Phytopathology Research

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Biocontrol ability of *Arcopilus aureus* YZXR against *Fusarium fujikuroi* causing leaf spot on *Polygonatum odoratum*

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Abstract

Arcopilus aureus is a plant-beneficial fungal species showing remarkable biocontrol capabilities. We investigated the antifungal activity of *A. aureus* YZXR strain and found that it strongly suppressed *Polygonatum odoratum* leaf spot disease caused by *Fusarium fujikuroi*. However, the antifungal substances and antifungal mechanisms remain unknown. To identify the antifungal substances, we extracted the fermentation liquid with ethyl acetate and found that the antifungal activity was mainly in the organic phase. Using column chromatography, semi-preparative HPLC, and GC–MS assays, we analyzed the compounds in the ethyl acetate phase and identified phenylethyl alcohol and 3,5-dihydroxytoluene as two compounds showing antifungal activity. The phenylethyl alcohol completely inhibited the growth of *F. fujikuroi* at a concentration of 0.25% (v/v), whereas the inhibition rate of 10.00 mg/mL 3,5-dihydroxytoluene on fungal growth was 80.60%. The fermentation broth of strain YZXR efficiently inhibited mycelial growth and conidiation of *F. fujikuroi*. Thus, this study uncovered the antifungal metabolites of *A. aureus* and the potential of *A. aureus* as a biological fungicide for managing *P. odoratum* leaf spot disease, providing a foundation for further research on its biocontrol mechanisms and field applications.

Keywords Biological control, *Arcopilus aureus*, Leaf spot disease, *Polygonatum odoratum*, *Fusarium fujikuroi*

Background

Polygonatum odoratum (Mill.) Druce, also known as fragrant Solomon's seal, is a species of flowering plant in the Asparagaceae family (Liu et al. 2023). *P. odoratum* is native to Europe, China, Caucasus, Siberia, the Russian Far East, Mongolia, Korea, and Japan. In China, it is widely distributed in central and southwest areas (Zhou et al. 2015). The fleshy rhizomes of *P. odoratum* have been extensively used in traditional medicine, from which analyses have revealed multiple medicinal compounds and bioactive substances, including polysaccharides, steroidal saponins, and lectins (Zhao et al. 2018). These components exhibit significant hypotensive, hypoglycemic, anticancer, and antioxidant effects (Deng et al. 2012; Bai et al. 2014; Quan et al. 2015). With the expansion of planting areas, leaf spot has gradually become one

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of the major diseases affecting *P. odoratum*. *P. odoratum* leaf spot disease, caused by *F. fujikuroi*, is categorized as a soil-borne disease with a notable degree of severity. Upon infection, *P. odoratum* plants may exhibit a disease incidence ranging from 60 to 70%, ultimately leading to diminished yield and compromised quality (Xu et al. 2023). This disease significantly hinders *P. odoratum* production in China and hence heightens the importance of disease prevention and control.

At present, the prevention and control of *P. odoratum* leaf spot disease mainly include agricultural control, chemical control, and biological control, but chemical control is mostly used in actual production. However, over-reliance on chemical pesticides makes it easy to produce pesticide residues and environmental pollution. With the increasing concern about food safety and the environment, the use of biocontrol agents to reduce the input of agricultural chemicals in agricultural production is an effective means of sustainable plant disease control (Herman et al. 2008; Sairanen et al. 2012; Nautiyal et al. 2013; Chowdhury et al. 2015).

We have isolated a strain of *Arcopilus aureus* YZXR from the rhizome of *P. odoratum*, which demonstrates robust antagonistic effects and biocontrol potential against plant disease. Ghora and Chaudhuri (1975) investigated the cellulolytic activity of *A. aureus* in earlier studies. *A. aureus* has been harnessed for the control of *Phytophthora capsici* in chili peppers (Liu et al. 2019) and the blast disease *Magnaporthe grisea* and sheath blight pathogen *Rhizoctonia solani* in rice plants (Wang et al. 2013), exhibiting significant fungal inhibition activity. In addition, *Arcopilus aureus* can produce metabolite resveratrol, which is extensively used as a therapeutic moiety. Resveratrol has been linked to various benefits like anti-inflammatory and anti-oxidant activity, preventing diabetes, cardiovascular diseases, and cancer, and as an anti-aging substance (Dwibedi et al. 2018). *A. aureus* is also able to produce cochlioquinol II and riboflavin for further development and industrial application (Amaral et al. 2022).

However, the active factors and antifungal mechanism of *A. aureus* have yet to be reported. In order to fully isolate and identify the active substances in *A. aureus* fermentation solution, two different methods were used. GC–MS and semipreparative HPLC were used to analyze the active substances from the complex fermentation broth. In addition, the antifungal activity of *A. aureus* against different pathogens, the influence of the fermentation broth on mycelial growth and spore formation/release in *F. fujikuroi*, and the disease control effect of the fermentation broth on the leaf spot disease of *P. odoratum* were studied. This research contributes to the understanding of the biocontrol mechanism of *A. aureus*. It provides a foundation for further developing a new strategy for preventing and managing leaf spot disease caused by *F. fujikuroi* on *P. odoratum*, supporting further research into field applications.

Results

The *A. aureus* YZXR showed stronger inhibition ability on plate experiment

Plate standoff assays showed that the strain YZXR strongly inhibited the growth of different pathogens. The rate of inhibition was 62.80% against *F. fujikuroi*, 71.25% on *Colletotrichum spaethianum*, 80.00% on *Alternaria tenuissima*, 69.25% on *Nigrospora oryzae*, 59.25% on *Phytophthora capsici*, 64.00% on *Fusarium oxysporum*, 56.25% on *Fusarium moniliforme*, 82.00% on *Peniophora incarnata*, and 75.50% on *Pestalotiopsis* (Fig. 1). The colony of tested pathogens exhibited irregular and distorted growth patterns (Fig. 1a, b), indicating strong antagonistic activity against the pathogens.

Inhibitory activity of strain YZXR fermentation broth on the growth and conidiation of *F. fujikuroi*

To investigate the inhibitory effect of the fermentation broth on the mycelial growth and spore formation/release in *F. fujikuroi*. The culture dish was supplemented with 19 mL of V8 (ELITE-MEDIA) nutrient solution. To the experimental groups, 1 mL of strain YZXR fermentation broth was added, while 1 mL of sterile water was added to the control group. Blend the nutrient solution independently, and 5 fungal plugs (8 mm in diameter) were placed in each culture dish. After cultivating the experimental and control groups under identical conditions for 3 days, vigorous growth of mycelia could be observed in the control medium, whereas much smaller colonies could be seen in the medium supplemented with 1 mL of strain YZXR fermentation broth (Fig. 2a). We subsequently collected the culture's mycelia and measured the fungal dry weight. Results showed that the dry weight of the mycelia grown in the control medium was 2.59 times more than that in the treated medium (Fig. 2b). Consequently, the inhibition rate of strain YZXR fermentation broth on *F. fujikuroi* mycelial growth was 61.43%. This indicates the potent inhibitory effect of the strain YZXR fermentation broth on *F. fujikuroi* growth.

Microscopic examination of fungal conidiation and mycelial morphology showed that the spores were more dispersed and much less in number in the medium treated with strain YZXR fermentation broth. In contrast, in the control medium, the spores were more concentrated and significantly more than those in the treatment group (Fig. 2c, d). In addition, fungal mycelia grown in the treated medium were enlarged and ruptured (Fig. 2d) compared to the control. These findings suggest that the

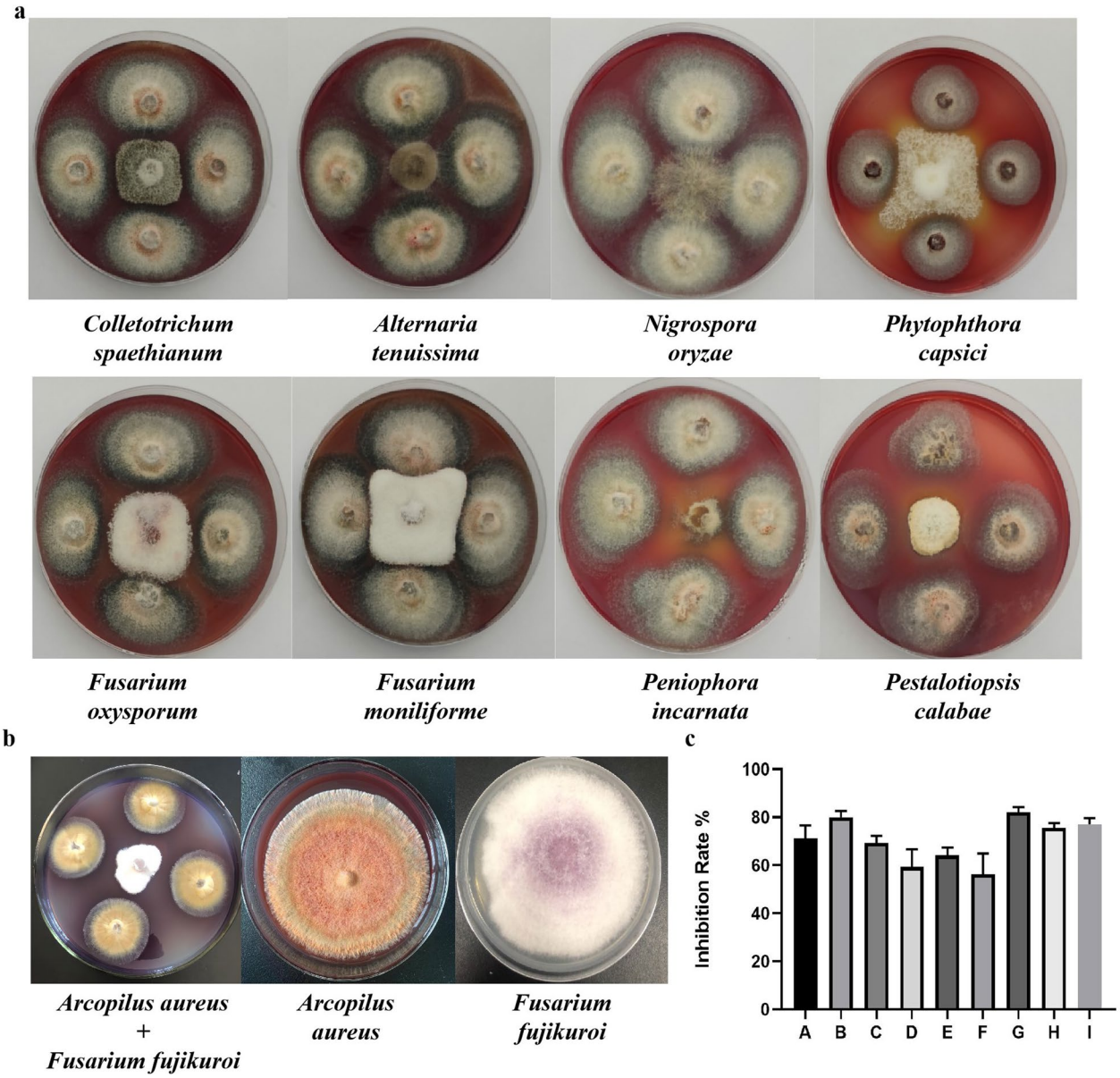

Fig. 1 Antimicrobial activity tests of strain YZXR. **a, b** Inhibitory effect of strain YZXR on the growth of nine filamentous phytopathogens, and the culture plate of *A. aureus* YZXR strain and *Fusarium fujikuroi*. **c** Inhibition rate data of **a, b**. A: *Colletotrichum spaethianum*; B: *Alternaria tenuissima*; C: *Nigrospora oryzae*; D: *Phytophthora capsici*; E: *Fusarium oxysporum*; F: *Fusarium moniliforme*; G: *Peniophora incarnata*; H: *Pestalotiopsis calabae*; I: *Fusarium fujikuroi*

fermentation broth of strain YZXR can inhibit the mycelial growth and conidiation of *F. fujikuroi*.

The effect of strain YZXR fermentation broth on disease control in *P. odoratum*

To test the impact of *A. aureus* strain YZXR on *P. odoratum* leaf spot disease, *P. odoratum* seedlings were sprayed with YZXR fermentation broth (1.8×10^8 spores/mL), and the treated plants were inoculated with *F. fujikuroi* conidial suspension (1.5×10^7 spores/mL) 48 h after treatment. Ten days after the inoculation, the disease incidence, disease index, and biocontrol effect were calculated by observing the leaf spot size. As shown in Fig. 3 and Table 1, the YZXR fermentation broth-treated plants had very limited size of lesion spots, while the control plants had obviously larger disease lesions. The treatment suppressed the disease by 60.55%. These observations indicate that the strain YZXR fermentation broth

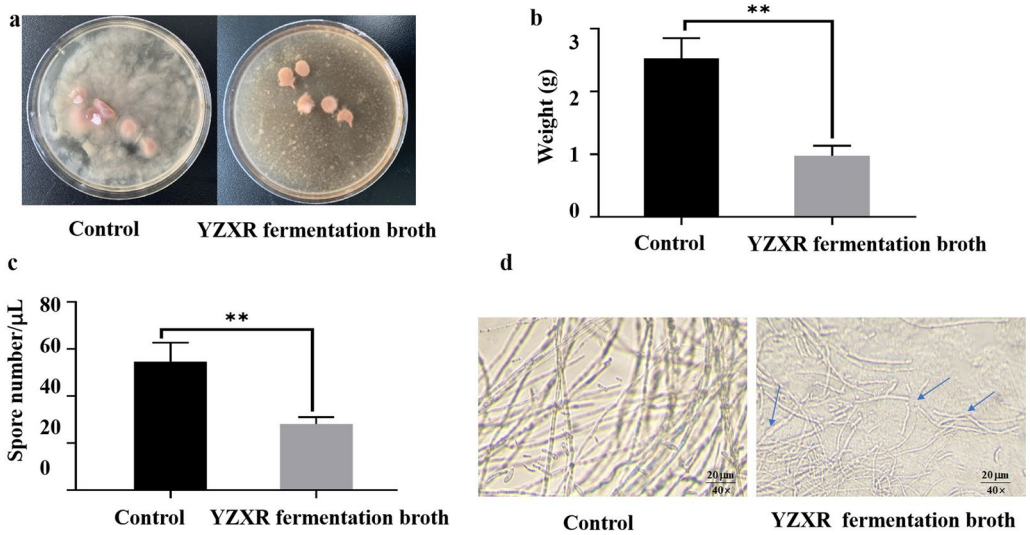

Fig. 2 Inhibitory effect of the fermentation broth on the growth and conidiation of *F. fujikuroi*. **a** Growth status of *F. fujikuroi* under different conditions. Five mycelial plugs (8 mm in diameter) of *F. fujikuroi* were inoculated into V8 nutrient solution supplemented with YZXR fermentation broth and the culture dishes were cultured at 28°C for 3 days. V8 solution supplemented with sterile water was used as a control. **b** Analysis of mycelial dry weight data. The mycelia were harvested, and the excessive nutrient solution was removed before drying at 80°C in an oven for 2 h. **c** The inhibition of conidiation by the fermentation broth. An aliquot of 100 μL of solution near the mycelium was taken to count the spore number under a microscope. **d** The impact of YZXR fermentation broth on the mycelial morphology of *F. fujikuroi*. The arrows showed the mycelium undergoing breakage and expansion. Data shown are mean ± SE; ** indicate significant difference (F-test, $P < 0.01$)

Fig. 3 Biocontrol efficacy of YZXR fermentation broth on *P. odoratum* leaf spot disease. The treatment group (YZXR group sprayed with YZXR fermentation broth followed by inoculation with *F. fujikuroi* conidial suspension) and the control group (sprayed with sterile water followed by inoculation with *F. fujikuroi* conidial suspension) were given a 12-h/12-h light/dark cycle at 25°C, after 10 days to observe the formation of leaf spots. Each group had five pots as a repetition, and each pot had one seedling. The experiment was repeated three times

Table 1 Biocontrol efficacy of YZXR fermentation broth on *P. odoratum* leaf spot disease

Treatment	Disease incidence (%)	Disease index	Biocontrol effect (%)
Control	83.32 ± 5.56 b	60.16 ± 4.70 b	0.00
YZXR	60.37 ± 4.49 a	23.65 ± 0.57 a	60.55 ± 2.69

Statistical analysis was performed using the SPSS 21.0 software by one-way ANOVA with Tukey's test. Different letters in the columns represent significant differences ($P < 0.05$). The data in the table represent means ± SE

has significant disease control effect on *P. odoratum* leaf spot disease.

Identification of the bioactive compounds in strain YZXR fermentation broth by GC–MS

The ethyl acetate and aqueous crude extracts of the fermentation broth of strain YZXR were prepared and subsequently subjected to plate assay. Results showed that the ethyl acetate crude extracts (60 mg/mL) substantially inhibited the growth of *F. fujikuroi*. In contrast, the extracts of the aqueous phase had no effect to *F. fujikuroi* on the plate (Fig. 4). Thus, the inhibitory compounds are contained within the ethyl acetate crude extracts, which warrants further investigation. GC–MS analysis identified several secondary metabolites of interest from the ethyl acetate crude extracts based on factors such as peak area, retention time, molecular weight, molecular

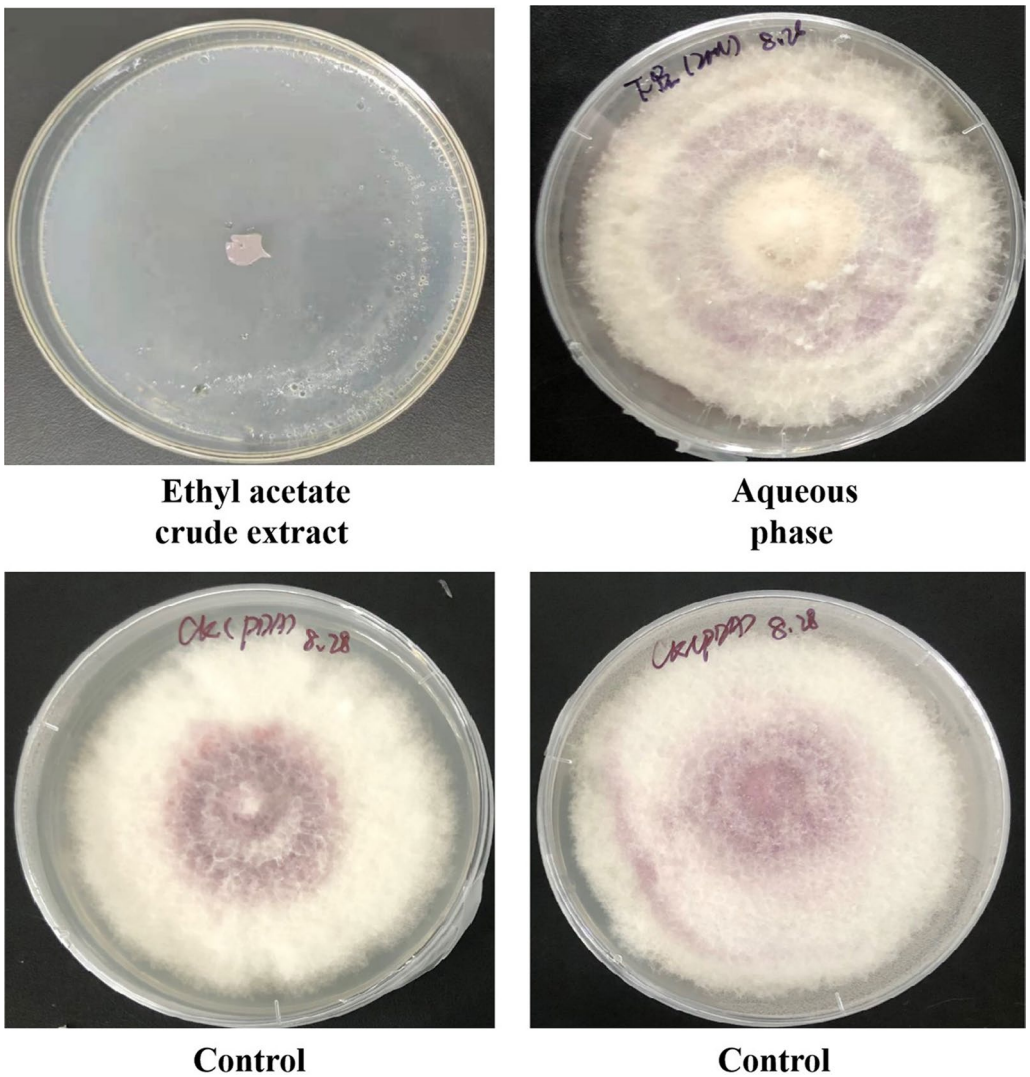

Fig. 4 Growth inhibition activity of the crude extracts of strain YZXR on *F. fujikuroi*. The fungus was cultivated on PDA supplemented with crude extracts of ethyl acetate phase and aqueous phase (10%, v/v) at 25°C for 7 days. The ethyl acetate extract of PDB medium (10%, v/v) was used as a control

formula, and antimicrobial properties. These compounds include p-xylene, ethyl pivaloylacetate, undecane, phenylethyl alcohol, dodecane, tridecane, and 3-methoxy-5-methylphenol, as illustrated in Fig. 5a and Table 2.

We obtained the listed compounds from the China Standard Materials Network and tested their activity against *F. fujikuroi*. Results showed that phenylethyl alcohol exhibited significant fungicidal effects, and the other compounds p-xylene, ethyl pivaloylacetate, undecane, dodecane, tridecane, and 3-methoxy-5-methylphenol had no antifungal activity. Specifically, 400-fold dilution (0.25%, v/v) of phenylethyl alcohol completely inhibited the growth of *F. fujikuroi* on plates, while the inhibition rate gradually dropped to 74.80% and 38.70% when the fold of dilution increased to 800 and 1000, respectively, compared to the sterile water control (Fig. 5b, c). We compared the retention time of the phenylethyl alcohol standard with the peak detected in the fermentation broth of YXZR and results showed that both of them were eluted at approximately 12 min under the same assay conditions (Figs. 5a, 6a). Moreover, the MS results showed that the sample extracted from YXZR fermentation broth had fragment peaks at m/z 122 and m/z 91, which correlate well with those of phenylethyl alcohol (Fig. 6b). These results confirmed that phenylethyl alcohol is one of the compounds that inhibit pathogen growth in the strain YZXR fermentation broth.

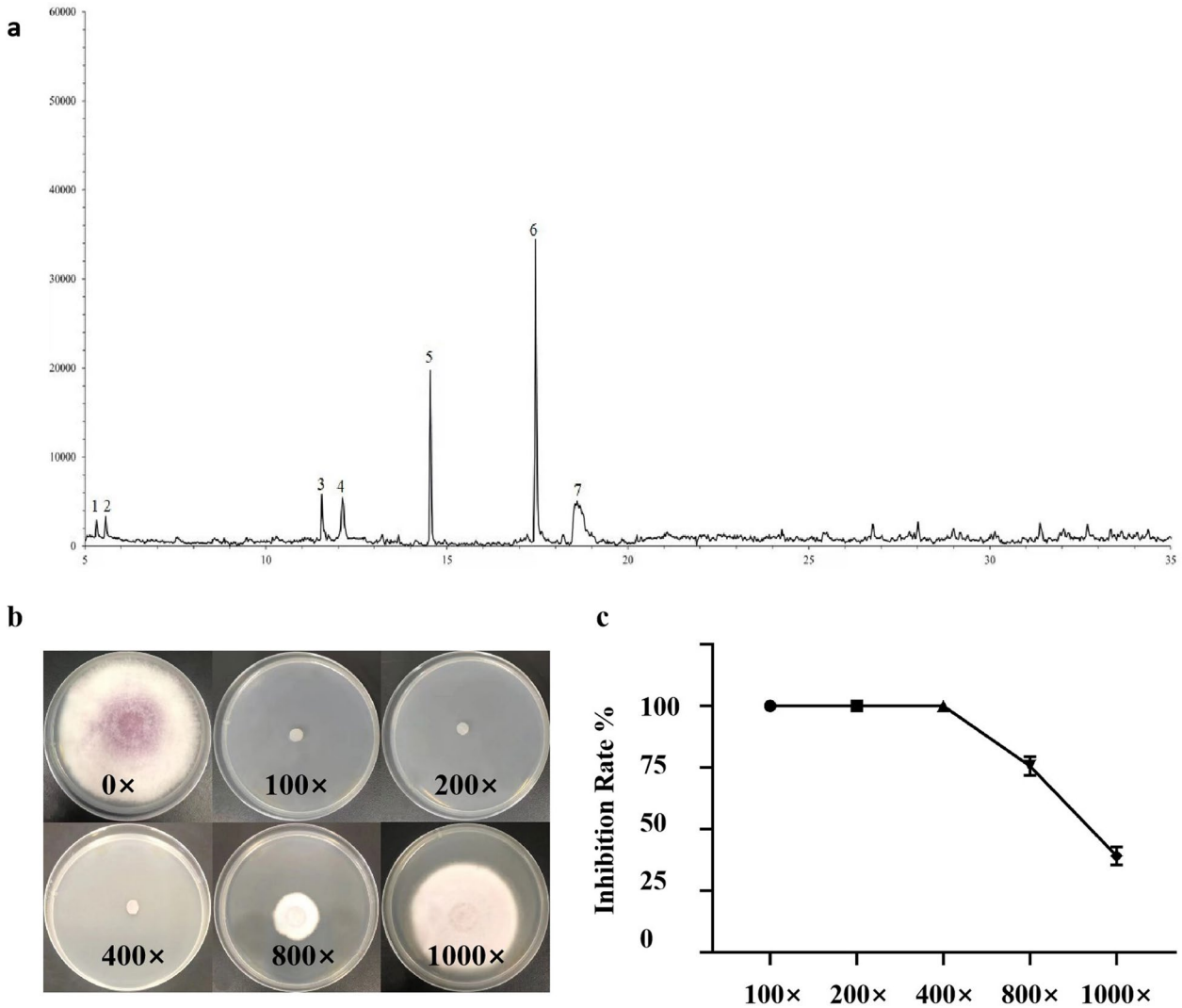

Fig. 5 Identification of bioactive compounds by GC–MS. **a** GC–MS chromatogram, peaks 1, 2, 3, 4, 5, 6, and 7 correspond to p-xylene, ethyl pivaloylacetate, undecane, phenylethyl alcohol, dodecane, tridecane, and 3-methoxy-5-methylphenol, respectively. **b** The inhibitory effect of phenylethanol on *F. fujikuroi*. **c** Inhibition rate of phenylethanol on *F. fujikuroi* at different dilutions

Table 2 Candidate functional compounds identified by GC–MS analysis

S. no	Retention time	Compound name	Mol. weight (g/mole)	Formula	Antifungal biological activity
1	5.316	p-Xylene	106	C_8H_{10}	–
2	5.578	Ethyl pivaloylacetate	172	$C_9H_{16}O_3$	–
3	11.54	Undecane	156	C11H24	–
4	12.116	Phenylethyl Alcohol	122	$C_8H_{10}O$	+
5	14.548	Dodecane	170	$C_{12}H_{26}$	–
6	17.45	Tridecane	184	$C_{13}H_{28}$	–
7	18.601	3-Methoxy-5-methylphenol	138	$C_8H_{10}O_2$	–

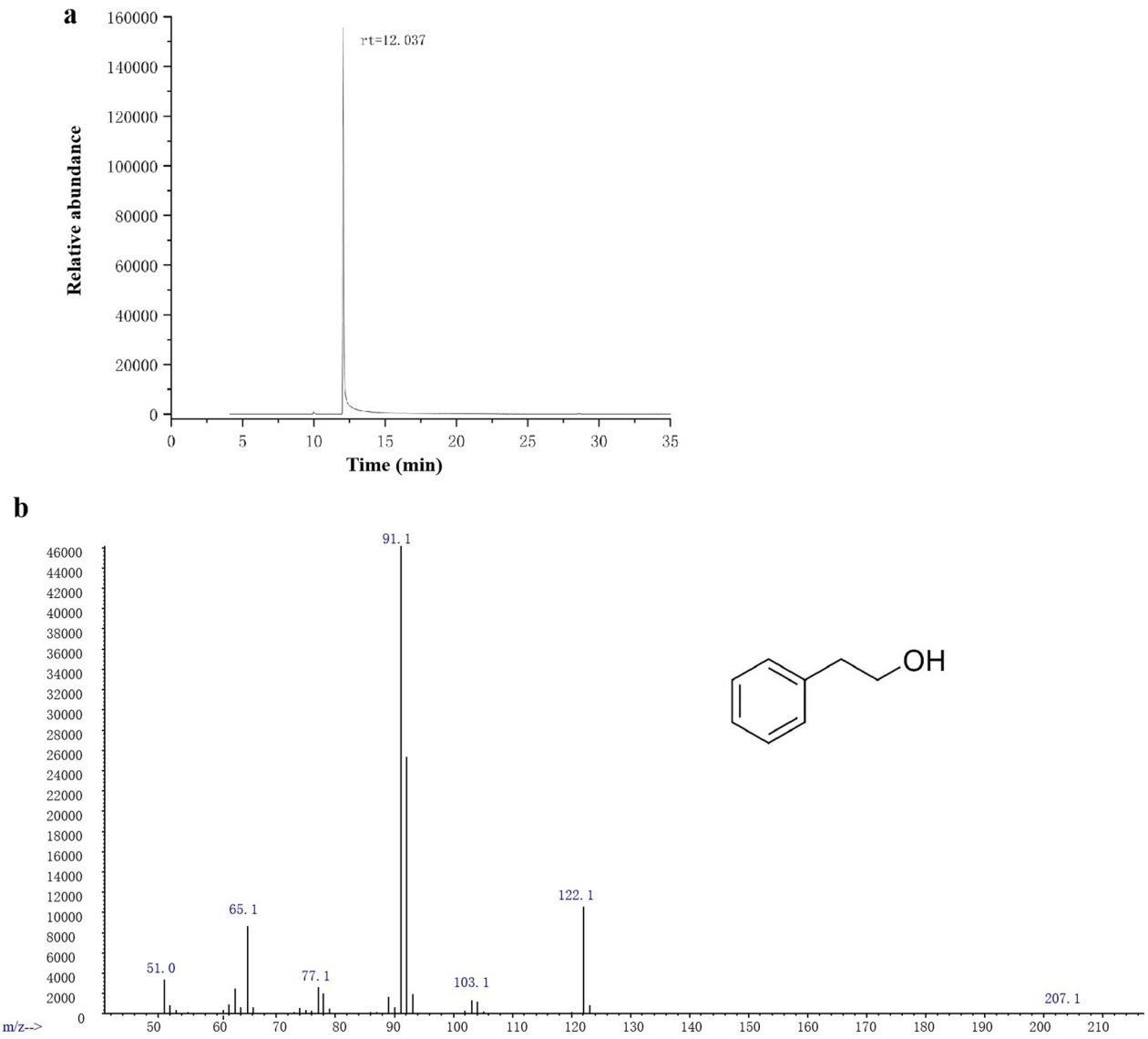

Fig. 6 Validation of the active compound as phenethyl alcohol. **a** The retention time of the phenylethyl alcohol standard (compare with peak 4 in Fig. 5a). **b** MS results of peak 4 in YXZR fermentation broth**.** The fragments peak at m/z 122 and m/z 91, which correlate well with those of phenylethyl alcohol

Isolation and identification of the bioactive compounds with column chromatography and preparative HPLC

The ethyl acetate crude extracts (1.0 g) was separated using column chromatography (silica gel) with a solvent of dichloromethane and methanol (dichloromethane: methanol = 19:1, 9:1, 4:1, 3:2, 0:10), a total of 14 fractions (XR1-5–1 to XR1-5–14) were obtained, with a collective weight of 994.6 mg. These 14 fractions were analyzed by TLC, and only XR1-5–7 exhibited a single spot on TLC plates with dispersing agents (dichloromethane: methanol = 10:1), while the other 13 fractions didn't exhibit spots on TLC plates. This analysis showed that the majority of the extracts were XR1-5–7. Then the semi-preparative liquid chromatography was used to further purify the fraction of XR1-5–7, and finally the pure substance XR1-10–1 (tR = 15.6 min) was obtained. The pure substance XR1-10–1 was analyzed using proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopy on a superconducting NMR instrument. The structure of XR1-10–1, identified as 3,5-dihydroxytoluene, was determined based on the chemical shifts and coupling patterns of carbon and hydrogen atoms (Fig. 7a). The detailed analysis and assignments of the positions of the carbon and hydrogen atoms are presented in Table 3.

The 3,5-dihydroxytoluene was obtained from the China Standard Materials Network and tested its activity against *F. fujikuroi*. The assessment of the activities of 3,5-Dihydroxytoluene was used at 100-fold dilution (10.00 mg/mL), 200-fold dilution (5.00 mg/mL), 400-fold dilution (2.50 mg/mL), 800-fold dilution (1.25 mg/mL), and 1000-fold dilution (1.00 mg/mL), and the fungicidal effects against *F. fujikuroi* were 80.60%, 50.00%, 25.60%, 19.00%, and 0.00% respectively. The control group (sterile water), with an inhibition rate of 0.00%, as depicted in Fig. 7b, c.

Table 3 ^{1}H and ^{13}C NMR chemical shifts of orcinol (XR1-10-1) in CD_3OD (600 MHz and 125 MHz, TMS as an internal standard, chemical shifts in δ values)

Num	^{13}C	^{1}H
1	108.6	6.11
2	159.4	
3	100.7	6.05
4	159.4	
5	108.6	6.11
6	141.2	
7	21.6	2.16

Discussion

The bioactive compounds obtained through column chromatography with preparative HPLC and GC–MS analysis are different. The bioactive compound obtained by GC–MS was phenylethyl alcohol, while 3,5-dihydroxytoluene was obtained by column chromatography with preparative HPLC. The discrepancy arises from the distinct principles underlying these two methods. While column chromatography with preparative HPLC yields a single compound (Latif et al. 2012), a more comprehensive range of active components can be separated through further investigations.

Phenylethyl alcohol is widely used in pharmaceutical, chemical, and other industries, with a global output of more than 10,000 tons, and is the second-largest spice substance in the world (Zhang et al. 2014). Phenylethyl alcohol has many biological functions and can be used as a fresh-keeping agent and a natural fungicide (Sun et al. 2023). Studies on the biological control function of phenylethyl alcohol have found that phenylethyl alcohol can destroy the cell structure of *Fusarium graminearum* and inhibit its production of mycotoxins, thus controlling wheat disease (Lu et al. 2023). Phenylethyl alcohol was found to be one of the main VOCs emitted by the yeast strains, which were proven to efficiently suppress

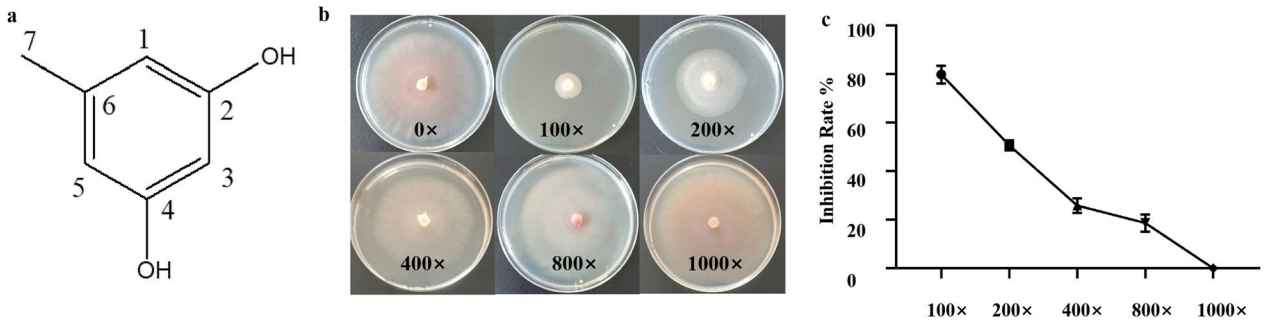

Fig. 7 Identification of bioactive compounds by column chromatography and preparative HPLC. **a** Structure of 3,5-dihydroxytoluene. **b** Inhibitory effect of different concentrations of 3,5-dihydroxytoluene on *F. fujikuroi*. **c** The inhibition rate of 3,5-dihydroxytoluene to *F. fujikuroi* at different dilutions

the growth of fungi. An antifungal substance, 2-phenylethanol (PEA), was isolated from *K. apiculata* and demonstrated to have antimicrobial activity against selected phytopathogenic fungi (Liu et al. 2014). It has been reported that the inhibition of aflatoxin production by the low level of 2-PE is due to its effect on promoting the active growth of *A. flavus* (Chang et al. 2015). Phenylethyl alcohol can also control *Phytophthora infestans* by blocking oxidative phosphorylation pathway, inhibiting ATP production of *P. infestans* and causing the death of the pathogen (Zou et al. 2023). Phenylethyl alcohol can also be used as a substrate to synthesize other high-value compounds, such as ethyl phenylacetate, which can be used as a fragrance and neuropathic drug, 3,5-dihydroxytoluene (Scognamiglio et al. 2012).

3,5-Dihydroxytoluene is a highly sensitive analytical chemical reagent, mainly found in lichen plants. 3,5-Dihydroxytoluene has strong oxidase inhibition activity and can be used in the processing and storage of fruit and vegetable products to prevent browning. Because the molecule contains two hydroxyl groups, it is easy to oxidize and deteriorate, which makes it difficult to synthesize (Zhang et al. 2022).

Phenylethyl alcohol and 3,5-dihydroxytoluene have great market potential as fresh-keeping agents and natural fungicides. This study found that *A. aureus* (YZXR) can synthesize phenylethyl alcohol and 3,5-dihydroxytoluene, which provides an additional research basis for the biosynthesis of phenylethyl alcohol and 3,5-dihydroxytoluene.

Conclusions

This study provides more theoretical backgrounds for the further development and utilization of the biocontrol fungus *A. aureus.* The main findings of this study are as follows: (1) *A. aureus* (YZXR) has good antifungal activity against different pathogens; (2) The fermentation broth of strain YZXR strongly inhibits mycelial growth and spore formation and release in *F. fujikuroi*; (3) Two different methods (GC–MS and semipreparative HPLC) are used to isolate and identify complex fermentation broth of active compounds. Phenylethyl alcohol and 3,5-dihydroxytoluene have strong biological activity, which can effectively inhibit the growth of *F. fujikuroi*; (4) The YZXR fermentation broth has a significant disease control effect on *P. odoratum* leaf spot disease.

Methods

Strains and culture media

Tested strains: *A. aureus* (YZXR) (isolated from the rhizome of *P. odoratum*), *F. fujikuroi* strains (isolated from the leaves of *P. odoratum*), *Colletotrichum spaethianum* (isolated from the leaves of *Polygonatum sibiricum*), *Alternaria tenuissima* (isolated from the leaves of *Peucedanum praeruptorum*), *Nigrospora oryzae* (isolated from the leaves of *Gardenia jasminoides*), *Phytophthora capsici* (isolated from *Capsicum annuum*), *Fusarium oxysporum* (isolated from the stem of *P. odoratum*), *Fusarium moniliforme* (isolated from the rhizome of *P. odoratum*), *Peniophora incarnata* (isolated from the leaves of *Paeonia lactiflora*), and *Pestalotiopsis calabae* (isolated from the leaves of *Lilium brownii* var. *viridulum*) were procured from the Institute of Agricultural Environment and Ecology, Hunan Academy of Agricultural Sciences.

Culture media: PDA (potato 200 g, dextrose 20 g, agar 15 ~ 20 g, distilled water 1000 mL, natural pH) served as the medium for fungal culture and inhibition tests, while PDB (potato 200 g, dextrose 20 g, distilled water 1000 mL, natural pH) was used for the fermentation culture of *A. aureus*.

Experiments on plate standoff of *A. aureus* (YZXR) against different pathogens

To test the inhibitory effect of *A. aureus* YZXR against different pathogens, we analyzed the antagonistic activity against a panel of filamentous plant pathogens. The mycelial plug (8 mm) of tested pathogenic strains was placed at the center of a PDA plate and four plugs (8 mm) of *A. aureus* were positioned 2 cm away from the tested pathogenic strains (Balouiri et al. 2016). The inoculated plates were sealed and inverted before they were incubated at 28°C. The plates inoculated with only tested strains were used as controls. Each treatment was repeated three times (Berendsen et al. 2018). The colony diameter was measured when the control plate was fully covered by colonies. The inhibition rate was calculated by the following formula: Inhibition rate = (D1 − D2)/D1 × 100%, where D1 is the colony diameter of the control and D2 is that of the treatment (Kamaruzzaman et al. 2021).

Antimicrobial activity of the fermentation broth of strain YZXR

Ten mycelial plugs from vigorously growing colonies on PDA plates were inoculated into flasks containing PDB medium. The flasks were shaken at 180 rpm under 28°C for 3–4 days. The fermentation broth of YZXR was centrifuged at 10,000 rpm for 10 min, the supernatant was collected, then pumped and filtered. Further impurities were removed using 0.22 μm bacterial filters to obtain the YZXR filtrate.

To investigate the inhibitory effect of the fermentation broth on the growth of *F. fujikuroi* mycelia, five mycelial plugs (8 mm in diameter) from colonies grown for 2–3 days at 28°C were inoculated into a culture dish containing 19 mL of V8 (ELITE-MEDIA) nutrient solution

supplemented with 1 mL of YZXR fermentation broth. The V8 solution supplemented with 1 mL of sterile water was inoculated as a control. The inoculated culture dishes were incubated at 28°C for 3 days and the mycelium of *F. fujikuroi* was picked for observation of the morphology (El-Dawy et al. 2021). The mycelia were harvested and excessive nutrient solution was removed before drying at 80°C in an oven for 2 h (Antinori et al. 2021). The inhibition rate of mycelial growth was calculated using the following formula: Inhibition rate = (W1 − W2) / W1 × 100%, where W1 and W2 are dry weight of mycelial samples collected from control and treatment, respectively. The experiment was repeated three times.

To examine the inhibitory effect of fermentation broth on fugal conidiation, five *F. fujikuroi* mycelial plugs were inoculated into a culture dish containing 20 mL of V8 nutrient solution and cultured at 28°C for 3 days. The liquid medium was removed. An aliquot of 19 mL of sterile water and 1 mL of fermentation broth was added to treat the mycelia and 20 mL of sterile water was added as control. The dishes were incubated at 28°C for another day and a small amount of mycelia was collected for morphological observation. The remaining culture was incubated at 4°C for 30 min, followed by an additional 30 min incubation at 28°C. An aliquot of 100 µL of solution near the mycelium was taken to count the spore number under a microscope (Lobb et al. 2015). The inhibition rate on conidiation by the fermentation broth was calculated using the following formula: Inhibition rate = (N1 − N2) / N1 × 100%, where N1 and N2 are spore numbers in the control and treatment, respectively. The experiment was repeated three times.

Analysis of the effect of strain YZXR fermentation broth on disease control in *P. odoratum*

Healthy *P. odoratum* potted plants were selected as the treatment group (YZXR) and control group. The treatment group seedlings were sprayed with YZXR fermentation broth (1.8×10^8 spores/mL) and the control group seedlings were sprayed with sterile water. Each seedling was bagged for 48 h, and then the leaves of *P. odoratum* seedlings (each seedling picked 4 leaves) of the two groups were inoculated with *F. fujikuroi* conidial suspension (1.5×10^7 spores/mL). The inoculated plants were cultured in a 12-h/12-h light/dark cycle at 25°C for 10 days before the observation of the leaf spots caused by *F. fujikuroi*. Each group had five replicates with one seedling in each pot. The experiment was repeated three times.

Disease severity was rated on a scale from 0 to 6 according to the percentage of symptomatic areas in each leaf: scale 0, no symptoms; scale 1, 10% or less symptomatic areas; scale 2–6 represent 11–25%, 26–50%, 51–75%, 76–90%, and > 90% of symptomatic areas, respectively (Gu et al. 2021). The disease index, disease incidence, and disease control effect were calculated as follows:

Disease index = ∑(Rating × Number of diseased leaves rated) / (Total number of investigated leaves × Highest rating) × 100

Disease incidence (%) = (Total number of diseased leaves / Total number of investigated leaves) × 100

Disease control effect (%) = [(Disease incidence of the control − Disease incidence of the treatment) / Disease incidence of the control] × 100

Identification of the active components of strain YZXR

Crude extraction from strain YZXR

The fermentation broth prepared as described was mixed with an equal volume of ethyl acetate and vigorously shaken in a separatory funnel. The organic phase and the aqueous phase were collected and subjected to rotary evaporation (60 rpm) at 50–60°C and 60–65°C, respectively. The resulting solid ethyl acetate layer was recovered and labeled; the aqueous phase was collected when a nearly constant volume was reached, after which the mixture was freeze-dried to harvest the product. The PDB ethyl acetate-extracted concentrate was also processed in the same way.

Growth inhibition activity of the crude extract of strain YZXR

The upper organic phase and the lower aqueous phase were sterilized using 0.22 µm bacterial filters (Matyash et al. 2008). The 50 mL sterile upper organic and lower aqueous phases were separately mixed in 450 mL PDA agar media at 50–60°C, resulting in a final concentration of 10% (v/v). The medium was poured into Petri dishes. The ethyl acetate extract of PDB medium was used as a control (10%, v/v). Mycelial plugs of the tested pathogen (6 mm in diameter) were placed at the center of each plate. The plates were incubated at 25°C and colony diameter was measured when the control colonies covered the entire dish. The inhibition rate was calculated as follows: Inhibition Rate = (D3 − D4) / D3 × 100%, where D3 and D4 are colony diameters of the control and treatments, respectively.

GC–MS analysis of bioactive components

The metabolites were analyzed using a GC–MS system (Agilent 7890/5975B GC–MS; Agilent Technologies, Inc., Beijing, China). This system was equipped with an HP-5MS quartz capillary column measuring 30 mm in length, 0.25 µm in internal diameter, and 0.25 µm in film thickness. The initial temperature was 50°C for 1 min. Subsequently, the temperature was increased at a rate of 5°C/min until it reached 240°C, which was maintained for 2 min. The temperature was further increased at a

rate of 30°C/min, reaching 300°C and remaining constant for 5 min. Helium served as the carrier gas, flowing at a linear velocity of 27.4 cm/s. The MS parameters included electron ionization at 71 eV, an ion source temperature of 230°C, a quadrupole temperature of 150°C, and a mass range of 50–650 m/z (Amirav et al. 2021). Peaks representing various compounds eluted from the GC column were recorded along with their retention times. The data were subsequently cross-referenced with the mass spectra of these compounds, and a database search was conducted to identify similar compounds with matching retention times and molecular masses (Cottrell et al. 2011). All the peaks were identified by comparing their mass spectra with those of NIST 11 libraries (McLafferty, F.W. Wiley Registry of Mass Spectral Data, available at the National Institute of Standards and Technology, USA; http://www.nist.gov) (Hoffmann et al. 2022).

Bioactivity test of the isolated and identified compounds

The compounds detected through GC–MS were obtained from the China Standard Materials Network and subjected to activity verification. These compounds were then tested for their antimicrobial activity against *F. fujikuroi*. Under the same GC–MS conditions, compounds that have an obvious inhibitory effect on *F. fujikuroi* were selected for GC–MS analysis (Bömke et al. 2009). The peaks of standard compounds and fermentation broth were compared for their retention times, mass spectra, and structure to finally identify the active compounds (Ito et al. 2014).

Isolation of bioactive components by column chromatography and semi-preparative HPLC techniques

The dry crude extracts were dissolved in methanol (10 mg/mL). Dichloromethane: methanol (10:1, 10 mL) was employed as the mobile phase for thin layer chromatograph analysis. The resulting plate was observed under 254 nm UV light and then sprayed with vanillin-sulfuric acid reagent, followed by heating for coloration (Santiago et al. 2013). For column chromatography, a total of 30 g of silica gel was soaked in dichloromethane, and packed into a column. Furthermore, 1.0 g of the extracted sample was mixed with silica gel, and applied to the column. Solvent of dichloromethane: methanol = 19:1, 9:1, 4:1, 3:2, 0:10 (each gradient in volume of 500 mL) were sequentially used to eluted the sample (Arakawa et al. 2008). The fractions were analyzed by TLC and then combined. The target fraction was dissolved in methanol and passed through a 0.22 μm microporous filter for further separation using semi-preparative liquid-phase chromatography. Gradient elution with methanol/water as the mobile phase was conducted under the following conditions: 0–10 min, 0% methanol; 15 min, 10% methanol; 20 min, 30% methanol; 29 min, 95% methanol; and 30 min, 0% methanol; with a flow rate of 3 mL/min. Fractions with UV absorption peaks were collected, concentrated, and dried using a rotary evaporator under reduced pressure.

Structural identification and verify the biological activity of the compounds

The individual compounds were analyzed using deuterated methanol on a 600 MHz superconducting nuclear magnetic resonance (NMR) spectrometer (Bruno et al. 2024). The structures of the individual compounds were determined based on the NMR results (Fowler et al. 2020). The compounds detected through semi-preparative HPLC techniques were obtained from the China Standard Materials Network. These compounds were then tested for their antimicrobial activity against *F. fujikuroi*.

Abbreviations

ATP	Adenosine triphosphate
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid Chromatography
NMR	Nuclear magnetic resonance
PDA	Potato dextrose agar
PDB	Potato dextrose broth
TLC	Thin-layer chromatography
VOCs	Volatile organic compounds

Acknowledgements

We would like to thank Dr. Kailong Li for sharing the GC-MS analytical instruments.

Author contributions

RX, YH, and RS contributed to the study conception and design. SS, XW, SP, and YX performed the experiments. LC, JX, and YY analysed the data. RX wrote the first draft of the manuscript. GW, SZ, and BH commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Changsha Natural Science Foundation of China (kq2208106); Research and application of early diagnosis and biological control of tobacco leaf spot disease (HYYC2023KJ34).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interests.

Received: 6 March 2024 Accepted: 26 August 2024
Published online: 05 December 2024

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