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Biological Control of Pear Valsa Canker Caused by *Valsa pyri* Using *Penicillium citrinum*

Hongbo Yuan, Bingke Shi, Tianxiang Huang, Zengqiang Zhou, Li Wang *, Hui Hou * and Hongtao Tu *

Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou 450009, China; yuanhongbo@caas.cn (H.Y.); 18337613180@163.com (B.S.); tianxianghug@163.com (T.H.); zhouzengqiang@caas.cn (Z.Z.)

* Correspondence: wangli06@caas.cn (L.W.); houhui@caas.cn (H.H.); tuhongtao@caas.cn (H.T.)

Abstract: Valsa canker caused by *Valsa pyri* is one of the most destructive diseases of commercial pear. For the present analysis, 29 different endophytic fungal strains were isolated from the branches of a healthy pear tree. In dual culture assays, strain ZZ1 exhibited robust antifungal activity against all tested pathogens including *Valsa pyri*. Microscopic analyses suggested that following co-culture with ZZ1, the hyphae of *V. pyri* were ragged, thin, and ruptured. ZZ1 also induced significant decreases in lesion length and disease incidence on detached pear branches inoculated with *V. pyri*. ZZ1 isolate-derived culture filtrates also exhibited antifungal activity against *V. pyri*, decreasing mycelial growth and conidium germination and inhibiting *V. pyri*-associated lesion development on pear branches. These results suggest that the ZZ1 isolate has the potential for use as a biological control agent against *V. pyri*. The strain was further identified as *Penicillium citrinum* based on its morphological characteristics and molecular analyses. Overall, these data highlight a potentially valuable new biocontrol resource for combating pear Valsa canker.

Keywords: Valsa canker; *Valsa pyri*; biological control; antifungal activity; *Penicillium citrinum*

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1. Introduction

Pear is one of the most important fruits in China, which is the leading global producer of pear. However, pear trees are susceptible to a number of microbial pathogens, with Valsa canker being among the most destructive of these diseases, posing a major threat to pear production [1,2]. Pear Valsa canker is caused by *Valsa pyri* [3,4], which can infect plants through bark injuries, leading infected tissue to turn reddish-brown and to soften and decay, potentially resulting in the death of the entire tree in some cases [2,3]. As such, Valsa canker disease severely impacts the pear industry. Currently, chemical control measures are the most common and effective approaches to controlling this disease, but environmental and food safety concerns can markedly constrain the use of these fungicides. In addition, the prolonged use of chemical fungicides has the potential to select for fungicide-resistant *V. pyri* isolates. There is thus an urgent need for the development of alternative or complementary approaches capable of controlling this economically important plant disease.

The biological control of plant diseases using endophytes or derivatives thereof has been shown to be safe and largely nontoxic, and as such, this is a major focus of active research efforts. A diverse array of microorganisms have been identified as potential biocontrol resources to date [5–7], some of which have been implemented in commercial production [8,9]. However, there have been relatively few studies evaluating the biological control of pear Valsa canker using antagonistic endophytes, limiting the available biocontrol options for this disease. Song et al. (2020) recently demonstrated that dipicolinic acid (DPA) derived from endophytic *Bacillus subtilis* exhibited antifungal activity against different canker pathogens including *V. pyri*. DPA was able to suppress chitin biosynthesis and cause *V. pyri* cell lysis [10]. Two endophytic *Bacillus velezensis* strains isolated from pear

branches were also shown to be antagonistic against pear Valsa canker [11]. However, overall, endophytes or bioactive molecules derived there from exhibiting antifungal activity against *V. pyri* remain rare.

Plant endophytic fungi are natural plant colonizers that live within plant tissues without causing any apparent damage [7]. The use of fungal endophytes for the biological control of plant diseases has been a focus of growing research in recent years [5,12], highlighting the promise of this approach. These endophytes can both directly inhibit pathogen growth and can indirectly prevent disease development by inducing host resistance mechanisms [5,13,14].

As there have been few reported endophytic fungi exhibiting antagonistic activity against *V. pyri*-induced pear Valsa canker disease to date [10,11], the present study was designed to identify novel biocontrol agents for this pear disease in order to expand the available biocontrol supply. To that end, we isolated endophytic fungi from the bark of healthy branches of pear trees from Xinjiang province, China, and demonstrated their ability to antagonize *V. pyri* and to prevent pear Valsa canker disease.

2. Materials and Methods

2.1. Pathogenic Fungal Isolates

The pathogens *Valsa pyri*, *Valsa mali*, *Botryosphaeria dothidea* and *Colletotrichum gloeosporioides*were used in this study and stored at Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou, China [15,16]. The fungal strains used in this study were cultured in potato dextrose agar (PDA) (200 g potato extracts L^{-1}, 2% glucose and 2% agar) at 25 °C.

2.2. Isolation of Endophytic Fungi from the Branches of Pear

One-year-old branches (about 1 cm in diameter) without any canker disease symptoms were collected from ten-year-old pear tree at Korlaheung city, Xingjiang province, China in June 2020. The endophytic fungi were isolated using a tissue separation method according to a previous study [17]. In brief, the bark of pear branches was peeled off and cut into small pieces (5 mm × 5 mm) and put into 75% ethyl alcohol for 1 min. The small pieces were then placed into 1% sodium hypochlorite for 5 min. After disinfection, the small pieces were washed with sterilized ddH_2O five times and put onto sterilized filter paper to absorb water. Subsequently, the small pieces were put onto PDA medium. The plates were incubated at 25 °C for 2–3 weeks, after which emerged fungal colonies were sub-cultured onto new plates to isolate individual colonies.

2.3. Dual Culture Test for Screening Potential Biocontrol Fungi

In total, 29 different endophytic fungal strains were isolated from the branches of a healthy pear tree and were screened for antagonistic activity against *V. pyri* strain lfl-XJ by dual culture test. After the preliminary screening, the antagonistic strains were further tested antifungal activity against *V. mali*, *B. dothidea* and *C. gloeosporioides*. The method of dual culture for screening potential biocontrol fungi was based on a pervious study with some modifications [18]. Four mycelial plugs (5 mm in diameter) of endophytic fungi were placed at each side 2 cm from the center on PDA medium. After inoculation for 2 days, a mycelial plug (5 mm in diameter) of the pathogenic fungal isolate was placed in the center on PDA medium. Plates were incubated at 25 °C for 6 days. Then, the colony diameter of the pathogenic fungal isolate was measured. The negative control was just inoculated with the pathogenic fungal isolate. Inhibition = (colony diameter of control − colony diameter of treated)/(colony diameter of control − 0.5 cm) × 100%.

2.4. Assessment of the Impact of Antagonistic Strains on V. pyri Hyphal Morphology

Following two days of dual culture, the morphological characteristics of hyphae were assessed using an ultra-depth three-dimensional microscope (KEYENCE), with hyphal diameters being measured. A total of three replicates were analyzed per treatment, with a minimum of 10 hyphae per replicate. The assay was repeated two times.

2.5. Assessment of Antagonistic Strain-Derived Culture Filtrates on V. pyri Mycelial Growth

Three 5 mm diameter mycelial plugs from antagonistic fungi were added to 100 mL of potato dextrose broth (PDB) (200 g potato extracts L^{-1}, 2% glucose) for 7 days (25 °C, 200 rpm). Culture filtrates were collected by centrifuging these samples for 10 min at 5000 rpm and then passing the supernatant through a filter with a 0.22 μm pore size to remove remaining hyphal fragments and spores. After sterilization, the PDA medium was then mixed with different culture filtrate volumes to yield PDA containing 10% or 20% culture filtrate. Each plate was inoculated with one 5 mm diameter plug of *V. pyri*. Following a 6-day inoculation period, *V. pyri* colony diameters were measured, with untreated PDA medium serving as a control.

2.6. Assessment of Antagonistic Strain-Derived Culture Filtrates on V. pyti Conidial Germination

V. pyri conidia were isolated as in prior studies [19] and counted with a hemocytometer, after which they were diluted to 10^6 conidia/mL. This conidial suspension was mixed with the culture filtrate derived from the antagonistic endophytic strain at a 50% concentration (*v*/*v*), with an equivalent volume of PB media being used as a control. Next, 15 μL volumes of this liquid were added to 1 cm^2 of PDA medium on glass slides in Petri dishes. Samples were incubated at 25 °C for 36 h. Numbers of conidia were then counted with an optical microscope, with three replicates and a minimum of 100 conidia being counted per replicate. The assay was repeated twice.

2.7. Disease Suppression Assay

One-year-old detached branches from a healthy pear tree (Zhongli no.1) were utilized to assess the ability of isolated strains to suppress pear Valsa canker disease development. Branches were cut into 10 cm lengths, washed with sterilized water, disinfected with 75% ethanol, and punched at the midpoint of each branch (5 mm in diameter) using a puncher that had been burned with an alcohol burner. Conidial suspensions (10^8 conidia/mL) or culture filtrates prepared from antagonistic strains were sprayed evenly onto the surface of these branches using a small spray bottle (1 mL/branch), after which the branches were inoculated with *V. pyri* mycelial plugs immediately after culture filtrate treatment or two days following conidial suspension treatment. *V. pyri* mycelial plugs were obtained from the edge of PDA medium after 4 days' inoculation. The branches were sprayed with sterile water or tebuconazole (86 μg/mL) (Anhui Huilong Group Youngsun Pesticides Co., Ltd, China) as negative and positive controls, respectively. Mycelial plugs were covered with medical absorbent cotton (about 1 cm × 2 cm) (Wellday) wetted with sterile water to maintain appropriate moisture levels. The inoculated branches were kept horizontal in a transparent plastic box. Samples were then incubated for 7 days at 25 °C, after which vernier calipers were used to measure lesion length. Assays were repeated three times, with 10 inoculation sites per experiment. Disease incidence was calculated as follows: (number of symptomatic inoculation sites)/total number of inoculation sites × 100%.

2.8. Antagonistic Strain Identification

The endophytic ZZ1 fungal strain, which exhibited the most robust antagonistic activity against *V. pyri*, was identified via morphological and molecular identification.

For morphological identification, isolate ZZ1 was inoculated onto PDA medium and cultured for 7 days at 25 °C, after which colony morphology including colony color and colony shape were visually observed. In addition, conidia were eluted from the colony using sterile water and the conidial morphology was observed with a light microscope

(NIKON). For molecular identification, DNA from the ZZ1 isolate was extracted with a DNA extraction kit (Omega), and the ITS-rDNA,β-tubulin, nuclear small subnit rDNA (ssu) and elongation factor 1-a (EF-1a) sequences were amplified with appropriate primers. ITS1: 5′-CCGTAGGTGAACCTGCGG-3′, ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ [20]; Bt2a: 5′-GGTAACCAAATCGGTGCTGCTTTC-3′, Bt2b: 5′-ACCCTCAGTGTAGTGACCCTTGGC-3′ [21]; NS1: 5′-GTAGTCATATGCTTGTCTC-3′, NS6: 5′-GCATCACAGACCTGTTATTGCCTC-3′ [20]; EF6: 5′-CTTSTYCCARCCCTTGTACCA-3′, EF-1b: 5′-CACATCAACATCGTCGTTAT-3′ [22]. All PCR reactions were conducted in a reaction containing 1.5 µL of 10 × Taq buffer, 1 µL of 2.5 mM dNTPs, 1 µL of 100 mM Mg^{2+}, 0.25 µL of 5 U/µL Taq DNA polymerase, 0.25 µL of each primer (10 µM), 1 µL of 10 ng/µL fungal DNA, and ddH_2O to a final 15 µL volume. Thermo cycler settings were: 3 min at 94 °C; 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; 72 °C for 5 min. PCR products were sequenced by Bgi Genomics Co., Ltd., China, and the resultant sequences were blasted against the NCBI nucleotide collection database. Highly homologous sequences were selected for multiple sequence alignment with the MEGA 7.0 software. A tree was constructed via a Neighbor-Joining approach, with 1000 replicate Bootstrap analyses being used to calculate node support.

3. Results

3.1. Isolation and Assessment of Antifungal Activity of ZZ1 against Phytopathogenic Fungi

In total, 29 different endophytic fungal strains were isolated from the bark of branches of a healthy pear tree, and were screened for antagonistic activity against *V. pyri* train lfl-XJ in vitro. The inhibition of endophytic fungal strains against *V. pyri* is shown in Table 1. Of these, strain ZZ1 exhibited the most pronounced antagonistic activity, inhibiting ~95% of *V. pyri* mycelial growth in a dual culture assay (Figure 1, Table 1). Most of the endophytes did not show clear inhibition zone (Supplementary Figure S1), which indicated that these endophytes did not have antibiotic activity to *V. pyri*.

The ability of strain ZZ1 to inhibit the growth of other fungal fruit tree pathogens was additionally assessed. In a dual culture assay system, isolate ZZ1 was able to strongly inhibit all tested pathogens including *Valsa mali*, *Botryosphaeria dothidea,* and *Colletotrichum gloeosporioides*, with respective inhibition of 96%, 94% and 92% (Figure 1). Together, these data indicated that isolate ZZ1 possessed broad-spectrum antifungal activity.

Table 1. The inhibition of endophytic fungal strains against *V. pyri*.

Endophytic Strains	Inhibition (%)
ZZ1	95.2 ± 2.1
ZZ2	7.4 ± 0.8
ZZ3	3.9 ± 0.7
ZZ4	5.7 ± 1.1
ZZ5	2.7 ± 0.8
ZZ6	3.5 ± 1.2
ZZ7	12.3 ± 1.4
ZZ8	32.5 ± 3.1
ZZ9	13.5 ± 2.3
ZZ10	5.3 ± 1.6
ZZ11	3.5 ± 0.5
ZZ12	55.0 ± 2.6
ZZ13	22.3 ± 3.1
ZZ14	15.4 ± 2.4
ZZ15	5.9 ± 1.5
ZZ16	14.6 ± 2.4

Table 1. *Cont.*

Endophytic Strains	Inhibition (%)
ZZ17	58.7 ± 3.2
ZZ18	8.6 ± 0.9
ZZ19	5.4 ± 0.4
ZZ20	23.5 ± 3.5
ZZ21	16.5 ± 2.6
ZZ22	7.4 ± 1.8
ZZ23	8.9 ± 1.1
ZZ24	3.3 ± 0.8
ZZ25	5.4 ± 1.3
ZZ26	8.4 ± 2.1
ZZ27	4.9 ± 0.5
ZZ28	9.4 ± 1.7
ZZ29	10.5 ± 2.1

Note: Values are means ± SD from three replicates.

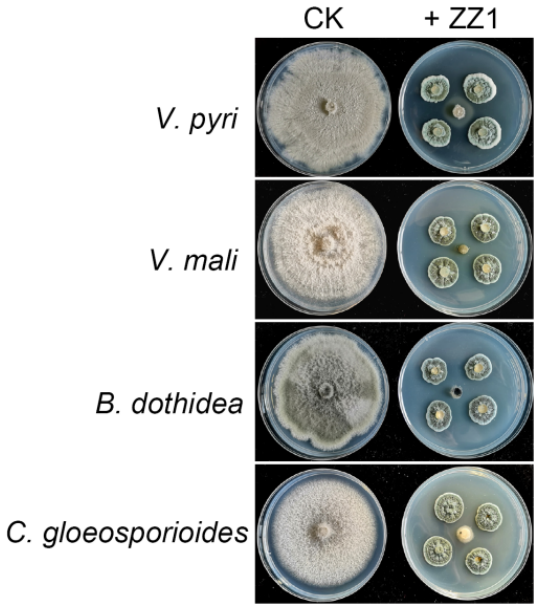

Figure 1. Antifungal activity of strain ZZ1 against different phytopathogenic fungi. After inoculation with ZZ1 for 2 days, a mycelial plug (5 mm in diameter) of the pathogenic fungal isolate was placed in the center on PDA medium. The pictures were taken at 6 days after inoculation.

3.2. Assessment of the Antagonistic Effects of Isolate ZZ1 on V. pyri Hyphal Morphology

To further examine the antifungal activity of endophytic strain ZZ1, we utilized *V. pyri* as a model pathogen in follow-up experiments. We examined the antagonistic effects of isolate ZZ1 on *V. pyri* hyphae. Under control conditions, *V. pyri* hyphae appeared smooth and transparent with branches, whereas following ZZ1 isolate exposure these hyphae were rough, tenuous, and ruptured (Figure 2a). Average *V. pyri* hyphal diameter following ZZ1 exposure (2.3 μm) was significantly smaller than for the control treatment (Figure 2b).

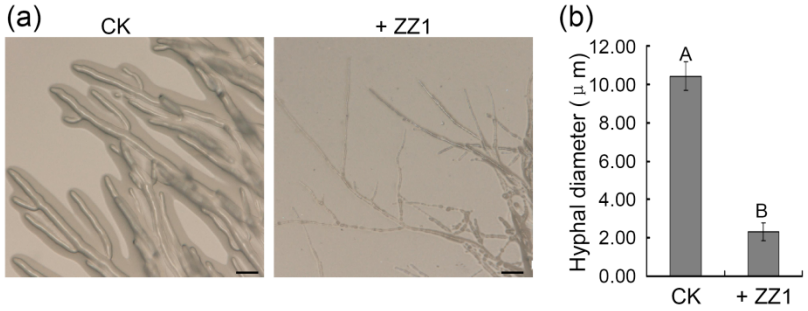

Figure 2. Effect of ZZ1 isolate on hyphal morphology of *V. pyri*. (**a**) Hyphae morphology of *V. pyri* after antagonism with ZZ1. The picture was taken at 2 days after antagonism with ZZ1 with an ultra-depth three-dimensional microscope. Bar = 10 μm. (**b**) Hyphal diameter of *V. pyri* after antagonism with ZZ1. Values are means ± SD from ten replicates. Letters above the bars indicate statistical significance ($p < 0.01$) based on Tukey's test.

3.3. Analysis of the Ability of ZZ1 Conidial Suspensions to Control Pear Valsa Canker Disease

Conidial suspensions prepared from the ZZ1 isolate were next used to pre-treat detached branches from healthy pear trees to assess the resultant suppression of *V. pyri*-induced Valsa canker disease development. As of day 7 post-*V. pyri* inoculation, ZZ1 conidial suspensions had significantly suppressed disease development (Figure 3a), reducing disease incidence to 32% as compared to the 95% incidence observed under control conditions (Figure 3b). Branches treated with tebuconazole as a positive control remained disease-free (Figure 3a,b). The average lesion length on detached branches was also significantly reduced following treatment with a ZZ1 conidial suspension as compared to the control, with these reductions being comparable to those observed following tebuconazole treatment (Figure 3c). These data thus indicated that strain ZZ1 was able to suppress the incidence of pear Valsa canker disease caused by *V. pyri*.

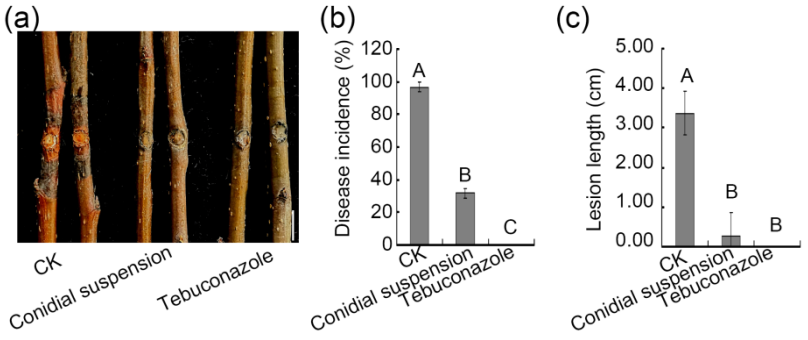

Figure 3. Effect of conidial suspension of ZZ1 on pear Valsa canker disease caused by *V. pyri*. (**a**) Conidial suspension of ZZ1 suppressed pear Valsa canker. Bar, 1 cm. The picture was taken at 7 days after inoculation. (**b**) Statistical analysis of the disease incidence. (**c**) Statistical analysis of disease lesion length. Values are means ± SD from ten replicates. Letters above the bars indicate statistical significance ($p < 0.01$) based on Tukey's test.

3.4. Assessment of the Ability of ZZ1 Culture Filtrates to Suppress V. pyri Mycelial Growth and Spore Germination

We next collected culture filtrates from isolate ZZ1 and applied these filtrates to PDA medium prior to *V. pyri* mycelial plug inoculation to determine whether these culture filtrates retained the antifungal activity of the strain from which they were derived. The growth of *V. pyri* mycelia was significantly suppressed by 47.9% and 71.3% on PDA medium containing 10% and 20% ZZ1 culture filtrate, respectively, relative to control medium (Figure 4a–c). These ZZ1 culture filtrates similarly suppressed *V. pyri* conidial germination (Figure 5a), with conidial germination in the control and ZZ1 culture filtrate-

treated groups of 85% and 4%, respectively (Figure 5b). These results indicated that ZZ1 culture filtrates possess antifungal activity against *V. pyri*.

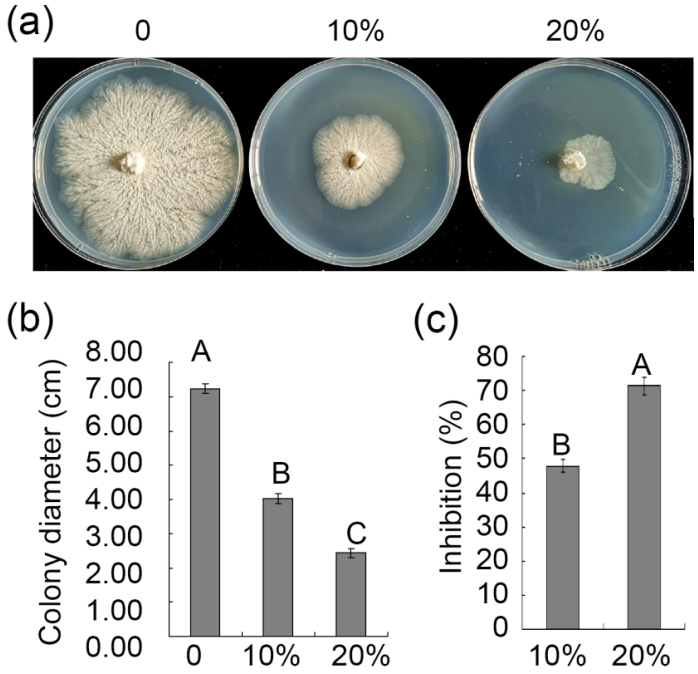

Figure 4. Effect of culture filtrate of ZZ1 on mycelial growth of *V. pyri*. (**a**) Inhibition of culture filtrate of ZZ1 on mycelial growth of *V. pyri*. The picture was taken at 6 days after inoculation on PDA medium. (**b**) Statistical analysis of colony diameter. (**c**) Inhibition of culture filtrate of ZZ1 on mycelial growth of *V. pyri*. Values are means ± SD from three replicates. Letters above the bars indicate statistical significance ($p < 0.01$) based on Tukey's test.

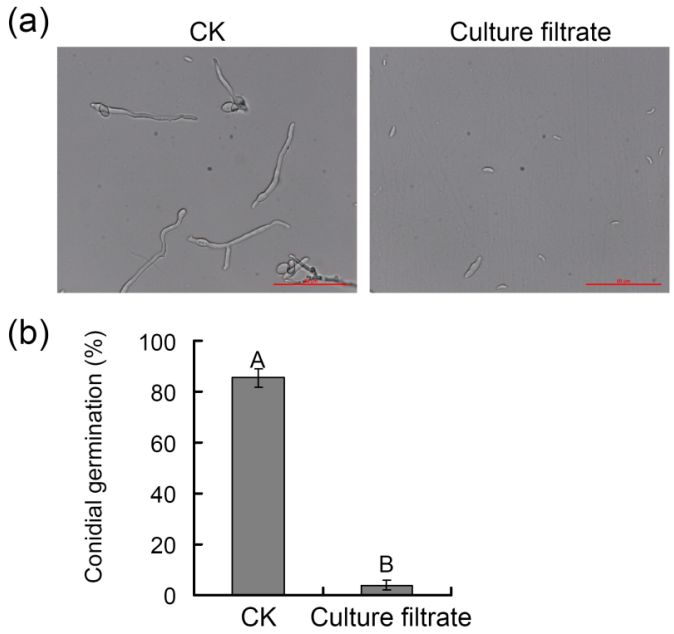

Figure 5. Effect of culture filtrate of ZZ1 on conidial germination of *V. pyri*. (**a**) Optical photomicrograph of conidial germination of *V. pyri* after treatment with culture filtrate of ZZ1 isolate. Bar, 50 μm. The picture was taken at 36 h after inoculation (**b**) Inhibition of culture filtrate of ZZ1 on conidial germination of *V. pyri*. Conidial germination was counted at 36 h after inoculation. Values are means ± SD from three replicates. Letters above the bars indicate statistical significance ($p < 0.01$) based on Tukey's test.

3.5. Assessment of the Ability of ZZ1 Culture Filtrates to Control Pear Valsa Canker Disease

To determine whether ZZ1 culture filtrates were able to inhibit pear Valsa canker disease, detached branches from healthy pear trees were sprayed with these culture filtrates, followed by inoculation with a *V. pyri* mycelial plug. The results indicated that ZZ1 culture filtrates strongly impacted disease development (Figure 6a). Specifically, while culture filtrates had no effect on disease incidence, the average lesion length decreased significantly relative to that observed following control treatment (Figure 6b,c). This thus suggested that ZZ1culture filtrates were able to inhibit *V. pyri*-induced lesion development on pear branches.

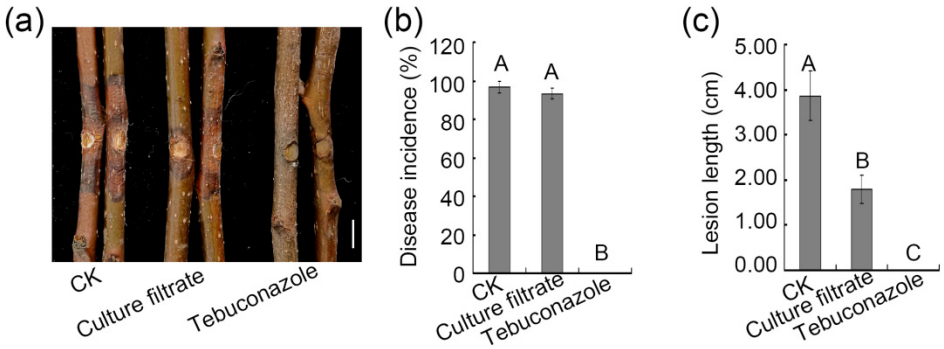

Figure 6. Effect of culture filtrate of ZZ1 on pear Valsa canker disease caused by *V. pyri*. (**a**) Culture filtrate of ZZ1 could antagonize pear Valsa canker. Bar, 1 cm. The picture was taken at 7 days after inoculation. (**b**) Statistical analysis of the disease incidence. (**c**) Statistical analysis of disease lesion length. Values are means ± SD from ten replicates. Letters above the bars indicate statistical significance ($p < 0.01$) based on Tukey's test.

3.6. Identification of Strain ZZ1

When grown on PDA, ZZ1 exhibited gray-olive colored colonies containing distinctive folds. These colonies had white margins, and appeared grayish-yellow from the underside (Figure 7a). Colonies exhibited numerous ovoid conidia (Figure 7b). These morphological characteristics were consistent with those of *Penicillium* sp.

Figure 7. Morphological characteristics of the antagonistic strain ZZ1. (**a**) Colony characteristics of ZZ1 isolate. The picture was taken at 7 days after inoculation on PDA at 25 °C. (**b**) Conidia of ZZ1 isolate. Bar, 20 μm.

ITS sequences from isolate ZZ1 were next amplified and used to construct a phylogenetic tree with other closely related sequences using the MEGA 7.0 software. In this analysis, isolate ZZ1 was included in the same branch as *P. citrinum* strains (Figure 8a). When we similarly constructed a tree based upon the β-tubulin sequence, ssu or EF-1a,isolate ZZ1 similarly exhibited the greatest homology to *P. citrinum* strains (Figure 8b–d).

Based upon these morphological and molecular analysis results, strain ZZ1 was thus identified as *P. citrinum*. strain ZZ1 has been preserved at the China General Microbiological Culture Collection Center (CGMCC), a typical culture preservation center in China, under preservation number CGMCC No. 22436; the deposit date is 26 May 2021.

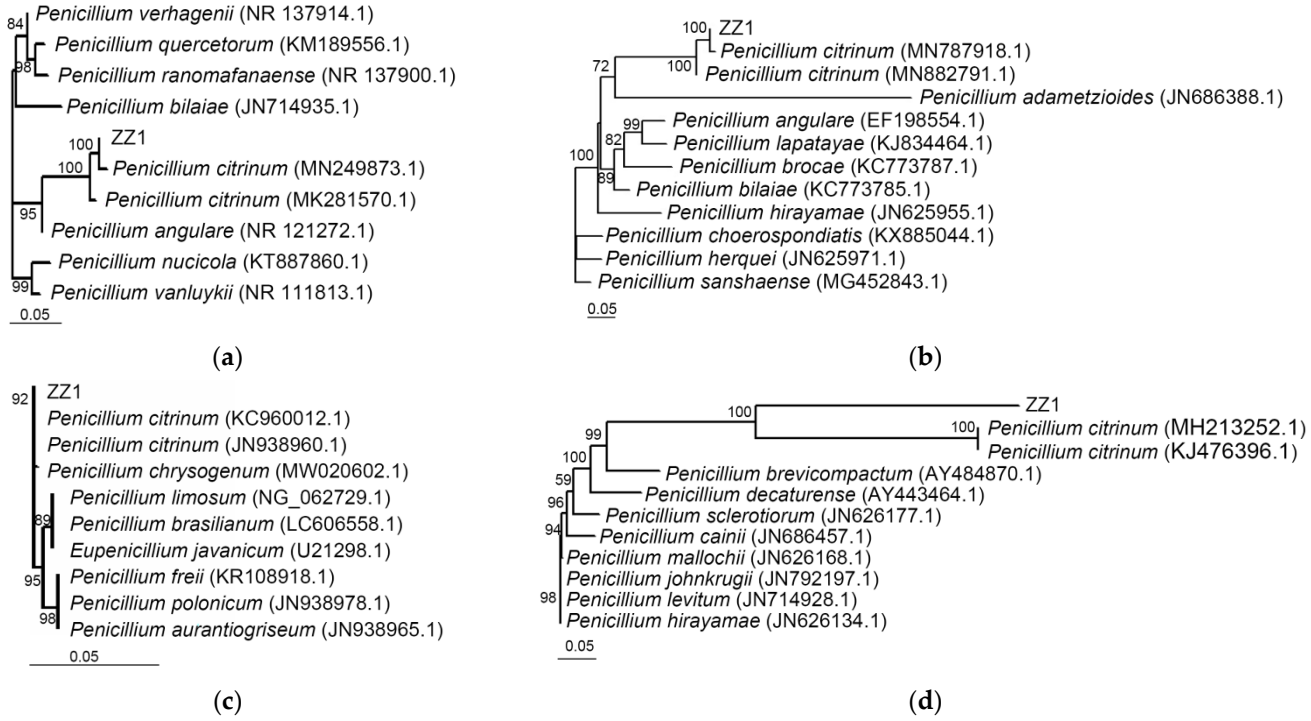

Figure 8. Phylogenetic analysis of strain ZZ1 and its relatives based on the nucleotide sequences of ITS (**a**) the genes encoding β-tubulin (**b**), ssu (**c**) and EF-1a (**d**).

4. Discussion

Valsa canker caused by *V. pyri* is one of the greatest threats to global pear production. Endophyte-based biocontrol of plant diseases has emerged as a viable and sustainable alternative to chemical fungicides [23]. However, there have been relatively few endophytes reported to date that exhibit antagonistic activity against pear Valsa canker, thus necessitating the use of agrochemicals to control the spread of this disease. Nowadays, many chemical fungicides have been registered for control of Valsa canker, such as tebuconazole, carbendazim and thiophanate-methyl, while only one biological control agent (*Bacillus* methylotrophicus) is registered for this disease in China. Herein, we isolated 29 endophytic fungal strains from the branches of healthy pear trees and determined that strain ZZ1 possessed significant antagonistic activity against a range of pathogenic fungi. Notably, strain ZZ1 was able to inhibit the development of *V. pyri*-induced pear Valsa canker disease, suggesting that this strain represents a promising resource for the biocontrol of this destructive disease.

Through a series of morphological and molecular analyses, the isolate ZZ1 was ultimately identified as a strain of *Penicillium citrinum*. *Penicillium* species are common fungi that are often studied in the context of biocontrol, with many such isolates having been shown to exhibit antifungal activities against a range of plant pathogens [24,25]. For example, *P. striatisporum* isolate Pst10 exhibited robust antagonistic activity against *Phytophthora* spp. and was able to significantly suppress chili pepper *Phytophthora* root rot disease incidence [26]. Similarly, a *P. citrinum* isolate derived from Egyptian henbane was able to significantly inhibit a range of fungi, secreting specific extracellular antifungal compounds [27]. *P. citrinum* isolate BTF08 from wild bananas was also shown to elicit host defenses against *Fusarium oxysporum* f. sp. *cubense* in banana plantlets [28]. However, there have been no prior reports regarding the biocontrol of Valsa canker using *Penicillium* species, and as such, this study is the first to describe the use of a pear tree-derived *P. citrinum* isolate as a resource capable of suppressing *V. pyri*-induced pear Valsa canker. Although there are a few studies showing that some *P. citrinum* strains were pathogenic [29–31], our result revealed that *P. citrinum* strain ZZ1 did not cause disease symptom on pear

branch (Supplementary Figure S2). This indicated that strain ZZ1 is an endophyte, not a pathogen on pear. We further found that strain ZZ1 exhibited broad-spectrum antifungal activity against other fruit tree pathogens such as *V.mali*, *B. dothidea* and *C. gloeosporioides*, suggesting that it represents a valuable resource with the potential to be leveraged for the biocontrol of a range of fruit tree diseases.

As biocontrol agents, endophytes can produce an array of bioactive secondary metabolites that can enhance the growth of plants under conditions of biotic stress [6,32,33]. Recently, there have been several research efforts aimed at isolating and identifying antimicrobial compounds derived from *Penicillium* species. Three are certain compounds with antagonistic activity against plant pathogen species, including citrininand emodin derived from the fermentation of *P. citrinum* [32], and ent-homocyclopiamine B derived from *P. concentricum* [34]. Our data indicated that *V. pyri* mycelial growth and conidium germination were significantly reduced following treatment with ZZ1 culture filtrates. Additionally, we found that ZZ1 culture filtrates were able to significantly inhibit the development of *V. pyri* lesion on pear branches. As such, these data suggested that ZZ1-derived compounds with antimicrobial activity against *V. pyri* were present within these culture filtrates. Therefore, isolating and identifying the bioactive compounds in these culture filtrates will be an important focus of our future research.

Herein, detached pear branches were used to evaluate the biocontrol potential of the ZZ1 isolate as an inhibitor of *V. pyri*-induced pear Valsa canker disease. This analysis revealed that both conidium suspensions and culture filtrates from the ZZ1 isolate were able to suppress such disease, with the biocontrol efficiency of conidial suspensions being comparable to that of the fungicide tebuconazole. As such, these results offer new evidence that the ZZ1 isolate may be used as an effective new antifungal agent for controlling pear Valsa canker disease.

5. Conclusions

In summary, we successfully isolated and identified a *P. citrinum* isolate ZZ1 from the bark of a pear tree as a potential resource for the biocontrol of pear Valsa canker caused by *V. pyri*. ZZ1-derived culture filtrates retained antifungal activity against *V. pyri*, inhibiting associated mycelial growth and conidium germination. Both ZZ1 conidium suspensions and culture filtrates were additionally able to suppress pear Valsa canker caused by *V. pyri*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7070198/s1, Figure S1: The endophyte strain ZZ5 did not show antagonistic activity to *V. pyri*, Figure S2: Strain ZZ1 did not cause disease symptoms on pear branch.

Author Contributions: H.Y., B.S. and T.H. carried out experiments; Z.Z. gave instructions in experiments; H.Y. designed experiments and wrote the manuscript; L.W., H.H. and H.T. supervised the project. All authors have read and agreed to the published version of the manuscript.

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