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Identification of a novel strain, *Streptomyces blastmyceticus* JZB130180, and evaluation of its biocontrol efficacy against *Monilinia fructicola*[*#]

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Abstract: Peach brown rot, caused by *Monilinia fructicola*, is one of the most serious peach diseases. A strain belonging to the Actinomycetales, named *Streptomyces blastmyceticus* JZB130180, was found to have a strong inhibitory effect on *M. fructicola* in confrontation culture. Following the inoculation of peaches in vitro, it was revealed that the fermentation broth of *S. blastmyceticus* JZB130180 had a significant inhibitory effect on disease development by *M. fructicola*. The fermentation broth of *S. blastmyceticus* JZB130180 had an EC_{50} (concentration for 50% of maximal effect) of 38.3 μg/mL against *M. fructicola,* as determined in an indoor toxicity test. Analysis of the physicochemical properties of the fermentation broth revealed that it was tolerant of acid and alkaline conditions, temperature, and ultraviolet radiation. In addition, chitinase, cellulase, and protease were also found to be secreted by the strain. The results of this study suggest that *S. blastmyceticus* JZB130180 may be used for the biocontrol of peach brown rot.

Key words: *Streptomyces blastmyceticus*; Peach brown rot; *Monilinia fructicola*; Biocontrol efficacy
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1 Introduction

The peach is one of the most popular fruits worldwide, and the brown rot disease caused by fungus *Monilinia fructicola* severely reduces the yield of peach and other stone fruits (Hu et al., 2011; Poniatowska et al., 2013; Pei et al., 2019). Currently, the application of chemical pesticides can result in many problems, such as environmental issues and food security. Thus, the identification of "green" microbial pesticides has become a major research focus.

Actinomycetes are an important microbial resource and are widely distributed in nature (Sharma et al., 2014). To date, among the active compounds

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identified from microbial sources, about two-thirds are produced by actinomycetes, including validamycin (Yu et al., 2005), kasugamycin (Ikeno et al., 2002), and qingfengmycin (Wu et al., 1994), which are commonly used in agriculture (Bérdy, 2005). Many secondary metabolites in actinomycetes can be classified into different groups, such as polyketones, non-ribosomal peptides, terpenes, and aldehydes. The synthesis of most secondary metabolites is regulated by gene clusters (Liu et al., 2013). The exploration and utilization of products with antibacterial activity from actinomycetes are important for the biocontrol of plant diseases.

Actinomycetes, especially of the genus *Streptomyces*, can also produce a series of cell wall-degrading enzymes (CWDEs), such as chitinase, cellulase, and protease (Yuan and Crawford, 1995). These enzymes have an antagonistic effect by degrading the cell walls of plant-pathogenic fungi, highlighting the potential importance of *Streptomyces* in the biological control of fungi. Evaluation of the activity of CWDEs may serve as a simple and powerful means to screen for strains of *Streptomyces* for use in biocontrol (Kubicek et al., 2014).

In this study, an actinomycete strain was isolated from soil collected from Thunder Mountain in Guizhou Province, China, and has been identified as *Streptomyces blastmyceticus* and designated as JZB 130180. Through dual-culture and an in vitro peach fruit experiment, the inhibitory effect of *S. blastmyceticus* JZB130180 on the peach brown rot pathogen was investigated. This study provides a theoretical foundation for the biocontrol of peach brown rot disease using *S. blastmyceticus* JZB130180.

2 Materials and methods

2.1 Isolation and morphological and molecular identification of a novel strain

A soil sample was collected at Thunder Mountain, Guizhou Province, China via the diagonal method. No specific permissions were required for activities related to the collection of a soil sample at this location as the land owner gave their permission to conduct the study on this site. Further, the field studies in this work did not involve endangered or protected species.

Via dilution plating, Gause's medium was used to isolate *Streptomyces* spp., among which a novel strain, *S. blastmyceticus* JZB130180, was discovered. Its morphology was observed on King's broth agar (KBA), lysogeny broth agar (LBA), potato dextrose agar (PDA), and Gause's medium.

The classification was confirmed by sequencing of the 16S ribosomal DNA (rDNA), *atpD*, and *recA* genes. *S. blastmyceticus* JZB130180 was cultured in potato dextrose (PD) broth in a rotary shaker for 48 h at 28 °C and 180 r/min. The supernatant was removed by centrifugation, pelleted cells were collected, and genomic DNA was extracted by cetyltrimethyl ammonium bromide (CTAB) method (Wang et al., 2011). Using the genomic DNA of *S. blastmyceticus* JZB130180 as the template, the 16S rDNA, *atpD*, and *recA* sequences were amplified by polymerase chain reaction (PCR) using corresponding primers (Table S1) (Weisburg et al., 1991; Guo and Huang, 2007). The resulting sequences were compared with those of known strains using the National Center for Biotechnology Information (NCBI) BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree based on the concatenated 16S rDNA, *atpD*, and *recA* sequences was constructed (Vaidya et al., 2011) using the maximum parsimony (MP) method in PAUP* 4.0b10, and the support of branching topologies was derived from 1000 replicates with 10 random additions (Wilgenbusch and Swofford, 2003).

2.2 Detection of biocontrol efficacy of *S. blastmyceticus* JZB130180 against *Monilinia fructicola*

Discs were removed from 4-d-old cultures of *M. fructicola* using a sterilized hole-punch. Each disc was inverted and placed in the center of a 9-cm Petri dish containing PDA. Discs of *S. blastmyceticus* JZB130180 were also cut and placed on either side of the *M. fructicola* disc. The control plate contained only pathogen discs. All plates were incubated at 25 or 28 °C for 5 d (Oldenburg et al., 1996), and inhibition bands were then measured. Each treatment had three replicates, and the experiment was repeated at least three times.

2.3 Inhibitory effect of *S. blastmyceticus* JZB130180 fermentation broth on *M. fructicola*

S. blastmyceticus JZB130180 was inoculated into 50 mL fermentation broth (corn starch 30.0 g,

glucose 10.0 g, soybean meal 20.0 g, peptone 6.0 g, $(NH_4)_2SO_4$ 2.50 g, $MgSO_4·7H_2O$ 1.50 g, KH_2PO_4 0.30 g, NaCl 7.50 g, $CaCO_3$ 4.0 g, thermostable amylase 0.05 g, distilled water 1000 mL, pH 7.0–7.4) and cultured at 28 °C and 200 r/min for 5 d.

M. fructicola was cultured on a PDA plate at a constant temperature of 25 °C for 4–5 d. A sterile spatula was used to gently scrape off the mycelium and conidia into sterile water to create a suspension.

The suspension of *M. fructicola* was mixed thoroughly with PDA at 50 °C and was then poured into a Petri plate. A hole was cut in the center with a hole-punch. Then, 100 μL of the original fermentation broth of *S. blastmyceticus* JZB130180 or 100 μL of its centrifuged supernatant was placed into the hole, with sterile deionized water used as the control (Wang et al., 2010). The diameter of each inhibition zone was measured after 3 d of cultivation at 25 °C. Each treatment had three replicates, and the experiment was repeated at least three times.

Peach fruits with uniform morphology were selected and sterilized with 2% NaClO solution to conduct the inoculation experiment in vitro. Next, 100 μL of the original fermentation broth of *S. blastmyceticus* JZB130180, a 5-fold dilution of the fermentation broth, or sterilized water (CK) was injected into three peach fruits. After 2 h, peaches were inoculated with 30 μL of *M. fructicola* suspension and then stored at room temperature and ambient moisture conditions. Disease incidence and lesion diameter on the fruits were recorded every 24 h. Each treatment had three replicates, and the experiment was repeated at least three times.

2.4 Crude extraction and full wavelength scanning of fermentation broth

After 6 d of fermentation, the morphology of the fermentation broth of *S. blastmyceticus* JZB130180 was observed under a microscope at 400×.

Fermentation broth (20 mL) and organic reagents, including methanol, chloroform, petroleum ether, acetone, *n*-butanol, and ethyl acetate, were mixed in a ratio of 1:2 (v/v) separately, and sonicated for 40 min. Sterilized water was used as a control. After overnight extraction, the inhibitory effect of each extract on *M. fructicola* was detected, and the solubility of the antimicrobial substance in the fermentation broth was analyzed in the different organic reagents.

Next, 1 L of fermentation broth and methanol mixed in a volume ratio of 1:6 was stirred on a magnetic stirrer at room temperature for 2 h, and the pH was adjusted to 11.0 with NaOH. The mixture was then centrifuged at 6000 r/min at 4 °C for 15 min. The supernatant was stirred to adjust the pH to 7.0 with hydrochloric acid solution, followed by stirring at room temperature for 2 h and centrifugation at 6000 r/min at 4 °C for 15 min. The supernatant was concentrated and dried to obtain the active crude extract. The crude extract was dissolved in a small amount of methanol, and the typical absorption peaks were scanned via ultraviolet (UV)-visible spectrophotometry (Yu et al., 2015).

2.5 Effects of physicochemical conditions on the inhibitory ability of *S. blastmyceticus* JZB130180 fermentation broth

S. blastmyceticus JZB130180 fermentation broth (15 mL) was collected and incubated for 30 min or 1 h in a water bath at temperatures of 40, 50, 60, 70, 80, 90, and 100 °C, with fermentation broth held at room temperature (25 °C) as the control. The fermentation broth was centrifuged at 4000 r/min for 10 min at room temperature, and the supernatant was filtered with a Millipore filter (0.45 μm). *M. fructicola* was used as the target pathogen; bioactivity was measured by the disc method as described above, and the relative bacteriostatic activity was determined by comparison to fermentation broth at room temperature, which was considered to be 100% (Wu et al., 2013b).

Then, 3 mL of *S. blastmyceticus* JZB130180 fermentation broth was collected, and the pH of the broth was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0 with a pH meter. After 2 h, the pH of the fermentation broth was adjusted back to 7.0. The fermentation broth was centrifuged at room temperature for 10 min at 4000 r/min. Next, the supernatant was filtered with a 0.45-μm Millipore filter. Bioactivity against *M. fructicola* was measured by the disc method as described above, and the relative bacteriostatic activity was determined by comparison to a pH of 7.0, which was considered to be 100% (Wu et al., 2013b).

Finally, 3 mL of *S. blastmyceticus* JZB130180 fermentation broth was collected and exposed to UV radiation for 30 min, 1.0, 1.5, 2.0, 2.5, and 3.0 h with the control treatment being no exposure to UV radiation. The broth was then centrifuged for 10 min at

room temperature at 4000 r/min, and the supernatant was filtered with a 0.45-μm Millipore filter. Bioactivity against *M. fructicola* was determined using the disc method mentioned above, and the relative bacteriostatic activity was assessed in comparison to that of the unexposed fermentation broth, which was considered to be 100%.

Each treatment was replicated three times in a 25 °C incubator for 3 d, and each experiment was repeated at least three times. To observe the antibacterial effect, a crossing method was used to measure the diameter of the inhibition zone. Statistical analysis was performed using SPSS software.

2.6 Indoor toxicity of *S. blastmyceticus* JZB130180 fermentation broth

The fermentation broth of *S. blastmyceticus* JZB130180 was centrifuged at 4000 r/min at room temperature for 10 min, and the supernatant was filtered with a 0.45-μm Millipore filter. Different volumes of fermentation filtrates were thoroughly mixed with PDA to produce the following concentrations of fermentation broth: 9.9, 19.5, 38.1, 72.7, and 133.3 μg/mL (1 L fermentation broth could be concentrated into 0.8 g of toxic compound). These solutions were made into toxic medium plates, and PDA without fermentation supernatant was used as the control. A disc was cut with a hole-punch from a plate of *M. fructicola* and was then placed upside-down in the center of a toxic medium plate and cultured for 5 d in a 25 °C incubator. A crossing method was used to measure colony diameter, and regression analysis was conducted to calculate the EC_{50} (concentration for 50% of maximal effect) of each fermentation broth. Average colony diameters were used in the following equation: relative inhibition rate=[(colony diameter of control–colony diameter of treatment)/colony diameter of control]×100%. Each treatment was repeated three times, and the experiment was repeated at least three times.

2.7 Assay of enzyme activity and siderophore of *S. blastmyceticus* JZB130180

From plates of *S. blastmyceticus* JZB130180, discs were obtained with a hole-punch and were then placed upside-down on a chitinase-inducing medium (0.3% colloidal chitin, 0.4% NH_4NO_3, 0.05% K_2HPO_4, 0.05% $MgSO_4·7H_2O$, 0.05% KCl, 0.001% $FeSO_4·7H_2O$, and 2% agar). Plates were incubated at 37 °C for 5 d, after which the diameters of the chitin hydrolysis zones were measured (Gao et al., 2015).

From plates of *S. blastmyceticus* JZB130180, discs were obtained with a hole-punch and were then placed upside-down on cellulose-inducing medium (0.025% $MgSO_4·7H_2O$, 0.05% K_2HPO_4, 0.188% cellulose, 0.02% Congo red, 0.2% gelatin, 0.4% agar). Plates were incubated at 28 °C for 5 d, after which the diameters of the cellulose hydrolysis zones were measured (Hankin and Anagnostakis, 1977).

From plates of *S. blastmyceticus* JZB130180, discs were obtained with a hole-punch and were then placed upside-down on a protease-inducing medium (1.5% skim milk and 2.5% agar). Plates were incubated at 28 °C for 5 d, after which the diameters of the protein hydrolysis zones were measured (Chen et al., 2009).

Three discs were inverted and placed on a 9-cm Petri dish containing 25 mL of siderophore-detecting medium (Schwyn and Neilands, 1987). The reaction was observed and measured after 5 d of cultivation.

Each treatment was performed in triplicate, and each experiment was repeated at least three times.

3 Results

3.1 Colony morphology of *S. blastmyceticus* JZB 130180

Colonies of *S. blastmyceticus* JZB130180 on KBA, LBA, PDA, and Gause's plates are shown in Fig. 1. Physiological and biochemical characteristics showed that this was a Gram-positive strain that could liquefy gelatin, coagulate and peptonize milk, hydrolyze starch, and weakly use citrate; in contrast, the strain did not produce melanin or hydrogen sulfide (data not shown).

3.2 Identification and phylogenetic analysis of *S. blastmyceticus* JZB130180

Through PCR amplification, 16S rDNA, *atpD*, and *recA* genes of *S. blastmyceticus* JZB130180 were sequenced (Fig. 2a). Based on comparisons of the 16S rDNA sequence with the NCBI database, this strain had the highest homology (99%) to *S. blastmyceticus* SMD11 (NZ_CP021744.1). Further, a phylogenetic tree based on the concatenated 16S rDNA, *atpD*, and

recA sequences was constructed and showed that the strain clustered on the same evolutionary branch as *S. blastmyceticus* (Fig. 2b).

3.3 Inhibitory effect of *S. blastmyceticus* JZB 130180 against *M. fructicola*

The results of inhibition tests showed that *S. blastmyceticus* JZB130180 had clear inhibitory effects against *M. Fructicola*. The width of the antifungal band was (0.4±0.1) cm (Fig. 3).

3.4 Inhibitory effect of *S. blastmyceticus* JZB 130180 fermentation broth against *M. fructicola*

Experiments on PDA plates revealed that the fermentation broth of *S. blastmyceticus* JZB130180 and its cell-free supernatant both had relatively good inhibitory activity against *M. fructicola* (Fig. 4a).

Moreover, the results of the in vitro peach fruit experiment indicated that the original fermentation

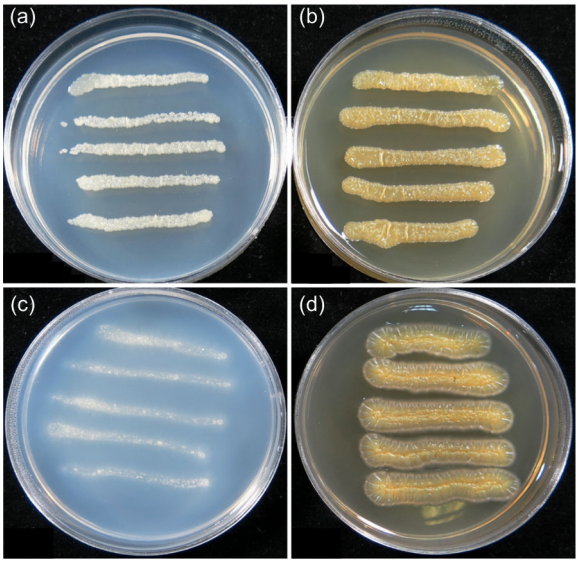

Fig. 1 Colony morphology of *S. blastmyceticus* JZB130180
(a) King's broth agar (KBA) medium; (b) Lysogeny broth agar (LBA) medium; (c) Gause's medium; (d) Potato dextrose agar (PDA) medium

Fig. 3 Inhibitory effect of *S. blastmyceticus* JZB130180 against *M. fructicola*

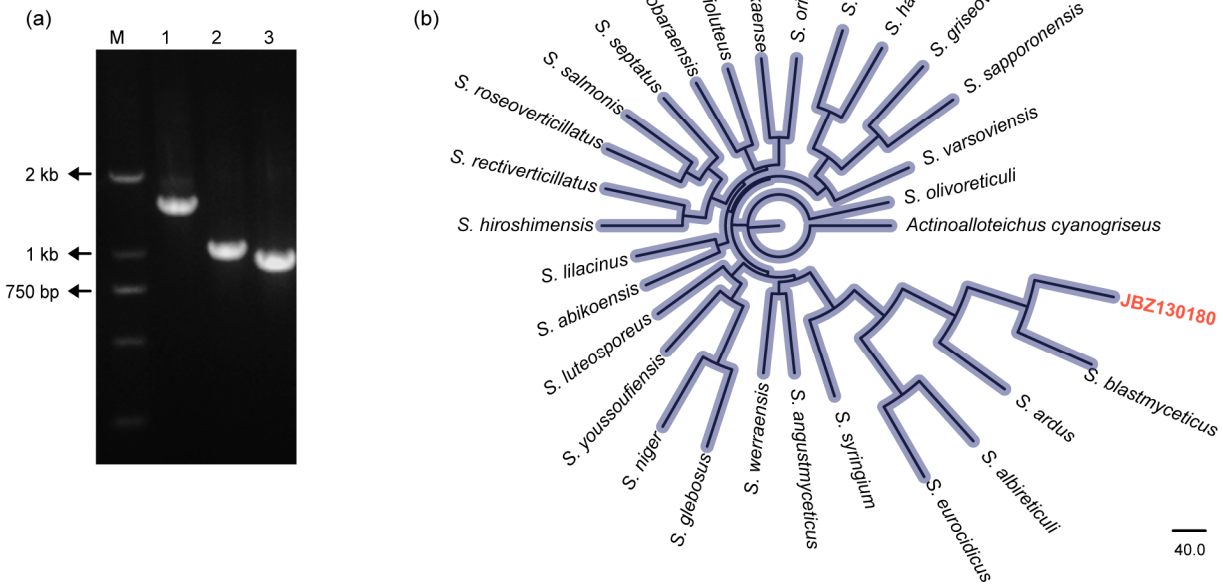

Fig. 2 Identification of *S. blastmyceticus* JZB130180
(a) PCR amplification of 16S rDNA, *atpD*, *and recA* genes; (b) Concatenated phylogenetic tree. M: DL 2000 DNA Marker; 1: PCR products of 16S rDNA; 2: PCR products of *atpD*; 3: PCR products of *recA*

broth of *S. blastmyceticus* JZB130180 and its 5-fold dilution had good inhibitory activity against *M. fructicola*. The inhibitory effects on fruits treated with the original fermentation broth were obvious during the first 2 d; after the third day, the disease incidence of fruit treated with the original broth was basically the same as that of fruit treated with the 5-fold dilution, but both were significantly lower than that of the CK fruit (Fig. 4b).

Several polygonal crystals appeared in the fermentation broth but not in the blank broth. These may have been caused by the active metabolites secreted by *S. blastmyceticus* JZB130180 (Fig. 4c). After dissolving in different solvents, the inhibitory effects of the crude extracts against *M. fructicola* varied. The solubility of the active metabolites from the fermentation broth was at its highest in methanol, followed by *n*-butanol and acetone, while the solubility was

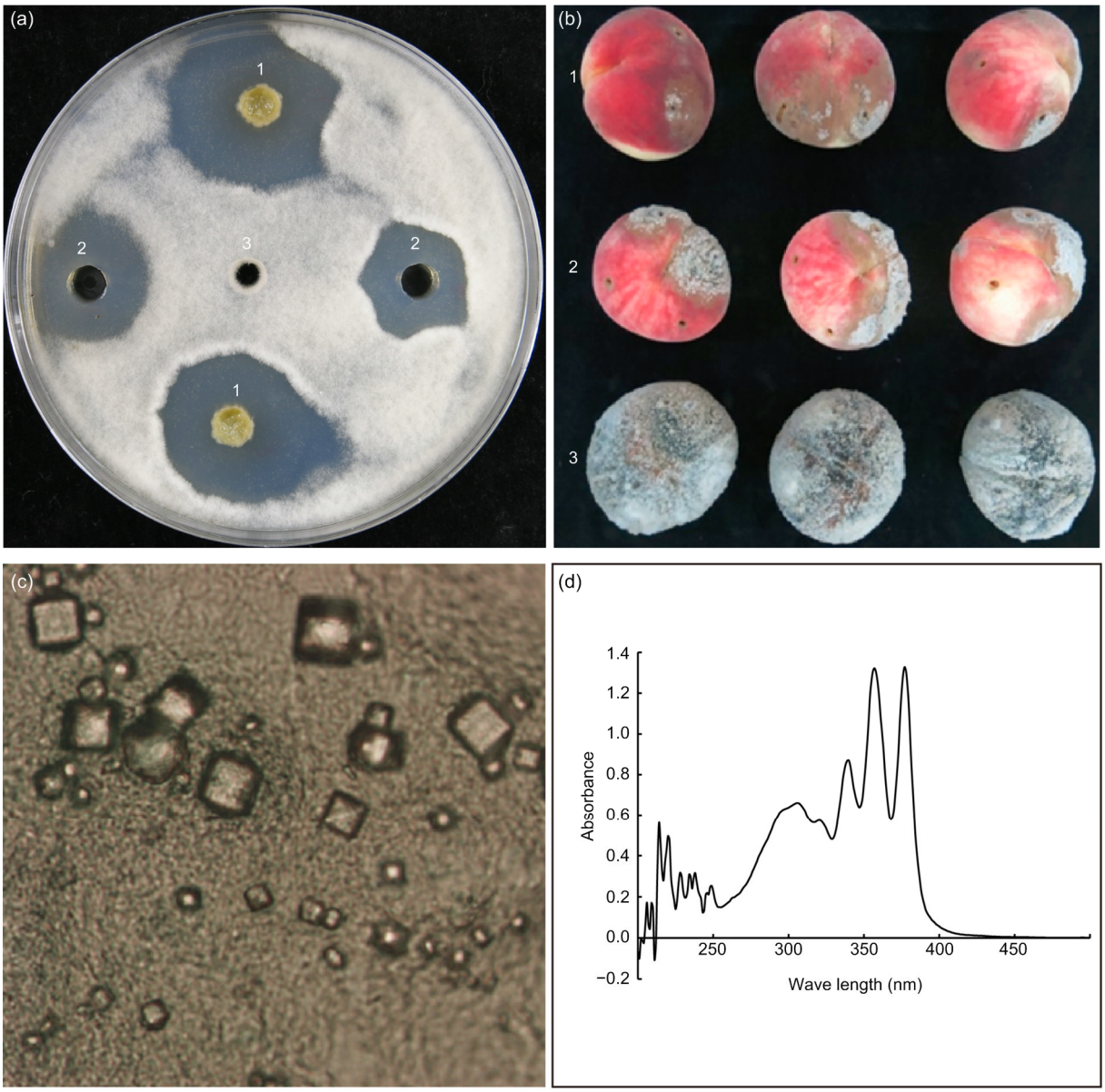

Fig. 4 Characteristics of fermentation broth of *S. blastmyceticus* JZB130180

(a) Inhibitory effect of fermentation broth against *M. fructicola* on PDA medium; (b) Inhibitory effect of fermentation broth against *M. fructicola* in peach fruit; (c) Crystallization of fermentation broth; (d) Scanning micrograph of UV wavelength of fermentation broth after extraction with methanol. 1: the treatment of the original fermentation broth; 2: the treatment of the 5-fold dilution of original fermentation broth; 3: the treatment of the sterilized water (CK)

considerably lower in water, chloroform, petroleum ether, and ethyl acetate (Table S2). The fermentation broth was concentrated to give a light-yellow crystalline substance (data not shown), and three typical absorption peaks were observed at 340, 357, and 378 nm after UV scanning (Fig. 4d).

3.5 Physicochemical properties of *S. blastmyceticus* JZB130180 fermentation broth

Compared with the control group, the relative antifungal activity of *S. blastmyceticus* JZB130180 fermentation broth showed a significantly reduced inhibitory effect after both 30 and 60 min of treatment ($P<0.05$) at 40 °C. Other temperature treatments ranging from 50 to 100 °C showed no significantly changed inhibitory effect ($P>0.05$), except for after 60 min at 70 °C, which resulted in a significantly reduced effect. These findings indicated that temperature had only a minimal effect on the antifungal activity of *S. blastmyceticus* JZB130180 fermentation broth (Table 1).

Compared with the control treatment, after exposure to UV radiation for 0.5 h, the relative antifungal activity of *S. blastmyceticus* JZB130180 fermentation broth was 78.57%, which was significantly different from those in the other experimental groups ($P<0.05$). After exposure to UV radiation for 1–3 h, the relative antifungal activities were all above 80%, and there was no significant difference among these groups. This experiment showed that UV radiation had virtually no effect on the antifungal substances in the fermentation broth (Table 2).

Compared with the control, after *S. blastmyceticus* JZB130180 fermentation broth was treated for 2 h in media with a range of pH values from 3 to 12, the variation in relative antifungal activity was minimal, and there were no significant differences among different pH treatments ($P>0.05$). These results demonstrated that the antifungal substances in the fermentation broth could tolerate both acidic and alkaline conditions (Table 3).

3.6 Indoor toxicity of *S. blastmyceticus* JZB130180 fermentation broth

Based on the toxicity regression equation of *S. blastmyceticus* JZB130180 fermentation broth, the

Table 1 Effect of different temperatures on the activity of antimicrobial substances

Temperature (°C)	Mean diameter of inhibition zone (mm)	Relative activity (%)
30 min		
40	22	78.57[b]
50	23	82.14[a]
60	25	89.28[a]
70	23	82.14[a]
80	23	82.14[a]
90	23	82.14[a]
100	29	103.57[a]
RT	28	100.00[a]
60 min		
40	21	75.00[b]
50	23	82.14[a]
60	25	89.28[a]
70	21	75.00[b]
80	23	82.14[a]
90	27	96.42[a]
100	23	82.14[a]
RT	28	100.00[a]

[a, b] The same letter indicates that the difference is not significant ($P>0.05$). RT: room temperature

Table 2 Effect of UV irradiation on the activity of antimicrobial substances

Irradiation time of UV (h)	Mean diameter of inhibition zone (mm)	Relative activity (%)
0.5	22	78.57[b]
1.0	24	85.71[a]
1.5	27	96.42[a]
2.0	28	100.00[a]
2.5	23	82.14[a]
3.0	24	85.71[a]
0.0	28	100.00[a]

[a, b] The same letter indicates that the difference is not significant ($P>0.05$)

Table 3 Effect of pH on the activity of antimicrobial substances

pH	Mean diameter of inhibition zone (mm)	Relative activity (%)
3	23	104.55
4	22	100.00
5	23	104.50
6	23	104.55
7	22	100.00
8	23	104.55
9	23	104.50
10	22	100.00
11	22	100.00
12	24	109.09

EC_{50} of the fermentation broth against *M. fructicola* was 38.3 μg/mL, indicating that the fermentation broth had a relatively strong inhibitory effect against *M. fructicola* (Fig. 5).

3.7 Detection of CWDEs and siderophore

Following growth on enzyme-inducing plates for 5 d, hydrolytic circles were obvious, showing that *S. blastmyceticus* JZB130180 excreted chitinase, cellulase, and protease (Figs. 6a–6c). Furthermore, a siderophore was secreted by *S. blastmyceticus* JZB130180 (Fig. 6d). The results showed that the activity of the protease excreted by *S. blastmyceticus* JZB130180 was the highest, followed by the chitinase, protease, and siderophore (Fig. 6e).

4 Discussion

In the agricultural field, actinomycetes, such as *Streptomyces hygroscopicus* (Prapagdee et al., 2008), *Streptomyces lydicus*, and A01 (Lu et al., 2008; Wu et al., 2013a), are widely used in disease resistance research. The novel *S. blastmyceticus* JZB130180 strain isolated in this study and its fermentation broth had strong inhibitory effects against peach brown rot (Fig. 3). Thus, the discovery of *S. blastmyceticus* JZB130180 provides a foundation for the biocontrol of *M. fructicola*.

Many secondary metabolites with anti-microbial effects, such as polyketones, non-ribosomal peptides, terpenes, and aldehydes, can be found in actinomycetes and their fermentation broths. The synthesis of secondary metabolites by actinomycetes is generally regulated by gene clusters, which are made up of core genes and modifier genes with sizes varying from dozens to 100 kb (Foulston and Bibb, 2010; Liu et al., 2013; Miyamoto et al., 2014; Castro et al., 2015). Currently, several complete gene clusters have been cloned and associated with compounds (Wu et al., 2012), with most studies focusing on polyketone (Thanapipatsiri et al., 2015) and peptide gene clusters (Foulston and Bibb, 2010). In our study, it was

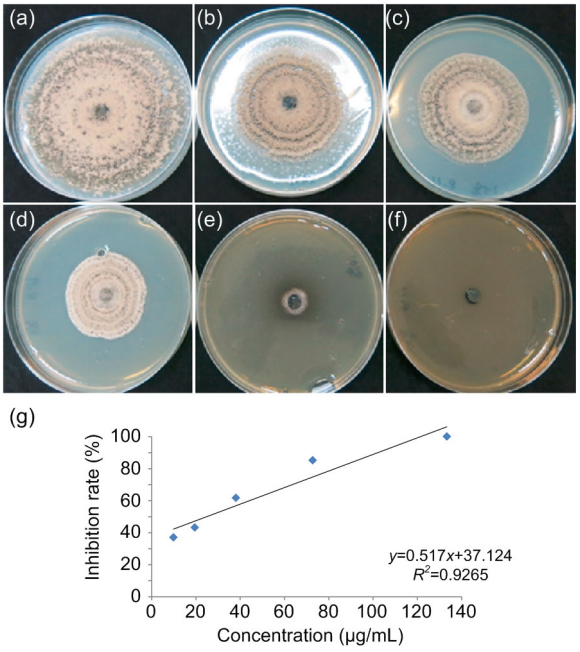

Fig. 5 Toxicity of fermentation broth of *S. blastmyceticus* JZB130180 against *M. fructicola*
(a–f) Growth of *M. fructicola* on toxic medium; (g) Toxicity regression equation for EC_{50} calculation

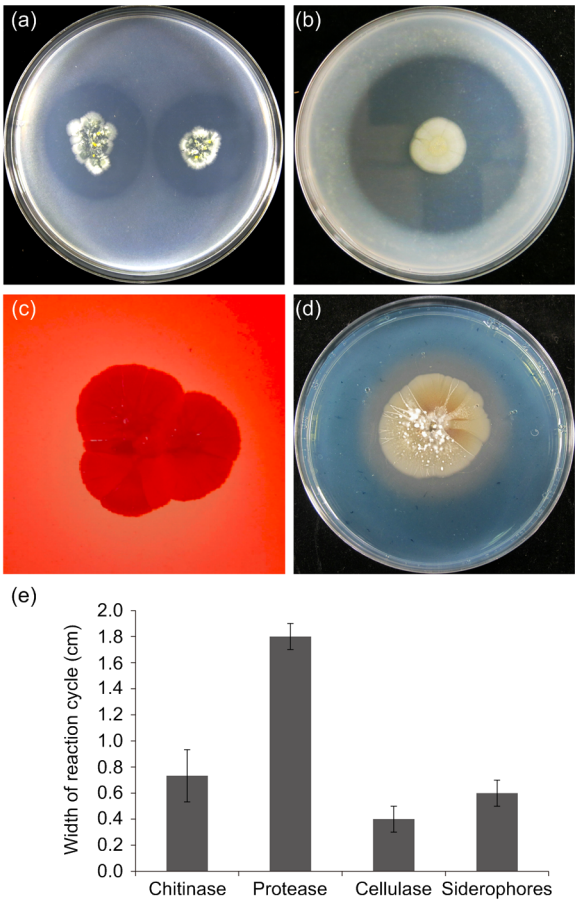

Fig. 6 Detection of biocontrol factors
(a) Chitinase; (b) Protease; (c) Cellulase; (d) Siderophores; (e) Statistical results. Data are expressed as mean±standard deviation (n=3)

discovered that the fermentation broth of *S. blastmyceticus* JZB130180 has a strong inhibitory effect against *M. fructicola*, with an EC_{50} of 38.3 μg/mL (Fig. 5). The fermentation broth showed tolerance to acidity and alkalinity, as well as temperature and UV radiation (Tables 1–3). The fermentation broth was concentrated to give a light-yellow crystalline substance (data not shown), and three typical absorption peaks were observed at 340, 357, and 378 nm after UV scanning (Fig. 4d). In the future, additional chemical techniques, such as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR), should be used to isolate and identify the secondary metabolites in the fermentation broth of *S. blastmyceticus* JZB130180 (Jung et al., 2003; Cho et al., 2007). Related gene clusters should also be analyzed. If some of these gene clusters are silenced, their expression can be induced through epigenetic gene knockouts (Liu et al., 2013); overexpression of transcriptional regulatory factors and heterologous expression can lead to the synthesis of new compounds (Castro et al., 2015).

Actinomycetes can produce a wide range of chitinases, cellulases, and proteases (Yuan and Crawford, 1995; Kubicek et al., 2014). These enzymes degrade the cell walls of plant-pathogenic fungi, producing an antagonistic effect. In this study, *S. blastmyceticus* JZB130180 produced a series of CWDEs, such as chitinase, cellulase, and protease (Fig. 6). In future work, through local BLAST and PCR amplification, several relevant and novel genes encoding CWDEs may be identified in *S. blastmyceticus* JZB130180. Moreover, these CWDE genes could be expressed in *Escherichia coli* and yeast to further study their antifungal capacities and physicochemical properties (Wu et al., 2006; Turumtay, 2015), providing a theoretical foundation for the development of new biocontrol agents.

In summary, *S. blastmyceticus* JZB130180 should be further studied from the perspective of the identification and separation of novel active substances and the analysis and expression of related genes and gene clusters.

5 Conclusions

This study isolated a novel strain, *S. blastmyceticus* JZB130180. This strain has an extremely strong inhibitory effect against the common peach-pathogenic fungus *M. fructicola*. By inoculating peaches in vitro, it was revealed that the fermentation broth of *S. blastmyceticus* JZB130180 had a significant inhibitory effect against *M. fructicola*. Further, the indoor toxicity test determined that the EC_{50} of the fermentation broth for *M. fructicola* was 38.3 μg/mL. The strain was also found to secrete chitinase, cellulase, and protease. This study of *S. blastmyceticus* JZB130180 provides a theoretical foundation for the biocontrol of peach brown rot disease.

Contributors

Cai-ge LU and Wei-cheng LIU conceived the project. Cai-ge LU, Mi NI, Qiong WU, and Hong-li WANG performed the experiments. Mi NI, Qiong WU, Cai-ge LU, and Hong-li WANG analyzed the data. De-wen LIU, Dian-peng ZHANG, Juan ZHAO, and Bin HU contributed reagents, materials, and analysis tools. Mi NI and Qiong WU wrote the manuscript. Cai-ge LU and Wei-cheng LIU polished the English.

Compliance with ethics guidelines

Mi NI, Qiong WU, Hong-li WANG, Wei-cheng LIU, Bin HU, Dian-peng ZHANG, Juan ZHAO, De-wen LIU, and Cai-ge LU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Primers used in this study
Table S2 Solubility determination of active substances

中文概要

题　目：一株新型稻瘟霉素链霉菌 JZB130180 的鉴定及其对桃褐腐病的生防效果评价

目　的：筛选对桃褐腐病有显著防效的链霉菌并解析其生防因子。

创新点：本研究筛选到一株新型稻瘟霉素链霉菌 JZB 130180，其对桃褐腐病具有显著防效。通过对其生防因子解析及发酵液分析，为后续分离抑菌次生代谢物奠定了基础。

方　法：通过对峙培养和离体实验，检测链霉菌对桃褐腐病的抑制作用。通过平皿抑菌实验，计算链霉菌发酵液的室内毒力；随后进行了发酵液的理化性质检测。通过酶活力测定等，解析链霉菌中的生防因子。

结　论：链霉菌 JZB130180 及其发酵液对桃褐腐病具有显著的抑制作用。发酵液对桃褐腐病菌的半数有效抑制浓度（EC_{50}）为 38.3 μg/mL，且对酸碱、温度和紫外线都有一定的耐受能力。此外，该菌株可以分泌几丁质酶、纤维素酶、蛋白酶等重要生防因子。

关键词：稻瘟霉素链霉菌；桃褐腐病；褐腐病菌；生防效果