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Toxigenic Fungi and Aflatoxins as Food Biochemistry Hazard: Biosynthesis, Factor Affecting, and Gene Regulation (A review)

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Abstract

Food is a principal need for the continued survival of all living organisms. Food safety is an essential demand that must be achieved to avoid its refusal. The contamination of food by the toxigenic fungi threatens food safety and security. This review aims to get an overview of the pathway of AFB1 enzyme biosynthesis and their interaction with the genes relating to the cluster genes of AFB_1. It also aims to demonstrate the impact of the global environmental conditions that influence the secretion process of aflatoxin and the current information indicating that genes regulated by such environmental signals are interconnected with aflatoxin biosynthesis. Various fungi exist in food commodities, but not all can secret mycotoxins. *Aspergillus* fungi species are one of the dangerous enemies that have caused food condemnation. *Aspergillus* strains mainly produce aflatoxins as a secondary metabolite during their bioactivities. Aflatoxins are classified by the International Agency for Cancer Research, where aflatoxin B_1 (AFB_1) is considered a class I carcinogen. *Aspergillus* fungi may biosynthesis the aflatoxin through about 30 genes, with principal ones including *aflR, ver-1, verA, avfA,* and *nadA*. These genes cover about 75 kb of the fungal genome. With the advancement of molecular tools, the research of filamentous fungi developed dramatically and offered valuable opportunities to explain specific fungal pathways, such as the formation of secondary metabolites. Using the advantage of these technologies to enhance food safety and security, the molecular analysis of toxigenic fungi will hopefully understand the role influencing the formation of toxins and allow the creation of new successful fungal toxicity control strategies. Extensive research has been done on the genes that are implicated in the formation of AFB1, which is one of the most harmful human and animal carcinogenic toxins. The existing review discusses the responsibilities of these genes and their potential effect on forming AFB_1. The emphasis was on the *Aspergillus flavus* and *Aspergillus parasiticus*, deemed the critical pathogens characterized as the significant AFB1-generator in crops. This review perfectly understands factors, related genes, mechanisms, and pathways of aflatoxin production as a critical hazard that threatens food safety production. This review provided complete knowledge about reducing aflatoxin in the food chain supply. It supported the idea of eliminating aflatoxin secretion using novel strategies, leading to more safety in food production.

Keywords: Toxigenic fungi; food safety; aflatoxin B_1; AFB_1 enzymes biosynthesis; Aflatoxin genes; toxin formation.

1. Introduction

Food is considered the principal need for the continued survival of all living organisms, including humans, animals, plants, and microbial organisms. Food supports the biological activities of living organisms during their life cycles, which requests to be offered in suitable amounts according to global requirements. Food safety is a worldwide demand, and it assists in food availability without health problems or refusal issues of food products. Besides, food security is a general concern in ensuring political stability in developing countries [1]. Although food is essential for the continuation of human life, it is also crucial in maintaining vitality and meeting human needs [2]. So, food should reach the final consumers at a high level of safety and ensure its availability wherever they need it. Food contamination is a real challenge that faces food safety and security. Food could be threatened by such contamination sources, which vary between chemical, physical, and biological hazards. In some cases, the risk occurs double through the existence of microorganisms that can produce metabolites with health-hazard impacts [3]. Two types of microorganisms are eligible to achieve these

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risks in food commodities. The first one is related to the bacterial strain, which can secrete bacterial toxins [4]. A second hazard is toxigenic fungi's appearance on food products [5].

The risk of these fungi relates to their potential for producing mycotoxins, which could happen within its growth in food raw-materials and the final-food products [6]. It is significant to know that

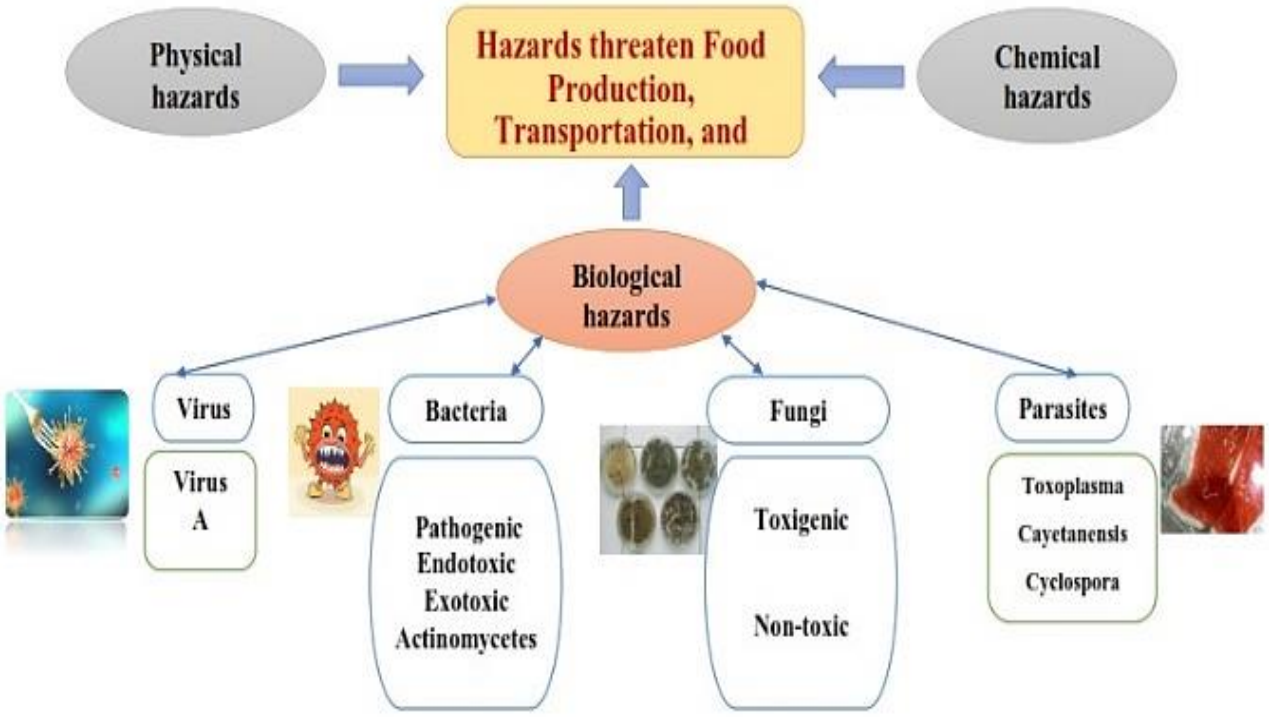

Figure 1: a diagram of Food hazard that are threating food safety and security

consumers are fighting against a shadow-hazardous kind of toxicology [7]. This happens according to the tasteless, colorless, and odorless mycotoxin compounds. It is concluded that fungi's presence does not necessarily mean mycotoxin's existence, but its potential presence still exists [8]. Consequently, it is essential to know about fungal-producing toxins, mycotoxin excretion, and the mechanism of fungi's secretion [9]. Various types of toxigenic fungal strains are reported by their capability to produce hazardous compounds through their metabolic cycles. These compounds are designated as mycotoxins and were recognized by more than 400 hundreds of types [10].

Based on sclerotia size, A. flavus can be categorized into a pair of types. Type L and S. type L produce a few vast sclerotia sizes of more than 400µm. Reproducible conidia and inconstant levels of aflatoxin secretion also distinguish it. At the same time, type S can produce few conidial numbers, with a considerably small sclerotia size of less than 400µm and a high-consistent level of aflatoxin secretion in media [11, 12].

Some strains of type L could not produce aflatoxin due to damage to the aflatoxin gene cluster, such as a toxigenic fungus [13].

Fungal strains are joined to a class of living organisms, which turns mycotoxin production into a process wholly controlled by the fungal preference. Also, this preference is affected by the growth condition of the fungal strain [14]. Usually, suitable conditions for fungal vegetative growth differ from those of mycotoxin excretion. Mycotoxins are classified as secondary metabolic compounds of fungal metabolism, which refers to their production occurrence under stress exercised on the fungal strain [15].

2. The occurrence of the aflatoxins

Aspergillus sp. is the predominant strain of fungi, widespread in most food commodities. This strain is familiar for its capacity to secrete a compound described as aflatoxins [16].

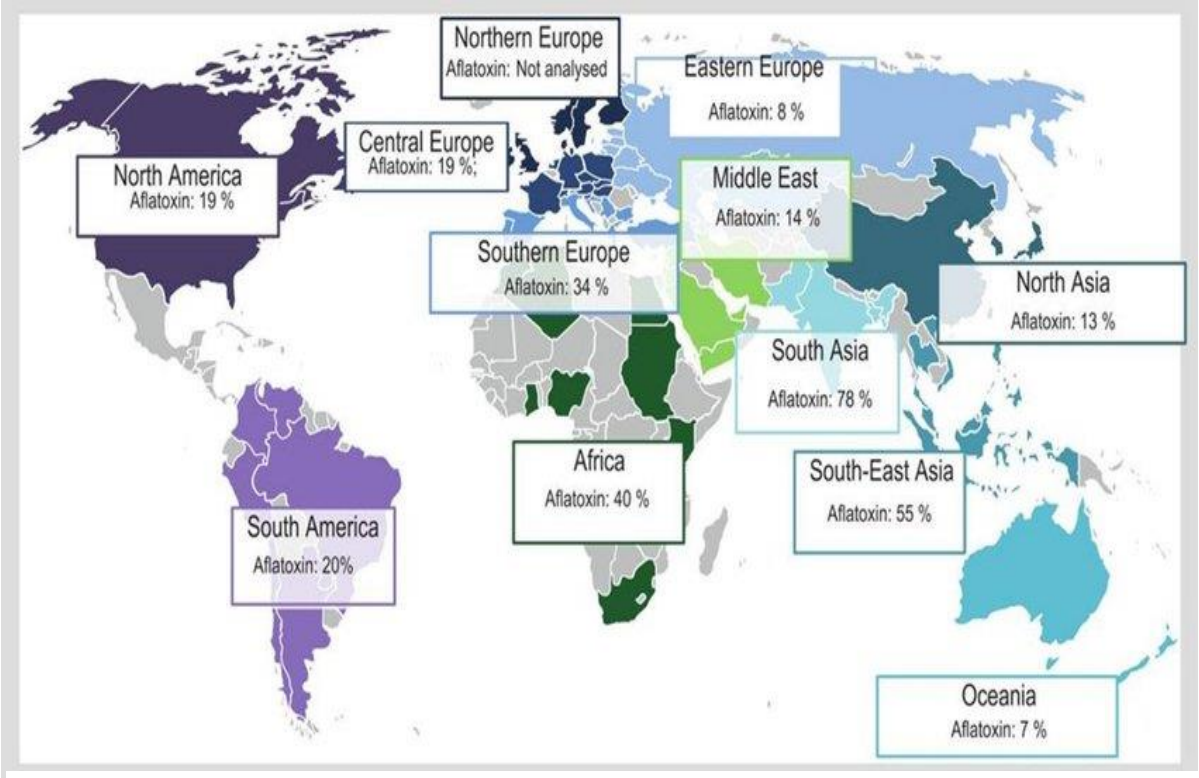

Figure 2: incidence of the global aflatoxins measured in percentile ration of total cultivated crops [22].

Aspergillus strains vary between two classes; the first category can secret just two types of aflatoxins (Aflatoxin B1 and aflatoxin B2). The second category of *Aspergillus* strains is secreting several aflatoxin-types (Aflatoxin B1; B2; Aflatoxin G1; and aflatoxin G2). The first category mainly contains *Aspergillus flavus* subspecies *flavus*, while the second category, *Aspergillus flavus* subspecies *parasiticus*, is involved. Generally, the last strain is known as *Aspergillus parasiticus* and is more common in cereal-based products, with high secretion levels of aflatoxins [17]. *Aspergillus* strains are known to contaminate cereals, threatening the essential food products in Egypt (bread and bakeries). The occurrence of aflatoxin contamination on a food product commonly means it should be condemned.

The most dangerous mycotoxins are recognized as aflatoxins, particularly the aflatoxin B1 (AFB1). Usually, the molecule AFB_1 is the primary metabolite secreted by most aflatoxigenic fungi, with other derivatives secreted in fewer levels [18]. The sixties and seventies decades of the twentieth century were named the era of aflatoxin, as this pointed to many investigations. Hydroxyl derivatives of aflatoxins are just secreted in lower media-pH and manifest like minor metabolites [19].

The risk occurred due to the existence of the AFB1 in metabolic cycles. The AFB_1 considered a pre-carcinogenic substance, causes several health issues by its entering the living tissues. Aflatoxins are present in global agro-food products, and they cause a significant loss of agricultural food products [20]. Aflatoxins are a natural hazard of Egyptian cereals during post-harvest handling [21].

2. Aflatoxins classification

However, the fungal strain of *Aspergillus* could secret the aflatoxins of B-types and G-types [23]. Furthermore, other types of aflatoxins, known as derivatives, result from metabolic transformation inside the body systems. Aflatoxin M1 and Aflatoxin M2 are the common types of these metabolites. Aflatoxin M is a hydroxyl derivative of Aflatoxin B, mainly occurring if ruminants and lactating animals feed on contaminated diets. Aflatoxin M derivatives may be excreted in bio-fluids like blood, milk, and urine [18]. Generally, the metabolic pathway of aflatoxins in the biological systems is so close. However, the active group of different aflatoxin molecules is related to the biological damage that occurred. In contrast, a change in the derivative active-group joins to the degree of damage.

3. Aflatoxin metabolites

The research of aflatoxin detection and their determination uncovered several metabolites that resulted under several conditions from the principal aflatoxin structure. The conditions of each metabolite may differ from the other one, like the media's pH change. Aflatoxin derivatives are varying due to the related conversion [24]. Other derivatives like Aflatoxicol, aflatoxin Q, aflatoxin B2a, aflatoxin P, and aflatoxin B23. The epoxide derivatives of aflatoxin B_1, including AFB1-8, 9-endo-epoxide, and AFB1-8, 9-endo-epoxide, were reported to form AF-adduct with protein and DNA molecules [25]. The data in Figure 4 represents the familiar derivatives of aflatoxins known to be more existence and detectable.

4. Aflatoxin structure and cause for the name

Aflatoxins are di-furanocoumarin derivatives in which a lactone ring is part of the coumarin compound. Coumarin is a crystalline, colorless solid with a pleasant odor. This molecule can be defined as a benzene derivative substituted by a lactone-like chain with two neighboring hydrogens. Coumarins can act as chemical protection in plants in the defense system [26]. Two types of aflatoxins are different according to their fluorescence emission. The emission lights are identified as green and blue fluorescent. This is noticed if the aflatoxin is detected using thin-layer chromatography through UV determination. This explains the reason for the aflatoxin capital name-letters [27]. The aflatoxin B (AFB_1 and AFB_2) refers to their blue fluorescent, and the aflatoxin G (AFG_1 and AFG_2) refers to their green fluorescent. The structure of aflatoxin B is a ring of bifuran merged with a molecule of coumarin containing a Penta-shaped ring [28]. The coumarin here attaches to a Hexa-shaped ring for the aflatoxin G structure. Aflatoxin M is considered a metabolite of aflatoxin B, so it has the same structure by adding a hydroxyl group to the bifuran rings.

The first biotransformation product of the aflatoxins known was the AFM type, which was discovered in animals' body fluids [29, 30], while the other two types were known as AFGM types and were isolated from the sheep-urine [31]. Besides the AFM and AFGM types, hydroxyl-aflatoxin types were identified as metabolic aflatoxins such as AFB2a, AFG_2a, AFB_3, Aflatoxicol, Aflatoxin Q_1, AFR_0, and AFP [32]. By excluding the AFB_{2a} and AFB-epoxide derivatives, the previous metabolic compounds are deemed as biotransformation detoxification forms of the most dangerous aflatoxin (AFB_1) [33]. The AFB_1 is metabolized mainly via the P450 mono-oxygenase mechanism into a reactive 8, 9-epoxide derivative of aflatoxin. This epoxide will react to form DNA or protein adducts, which their formation leads to mutations and carcinogenicity.

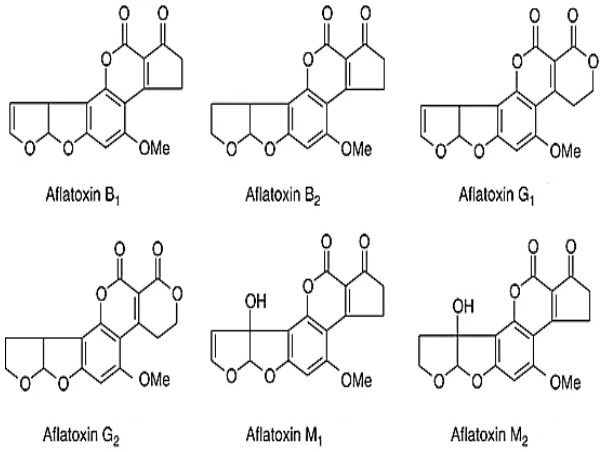

Figure 3; chemical composition of aflatoxin compounds derivatives (Aflatoxins B_1; B_2; G_1; G_2; M_1, M_2) [34]

5. The global ratio of Aflatoxin occurrence

Mycotoxin's global occurrence varies throughout the places of determination. This variation depends on several factors affecting the ratios of mycotoxins-contamination recorded in food commodities. Recently, Climate change has been considered the main factor controlling the dominant fungi inoculation of agro-food products, which consequently relates to the existing type of mycotoxin. Generally, each fungus has optimum conditions for both vegetative growth and secretion of secondary metabolites. Depending on these conditions, the biological activities of the fungi will take the principal pathway. The fungi can produce the toxic metabolites of mycotoxin, termed as toxigenic fungi. These fungi could grow on a broad spectrum of agro-food materials, lead to their spoilage, and may turn them to condemn.

The ratio of mycotoxins present in a world place did not mean the absence of other mycotoxins in this place. Each place worldwide has a dominant mycotoxin, which presents a high ratio of food commodities. However, the contamination by other mycotoxin types is recorded by low ratios.

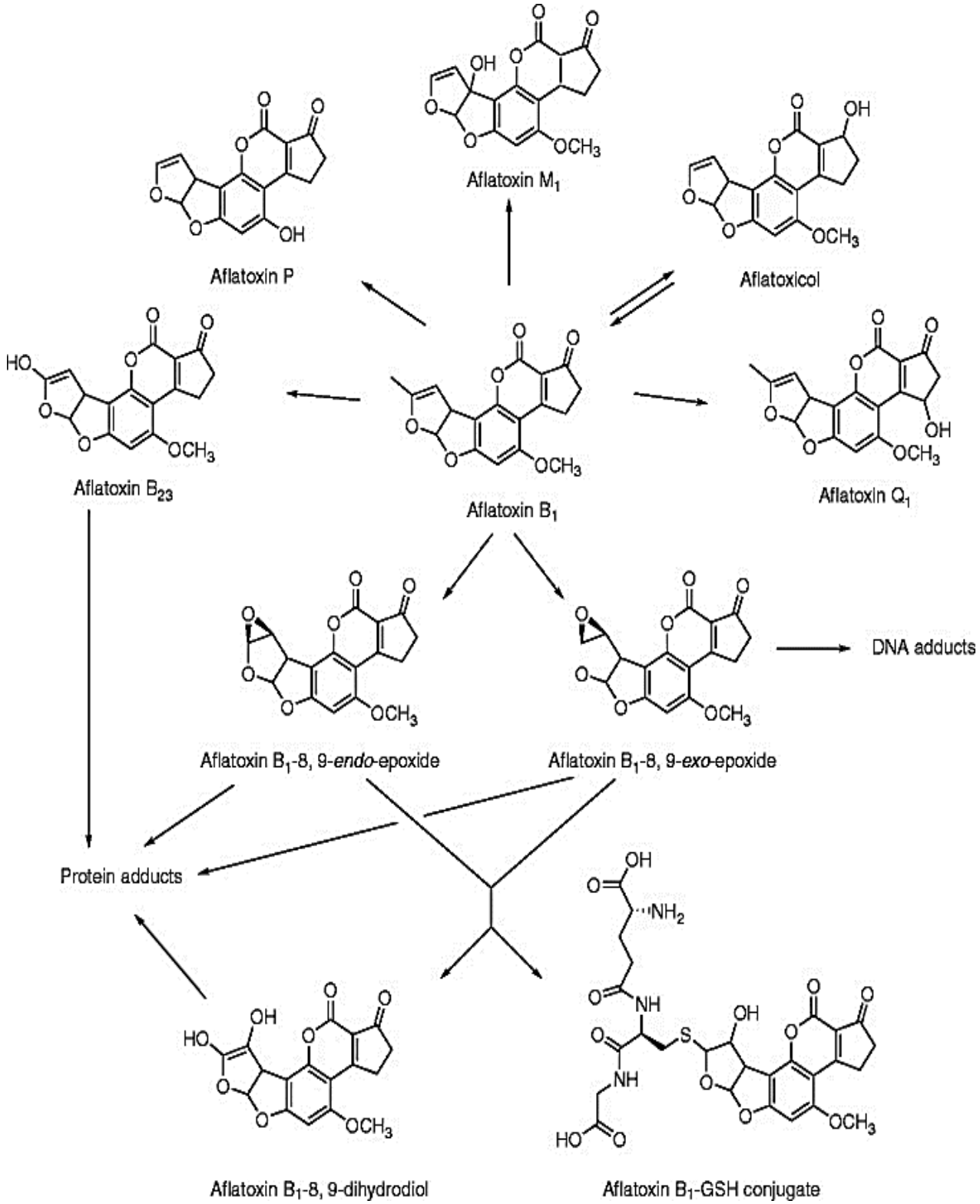

Figure 4; the familiar derivatives of aflatoxin metabolites that could be resulted during *In-vivo* aflatoxin metabolism [35]

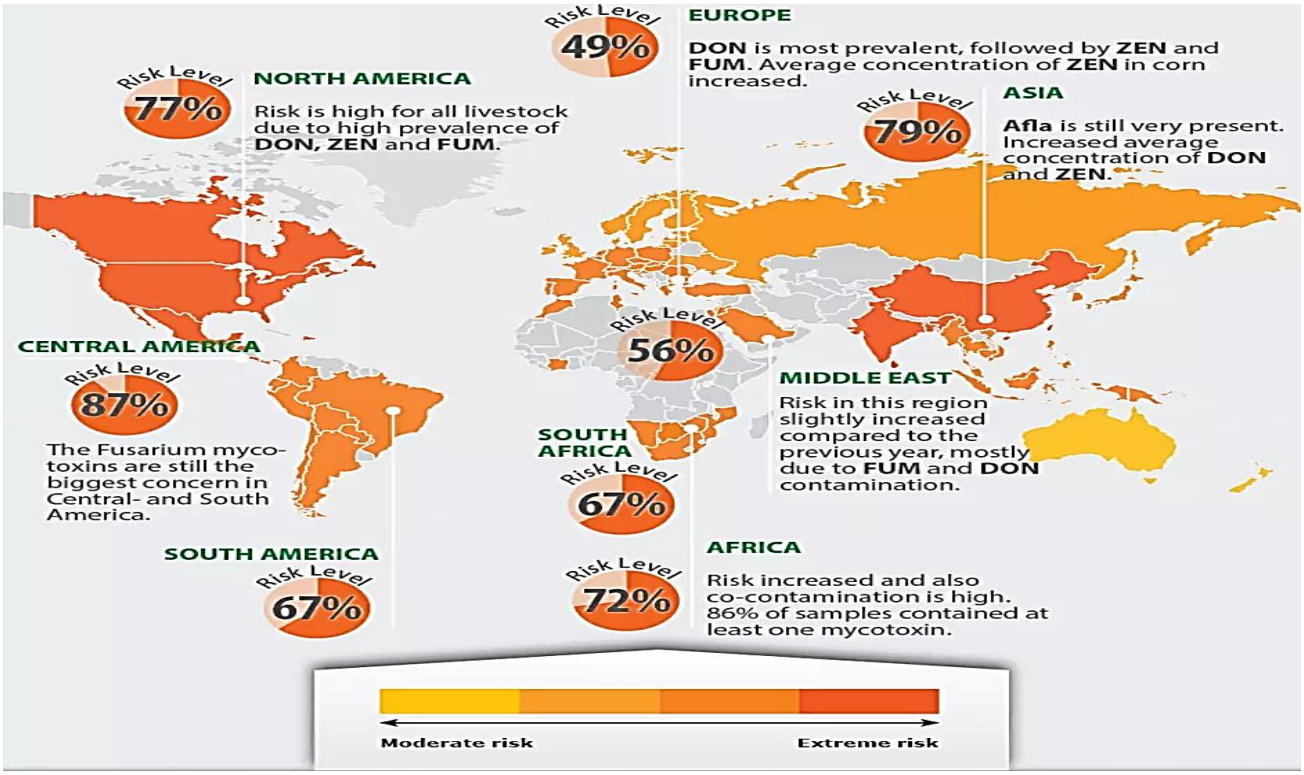

Figure 5; the risk degree and mycotoxins existence ratios in various global-places https://www.biomin.net/fileadmin/_processed_/0/b/csm_IG_MTXSurvey_2021_Global_EN_5cf2fd1eb6.png [36]

Figure (5) represents the dominant mycotoxin of each world area and the ratio of the globally-mycotoxin existences. Based on the data displayed in this figure, Africa and Southern-east of Asia are the highly contaminated areas by aflatoxins. In this regard, a demand for the search for novel strategies that can limit this type of risk is an urgent need.

5. Biosynthesis of Aflatoxins

The presence of secondary metabolism is considered a significant signal that is known in toxigenic fungi. The differences in secondary metabolites (SMs) are notable, where *Aspergillus* species have been known to be rich in the SMs genes [37-39]. It is known commonly that the production of secondary metabolite molecules is expressed as non-essential for the vegetative growth of fungi. These metabolites participate in the fungal adaptation to the surrounding growth environment during their life cycle. More than 48 kinds of the protein of the NRPS and PKS were predicted in *Aspergillus* oryzae, while at least 26 specific SMs clusters in *Aspergillus* fumigatus were identified [37]. It is indeed hard to identify the SMs, and much more complicated to attribute the biological functions of such molecules [40].

Until the discovery of penicillin at the beginning of the last century by Alexander Fleming, awareness and respect of fungal-SMs stayed relatively unclear. They have been extensively studied because of these metabolites' naturally biologically active existence and commercial benefits. Whether as pollutants or as bioactive components, the molecules of the SMs affect peoples' lives. The molecules of food additives (like Kojic acid), antibiotics (like penicillin), and even lipids-reducers (including lovastatin) are effective SMs produced by *Aspergillus* fungi with benefit impacts [41]. The metabolites of fungi, further including harmful outputs, are identified as mycotoxins. Aflatoxins are known as stable molecules produced by *Aspergillus*-species fungi, which mainly spoil cereals, legumes, and nuts. It is both toxigenic and causes carcinogenicity, where aflatoxins-exposure was reported to be associated with toxic syndromes [42]. The AFs have shown to connect with hepatocellular carcinoma [43]; this was led to a parallel for understanding factors that affect their synthesis besides the mechanism to avoid their toxicity.

Several pathways are involved in the SMs biosynthesis, including the large multifunctional

fluctuating proteins. This was reported clearly in the PKSs and non-ribosomal peptide synthases (NRPSs), which contribute to complex structures towards the SMs. Many pathways contribute to the formation of the SMs, with the most abundant class of polyketides being [40]. Generally, Mycotoxins, including the AFs group, are typical polyketide and non-ribosomal peptides formed by *Aspergillus* fungal species [40, 44]. AFs are still the best-distinguished fungal SMs. The AFs-biosynthesis genes accompanying the aflR specific-pathway regulator were established within a 70 kb DNA cluster close to a telomere of chromosome 3 in fungi strain [45, 46]. Simple research has investigated the AFs-production precursor (sterigmatocystin) and has contributed considerably to describing the AFs production pathways. It also significantly assists in realizing AF- the regulative mechanism, which has lately been addressed.

Considerable efforts have been made, and expenditures have been sustained globally since the recognition of aflatoxins to track their incidence and improve preventive and management strategies. A cornerstone in discovering the chemistry of aflatoxin biosynthesis was monitoring a color mutant accumulating the dark-red dye of the norsolorinic acid (nor) during the inspection of A. parasiticus fungi [47-50]. Since the nor is an initial stable precursor of aflatoxin throughout the biosynthetic pathway of aflatoxin, such finding has enabled the identification of more primary intermediates of aflatoxin. It has established preliminary stage derivatives in the pathway of aflatoxin [33, 51, 52].

The pathway of the aflatoxin gene cluster is identified in *Aspergillus* (flavus and parasiticus) following the cloning of many other significant biosynthesis pathways [53-57]. The increment in awareness about clusters promoted is a globally rising interest among investigators who have encouraged the complete understanding of aflatoxin biosynthesis. Throughout the explanation of the biosynthetic pathway, the regulatory mechanism, an intermediate pathway, related enzymes, and genes were more clarified to explore the critical steps in the synthesis process [54-62]. In aflatoxin biosynthesis, as many as about 30 genes are possibly implicated. The aflatoxins-pathway gene in *Aspergillus* (*flavus* and *parasiticus*) is concentrated on chromosome 4 [63-68]. The outdated gene terms are listed above the line, and the modern gene terms are consistently designated just below the line according to gene convention [68].

6. Introduction to Genetic Biosynthesis of Aflatoxin

Earlier investigations have shown that aflatoxins are reproduced via the pathway of the metabolic polyketide [45, 68-70]. The *A. parasiticus* and *A. flavus* strains showed that genomes are aligned throughout the aflatoxin biosynthesis throughout their mapping of super-positioning DNA cosmid clones [45, 65, 69]. The genetic cluster for aflatoxin synthesis within *Aspergillus* strains of *A. flavus* side to *A. parasiticus* typically consists of 25 genes covering about 70 kb. The aflR gene, as a positive regulator, is located in the cluster, coding for a specific sequence of the DNA and zinc-finger protein binding, and also is necessary for transcriptional activation of most of the target aflatoxin gene [69]. The database declared that the aflJ gene is divergently and adjacent to the transcribed from the aflR gen. No noticeable similarity with any other genes/ proteins has been reported for the aflJ gene. Although the exact role of the aflJ is not fully demonstrated yet, other aflatoxin cluster genes are essential for expression [22, 23]. The activity of most aflatoxin genes has been postulated through genetic or biochemical means [45, 71, 72]. Only four genes, out of 25 genes found in the AF pathway, have to experimentally understand the purpose of their protein product: norA, norB, aflT, and ordB.

7. The main steps in aflatoxin-biosynthesis

7.1. The Gal 4-E47

The primary step of aflatoxin biosynthesis is connecting to the Gal4, a transcriptional activator bound to Upstreaming Activator Sequences enhancer sequences found in DNA. Also, it is deemed a short oligonucleotide that responds to DNA binding to zinc-finger protein. Moreover, Gal47 has biological activities, including Carbohydrate metabolism, Galactose metabolism, Transcription, and Transcription regulation. It also has a metal-binding functionality besides its function in positive RNA transcription regulation during aflatoxin biosynthesis. The E47 is a fundamental helix protein essential for lymphocyte development [73, 74]. It is adequate for activating the transcription and gene encoding terminal if overexpression occurs [75, 76]. Therefore, the E47 holds over specific features demanded regarding a master regulatory protein like the MyoD features, but E47 is broadly formulated [77]. It is also important to note that E47 knows a specific mechanism in the DNA-binding function. The Gal4 was involved in plasmid encoding, namely as GAL4-E47, a short oligonucleotide that binds to DNA and energizes the gene transcribing.

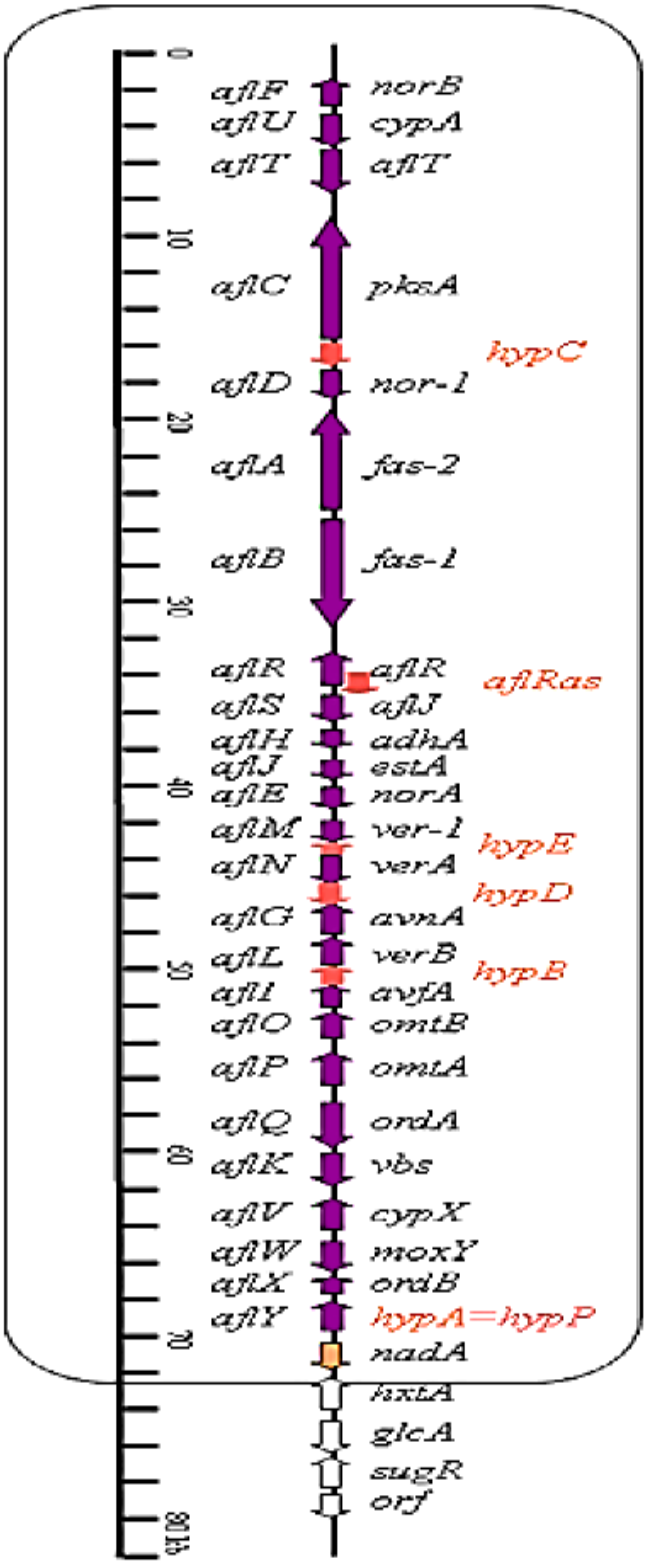

Figure 6. The aflatoxin pathway gene-cluster of *Aspergillus flavus* strain. [48].

During the aflatoxin- biosynthesis, the regulator gene was aflR, recognized as a type of Gal4 type 47-KDa joined to zinc-finger protein. The aflR gene connects to a palindromic 5/-TCGN5 CGA-3/ sequence motif through the AF-gene promoter section. At least one region of this type is ready to bind, mostly in promoter regions of aflatoxin genomes throughout 200bp of the transcription initiation region. In contrast, little putative linking regions were recognized more upstream [69, 73]. The distinguished point of the Gal4-type protein of the aflR was related to its recognition of its palindromic-binding sites as a dimer [78]. Under the control of negative regulators, the aflR perhaps does self-regulation [53, 79-82]. Upstream components could be interested in adverse control of aflR promoter activity [53, 56].

A) Acetate conversion to norsolorinic acids (nor)

Norsolorinic acids (nor) were reported as the initial equiponderant precursor of aflatoxin [44, 45, 60]. The starter substrate for aflatoxin synthesis is a hexanoyl initiator unit [50]. The synthesis of polyketide from a hexanoyl initiator unit requires two fatty acid synthases (fas) and polyketide synthases (nr-PKS, pksA). Norsolorinic acid anthrone (nor-anthrone) formation requires extensions of seven iterative, malonyl-derived ketides [83-88].

According to the investigation by Mahanti et al., [89] cloned a 7.5-kb is a board transcription by complementarity genetic needed for the nor synthesis in mutation-blocked A. parasiticus. The identity and similarity of its protein were reported highly (48% and 67%, respectively) towards the beta sub-unit of the fatty acid synthesis (fas1) in two kinds of microorganisms. The updating term of the fas-1A gene was fas-1, which is represented in the aflatoxin biosynthesis of the DNA genome. A further large transcript (fas-2A) encoding the fatty acid synthase α-subunit throughout the aflatoxin gene cluster has also been recorded [85, 89, 90].

Biochemical evidence has been shown to illustrate the function of the FAS and the PKS in aflatoxin biosynthesis [91, 93]. More data have been published on the preliminary stage of the aflatoxin biosynthetic pathway, including the synthetase of fatty acids and polyketide synthases [83, 84, 86, 87, 92].

In a fas-1 disrupted transforming, the N-acetyl-cysteamine thioester of hexanoic acid was integrated into the nor. A synthase gene of polyketide (pksA) in A. Parasiticus is necessary for aflatoxin biosynthesis via gene disruption [53, 54]. Noranthrone is the expected substance converted by pksA. Transformation of noranthrone to the NOR, the initial equilibrium intermediate pathway [48, 83, 94-97], is poorly described, but it has been suggested that

noranthrone oxidase, or monooxygenase, should be catalyzed or spontaneously occur [49]. The in-depth investigations prove that the gene product hypC is a necessary nor-anthrone oxidase that activates nor-oxidation [60]. The new fas-2, pksA, and fas-1 terms were changed to aflB, aflC, and aflA, respectively [63, 67, 98]. A gene homolog of afl-A, afl-B, and afl-C in A. nidulans are the stc-J, the stc-K, and the stc-A, respectively [92].

B) Norsolorinic acid transformation to Averantin (AVN)

The initial equilibrium intermediate of aflatoxin biosynthesis pathways was recognized as NOR, resulting from the UV-generated disruption mutants in A. parasiticus and A. flavus [96, 99]. The NOR-accumulating mutants, whose aflatoxin biosynthetic pathway is not fully suppressed, are Leaky mutants. The aflD (nor-1) gene, which encodes a reductase, has been cloned via genetic complementation [100]. The NOR reduction was catalyzed by a recombinant Nor-1 protein generated in E. coli bacteria. Consequently, aflD (nor-1) encodes the ketoreductase necessary for transforming the NOR 1'-keto group to the Averantin 1'-hydroxyl group [101].

Disruption of the gene nor-1 has verified the aflatoxin-biosynthetic pathways' participation in NOR-to-AVN transformation [102]. The homologous gene nor-1 in A. nidulans is the stcE [85]. In the AF cluster, genes sequence homology to nor-1, such as norA and norB, are expected to encode dehydrogenases of aryl-alcohol. Also, such types of protein can catalyze NOR-to-AVN decreases depending on the cell's reductive condition and may clarify the leakiness of the nor-1 mutation and whether they are capable of complementing the function of nor-1 [103].

C) The transformation of the AVN to 5'-Hydroxy-averantin (HAVN)

Experimental studies on radioisotope integration provide the most substantial evidence to define the transformation of AVN to HAVN [104, 105]. The transformation of the nor to averufin (avf) is controlled through three enzymatic paths: (1) a reductase-catalyzed nor to AVN, (2) a monooxygenase-catalyzed the nor to HAVN, and (3) a second dehydrogenase-catalyzed the HAVN to the avf [106]. The oxidation reactions were also considered reversible, and NADPH was the favored cofactor [107]. The P-450 monooxygenase was encoded and disrupted by the gene initially called ord-1 [108]. The substrate-feeding experiments of the ord-1 mutant stated that HAVN seems to be intermediate in AVN to AVF transformation. The ord-1 gene is strongly similar to A. nidulans in the StcF sequence, designated to be the aflG (avnA) gene [92].

D) The HAVN Transformation to Oxoaverantin and Averufin

One of the primary intermediates in aflatoxin synthesis is averufin (avf) [109-114]. A few other intermediates are being confirmed to be implicated in the AVN to AVF transformation [113], where the Averufanin (AVNN) is deemed one of them. Later, the experiments revealed that it is a shunt metabolite but not a genuine intermediate aflatoxin [94,120]. To encode alcohol dehydrogenase, the gene cluster adhA was recognized [113, 115]. The AdhA deletion mutants have been shown to accumulate primarily the HAVN. After extended development, the mutants could generate minimal quantities of the AVNN that were inconsistent with the AVNN as a shunt metabolite.

Therefore, an appropriate cytosolic enzyme may transform the HAVN directly or indirectly into the AVF. Earlier in the 21st century, two cytosolic enzymes and a novel intermediate of the aflatoxin pathway called 5'-oxoaverantin (OAVN) were identified between the HAVN and the AVF [116]. The adhA gene controls the HAVN-to-OAVN transformation enzyme. The deletion mutant of gene adhA is leaky, suggesting that the transformation from the OAVN to the AVF can require extra enzymes or genes. Enzymatic processes were also suggested for the aflatoxin biosynthetic pathway and the potential participation of specific enzymes [117].

E) The AVF Transformation to Versiconal Hemiacetal Acetate (VHA)

A cytochrome P450 mono-oxidase, CypX, and aflI (avfA) gene are concerned with transforming AVF to VHA. While this transformation demands the aflI gene activity, the gene oxidative function is obscure [118]. The aflI gene is also included in a strain of A. nidulans as a homolog stcO gene [92, 118]. The integration of the mutant accumulation of the averufin from strain *Aspergillus* SRRC strain to the *A. flavus* gene (aflI) reactivates the strain's capability to transform AVF to VHA and secrete aflatoxins [118]. The aflI (avfA) encoded protein will likely participate in the ring-closure process of hydroxy Versicolor-bone formation with the CypX-specific gene. To carry out the transformation, it was likely that gene-avfA is correlated to the P450 monooxygenase as no specific intermediates other than AVF arise from the degradation of either gene.

F) Transformation of the VHA to Versiconal (VAL)

The esterase enzyme is interested in transforming the VHA to the VAL, where it was purified in A. parasiticus [119-122]. The aflJ esterase gene (estA) was recognized as a section in the aflatoxin gene cluster [123]. In the biosynthetic gene cluster of A.

nidulans ST fungi, the homologous gene is the stcI gene. The cumulative metabolites of the estA gene suppress-mutants were predominantly the VHA and the versicolorin A (verA) [124]. The versiconol acetate accumulation and other intermediates of aflatoxin synthesis compounds were recorded, such as the VAL and versicolorin B [106, 125]. Afterward, more versicolorone (VONE), VOAc, and VHA-containing metabolic grids were recognized [116]. Admittedly, it is now established that the estA-encoded esterase catalyzes the transformation throughout the aflatoxin biosynthetic pathway of both VHA to VAL and VOAc to VOH [126].

G) Transformation of the VAL to the Versicolorin B (ver B)

The investigation of Lin and Anderson [127] was the first to offer enzymatic confirmation that VAL is translated to the verB by such a cyclase. Such an enzyme is established as versicolorin B synthase [128-130]. The gene was cloned and referred to as the vbs gene. Revealed recombinant protein from the vbs gene is shown to demonstrate the predicted cyclase activity [129-131]. The VAL cyclase and the verB synthase were isolated separately from the *Aspergillus* fungal strain. An enzyme catalyzes the cyclodehydration VHA-racemic side chain to the verB. This is another crucial step towards creating aflatoxin as it closes the aflatoxin bisfuran ring, the moiety essentially connected to the toxigenic and carcinogenic actions of aflatoxin. The vbs gene was called aflK [132]. In the A. nidulans ST fungi, the stcN is known as a homologous gene biosynthetic cluster.

H) The verB transformation to Versicolorin A (verA)

The verB is the essential branch point contributing to establishing either AFB_1/ AFG_1 or AFB_2/AFG_2. Close to AFB2/AFG2, the tetrahydro-bisfuran ring is located in verB, and similar to AFB_1/ AFG_1, a dihydro-bisfuran ring is also included in the verA. The translation of verB to verA demands the de-saturation of the difuran ring by the unstable enzyme of microsom in verB [133]. The instability of the stcL in A. nidulans has abolished ST synthesis and culminated in the verB [94, 134].

I) The verA Transformation to Demethyl-sterigmatocystin (DMST)

The verB Transformation to Demethyl-dihydro-sterigmatocystin (DMDHST). Much detail has been mentioned in the biochemical transition steps from the verA to the DMST [135]. The aflM (ver-1) gene transcription by gene integration with verA accumulating in A. parasitic CS10 was considered responsible for transforming the verA to a non-isolated intermediate gene. A ketoreductase, identical to Nor-1, is expected to encode the aflM (ver-1) gene. The ver-1 homolog, stcU, was detected—the double mutation of the stcU and the stcL accumulated in only verA [136]. The stcS gene (previously referred to as verB), also known to be the similar gene of the P-450 monooxygenase, has also been characterized, and analyses have shown that it also engages in verA-transformation into DMST-formation. The StcS interference culminated in verA, as did Ver-1 disruption [137].

The stcU and the stcS are necessary to transform verA to the DMST. The stcS-homologue, aflN (verA), has also been described in the A. parasiticus strain [81, 87]. A third enzyme, known as hypA (aflY), is required for the transformation. A Baeyer-Villiger monooxygenase is expected to encode such genes. The instability of such an allele sometimes resulted in the concentration of verA, such as ver-1, suggesting that it acts without allowing intermediate formation as part of the enzyme complex. The OrdB, known as a fourth enzyme, has also been transforming. Like the AvfA, the homolog CypX is deemed an assistance monooxygenase protein.

J) The DMST Transformation to Sterigmatocystin (ST),

* *The DHDMST Transformation to dihydro-sterigmatocystin (DHST)*

Two O-methyltransferases are reportedly participating in the biosynthesis of aflatoxins [138]. The O-methyl-transferase I catalyzes methyl transfer from S-adenosyl-methionine (SAM) to DMST and DHDMST hydroxyls for processing the ST and the DHST, respectively. The enzyme of 43-kDa is isolating and characterizing of the A. parasiticus [139, 140]. Centered upon a partial sequence of amino acids, the refined dmtA-enzyme was segregated from A parasiticus as the corresponding gene [141]. The recent investigation simultaneously isolated the same gene from other *Aspergillus* species but called it the omtB gene [118]. The consensus SAM-binding motif contains the expected dmtA-encoded protein [141]. The omtB homolog was recognized as the stcP, which was recognized as being necessary for DMST-transformation to the ST in A. nidulans, as shown by gene disruption [142].

K) The ST Transformation to the OMST

* *The DHST Transformation to the DHOMST*

The O-methyltransferase gene is needed to transform the ST to OMST and DHST to DHOMST. It was first cloned by reverse genetics from A. parasiticus using antibodies against clarified O-methyltransferase A [59, 143]. Such a gene was initially called omt-1, then omtA, and eventually designated as aflP [143]. The enzyme recombination was generated by *E. coli*, where substrate-feeding

studies showed its efficiency in transforming ST to OMST [143]. O-methyltransferase A has strong specificity for the substrate but cannot methylate the DMST or the DHDMST.

Therefore, the O-methyltransferases A encoded by the aflP gene is the enzyme trustworthy for ST to OMST transforming and the DHST to DHOMST. This gene's (aflP) genomic DNA sequence was cloned from the two main *Aspergillus* strains [144]. This homolog of the aflP gene was also observed in other *Aspergillus* strains of non and aflatoxigenic strains [145]. The disappearance of the aflP orthologue was counted as the reason why A. nidulans generate ST as the final result instead of aflatoxins.

L) The OMST Transformation to AFB1 & AFG1

**The DHOMST transformation to AFB2 & AFG2*

Based on feeding studies, the association between aflatoxin B and G groups' formation has been suggested [146]. For this reaction, a P-450 mono-oxygenase gene in A. flavus called ord-1 existed significantly [147, 148]. This P-450 mono-oxygenase gene, the ordA, has been cloned in A. parasiticus and participated in the transformation of OMST and DHOMST to (AFB_1/AFG_1) and (AFB2/AFG2), respectively, and was demonstrated in the yeast system [154]. In the late stages of aflatoxin biosynthesis, whether the aflQ (ordA) gene is producing is unclear, where the ordA gene catalyzes two successive mono-oxygenase reactions. Recent investigations have proposed synthesizing G-group aflatoxins includes additional enzymes [149]. Once the cypA gene has been cloned and characterized, it is evident how cypA has encoded the P450 monooxygenase for aflatoxin G-group production [71].

The nadA gene has recently played an important role in producing AFG1/ AFG2 [43, 150]. The gene of nadA was stated as an enzyme for AFG1-translation of modern intermediate aflatoxin called nadA [159]. The norA gene was previously assumed to be active in the synthesis of the nor gene, leading to a degree of sequence resemblance to the nor-1 gene. [103]. Nevertheless, recent studies prop the hypothesis that aflE (norA) is implicated in the AFB1 production throughout the two final stages [60]. An oxidation transaction in OMST-transformation to aflatoxin is perhaps included in the transcript hypB gene, a homolog of hypC. Conveniently, there are incomplete nadA and norB genes in only the G-group aflatoxin maker. Tentative findings suggest a relation refers to the norB function in the transcription of other proteins, which mainly creates AFG_1 / AFG_2 [151].

M) Other biotransformations of aflatoxins (Aflatoxins M)

Aflatoxins M (AFM1 and AFM2) are deemed bio-conversion mammalian products of Aflatoxin B; these derivatives are isolated and described from bovine milk [152-154]. Since entering the mammalian body, the liver P450 enzymes convert aflatoxins into reactive-epoxide intermediates, which are much more mutants or hydrolyzed and have become a little dangerous for the AFM1 and AFM2 molecules. Even then, recently, the aspertoxin feeding studies (12c-hydroxy-OMST) have shown that A. parasiticus develops AFM1, AFM2, AFGM1, and AFGM2 (as minor aflatoxins), also develops the AFB1, AFB2, AFG1, and AFG2 (as principal aflatoxins) [155].

8. Genetic Regulation Implemented for Aflatoxin Biosynthesis

The genes of the aflatoxin pathway are arranged in clusters; this was very clear in the genome of A. flavus and A. parasiticus, where they are simultaneously expressed [67, 98]. The aflR, considered a positive regulation gene, exists at the center of the gene cluster. The aflJ is adjacent to aflR and was found to be implicated in the regulation of transcription [126, 156]. Other physically unrelated genes of the veA or the laeA are displayed as having a regulation role in aflatoxin metabolic pathways [157, 158].

9. Pathway-Specific Transcription Factor Encoding Genetic Control by aflR Gene

For highly energetic transcription of aflatoxin structural genes, a specific sequence of 47KDa zinc-finger protein is required to link to DNA encoded by the aflR gene [132]. The AflR links are a potential functional dimer, similar to the other regulatory protein (Gal4-type), which can link with palindromic sequences. In the promoter sites of gene-structural, it connects to the 5'-TCGN5CGR-3 'palindromic sequence [58, 159]. The AflR-linking motifs are between -80 and -600 positions, with the preponderance relative to the translation start site at -100 to -200 positions. In some instances, AflR attaches to a deviated sequence instead of the usual motif, as seen in the situation of the avnA.

Only one available binding motif is chosen as the linking region if there is more than one being active [58, 159]. The more upstream motif, for turning on the expression of hypC, is found to belong to another gene. Regulation of other aflatoxin pathway genes is abolished by deleting aflR in A. parasiticus [160]. In A. flavus and A. parasiticus, the high expression of aflR re-arranges the aflatoxin pathway gene expression and aflatoxin accumulation. [79, 132]. The present findings indicate the direct participation of AflR in controlling aflatoxin-biosynthesis. Surly, in their promoter regions, a total of the up-regulating genes found by encoding-profiling utilizing the microarray assays of the DNA in comparison to wild-

style and aflR-suppressed strains of A. parasiticus possess an assent AflR linking motif [161, 162].

10. Encoding a Putative Transcriptional Co-activator Genetic Regulation by aflS gene

Bidirectionally, aflR-translated resulted in the aflS gene essential for the aflatoxin synthesis while not exhibiting substantial homologous recombination by encoded protein contained in databases [156]. A region of the 737 bp internal transcribed is shared between aflS and aflR genes. In the gene conversion aflR of A. parasiticus, the performance of intermediate aflatoxin pathways was substantially enhanced in transformants containing more additional of the aflR and aflS [81].

Quantitative PCR showed that the absence of aflS transcription is correlated with a five to twentyfold decrease in the production of specific aflatoxin biosynthesis pathways of the aflS knockout mutants. Mutants could not generate intermediates of aflatoxin, and no aflatoxins were secreted [156]. Deactivating the aflJ gene did not have a discernible impact on the transcription of aflR. The over-expression in the *A. flavus* aflJ gene was recorded by non-raising of the aflR, omtA, or ver-1 transcription. Otherwise, it appears to have some effect on aflC (pksA), aflD (nor-1), aflA (fas-1), and aflB (fas-2) genes [163]. These latter genes are necessary for early intermediates of the aflatoxin biosynthetic pathway. The aflS modulates the transcription mechanism in conjunction with aflR pathway genes, is still needs more investigation.

11. Genetic Regulation by the laeA gene on Secondary Metabolism

The strain of A. nidulans fungi was the first to recognize the innovative global regulatory gene of the laeA [157]. As shown by its inclusion in the genomes of all fungi sequenced, this gene is well maintained in fungi. Besides the AF-cluster, the LaeA gene is a nuclear protein that contains a binding motif of S-adenosyl-methionine and transcription activities. Instances have included a reference to A. nidulan sterigmatocystins and penicillins cluster, A. fumigatus gliotoxin cluster, and A. flavus aflatoxin cluster [157, 164]. The full-genome analysis of the transcription profiles of wild style with the laeA-suppressed of the A. fumigatus was also performed [165], where the results were expressed that LaeA positively regulates the expression of twenty to forty percent of main categories of secondary genes for metabolite biosynthetic pathways. It also controls specific genes not associated with clusters of secondary metabolites [166]. Regarding the suspected regulatory mechanism, the LaeA methylated histone-proteins differentially change the chromatin template for the expression. A primary function of LaeA is to control gene-cluster metabolism established toward observation whose conidial amounting to a wild-style level was generated by laeA-deleted strains [157]. The latest studies of non-aflatoxigenic A. parasiticus (sec-) variants developed by serial mycelia transfer of parents (sec+) have shown that laeA was encoded in both strains [167].

12. Encoding a Regulator VeA gene impacts (fungal development and mycotoxin formation)

In the strain of A. nidulans, the veA gene was initially important for light-conditional requests [168]. In comparing the light impact of the veA+ and veA1 on sterigmatocystin formation, the result displayed the ability of the two strain types to generate sterigmatocystin. In contrast, the veA+ strain grown in darkness produced the highest amount. Furthermore, the VeA-absence in the two *Aspergillus* strains (parasiticus and flavus) resulted in a wholly lost ability to generate aflatoxins irrespective of the illumination [41]. The VeA gene contains the motif of a bipartite nuclear localization allusion, and its relocation to the nucleus is light-dependent. It also includes the alpha-carrier protein of importin [169]. The VeA gene is primarily found in the nucleus in the dark; it is present in both the cytoplasm and the nucleus under the light. The VeA has no identifiable DNA-binding thresholds and is likely to affect the development of the ST and AF via protein-protein interactions with some other regulation factors. Its activity can be modulated by post-translational modifications such as phosphorylation and dephosphorylation.

13. Aflatoxin Biosynthesis and their Factors Affecting

13.1. Carbon sources

Aflatoxin production has long been influenced by carbon, nitrogen, amino acids, lipids, trace elements, and other nutritional factors [170]. The media with a provenance of nitrogen and carbon are considered the best nutrition for the AF-formation [171]. The association between the carbon source and aflatoxin formation has been well established. The production of aflatoxin is facilitated by simple types of sugar [91]. Connexion has been documented between alpha-amylase activity and the development of aflatoxin in A. flavus [172]. A gene cluster associated with sugar-utilizing was found in A. parasiticus near the aflatoxin gene cluster, as described in a study by Yu et al. [118]. The near physical connection between the two gene clusters might indicate the relationship between the carbohydrate processing clusters leading to aflatoxin biosynthesis induction. A good carbon source for promoting aflatoxin production is the lipid substrate. In A parasiticus and A. flavus, the lipase gene, lipA, was cloned.

13.2. Nitrogen

Nitrogen has a very close relation to AF production [91]. Media-containing amino acids help the development of aflatoxin, whereas the medium-containing NaNo2 and NaNo3 did not show any impact [173]. Nitrate has also been proposed to suppress the development of averufin and aflatoxin [174, 175]. It has been documented that nitrate seems to have a suppressive impact on the formation of aflatoxin, and aflR over-expression overcomes the regulation by a negatively-influence of aflatoxin pathway gene transcription [81]. Nitrogen use efficiency genes and a nitrogen-regulation gene have been cloned in as an area gene of A. parasiticus. Numerous AreA binding motifs were recognized in the aflR-aflS intergenic site [82, 176]. The binding of the areA gene may protect AflR linking. The amino acids may have various effects on the development of aflatoxin. The investigations show that tryptophan suppresses the aflatoxin-formation process, but the tyrosine of A. flavus speeds up the process of aflatoxin formation [162].

13.3. Temperature

The aflatoxin formation was impacted directly by temperature. The optimum development of aflatoxin is observed at temperatures close to 30 °C [177]. The production of aflatoxin is almost completely inhibited when the temperature rises above 36 °C. Using microarray gene profiling and RT-PCR verification, genome-wide gene profiling suggested that elevated temperature was connected to a substrate in aflatoxin pathway gene expression. A large number of transcripts of both the regulatory genes aflR and aflJ were found by RT-PCR [177]. It has, therefore, been hypothesized that the behavior of AflR or some other unknown regulatory factor could be influenced by temperature. The AflJ is more influenced by the high temperature than the aflR. A change in the aflJ to aflR ratio makes aflR unfunctional for transcription activation.

13.4. Water Activity

Extreme outbreaks of aflatoxin in maize were reported due to warm climate conditions [178, 179]. The mechanism of infestation of A. flavus in maize under these circumstances is not well known. A combination of these factors may be included in potential scenarios: (1) the plant defense system is weak under conditions of water pressure; (2) plant tissues damaged, which were infected by the insects, thereby creating chances of the mold spores to enter; (3) enrichment by the spores of fungi spread during dry weather into the air.

13.5. The pH value of the culture

In acidic media, aflatoxin synthesis by *A. flavus* happens but is inhibited with alkaline media. The PAC-C gene was recognized as a transcription regulatory factor of pH [134, 180]. The pacC linking region has been recorded at the aflR promoter region [58]. A putative pacC linking region near the aflR transcription's starting region effectively regulates the pH of aflatoxin excretion [181]. This region was displayed as a suppressing area in the non-aflatoxin-conducive peptone medium [180]. The action of regulation could connect with the pacC linking to that region under alkaline properties of media to suppress the transcription of the aflR acid-gene and hence the production of aflatoxin. The PacC and areA linking regions of intergenic sites in the aflR-aflJ indicate that environmental signals (like pH) control gene expression [82].

13.6. Fungal development stage

Secondary metabolism is associated with sporulation and sclerotial development. At around the same time, spore formation and secondary metabolite formation occur. Some sporulation-deficient mutants cannot develop aflatoxins, and some sporulation-inhibiting compounds in A. parasiticus even inhibit the production of aflatoxins. The aflatoxin-producing potential was progressively reduced in reaction to a series of subcultures. The increases in the development potential of aflatoxin have been followed by marked morphological changes [182].

13.7. The impact of oxidative Stress

In *A. parasiticus* fungal strain, oxidative stress causes the development of aflatoxin. However, the oxidative stress and biosynthesis of aflatoxin were associated, as was previously mentioned. Significant increases in aflatoxin output were reported in *A. flavus* regarding treating tert-butyl hydroperoxide or gallic acid. Similar treatment with *A. parasiticus* also induced the development of aflatoxin [183]. Hydrolyzable tannins significantly inhibit the biosynthesis of aflatoxin, with gallic acid being the principal anti-aflatoxigenic constituent of these tannins. Gallic acid decreases structural gene expression within the biosynthetic aflatoxin cluster. Gallic acid tends to block the signal transduction pathways.

Applying phenolic acids and other antioxidant molecules to A. flavus with oxidative stress decreases the production of aflatoxin without affecting the growth of fungi. Another antioxidant that prevents aflatoxigenesis is caffeic acid. A. flavus treated with caffeic acid microarray analysis identified a gene called ahpC2, an alkyl hydroperoxide reductase potentially involved in quelling the aflatoxin production signal. However, when treated with caffeic

acid, there was non-observed impact on the expression of laeA [172].

13.8. Effect of plant metabolisms

The metabolites of plants play some role in the production of aflatoxin. The N-decyl aldehyde reduces, under some conditions, both the fungal growth of *A. parasiticus* and its aflatoxin secretion are affected by more than 95 percent compared to control [184]. Octanal decreases the vegetative growth of the fungi by 60 percent, but it raises the excretion of aflatoxin by five hundred percent. However, hexanal decreases the vegetative growth of the fungi by 50 percent, but it has no impact on the production of aflatoxin. The linoleic acid derivatives have been reported to reduce the development of aflatoxin [185]. Regulation of aflatoxin levels in goods should include pre and post-harvest stages and routine identification and screening.

14. Future outlook regarding the management the aflatoxin-contamination issues

Aflatoxins ' economic effects and possible dangers to human health have displayed the requirements to prevent or mitigate their food and feed contamination. The initiative includes tracking, managing, and regulating the amount of Agro-Food products from farms to the markets.

A) Future prospective in detection and screening

Surveillance programs have been developed to influence by reducing the aflatoxin hazard ingestion. Analytical test assays were improved to detect many food samples [63] rapidly. The TLC, HPLC, and GC-MSMS are existing determination strategies for accurately identifying and measuring the quantities of aflatoxins. The ELISA technique is also considered a precise method determined at 0.1 ng/mL for rapidly analyzing aflatoxins [186].

15. Future prospective in pre-harvest

Recently, the main direction in controlling the aflatoxin contamination in food crops, particularly cereals, has been achieved by utilizing atoxigenic *Aspergillus* strains to cause a mutation in the producer strains. Two major projects are still being worked on at this point. In Africa, the project was known as "Afla-Safe." Later, it had a factory producing a commercial product with a trademark. The website of this project is https://.a.flasafe.com/a.flasafe/, and the project has succeeded in limiting the aflatoxin contamination through the bio-controlling stage using a non-producer strain that affect the metabolic pathways of aflatoxin biosynthesis in the field. The second project was established in Europe, incorporating eight countries. The website of this project is https://.mcoykey.eu. The project is also interested in discovering and exploring a new strain of fungi as a control agent that can regulate the aflatoxin gene as a novel methodology for stopping the AF hazard.

16. Modern-prospect of food bio-preservation

Some modern investigations were interested in limiting the aflatoxin contamination of food materials. These critical studies are interested in the impact of lactic acid bacterial metabolites [187, 188]. These investigations reflect various bio-active molecules [189, 190], which could regulate the aflatoxin excretion in liquid media using high-producer strains of aflatoxins. The In-vitro studies displayed the valuable impact of these molecules on inhibiting the fungal ability to secrete aflatoxins [191]. The determination of aflatoxin amounts in the growth media reflects decreases in aflatoxin-producing compared to the control. Moreover, the bacterial cells and their postbiotics were also capable to reduce metabolites of aflatoxin such as aflatoxin M1 [192].

Knowledge of the biological and genetic pathway for synthesizing mycotoxins, especially aflatoxins, has helped lead to a new aspect of development in limiting the production of these toxins by fungi. Recently, molecular docking (modeling) and molecular dynamics have been used to predict inhibition pathways for producing aflatoxins by applying some active ingredients in fungal growth environments, mainly phenolic compounds [193-196]. The results revealed the ability of several phenolic compounds, essential oils [195], and some alkaloids [194] to influence the production pathways of mycotoxins through genetic prediction of the nature and stability of the association between these substances and the genes responsible for the secretion of aflatoxins. However, further studies need to discover these molecules' genomic impact. Utilization of novel types of non-traditional oils that possess a unique oxidative stability may have a critical function to inhibit the fungal producing of toxin [197-200]. This point can open a new vision of research to limit the mycotoxin contamination through the oxidative effect of special oils type.

Conclusion

Aflatoxin is a mutant and carcinogenic compound secreted by fungi on food commodities. Aflatoxin is produced through complicated pathways of *Aspergillus* gene clusters. More than twenty genes participate in aflatoxin-metabolic pathways. These genes vary between principles and intermediate ones, which regulate the sequenced steps of aflatoxin bio-synthetic stages. Several environmental and growing conditions contributed to the influence of aflatoxin secretion and its related genes. The perfect understanding of these factors and their related genes, including their mechanisms and pathways, will

facilitate the modern steps and efforts that target eliminating aflatoxin secretion, leading to more safety in food production. The limitation of aflatoxin secretion throughout affecting the synthesis gene-clusters should consider the pivotal genes that count as critical points through the synthesis steps. Moreover, molecular docking and dynamics could be applied for enhancing toxin suppression process. This review is uncovering the relation between the gene clusters of aflatoxins and how to avoid AF accumulation.

Conflicts of interest

"There are no conflicts to declare".

References

1. Allouche, J., The sustainability and resilience of global water and food systems: Political analysis of the interplay between security, resource scarcity, political systems, and global trade. Food Policy, 2011. **36**: p. S3-S8. DOI|10.1016/j.foodpol.2010.11.013.

2. Medicine, I.o., Providing Healthy and Safe Foods As We Age: Workshop Summary, ed. L. Pray, C. Boon, E.A. Miller, and L. Pillsbury. Washington, DC: The National Academies Press. 192. DOI:10.17226/129672010.

3. Uyttendaele, M., E. Franz, and O. Schlüter, Food Safety, a Global Challenge. Int J Environ Res Public Health, 2016. **13**(1): p. 67. DOI:10.3390/ijerph13010067.

4. Hernández-Cortez, C., Palma-Martínez, I., Gonzalez-Avila, L.U., Guerrero-Mandujano, A., Solís, R.C. and Castro-Escarpulli, G., 2017. Food poisoning is caused by bacteria (food toxins). Poisoning: From specific toxic agents to novel rapid and simplified techniques for analysis, 33.

.

5. Adeyeye, S.A., and Y. Fathi, Fungal mycotoxins in foods: A review. Cogent Food & Agriculture, 2016. **2**(1): p. 121-127. DOI|10.1080/23311932.2016.1213127.

6. Eskola, M., G. Kos, C.T. Elliott, J. Hajšlová, S. Mayar, and R. Krska, Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. Critical Reviews in Food Science and Nutrition, 2020. **60**(16): p. 2773-2789. DOI 10.1080/10408398.2019.1658570.

7. Badr, A.N., A.F. Logrieco, H.A. Amra, and T. Hussein, Ochratoxin is an occurrence in Egyptian wheat during seasons (2009-2014). Asian Journal of Scientific Research, 2017. **10**(3): p. 178-185,

8. Amend, A., G. Burgaud, M. Cunliffe, V.P. Edgcomb, C.L. Ettinger, M.H. Gutiérrez, J. Heitman, E.F.Y. Hom, G. Ianiri, A.C. Jones, M. Kagami, K.T. Picard, C.A. Quandt, S. Raghukumar, M. Riquelme, J. Stajich, J. Vargas-Muñiz, A.K. Walker, O. Yarden, and A.S. Gladfelter, Fungi In the Marine Environment: Open Questions and Unsolved Problems. mBio, 2019. **10**(2): p. e01189-18. DOI 10.1128/mBio.01189-18 |.

9. Lahiani, A., E. Yavin, and P. Lazarovici, The Molecular Basis of Toxin'' Interactions with Intracellular Signaling via Discrete Portals. Toxins (Basel), 2017. **9**(3): p. 107. DOI 10.3390/toxins9030107 |.

10. De Saeger, S. and A. Logrieco, Report from the 1st MYCOKEY International Conference Global Mycotoxin Reduction in the Food and Feed Chain Held in Ghent, Belgium, 11-14 September 2017. Toxins (Basel), 2017. **9**(9): p. 276. DOI 10.3390/toxins9090276 |.

11. Hernández-Cortez, C., Palma-Martínez, I., Gonzalez-Avila, L.U., Guerrero-Mandujano, A., Solís, R.C. and Castro-Escarpulli, G., 2017. Food poisoning caused by bacteria (food toxins). Poisoning: From specific toxic agents to novel rapid and simplified techniques for analysis, 33.

12. Luque, M.I., Rodriguez, A., Andrade, M.J., Martin, A. and Cordoba, J.J., Development of a PCR protocol to detect aflatoxigenic molds in food products. Journal of Food Protection, 2012. 75(1), pp.85-94. https://doi.org/10.4315/0362-028x_jfp-11-268

13. Adhikari, B.N., R. Bandyopadhyay, and P.J. Cotty, Degeneration of aflatoxin gene clusters in *Aspergillus* flavus from Africa and North America. AMB Express, 2016. **6**(1): p. 62-62. DOI 10.1186/s13568-016-0228-6 |.

14. Pinotti, L., M. Ottoboni, C. Giromini, V. Del''Orto, and F. Cheli, Mycotoxin Contamination in the EU Feed Supply Chain: A Focus on Cereal Byproducts. Toxins (Basel), 2016. **8**(2): p. 45-45. DOI 10.3390/toxins8020045

15. Godswill Awuchi C, Nyakundi Ondari E, Josiah Eseoghene I, et al. (2022) Fungal Growth and Mycotoxins Production: Types, Toxicities, Control Strategies, and Detoxification. Fungal Reproduction and Growth. IntechOpen. Available at: http://dx.doi.org/10.5772/intechopen.100207.

16. Pfliegler, W.P., I. Pócsi, Z. Győri, and T. Pusztahelyi, The Aspergilli and Their Mycotoxins: Metabolic Interactions With Plants and the Soil Biota. Front Microbiol, 2020. **10**: p. 2921-2921. DOI 10.3389/fmicb.2019.02921

17. Nadumane, V.K., P. Venkatachalam, and B. Gajaraj, Chapter 19—- *Aspergillus* Applications in Cancer Research, in New and Future Developments in Microbial Biotechnology and Bioengineering, V.K. Gupta, Editor. 2016, Elsevier: Amsterdam. p. 243-255. https://doi.org/10.1016/B978-0-444-63505-1.00020-8

18. Marchese, S., A. Polo, A. Ariano, S. Velotto, S. Costantini, and L. Severino, Aflatoxin B1 and M1: Biological Properties and Their Involvement in Cancer Development. Toxins (Basel), 2018. **10**(6): p. 214. DOI 10.3390/toxins10060214 |.

19. Okechukwu, V.O., Adelusi, O.A., Kappo, A.P., Njobeh, P.B. and Mamo, M.A., 2023. Aflatoxins: Occurrence, biosynthesis, mechanism of action and effects, conventional/emerging detection techniques. Food Chemistry, p.137775. https://doi.org/10.1016/j.foodchem.2023.137775.

20. Giray, B., G. Girgin, A.B. Engin, S. Aydın, and G. Sahin, Aflatoxin levels in wheat samples consumed in some regions of Turkey. Food Control, 2007. **18**(1): p. 23-29. https://doi.org/10.1016/j.foodcont.2005.08.002|.

21. Selim, M.I., W. Popendorf, M.S. Ibrahim, S.E. Sharkawy, and E.S.E. Kashory, Anatoxin B1 in Common Egyptian Foods. Journal of AOAC International, 2020. **79**(5): p. 1124-1129. DOI|10.1093/jaoac/79.5.1124 |.

22. Wacoo, Alex. Detection and reduction of aflatoxin in the food chain. PhD thesis. Makerere University, Department of Medical Biochemistry, Ughanda. 2020

23. Kumar, P., D.K. Mahato, M. Kamle, T.K. Mohanta, and S.G. Kang, Aflatoxins: A Global Concern for Food Safety, Human Health, and Their Management. Front Microbiol, 2017. **7**: p. 2170-2170. DOI 10.3389/fmicb.2016.02170 |.

24. Loveland, P.M., J.S. Wilcox, J.D. Hendricks, and G.S. Bailey, Comparative metabolism and DNA binding of aflatoxin B_1, aflatoxin M1, aflatoxicol and aflatoxicol-M_1 in hepatocytes from rainbow trout (Salmo gairdneri). Carcinogenesis, 1988. **9**(3): p. 441-446. DOI|10.1093/carcin/9.3.441|.

25. Benkerroum, N., Chronic and Acute Toxicities of Aflatoxins: Mechanisms of Action. Int J Environ Res Public Health, 2020. **17**(2): p. 423. DOI 10.3390/ijerph17020423 |.

26. Information, N.C.f.B., PubChem Compound Summary for CID 186907, Aflatoxin B1, in National Center for Biotechnology Information. 2020, National Center for Biotechnology Information, 8600 Rockville Pike, Bethesda, MD, 20894 USA: The USA. p. PubChem Blog. https://pubchem.ncbi.nlm.nih.gov/compound/Aflatoxin-B1

27. Iqbal, S.Z., M.R. Asi, and A. Ariño, Aflatoxins, in Brenne''s Encyclopedia of Genetics (Second Edition), S. Maloy and K. Hughes, Editors. 2013, Academic Press: San Diego. p. 43-47. https://doi.org/10.1016/B978-0-12-374984-0.00022-X

28. Dhanasekaran, D., S. Shanmugapriya, N. Thajuddin, and A. Panneerselvam, Aflatoxins, and aflatoxicosis in human and animals. Aflatoxins-Biochemistry Molecular Biology, 2011. **1**: p. 221-254,

29. Holzapfel, C.W., P.S. Steyn, and I.F. Purchase, Isolation and structure of aflatoxins MI and M2. Tetrahedron Lett, 1966. **25**: p. 2799-2803

30. Allcroft, R., B.A. Roberts, and M.K. Lloyd, Excretion of aflatoxin in a lactating cow. Food Cosmetics Toxicology, 1968. **6**(5): p. 619-625,

31. Nabney, J., M.B. Burbage, R. Allcroft, and G. Lewis, Metabolism of aflatoxin in sheep: Excretion pattern in the lactating ewe. Food cosmetics toxicology, 1967. **5**: p. 11-17,

32. Heathcote, J.G. and J. Hibbert, Aflatoxins: chemical and biological aspects. 1978: Elsevier Scientific Publishing Co.

33. Caceres I, Khoury AA, Khoury RE, Lorber S, Oswald IP, Khoury AE, Atoui A, Puel O, Bailly JD. Aflatoxin Biosynthesis and Genetic Regulation: A Review. Toxins (Basel). 2020 Feb 28;12(3):150. doi: 10.3390/toxins12030150.

34. Krzyczkowska, J., Phan-Thi, H., Waché, Y. (2017). Lactone Formation in Yeast and Fungi. In: Mérillon, JM., Ramawat, K. (eds) Fungal Metabolites. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-25001-4_13.

35. Diaz, Gonzalo. (2011). Biotransformation of Aflatoxin B1 and Its Relationship with the Differential Toxicological Response to Aflatoxin in Commercial Poultry Species. https://doi.org/10.5772/22109.

36. World-mycotoxin-survey-impact-2021. Biomin Company for mycotoxin analysis. World Mycotoxin Survey: Impact 2021 | biomin.net.

37. Nierman, W.C., A. Pain, M.J. Anderson, J.R. Wortman, H.S. Kim, J. Arroyo, M. Berriman, K. Abe, D.B. Archer, C. Bermejo, J. Bennett, P. Bowyer, D. Chen, M. Collins, R. Coulsen, R. Davies, P.S. Dyer, M. Farman, N. Fedorova, N. Fedorova, T.V. Feldblyum, R. Fischer, N. Fosker, A. Fraser, J.L. García, M.J. García, A. Goble, G.H. Goldman, K. Gomi, S. Griffith-Jones, R. Gwilliam, B. Haas, H. Haas, D. Harris, H. Horiuchi, J. Huang, S. Humphray, J. Jiménez, N. Keller, H. Khouri, K. Kitamoto, T. Kobayashi, S. Konzack, R. Kulkarni, T. Kumagai, A. Lafton, J.-P. Latgé, W. Li, A. Lord, C. Lu, W.H. Majoros, G.S. May, B.L. Miller, Y. Mohamoud, M. Molina, M. Monod, I. Mouyna, S. Mulligan, L. Murphy, S. ''Neil, I. Paulsen, M.A. Peñalva, M. Pertea, C. Price, B.L. Pritchard, M.A. Quail, E. Rabbinowitsch, N. Rawlins, M.-A. Rajandream, U. Reichard, H. Renauld, G.D. Robson, S.R. de Córdoba, J.M. Rodríguez-Peña, C.M. Ronning, S. Rutter, S.L. Salzberg, M. Sanchez, J.C. Sánchez-Ferrero, D. Saunders, K. Seeger, R. Squares, S. Squares, M. Takeuchi, F. Tekaia, G. Turner, C.R.V. de Aldana, J. Weidman, O. White, J. Woodward, J.-H. Yu, C. Fraser, J.E. Galagan, K. Asai, M. Machida, N. Hall, B. Barrell, and D.W. Denning, Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus* fumigatus. Nature, 2005. **438**(7071): p. 1151-1156. DOI 10.1038/nature04332 |.

38. Kobayashi, J.i. and T. Kubota, Bioactive Macrolides and Polyketides from Marine Dinoflagellates of the Genus Amphidinium. Journal of Natural Products, 2007. **70**(3): p. 451-460. DOI 10.1021/np0605844 |.

39. Rokas, A., G. Payne, N.D. Fedorova, S.E. Baker, M. Machida, J. Yu, D.R. Georgianna, R.A. Dean, D. Bhatnagar, T.E. Cleveland, J.R. Wortman, R. Maiti, V. Joardar, P. Amedeo, D.W. Denning, and W.C. Nierman, What can comparative genomics tell us about species concepts in the genus *Aspergillus*? Studies in Mycology, 2007. **59**: p. 11-17. https://doi.org/10.3114/sim.2007.59.02 |.

40. Keller, N.P., G. Turner, and J.W. Bennett, Fungal secondary metabolism — from biochemistry to genomics. Nature Reviews Microbiology, 2005. **3**(12): p. 937-947. DOI: 10.1038/nrmicro1286

41. Adrio, J.L. and A.L. Demain, Fungal biotechnology. International Microbiology, 2003. **6**(3): p. 191-199. DOI 10.1007/s10123-003-0133-0 |.

42. Scheidegger, K.A. and G.A. Payne. Unlocking the Secrets Behind Secondary Metabolism: A Review of *Aspergillus* flavus from Pathogenicity to Functional Genomics. Journal of Toxicology: Toxin Reviews, 2003. **22**(2-3): p. 423-459. DOI 10.1081/TXR-120024100 |.

43. Groopman, J.D. and T.W. Kensler, Role of metabolism and viruses in aflatoxin-induced liver cancer. Toxicology and Applied Pharmacology, 2005. **206**(2): p. 131-137.https://doi.org/10.1016/j.taap.2004.09.020.

44. Duran, R.M., J.W. Cary, and A.M. Calvo, Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* are regulated by veA, a gene necessary for sclerotial formation. Applied Microbiology and Biotechnology, 2007. **73**(5): p. 1158-1168 DOI..10.1007/s00253-006-0581-5

45. Yu, J., D. Bhatnagar, and T.E. Cleveland, the Completed sequence of aflatoxin pathway gene cluster in *Aspergillus* parasiticus11The nucleotide sequence data in A. parasiticus reported here are available in the NCBI GenBank database under the accession number AY371490. FEBS Letters, 2004. **564**(1): p. 126-130. https://doi.org/10.1016/S0014-5793(04)00327-8|.

46. Price, M.S., J. Yu, W.C. Nierman, H.S. Kim, B. Pritchard, C.A. Jacobus, D. Bhatnagar, T.E. Cleveland, and G.A. Payne, The aflatoxin pathway regulator AflR induces gene transcription inside and outside of the aflatoxin biosynthetic cluster. FEMS Microbiology Letters, 2006. **255**(2): p. 275-279. DOI 10.1111/j.1574-6968.2005.00084.x |.

47. J. W. Bennett, F. G. Kronberg, L. A. Goodman & M. A. Seltman (1983) Isolation of an Anthraquinone-Accumulating Mutant of *Aspergillus* Parasiticus and Partial Characterization by Dry Column Chromatography, Mycologia, 75:2, 202-208, DOI: 10.1080/00275514.1983.12021656

48. Muaz, K. et al. (2021). Aflatoxin Biosynthesis. In: Hakeem, K.R., Oliveira, C.A.F., Ismail, A. (eds)

Aflatoxins in Food. Springer, Cham. https://doi.org/10.1007/978-3-030-85762-2_2

49. Okafor, S. E., and A. O. Eni.“"Microbial quality and the occurrence of aflatoxins in Plantain/Yam and wheat flours in Ado-Odo Ota”" In IOP conference series: earth and environmental science, vol. 210, no. 1, p. 012017. IOP Publishing, 2018. DOI 10.1088/1755-1315/210/1/012017

50. Arora, Dilip K., and Randy Berka. Genes and genomics. Elsevier, 2005.

51. Yu, J. Current Understanding on Aflatoxin Biosynthesis and Future Perspective in Reducing Aflatoxin Contamination. Toxins 2012, 4, 1024-1057. https://doi.org/10.3390/toxins4111024

52. Cary, J.W., Ehrlich, K.C., Beltz, S.B., Harris-Coward, P. and Klich, M.A., 2009. Characterization of the *Aspergillus* ochraceoroseus aflatoxin/sterigmatocystin biosynthetic gene cluster. Mycologia, 101(3), pp.352-362. DOI: 10.3852/08-173

53. Yu, Jiujiang, Deepak Bhatnagar, and Thomas E. Cleveland.“"Completed sequence of aflatoxin pathway gene cluster in *Aspergillus* parasiticus”" FEBS letters 564, no. 1-2 (2004): 126-130. DOI: https://doi.org/10.1016/S0014-5793(04)00327-8

54. Chang, P.K., J. Yu, D. Bhatnagar, and T.E. Cleveland, The Carboxy-Terminal Portion of the Aflatoxin Pathway Regulatory Protein AFLR of *Aspergillus* parasiticus ActivatesGAL1:: lacZ Gene Expression in *Saccharomyces cerevisiae*. Applied Environmental Microbiology, 1999. **65**(6): p. 2508-2512,

55. Cleveland, T., A. Lax, L. Lee, and D. Bhatnagar, Appearance of enzyme activities catalyzing the conversion of sterigmatocystin to aflatoxin B1 in late-growth-phase *Aspergillus* parasiticus cultures. Applied environmental microbiology, 1987. **53**(7): p. 1711-1713,

56. Crawford, J.M., P.M. Thomas, J.R. Scheerer, A.L. Vagstad, N.L. Kelleher, and C.A. Townsend, Deconstruction of iterative multidomain polyketide synthase function. Science, 2008. **320**(5873): p. 243-246,

57. Crawford, J.M., A.L. Vagstad, K.C. Ehrlich, and C.A. Townsend, Starter unit specificity directs genome mining of polyketide synthase pathways in fungi. Bioorganic Chemistry, 2008. **36**(1): p. 16-22 .https://doi.org/10.1016/j.bioorg.2007.11.002 |.

58. Crawford, J.M., A.L. Vagstad, K.P. Whitworth, K.C. Ehrlich, and C.A. Townsend, Synthetic Strategy of Nonreducing Iterative Polyketide Synthases and the Origin of the Classical “Starter-Unit Effect”. ChemBioChem, 2008. **9**(7): p. 1019-1023. DOI|10.1002/cbic.200700702 |.

59. Ehrlich, K.C., J.W. Cary, and B.G. Montalbano, Characterization of the promoter for the gene encoding the aflatoxin biosynthetic pathway regulatory protein AFLR. Biochimica et Biophysica Acta (BBA)–- Gene Structure and Expression, 1999. **1444**(3): p. 412-417.https://doi.org/10.1016/S0167-4781(99)00022-6 |.

60. Zhao, L.H., Guan, S., Gao, X., Ma, Q.G., Lei, Y.P., Bai, X.M. and Ji, C. (2011), Preparation, purification and characteristics of an aflatoxin degradation enzyme from *Myxococcus fulvus* ANSM068. Journal of Applied Microbiology, 110: 147-155. https://doi.org/10.1111/j.1365-2672.2010.04867.x

60. Ehrlich, K.C., Predicted Roles of the Uncharacterized Clustered Genes in Aflatoxin Biosynthesis. Toxins (Basel), 2009. **1**(1): p. 37-58. DOI|10.3390/toxins1010037 |.

61. Ehrlich, K.C. and J. Yu, Aflatoxin-like Gene Clusters and How They Evolved, in Mycotoxins in Food, Feed, and Bioweapons, M. Rai and A. Varma, Editors. 2010, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 65-75. DOI:10.1007/978-3-642-00725-5_5

62. Chang, P.-K., B.W. Horn, and J.W. Dorner, Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus* flavus isolates. Fungal Genetics and Biology, 2005. **42**(11): p. 914-923.https://doi.org/10.1016/j.fgb.2005.07.004|.

63. Wilson, D.M., Analytical methods for aflatoxins in corn and peanuts. Archives of Environmental Contamination and Toxicology, 1989. **18**(3): p. 308-314. DOI 10.1007/BF01062353 |.

64. Rao, K.R., Vipin, A.V. and Venkateswaran, G., 2020. Mechanism of inhibition of aflatoxin synthesis by non-aflatoxigenic strains of *Aspergillus* flavus. Microbial pathogenesis, 147,

p.104280. https://doi.org/10.1016/j.micpath.2020.104280.

65. Xu, J. and Luo, X., 2003. Molecular biology of aflatoxin biosynthesis. *Wei Sheng yan jiu= Journal of Hygiene Research*, *32*(6), pp.628-631.

66. Madbouly AK, Rashad YM, Ibrahim MIM, Elazab NT. Biodegradation of Aflatoxin B1 in Maize Grains and Suppression of Its Biosynthesis-Related Genes Using Endophytic Trichoderma harzianum AYM3. Journal of Fungi. 2023; 9(2):209. https://doi.org/10.3390/jof9020209

67. Yu, J., D. Bhatnagar, and T.E. Cleveland, a Completed sequence of aflatoxin pathway gene cluster in *Aspergillus* parasiticus. FEBS Letters, 2004. **564**(1-2): p. 126-130. DOI 10.1016/s0014-5793(04)00327-8 |.

68. Bhatnagar, D., K.C. Ehrlich, and T.E. Cleveland, Molecular genetic analysis and regulation of aflatoxin biosynthesis. Applied Microbiology and Biotechnology, 2003. **61**(2): p. 83-93. DOI 10.1007/s00253-002-1199-x |.

69. Cary, J.W., D. Bhatnagar, and J.E. Linz, Aflatoxins: Biological significance and regulation of biosynthesis, in Microbial Foodborne Diseases., J.W. Cary, et al., Editor. 2000, Technomic Publishing Co.: Lancaster, Pennsylvania. p. 317–362,

70. Yabe, K., Nakajima, H. Enzyme reactions and genes in aflatoxin biosynthesis. Appl Microbiol Biotechnol 64, 745–755 (2004). https://doi.org/10.1007/s00253-004-1566-x

.

71. Ehrlich, K.C., P.K. Chang, J. Yu, and P.J. Cotty, Aflatoxin Biosynthesis Cluster Gene cypA Is Required for G Aflatoxin Formation. Applied and Environmental Microbiology, 2004. **70**(11): p. 6518-6524. DOI 10.1128/AEM.70.11.6518-6524.2004 |.

72. Cary, J.W., K.C. Ehrlich, J.M. Bland, and B.G. Montalbano, The Aflatoxin Biosynthesis Cluster Gene, aflX, Encodes an Oxidoreductase Involved in Conversion of Versicolorin A to Demethylsterigmatocystin. Applied and Environmental Microbiology, 2006. **72**(2): p. 1096-1101. DOI 10.1128/AEM.72.2.1096-1101.2006 |.

73. Bain, G., E.C. Maandag, D.J. Izon, D. Amsen, A.M. Kruisbeek, B.C. Weintraub, I. Krop, M.S. Schlissel, A.J. Feeney, M. van Roon, and et al., E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. Cell, 1994. **79**(5): p. 885-92. DOI|10.1016/0092-8674(94)90077-9|.

74. Kwon, K., Hutter, C., Sun, Q., Bilic, I., Cobaleda, C., Malin, S. and Busslinger, M., 2008. Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. Immunity, 28(6), pp.751-762. https://doi.org/10.1016/j.immuni.2008.04.014.

75. Frasca D, Nguyen D, Riley RL, Blomberg BB. Effects of aging on DNA-binding activity of the E47 transcription factor in splenic B cells. Mechanisms of Ageing and Development. 2004. 125(2):111-112. DOI: 10.1016/j.mad.2003.11.004.

76. Kim S, Lahmy R, Riha C, Yang C, Jakubison BL, van Niekerk J, Staub C, Wu Y, Gates K, Dong DS, Konieczny SF, Itkin-Ansari P. The basic helix-loop-helix transcription factor E47 reprograms human pancreatic cancer cells to a quiescent acinar state with reduced tumorigenic potential. Pancreas. 2015 Jul;44(5):718-27. DOI: 10.1097/MPA.0000000000000328.

77. Xu, J., Li, F., Gao, Y. et al. E47 upregulates ΔNp63α to promote growth of squamous cell carcinoma. Cell Death Dis 12, 381 (2021). https://doi.org/10.1038/s41419-021-03662-3

78. Bhatnagar, D., J.W. Cary, K. Ehrlich, J. Yu, and T.E. Cleveland, Understanding the genetics of regulation of aflatoxin production and *Aspergillus* flavus development. Mycopathologia, 2006. **162**(3): p. 155,

79. Wang P, Xu J, Chang PK, Liu Z, Kong Q. New Insights of Transcriptional Regulator AflR in *Aspergillus* flavus Physiology. Microbiol Spectr. 2022 Feb 23;10(1):e0079121. doi: 10.1128/spectrum.00791-21.

80. Junhua Liao, Zemin He, Youlin Xia, Yong Lei, Boshou Liao. A review on biosynthesis and genetic regulation of aflatoxin production by major *Aspergillus* fungi, Oil Crop Science.2020. 5(4):166-173.https://doi.org/10.1016/j.ocsci.2020.11.001.

81. Akinola, S.A., Ateba, C.N. and Mwanza, M., 2021. Behaviour of *Aspergillus* parasiticus in aflatoxin production as influenced by storage parameters using response surface methodology approach. International Journal of Food Microbiology, 357, p.109369. https://doi.org/10.1016/j.ijfoodmicro.2021.109369.

82. Chang, P.-K., J. Yu, D. Bhatnagar, and T.E. Cleveland, Characterization of the *Aspergillus* parasiticus major nitrogen regulatory gene, areA. Biochimica et Biophysica Acta (BBA)–- Gene Structure and Expression, 2000. **1491**(1): p. 263-266. DOI 10.1016/S0167-4781(00)00004-X |.

83. Mylroie JE, Ozkan S, Shivaji R, Windham GL, Alpe MN, Williams WP. Identification and Quantification of a Toxigenic and Non-Toxigenic *Aspergillus flavus* Strain in Contaminated Maize Using Quantitative Real-Time PCR. Toxins. 2016; 8(1):15. https://doi.org/10.3390/toxins8010015

84. Saruwatari, T., Praseuth, A., Sato, M. et al. A comprehensive overview on genomically directed assembly of aromatic polyketides and macrolide lactones using fungal mega synthases. J Antibiot 64, 9–17 (2011). https://doi.org/10.1038/ja.2010.130

85. Brown, D.W., T.H. Adams, and N.P. Keller, *Aspergillus* has distinct fatty acid synthases for primary and secondary metabolism. Proceedings of the National Academy of Sciences, 1996. **93**(25): p. 14873-14877. DOI|10.1073/pnas.93.25.14873|.

86. Barajas, J.F., Shakya, G., Moreno, G., Rivera Jr, H., Jackson, D.R., Topper, C.L., Vagstad, A.L., La Clair, J.J., Townsend, C.A., Burkart, M.D. and Tsai, S.C., 2017. Polyketide mimetics yield structural and mechanistic insights into product template domain function in nonreducing polyketide synthases. *Proceedings of the National Academy of Sciences*, *114*(21), pp.E4142-E4148.

87. Watanabe, C.M.H. and C.A. Townsend, Initial Characterization of a Type I Fatty Acid Synthase and Polyketide Synthase Multienzyme Complex *NorS* in the Biosynthesis of Aflatoxin B1. Chemistry & Biology, 2002. **9**(9): p. 981-988. https://doi.org/10.1016/S1074-5521(02)00213-2 |.

88. Crawford, J.M., B.C.R. Dancy, E.A. Hill, D.W. Udwary, and C.A. Townsend, Identification of a starter unit acyl-carrier-protein transacylase domain in an iterative type I polyketide synthase. Proceedings of the National Academy of Sciences, 2006. **103**(45): p. 16728-16733. DOI|10.1073/pnas.0604112103|.

89. Chang, PK., Bennett, J.W. & Cotty, P.J. Association of aflatoxin biosynthesis and sclerotial development in *Aspergillus* parasiticus. Mycopathologia 153, 41–48 (2002). https://doi.org/10.1023/A:1015211915310.

90. Yabe, K. and H. Nakajima, Enzyme reactions and genes in aflatoxin biosynthesis. Applied Microbiology and Biotechnology, 2004. **64**(6): p. 745-755. DOI|10.1007/s00253-004-1566-x|.

91. Payne, G.A., Process of Contamination by Aflatoxin-Producing Fungi and Their Impacts on Crops., in Mycotoxins in Agriculture and Food Safety, K.K. Sinha and D. Bhatnagar, Editors. 1998, Marcel Dekker: New York, NY, USA. p. 279–306.,

92. Brown, D.W., J.H. Yu, H.S. Kelkar, M. Fernandes, T.C. Nesbitt, N.P. Keller, T.H. Adams, and T.J. Leonard, Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus* nidulans. Proceedings of the National Academy of Sciences, 1996. **93**(4): p. 1418-1422. DOI|10.1073/pnas.93.4.1418|.

93. Hitchman, T.S., E.W. Schmidt, F. Trail, M.D. Rarick, J.E. Linz, and C.A. Townsend, Hexanoate Synthase, a Specialized Type I Fatty Acid Synthase in Aflatoxin B1 Biosynthesis. Bioorganic Chemistry, 2001. **29**(5): p. 293-307.https://doi.org/10.1006/bioo.2001.1216|.

94. Papa, K.E., Genetics of *Aspergillus* flavus: complementation and mapping of aflatoxin mutants. Genetical Research, 1979. **34**(1): p. 1-9. DOI|10.1017/S0016672300019236|.

95. Bennett, J.W., Aflatoxins, and Anthraquinones from Diploids of *Aspergillus* parasiticus. Microbiology, 1979. **113**(1): p. 127-136.https://doi.org/10.1099/00221287-113-1-127|.

96. Papa, K.E., Norsolorinic Acid Mutant of *Aspergillus* flavus. Microbiology, 1982. **128**(6): p. 1345-1348.https://doi.org/10.1099/00221287-128-6-1345|.

97. Lee, L.S., J.W. Bennett, L.A. Goldblatt, and R.E. Lundin, Norsolorinic acid from a mutant strain of *Aspergillus* parasiticus. J Am Oil Chem Soc, 1971. **48**(2): p. 93-94. DOI|10.1007/bf02635696|.

98. Yu, J., P.-K. Chang, K.C. Ehrlich, J.W. Cary, D. Bhatnagar, T.E. Cleveland, G.A. Payne, J.E. Linz, C.P. Woloshuk, and J.W. Bennett, Clustered Pathway Genes in Aflatoxin Biosynthesis. Applied and Environmental Microbiology, 2004. **70**(3): p. 1253-1262. DOI|10.1128/AEM.70.3.1253-1262.2004|.

99. Bennett, J.W., D. Bhatnagar, and P.K. Chang, The Molecular Genetics of Aflatoxin Biosynthesis, in The Genus *Aspergillus*: From Taxonomy and Genetics to Industrial Application, K.A. Powell, A. Renwick, and J.F. Peberdy, Editors. 1994, Springer US: Boston, MA. p. 51-58. DOI|10.1007/978-1-4899-0981-7_3
100. Chang, P.-K., C.D. Skory, and J.E. Linz, Cloning of a gene associated with aflatoxin B1 biosynthesis in *Aspergillus* parasiticus. Current Genetics, 1992. **21**(3): p. 231-233. DOI|10.1007/BF00336846|.
101. Zhou, R. and J.E. Linz, Enzymatic Function of the Nor-1 Protein in Aflatoxin Biosynthesis in *Aspergillus parasiticus*. Applied and Environmental Microbiology, 1999. **65**(12):p.5639-5641. DOI|10.1128/AEM.65.12.5639-5641.1999|.
102. Trail, F., P.K. Chang, J. Cary, and J.E. Linz, Structural and functional analysis of the nor-1 gene involved in the biosynthesis of aflatoxins by *Aspergillus* parasiticus. Appl Environ Microbiol, 1994. **60**(11): p. 4078-4085, https://aem.asm.org/content/aem/60/11/4078.full.pdf.
103. Cary, J.W., M. Wright, D. Bhatnagar, R. Lee, and F.S. Chu, Molecular characterization of an *Aspergillus* parasiticus dehydrogenase gene, norA, located on the aflatoxin biosynthesis gene cluster. Appl Environ Microbiol, 1996. **62**(2): p. 360-366, https://aem.asm.org/content/aem/62/2/360.full.pdf
104. Bennett, J.W., L.S. Lee, S.M. Shoss, and G.H. Boudreaux, Identification of averantin as an aflatoxin B1 precursor: placement in the biosynthetic pathway. Appl Environ Microbiol, 1980. **39**(4): p. 835-839, https://aem.asm.org/content/aem/39/4/835.full.pdf.
105. McCormick, S.P., D. Bhatnagar, and L.S. Lee, Averufanin is an aflatoxin B1 precursor between averantin and averufin in the biosynthetic pathway. Appl Environ Microbiol, 1987. **53**(1): p. 14-16, https://aem.asm.org/content/aem/53/1/14.full.pdf .
106. Yabe, K., Y. Ando, and T. Hamasaki, A metabolic grid among versiconal hemiacetal acetate, versiconol acetate, versiconol and *versiconal* during aflatoxin biosynthesis. J. Gen. Microbiol, 1991. **137**(10): p. 2469-2475. https://doi.org/10.1099/00221287-137-10-2469.
107. Yabe, K., Y. Nakamura, H. Nakajima, Y. Ando, and T. Hamasaki, Enzymatic conversion of norsolorinic acid to averufin in aflatoxin biosynthesis. Appl Environ Microbiol, 1991. **57**(5): p. 1340-1345, https://aem.asm.org/content/aem/57/5/1340.full.pdf.
108. Yu, J., P.K. Chang, J.W. Cary, D. Bhatnagar, and T.E. Cleveland, avnA, a gene encoding a cytochrome P-450 monooxygenase, is involved in the conversion of averantin to averufin in aflatoxin biosynthesis in *Aspergillus* parasiticus. Appl Environ Microbiol, 1997. **63**(4): p. 1349-1356, https://aem.asm.org/content/aem/63/4/1349.full.pdf.
109. Hsieh, D.P.H., Inhibition of aflatoxin biosynthesis of dichlorvos. Journal of Agricultural and Food Chemistry, 1973. **21**(3): p. 468-470. DOI: 10.1021/jf60187a035 |.
110. Singh, R. and D.P.H. Hsieh, Aflatoxin biosynthetic pathway: Elucidation by using blocked mutants of *Aspergillus* parasiticus. Archives of Biochemistry and Biophysics, 1977. **178**(1): p. 285-292. DOI|10.1016/0003-9861(77)90193-X|.
111. Fitzell, D.L., D.P.H. Hsieh, R.C. Yao, and G.N. La Mar, Biosynthesis of averufin from acetate by *Aspergillus* parasiticus. Journal of Agricultural and Food Chemistry, 1975. **23**(3): p. 442-444. DOI 10.1021/jf60199a039 |.
112. Lin, M.T., D.P.H. Hsieh, R.C. Yao, and J.A. Donkersloot, Conversion of averufin into aflatoxins by *Aspergillus* parasiticus. Biochemistry, 1973. **12**(25): p. 5167-5171. DOI|10.1021/bi00749a023|.
113. Lin, M.T. and D.P.H. Hsieh, Averufin in the biosynthesis of aflatoxin B1. Journal of the American Chemical Society, 1973. **95**(5): p. 1668-1669. DOI|10.1021/ja00786a056|.
114. Keller, N.P., C.M.H. Watanabe, H.S. Kelkar, T.H. Adams, and C.A. Townsend, Requirement of Monooxygenase-Mediated Steps for Sterigmatocystin Biosynthesis by *Aspergillus nidulans*. Applied and Environmental Microbiology, 2000. **66**(1): p. 359-362. DOI|.10.1128/AEM.66.1.359-362.2000
115. Chang, P.-K., J. Yu, K.C. Ehrlich, S.M. Boue, B.G. Montalbano, D. Bhatnagar, and T.E. Cleveland, adhA in *Aspergillus* parasiticus Is Involved in Conversion of 5′-Hydroxyaverantin to Averufin. Applied and Environmental Microbiology, 2000. **66**(11): p. 4715-4719. DOI|10.1128/AEM.66.11.4715-4719.2000.
116. Sakuno, E., K. Yabe, and H. Nakajima, Involvement of Two Cytosolic Enzymes and a Novel Intermediate, 5′-Oxoaverantin, in the Pathway from 5′-Hydroxyaverantin to Averufin

in Aflatoxin Biosynthesis. Applied and Environmental Microbiology, 2003. **69**(11): p. 6418-6426. DOI|10.1128/AEM.69.11.6418-6426.2003.

117. Ehrlich, K.C., P.-K. Chang, L.L. Scharfenstein, Jr, J.W. Cary, J.M. Crawford, and C.A. Townsend, Absence of the aflatoxin biosynthesis gene, norA, allows accumulation of deoxyaflatoxin B1 in *Aspergillus* flavus cultures. FEMS Microbiology Letters, 2010. **305**(1): p. 65-70. DOI|10.1111/j.1574-6968.2010.01914.x.

118. Yu, J., C.P. Woloshuk, D. Bhatnagar, and T.E. Cleveland, Cloning and characterization of avfA and omtB genes involved in aflatoxin biosynthesis in three *Aspergillus* species. Gene, 2000. **248**(1): p. 157-167. DOI:10.1016/S0378-1119(00)00126-8|.

119. Ahmed, Y.L., J. Gerke, H.-S. Park, Ö. Bayram, P. Neumann, M. Ni, A. Dickmanns, S.C. Kim, J.-H. Yu, G.H. Braus, and R. Ficner, The Velvet Family of Fungal Regulators Contains a DNA-Binding Domain Structurally Similar to NF-κB. PLOS Biology, 2014. **11**(12): p. e1001750. DOI|10.1371/journal.pbio.1001750|.

120. Bayram, Ö. and G.H. Braus, Coordination of secondarymetabolism and development in fungi: the velvet familyof regulatory proteins. FEMS Microbiology Reviews, 2012. **36**(1): p. 1-24. DOI|10.1111/j.1574-6976.2011.00285.x.

121. Bayram, Ö., S. Krappmann, M. Ni, J.W. Bok, K. Helmstaedt, O. Valerius, S. Braus-Stromeyer, N.-J. Kwon, N.P. Keller, J.-H. Yu, and G.H. Braus, VelB/VeA/LaeA Complex Coordinates Light Signal with Fungal Development and Secondary Metabolism. Science, 2008. **320**(5882): p. 1504-1506. DOI|10.1126/science.1155888.

122. Sarikaya-Bayram, Ö., J.M. Palmer, N. Keller, G.H. Braus, and Ö. Bayram, One Juliet and four Romeos: VeA and its methyltransferases. Front Microbiol, 2015. **6**: p. 1-1. DOI|10.3389/fmicb.2015.00001.

123. Palmer, J.M., J.M. Theisen, R.M. Duran, W.S. Grayburn, A.M. Calvo, and N.P. Keller, Secondary Metabolism and Development Is Mediated by LlmF Control of VeA Subcellular Localization in *Aspergillus* nidulans. PLOS Genetics, 2013. **9**(1): p. e1003193. DOI|10.1371/journal.pgen.1003193|.

124. Purschwitz, J., S. Müller, and R. Fischer, Mapping the interaction sites of *Aspergillus* nidulans phytochrome FphA with the global regulator VeA and the White Collar protein LreB. Molecular Genetics and Genomics, 2009. **281**(1): p. 35-42. DOI|10.1007/s00438-008-0390-x.

125. Fasoyin, O.E., K. Yang, M. Qiu, B. Wang, S. Wang, and S. Wang, Regulation of Morphology, Aflatoxin Production, and Virulence of *Aspergillus* flavus by the Major Nitrogen Regulatory Gene areA. Toxins (Basel), 2019. **11**(12): p. 718. DOI|10.3390/toxins11120718|.

126. Chang, P.-K., Lack of interaction between AFLR and AFLJ contribute to non aflatoxigenicity of *Aspergillus* sojae. Journal of Biotechnology, 2004. **107**(3): p. 245-253.https://doi.org/10.1016/j.jbiotec.2003.10.012 .

127. Lin, B.-K. and J.A. Anderson, Purification and properties of versiconal cyclase from *Aspergillus* parasiticus. Archives of Biochemistry and Biophysics, 1992. **293**(1): p. 67-70. https://doi.org/10.1016/0003-9861(92)90366-5.

128. McGlynn, K.A., K. Hunter, T. LeVoyer, J. Roush, P. Wise, R.A. Michielli, F.-M. Shen, A.A. Evans, W.T. London, and K.H. Buetow, Susceptibility to aflatoxin B1-related primary hepatocellular carcinoma in mice and humans. Cancer research, 2003. **63**(15): p. 4594-4601,

129. Silva, J.C., R.E. Minto, C.E. Barry, K.A. Holland, and C.A. Townsend, Isolation and Characterization of the Versicolorin B Synthase Gene from *Aspergillus* parasiticus Expansion of the Aflatoxin B1 Biosynthetic Gene Cluster. Journal of Biological Chemistry, 1996. **271**(23): p. 13600-13608. DOI|10.1074/jbc.271.23.13600.

130. Ren S, Yue Y, Li Y, Guo X, Wang S. Functional analyses of the versicolorin B synthase gene in *Aspergillus* flavus. Microbiologyopen. 2017 ;6(4):e00471. DOI: 10.1002/mbo3.471.

131. McGuire, S.M., J.C. Silva, E.G. Casillas, and C.A. Townsend, Purification and Characterization of Versicolorin B Synthase from *Aspergillus* parasiticus. Catalysis of the Stereodifferentiating Cyclization in Aflatoxin Biosynthesis Essential to DNA Interaction. Biochemistry, 1996. **35**(35): p. 11470-11486. DOI|10.1021/bi960924s.

132. Linnemannstöns, P., Schulte, J., del Mar Prado, M., Proctor, R.H., Avalos, J. and Tudzynski, B., 2002. The polyketide synthase gene pks4 from Gibberella fujikuroi encodes a key enzyme in the biosynthesis of the red pigment bikaverin. Fungal

Genetics and Biology, 37(2), pp.134-148. https://doi.org/10.1016/S1087-1845(02)00501-7

133. Muaz, K. et al. (2021). Aflatoxin Biosynthesis. In: Hakeem, K.R., Oliveira, C.A.F., Ismail, A. (eds) Aflatoxins in Food. Springer, Cham. https://doi.org/10.1007/978-3-030-85762-2_2

134. Kelkar, H.S., T.W. Skloss, J.F. Haw, N.P. Keller, and T.H. Adams, *Aspergillus nidulans* stcL encodes a putative cytochrome P-450 monooxygenase required for bisfuran desaturation during aflatoxin/sterigmatocystin biosynthesis. Journal of Biological Chemistry, 1997. **272**(3): p. 1589-1594,

135. Henry, K.M. and C.A. Townsend. Ordering the reductive and cytochrome P450 oxidative steps in demethyl sterigmatocystin formation yields general insights into the biosynthesis of aflatoxin and related fungal metabolites. . J. Am. Chem. Soc., 2005. **127**(5): p. 3724–3733.,

136. Calvo AM, Bok J, Brooks W, Keller NP. veA is required for toxin and sclerotial production in *Aspergillus parasiticus*. Appl Environ Microbiol. 2004 Aug;70(8):4733-9. doi: 10.1128/AEM.70.8.4733-4739.2004.

137. Keller, N.P., S. Segner, D. Bhatnagar, and T.H. Adams, stcS, a putative P-450 monooxygenase, is required for the conversion of versicolorin A to sterigmatocystin in *Aspergillus nidulans*. Appl Environ Microbiol, 1995. **61**(10): p. 3628-3632, https://aem.asm.org/content/aem/61/10/3628.full.pdf.

138. Yabe, K., Y. Ando, J. Hashimoto, and T. Hamasaki, Two distinct O-methyltransferases in aflatoxin biosynthesis. Appl Environ Microbiol, 1989. **55**(9): p. 2172-2177, https://aem.asm.org/content/aem/55/9/2172.full.pdf.

139. Yabe, K., K.-i. Matsushima, T. Koyama, and T. Hamasaki, Purification and Characterization of O Methyltransferase I Involved in Conversion of Demethylsterigmatocystin to Sterigmatocystin and of Dihydrodemethylsterigmatocystin to Dihydrosterigmatocystin during Aflatoxin Biosynthesis. Appl Environ Microbiol, 1998. **64**(1): p. 166-171. DOI|10.1128/AEM.64.1.166-171.1998.

140. Yabe, K., M. Nakamura, and T. Hamasaki, Enzymatic Formation of G-Group Aflatoxins and Biosynthetic Relationship between G- and B-Group Aflatoxins. Appl Environ Microbiol, 1999. **65**(9): p. 3867-3872. DOI|10.1128/AEM.65.9.3867-3872.1999.

141. Motomura, M., N. Chihaya, T. Shinozawa, T. Hamasaki, and K. Yabe, Cloning and Characterization of theO-Methyltransferase I Gene (dmtA) from *Aspergillus parasiticus* Associated with the Conversions of Demethylsterigmatocystin to Sterigmatocystin and Dihydrodemethylsterigmatocystin to Dihydrosterigmatocystin in Aflatoxin Biosynthesis. Applied and Environmental Microbiology, 1999. **65**(11): p. 4987-4994. DOI|10.1128/AEM.65.11.4987-4994.1999.

142. Kelkar, H.S., N.P. Keller, and T.H. Adams, *Aspergillus nidulans* stcP encodes an O-methyltransferase that is required for sterigmatocystin biosynthesis. Appl Environ Microbiol, 1996. **62**(11): p. 4296-4298, https://aem.asm.org/content/aem/62/11/4296.full.pdf.

143. Yu, J., J.W. Cary, D. Bhatnagar, T.E. Cleveland, N.P. Keller, and F.S. Chu, Cloning and characterization of a cDNA from *Aspergillus parasiticus* encoding an O-methyltransferase involved in aflatoxin biosynthesis. Appl Environ Microbiol, 1993. **59**(11): p. 3564-3571, https://aem.asm.org/content/aem/59/11/3564.full.pdf.

144. Yu, J., P.-K. Chang, G.A. Payne, J.W. Cary, D. Bhatnagar, and T.E. Cleveland, Comparison of the omtA genes encoding O-methyltransferases involved in aflatoxin biosynthesis from *Aspergillus parasiticus* and A. flavus. Gene, 1995. **163**(1): p. 121-125. DOI|10.1016/0378-1119(95)00397-O.

145. Klich, M.A., J. Yu, P.K. Chang, E.J. Mullaney, D. Bhatnagar, and T.E. Cleveland, Hybridization of genes involved in aflatoxin biosynthesis to DNA of aflatoxigenic and non-aflatoxigenic aspergilli. Applied Microbiology and Biotechnology, 1995. **44**(3): p. 439-443. DOI|10.1007/BF00169941.

146. Yabe, K., Y. Ando, and T. Hamasaki, Biosynthetic relationship among aflatoxins B1, B2, G1, and G2. Applied and Environmental Microbiology, 1988. **54**(8): p. 2101-2106, https://aem.asm.org/content/aem/54/8/2101.full.pdf.

147. Prieto, R. and C.P. Woloshuk, ord1, an oxidoreductase gene responsible for conversion of O-methyl sterigmatocystin to aflatoxin in

Aspergillus flavus. Appl Environ Microbiol, 1997. **63**(5): p. 1661-1666, https://aem.asm.org/content/aem/63/5/1661.full.pdf.

148. Prieto, R., G.L. Yousibova, and C.P. Woloshuk, Identification of aflatoxin biosynthesis genes by genetic complementation in an *Aspergillus flavus* mutant lacking the aflatoxin gene cluster. Appl Environ Microbiol, 1996. **62**(10): p. 3567-3571, https://aem.asm.org/content/aem/62/10/3567.full.pdf.

149. Yu, J., P.-K. Chang, K.C. Ehrlich, J.W. Cary, B. Montalbano, J.M. Dyer, D. Bhatnagar, and T.E. Cleveland, Characterization of the Critical Amino Acids of an *Aspergillus parasiticus* Cytochrome P-450 Monooxygenase Encoded by ordA. That Is Involved in the Biosynthesis of Aflatoxins B1, G1, B2, and G2. Applied and Environmental Microbiology, 1998. **64**(12): p. 4834-4841. DOI|10.1128/AEM.64.12.4834-4841.1998.

150. Ehrlich, K.C., P.K. Chang, J. Yu, J.W. Cary, and D. Bhatnagar, Control of aflatoxin biosynthesis in Aspergilli. Aflatoxins–- Biochemistry and Molecular Biology. Vol. 2. 2011, Rijeka, Croatia: InTech-open, https://www.intechopen.com/books/aflatoxins-biochemistry-and-molecular-biology/control-of-aflatoxin-biosynthesis-in-aspergilli.

151. Ehrlich, K.C., L.L. Scharfenstein, B.G. Montalbano, and P.-K.J.I.J.o.M.S. Chang, Are the genes nadA and norB involved in formation of aflatoxin G1? Int. J. Mol. Sci., 2008. **9**(9): p. 1717-1729. DOI|10.3390/ijms9091717.

152. Rice, D.W. and D.P. Hsieh, Aflatoxin M1: in vitro preparation and comparative in vitro metabolism versus aflatoxin B1 in the rat and mouse. Res Commun Chem Pathol Pharmacol, 1982. **35**(3): p. 467-490, http://europepmc.org/abstract/MED/6805048.

153. Garrido, N.S., M.H. Iha, M.R. Santos Ortolani, and R.M. Duarte Fávaro, Occurrence of aflatoxins M1 and M2 in milk commercialized in Ribeirão Preto-SP, Brazil. Food Additives & Contaminants, 2003. **20**(1): p. 70-73. DOI|10.1080/0265203021000035371.

154. Hsieh, D.P., J.M. Cullen, L.S. Hsieh, Y. Shao, and B.H. Ruebner, Cancer risks posed by aflatoxin M1. Princess Takamatsu symposia, 1985. **16**: p. 57-65,

155. Yabe, K., N. Chihaya, H. Hatabayashi, M. Kito, S. Hoshino, H. Zeng, J. Cai, and H. Nakajima, Production of M-/GM-group aflatoxins catalyzed by the OrdA enzyme in aflatoxin biosynthesis. Fungal Genetics and Biology, 2012. **49**(9): p. 744-754. https://doi.org/10.1016/j.fgb.2012.06.011.

156. Meyers, D.M., G. Obrian, W.L. Du, D. Bhatnagar, and G.A. Payne, Characterization of aflJ, a Gene Required for Conversion of Pathway Intermediates to Aflatoxin. Appl Environ Microbiol, 1998. **64**(10): p. 3713-3717. DOI: 10.1128/AEM.64.10.3713-3717.1998 |.

157. Bok, J.W., and N.P. Keller, LaeA, a Regulator of Secondary Metabolism in *Aspergillus* spp. Eukaryotic Cell, 2004. **3**(2): p. 527-535. DOI|.10.1128/EC.3.2.527-535.2004

158. Calvo, A.M., J. Bok, W. Brooks, and N.P. Keller, veA. Is Required for Toxin and Sclerotial Production in *Aspergillus parasiticus*. Appl Environ Microbiol, 2004. **70**(8): p. 4733-4739. DOI|10.1128/AEM.70.8.4733-4739.2004.

159. Fernandes, M., Keller, N.P. and Adams, T.H. (1998), Sequence-specific binding by *Aspergillus nidulans* AflR, a C6 zinc cluster protein regulating mycotoxin biosynthesis. Molecular Microbiology, 28: 1355-1365. https://doi.org/10.1046/j.1365-958.1998.00907.x

160. Cary, J.W., K.C. Ehrlich, M. Wright, P.K. Chang, and D. Bhatnagar, Generation of aflR disruption mutants of *Aspergillus parasiticus*. Applied Microbiology and Biotechnology, 2000. **53**(6): p. 680-684. DOI|10.1007/s002530000319.

161. Wilkinson, J.R., J. Yu, H.K. Abbas, B.E. Scheffler, H.S. Kim, W.C. Nierman, D. Bhatnagar, and T.E. Cleveland, Aflatoxin formation and gene expression in response to carbon source media shift in *Aspergillus parasiticus*. Food Additives & Contaminants, 2007. **24**(10): p. 1051-1060. DOI|.10.1080/02652030701579454

162. Wilkinson, J.R., J. Yu, J.M. Bland, W.C. Nierman, D. Bhatnagar, and T.E. Cleveland, Amino acid supplementation reveals differential regulation of aflatoxin biosynthesis in *Aspergillus flavus* NRRL 3357 and *Aspergillus parasiticus* SRRC 143. Applied Microbiology and Biotechnology, 2007. **74**(6): p. 1308-1319. DOI|.10.1007/s00253-006-0768-9

163. Du, W., G.R. Obrian, and G.A. Payne, Function and regulation of aflJ in the accumulation of aflatoxin early pathway intermediate in

Aspergillus flavus. Food Additives & Contaminants, 2007. **24**(10): p. 1043-1050. DOI|.10.1080/02652030701513826

164. Bouhired, S., M. Weber, A. Kempf-Sontag, N.P. Keller, and D. Hoffmeister, Accurate prediction of the *Aspergillus nidulans* terrequinone gene cluster boundaries using the transcriptional regulator LaeA. Fungal Genetics and Biology, 2007. **44**(11): p. 1134-1145. DOI|10.1016/j.fgb.2006.12.010.

165. Perrin, R.M., N.D. Fedorova, J.W. Bok, R.A. Cramer, Jr., J.R. Wortman, H.S. Kim, W.C. Nierman, and N.P. Keller, Transcriptional Regulation of Chemical Diversity in *Aspergillus fumigatus* by LaeA. PLOS Pathogens, 2007. **3**(4): p. e50. DOI 10.1371/journal.ppat.0030050 |.

166. Bok, J.W., D. Noordermeer, S.P. Kale, and N.P. Keller, Secondary metabolic gene cluster silencing in *Aspergillus nidulans*. 2006. **61**(6): p. 1636-1645. DOI|. 10.1111/j.1365-2958.2006.05330.x

167.Kale, S.P., J.W. Cary, N. Hollis, J.R. Wilkinson, D. Bhatnagar, J. Yu, T.E. Cleveland, and J.W. Bennett, Analysis of aflatoxin regulatory factors in serial transfer-induced non-aflatoxigenic Aspergillus parasiticus. Food Additives & Contaminants, 2007. **24**(10): p. 1061-1069. DOI|.10.1080/02652030701564563

168. Mooney, J.L., and L.N. Yager, Light is required for conidiation in *Aspergillus nidulans*. Genes & development, 1990. **4**(9): p. 1473-82. DOI|.10.1101/gad.4.9.1473

169. Stinnett, S.M., E.A. Espeso, L. Cobeño, L. Araújo-Bazán, and A.M. Calvo, *Aspergillus nidulans* VeA subcellular localization is dependent on the importin α carrier and on light. Molecular Microbiology, 2007. **63**(1): p. 242-255. DOI|.10.1111/j.1365-2958.2006.05506.x

170. Feng, G.H. and T.J. Leonard, Culture Conditions Control Expression of the Genes for Aflatoxin and Sterigmatocystin Biosynthesis in *Aspergillus parasiticus* and A. nidulansAppl Environ Microbiol, 1998. **64**(6): p. 2275-2277. DOI:10.1128/AEM.64.6.2275-2277.1998|.

171 Ahmad MM, Qamar F, Saifi M, Abdin MZ. Natural inhibitors: A sustainable way to combat aflatoxins. Front Microbiol. 2022 Dec 8;13:993834. doi: 10.3389/fmicb.2022.993834.

172. Woloshuk, C.P., J.R. Cavaletto, and T.E. Cleveland, Inducers of Aflatoxin Biosynthesis from Colonized Maize Kernels Are Generated by an Amylase Activity from Aspergillus flavus. 1997. **87**(2): p. 164-169. DOI|.10.1094/phyto.1997.87.2.164

173. Benkerroum N. Aflatoxins: Producing-Molds, Structure, Health Issues and Incidence in Southeast Asian and Sub-Saharan African Countries. International Journal of Environmental Research and Public Health. 2020; 17(4):1215. https://doi.org/10.3390/ijerph17041215

174. Wang, B., Han, X., Bai, Y., Lin, Z., Qiu, M., Nie, X., Wang, S., Zhang, F., Zhuang, Z., Yuan, J. and Wang, S., 2017. Effects of nitrogen metabolism on growth and aflatoxin biosynthesis in Aspergillus flavus. Journal of hazardous materials, 324, pp.691-700. https://doi.org/10.1016/j.jhazmat.2016.11.043.

175. Niehaus, W.G., and W. Jiang, Nitrate induces enzymes of the mannitol cycle and suppresses versicolorin synthesis in *Aspergillus parasiticus*. Mycopathologia, 1989. **107**(2-3): p. 131-137,

176. Chang, P.-K., K.C. Ehrlich, J.E. Linz, D. Bhatnagar, T.E. Cleveland, and J.W. Bennett, Characterization of the Aspergillus parasiticus niaD and niiA gene cluster. Current Genetics, 1996. **30**(1): p. 68-75. DOI|.10.1007/s002940050102

177. Obrian, G.R., D.R. Georgianna, J.R. Wilkinson, J. Yu, H.K. Abbas, D. Bhatnagar, T.E. Cleveland, W. Nierman, and G.A. Payne, The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. Mycologia, 2007. **99**(2): p. 232-239 DOI|..10.1080/15572536.2007.11832583

178. Sanders, T.H., P.D. Blankenship, R.J. Cole, and R.A. Hill, Effect of soil temperature and drought on peanut pod and stem temperatures relative to *Aspergillus flavus* invasion and aflatoxin contamination. Mycopathologia, 1984. **86**(1): p. 51-54,

179. Cotty, P.J. and R. Jaime-Garcia, Influences of climate on aflatoxin producing fungi and aflatoxin contamination. International Journal of Food Microbiology, 2007. **119**(1): p. 109-115. DOI 10.1016/j.ijfoodmicro.2007.07.060.

180.Cotty, P.J., Aflatoxin, and sclerotial production by *Aspergillus flavus*: influence of pH. growth, 1988. **4**: p. 11,

181. Keller, N.P., C. Nesbitt, B. Sarr, T.D. Phillips, and G.B. Burow, pH Regulation of Sterigmatocystin and Aflatoxin Biosynthesis in

Aspergillus spp. Phytopathology, 1997. **87**(6): p. 643-648. DOI|.10.1094/phyto.1997.87.6.643

182. Torres, J., J. Guarro, G. Suarez, N. Sune, M. Calvo, and C. Ramirez, Morphological changes in strains of Aspergillus flavus Link ex Fries and *Aspergillus parasiticus* Speare related with aflatoxin production. Mycopathologia, 1980. **72**(3): p. 171-174,
183. Jayashree, T. and C. Subramanyam, Oxidative stress as a prerequisite for aflatoxin production by *Aspergillus parasiticus.* Free Radical Biology and Medicine, 2000. **29**(10): p. 981-985. DOI|10.1016/S0891-5849(00)00398-1.
184. Wright, M.S., D.M. Greene-McDowell, H.J. Zeringue, D. Bhatnagar, and T.E. Cleveland, Effects of volatile aldehydes from *Aspergillus*-resistant varieties of corn on *Aspergillus parasiticus* growth and aflatoxin biosynthesis. Toxicon, 2000. **38**(9): p. 1215-1223. https://doi.org/10.1016/S00410101(99)00221-4.
185. Criseo, G., A. Bagnara, and G. Bisignano, Differentiation of aflatoxin-producing and non-producing strains of *Aspergillus flavus* group. Letters in Applied Microbiology, 2001. **33**(4): p. 291-295. DOI|10.1046/j.1472-765X.2001.00998.x.
186. Kraft S, Buchenauer L, Polte T. Mold, Mycotoxins and a Dysregulated Immune System: A Combination of Concern? International Journal of Molecular Sciences. 2021; 22(22):12269. https://doi.org/10.3390/ijms222212269.
187. Abdel-Nasser, A., Hathout, A.S., Badr, A.N., Barakat, O.S. and Fathy, H.M., 2023. Extraction and characterization of bioactive secondary metabolites from lactic acid bacteria and evaluating their antifungal and antiaflatoxigenic activity. Biotechnology Reports, 38, p.e00799. https://doi.org/10.1016/j.btre.2023.e00799.
188. Shehata, M.G., Badr, A.N., Abd El-Aziz, N.M., Abd-Rabou, H.S. and El-Sohaimy, S.A., Optimization, Partial Purification, and Characterization of Bioactive Peptides of *Lactobacillus paracasei* Isolated from Traditional Egyptian Cheese. Journal of food quality and hazards control. 2022. 9 (4):199 – 214. https://doi.org/10.18502/jfqhc.9.4.11375.
189. Mogahed Fahim K, Noah Badr A, Gamal Shehata M, Ibrahim Hassanen E, Ibrahim Ahmed L. Innovative application of postbiotics, parabiotics and encapsulated Lactobacillus plantarum RM1 and Lactobacillus paracasei KC39 for detoxification of aflatoxin M1 in milk powder. Journal of Dairy Research. 2021;88(4):429-435. https://doi.org/10.1017/S002202992100090X.
190. Shehata, M.G., Badr, A.N., El Sohaimy, S.A., Asker, D. and Awad, T.S., 2019. Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. Annals of Agricultural Sciences, 64(1), pp.71-78. https://doi.org/10.1016/j.aoas.2019.05.002.
191. Shehata MG, Alsulami T, El-Aziz NMA, Abd-Rabou HS, El Sohaimy SA, Darwish AMG, Hoppe K, Ali HS, Badr AN. Biopreservative and Anti-Mycotoxigenic Potentials of Lactobacillus paracasei MG847589 and Its Bacteriocin in Soft White Cheese. Toxins. 2024; 16(2):93. https://doi.org/10.3390/toxins16020093.
192. Hassanen, E.I., Ahmed, L.I., Fahim, K.M. et al. Chitosan nanoparticle encapsulation increased the prophylactic efficacy of *Lactobacillus plantarum* RM1 against AFM1-induced hepatorenal toxicity in rats. Environ Sci Pollut Res 30, 123925–123938 (2023). https://doi.org/10.1007/s11356-023-31016-3
193. Farouk A, Abdel-Razek AG, Gromadzka K, Badr AN. Prevention of Aflatoxin Occurrence Using Nuts-Edible Coating of Ginger Oil Nanoemulsions and Investigate the Molecular Docking Strategy. *Plants*. 2022; 11(17):2228. https://doi.org/10.3390/plants11172228.
194. Farouk A, Alsulami T, Ali HS, Badr AN. *In-Vitro and In-Silico* Investigation for the Spent-Coffee Bioactive Phenolics as a Promising Aflatoxins Production Inhibitor. Toxins. 2023; 15(3):225. https://doi.org/10.3390/toxins15030225.
195. Boudechicha A, Aouf A, Farouk A, Ali HS, Elkhadragy MF, Yehia HM, Badr AN. Microfluidizing Technique Application for Algerian *Cymbopogon citratus* (DC.) Stapf Effects Enhanced Volatile Content, Antimicrobial, and Anti-Mycotoxigenic Properties. Molecules. 2023; 28(14):5367. https://doi.org/10.3390/molecules28145367
196. Sabry, B.A., Badr, A.N., Mohammed, D.M., Desoukey, M.A. and Farouk, A. Validating the protective role of orange and tangerine peel extracts foramending food safety against microorganisms' contamination using molecular docking. Heliyon. 2024. 10(6): e27737. https://doi.org/10.1016/j.heliyon.2024.e27737.

197. Abdel-Razek, A. G., Hassanein, M. M., Ozçelik, B., Baranenko, D. A., & El-Messery, T. M. (2022). Omega fatty acid-balanced oil formula and enhancing its oxidative stability by encapsulation with whey protein concentrate. Food Bioscience, 50, 101975.

198. Selim, K. A., Alharthi, S. S., Abu El-Hassan, A. M., Elneairy, N. A., Rabee, L. A., & Abdel-Razek, A. G.. The effect of wall material type on the encapsulation efficiency and oxidative stability of fish oils. Molecules, 2021. 26 :(20), 6109.

199. Abdel-Salam, A. M., Badr, A.N., Zaghloul, A. H.and Farrag, A. H. "Functional yogurt aims to protect against the aflatoxin B1 toxicity in rats." Toxicology Reports 7 (2020): 1412-1420. https://doi.org/10.1016/j.toxrep.2020.10.012

200. Badr A.N., El-Said M.M., Elmessery, T.M., Abdel-Razek, A.G., "Non-traditional oils encapsulation as novel food additive enhanced yogurt safety against aflatoxins." Pakistan Journal of Biological Sciences: PJBS 22, no. 2 (2019): 51-58. https://doi.org/10.3923/pjbs.2019.51.58