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Biological control of poplar anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

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Abstract

Poplar anthracnose is one of the most serious diseases caused by the fungal pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Biocontrol is an efficient green way for the disease control, and numerous researches have focused on exploring the potential biocontrol bacteria strains against *C. gloeosporioides*. In this study, antifungal activities against *C. gloeosporioides* of 108 rhizosphere soil isolates from healthy polar plants were investigated in vitro by the dual culture assay. The results suggested that strain ZSH-1 showed the highest level of antifungal activity, as it inhibited *C. gloeosporioides* at a distance of 10.00 mm. Based on the morphological, physiological-biochemical characteristics, and phylogeny analysis, strain ZSH-1 was identified as *Bacillus subtilis*. The sterile culture filtrate, crude protein, and crude lipopeptide extracts from the culture filtrate, and volatile compound(s) of ZSH-1 displayed a strong antagonism towards 7 fungal phytopathogens (*C. gloeosporioides*, *Fusarium oxysporum*, *Alternaria tenuissima*, *Cytospora chrysosperma*, *Botryosphaeria dothidea*, *Mucor* sp., and *Absidia* sp.), with inhibition rates ranging from 44.0 to 89.1%, 26.7 to 85.4%, 11.6 to 89.7%, and 7.8 to 63.2%, respectively. Moreover, ZSH-1 exhibited cell wall-degrading traits by producing 3 lytic enzymes (cellulose, β-1,3-glucanase, and protease). Finally, the greenhouse studies also revealed that strain ZSH-1 had a 47.6% (12 days) efficacy in controlling poplar anthracnose when compared with the control. In concluding, obtained results demonstrate the potential biocontrol effect of *B. subtilis* ZSH-1, and it can be used as a promising biocontrol agent against poplar anthracnose and other fungal phytopathogens.

Keywords: *Colletotrichum gloeosporioides*, Poplar anthracnose, Fungal phytopathogens, Antifungal activity, *Bacillus subtilis*

Background

Poplar is one of the most important trees in China where it has a major role in farmland shelterbelts, soil conservation, and environmental protection. Poplar anthracnose, as a serious branch and leaf disease of poplar, is mainly caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. The disease generally occurs in various poplar cultivated areas in the north and south of the country (Li et al. 2012). Currently, applications of chemical fungicides are still the effective means to prevent the infection and dispersal of poplar anthracnose (Song et al. 2016). However, the excessive and long-term use of chemical fungicides has led to the evolution of drug resistance of pathogenic fungi. Even more, chemical fungicides result in serious environmental pollution because of the difficult degradation and non-target toxicity (Furuya et al. 2011). Therefore, more and more researches have focused on developing and applying alternative methods that are less reliant on fungicides and more environmentally friendly. Biological control is high efficiency and safer for the environment to be used in disease management (Shafi et al. 2017).

Many microorganisms have been used for the control of fungal phytopathogens. Among them, the *Bacillus*

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genus is recognized as a promising group with a wide distribution in soils. Due to their strong antagonistic activity, broad inhibitory spectrum, and high viability, members of the *Bacillus* species have been effectively used in controlling soil-borne, air-borne, and post-harvest diseases (Pérez-García et al. 2011; Shafi et al. 2017). Antagonistic strains control fungal phytopathogens through several mechanisms, such as competition, mycoparasitism, the production of antibiotics, and the induction of defense responses (Guo et al. 2019; Jinal and Amaresan 2020). Production of a variety of metabolites is an important mechanism by which the *Bacillus* genus exerts biocontrol. These inhibitory metabolites are mainly including lipopeptides, proteins, and volatile compound(s) (Baysal et al. 2013; Shafi et al. 2017). In addition, lysates of antagonistic bacteria have also been reported to possess antifungal activity (Yu et al. 2013).

The objectives of this study were to explore the potential biocontrol antagonistic bacteria from rhizosphere soil against the poplar anthracnose pathogen *C. gloeosporioides* and to identify the strain, characterize the main ingredients for antagonistic, and clarify the cell wall-degrading traits.

Materials and methods

Fungal phytopathogens

Seven fungal phytopathogens (*Colletotrichum gloeosporioides* CFCC80308, *Fusarium oxysporum* CFCC82468, *Alternaria tenuissima* CFCC84533, *Cytospora chrysosperma* CFCC 89600, *Botryosphaeria dothidea* CFCC82975, *Mucor* sp. CFCC80870, and *Absidia* sp. CFCC80375) were provided by China Forestry Culture Collection Center (CFCC). These fungi were maintained on potato dextrose agar (PDA) slants at 4 °C and grown on PDA plates at 28 °C when used.

Isolation of rhizobacteria

Rhizobacteria were isolated from the rhizosphere soil of healthy poplar plants in Haidian and Changping districts of Beijing City, China. Five grams of soil sample was transferred to 45 ml of sterilized water and shaken at 200 rpm for 20 min prior to use (Kurabachew and Wydra 2013). Gradient dilutions of the soil suspension were spread onto Luria-Bertani (LB) agar plates, and the plates were incubated at 30 °C in darkness. A single bacterial colony that is well separated from the other colonies were selected and re-streaked onto a new LB agar plate with an inoculating loop. Then, the plate was incubated at 30 °C for 24 h, and then stored at − 80 °C in LB with 30% glycerol (v/v).

In vitro screening of rhizobacteria against *C. gloeosporioides*

The rhizobacteria isolates were screened for antifungal activity against *C. gloeosporioides* using the dual culture assay described by Huang et al. (2014). The inhibition distance was defined as the width of the inhibition zone between the bacterial and fungal strains. The strain showed the highest antifungal activity was selected for further study.

Morphology, culture conditions, and physiological-biochemical characteristics of selected strain

Morphology, culture conditions, and physiological-biochemical characteristics were performed according to previously published methods of Dong et al. (2001) and Furuya et al. (2011).

Phylogenetic analysis of selected strain

The 16S rRNA gene was amplified and sequenced, using a single intact colony of selected strain according to a previously described method (Huang et al. 2014). The primers 63f (5′-CAGGCCTAACACATGCAAGTC-3′) and 1387r (5′-GGGCGGWGTGTACAAGGC-3′) were used for amplification. Polymerase chain reaction (PCR) conditions were as follows: 94 °C for 4 min, 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The PCR products were sequenced by Invitrogen Corporation (Beijing, China). The sequences were blasted in the GenBank database, using a blastn search and aligned using Clustal W (ver. 1.82). Phylogenetic analysis was performed using MEGA (ver. 5.0) and a neighbor-joining phylogenetic tree was constructed by bootstrap analysis with 1000 replicates.

Evaluation of the effects of sterile culture filtrate

The strain selected from the in vitro test was grown in 100 ml modified medium no. 3 (g L^{-1}; glucose 10.0, peptone 5.0, soybean meal 5.0, $KH_2PO_4$1.0, $MgSO_4{\cdot}7H_2O$ 0.5, NH_4Cl 3.0, $Na_2HPO_4$1.0, and yeast extract 0.5; pH 7.0–7.2) in a 500-ml Erlenmeyer flask at 28 °C with 200 rpm. Culture broth was incubated for 3 days and centrifuged with 10,000 rpm at 4 °C for 20 min. Sterile culture filtrate (SCF) was obtained by passing the supernatant through a sterile membrane filter (0.22 μm; Pall, Ann Arbor, USA). The antifungal effects of SCF against 7 fungal phytopathogens were assayed by measuring the mycelial growth inhibition rates (Song et al. 2012). Briefly, 2 ml of SCF was mixed with 20 ml of PDA at 40–45 °C in a culture plate; no SCF was added for the control. After the medium was solidified, freshly growing fungal mycelial plugs (6 mm) were collected and inoculated onto the center of the plate using a sterile steel borer. The plates were incubated at 28 °C. The diameters of the fungal colonies were measured when those of the control colonies covered more than three quarters of the diameter of the dish according to the crisscross method. All experiments were repeated 3 times. The percentage

of growth inhibition was calculated using the following formula (Yang et al. 2011):

$$P(\%) = 100 \times [(C - - d) - - (T - - d)]/(C - - d)$$

where P is the inhibitory rate, C is the diameter of a control colony, T is the diameter of a treated colony, and d is the diameter of the mycelial plug.

Evaluation of the effects of crude protein and crude lipopeptide extracts

Crude protein and crude lipopeptide extracts were obtained respectively from SCF according to a previously described method (Huang et al. 2014). Then, the extracts were sterilized by passing through a sterile membrane filter (0.22 μm; Pall). The antifungal effects of crude protein and crude lipopeptide extracts against 7 fungal phytopathogens were assayed by measuring the mycelial growth inhibition rates using the procedure described above.

Evaluation of the effects of volatile compound(s)

The antifungal effects of the volatile compound(s) (VOCs) against the 7 fungal phytopathogens were tested by co-cultivating the fungi and bacteria on two-compartment plates (Yuan et al. 2012). One half of each plate was filled with PDA, while the other half contained TYB medium. A 6-mm diameter fungal plug was placed on the PDA side, and the candidate antagonistic strain was inoculated on the other side 24 h later. Plates not inoculated with the test strain served as the controls. Then, the plates were sealed with parafilm and incubated at 28 °C. The diameters of fungal colonies and percentages of growth inhibition were measured as described above. All experiments were repeated three times.

Characterization of cell wall-degrading traits

The cellulase activity and β-1, 3-glucanase activity of the test strain was determined, using a method described previously (Essghaier et al. 2009). The activities of β-1,3-glucanase, chitinase, protease, and lipase were determined using aniline blue pachyman agar plate, 0.2% colloidal chitin plate, skim milk agar plate, and 1% Tween 80 agar plate, respectively (Dong et al. 2001; Gao et al. 2009; Ren et al. 2013). Briefly, a single colony of test strain collected from an overnight culture was seeded onto the center of the plates; then, the plates were monitored for the appearance of a clear zone around the developing bacterial colonies at 28 °C.

Evaluation of biocontrol activity of the candidate strain under greenhouse conditions

To evaluate the biocontrol activity of the candidate strain towards poplar anthracnose caused by *C. gloeosporioides*, a pot assay was performed using 1-year-old poplar seedlings (*Populus* ×*euramericana* cv. '74/76') with 7–10 expanded leaves (Huang et al. 2014). Each treatment consisted of 15 plants with 3 replicates (5 plants per replicate). Three treatments were set up as follows: SZ, the candidate strain and *C. gloeosporioides* sprayed on leaves at the same time; CK1, only sterile distilled water sprayed on leaves; and CK2, leaves only sprayed with *C. gloeosporioides*. For the phytopathogen inoculation, a spore suspension (1.0×10^5 spores ml^{-1}) of *C. gloeosporioides* was sprayed on both sides of the leaves (2 ml per leaf) using an artist's airbrush (YD12-F111, Yudi, Zhejiang, China). Sterile distilled water and the suspension (1.0×10^9 cfu ml^{-1}) of the candidate strain were sprayed on the leaves using the same method as for *C. gloeosporioides*. The diseased leaves rate (DLR), disease severity (DS), disease index (DI), and greenhouse control efficacy (GCE) were investigated 12 days after *C. gloeosporioides* inoculation, respectively. The DS was expressed as a percentage of lesion area over the total surface area per leaf and divided into 6 ratings: 0, no lesion; 1, lesion area ≤ 5%; 2, lesion area 5–25%; 3, lesion area 25–45%; 4, lesion area 45–65%; and 5, lesion area > 65%. The DLR, DI, and GCE were calculated using the following formulas:

$$\mathrm{DLR}\ (\%) = 100 \times (\text{No.of affected leaves}/\text{No.of total leaves})$$

$$\mathrm{DI} = \left[100 \times \sum(\text{No.of affected leaves} \times \text{corresponding DS})\right]/(\text{No.of total leaves} \times 5)$$

$$\mathrm{GCE}\ (\%) = 100 \times [(\text{DI of CK2} - - \text{DI of CK1}) - - (\text{DI of SZ treatment} - - \text{DI of CK1})]/(\text{DI of CK2} - - \text{DI of CK1})$$

Statistical analysis

The mean values and standard deviations were calculated, and statistically compared using analysis of variance (ANOVA) and Duncan's multiple range tests ($p \leq 0.05$) using SPSS software version 20.0 (SPSS Inc., Chicago, Illinois).

Results and discussion

Isolation and screening of antifungal bacteria in vitro

A total of 108 bacterial isolates were obtained from 5 rhizosphere soil samples, and 15 of them showed antifungal activity in vitro. Of the 15 bacterial strains, ZSH-1 showed the highest level of antifungal activity, as it inhibited *C. gloeosporioides* at a distance of 10.00 mm (Table 1). Strain ZSH-1 was deposited in China General Microbiological Culture Collection Center (no. 9025). Microorganisms that grow in plant rhizosphere are widely used as biocontrol agents. Islam et al. (2018) reported that *Pseudomonas aeruginosa* BA5 isolated from rhizosphere of plants suppressed the growth of *F. oxysporum* (the causal agent of Fusarium wilt). Karthika et al. (2020) reported that *B. cereus* KTMA4 from tomato rhizosphere showed the highest inhibition against major phytopathogens of tomato.

Table 1 Antifungal activities of rhizobacteria isolates against *C. gloeosporioides* in vitro

Isolate	Location	Inhibition distance (mm)
ZSH-1	Haidian district of Beijing City, China	10.00 ± 0.00a
ZSH-3		8.1 ± 0.20b
ZSH-9		4.83 ± 0.42 fg
ZSH-14		3.93 ± 0.65 h
HKB-5		5.97 ± 0.95e
HKB-12		2.17 ± 0.29i
HKB-17		7.63 ± 0.15b
HHY-7	Changping district of Beijing City, China	7.43 ± 0.57bc
HHY-9		5.37 ± 0.06efg
HHY-13		4.70 ± 0.20 g
HSL-9		3.60 ± 0.10 h
HSL-12		7.93 ± 0.21b
CYS-1		6.73 ± 0.21d
CYS-7		5.50 ± 0.10ef
CYS-9		6.80 ± 0.44 cd

Values with the different letter within the same column are significantly different at $p \leq 0.05$ according to Duncan's test. Numbers followed by the "±" are standard errors (SE)

Morphology, culture conditions, and physiological-biochemical characteristics of strain ZSH-1

The colonies of strain ZSH-1 grown on LB agar medium were off-white to gray-yellow, oblong, smooth, moist, and slightly convex and had an entire margin. The cells were rod-shaped, 0.5–0.8 μm wide, and 1.2–2.5 μm long. Strain ZSH-1 can grow at temperatures ranging from 10 to 50 °C, in pH values ranging from 5 to 10, and in NaCl concentrations ranging from 0.2 to 10% (w/v) (Table 2). The morphological and physiological-biochemical characteristics of strain ZSH-1 were found to be in complete accordance with *B. subtilis*, using interpreting software cluster analysis (Todorova and Kozhuharova 2010).

Phylogenetic analysis of strain ZSH-1

Based on the morphological and physiological-biochemical characteristics, strain ZSH-1 was identified as *B. subtilis*. To precisely determine the species of strain ZSH-1, 1281 bp (GenBank accession KM114000.1) of the 16S rDNA sequence was aligned with those of the most closely related *B. subtilis* by the Clustalx program. The 16S rDNA sequence of *Escherichia coli* U5/41 (GenBank accession NR_024570.1) was used as an out group. Bootstrap analysis showed strain ZSH-1 was grouped with *B. subtilis* (GenBank accession HE582781.1) with a sequence similarity of 89% (Fig. 1). The phylogenetic analysis of 16S rDNA sequence revealed that strain ZSH-1 belongs to *B. subtilis*.

Table 2 Culture conditions and physiology-biochemical characteristics of strain ZSH-1

Indicator	Result
Gram staining	+
Mobility	+
Growth at 4 °C	–
Growth at 10 °C	+
Growth at 50 °C	+
Growth at 55 °C	–
Growth at pH 4	–
Growth at pH 5	+
Growth at pH 5.7	+
Growth at pH 10	+
Growth at pH 11	–
Growth with 0.2% (w/v) NaCl	+
Growth with 5% (w/v) NaCl	+
Growth with 10% (w/v) NaCl	+
Growth with 15% (w/v) NaCl	–
Liquefication of gelatin	+
Phenylalanine deaminase test	–
Hydrolysis of casein	+
Hydrolysis of starch	+
Methyl red reaction	–
Catalase test	+
Lecithinase test	+
Oxidase test	+
Reduction of nitrates	+
Production of indole	–
Production of hydrogen sulfide	+
Voges-Proskauer reaction	+
pH Voges-Proskauer broth	6.89–7.13
Decomposition of tyrosine	–
Utilization of glucose	+
Utilization of D-xylose	+
Utilization of L-arabinose	+
Utilization of mannitol	+
Utilization of D-sorbitol	+
Utilization of sodium malonate	+
Utilization of sodium citrate	–
Production of gas from glucose	–
Anaerobic growth in glucose agar	–
Production of acid through the decomposition of milk	+

+, positive; –, negative

Antifungal activities of sterile culture filtrate

The SCF of ZSH-1 exhibited strong antifungal activities against 7 fungal phytopathogens (*C. gloeosporioides*, *F.*

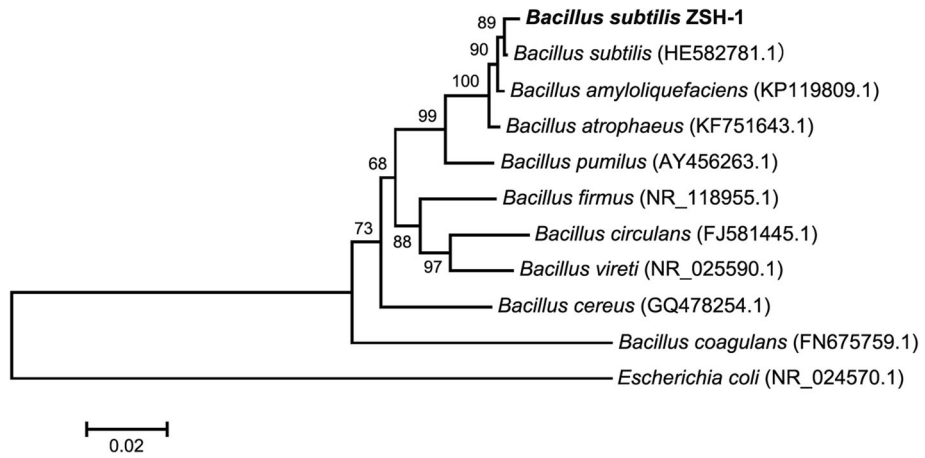

Fig. 1 Phylogenetic tree of strain ZSH-1 based on 16S rRNA sequence analysis

oxysporum, *A. tenuissima*, *C. chrysosperma*, *B. dothidea*, *Mucor* sp., and *Absidia* sp.) belonging to various taxonomic groups. The inhibition rates ranged from 44.0 to 89.1% (Fig. 2a). Moreover, when the colonies of *C. gloeosporioides* were measured every day, the diameters were significantly reduced by treatment with SCF of ZSH-1 than the control. The inhibition rate was gradually increased over time from day 1 to day 6 (Fig. 2b). The antifungal activity of sterile culture filtrate was also stable after treatment at temperatures from 40°C to 100°C for 30 min, or autoclaved at 121°C for 20 min, as determined by inhibition of mycelial growth of *C. gloeosporioides* (Fig. S1). Numerous studies have reported that the culture filtrates of *Bacillus* genus have a broad spectrum of activities. The culture filtrate of *B. safensis* B21 showed antifungal activity against *Magnaporthe oryzae*, which causes rice blast disease (Rong et al. 2020). Dong et al. (2019) reported that culture filtrate of *B. amyloliquefaciens* Rdx5 strongly inhibited the growth of *M. oryzae*. These indicated the great potential of *Bacillus* genus in control plant disease.

Antifungal activities of crude protein and crude lipopeptide extracts from sterile culture filtrate

The 7 fungal phytopathogens that were strongly inhibited by the SCF were used to determine the antifungal activities of the crude protein extracts and crude lipopeptide extracts from the SCF. The crude protein and crude lipopeptide extracts strongly inhibited the growth of all 7 fungal phytopathogens, with inhibition rates in the range of 26.7 to 85.4% and 11.6 to 89.7%, respectively (Fig. 2c). Many reports have shown that members of *Bacillus* genus can produce different antibiotics from culture filtrates that have strong antifungal activities. Proteins and lipopeptides are widely found in culture filtrates secreted by *Bacillus* genus. Lipopeptides and proteins in the culture filtrate produced by *B. atrophaeus* CAB-1 inhibited various fungal pathogens (*F. oxysporum*, *Verticillium dahlia*, *Rhizoctonia solani*, and *B. cinerea*) (Zhang et al. 2013). Rong et al. (2020) reported that *B. safensis* B21 produced iturin A2 and A6 that inhibited the growth of hyphae of *M. oryzae* by changing membrane permeability. Ren et al. (2019) reported that a novel protein obtained from the culture filtrate of *B. subtilis* XB-1 inhibited the mycelial growth of *M. fructicola*.

Antifungal activities of volatile compound(s)

Strain ZSH-1 produced antifungal VOCs that reduced the mycelial growth of the 7 fungal phytopathogens with inhibition rates in the range of 7.8 to 63.2% (Fig. 2a). The result consists with previous findings that VOCs produced by *Bacillus* genus had antifungal activities. Yuan et al. (2012) reported that the VOCs produced by *B. amyloliquefaciens* NJN-6 inhibited the growth and spore germination of *F. oxysporum*. Currently, the VOCs produced by *Bacillus* genus are generally grouped into amides, aldehydes, alcohols, ketones, and phenols. Zhang et al. (2013) reported that *O*-anisaldehyde is one of the most abundant VOCs produced by *B. atrophaeus* CAB-1 with the highest inhibitory effect on the mycelial growth of *B. cinerea*. Three VOCs (2-nonanone, β-benzeneethanamine, and 2-decanone) produced by *B. pumilus* and *B. thuringiensis* had great inhibitory effects on *C. gloeosporioides* (Zheng et al. 2013). VOCs of ZSH-1 probably contain many kinds of antifungal components, and different fungi have different sensitivities to the antifungal action of a same component, so there is difference in the inhibitory effects of VOCs from ZSH-1 against different fungal phytopathogens. Moreover, when the colony diameters of *C. gloeosporioides* were measured every 12 h, the mycelial growth was significantly inhibited after 24 h by the

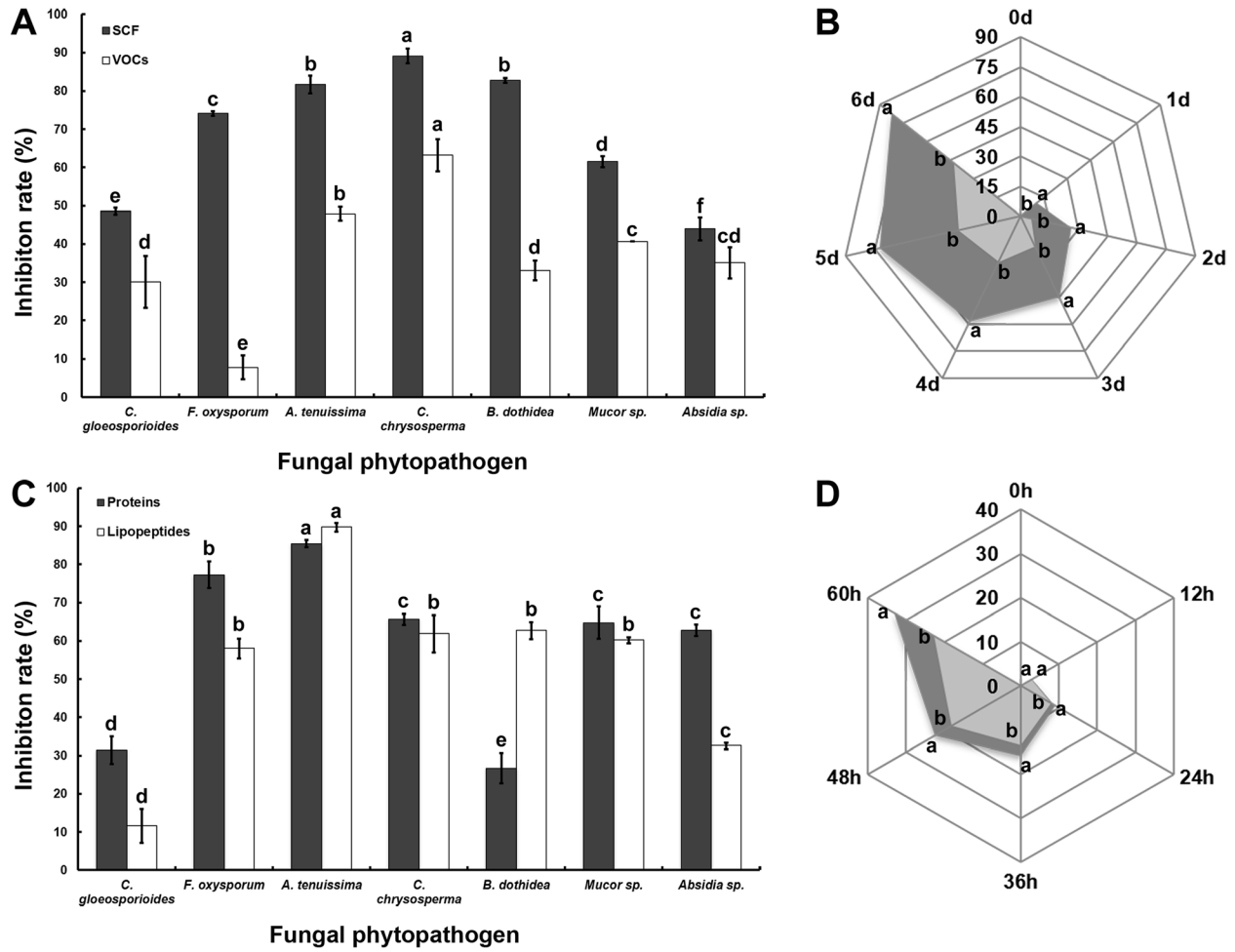

Fig. 2 Antifungal activities of strain ZSH-1 against fungal phytopathogens. **a**, **c** Antifungal activities of SCF, VOCs, protein, and lipopeptide extracts from SCF against fungal phytopathogens, Values with the different letter within the same color shaded box are significantly different at the p pro.2004.03.014vity of the test. **b**, **d** Inhibition patterns as determined by colony diameter of *C. gloeosporioides* by the SCF (**b**) and VOCs (**d**) (inner shaded area) compared to each control (outer shaded area). The unit for all axes in the chart is millimeters. Different letters within the same axis in the spider chart are significantly different each other at the $p \leq 0.05$ level of the *t* test

VOCs produced by strain ZSH-1, and there was a gradual increase in the inhibition rate from 0 to 60 h (Fig. 2d). This result is in agreement with the study that the inhibitory effect of volatiles produced by *B. amyloliquefaciens* NJN-6 gradually increased with treatment time (Yuan et al. 2012). The increasing inhibition rate may be due to the creation of a hypoxic environment by the antifungal bacteria (Boukaew et al. 2013).

Cell wall-degrading traits

The cell wall-degrading traits of strain ZSH-1 was determined by the activities of 5 lytic enzymes (cellulose, β-1, 3-glucanase, chitinase, protease, and lipase). When the colony of ZSH-1 was cultured after 4 days, clear zones around the colonies on different culture plates were observed. This indicated that ZSH-1 have activities of cellulase (Fig. 3a), β-1,3-glucanase (Fig. 3b), and protease (Fig. 3c). No clear zone was found in the culture plates containing chitinase or lipase (not shown). Several cell wall-degrading enzymes such as chitinase, β-1,3-glucanase, cellulase, protease, and lipase are produced by *Bacillus* species which are involved in the antifungal activity against fungal phytopathogens. The enzymes mainly play an important role in the decomposition of fungal cell walls and subsequent cell death (Tseng et al. 2008). Ren et al. (2013) reported that *B. pumilus* JK-SX001 produced cellulase and protease. Dong et al. (2019) demonstrated that cellulase and protease were secreted by *B. amyloliquefaciens* Rdx5. In the present study, the cell wall-degrading traits of strain ZSH-1 may be one reason that this bacterium is equally effective against different fungal phytopathogens with variable cell wall compositions.

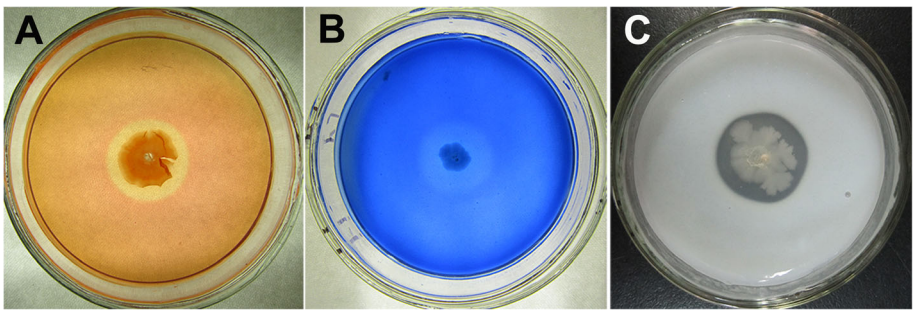

Fig. 3 Cell wall-degrading traits of strain ZSH-1. Production of cellulase (**a**), β-1,3-glucanase (**b**), and protease (**c**) is indicated by clear zones around the colonies on the plates

Evaluation of biocontrol activity of strain ZSH-1 under greenhouse conditions

At present, the main disease control measure for poplar anthracnose is done by applying with fungicides (Song et al. 2016). Many *Bacillus* species have been used as biocontrol agents for *C. gloeosporioides* that causes plant anthracnose (Fira et al. 2018; Guerrero-Barajas et al. 2020). However, used *Bacillus* species for control poplar anthracnose that caused by *C. gloeosporioides* is rarely reported. In the previous study, *B. atrophaeus* XW2 was used as an effective biocontrol agent against *C. gloeosporioides*, it had a 49.1% efficacy in controlling poplar anthracnose under controlled greenhouse conditions (Huang et al. 2014). In the present study, strain ZSH-1 obviously inhibited the development of poplar anthracnose in greenhouse experiments (Table 3). After 12 days inoculation, the diseased leaves rate and the disease index of plants inoculated with ZSH-1 were significantly lower than the values of plants un-inoculated with ZSH-1. ZSH-1 had 47.6% control effect against poplar anthracnose that was caused by *C. gloeosporioides*. The leaves treated with sterile distilled water had no disease symptoms. The pot experiments in greenhouse indicated that strain ZSH-1 has a good biocontrol efficacy against *C. gloeosporioides* in vivo and can be used as a potential biocontrol agent for more study. However, biocontrol of the poplar anthracnose in the greenhouse may not effectively control the disease in the field. Additional studies about the biological characteristics of strain ZSH-1, such as colonization, survival ability, and product formulation, are needed to confirm the biocontrol effect of strain ZSH-1 in the field (Liu et al. 2019). Meanwhile, the abilities of strain ZSH-1 to promote plant growth and activate induced systemic resistance (ISR) of plant in the field should also be studied in the future (Lyu et al. 2019; Tiwari et al. 2019, and Jinal and Amaresan 2020).

Table 3 Efficacy of strain ZSH-1 in controlling poplar anthracnose under greenhouse conditions

Treatment	Diseased leaves rate (%)	Disease index	Greenhouse control efficacy (%)
CK1	0 ± 0c	0 ± 0c	\
CK2	54.3 ± 3.0a	25.0 ± 3.0a	\
SZ	37.5 ± 3.0b	13.1 ± 1.3b	47.6

Values with the different letter within the same column are significantly different at $P \leq 0.05$ according to Duncan's test. Numbers followed by the "±" are standard errors (SE). "\" indicates the data is incalculable

Conclusion

In the present study, an antagonistic bacteria *B. subtilis* ZSH-1 against *C. gloeosporioides* from the rhizosphere soil of poplar was evaluated. The sterile culture filtrate, crude protein, and crude lipopeptide extracts from the culture filtrate and volatile compound(s) of ZSH-1 strongly inhibited the fungal phytopathogens growth in vitro. Moreover, the greenhouse trial revealed that strain ZSH-1 had a good biocontrol efficacy in controlling *C. gloeosporioides* in vivo. Therefore, the results proved that *B. subtilis* ZSH-1 can be used as a promising biocontrol agent against *C. gloeosporioides* and other fungal phytopathogens.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s41938-020-00301-5.

Additional file 1: Fig. S1. Effect of temperature on antifungal activity of sterile culture filtrate of strain ZSH-1.

Abbreviations

DI: Disease index; DS: Disease severity; DLR: Diseased leaves rate; GCE: Greenhouse control efficacy; LB: Luria-Bertani; PDA: Potato dextrose agar; SCF: Sterile culture filtrate; TYB: Tryptone yeast extract broth; VOCs: Volatile compound(s)

Acknowledgements

Not applicable.

Authors' contributions

First author HH participated in the planning and designing of the experiments, implementation of the experiments, and writing of the manuscript. CM conceived of the study and participated in its design and coordination. YH participated in drafting the manuscript. The fourth author HH participated in

revising the paper scientifically and checking analysis. All authors read and approved the final manuscript.

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Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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