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Mycophenolic Acid as a Promising Fungal Dimorphism Inhibitor to Control Sugar Cane Disease Caused by *Sporisorium scitamineum*

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Supporting Information

ABSTRACT: The morphological changes from single-cell yeast to filamentous hypha form are critical in plant pathogenic smut fungi. This dimorphic switch is tightly regulated by complex gene pathways in pathogenic development. The phytopathogenic basidiomycetes *Sporisorium scitamineum* displays a morphological transition from budding growth of haploid cells to filamentous growth of the dikaryon, which enables fungi to forage for nutrients and evade the host plant immune system. In the search for compounds that affect dimorphic switch instead of killing the cell directly, a natural product, mycophenolic acid (MPA), was purified and exhibited significant dimorphism inhibitory activities with minimum effective concentrations of 0.3 μg/mL. RNA sequencing and real-time quantitative transcription-PCR analysis showed that treatment of 100 μg/mL MPA dramatically repressed the expression of the ammonium transporter gene *Ssa2* . A further subcellular localization experiment, ammonium response assay, and Western blot assay confirmed that *Ssa2* could be one of the most important molecular targets of MPA in regulating dimorphism of *S. scitamineum*. These observations suggest that *Ssa2* serves as a molecular target of MPA and could be used in the treatment of sugar cane smut diseases caused by *S. scitamineum*.

KEYWORDS: *Sporisorium scitamineum, sugar cane smut, dimorphism, mycophenolic acid, inhibitor*

INTRODUCTION

Sugar cane is a giant grass grown in tropical or subtropical areas of the world, from which white sugar has a very high purity, making it one of the purest organic substances that is produced on the industrial scale. In many parts of the world, natural sugar (sucrose) from sugar cane is the main dietary source of carbohydrate.[1] Sugar cane smut is a serious disease of sugar cane caused by the basidiomycete fungus *Sporisorium scitamineum*, which causes substantial losses in cane yield and the sugar industry. Typical symptoms of sugar cane smut disease are a whip structure in the stalk apex containing a mixture of fungal and plant tissues, named "smut whip". The pathogen produces two types of haploid sporidia, then fused to form dikaryon, and finally developed into hyphae to penetrate the bud scales of the sugar cane plant and infect host meristematic tissues. During this process, *S. scitamineum* switches from a non-pathogenic yeast-like form to a pathogenic filamentous form, which are critical to pathogenicity in dimorphism fungus.[2−4] A recent study indicates that the MAP kinase SsKpp2 is required for *S. scitamineum* filamentation, likely through regulating the conserved pheromone signal transduction pathway and tryptophol production.[5] However, control of sugar cane smut disease remains a big challenge, and traditional ways of controlling the disease mainly rely on the breeding of resistant cultivars and soaking seed canes with toxic chemical fungicides, which may cause toxic effects through the food cycle in various life forms.[6,7]

Since the dimorphic switch plays an important role in the pathogenicity development of *S. scitamineum* and no reported chemicals targeted on it, we started searching for a chemical candidate with dimorphic switch inhibitory activity. In our continuing search for structurally novel compounds with diverse bioactivities from marine fungi,[8−11] mycophenolic acid (MPA, Figure 1) showed significant inhibitory activity toward dimorphic switch of *S. scitamineum*. Instead of killing the cell directly to control sugar cane smut diseases, MPA possibly targeted on the amino transporter gene *Ssa2* (SSCI39B01222) and inhibited the dimorphic switch and teliospores germination of *S. scitamineum*. These findings suggest that MPA has the potential of being a chemical candidate for sugar cane smut diseases with significant biocontrol activity and relatively lower toxicity.

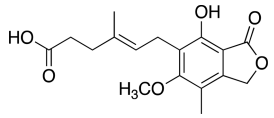

Figure 1. Chemical structure of MPA.

MATERIALS AND METHODS

Fungal Strains and Culture Conditions. The strains used in this study are listed in Table S1. *S. scitamineum* and fluorescent

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mutants were used and cultured as previously reported.[12] The assay used the YePS medium supplemented with 100 μg/mL ampicillin and 2% agar at 28 °C for 3 days. *Escherichia coli* strains DH5α (TransGen Biotech, China) were used for preparation of plasmid DNA and cultured in LB medium with 50 μg/mL spectinomycin. MPA was purified from the strain *Penicillium biourgeianum* MCCC 3A00078, which was identified by Dr. Zhongze Shao, and a voucher specimen is deposited in the Marine Culture Collection of China.

Dimorphic Switch Inhibitory Assay. Tested compounds were dissolved in DMSO and prepared in different concentrations. MAT-1 and MAT-2 colonies were cultured in 5 mL YePSA overnight (28 °C, 200 rpm), respectively. Next, 1 mL of YePSA medium (agar) with different concentrations of compounds were added to a 24-well plate. Then, 1 μL of mixture of MAT-1and MAT-2 was added to each well. The well without compounds was used as a negative control. The 24-well plate was incubated in a 28 °C incubator for 2 days by observing the formation of fermentation, and the MIC value was calculated accordingly.

Effect of MPA on Growth of *S. scitamineum*. MAT-1 at 5 mL of YePSA medium was cultured at 28 °C and 200 rpm overnight, and then the medium OD_{600} was adjusted to 0.1. The OD_{600} values of the fungi culture with different concentrations of MPA were measured every 4 h until the cell reached to the stationary phase, and then the growth curve was drawn.

Effect of MPA on Teliospore Germination. MPA was added into YePSA medium with different concentrations (from 0.5 to 100 μg/mL). Then, 100 μL of aliquot of chlamydospore (1×10^6 chlamydospore mL^{-1}) of *S. scitamineum* was spread in each Petri dish. After 12 h incubation (28 °C), teliospore germination was checked under a light stereoscope (Axioplan 2 imaging, ZEISS) and a stereo microscope (Stemisv 11, ZEISS).

Greenhouse Trial. These procedures were followed as previously reported.[13]

RNA Extraction and Sequencing. These procedures were followed as previously reported.[14]

Real-Time qPCR Analysis. Transcript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech, Beijing, China) were used to synthesize cDNA. The constitutively expressed *ppi* gene was used as an internal control. The qPCR was performed with a Talent qPCR PreMix SYBR Green Kit (TIANGEN, Beijing, China) on a 7500 real-time qPCR system (Applied Biosystems, Carlsbad, CA, USA). The reaction mixture contained 10 μL of 2x Talent qPCR PreMix, 1.0 μL of template (100 × diluted cDNA), 2.0 μL of 50x ROX Reference Dye^{Δ}, 5.8 μL of RNase -free ddH_2O, and 0.6 μM of each primer in a 20 μL total volume. The conditions were used as follows: 95 °C for 3 min, 95 °C for 5 s, 45 cycles at 60 °C for 15 s, and 95 °C for 15 s. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative changes in gene expression from real-time qPCR experiments.

Subcellular Localization Experiment. Fungi hyphae from YePS medium was ground in liquid nitrogen and lysed in DNA extracting buffer (50 mM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS) at 65 °C. Genomic DNA was extracted twice with chloroform (1:1, v/v) and subsequently precipitated with isopropanol and 3 M sodium acetate. Genomic DNA was dissolved in RNase A (Omega Bio-Tek, America) treated sterile water and stored at −20 °C. Strains and plasmids generated and used in this study are listed in Tables 1 and 2. Primers used in this study are listed in Table 1. All primers were synthesized by Invitrogen Trading Co., Ltd. (Shanghai, China). A 3.8 kb flanking fragment A (containing *Ssa2* and upstream of target genes) was amplified from genomic DNA by PCR using the *Ssa2*-F/*Ssa2*-R primer set. A eGFP fragment was driven from plasmid pEX2-eGFP by PCR using the eGFP-F/eGFP-R primier set and was fused to fragment A via fusion PCR using primers *Ssa2*-F and eGFP-R and generated fragment B. Then, fragment B was digested with restriction enzymes *Pme*I (New England Biolabs, Ipswich, MA) and ligated with vector pEX2 to form new vector pEX2MAT1*Ssa2*-eGFP.[4] The PCR-amplified fragment and recombinant plasmid pEX2MAT1*Ssa2*-eGFP were sequenced by Invitrogen Trading Co., Ltd. (Shanghai, China). The pEX2MAT1*Ssa2*-eGFP was transformed into *Agrobacterium tumefaciens* by electroporation, and the positive transformants grew on LB agar with spectinomycin (100 μg/mL) and rifampicin (75 μg/mL) at 28 °C for 2 days. Transformation in *S. scitamineum* was performed by *Agrobacterium tumefaciens*-mediated method.[15] The transformants of *Ssa2*-GFP were verified by microscope examination and Southern blot analyses.

Table 1. Fungal and Bacterial Strains Used in This Study

strain name	description[a]	ref
Sporisorium scitamineum MAT-1	wild-type	4
Sporisorium scitamineum MAT-2	wild-type	4
MAT-1G	*MAT-1* strain with the strongest GFP signal	12
MAT-2R	*MAT-2* strain with the strongest RFP signal	12
Ssa2-GFP	MAT-1*Ssa2*□eGFP□Hm^r	this study
*Agrobacterium tumefaciens*Strain *AGL 1*	transformation	15
E. coli DH5α	transformation	TransGen Biotech

[a]Hm^r, hygromcin resistance

Table 2. Plasmids Used in This Study

plasmid	description[a]	ref
pEX2	Gpda□Hm^r	4
pEX2-eGFP	pAngpd□Hm^r□eGFP	4
pEX2MAT1*Ssa2*-eGFP	*Ssa2*□eGFP□gpda□Hm^r	this study

[a]Hm^r, hygroycin resistance

Western Blotting Assay. YePS medium was used to exact protein from *S. scitamineum*. *Ssa2*-GFP was inoculated into 100 mL of liquid YePS, and the mixture was incubated at 28 °C (200 rpm) for 24 h. After low speed centrifugation, cells were suspended with sterile water in a new flask, to which MPA was added with different concentrations (5, 10, 25, 50, and 100 μg/mL), and the mixture was continued to be incubated for another 6 h (DMSO was used as the control). Haploid spores were harvested and ground in liquid nitrogen. The frozen pellet was resuspended in 300 μL of extraction buffer (40 mM HEPES-NaOH, pH 7.4, 350 mM NaCl, 0.1% NP40, 10% glycerol, 1 mM PMSF, 1 μg/mL Pepstatin A, 1 μg/mL Bestatin, EDTA-free protease inhibitor cocktail) and transferred into 1.5 mL tubes. After incubation on ice for 30 min, samples (supernatant and any pellet) were centrifuged at 13000*g* for 10 min at 4 °C and transferred into 1.5 mL tubes. For Western experiments, the resulting proteins were separated by PAGE and transferred onto a nitrocellulose membrane (Pall Corporation, New York, USA) by electroblotting. First antibody Anti-GFP Mouse Monoclonal (TransGen Biotech, Beijing, China) and second antibody Anti-Mouse IgG (H+L) HRP Conjugate (TransGen Biotech, Beijing, China) were used to detect *Ssa2* with GFP. GAPDH was detected in the samples using anti-GAPDH antibody (TransGen Biotech, Beijing, China) as reference. Incubation with an antimouse HRP-conjugated secondary antibody (TransGen Biotech, Beijing, China) was performed. As a detection reagent, the ECL technology (GE Healthcare Life Sciences, Munich, Germany) products were used.

RNA-seq Data Accession Number and Sequence Data. All of the RNA sequencing data were deposited to the NCBI database under Bioproject accession number PRJNA472153. DNA sequence information could also be found in https://figshare.com/s/4c45e5ba205f49e4cd6d.

RESULTS

MPA Inhibited Dimorphic Switch of *S. scitamineum*. Among all the tested compounds, MPA showed a significant inhibitory effect on dimorphic switch of *S. scitamineum* with

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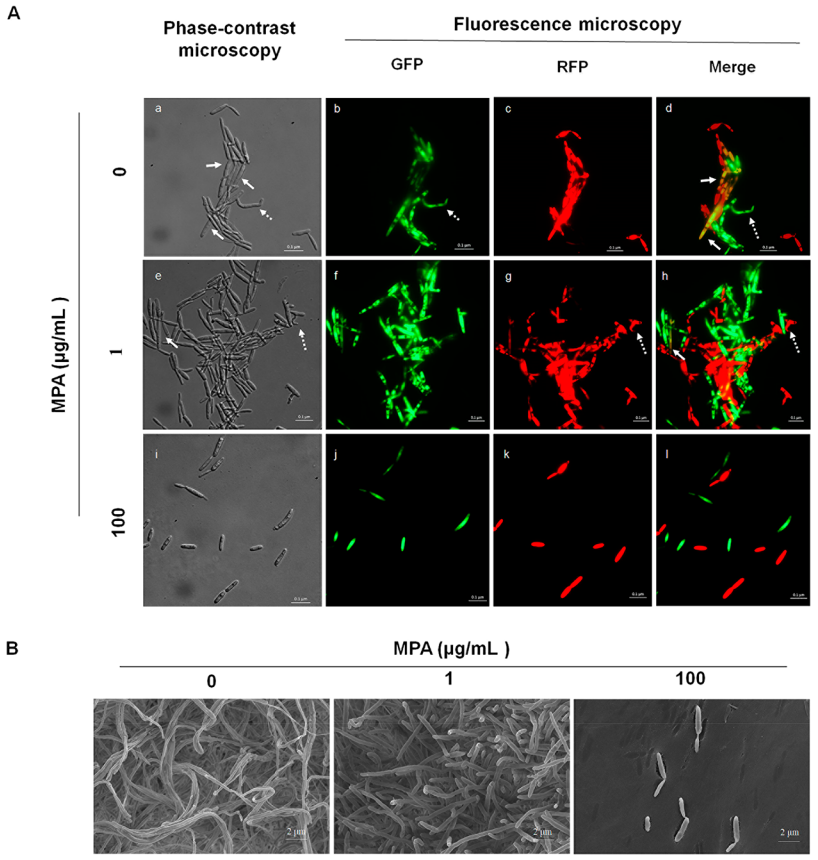

Figure 2. (A) Effects of MPA on *S. scitamineum* dimorphic switch in fluorescence microscopy images: (a–d) control, (e–h) 1 μg/mL MPA treatment group, (i–l) 100 μg/mL of MPA treatment group. (a, e, and i) Phase-contrast microscopy. Arrows and the dotted arrows indicate the filamentous and conjugate tubes formed by cell fusion. (B). SEM images of *S. scitamineum* cells under different MPA concentrations.

MIC values at 0.3 μg/mL (Table S2). To make further observations of morphological changes after treatment with MPA, we used the red and green fluorescence mutants MAT-1 (expression GFP) and MAT-2 (expression RFP).[12] Under bright field as well as under epifluorescence microscopy, the fusion of sporidia was observed (Figure 2Aa–Ad, arrows). GFP and RFP fluorescence were merged and resulted in forming an orange-yellow color (Figure 2Ad, arrows). When treated with 1 μg/mL MPA, few dikaryon hyphae and conjugation tubes could be found (Figure 2Ae–Af, arrows). The fungal cells were cigar-like with cytosolic GFP or RFP when treated with 100 μg/mL MPA (Figure 2Ai–Al). Similar results were observed when preparing fungal cells to scanning electron microscope (SEM) imaging. After 1 μg/mL MPA was added, the produced sporidium appeared to be swollen. When treated with 100 μg/mL of MPA, cells were inhibited to budding yeasts stage (Figure 2B). The results mentioned above showed that MPA inhibited filamentation of *S. scitamineum* significantly.

Effect of MPA on *S. scitamineum* Growth. To test whether the inhibition of MPA on hyphal growth was due to its toxic characteristic of killing the cells directly, we checked the coculture results on a 24-well plate for 15 days (Figure S5). With 5–50 μg/mL MPA treatment, MAT-1 and MAT-2 haploid cells grew slowly at the beginning 4 days, but dikaryon cells were formed and observed after 15 days, which indicated that the growth rate might be affected with relatively high concentrations of MPA. We also measured the growth curve of *S. scitamineum* treated with different concentrations of MPA. The cell growth rate under 2–10 μg/mL MPA treatments were not obviously affected, and they all reached 0.4 of the similar OD_{600} value after culturing for 72 h. It showed that the cell growth rate was very slow with 50 μg/mL MPA (Figure 3).

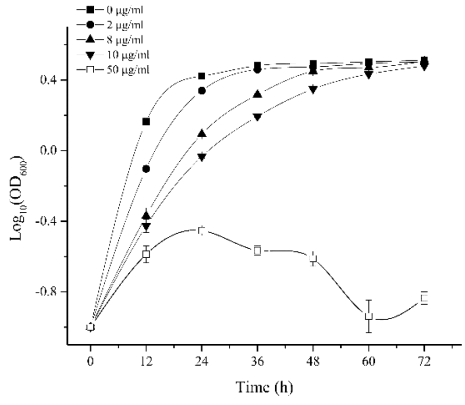

Figure 3. Grow curves of MPA against *S. scitamineum* strain MAT-1.

This result is consistent with 24-well plate assay. MPA has no growth inhibitory activity toward *S. scitamineum* cells at low concentration (<10 μg/mL) but may affect the cell growth at high concentration (>50 μg/mL).

Effects of MPA on *S. scitamineum* Infected Sugar Cane Plants. We hypothesized that if MPA inhibited the dimorphism of *S. scitamineum*, treatment of sugar cane with MPA could prevent sugar cane smut. Therefore, we tested the effects of MPA on the *S. scitamineum* infected sugar cane plant

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J. Agric. Food Chem. 2019, 67, 112–119

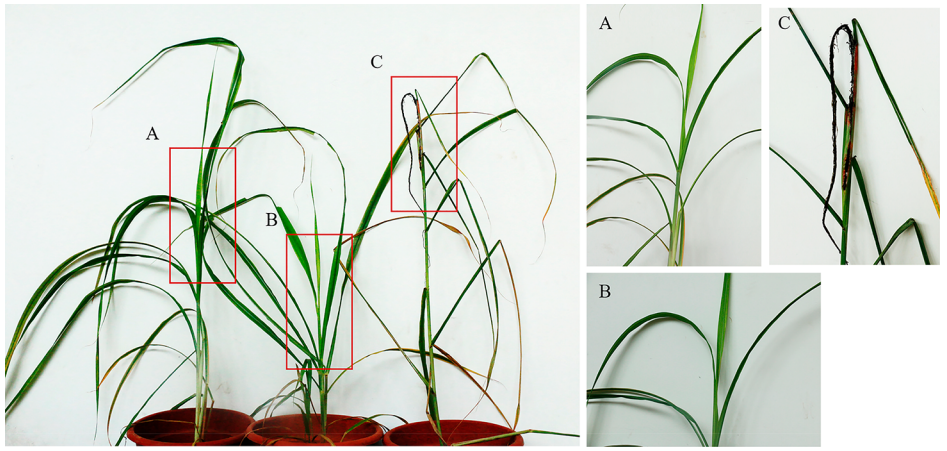

Figure 4. MPA effects on *S. scitamineum* infection of sugar cane. Sugar cane variety ROC22 was inoculated with the mixture of fungal cells through injection at the seeding stage. Symptoms of whole plants treated with 320 μg/mL MPA + *S. scitamineum*, 32 μg/mL MPA + *S. scitamineum*, and *S. scitamineum* are presented from left to right. (A, B, and C) show the enlarged boxed regions of the sugar cane.

Table 3. List of Genes Up- and Down-Regulated Across All Time Points in the Comparison between Treatment and Control Samples by RNA-seq[a]

	$\log_2$ fold change				
Ssci39b gene ID	4 h	8 h	12 h	Umay gene ID	description
SSCI39B01085	1.39	1.42	1.73	UMAG02333	hypothetical protein
SSCI39B02003	1.38	1.74	1.17	UMAG15028	probable arginine-specific carbamoyl-phosphate ammonia chain
SSCI39B02631	1.35	1.52	1.38	UMAG01165	glycoside hydrolase
SSCI39B00835	−1.12	−1.30	−1.11	UMAG15020	kelch repeat-containing
SSCI39B01222	−2.63	−2.76	−5.34	UMAG05889	high affinity ammonium transporter
SSCI39B02366	−1.94	−2.60	−4.23	UMAG01425	homogentisate 1,2-dioxygenase
SSCI39B02367	−3.76	−4.07	−5.47	UMAG01424	probable cytochrome P450 monooxygenase phenylacetate hydroxylase
SSCI39B02541	−1.43	−2.66	−2.44	UMAG11855	transcription factor MBF1
SSCI39B02713	−1.63	−2.60	−3.16	NA	monooxygenase, tryosinase
SSCI39B04169	−1.81	−2.55	−1.57	UMAG10837	serine-threonine phosphatase
SSCI39B04563	−1.34	−1.73	−1.05	UMAG12340	C-4 methylsterol oxidase
SSCI39B04693	−2.24	−1.52	−1.67	UMAG04211	DEAD-box-containing helicase
SSCI39B05236	−1.00	−1.93	−1.63	UMAG05421	related to multidrug resistance
SSCI39B05268	−2.31	−1.11	−3.58	UMAG03643	glycosyltransferase family 2
SSCI39B06112	−1.66	−1.93	−1.82	UMAG04479	4-coumarate- ligase

[a]Note: "−" means down-regulated.

with 32 and 320 μg/mL. Consistent with the above in vitro experiment data, MPA treated plants showed no characteristic symptoms of black "whip", indicating the protective activity of MPA (Figure 4A and B).

Effects of MPA on *S. scitamineum* Transcriptome. Based on the inhibitory effect of MPA on hypha growth and reduced virulence of *S. scitamineum*, it was necessary to check the transcriptional response in *S. scitamineum* with MPA treatment. By using the threshold of significance as $\log_2$ fold change >1, we found that MPA treatment (100 μg/mL) significantly affected 441 genes (DEGs; Table S5), with 192 up-regulation and 249 down-regulation genes. Comparing the up- and down-regulation genes in three time points samples, we identified that 12 down-regulation and 3 up-regulation genes consisted, and their predicted functional annotations are listed in Table 3, including ammonium transporter (*Ssa2*, regulating dimorphic growth in fungi and involved in cell growth and development in yeast),[16,17] homogentisate 1,2-dioxygenase (HGD, involved in melanin biosynthesis pathway),[18,19] tyrosinase (TYR, associated with the formation and stability of spores, browning, and pigmentation),[20] and cytochrome P450 monooxygenase (PHACA, plays a critical role in colonization, degradation of plant material, and detoxification of plant defense chemicals).[21,22] To validate the RNA-seq results, four DEGs were chosen for qPCR, including *Ssa2*, HGD, TYR, and PHACA. The results were consistent with RNA-seq analysis (Figure 5). *Ssa2* was down-regulated by 2.63-, 2.76-, and 5.34-fold at three time points; HGD was down-regulated by 1.94-, 2.60-, and 4.32-fold; TYR was down-regulated by 1.63-, 2.60- and 3.16-fold; and PHACA was down-regulated by 3.76-, 4.07-, and 5.47-fold. Although the function of many other affected genes were unknown, down-regulated these genes might be the reason for reduced virulence factors of *S. scitamineum*.

Effects of MPA on *Ssa2* Expression. In *S. cerevisiae*, *C. albicans*, and *U. maydis*, the AMT family is involved in morphological changes and cell growth.[23] Looking for detailed effects of MPA on *Ssa2*, we constructed the N-terminal tagged *Ssa2*-GFP fused protein in a *MAT-1* background (Figure S6). Subcellular localization of *Ssa2* indicated the location in the cell membrane by visualizing the *Ssa2*-GFP fluorescence signals (Figure 6). In the Western blotting assay, *Ssa2*-GFP expression levels were significantly decreased when treated with MPA

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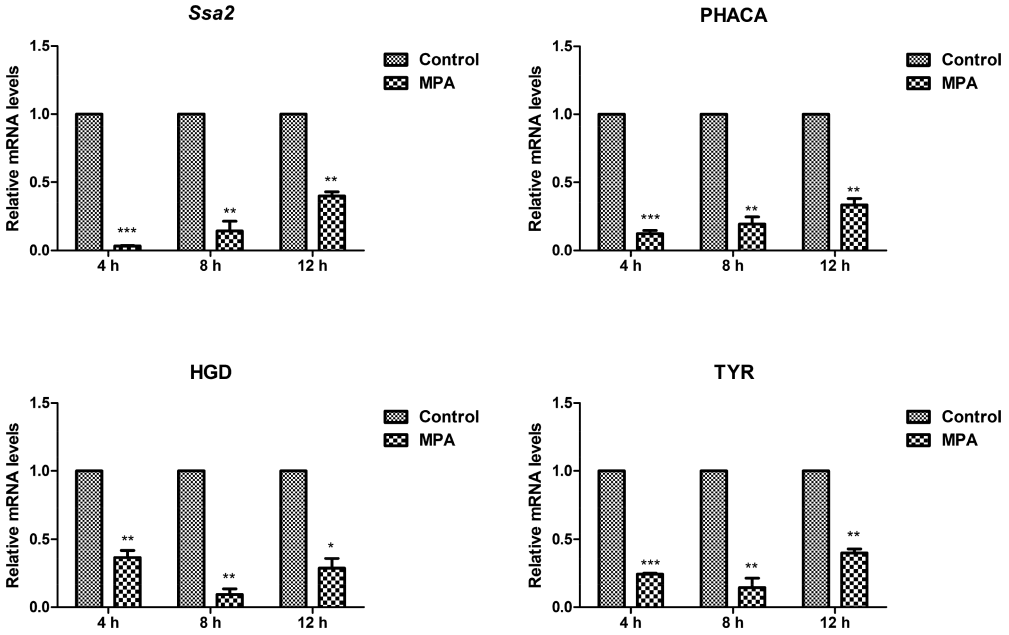

Figure 5. Gene expression levels after MPA treatment. RNA concentrations were normalized by *ppi* expression. Data are means ± SD from three experiments. The relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. The normalized value of mRNA for the control was 1.0, where $*p < 0.01$, $**p < 0.001$, and $***p < 0.0001$.

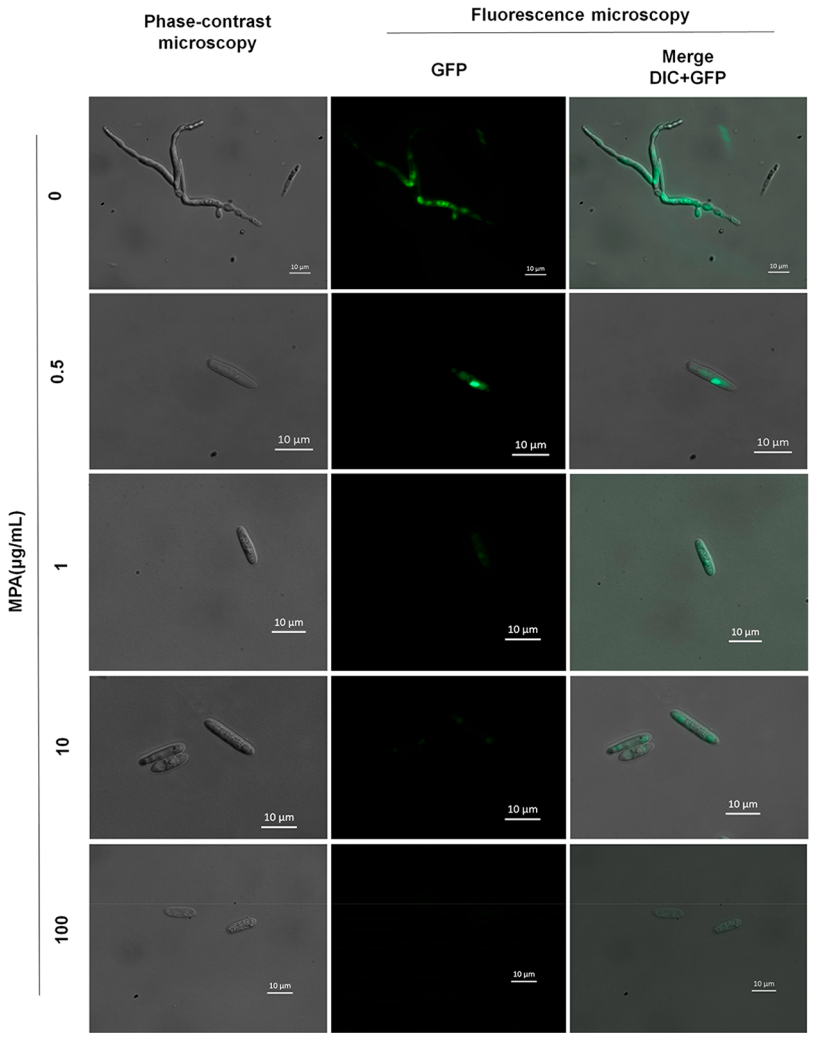

Figure 6. Results of subcellular localization and the effect of MPA on *Ssa2*-GFP.

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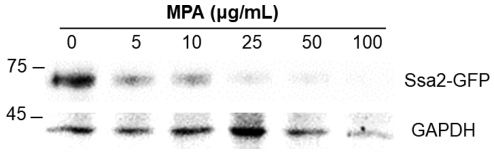

Figure 7. MPA reduces the expression of *Ssa2*-GFP.

(Figure 7). It has been known that the availability and sensing of nitrogen are essential to the diverse processes in animal and plant fungal pathogens, including dimorphic switch and pathogenicity. In our study, the expression levels of an ammonium transporter *Ssa2* was down-regulated by MPA, indicating the important role of *Ssa2* in controlling *S. scitamineum* dimorphic switch, and might be an important target of MPA.

MPA Inhibits Teliospore Germination of *S. scitamineum*. Teliospore germination was totally inhibited with 100 μg/mL MPA (Figure 8A). The control was 68.9%. Microscopic and SEM observations also showed MPA inhibitory activity of teliospores germination (Figure 8B). MPA showed inhibitory activities not only toward dimorphic switch but also to teliospore germination. It is tempting to speculate that MPA might become a promising drug candidate for sugar cane smut disease.

DISCUSSION

In this paper, we reported the first natural products MPA that showed potent inhibitory activity against the dimorphic switch of *S. scitamineum* as well as significant protective effect on *S. scitamineum* infected sugar cane plants. Additionally, transcriptome and real-time PCR analysis showed *Ssa2* might be one of the most important targets of MPA and inhibitory of *Ssa2* expression would reduce hypha growth and teleocidins germinations. Previously, MPA was reported as having antifungal activity toward *C. albicans*, which inhibited *C. albicans* morphological switch and a cell cycle at 20 μg/mL and 8.4 μg/mL, repectively.[23−25] In our study, MPA showed significant inhibitory activity toward morphological changes of *S. scitamineum* at 0.3 μg/mL. After further SEM imaging as well as fluorescence microscope examinations, no conjugation tube was observed at 100 μg/mL MPA, whereas the formation of germ tubes was greatly suppressed at lower concentrations of MPA. Moreover, MPA also showed an inhibitory effect on haploid cells of *S. scitamineum*. Given these desirable properties of MPA, it would be of great interest to understand the potential mechanism underlying its inhibitory activity. In dimorphic fungi, the morphological change from yeast-like cells to filamentous hypha is regulated by a number of mating-related genes, especially loci *a* and *b*.[14] The *a* loci encodes the pheromone and pheromone−receptor gene system required for recognition and hyphal fusion between cells, whereas the *b* loci encodes two subunits of a heterodimeric transcription factor (*bE*/*bW*) which is a major regulator of mating and pathogenicity in *S. scitamineum*.[26] The formation of conjugation tubes is mainly regulated by the *a* loci.

To further investigate these possibilities, we assessed the transcriptional modulations caused by MPA treatment using RNA-seq and qRT-PCR. The two independent approaches revealed that the expression levels of *Ssa2*, HGD, TYR, and PHACA were greatly decreased in response to MPA. The gene *Ssa2* is of particular interest, which is a member of the AMT gene family encoding ammonium transporters in the transport and uptake of ammonium. It has been known that the availability and sensing of nitrogen are essential to the diverse processes in animal and plant fungal pathogens, including dimorphic switch and pathogenicity. RNA-seq and qPCR approaches revealed that MPA could reduce the expression levels of *Ssa2*. Subcellular localization was used to visualize the expression of *Ssa2*-GFP. The results indicated that *Ssa2* was located in the cell membrane, which was consistent with previous findings.[27] Western blot results further demonstrated that MPA down-regulated the expression levels of *Ssa2* at

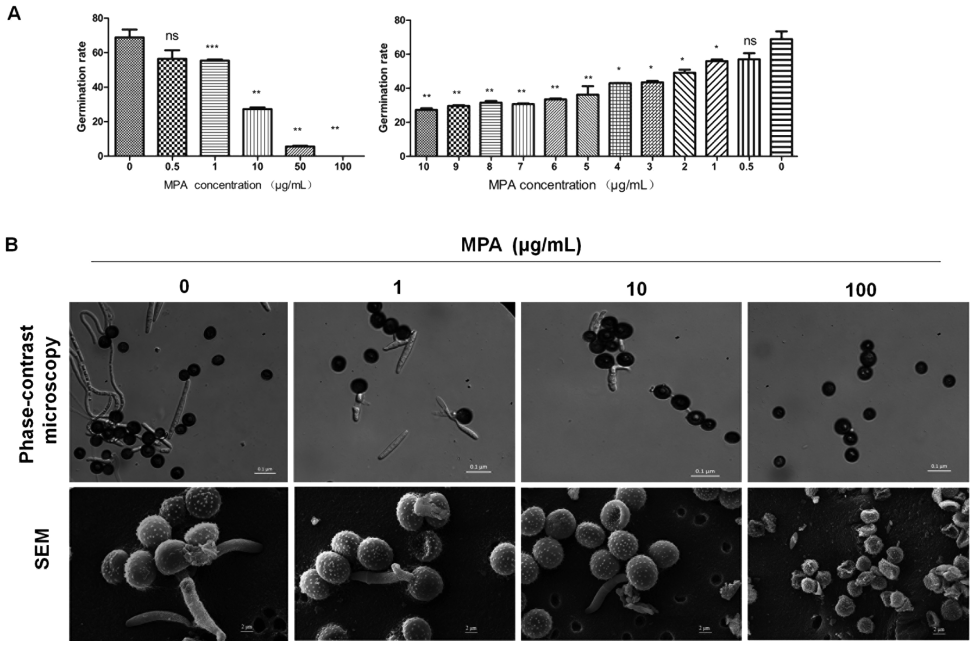

Figure 8. Effects of MPA on *S. scitamineum* teliospore development. Teliospores were incubated on 2% agar plates and imaged at 12 h. (A) The teliospore germination rate was calculated; line bars in each column denote standard errors of three repeated experiments. Statistically significant (*) and not significant (ns) differences are shown, where *$p < 0.05$ and ** $p < 0.01$. (B) The teliospores were inoculated in 2% agar with the 100 μg/mL MPA treatment group, 10 μg/mL MPA treatment group, and 1 μg/mL MPA treatment group. The figure was imaged in phase-contrast microscopy.

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different concentrations. Moreover, MPA also exhibited a significant inhibitory effect on teliospore germinations, indicating the protective possibility of MPA at an early stage of sugar cane infection by *S. scitamineum* teliospores.

In conclusion, MPA strongly inhibited the dimorphic switch of *S. scitamineum*. Although there is a report of synthesized compounds that exhibited a lethal effect on whip smut of sugar cane and could easily be degraded in the environment to nontoxic residues,[28] MPA is the first case of a natural product that act as a dimorphic switch inhibitor in sugar cane smut with low toxicity. Our results also shed light on the role of ammonium transporter in the pathogenicity of *S. scitamineum*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b04893.

Extraction and isolation of MPA; list of primers used in this study (Table S1); list of chemical structures for in vitro assay (Table S2); HPLC analysis of MPA (Figure S1); ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) data of MPA in CD_3OD (Table S3); ^{1}H NMR spectrum of MPA (Figure S2); ^{13}C NMR spectrum of MPA (Figure S3); inhibitory effect of MPA and compounds 2, 5, and 14 on dimorphic switch in *S. scitamineum* (Figure S4); inhibitory effect of MPA on dimorphic switch in *S. scitamineum* (Figure S5); read filter information statistics (Table S4); list of genes up- and down-regulated across three time points in the comparison between treatment and control samples (Table S5); and Southern blot assay (Figure S6) (PDF)

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Notes

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