BMC Microbiology

RESEARCH

Open Access

The antagonistic activity of *Streptomyces spiroverticillatus* (No. HS1) against of poplar canker pathogen *Botryosphaeria dothidea*

Qingzhen Liu[1†], Xin Li[2†], He Mao[1], Tongtong Zuo[1], Yang Zhang[1], Tianbing Gou[1], Jingsheng Chen[3*] and Limei Li[1*]

Abstract

Background Poplar canker caused by *Botryosphaeria dothidea* is one of the most severe plant disease of poplars worldwide. In our study, we aimed to investigate the modes of antagonism by fermentation broth supernatant (FBS) of *Streptomyces spiroverticillatus* HS1 against *B. dothidea*.

Results In vitro, the strain and FBS of *S. spiroverticillatus* HS1 significantly inhibited mycelial growth and biomass accumulation, and also disrupted the mycelium morphology of *B. dothidea*. On the 3rd day after treatment, the inhibition rates of colony growth and dry weight were 80.72% and 52.53%, respectively. In addition, FBS treatment damaged the plasma membrane of *B. dothidea* based on increased electrical conductivity in the culture medium, and malondialdehyde content of *B. dothidea* mycelia. Notably, the analysis of key enzymes in glycolysis pathway showed that the activity of hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK), $Ca^{2+}Mg^{2+}$-ATPase were significantly increased after FBS treatment. But the glucose contents were significantly reduced, and pyruvate contents were significantly increased in *B. dothidea* after treatment with FBS.

Conclusions The inhibitory mechanism of *S. spiroverticillatus* HS1 against *B. dothidea* was a complex process, which was associated with multiple levels of mycelial growth, cell membrane structure, material and energy metabolism. The FBS of *S. spiroverticillatus* HS1 could provide an alternative approach to biological control strategies against *B. dothidea*.

Keywords *Streptomyces spiroverticillatus* HS1, *Botryosphaeria dothidea*, Poplar canker, Glucose metabolism, Antagonistic activities, Fermentation broth supernatant (FBS)

Background

Poplars (*Populus L.*) are the main plants in the plains and sandy areas of northern China [1]. They contribute to protect and improve environment, such as protecting farmland from soil erosion caused by wind and water, providing forest products, and park land for recreation [2, 3]. Poplar canker is a major disease affecting poplars worldwide. It mainly involves infections in the trunk and branches, which can lead to tree death in severe cases. At present, there are more than ten pathogens that can cause poplar canker worldwide [4]. Among them, *B. dothidea* is widely distributed worldwide and has been identified

†Qingzhen Liu and Xin Li contributed equally to this work.

*Correspondence:
Jingsheng Chen
jingsheng6673182@163.com
Limei Li
lilimei19820406@163.com
[1] Jilin Provincial Academy of Forestry Science, Changchun 130033, China
[2] Dalian Customs Technology Center, Dalian 116001, China
[3] College of Biology and Food Engineering, Chongqing Three Gorges University, Wanzhou 404010, China

as a pathogen of many woody plants. It also shows high genetic diversity in the same host, which is one of the major reasons for the outbreak of poplar canker [5, 6]. Using chemical fungicides, such as benomyl, tebuconazole, carbendazim, thiophanate-methyl, is effective against pathogens [7, 8]. However, chemical control is not a sustainable solution owing to the potential human health hazards, environmental pollution, and enhanced pathogen resistance [9]; therefore, the use of biological control has attracted more attention in recent years.

At present, many studies mainly focus on the development of biocontrol agents (BCAs) and antagonistic mechanisms. Some studies have shown that the extracts of plants such as *Cymbopogon*, *Curcuma longa*, and *Notopterygium incisum*, as well as bioactive components such as cuminaldehyde, geraniol, β-citronellol, and lemon essential oil can inhibit *B. dothidea* mycelial growth, and the germination and formation of spores [10–12]. The screening of antagonistic microorganisms generally focuses on *Bacillus* spp. and *Streptomyces* spp. from soil or healthy hosts. Fengycin is produced by *Bacillus subtilis* isolated from healthy apples, and it has a good control effect on apple ring rot caused by *B. dothidea* [13]. *B. amyloliquefaciens* subsp. *plantarum* (CGMCC 11640) isolated from soil can inhibit *B. dothidea* growth and provides a bio-resource for the biocontrol of *Carya cathayensis* canker [14]. In addition, a few studies provide a new strategy of using varieties of effective antagonistic substances in combination for inhibiting pathogen growth instead of using a single biocontrol strain metabolite. For example, ε-polylysine, chitosan oligomers, their conjugates, *S. rochei*, *S. lavendofoliae* culture filtrates, and their secondary metabolites with chitosan oligomers have been used to inhibit the growth of *B. dothidea*, *Neofusicoccum parvum* and *Diplodia seriata* that cause grapevine trunk diseases [15].

Streptomyces are the most widely studied and notable species among the Actinomycetes and they produce many naturally occurring antibiotics [16], which are widely used as BCAs owing to their ability to produce various secondary metabolites. *S. violaceusniger* has antagonistic activity against *Fusarium oxysporum* that causes banana wilt; antagonistic activity involves disrupting cell membranes and inhibiting mycelial growth and spore germination [17]. A low-concentration culture filtrate (diluted to 10^{-3}) of *Streptomyces* spp. can inhibit the spore germination of *Botrytis cinerea* [18]. It was reported that antibiotic compounds consisting of heterologous small-molecular weight molecules, could affect pathogen growth and metabolism at relatively low concentrations and play a significant role in plant disease control [19, 20]. Also, some studies have reported that the antibiotic production of *Streptomyces* sp. had a negative impact on normal pathogen metabolism by altering the key enzyme activity [21]. In addition to the antagonistic effect of *Streptomyces* applied to inhibit plant pathogen, *Streptomyces* could promote plant growth [22–24] and induce systemic resistance against pathogen invasion [25]. For example, *S. albus* CAI-21 can promote the growth of rice, sorghum, and certain legumes, and it can also inhibit the growth of *Macrophomina phaseolina* in sorghum anthracnose infections [26]. In summary, *Streptomyces* and their secondary metabolites are important sources of biocontrol agents, because it could affect pathogen growth and cell metabolism, and also may induce systemic resistance.

In our previous study, *S. spiroverticillatus* isolated from forest soil in Jilin Province, China, has been found to display broad-spectrum antifungal activity against a range of plant pathogens. The preventive and therapeutic effects of *S. spiroverticillatus* HS1 fermentation broth supernatant (FBS) on poplar canker have been reported to be 60.87% and 71.74%, respectively. It efficiently prevented and control the occurrence and spread of canker [27]. However, the antifungal mechanism of *S. spiroverticillatus* HS1 against *B. dothidea* are unclear. In our study, we aimed to systematically test the antagonistic mechanisms of *S. spiroverticillatus* HS1, it will provide a new method to control the poplar canker disease.

Results

Antagonistic activity of *S. spiroverticillatus* HS1 against *B. dothidea* in vitro

Live *S. spiroverticillatus* HS1 exerted a strong inhibitory effect on *B. dothidea* with an inhibition band of 20.66 mm (Fig. 1a), and the FBS displayed an inhibition zone diameter of 40.26 mm (Fig. 1b). The mycelia of *B. dothidea* in the control group showed fast growth and covered the entire petri dish (Fig. 1a-C). *B. dothidea* mycelia in the treatment group showed slow growth away from the *S. spiroverticillatus* HS1, and the growth area was less than 1/3rd of the petri dish area (Fig. 1a-T). Compared with the control (Fig. 1b-C), the inhibition zone created by FBS of *S. spiroverticillatus* HS1 was large and clear (Fig. 1b-T). The anti-fungal effect of live strain and FBS of *S. spiroverticillatus* HS1 were long-lasting and stable, and the experiment was reproducible.

Antagonistic effect of *S. spiroverticillatus* HS1-FBS against *B. dothidea* mycelia

Effect of FBS on the mycelial morphology of B. dothidea

Microscopic observation revealed that the mycelia of *B. dothidea* in the control group showed dense and vigorous growth with smooth colonies (Fig. 2-CK). In contrast, the mycelia were deformed, sparse, and shrunk, and the

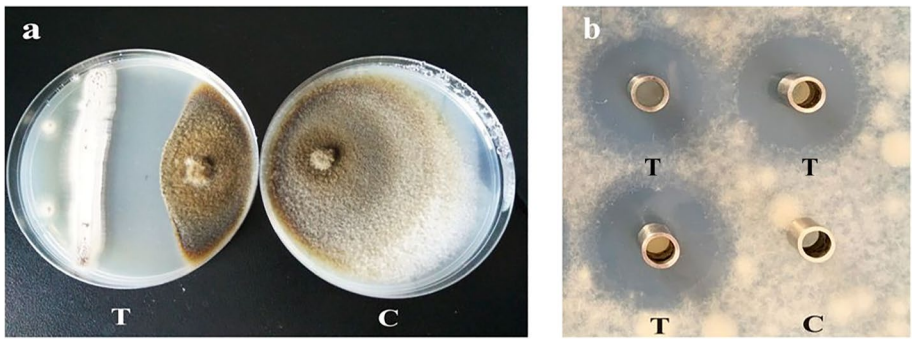

Fig. 1 Antagonistic activity of *S. spiroverticillatus* HS1 vs. *B. dothidea* in vitro (T-treatment, C-control). **a** Dual cultures of *S. spiroverticillatus* HS1 and *B. dothidea*; **b** Dual cultures of *S. spiroverticillatus* HS1-FBS and *B. dothidea*

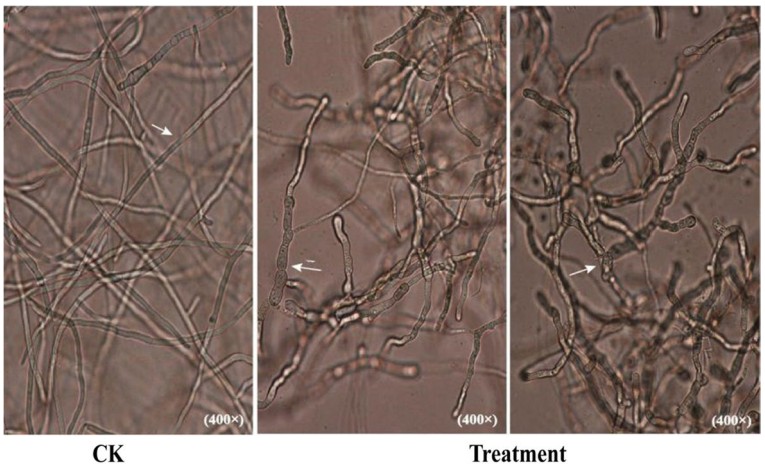

Fig. 2 Monitoring of mycelial morphology of *B. dothidea* using an optical microscope. The mycelia of *B. dothidea* exposed to FBS (Treatment) or to equivalent sterile fermentation medium (CK). The magnification was 400 times

internodes were shortened, twisted, enlarged and broken after treatment with FBS (Fig. 2-Treatment).

Effect on the dry weight of B. dothidea mycelia

At 1–3 d after FBS treatment, the dry weight of mycelia in all treatment groups was significantly lower than that in the control group. Undiluted FBS showed the best antagonistic effect on *B. dothidea* mycelium biomass, and the rates of inhibition of *B. dothidea* growth based on dry weight of mycelia were 58.42% (1 d) and 52.53% (3 d). The dry weight of mycelia in the control group was 0.42 g and 0.46 g for 1d and 3d respectively, and 0.31 g and 0.32 g after treatment with 400 times diluted fermentation solution separately. The FBS diluted by 400 time retained the antagonistic effect, and treated *B. dothidea* was significantly different from that in the control group (Fig. 3).

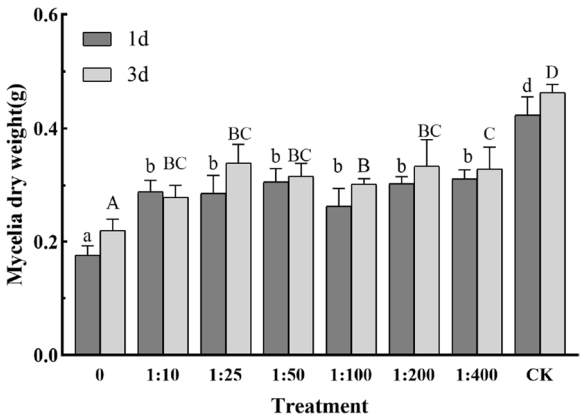

Fig. 3 The influence of the *S. spiroverticillatus* HS1-FBS on the dry weight of *B. dothidea*. Data represent the mean ± standard deviation ($n = 3$). The numbers on X axis represent the different dilution ratio of *S. spiroverticillatus* HS1-FBS treatments. CK, equivalent volume of sterile fermentation medium. The different letters indicate significant differences among treatments using Duncan's test at $p < 0.05$. The lower-case letters and the upper-case letters represent the significant difference on Day1and Day 3, respectively

Effect of FBS on growth of B. dothidea

The effects of different concentrations of *S. spiroverticillatus* HS1-FBS on *B. dothidea* colony growth are shown

in Table 1. The growth rate of *B. dothidea* in medium containing FBS was slow. The growth rate gradually decreased with an increase in inhibition rate as the FBS concentration increased. The inhibition rate after treatment with undiluted FBS was the highest at 80.72% on day 3, followed by 67.99% and 72.11% on days 5 and 7, respectively, when compared to that of diluted FBS treatments ($P<0.05$). The inhibition rate on the 7th day was higher than those on the 3rd and 5th days when the dilution was less than 100-fold, indicating that the antagonistic effect of FBS was efficient, durable, and stable (Table 1).

Antagonistic effect of FBS on *B. dothidea* cell membrane

Effect of FBS on membrane permeability of B. dothidea

After treatment of *B. dothidea* with FBS, the conductivity was significantly higher than that of the control ($P<0.001$), indicating that the permeability of *B. dothidea* mycelial cell membrane was increased and electrolyte leakage occurred. In the FBS treatment group, electrolyte leakage continued as the experiment was prolonged, and there was no decrease. The highest value was 3.693 ms/cm at 12 h, which was 5.77 times that of the control group, indicating that electrolyte leakage was severe (Fig. 4a). It can be presumed that FBS had a considerably negative impact on the cell membrane permeability of mycelia.

Table 1 The effect of *S. spiroverticillatus* HS1 fermentation broth on the growth of mycelia of *B.dothidea*

Dilution ratio	Inhibition rate		
	3d	5d	7d
0	80.72 ± 0.001a	67.99 ± 0.010a	72.11 ± 0.031a
10×	32.83 ± 4.542b	65.56 ± 0.068a	72.24 ± 0.014a
25×	33.09 ± 3.430b	44.18 ± 0.028b	56.51 ± 0.085b
50×	31.05 ± 3.545bc	31.12 ± 0.028c	41.43 ± 0.067c
100×	25.70 ± 2.572bc	35.96 ± 0.043c	44.20 ± 0.106c
200×	24.12 ± 7.096c	7.86 ± 0.058d	12.89 ± 0.043d
400×	15.96 ± 6.369d	12.30 ± 0.032d	8.16 ± 0.054d

Data in the table are mean ± SD. Different lowercase letters in the same column indicate significant difference at $P<0.05$ levels by Duncan's new multiple range test, respectively

Effect of FBS on membrane lipid peroxidation of B. dothidea

Malondialdehyde (MDA) is the product of cell membrane lipid peroxidation, and its level reflects the degree of damage caused to the cell membrane. The MDA content in the mycelia of *B. dothidea* treated with FBS was significantly higher than that in the control group ($P<0.05$), and lipid peroxidation of the cell membrane was increased, indicating that the cell membrane was severely damaged. The degree of lipid peroxidation gradually increased with an increase in treatment time. At 48 h, the MDA content in the FBS treatment group was 2.88 times that of the control group (Fig. 4b), further indicating that the FBS had a considerably adverse effect on the mycelial cell membrane.

Antagonistic effect of *S. spiroverticillatus* HS1-FBS on glycolysis pathway in *B. dothidea*

Effect of FBS on HK, PFK, PK, and $Ca^{2+}Mg^{2+}$-ATP activity

The activities of three key enzymes (HK, PFK, and PK) in the glycolysis pathway were determined to evaluate the effect of FBS on the metabolism of *B. dothidea*. The HK activity in all groups of *B. dothidea* showed a downward trend in general (Fig. 5a). However, the activity of HK in the FBS treatment group was slightly higher than that in the control, especially within 2–4 h, and the difference was considerable ($P<0.001$). After treatment with FBS for 4 h, the activity of PFK in the cells was higher than that in the control group, and the difference was significant at 6, 12, and 48 h ($P<0.001$). Notably, PFK activity in the FBS treatment group was

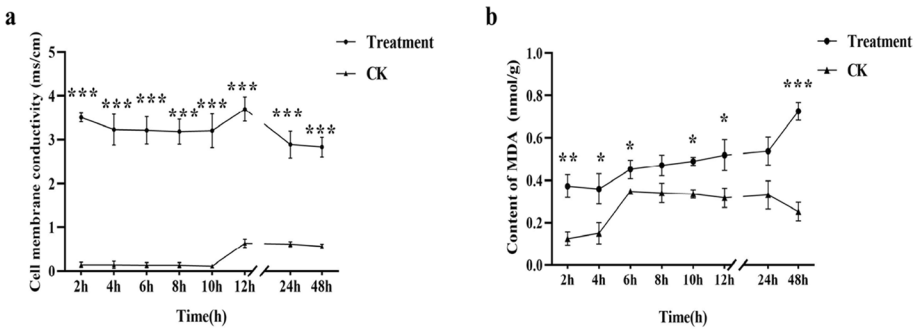

Fig. 4 Influence of *S. spiroverticillatus* HS1-FBS on the membrane of *B. dothidea*. **a** Cell membrane conductivity of *B. dothidea*; **b** The level of MDA in *B. dothidea*. Data are expressed as mean ± SD of three biological replicates. Differences between samples were determined by a two-tailed Student's t-test at $P<0.05$. *, $0.01<P<0.05$; **, $0.01<P<0.001$; ***, $P<0.001$

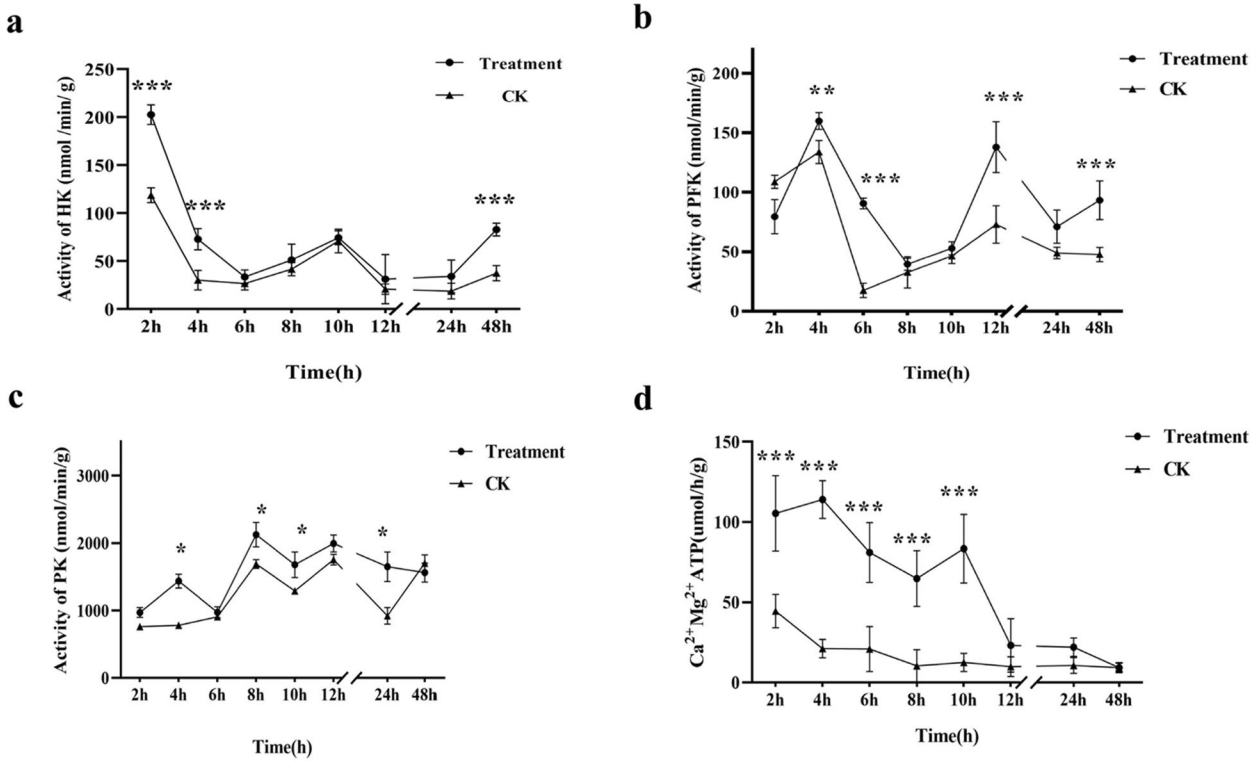

Fig. 5 Influence of *S. spiroverticillatus* HS1-FBS on HK (**a**), PFK (**b**), PK (**c**), Ca^{2+} Mg^{2+}-ATPase (**d**) activity in *B. dothidea*. Differences between samples were determined by two-tailed Student's t-test at $P < 0.05$. *, $0.01 < P < 0.05$; **, $0.01 < P < 0.001$; ***, $P < 0.001$

5.2 times that of the control group at 6 h (Fig. 5b). Similarly, PK activity in the FBS treatment group was slightly higher than that in the control group, and the difference was evident at 4, 8, 10, and 24 h ($P < 0.05$) (Fig. 5c). The activity of $Ca^{2+}Mg^{2+}$-ATPase reflected the energy metabolism of *B. dothidea*. As time increased, the enzyme activity of all groups showed a downward trend. Moreover, the $Ca^{2+}Mg^{2+}$-ATPase activity in the FBS treatment group was higher than that of the control group, especially within 2–10 h (Fig. 5d), and the difference was highly significant ($P < 0.001$). FBS treatment enhanced Ca^{2+} Mg^{2+}-ATPase activity, accelerated the utilization of ATP, and affected the regular energy supply of cells.

Effect of FBS on total glucose and total pyruvate levels

The total glucose level in the control group was stable at first and then decreased sharply at 12 h. The FBS treatment group showed an upward trend as the total glucose level increased sharply at 10 h, and the two groups showed opposite trends (Fig. 6a). The total glucose level of the control group was markedly higher than that of the FBS treatment group for 2–8 h ($P < 0.05$). Especially at 4 h, the total glucose level in the control group was 3-fold

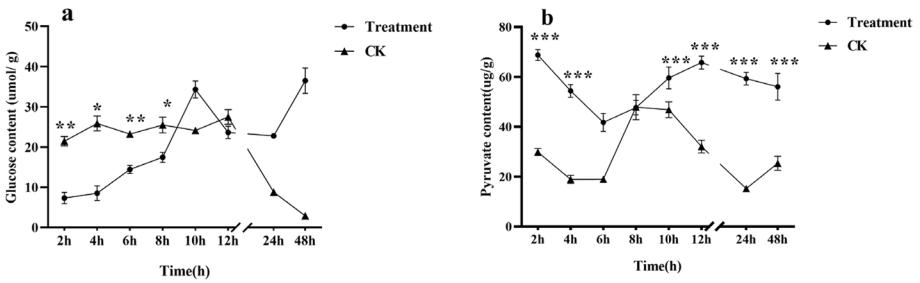

Fig. 6 Influence of *S. spiroverticillatus* HS1-FBS on total glucose and total pyruvate level in *B. dothidea*. **a** Total glucose level; **b** Total pyruvate level. Differences between samples were determined by two-tailed Student's t-test at $P < 0.05$. *, $0.01 < P < 0.05$; **, $0.01 < P < 0.001$; ***, $P < 0.001$

higher than that in the FBS treatment group. Therefore, glucose metabolism in the cells of *B. dothidea* was accelerated via FBS treatment at this time, and the glucose content could not accumulate, which was mutually verified based on the enhanced activity of the three carbohydrate-metabolizing enzymes. In contrast, the total pyruvate level in the FBS treatment group was significantly higher than that in the control group ($P < 0.001$). Notably, the pyruvate level in the FBS treatment group was 3.9 times that of the control group at 24 h (Fig. 6b). This indicated that pyruvate gradually accumulated after FBS treatment, which further indicated abnormal glucose metabolism and confirmed the severe adverse effects on the growth of the *B. dothidea*.

Materials and methods

The identification of *S. spiroverticillatus* HS1 in vitro

S. spiroverticillatus HS1 was previously isolated from the soil at Hengshan Protection Station, Changbai Mountain National Nature Reserve, Jilin Province, China. It was identified based on the morphology, physiological and biochemical characteristics, and 16S rDNA sequence (GenBank accession number: MN636764). *B. dothidea*, the causal agent of poplar canker, was isolated, identified, maintained, and stored by the Jilin Provincial Academy of Forestry Sciences. The *S. spiroverticillatus* HS1 fermentation medium and FBS has been optimized and widely used [28].

Antagonistic activities of *S. spiroverticillatus* HS1-FBS against mycelial growth of *B. dothidea* in vitro

Antifungal activity

In vitro antifungal activity of *S. spiroverticillatus* HS1 against *B. dothidea* was tested by the dual culture technique. The mycelia plug (5 mm in diameter) of *B. dothidea* taken from 5-day-old target fungus on Potato Dextrose Agar (PDA) was placed 5 cm away from the bacterial streak line of *S. spiroverticillatus* HS1. The dual culture plates were incubated at 28 °C, and the inhibition of fungal growth antifungal activity was measured every day until 7 days after inoculation.

Antifungal activity of FBS

In vitro antifungal activity of *S. spiroverticillatus* HS1-FBS against *B. dothidea* was also tested by the dual culture technique. The mycelia of *B. dothidea* was mixed with PDA which was under 45℃, and the oxford cups containing 200ul of *S. spiroverticillatus* HS1-FBS were placed in the center of the cooled PDA. The dual culture plates were incubated at 28 °C, and the inhibition of fungal growth antifungal activity was measured every day until 7 days after inoculation.

Mycelial morphology

FBS was added to potato dextrose agar (PDA) medium at 50 °C at a volume ratio of 1:4, and the mycelial plug of *B. dothidea* (5 mm in diameter) was placed in the center of the plate following culturing at 28 °C for 5 d. The medium supernatant without bacteria was used as a control. The mycelial morphology was observed and compared with that of the control using an optical microscope (BX53; Olympus, Tokyo, Japan).

Mycelial dry weight

Briefly, three mycelial plugs (5 mm) of *B. dothidea* were inoculated in 100 ml potato dextrose broth (PD) medium at 28 °C with shaking at 150 rpm for 48 h. Subsequently, 20 ml of the undiluted or different fold diluted FBS (10-, 25-, 50-, 100-, 200-, and 400-fold) were added into erlenmeyer flasks as different treatment. Cultures of pathogen conducted in PD and supplemented with equivalent fermentation medium were used as control. After 1 and 3 d of culture, the mycelia were collected and rinsed with sterile water and then dried in an oven at 80 °C for 3 h. The dry weight of mycelia was determined, and each treatment was performed in triplicate.

Mycelial growth

The PDA medium was adjusted with undiluted FBS or the FBS diluted by 10-, 25-, 50-, 100-, 200-, or 400-fold at 50 °C in a volume ratio of 1:4, and the mycelial plug of *B. dothidea* (5 mm in diameter) was placed in the center of the plate following culturing at 28 °C for 5 d. Cultures of pathogen grown in PDA mixing with equivalent fermentation medium were used as control. The diameter of the mycelial colony was measured using the crosshairs at 3, 5, and 7 d. Each treatment was performed in triplicate. Rate of inhibition of growth was calculated as follows:

$$\text{Inhibition rate (\%)} = (\text{control net growth} - \text{treatment net growth}) / \text{control net growth} \times 100\%.$$

Effect of *S. spiroverticillatus* HS1-FBS on *B. dothidea* membrane

Electrolyte leakage

Briefly, 15 *B. dothidea* mycelial plugs (5 mm) were inoculated into PD medium (100 ml) and incubated at 28 °C with shaking at 150 rpm for 48 h. FBS solution (20 mL) was added separately. The conductivity was measured using a conductivity meter (FC20; OHAUS, Shanghai,

China) at 0, 2, 4, 6, 8, 10, 12, 24, and 48 h [29]. Each treatment was performed in triplicate. Cultures of *B. dothidea* supplemented with equivalent fermentation medium were used as a control.

Malondialdehyde (MDA) content

B. dothidea mycelial plugs were cultured at 28 °C with shaking at 150 rpm. Briefly, 0.5 g of wet mycelia was collected at 0, 2, 4, 6, 8, 10, 12, 24, and 48 h and then rinsed with sterile water several times, after which it was ground in liquid nitrogen for homogenization [29]. Subsequently, 4 mL of 0.5% barbituric acid and 20% trichloroacetic acid was added to the mixture and mixed well. The suspension was centrifuged at 12,000 g for 20 min, followed by treatment in boiling water bath for 25 min and cooling in an ice bath. The absorbance at 450 nm, 532 nm, and 600 nm was measured using an ultraviolet spectrophotometer (UV-2100; UNICO, Shanghai, China), each treatment and control was replicated for three times. The MDA level was measured as follows:

$$\text{MDA (nmol/g)} = 6.45 * (\text{OD532} - \text{OD600}) - 0.56 * \text{OD450}$$

Determination of enzyme activity, total glucose, and pyruvate content in glycolysis metabolism pathway

B. dothidea mycelial plugs were cultured at 28 °C with shaking at 150 rpm. Briefly, 0.1 g of wet mycelia was collected at 0, 2, 4, 6, 8, 10, 12, 24, and 48 h [29], rinsed with sterile water and ground in liquid nitrogen. Then, the activities of hexokinase (HK), 6-phosphofructokinase (PFK), Ca^{2+} Mg^{2+}-ATPase, and pyruvate kinase (PK) in the glycolysis pathway as well as the total glucose and total pyruvate levels were measured using commercial assay kits (Comin log, Suzhou, China, http://www.cominbio.com/index.html).

Statistical analysis

All statistical analyses were performed using the IMB SPSS statistics software (Version 20.0; IBM, Armonk, NY, United States); values of $P < 0.05$ were considered significant. One-way analysis of variance followed by Duncan's post-hoc test was used to compare the means among treatments. A two-tailed Student's t-test was used to determine significant differences between samples.

Discussion

It is essential to study the function of BCAs along with their limitations and requirements to exploit their potential in plant disease management. The mechanisms of action of BCAs vary extensively. As one of the most effective and popular BCAs, *Streptomyces* fermentation broth represents the main source of antibiotics or antagonistic substances that inhibit the growth of pathogenic bacteria and fungi [30–33]. By studying the appearance, cell membrane permeability, other physiological indicators of mycelia, and the impact on related enzymes in metabolic pathways, we can understand the specific mode of action of *Streptomyces*-induced inhibition of pathogen growth and clarify the disease resistance mechanism of the strain.

The FBS of *S. spiroverticillatus* HS1 exerted a significant inhibitory effect on the growth of *B. dothidea* mycelia. Morphologically, the mycelia treated with FBS were sparsely shrunk and broken, and the internodes were shortened, twisted, and enlarged, which is consistent with the findings of Jiang et al. [34], which showed that the mycelia of *B. dothidea* exhibit premature aging and wrinkling. Except for the shrinkage and deformation of mycelial morphology, the growth of mycelia was also inhibited with significant decrease in growth rate and dry weight of mycelia. The undiluted FBS of *S. spiroverticillatus* HS1 had the best antagonistic effect. The inhibition rates of *B. dothidea* growth volume were 58.42% (1 d) and 52.53% (3 d), and inhibition of growth rate were 80.72% (3 d), 67.99% (5 d), and 72.11% (7 d). The changes in mycelia morphology, mass and volume mutually verify that the growth of *B. dothidea* was seriously damaged, thus it can be inferred that the cell structure and metabolism of *B. dothidea* may also be affected. We suggested that the decrease of growth may indicate the mycelial cells may be disrupted.

By measuring the electrical conductivity of the culture medium of pathogens, we could understand the membrane permeability [35]. The FBS-treated mycelium of *B. dothidea* resulted in a significant increase in electrical conductivity. We suggested that the leakage of cell contents from the *B. dothidea* after FBS-treated, and the permeability of the *B. dothidea* cell membrane may be increased. Some studies proved that the antagonistic mycin N2 derived from *Streptomyces sp.* N2 exerted its antagonistic activity by inducing cell wall degradation and oxidative stress in *Rhizoctonia solani*, thus leading to fungal morphogenesis and autolysis [36]. MDA was accumulated in large amounts in the cells indicating severe lipid peroxidation of the cell membrane. The fluidity and permeability of cell membranes were altered, which ultimately led to changes in cell structure and function. The decreased ergosterol formation and increased MDA levels after treatment of *F. oxysporum* f. sp. *niveum* with *S. corchorusii* strain AUH-1, which is attributed to adverse effects on the structure and function of cell membranes [37]. Similarly, Woo et al. showed that an anti-pythium protein from *Streptomyces* sp. strain AP77

can significantly inhibit the hyphal growth of *Pythium porphyrae* due to alteration of the membrane permeability in *P. porphyrae* [38]. Therefore, we hypothesized that the cell membrane represents one of the sites of action of FBS in pathogenic mycelium characterized by disruption of the cell membrane structure and inability to maintain cell proliferation and growth, resulting in abnormal atrophy and fracture of the mycelium.

Glycolysis is the main process via which eukaryotes obtain energy. PFK and PK are often described as "key regulatory enzymes" in glycolysis since under physiological conditions, they catalyze irreversible reactions and their activities are controlled allosterically by ATP and ADP [39]. *Saccharomyces cerevisiae* Y-912 can significantly inhibit the growth, spore germination rate, and germ tube length of *Fusarium graminearum* [40]. Proteomics-based analyses have shown that few essential proteins and enzymes related to basal metabolism in the glycolytic pathway and tricarboxylic acid cycle, such as glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, fructose diphosphate aldolase, and other enzymes are downregulated. Similarly, the related proteins and genes involved in amino acid metabolism have been reported to be downregulated [40]. The glycolysis pathway involves a series of reactions that convert glucose into pyruvate and is accompanied by ATP generation. Therefore, to verify the inhibitory effect of *S. spiroverticillatus* HS1 on *B. dothidea*, it is necessary to evaluate the effect of FBS on the activity of enzymes involved in the glycolysis pathway. After treatment with the FBS, the three key enzymes of the glycolysis pathways in *B. dothidea*, including HK, PFK, and PK, showed significantly increased activities; the enzyme activity of the FBS group was several times that of the control group, such as that of PFK at 6 h. We speculated that the increased activity of key enzymes will accelerate the conversion of glucose. The $Ca^{2+}Mg^{2+}$-ATPase activity was also enhanced, accelerating energy consumption. The increase in activity of these key enzymes in the glycolysis pathway may seriously affect the accumulation of nutrients and energy in the mycelia. The reduction in the total glucose level and the accumulation of total pyruvate content in the cell will disrupt basal metabolism and reduce cell function, accelerating the senescence and death of pathogen cells.

Conclusions

B. dothidea treated with live *S. spiroverticillatus* HS1-FBS showed a clear zone of inhibition, retarded mycelial growth, and other antagonistic effects. *B. dothidea* showed abnormal and broken mycelia after treatment with FBS. The cell mass and growth rate of *B. dothidea* reduced after FBS treatment. Further, the mycelial cell membrane was damaged, the permeability increased, and lipid peroxidation was severe. In mycelial cells, the activities of key enzymes of the glycolysis pathway, including HK, PFK, PK, and $Ca^{2+}Mg^{2+}$-ATP, increased, the total glucose content decreased, and the total pyruvate content increased. Glycolysis was abnormal and accompanied by cell dysfunction.

Collectively, the inhibitory effect of *S. spiroverticillatus* HS1-FBS on *B. dothidea* was multifaceted and the different mechanisms complement each other. However, the effective antagonistic substances in FBS need to be further identified. The FBS of *S. spiroverticillatus* HS1 can effectively prevent the occurrence and damage of poplar canker, and has a good development and application prospects.

Authors' contributions

Q.L. and X.L. Writing - review & editing, Validation, Writing-original draft. H.M. and T.Z. Investigation, Writing - review, Y.Z. and T.G., Editing. J.C., Writing-review, Software and Formal analysis. L.L., Conceptualization, Writing-review & editing, Supervision, Funding acquisition.

Funding

This work was supported financially by the department of Science and Technology of Jilin Province [20230508105RC] and the department of Science and Technology of Jilin Province[20190301043NY].

Availability of data and materials

No applicable.

Data availability

The authors will supply the relevant data in response to reasonable requests.

Declarations

Ethics approval and consent to participate

No applicable.

Consent for publication

No applicable.

Competing interests

The authors declare no competing interests.

Received: 23 August 2023 Accepted: 3 September 2024
Published online: 14 September 2024

References

1. Huang GW, Su XH, Huang QJ. Differences in growth and physiological characteristics in different growth Vigor clones of *Populus deltoides*. Sci Silvae Sin. 2012;48:27–34. https://en.cnki.com.cn/Article_en/CJFDTOTAL-LYKE201204006.htm.
2. Martín-García J, Jactel H, Oria-De-Rueda J. The effects of poplar plantations on vascular plant diversity in riparian landscapes. Forests. 2016;7:50–63. https://doi.org/10.3390/f7030050.
3. Boothroyd-Roberts K, Gagnon D, Truax B. Hybrid poplar plantations are suitable habitat for reintroduced forest herbs with conservation status. Springer Plus. 2013;2:1–13. https://doi.org/10.1186/2193-1801-2-507.

4. Yang L, Zhou GY, Liang J. Inhibitory effects of two biocontrol fungous strains on poplar canker *Botryosphaeria dothidea*. Sci Silvae Sin. 2015;51:67–73. https://doi.org/10.11707/j.1001-7488.20150809.
5. Nagel JH, Wingfield MJ, Slippers B. Next-generation sequencing provides important insights into the biology and evolution of the Botryosphaeriaceae. Fungal Biol Rev. 2021;38:25–43. https://doi.org/10.1016/j.fbr.2021.09.002.
6. Manawasinghe IS, Zhang W, Li XH, Zhao WS, Chethana KWT, Xu JP, Chen Z, Dissanayaka AJ, Mugnaie L, Úrbez-Torres J, Savocchia S, Hyde KD, Yan JY. Novel microsatellite markers reveal multiple origins of *Botryosphaeria dothidea* causing the Chinese grapevine trunk disease. Fungal Ecol. 2018;33:134–42. https://doi.org/10.1016/j.funeco.2018.02.004.
7. Bester W, Crous PW, Fourie PH. Evaluation of fungicides as potential grapevine pruning wound protectants against *Botryosphaeria* species. Australas Plant Path. 2007;36:73–7. https://doi.org/10.1071/AP06086.
8. Tennakoon KMS, Ridgway HJ, Jaspers MV, Langford G, Jones EE. Evaluation of fungicide efficacy against *Neofusicoccum* species causing dieback disease of blueberries in New Zealand. Australas Plant Path. 2019;48:75–84. https://doi.org/10.1007/s13313-018-0565-9.
9. Ishii H. New chemical fungicides in relation to risk for resistance development. Trop Plant Pathol. 2023. https://doi.org/10.1007/s40858-023-00596-3.
10. Faiza A, Oussama M, Abdelbaset G, Salam O, Kato-Noguchi H, Bachar Z, Othmane M. The potency of lemon (*Citrus limon* L.) essential oil to control some fungal diseases of grapevine wood. CR Biol. 2018;341:97–101. https://doi.org/10.1016/j.crvi.2018.01.003.
11. Xiao L, Zhou YM, Zhang XF, Du FY. *Notopterygium incisum* extract and associated secondary metabolites inhibit apple fruit fungal pathogens. Pestic Biochem Physiol. 2018;150. https://doi.org/10.1016/j.pestbp.2018.07.001.
12. Zhang ZL, Xie YJ, Hu X, Shi HA, Wei M, Lin ZF. Antifungal activity of monoterpenes against *Botryosphaeria dothidea*. Nat Prod Commun. 2018;13:1934578X1801301234. https://doi.org/10.1177/1934578X1801301234.
13. Fan H, Ru JJ, Zhang YY, Wang Q. Fengycin produced by *Bacillus subtilis* 9407 plays a major role in the biocontrol of apple ring rot disease. Microbiol Res. 2017;199. https://doi.org/10.1016/j.micres.2017.03.004.
14. Cheng MWL, Xu QF, Li YC, Qin H, Chen JH. Antifungal activity and identification of active compounds of *Bacillus amyloliquefaciens* subsp. *plantarum* against *Botryosphaeria dothidea*. Forest Pathol. 2016;46:561–8. https://doi.org/10.1111/efp.12309.
15. Laura BD, Jesús MG, Eduardo PL, David RR, José LR, José CG, M.Carmen RS, Pablo MR. Antifungal agents based on chitosan oligomers, ε-polylysine and *Streptomyces* spp. Secondary metabolites against three Botryosphaeriaceae species. Antibiotics. 2019;8:99. https://doi.org/10.3390/antibiotics8030099.
16. Subramani R, Aalbersberg W. Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res. 2012;167(10):571–80. https://doi.org/10.1016/j.micres.2012.06.005.
17. Jing T, Zhou DB, Zhang MY, Tian YY, Qi DF, Wei YZ, Chen YF, Zang XP, Wang W, Xie JH. Newly isolated *Streptomyces* sp. JBS5-6 as a potential biocontrol agent to control banana Fusarium wilt: genome sequencing and secondary metabolite cluster profiles. Front Microbiol. 2020;11. https://doi.org/10.3389/fmicb.2020.602591.
18. Boukaew S, Prasertsan P, Troulet C, Bardin M. Biological control of tomato gray mold caused by *Botrytis cinerea* by using *Streptomyces* spp. Biocontrol. 2017;62:793–803. https://doi.org/10.1007/s10526-017-9825-9.
19. Cohen NR, Lobritz MA, Collins JJ. Microbial persistence and the road to drug resistance. Cell Host Microbe. 2013;13:632–42. https://doi.org/10.1016/j.chom.2013.05.009.
20. Martinez KE, Rodríguez PK, Sánchez S. Endophytes as sources of antibiotics. Biochem Pharmacol. 2017;134:1–17. https://doi.org/10.1016/j.bcp.2016.10.010.
21. Duan Y, Chen J, Pang Z, Ye X, Zhang C, Hu H, Xie J. Antifungal mechanism of *Streptomyces* ma. FS-4 on fusarium wilt of banana. J Appl Microbiol. 2020;130:196–207. https://doi.org/10.1111/jam.14784.
22. Chen Y, Zhou D, Qi D, Gao Z, Xie J, Luo Y. Growth promotion and disease suppression ability of a *Streptomyces* sp. CB-75 from banana rhizosphere soil. Front Microbiol. 2018;8:2704. https://doi.org/10.3389/fmicb.2017.02704.
23. Jog R, Pandya M, Nareshkumar G, Rajkumar S. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology. 2014;160:778–88. https://doi.org/10.1099/mic.0.074146-0.
24. Sadeghi A, Koobaz P, Azimi H, Karimi E, Akbari AR. Plant growth promotion and suppression of *phytophthora drechsleri* damping-off in cucumber by cellulase-producing *streptomyces*. Biocontrol. 2017;62:805–19. https://doi.org/10.1007/s10526-017-9838-4.
25. Lin L, Ge HM, Yan T, Qin Y, Tan R. Thaxtomin A-deficient endophytic *Streptomyces* sp. enhances plant disease resistance to pathogenic *Streptomyces scabies*. Planta. 2012;236:1849–61. https://doi.org/10.1007/s00425-012-1741-8.
26. Subramaniam G, Rajan S, Vadlamudi S, Nimmala N, Suraj P, Sravani A, Sambangi P, Mahalingam G, Susana VG, Sondre N, Nebojsa S. Identification and characterization of a *Streptomyces albus* strain and its secondary metabolite Organophosphate against charcoal rot of sorghum. Plants. 2020;9:17–27. https://doi.org/10.3390/plants9121727.
27. Chen Y, Liu Q, Li L, Zhang Y, Han J, Zhang Y. Screening and identification of antagonistic *Streptomyces* for biocontrol of poplar canker. Sci Silvae Sin. 2021;57:92–100. https://doi.org/10.11707/j.1001-7488.20210710 http://www.linyekexue.net/EN/10.11707/j.1001-7488.20210710.
28. Liu QZ, Chen YQ, Yao MY, Zuo TT, Mao H, Li LM. Optimization for fermentation process of *Streptomyces scopuliridis* HS1. For Pest Dis. 2022;1–10. https://doi.org/10.19688/j.cnki.issn1671-0886.20220024.
29. Li LM, Zheng TT, Chen YQ, Sui Y, Ding RH, Hou LW, Zheng FL, Zhu CY. The antagonistic mechanisms of *Streptomyces sioyaensis* on the growth and metabolism of poplar canker pathogen *Valsa sordida*. Biol Control. 2020;151:104392. https://doi.org/10.1016/j.biocontrol.2020.104392.
30. Atta HM. Biochemical studies on antibiotic production from Streptomyces sp.: taxonomy, fermentation, isolation and biological properties. J Saudi Chem Soc. 2015;19(1):12–22. https://doi.org/10.1016/j.jscs.2011.12.011.
31. Boukaew S, Plubrukam A, Prasertsan P. Effect of volatile substances from *Streptomyces philanthi* RM-1-138 on growth of *Rhizoctonia solani* on rice leaf. Biocontrol. 2013;58:471–82. https://doi.org/10.1007/s10526-013-9510-6.
32. Choudoir M, Rossabi S, Gebert M, Helmig D, Fierer NA. Phylogenetic and functional perspective on volatile organic compound production by *Actinobacteria*. mSystems. 2019;4:295–318. https://doi.org/10.1128/mSystems.00295-18.
33. Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus *Streptomyces*. Arch Microbiol. 2001;176:386–90. https://doi.org/10.1007/s002030100345.
34. Jiang H, Rao YX, Mei L, Wang YJ. Antifungal activity of rapamycin on *Botryosphaeria dothidea* and its effect against Chinese hickory canker. Pest Manag Sci. 2020;77:425–31. https://doi.org/10.1002/ps.6035.
35. Nandi M, Selin C, Brawerman G, Fernando WGD, Kievit TD. Hydrogen cyanide, which contributes to *Pseudomonas chlororaphis* strain PA23 biocontrol, is upregulated in the presence of glycine. Biol Control. 2017;108:47–54. https://doi.org/10.1016/j.biocontrol.2017.02.008.
36. Yang Y, Wu ZM, Li KT. The peculiar physiological responses of *Rhizoctonia solani* under the antagonistic interaction coupled by a novel antifungalmycin N2 from *Streptomyces* sp. N2. Arch Microbiol. 2019;201:787–94. https://doi.org/10.1007/s00203-019-01645-9.
37. Yang Y, Zhang SW, Li KT. Antagonistic activity and mechanism of an isolated *Streptomyces corchorusii* stain AUH-1 against phytopathogenic fungi. World J Microbiol Biotechnol. 2019;35. https://doi.org/10.1007/s11274-019-2720-z.
38. Woo JH, Kamei Y. Antifungal mechanism of an anti-*Pythium* protein (SAP) from the marine bacterium *Streptomyces* sp. strain AP77 is specific for *Pythium porphyrae*, a causative agent of red rot disease in *Porphyra* spp. Appl Microbiol Biotechnol. 2003;62:407–13. https://doi.org/10.1007/s00253-003-1235-5.
39. Jojima T, Inui M. Engineering the glycolytic pathway: a potential approach for improvement of biocatalyst performance. Bioengineered. 2015;6:328–34. https://doi.org/10.1080/21655979.2015.1111493.
40. Zhao L, Cheng YY, Li B, Gu XY, Zhang XY, Boateng NAS, Zhang HY. Integration of proteome and transcriptome data reveals the mechanism involved in controlling of *Fusarium graminearum* by *Saccharomyces cerevisiae*. J Sci Food Agric. 2019;99:5760–70. https://doi.org/10.1002/jsfa.9844.

Publisher's Note