Identification of a Chitinase-modifying Protein from *Fusarium verticillioides*

**TRUNCATION OF A HOST RESISTANCE PROTEIN BY A FUNGALYSIN METALLOPROTEASE**

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**Background:** *Fusarium* fungi manipulate plant defenses to cause disease.

**Results:** *Fusarium* fungi secrete fungalysin proteases that truncate maize class IV chitinases.

**Conclusions:** *Fusarium* fungalysins target nonstructural defense proteins associated with plant disease resistance.

**Significance:** Manipulating the fungalysin-chitinase interaction might improve fungal disease resistance of plants.

Chitinase-modifying proteins (cmps) are proteases secreted by fungal pathogens that truncate the plant class IV chitinases ChitA and ChitB during maize ear rot. cmp activity has been characterized for *Bipolaris zeicola* and *Stenocarpella maydis*, but the identities of the proteases are not known. Here, we report that cmps are secreted by multiple species from the genus *Fusarium*, that cmp from *Fusarium verticillioides* (Fv-cmp) is a fungalysin metalloprotease, and that it cleaves within a sequence that is conserved in class IV chitinases. Protein extracts from *Fusarium* cultures were found to truncate ChitA and ChitB in vitro. Based on this activity, Fv-cmp was purified from *F. verticillioides*. N-terminal sequencing of truncated ChitA and MALDI-TOF-MS analysis of reaction products showed that Fv-cmp is an endoprotease that cleaves a peptide bond on the C-terminal side of the lectin domain. The N-terminal sequence of purified Fv-cmp was determined and compared with a set of predicted proteins, resulting in its identification as a zinc metalloprotease of the fungalysin family. Recombinant Fv-cmp also truncated ChitA, confirming its identity, but had reduced activity, suggesting that the recombinant protease did not mature efficiently from its propeptide-containing precursor. This is the first report of a fungalysin that targets a nonstructural host protein and the first to implicate this class of virulence-related proteases in plant disease.

Fungalysins are a family of zinc metalloproteases (M36) that occur in pathogenic fungi and some bacteria. Fungalysins contain peptide motifs similar to those of bacterial thermolysins but have little sequence homology to this well studied class of proteases. Like thermolysins and most secreted bacterial proteases, fungalysins are expressed as inactive precursors that contain N-terminal propeptides. Propeptides are intermolecular chaperones that both inhibit activity and guide the correct folding of the protease (1). The first fungalysin described was MEP42, a protease secreted by *Aspergillus fumigatus*, a human pathogen that causes respiratory infections (2, 3). MEP42 was isolated because of its ability to degrade a nonspecific protease substrate (azocasein). The purified protein was then shown to degrade the structural protein elastin, which is a component of human lungs, suggesting that fungalysins promote pathogenicity by degrading extracellular matrix proteins. This model was strengthened by the observation that a fungalysin from *Microsporum canis*, which causes skin diseases in cats and dogs, is able to degrade the structural protein keratin (4). Besides these examples, few protein-based studies of fungalysin proteins or their substrates have been reported. Molecular studies of fungalysins also suggest that their expression is involved in fungal diseases of insects (5) and amphibians (6), but the host target of their activity is unknown. Analysis of genome sequences has shown that the number of different fungalysins that fungi have varies. As noted previously (7), the model mushroom *Coprinopsis cinerea* has eight predicted fungalysins, whereas bakers’ yeast (*Saccharomyces cerevisiae*) and bread mold (*Neurospora crassa*) have none. Genome sequences from four plant pathogenic species of filamentous fungi from the genus *Fusarium* predict that they each have one fungalysin (8–10). Their biological role is not known, and to date, fungalysins have not been linked to plant pathogenesis.

*Fusarium* is a filamentous fungus genus that contains many species that grow on plants and cause diseases of cereal crops (11). Some diseases, such as scab of wheat and ear rot of maize, result in contamination of grain with fungus-produced mycotoxins (12). Because of the potential dangers associated with mycotoxin contamination of grain and animal feed, studies of *Fusarium* plant pathogens initially focused on the genetics of mycotoxin synthesis (13) and the toxicity of mycotoxins to humans and animals (14, 15). Spurred by genome sequencing (8–10) and microarray and proteomics studies, research into *Fusarium* plant pathogens has broadened to include the search for pathogenicity factors. The expression of many genes has been reported to correlate with pathogenicity, but, with exception (16), few observations of secreted proteins or small molecules that directly manipulate host plant biology have been reported.

Previously, we surveyed the chitinase content of maize ears rotted by fungal pathogens. Maize seeds contain abundant...
amounts of the class IV chitinases ChitA and ChitB, which, in purified form, were shown to inhibit the growth of some fungi growing on agar plates (17). We observed that when ears were rotted by Bipolaris zeicola (G. L. Stout) Shoemaker (holomorph, Cochliobolus carbonum R. R. Nelson) or Stenocarpella maydis (Berkeley) B. Sutton (synonym, Diplodia maydis (Berkeley) Saccardo), the maize class IV chitinases ChitA and ChitB were truncated (18, 19). This resulted from the activity of unidentified proteases termed chitinase-modifying proteins (cmps).\(^2\) cmps are also secreted by these fungi when grown in culture, and their activity can be detected by incubating secreted proteins with purified ChitA and ChitB. We hypothesized that cmps are likely to represent a broader pathogenic mechanism that would be conserved among fungal species differing in other lineage-specific pathogenic innovations (20). To test this hypothesis, we grew cultures of isolates from five different species of Fusarium distributed among three phylogenetically distinct Fusarium species complexes that are associated with maize diseases. We extracted proteins and determined that these pathogens do indeed secrete cmps. We demonstrate that this cmp activity is due to their fungalysin proteases.

**EXPERIMENTAL PROCEDURES**

**Fungal Cultures and Preparation of Secreted Protein Extracts—**Fusarium isolates (see Table 1) were grown as potato dextrose-agar slant cultures (14 days, 25 °C). For each solid substrate culture, whole maize seed (50 g) was combined with water (50 ml) in a 500-ml Erlenmeyer flask. The seed/water mixture was incubated overnight, autoclaved (15 p.s.i., 30 min), and inoculated with a suspension of hyphal cells (propagation 1). Cultures were incubated for 7 days at 25 °C, transferred to plastic containers, and stored at −80 °C. Secreted protein extracts were prepared by combining 5 g of culture with 5 ml of extraction buffer (10 mM sodium acetate (pH 5.2), 0.2 mM PMSF, 1 mM EDTA, and 2 mM ascorbic acid) in a 50-ml conical tube. Cultures were incubated with buffer for 30 min at 4 °C, followed by centrifugation at 20,000 g for 5 min at 4 °C. The protein extracts were then diluted to a concentration of 1 mg/ml protein as determined by the DC \(^\text{TM}\) protein assay (Bio-Rad). The supernatants were assayed by adding 1 μl to a 10-μl reaction.

**Recombinant ChitB and Fusarium verticillioides cmp (Fv-cmp) Expression Strains—**The ChitB-encoding cDNA was made by removing the intron (21) from a plasmid containing the chiB gene (GRMZM2G005633) (19). The cDNA was then amplified by PCR and cloned into expression vector pPICZαA (Invitrogen). Two Fv-cmp expression strains were created. One encoded secreted expression of the full-length recombinant protein (fFv-cmp(FL)), whereas the other encoded only the protease (rFv-cmp(PROT)). Both cDNAs were amplified from a plasmid cDNA library (22) and cloned into pPICZαA. For each expression strain and a vector-only control, plasmid DNA was purified from Escherichia coli cultures, linearized, and transformed into Pichia pastoris X-33 by electroporation. Protein-expressing isolates were selected by plating transformed cells on yeast/peptone/dextrose-agar supplemented with sorbitol and Zeocin (1% yeast extract, 2% peptone, 2% glucose, 1 M sorbitol, 2% agar, and 100 μg of Zeocin). Oligonucleotide primers are listed in supplemental Table 1.

**Expression and Purification of Recombinant Chitinases—**Expression of recombinant ChitA-LHB2 and ChitA-B73 and their purification from heterologous P. pastoris were as described previously (23). Recombinant ChitB was expressed and purified by the same method.

**Expression of rFv-cmp(FL) and rFv-cmp(PROT)—**Heterologous P. pastoris strains that express Fv-cmp and negative control strains with integrated vectors were grown in 25 ml of buffered glycerol complex medium at 30 °C using an EasySelect Pichia expression kit (Invitrogen) as recommended by the manufacturer. Cultures were grown to an absorbance of 10. Cells were pelleted by centrifugation and resuspended in 25 ml of buffered methanol complex medium at 30 °C. Samples were tested after 1 day and after 4 days. For testing, 1 ml of culture was removed, cells were removed by centrifugation, and the medium was concentrated 10-fold by ultrafiltration.

**cmp Assays—**Reactions consisted of recombinant maize chitinase (20 μg) and various amounts of fungal protein solution in 10 μl of 10 mM sodium acetate (pH 5.2). Reactions with purified Fv-cmp contained 10 ng of protease. Reactions were incubated for 1 h at 25 °C and stopped by the addition of SDS-PAGE loading dye, followed by heat denaturation (1 min in boiling water). All reactions were analyzed by SDS-PAGE.

**Purification of Fv-cmp—**Fv-cmp was purified from 32 flasks of F. verticillioides (NRRL 20956). 95 ml of extraction buffer was added to each flask and mixed. The extract was filtered through cheesecloth and centrifuged at 15,000 × g for 10 min at 4 °C. Ethanol was added to the extract to 35%. The solution was stirred at room temperature for 10 min and then centrifuged at 15,000 × g for 15 min at 4 °C. The supernatant was discarded, and precipitated proteins were dried overnight at 4 °C. Dried precipitated proteins were resuspended in 40 ml of buffer containing 50 mM sodium acetate (pH 4.7) and 250 mM NaCl; the solution was centrifuged and filtered through a 0.45-μm filter.

The resuspended pellet was loaded onto a mixed-mode cation exchange column (HiTrap Capto MMC, 5 ml). Bound proteins were eluted by increasing the pH and ionic strength with a linear gradient in 100 mM MES (pH 6.0) and 1 x NaCl. Fractions of 1 ml were collected. The activity of each elution fraction was tested by cmp assay. The two fractions with the highest activity were combined, and saturated ammonium sulfate was added to 35%. This solution was loaded onto a hydrophobic interaction chromatography column (HiTrap butyl HP, 1 ml) in 50 mM MES (pH 6.0) and 35% ammonium sulfate bound protein were eluted with a linear gradient in buffer with reduced ionic strength (50 mM sodium acetate (pH 5.2) and 250 mM NaCl). Elution fractions of 0.5 ml were collected, and 1 μl of each was tested by cmp assay. Based on the results, seven elution

\(^2\) The abbreviations used are: cmp, chitinase-modifying protein; Fv-cmp, F. verticillioides cmp; rFv-cmp(FL), full-length recombinant Fv-cmp; rFv-cmp(PROT), recombinant Fv-cmp protease; CAPS, 3-(cyclohexylamino)-propanesulfonic acid; CFM, cell-free medium.
fractions were concentrated in Centricon YM-30 concentrators (Millipore).

**RESULTS**

cmp Activity Assay of Fusarium Fermentation Cultures—Solid substrate fermentation cultures were grown for four isolates from each of five species from the genus *Fusarium* reported to cause maize seed disease: *F. verticilloides*, *F. oxysporum*, *F. graminearum*, *F. proliferatum*, and *F. subglutinans* (Table 1). Isolates Fv1 (NRRL 20956), Fo1 (NRRL 34936), and Fg1 (NRRL 31084) were chosen because they have complete genome sequences (8, 10). The remaining 17 were chosen because they were isolated from diseased maize. Secreted proteins were extracted from each of the 20 cultures, and each extract was incubated with three different class IV chitinases from maize: ChitA-LH82 and ChitA-B73 (alloforms of the ChitA protein produced by maize inbreds LH82 and B73) and ChitB (an isoform of ChitA encoded by a different gene). Reactions were incubated for 16 h and analyzed by SDS-PAGE (Fig. 1).

All 20 isolates secreted cmps that converted the three chitinase substrates into a form with increased electrophoretic mobility. The *F. oxysporum* and *F. proliferatum* extracts converted all three substrates without preference. The *F. verticilloides* and *F. subglutinans* extracts degraded a higher percentage of the ChitA-B73 substrate, whereas the other two were degraded equally. In contrast, the *F. graminearum* extracts degraded a lower percentage of the ChitA-B73 substrate than...
the other two. In all extracts, ChitA-LH82 and ChitB were equivalent as substrates, whereas ChitA-B73 was sometimes recognized differently, leading to either a higher (F. verticillioides and F. subglutinans) or lower (F. graminearum) percentage of product. Although these subtle substrate preferences were observed, all protein extracts efficiently truncated each of the three chitinases.

Purification of Fv-cmp—Fv-cmp was purified from F. verticillioides Fv1, an isolate with a sequenced genome. Secreted proteins were extracted from solid substrate cultures. Proteins were precipitated by the addition of ethanol, followed by centrifugation. Precipitated proteins were resuspended in buffer and subjected to chromatography by mixed-mode cation exchange (supplemental Fig. 1). Analysis of elution fractions by cmp assay indicated that the majority of the activity was confined to two of the 12 fractions. These fractions were combined, ammonium sulfate was added, and proteins were separated by hydrophobic interaction chromatography (Fig. 2a). The flow-through and elution fractions were tested for activity (data not shown). The results indicated that Fv-cmp bound the column and eluted near the end of the elution gradient. The final seven elution fractions and a sample of the flow-through were concentrated by ultrafiltration. Each concentrated sample was analyzed for protein content by SDS-PAGE and for activity by cmp assay (Fig. 2b). cmp activity correlated with a protein band of ~45 kDa (Fig. 2b, star).

Fv-cmp Target Site Analysis—To determine their N termini, the ChitA-LH82 substrate and the truncated Fv-cmp-cleaved product were analyzed by Edman degradation (Fig. 3a) (24). For the substrate, the first four amino acids were glutamate, phenylalanine, glutamine, and asparagine, whereas the fifth amino acid did not yield a signal (consistent with the presence of cysteine), as reported previously for ChitA-B73 (23). For the product, the first amino acid did not yield a signal, suggesting that it was cysteine, whereas the next four amino acids were glutamine, serine, glycine, and proline. This matches an internal ChitA-LH82 sequence that is after the region that is predicted to be critical for chitin binding (25) but prior to a polyglycine hinge that separates the chitin-binding and chitinase domains. To determine whether the amino acids surrounding the cleavage site are conserved in plant class IV chitinases, the sequences of the maize chitinases were compared with those of four class IV chitinases from dicotyledonous angiosperms (carrot, common bean, thale cress, and tobacco) and one class IV chitinase from a gymnosperm (Norway spruce) (Fig. 3b) (26–30). A comparison of the sequences revealed that seven of the 14 amino acids that flank the targeted peptide bond are conserved (~4, ~3, ~1, +1, +4, +5, and +6), whereas two additional amino acids differ in only one protein (~5 and +2). To augment this analysis, the target sequence was used to search for hypothetical proteins with similar sequences in the GenBank™ nr Database (supplemental Fig. 2). This analysis identified additional chitinases with these conserved residues from monocots, dicots, gymnosperms, and a bryophyte (moss).

Visualization of the C-terminal cleavage product by SDS-PAGE and sequencing of its N terminus suggested that Fv-cmp
is an endoprotease that converts ChitA-LH82 into two products by cleaving a specific peptide bond (Fig. 4, a and b). To confirm this mechanism, the reaction products were analyzed by mass spectrometry. ChitA-LH82 was incubated in the presence or absence of Fv-cmp. Samples were mixed with matrix, ionized by MALDI, and detected with a TOF mass analyzer. In the control reaction (Fig. 4, lower left panel), ChitA-LH82 was observed as a singly charged ion. In the Fv-cmp reaction, the substrate and both peptide products were observed. Both the substrate and C-terminal product were visible in the high mass range (upper left panel). The smaller N-terminal product, not detected by SDS-PAGE, was visible in the low mass range as both protonated and sodium adduct ions (upper right panel). Multiply charged ions were also detected (supplemental Fig. 3).

**Fv-cmp Identification and Analysis**—The N-terminal sequence of purified Fv-cmp was determined by Edman degradation (supplemental Fig. 4). Ten amino acids were analyzed; nine were identified. The sequence was compared with a list of predicted proteins that is available from the *Fusarium* Comparative Database (Broad Institute of MIT and Harvard). The deduced sequence of the purified protein, ATYKVYPXGV, matched a single predicted protein, fungalysin protease FVEG_13630.0. The N-terminal sequence of purified Fv-cmp is

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**FIGURE 2. Second chromatography step: hydrophobic interaction chromatography.** a, chromatograph. Proteins were loaded at high ammonium sulfate (AS) concentration, and the column was washed. Bound proteins were eluted with a linear descending ammonium sulfate gradient. cmp assays determined that activity was present in late elution fractions (thick line). These active fractions were concentrated and analyzed. b, SDS-PAGE analysis. The protein content of the concentrated flow-through and active fractions was analyzed (upper panel); the activity of each was determined by cmp assay (lower panel; 1-h reactions). The fraction indicated by a star was used for biochemical tests and protein identification. Lane M, molecular mass markers.

**FIGURE 3. Determination of Fv-cmp cleavage site.** a, N-terminal sequencing. ChitA-LH82 was incubated with purified Fv-cmp (1 h), subjected to SDS-PAGE, transferred to a PVDF membrane, and stained (left panel). Both the substrate (upper) and product (lower) bands were removed with a scalpel (right panel) and analyzed by Edman degradation. The analysis showed that Fv-cmp cleaved a peptide bond between the predicted chitin-binding region and the polyglycine hinge region. b, multiple sequence alignment. The sequence near the Fv-cmp-cleaved peptide bond of ChitA-LH82 was aligned with sequences from other plant class IV chitinases. Dots indicate homology. Gray boxes mark amino acids that are conserved in all eight chitinases.
FIGURE 4. Confirmation of cleavage site by mass spectrometry. a, N-terminal sequencing data suggest that Fv-cmp cleaves ChitA-LH82 into a small N-terminal protein (gray) and a large C-terminal protein (black). b, monomeric masses of the substrate and both predicted products. c, MALDI-TOF-MS analysis of ChitA-LH82 (lower panels) and Fv-cmp-cleaved ChitA-LH82 (upper panels). In the high mass range (left panels), ChitA-LH82 and the C-terminal product were observed as singly charged ions. In the low mass range (right panels), the N-terminal product was observed as singly charged protonated ions and sodium adduct ions.
homologous to that reported for MEP42 isolated from *A. fumigatus* (2).

To identify homologous proteins in *F. oxysporum* and *F. graminearum*, the peptide sequence of Fv-cmp was used to search predicted proteins (32). A single fungalysin from each organism was identified as a putative protein responsible for the *F. oxysporum* cmp (FOXG_16612.2) and *F. graminearum* cmp (FGSG_03467.3) activity that was observed in the secreted protein extracts (Fig. 1). The peptide sequences of these putative cmps and *A. fumigatus* MEP42 were individually compared with that of Fv-cmp by global alignment, as shown in Fig. 5a (33). Each sequence was compared three different ways: using the full-length protein, using the propeptide region, or using the protease region. The full-length analysis showed that the *F. oxysporum* homolog has a higher percentage identity to Fv-cmp than the *F. graminearum* homolog and that both *Fusarium* proteins have higher identity than *A. fumigatus* MEP42. Separate analysis of the propeptide and protease domains showed that the propeptide region of Fv-cmp and MEP42 are less conserved than their protease regions, but comparison of Fv-cmp and the *F. graminearum* homolog showed the opposite, that the protease is less conserved than the propeptide.

To confirm the identity of Fv-cmp, recombinant protein was produced from fungalysin cDNA and tested for activity. One recombinant protein, rFv-cmp(FL), was expressed from cDNA encoding both the propeptide and protease, whereas a second protein, rFv-cmp(PROT), was expressed from cDNA encoding the protease but not the propeptide (Fig. 5b). For each strain and a negative control with an integrated empty vector, two expression cultures were grown, and cmp activity was assayed from concentrated cell-free medium (CFM). After 1 day of induction, CFM from both rFv-cmp(FL) cultures truncated the
ChitA substrate, forming three peptide products. One (p1) was consistent with that produced by the native protein at earlier time points (Figs. 2b and 3a). The remaining two (p2 and p3) were larger and matched minor products produced by the native protein at a later time point (Fig. 5b, rightmost lanes). After 4 days of induction, CFM from the rFv-cmp(FL) cultures truncated ChitA and formed the same three products. CFM from one of the rFv-cmp(PROT) cultures also truncated ChitA, producing two of these products (Fig. 5b, right panel, p1 and p2). Truncation of ChitA by CFM from the vector-only negative controls was not observed, demonstrating that cmp activity was from recombinant protein encoded by the fungalysin cDNA.

DISCUSSION

We reported previously that B. zeicola and S. maydis secrete cmps, proteases that cleave maize ChitA and ChitB. These activities were discovered by analyzing the chitinase content of ears rotted by various fungal pathogens. In this study, we tested secreted protein extracts from Fusarium cultures for cmp activity and found that 20 isolates representing five species and distributed among three phylogenetically distinct species complexes (F. graminearum, F. oxysporum, and Gibberella fujikuroi (=F. proliferatum, F. subglutinans, and F. verticillioides)) (10, 34) also secreted cmps. Based on this activity, Fv-cmp was purified from an isolate of F. verticillioides and identified as a fungalysin protease. Cleavage of ChitA by purified Fv-cmp was analyzed by N-terminal sequencing and MALDI-TOF-MS. This analysis showed that Fv-cmp cleaved ChitA in a region that is conserved in other class IV chitinases, suggesting that Fv-cmp and other fungalysins may truncate class IV chitinases from other plants.

The identity of Fv-cmp as a fungalysin was confirmed by expressing two different recombinant proteins from fungalysin cDNAs, followed by detection of cmp activity. Both rFv-cmp(FL) and rFv-cmp(PROT) truncated ChitA, forming the same product as the native protein (Fig. 5b, p1). Recombinant proteins also produced two other products that were produced by the native protein as minor products (p2 and p3). It seems probable that the major product results from correctly folded Fv-cmp and that the other two are due to Fv-cmp that is in an altered conformation. Although the maturation pathway of fungalysins in unknown, studies of protease maturation guided by propeptide intermolecular chaperones have shown that it is a complex process (35) that can produce proteases with identical sequence but altered activity (36, 37).

Our description of Fv-cmp, a fungalysin from a filamentous fungus that is adapted for growth on plants, expands the knowledge of fungalysins, which was based largely on the study of a single protein (2, 3, 38). This previous work suggests that fungalysins from human and animal pathogens promote pathogenicity by degrading structural proteins. In our study, we report that fungalysins from plant pathogens truncate the seed chitinases ChitA and ChitB, a pair of nonstructural maize defense proteins. Identification of the target site cleaved by mature Fv-cmp also contradicts the predicted preference for cleavage by fungalysins on the N-terminal side of hydrophobic residues with bulky side chains: Fv-cmp cleaves next to the polar uncharged residue cysteine. Therefore, fungalysins likely have a wide variety of activities that will be hard to predict. This is not surprising considering that they have a single active site that must perform different functions during processing and maturation and yet another when mature.

Understanding the methods employed by plant pathogens to manipulate and evade plant defenses is an important area of research that has led to practical results, such as the development of maize hybrids that are resistant to northern corn leaf blight (39). Although few of the likely many mechanisms employed by eukaryotic plant pathogens are currently known (40), these results and others (31, 41–43) indicate that chitin and proteins that have chitin-binding domains, such as ChitA and ChitB, play a prominent role in plant-fungal interactions.

Our finding that Fv-cmp targets a region of ChitA that is conserved in plant class IV chitinases suggests that the interaction between Fv-cmp and ChitA represents a specific example of a general mechanism involved in plant-fungal interactions, speculation that is strengthened by our observation that an isolate of F. oxysporum that was isolated from a diseased tomato plant (Fo1), which we selected because it has a sequenced genome, truncated maize ChitA and ChitB (Fig. 1 and Table 1). Further understanding of the interaction between fungalysins and plant class IV chitinases may lead to new ways to reduce Fusarium ear rot and the resulting mycotoxin contamination of maize and will improve knowledge of the molecular basis of fungal disease resistance in plants.

Acknowledgments—We thank Robert Proctor for providing F. verticillioides plasmid cDNA libraries and Kurt Sollenberger, Jacob Brown, Trina Hartman, and Mark Berhow for technical assistance.

REFERENCES
1. Shinde, U., and Inouye, M. (1993) Trends Biochem. Sci. 18, 442–446
2. Markaryan, A., Morozova, I., Yu, H., and Kolattukudy, P. E. (1994) Infect. Immun. 62, 2149–2157
3. Sirakova, T. D., Markaryan, A., and Kolattukudy, P. E. (1994) Infect. Immun. 62, 4208–4218
4. Brouta, F., Descamps, F., Monod, M., Vermout, S., Losson, B., and Mignon, B. (2002) Infect. Immun. 70, 5676–5683
5. Xu, J., Baldwin, D., Kindrachuk, C., and Hegenus, D. D. (2006) Can. J. Microbiol. 52, 550–559
6. Rosenblum, E. B., Stajich, J. E., Maddox, N., and Eisen, M. B. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 17034–17039
7. Lilly, W. W., Stajich, J. E., Pukkila, P. I., Wilke, S. K., Inoguchi, N., and Gathman, A. C. (2008) Mycol. Res. 112, 389–398
8. Cuomo, C. A., Guldener, U., Xu, J. R., Trail, F., Turgeon, B. G., Di Pietro, A., Walton, J. D., Ma, L. J., Baker, S. E., Rep, M., Adam, G., Antoniou, I., Baldwin, T., Calvo, S., Chang, Y. L., Decaprio, D., Gale, L. R., Gnerre, S., Goswami, R. S., Hammond-Kosack, K., Harris, L. J., Hibburn, K., Kennell, J. C., Kroken, S., Magnuson, J. K., Mannhaupt, G., Mauceli, E., Mewes, H. W., Mittberbauer, R., Muelhaber, G., Münksterkötter, M., Nelson, D., O’donnell, K., Ouellet, T., Qi, W., Quesneville, H., Roncero, M. I., Seong, K. Y., Tetko, I. V., Urban, M., Waalwijk, C., Ward, T. J., Yao, J., Birren, B. W., and Kistler, H. C. (2007) Science 317, 1400–1402
9. Coleman, J. J., Rounsley, S. D., Rodriguez-Carres, M., Kuo, A., Wassmann, C. C., Grimwood, J., Schmutz, J., Taga, M., White, G. J., Zhou, S., Schwartz, D. C., Freitag, M., Ma, L. J., Danchin, E. G., Henriissat, B., Coutinho, P. M., Nelson, D. R., Straney, D., Napoli, C. A., Barker, B. M., Gribskov, M., Rep, M., Kroken, S., Molnár, I., Rensing, C., Kennell, J. C., Zamora, J., Farman, M. L., Selker, E. U., Salamov, A., Shapiro, H., Pangilinan, J., Lindquist, E.,
Fusarium Fungalysins

Lamers, C., Grigoriev, I. V., Geiser, D. M., Covert, S. F., Temporini, E., and Vanetten, H. D. (2009) PLoS Genet. 5, e1000618

10. Ma, L. J., van der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M. J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., Houterman, P. M., Kang, S., Shim, W. B., Woloshuk, C., Xie, X., Xu, J. R., Antoniw, J., Baker, S. E., Bluhm, B. H., Breakspear, A., Brown, D. W., Butchko, R. A., Chapman, S., Coulson, R., Coutinho, P. M., Danchin, E. G., Diener, A., Gale, L. R., Gardiner, D. M., Goff, S., Hammond-Kosack, K. E., Hliburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C. D., Koehrsen, M., Kumar, L., Lee, Y. H., Li, L., Manners, J. M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S. Y., Proctor, R. H., Regev, A., Ruiz-Roldan, M. C., Sain, D., Sakthikumar, S., Sykes, S., Schwartz, D. C., Turgeon, B. G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan, J., Cuomo, C. A., Kistler, H. C., and Rep, M. (2010) Nature 464, 367–373

11. Agrios, G. N. (2005) Plant Pathology, 5th Ed., Academic Press, New York

12. Stepień, L., and Chelkowski, J. (2010) World Mycotoxin J. 3, 107–119

13. Desjardins, A. E. (2006) Fusarium Mycotoxins: Chemistry, Genetics, and Biology, American Phytopathological Society, St. Paul, MN

14. Gelderblom, W. C., Jaskiewicz, K., Marasas, W. F., Thiel, P. G., Horak, R. M., Vleggaar, R., and Kriek, N. P. (1988) Appl. Environ. Microbiol. 54, 1806–1811

15. Desai, K., Sullards, M. C., Allegood, J., Wang, E., Schmelz, E. M., Hartl, M., Humph, H. U., Liotta, D. C., Peng, Q., and Merrill, A. H., Jr. (2002) Biochim. Biophys. Acta 1585, 188–192

16. Takken, F., and Rep, M. (2010) Mol. Plant Pathol. 11, 309–314

17. Huynh, Q. K., Hironaka, C. M., Levine, E. B., Smith, C. E., Borgmeyer, J. R., and Shah, D. M. (1992) Mol. Plant Pathol. 3, 107–119

18. Naumann, T. A., Wicklow, D. T., and Kendra, D. F. (2009) Physiol. Mol. Plant Pathol. 74, 134–141

19. Naumann, T. A., and Wicklow, D. T. (2010) Phytopathology 100, 645–654

20. Soanes, D. M., Richards, T. A., and Talbot, N. J. (2007) Plant Cell 19, 3318–3326

21. Horton, R. M., Cai, Z. L., Ho, S. N., and Pease, L. R. (1990) BioTechniques 8, 528–535

22. Brown, D. W., Cheung, F., Proctor, R. H., Butchko, R. A., Zheng, L., Lee, Y., Utterback, T., Smith, S., Feldblum, T., Glenn, A. E., Plattner, R. D., Kendra, D. F., Town, C. D., and Whitelaw, C. A. (2005) Fungal Genet. Biol. 42, 848–861

23. Naumann, T. A. (2011) Mol. Plant Pathol. 12, 365–372

24. Edman, P., and Begg, G. (1967) Eur. J. Biochem. 1, 80–91

25. Aboitiz, N., Vila-Perello, M., Groves, P., Asensio, J. L., Andreu, D., Cañada, F. J., and Jiménez-Barbero, J. (2004) ChemBioChem 5, 1245–1255

26. De Jong, A. J., Cordewener, J., Lo Schiavo, F., Terzi, M., Vandekerckhove, J., Van Kammen, A., and De Vries, S. C. (1992) Plant Cell 4, 425–433

27. Lange, J., Mohr, U., Viemken, A., Boller, T., and Vögeli-Lange, R. (1996) Plant Physiol. 111, 1135–1144

28. Gerhardt, L. B., Sachetto-Martins, G., Contarini, M. G., Sandroni, M., Ferreira, R., de Lima, V. M., Cordeiro, M. C., de Oliveira, D. E., and Margis-Pinheiro, M. (1997) FEBS Lett. 419, 69–75

29. Shinya, T., Haniu, K., Gális, I., Suzuki, K., Matsuoka, K., Matsuoka, H., and Saito, M. (2007) Biochem. Biophys. Res. Commun. 353, 311–317

30. Wiweger, M., Farbos, I., Ingouff, M., Lagercrantz, U., and Von Arnold, S. (2003) J. Exp. Bot. 54, 2691–2699

31. Petutschnig, E. K., Jones, A. M., Serazetdinova, L., Lipka, U., and Lipka, V. (2010) J. Biol. Chem. 285, 28902–28911

32. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402

33. Needleman, S. B., and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443–453

34. Geiser, D. M., Jimenez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A., and O’Donnell, K. (2004) Eur. J. Plant Pathol. 110, 473–479

35. Gao, X., Wang, J., Wu, J. W., and Zhang, Y. Z. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 17569–17574

36. Shinde, U., Fu, X., and Inouye, M. (1999) J. Biol. Chem. 274, 15615–15621

37. Shinde, U. P., Liu, J. J., and Inouye, M. (1997) Nature 389, 520–522

38. Markaryan, A., Lee, J. D., Sirakova, T. D., and Kolattukudy, P. E. (1996) J. Bacteriol. 178, 2211–2215

39. Johal, G. S., and Briggs, S. P. (1992) Science 258, 985–987

40. Kamoun, S. (2007) Curr. Opin. Plant Biol. 10, 358–365

41. van den Burg, H. A., Westerink, N., Francoijs, K. J., Roth, R., Wosten, E., Boeren, S., de Wit, P. J., Joosten, M. H., and Vervoort, J. (2004) J. Biol. Chem. 279, 27340–27346

42. van den Burg, H. A., Spronk, C. A., Boeren, S., Kennedy, M. A., Vissers, J. P., Vlieg, G. W., de Wit, P. J., and Vervoort, J. (2004) J. Biol. Chem. 279, 16786–16796

43. Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiya, C., Doehma, N., Takio, K., Minami, E., and Shibuya, N. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 11086–11091