Wageningen Academic Publishers

Microbial transformation of trichothecene mycotoxins

T. Zhou, J. He and J. Gong

Food Research Program, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, N1G 5C9 Ontario, Canada; zhout@agr.gc.ca

Received: 9 September 2007 / Accepted: 26 November 2007

Abstract

Trichothecene mycotoxins produced by the *Fusarium* genus are highly toxic to humans and animals. They are commonly found in cereals worldwide, which is not only a concern for food safety, but also highly relevant to the livestock industry. Controlling trichothecenes in food and feed has been a challenge since the toxins are markedly stable under different environmental conditions. Thermal processing is usually ineffective, and chemical treatments generally are expensive and often result in side effects. Previous studies on innovative biological approaches, such as the use of microorganisms and enzymes, to convert the toxins into non or less toxic compounds have shown promise. This review will briefly describe the chemical structures and toxicity of trichothecenes, and examine the microorganisms, including both bacteria and fungi, from various natural sources that are able to detoxify the toxins as either mixed cultures or a pure culture of single isolates. Finally, challenges and innovative strategies in the development of technology to detoxify trichothecenes by microorganisms are described.

Keywords: biodetoxification, deacetylation, deepoxidation, degradation, *Fusarium*

1. Introduction

Trichothecene mycotoxins are a large family of structurally related fungal secondary metabolites produced by various species of *Fusarium, Myrotecium, Trichoderma, Cephalosporium, Verticimonosporium,* and *Stachybotrys*. The most important trichothecene mycotoxins are produced mainly by species of *Fusarium* (Desjardins, 2006). Trichothecenes are toxic to human beings and animals and can cause both acute and chronic diseases including vomiting, diarrhoea, skin irritation, feed refusal, nausea, neural disturbances and abortion (Desjardins, 2006; Eriksen *et al.*, 2004; Morgavi and Riley, 2007). In addition, high doses of trichothecenes promote rapid onset of leukocyte apoptosis, which is manifested as immunosuppression (Pestka and Smolinski, 2005). The toxicity mechanisms of trichothecenes are inhibition of protein, DNA and RNA synthesis, inhibition of mitochondrial functions, cell division and membrane effects, and immunosuppression (Azcona-Olivera *et al.*, 1995; Nelson, 2002; Avantaggiato *et al.*, 2004; Eriksen *et al.*, 2004; Kouadio *et al.*, 2005; Pestka and Smolinski, 2005; Rocha *et al.*, 2005; Stark, 2005; Zhou *et al.*, 2005).

Contamination of feeds and feed ingredients with trichothecene mycotoxins is a major problem in livestock production worldwide. A survey conducted in Eastern Canada between 1991 and 1998 found that maize had the highest ratio of deoxynivalenol (DON) in contaminated samples (0.1 mg/kg and over), amounting to 90%, followed by wheat, 82%, and barley, 73%. In a recent report, of the samples analysed in Asia, 70% maize and 36% finished feed were found to be positive for DON; among the European samples, 81% of maize and 56% of finished feed contained DON (Binder *et al.*, 2007). In addition to their effect on animal performance, some mycotoxins are readily transferred to milk, meat or eggs, and pose a risk to consumers' health (Fink-Gremmels, 1989; Hollinger and Ekperigin, 1999).

Trichothecene mycotoxins, particularly DON, are frequently encountered in human foods such as cornmeal

and granola worldwide (EFSA, 2004; Pestka and Smolinski, 2005). Many outbreaks of acute human diseases have been attributed to consumption of *Fusarium*–contaminated grains and, more recently, to the presence of DON at reported concentrations of 3-93 mg/kg in grain for human consumption (Canady *et al.*, 2001). In 2003, DON was detected in 63% of the samples taken from cereal-based infant foods in the retail market (Lombaert *et al.*, 2003).

Extensive efforts have been made to prevent the spread of trichothecene mycotoxins during plant growth, harvest, storage and processing, however, the presence of mycotoxins in agricultural commodities appears unavoidable. Prevention of mycotoxin contamination within the framework of pre-harvest management, including breeding for resistance and use of fungicides, has not been efficacious in eliminating mycotoxins from crop products. Cleaning methods, such as gravity separation and washing procedures, can only reduce the amount of DON in wheat and maize. The effectiveness of milling practices in reducing the concentration of DON in flour depends to a large extent on the degree of fungal penetration into the endosperm (Shapira and Paster, 2004). The trichothecenes are markedly stable under different environmental conditions. Thermal processing is usually ineffective, and chemical treatments generally are expensive and often result in side effects. It is expected that progress in the control of mycotoxin contamination will depend on the introduction of technologies for specific, efficient, and environmentally sound detoxification. The utilization of microorganisms to detoxify trichothecene mycotoxins in contaminated food and feed may be one such choice of technology and is the focus of this review.

2. Trichothecenes and major toxicity functional groups

The trichothecenes are characterized by the 12,13-epoxy-trichothec-9-ene ring structure as shown in Figure 1 and are grouped into four categories according to their chemical differences (Cole and Cox, 1981; Ueno, 1977). The type A trichothecenes do not contain carbonyl function at C-8, including T-2 toxin, HT-2 toxin, T-2 tetraol, T-2 triol, 15-monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), neosolaniol (NEO) and scirpentriol (SCP); the type B trichothecenes have a carbonyl function at C-8, and deoxynivalenol (DON) and its acetylated derivatives, nivalenol (NIV), fusarenon X (4-acetylnivalenol, FUS) are members of this group; the type C trichothecenes are characterized by a second epoxide group at C-7, 8 or C-9,10 with crotocin and bocchorin as examples; the type D trichothecenes contain a macrocyclic ring system between C-4 and C-15, represented by roridins and verrucorins. More than 200 trichothecenes have been identified to date (Frisvad *et al.*, 2006), however, major trichothecenes that have been found to contaminate food or animal feed include DON, NIV, DAS, and T-2 toxin as well as less frequently detected derivatives of these toxins, such as 3-acetyldeoxynivalenol (3ADON), 15- acetyldeoxynivalenol (15ADON), FUS and HT-2 toxin. DON is the most commonly encountered trichothecene mycotoxin in food and animal feed thus far (EFSA, 2004).

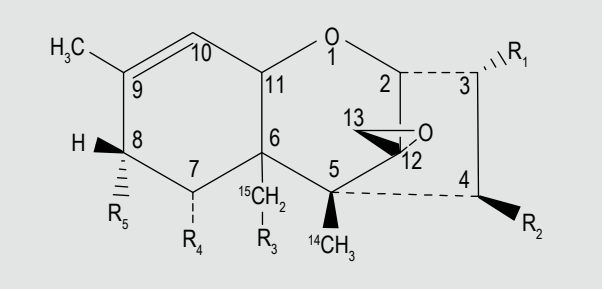

Figure 1. Basic structure of trichothecenes (R_1, R_2, R_3, R_4 and R_5 can be -H, -OH, -OAC, $-COCH_2CH(CH_2)_2$, and/or =O functional groups).

The toxicity of trichothecene mycotoxins varies significantly and is determined by their molecular structures, particularly, toxic functional groups (Betina, 1989; Nagy *et al.*, 2005). Some of the functional groups have been the targets of microbial detoxifications.

Epoxy group

The 12,13-epoxy ring in the trichothecene structure is an essential functional group for toxicity; opening the ring, i.e. de-epoxidation, can result in non or less toxic products for both type A and type B trichothecenes. When tested in a rat skin irritation assay, de-epoxy T-2 toxin proved 400 times less toxic than T-2 toxin (Swanson *et al.*, 1988). In a test against brine shrimp (*Artemia salina*), de-epoxy T-2 toxin and its metabolites were significantly less toxic than their corresponding T-2 toxin metabolites with an intact epoxy ring (Swanson *et al.*, 1987a,b). The cytotoxicities of several type B trichothecenes were compared using a 5-bromo-2'-deoxyuridine (BrdU) incorporation assay by assessing DNA-synthesis. The IC_{50} value of de-epoxy DON was 55 times higher than that of DON; similarly, the IC_{50} of de-epoxy NIV was 54 times higher than that of NIV (Eriksen *et al.*, 2004). Therefore, de-epoxidation is an efficient detoxification reaction.

Ester group

Although the 12,13-epoxy ring and C9,10 double bond are essential for toxic effects, the number and position of the acetyl groups also greatly affect the toxicity of trichothecenes. In a cytotoxicity test performed by assessing DNA-synthesis using Swiss mouse 3T3 fibroblasts, 15ADON was found to be as toxic as DON, but 3ADON was 10 times less toxic than DON (Eriksen *et al.*, 2004). In

a different test using a yeast bioassay, however, 15ADON was more toxic than DON (Madhyastha *et al.*, 1994). In the same test, HT-2 toxin (one acetyl group at C15) was much less toxic than T-2 toxin (two acetyl groups at C4 and C15), but more toxic than triacetyl T-2 toxin (three acetyl groups at C3, C4 and C15). The reduced toxicity of HT-2 toxin in comparison to T-2 toxin was also obtained when they were applied to the midbrain of albino rats in solid form (Bergmann *et al.*, 1988) and tested with brine shrimp (Swanson *et al.*, 1987b). Both acetylation and deacetylation may reduce the toxicity of trichothecenes, which is specific and determined by the number and the position of the acetyl groups in the trichothecene molecules.

Hydroxyl groups

The toxicity (lymphotoxicity) of 8-ketotrichothecene varies, for example, according to the C-4 substitutes, which follow the order of toxicity: acetyl > hydroxyl > hydrogen (Betina, 1989). The presence and the position of hydroxyl groups on the trichothecene molecules can also influence their toxicity (Betina, 1989). NIV is ten times toxic compared to DON; their only structural difference is the presence of C-4 hydroxyl group in NIV (Ueno, 1985). However, C-3 hydroxyl group may affect trichothecene toxicity differently. The toxicity of the resulting compounds decreased when the C-3 hydroxyl in T-2 toxin, HT-2 toxin and T-2 triol was substituted with an acetyl to form T-2 acetate, iso-T-2 toxin and T-2 tetraol tetraacetate, respectively (Jarvis and Mazzola, 1982). When the C-3 hydroxyl in DON was transformed to oxygen forming 3-keto-DON, the immunosuppressive toxicity of the latter decreased remarkably, indicating the significance of the C-3 hydroxyl group in the toxicity of DON (Shima *et al.*, 1997).

3. Trichothecene transforming microorganisms

Reports on the transformation of trichothecene mycotoxins by microorganisms are summarized in Table 1. Although trichothecene transformation by microorganisms has been evidenced since the early 1960's (Horvath and Varga, 1961), studies in this topic have been limited. In addition, the majority of these studies used mixed cultures of microorganisms from various sources such as animal guts, soils and plants; few active microorganisms have been identified.

Microorganisms from ruminant animals

There have been numerous studies on the transformation of trichothecenes by microorganisms in rumen fluid from different ruminants (Table 1). Microorganisms from sheep rumen fluid were found to have a deacetylation function (Kiessling *et al.*, 1984). DAS and T-2 toxin were transformed into MAS and HT-2 toxin, respectively, after incubation with rumen fluid under anaerobic conditions; however, DON was not changed (Kiessling *et al.*, 1984). In contrast, Yoshizawa *et al.* (1983) reported that microorganisms in the rumen fluid from a dairy cow transformed DON into 3a,7a,l5-trihydroxytrichothec-9,12-dien-8-one (de-epoxy DON), which has been named DOM-1 (Yoshizawa *et al.*, 1983). The deepoxidation of DON by rumen fluid was also described by Côté *et al.* (1986) and He *et al.* (1992). It appeared that microorganisms from rumen fluid could have both deacetylation and deepoxidation functions, transforming 3ADON to DON and DOM-1 (King *et al.*, 1984). These transformations by rumen fluid were also observed when animal feed containing DON and 3ADON was used in a test (Binder *et al.*, 1998).

Eubacterium strain BBSH 797 is by far the most intensively studied bacterial isolate that is capable of transforming trichothecenes. The strain was isolated from the bovine rumen fluid and its primary function was to transform DON into DOM-1 *in vitro* and *in vivo* (Binder *et al.*, 1998; Schatzmayr *et al.*, 2006). It was subsequently proved that the strain BBSH 797 was also capable of transforming type A trichothecenes either by deepoxidation such as from SCP to de-epoxy SCP or deacetylation from T-2 toxin to HT-2 toxin (Fuchs *et al.*, 2002). A BBSH 797 –based commercial feed additive product is available on the market.

Bacterial mixtures originating from the rumen of a fistulated dairy cow showed both deacetylation and deepoxidation functions (Swanson *et al.*, 1987a,b). Microbial transformations were obtained after the tested mycotoxins were incubated within the bacterial suspensions at 4.9 x 10^7 per ml anaerobically at 38 °C for 24 or 48 hours. DON was transformed into DOM-1; DAS was converted to MAS, SCP, 15-acetoxy-3α,4β-dihydroxytrichothec-9,12-diene (de-epoxy MAS) and 3α,4β,15-trihydroxytrichothec-9,12-diene (de-epoxy SCP); T-2 toxin was transformed into HT-2, T-2 trio1, 15-acetoxy-3α,4β-dihydroxy-8α-(3-methylbutyryloxy) trichothec-9,12-diene (de-epoxy HT-2) and 3α,4β,15-trihydroxy-8α-(3-methylbutyryloxy) trichothec-9,12-diene (de-epoxy T-2 trial).

Several single strains of rumen bacteria known to have esterase activity were tested for their capability of deacetylating T-2 toxin, and showed different activities (Westlake *et al.*, 1987). *Butyrivibrio fibrisolvens* strains CE51 and CE56 transformed T-2 toxin into HT-2 toxin (22 and 38%, respectively), T-2 triol (3 and 9%, respectively) and NEO (10 and 14%), whereas *Anaerovibrio lipolytica* and *Selenomonas ruminantium* converted T-2 toxin only to HT-2 toxin (22 and 18%, respectively) and T-2 triol (7 and 10%, respectively). The transformation activities of the bacterial cultures were similar in both media with glucose and cellobiose (Westlake *et al.*, 1987). In a different study, *B. fibrisolvens* M-14a deacetylated DAS to 15-acetoxyscirpenol (15ASCP). The deacetylation activity was also achieved

Table 1. Microbial transformation of trichothecene mycotoxins.

Trichothecene[1]	Transforming reaction	Transformed product	Microorganism	Origin	Reference
15ADON	Deepoxidation	de-epoxy 15ADON	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
	Deacetylation Deepoxidation	DOM-1	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
3ADON	Deacetylation	DON	*Fusarium nivale, F. roseum and F. solani*	Fungus	Yoshizawa *et al.*, 1975
			Rumen Microorganisms	Cow	King *et al.*, 1984
			Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
	Deacetylation	DOM-1	Rumen Microorganisms	Cow	King *et al.*, 1984
	Deepoxidation		Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
3MAS	Deepoxidation	de-epoxy 3MAS	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
A_3DON	Deacetylation	7,15DADON, 7ADON	*F. solani*	Fungus	Yoshizawa *et al.*, 1975
DAS	Deacetylation	MAS	*Butyrivibrio fibrisolvens, Lactobacillus* spp.	Ovine rumen fluid	Matsushima *et al.*, 1996
			Rumen bacteria	Sheep	Kiessling *et al.*, 1984
		MAS, SCP	Faecal microorganisms	Horse, dog, chicken	Swanson *et al.*, 1988
		MAS, SCP	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
		SCP	*Curtobacterium* spp. Strain 114-2	Soil	Ueno *et al.*, 1983
	Deacetylation	MAS, de-epoxy MAS	Rumen microorganisms	Dairy cow	Swanson *et al.*, 1987a,b
	Deepoxidation	SCP, de-epoxy SCP	Rumen microorganisms	Dairy cow	Swanson *et al.*, 1987a,b
		de-epoxy MAS and de-epoxy SCP	Faecal microorganisms	Rat, swine, cattle	Swanson *et al.*,. 1988
		de-epoxy MAS, de-epoxy SCP and SCP	Intestinal microorganismes	Rats	Swanson *et al.*, 1988
DON	Acetylation	3ADON	*F. nivale*	Fungus	Yoshizawa and Morooka, 1975
	Deepoxidation	DOM-1	Rumen Microorganisms	Cow	King *et al.*, 1984
			Rumen microorganisms	Dairy cow	Swanson *et al.*, 1987a,b
			Chicken gut microorganisms	Chicken	He *et al.*, 1992
			Pig gut microorganisms	Pig	Kollarczik *et al.*, 1994
			Bacterial strain BBSH 797	Rumen fluid	Fuchs *et al.*, 2002
			Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
	Oxidation	3-keto-DON	*Agrobacterium-Rhizobium* group strain E3-39	Enriched soil	Shima *et al.*, 1997
			Mixed culture D107	unknown	Völkl *et al.*, 2004
FUS	Deacetylation	NIV	*Curtobacterium* spp. strain 114-2	Soil	Ueno *et al.*, 1983
	Deacetylation Deepoxidation	NIV, de-epoxy NIV	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
HT-2 toxin	Deepoxidation	de-epoxy HT-2 toxin	Bacterial strain BBSH 797	Rumen fluid	Fuchs *et al.*, 2002
			Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
MAS	Deepoxidation	de-epoxy MAS	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
NEO	Deacetylation	T-2 tetraol	*Curtobacterium* spp. strain 114-2	Soil	Ueno *et al.*, 1983
		NEO-dA, NEO-dA_2	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
NIV	Deepoxidation	de-epoxy NIV	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
SCP	Deepoxidation	de-epoxy SCP	Intestinal microorganismes	Rat	Swanson *et al.*,. 1988
			Bacterial strain BBSH 797	Rumen fluid	Fuchs *et al.*, 2002
T-2 tetraol	Deepoxidation	de-epoxy T-2 tetraol	Intestinal microorganismes	Rat	Swanson *et al.*, 1988
			Bacterial strain BBSH 797	Rumen fluid	Fuchs *et al.*, 2002
T-2 toxin	Deacetylation	HT-2 toxin	Rumen bacteria	Sheep	Kiessling *et al.*, 1984
			Intestinal microorganisms	Rat	Conrady-Lorck *et al.*, 1988
			Bacterial strain BBSH 797	Rumen fluid	Fuchs *et al.*, 2002
			Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007

Table 1. Continued.

Trichothecene[1]	Transforming reaction	Transformed product	Microorganism	Origin	Reference
T-2 toxin (Continued)	Deacetylation (Continued)	HT-2 toxin, T-2 triol	*Curtobacterium* spp. strain 114-2	Soil	Ueno *et al.*, 1983
			Bacterial communities	Soil, water	Beeton and Bull, 1989
		HT-2 toxin, T-2 triol, NEO	*Butyrivibrio fibrosolvents* CE51	Sheep rumen fluid	Westlake *et al.*, 1987
	Deacetylation	HT-2 toxin, T-2 triol,	Rumen microorganisms	Dairy cow	Swanson *et al.*, 1987a,b
	Deepoxidation	de-epoxy HT-2 toxin, de-epoxy T-2 triol	Intestinal microorganisms	Rat	Swanson *et al.*, 1988
T-2 triol	Deepoxidation	de-epoxy T-2 triol	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007
	Deacetylation	T-2 tetraol, de-epoxy T-2 tetraol	Bacterial strain BBSH 797	Rumen fluid	Fuchs *et al.*, 2002
	Deepoxidation				
VER	Deepoxidation	de-epoxy VER	Bacterial isolates LS100 & SS3	Chicken digesta	Young *et al.*, 2007

[1] 15ADON = 15-acetyldeoxynivalenol; 3ADON = 3-acetyldeoxynivalenol; A3DON=3α,7α,15α-triacetoxy-deoxynivalenol; DADON=diaacetoxy-deoxynivalenol; DAS = diacetoxyscirpenol; DOM-1 = de-epoxy deoxynivalenol; DON = deoxynivalenol; FUS = fusarenon X; 15MAS = 15-acetylscirpenol; NEO = neosolaniol; NIV = nivalenol; SCP = Scirpentriol; VER = verrucarol.

with *Lactobacillus* spp. and an unidentified bacterium from the ovine rumen (Matsushima *et al.*, 1996).

Microorganisms from nonruminant animals

Different nonruminant animals may have different communities of intestinal microorganisms. When trichothecene mycotoxins were incubated anaerobically with suspensions of microorganisms from the faeces of various types of animals, different transformations were achieved (Swanson *et al.*, 1988). Both deacetylation and deepoxidation of DAS were observed with microorganisms from rats and swine, resulting in deacetylated de-epoxy products, de-epoxy MAS and de-epoxy SCP (Swanson *et al.*, 1988). Intestinal microorganisms of chickens, on the other hand, showed only deacetylation activity, and transformed DAS into MAS and SCP (Swanson *et al.*, 1988). However, in a separate study, very effective deepoxidation activity was achieved when DON was mixed with the digesta of the large intestines of chickens; DON was completely transformed to DOM-1 (He *et al.*, 1992). It was found that the deepoxidation function of the intestinal microorganisms of chickens may vary greatly among the samples collected from different chicken varieties, individuals and intestinal regions. The variation was also noticed in DON deepoxidation activity by microorganisms from swine intestine, either no activity (He *et al.*, 1992) or with activity (Kollarczik *et al.*, 1994).

In the attempt to develop the technology for detoxifying DON with microorganisms, our research team has successfully identified and purified single bacterial isolates from chicken intestinal digesta, which are able to transform DON into DOM-1. Two of these bacterial isolates, namely LS100 (IDAC 180507-1) and SS3, were examined for their ability to transform 12 trichothecenes. The types of transformation appeared to be trichothecene structures dependent. To non-acetylated trichothecenes such as DON, NIV and verrucarol, de-epoxy metabolites were the major transforming products. However, monoacetyl trichothecenes 3-acetyl DON, 15-acetyl DON, and fusarenon X were mainly transformed to deacetylated products. Diacetylated trichothecenes DAS and NEO exhibited only deacetylation. T-2 toxin was also only deacetylated, whereas deepoxidation was the prevalent reaction in HT-2 toxin and T-2 triol (Young *et al.*, 2007). However, microorganisms from the rats intestine transformed T-2 toxin into its de-epoxy products, de-epoxy HT-2, de-epoxy T-2 triol, and further to T-2 tetraol and SCP (Swanson *et al.*, 1988).

Microorganisms from environments

Environmental samples from soil, water and plants have also been used as sources for selecting trichothecene transforming microorganisms. A bacterial strain able to transform type A trichothecenes has been identified from soil. *Curtobacterium* spp. strain 114-2, using T-2 toxin as its sole carbon source, transformed T-2 toxin firstly into HT-2 toxin, and then HT-2 toxin was further transformed into T-2 triol in an aerobic condition. In a prolonged culture, T-2 toxin was completely converted and no trichothecenes could be detected in the culture. The isolate was also able to degrade DAS, NEO, NIV, and FUS. The degradation involved hybridization and the hydrolyzing enzymes which appeared in whole cells, cell-free soluble fraction and culture filtrate (Ueno *et al.*, 1983). Microflora (microbial

communities) of fresh water has also been found to be able to degrade T-2 toxin (Beeton and Bull, 1989).

DON is the most common mycotoxin produced on cereal crops and is chemically stable but does not appear to accumulate in agriculture soils, implying the possibility of biological mineralization of DON in nature (Völkl *et al.*, 2004). Among 1285 microbial cultures obtained from farmland soils, cereal grains, insects and other sources, one mixed culture was able to transform DON into 3-keto-DON (Völkl *et al.*, 2004). The culture was also capable of transforming 3ADON, 15ADON and FUS. However, the microorganisms responsible for the biotransformation have not been identified. In addition, mixed soil microorganisms have been reported as transforming DON and 3ADON into five unidentified metabolites that showed toxicity reduction in the bioassay with *Saccharomyces cerevisae* (Binder *et al.*, 1998).

With an enrichment strategy that used DON as the sole carbon source in a medium containing mineral salts. *Agrobacterium-Rhizobium* strain E3-39 was isolated from soil samples (Shima *et al.*, 1997). The strain transformed DON into 3-keto DON under aerobic conditions. The transformation activity of the strain was considerably persistent and the enzyme(s) responsible for the microbial transformation was found to be in cells-culture, cell-free filtrate, but not in the cell extract (Shima *et al.*, 1997).

Six agricultural soils capable of transforming DON into several products were obtained after enhancement using *F. graminearum* infected mouldy maize. These enhanced soils transformed DON by more than 87%, and two of them completely removed DON from culture media after incubation at 28 °C for 3 days (He and Zhou, 2007, unpublished results). A bacterium strain Barpee was isolated from one of the soil samples and showed strong DON transforming activity. Under aerobic conditions, the bacterial strain transformed DON into at least two products: the major one was identified as a stereo-isomer of DON, and the minor one as 3-keto-DON.

Trichothecene transformation by plant fungal pathogens

The possible occurrence of deacetylation of crotocin by *Penicillium chrysogenum* reported as early as 1961 appeared to be the first example of trichothecene transformation by microorganisms in literature (Horvath and Varga, 1961).

Three trichothecene-producing species *Fusarium nivale, F. roseum* and *F. solani* were found to be able to transform trichothecenes into their acetylated or deacetylated products. *Fusarium roseum* produced 3ADON in Czapek-Dox medium supplemented with peptone and the production of 3ADON increased with the growth of this fungus. The deacetylation of 3ADON coincided with the increase of DON after the fungus reached its maximum growth. The deacetylation was also observed when these three species were cultured in sugar-free Czapek-Dox medium, although this reaction differed significantly among the species. When incubated with *F. solani* growing mycelia, DON triacetate (3α,7α,15-triacetoxy-deoxynivalenol, A_3DON) was deacetylated to 7α,15-diacetyl- deoxynivalenol (7,15A_2DON) and then 7α-acetyl- deoxynivalenol (7ADON). On the other hand, *F. nivale* transformed 5% DON into 3ADON in a 24-hour period; however, the acetylation by the other two species was hardly observed (Yoshizawa and Morooka, 1975). The acetylation and deacetylation functions have also been observed in other *Fusarium* species, and the gene (*Tri101*) that encodes trichothecene 3-O-acetyltransferase has been cloned from *F. graminearum* (Kimura *et al.*, 1998). Also, isolation of other genes involved in trichothecene toxin biosynthesis in *Fusarium* species has been reported (Desjardins, 2006).

4. Perspective

A fundamental solution to the problems of trichothecene mycotoxin contamination is to completely eliminate toxin production through a truly effective disease management system consisting of multiple approaches, including the use of resistant crop varieties. However, lack of appropriate genes has limited the development of resistant crop varieties and such effective varieties currently are still not available. Further studies on the trichothecene transforming microorganisms should result in identification of the genes responsible for trichothecene detoxifications. These identified genes can be used not only in genetically modifying crop varieties for improved *Fusarium* resistance but also in developing effective biological/enzymatic detoxification strategies.

Since mycotoxin contamination can not be avoided in current agricultural practices, detoxification strategies are very much needed. Microbial detoxification should be a promising choice since it can be a specific, effective and environmental friendly strategy for reducing and/or eliminating possible contaminations of trichothecenes in food and in feed. Previous research has clearly indicated that microorganisms capable of transforming/ detoxifying trichothecene mycotoxins exist widely in nature (Table 1). However, only countable detoxification microbial isolates have been identified to date. Screening detoxification microorganisms from more diversified sources should increase the potential for success. Innovative strategies and techniques such as enrichments, highly selective media and sensitive and effective molecular techniques to guide each selecting step can significantly increase the opportunities of selecting target microorganisms from a complex microflora. An understanding in microbial ecology and the mode of

actions of detoxification microorganisms is essential to the development of these strategies and techniques.

Applications of detoxification microorganisms may also face great challenges. The fate of a microorganism is first determined by its safety including safety for the target species, for the consumers, for the users and for the environment (EFSA, 2005). Anaerobic microorganisms isolated from animal guts are generally suitable for developing feed additives, which will act in the intestines of the targeting animals. Survival and adaptation of the microorganisms in the animal guts are key factors for successful detoxification. On the other hand, aerobic microorganisms mainly isolated from environmental samples will have great potential to be developed for detoxifying feed and food materials through fermentations. Although no pure enzyme product is currently available for detoxifying trichothecenes, utilization of active enzyme(s) should be an optimal detoxification strategy. Many enzymes from other microbial systems have been purified and applied in food, feed and other industries. In this regard, enzyme products for the effective detoxification of trichothecene mycotoxins can be developed through isolation of the microorganisms from different sources and optimization of their fermentation conditions and enzyme purification.

References

Avantaggiato, G., Havenaar, R. and Visconti, A., 2004. Evaluation of the intestinal absorption of deoxynivalenol and nivalenol by an in vitro gastrointestinal model, and the binding efficacy of activated carbon and other adsorbent materials. Food and Chemical Toxicology 42: 817-824.

Azcona-Olivera, J.I., Ouyang, Y., Murtha, J., Chu, F.S. and Pestka, J.J., 1995. Induction of cytokine mRNAs in mice after oral exposure to the trichothecene vomitoxin (deoxynivalenol): relationship to toxin distribution and protein synthesis inhibition. Toxicology and Applied Pharmacology 133: 109-120.

Beeton, S. and Bull, A.T., 1989. Biotransformation and detoxification of T-2 toxin by soil and freshwater bacteria. Applied and Environmental Microbiology 55: 190-197.

Bergmann, F., Soffer, D. and Yagen, B., 1988. Cerebral toxicity of the trichothecene toxin T-2, of the products of its hydrolysis and of some related toxins. Toxicon 26: 923-930.

Betina, V., 1989. Structure-activity relationships among mycotoxins. Chemico-Biological Interactions 71: 105-146.

Binder, E.M., Binder, J., Ellend, N., Schaffer, E., Kriska, R. and Braun, R., 1998. Microbiological degradation of deoxynivalenol and 3-acetyl-deoxynivalenol. In: Miraglia, M., van Egmond, H., Brera, C. and Gilbert, J. (eds.) Mycotoxins and Phycotoxins-Developments in Chemistry, Toxicology and Food Safety. Alaken, Inc., Ft. Collins, Colo., pp. 279-285.

Binder, E.M., Tan, L.M., Chin, L.J., Handl, J. and Richard, J., 2007. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Animal Feed Science and Technology 137: 265-282.

Canady, R., Coker, R., Egan, S.K., Krska, R., Kuiper-Goodman, T. and Olsen, M., 2001. Deoxynivalenol (DON), Safety evaluation of certain mycotoxins in food. Joint FAO/WHO Expert Committee on Food Additives (JECFA), FAO Food and Nutrition Paper 74: 419-555.

Cole, R.J. and Cox, R.H., 1981. Handbook of Toxic Fungal Metabolites. Academic Press, New York, pp. 152-263.

Conrady-Lorck, S., Gareis, M., Feng, X.C., Amselgruber, W., Forth, W. and Fichtl, B., 1988. Metabolism of T-2 toxin in vascularly autoperfused jejunal loops of rats. In: Toxicology and Applied Pharmacology 94: 23-33.

Côté, L.-M., Nicoletti, J., Swanson, S.P., and Buck, W.B., 1986. Production of deepoxydeoxynivalenol (DOM-l), a metabolite of deoxynivalenol, by *in Vitro* rumen incubation. Journal of Agriculture and Food Chemistry 34: 458-460.

Desjardins, A.E., 2006. *Fusarium* Mycotoxins: Chemistry, Genetics, and Biology. American Phytopathological Society, St. Paul, MN, USA, 260 pp.

European Food Safety Authority (EFSA), 2004. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to deoxynivalenol (DON) as undesirable substance in animal feed (Question N° EFSA-Q-2003-036) Adopted on 2 June 2004. The EFSA Journal 73: 1-42.

European Food Safety Authority (EFSA), 2005. Opinion of the scientific panel on additives and products or substances used in animal feed on a request from the commission on the safety of the product "Biomin BBSH 797" for piglets, pigs for fattening and chickens for fattening (Question NO EFSA-Q-2003-052). The EFSA Journal 169: 1-14.

Eriksen, G.S., Pettersson, H. and Lundh, T., 2004. Comparative cytotoxicity of deoxynivalenol, nivalenol, their acetylated derivatives and de-epoxy metabolites. Food and Chemical Toxicology 42: 619-624.

Fink-Gremmels, J., 1989. The significance of mycotoxin assimilation for meat animals. Deutsche Tierarztliche Wochenschrift 96: 360-363.

Frisvad, J.C., Thrane, U., Samson, R.A. and Pitt, J.I., 2006. Important mycotoxins and the fungi which produce them. In: Hocking, A.D., Pitt, J.I., Samson, R.A. and Thrane, U. (eds.) Advances in Food Mycology. Springer, New York, USA, pp. 3-31.

Fuchs, E., Binder, E.M., Heidler, D. and Krska, R., 2002. Structural characterization of metabolites after the microbial degradation of type A trichothecenes by the bacterial strain BBSH 797. Food Additives and Contaminants 19: 379-386.

He, P., Young, L.G. and Forsberg, C., 1992. Microbial transformation of deoxynivalenol (vomitoxin). Applied and Environmental Microbiology 58: 3857-3863.

Hollinger, K. and Ekperigin, H.E., 1999. Mycotoxicosis in food producing animals. The Veterinary Clinics of North America: Food Animal Practice 15: 133-165.

Horvath, I. and Varga, M., 1961. Enzymatic Inactivation of trichothecin and crotocin. Nature 192: 88.

Jarvis, B.B. and Mazzola, E.P., 1982. Macrocyclic and other novel trichothecenes: Their structure, synthesis, and biological significance. Accounts of Chemical Research 15: 388-395.

Kiessling, K.H., Pettersson, H., Sandholm, K. and Olsen, M., 1984. Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa and rumen bareria. Applied and Environmental Microbiology 47: 1070-1073.

Kimura, M., Matsumoto, G., Shingu, Y., Yoneyama, K. and Yamaguchi, I., 1998. The mystery of the trichothecene 3-O-acetyltransferase gene: Analysis of the region around Tri101 and characterization of its homologue from Fusarium sporotrichioides. FEBS Letters 435: 163-168.

King, R.R., McQueen, R.E., Levesque, D. and Greenhalgh, R., 1984. Transformation of deoxynivalenol (vomitoxin) by rumen microorganisms. Journal of Agriculture and Food Chemistry 32: 1181-1183.

Kollarczik, B., Gareis, M. and Hanelt, M., 1994. *In vitro* transformation of the Fusarium mycotoxins deoxynivalenol and zearalenone by the normal gut microflora of pigs. Natural Toxins 2: 105-110.

Kouadio, J.H., Mobio, T.e.A., Baudrimont, I., Moukha, S., Dano, S.D. and Creppy, E.E., 2005. Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B_1 in human intestinal cell line Caco-2. Toxicology 213: 56-65.

Lombaert, G.A., Pellaers, P., Roscoe, V., Mankotia, M., Neil, R. and Scott, P.M., 2003. Mycotoxins in infant cereal foods from the Canadian retail market. Food Additives and Contaminants 20: 494-504.

Madhyastha, M.S., Marquardt, R.R. and Abramson, D., 1994. Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by yeast bioassay. Toxicon 32: 1147-1152.

Matsushima, T., Okamoto, E., Miyagawa, E., Matsui, Y., Shimizu, H. and Asano, K., 1996. Deacetyllation of diacetoxyscirpenol to 15-acetoxyscirpenol by rumen bacteria. Journal of General and Applied Microbiology 42: 225-234.

Morgavi, D.P. and Riley, R.T., 2007. Fusarium and their toxins: Mycology, occurrence, toxicity, control and economic impact. Animal Feed Science and Technology 137: 199-200.

Nagy, C.M., Fejer, S.N., Berek, L., Molnar, J. and Viskolcz, B., 2005. Hydrogen bondings in deoxynivalenol (DON) conformations – A density functional study. Journal of Molecular Structure: Theochem 726: 55-59.

Nelson, P.E., 2002. *Fusarium*-Paul E. Nelson Memorial Symposium. The American Phytopathological Society, St. Paul, MN, USA, 392 pp.

Pestka, J. and Smolinski, A., 2005. Deoxynivalenol: Toxicology and potential effects on humans. Journal of Toxicology and Environmental Health Part B 8: 39-69.

Rocha, O., Ansari, K. and Doohan, F.M., 2005. Effects of trichothecene mycotoxins on eukaryotic cells: A review. Food Additives and Contaminants 22: 369-378.

Schatzmayr, G., Zehner, F., Taubel, M., Schatzmayr, D., Klimitsch, A., Loibner, A.P. and Binder, E.M., 2006. Microbiologicals for deactivating mycotoxins. Molecular Nutrition and Food Research 50: 543-551.

Shapira, R. and Paster, N., 2004. Control of mycotoxins in storage and techniques for their decontamination. In: Magan, N. and Olsen, M. (eds.) Mycotoxins in Food: Detection and Control, CRC Press, Woodhead Publishin Limited, England, pp. 190-223.

Shima, J., Takase, S., Takahashi, Y., Iwai, Y., Fujimoto, H., Yamazaki, M. and Ochi, K., 1997. Novel detoxification of the trichothecene mycotoxin deoxynivalenol by a soil bacterium isolated by enrichment culture. Applied and Environmental Microbiology 63: 3825-3830.

Stark, A.-A., 2005. Threat assessment of mycotoxins as weapons: Molecular mechanisms of acute toxicity. Journal of Food Protection 68: 1285-1293.

Swanson, S.P., Helaszek, C., Buck, W.B., Rood Jr., H.D. and Haschek, W.M., 1988. The role of intestinal microflora in the metabolism of trichothecene mycotoxins. Food and Chemical Toxicology 26: 823-829.

Swanson, S.P., Nicoletti, J., Rood Jr., H.D., Buck, W.B., Cote, L.M. and Yoshizawa, T., 1987a. Metabolism of three trichothecene mycotoxins, T-2 toxin, diacetoxyscirpenol and deoxynivalenol, by bovine rumen microorganisms. Journal of Chromatography 414: 335-342.

Swanson, S.P., Rood Jr., H.D., Behrens, J.C., and Sanders, P.E., 1987b. Preparation and characterization of the deepoxy trichothecenes: deepoxy HT-2, deepoxy T-2 triol, deepoxy T-2 tetraol, deepoxy 15-monoacetoxyscirpenol, and deepoxy scirpentriol. Applied and Environmental Microbiology 53: 2821-2826.

Ueno, Y., 1977. Trichothecenes: overview address. In: Rodricks, J.V., Hesseltine, C.W. and Mehlman, M.A. (eds.) Mycotoxins in Human and Animal Health. Pathotox Publishers, Park Forest, IL, USA, pp. 189-207.

Ueno, Y., 1985. The toxicology of mycotoxins. Toxicology 14: 99-133.

Ueno, Y., Nakayama, K., Ishii, K., Tashiro, F., Minoda, Y., Omori, T. and Komagata, K., 1983. Metabolism of T-2 toxin in *Curtobacterium* sp. strain 114-2. Applied and Environmental Microbiology 46: 120-127.

Völkl, A., Vogler, B., Schollenberger, M. and Karlovsky, P., 2004. Microbial detoxification of mycotoxin deoxynivalenol. Journal of Basic Microbiology 44: 147-156.

Westlake, K., Mackie, R.I. and Dutton, M.F., 1987. T-2 toxin metabolism by ruminal bacteria and its effect on their growth. Applied and Environmental Microbiology 53: 587-592.

Yoshizawa, T. and Morooka, N., 1975. Biological modification of trichothecene mycotoxins: acetylation and deacetylation of deoxynivalenols by *Fusarium* spp.. Applied Microbiology 29: 54-58.

Yoshizawa, T., Hiroaki, T. and Ohi, T., 1983. Structure of a novel metabolite from deoxynivalenol, a trichothecene mycotoxin, in animals. Agricultural and Biological Chemistry 47: 2133-2135.

Young, J.C., Zhou, T., Yu, H., Zhu, H. and Gong, J., 2007. Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food and Chemical Toxicology 45: 136-143.

Zhou, H.-R., Islam, Z. and Pestka, J.J., 2005. Induction of competing apoptotic and survival signaling pathways in the macrophage by the ribotoxic trichothecene deoxynivalenol. Toxicological Sciences 87: 113-122.