Available online at www.sciencedirect.com
ScienceDirect
International Journal of Food Microbiology 119 (2007) 47–50
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Molecular biology of *Fusarium* mycotoxins ☆

A.E. Desjardins *, R.H. Proctor

National Center for Agricultural Utilization Research, United States Department of Agriculture, Peoria, Illinois, 61604, USA

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.
Published by Elsevier B.V.

Keywords: *Fusarium*; Gene cluster; Mycotoxin

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

☆ Disclaimer: Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

* Corresponding author. Tel.: +1 309 6816378; fax: +1 309 681 6665.
E-mail address: anne.desjardins@ars.usda.gov (A.E. Desjardins).

0168-1605/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2007.07.024

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

experimental animals. Zearalenones belong to a very large family of fungal metabolites derived by different cyclizations and modifications of nonaketide precursors. Zearalenones are derived by cyclization to form a resorcyclic acid lactone, and have a close structural relationship to antibiotic metabolites produced by a number of fungi.

A 25-kb zearalenone biosynthetic gene cluster has been located on contigs 1.118–1.120 of the *F. graminearum* genome (Kim et al., 2005; Gaffoor and Trail, 2006). Gene-disruption studies have determined that four tightly linked genes are required for zearalenone biosynthesis. The *PKS4* (*ZEA2*) gene encodes an iterative Type I, reducing, polyketide synthase with six functional domains (ketoacyl synthase, acyl transferase, dehydratase, enoyl reductase, keto reductase, and acyl carrier protein). The *PKS13* (*ZEA1*) gene also encodes an iterative Type I, non-reducing, polyketide synthase, but has only four functional domains (ketosynthase, acyl transferase, acyl carrier protein, an thiolesterase) and lacks the domains responsible for reduction of the carbonyl moieties of the polyketide. Although both *PKS4* and *PKS13* polyketide synthases are required for zearalenone biosynthesis, their specific roles are unknown. The cluster also contains a gene (*ZEB1*) that controls oxidization of zearalenol to zearalenone and a gene (*ZEB2*) that regulates transcription of the other three genes.

5. Beauvercin and enniatins

Beauvericin and enniatins are non-ribosomal, cyclic hexadepsipeptides with general cation chelating, ionophore, and antibiotic activities. Studies of the biological effects of enniatins on animal and plant systems have been very limited, but indicate only low to moderate toxicity. Although they can accumulate in *Fusarium*-infected grain, beauvericin and enniatins have not been associated with any animal disease outbreaks nor been shown to cause disease in experimental animals. Beauvericin and enniatins are members of a family of fungal *N*-methylated cyclic hexadepsipeptides. Enniatins consist of three residues of D-2-hydroxyisovaleric acid alternating with three of *N*-methyl branched-chain L-amino acids such as valine, leucine, or isoleucine. Enniatin A contains only *N*-methyl isoleucine residues, whereas other enniatin homologues contain other patterns of branched-chain amino acids, and beauvericin contains only phenylalanine.

In *Fusarium*, enniatins are synthesized by enniatin synthetase, which is a member of a class of multifunctional enzymes known as non-ribosomal peptide synthetases. The enniatin synthetase gene (*ESYN1*) of *F. scirpi* comprises two modules, one for an L-amino acid and the other for hydroxyisovaleric acid. Both modules contain condensation, adenylation, and thiolation domains. In addition, the amino acid module includes a second thiolation domain and a methyl transferase domain. After *N*-methylation, the amino acid and hydroxyisovaleric acid form a dipeptol. The depsipeptide chain is then formed by three successive condensations of dipeptidol units in an interative manner by one molecule of enniatin synthase.

6. Butenolide

Butenolide is a 4-acetamido-4-hydroxy-2-butenoic acid lactone that is produced by *F. graminearum* and a number of other trichothecene-producing *Fusarium* species. Butenolide was reported as moderately toxic to mice, but also has been associated with a noninfectious condition of cattle called fescue foot, which is characterized by edema, lameness and gangrenous loss of extremities.

A putative butenolide gene cluster has been identified on *F. graminearum* contig 1.324. Gene-disruption and complementation experiments determined that a gene (FG08079) in the cluster encodes a cytochrome P450 monooxygenase that is required for butenolide production (Harris et al., 2007). Gene FG08079 is clustered and co-regulated with seven other genes that encode predicted proteins with similarities to the types of enzymes involved in secondary metabolite biosynthesis, but their roles in butenolide biosynthesis remain to be established.

7. Equisetin

Equisetin is a derivative of *N*-methyl-2,4-pyrollidone (1-methyl-3-acyl-5-hydroxymethyl-2,4-dione) and is of particular interest due to its activity against the human immunodeficiency virus. Equisetin was reported as a metabolite of *F. equiseti* and *F. semitectum*, with weak activity against Gram-positive bacteria and moderate toxicity to mice, but also has been isolated from marine fungi.

A hybrid polyketide synthase-non-ribosomal peptide synthetase (*EQIS*) that is required for equisetin biosynthesis has been isolated from *F. heterosporum* (Sims et al., 2005). Equisetin synthase is a hybrid of an iterative Type 1, reducing, polyketide synthase with six functional domains (ketoacyl synthase, acyl transferase, dehydratase, methyl transferase, keto reductase, and acyl carrier protein) that is fused to a complete non-ribosomal synthetase with four functional domains (condensation, adenylation, thiolation, and reduction). Disruption of the equisetin synthase gene caused complete reduction of equisetin production. Adjacent to *EQIS* are a number of open reading frames that encode predicted proteins with similarities to the types of enzymes involved in secondary metabolite biosynthesis, but their roles in equisetin biosynthesis remain to be established.

8. Fusarins

Fusarin C is mutagenic in the Ames test, which measures reverse mutations in *Salmonella typhimurium*, but only after pretreatment with a liver homogenate prepared from phenobarbital-induced rats. Based on dose–response curves for mutagenicity under these experimental conditions, fusarin C was one-half as potent as aflatoxin. Fusarin C, however, was not active in cancer initiation/promotion assays in rat liver, possibly due to inactivation by glutathione. Consumption of grain infected with fusarin-producing *Fusarium* species such as *F. verticillioides* and *F. graminearum* has been associated epidemiologically with human diseases. However, a specific role for fusarins in

human or animal disease outbreaks has not been established. Fusarins are 2-pyrrolidones with a methylated, polyunsaturated side chain, but differ in the structure and substitution of the 2-pyrrolidone moiety. In fusarin C, the pyrrolidone contains a C-13,14 epoxide and an ethanolic side chain. In other fusarin homologues, the epoxide is shifted or opened, and the ethanolic side chain forms a ring structure. Fusarins A and D, which lack the C-13,14 epoxide group, were not mutagenic in the Ames test.

Precursor incorporation experiments supported the biosynthesis of fusarins *via* condensation of a fourteen-carbon polyketide and a four-carbon intermediate of the tricarboxylic acid cycle. A hybrid polyketide synthase-non-ribosomal peptide synthetase gene (*FUSA/FUS1*) that is required for biosynthesis of fusarin C has been isolated from *F. verticillioides, F. venenatum,* and *F. graminearum* (Gaffoor et al., 2005). Fusarin polyketide synthase is an iterative Type I, reducing, polyketide synthase with seven functional domains (ketosynthase, acyltransferase, dehydratase, methyl transferase, enoylreductase, ketoacylreductase, and acyl carrier protein), plus a non-ribosomal synthetase with four functional domains (condensation, adenylation, thiolation, and thiolester reductase). Disruption of the fusarin polyketide synthase gene caused complete reduction of fusarin production in *F. graminearum* and in *F. verticillioides* (R. Proctor and R. Plattner, unpublished results) and partial or complete reduction of fusarin production in *F. venenatum* (Song et al., 2004). Adjacent to *FUSA* in *F. graminearum* and *F. verticillioides* are a number of open reading frames that are predicted to encode proteins with similarities to the types of enzymes involved in secondary metabolite biosynthesis, but their roles in fusarin biosynthesis remain to be established.

9. Conclusions

Fusarium species produce an extraordinary diversity of biologically active secondary metabolites, some of which are harmful to animals and, thus, mycotoxins. If mycotoxins are classified based on their biogenetic origins, then polyketides are the largest class of mycotoxins produced by *Fusarium* species, as well as the class whose molecular biology is most advanced. Of the seven mycotoxin biosynthetic genes or gene clusters identified in *Fusarium* to date, four are polyketide synthase gene clusters for equisetin, fumonisins, fusarins, and zearalenones. This dominance of polyketides may reflect their intrinsic biological activity or may simply reflect the relative ease of discovering polyketides due to their structural complexity and polyketide synthase genes due to their several distinctive and highly conserved domains. Other classes of *Fusarium* mycotoxin biosynthetic genes discovered to date include a terpene cyclase gene cluster for trichothecenes and a cyclic peptide synthetase for enniatins. The biogenesis of butenolide remains unknown since only one biosynthetic gene, a cytochrome P450, has been confirmed to date. Other *Fusarium* mycotoxins awaiting gene discovery include the putative polyketide mycotoxins fusaric acid, moniliformin, and sambutoxin, the terpenoids culmorin and fusaproliferin, several cyclic peptides such as acuminatum, the substituted chromanone fusarochromanone, and the furanocyclohexadiene lactone wortmannin.

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. For example, mycotoxin gene knock-out mutants have been used to demonstrate the importance of enniatins, fumonisins, and trichothecenes in plant pathogenesis. Trichothecene biosynthetic genes have been exploited in marker-based mapping and genetic engineering of wheat for resistance to *Fusarium* head blight. New information on fungal and plant genomes and gene expression will continue to provide information on genes important for fungal-plant interactions and facilitate the development of targeted approaches for breeding and engineering crops for resistance to *Fusarium* infection and mycotoxin contamination.

References

Desjardins, A.E., 2006. *Fusarium* Mycotoxins. Chemistry, Genetics, and Biology, American Phytopathological Society Press, St. Paul, MN.

Gaffoor, I., Trail, F., 2006. Characterization of two polyketide synthase genes involved in zearalenone biosynthesis in *Gibberella zeae*. Applied Environmental Microbiology 72, 1793–1799.

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

Harris, L.J., Alexander, N.J., Saparno, A., Blackwell, B., McCormick, S.P., Desjardins, A.E., Robert, L.S., Tinker, N., Hattori, J., Piche, C., Schernthaner, J., Watson, R., Ouellet, T., 2007. A novel gene cluster in *Fusarium graminearum* contains a gene that contributes to butenolide biosynthesis. Fungal Genetics and Biology 44, 293–306.

Kim, Y.-T., Lee, Y.-R., Jin, J., Han, K.-H., Kim, H., Kim, J.-C., Lee, T., Yun, S.-H., Lee, Y.-W., 2005. Two different polyketide synthase genes are required for synthesis of zearalenone in *Gibberella zeae*. Molecular Microbiology 58, 1102–1113.

Proctor, R.H., Plattner, R.D., Desjardins, A.E., Busman, M., Butchko, R.A.E., 2006. Fumonisin production in the maize pathogen *Fusarium verticillioides*: genetic basis of naturally occurring chemical variation. Journal of Food and Agricultural Chemistry 54, 2424–2430.

Sims, J.W., Fillmore, J.P., Warner, D.D., Schmidt, E.W., 2005. Equisetin biosynthesis in *Fusarium heterosporum*. Chemical Communications 186–188.

Song, Z., Cox, R.J., Lazarus, C.M., Simpson, T.J., 2004. Fusarin C biosynthesis in *Fusarium moniliforme* and *Fusarium venenatum*. Chembiochem 5, 1196–1203.