Identification and Characterization of a New Fungal Pathogen Causing Twisted Leaf Disease of Sugarcane in China

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Abstract

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Sugarcane twisted leaf disease, caused by *Phoma* sp., was first reported in Guangxi, China, in 2012, when more than 5% of sugarcane was infected in the field. Three single-spore isolates were recovered from symptomatic leaves. Sequences from five fungal loci, 28S nrDNA (LSU), 18S nrDNA (SSU), the internal transcribed spacer regions 1 and 2 and 5.8S nrDNA (ITS), β -tubulin (TUB), and the translation elongation factor alpha (TEF- α) were amplified from the disease-associated isolates. The twisted leaf disease pathogen was identified and formally described as *Phoma sorghina* var. *saccharum* through phylogenetic analyses, morphological observations, and the pathogenicity of the isolates on sugarcane. *P. sorghina* var. *saccharum* can be differenti-

ated from related species based on the morphology of pycnidia and chlamydospores that formed regular, glabrous, papillate ostioles. Chlamydospore-anamorph was unicellular, botryoid-alternarioid shape, as well as the binucleate, frequently branched hyphae. We also showed that mycelial growth of *P. sorghina* var. *saccharum* was optimal at pH 4.0 and 20 to 25°C. Additionally, among 13 chemical compounds tested, carbendazim was found to be the most effective in suppressing the radial growth of the fungus. Mycelial growth in vitro was completely inhibited at concentrations of 100 and 50 ppm, and 87.6% of mycelial growth was inhibited at 10 ppm. Carbendazim is therefore a potentially effective fungicide to control this disease in China.

Sugarcane is one of the major sources of sugar for human consumption and a leading energy crop worldwide. Guangxi is one of the largest sugarcane production areas in China. During a survey of sugarcane crops conducted in September 2012 and June 2013, approximately 5 to 10% of the sugarcane crop (cvs. FN-40 and ROC22) planted in Chongzuo and Laibing, Guangxi Zhuang Autonomous Region, China, had twisting and curling symptoms of crown sugarcane leaves similar to sugarcane pokkah boeng disease (caused by *Fusarium moniliforme* Sheldon). Similar symptoms were also observed in December 2012 at Fuzhou, Fujian, within sugarcane seedling nurseries. The symptoms started appearing as yellowing on midribs and leaf margins, which spread further to the entire leaf, along with twisting and curling of crown leaves. This was the first report of *Phoma* sp. associated with sugarcane twisted leaf disease by morphological observation and ITS identification in the mainland of China (18).

The genus *Phoma* is one of the largest fungal genera, with more than 3,000 infrageneric taxa described (22). Approximately 50% of the *Phoma* taxa redescribed by Boerema were recognized as relevant phytopathogenic fungi (2,4). Morphological characteristics have been used for the identification of *Phoma* species, including conidial shape, size, septation, ornamentation, and culture characteristics (11). However, some of these characteristics overlap among species and vary depending on culture conditions such as

temperature, pH, and medium (15). To determine the taxonomic distribution of *Phoma* species, both morphological and molecular phylogenetic analyses are complementary in modern fungal systematics (26). Molecular phylogenetic studies are often conducted using multilocus sequence typing (2). These analyses make use of sequences derived from 28S nrDNA (LSU) (28), 18S nrDNA (SSU) (37), the internal transcribed spacer regions 1 and 2 and 5.8S nrDNA (ITS) (37), β -tubulin (TUB) (14; 38), actin (ACT) (6), and the translation elongation factor alpha (TEF- α) (3). In addition, growth characteristics on media, such as growth rate, pigment formation, and colony outline and pattern are also aids for fungal identification (1). Temperature, pH, water activity, and light are considered as the major environmental factors affecting fungal growth and development in plants and culture media (20). However, the taxonomy of the *Phoma* section is still not fully understood (3).

Species belonging to the genus *Phoma* and related coelomycetes are often encountered as serious plant pathogens, mostly causing leaf and stem spots. *Phoma* comprises large taxa whose members appear to be different in their host specificity (38). The host ranges associated with the genus *Phoma* are diverse, including corn, grain, citrus, and sorghum. *Phoma* sp. on sugarcane leaves has been reported in India, Pakistan, Hawaii, and China (12,17,18,31). In order to further understand the pathology of sugarcane twisted leaf disease, we identified *Phoma* sp. from sugarcane isolates by morphological observation, molecular identification, and phylogenetic analyses. We also conducted pathogenicity tests and characterized fungal growth at different temperatures and pH, and tested the fungal responses to chemical compounds in vitro. Our studies lay the groundwork for early identification of *Phoma* in sugarcane twisted leaf disease and point to strategies for management of this disease.

Materials and Methods

Fungal cultures and growth. The symptomatic leaves of sugarcane, manifesting twisting and curling, were collected at Nanning

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*The e-Xtra logo stands for "electronic extra" and indicates that two supplementary tables are available online.

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(E108°42', N22°88'), Guangxi, in September 2012, and Fuzhou (E119°23', N26°09'), Fujian, China, in January 2013. Leaf tissue (5 × 5 mm) was surface-disinfected and cultured, and then single spores were isolated from the growing cultures. Briefly, the tissues were first surface-sterilized by dipping in 70% ethanol, followed by 30 s in 0.1% HgCl₂ solution, rinsed three times in sterile water, placed on potato dextrose agar (PDA), and then incubated in darkness at 28°C. The single conidium-derived isolates were selected from the plates according to a previously described method (16). All cultures were stored at -80°C in the Key Lab of Sugarcane Biology and Genetic Breeding at the Fujian Agriculture and Forestry University, Fuzhou, China.

Morphological observation and pathogenicity test. Microscopic examinations were carried out after 2 weeks of hyphal tip growth on malt extract agar (MEA), oatmeal agar (OA), and PDA plates at 28°C in darkness. Sterile water was used as a mounting medium for microscopy. The shape, size, color, opening of ascospores and conidiomata, as well as zone lines were observed under a microscope (ZEISS Scope A1, Carl Zeiss AG, Germany) using standard protocols (1,2,4). One hundred microliters of conidial suspensions (10⁷ CFU.mL⁻¹) of three representative isolates (Fn40, FJ-1, and FJ-2) were micro-injected using a sterile inoculator into 20 seedlings (20 days old) of sugarcane cultivar FN-40. Control seedlings were injected with water without conidia. The inoculated plants were grown in a growth chamber at 28°C with a photoperiod of 16 h light, 8 h dark.

Effects of temperature and pH on mycelial growth in vitro. *Phoma* isolate Fn40 was grown on standard PDA medium (pH = 5.6) and used for assessing the effect of temperature on radial growth. Temperatures ranged from 5 to 35°C at intervals of 5°C. The pH effects on the radial growth were investigated on modified PDA medium at different pH values ranging from 4.0 to 8.0 adjusted by McIlvaine buffers at 25°C in darkness (21,29). A 5-mm PDA plug of mycelium of isolate Fn40 was transferred to the center of medium plates for radial growth measurements. For each temperature and pH tested, four replicate observations were performed. Colony diameter was measured after 7 days of incubation and the isolates were also scored for pigmentation of the mycelia.

Responses to chemical compounds in vitro. Eight bactericidal or viricidal compounds (rifaximin, lincomycin hydrochloride, cinoxacin, cephalaxin 2-methyl-4-isothiazolin-3-one, 2-amino-5-chloro-benzoxazole, pasiniazid, and berberine chloride) were purchased from Sigma-Aldrich Co. Ltd (St. Louis, MO). Other registered fungicides were purchased in China, including oxine-copper and emamectin benzoate from Zhejiang Hisen Chemical Co. Ltd. (Zhejiang, China), as well as thiophanate-methyl, mancozeb, and carbendazim from Jiangsu Lanfeng Biochemical Co. Ltd (Jiangsu, China). Different concentrations (100, 50, and 10 ppm) of the chemical compounds were prepared by dissolving the requisite quantity of each compound in warm PDA medium before autoclaving. After autoclaving, the medium was dispensed uniformly into 90-mm-diameter petri plates and inoculated with 5-mm mycelial discs of *Phoma* isolate Fn40. A non-supplemented PDA medium served as a control and each treatment was repeated in triplicate. The plates were incubated at 25°C for 3 days in darkness

and the diameter of the fungal colony was measured. The inhibitory rate was calculated as follows:

$$\% \text{ inhibition} = [(G_c - G_t)/G_c] \times 100$$

where G_c = growth in control and G_t = growth in treatment.

All data were analyzed as a generalized linear mixed model using the SAS procedure GLIMMIX. Differences among treatment levels were determined with the LINES option of the LSMEANS statement.

PCR amplification and sequence analysis. DNA from isolates Fn 40, FJ-1, and FJ-2 were extracted with a modified Fungal DNA Midi Kit (Omega Bio-Tek, Inc., USA). DNA derived from TUB, LSU, SSU, ITS, and TEF- α loci were amplified using primers specific for sequences conserved in fungi (Table 1). Each amplification reaction included 25 μ l of Dream Taq Green PCR Master MIX (Thermo Fisher Scientific, Waltham, MA), 5 μ l (10 μ M) of each primer set, and 14 μ l of ddH₂O in a final volume of 50 μ l. Conditions for amplification included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation (94°C for 30 s), annealing (48 to 57°C for 1 min depending on the locus), and extension (72°C for 3 min). A final extension was conducted at 72°C for 10 min. Annealing temperatures were 48°C for LSU and SSU, 56°C for ITS and TUB, and 57°C for TEF- α , respectively. All PCR reactions were performed on a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA). Amplified products were sequenced by Sangon Biotech Co., Ltd (Shanghai, China).

Phylogenetic analyses. TUB, LSU, SSU, ITS, and TEF- α locus sequence data from the three isolates as well as reference sequences obtained from CBS or NCBI are summarized in Supplementary Table 1 (2) and Supplementary Table 2 (3), respectively. Sequences were first analyzed using DNAMAN version 4.0 (DNAMAN, Lynnon Biosoft, San Ramon, CA). All sequences derived from different loci in each isolate were concatenated for a single nucleotide alignment. Nucleotide sequences were aligned using CLUSTAL W implemented in MEGA 6.0 followed by manual modification, and gaps were coded as missing data (23,35). Phylogenetic trees were generated using maximum likelihood analysis with the Kimura 2 parameter method on the concatenated alignments. Statistical significance of the tree branches was assessed using 1,000 bootstrap replications (13,33). Phylograms with the representative bootstrap values ($\geq 50\%$) above each branch were generated using the tree explorer available in MEGA 6.0 (34).

Results

Species identification within the genus *Phoma*. *Molecular phylogenetic analysis.* Amplified PCR products were 380 bp (TUB locus), 1,350 bp (LSU), 1,070 bp (SSU), 540 bp (ITS), and 930 bp (TEF- α), respectively. The sequences obtained from the three isolates (Fn40, FJ-1, and FJ-2) were deposited in GenBank. In order to accurately identify the disease-associated fungal isolates, a phylogenetic tree for the *Phoma* species complex was constructed using the maximum likelihood method. Input for this analysis was based on the LSU+ITS+SSU+TEF- α loci of the disease-associated isolates and other reference sequences. Nine clades were revealed in the combined phylogenetic tree based on the LSU-ITS-SSU-TEF- α phylogeny, when the tree was rooted to *Pseudorobillarda*

Table 1. Primers used for the identification of *Phoma sorghina* var. *saccharum*

Locus	Primer	Primer sequence 5'-3'	References
TUB	TUB2Fd	GTBCACCTYCARACCGGYCARTG	Groenewald et al. (14); Woudenberg et al. (38)
	TUB4Rd	CCRGAYTGRCCRAARACRAAGTTGTC	
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. (37)
	ITS4	TCCCTCCGCTTATTGATATGC	
LSU	LR0R	GTACCCGCTGAACCTTAAGC	Rehner and Samuels (28); Vilgalys and Hester (36)
	LR7	TACTACCACCAAGATCT	
SSU	NS1	GTAGTCATATGCTTGTCTC	White et al. (37)
	NS4	CTTCCGTC AATTCCTTTAA	
TEF- α	Efd	AAGGAYGGNCARACYCGNGARCA YGC	www.aftol.org/pdfs/EF1primer.pdf
	EF1-2218R	ATGACACCRACRGCRACRGTYTG	

phragmitis (CBS 398.61) as an outgroup specimen (Fig. 1). These findings were largely in congruence with the observations of Aveskamp et al. (2). Ten previously described species in the genus *Phoma* as well as our disease-associated isolates (Fn40, FJ-1, and FJ-2) formed a monophyletic clade in Didymellaceae with 99%

bootstrap support, indicating that our three isolates were closely related to *Chaetabolisia erysiphoides* (CBS 148.94) and *Epicoccum nigrum* (CBS 173.73) in the clade Didymellaceae. The phylogenetic tree clearly showed that the disease-associated fungus was a species of the genus *Phoma* (Fig. 1).

Fig. 1. Phylogenetic tree constructed using the maximum likelihood method and inferred from the combined four locus, LSU-ITS-SSU-TEF- α , taxon data set rooted with sequences derived from *Pseudorobillarda phragmitis* (CBS 398.61). Bootstrap values $\geq 50\%$ from 1,000 replicates of the data are indicated above nodes. The positions of the Fn40, FJ-1, and FJ-2 isolates are indicated in bold text.

Fig. 2. Phylogenetic tree constructed using the maximum likelihood method and inferred from the combined LSU-ITS-TUB sequences. The taxon data set was rooted with sequences derived from *P. paspali* (CBS 560.81). Bootstrap values $\geq 50\%$ from 1,000 replicates of the data are indicated above nodes. The positions of the Fn40, FJ-1, and FJ-2 isolates are indicated in bold text.

To further assess evolutionary relationships of the disease-associated *Phoma* isolates within the Didymellaceae clade, the nucleotide sequences derived from LSU, ITS, and TUB loci were aligned for 59 taxa in the *Peyronellaea* section of *Phoma*, including one outgroup taxon (CBS 560.81 *P. paspali*). Maximum likelihood analysis was used to construct a second phylogenetic tree of these *Phoma* species. The synonymy on the *Phoma* species was referred to in Aveskamp et al. (3). Ten isolates of *P. sorghina* and our *Phoma* isolates (Fn40, FJ-1, and FJ-2) formed a monophyletic group, indicating that our *Phoma* isolates were ascribed to *P. sorghina* (Fig. 2). The results presented here revealed little genetic variation within isolates of *P. sorghina* from *Lycopersicon esculentum* (CBS 301.89), *Sorghum vulgare* (CBS 179.80), *Zea mays* (CBS 180.80), and *Citrus* sp. (CBS 627.68) (3).

Morphological observations. The pycnidia of isolates produced on the agar surface were regular glabrous, solitary or confluent, globose to subglobose, 50 to 200 × 75 to 250 μm, with papillate ostioles, brown/brown-black (Fig. 3I). The pycnidial wall was

pseudoparenchymatous and composed of oblong to isodiametric cells in one layer (Fig. 3H). Conidiogenous cells were phialidic, hyaline, simple, smooth, fusiform, and relatively small, approx. 3.5 to 5 × 3 to 4 μm (Fig. 3G). Conidia were ellipsoidal to cylindrical, thin-walled, smooth, hyaline, and aseptate, 3 to 5 × 1.5 to 3 μm, with a creamy white matrix. Chlamydospores with a diameter of 5 to 10 μm were dark brown or olivaceous, terminal on erect aerial hyphae, and solitary. A single chlamydospore was below the colony, with a reddish or yellowish discoloration. Chlamydospore-anamorph was unicellular, botryoid-alternarioid shape (Fig. 3E). Hyphae were thick-walled, septate, binucleate, and hyaline (Fig. 3D). The mycelia were thick and septate. Conidiophores formed from buds on the hyphae and were unbranched to rarely branched, straight or flexuous, globose, pale brown to medium brown, smooth, 10 to 20 × 10 to 15 μm in size, and had 1 to 2 percurrent proliferations (Fig. 3F).

Culture characteristics. Colonies on OA medium had irregular margins and were 50 to 60 mm in diameter after 7 days of incuba-

Fig. 3. Morphological observation of *Phoma sorghina* var. *saccharum*. **A-C**, Seven-day-old colonies on OA (**A**), MEA (**B**), and PDA (**C**). **D**, Septate hyphae. **E**, Chlamydospore. **F**, Asexual conidia formation. **G-I**, Pycnidia and conidiophores.

tion. Immersed mycelia were rarely visible due to the aerial mycelia. Mycelia were hyaline or white with many pycnidia from the top, while it was grayish to olivaceous-buff at the bottom (Fig. 3A). Colonies on MEA medium had regular margins and were 45 to 55 mm in diameter after being cultured for 7 days. Immersed mycelia were hyaline or amber. Only the sparsely small white tufts of aerial mycelia were produced in the older cultures from the top, while it was black at the bottom (Fig. 3B). Colonies on PDA had regular margins and were 45 to 55 mm in diameter after being cultured for 7 days. Immersed mycelia were hyaline and white, and the colonies appeared as white, villous, spherical, radial, and densely packed colorless mycelia from the top, while it was reddish-brown at the bottom and later changed to a grayish color (Fig. 3C).

As characterized by Boerema et al. (4) and Aveskamp et al. (2), *P. sorghina* occasionally produced micropycnidia that developed from a hyphal cell or from a single chlamydospore cell. Chlamydospore-anamorph was unicellular or multicellular, botryoid-alternarioid shape. However, the various strains of *Phoma* section *Peyronellaea*, including *P. sorghina*, showed extreme variability in the shape and size of the chlamydospore (4). In our current study, fungal isolates had a unique characteristic, whereby the produced chlamydospores were botryoid or epicoccoid with a diameter of 5 to 10 μm . These chlamydospores were unicellular with dark brown or olivaceous pigmentation, terminal on erect aerial hyphae, and individual with papillate pycnidia and conidia aseptate. Through studies of phylogenetic analysis together with morphological observations, we formally describe our isolates as *P. sorghina* var. *saccharum* (Mycobank: MB808056) based on the genus of the host plant (*Saccharum officinarum* L.).

Pathogenicity test of *P. sorghina* var. *saccharum* from China.

Twisted and curly leaf symptoms similar to those observations of sugarcane plants growing in the field appeared on the inoculated sugarcane leaves at 10 days after inoculation. The symptoms started as yellowing on midribs and leaf margins that spread fur-

ther to the entire leaf, along with twisting and curling of crown leaves (Fig. 4A, 4B). Uninoculated control leaves remained asymptomatic (Fig. 4C). The pathogens that were inoculated onto the sugarcane seedlings were reisolated from representative lesions and their identity confirmed, completing Koch's postulates. The *Phoma* sp. pathogen associated with sugarcane leaf twisting disease was formally described herein as *P. sorghina* var. *saccharum*.

Effects of temperature and pH on mycelial growth in vitro.

Results of variance analysis showed a significant effect of temperature ($P < 0.0001$) on the mycelial growth of fungal isolate Fn40. Isolate Fn40 grew most slowly at 5 and 35°C and the optimum temperature ranged from 20 to 25°C (Fig. 5A). For all tested pH values, pH 4.0 was optimum for the mycelial growth of *P. sorghina* var. *saccharum* (Fig. 5B).

Responses of *P. sorghina* var. *saccharum* to chemical compounds in vitro.

Thirteen compounds were tested at three concentrations (100, 50, and 10 ppm) for their ability to inhibit mycelial growth of *P. sorghina* var. *saccharum* (Fig. 6). Among the tested compounds, carbendazim was the most effective in inhibiting the radial growth. Mycelial growth was completely inhibited at 100 and 50 ppm, and 87.6% of mycelial growth was inhibited at 10 ppm. Three compounds including mancozeb, carbendazim, and copper 8-hydroxyquinoline inhibited mycelial growth by more than 50% at all tested concentrations. Six antibacterial compounds (rifaximin, lincomycin hydrochloride, cinoxacin, cephalixin, berberine chloride, and emamectin benzoate) and one antiviral compound (pasiniazid) had no effect on the mycelial growth of *P. sorghina* var. *saccharum*. Two fungicides, including 2-amino-5-chloro-benzoxazole and thiophanate-methyl, partially inhibited mycelial growth only at the concentration of 100 ppm (Fig. 6).

Discussion

Species within the coelomycete genus *Phoma* are ubiquitous in the environment and occupy numerous ecological niches (1).

Fig. 4. Twisted and curly symptoms of sugarcane (cultivar FN40) leaves in the field (A) and on plants inoculated with *Phoma sorghina* var. *saccharum* (B). No symptoms were observed on the healthy control plants inoculated with water (C).

Phoma is an important pathogen of many plants and crops, and causes significant yield losses. In Australia in the 1970s, rapeseed yield was drastically reduced by epidemics of blackleg caused by *Phoma lingam* (anamorph) (30). Sugarcane twisted leaf disease, caused by *Phoma* sp., was first reported in Guangxi, China, in 2012 (18). On the basis of disease incidence and severity observed in several regions in China, we believe that this disease may pose a serious threat to cane production and management in the future. *P. sorghina* on sugarcane leaves or sugarcane disease caused by *Phoma* sp. has been reported in Pakistan, Hawaii, and Taiwan (12,17,31). According to our morphological observations, molecular phylogenetic trees, and culturing experiments, we formally described and established our disease-associated isolates as *P. sorghina* var. *saccharum*. The isolates described here were pathogenic on inoculated sugarcane, and produced identical symptoms to those observed in the field, thus confirming their role as the causal agent of twisted leaf disease of sugarcane in China. To the best of our knowledge, it is the first report to identify the pathogen causing this disease.

Based on detailed studies of its morphology, *P. sorghina* var. *saccharum* can be differentiated from other known species of *Phoma* by the morphology of the pycnidia and chlamydospores. The chlamydospores of *P. sorghina* var. *saccharum* were unicellular, botryoid or epicoccoid, terminal on erect aerial hyphae, and individual with papillate of pycnidia and conidia aseptate. Species linked to *Phoma* section *Peyronellaea* are typified as chlamydospore-producing, which is a useful characteristic to use for taxon delineation (3). However, the various strains of *Peyronellaea* show extreme variability in the shape and size of the chlamydospores (4). In addition, the reddish tint of the culture may serve as an additional distinguishing morphological feature for this species.

The availability of DNA sequence data at multiple loci led to major developments in systematics and the ability to correctly identify coelomycetous fungi (5). Phylogenetic studies based on

LSU+ITS+SSU+TEF- α loci were conducted for the *Phoma* species complex as described by Aveskamp (2). When our isolates (FN40, FJ1, and FJ2) were included in this analysis, results showed that *P. sorghina* var. *saccharum* is classified in the Didymellaceae clade. The Didymellaceae clade is one of the major clusters observed in the genetic phylogeny, such that five out of nine *Phoma* sections are related to the Didymellaceae (9). Furthermore, the results obtained from a phylogenetic tree based on LSU+ITS+TUB loci for the *Phoma* section *Peyronellaea* were consistent with a previous report by Aveskamp (3). *P. sorghina* var. *saccharum* was grouped with other *P. sorghina* species, but the host ranges associated with *P. sorghina* revealed little genetic variation (25). The position of *P. sorghina* relative to *P. epicoccum* was of particular interest because *P. sorghina* was closely related to *P. epicoccum* (2). *P. epicoccum* occurs in a wide variety of plants, soil, and other substrates (4). It is also encountered as a facultative plant pathogenic fungus with a preference for Gramineae in tropical regions, and causes leaf spot (25). *P. sorghina* var. *saccharum* causes symptoms of twisting and curling on crown leaves of sugarcane, and this specialization is a new characteristic.

Environmental factors, such as temperature and pH, are key factors that influence the growth and development of disease-associated fungal mycelia in plants. We observed in vitro growth of *P. sorghina* var. *saccharum* mycelia under a wide range of temperatures (5 to 35°C). The optimum temperature for mycelial growth was between 20 and 25°C. These findings were in agreement with those observed for *P. macdonaldii* and *P. lingam* (29), indicating that *P. sorghina* var. *saccharum* has a preference for growth in subtropical climates. The radial growth rate of the mycelium decreased at temperatures above 30°C.

Most phytopathogenic fungi grow optimally in a pH range between 5 and 6.5 (8). However, *P. sorghina* var. *saccharum* exhibited growth typical of a fungus specialized for acidic habitats. The isolate of *P. sorghina* var. *saccharum* exhibited optimal growth at pH 4. Preference for acidic conditions was also reported for *Acidomyces acidophilus* (32). However, *P. sorghina* var. *saccharum* had a lower growth rate at pH 7.0 and 8.0 (Fig. 5B). Our results could be important for future studies aiming to control the incidence of *P. sorghina* var. *saccharum* in the field.

Fungicides are especially valuable because their toxicity to non-target animals and humans is lower (10). Effective chemical control of sugarcane fungal diseases caused by *P. sorghina* var. *saccharum* requires appropriate information on the sensitivity of this fungus to a range of fungicidal compounds. Although it is difficult to correlate the effects of fungicides in vitro with their performance in the field, such studies must serve as the first step for designing strategies for disease control (24). A total of 13 chemical com-

Fig. 5. Radial growth of *P. sorghina* var. *saccharum* in response to different temperatures (A) and pH (B) on standard PDA medium.

Fig. 6. Inhibition rate (%) of fungicides on *Phoma sorghina* var. *saccharum* in vitro. Data are presented as the mean of three independent experiments $\pm$ SD.

pounds, including some commonly used fungicides, were used to evaluate their ability to inhibit the growth of *P. sorghina* var. *saccharum* mycelia in vitro. The strongest inhibition of mycelial growth was observed in cells treated with carbendazim followed by mancozeb. Of the other fungicides tested, copper 8-hydroxyquinoline, 2-methyl-4-isothiazolin-3-one, 2-amino-5-chloro-benzoxazole, and thiophanate-methyl were moderately to highly inhibitory of fungal growth. However, six antibacterial compounds and one antiviral compound were not effective against mycelial growth of *P. sorghina* var. *saccharum*. Benzimidazoles, particularly carbendazim, are site-specific fungicides that interfere with cell division (7), and comprise a well-known group of β -tubulin disruptors that affect micro-tubule function in many fungi (19,27). In our study, mycelial growth was inhibited by low concentrations of carbendazim, which might decrease the virulence of this pathogen in nature. Therefore, carbendazim is a potentially useful fungicide against *P. sorghina* var. *saccharum*, the causal agent of sugarcane twisted leaf disease, and may be effective in managing the disease in China and preventing its spread. The findings of this study will provide basic information for *P. sorghina* var. *saccharum* management in the field.

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