Fusarium Secondary Metabolism Biosynthetic Pathways: So Close but So Far Away

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Abstract

Fusarium species are casual filamentous fungi, including opportunistic pathogens infecting plants worldwide, but also able to grow as saprotrophs in a range of climatic zones. The genus is extremely variable in terms of genetics, biology, ecology, and, consequently, secondary metabolism, which directly relates to ecological conditions and niches occupied by individual species. Fungal secondary metabolites are the main "weapon" of the pathogenic species before, during, and after the infection process, allowing for the communication with the organism that is being attacked. Many of secondary metabolites are common for diverse fungal microorganisms, and their mode of action is similar for various plant-pathogen systems. *Fusaria* are able to produce a range of quite specific metabolites, some of which have yet unknown biological functions.

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Nevertheless, genetic and biochemical pathways responsible for their biosynthesis remain under strong selection pressure, which keeps their structures and functions relatively stable, regardless of the producing organism. Here, we summarize the data available in recent literature reports on genetic and biochemical diversity occurring in the studies of main secondary metabolites produced by *Fusarium* species differing in origin and ecology.

Keywords
Fumonisins · Fungal ecology · Metabolic pathways · Mycotoxins · Phylogeny · Trichothecenes · Zearalenone

List of Abbreviations

AcDON	Acetylated DON derivatives
BEA	Beauvericin
bik	Bikaverin biosynthetic gene cluster
c*ar*	Carotenoid biosynthetic gene cluster
DAS	Diacetoxyscirpenol
DMATS	Dimethylallyltryptophan synthase
DON	Deoxynivalenol
ENN	Enniatin
eqx	Equisetin biosynthetic gene cluster
FA	Fusaric acid
FB	Fumonisin B
FESC	*F. equiseti* species complex
FFSC	*F. fujikuroi* species complex
FGSC	*F. graminearum* species complex
FOSC	*F. oxysporum* species complex
FPP	Farnesyl pyrophosphate
Fsr	Fusarubin biosynthetic gene cluster
FSSC	*F. solani* species complex
FUB	Fusaric acid biosynthetic gene cluster
FUM	Fumonisin biosynthetic gene cluster
FUS	Fusarin C biosynthetic gene cluster
GA	Gibberellins
GGPP	Geranylgeranyl pyrophosphate
MAPK	Mitogen-activated protein kinase
MON	Moniliformin
NIV	Nivalenol
NRPS	Nonribosomal peptide synthetase
PKS	Polyketide synthase
PM	Primary metabolism
SM	Secondary metabolite
TC	Terpene cyclase
TF	Transcription factor
TRI	Trichothecene biosynthetic gene cluster
ZEA	Zearalenone

1 Introduction

Secondary metabolites (SMs) are universal messengers between plants and pathogens, of which the most widespread are filamentous fungi. SMs are responsible for pathogen recognition by the plant host and for pathogen actions during host infection. They belong to multiple classes concerning their chemical structures and influence diverse biochemical processes exhibiting signaling, toxic, eliciting, priming, growth-promoting, or defense response-inducing actions [1]. The ability to produce the SMs is often governed by the presence and activity of the specific gene clusters present in fungal genomes, which usually contain several enzyme-encoding genes devoted exclusively to biosynthesize specific group of compounds [2, 3]. The distribution of these gene clusters among fungal taxa generally resembles their phylogenetic relationships but sometimes may serve as the evidence of the past horizontal gene transfer events, since the same biosynthetic pathways may be found in species that not share close relationship [4, 5]. Moreover, it seems that SM biosynthetic gene clusters not only undergo common regulation and expression patterns but also share evolutionary fate, which often depends strongly on the genomic context and differs from main primary metabolism (PM) regions. Many SMs are universal for diverse fungal microorganisms, and their actions are similar for various plant-pathogen systems; nevertheless, significant level of specificity may be observed in comparative metabolomic analyses of pathogenic fungi.

Fusarium genus consists of a large number of diverse species of different lifestyles. Many of them are opportunistic pathogens infecting multiple plant species in a range of climatic zones (e.g., *F. graminearum* species complex (FGSC), *F. fujikuroi* species complex (FFSC), *F. equiseti* species complex (FESC), *F. avenaceum*, and *F. culmorum*), and some are more typical soil-borne pathogens and are more likely isolated from the rhizosphere of plants (mainly *F. oxysporum* species complex (FOSC) and *F. solani* species complex (FSSC)).

Fusaria are extremely variable in terms of genetics, biology, and ecology; thus, they produce also very diverse repertoire of SMs. This divergence relates partially to the ecological niches occupied by individual species but also seems to play some, yet unknown role in the organism ecological flexibility. On the other hand, closely related species may vary in biosynthetic potentials. Fumonisins may serve as an example. *F. verticillioides* and *F. proliferatum* are the main producers of fumonisins, both capable of infecting maize as the typical host. Yet, the sequence divergence of the *FUM* biosynthetic cluster responsible for fumonisin biosynthetic ability reaches 20% when those two species are compared [2, 6–8]; in *F. oxysporum*, *FUM* cluster has been found and characterized for just one strain O-1890 [9], and another maize pathogens from the FFSC – *F. subglutinans* and *F. temperatum* – are essentially fumonisin nonproducers [10, 11].

Genes inside the clusters responsible for the SMs' biosynthesis remain under strong selection pressure, exerted by ecological factors (environment, competitive organisms, host availability, and resistance) which keep the structures and functions of encoded enzymes relatively stable. Still, even intraspecific polymorphism can be observed for some of the pathways, like *FUM* cluster divergence in populations of *F. proliferatum* [12, 13]. Similar examples of discrepancies in phylogenetic

relationships between closely related taxa and their SM biosynthetic abilities are frequent in *Fusarium* genus and are presented and discussed in this chapter. Main *Fusarium*-produced mycotoxin pathways were reviewed in terms of genetic divergence and biochemical and chemotypic population shifts. We also summarized the data on genetic and biochemical diversity occurring in the studies of main secondary metabolites produced by *Fusaria* differing in origin and ecology.

2 *Fusarium*: Clades and Species

First description of *Fusarium* was reported in 1809 by Link and since than more than a thousand species have been identified, of which 70 is well-known. The first taxonomic classifications have been created based on morphological characters of species and test crosses [14]. Later, thanks to the genetic and bioinformatic tools, species became classified using phylogenetic analyses. Aoki et al. in 2014 divided *Fusarium* species into four complexes based on RNA polymerase II subunit gene sequences (*Fusarium fujikuroi* species complex (FFSC), *Fusarium graminearum* species complex (FGSC), *Fusarium oxysporum* species complex (FOSC), and *Fusarium solani* species complex (FSSC)), but some well-known species were not assigned to any of these [15]. In 2011, Watanabe et al. used maximum likelihood method for reconstruction of the phylogenetic relationships using the following genetic markers: rDNA cluster region, *β*-tubulin (*β-tub*), translation elongation factor 1*α* (*EF*-1α), and aminoadipate reductase (*lys2*). Based on the resulting phylogenetic tree, they proposed a new classification divided into seven clades (Table 1) [16].

Obviously, this classification contained some flaws, related to the limited number of strains used, but mainly followed earlier dividing *Fusarium* into "sections" which is no longer used. More detailed studies allowed to discriminate closely related species inside the clades, and currently, many reports describing new species or chemotypes are becoming available, particularly concerning trichothecene producers from the FGSC and fumonisin producers from the FFSC.

3 Ecological Niches: From Saprotrophs to Human Pathogens

As a worldwide occurring genus, *Fusaria* are adapted to survive and spread in a wide spectrum of environmental conditions. The genus is known at best as a plant pathogen that causes yearly huge economic losses in yields of almost all crops cultivated all over the world. Spores of *Fusarium* infect plants and then develop hyphae within plant organs (e.g., leaves, stems, seeds, flowers, roots) which cause changes in host cells' metabolism, tissue destruction, and, eventually, the development of numerous diseases.

Some *Fusarium* species complexes are still classified as specialized groups within the species, so-called *formae specialis* (f.sp.) based on specific host that they are able to infect. *F. oxysporum* is the species with the largest number of *formae*

Table 1 *Fusarium* clade classification based on the multilocus sequence analysis [according to Ref. 16]

Clade	*Fusarium* species
Clade I	*F. larvarum*
	F. merismoides
Clade II	*F. dimerium*
Clade III	*F. solani*
Clade IV	*F. decemcellulare*
Clade V	*F. oxysporum*
	F. proliferatum
	F. subglutinans
	F. verticillioides
Clade VI	*F. avenaceum*
	F. lateritium
	F. tricinctum
Clade VII	*F. acuminatum*
	F. culmorum
	F. graminearum
	F. kyushuense
	F. langsethiae
	F. poae
	F. sporotrichioides

specialis. For instance, *F. oxysporum* f.sp. *lycopersici* causes wilt in tomato, while *F. oxysporum* f.sp. *cubense* causes Panama disease on banana. Other specific examples of *Fusarium* plant diseases (also called fusiariosis) are ear rot of maize (*F. verticillioides*), *Fusarium* head blight of wheat and barley (*F. avenaceum*, *F. culmorum*, *F. graminearum*), and root rot of soybean (*F. solani*) [17].

Species belonging to *Fusarium* genus are generally saprotrophic, and necrotrophs, being potentially pathogenic, are not obligatory pathogens, like biotrophic *Puccinia* spp. causing rusts in small grain cereals (mainly wheat, barley, and triticale). After the harvest, fragments of infected tissues get into the soil, where the fungi develop feeding on decayed organic matter and can survive unfavorable environmental conditions. Some of the species can form fruiting bodies which can remain viable for a very long time and then germinate at the appearance of appropriate conditions to infect plant roots. This process is a part of the vegetative development of some *Fusaria*, for example, *F. culmorum*, *F. oxysporum*, and *F. graminearum* [18–21].

Animal and human fusariosis are not as common as plant fusariosis. Human fusariosis usually occur in people with tissue breakdown or patients with impaired immune system. The symptoms of these diseases are usually keratitis and onychomycosis which are caused by *F. verticillioides*, *F. oxysporum*, and *F. solani* [22]. Mycotoxicoses occur more often than fusariosis and are the effects of ingestion of toxic fungal secondary metabolites. Exposure to mycotoxins occurs

mainly through the consumption of contaminated food, but inhalation with the air is also possible. Many of these compounds do not degrade during technological processes even under high temperature and pressure conditions. Mycotoxins are biosynthesized by fungi and secreted into host tissues where they are accumulated and transferred into food and feedstuffs. Exposure to mycotoxins may also occur through the consumption of contaminated animal products such as meat, milk, or eggs because some compounds pass from plant-derived materials to animal tissues and may be excreted with milk (e.g., aflatoxins). Numerous reports on diseases caused by *Fusarium* mycotoxins are available. Fumonisins B_1 and B_2 causing equine encephalomalacia, deoxynivalenol causing vomiting as well as diarrhea, and zearalenone causing breast cancer are among the most frequent ones [1, 23, 24].

4 Phylogeny and New Species Discovery

Exact taxonomic positioning of the *Fusarium* genotype studied is one of the most basic questions faced by researchers interested in *Fusarium* research, because mistake at this stage may have serious consequences. During last decades, three kinds of species concepts were proposed to identify *Fusarium* species:

- Morphological species concept (defined by the morphological characters of pure fungal cultures on standardized laboratory media)
- Biological species concept (using sexual crosses, aggressiveness tests)
- Phylogenetical species concept (molecular analyses defining similarities between related strains)

Sometimes, the combination of two or three of these species' concepts can be found [25–27]. Nevertheless, the use of morphological species concept for identifying species requires a skilled and experienced researcher with wide knowledge of classical taxonomy, which is nowadays more and more difficult to find. Additional problem bears in overlapping morphological characters among closely related species and new species described practically each year.

The biological characteristics based on sexual compatibility show numerous problems, such as environmental factors suppressing sexual reproduction, unequal frequencies of mating-type alleles in different populations, or failure of compatible isolates to reproduce due to male or female dominance. Moreover, the environmental conditions and genetics of the host may play significant roles in aggressiveness tests on specific host plant [28]. According to Moretti (2009), it is a great challenge to determine the taxonomic status of *Fusarium* species on the basic of their phenotypic characteristics alone, including pathogenicity and toxigenicity [29].

After the year 2000, most scientists have utilized the molecular phylogenetic approaches to ascertain the taxonomy of *Fusarium* species and have proposed new taxonomic systems based on the phylogenetic species concept [3, 7, 10, 12, 30–35]. This was a requirement that arose from the fact that several of the "traditional"

species based on morphological identification are now considered to be species complexes composed of many species [33, 36, 37].

Phylogenetic analyses of *Fusarium* isolates are being performed based on numerous diagnostic marker sequences. Among them the most common are calmodulin (*cmd*) [38], histone 3 (*HIS3*), *Tri101* [39], mating-type (*MAT*) locus [36], internally transcribed spacer regions in the ribosomal repeat region (ITS1 and ITS2) [40, 41], the intergenic spacer region (IGS) [42], the nuclear ribosomal RNA large subunit (28S or LSU rDNA), and the mitochondrial small subunit (mtSSU rDNA) [27]. Protein-coding genes are also in use, such as RNA polymerase (*RPB2*), β-tubulin (*tub2*) [43], translation elongation factor (*EF*-1α) [43–45], and ATP citrate lyase (*ACL1*) [46]. Notably, not all of these sequences work equally well showing significant polymorphism for all *Fusarium* species. For instance, the ITS regions have shown its limited usefulness within many *Fusarium* species, such as *F. avenaceum*, *F. arthrosporioides*/*F. tricinctum*, *F. sporotrichioides*/*F. langsethiae*, and the lineages of *F. graminearum* species complex, due to the occurrence of non-orthologous copies [25, 38]. Correspondingly, *β*-tubulin gene is not discriminative for genotypes from the *Fusarium solani* species complex [47]. Nevertheless, *EF*-1α, *RPB1*, and/or *RPB2* gene fragments have gained the most of the researcher's interest for the following reasons: (i) highly informative at the species level, (ii) non-orthologous copies, (iii) amplified from all species of the genus using single pairs of universal primers, and (iv) sequences from these three genes are well represented in the reference database (i.e., FUSARIUM-ID, Fusarium MLST, and NCBI GenBank) [48–50].

Phylogenetical characterization based on genealogical concordance (GCPSR), a robust method for determined species boundaries [31], has shown the severe limitations of morphological and biological species identification in *Fusarium* and accelerated species discovery inside the genus. To date, approximately two-thirds of the 300 phylogenetically distinct species-level *Fusaria* were discovered using GCPSR-based studies [51]. Moreover, continuous research investments have provided tremendous insight into evolutionary relationships within the *Fusarium* genus inferred from partial *RPB1* and *RPB2* sequences. The study determined 20 monophyletic species complexes and 9 monotypic lineages, which were named informally to facilitate the communication of an isolate's clade membership and genetic diversity [24, 52]. Based on newly discovered species, two of these monotypic lineages are currently considered as species complexes [24, 53, 54].

5 Secondary Metabolism Biosynthetic Pathways

Recently, it appeared that genomic regions involved in secondary metabolism present similarly useful or sometimes better targets for designing phylogenetic markers and their analysis [3]. The weak side of such approach is that only some of *Fusarium* species may possess the gene cluster of interest but the resolution of the genotypes obtained with SM biosynthetic sequences may be higher than that

of PM ones. Therefore, each of the clusters should be carefully and individually checked for its usefulness in the species studied.

5.1 Mycotoxins

Mycotoxins are SMs produced by vast majority of filamentous fungi, mostly under favorable environmental conditions. *Fusarium* species have the genetic potential to produce hundreds of structurally diverse SMs, most of which have poorly understood or completely unknown ecological functions [24, 55–57]. These substances are usually produced in complex biochemical processes, including polyketide, terpenoid, and amino acid metabolic pathways, and can be accumulated in crop plants. Thereby, they pose a health risk to human and livestock [4, 58–60]. Many known mycotoxins are the virulence factors related to plant disease development [61], or they might play a role in improvement of the survival of the spores and, consequently, influence the development of the producing organism by enhancing the fitness of a given community/species [59].

Throughout the past two decades, numerous studies have been made to better understand the molecular mechanisms of mycotoxin biosynthesis and the direct and indirect regulatory agents and patterns controlling these processes. Mycotoxin biosynthetic pathways involve several coordinately regulated and functionally related genes physically grouped into clusters that can be co-expressed under specified conditions. Generally, these genes can be identified through the presence of four classes of enzymes: terpene cyclases (TCs), dimethylallyltryptophan synthases (DMATSs), polyketide synthases (PKSs), and nonribosomal peptide synthetases (NRPSs) [55, 62, 63], which catalyze the condensation or rearrangement of simple molecules to form more complex structures. Typically, the clusters contain also the core genes responsible for structural modifications of the initial metabolite, transporters for metabolite transport, and transcription factors for coordinated transcriptional regulation of genes in the cluster. These chemical products undergo multiple enzymatic modifications to form biologically active SMs and are transported to their site of activity [64, 65].

The release of the full genomic sequences of *F. fujikuroi* [62] and closely related *Fusarium* species, such as *F. verticillioides* [66], *F. mangiferae*, and *F. proliferatum* [63], revealed that the species have the genetic capacity of producing even more SMs than previously thought. Before the publication of the first *Fusarium* genomic sequence, the members of the entire genus were believed to produce about 40 structurally distinct families of SMs, while some groups, for instance, fumonisins and trichothecenes, contain tens of different analogs [67, 68]. Regardless of this metabolic diversity within the genus, single species and isolates were reported to produce a relatively low number of metabolites, e.g., there is some evidence showing that *F. graminearum* produced eight secondary metabolite families, such as aurofusarin, butenolide, fusarins, trichothecenes, culmorin, cyclonerodiol, chlamydosporol, and zearalenones. However, the study of the *F. graminearum* genome sequence identified 16 PKSs, 19 NRPSs, and 8 TSs, which suggests that

a single species has the genetic potential to produce about the equal number of the SMs to that earlier claimed for the entire genus [65].

The in silico analyses of genomic sequences of a wide range of *Fusarium* species revealed surprisingly high level of differences in the distribution of secondary metabolite biosynthetic genes and, therefore, differences in the genetic potential of individual species to produce SMs [52, 66, 69]. Namely, the PKS gene *PGL1*, which is necessary for the production of a blackish perithecial pigment and a family of reddish mycelial pigments (fusarubins), was occurring in all *Fusaria* examined in multiple studies [52, 64, 70, 71]. Moreover, there are some reports indicating that the SM's biosynthetic gene clusters are well-conserved among organisms. From the evolutionary point of view, their maintenance could only be beneficial for the fungus if the final product would confer any advance to the producing organism, even if the effect of their action is subtle or not directly obvious [59, 60]. This statement applies for mycotoxins, like the narrowly distributed fumonisin and gibberellin gene clusters that are exhibited in only some species of the *F. fujikuroi* and *F. oxysporum* species complexes [72, 73]. Additionally, the fusarin biosynthetic genes, which are extensively spread in *Fusarium*, are occurring in all *F. oxysporum* isolates that have been analyzed [52].

5.1.1 Trichothecenes

Trichothecenes are the major group of mycotoxins produced by various *Fusarium* plant pathogens [61, 64, 74]. Due to their toxicity and economic significance, trichothecenes are among the best characterized mycotoxins. Structurally, they are sesquiterpenoid compounds with a tricyclic 12,13-epoxytrichothec-9-ene ring that can be chemically substituted at several positions, which result in multiple derivatives [75, 76].

There are over 200 trichothecene derivatives which can be grouped into four main groups: types A, B, C, and D. Type A trichothecenes characterized by hydroxyl, or ester substitution at C-8, contain diacetoxyscirpenol (DAS), T-2 toxin, HT-2 toxin, and neosolaniol, and T-2 toxin is the most toxic trichothecene in animals. Recently, a new chemotype has been discovered among type A trichothecenes and designed NX-2. Surprisingly, it can be produced by *F. graminearum*, which is a typical type B trichothecene producer [77, 78]. The most important producers of type A trichothecenes are *F. sporotrichioides*, *F. langsethiae*, *F. poae*, *F. sambucinum*, *F. armeniacum*, and *F. venenatum*. They may develop on variety of cereal grains especially in cold climate regions or during storage conditions [76, 79]. Type B trichothecenes contain a C-8 keto group and are produced by various *Fusarium* species, particularly from the *Fusarium graminearum* species complex: *F. graminearum* sensu lato, *F. culmorum*, *F. pseudograminearum*, and *F. cerealis*. The most common type B trichothecenes are deoxynivalenol (DON), nivalenol (NIV), and the DON-acetylated derivatives AcDONs. Type C trichothecenes are a minor group of toxins produces by several other genera of fungi, and type D includes compounds produced by *Stachybotrys* species that are considered as important indoor mold hazards [17, 74, 80].

Alongside this major metabolite, type B trichothecenes are among the most toxic mycotoxin compounds and best-studied virulence factors. The mechanism of action of this mycotoxin is based on the inhibition of protein synthesis in eukaryotes. Trichothecenes interact with peptidyl transferase enzyme binding the 60S ribosomal subunit, thus causing the inhibition of translation. Alternative mechanism of action involves the activation of numerous mitogen-activated protein kinases (MAPKs) [60]. Humans and animals that have consumed trichothecene mycotoxins present various symptoms, such as vomiting, dizziness, diarrhea, and spontaneous abortion [81]. Moreover, the potential of trichothecenes to act as virulence factors in plant-fungal interactions and elicit plant defense responses has been investigated [82]. While trichothecene production is not required for *Fusarium* to develop on the host and penetrate its tissues, they still are essential compounds for the exposure of the pathogen after initial colonization [60, 83].

The trichothecene biosynthetic (*TRI*) gene cluster is responsible for trichothecene biosynthesis and was first characterized in *F. graminearum* and *F. sporotrichioides* [84–86]. Trichothecene biosynthetic enzymes and direct regulatory proteins are encoded by 15 genes which are located at three different loci on different chromosomes: a 12-gene core *TRI* cluster [80, 87]; the two-gene locus, *TRI1* which encodes a cytochrome P450 monooxygenase and *TRI16* which encodes an acyl transferase; and a single acyl transferase gene *TRI101* locus that is responsible for esterification of acetate to the hydroxyl function at carbon atom 3 (C-3) of trichothecenes [88]. In *F. sporotrichioides*, the TRI1 enzyme catalyzes the hydroxylation of trichothecenes at C-8, and the TRI16 enzyme catalyzes esterification of a five-carbon carboxylic acid, isovalerate, to the C-8 oxygen [89, 90]. Analysis of the *TRI* loci in 16 species of *Fusarium* exposed that *TRI1* and *TRI101* are in the core *TRI* cluster in four species of *Fusarium* that are members of the *F. incarnatum-equiseti* species complex [91, 92]. It was shown that *TRI16* and *TRI10* are major transcriptional regulators of *TRI* expression [93].

The trichothecenes have a skeleton resulting from the farnesyl pyrophosphate (FPP) [94, 95]. The first step in the biosynthesis pathway is the conversion of FPP to trichodiene. This reaction is governed by *TRI5*-encoded trichodiene synthase [96]. Subsequently nine reactions follow, catalyzed by the enzymes encoded by *TRI4*, *TRI101*, *TRI11*, and *TRI3*, correspondingly, and leading to the formation of calonectrin [80]. All these steps are common for type A trichothecenes (T-2 toxin) and type B trichothecenes (NIV and DON) producing *Fusaria* [76, 80].

A comparative study showed that similar genes are functioning in *F. graminearum* and *F. sporotrichioides* [85]. For instance, *TRI7* and *TRI13* are functional only in *F. sporotrichioides* and in *F. cerealis* as well as in the strains of *F. graminearum* and *F. culmorum* producing NIV [86]. In *F. graminearum* DON producers, *FgTri7* and *FgTri13* are not functioning [86]; therefore, the biosynthesis continues directly from calonectrin with the products of *FgTri1* and *FgTri8* and leads to the formation of either 3AcDON or 15AcDON followed by DON [97]. In contrast, in NIV producers, the pathway proceeds with the product of *FgTri1* to generate 4AcNIV and the last step with *FgTri8* product giving NIV [76]. Alexander

et al. (2011) demonstrated that polymorphisms of *TRI8* resulted in the chemotype of AcDON [98]. Moreover, in *F. sporotrichioides*, which is a T-2 toxin producer, the biosynthesis pathway proceeds with the products *FsTri1*, *FsTri16*, and *FsTri8* [89, 97]. In most *F. graminearum* strains, *TRI1* is responsible for trichothecene oxygenation at both C-7 and C-8, which leads to the formation of variants like DON or NIV [99]. Nevertheless, in some *F. graminearum* strains, *TRI1* adds a hydroxyl group at C-7 only, leading to the formation of the T-2 toxin [77].

5.1.2 Fumonisins

Fumonisins are a group of mycotoxins primarily produced by *F. verticillioides*, *F. fujikuroi*, and *F. proliferatum*, worldwide pathogens of rice and maize but also found on a wide range of other agro-food crops [4, 12, 60]. Other species from the *F. fujikuroi* species complex also produce fumonisins, but they are of minor importance. Interestingly, there are also species that are fumonisin nonproducers, and for some the status is ambiguous, as at least some strains were found to produce fumonisins for *F. oxysporum*, *F. temperatum*, or *F. subglutinans* [4, 7, 8]. The synthesis of FBs in association with disease symptoms differs markedly depending on the host conditions and infected tissue type [100]. It has been shown that fumonisins produced by *F. verticillioides* have a slight impact on maize ear rot development and significant effect on maize seedling blight. The successful transformation of the fumonisin-producing genes into an endophytic, fumonisin-nonproducing *F. verticillioides* strain has converted this endophyte into a pathogen that causes seedling blight disease in maize [101, 102], strongly supporting the hypothesis that fumonisin is a pathogenicity factor during maize seedling infection [102].

At least 28 different analogs of fumonisins were described and divided into four main categories: A, B, C, and P series [103, 104]. The most important group are the B fumonisins, B_1, B_2, B_3, and B_4, mainly due to their toxicity to humans and animals. Fumonisin B_1 is also, apart from *Aspergilli*-produced aflatoxins, the most abundant and important contaminant of maize and maize-derived products. The structures of FBs were first described in 1988 and 1989 by the researchers in South Africa, New Caledonia, and France [105, 106]. The B series fumonisins have a 20-carbon polyketide backbone with terminal amine residue, several hydroxyl groups, and two propane-1,2,3-tricarboxylate esters at various positions. The A and P series fumonisins differ due to alteration or replacement of the terminal amine group, while the C series fumonisins have a 19-carbon backbone [1, 2, 17]. Fumonisins that are characterized by an unsubstituted primary amino group at the C-2, and structurally close to sphingolipids, actually can disturb the sphingolipid metabolism by inhibiting the enzyme ceramide synthase and consequently lead to the degeneration of the sphingolipid-rich tissues and disruption of cell membrane integrity [107].

Fumonisin biosynthetic (*FUM*) gene cluster has been first described in *F. verticillioides* belonging to the *Fusarium fujikuroi* species complex (FFSC), containing 17 genes encoding biosynthetic enzymes, a transcription factor, and an ABC transporter [4, 80, 103, 108]. The *FUM1* gene encodes a polyketide

synthase that catalyzes the synthesis of a linear polyketide that forms the backbone structure of fumonisins. Additionally, the *FUM8* gene runs the condensation of the linear polyketide with alanine to produce fumonisins [9], and *FUM21* encodes a Zn (II)2Cys6 DNA-binding transcription factor that positively regulates *FUM* expression [108]. The cluster also encodes an ABC transporter (*FUM19*) that provides a sort of self-protection by exporting the toxin from the cell and reducing its cellular concentration. The number, order, and orientation of genes within *FUM* cluster were specified to be similar for closely related *F. verticillioides* and *F. proliferatum* but also for *F. oxysporum*; however, only one fumonisin-producing strain O-1890 has been described in detail [6, 9, 103]. Nevertheless, the sequences flanking the *FUM* cluster seem to alter in *F. verticillioides*, *F. proliferatum*, and *F. oxysporum*, showing different genomic contexts of the *FUM* cluster in these three species and, possibly, also in other producers, like *F. nygamai* [7]. Proctor et al. (2003) determined the genomic context of the *FUM* cluster by the sequence analysis of the DNA regions flanking each side of the cluster. The analysis shows five different genomic context or genetic environments (GE), namely, GC1, GC2, GC3a, GC3b, and GC4. The one designed GC1 is devoted to the full *FUM* cluster in *F. verticillioides* [103] and for *FUM* cluster remnant in *F. musae* [109], where *ORF20* and *ORF21* represent pseudogenes, most likely the homologs of the *F. graminearum* gene FGSG_00274, and are flanking the *FUM21* side, whereas *ZBD1* and *ZNF1* are flanking the *FUM19* side. The GC2 was detected in all African clade species examined, where *ANK1* and *GAT1* are flanking the *FUM19* side and *ZBD1* and *MFS1* are flanking the *FUM21* side. The GC3a and GC3b were shown in American-clade species *F. anthophilum* and *F. bulbicola*, respectively. They have a similar structure with three genes (*CPM1*, *MF2*, and *DOX1*) flanking the *FUM19* side, differing in the *FUM21*-flanking region: in the GC3a, *FUM21* is flanked by *CPM2* and *TSP1*, while in the GC3b, there was no evidence for these genes. The GC4 was observed in *F. oxysporum* (FRC O-1890 strain), where there was no full-length gene within the Σ2800 bp region upstream of *FUM21* and a homolog of *CPM1* was flanking the *FUM19* side [59].

The fumonisin biosynthesis starts when the *FUM1* product catalyzes the condensation of nine acetate and two methyl units to form a linear, 18-carbon-long polyketide. The polyketide should be identical or similar in structure to 10,14-dimethyl octadecanoic acid. However, it is possible that the polyketide does not exist as a free acid but remains covalently attached to the phosphopantetheinyl cofactor of the PKS instead [110]. In the second step, the *FUM8*-encoded protein Fum8p catalyzes the condensation of the linear polyketide and alanine to yield a linear molecule that is 20 carbons long and has an amine at C-2, a carbonyl at C-3, and methyl residues at the C-12 and C-16 [9, 111, 112]. A third step of the pathway is catalyzed by the *FUM6*-encoded Fum6p protein and consists of the hydroxylation of the polyketide-amino acid condensation product at the C-14 and C-15 [9, 113]. The fourth, fifth, and sixth steps are the following reactions: C-3 carbonyl reduction, C-10 hydroxylation, and C-14/C-15 esterification, respectively. Metabolic profiling of numerous *F. verticillioides* mutants indicated that each of these reactions can occur independently from the others. The C-3 carbonyl reduction

is catalyzed by Fum13p [114], fumonisin C-10 hydroxylation is most likely catalyzed by Fum2p [2], and esterification of the tricarballylic moieties to the hydroxyls at C-14 and C-15 of fumonisins is catalyzed by Fum14p [115]. Although Fum14p catalyzes the C-14/C-15 esterification, analysis of gene deletion mutants indicated that Fum7p, Fum10p, and Fum11p also contribute to the formation of the tricarballylic esters [116]. The final step in the fumonisin biosynthesis is the Fum3p-catalyzed hydroxylation of the fumonisin backbone at the C-5. Fum3p is predicted to encode a dioxygenase, and its role in fumonisin biosynthesis was confirmed using enzyme assay in which the purified protein catalyzed the C-5 hydroxylation [117].

Phylogenetic discord of the *FUM* gene-based and primary metabolism gene genealogies was demonstrated, and it coincides with the differences in the *FUM* cluster genomic context, whereas it was not compatible with fumonisin chemotype differences [59]. Proctor et al. (2013) proposed that combination of a variety of dynamic processes, such as cluster duplication and loss, balancing selection, shifts in functional contrast, translation, and horizontal transfers, has shaped the evolution and distribution of some secondary metabolite biosynthetic gene cluster, as well as contributed to the metabolic diversity in fungi [59, 87, 118–121].

5.1.3 Zearalenone

Zearalenone (ZEA) is a phenolic resorcylic acid lactone mycotoxin with low acute toxicity that does not cause fatal toxicosis. It is associated mainly with maize but also occur in wheat, barley, and sorghum. Moreover, it can cause reproductive problems in farm animals, particularly in pigs. Zearalenone was first purified from a culture of *F. graminearum* and originally was designated as fermentation estrogenic substance F-2. Then, it was structurally characterized and named zearalenone [122]. ZEA is produced by several *Fusarium* species that usually also produce type B trichothecenes, and therefore it is found together with DON and NIV. Fungi belonging to the *F. graminearum* species complex are the most significant ZEA producers; however, there are other species that have been reported to produce ZEA, such as *F. culmorum* [123] and *F. cerealis* [124]. Fungi from the *F. oxysporum*, *F. solani*, and *F. fujikuroi* species complexes are not able to produce ZEA [1]. *F. equiseti-incarnatum* species complex is an exception here, as these fungi are able to produce ZEA but produce type A trichothecenes instead of type B [45, 125].

ZEA may undergo various modifications in the organisms of plants, fungi, and animals by phase I and phase II metabolism. Modified forms of ZEA found in animal feed include its reduced phase I metabolites (e.g., α-zearalenol, β-zearalenol, α-zearalanol, β-zearalanol) and its phase II conjugate forms with glucose, sulfate, and/or glucuronic acid [60]. Early chemical studies have proposed that ZEA is derived from the acetate through the polyketide synthesis pathway [126]. More recent research contributed to the development of the zearalenone biosynthetic gene cluster with two polyketide synthases, *PKS4* and *PKS13*, which have been characterized later [123, 127]. Despite the fact that biological functions of these genes have still been relatively poorly understood and some strains do not produce ZEA while still carry at least one of the genes, some evidence has been reported that the *PKS* genes

have accumulated enough intraspecific polymorphisms to be explored as promising targets for phylogenetic studies [3, 125].

5.1.4 Enniatins and Beauvericins

Enniatins (ENNs) and beauvericin (BEA) belong to a structurally and genetically related group of nonribosomal cyclic hexadepsipeptides consisting of alternating D-2-hydroxyisovaleric (d-HIV) acid and *N*-methyl-L-amino acids. The subunits are linked by peptide bonds and intramolecular ester (lactone) bonds, forming a cyclic depsipeptide [128, 129]. In the type A and B enniatins, these building blocks are typically either aliphatic *N*-methyl-valine, *N*-methyl-isoleucine, or a mixture of these amino acids [130]. In canonical beauvericin molecule, the three amino acid substituents are all aromatic *N*-methyl-phenylalanines instead of aliphatic residues [129, 131]. Also the identified three analogs of beauvericin (A, B, C) contain one, two, or three groups of 2-hydroxyisocaproic acid (HMP) instead of HIV group, respectively [132].

To date, 29 naturally occurring enniatin analogs have been identified. The most frequent variants detected in foods and feeds, especially in cereals, are enniatin A, A_1 (ENN A_1), B (ENN B), B_1 (ENN B_1), and B_4 (ENN B_4), together with smaller amounts of enniatins C, D, E, and F [128]. Enniatins are of high interest, because of their wide range of biological activities. Structural differences related to the *N*-methyl-L-amino acid are responsible for the different bioactivities of these mycotoxins. A mixture of ENNs can cause cytotoxic effects of various severities at low concentrations and on different types of cells [133]. Affected cells frequently include human cancer cells, implicating the potential use of ENNs as anticancer drugs [134]. In particular, ENNs A_1 and B_1 induce apoptotic cell death and disrupt the extracellular signal-regulated protein kinase's (ERK) activity associated with cell proliferation. This bioactivity has long been assumed to be associated with their ionophoric properties [135]. Today, the depsipeptides are known to incorporate into cell membranes and form pores with a high affinity for K^+, Na^+, Mg^{2+}, and Ca^{2+} [136]. ENNs also exhibit different biological properties, such as insecticidal and antibiotic activity against *Mycobacterium* sp. and *Plasmodium falciparum.* Of particular interest is the proven action of identified ENNs as inhibitors of major drug efflux pumps in *Saccharomyces cerevisiae* [137–140].

The chemical properties of depsipeptide compounds allow for their application in pharmaceutical products with anti-inflammatory and antibiotic properties in targeted treatment of diseases of the upper respiratory tract [141]. A mixture of enniatins was shown by Gaumann et al. (1960) to act synergistically as complex phytotoxin in causing wilt and necrosis to leaves of plants affected by *Fusarium* [142]. Pertinently, enniatins are often found in cereal grain at high concentrations, as a result of fungal infection. This fact has yet unknown implications for human and animal health, which leads to depsipeptide perception as emerging mycotoxins [143, 144].

Beauvericin (BEA) is a cyclodepsipeptide ionophore transporting monovalent cations across membranes as a free carrier uncoupling oxidative phosphorylation. BEA displays a diverse array of biological activities in vitro [145] and is one of the most potent cholesterol acyltransferase inhibitors of microbial origin. It shows

moderate antibiotic and antifungal activities; the combined use of beauvericin with ketoconazole (an antifungal drug) was found to enhance the antifungal effect, suggesting the potential use of beauvericin as a co-drug for antifungal infections in human [146, 147]. BEA has shown strong cytotoxicity to various human cancer cell lines and induced the apoptosis of some cancer cell lines by activating calcium-sensitive cell apoptotic pathways [148]. It also inhibits the directional cell motility (haptotaxis) of cancer cells at subcytotoxic concentrations [147].

ENN production is catalyzed by large multidomain protein (M = 347 kDa) – the nonribosomal peptide synthase (NRPS), known as enniatin synthetase (abbreviated as *Esyn1*) [149]. As a family of related enzymes, the fungal NRPSs are all modularly organized multienzyme complexes in which each module, located on the same protein chain, is responsible for the initiation, elongation, and termination of growing polypeptide (in this case – by ring closure). Each module of the NRPS system is composed of distinctly folded catalytic domains with highly conserved core motifs, important for their catalytic activities. A minimal (inexactly) repeated unit consists of three core domains in succession: an adenylation (A) domain which recognizes and activates the substrate via adenylation with ATP and a thiolation/transferase (T) or peptidyl carrier protein (PCP) domain which binds the activated substrate to a 4′-phosphopantetheine (PP) cofactor via a thioester bond and transfers the substrate to a condensation (C) domain which catalyzes peptide bond formation between adjacent substrates on the megasynthase complex. Several other specialized C-terminal domains involved in chain termination and release of the final peptide product have also been identified. Optional domains include methyltransferase (M), epimerization (E), heterocyclization (Cy), and oxidation (Ox) domains, which may alter the enzyme-bound precursors or growing peptide intermediates at various stages of the process. The full-length NRPS product is normally released by a thioesterase (TE) domain giving rise to free acids, lactones, or lactams. Eukaryotic NRPSs that synthesize cyclooligomer peptides assemble oligopeptide monomer intermediates by the programmed iterative reuse of their modules, which differs from the classical NRPS paradigm described in bacteria, and the resultant monomers are frequently employed in further recursive oligomerization and cyclization process [129, 150–152].

The *Esyn1*-encoded protein was previously purified and characterized by Zocher and co-workers (1982) from *Fusarium oxysporum* [153]. Biosynthesis proceeds through the condensation of three dipeptidol units followed by ring closure. The ENNs are synthesized from their primary precursors, i.e., valine, leucine, or isoleucine, D-2-hydroxyisovaleric acid, and S-adenosylmethionine. The NRPS domain architecture is composed of three functional modules: C-A-T-M (C, condensation domain; A, adenylation domain; T, thiolation/transferase domain; M, methyltransferase domain). The two adenylation domains are responsible for the specific activation of the primary substrates D-2-hydroxyisovaleric acid and L-amino acid as acyl adenylate intermediates [153–158].

A genomic locus containing the gene cluster related to beauvericin (BEA) biosynthesis in the entomopathogenic fungus, *Beauveria bassiana*, has also been cloned. Beauvericin synthetase (*bbBEAS*) consists of a single polypeptide chain with

a molecular mass of about 351 kD [159]. Similar to enniatin biosynthesis, beauvericin is also produced by a thiol template mechanism [160, 161]. However, the two depsipeptide synthetases differ in their substrate selectiveness. Beauvericin synthetase preferably accepts *N*-methyl-L-phenylalanine and some other aliphatic hydrophobic amino acids. The efficiency of incorporation into the cyclodepsipeptide framework decreases with the length of the side chain: *N*-methyl-L-phenylalanine was easily replaced by ortho-, meta-, and para-fluoro-substituted phenylalanine derivatives, as well as by *N*-methyl-L-leucine, *N*-methyl-L-norleucine, and *N*-methyl-L-isoleucine residues [149]. Consequently, significant sequence homologies to some of the *Fusarium* enzymes were found [150], establishing a common genetic background to depsipeptide biosynthesis. Previously, some *Fusarium* species like *Fusarium poae* have been reported to produce ENNs and BEA simultaneously [162, 163], which is justified by the fact that both mycotoxins share a common metabolic pathway and the co-occurrence of ENNs and BEA in field samples infected by *Fusarium* spp. has been observed [164, 165]. Previous works demonstrate high probability that even a single PCR-based *esyn1*-specific marker can detect potential producers of both toxins among *Fusarium* isolates originating from contaminated plant material [130, 163].

5.1.5 Fusaric Acid

Fusaric acid (FA) is a picolinic acid derivative which was isolated for the first time from *Fusarium heterosporum* strains. Further research have proven that other *Fusarium* species, e.g., *F. verticillioides*, *F. fujikuroi*, and *F. oxysporum*, are also able to produce this mycotoxin [166, 167]. FA shows moderate impact on mammalian health, but its high toxicity to plants is documented. It is responsible for "fusarium wilt" development through the lipid peroxidation, increase of reactive oxygen species, and, finally, host cells' death [168]. FA also causes bakanae disease in rice seedlings and has a strong antimicrobial activity, inhibiting *quorum sensing* in Gram-negative bacteria [169, 170].

Biosynthetic pathway of fusaric acid remains largely unexplored; however, the gene cluster responsible for encoding of proteins involved in this process has been identified in *F. verticillioides*. Initially, only 5 genes were described in fusaric acid biosynthetic (*FUB*) gene cluster, but just a few years later, additional 7 contiguous genes were located 14.6 kb upstream of the previous five genes identified [171, 172]. These genes are conserved in genomes of all FA-producing *Fusarium* strains, and no significant differences in cluster organization between the species have been found [167]. Functions of all 12 *FUB* genes were predicted using BLAST analysis (Table 2) [172, 173].

Fusaric acid synthase encoded by *FUB1* is responsible for the synthesis of six-carbon polyketide chain using three acetyl-CoA molecules. Fusion of polyketide chain, oxaloacetate, and amino group is catalyzed by amino acid kinase (*FUB3*). Hydrolase encoded by *FUB4* transform the product of this reaction to fusarate [171]. *FUB1* gene (designed also as *PKS21* according to the nomenclature proposed by Hansen et al. [55]) plays a significant role in FA biosynthesis. Orthologs of this gene were found in *F. fujikuroi*, *F. verticillioides*, *F. oxysporum*, *F. circinatum*, and *F.*

Table 2 Fusaric acid biosynthetic gene cluster structure – genes and their predicted functions [according to Ref. 172]

Functional gene name	Predicted function
FUB1	Polyketide synthase (PKS)
FUB2	Unknown protein
FUB3	Aspartate kinase
FUB4	Serine hydrolase
FUB5	Homoserine *O*-acetyltransferase
FUB6	NAD(P)-dependent dehydrogenase
FUB7	*O*-Acetylhomoserine (thiol-)lyase
FUB8	Nonribosomal peptide synthetase (NRPS)-like enzyme
FUB9	FMN-dependent dehydrogenase
FUB10	Fungal-type $Zn(II)_2Cys_6$ transcription factor
FUB11	Major facilitator superfamily transporter
FUB12	Fungal-type $Zn(II)_2Cys_6$ transcription factor

mangiferae [171, 173]. Deletions of *FUB1*, as well as *FUB4*, cause complete cessation of FA biosynthesis, while *FUB3* and *FUB5* silencing results in 20 to 40% drop in FA production [171, 174]. Although the processes related to FA biosynthesis are still not sufficiently understood, it is known that *FUB6*, *FUB7*, and *FUB8* genes are also crucial for the process [173].

FUB gene cluster contains two genes (*FUB10* and *FUB12*) responsible for the expression of $Zn(II)_2Cys_6$-pathway-specific transcription factors (TFs) which control the FA biosynthesis. The *FUB10* TF is directly linked to FA production, while *FUB12* TF is involved in effective FA conversion into fusarinolic acid and dehydrofusaric acid. Deletion of any of the TF genes results in decreased production of FA and its derivatives [171, 173, 174]. This process is also controlled by the global regulators, which build up complex regulatory network controlling life processes including SM biosynthesis [175]. For instance, culture medium of pH = 8 acts like a positive regulator of *FUB1* [PacC regulator], while copper, zinc and iron are negative regulators [176]. FA belongs to the nitrogen-induced SMs. High nitrogen concentrations affect the GATA-type TFs (AreA and AreB) which cause *FUB1* overexpression and, hence, increase in FA production [177]. The *Sge1* gene is another global regulator important in nitrogen-dependent FA biosynthesis. The function of *Sge1* differs between *Fusarium* species, for example, *FoSge1* regulates the conidiation and pathogenicity of *F. oxysporum*, while *F. fujikuroi FfSge1* is required for SM biosynthesis [178, 179]. *ΔSge1* mutants show reduced FA production [173, 175, 180]. *Fusarium velvet*-like complex is also involved in the regulation of the differentiation as well as the pathogens' virulence and FA biosynthesis. *Vel1*, *Vel2*, and *Lae1* genes are primary components of this complex. In *ΔVel1* and *ΔLae1* mutants, FA production was significantly lower than in the wild-type strains [180, 181]. Some reports suggest the epigenetic modifications like histone acetylation to influence these processes. Deletions in *Hda1* and *Hda2* genes, which are responsible for the expression of histone deacetylases, cause reduced FA biosynthesis in *F. fujikuroi* [180, 183].

5.1.6 Fusarins

Fusarins A, C, and D are a group of SMs built of a polyene chain linked to the 2-pyrrolidone ring. Additionally, fusarin C contains an epoxide group on the pyrrolidone ring, unlike fusarins A and D. First report on fusarin C produced by maize pathogen *F. moniliforme* (now *F. verticillioides*) has been published in 1981 in North America [184]. These mycotoxins are also produced by other *Fusaria*, e.g., *F. fujikuroi*, *F. graminearum*, and *F. venenatum* [167, 171, 185, 186]. Toxicity of fusarin C was not very widely investigated, but it was recognized by the International Agency for Research on Cancer as possible carcinogenic for human [187, 188]. Its mutagenic effect is probably related to the interaction of the epoxide group with DNA [189].

Enzymes involved in fusarin C biosynthesis are encoded by nine genes included in the *FUS* cluster. There are two versions of the *FUS* cluster organization. The first scheme is represented by *F. fujikuroi*, *F. verticillioides*, and *F. graminearum*, where the *FUS1-FUS9* genes are arranged one after another. The second one occurs in *F. solani* and in *F. circinatum* (which does not produce fusarins), where the genes *FUS9-FUS6* and *FUS2-FUS5* are separated by *FUS1* [167]. Predicted gene functions were presented in Table 3.

Fusarin C gene cluster consists of nine genes, but only four (*FUS1*, *FUS2*, *FUS8*, and *FUS9*) are essential for fusarin C biosynthesis [182]. *FUSS* was the first gene participating in fusarin biosynthesis identified, and it was described in *F. venenatum* and *F. verticillioides*. Its orthologs, *GzFUS1* and *fusA*, were identified in *F. graminearum* and *F. fujikuroi*, respectively [167, 171]. The *FUSS*-encoded protein is a combination of the polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS), an enzyme which plays a key role in fusarin biosynthetic pathway. The PKS-NRPS uses malonyl-CoA, six moieties of acetyl-CoA, and homoserine as substrates which are transformed into prefusarin [190]. Subsequently, prefusarin is oxidized by monooxygenase (*FUS8*) to form 20-hydroxy-prefusarin which undergoes epoxidation by α-/β-hydrolase (*FUS2*) to 20-hydroxy-fusarin. This product also undergoes oxidation by monooxygenase to the 20-carboxy-fusarin. Methyltransferase encoded by *FUS9* is responsible for the last substrate methylation and obtaining final product – fusarin C [167, 182].

Table 3 Fusarin biosynthetic gene cluster – gene designations and predicted functions [according to Ref. 182]

Functional gene name	Predicted function
FUS1	Polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS10)
FUS2	α-/β- Hydrolase
FUS3	Glutathione S-transferase
FUS4	Peptidase A1
FUS5	Serine hydrolase
FUS6	Major facilitator superfamily (MFS) transporter
FUS7	Aldehyde dehydrogenase
FUS8	Cytochrome P450 monooxygenase
FUS9	Methyltransferase

So far, no fusarin pathway-specific transcriptional factors have been identified, but the impact of some global regulators on fusarin biosynthesis has been well-established [180]. The expression of the *FUS* genes is pH-dependent and is upregulated in acidic conditions, but PacC TF is not involved in this process. *FUS* expression is also nitrogen-dependent. The expression of the *velvet*-like complex is increased in response to high nitrogen concentrations. *Δvel1*, *Δvel2*, and *Δlae1* mutants produce significantly lower amounts of fusarins compared to the wild-type strain. The deletion of a glutamine synthetase transcriptional factor *gln1* dramatically decreases *FUS* genes' expression [182]. On the other hand, the epigenetic modifications of histones like acetylation positively influence the expression of *FUS* gene cluster [182].

5.1.7 Moniliformin

In 1973, Cole and co-workers have isolated a compound from *F. moniliforme* cultures (later properly identified as *F. proliferatum*) which they called moniliformin (MON) [191]. MON has a very simple chemical structure (3-hydroxycyclobut-3-ene-1,2-dione) and is biosynthesized also by other *Fusarium* species, e.g., *F. avenaceum*, *F. oxysporum*, *F. fujikuroi*, and *F. subglutinans* [192, 193]. This SM shows moderate toxicity toward plants and animals [144]. Moniliformin biosynthesis is a very short and simple process. Condensation of two units of acetate leads to the formation of cyclobutadione moiety, which after oxidation and dehydration results in MON synthesis [194]. Presumably due to the uncomplicated biosynthetic pathway, until now all attempts to identify specific gene cluster devoted to MON biosynthesis, as well as the pathway-specific regulators, have failed.

5.2 Pigments

Fusaria produce a wide range of pigments, with the colors from pink, through carmine red, to purple, but some species may also produce yellow and brown pigments. Pigments can be best seen during the incubation of the fungus on rich microbiological media on the plate reverse. Colors of fungal pigmentation depend on the applied medium, its composition and pH.

Most of the *Fusarium*-produced pigments are naphthoquinones and javanicin, anhydrojavanicin, fusarubin, anhydrofusarubin, bikaverin, bostricoidin, novarubin, and naphthoquinone dimer – aurofusarin belong to this group. Many of these compounds have antifungal and antibacterial properties which sometimes inhibit the development of laboratory cell lines (e.g., HeLa). In this section we present the most common *Fusarium*-produced pigments with known gene clusters: carotenoids, bikaverin, and fusarubin [195].

5.2.1 Carotenoids

Carotenoids are characteristic yellow and orange pigments produced by plants, algae, bacteria, and fungi including *Fusaria*. These pigments are tetraterpenoids

Table 4 The designations and predicted functions of the *car* gene cluster and enzymes involved in carotenoid synthesis [according to Ref. 197]

Functional gene name	Predicted function
carX	Oxygenase
carRA	Cyclase
carB	Desaturase
carO	Rhodopsin
ggs1	Geranylgeranyl pyrophosphate synthase 1
carT	Oxygenase
carD	Oxygenase
carS	Unknown

and play a role in photosynthesis, photoprotection, and plant signaling, but no other significant function besides pigmentation has been found in fungi. Carotenoids were identified for the first time in cultures of *F. aquaeductum* but later also in *F. fujikuroi* and *F. oxysporum*. *Fusarium* species are able to produce *β*-carotene, lycopene, and neurosporaxanthin thanks to the *car* gene cluster encoding enzymes involved in carotenoid biosynthesis [196, 197].

Carotenoid biosynthesis is basically a continuation of the mevalonic acid biosynthesis because of the use of geranylgeranyl pyrophosphate (GGPP) as a first substrate. *carRA* and *carB* were the first genes involved in carotenoid biosynthesis that were discovered [196]. The cyclase encoded by *carRA* catalyzes the transformation of two GGPP units into 15-*cis*-phytoene which is converted into neurosporene by desaturase encoded by *carB*. Then, this compound serves as a substrate for the *γ*-carotene formation. Two intermediate products of this reaction are possible, and the outcome depends on which enzyme (cyclase or desaturase) acts first. If desaturase is the first acting enzyme, the intermediate product will be lycopene, and *β*-zeacarotene is a product of the cyclase. Carotenoidogenesis may diverge into two ways at this point. Using the first, cyclase converts *γ*-carotene into *β*-carotene which can be transformed by oxygenase (encoded by *carX*) into two retinol units. Using the second route, *γ*-carotene is desaturated into torulene by the first oxygenase (encoded by *carT*) into *β*-apo-4′-carotenol which is finally converted into neurosporaxanthine thanks to the oxygenase action (encoded by *carD*) [196, 197]. The summary of the c*ar* gene cluster and their predicted functions is presented in Table 4.

In carotenoidogenesis light-dependent and light-independent regulators can participate. Long-lasting exposure to light stimulates expression of *carRA*, *carB*, *carO*, *carX*, and *carT* and led to pigment accumulation, while *carD* gene is insensitive to photoinduction. In turn, high nitrogen conditions repress carotenoids biosynthesis. There seems to be a significant impact of *carS* on nitrogen-dependent regulation. *ΔcarS* mutants produce higher amounts of carotenoids than wild-type in media containing high amounts of nitrogen, but this mechanism is yet not clear [197].

Table 5 Designations and predicted functions of the genes from the *bik* gene cluster responsible for the biosynthesis of bikaverin [according to Ref. 198]

Functional gene name	Predicted function
bik1	Polyketide synthase
bik2	FAD-dependent monooxygenase
bik3	*O*-Methyltransferase
bik4	NmrA-like transcriptional regulator
bik5	Fungal-type $Zn(II)_2Cys_6$ transcription factor
bik6	Major facilitator superfamily transporter

5.2.2 Bikaverin

Bikaverin is a red pigment of polyketide structure produced by a number of *Fusarium* species (*F. oxysporum*, *F. solani*, *F. fujikuroi*, *F. proliferatum*, and *F. verticillioides*), and *F. oxysporum* was the first species from which bikaverin was isolated. As with most pigments, it acts as a stress protection, for example, against UV light. Bikaverin gene cluster has been found and characterized for *F. fujikuroi* [198]. It consists of six genes, among which only three are essential for bikaverin biosynthesis. Acetyl-CoA units are condensed into prebikaverin by multifunctional polyketide synthase encoded by *bik1*. Transformation of this compound into nor-bikaverin is catalyzed by FAD-dependent monooxygenase and *O*-methyltransferase. Rework of *O*-methyltransferase leads to the final product – bikaverin. The *bik* cluster contains a gene *bik4* responsible for the expression of pathway-specific NmrA-like transcription factor [198, 199]. The organization of the *bik* cluster was presented in Table 5.

Bikaverin biosynthetic pathway is another one regulated in a nitrogen-dependent way. During nitrogen starvation, the bikaverin biosynthesis is stimulated at first, but after a few days, this process is abolished. Experiments with *ΔareA* and *ΔpacC* mutants deficient in these global regulators did not show any significant effect on the bikaverin biosynthesis in *F. fujikuroi*, suggesting the existence of other regulatory mechanisms for this process [198, 199].

5.2.3 Fusarubins

Red pigments fusarubins are produced by *F. verticillioides*, *F. graminearum*, *F. fujikuroi* as well as other *Fusaria*. Few works on fusarubin are available, and only biosynthesis of 8-*O*-methylfusarubin is clear [71]. The other compounds synthesized in the course of this biosynthetic pathway include 8-*O*-methylnectriafurone, 8-*O*-methyl-13-hydroxynorjavanicin, 8-*O*-methylanhydrofusarubinlactol, and 13-hydroxynorjavanicin and require extensive further research.

Fusarubin gene cluster contains six genes among which *fsr1–fsr3* play essential roles in 8-*O*-methylfusarubin biosynthesis. The condensation of seven acetyl-CoA units results in the formation of a heptaketide which is transformed into 6-*O*-demethylfusarubinaldehyde. These reactions are catalyzed by a polyketide synthase encoded by *fsr1*. The resulting substrate undergoes further transformation

Table 6 Fusarubin biosynthetic gene cluster's organization [according to Ref. 71]

Functional gene name	Predicted function
fsr1	Polyketide synthase
fsr2	*O*-Methyltransferase
fsr3	FAD-dependent monooxygenase
fsr4	Alcohol dehydrogenase superfamily
fsr5	Short-chain dehydrogenase/reductase
fsr6	Fungal-type $Zn(II)_2Cys_6$ transcription factor

to 8-*O*-methylfusarubin by FAD-dependent monooxygenase. Unfortunately, molecular mechanisms of fusarubin biosynthesis regulation remain unrevealed. It is only known that this process is stimulated under alkaline pH and nitrogen limitation conditions [71]. *Fsr* cluster genes were presented in Table 6.

5.3 Antimicrobials and Hormones

Fusarium SMs affect plant, animal, and human health. They very often show antimicrobial properties as well (both antifungal and antibacterial), for example, DAS, DON, and T-2 toxin. Some mycotoxins have only antibacterial effect. Beauvericin, enniatins, and fusaric acid belong to this group [200]. The role, biosynthetic pathways, and gene clusters of the abovementioned mycotoxins have been described in previous subsections. Similarly, naphthoquinones such as bikaverin and fusarubins and their derivatives having antimicrobial activities were discussed previously. Javanicin and anhydrofusarubin are antibiotics against Gram-positive bacteria, e.g., *S. aureus* and *Corynebacterium poinsettiae*. Gram-negative bacteria and filamentous fungi are resistant to naphthoquinones [201, 202]. Chemical properties and biosynthesis of these pigments were described in previous subsection. Its antibacterial properties probably are caused by electron-releasing group substitution at 2 or 3 position of the moiety [202]. Here, other metabolites with antibacterial activities were considered: antibiotic Y, equisetin, and gibberellins.

5.3.1 Antibiotic Y

Unfortunately only few reports from 1980s about the antibiotic Y are available. Antibiotic Y was isolated from *F. avenaceum*, and, hence, also avenacein Y is known [203]. The activity of antibiotic Y was investigated toward *Bacillus subtilis* and *Erwinia carotovora*, and the results show very strong antibacterial activity especially at slightly acidic pH = 6.2. This activity was even stronger than the activity of streptomycin against *Staphylococcus aureus* [204]. Notably, only minor inhibition of other fungal genera, like *Alternaria*, *Penicillium*, *Aspergillus*, and *Botrytis* was reported [205].

Table 7 *Eqx* genes and their names according to the *eqx* gene cluster as well as genes' predicted functions [according to Refs. 210, 213]

Functional gene name	*Eqx* gene cluster name	Predicted function
eqxS	*fsa1*	Polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS10)
eqx3	*fsa2*	Diels-Alderase
eqxC	*fsa3*	*Trans*-acting enoylreductase
eqxD	*fsa4*	Methyltransferase
eqxR	*fsa5*	C6 transcription factor
eqxF	*fsa6*	C6 transcription factor
eqxG	*fsa7*	Major facilitator family (MFS) transporter
eqx9	*orf1*	Von Willebrand factor type A
eqxH	*orf2*	Cytochrome P450
eqx11	*orf3*	Short-chain reductases family protein
eqx11	*orf4*	Unknown protein
	orf5	Cell wall protein

5.3.2 Equisetin

Equisetin has been isolated for the first time from *F. equiseti* which gave rise to its name. This mycotoxin with antimicrobial properties inhibits the development of Gram-positive bacteria [206]. So far, equisetin was also found in cultures of *F. solani* and *F. heterosporum*. Equisetin is a phytotoxin causing seed deterioration, which simultaneously has an important pharmacological importance for human [207]. The interest in this compound increased dramatically because of its ability to inhibit the enzyme responsible for human DNA infection by human immunodeficiency virus type 1 (HIV-1) [208, 209].

Equisetin is a tetramic acid composed of octaketide linked with serine moiety. In 2005, Sims and colleagues described *eqx* gene cluster responsible for equisetin biosynthesis [210]. Eight units of malonyl-CoA are condensed and transformed by the *eqxC*-encoded enoylreductase and PKS-NRPS hybrid, encoded by *eqxS* gene. Heptaketide formed in these reactions undergoes a Diels-Alder cyclization carried out by *eqx3*-expressed protein. Further conversions are accomplished by PKS-NRPS and lead to the formation of trichosetin, which after N-methylation becomes equisetin [210–212]. Ten years after the results of Sims et al. were published [210], Kato et al. reported equisetin as an intermediate substrate in fusarisetin production and proposed new genes' designations [213]. As in other examples of PKS-NRPS-dependent biosynthetic pathways, also in this case, PKS-NRPS hybrid plays a key role for equisetin production, as *ΔeqxS* mutants completely lose their ability to produce equisetin [210, 212]. Unfortunately, there are no reports on detailed data on the regulation of *eqx* cluster are available. *Eqx* genes and their names according to the *eqx* gene cluster as well as genes' predicted functions were presented in Table 7.

5.3.3 Gibberellins

Gibberellins (GAs) are a group of well-known growth-promoting phytohormones, which are also secondary metabolites produced by some bacteria and filamentous fungi including *Fusarium*. GAs are tetracyclic diterpene acids containing 20 or 19 carbons in the cases where lactone bridge is present. Despite the fact that gibberellins are essentially plant hormones, for the first time, they were identified in *Gibberella fujikuroi* (*F. fujikuroi*), and this is where their name came from. Other *Fusarium* species have also the capacity to synthesize GAs, for instance, *F. circinatum*, *F. mangiferae*, and *F. oxysporum* produce abundant amounts of GAs. From the economical point of view, the most important gibberellins are GA_1, GA_3, GA_7, and GA_{14}, all produced by *F. fujikuroi* – the strain used most frequently in biotechnological production of GAs [62, 73, 214].

GA biosynthetic gene cluster was identified in *F. fujikuroi*, and it consists of seven genes (Table 8). The presence of this cluster was explained as a horizontal gene transfer from host plant to the pathogen [73, 215, 216]. Gibberellin biosynthesis starts from farnesyl pyrophosphate arising from the mevalonic acid biosynthetic pathway. This compound is transformed into geranylgeranyl diphosphate and then into *ent*-kaurenoic acid. These reactions are catalyzed by the enzymes encoded by *GGS2* as well as bifunctional *CPS/KS* and *P450-4* genes, accordingly. GA_{14} synthase leads to the formation of GA_{14} which is a substrate for C-20 oxidase, which forms GA_4. In turn, GA_4 may be further transformed into two ways. The first reaction is catalyzed by 13-hydroxylase and results in GA_1 production. The second one is catalyzed by desaturase and optionally followed by 13-hydroxylase, which leads to the formation of GA_7 and GA_3, respectively [73, 179, 214]. GA gene clusters of *Fusarium* species differ from each other. *F. fujikuroi*, *F. circinatum*, and *F. mangiferae* have the whole GA cluster consisting of all seven genes. Some strains of *F. oxysporum* contain also complete cluster, while in others some genes have been deleted (e.g., *P450-2*, *GGS2*, *CPS/KS*, and *P450-3* in II5 strain) or pseudogenes are present (*P450-2* pseudogene in PHW815 strain) [62].

Gibberellin biosynthesis is regulated in many ways. High nitrogen concentrations repress the production of GAs through decreased expression of nitrogen-dependent global regulators *areA*, *nmr*, and *meaB* [177]. Global regulator *Lae1* belonging to the v*elvet*-like complex is essential for GA biosynthesis. *Δlae1* mutant has abolished GA production, but, interestingly, the overexpression of histone acetyltransferase gene *HAT1* restores the GA biosynthesis in *Δlae1* mutants [180, 181]. Additional research is needed to explain this issue.

Table 8 Gibberellin biosynthetic gene cluster's organization in *F. fujikuroi* [according to Ref. 73]

Functional gene name	Predicted function
DES	Desaturase
P450-4	*Ent*-kaurene oxidase
P450-1	GA_{14} synthase
P450-2	C20 oxidase
GGS2	Geranylgeranyl diphosphate synthase 2
CPS/KS	*Ent*-copalyl diphosphate synthase/*ent*-kaurene synthase
P450-3	13-hydroxylase

6 Population and Chemotype Shifts

Combined analyses of multilocus genotyping and neutral molecular markers permit a large-scale analysis of the diversity, mycotoxigenic potential, and population structure among *Fusarium* species [217–223]. For instance, such analyses have exposed two dominant populations of *F. graminearum* in North America – NA1 and NA2 populations. The NA1 population is genetically diverse and comprises of native isolates which typically represent the 15-AcDON chemotype, whereas the NA2 population characterizes an invasive population that has undergone a bottleneck and is related with the 3-AcDON chemotype [24]. Recently, isolates possessing a novel NX-2 chemotype have been found in *F. graminearum* populations in southern Europe and in the north of the USA, which are sympatric with the NA1 and NA2 populations [77, 78, 223]. *F. graminearum* with NX-2 chemotype has undergone toxin diversification in response to the variations in selection pressure acting on the cytochrome P450 enzyme which is encoded by *TRI1* [24]. Kelly et al. (2016) suggested that adaptive constrains on the molecular evolution of trichothecene biosynthetic genes might be population- or niche-specific and, moreover, have shown that the variation of particular mycotoxins might be significant in niche adaptation [78].

Extensive research has provided tremendous insight into the genetic basis of the chemotype variation among *Fusarium* strains. On one hand, chemotype variation relates to the differences in the presence and/or absence of biosynthetic genes. For example, *TRI16* is present and functional in T-2 toxin-producing *Fusarium* species (*F. sporotrichioides* Sherb.), whereas it is not occurring or pseudogenized in the species producing NIV or DON. Similarly, the presence or absence of a functional *TRI13* is responsible for the DON and NIV chemotype polymorphism observed in *F. graminearum* and associated species [85, 86]. However, on the other hand, trichothecene chemotype variation results from the differences in function of allelic variants of the same *TRI1* gene [77]. In some *F. graminearum* strains, *TRI1* adds a hydroxyl group both at C-7 and C-8, resulting in the formation of DON and NIV [99], whereas in *F. sporotrichioides*, *TRI11* adds a hydroxyl group at C-8 only, leading to the formation of the T-2 toxin [89, 90].

The particular mycotoxin variant (chemotype) produced by an unknown isolate or a novel *Fusarium* species can readily be inferred using DNA-based methods. For instance, the *TRI5* gene which encodes trichodiene synthase [74] was one of the first ones to be used in designing the "generic trichothecene" marker [224]. Based on this knowledge, gene-specific markers were designed for identifying the particular chemotype variants of *F. culmorum*, *F. cerealis*, and *F. graminearum*. *TRI3*, *TRI7*, and *TRI13* genes were the targets in designing chemotype-specific markers which are helpful in detecting the DON, 3-AcDON, 15-AcDON, and NIV chemotypes, as well as the *TRI5* and *TRI4* for the discriminating type A versus type B trichothecene producers [224–228]. Moreover, the zearalenone chemotype has been detected in *F. culmorum* and *F. equiseti* populations using *PKS4* and *PKS13* genes from the ZEA gene cluster [125, 229, 230]. Additionally, the fumonisin chemotype was identified based on *FUM1* and *FUM8* gene-based markers among *F. verticillioides*, *F. anthophilum*, *F. fujikuroi*, and *F. proliferatum* species [2, 7, 12, 102, 231]. The

FRC O-1890 *F. oxysporum* strain has been used for the cloning and sequencing of the *FUM* gene cluster [9], although it is supposed to be the only strain of the species proven to produce fumonisins. Generally, *F. oxysporum* genotypes are regarded as able to produce fumonisins in low amounts [232, 233]; nevertheless, Stępień et al. [7] indicated that it was not possible to confirm the presence of *FUM* genes in any of the strains originating from natural *F. oxysporum* populations.

7 Conclusions

Fusarium genus appears to be very diverse, flexible, and dynamic group of fungi, able to grow and spread to new environments which includes infecting new hosts. Moreover, when climatic changes are taken into account, the population shifts and colonizing new areas become even more obvious. This unique ability depends often on the secondary metabolites produced by the fungi under specific conditions. Although the ecological roles of many of the SMs are still blurred or completely unknown, more and more researchers show their interest in revealing these issues. Apart from pure scientific curiosity, one has to keep in mind the possible use of the SMs in biotechnology, pharmacy, and medicine.

The SM biosynthetic gene clusters are an excellent model for evolutionary studies. Numerous reports on the divergence of the main pathways (e.g., trichothecenes, fumonisins, zearalenone) show that their history may be quite independent of the primary metabolic processes, implicating horizontal transfers, functional differentiations, and other rearrangements in adapting the microorganism to changing external conditions. Also, the discovery of new mycotoxin analogs is a proof for the dynamics that drives the *Fusarium* populations to develop and spread. Finally, the regulatory mechanisms of the SMs' biosynthesis are becoming much clearer each year, improving our understanding of fungal biology and biochemistry, which is particularly important in the context of the host-pathogen interactions on genetic and molecular levels. All this aspects make the future research of fungal secondary metabolism even more exciting and promising.

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