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Direct Evidence for the Function of *FUM13* in 3-Ketoreduction of Mycotoxin Fumonisins in *Fusarium verticillioides*

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Fumonisins are mycotoxins produced by *Fusarium verticillioides*, a widespread pathogen of corn. Although the gene cluster for the biosynthesis of fumonisins has been cloned, the majority of the genes have not been biochemically characterized. Here, we report the biochemical characterization of *FUM13*, a gene that encodes a short-chain dehydrogenase/reductase required for fumonisin biosynthesis. *FUM13* has been expressed in *E. coli*, and the produced protein, Fum13p, has been purified. When the protein was incubated with 3-keto fumonisin B_3 (FB_3) in the presence of NADPH, FB_3 was produced. The data provide direct evidence for the role of *FUM13* in the 3-ketoreduction of fumonisins. In a functional complementation experiment, *FUM13* gene was introduced into *tsc10* mutants of the yeast *Saccharomyces cerevisiae*, which carry a mutation in the 3-ketosphinganine reductase gene in the sphingolipid pathway. The *tsc10* mutants were not able to grow on the selection medium, but the same mutants transformed with *FUM13* were able to grow. The results further confirm the function of *FUM13* in 3-ketoreduction in vivo.

KEYWORDS: Fumonisin; 3-ketoreductase; *Fusarium verticillioides*; mycotoxin

INTRODUCTION

Fumonisins are a group of mycotoxins produced by the filamentous fungus *Fusarium verticillioides* (*1*, *2*). The fungus is a widespread pathogen of corn, which causes ear and stalk rots and impairs agricultural productivity and food quality (*3*). More importantly, the fungus produces harmful secondary metabolites, fumonisins. These toxins cause several fatal diseases in animals, including leukoencephalomalacia in horses, pulmonary edema in swine, and cancer in rats (*1*, *4*, *5*). In addition, fumonisins are suspected to cause human esophageal cancer (*1*).

The B-series (B_1, B_2, B_3, and B_4) are the predominant analogues of fumonisins found in wild-type strains of *F. verticillioides* (*2*, *6*). These compounds have a 20-carbon backbone, among which the carbons 3−20 are derived from acetate by a polyketide biosynthesis mechanisim. C-1 and C-2 and the amino group on C-2 are derived from alanine (*7*, *8*), whereas the methyl groups on C-12 and C-16 are derived from methionine (*9*). The difference among the fumonisin analogues is the number of hydroxyl groups on the backbone. All analogues have a hydroxyl group on C-3 and tricarballylic esters on C-14 and C-15 (*10*). In addition, FB_1 has hydroxyl groups on both C-5 and C-10, FB_2 has a hydroxyl on C-5, FB_3 has a hydroxyl on C-10, and FB_4 has no hydroxyl on either of the carbons. The tricarballylic acids on C-14 and C-15 hydroxyl are likely derived from the citric acid cycle (*7*).

Using three gene-disrupted mutants, we studied the biosynthetic sequence in the early steps of the fumonisin pathway (*11*). The biosynthesis starts with the carbon backbone assembly, which is likely catalyzed by the polyketide synthase encoded by *FUM1*. The dimethylated C_{18} polyketide chain is released from the synthase by the attack of alanine on the carbonyl carbon of the polyketide acyl chain. This step is most likely catalyzed by the putative aminoacyl transferase encoded by *FUM8* (*12*, *13*). Fum8p is a homologue of serine palmitoyltransferase, which is the first enzyme in the sphingolipid pathway to produce 3-ketosphinganine from serine and palmitoyl-CoA (*14*). The resultant 3-keto intermediate (2-amino-3-oxo-12,16-dimethylicosane) is then reduced to a 3-hydroxyl product (2-amino-3-hydroxy-12,16-dimethylicosane) in a manner similar to the reduction of 3-ketosphinganine to sphinganine (*12*, *15*). Subsequent oxidations at C-14, C-15, C-10, and C-5 and tricarballylic esterification of the hydroxyl groups on C-14 and C-15 furnish the biosynthesis of FB_1 (*11*, *16*, *17*). Except the enzyme (Fum3p) for C-5 hydroxylation (*16*), the enzymes for fumonisin biosynthesis have not been biochemically characterized.

Recently, Butchko et al. (*12*) used a gene deletion approach to demonstrate that *FUM13* is required for 3-ketoreduction of fumonisins. *FUM13* deletion mutants produced the C-3 keto form of FB_3 and FB_4, indicating that the *FUM13* protein, Fum13p, catalyzes 3-ketoreduction in the biosynthesis of fumonisins (*12*). However, the mutants also produced approximately 10% of wild-type levels of B-series fumonisins (with a C-3 hydroxyl). Thus, the function of *FUM13* in 3-ketoreduction is not totally clear. Moreover, since these two 3-keto compounds are related to the penultimate products FB_3 and FB_4, not to the intermediates in the earlier steps of the pathway (*11*), the results suggest that the 3-ketoreduction could

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also take place at the later stage of the pathway. An alternative interpretation is that 3-ketoreduction occurs early in fumonisin biosynthesis and that enzymes downstream in the pathway have a relatively broad substrate specificity that enables them to process 3-keto intermediates to the final stage (up to FB_3 and FB_4). Previous sequence analysis has predicted that *FUM13* encodes an enzyme belonging to the superfamily of short-chain dehydrogenases/reductases (SDR) (*12*, *18*). To obtain direct evidence for the function of *FUM13*, we have expressed the gene in *Esherichia coli* and purified the produced protein.

MATERIALS AND METHODS

Materials, Strains, and Vectors. Standards FB_1, FB_2, FB_3, and FB_4 were gifts from Dr. Ronald D. Plattner (USDA, Peoria, IL). Wild-type strain *F. verticillioides* A0149 was provided by Dr. Gilchrist (University of California, Davis). GYP medium (2% glucose, 1% peptone, and 0.3% yeast extract) was used for mycelium growth, V-8 juice agar plates for production of conidia, and CMK (cracked maize kernels) medium for fumonisin production (*10*, *19*). Qiagen kits (Santa Clarita, CA) were used for plasmid DNA preparation and DNA extraction from agarose gels. The pGEM-zf plasmid series from Promega (Madison, WI) were used as cloning vectors with *Escherichia coli* strain DH5α as the host. For protein expression, pET series from Novagen (Madison, WI) were used with *E. coli* BL21(DE3) as the host. All other DNA manipulations were carried out according to standard methods (*20*).

FUM13* Expression in *E. coli*.** The *FUM13* coding region was amplified by PCR using the forward primer 5′-CG ***GAA TTC AGT CGG GGT CAA GAG CTT G-3′ (italic letters indicating *Eco*RI site) and reverse primer 5′-GCG ***GTC GAC*** GTT ATA GCT TCT TGT GGT-3′ (italic letters indicating *Sal*I site). PCR employed genomic DNA isolated from wild-type *F. verticillioides* as template and Pfu-Ultra (Stratagene, La Jolla, CA) as the DNA polymerase. The 1.1 kb PCR product was purified using a Qiagen kit and digested with *Eco*RI and *Sal*I. The PCR-amplified *FUM13* coding region was cloned into pGEM 3zf and sequenced on a Li-Cor Model 4000 DNA sequencer at the Core Research Facilities at the University of Nebraska-Lincoln to confirm that PCR did not introduce any errors. *FUM13* was then transferred as an *Eco*RI/*Sal*I fragment to the expression vector pET28a (Novagen). This construct was introduced into *E. coli* BL21(DE3) (Novagen) for protein production following the protocol provided by the manufacturer. The expression of *FUM13* was induced by 0.1 mM IPTG, and the cells were allowed to grow in a shaker at 250 rpm, 25 °C for 10−12 h. The soluble fraction of total protein extract from the cells was loaded to an Ni−NTA affinity column (Qiagen) preequilibrated with PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.4), and Fum13p was eluted in PBS buffer containing 250 mM imidazole. The eluted fractions were analyzed by SDS−PAGE, and the fractions containing pure Fum13p were combined and desalted on a PD-10 column (Sephadex G-25, Pharmacia Biotech, Piscataway, NJ). Finally, the protein was eluted in 50 mM sodium phosphate buffer, pH 7.8, containing 1 mM dithiothreitol (DTT) and 5% glycerol, and stored at − 80 °C until used for in vitro assays.

Isolation of 3-Keto FB_3 from *FUM13* Mutant. Five hundred grams of CMK (divided in 20 flasks, each with 25 g) was soaked in water overnight to remove any potential fumonisin contaminant as previously described (*11*). After autoclaving, the medium was inoculated with the *FUM13*-deleted mutant of *F.* verticillioides (*12*). The fungus was allowed to grow in the dark for 4−6 weeks. Fumonisins were extracted from the culture by adding approximately 500 mL of 50% acetonitrile following the procedure described in ref (*12*). The extracts were first analyzed by high-performance liquid chromatography−evaporative light scattering detection (HPLC-ELSD) to examine if the extracts contained 3-keto fumonisins. The conditions for HPLC-ELSD were identical to those published earlier (*11*). 3-Keto FB_3 was then purified from the extract using a 250 × 10.0 mm i.d., 5 μ, Nucleosil 100A C18 preparative HPLC column (Alltech, Deerfield, IL). The mobile phases were (A) water-TFA (100:0.025, v/v) and (B) 95% acetonitrile-TFA (100:0.025, v/v) with a similar gradient to that previously described (*11*). Fractions were collected and analyzed by liquid chromatography−mass spectrometry (LC-MS) to identify those containing the pure 3-keto FB_3. LC-MS was performed on a 250 × 1 mm i.d., 5 μ, RFC_{18} Vydac column (Grace Vydac, Hesperia, CA). The solvent systems were (A) water-formic acid (100:0.1, v/v) and (B) 95% acetonitrile-formic acid (100:0.1, v/v). The flow rate was 0.05 mL/min, and the injection volume was 50 μL. All positive electrospray spectra were acquired using a VG Platform II mass spectrometer or a Micromass Q-Tof (Manchester, U.K.).

In Vitro Assay for Fum13p Activity. The reaction included approximately 350 ng 3-keto FB_3, 50 μM NADPH, 10 μg Fum13p, and 100 mM Tris-HCl, pH 7.4, in a final volume of 100 μL. The reaction was initiated by adding the substrate to the mixture. As controls, Fum13p was boiled in water for 10 min and used in the reaction. After incubation at 37 °C for 15 min, the reaction was stopped by adding 300 μL of 100% ethanol. The mixture was put on ice for 30 min and then centrifuged at 16 000*g* (Eppendorf Centrifuge 5415D) at 4 °C for 10 min to remove precipitates. The supernatant was transferred to a new tube and dried in a SpeedVac concentrator. The reaction mixture was redissolved in 100 μL of water and was used for HPLC-ELSD and LC-MS analyses.

Complementation of Yeast *tsc10* Mutants. *Saccharomyces cerevisiae* strain LHYa56 tsc10-csg2 (*Mata ade2-101 ura3-52 trp1*Δ *leu2*Δ *csg2::LEU2 tsc10-1*) and strain LHYa56 tsc10 were obtained from Dr. T. Dunn (Uniformed Services University of the Health Sciences, Bethesda, MA) (*15*). LHYa56 tsc10-csg2 was initially generated from a Ca^{2+}-sensitive mutant strain (because of a mutation in *CSG2* gene), and LHYa56 tsc10 was created by backcrossing LHYa56 tsc10-csg2 with a wild-type strain to obtain the *tsc10* mutation in a wild-type *CSG2* genetic background. Both strains carry a temperature-sensitive mutation at *TSC10*, a gene coding for the sphingolipid biosynthetic enzyme 3-ketosphinganine reductase (*15*). Thus, a functional complementation of *tsc10* would restore the growth of the mutants at 37 °C. To perform the complementation, a yeast expression vector was constructed by transferring *FUM13* as a *Bam*HI/*Not*I fragment from pET28a/*FUM13* into pYES2/NT C vector (Invitrogen, Carlsbad, CA). The construct was introduced into yeast cells using a transformation method provided by the manufacturer. The transformants were selected on SC minimal medium (Invitrogen) for uracil prototrophy at 26 °C according to the protocol provided by the manufacturer. After the transformants were identified, single colonies were replica-streaked to SC plates and placed at both 26 °C and 37 °C. Control experiments using colonies from INVSc1 strain (Invitrogen, Carlsbad, CA), nontransformed *tsc10* mutants, and *tsc10* mutants transformed with empty pYES2/NT C vector were also similarly conducted.

RESULTS AND DISCUSSION

Expression of *FUM13* in *E. coli* and In Vitro Assays of Activity of Fum13p. *FUM13* has a 1,110-bp open reading frame coding for 369 amino acid residues. When it was cloned into pET28a and expressed in *E. coli* BL21(DE3), the calculated size of the His-tagged protein is 45.6 kD. Using Ni−NTA affinity column, we purified Fum13p from *E. coli* carrying pET28a/*FUM13*. SDS−PAGE analysis showed that the protein had a size of approximately 45 kD (**Figure 1**), which is consistent with the expected size of Fum13p. The protein was used for in vitro 3-ketoreductase assays. To assay the activity of Fum13p, we prepared 3-keto FB_3 from a *FUM13*-deleted mutant, which was generated by replacing the ORF of *FUM13* in *F. verticillioides* with a hygromycin B resistance gene (*HygB*) (*12*). The mutant strain was grown on CMK medium for 4−6 weeks and was extracted with 50% acetonitrile. 3-Keto FB_3 was prepared by collecting individual fractions from a preparative HPLC column, followed by analyzing each of the fractions using LC-MS. Under the experimental conditions used, the 3-keto FB_3 peak had a retention time of 17.20 min on HPLC-ELSD and a

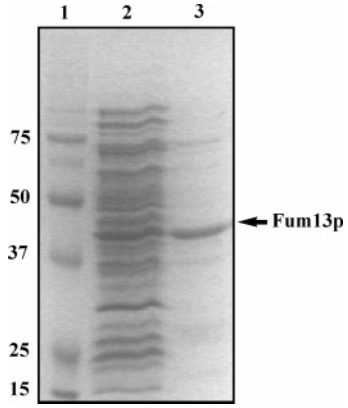

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel analysis of Fum13p expressed in *E. coli*. Lane 1, molecular mass markers; lane 2, total soluble proteins extracted from cells 10 h after induction by isopropyl β-D-thiogalactopyranoside; lane 3, a fraction eluted from Ni–NTA affinity column by 250 mM imidazole.

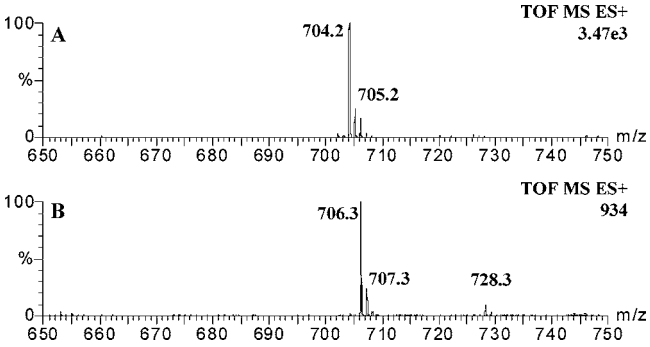

Figure 2. Mass spectrometry of (A) 3-keto fumonisin B_3 isolated from the culture of a *FUM13*-deleted mutant strain and (B) fumonisin B_3 present in the reaction mixture containing enzyme Fum13p, 3-keto fumonisin B_3, and NADPH. A $[M + Na]^+$ of *m*/*z* 728.3 was also present in the reaction mixture in addition to the $[M + H]^+$ of *m*/*z* 706.3 for fumonisin B_3.

$[M + H]^+$ of *m*/*z* 704.2 on LC-MS (**Figure 2A**). The yield of 3-keto FB_3 appeared very low (lower than 1 mg from 500 g CMK medium).

When 3-keto FB_3 was incubated with Fum13p in the presence of NADPH, the 3-keto FB_3 peak at 17.20 min disappeared and a new peak at 17.0 min appeared on HPLC-ELSD (data not shown). In the control experiment where boiled Fum13p was included in the reaction, the new peak was not observed. The new peak comigrated with standard FB_3. When the reaction mixture was analyzed by LC-MS, a $[M + H]^+$ of *m*/*z* 706.3 was observed (**Figure 2B**), which is identical to standard FB_3. A small peak corresponding to $[M + Na]^+$ of FB_3 (*m*/*z* 728.3) was also present in the reaction mixture. The results show that Fum13p is able to reduce the 3-keto group to a 3-hydroxyl group using 3-keto FB_3 as substrate. Previous sequence analysis predicted that Fum13p belongs to the short-chain dehydrogenase/reductase family (*15*, *18*). The in vitro results confirm that Fum13p is a reductase.

Functional Complementation of *TSC10* Mutants by *FUM13*. *TSC10* is a gene encoding 3-ketosphinganine reductase, which is an enzyme involved in the biosynthesis of sphingolipids in yeast (*15*). It has been proposed that the early steps in the biosynthetic pathways for fumonisins and sphingolipids are very similar (*11*, *12*). For example, the first enzyme in the sphingolipid pathway, serine palmitoyltransferase, catalyzes the condensation between serine and palmitoyl-CoA to form 3-ketosphinganine (*14*). The 3-keto group is reduced to 3-hydroxyl

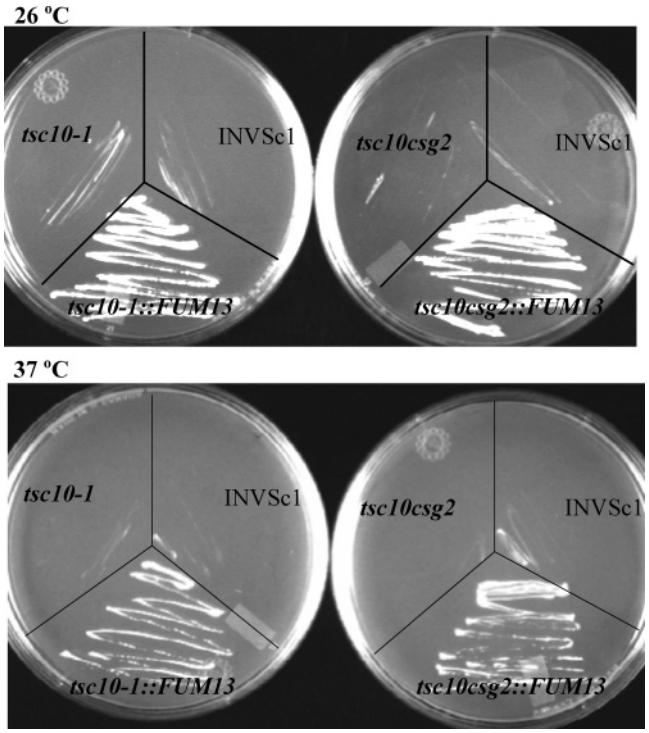

Figure 3. Complementation of yeast *tsc10* mutants by *FUM13*. *FUM13* was cloned into pYES2/NT C vector, which carries a uracil auxotrophic selection marker. The yeast cells were streaked on SC medium without uracil and were allowed to grow at 26 °C or 37 °C for 3 days. All strains, including INVSc1, are uracil auxotroph.

to generate sphinganine (dihydrosphingosine), which is catalyzed by the *TSC10*-encoded 3-ketosphinganine reductase (*15*). In the fumonisin pathway, Fum8p is a homologue of serine palmitoyltransferase and is predicted to catalyze the condensation between alanine and 10,14-dimethyl octadecyl-thioester to form a 3-keto intermediate (*13*). This 3-keto intermediate is then reduced to a 3-hydroxyl intermediate in the fumonisin pathway (*11*), which is catalyzed by Fum13p. We used both LHYa56 tsc10-csg2 and LHYa56 tsc10 (*15*) in the experiments. The *tsc10* mutation is temperature sensitive, such that *tsc10* mutants grow well at the permissive temperature (26 °C) but do not grow at the restrictive temperature (37 °C) (*15*). In addition, all the strains, including the control strain (INVSc1, from Invitrogen), carry a uracil auxotroph, which can be used to select for transformants that contain pYES2/NT C vector on SC medium without uracil. As expected, INVSc1, LHYa56 tsc10-csg2, or LHYa56 tsc10 was not able to grow on SC medium without uracil at 26 °C or 37 °C (**Figure 3**). When *FUM13* (on pYES2/NT C vector) was introduced into LHYa56 tsc10-csg2 or LHYa56 tsc10, the mutants were able to grow on the SC medium without uracil at both 26 °C and 37 °C (**Figure 3**). The transformant colonies were clearly visible after 3 days of growth at 26 °C or 37 °C, while no colony appeared from INVSc1 or the mutant strains without *FUM13*. Since previous studies have demonstrated that *TSC10* is essential for the growth of the yeast in the absence of exogenous sphinganine (*15*), our results suggest that the biosynthesis of sphinganine has been restored after the introduction of *FUM13* into the mutants. Thus, the data support that Fum13p functions as a 3-ketoreductase in vivo.

We have shown that purified Fum13p is able to convert 3-keto FB_3 to FB_3 in vitro. The data provide direct evidence for the function of *FUM13* in the biosynthesis of fumonisins (**Figure 4**). The in vitro result is consistent with that obtained from the

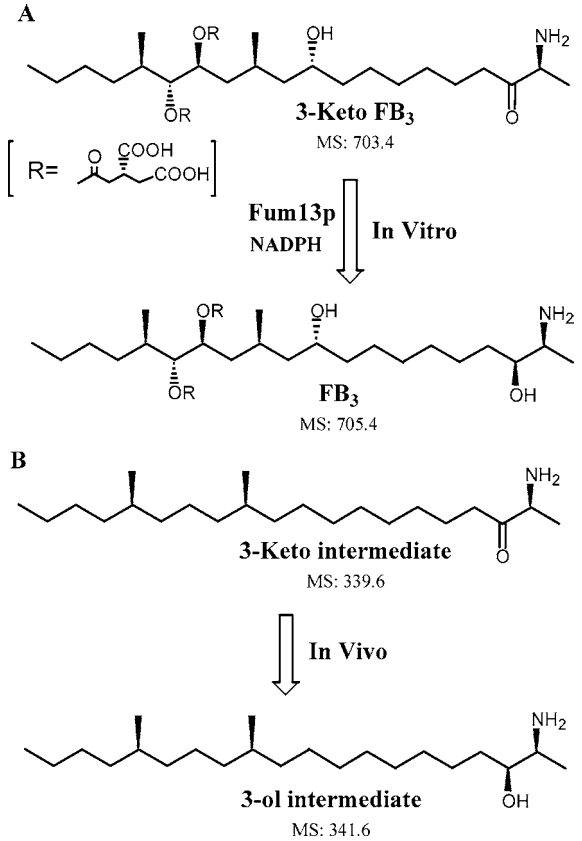

Figure 4. Chemical reactions catalyzed by Fum13p. (A). Observed in vitro reaction of Fum13p using 3-keto fumonisin B_3 as substrate. (B) Proposed in vivo reaction of Fum13p using a 3-keto intermediate in the early stage of the biosynthetic pathway as the substrate.

gene-deletion study conducted by Butchko et al. (*12*). Despite the small amount of B-series fumonisins produced by the *FUM13* deletion mutant, both biochemical data and genetic data support that *FUM13* is responsible for the reduction of the 3-keto group of fumonisins. More studies are needed to prove if other reductases are responsible for the low level of B-series fumonisins in the *FUM13*-deleted mutant. A possible candidate for this activity is 3-ketosphinganine reductase of *F. verticillioides* as proposed previously (*12*).

The conversion of 3-keto FB_3 to FB_3 by purified Fum13p also suggests that this 3-ketoreduction step could take place at the final stage of the biosynthesis of fumonisins (**Figure 4A**), because FB_3 is one of the final products produced by the fungus. However, previous mass spectrometric data suggested that biosynthetic intermediates with a 3-keto group (2-amino-3-oxo-12,16-dimethylicosane, **Figure 4B**) and a 3-hydroxyl group (2-amino-3-hydroxy-12,16-dimethylicosane, **Figure 4B**) were present in the co-cultures of *FUM1-FUM6* mutants and *FUM8-FUM6* mutants (*11*). The MS data strongly suggests that the reduction of 3-keto group is an early step in the pathway. Because of the extremely low yield and instability of this 3-keto intermediate in the cultures, we were not able to test this intermediate in the in vitro assays. Nevertheless, the 3-keto intermediate could be the natural substrate of this enzyme (**Figure 4B**). This is supported by the functional complementation of yeast *tsc10* mutants by *FUM13*. *TSC10* gene is responsible for the conversion of 3-ketosphinganine to sphinganine, which is the second step of the sphingolipid pathway. Since *FUM13* can complement *tsc10* mutants, the result not only supports that *FUM13* functions in 3-keto reduction but also suggests that it functions in the early stage of the biosynthesis. In summary, the current data demonstrate that Fum13p is a reductase catalyzing 3-ketoreduction in fumonisin biosynthesis in *F. verticillioides*.

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LITERATURE CITED

(1) Marasas, W. F. Discovery and occurrence of the fumonisins: A historical perspective. *Environ. Health Perspect.* **2001**, *109* (Suppl. 2), 239−243.

(2) 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.

(3) Desjardins, A. E.; Plattner, R. D. Fumonisin B(1)-nonproducing strains of *Fusarium verticillioides* cause maize (*Zea mays*) ear infection and ear rot. *J. Agric. Food Chem.* **2000**, *48*, 5773−5780.

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

(5) Marasas, W. F.; Kellerman, T. S.; Gelderblom, W. C.; Coetzer, J. A.; Thiel, P. G.; van der Lugt, J. J. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* **1988**, *55*, 197−203.

(6) Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; 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.

(7) Blackwell, B. A.; Edwards, O. E.; Fruchier, A.; ApSimon, J. W.; Miller, J. D. NMR structural studies of fumonisin B1 and related compounds from *Fusarium moniliforme*. *Adv. Exp. Med. Biol.* **1996**, *392*, 75−91.

(8) Branham, B. E.; Plattner, R. D. Alanine is a precursor in the biosynthesis of fumonisin B1 by *Fusarium moniliforme*. *Mycopathologia* **1993**, *124*, 99−104.

(9) Plattner, R. D.; Shackelford, D. D. Biosynthesis of labeled fumonisins in liquid cultures of *Fusarium moniliforme*. *Mycopathologia* **1992**, *117*, 17−22.

(10) Caldas, E. D.; Sadilkova, K.; Ward, B. L.; Jones, A. D.; Winter, C. K.; Gilchrist, D. G. Biosynthetic Studies of Fumonisin B1 and AAL Toxins. *J. Agric. Food Chem.* **1998**, *46*, 4734−4743.

(11) 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.

(12) Butchko, R. A.; Plattner, R. D.; Proctor, R. H. *FUM13* encodes a short chain dehydrogenase/reductase required for C-3 carbonyl reduction during fumonisin biosynthesis in *Gibberella moniliformis*. *J. Agric. Food Chem.* **2003**, *51*, 3000−3006.

(13) 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.

(14) Hanada, K. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim. Biophys. Acta* **2003**, *1632*, 16−30.

(15) Beeler, T.; Bacikova, D.; Gable, K.; Hopkins, L.; Johnson, C.; Slife, H.; Dunn, T. The *Saccharomyces cerevisiae* TSC10/YBR265w gene encoding 3-ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca2+-sensitive *csg2*Δ mutant. *J. Biol. Chem.* **1998**, *273*, 30688−30694.

(16) Ding, Y.; Bojja, R. S.; Du, L. Fum3p, a 2-ketoglutarate-dependent dioxygenase required for C-5 hydroxylation of fumonisins in *Fusarium verticillioides*. *Appl. Environ. Microbiol.* **2004**, *70*, 1931−1934.

(17) Butchko, R. A.; Plattner, R. D.; Proctor, R. H. *FUM9* is required for C-5 hydroxylation of fumonisins and complements the meitotically defined Fum3 locus in *Gibberella moniliformis*. *Appl. Environ. Microbiol.* **2003**, *69*, 6935−6937.

(18) 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.

(19) Proctor, R. H.; Desjardins, A. E.; Plattner, R. D.; Hohn, T. M. A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. *Fungal Genet. Biol.* **1999**, *27*, 100−112.

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

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