JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 5089–5094 5089
DOI:10.1021/jf900783u

FvVE1 Regulates Biosynthesis of the Mycotoxins Fumonisins and Fusarins in *Fusarium verticillioides*

Kyung Myung,[†] Shaojie Li,[†] Robert A. E. Butchko,[‡] Mark Busman,[‡] Robert H. Proctor,[‡] Hamed K. Abbas,[§] and Ana M. Calvo*,[†]

[†]Department of Biological Sciences, Northern Illinois University, 1425 West Lincoln Highway, Dekalb, Illinois 60115, [‡]Mycotoxin Research Unit, United States Department of Agriculture−Agricultural Research Service (ARS), 1815 North University Street, Peoria, Illinois 61604, and [§]Crop Genetics and Production Research Unit (CG and PRU), United States Department of Agriculture−Agricultural Research Service (ARS), Post Office Box 345, Stoneville, Mississippi 38776

The *veA* gene positively regulates sterigmatocystin production in *Aspergillus nidulans* and aflatoxin production in *Aspergillus parasiticus* and *Aspergillus flavus*. Whether *veA* homologues have a role in regulating secondary metabolism in other fungal genera is unknown. In this study, we examined the role of the *veA* homologue, *FvVE1*, on the production of two mycotoxin families, fumonisins and fusarins, in the important corn pathogen *Fusarium verticillioides*. We found that *FvVE1* deletion completely suppressed fumonisin production on two natural substrates, corn and rice. Furthermore, our results revealed that *FvVE1* is necessary for the expression of the pathway-specific regulatory gene *FUM21* and structural genes in the fumonisin biosynthetic gene (*FUM*) cluster. *FvVE1* deletion also blocked production of fusarins. The effects of *FvVE1* deletion on the production of these toxins were found to be the same in two separate mating types. Our results strongly suggest that *FvVE1* plays an important role in regulating mycotoxin production in *F. verticillioides*.

KEYWORDS: ***Fusarium verticillioides*; fumonisin; fusarins; *FvVE1*; *veA*; secondary metabolism**

INTRODUCTION

The filamentous fungus *Fusarium verticillioides* (syn. *Fusarium moniliforme*, teleomorph *Gibberella moniliformis*) is one of the most common causes of corn ear rot worldwide and can produce multiple families of mycotoxins (*1*, *2*). Consequently, *F. verticillioides* mycotoxins are commonly detected in corn (maize, *Zea mays* L.) and often contaminate corn-based human food and animal feed (*1*, *3*). Fumonisins are currently considered the most agriculturally significant *F. verticillioides* mycotoxins because they can cause several animal diseases and are epidemiologically associated with some human diseases (*4*, *5*). Fumonisins are polyketide-derived metabolites that can inhibit ceramide synthase, a key enzyme in sphingolipid metabolism, and induce apoptosis (*1*, *5*). Fumonisin B_1 (FB_1) is typically the most abundant fumonisin in contaminated corn and accounts for approximately 70% of the total fumonisin content. Fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) are also common in corn but typically comprise 10−20% of the total fumonisin content (*1*).

In *F. verticillioides*, fumonisin biosynthetic genes (*FUM*) are clustered. The cluster consists of 17 genes, designated as *FUM1*−*FUM3* and *FUM6*−*FUM21* (*6*−*8*). Disruption of *FUM1*, *FUM6*, and *FUM8* has been shown to abolish fumonisin production (*7*, *8*). In many cases, genes responsible for the synthesis of fungal secondary metabolites, such as sterigmatocystin, gibberellins, aurofusarin, trichothecenes, and lovastatin, are also found clustered and specific regulatory genes are located within these gene clusters (*9*−*11*). *FUM21*, a predicted $Zn^{II}2Cys6$ DNA-binding transcription factor located in the *FUM* cluster, positively regulates *FUM* gene expression and is required for fumonisin synthesis (*12*). Nevertheless, the regulatory mechanism controlling fumonisin biosynthesis is poorly understood. Among the genes involved in fumonisin gene regulation are *FCC1*, *PAC1*, and *ZFR1* (*13*−*15*). *FCC1* encodes a cyclin-like protein (Fcc1) that positively regulates fumonisin biosynthesis and conidiation (*13*) and interacts with *FCK1*, a cyclin-dependent kinase (Fck1) (*16*). *PAC1* is required for growth at alkaline pH and may act as a repressor of fumonisin biosynthesis (*14*). *ZFR1* encodes a zinc binuclear cluster-type protein (Zfr1), which functions as a positive regulator of fumonisin biosynthesis (*15*). Studies by Flaherty and Woloshuk (*15*) indicated that Fcc1 is required for Zfr1 function. On the other hand, Pac1 and Fcc1 seem to act independently of each other in regulating fumonisin biosynthesis (*15*).

In addition to fumonisins, *F. verticillioides* produces other mycotoxins. Among them are the polyketide compounds fusarins (*17*−*19*). Fusarins have been reported to induce mutagenesis in mammalian cells *in vitro* (*17*) and to cause immunosuppression (*18*). Although a polyketide synthase gene required for fusarin biosynthesis has been identified in several *Fusarium* species (*20*, *21*), nothing is known about how fusarin biosynthesis is regulated.

In *Aspergillus* spp., the velvet gene (*veA*) regulates the biosynthesis of several secondary metabolites, including the polyketide toxins sterigmatocystin and aflatoxin (*22*−*24*). Whether *veA*

*To whom correspondence should be addressed. Telephone: (815) 753-0451. Fax: (815) 753-0461. E-mail: amcalvo@niu.edu.

 Published on Web 04/21/2009

homologues have a similar role in regulation of toxin production in other fungal genera has not been investigated. Previously, we identified *FvVE1*, a *veA* homologue in *F. verticillioides*, and demonstrated that it functions in regulation of morphogenesis (*25*). In this study, we investigate the role of *FvVE1* in secondary metabolism in *F. verticillioides*, specifically in the biosynthesis of fumonisin and fusarins. Our results suggest that *FvVE1* regulates biosynthesis of both fumonisin and fusarins in this important plant pathogenic fungus.

MATERIALS AND METHODS

Strains and Media. The strains used in this study are M-3125 (*MAT1-1*, *FvVE1*), M-3120 (*MAT1-2*, *FvVE1*), M312501 (*MAT1-1*, Δ*Fvve1::HygB*), M31206 (*MAT1-2*, Δ*Fvve1::HygB*), M312501C1 (*MAT1-1*, Δ*Fvve1::HygB*, *FvVE1::GenR*), and M31206C5 (*MAT1-2*, Δ*Fvve1::HygB*, *FvVE1::GenR*). *MAT1-1* and *MAT1-2* are the two different mating type idiomorphs (alleles) in *F. verticillioides*. M-3120 and M-3125 are strain designations from the Fusarium Research Center culture collection (Pennsylvania State University, University Park, PA). The *FvVE1* deletion strains and complementation strains were generated in both mating types as described by Li et al. (*25*). In brief, the Δ*Fvve1* mutant strains were generated by gene replacement via double homologous recombination events using the hygromycin B resistance gene (*HygB*) as a selectable marker (*25*). Complementation strains were obtained by transformation of the Δ*Fvve1* mutants with the wild-type *FvVE1* allele using the Geneticin-resistant gene, *GenR*, as a selectable marker (*25*).

V8 agar medium (10% V8 juice, 0.1% $CaCO_3$, and 1.5% agar) was used for production of conidia. Corn and rice media were prepared as previously described (*26*, *27*), with some modifications. In this study, we mixed 25 g of corn kernels and 40 mL of distilled water in 250 mL flasks and 50 g of long-grain rice and 60 mL of distilled water in 250 mL flasks. For RNA experiments, cracked-corn kernel cultures were prepared by thoroughly mixing 250 g of cracked-corn kernels and 100 mL of water and autoclaving. After cooling, the moistened kernels were combined with 25 mL of a suspension of *F. verticillioides* conidia (1×10^7 conidia per mL of water) prepared from 7-day-old V8 agar cultures of the fungus. This mixture was then distributed among eight 100 mm plastic Petri plates and incubated in the dark at 22 °C.

RNA Preparation and Northern Blots. At 36, 48, and 72 h of incubation, 10 g of cracked-corn culture was frozen in liquid nitrogen, placed at −80 °C until the nitrogen evaporated, and then lyophilized. The total RNA was isolated from the lyophilized material with TRIzol (Invitrogen Life Technologies, Carlsbad, CA) using the protocol for samples with high polysaccharide content, as described by the manufacturer. For Northern Blot analysis, 5 μg of total RNA for each sample was subjected to electrophoresis in a 1.3% agarose gel containing 1.8% formaldehyde and then transferred to a nylon membrane following standard protocols (*28*). ^{32}P-Labaled hybridization probes were prepared with the Ready-to-Go DNA labeling kit (Amersham Biosciences, Little Chalf-ont, Buckinghamshire, U.K.), and the hybridization, wash, and autoradiography procedures followed standard protocols (*28*). Templates for hybridization probes corresponding to *FUM1*, *FUM8*, and *TEF1* were prepared by polymerase chain reaction (PCR) that employed genomic DNA from wild-type *F. verticillioides* strain M-3120 and the following primer pairs. The primers used to amplify DNA templates for Northern Blot hybridization probes were, for *FUM1*, rp405 (5′-TGGGACA-CAGTTCTCAAGGAGA-3′) and rp408 (5′-CAAGCTCCTGTGACA-GAGATAC-3′), for *FUM8*, rp679 (5′-CGTAGTAGGAATGAGAAG-GATG-3′) and rp680 (5′-GCAAGCTTTGTGGCTGATTGTC-3′), and for *TEF1*, rp992 (5′-ATGGGTAAGGARGACAAGAC-3′) and rp993 (5′-GGARGTACCAGTSATCATGTT-3′). *TEF1*, encoding the transcription elongation factor 1α, was used as a loading control.

Reverse Transcription PCR. Total RNA was treated with Turbo DNA-free DNase (Applied Biosystems, Carlsbad, CA) following the recommended protocol of the manufacturer. RNA from DNase-treated samples was quantified on a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and diluted to a concentration of 40 ng/μL. A total of 60 ng of RNA was used per reverse transcription (RT)-PCR reaction. RT-PCR was accomplished with the Easy-A One-Tube RT-PCR System (Stratagene, La Jolla, CA) following the recommended protocol of the manufacturer. Primers used are, for *TEF1*, rb291 (5′-ATGGGTAAG-GAGGACAAGAC-3′) and rb292 (5′-GGAAGTACCAGTGATCAT-GTT-3′), for *FUM21*, rb373 (5′-TAAATGCGAGACAGCATTTGC-GGG-3′) and rb374 (5′-TGCATCTTGCCCTACTCAATCGGA-3′), for *FUM8*, rb379 (5′-TCCATGTTTACGGGCGCATTTGTC-3′) and rb380 (5′-TCGTGAAACCTAGACGCTTGCTGA-3′), for *ZFR1*, rb384 (5′-ATCCACGAAGGAGGCATGTTGGTA-3′ and rb385 (5′-AGGCGGATACAAAGAACGACAGGT-3′), and for *FCC1*, rb391 (5′-AATGTTTCCGCTTCCGCA-3′) and rb394 (5′-TGCCGCTTCT-CCTTAGGTTCT-3′). When possible, primers were designed to amplify different size fragments from genomic DNA and cDNA. *TEF1* was used as control reference to indicate amounts of total RNA. Primers for *TEF1* amplify a 771 bp fragment from genomic DNA and a 324 bp fragment from cDNA. *FUM21* primers amplify 920 and 707 bp fragments from genomic DNA and cDNA, respectively. *FUM8* primers amplify 789 and 638 bp fragments from genomic DNA and cDNA, respectively. Primer pairs for *ZFR1* and *FCC1* amplify the same size fragments from genomic DNA and cDNA (566 and 725 bp, respectively) because of the absence of introns in these genes.

Fumonisin Analysis. A plug (1.6 cm in diameter) containing mycelia and conidia from a 7-day-old V8 agar culture was used as inoculum. In each case, the cultures were mixed twice during the first 3 days of incubation by shaking for 30 s to ensure homogenization. Cultures were incubated for a total period of 2 weeks. After that time, the samples were lyophilized and ground to powder. Fumonisin analysis was performed as previously described by Abbas et al. (*29*). Samples were analyzed by liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS). Samples (10 g) from the ground corn and rice cultures were extracted with 50 mL of 70% methanol and filtered through number 1 Whatman filter paper. An aliquot (10 mL) was applied to a SAX cleanup column (Varian, Harbor City, CA). The sample was reconstituted in 1 mL of acetonitrile/water (1:1) and diluted if necessary. The LC/ESI/MS analysis was performed on a Thermo Finnigan LCQ Advantage, coupled to a Thermo Finnigan Surveyor MS and a Thermo Finnigan Surveyor MS Pump (Thermo Electron Corp., West Palm Beach, FL). A 10 μL aliquot was injected, and each sample was evaluated in full-scan mode, using the appropriate mass ranges: fumonisin B1, 722 (M + H); fumonisin B2 and B3, 706 (M + H); fumonisin B4, 690 (M + H); fumonisin (FA1, FA2, and FA3), 764, 748, and 748 (M + H); and fumonisin C1, 708 (M + H). MS/MS was performed on 722 (M + H) for further confirmation of FB1. The column used for fumonisin analysis was a 3.0 × 150 mm inner diameter, 5 μm, Intersil ODS-3 column (MetaChem Technologies, Inc., Torrance, CA). The mobile phase at initial elution starting condition consisted of water/1% acetic acid in methanol (65:35) at 0.3 mL/min, followed by water/1% acetic acid in methanol/methanol (5:35:65) at 10 min. The gradient was held constant for 10 min and returned to the initial starting conditions for 4 min for column equilibration. Quantitation of FB1, FB2, and FB3 was carried out by the external standard method, where FB4 was calculated as a percentage of FB1. Other derivatives of fumonisin were monitored for qualitative purposes only.

Fumonisin and fusarin analysis was also carried out on crack-corn cultures used for RNA analysis to further investigate the correlation between fumonisin production and gene expression levels. A total of 10 g of cracked-corn kernel culture was extracted in 25 mL acetonitrile/water (1:1, v/v) on a rotary shaker set at 250 rpm. After 2.5 h of shaking, the mixture was centrifuged at 500*g* for 5 min and the supernatant was recovered for analysis by reversed-phase liquid chromatography−mass spectrometry (LC−MS) in electrospray mode, as previously described (*30*). Briefly, the LC−MS system consisted of a ThermoFinnigan LCQ Deca mass spectrometer coupled to a ThermoSpectraPhysics high-performance liquid chromatography (HPLC) with a C18 column. The column used for fumonisin analysis was the same as the one described above (Intersil ODS-3 column MetaChem Technologies, Inc., Torrance, CA). Samples were run on a gradient of 35−95% (v/v) methanol over 35 min, at a flow rate of 0.3 mL/min. Fumonisins and fusarins were detected by monitoring masses of 240−1000. The identities of fumonisins and fusarins were confirmed by retention time and the presence of appropriate $[M + H]^+$ ions. Quantification was accomplished by a comparison of the integrated intensity of ions corresponding to fumonisin and fusarin standards (*30*).

RESULTS AND DISCUSSION

Fumonisin Production on Natural Substrates. Fumonisins are currently considered the most economically important mycotoxins produced by the corn pathogen *F. verticillioides* because of their widespread occurrence in corn and their potential health effects on humans and animals (*1*, *2*, *4*, *5*). Our current studies indicated that fumonisin production is affected in the Δ*Fvve1* mutants. In corn and rice cultures (**Figure 1**), FB_1 was produced by the wild-type strains as well as the complemented strains (**Figure 2**). Trace amounts of FB_2 and FB_3 were also detected in corn and rice cultures of the wild-type and complemented strains. In contrast, neither FB_1, FB_2, nor FB_3 was detected in rice or corn cultures of the Δ*Fvve1* mutants (**Figure 2**). These results were consistent in both mating-type genetic backgrounds. Our findings reveled that the novel regulatory factor *Fv*Ve1 encoded by the *FvVE1* gene is required for fumonisin production when the fungus grows on the natural substrates corn and rice (**Figures 1** and **2**).

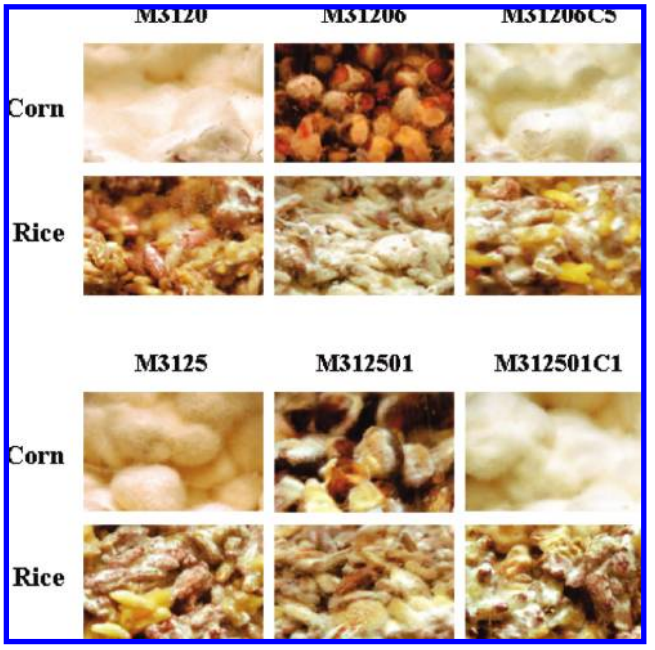

Figure 1. Photographs of wild-type (WT, M-3120 and M-3125), *FvVE1*-deletion mutant (Δ*Fvve1*, M31206 and M312501), and complemented (Com, M31206C5 and M312501C1) strains of *MAT1-2* and *MAT1-1*, respectively, in corn and rice cultures.

Expression of Fumonisin Biosynthetic Genes. Previous studies showed that VeA, the *Fv*Ve1 homologue in *Aspergillus* species, is required for expression of sterigmatocystin/aflatoxin biosynthetic genes and concomitant production of the toxins (*22*−*24*). However, the possible role of VeA homologues in activation of mycotoxin biosynthetic genes in other fungal genera was not known until now. To investigate whether the expression of fumonisin biosynthetic genes is regulated by the *FvVE1* gene, transcription levels of *FUM1* and *FUM8* (essential fumonisin biosynthetic genes) were examined in the wild-type, Δ*Fvve1* mutant, and complemented strains grown on cracked-corn medium. The Northern analysis in **Figure 3** shows that transcription of *FUM1* was first detected at 48 h after inoculation in both wild-type and complemented strains. *FUM8* expression followed a similar pattern, where transcripts started to accumulate slightly earlier in the wild-type strain. However, *FUM1* and *FUM8* transcripts were not detected in the Δ*Fvve1* mutant (**Figure 3**). The lack of fumonisin biosynthetic gene expression in the *FvVE1*

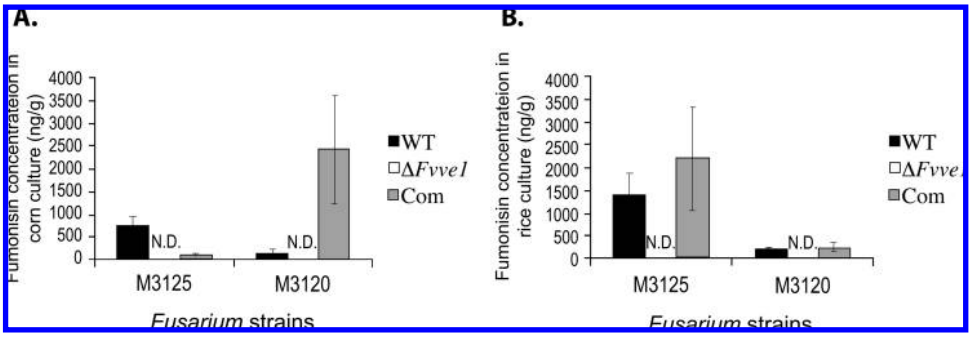

Figure 2. Production of fumonisins in wild-type (WT), *FvVE1* deletion mutant (Δ*Fvve1*), and complemented (Com) strains in both the *MAT1-1* (M3125) and *MAT1-2* (M3120) genetic background grown in corn (A) and rice (B) cultures. Total fumonisins (B_1, B_2, and B_3 combined) were analyzed at 2 weeks after inoculation. Bars indicate standard errors of three independent cultures. N.D. = not detected.

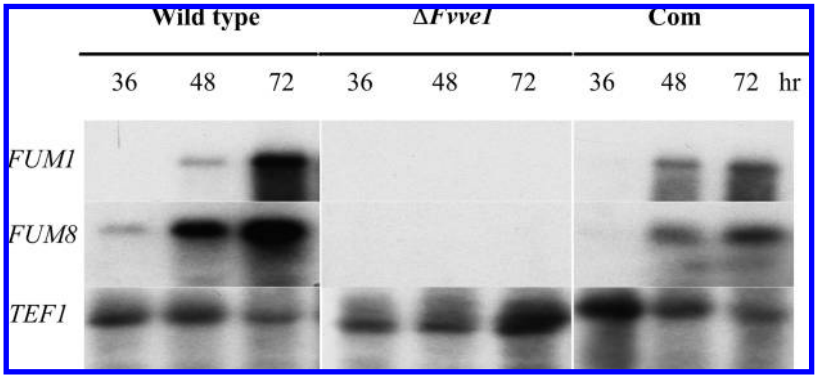

Figure 3. Northern analysis of *FUM1* and *FUM8* gene expression from wild-type M-3120, *FvVE1* deletion mutant M31206 (Δ*Fvve1*), and complementation (Com) strain M31206C5. Total RNA was isolated from mycelial tissue grown on cracked-corn medium at 36, 48, and 72 h after inoculation. *TEF1* was used as a loading control.

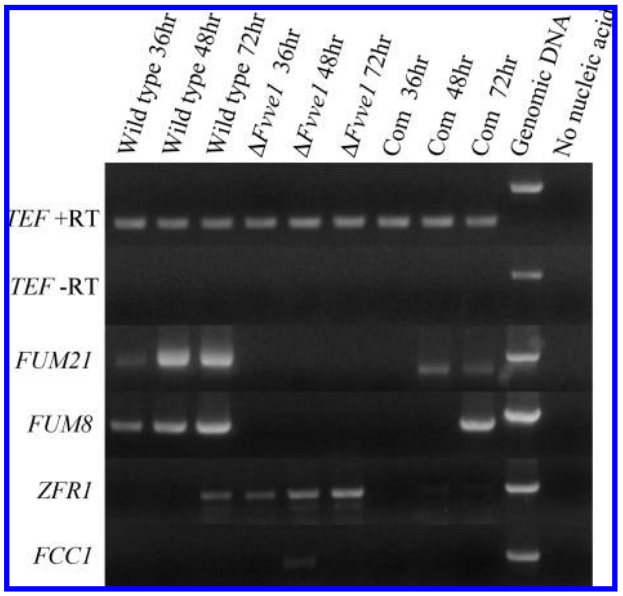

Figure 4. RT-PCR analysis of *FUM21*, *FUM8*, *ZFR1*, and *FCC1* gene expression from wild-type M-3120, *FvVE1* deletion mutant M31206 (Δ*Fvve1*), and complementation (Com) strain M31206C5. Total RNA was isolated from mycelial tissue grown on cracked-corn medium at 36, 48, and 72 h after inoculation. *TEF1* was used as a loading control. A no reverse transcriptase control reaction is shown for *TEF1* primers, indicating that no genomic DNA remained after DNase treatment.

deletion mutant is most likely responsible for the lack of fumonisin production in this strain.

Expression of *FUM21*, *FCC1*, and *ZFR1*. With the goal of further elucidating the mechanism through which *FvVE1* regulates the expression of the *FUM* gene cluster and the concomitant fumonisin production, we investigated whether expression of the *FUM21* gene was altered by *FvVE1* deletion. *FUM21* gene encodes a putative $Zn^{II}2Cys6$ DNA-binding transcriptional activator that is likely specific for *FUM* cluster genes (*12*). Furthermore, a functional *FUM21* is necessary for fumonisin production in *F. verticillioides*. Genes encoding cluster-specific regulatory proteins have been previously found in other secondary metabolism gene clusters. Well-known examples of these regulators are *aflR* and *aflJ*, demonstrated to govern the expression of sterigmatocystin/aflatoxin gene clusters in *Aspergillus* spp. (*31*−*33*). We have previously shown that *veA* is necessary for the expression of *aflR* and *aflJ* in *Aspergillus* (*22*−*24*). In the present study, to investigate whether *FvVE1* plays a role in regulating the expression of *FUM21*, we chose RT-PCR analysis because of its ability to detect transcripts that are present at low levels. *FUM21* transcripts were absent in the Δ*Fvve1* mutant cultures under conditions that allow for the expression of this gene in the wild-type and complementation strains (**Figure 4**). *FUM8* was also included in the RT-PCR analysis as an internal control for a comparison of the RT-PCR and Northern experiments, which yielded essentially the same results. The absence of *FUM21* transcripts in the Δ*Fvve1* mutant indicates that a functional *FvVE1* is necessary for *FUM21* expression.

Genes outside the *FUM* cluster can also regulate fumonisin production in *F. verticillioides*. For example, deletion of the C-type cyclin-like gene, *FCC1*, abolished fumonisin production on corn kernels and in a defined medium at high pH (*14*). To test whether *FvVE1* controls this regulatory gene, the transcription levels of *FCC1* were examined in wild-type, Δ*Fvve1* mutant, and complemented strains grown on cracked-corn medium (**Figure 4**). Our results showed that *FCC1* expression was very low and only detected in the *FvVE1* deletion mutant at 48 h after inoculation.

Table 1. Fumonisin and Fusarin Analysis of Cracked-Corn Cultures

time (h)	strain	fumonisins[a]	fusarins[b]
36	wild type	0	0
	Δ*Fvve1*	0	0
	complementation	0	196
48	wild type	1	0
	Δ*Fvve1*	0	0
	complementation	0	864
72	wild type	98	330
	Δ*Fvve1*	0	0
	complementation	127	1709
96	wild type	178	673
	Δ*Fvve1*	0	0
	complementation	316	2066
144	wild type	281	1945
	Δ*Fvve1*	0	0
	complementation	334	2246

[a] In units of micrograms of fumonisins B_1, B_2, and B_3 combined per gram of cracked-corn culture. [b] In units of micrograms of fusarins C_1, C_2, and C_3 combined per gram of cracked-corn culture.

Although this result differs from those previously reported (*13*), where *FCC1* expression was detected at higher levels in cracked-corn cultures, under the experimental conditions assayed in our study, fumonisin production was detected as well as *FUM1* and *FUM8* expression, indicating that our culture system yielded reliable results. Fumonisin production in the *fcc1* mutant is not blocked in the defined medium at low pH (*14*). Changes in pH did not rescue fumonisin production in the Δ*Fvve1* mutant (data not shown) as in the case of the *FCC1* mutant (*14*). *FCC1* is required for function of the Zfr1, a putative $Zn^{II}2Cys6$ transcription factor postulated to control fumonisin production by regulating genes involved in the perception or uptake of carbohydrates (*15*, *34*). In our study, *ZFR1* transcripts were detected in the wild-type, Δ*Fvve1* mutant, and complementation strains, particularly in the Δ*Fvve1* mutant, where *ZFR1* transcription occurred earlier and was more abundant than in strains with a functional *FvVE1* (**Figure 4**). This suggests that *FvVE1* negatively influences *ZFR1* expression. Further studies will focus on elucidating possible interactions between *ZFR1* and *FvVE1* and whether *FvVE1* has a role in carbohydrate metabolism in *F. verticillioides*.

Cultures used for RNA studies were also analyzed for fumonisins. In agreement with previous experiments (**Figures 1** and **2**), wild-type and complementation strains produced fumonisins but the Δ*Fvve1* mutant did not (**Table 1**). Even when the incubation time was increased to 144 h after inoculation of the crack-corn medium, fumonisin were not detected in Δ*Fvve1* cultures.

Fusarin Analysis. The VeA homologues in *Aspergillus* regulate not only production of sterigmatocystin and aflatoxin but also production of other secondary metabolites, such as aflatrem, cyclopiazonic acid, and penicillin (*22*−*24*). In addition to fumonisins, *F. verticillioides* produces an array of other secondary metabolites, including the mycotoxins fusarins. These toxins have been reported to be mutagenenic as well as immunosuppressive (*17*−*19*). To evaluate the role of *FvVE1* in fusarin biosynthesis, we examined the production of this compound in the wild-type, *FvVE1* deletion mutant, and complementation strains on cracked-corn medium. Fusarins were detected in extracts from cultures of wild-type and complementation strains but were not detected in extracts of Δ*Fvve1* cultures (**Table 1**), indicating that *FvVE1* is also necessary for fusarin biosynthesis in *F. verticillioides*. To our knowledge, this is the first report of a gene described to regulate fusarin production in *F. verticillioides*. Interestingly, a recent report by Estrada and Avalos (*35*) showed that the white-collar gene *wcoA* modulates fusarin production in a

light-dependent manner in *F. fujikuroi*. In *Aspergillus nidulans*, VeA forms a nuclear protein complex that includes light-sensing proteins, such as the red phytochrome-like FphA and the white-collar LreA and LreB proteins responsive to blue light (*36*). In future studies, we will investigate if a similar protein complex that includes *Fv*Ve1 also exists in *F. verticillioides*.

As in the case of *veA* regulation of secondary metabolism in *Aspergillus* (*22*−*24*), the effect of *FvVE1* on secondary metabolism in *F. verticillioides* could also be broad. The differences in pigmentation observed in the natural substrate cultures (**Figure 1** and data not shown) indicate that the synthesis of other unknown metabolites is also regulated by *FvVE1*.

In conclusion, we have demonstrated that *F. verticillioides FvVE1* is required for fumonisin and fusarin production on the natural substrates corn and rice. We also showed that *FvVE1* is necessary for the expression of the fumonisin biosynthetic enzyme-encoding genes *FUM1* and *FUM8* as well as the transcription factor gene *FUM21*. This study also revealed that *FvVE1* also affects production of fusarins, a second family of *F. verticillioides* secondary metabolites. The consistent blockage of fumonisin production in corn suggests that *FvVE1* is a potential target to control fumonisin contamination in corn-based food and feed. We are currently exploring this possibility by investigating whether the *FvVE1* deletion affects the ability of *F. verticillioides* to produce fumonisins in living corn plants.

ABBREVIATIONS USED

FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_3, fumonisin B_3; *veA*, velvet gene; *FvVE1*, *Fusarium verticillioides veA*.

ACKNOWLEDGMENT

We thank Bobbie Johnson and Jennifer Tonos for their help in analyzing fumonisins and Marcie Moore for the Northern Blot analysis. We also thank Dapeng Bao for his technical support.

LITERATURE CITED

(1) Nelson, P. E.; Desjardins, A. E.; Plattner, R. D. Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. *Annu. Rev. Phytopathol.* **1993**, *31*, 233–252.

(2) Rheeder, J. P.; Marasas, W. F. O.; Vismer, H. F. Production of fumonisin analogs by *Fusarium* species. *Appl. Environ. Microbiol.* **2002**, *68*, 2101–2105.

(3) Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Marthinus Horak, R.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun.* **1988**, 743–745.

(4) Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, M. J.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806–1811.

(5) Desai, K.; Sullards, M. C.; Allegood, J.; Wang, E.; Schmelz, E. M.; Hartl, M.; Humpf, H. U.; Liotta, D. C.; Peng, Q.; Merrill, A. H.Jr. Fumonisins and fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. *Biochim. Biophys. Acta* **2002**, *1585*, 188–192.

(6) Proctor, R. H.; Brown, D. W.; Plattner, R. D.; Desjardins, A. E. Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. *Fungal Genet. Biol.* **2003**, *38*, 237–249.

(7) Bojja, R. S.; Cerny, R. L.; Proctor, R. H.; Du., L. Determining the biosynthetic sequence in the early steps of the fumonisin pathway by use of three gene-disruption mutants of *Fusarium verticillioides*. *J. Agric. Food Chem.* **2004**, *52*, 2855–2860.

(8) Seo, J. A.; Proctor, R. H.; Plattner, R. D. Characterization of four clustered and coregulated genes associated with fumonisin biosynthesis in *Fusarium verticillioides*. *Fungal Genet. Biol.* **2001**, *34*, 155–165.

(9) Hohn, T. M.; Krishna, R.; Proctor, R. H. Characterization of a transcriptional activator controlling trichothecene toxin biosynthesis. *Fungal Genet. Biol.* **1999**, *26*, 224–235.

(10) Kennedy, J.; Auclair, K.; Kendrew, S. G.; Park, C.; Vederas, J. C.; Hutchinson, C. R. Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis. *Science* **1999**, *284*, 1368–1372.

(11) Malz, S.; Grell, M. N.; Thrane, C.; Maier, F. J.; Rosager, P.; Felk, A.; Albertsen, K. S.; Salomon, S.; Bohn, L.; Schafer, W.; Giese, H. Identification of a gene cluster responsible for the biosynthesis of aurofusarin in the *Fusarium graminearum* species complex. *Fungal Genet. Biol.* **2005**, *42*, 420–433.

(12) Brown, D. W.; Butchko, R. A. E.; Busman, M.; Proctor, R. H. The *Fusarium verticillioides FUM* gene cluster encodes a Zn(II)2Cys6 protein that affects *FUM* gene expression and fumonisin. *Eukaryotic Cell* **2007**, *6*, 1210–1218.

(13) Shim, W. B.; Woloshuk, C. P. Regulation of fumonisin B1 biosynthesis and conidiation in *Fusarium verticillioides* by a cyclin-like (C-type) gene, *FCC1*. *Appl. Environ. Microbiol.* **2001**, *67* 1607–1612.

(14) Flaherty, J. E.; Pirttila, A. M.; Bluhm, B. H.; Woloshuk, C. P. *PAC1*, a pH-regulatory gene from *Fusarium verticillioides*. *Appl. Environ. Microbiol.* **2003**, *69*, 5222–5227.

(15) Flaherty, J. E.; Woloshuk, C. P. Regulation of fumonisin biosynthesis in *Fusarium verticillioides* by a zinc binuclear cluster-type gene, *ZFR1*. *Appl. Environ. Microbiol.* **2004**, *70*, 2653–2659.

(16) Bluhm, B. H.; Woloshuk, C. P. Fck1, a C-type cyclin-dependent kinase, interacts with Fcc1 to regulate development and secondary metabolism in *Fusarium verticillioides*. *Fungal Genet. Biol.* **2006**, *43*, 146–154.

(17) Cheng, S. J.; Jiang, Y. Z.; Li, M. H.; Lo, H. Z. A mutagenic metabolite produced by *Fusarium moniliforme* isolates in Linxian County, China. *Carcinogenesis* **1985**, *6*, 903–905.

(18) Dong, Z. Y.; Zhan, Y. H. Inhibitory effect of a mycotoxin, fusarin C, on macrophage activation and macrophage mediated cytotoxicity to tumor cells in mice. *J. Exp. Clin. Cancer Res.* **1987**, *6*, 31–38.

(19) Desjardins, A. E.; Proctor, R. H. Molecular biology of *Fusarium* mycotoxins. *Int. J. Food Microbiol.* **2007**, *119*, 47–50.

(20) Gaffoor, I.; Brown, D. W.; Plattner, R.; Proctor, R. H.; Qi, W.; Trail, F. Functional analysis of the polyketide synthase genes in the filamentous fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). *Eukaryotic Cell* **2005**, *4*, 1926–1933.

(21) Song, Z. S.; Cox, R. J.; Lazarus, C. M.; Simpson, T. J. Fusarin C biosynthesis in *Fusarium moniliforme* and *Fusarium venenatum*. *ChemBioChem* **2004**, *5*, 1196–1203.

(22) Kato, N.; Brooks, W.; Calvo, A. M. The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by *veA*, a gene required for sexual development. *Eukaryotic Cell* **2003**, *2*, 1178–1186.

(23) Calvo, A. M.; Bok, J.; Brooks, W.; Keller, N. P. *veA* is required for toxin and sclerotial production in *Aspergillus parasiticus*. *Appl. Environ. Microbiol.* **2004**, *70*, 4733–4739.

(24) Duran, R. M.; Cary, J. W.; Calvo, A. M. Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* is regulated by *veA*, a gene necessary for sclerotial formation. *Appl. Microbiol. Biotechnol.* **2007**, *73*, 1158–1168.

(25) Li, S.; Myung, K.; Guse, D.; Donkin, B.; Proctor, R. H.; Grayburn, W. S.; Calvo, A. M. FvVE1 regulates filamentous growth, the ratio of microconidia to macroconidia and cell wall formation in *Fusarium verticillioides*. *Mol. Microbiol.* **2006**, *62*, 1418–1432.

(26) Abbas, H. K.; Mirocha, C. J.; Shier, W. T. Mycotoxins produced from fungi isolated from foodstuffs and soil: Comparison of toxicity in fiberblasts and rat feeding test. *Appl. Environ. Microbiol.* **1984**, *48*, 654–661.

(27) Abbas, H. K.; Cartwright, R. D.; Xie, W.; Mirocha, C. J. Richard, J. L.; Dvorak, T. J.; Sciumbato, G. L.; Shier, W. T. Mycotoxins production by *Fusarium proliferatum* isolates from rice

with *Fusarium* sheath rot disease. *Mycopathologia* **1999**, *147* 97–104.

(28) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

(29) Abbas, H. K.; Cartwright, R. D.; Xie, W.; Shier, W. T. Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. *Crop Prot*. **2006**, *25*, 1–9.

(30) Plattner, R. D.; Weisleder, D.; Poling, S. M. Analytical determination of fumonisins and other metabolites produced by *Fusarium moniliforme* and related species on corn. *Adv. Exp. Med. Biol*. **1996**, *392*, 57–64.

(31) Yu, J. H.; Butchko, R. A.; Fernandes, M.; Keller, N. P.; Leonard, T. J.; Adams, T. H. Conservation of structure and function of the aflatoxin regulatory gene *aflR* from *Aspergillus nidulans* and *A. flavus*. *Curr. Genet*. **1996**, *29*, 549–555.

(32) Payne, G. A.; Nystrom, G. J.; Bhatnagar, D.; Cleveland, T. E.; Woloshuk, C. P. Cloning of the *afl*-2 gene involved in aflatoxin biosynthesis from *Aspergillus flavus*. *Appl. Environ. Microbiol*. **1993**, *59*, 156–162.

(33) Chang, P. K. The *Aspergillus parasiticus* protein AFLJ interacts with the aflatoxin pathway-specific regulator AFLR. *Mol. Genet. Genomics* **2003**, *268*, 711–719.

(34) Bluhm, B. H.; Kim, H.; Butchko, R. A.; Woloshuk, C. P. Involvement of ZFR1 of *Fusarium verticillioides* in kernel colonization and the regulation of FST1, a putative sugar transporter gene required for fumonisin biosynthesis on maize kernels. *Mol. Plant Pathol*. **2008**, *9*, 203–211.

(35) Estrada, A. F.; Avalos, J. The White Collar protein WcoA of *Fusarium fujikuroi* is not essential for photocarotenogenesis, but is involved in the regulation of secondary metabolism and conidiation. *Fungal Genet. Biol*. **2008**, *45*, 705–718.

(36) Calvo, A. M. The VeA regulatory system and its role in morphological and chemical development in fungi. *Fungal Genet. Biol*. **2008**, *45*, 1053–1061.

Received for review March 8, 2009. Revised manuscript received April 3, 2009. Accepted April 6, 2009. This work was funded by National Institutes of Health (NIH) GM074267-01A1.