ELSEVIER

International Journal of Food Microbiology 43 (1998) 141–158

International Journal of Food Microbiology

Review

Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species

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Received 29 March 1998; received in revised form 15 June 1998; accepted 27 July 1998

1. Introduction

Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in foods as a result of fungal growth (Table 1). They cause a toxic response, termed a mycotoxicosis, when ingested by higher vertebrates and other animals. Mycotoxin ingestion by humans, which occurs mainly through plant-based foods and the residues and metabolites present in animal-derived foods; can lead to deterioration of liver or kidney function. Other mycotoxins are neurotoxins, while others act by interfering with protein synthesis, and produce effects ranging from skin sensitivity or necrosis to extreme immunodeficiency. The mycotoxigenic fungi involved with the human food chain belong mainly to three genera: *Aspergillus*, *Fusarium* and *Penicillium*. While *Fusarium* species are destructive plant pathogens producing mycotoxins before, or immediately post harvesting, *Penicillium* and *Aspergillus* species are more commonly found as contaminants of commodities and foods during drying and subsequent storage. In this review the mycotoxins produced by the most significant species of these three genera in relation to food safety will be discussed.

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2. Chemical structures and biosynthetic pathways

2.1. The Aspergillus mycotoxins

The mycotoxins of greatest significance in foods and feeds are aflatoxins which are produced mainly by *A. flavus*, *A. parasiticus* and *A. nominus*. They are difuranocoumarin derivatives, [Fig. 1]. The four main naturally produced aflatoxins are B_1, B_2, G_1 and G_2; with B_1 usually being the aflatoxin found at the highest concentration in contaminated food and feed. The nomenclature of B and G is derived from the blue and green fluorescent colours produced under UV light on thin layer chromatography plates, with the subscript numbers indicating major and minor compounds respectively. Aflatoxin B_1 is regarded by many to be the most potent liver carcinogen known for a wide variety of animal species, including humans (Dragan and Pitot, 1994). Aflatoxin M_1 and M_2 are monohydroxylated derivatives of aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) which are formed and excreted in the milk of lactating animals including humans that have consumed AFB_1 or AFB_2 contaminated material (Frobish et al., 1986). Aflatoxin M_1 has been widely found in a number of food products including infant formula, dried milk, cheese and yoghurt (Galvano et al., 1996). Consumption of aflatoxin M_1 contami-

0168-1605/98/$19.00
PII: S0168-1605(98)00112-3

Table 1
Mycotoxins found in foods and feedstuffs

Commodity	Situation	Potential mycotoxins
Cereals	pre-harvest fungal infection	deoxynivalenol, T2 toxin, nivalenol, zearalenone, alternariol, alternariol monomethyl ether, tenuazonic acid fumonisins
Maize and peanuts	pre-harvest fungal infection	aflatoxins
Maize and sorghum	pre-harvest fungal infection	fumonisins
Stored cereals, nuts, spices	damp storage conditions (storage abuse)	aflatoxins and ochratoxin
Fruit juice	mould growth on fruit	patulin
Dairy products	animal consumption of mould contaminated feeds	aflatoxin M_1, cyclopiazonic acid, ochratoxin, compactin, cyclopaldic acid
Meat and eggs	animal consumption of mould contaminated feeds	patulin, citrinin, ochratoxin, cyclopiazonic acid, cyclopaldic acid, citromycetin, roquefortine fumonisins
Oilseeds	pre-harvest fungal infection	tenuazonic acid, alternariol,

Taken from (Prelusky, 1994; Smith et al., 1994; Bullerman, 1995; Smith and Thakur, 1995; Webley et al., 1997).

nated infant milk and milk products by infants is to be avoided and very low limits have been set (0.01 to 0.05 μg/kg) for infant foods, given the relatively high consumption rate of these products by infants, their low body weight and the possible higher susceptibility of younger children to aflatoxins (Aksit et al., 1997).

Other important mycotoxins produced by *Aspergillus* species include ochratoxin A (OTA), which is produced by *A. ochraceus* and related species. OTA is a derivative of isocoumarin linked to L-phenylalanine and is also widely produced by *Penicillium* species and in particular *Penicillium verrucosum*. OTA is a potent nephrotoxin, teratogen, and carcinogen (Krogh, 1987), and inhalation can lead to renal failure (Dipaulo et al., 1994). Another of these is Sterigmatocystin, a 7,8-dihydrofuro[2,3-β]furan which is a precursor of the aflatoxins and is produced mainly by *A. versicolor*. It is characterized by a xanthone moiety fused to a dihydrodifuran or tetrahydrofuran moiety. While acutely toxic and carcinogenic (Terao, 1983) it is not as toxic as aflatoxin B_1. Finally there is cyclopiazonic acid (CPA), which is an indole-tetramic acid mycotoxin which is produced primarily by *A. flavus*, but it can also be produced by *Penicillium* spp. (Pitt, 1997). CPA causes liver necrosis or gastrointestinal tissue necrosis (Cole, 1986).

The biosynthetic pathway of the aflatoxins is quite well understood and has recently been described in a number of reviews (Bhatnagar et al., 1994; Trail et al., 1995a; Bennett et al., 1997; Minto and Townsend, 1997). Firstly acetate and malonyl CoA are converted to a hexanoyl starter unit by a fatty acid synthase, which is subsequently extended, by a polyketide synthase to the decaketide norsolorinic acid; the first stable precursor in aflatoxin biosynthesis. The polyketide then undergoes approximately 12 to 17 enzymatic transformations, through a series of pathway intermediates, which are summarized in Fig. 2; including averantin, 5′-hydroxyaverantin, averufanin, averufin, 1′-hydroxyversicolorone, versiconal acetate, versiconal and veriscolorin B. The pathway then branches to form AFB_1 and AFG_1 which contain dihydrobisfuran rings and are produced from demethylsterigmatocystin (DMST) and

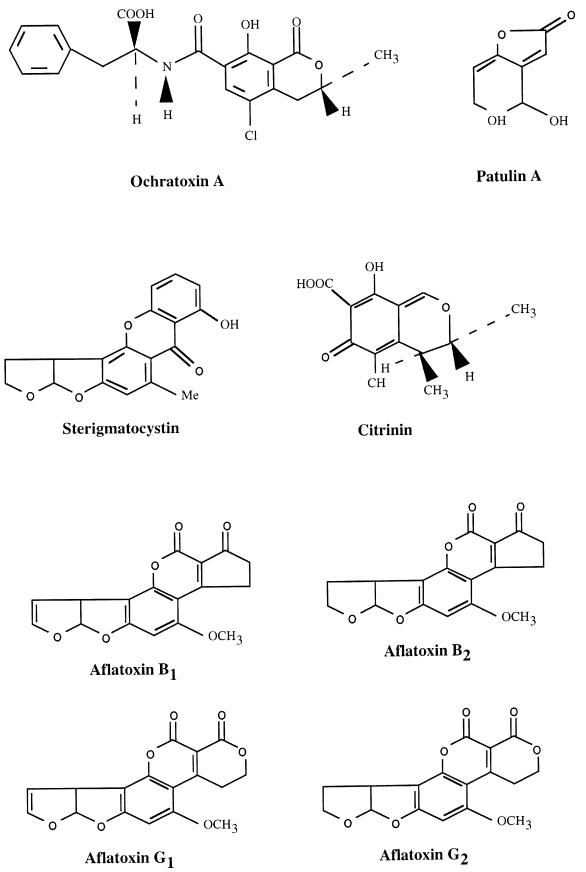


Fig. 1. Common toxins produced by *Aspergillus* and *Penicillium* species.

in turn the other branch forms AFB_2 and AFG_2, containing tetrahydrobisfuran rings which are produced from dihydrodemethylsterigmatocystin (DHDMST). The proposed pathway is based mainly on evidence from enzymatic and genetic analysis. The genetics of aflatoxin biosynthesis will be discussed later.

Regarding the enzymology of the system several of the enzymes involved have been purified to homogeneity. A 168 kDa *O*-methyltransferase (Bhatnagar et al., 1988) and a 40 kDa methyltransferase (Keller et al., 1992) corresponding to MT-II have been purified and have been shown to be involved in the conversion of sterigmatocystin to *O*-methylsterigmatocystin and dihydrosterigmatocystin to dihydro-*O*-methylsterigmatocystin respectively (Yabe et al., 1989). Recently another *O*-methyltransferase (MT-I), with an estimated molecular weight of 150 kDa has been purified which is involved in the conversion of DMST to sterigmatocystin and of DHDMST to dihydrosterigmatocystin (Yabe et al., 1998). These methyltransferases differ not only in molecular weight but in their sensitivity to the chemical agent N-ethylmaleimide, with MT-I being sensitive and MT-II resistant to this reagent. At least three different enzymes have been characterized which may be involved in the conversion of norsolorininc acid to averantin. A 38 kDa norsolorinic acid reductase (NAR) (Bhatnagar and Cleveland, 1990); a 43 kDa isozyme of the reductase (Yabe et al., 1993) and a 140 kDa NAR (Chuturgoon and Dutton, 1991). In addition an alcohol dehydrogenase capable of norsolorinic reduction has also been reported (Yabe et al., 1991). A tetrahydrobisfuran cyclizing enzyme namely vericonyl cyclase has been purified which is responsible for the conversion of versiconal to versicolorin B (Lin and Anderson, 1992; Yabe and Hamasaki, 1993). A versicolorin B synthase enzyme (VBS) has also been isolated which catalyzes the cyclization of versiconal to versicolorin B (McGuire et al., 1996). This 78 kDa enzyme is believed to be the pivotal enzyme responsible for setting the stereochemistry of the bisfuran ring system present in all the aflatoxins (Minto and Townsend, 1997). Two versiconal hemiacetal acetate (VHA) reductases (VHA I and II), which catalyze the conversion of VHA to versiconal acetate, have been purified and characterized from *A. parasiticus* mutant NIAH-26 (Matsushima et al., 1994). In addition three esterases which catalyze the conversion of versiconal acetate to versiconal have recently been isolated (Kusumoto and Hsieh, 1996). One of these which has been partially purified consists of two isomeric subunits each of 32 kDa.

The purification of many of the enzymes described has facilitated the design of degenerate codon-derived oligonucleotide primers which has lead to the cloning of a number of the genes encoding these enzymes (Trail et al., 1995a; Minto and Townsend, 1997). This approach together with other aspects of the genetics of mycotoxin aflatoxin production will be discussed later.

2.2. The Penicillium mycotoxins

The genus *Penicillium* contains many toxigenic species, (approximately 100), and the range of

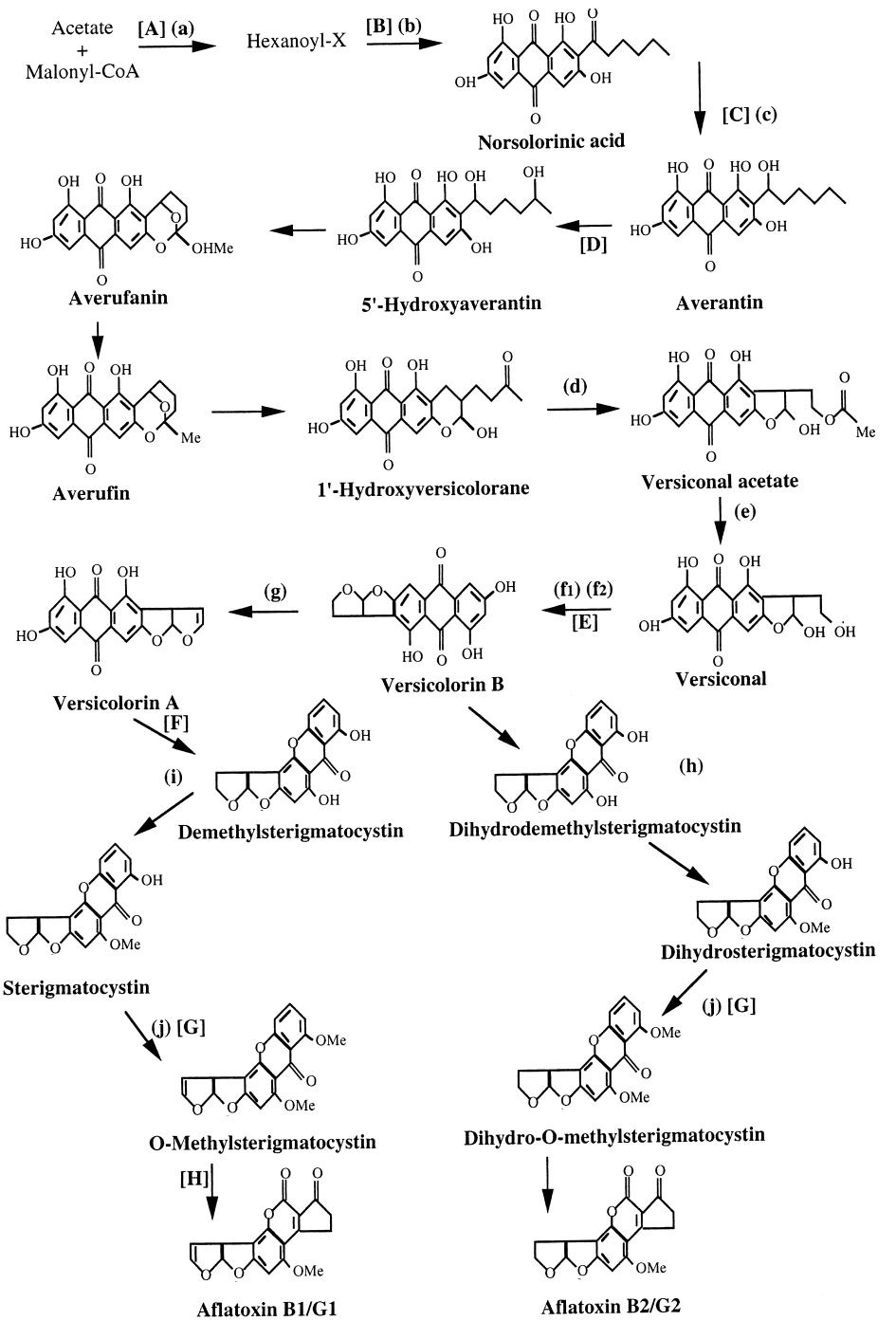


Fig. 2. Aflatoxin biosynthetic pathway. Enzymes involved: (a) fatty acid synthase, (b) polyketide synthase, (c) norsolorininc acid reductase, (d) versiconal hemiacetal acetate reductase, (e) esterase, (f_1) versicolorin B synthase, (f_2) versiconyl cyclase, (g) desaturase, (h) O-methyltransferase (MT-II), (i) O-methyltransferase, (j) O-methyltransferase (MT-I). Genes involved: [A] *fas*-1A and *fas*-2A, [B] *pks*A, [C] *nor*-1, *nor*A, [D] *avn*A, [E] *vbs*, [F] *ver*-1, [G] *omt*A and [H] *ord*-1. (Compiled from Trail et al., 1995a; Bennett et al., 1997 and Minto and Townsend, 1997).

mycotoxin classes produced is much broader than that of any other genus. Much confusion has existed in the past regarding mycotoxin production by *Penicillium* spp. due mainly to incorrect identification of producing species and many of the toxic compounds reported are produced by *Penicillium* spp. not commonly found in food commodities. A more accurate reflection of the mycotoxin producing capabilities of the genus comes in a review by Pitt and Leistner (1991), where they list 27 mycotoxins, produced by 32 species which possess demonstrated toxicity. *Penicillium* toxins can be placed in two broad groups based on effect: those that affect liver and kidney function and those that are neurotoxins (Pitt, 1997). In this review three of the more important *Penicillium* mycotoxins will be discussed, namely OTA, patulin and citrinin. (For structures see Fig. 1). As previously mentioned OTA is a derivative of isocoumarin linked to L-phenylalanine and is the most toxic of the isocoumarin compounds. It is produced by *Penicillium verrucosum*, as well as by *A. ochraceus* and related species. *Penicillium* species primarily produce OTA in temperate climates while *A. ochraceus* strains are more commonly associated with warmer climates. Patulin is produced mainly by *P. expansum*, a fruit pathogen, which causes apple rot. It has been reported to have adverse effects on rodent fetuses, together with immunological, neurological and gastrointestinal effects (Smith et al., 1994; Canas and Aranda, 1996).

Citrinin is produced mainly by *P. citrinum*, but *P. expansum* and *P. verrucosum* are also reported producers (El-Banna et al., 1987). It is a quinone methide with two intramolecular hydrogen bonds. Citrinin is a well established renal toxin affecting monogastric domestic animals such as pigs and dogs. The effect on humans is as yet unknown.

Biosynthetically the ochratoxins are formed via a number of combined pathways. The isocoumarin group is a pentaketide skeleton formed from acetate and malonate via the polyketide pathway. The heterocyclic portion of the ochratoxins is structurally related to the widespread fungal metabolite mellein. A chlorine atom is incorporated into the pentaketide backbone through the action of a chloroperoxidase. A C1 unit is added and oxidized to a carboxyl group at C-8. L-phenylalanine derived from the shikimic acid pathway is linked through this additional carboxyl group (Moss, 1996). The ochratoxin pentaketide skeleton can also give rise to the mycotoxin citrinin, through the addition of three C1 units, the C1 units being derived from methionine (Moss, 1977).

2.3. The Fusarium mycotoxins

The trichothecenes are a group of mycotoxins which are produced by a variety of different *Fusarium* species, however a number of other fungal genera are also known to produce trichothecenes (Table 2). Trichothecenes are known to be produced by at least 24 different species (Marasas et al., 1984). The more common producers are listed in Table 3. The four species of most importance namely, *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F. sporotrichioides* will be discussed here.

The trichothecenes are chemically the most diverse of all the mycotoxins. They are all tricyclic

Table 2
Trichothecene producing fungal genera

Genus
Cephalosporium
Cylindrocarpon
Fusarium
Myrothecium
Trichoderma
Trichothecium
Stachybotrys
Verticimonosporium

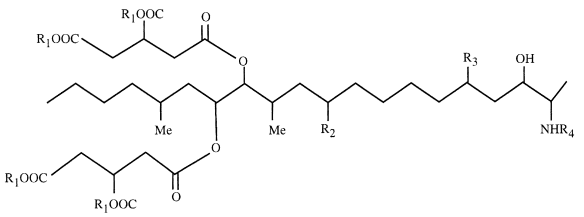
Taken from Smith et al. (1994).

Table 3
Common Trichothecene producing *Fusarium* strains

F. acuminatum	*F. oxysporium*
F. avenaceum	*F. poae*
F. camptoceras	*F. proliferatum*
F. chlamydosporium	*F. sambucinum*
F. compactum	*F. scirpi*
F. crookwellense	*F. semitectum*
F. culmorum	*F. solani*
F. equesiti	*F. sporotrichioides*
F. graminearum	*F. subglutinans*
F. moniliforme	*F. tricinctum*
F. nivale	*F. tumidum*
F. nygamai	*F. venenatum*

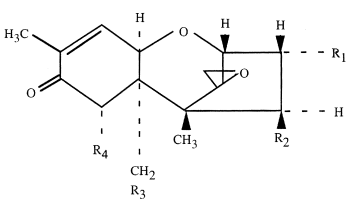
Taken from Smith et al., 1994; Bullerman, 1997; Mule et al., 1997; Pitt and Hocking, 1997.

sesquiterpenes with a basic 12,13-epoxy-trichothec-9-ene ring system (Fig. 3). They can be designated into four subclasses: Type A having a functional group other than a ketone at position C-8; Type B having a ketone at position C-8; Type C having a second epoxy group at C-7, 8 or C-9, 10 and Type D containing a macrocyclic ring between C-4 and C-5 with two ester linkages (Smith et al., 1994). From a toxicity standpoint, because of the large numbers of trichothecenes produced by *Fusarium* spp, together with the fact that they are produced in mixtures even under pure culture conditions; it is often difficult to identify the causative toxins, even though the infecting fungal species is known. However trichothecenes are known to cause alimentary toxic aleukia, fusariotoxicoses and to be cytotoxic to mammalian cells. They are immunotoxic and potent



	R_1	R_2	R_3	R_4
Fumonisin B_1	H	OH	OH	H
Fumonisin B_2	H	H	OH	H
Fumonisin B_3	H	OH	H	H
Fumonisin A_1	H	OH	OH	$COCH_3$

Structure of some Fumonisins



	R_1	R_2	R_3	R_4	R_5
Deoxynivalenol	OH	H	OH	OH	
Nivalenol	OH	OH	OH	OH	
Diacetoxyscirpenol	OH	OAc	OAc	H	H
T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
Neosolaniol	OH	OAc	OAc	H	OH

Structure of some Trichothecenes

Fig. 3. Important toxins produced by *Fusarium* species.

inhibitors of protein synthesis, which can predispose animals to other diseases, and mask the underlying toxicoses (Prelusky et al., 1994). Dietary exposure to trichothecenes can lead to an increased susceptibility to other microbial infections (Pestka and Bondy, 1994). Pigs and other monogastric animals including humans appear to display the greatest susceptibility to these toxins.

The trichothecene biosynthetic pathway (Fig. 4), begins with the cyclization of the isoprenoid biosynthetic intermediate farnesyl pyrophosphate to trichodiene by the enzyme trichodiene synthase. The pathway subsequently involves a number of oxygenations, isomerizations cyclizations and esterifications leading from trichodiene to diacetoxyscirpenol, T-2 toxin and 3-acetyldeoxynivalenol. *F. culmorum*, *F. sporotrichioides* and *Gibberella pulicaris* (anamorph, *F. sambucinum*) appear to share most of the initial oxygenations and cyclizations in the biosynthetic pathway with a branch point occuring after decalonectrin. The pathway has largely been elucidated through whole cell feeding experiments and mutant characterization on a number of *Fusarium* species (Desjardins et al., 1993). The initial enzyme in the pathway, trichodiene synthase has been purified and consists of a homodimer with a subunit of 45 kDa (Hohn and van Middlesworth, 1986). The isolation of other biosynthetic enzymes has proven more difficult, although cytochrome P-450 monooxygenases are known to be involved (Gledhill et al., 1991).

Another important group of *Fusarium* mycotoxins are the fumonisins, produced primarily by *Fusarium moniliforme*; however *F. proliferatum* (Keller and Sullivan, 1996), *F. napiforme* (Nelson et al., 1992) and *F. nygamai* (Thiel et al., 1991; Smith et al., 1994), together with *Gibberella fujikuroi* (Desjardins et al., 1997), are also known to produce these toxins. Their basic chemical structure is a C-20, diester of propane-1,2,3-tricarboxylic acid and a pentahydroxyicosane containing a primary amino group (Fig. 3) (Savard and Blackwell, 1994; Musser, 1996). They have a similar structure to sphingosine, which forms the backbone of sphingolipids. Six fumonisin structures have been identified to date: FB_1, FB_2, FB_3, FB_4, FA_1 and FA_2. However of these only the B-series have been confirmed as natural products, with FB_1 usually being the most abundant (Marasas, 1996; Pitt and Hocking, 1997). Two new fumonisins,

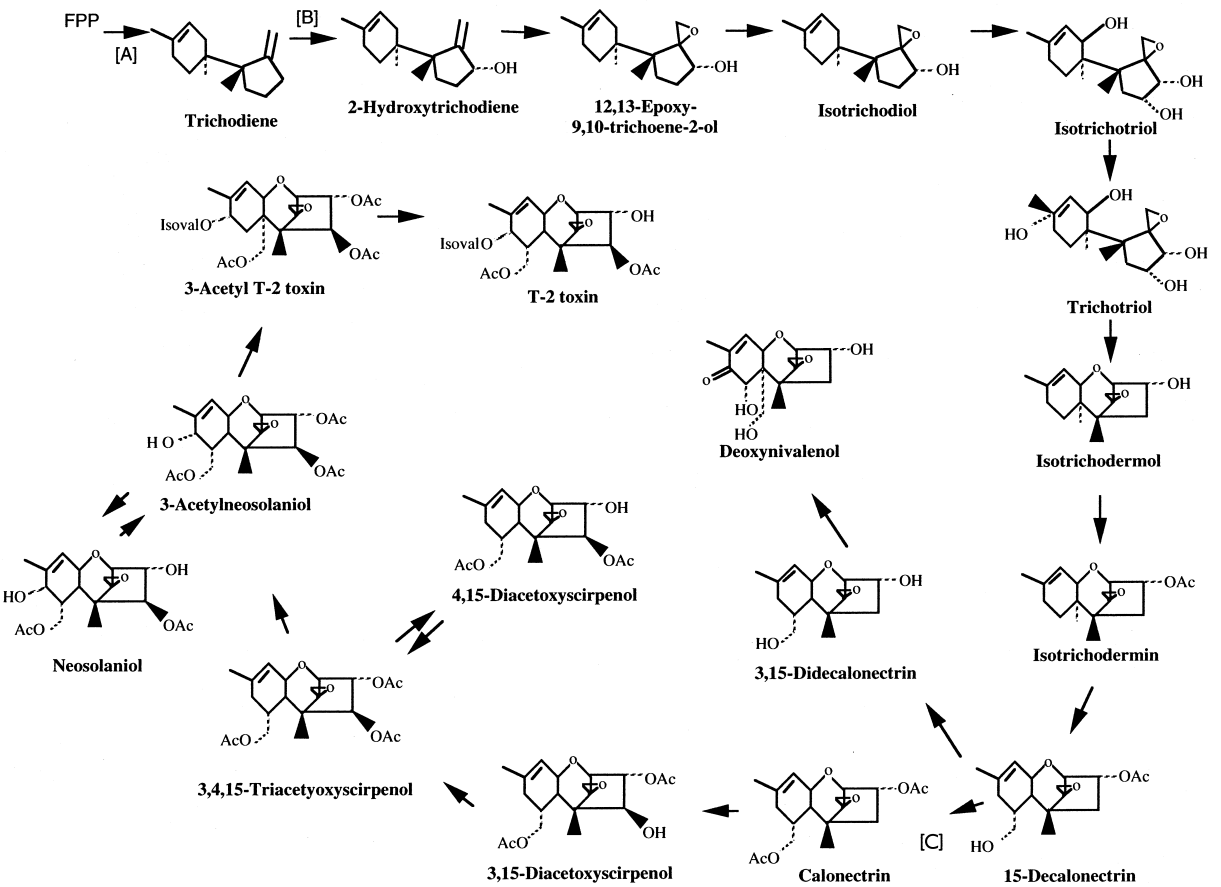


Fig. 4. Trichothecene biosynthetic pathway in *Fusarium* species. Genes involved [A] *Tri* 5, [B] *Tri* 4 and [C] *Tri* 3. (Compiled from Desjardins et al., 1993 and Proctor et al., 1995a).

hydroxylated fumonisin C_1 and fumonisin C_3 have been isolated from cultures of *F. oxysporum* (Seo-Jeong et al., 1996). Both compounds are structurally similar except for an additional hydroxy group at C-3 of hydroxylated fumonisin C_1. Fumonisin C_3 is structurally similar to fumonisin B_3 except that the C-1 terminal methyl group is missing. Fumonisin B_1 (FB_1) is hepatotoxic and hepatocarcinogenic in rats (Gelderblom et al., 1996) and in addition has been shown to inhibit sphingolipid biosynthesis, which has been linked as a contributing factor in both its toxicity and carcinogenicity (Merril et al., 1993; Riley et al., 1996). FB_1 has also been associated with an increased risk of human oesophageal cancer (Sydenham et al., 1990; Chu and Li, 1994).

Fumonisins are believed to be synthesized through the condensation of the amino acid alanine to an acetate-derived precursor (Branham and Plattner, 1993).

Branched-chain methyl groups are added at C-12 and C-16 by an S-adenosyl methionine transferase. The order and number of the subsequent steps in the biosynthetic pathway involved in the oxygenation and subsequent esterification of the acetate-derived backbone are as yet unknown. However if fumonisin biosynthesis is similar to the synthesis of aflatoxins and trichothecenes, less oxygenated homologues, such as FB_2, FB_3 and FB_4 are likely precursors of the most highly oxygenated homologue, FB_1 (Fig. 5), (Desjardins et al., 1996a). It is still not clear however whether oxygenation and methylation occur before or after condensation with alanine.

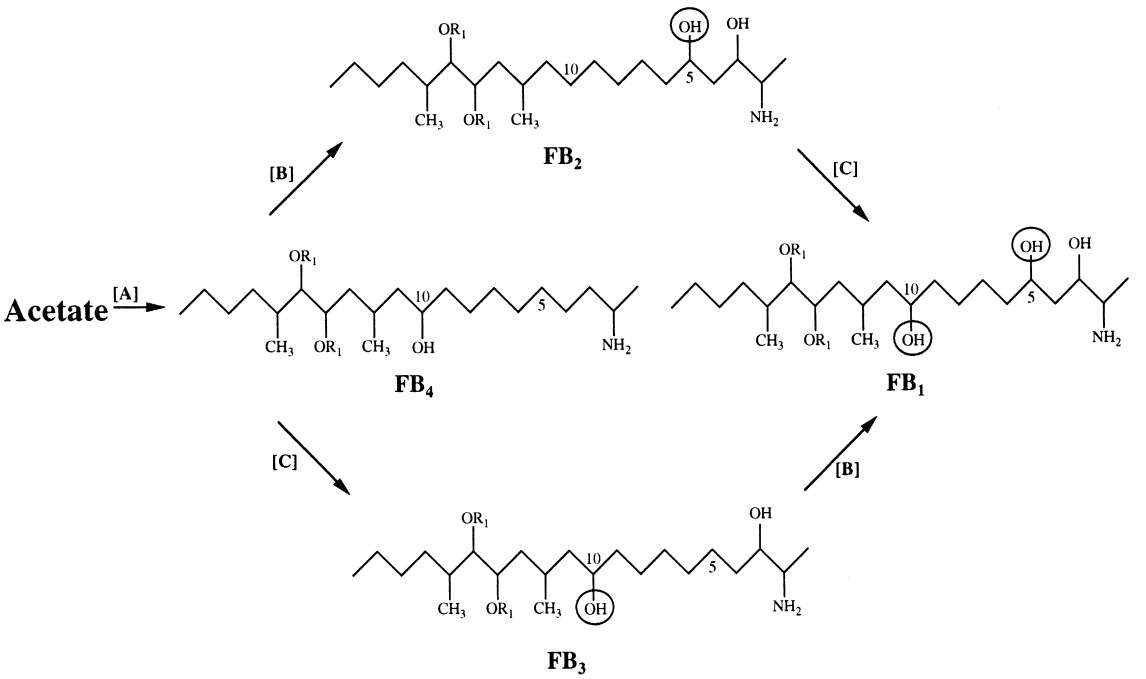


Fig. 5. Proposed pathway for fumonisin biosynthesis. R_1 designates tricarballylic acid esters. Genes involved [A] *fum* 1, [B] *fum* 3 and [C] *fum* 2. (Taken from Desjardins et al., 1996a).

3. Physiological factors affecting mycotoxin production

Mycotoxin production is influenced by a number of different parameters including, nutrient avaliability and environmental factors such as water activity and temperature.

3.1. The Aspergillus mycotoxins

3.1.1. Aflatoxins

Both the main aflatoxin producing *Aspergillus* strains *A. flavus* and *A. parasiticus* have similar growth patterns (ICMSF, 1996). Both grow in the temperature range from 10–12°C to 42–43°C, with an optimum in the 32 to 33°C range (Pitt and Hocking, 1997). Aflatoxins are produced at temperatures ranging from 12 to 40°C (Koehler et al., 1985). Both *A. flavus* and *A. parasiticus* can grow over the pH range 2.1 to 11.2, with an optimum between pH 3.5 and 8 (Wheeler et al., 1991). Aflatoxins can also be produced between pH 3.5 and 8.0 (ICMSF, 1996), with an optimum near pH 6.0 (Buchanan and Ayres, 1976). The minimum a_w for growth and toxin production in some common toxigenic *Aspergillus* species is shown in Table 4. The optimum water activity (a_w) for growth is 0.99 with minimum values reported as 0.80 to 0.83 (Northolt et al., 1977; Pitt and Miscamble, 1995). Aflatoxins are produced at a_w values ranging from 0.95 to 0.99, with a minimum a_w value of 0.82 being reported for *A. flavus* (ICMSF, 1996). The co-production of mycotoxins by *A. flavus* has demonstrated that the fungus produces both aflatoxin and cyclopiazonic acid at an a_w of

Table 4
Comparison of minimum a_w for growth and toxin production in some common *Aspergillus* species

Species	Mycotoxin	Water activity (a_w)	
		Growth	Toxin production
A. flavus	Aflatoxin	<0.80	0.82
A. parasiticus	Aflatoxin	0.84	0.87
A. ochraceus	Ochratoxin A	0.77	0.85
A. ochraceus	Penicillic acid	0.77	0.88
A. clavatus	Patulin	0.88	0.99

Taken from Kozakiewicz and Smith, 1994.

0.996 (Gqaleni et al., 1997). Differences exist in aflatoxin production between *A. flavus* and *A. parasiticus*. *A. parasiticus* produces G and B aflatoxins and in addition isolates tend to produce aflatoxins in higher concentrations than *A. flavus* which contains a greater percentage of non-aflatoxigenic strains and produces only B aflatoxins (Pitt, 1993). Inhibition of aflatoxin production by *A. flavus* and *A. parasiticus* has been reported to occur by the reduction of avaliable oxygen through modified atmosphere packaging of foods in barrier film or by using oxygen scavangers (Ellis et al., 1994a,b).

A number of nutritional factors are known to affect aflatoxin production including the presence of various carbohydrate and nitrogen sources, phosphates, lipoperoxides and trace metals in the growth medium (Luchese and Harrigan, 1993). How these different factors act to regulate aflatoxin biosynthesis is unclear. Many of the effects may in fact be exerted indirectly through interference with primary metabolism. This however appears unlikely given that direct control of aflatoxin biosynthesis does occur at the transcriptional level (see Section 4). The twenty five genes involved in sterigmatocystin biosynthesis in *A. nidulans* are co-regulated (Brown et al., 1996). In addition the *aflR* gene product has been shown to be involved in the transcriptional regulation of aflatoxin production in *A. flavus* and *A. parasiticus* respectively (Trail et al., 1995a). Recently mycotoxin production has been shown to be linked to asexual sporulation in *A. nidulans* and *A. parasiticus* (Hicks et al., 1997; Guzman-de-Pena and Ruiz-Herrera, 1997).

3.1.2. Ochratoxins

A. ochraceus which grows more slowly than *A. flavus* or *A. parasiticus*; will grow at temperatures ranging from 8 to 37°C with an optimum between 24 and 37°C (ICMSF, 1996), with ochratoxin being produced at 12 to 37°C with an optimum at 31°C. *A. ochraceus* grows well between pH 3 and 10, and minimally at pH 2.2 (Wheeler et al., 1991). While the optimum a_w for growth is 0.95–0.99, *A. ochraceus* is quite xerophilic growing at an a_w as low as 0.77 (Pitt and Christian, 1968). The optimum a_w for ochratoxin production mirrors that for growth but OTA can be produced at an a_w as low as 0.80 (Adebajo et al., 1994).

3.1.3. Sterigmatocystin

The growth characteristics of the main sterigmatocystin producer *A. versicolor* are similar to those of *A. ochraceus*, the main difference being that *A. versicolor* is less tolerant of low pH. *A. versicolor* is a xerophile which has been reported to grow at an a_w of 0.76 (ICMSF, 1996). Little is known about factors affecting toxin production in *A. versicolor*.

3.2. The Penicillium mycotoxins

3.2.1. Ochratoxin

P. verrucosum the principal producer of OTA in temperate climates, grows from 0 to 31°C, with an optimum at 20°C (ICMSF, 1996). It is a xerophile, capable of growth at an a_w of 0.80 (Pitt and Hocking, 1997). It will grow in the pH range of 2.1 to 10, with an optimum pH of between 6.0 and 7.0. These factors taken together allow this strain to grow in a wide variety of habitats in cooler climates. OTA can be produced over the entire temperature range, with an optimum at 20°C (ICMSF, 1996). In fact significant levels of OTA production can occur at 4°C and at an a_w as low as 0.86.

3.2.2. Patulin

P. expansum is a psychrophile which grows quite strongly at 0°C, but which can also grow at -2 to -3°C. The optimum growth temperature is 25°C and the maximum is 35°C (Pitt and Hocking, 1997). The minimum a_w for germination is 0.82 to 0.83 (Hocking and Pitt, 1979). Little is known about factors affecting toxin production, but one report cites a minimum a_w of 0.95 for patulin production (Mortimer et al., 1985). Another reports on patulin production in the narrow pH range of 3.2 to 3.8 in apple juice (Damoglou and Campbell, 1986).

3.2.3. Citrinin

P. citrinum is a mesophile growing in the temperature range of 5 to 40°C, with an optimum of between 26 and 30°C (ICMSF, 1996). It grows over the pH range of 2 to 10, with an optimum of pH 5.0 to 7.0. It is a xerophile, with a minimum a_w for growth of between 0.8 and 0.84 (Pitt and Hocking, 1997). Citrinin is produced at temperatures ranging from 15 to 37°C with an optimum at 30°C, but no information

exists regarding the effect of a_w on toxin production (Pitt, 1997).

3.3. The Fusarium mycotoxins

3.3.1. Trichothecenes

Fusarium graminearum, a plant pathogen of gramineous plants, especially wheat, is known to produce deoxynivalenol, nivalenol and zearalenone (Marasas et al., 1984). It is the most widely distributed toxigenic *Fusarium* species (IARC, 1993). It has an optimal growth range of between 24 to 26°C and grows at a minimum a_w of 0.90. The affect of pH on growth is temperature dependant, with minimum values of 2.4 at 30°C and 3.0 at 25°C and 37°C being reported (Wheeler et al., 1991). Toxin production largely mirrors growth conditions (ICMSF, 1996).

F. sporotrichioides which produces T-2 toxin, as well as deoxynivalenol, nivalenol and zearalenone has an optimum growth temperature of between 22.5 and 27.5°C, a maximum of 35°C and a reported minimum of −2°C. Little is known about the physiological factors affecting toxin production in this strain.

F. culmorum a psychrotrophic strain which is an important producer of deoxynivelanol, is capable of growth at 0°C has an optimum growth temperature of 21°C, and a maximum of 31°C. It is however capable of producing zearalenone at temperatures above 25°C (Bottalico et al., 1982).

3.3.2. Fumonisins

F. moniliforme which produces fumonisin B_1, has an optimum growth range of between 22.5 to 27.5°C, a minimum of 2 to 5°C and a reported maximum of 32 to 37°C (Pitt and Hocking, 1997). It has a minimum a_w of 0.87 for growth and has been reported to produce toxin at an a_w of 0.92 (Marin et al., 1995). At a threshold a_w of 0.85–0.86, there is no toxin production by *F. moniliforme* (Cahagnier et al., 1995). *F. proliferatum* a major producer of fumonisins B_1, B_2 and B_3 has a similar growth profile to *F. moniliforme*. Toxin production is higher at 25 than 30°C and can be produced at the 0.97 to 0.92 a_w range. In addition *F. subglutinans* a psychrotrophic strain which produces fumonisin B_1 (Visconti and Doko, 1994) and moniliformin is capable of growth at 5 but not at 37°C. Fumonisin production in *Fusarium* species appears to be enhanced under anaerobic growth conditions (Musser and Plattner, 1997).

4. Genetics of mycotoxin production

4.1. Aflatoxins

To date many of the genes involved in the aflatoxin biosynthetic pathway have been cloned and their functions identified (Fig. 2). These include, in stepwise order, the fatty acid synthase genes, *fas-1A* and *fas-2A* (Mahanti et al., 1996; Trail et al., 1995b; Watanabe et al., 1996); *pksA*, (*pksL1*) which encodes a polyketide synthase (Chang et al., 1995a; Feng and Leonard, 1995); *nor-1* which encodes for a reductase involved in the conversion of norsolorinic acid to averantin and *norA* which encodes an aryl-alcohol dehydrogenase involved in the same biosynthetic step (Trail et al., 1994; Cary et al., 1996). In addition many of the other genes involved in the pathway including the *avnA* gene (formerly *ord-1*), encoding a cytochrome P-450 monooxygenase involved in the conversion of averantin (Yu et al., 1997); the *vbs* gene, which encodes a versicolorin B synthase involved in the conversion of versiconal hemiacetal to versicolorin (Silva et al., 1996); the *ver-1* gene which is involved in the conversion of versicolorin A to sterigmatocystin (Skory et al., 1992) and the *omt A* gene (formely *omt-1*) which encodes an *O*-methyltransferase involved in the conversion of sterigmatocystin and dihydrosterigmatocystin to *O*-methylsterigmatocystin and dihydro-*O*-methylsterigmatocystin respectively (Yu et al., 1995b), have also been cloned. Finally the *ord-1* gene encoding a cytochrome P-450-type monooxygenase putatively responsible for the conversion of *O*-methylsterigmatocystin to aflatoxin (Prieto and Woloshuk, 1997) and the *aflR* gene which encodes the positive regulatory protein AFLR (Chang et al., 1995b; Payne et al., 1993; Woloshuk et al., 1994) have also been cloned. Mapping studies indicate that all the cloned genes involved in the aflatoxin biosynthetic pathway are contained within a 75 kb cluster located on a single chromosome in *A. flavus* and *A. parasiticus* (Silva et al., 1996; Yu et al., 1995a). In addition the physical order of the genes in the cluster have been shown to largely

coincide with the enzymatic steps in the pathway although there are exceptions, namely (*avf-1* and *vbs*), (Yu et al., 1995a). This clustering of mycotoxin biosynthetic genes has also been reported for the trichothecene pathway in *Fusarium* spp. (Proctor et al., 1995a). The significance of this clustering in the regulation of the biosynthetic pathway is not known although the involvement of chromosome structure in gene regulation has been proposed (Wolffe, 1994).

A number of strategies including gene complementation in conjunction with gene disruption and 'reverse genetics' have been successfully employed to clone many of the structural and regulatory genes described above. The development of efficient transformation systems for both *A. flavus* (Woloshuk et al., 1989) and *A. parasiticus* (Skory et al., 1990) together with the isolation and characterization of mutants of these fungal strains, either partially or fully blocked in aflatoxin production has proven invaluable in elucidating the genes corresponding to the enzymatic activities in the pathway.

There is growing evidence to suggest that gene expression is involved in the regulation of multiple parts of the aflatoxin biosynthetic pathway. Chang et al. (1995b) have demonstrated that the transcription of *nor-1*, *ver-1* and *omtA* is activated by the *aflR* gene product, AFLR. The *aflR* gene itself also appears to be autoregulated (Chang et al., 1995b). The coordinate transcription of these genes suggests that they may be regulated at least in part, at the transcriptional level by a common positive regulatory factor. The predicted amino acid sequence derived from both the *apa-2* and *afl-2* clones contains a cysteine-rich zinc finger DNA-binding domain which is characteristic of some fungal transcriptional activators (Woloshuk et al., 1994). A second putative regulatory gene, *afl-1* has also been identified, which appears to positively regulate transcription of *nor-1*, *ver-1* and *omt-1* (Woloshuk et al., 1995).

Genes involved in the aflatoxin biosynthetic pathway may be used to design specific and sensitive PCR-based detection systems for aflatoxigenic strains in foods and feedstuffs. To date PCR has been successfully used to detect *A. flavus* and *A. parasiticus* in grains, using primers based on the coding regions of *ver-1*, *omt-1* (*omtA*) and *apa-2* (*aflR*), (Shapira et al., 1996) and also in the detection of *A. flavus* in figs (Färber et al., 1997). In addition sequence variability in the zinc finger and in the 5′ untranslated region of the *aflR* gene have provided distinct fingerprints which have allowed the non-aflatoxigenic species of *A. sojae* and *A. oryzae* in the *Aspergillus flavi* group to be distinguished from the aflatoxigenic species *A. parasiticus* and *A. flavus* (Chang et al., 1995c).

The genetics of the sterigmatocystin (ST) biosynthetic pathway have also been elucidated. The ST pathway is proposed to involve each enzyme activity from the aflatoxin pathway bar the penultimate steps involving the conversion of sterigmatocystin to aflatoxin. A 60 kb region of chromosome IV of *A. nidulans* has been identified which encodes twenty five co-regulated transcripts thought to encompass most or all of the genes involved in ST biosynthesis (Brown et al., 1996). To date, the functions of seven *stc* transcripts and homologues in *A. parasiticus* and *A. flavus* have been definitively assigned experimentally by gene disruption studies; *stcA* (*pksA*), *aflR*, *stcJ* and *stcK*, (*fas-1A* and *fas-2A*), *stcL*, *stcP*, *stcS* and *stcU* (*ver-1*); (Feng et al., 1992; Kelkar et al., 1996, 1997; Keller et al., 1994, 1995; Yu and Leonard, 1995; Yu et al., 1996). The remaining transcripts encode for four P-450 monooxygenases, an FAD monooxygenase and a peroxidase involved in various steps in the ST pathway (Brown et al., 1996).

4.2. Genetics of other mycotoxins

With respect to the genetics of the biosynthesis of other mycotoxins, the fumonisin biosynthetic genes have been identified in *Gibberella fujikuroi* (*Fusarium moniliforme*), by genetic crosses using naturally occurring fumonisin production variants. Three genes have been identified, *fum* 1, *fum* 2 and *fum* 3. *Fum* 1 appears to be involved in the regulation of fumonisin production. *Fum* 2 encodes a C-10 hydroxylase that can convert FB_4 to FB_3 and can also convert FB_2 to FB_1 (Fig. 5); while *fum* 3 encodes a C-5 hydroxylase that can convert FB_4 to FB_2 and FB_3 to FB_1 (Desjardins et al., 1996a). These genes are linked and appear to form a fumonisin biosynthetic gene cluster on chromosome 1 of *Gibberella fujikuroi* (Desjardins et al., 1996b).

Five genes involved in trichothecene biosynthesis which are clustered together within a 9 kb region of the *F. sporotrichioides* genome have also been

cloned and characterized. Two of these genes *Tri* 3 and *Tri* 4 were identified by complementation of UV-induced mutants of the fungus blocked in trichothecene T-2 toxin production. *Tri* 3 encodes a 15-*O*-acetyltransferase with a predicted molecular mass of approximately 57 kDa, that converts 15-decalonectrin to calonictrin (McCormick et al., 1996), (Fig. 4). *Tri* 4 encodes a cytochrome P-450 monooxygenase involved in the first oxygenation step in the pathway, that converts trichodiene to an unidentified oxygenated product (Hohn et al., 1995). A second cytochrome P-450 monooxygenase has also been identified, *Tri* 11 which appears to hydroxylate the trichothecene ring at the C-15 position (McCormick and Hohn, 1997; Alexander et al., 1998). The other gene in the cluster *Tri* 5 encodes trichodiene synthase which is involved in the cyclization of farnesyl pyrophosphate to trichiodiene (Fig. 4). Another gene involved in the pathway *Tri* 6 encodes a Cys_2, His_2 zinc finger protein, which appears to be involved in the transcriptional regulation of the biosynthetic pathway (Proctor et al., 1995b). More recently another gene involved in trichothecene biosynthesis has been isolated from *F. graminearum*. The gene *Tri* 101 encodes a 451 amino acid protein which catalyzes the acetyl Co-A dependent *O*-acetylation of the trichothecene ring at the C-3 position (Kimura et al., 1998).

Thus much progress has been made on the molecular characterization of the genes involved in the biosynthesis of various mycotoxins. The knowledge gained through this work will ultimately aid in our overall understanding of both the type of enzymes and the order of the enzymatic steps involved in a number of the biosynthetic pathways. In addition the use of molecular techniques, such as PCR-based detection methods and reporter gene fusions; based on the genetic information currently available, will allow us gain a fuller understanding of the physiological factors affecting mycotoxin production in a wide variety of mycotoxigenic fungal strains. Finally this knowledge will also allow the development of atoxigenic biocontrol fungal strains, through the use of techniques such as gene disruption, resulting in strains with specifically deleted mycotoxin genes, such as those developed by Proctor et al. (1995a) for potential field application, in the overall quest to help eliminate mycotoxins from the food chain.

5. Microbial degradation of mycotoxins

While a number of physical methods including extraction, adsorption, irradiation, heat treatment, ultraviolet light, ozone gas, solar irradiation and gamma rays can be used to help eliminate mycotoxin contamination, (for review see Ruston, 1997); the biological detoxification of mycotoxins is now being widely studied. Biological detoxification is regarded as any microbial based, either whole cell or enzyme system; which results in the biotransformation or degradation of the toxin to produce metabolites that are either non-toxic when ingested by animals or less toxic than the parent toxin molecule. A number of microorganisms are known to degrade mycotoxins, and this area has recently been reviewed (Basappa and Shantha, 1996).

The bacterium *Flavobacterium aurantiacum* has been extensively studied in this regard and has been shown to be capable of metabolizing aflatoxin B_1 (AFB_1), with ^{14}C labelled AFB_1 being degraded to water soluble degradation products, and $^{14}CO_2$, in various food; including milk vegetable oil and peanut butter (Line et al., 1994). The aflatoxin degradation process in *F. aurantiacum* is likely to be a mineralization process, as opposed to a co-metabolic event (Line and Brackett, 1995a,b). Line et al. (1994) have also shown that older cultures of the bacterium, grown for up to 72 hours, remove more AFB_1 from the growth medium than 24 hour cultures. A similar phenomenon has been reported with ochratoxin A (OTA) transformation by *Acinetobacter calcoaceticus*, where the peak of OTA transformation occurred following the log phase of cell growth (Hwang and Draughton, 1994).

Other microorganisms including *Butyrivibrio fibrisolvens*, *Lactobacillus* spp. and an as yet unidentified bacterium, which were all isolated from ovine rumen fluid, have been demonstrated to be capable of transforming the trichothecene mycotoxin diacetoxyscirpenol to its deacetylated derivative 15-acetoxyscirpenol (Matsushima et al., 1996). In addition biotransformation of deoxynivalenol has been demonstrated to varying degrees using various microbial inocula; including rumen fluid from a cannulated cow, soil from a *F. graminearum* infected corn field (50%) and a microbial consortium from the large intestine of chickens (He et al., 1992). The

application of a *Bacillus subtilis* strain (FERM BP-3418), which has aflatoxin degrading ability as an active ingredient, in a livestock fattening feed product, has recently been patented (Kubo and Kazuhiro, 1996). Recently a microbial consortium isolated from soil at a coal gasification site has been shown to completely degrade zearalenone (ZEA), using the mycotoxin as a sole carbon source (Megharaj et al., 1997). Interestingly the degradative capability was lost upon purification and recombination of the bacteria within the consortium.

With respect to yeasts and fungi; a number of fungal species have been shown to be capable of degrading mycotoxins. *Rhizopus* spp. and *Aspergillus flavus* are both capable of degrading aflatoxins, and reducing their inherent toxicity and potential mutagenicity (Bol and Smith, 1990; Knol et al., 1990); with the monooxygenase enzyme system in *A. flavus* reportedly being involved. The dual cultivation of *Apergillus flavus*, together with *A. niger*, *Rhizopus oryzae* and *Bacillus stearothermophilus*, isolated as natural contaminants of maize in Thailand; has been shown to result in 80, 70 and 87% aflatoxin degradation respectively (Faraj et al., 1993). The biotransformation of ZEA has also been reported by yeast cultures, where *Candida tropicalis*, *Torulaspora delbruckii*, *Zygosaccharomyces rouxii* and several *Saccharomyces* strains reduced ZEA to α and β-ZEA (Boswald et al., 1995). Mycotoxin have been shown to be degraded during fermentations by both fungi and yeasts. During solid substrate fermentations *Neurospora sitophilia*, which is used to produce 'oncom'; has been shown to reduce the total aflatoxin content of groundnut press cake by approximately 80% (Fardiaz, 1991). Reductions in aflatoxin levels have been reported during fermentation and baking in the manufacture of balady and white bread (Hassanin and Kheirella, 1995). On the other hand Kpodo and co-workers report that aflatoxins are not destroyed during the kenkey fermentation process (Kpodo et al., 1996). There is also conflicting data on the reduction of mycotoxin levels due to active yeast fermentations. In one report, ethanol distilled following a fermentation from contaminated corn did not contain any fumonisins, but fumonisins were present in all the other products of the fermentation; indicating that the distillation process rather than the fermentation process resulted in the elimination of the mycotoxin (Bothast et al., 1992). Bennett and co-workers report that zearalenone, deoxynivalenol and fumonisins are stable during ethanol fermentations (Bennett and Richard, 1996). However in another report three *Saccharomyces cerevisiae* strains have been reported to decrease the ochratoxin A, fumonisin B_1 and B_2 levels in wort during alcohol fermentations, resulting in reduced mycotoxin levels in the finished beer (Scott et al., 1995). In addition trichothecenes have been reportedly degraded during the alcoholic fermentation of grape juice (Flesch and Voight-Scheuermann, 1994).

So while it is clear that microbial metabolism of mycotoxins does occur and that there is evidence of complete mineralization of certain mycotoxins, the enzymatic pathways employed, together with the degradative products produced are as yet not fully known. Thus a biotechnological approach to the detoxification of mycotoxins while feasible, still requires additional input from a research standpoint.

6. Conclusions

The biochemistry, physiology and genetics of mycotoxins produced by *Aspergillus*, *Fusarium* and *Penicillium* species have been discussed. It is clear that much progress has been made both in the elucidation of the biochemical pathways involved in mycotoxin production and in identifying some of the physiological factors controlling the process. Most studies on mycotoxin production to date have however been undertaken under laboratory conditions, using defined laboratory media or autoclaved cereal based media as substrate. In addition few studies have addressed the ability of mycotoxigenic strains to produce toxins when they are co-cultured together with other competing fungi, whether they be toxigenic or nontoxigenic strains. Studies which mimic more closely the environmental factors likely to be encountered by the fungi in nature need to be undertaken if we are to gain further insights into additional factors likely to influence mycotoxin production. This apparently complex task has now become somewhat easier given the recent progress that has been made in the genetics of mycotoxin production; which allow for the development of strain specific DNA probes together with the use of

mycotoxin specific gene probes, both vital tools in this type of study.

Acknowledgements

The authors acknowledge the Irish Department of Agriculture Food and Forestry for support under the Food Industry Sub-programme of the EU Structural Funds 1994–9.

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