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Molecular biology of *Fusarium* mycotoxins ☆

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Abstract

As the 20th century ended, *Fusarium* mycotoxicology entered the age of genomics. With complete genomes of *Fusarium graminearum* and *F. verticillioides* and several *Fusarium* gene expression sequence databases on hand, researchers worldwide are working at a rapid pace to identify mycotoxin biosynthetic and regulatory genes. Seven classes of mycotoxin biosynthetic genes or gene clusters have been identified in *Fusarium* to date; four are polyketide synthase gene clusters for equisetin, fumonisins, fusarins, and zearalenones. Other *Fusarium* mycotoxin biosynthetic genes include a terpene cyclase gene cluster for trichothecenes, a cyclic peptide synthetase for enniatins, and a cytochrome P450 for butenolide. From the perspective of the United States Department of Agriculture, the ultimate goal of research on *Fusarium* molecular biology is to reduce mycotoxins in cereal grains. With this goal in mind, efforts have focused on identifying aspects of mycotoxin biosynthesis and regulation that can be exploited for mycotoxin control. New information on fungal and plant genomes and gene expression will continue to provide information on genes important for fungal-plant interactions and to facilitate the development of targeted approaches for breeding and engineering crops for resistance to *Fusarium* infection and mycotoxin contamination.
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1. *Fusarium* mycotoxins

As the 20th century ended, *Fusarium* mycotoxicology entered the age of genomics. Our research group at the United States Department of Agriculture reported the discovery of the trichothecene biosynthetic gene cluster in *F. sporotrichioides* in 1993 and the fumonisin biosynthetic gene cluster in *F. verticillioides* in 1999. During the 1990s, research groups in Germany cloned genes for biosynthesis of the mycotoxin enniatin and for other *Fusarium* secondary metabolites. During this decade, gene expression sequence databases of various mycotoxigenic *Fusarium* species also became available. The field of *Fusarium* genomics was accelerated when the United States Department of Agriculture and National Science Foundation jointly supported the sequencing and public release of the complete genomes of *F. graminearum* in 2003 and of *F. verticillioides* in 2006 (Broad Institute/MIT Center for Genome Research [www.broad.mit.edu/annotation/genome/fusarium_graminearum/Home.html] [www.broad.mit.edu/annotation/genome/fusarium_verticillioides/Home.html]). Access to these *Fusarium* genomes revealed the presence of dozens of candidate genes for polyketide synthases, non-ribosomal peptide synthetases, terpene cyclases, and other types of enzymes that synthesize mycotoxins and other biologically active metabolites. Comparison of DNA sequences *per se* cannot supply details of mycotoxin biosynthetic pathways; this information must be obtained by appropriate experimentation. Fortunately, *Fusarium* species are highly amenable to the techniques of biochemistry, classical genetics, and molecular genetics necessary to validate function of candidate genes. With two complete *Fusarium* genomes and several *Fusarium* gene expression sequence databases on hand, researchers worldwide are working at a rapid pace to identify biosynthetic and regulatory genes for individual mycotoxins and other biologically active metabolites.

This brief overview begins with molecular biology of three major classes of mycotoxins that have been proven to cause animal disease outbreaks: trichothecenes, fumonisins, and zearalenones. The review continues with minor mycotoxins, including metabolites that are carcinogenic or toxic in experimental systems (beauvericin and enniatins, equisetin, fusarins), and metabolites

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that have been implicated in animal mycotoxicoses (butenolide). In this review, genetic nomenclature has been standardized. Genes are indicated by three italicized uppercase letters followed by a number. This review is an adaptation and update of information from a recent text on *Fusarium* mycotoxins (Desjardins, 2006) and only research updates published since 2004 are cited directly.

2. Trichothecenes

Of all *Fusarium* mycotoxins discovered to date, trichothecenes have been most strongly associated with chronic and fatal toxicoses of humans and animals, including Alimentary Toxic Aleukia in Russia and Central Asia, Akakabi-byo (red mold disease) in Japan, and swine feed refusal in the central United States. The major mechanism of trichothecene toxicity is inhibition of ribosomal protein synthesis. *Fusarium* trichothecenes are tricyclic sesquiterpenes that contain a double bond between carbons 9 (C-9) and 10 and a 12,13-epoxide ring, and are thus designated as 12,13-epoxytrichothec-9-enes. *Fusarium* trichothecenes are also characterized by various patterns of oxygenation and esterification at positions C-3, C-4, C-7, C-8, and C-15. Trichothecenes comprise a large family of compounds, of which diacetoxyscirpenol, T-2 toxin, nivalenol, and deoxynivalenol are most important in cereal grains.

The trichothecene biosynthetic pathway in *Fusarium* species begins with a sesquiterpene cyclization catalyzed by the enzyme trichodiene synthase, followed by up to eight oxygenations and four esterifications. Trichothecene biosynthesis also requires expression of a transporter protein and a network of regulatory genes. Trichothecene biosynthetic and regulatory genes have been mapped to four unlinked loci in the *F. graminearum* genome and also have been localized to specific contigs of the *F. graminearum* genome sequence. Trichodiene synthase (*TRI5*) is notable not only as the first trichothecene gene to be cloned, but also as the topographic center of a 25-kb cluster of 12 co-regulated trichothecene biosynthetic genes on *F. graminearum* genomic contig 1.159. Gene-disruption studies have determined that 10 of these cluster genes are required for trichothecene biosynthesis. Adjacent to *TRI5* are two trichothecene regulatory genes, *TRI6* and *TRI10*; disruption of any of these three genes results in the complete reduction of trichothecene production. The product of *TRI6* is a classic zinc-finger protein, which regulates transcription of most known trichothecene biosynthetic genes, and is itself regulated by the master regulatory gene *TRI10*. The *TRI5* gene cluster also contains *TRI12*, which encodes a transporter of the major facilitator superfamily; three genes encoding cytochromes P450, which catalyze oxygenations at C-1, C-3, C-12,13 (*TRI4*), at C-15 (*TRI11*), and C-4 (*TRI13*); and three genes (*TRI3*, *TRI7*, and *TRI8*) encoding enzymes that add or remove ester groups. Although the majority of trichothecene biosynthetic genes are tightly linked in the *TRI5* cluster, four additional genes have been located at three other, unlinked loci. At one locus (on genomic contig 1.4) are *TRI1* and *TRI16*, which encode enzymes that catalyze C-8 oxygenation and *O*-acetyl transfer, respectively. At two other loci are *TRI101* (on genomic contig 1.321), which encodes a C-3 *O*-acetyl transferase and *TRI15* (on genomic contig 1.457), which encodes a zinc-finger transcription factor that may function as a negative regulator in trichothecene biosynthesis.

3. Fumonisins

Although fumonisins have a relatively simple chemical structure, their inhibition of sphingolipid metabolism can have diverse and complex effects in animal systems. Fumonisins cause leukoencephalomalacia, a brain lesion that can be fatal to horses after only a few days' consumption of contaminated feed. Fumonisins are carcinogenic in experimental rodents, and consumption of grain contaminated with fumonisins has been associated epidemiologically with human esophageal cancer and birth defects. Fumonisins are long-chain amino polyalcohols. The major fumonisin homologue in cereal grains is fumonisin B_1, a propane-1,2,3-tricarboxylic diester of 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyicosane. Minor fumonisin homologues, that lack hydroxyl groups at C-5 and C-10, can also occur naturally in cereal grains.

The fumonisin biosynthetic pathway in *Fusarium* species begins with formation of a linear dimethylated polyketide and condensation of the polyketide with alanine, followed by a carbonyl reduction, oxygenations, and esterification with two propane-1,2,3-tricarboxylic acids. To date, fumonisin biosynthetic genes have been mapped to one locus in the *F. verticillioides* genome. The entire 42-kb fumonisin biosynthetic gene cluster is absent from *F. graminearum*, but flanking genes map to *F. graminearum* genomic contig 1.159. Fumonisin polyketide synthase (*FUM1*) was the first fumonisin gene to be cloned and is the anchor of a cluster of 15 co-regulated fumonisin biosynthetic genes. Gene-disruption studies have determined that eleven of these genes are required for fumonisin biosynthesis. Fumonisin polyketide synthase is an iterative Type I, reducing, polyketide synthase with seven functional domains (ketoacyl synthase, acyl transferase, acyl carrier protein, ketoacyl reductase, dehydratase, methyl transferase, and enoyl reductase). The cluster also encodes an aminotransferase (*FUM8*), a C-3 carbonyl reductase (*FUM13*), and cytochromes P450 and other enzymes that catalyze oxygenations at C-5 (*FUM3*), C-10 (*FUM2*) and at an undetermined site (*FUM6*) (Proctor et al., 2006). Four genes (*FUM7, FUM10, FUM11, FUM14*) are required for tricarballylic acid esterification. At the opposite end of the cluster from *FUM1* are genes encoding a transporter protein (*FUM19*) and for two proteins (*FUM17* and *FUM18*) with predicted functions in fumonisin self-protection and sphingolipid metabolism. However, disruption of these genes had little or no effect on fumonisin production, indicating that their functions may be redundant or not required for fumonisin biosynthesis.

4. Zearalenones

Zearalenones are not acutely toxic and have not been associated with any fatal mycotoxicoses in humans or animals. Zearalenones are non-steroidal estrogenic mycotoxins and have been associated with estrogenic syndromes in swine and