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Research Article

Identification and Laboratory Fungicides Screening of the Pathogenic Fungus of Stem Spot of Pitaya (*Hylocereus* spp.) Stems

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In this study, the pathogenic fungus of stem spot disease of pitaya (*Hylocereus* spp.) stems was identified by isolation and purification, pathogenicity test, morphological characteristics, and analysis of rDNA-ITS sequences. The results turned out that the rDNA-ITS sequences of the H1 strain showed 100% of identity with *Botryosphaeria dothidea*, indicating that the pathogenic fungus of stem spot disease of pitaya stems was *Botryosphaeria dothidea*. Meanwhile, the H1 strain was then used as a reference strain to screen some commercial fungicides. The bioassay test results indicated that prochloraz had an obvious inhibitory effect on *Botryosphaeria dothidea* with the EC_{50} value of 0.0798 μg/mL. Our study could provide a theoretical basis for the effective control method of stem spot disease of pitaya.

1. Introduction

Pitaya (*Hylocereus* spp.), a famous fruit in tropical and subtropical regions of Central America, has been planted in Hainan, Guangdong, Guizhou, Guangxi, Yunnan, Fujian, and other regions, and has become one of the important sources of farmers' income in southern China. However, since cultivation and further domestication on commercial plantations, some symptoms of decay and spots have been observed in stems and fruits [1–4]. Meanwhile, the yields could be diminished due to such decay and spot diseases, which may induce economic losses reach 44% [1, 5]. For these reasons, since 1990s, some studies on stem spot disease and the crop protection had been initiated [6, 7]. Hong et al. reported that the stem rot on Wilford Swallowwort was caused by *Stemphylium lycopersici* in Korea [8]. Meanwhile, Edwards et al. found that *Fusarium agapanthi* sp. nov. was a novel bikaverin- and fusarubin-producing leaf and stem spot pathogen of *Agapanthus praecox* (African lily) from Australia and Italy [9]. Moreover, in 2008, Culbreath et al. showed that the incidence of stem rot for all penthiopyrad treatments was usually less than that of tebuconazole or azoxystrobin [10].

The aim of this work was to identify the pathogenic fungus of stem spot disease of pitaya stems and then screen some commercial fungicides on the pathogenic fungus to provide a theoretical basis for the effective control method of stem spot disease of pitaya stems.

2. Materials and Methods

2.1. Fungus Isolation and Purification. Pitaya stems with the symptoms of spots collected from Luodian county, Guizhou Province, China, were sterilized using sterile 75% ethanol and sterile distilled water for three times, excised the infected tissues with a sterile scalpel, plated the infected tissues on the sterile potato dextrose agar (PDA) plates, and then incubated the PDA plates in a sterile incubator at 28°C for 3 days. All isolations were cultured twice on a new PDA plate using a single spore technique to ensure purity [11]. Then, the pure cultures were maintained in a 4°C refrigerator for further use.

2.2. Morphological and Molecular Identification. Individual colony was inoculated on the PDA plate in a sterile incubator (28°C) for 7 days, and the morphology was

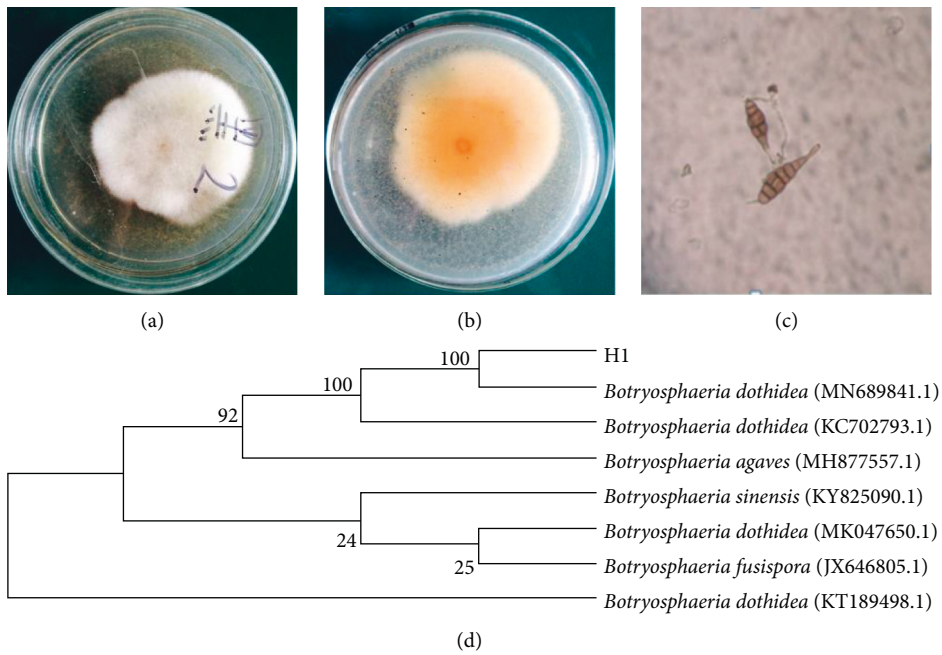


Figure 1: Morphology of H1 isolate cultures on PDA: (a) Observe surface of colony (front), (b) observe surface of colony (back), (c) microstructure of conidia, and (d) phylogenetic analysis based on sequence analysis.

identified by both eye and a Model EX30 inverted microscopy (Ningbo Shunyu Tech. Co. Ltd., Zhejiang, China) [12]. Approximately, 100 mg fungal mycelia were collected to extract DNA using a TIANamp fungal DNA kit (Tiangen-Biotech Co. Ltd., Beijing, China) [13–15]. Polymerase chain reactions (PCRs) were conducted using a Premix Taq ver. 2.0 plus dye kit (Takara, Dalian, China) according to the manufacturer's instructions with the universal primer ITS1 and ITS4 [16–18] and the following amplification program: 98°C for 5 min; 30 cycles of 95°C for 35 s, 55°C for 35 s and 72°C for 40 s; 72°C for 8 min. After that, the PCR products were sequenced at Sangon Corporation (Shanghai, China) and searched for sequence similarity with the NCBI database. The phylogenetic tree was constructed using the MEGA 7.0 software [19–21].

2.3. Pathogenicity Test. Pathogenicity tests were performed by injecting the 1.0×10^6 conidia/L conidial suspension on the surface of the pitaya stems, and the pitaya stems were incubated in an incubator at 28°C with 95% relative humidity for 14 days [22]. Pitaya stems inoculated with sterile water served as a control. After 14 days of inoculation, some symptoms of spots have been observed in stems. The causal fungus in the infected pitaya stems was reisolated on the PDA plates as described above. The characteristics of the reisolated fungus was used to compare with its original culture.

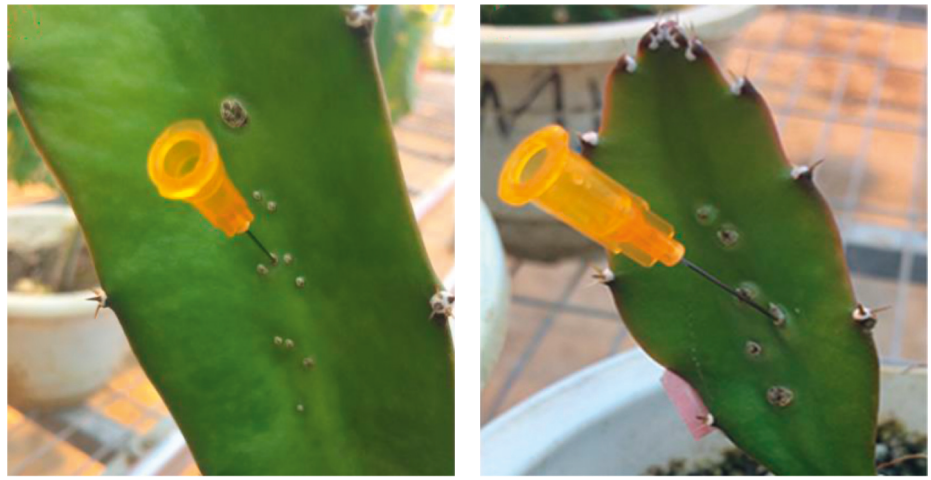
2.4. Laboratory Fungicides Screening. The *in vitro* antifungal activity of thiophanate-methyl (content: 99%), difenoconazole (content: 99%), pyraclostrobin (content: 98%), and prochloraz (content: 99%), which were mainly registered to control stem spot disease, against *Botryosphaeria dothidea* was tested according to the reported method [23, 24]. Each drug (5.0 mg) was dissolved in 1 mL DMSO, 9 mL Tween 20 aqueous solution (0.1%), 90 mL PDA medium, and poured into 6 sterilized dishes to prepare PDA plates. Mycelia dishes (0.4 cm diameter) were inoculated on the middle of PDA plates and fostered in an incubator at 28°C. After the mycelia diameter of control group (CK) reached 6–7 cm, the inhibition rates I (%) are calculated by the following formula, where C (cm) and T (cm) represent the fungi diameters of the CK and treated PDA plates, respectively. Meanwhile, the EC_{50} values of thiophanate-methyl, difenoconazole, pyraclostrobin, and prochloraz against *Botryosphaeria dothidea* were calculated with the SPSS 19.0 software (SPSS Inc., IL, USA).

$$\text{Inhibition rate } I\,(\%) = \frac{(C - T)}{(C - 0.4)} \times 100. \quad (1)$$

3. Results and Discussion

3.1. Fungal Isolation and Identification. Figure 1 showed that the fungal strain H1 appearance was white in front (Figure 1(a)) and claybank in back (Figure 1(b)). The conidia (Figure 1(c)) were fusiform and septate, with the length and width ranging of 18.00–30.00 μm and 4.00–11.00 μm, respectively. Meanwhile, based on the ITS sequences and phylogenetic analysis, as shown in Figure 1(d), the fungal strain H1 isolated from rotting pitaya stems was classified as *Botryosphaeria dothidea* (accession no. MN689841.1) with the similarity of 100%.

3.2. Pathogenicity Test. After 14 days of pathogenicity test, the symptoms of the stem disease (Figure 2) caused by H1



(a) (b)

Figure 2: The symptoms of H1 strain in the pathogenicity test. (a) CK group; (b) treatment group.

Table 1: The EC_{50} values of 5 fungicides against *Botryosphaeria dothidea*.

Fungicides	Toxicity regression equation	EC_{50} (μg/mL)
Thiobacillam-methyl	$y = 0.857x + 10.638$	0.2640
Difenoconazole	$y = 0.761x + 10.136$	0.1795
Pyraclostrobin	$y = 0.851x + 10.127$	0.9466
Prochloraz	$y = 1.212x + 13.606$	0.0798

strain were consistent with the initial symptoms appeared on the collected pitaya stems, and the strains isolated again had the same culture characteristics as the original strain, proving that the obtained strain was the pathogen of stem spot disease.

3.3. In Vitro Antifungal Activity. The antifungal activity against *Botryosphaeria dothidea* of thiophanate-methyl, difenoconazole, pyraclostrobin, and prochloraz were determined and listed in Table 1. The results showed that the EC_{50} values of four fungicides were in descending order as follows: prochloraz < difenoconazole < thiobacillam-methyl < pyraclostrobin, indicating that prochloraz had the best inhibitory effect on *Botryosphaeria dothidea* with an EC_{50} value of 0.0798 μg/mL.

4. Conclusion

In conclusion, our study demonstrated that the pathogen causing the stem disease of pitaya stems in Guizhou was *Botryosphaeria dothidea*. Bioassay results showed that prochloraz had the best in vitro inhibitory effect on *Botryosphaeria dothidea*. Our study could provide a theoretical basis for choosing the effective pesticide for controlling the stem spot disease of pitaya.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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