crude extract of *P. polymyxa* YF solution was formed, with a concentration of 1 mg mL^{-1}; then, a surfactin solution with the concentration of 5 mg mL^{-1} was formed. With these, the injection was repeated, with a 10 μL injection volume, with 6 consecutive needles; this allowed us to investigate the precision of the instrument.

2.12. Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA). All data are presented here as mean ± SEM. To detect the differences among the means in each group, one-way analysis of variance (ANOVA) was used, followed by Tukey's HSD (honest significant difference) test at a significance level of $p < 0.05$.

3. Results

3.1. Effect of *P. polymyxa* YF and *B. amyloliquefaciens* HT against *R. solani*

In order to screen the biocontrol bacteria, which can effectively control potato black scurf caused by *R. solani*, six bacterial strains were evaluated for their antagonistic properties. Among them, compared with the control group, the *P. polymyxa* YF and *B. amyloliquefaciens* HT strains exhibited the highest inhibitory activity against *R. solani*, with inhibition rates of 61.33% and 60.56%, respectively (Turkey HSD test, $p < 0.05$) (Figure 1 and Table 1). Due to their strong inhibitory effects, *P. polymyxa* YF and *B. amyloliquefaciens* HT were selected for further investigation.

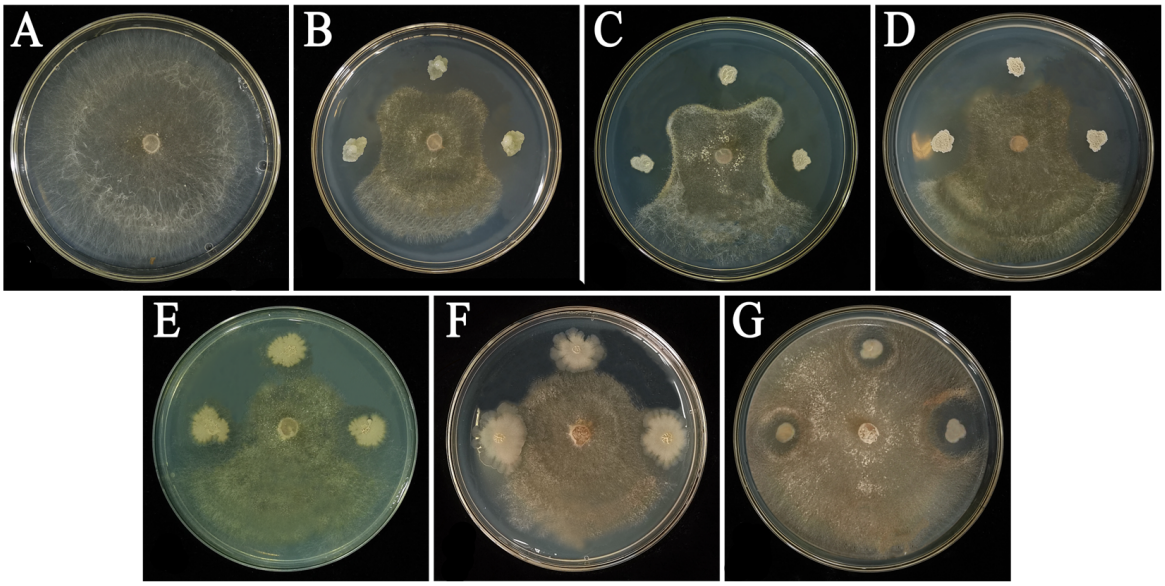

Figure 1. Antagonistic effect of various *Bacillus* strains against *Rhizoctonia solani*. (**A**) Only *R. solani* was inoculated; (**B**) *Paenibacillus polymyxa* YF and *R. solani*; (**C**) *Bacillus amyloliquefaciens* HT and *R. solani*; (**D**) *Bacillus subtilis* and *R. solani*; (**E**) *Bacillus megaterium* and *R. solani*; (**F**) *Bacillus mucilaginosus* and *R. solani*; (**G**) *Brevibacillus laterosporus* and *R. solani*.

Table 1. Evaluation of the inhibitory effect of six species of *Bacillus* against *Rhizoctonia solani*.

Bacillus Strains	Treatment Group Diameter (cm)	Control Diameter (cm)	Inhibition Ratio (%)
Bacillus subtilis	3.59 ± 0.07	9.00 ± 0.01	60.11 a
Paenibacillus polymyxa	3.48 ± 0.13	9.00 ± 0.01	61.33 a
Bacillus amyloliquefaciens	3.55 ± 0.09	9.00 ± 0.01	60.56 a
Bacillus megaterium	3.60 ± 0.10	9.00 ± 0.01	60.00 a
Bacillus mucilaginosus	3.78 ± 0.06	9.00 ± 0.01	58.00 b
Brevibacillus laterosporus	9.00 ± 0.01	9.00 ± 0.01	0.00 c

Note: ± represents the standard deviation of the three replicates (mean ± SD). According to the Tukey HSD test, different letters indicate significant differences at the 0.05 probability level.

The obtained cell-free culture filtrate (CF) was mixed with molten PDA medium (45 ± 5 °C) at final volume ratios of 10%, 20%, and 50% (*v*/*v*). Compared with the control, the inhibition of *P. polymyxa* YF was stronger than that of *B. amyloliquefaciens* HT in the three cases. Notably, at a 50% volume ratio, the inhibition rates of *P. polymyxa* YF and

B. amyloliquefaciens HT on *R. solani* were 85.55% and 82.86%, respectively. At this concentration, both *P. polymyxa* YF and *B. amyloliquefaciens* HT CF completely prevent *R. solani* growth under the test conditions (Tukey HSD test, $p < 0.05$) (Figure 2 and Table 2). The efficacy of the filtrates was consistent with the results of the plate confrontation test, highlighting their potential as biocontrol agents against *R. solani*.

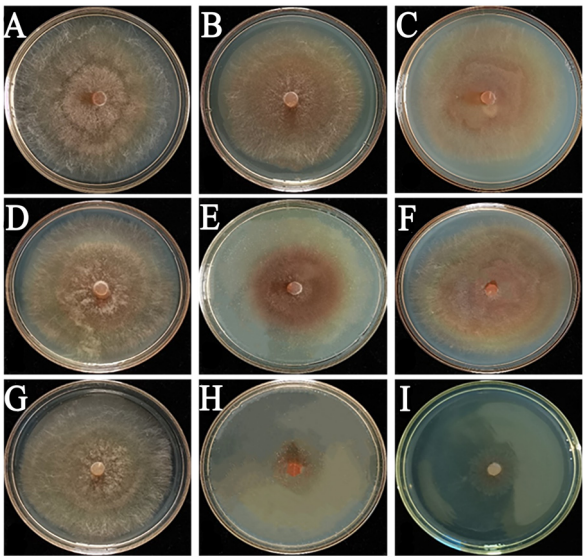

Figure 2. The inhibitory effect of fermentation filtrate of two biocontrol strains against *R. solani*. (**A**) Added 10 mL LB for 90 mL PDA; (**B**) added 10 mL *P. polymyxa* YF for 90 mL PDA; (**C**) added 10 mL *B. amyloliquefaciens* HT for 90 mL PDA; (**D**) added 20 mL LB for 80 mL PDA; (**E**) added 20 mL *P. polymyxa* YF for 80 mL PDA; (**F**) added 20 mL *B. amyloliquefaciens* HT for 80 mL PDA; (**G**) added 50 mL LB for 50 mL PDA; (**H**) added 50 mL *P. polymyxa* YF for 50 mL PDA; (**I**) added 50 mL *B. amyloliquefaciens* HT for 50 mL PDA.

Table 2. Evaluation of the inhibitory effect of two biocontrol bacterial fermentation filtrates against *R. solani*.

Biocontrol Bacteria		Fermentation Broth Concentration		
		10%	**20%**	**50%**
P. polymyxa YF	Control group	8.15 ± 0.06	7.95 ± 0.05	8.65 ± 0.02
	Treatment group	7.05 ± 0.04	4.35 ± 0.09	1.25 ± 0.07
	Inhibition rate (%)	13.50 c	45.28 b	85.55 a
B. amyloliquefaciens HT	Control group	7.85 ± 0.15	8.15 ± 0.16	8.75 ± 0.21
	Treatment group	7.50 ± 0.09	4.50 ± 0.18	1.50 ± 0.17
	Inhibition rate (%)	4.46 c	44.79 b	82.86 a

Note: ± represents the standard deviation of the three replicates (mean ± SD). According to the Tukey HSD test, different letters indicate significant differences at the 0.05 probability level.

3.2. Effect of P. polymyxa YF and B. amyloliquefaciens HT on Mycelial Morphology of R. solani

As shown in Figure 3, the hyphae of *R. solani* treated with *P. polymyxa* YF showed morphological alteration. The hyphae displayed pronounced distortion, shrinkage, atrophy, and even breakage. The control group hyphae maintained a healthy appearance, with an abundant, smooth surface, and a uniform size with a normal physiological structure. In the *B. amyloliquefaciens* HT-treated group, the hyphae of *R. solani* also exhibited significant morphological differences compared to the control group, with significant deformation and wrinkling.

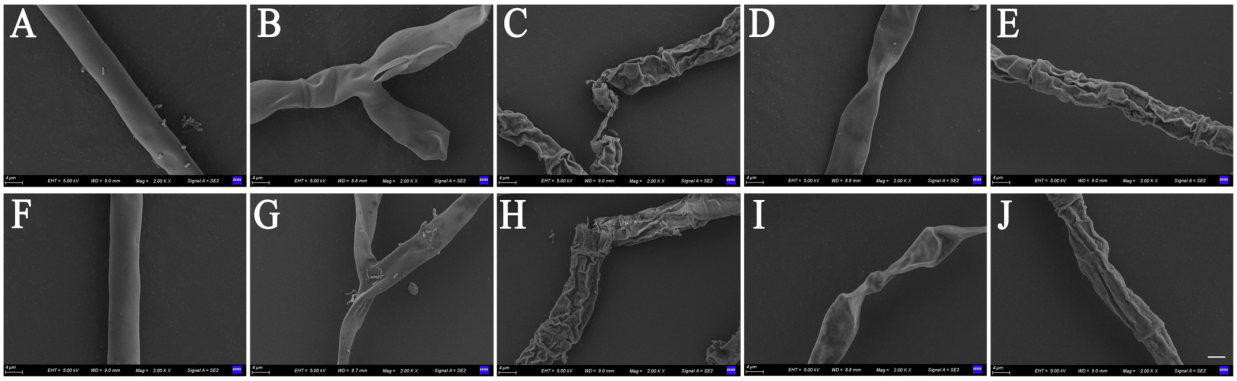

Figure 3. Observations of the morphologies of *R. solani* treated with *P. polymyxa* YF and *B. amyloliquefaciens* HT. (**A**) Blank control; (**B**) branching of *R. solani* mycelium after *P. polymyxa* YF treatment; (**C**) fracture of *R. solani* mycelium after *P. polymyxa* YF treatment; (**D**) constriction of *R. solani* mycelium after *P. polymyxa* YF treatment; (**E**) shrinkage of *R. solani* mycelium after *P. polymyxa* YF treatment; (**F**) blank control; (**G**) branching of *R. solani* mycelium after *B. amyloliquefaciens* HT treatment; (**H**) fracture of *R. solani* mycelium after *B. amyloliquefaciens* HT treatment; (**I**) constriction of *R. solani* mycelium after *B. amyloliquefaciens* HT treatment; (**J**) shrinkage of *R. solani* mycelium after *B. amyloliquefaciens* HT treatment. Each treatment had 3 replicates (n = 3) (Bar = 4 μm).

3.3. Effects of P. polymyxa YF and B. amyloliquefaciens HT Culture Supernatant on Membrane Permeabilization

The cell membrane permeability results showed a gradual increase in extracellular conductivity following the treatment with the biocontrol agents; meanwhile, those of the control group showed a marginal increase. Notably, the increase in conductivity was most rapid in the treatment groups and stabilized in 21 h. After 6 h of treatment, compared with the control group, the extracellular conductivity of *P. polymyxa* YF and *B. amyloliquefaciens* HT groups increased by 74.74 and 36.04 times, respectively. The results demonstrate that *P. polymyxa* YF and *B. amyloliquefaciens* HT could effectively disrupt the structural and functional integrity of *R. solani* cell membrane, contributing to their inhibitory effect on the growth and proliferation of *R. solani* (Figure 4).

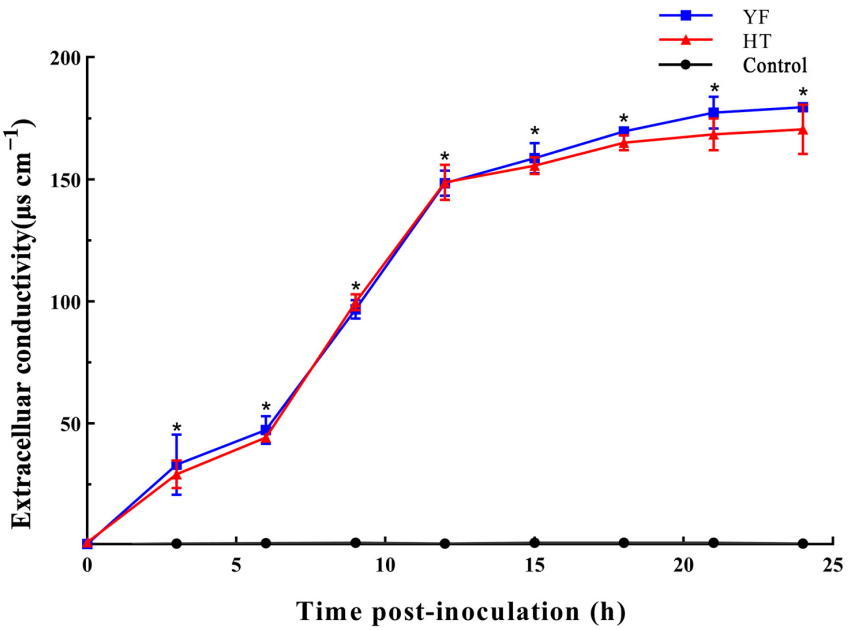

Figure 4. The effect of *P. polymyxa* YF and *B. amyloliquefaciens* HT on the cell membrane permeability of *R. solani*. Error bar represents the standard deviation of the three replicates (mean ± SD). * represented a significant difference ($p < 0.05$) at the same time point.

3.4. Determination of Biological Characteristics of Strains P. polymyxa YF and B. amyloliquefaciens HT

As shown in Figure 5, the plates inoculated with *P. polymyxa* YF and *B. amyloliquefaciens* HT showed a protease hydrolysis circle, a cellulase hydrolysis circle, an amylase hydrolysis circle, and a yellow halo formed by iron ions (Figure 5). This indicates that *P. polymyxa* YF and *B. amyloliquefaciens* HT could have the ability to produce protease, cellulase, amylase, and siderophores. The pink color of the antagonistic bacterial supernatant and colorimetric solution mixed for 30 min showed that both *P. polymyxa* YF and *B. amyloliquefaciens* HT could produce IAA. Compared with the absence of tryptophan, the pink color of *P. polymyxa* YF and *B. amyloliquefaciens* HT became darker in the presence of tryptophan, indicating that tryptophan can promote the production of more IAA in *P. polymyxa* YF and *B. amyloliquefaciens* HT. In the presence of tryptophan, the amounts of IAA produced by *P. polymyxa* YF and *B. amyloliquefaciens* HT were 13.83 mg/L and 17.54 mg/L, respectively (Figure 6).

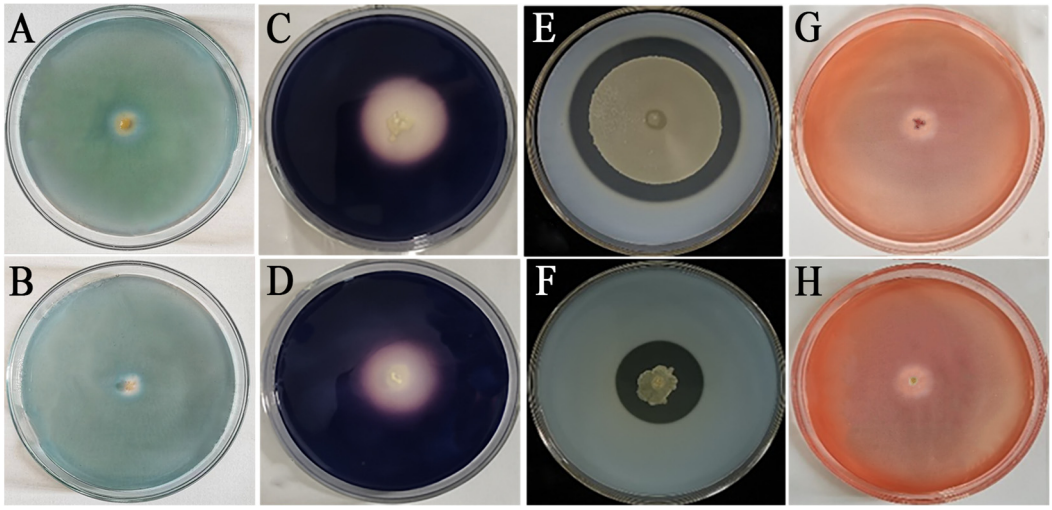

Figure 5. Detection of siderophore and extracellular enzymes produced by *P. polymyxa* YF and *B. amyloliquefaciens* HT. (**A**) Siderophores detection of *P. polymyxa* YF-produced siderophore; (**B**) siderophores detection of *B. amyloliquefaciens* HT-produced siderophore; (**C**) detection of amylase produced by *P. polymyxa* YF; (**D**) detection of amylase produced by *B. amyloliquefaciens* HT; (**E**) detection of protease produced by *P. polymyxa* YF; (**F**) detection of protease produced by *B. amyloliquefaciens* HT; (**G**) detection of cellulase produced by *P. polymyxa* YF; (**H**) detection of cellulase produced by *B. amyloliquefaciens* HT.

Figure 6. Qualitative and quantitative detection of IAA produced by *P. polymyxa* YF and *B. amyloliquefaciens* HT in the King's medium containing L-tryptophan and without L-tryptophan. (**A**) Qualitative detection of IAA produced by *P. polymyxa* YF: from left to right—control, tryptophan-free group, tryptophan-treated group; (**B**) qualitative detection of IAA produced by *B. amyloliquefaciens* HT: from left to right—control, tryptophan-free group, tryptophan-treated group; (**C**) IAA standard curve.

3.5. Observation on the Colonization of P. polymyxa YF in Potato Plants

After 5–10 d of inoculation, the colonization rate increased continuously, and the fluorescence intensity reached the strongest on the 10 d, indicating that a large number of antagonistic bacteria had penetrated the roots of potato, and the fluorescence intensity was stable at 15–20 d, indicating that *P. polymyxa* YF could be colonized continuously. The colonization rate was more than 10^4 CFU/g. At 25 d, the fluorescence intensity darkened obviously and *P. polymyxa* YF no longer permeated continuously (Figures 7 and 8A).

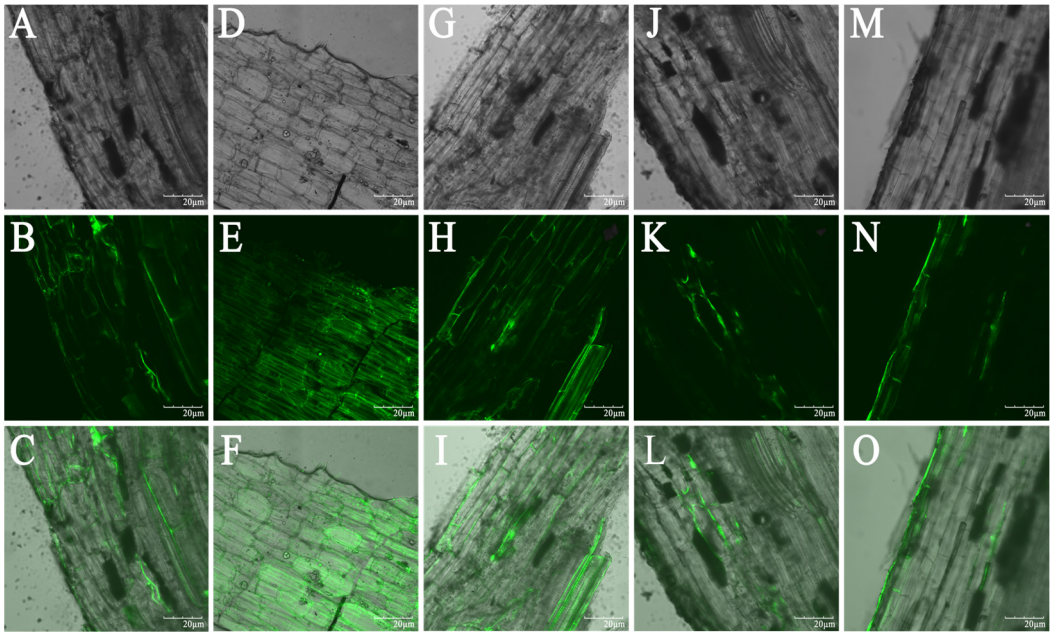

Figure 7. The observation of fluorescent cells of GFP-*P. polymyxa* YF; the potato roots after bacterial inoculation. (**A**–**C**) The colonization status of GFP-*P. polymyxa* YF (5 days); (**D**–**F**) the colonization status of GFP-*P. polymyxa* YF (10 days); (**G**–**I**) the colonization status of GFP-*P. polymyxa* YF (15 days); (**J**–**L**) the colonization status of GFP-*P. polymyxa* YF (20 days); (**M**–**O**) the colonization status of GFP-*P. polymyxa* YF (25 days). The first, second, and third lines are bright field, dark field, and overlap field, respectively.

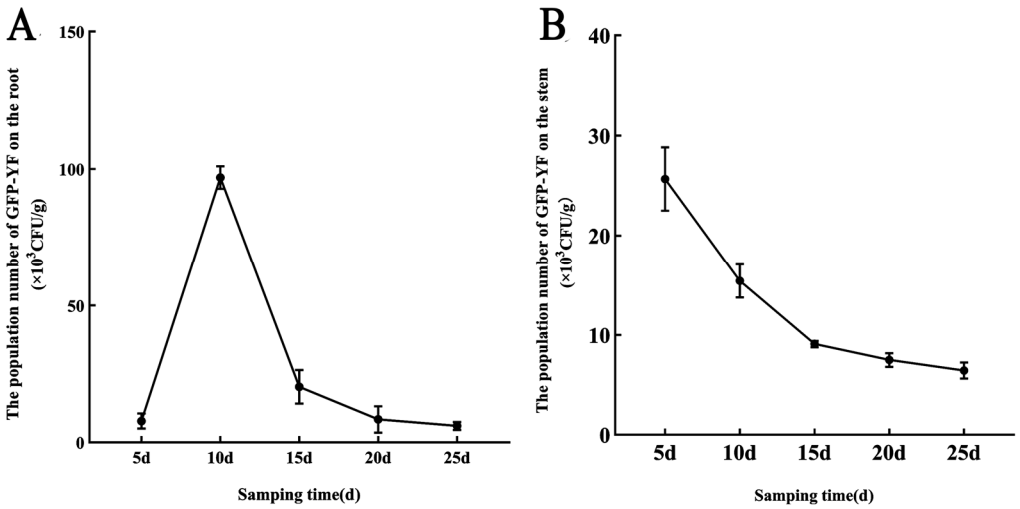

Figure 8. Colonization rate of GFP-*P. polymyxa* YF in potato roots and stems. (**A**) Colonization rate of GFP-*P. polymyxa* YF in potato roots; (**B**) colonization rate of GFP-*P. polymyxa* YF in potato stems.

On day 5 of inoculation, the fluorescence intensity in the stem was the strongest, indicating that many antagonistic bacteria had penetrated the potato stem at this time. The

fluorescence intensity was stable in the following 10–20 d, indicating that *P. polymyxa* YF could continue to colonize for 15 d, and the colonization rate was stable above 10^4 CFU/g. At day 20, the fluorescence intensity darkened obviously, and *P. polymyxa* YF no longer permeated continuously (Figures 8B and 9).

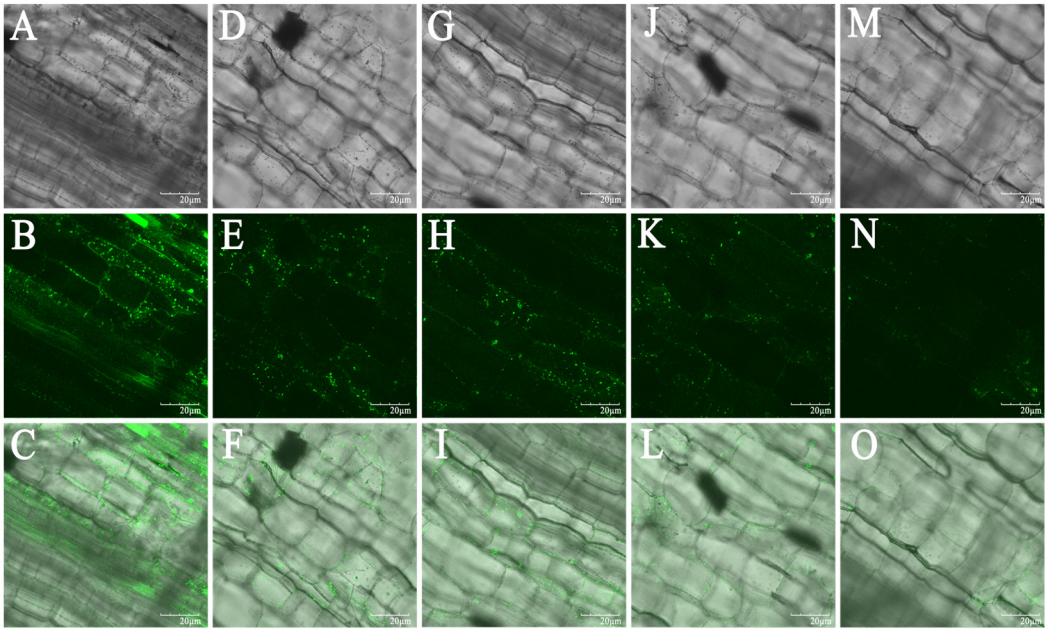

Figure 9. The observation of fluorescent cells of GFP-*P. polymyxa* YF; the potato stems after bacterial inoculation. (**A**–**C**) The colonization status of GFP-*P. polymyxa* YF (5 days); (**D**–**F**) the colonization status of GFP-*P. polymyxa* YF (10 days); (**G**–**I**) the colonization status of GFP-*P. polymyxa* YF (15 days); (**J**–**L**) the colonization status of GFP-*P. polymyxa* YF (20 days); (**M**–**O**) the colonization status of GFP-*P. polymyxa* YF (25 days). The first, second, and third lines are bright field, dark field, and overlap field, respectively.

3.6. Control Effect on R. solani and Growth Promotion of P. polymyxa YF in the Greenhouse

In the pot experiment, only potato plants inoculated with *R. solani* showed symptoms of fusarium wilt, including severe discoloration of vascular bundles and wilted leaves. However, the vascular bundles of potato plants treated with *P. polymyxa* YF were only slightly discolored, indicating a low severity of black scurf (Figure 10). These results prove that strain *P. polymyxa* YF exhibited good control effectiveness against potato black scurf. In the pot experiment, the *R. solani*-treated seedlings had the highest incidence rate; the negative control had the largest median disease severity scores (8.80), $\hat{p}$ij (0.88), and average grade $\bar{R}$ij (35.50) (Tukey's HSD test, $p < 0.05$); the *P. polymyxa* YF prevention group and *P. polymyxa* YF treatment group had the smallest median disease severity scores (1.40 and 1.20), $\hat{p}$ij (0.51 and 0.49), and average grade $\bar{R}$ij (21.00 and 20.00) (Tukey's HSD test, $p < 0.05$); the corresponding control effects were 59.23% and 52.76%, respectively (Tukey's HSD test, $p < 0.05$) (Table 3).

After 7 weeks, we determined the morphological indicators of the potato seedlings treated with *P. polymyxa* YF. Compared with the control, the plant height, stem thickness, root length, fresh weight, and dry weight of potato seedlings treated with *P. polymyxa* YF were significantly improved, increasing by 16.41%, 13.73%, 57.52%, 11.46%, and 56.03%, respectively (Tukey's HSD test, $p < 0.05$). In the *R. solani* + *P. polymyxa* YF and *P. polymyxa* YF + *R. solani* treatment, compared with the treatment with *R. solani*, the plant height, root length, stem thickness, fresh weight, and dry weight also significantly increased (Figure 11) (Tukey's HSD test, $p < 0.05$). The results demonstrate that *P. polymyxa* YF promotes the increase in the biomass of potato plants.

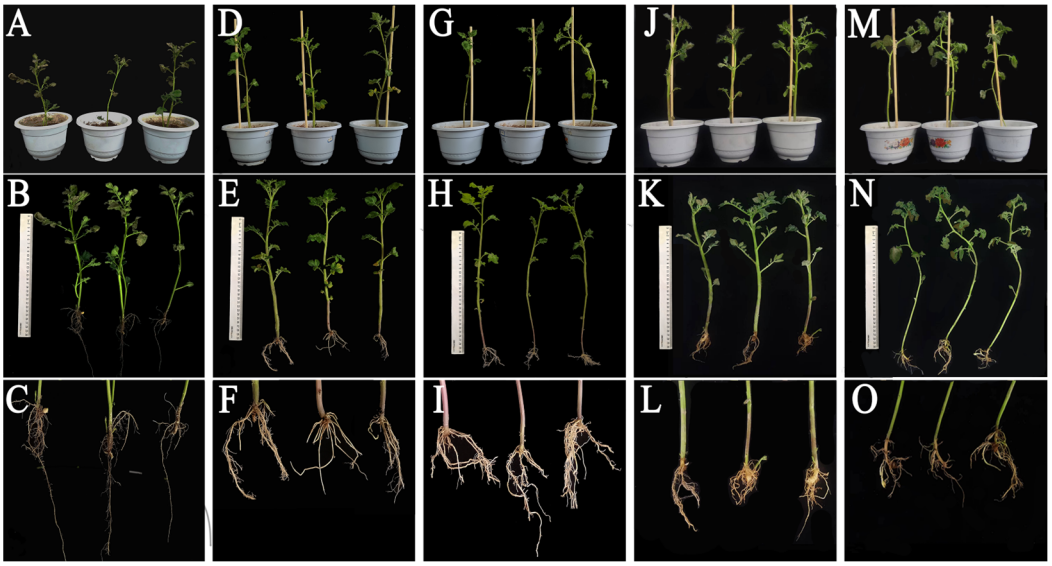

Figure 10. Control effect of *P. polymyxa* YF on potato black scurf in potted potato plants. (**A**–**C**) Disease incidence of the underground part after pathogen inoculation for 7 weeks; (**D**–**F**) blank control; (**G**–**I**) potato plant and underground part after *P. polymyxa* YF treatment; (**J**–**L**) disease incidence of the underground part after prevention; (**M**–**O**) disease incidence of the underground part after treatment.

Table 3. Evaluation of potted control effect of *P. polymyxa* YF on potato black scurf.

Treatment	MDR[x]	$\bar{R}ij$[y]	$\hat{p}ij$	Control Effect (%)	95%CI for $\hat{p}ij$	
					Lower	Upper
Blank Control	0.00	5.50	0.13	-	0.13	0.13
Negative Control	8.80	35.50	0.88	-	0.85	0.90
Prevention Group	1.40	21.00	0.51	59.23 ± 0.14 a	0.44	0.59
Treatment Group	1.20	20.00	0.49	52.76 ± 0.05 a	0.43	0.54

Note: The effects of *R. solani* on the disease severity were measured on a scale of zero to nine. Parameters include median, mean rank ($\bar{R}ij$), and relative treatment effect ($\hat{p}ij$) with 95% confidence intervals (CI) for disease severity. Different letters indicate significant differences at the 0.05 probability level.

For the excised tissue inoculation, the experiment indicates that potato slices inoculated with *R. solani* showed typical hyphal growth on the surface of the tuber slices compared to the control group. With time, the treatment group's hyphae continued accumulating on the surface of excised tissues. The surface is brown, and even the tuber tissue deteriorates and decays. In the treatment, compared with the control group, the mycelium growth of *R. solani* was more or less in the pathogen group, prevention group, and treatment group; among these, the pathogen group was the most, the treatment group was the second, and the prevention group was the least, indicating that the effect of *P. polymyxa* YF prevention was better than that of treatment. Moreover, the color of the tubers of *P. polymyxa* YF, prevention, and treatment groups are yellowish, and the symptoms of decay are significantly reduced (Figure 12). For the excised tissue inoculation, the negative control had the largest median disease severity scores (8.20), $\hat{p}ij$ (0.90), and average grade $\bar{R}ij$ (54.50) (Tukey's HSD test, $p < 0.05$); meanwhile, the *P. polymyxa* YF prevention group and *P. polymyxa* YF treatment group had the smallest median disease severity scores (4.00 and 5.80), $\hat{p}ij$ (0.43 and 0.68), and average grade $\bar{R}ij$ (26.50 and 41.00) (Tukey's HSD test, $p < 0.05$) (Table 4).

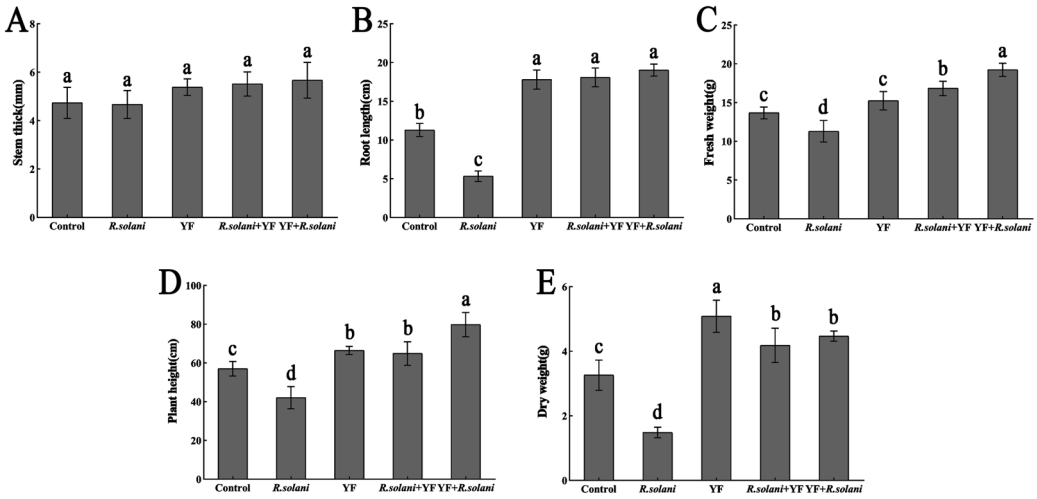

Figure 11. Evaluation of the growth promotion effect of *P. polymyxa* YF on potato plants. (**A**) Effect of *P. polymyxa* YF on stem thickness of potato plant (mm); (**B**) effect of *P. polymyxa* YF on root length of potato plant (cm); (**C**) effect of *P. polymyxa* YF on fresh weight of potato plant (g); (**D**) effect of *P. polymyxa* YF on plant height of potato plant (cm); (**E**) effect of *P. polymyxa* YF on dry weight of potato plant (g). Error bars represent the standard deviation of the three replicates (ten pots) (mean ± SD). Based on the Tukey HSD test, the different letters above the bar chart indicate significant differences at the 0.05 probability level.

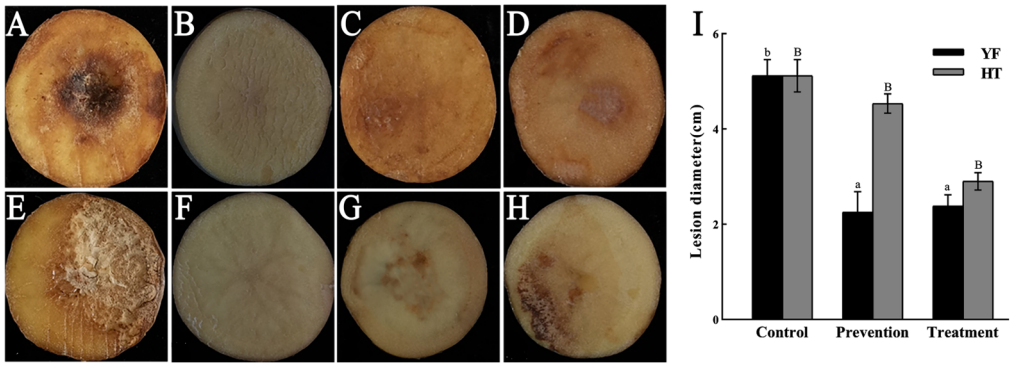

Figure 12. In vitro control effects of *P. polymyxa* YF and *B. amyloliquefaciens* HT on potato black scurf. (**A**) *R. solani*-treated negative control; (**B**) *P. polymyxa* YF-treated blank control; (**C**) prevention group for *P. polymyxa* YF treatment; (**D**) treatment group for *P. polymyxa* YF treatment; (**E**) *R. solani*-treated negative control; (**F**) *B. amyloliquefaciens* HT-treated blank control; (**G**) prevention group for *B. amyloliquefaciens* HT treatment; (**H**) treatment group for *B. amyloliquefaciens* HT treatment; (**I**) evaluation of potato tuber lesion. Error bars represent the standard deviation of the three replicates (mean ± SD). Based on the Tukey HSD test, the different letters above the bar chart indicate significant differences at the 0.05 probability level.

3.7. Detection of Antagonistic Enzyme Activities in Potato Plants

The POD, PPO, PAL, and CAT activities in the potato roots reflected the potatoes' resistance to pathogens. Compared to the control, the enzyme activities of POD showed significant improvements in the *P. polymyxa* YF group, the treatment group, and the prevention group, which increased by 4.04, 3.95, and 3.38 times, respectively (Tukey HSD test, $p < 0.05$) (Figure 13A). Compared to the control, the enzyme activities of PPO showed significant improvements in the *P. polymyxa* YF group, the treatment group, and the prevention group, which increased by 0.54, 0.33, and 0.33 times, respectively (Tukey HSD test, $p < 0.05$) (Figure 13B). Compared to the control, the enzyme activities of CAT showed significant improvements in the *P. polymyxa* YF group, the treatment group, and the prevention group, which increased by 3.10, 2.81, and 2.30 times, respectively (Tukey HSD

test, $p < 0.05$) (Figure 13C). Compared to the control, the enzyme activities of PAL showed significant improvements in the *P. polymyxa* YF group, the treatment group, and the prevention group, which increased by 0.46, 0.41, and 0.34 times, respectively (Tukey HSD test, $p < 0.05$) (Figure 13D).

Table 4. Evaluation of tuber control effect of *P. polymyxa* YF on potato black scurf.

Treatment	MDR[x]	$\bar{R}ij$[y]	$\hat{p}ij$	Control Effect (%)	95%CI for $\hat{p}ij$	
					Lower	Upper
Blank Control	0.00	5.50	0.08	-	0.08	0.08
Negative Control	8.20	54.50	0.90	-	0.85	0.95
YF Prevention Group	4.00	26.50	0.43	56.03 ± 0.43 a	0.33	0.54
YF Treatment Group	5.80	41.00	0.68	53.43 ± 0.24 ab	0.58	0.77
HT Prevention Group	3.40	22.40	0.37	50.50 ± 0.20 ab	0.26	0.47
HT Treatment Group	4.80	33.10	0.54	43.33 ± 0.18 b	0.43	0.65

Note: The mean and standard deviation (SD) of the data are shown (N = 3); different letters on the same line denote significant differences at the 0.05 level of *p*-value by the Kruskal–Wallis test and Dunn test as post hoc. The effects of *R. solani* on the disease severity of tubers were measured on a scale of zero to nine. Parameters include median, mean rank ($\bar{R}ij$), and relative treatment effect ($\hat{p}ij$), with 95% confidence intervals (CI) for disease severity.

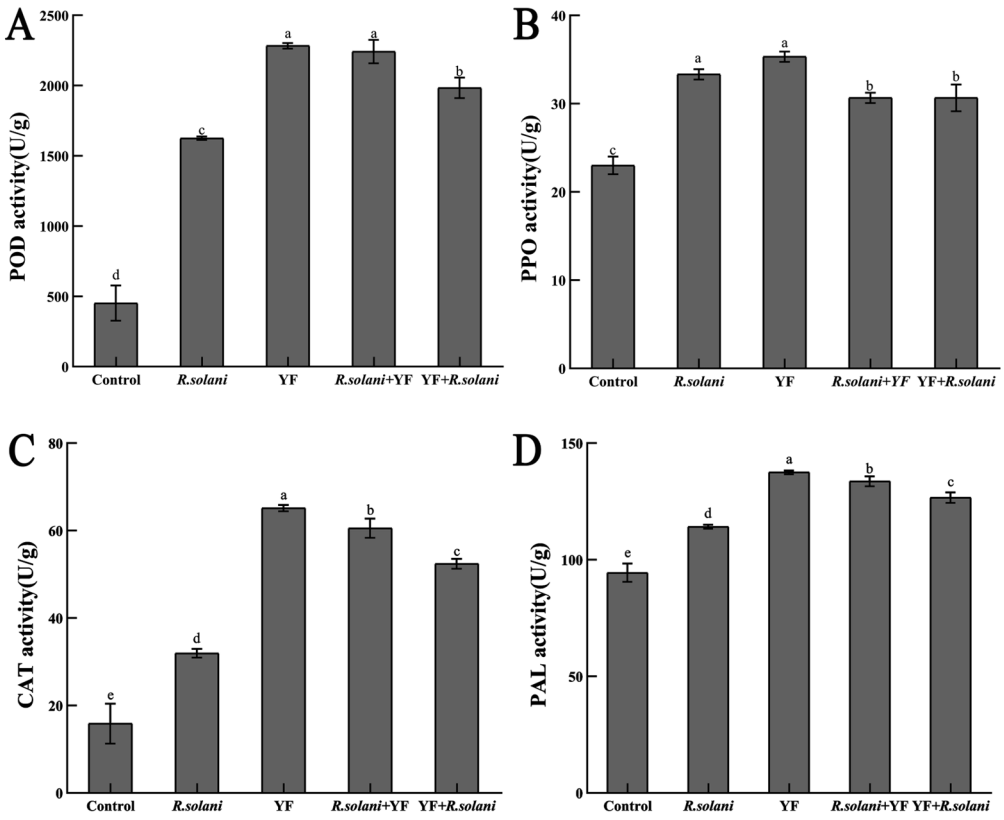

Figure 13. Induction of resistance enzyme activity of potato root. (**A**) The activities of POD; (**B**) the activities of PPO; (**C**) the activities of CAT; (**D**) the activities of PAL. Error bars represent the standard deviation of the three replicates (mean ± SD). Based on the Tukey HSD test, the different letters above the bar chart indicate significant differences at the 0.05 probability level.

After the roots of the potatoes were treated by strains *P. polymyxa* YF only, the content of POD, PPO, PAL, and CAT was significantly different (Tukey HSD test, $p < 0.05$). The results indicate that, even when potato seedlings are infected by *R. solani*, the strain of *P. polymyxa* YF can stimulate the production of defensive enzymes.

3.8. Detection of Synthesis Genes of Antimicrobial Lipopeptides of P. polymyxa YF and B. amyloliquefaciens HT

Using primer pairs designed to detect genes involved in the biosynthesis of iutirin, fengycin, suractin, polyketide synthase, and non-ribosomal peptide synthase, amplicons of the expected size were obtained (Figure S1). Through the inhibition test of the Oxford Cup, we obtained that the inhibition rates that the crude extract of *P. polymyxa* YF and standard of surfactin and polymyxin B sulfate had on *R. solani*: 35.26%, 51.87%, and 23.84 %, respectively (Figure 14). The liquid chromatography results showed that *P. polymyxa* YF produced surfactin and polymyxin B sulfate. At 205 nm, the peak value generated by YF coarse and Surfactin is 802,134 and 171,850; at 215 nm, the peak values produced by YF coarse and Polymyxin B Sulfate were 108,902 and 60,335, respectively. And there were no interfering peaks from the samples (Figure 15).

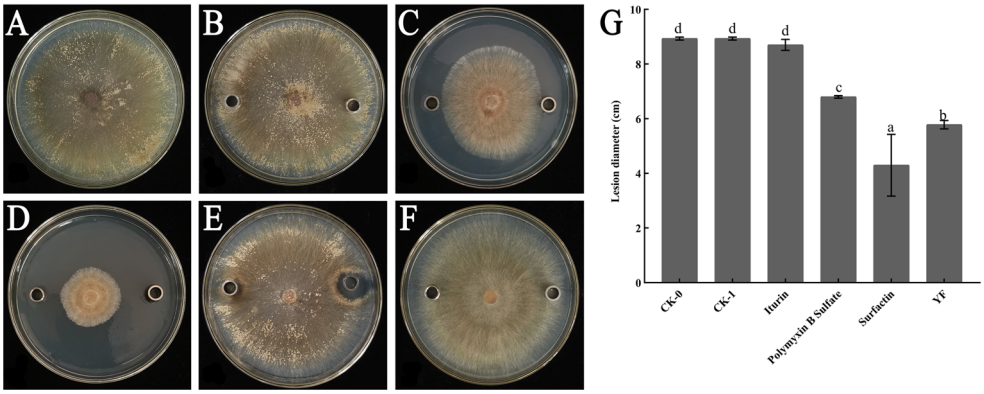

Figure 14. Determination of fungistatic of crude extract. (**A**) *R. solani*; (**B**) *R. solani* and the control of Oxford Cup without anything; (**C**) *R. solani* and the Oxford Cup with *P. polymyxa* YF cell filtrate; (**D**) *R. solani* and the Oxford Cup with surfactin standard; (**E**) *R. solani* and the Oxford Cup with polymyxin B sulfate standard; (**F**) *R. solani* and the Oxford Cup with iturin standard; (**G**) evaluation of antibacterial effect of A-F. Error bars represent the standard deviation of the three replicates (mean ± SD). Based on the Tukey HSD test, the different letters above the bar chart indicate significant differences at the 0.05 probability level.

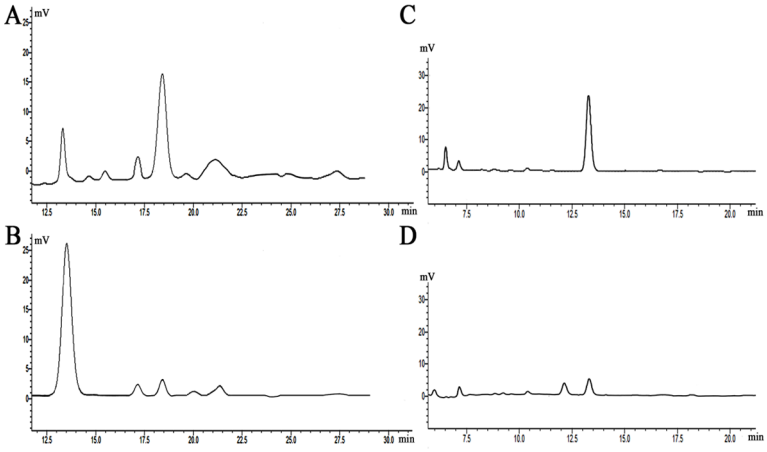

Figure 15. Identification of *P. polymyxa* YF Surfactin and polymyxin B sulfate components by HPLC. (**A**) Standard sample of surfactin; (**B**) crude extract of *P. polymyxa* YF; (**C**) standard sample of polymyxin B sulfate; (**D**) crude extract of *P. polymyxa* YF.

4. Discussion

R. solani is known for its broad host range of over 200 plant species, including important crops like rice, wheat, soybeans, potatoes, and tomatoes [7]. The potato black scurf caused by *R. solani* poses a significant challenge to global agriculture. Previous studies

have acknowledged the potential of Bacillus species as biological control agents against *R. solani*. Yet, reports addressing their role in combating potato black scurf and exploring their biocontrol mechanisms are limited [49]. Our study is the first extensive exploration of the biological control efficacy of *P. polymyxa* YF and *B. amyloliquefaciens* HT against *R. solani* for potatoes.

After treating biocontrol bacteria *P. polymyxa* YF and *B. amyloliquefaciens* HT, the hyphae of *R. solani* in the antagonistic zone were observed by SEM, and a great deformation occurred. The bacteria-free filtrates of YF and SY89 caused the hyphae of the pathogenics to rupture and shrink, thereby inhibiting the mycelial growth of *F. avenaceum* [33]. The results of the report are consistent with those of this study. *P. polymyxa* YF and *B. amyloliquefaciens* HT inhibited the growth of pathogens by increasing the lipid peroxidation of the pathogen hyphae and decreasing the synthesis of the metabolism products [50].

Compared to the inoculated and uninoculated pathogens, *P. polymyxa* YF-treated seedlings significantly suppressed the growth of the pathogen and enhanced the plant's growth and biomass. Under natural conditions, *P. polymyxa* YF could inhibit the disease and promote growth; these effects were more significant than those seen in the control. It was also observed that *P. polymyxa* YF was colonized more frequently in the potato rhizosphere. In greenhouse and field studies, *B. subtilis* MB14 and *B. amyloliquefaciens* MB101 can protect tomatoes from the pathogenic fungus (*R. solani*) [51,52]. Two *B. subtilis* strains, PCL1608 and PCL1612, producing iturin A, have exhibited the highest biocontrol and colonization capabilities [53]. The first step of bacterial cell attraction/adhesion is an efficient colonization phase and a further population decrease to reach a stable level at approximately 5×10^6 cells per gram of roots [54]. Following rhizosphere and rhizoplane colonization, some soil-borne microorganisms can enter roots and establish subpopulations ranging from 10^5 to 10^7 CFU g^{-1} FW [55]. The colonization process observed for *P. polymyxa* YF was similar to that reported for other plant-beneficial rhizobacteria. Plant-growth-promoting bacteria can be employed for their role in increasing the growth and yield of various crops such as wheat and rice. Among them, a series of laboratory experiments conducted on wheat under gnotobiotic (axenic) conditions demonstrated increases in root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 37.7%), and shoot dry weight (up to 36.3%) for the inoculated wheat seedlings. *Bacillus pantothenticus* P4 (MTCC 4695) and *Pseudomonas pieketti* Psd6 (MTCC 4715) increased rice grain yield by 55.5, 12.2, and 76.9% over the uninoculated control in micro-plot experiments [56,57].

Furthermore, *P. polymyxa* YF and *B. amyloliquefaciens* HT were found to amplify the genes of antimicrobial compounds, such as iturin, fengycin, surfactin, polyketide synthases, and nonribosomal polypeptide synthase biosynthesis genes. Antibiotics produced by *Bacillus* spp. play an essential role in the biocontrol of fungal diseases, and different types of metabolite production under in vitro assays showed that selected *Bacillus* spp. may be used in multiple modes of action against fungal pathogens [58]. A large number of antifungal peptides are reported by *Bacillus* spp. [59], including fengycin, surfactin, bacillomyci-ns D, F, and L, iturins A–E [60–62]. In addition, *P. polymyxa* YF also produces polymyxin B sulfate, which showed antibacterial activity both in vitro and in vivo, in this study. These antifungal peptides reduce the growth of many fungi, such as *Rhizoctonia*, *Fusarium*, *Aspergillus*, and *Penicillium* species [59]. In general, many antifungal peptides that produce bacteria or filamentous fungi have been reported to be biological control agents against different kinds of fungal diseases of plants [62–65]. Therefore, biocontrol bacteria could inhibit the growth of *R. solani* by secreting lipopeptides and other substances to achieve the purposes of prevention and control. These findings contribute significantly to our understanding of biocontrol effects and offer promising strategies for controlling potato black scurf and other fungal diseases in agriculture.

5. Conclusions

In summary, this study established *P. polymyxa* YF as a potent inhibitor of *R. solani*, the pathogen responsible for potato black scurf. Both *P. polymyxa* YF and *B. amyloliquefaciens* HT

have demonstrated the ability to produce extracellular enzymes, contributing significantly to the inhibition of *R. solani* growth. These strains are characterized by their ability to produce iron carriers and IAA, alongside their proficiency in hydrolyzing cellulase, amylase, and protease. On this basis, the efficient antagonism caused by *R. solani* can significantly inhibit the growth of mycelium. Moreover, *P. polymyxa* YF effectively mitigates the incidence of potato black scurf and exerts a notable growth-promoting effect on plants. It can induce plant stress enzyme activity and potentially secrete antibacterial lipopeptides to achieve efficient prevention and control effects. This study represents the first instance of *P. polymyxa* YF being used as a biocontrol agent against potato black scurf, providing a foundation for developing novel and effective biocontrol strategies for this agricultural challenge.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14020351/s1, Figure S1: Detection of synthesis genes of antimicrobial lipopeptides of antagonistic bacteria.; Table S1: Primer sequence of antimicrobial substance genes.

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References

1. Dennis, A.; Michael, K.T.; Jeffrey, S.M. Development of *Rhizoctonia solani* on Stems, Stolons and Tubers of Potatoes I. *Eff. Inoculum Source* **2010**, *87*, 374–381. [CrossRef]
2. Adams, G.C. Thanatephorus cucumeris (*Rhizoctonia solani*) a species complex of wide host range. *Adv. Plant Pathol.* **1988**, *6*, 535–552. [CrossRef]
3. Xie, K.Z.; Lu, L.Y.; Luo, A.H.; Hu, X.Y. Effect of long-term continuous cropping on soil-borne diseases and yield of potato. *China Seed Ind.* **2018**, *2*, 65–67. [CrossRef]
4. Liu, X.J.; An, J.H.; Mo, J.; Sun, X.M.; Wang, W.H.; Wei, Z.Q.; Yang, Y. Field test of 5 fungicides against *potato naevus* disease. *Chin. Potato J.* **2019**, *33*, 170–174. [CrossRef]
5. Ajayi-Oyetunde, O.O.; Bradley, C.A. *Rhizoctonia solani*: Taxonomy, population biology and management of rhizoctonia seedling disease of soybean. *Plant Pathol.* **2017**, *67*, 3–17. [CrossRef]
6. Li, X.C.; Hou, H.H.; Liu, H.; Wang, H.C.; Cai, L.T.; An, M.N.; Zhang, C.; Wu, Y.H. Identification of 3-Methoxyphenylacetic Acid as a Phytotoxin, Produced by *Rhizoctonia solani* AG-3 TB. *Molecules* **2023**, *28*, 790. [CrossRef] [PubMed]
7. Lehtonen, M.J.; Ahvenniemi, P.; Wilson, P.S.; German-Kinnari, M.; Valkonen, J.P.T. Biological diversity of *Rhizoctonia solani* (AG-3) in a northernpotato-cultivation environment in Finland. *Plant Pathol.* **2008**, *57*, 141–151. [CrossRef]
8. Ceresini, P.C. Genetic diversity of *Rhizoctonia solani* AG-3 from potato and tobacco in North Carolina. *Mycologia* **2002**, *94*, 437–449. [CrossRef]
9. Tuo, N.; Zhang, J.; Qiu, H.Z.; Zhang, W.M.; Zhang, C.H.; Liu, X.; Zhu, J. Pathogenic mechanism of *R.solani* potato blight I Micro-structure observation of the infection process and measurement of cell wall degradation enzyme activity. *Acta Prataculturae Sin.* **2015**, *24*, 74–82. [CrossRef]
10. Zhao, Y.Z.; Wang, D.; Xu, L.M.; Jia, R.F.; Guo, J.S.; Li, N.; He, Y.Q.; Yan, H.M. Comparison of field control effect of different microbial inoculants on *potato naevus* disease. *China Plant Prot. Guide* **2020**, *40*, 90. [CrossRef]
11. Larkin, R.P.; Honeycutt, C.W.; Griffin, T.S.; Olanya, O.M.; Halloran, J.M.; He, Z. Effects of different potato cropping system approaches and water management on soilborne diseases and soil microbial communities. *Phytopathology* **2011**, *101*, 58–67. [CrossRef]

12. Ismail, S.; Jiang, B.; Nasimi, Z.; Inam-Ul-Haq, M.; Yamamoto, N.; Ofori, A.D.; Khan, N.; Arshad, M.; Abbas, K.; Zheng, A.P. Investigation of *Streptomyces scabies* causing potato scab by various detection techniques, its pathogenicity and determination of host-disease resistance in potato germplasm. *Pathogens* **2020**, *9*, 760. [CrossRef]
13. Sakuma, F.; Maeda, M.; Takahashi, M.; Hashizume, K.; Kondo, N. Suppression of common scab of potato caused by *Streptomyces turgidiscabies* using lopsided oat green manure. *Plant Dis.* **2011**, *9*, 1124–1130. [CrossRef]
14. Larkin, R.P.; Halloran, J.M. Management effects of disease-suppressive rotation crops on potato yield and soilborne disease and their economic implications in potato production. *Am. J. Potato Res.* **2014**, *91*, 429–439. [CrossRef]
15. Bernard, E.; Larkin, R.P.; Tavantzis, S.; Hashizume, K.; Kondo, N. Rapeseed rotation, compost and biocontrol amendments reduce soilborne diseases and increase tuber yield in organic and conventional potato production systems. *Plant Soil* **2014**, *374*, 611–627. [CrossRef]
16. Jung, W.; Park, R.; Mabood, F.; Hashizume, K.; Kondo, N. Effects of *Pseudomonas aureofaciens* 63-28 on defense responses in soybean plants infected by *Rhizoctonia solani*. *J. Microbiol. Biotechnol.* **2011**, *21*, 379–386. [CrossRef] [PubMed]
17. Gutierrez, J.; Bakke, A.; Vatta, M.; Merrill, A.R. Plant natural products as antimicrobials for control of *Streptomyces scabies*: A causative agent of the common scab disease. *Front. Microbiol* **2021**, *12*, 4334. [CrossRef] [PubMed]
18. González, V.; Armijos, E.; Garcés-Claver, A. Fungal endophytes as biocontrol agents against the main soil-borne diseases of melon and watermelon in Spain. *Agronomy* **2020**, *10*, 820. [CrossRef]
19. Bustamante, M.I.; Elfar, K.; Eskalen, A. Evaluation of the Antifungal Activity of Endophytic and Rhizospheric Bacteria against Grapevine Trunk Pathogens. *Microorganisms* **2022**, *10*, 2035. [CrossRef] [PubMed]
20. Abo-Elyousr, K.A.M.; Saad, M.M.; Al-Qurashi, A.D.; Ibrahim, O.H.M.; Mousa, M.A.A. Management of *Cumin Wilt* Caused by *Fusarium oxysporum* Using Native Endophytic Bacteria. *Agronomy* **2022**, *12*, 2510. [CrossRef]
21. Samaras, A.; Roumeliotis, E.; Ntasiou, P.; Karaoglanidis, G. Bacillus subtilis MBI600 promotes growth of tomato plants and induces systemic resistance contributing to the control of soilborne pathogens. *Plants* **2021**, *10*, 1113. [CrossRef]
22. Ahmed, W.; Zhou, G.; Yang, J.; Munir, S.; Ahmed, A.; Liu, Q.; Ji, G. *Bacillus amyloliquefaciens* WS-10 as a potential plant growth-promoter and biocontrol agent for bacterial wilt disease of flue-cured tobacco. *Pest Control* **2022**, *32*, 25. [CrossRef]
23. Platel, R.; Sawicki, M.; Esmaeel, Q.; Randoux, B.; Trapet, P.; Guilli, M.E.; Chtaina, N.; Ségolène, A.; Alexandre, B.; Rochex, A.; et al. Isolation and Identification of Lipopeptide-Producing *Bacillus velezensis* Strains from Wheat Phyllosphere with Antifungal Activity against the Wheat Pathogen *Zymoseptoria tritici*. *Agronomy* **2022**, *12*, 95. [CrossRef]
24. Emre, D.D. Biological control of *Rhizoctonia solani* on potato by using indigenous Trichoderma spp. *AIP Conf. Proc.* **2016**, *1726*, 020020. [CrossRef]
25. Franziska, F.; Jana, L.; Rita, G.; Gabriele, B. Strategy to select and assess antagonistic bacteria for biological control of *Rhizoctonia solani* Khn. *Can. J. Microbiol.* **2004**, *50*, 811–820. [CrossRef]
26. Grosch, R.; Scherwinski, K.; Jana, L.; Berget, G. Fungal antagonists of the plant pathogen *Rhizoctonia solani*: Selection, control efficacy and influence on the indigenous microbial community. *Mycol. Res.* **2006**, *110*, 1464–1474. [CrossRef]
27. Moncef, M.; Naceur, D.; Salem, E.; Youssra, M.; Sabrine, S.; Belhassen, T.; Ridha, M. Efficacy of selected *Pseudomonas* strains for biocontrol of *Rhizoctonia solani* in potato. *Phytopathol. Mediterr.* **2013**, *52*, 449–456. [CrossRef]
28. Elham, K.; Naser, S.; Masoud, S.B.; Bagher, M. *Bacillus amyloliquefaciens* SB14 from rhizosphere alleviates Rhizoctonia damping-off disease on sugar beet. *Microbiol. Res.* **2016**, *192*, 221–230. [CrossRef]
29. Saoussen, B.K.; Olfa, K.F.; Mouna, D.; Hayfa, J.K.; Mejda, D.R.; Slim, T. Efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizoctonia solani* on potato. *C R Biol.* **2015**, *338*, 784–792. [CrossRef]
30. Bric, J.M.; Bostock, R.M.; Silverstone, S.E. Rapid in situ assay for indoleacetic Acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol.* **1991**, *57*, 535–538. [CrossRef] [PubMed]
31. Dorra, B.A.; Olfa, F.G.; Slim, T. Rizhospheric competence, plant growth promotion and biocontrol efficacy of *Bacillus amyloliquefaciens* subsp. plantarum strain 32a. *Biol. Control* **2018**, *124*, 61–67. [CrossRef]
32. Yang, Y.G.; Wu, X.H. First Report of *Potato Stem Canker* Caused by *Rhizoctonia solani* AG4 HGII in Gansu Province, China. *Plant Dis.* **2013**, *97*, 840. [CrossRef] [PubMed]
33. Zhang, Z.K.; Zhang, W.X.; Wang, X.F.; Kou, Z.A.; Wang, Y.L.; Rehmat, I.; Zhang, J.Q.; Liu, L.; Shen, T.; Tian, Y.Q. Isolation and identification of antagonistic bacteria of *Angelica root rot* and their mechanism as biological control. *Biol. Control* **2022**, *177*, 105120. [CrossRef]
34. Jiang, C.-H.; Liao, M.-J.; Wang, H.-K.; Zheng, M.-Z.; Xu, J.-J.; Guo, J.-H. *Bacillus velezensis*, a potential and efficient biocontrol agent in control of *pepper gray mold* caused by *Botrytis cinerea*. *Biol. Control* **2018**, *126*, 147–157. [CrossRef]
35. Chen, L.; Hao, S.; Junying, H.; Dianxuan, W.; Ke, B. Antimicrobial, plant growth-promoting and genomic properties of the peanut endophyte *Bacillus velezensis* LDO2. *Microbiol. Res.* **2019**, *218*, 41–48. [CrossRef] [PubMed]
36. Alsayed, A.; Floriane, L.; Eliane, A.M.; Laure, W. Disease Inhibiting Effect of Strain *Bacillus subtilis* EG21 and Its Metabolites Against Potato Pathogens *Phytophthora infestans* and *Rhizoctonia solani*. *Biol. Control Microb. Ecol.* **2022**, *112*, 1943–7684. [CrossRef]
37. Shin, S.H.; Lim, Y.; Lee, S.E.; Yang, N.W.; RheeSung, J.H. CAS agar diffusion assay for the measurement of siderophores in biological fluids. *J. Microbiol. Methods* **2001**, *44*, 89–95. [CrossRef]
38. Meng, X.-J.; Medison, R.G.; Cao, S.; Wang, L.-Q.; Cheng, S.; Tan, L.-T.; Sun, Z.-X.; Zhou, Y. Isolation, identification, and biocontrol mechanisms of endophytic *Burkholderia vietnamiensis* C12 from Ficus tikoua Bur against *Rhizoctonia solani*. *Biol. Control* **2023**, *178*, 105132. [CrossRef]

39. Zhao, P.; Quan, C.; Wang, Y.; Wang, J.; Fan, S. *Bacillus amyloliquefaciens* Q-426 as a potential biocontrol agent against *Fusarium oxysporum* f. sp. spinaciae. *J. Basic Microbiol.* **2014**, *54*, 448–456. [CrossRef]
40. Cao, Y.; Zhang, Z.; Ling, N.; Yuan, Y.; Zheng, X.; Shen, B.; Shen, Q. *Bacillus subtilis* SQR 9 can control *Fusarium wilt* in cucumber by colonizing plant roots. *Biol. Fertil. Soils* **2011**, *47*, 495–506. [CrossRef]
41. Wen, C.; Peng, H.; Shahzad, M.; Peng, H.; Xing, L.; Yong, L.; Jun, W.; Yi, W.; Li, Y.; Peng, H.; et al. Efficacy of plant growth promoting bacteria *Bacillus amyloliquefaciens* B9601-Y2 for biocontrol of southern corn leaf blight. *Biol. Control* **2019**, *139*, 104080. [CrossRef]
42. Wu, Y.C.; Zhou, J.Y.; Li, C.G.; Wu, Y.M. Antifungal and plant growth promotion activity of volatile organic compounds produced by *Bacillus amyloliquefaciens*. *MicrobiologyOpen* **2019**, *8*, e00913. [CrossRef]
43. Sharma, S.R.; Kolte, S.J. Effect of soil-applied NPK fertilizers on severity of black spot disease (*Alternaria brassicae*) and yield of oilseed rape. *Plant Soil* **1994**, *167*, 313–320. [CrossRef]
44. Lou, S.; Li Feng, Y.; Tian, G.; Wang, H.; Tian, Z.; Wang, L.; Liu, X.; Wang, H. Evaluation of Germplasms for Resistance to *Potato Late Blight* and Molecular Markers Assisted Screening. *Crops* **2021**, *37*, 196–201. [CrossRef]
45. Manoj, K.S.; Amrita, S.R.; Rajesh, K.S.; Sudheer, K.; Akhilesh, K.P.; Alok, K.S.; Dilip, K.A. Characterization of mycolytic enzymes of *Bacillus* strains and their bio-protection role against *Rhizoctonia solani* in tomato. *Curr. Microbiol.* **2012**, *65*, 330–336. [CrossRef]
46. Weinhold, A.R. Rhizoctonia disease of potato: Effect on yield and control by seed tuber treatment. *Plant Dis.* **1982**, *66*, 815. [CrossRef]
47. Xu, W.; Yang, Q.; Yang, F.; Xie, X.; Goodwin, P.H.; Deng, X.X.; Tian, B.M.; Yang, L.Y. Evaluation and genome analysis of *Bacillus subtilis* YB-04 as a potential biocontrol agent against *Fusarium wilt* and growth promotion agent of Cucumber. *Front. Microbiol.* **2022**, *13*, 885430. [CrossRef]
48. Saha, S.; Begum, H.H.; Nasrin, S.; Samad, R. Effects of drought stress on pigment and protein contents and antioxidant enzyme activities in five varieties of rice (*Oryza sativa* L.). *Bangladesh J. Bot.* **2020**, *49*, 997–1002. [CrossRef]
49. Huang, Y.; Wu, Z.; He, Y.; Ye, B.-C.; Li, C. Rhizospheric *Bacillus subtilis* Exhibits Biocontrol Effect against *Rhizoctonia solani* in Pepper (*Capsicum annuum*). *BioMed Res. Int.* **2017**, *2017*, 9397619. [CrossRef] [PubMed]
50. Huang, J.F.; Zhang, F.B.; Pang, Y.W.; Huang, Q.Y.; Tang, S.H.; Fu, H.T.; Yang, B.M. Inhibition of banana *Fusarium wilt* by two biocontrol agents. *Microbiol. China* **2017**, *44*, 835–844. [CrossRef]
51. Manoj, K.S.; Sudheer, K.; Akhilesh, K.P.; Supriya, S.; Rajesh, K.S.; Prem, L.K.; Srivastava, A.K.; Dilip, K.A. Diversity and antagonistic potential of *Bacillus* spp. associated to the rhizosphere of tomato for the management of *Rhizoctonia solani*. *Biocontrol Sci. Technol.* **2012**, *22*, 203–217. [CrossRef]
52. Hyun, M.W.; Yeo, H.Y.; Jun, Y.K.; Seong, H.K. Fungal and Plant Phenylalanine Ammonia-lyase. *Mycobiology* **2011**, *39*, 257–265. [CrossRef] [PubMed]
53. Cazorla, F.M.; Romero, D.; Pérez-García, A.; Lugtenberg, B.J.J.; Vicente, A.; Bloemberg, G. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. *J. Appl. Microbiol.* **2007**, *103*, 1950–1959. [CrossRef] [PubMed]
54. Nihorimbere, V.; Patrick, F.; Philippe, T.; Marc, O. Ecological fitness of *Bacillus subtilis* BGS3 regarding production of the surfactin lipopeptide in the rhizosphere. *Environ. Microbiol. Rep.* **2009**, *1*, 124–130. [CrossRef] [PubMed]
55. Stéphane, C.; Christophe, C.; Angela, S. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* **2009**, *42*, 669–678. [CrossRef]
56. Khalid, A.; Arshad, M.; Zahir, Z.A. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.* **2004**, *96*, 473–480. [CrossRef] [PubMed]
57. Thakuria, D.; Talukdar, N.C.; Goswami, C.; Hazarika, S.; Boro, R.C.; Khan, M.R. Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Curr. Sci.* **2004**, *86*, 978–985.
58. Ramarathnam, R.; Shen, B.; Yu, C.; Dilantha, F.W.G.; Gao, X.; Teresa, D.K. Molecular and biochemical detection of fengycin- and bacillomycin D-producing *Bacillus* spp., antagonistic to fungal pathogens of canola and wheat. *Can. J. Microbiol.* **2007**, *53*, 901–911. [CrossRef]
59. Marc, O.; Philippe, J. *Bacillus* lipopeptides: Versatile weapons for plant disease biocontrol. *Trends Microbiol.* **2007**, *16*, 115–125. [CrossRef]
60. Athukorala, S.N.P.; Dilantha, F.W.G.; Khalid, Y.R. Identification of antifungal antibiotics of *Bacillus* species isolated from different microhabitats using polymerase chain reaction and MALDI-TOF mass spectrometry. *Can. J. Microbiol.* **2009**, *55*, 1021–1032. [CrossRef]
61. Kim, P.I.; Jaewon, R.; Young, H.K.; Youn, T.C. Production of biosurfactant lipopeptides Iturin A, fengycin and surfactin A from *Bacillus subtilis* CMB32 for control of Colletotrichum gloeosporioides. *J. Microbiol. Biotechnol.* **2010**, *20*, 138–145. [CrossRef] [PubMed]
62. Raaijmakers, J.M.; Irene, D.B.; Ole, N.; Marc, O. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* **2010**, *34*, 1037–1062. [CrossRef] [PubMed]
63. Arguelles, A.A.; Marc, O.; Badre, H.; Yan, L.; Alain, B.; Bernard, J.; Patrick, F. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microb. Cell Factories* **2009**, *8*, 63. [CrossRef] [PubMed]

64. Ongena, M.; Emmanuel, J.; Akram, A.; Michel, P.; Alain, B.; Bernard, J.; Jean, L.A.; Philippe, T. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol.* **2007**, *9*, 1084–1090. [CrossRef]
65. Chen, X.H.; Scholz, R.; Borriss, M.; Junge, H.; Mögel, G.; Kunz, S.; Borriss, R. Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *J. Biotechnol.* **2008**, *140*, 38–44. [CrossRef]