Chapter 7
Enzymes for Degradation of *Fusarium* Mycotoxins

Md Shofiul Azam, Dianzhen Yu, and Aibo Wu

Abstract *The Fusarium* mycotoxins are one of the agro-economically important toxins that seriously and frequently contaminate cereal-derived food and feedstuffs. The currently biological enzymes are widely applied in practice with ubiquitous acceptance for the desirably specific degradation of target mycotoxin contamination. Simultaneously, biotransformation of target mycotoxins by application with the specific enzyme is a suitable and practically favorable prevention measure. Major *Fusarium* mycotoxins studied in this section are the following: trichothecene, zearalenone, and fumonisins groups. The possible bi- or trifunctional enzymes for degrading various target mycotoxins are also first mentioned as the trend. This section also discussed various resources of these degradation enzymes. In the future perspective, as predicted, this enzymatic control measure needs to target the actual co-occurring mycotoxins to strengthen its industrial and economic importance. From this control strategy, especially through the large-scale application, cereal-derived food and feedstuffs will be obviously protected and will minimize the food loss to ensure food security. Ultimately, food safety problems will be somewhat resolved worldwide.

Keywords Enzyme · Control · Contamination · Degradation · Transformation · Mycotoxins

7.1 Introduction

Fusarium mycotoxins are the most agro-economically important fungal toxins. Trichothecenes (DON, NIV, T-2, HT-2, etc.), zearalenone, and fumonisins are the major representatives and most studied *Fusarium* mycotoxins (Mankeviciene et al.

M. S. Azam · D. Yu · A. Wu (✉)
CAS Key Laboratory of Nutrition, Metabolism and Food Safety, Shanghai Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, People's Republic of China
e-mail: abwu@sibs.ac.cn

2006). *Fusarium* mycotoxins mostly contaminate cereal grains, a great variety of food products and feed, which seriously cause huge economic losses and pose a threat to animal production and human health worldwide. Depending on the type, toxicities and related mechanisms of *Fusarium* mycotoxins have been well investigated. More specifically, epigenetic modifications (DNA methylation, histone modifications, and regulation of noncoding RNA) have been implicated in various human diseases and the toxicities in animals caused by *Fusarium* mycotoxins, such ascarcinogenesis, genotoxicity, and reproductive disorders. Based on very recently documented data, this section discussed the relationship between epigenetic modifications and *Fusarium* mycotoxin-induced toxicities (Huang et al. 2019). The European Commission (EC) has already set legislative limits for DON and ZEN in cereal grains and cereal-based products intended for human consumption early in the year of 2006.

To the best knowledge we have right now, biodegradation of these mycotoxins has been considered as one of the best strategies to decontaminate food and feedstuffs. Biodegradation employs the application of microbes or functional enzymes to the contaminated food and feedstuffs. Several microbes from different niches have been previously reported to have a biotransformation capability. Biotransformation or cleaving and detoxifying mycotoxin molecules by microbes or enzyme is an effective and safer method for mycotoxin control (Upadhaya et al. 2010).

Mycotoxin biotransformation is defined as "the degradation of mycotoxins into nontoxic metabolites by using bacteria/fungi or enzymes." On some occassions, biotransformation referred to metabolism is the structural modification of a chemical by enzymes in the body. This represents a valid strategy, especially if multi-step reactions are required or if the microorganism is already implemented within industrial processes. On the other hand, in case of high levels of mycotoxin contamination, the increase and physiology of such microorganisms might be altered or inhibited, as a consequence requiringlonger time for adaptation before achieving satisfactory decontamination levels (Coward-Kelly and Chen 2007; Loi et al. 2017).

Enzymes are specific proteins that catalyze chemical reactions and extensively employed in biotechnological sectors. A protein is simply a polypeptide composed of amino acids linked by a peptide bond, while the term generally, but not always, refers to the folded conformation. To understand how an enzyme functions, including its binding and functional properties, it is necessary to know the properties of the amino acids and how the amino acids are linked together, including the torsion angles of the bonds and the space occupied. The interactions of the atoms lead to the final conformations of the folded protein. Only in the folded state can a protein function effectively as an enzyme to bind substrates and act as a catalyst (Lee and AJ 2003). This evidence proves the effective application of microbial enzymes for biodegradation of mycotoxins.

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} \ldots \xrightarrow{En} \text{metabolite}$$
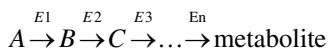
This section focuses on the biotransformation of mycotoxins performed with purified enzymes isolated from bacteria, fungi, and plants, while the enzymatic activity was validated via in vitro and in vivo assays (Loi et al. 2017).

7.2 Enzymes that Degrade *Fusarium* Mycotoxins

7.2.1 Zearalenone(ZEN)

Zearalenone (ZEN), mainly produced by *Fusarium* molds, has been associated with hyperestrogenism and other reproductive disorders in pigs, sheep, and other farm animals. Figure 7.1 illustrated the chemical structure of ZEN.

Enzymes from the *Acinetobacter* sp. SM04 extracellular extracts of liquid cultures were isolated by Sephadex G-100 column with oxidation process (Yu et al. 2011). Lactonase catalyzed the hydrolysis. For instances, a ZEN-degrading enzyme-encoding gene zhd101was isolated from *Clonostachys rosea*, and its encoding products could specifically cleave the lactone ring of ZEN (Wang et al. 2018; Takahashi-Ando et al. 2002). After incubation of the enzyme against ZEN, we detected the earlier elusive major reaction product of hydrolyzed ZEN (HZEN) by LC-MS/MS, after purification by pre-HPLC, and confirmed its postulated structure ((E)-2,4-dihydroxy-6-(10-hydroxy-6-oxo-1-undecen-1-yl)benzoic acid) by nuclear magnetic resonance (NMR) techniques. Spontaneous decarboxylation to DHZEN ((E)-1-(3,5-dihydroxyphenyl)-10-hydroxy-1-undecen-6-one) was observed (Vekiru et al. 2016). The maximal activity of ZHD101 toward ZEN was measured at approximately 37–45 °C and pH 10.5 (k cat at 30 °C, 0.51 s $^{-1}$). The enzyme was irreversibly inactivated at pH values below 4.5 or by treatment with serine protease inhibitors (Takahashi-Ando et al. 2004). The pathways for ZEN biodegradation were proposed as follows: ZEN underwent a cleavage of the lactone ring, followed by a decarboxylation, indicating that ZEN degradation may be partially contributed to esterase activities (Fig. 7.2).

In the previous study, a novel detoxifying agent which used rice husk (RH) to immobilize ZEN-degrading enzyme (ZDE) was produced to reduce ZEN from *Aspergillus niger* FS10 (He et al. 2016). Table 7.1 shows ZEN degradation enzyme and resource organism.
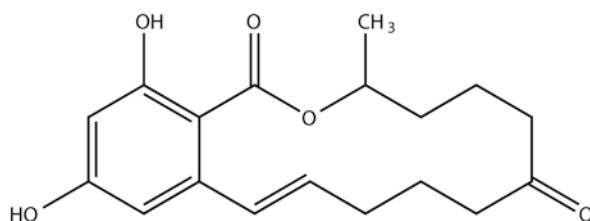

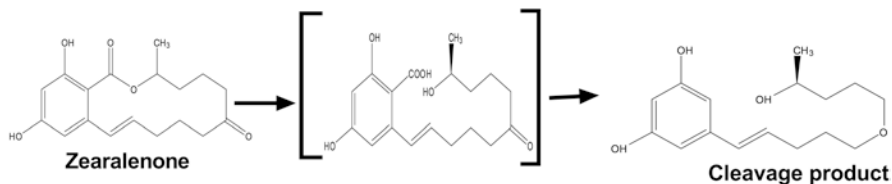**Fig. 7.1** The chemical structure of zearalenone (ZEN)

Fig. 7.2 Detoxification of ZEN. A hypothetical pathway for the detoxification of ZEN. Structures of ZEN (compound 1) and 1-(3,5-dihydroxyphenyl)-10h-hydroxy-1h-undecen-6h-one (compound 2) are indicated. A putative unstable intermediate is shown in square brackets

Table 7.1 ZEN controlling degradation enzymes

Enzyme	Producing organism	References
Laccase	*Trametes versicolor*	Novozymes (2009a)
Laccase	*Streptomyces coelicolor*	Novozymes (2009b)
Lactono hydrolase	*Clonostachys rosea*	Takahashi-Ando et al. (2002, 2005)
2cys-peroxiredoxin	*Acinetobacter* sp. SM04	Yu et al. (2012)
Lactonase	*Neurospora crassa*	Bi et al. (2018)

7.2.2   Fumonisins

Fumonisins are mainly produced by the phytopathogenic filamentous fungi of *Fusarium verticillioides* and *Fusarium proliferatum*. They frequently contaminate corn and corn-based products and cause, ingested with food or feed, several severe diseases in humans and animals. Fumonisins are associated with several mycotoxicoses, including equine leukoencephalomalacia, porcine pulmonary edema, and experimental kidney and liver cancer in rats. Chemically, fumonisins are diesters of propane-1,2,3-tricarboxylic acid and similar long-chain aminopolyol backbones. Structurally they are similar to the sphingoid bases sphinganine (Sa) and sphingosine (So), with tricarboxylic acid groups added at the C14 and C15 positions. This structural similarity is responsible for the action mechanism. It was once described to act using demanding the sphingolipids metabolism, with the aid of inhibiting the enzyme ceramide synthase and leading to accumulation of sphinganine in cells and tissues (Loi et al. 2017).

7.2.2.1   Fumonisin B1

Fumonisin B1 (FB1) is the most prevalent fumonisin and holds the highest risk for human and animal nutrition (Fig. 7.3). FB1 was shown to be carcinogenic and teratogenic and be linked with the etiology of esophageal cancer and neural tube defects in humans (Heinl et al. 2010).

Upadhaya et al. (2010) reported two genes, frame, encoding a carboxylesterase, and FumI encoding an aminotransferase which is responsible forFB1 degradation
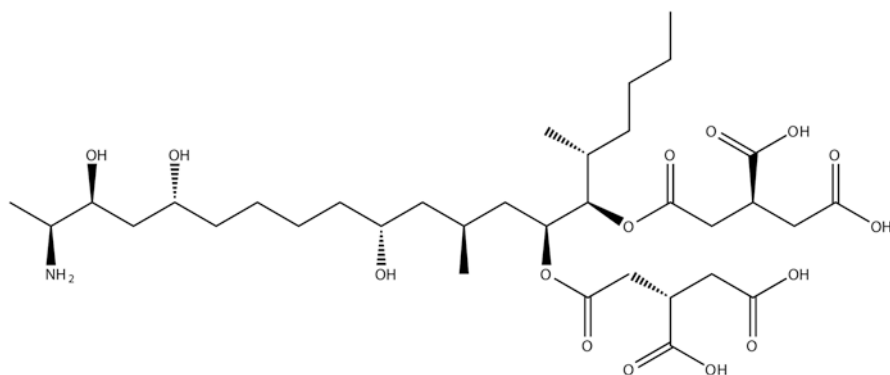
Fig. 7.3 The chemical structure of fumonisin B1 (FB1)

Table 7.2 The reported degrading enzymes of fumonisin B1 (FB1)

| Enzyme	Producing organism	References
Carboxylesterase and aminotransferase	*Sphingomonas* sp. ATCC55552	Pioneer (1985)
Carboxylesterase B and aminotransferase	*Sphingopyxis* sp. MTA144	Heinl et al. (2009)
Carboxylesterase FumD	Gastrointestinal tract of turkeys and pigs	Masching et al. (2016)
Fumonisin esterase	*Sphingopyxis* sp. MTA144	EFSA (2014)

by *Sphingopyxis* sp. MTA144 (Upadhaya et al. 2010). New fumonisin-metabolizing bacterial strains have been isolated and characterized. Recombinant enzymes from *Sphingopyxis* sp. caused hydrolysis of FB1 to HFB1 by carboxylesterase with loss of the two tricarballylic side chains, followed by deamination of HFB1 by aminotransferase in the presence of pyruvate and pyridoxal phosphate (Heinl et al. 2010).

Enzymatic characteristics of aminotransferase FumI of *Sphingopyxis* sp. MTA144 were assayed for deamination of hydrolyzed FB1. With this basis, a technological application of FumI, in combination with the fumonisin carboxylesterase FumD for hydrolysis of fumonisins, for decontamination and detoxification of hydrolyzed fumonisins seems possible, if the enzyme properties are considered (Hartinger et al. 2011). Table 7.2 shows FB1 biotransformation enzyme and its origin organism.

The interactive effects of combined DON, ZEN, and FB1 on the fungal growth of brewing yeasts were examined. Yeast growth was assessed by measurement of dry weight or relative growth, cell number, viability, and conductance change of the growth medium using direct and oblique techniques. The interactive effect of a combination of these mycotoxins was subject to the ratio of toxins as the mixture and the toxicity of individual toxin on yeast growth. When a combination of mycotoxins at low concentration was added into the growth media, no significant inhibitory effect on growth was observed when compared to controls. However, when a

combination of high concentrations of DON and ZEN which individually inhibited yeast growth was examined, the interactive effect was shown to pass from antagonism to synergism depending on the ratio of the mixed toxins. The ability to reduce *Fusarium* toxins by fermentative bacteria was evaluated in vitro (Niderkorn et al. 2006).

Initial steps of FB1 degradation pathway of *Sphingopyxis* sp. MTA144: FB1 (2-amino-12,16-dimethyl-3,5,10-trihydroxy-14,15-propan-1,2,3 tricarboxyicosane) are the substrate of the fumonisin carboxylesterase FumD, which catalyzes hydrolytic cleavage of both tricarballylic acid (TCA) chains off the core chain to produce HFB1 (2-amino-12,16-dimethylicosane-3,5,10,14,15-pentol) and tricarballylic acid (1,2,3-propanetricarboxylic acid). Aminotransferase FumI transfers the 2-amino group from HFB 1 to pyruvate, producing 2-keto-HFB 1(3,5,10,14,15-pentahydroxy-12,16-dimethylicosane-2-one) and alanine (Hartinger et al. 2011). The equation listed below shows the FB1 degradation process.

$$\text{FB1} \xrightarrow{\text{Carboxylesterase FumD}} \text{HFB1} \xrightarrow{\text{aminotransferase FumI}} 2\,\text{Keto HFB1}$$
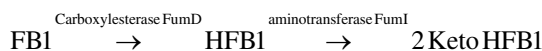
7.2.2.2   FB2

The empirical formula of Fumonisin B2 is C34H59NO14 and its molecular weight is 705.83. FB2 belongs to the family of toxins known as fumonisins. FB2 is a structural analog of FB1, but it is more cytotoxic than the latter, and it inhibits sphinganine-N-acetyltransferase (ceramide synthase). FB2 is a carcinogenic mycotoxin generally present on corn-based food and feedstuff, which is produced by *Fusarium verticillioides* and *Fusarium moniliforme*. FB2 could also be detected in *Aspergillus niger* (Frisvad et al. 2007). Presence of FB1 and FB2 was reported in cattle milk also due to the consumption of contaminated feed or fodder (Scott 2012). Figure 7.4 shows the chemical structure of fumonisin B2.
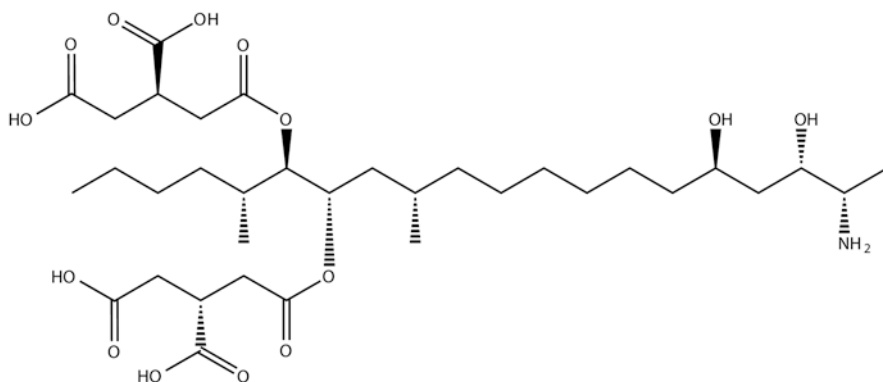

Fig. 7.4 The chemical structure of fumonisin B2 (FB2)

Cholestyramine, a bile acid sequestrant and preventative of diarrhea, was shown to be an effective binder for fumonisins – both in vivo, using the increase in the sphinganine/sphingosine (Sa/So) ratio in rat urine and tissues as a biomarker, and in vitro, using a dynamic gastrointestinal model. These results provide a basis for an enzymatic detoxification process. Lactic acid bacteria (*Bacillus subtilis* and *Micrococcus luteus*) bind FB1 and FB2. Peptoglycan is the likely binding site, and more FB2 is bound than FB1, with at least one tricarballylic acid moiety involved in the binding (Scott 2012; Niderkorn et al. 2006). FB2 was rapidly eliminated from the plasma of velvet monkeys dosed iv with 2 mg FB2/kg body mass. The concentration of FB2 in plasma after the iv dose was characterized by an initial distributional phase and a subsequent elimination phase with a mean half-life of 18 min (Shephard and Snijman 1999).

7.2.3   Trichothecenes

Trichothecenes are sesquiterpenoids mainly produced by the genera *Fusarium*, *Trichothecium*, *Myrothecium*, *Trichoderma*, and *Stachybotrys* fungi. Trichothecenes are produced by *Fusarium* species (*F. sporotrichioides*, *F. graminearum*, *F. poae*, and *F. culmorum*). It can also be generated by members of other genera via *Trichothecium* together with Tri101, which inactivates trichothecenes (Takahashi-Ando et al. 2002).

7.2.3.1   T-2 Toxin (T-2)

Poisoning of fungus toxin is based on the finding of T-2 toxin accumulation in grains, which may cause the disease. T-2 toxin is the major toxin produced by *Fusarium poae* and *F. sporotrichioides* in suitable environments and can endanger human health. T-2 toxin is present widely in nature and pollutes maize, wheat, barley, oat, and winter rye grain crops. When individuals eat the mildew food contaminated by T-2 toxin, the latter can reach the particular cartilage, where T-2 toxin can interfere with the DNA metabolism of chondrocytes and inhibit the synthesis of collagen and glycosaminoglycans, eventually leading to multiple articular cartilage lesions and chondrocyte necrosis. Moreover, T-2 toxin can provoke degenerative events in chicken embryo chondrocytes and alter the collagens and proteoglycans that are components of the cartilage matrix (Yu et al. 2017; Mankevičienė et al. 2006). Figure 7.5 shows the chemical structure of T-2 toxin.

T-2 causes cytotoxicity, which reduces cell viability and induces intracellular lactate dehydrogenase (LDH) release. It also leads to oxidative stress in cells via enhancing the generation of reactive oxygen species (ROS) (Yang et al. 2019). Table 7.3 shows T-2 toxin-controlling enzyme and its organism source.
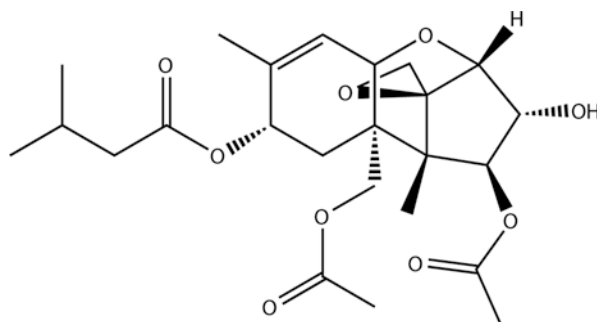
Fig. 7.5 The chemical structure of T-2 toxin (T-2)

Table 7.3 Detoxification enzymes of controlling T-2 toxin contamination

Enzyme	Producing organism	Final products	References
Acetyltransferases	*Blastobotrys proliferans*	3-acetyl T-2 toxin	McCormick et al. (2012)
Glucosyltransferase	*Blastobotrys muscicola*	T-2 toxin 3-glucoside	McCormick et al. (2012)
Cytochrome P450 (CYP 3A4)	*Homo sapiens*	3′-OH T-2	Lin et al. (2015)
Carboxylesterase	Tissue-infiltrating plasma cells	HT-2	Lin et al. (2015)
Hydrolyzing	*Curtobacterium* sp. strain 114-2	HT-2	Ueno et al. (1983)

7.2.3.2 HT-2 Toxin (HT-2)

HT-2 toxin is the main metabolite in vivo of T-2 toxin, where hepatic carboxylesterases are responsible for the specific deacetylation of T-2, resulting in HT-2 as the major metabolite (Medina and Magan 2011). Figure 7.6 shows the chemical structure ofHT-2 toxin.

7.2.3.3 Deoxynivalenol (DON)

Deoxynivalenol (DON), also known as vomitoxin, belongs to the large family of trichothecenes as potent inhibitors of protein synthesis. DON is mainly produced by *F. graminearum* and *F. culmorum*, which is a common contaminant of barley, wheat, oats, and corn all throughout the world (Mankevičienė et al. 2006). Deepoxidase was stated to be responsible for detoxifying DON (Upadhaya et al. 2010). Figure 7.7 shows the chemical structure of DON.

  3-O-acetylation of the trichothecene ring in DON leads to its inactivation. Gene Tri101 encoding trichothecene-3-O-acetyl-transferase from *F. graminearum*was characterized. The previous study cloned trichothecene 3-O-acetyl-transferases
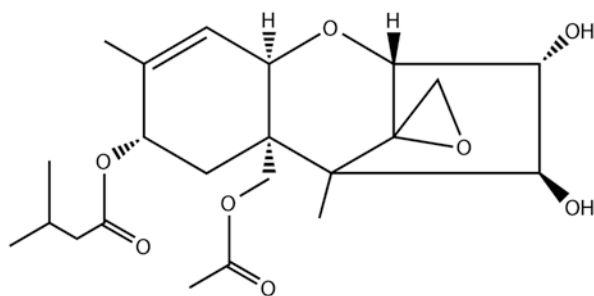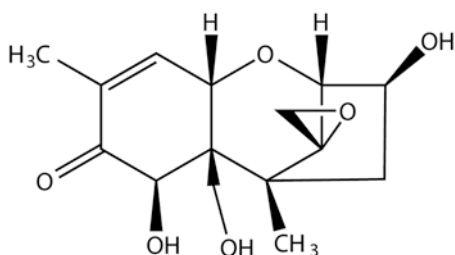
Fig. 7.6 The chemical structure of HT-2 toxin (HT-2)

Fig. 7.7 The molecular structure of deoxynivalenol (DON)

genes from *Fusarium* species and compared the properties of them to identify an optimal source of the enzyme for biotechnological applications. A UDP-glucosyltransferase from *Arabidopsis thaliana* catalyzed the transfer of glucose from UDP-glucose to the hydroxyl group at C3 of DON (Poppenberger et al. 2003). However, whether acetylation of C3-OH or conjugation by glycosylation can be considered as detoxification is controversial, because acetylated and conjugated mycotoxins may be hydrolyzed and regenerated the toxins in the digestive system of animals and human beings (Poppenberger et al. 2003).

DON degradation by *Aspergillus oryzae* and *Rhizopus oryzae* in a submerged fermentation system was found to correlate with the activity of oxydo-reductase enzymes (Garda-Buffon et al. 2011) and the catabolizingbacterial Cytochrome P450 system (Ito et al. 2013), as well as the peroxidase enzyme, which was extracted from rice bran (Feltrin et al. 2017).

As reported, the *Devosia* mutans 17-2-E-8 (*Devosia* spp. 17-2-E-8) was capable of transforming DON to the nontoxic stereoisomer 3-epi-deoxynivalenol, along with the earlier reported bacterial species capable of oxidizing DON to 3-keto-DON, generating great interests on the possible mechanism of recognition and enzyme(s) involved. An understanding of these details could pave the way for novel strategies to manage this widely present toxin. It was previously shown that DON epimerization proceeds through a two-step biocatalysis. Significantly, this report describes the identification of the first enzymatic step in this pathway. The enzyme, a dehydrogenase responsible for the selective oxidation of DON at the C3 position, was shown to readily convert DON to 3-keto-DON, a less toxic intermediate in the

DON epimerization pathway. DON detoxification enzymes have the following classification: de-epoxidation, oxidation, epimerization, and glycosylation (Tian et al. 2016). Table 7.4 shows the enzymes of DON detoxification and their origin. In addition, DON mitigation by the PQQ dependence of the enzyme could be a new feasible strategy (Carere et al. 2018).

7.2.3.4 Diacetoxyscirpenol (DAS)

Diacetoxyscirpenol (DAS), also called anguidine, is a mycotoxin from the group of type A trichothecene. It is a secondary metabolite of the genus *Fusarium* and may cause toxicosis in farm animals. DAS has been detected in agricultural products worldwide and persists in products after processing (Fig. 7.8). In humans as well as in animals, DAS consumption has been shown to induce hematological disorders (neutropenia, aplastic anemia). DAS is metabolized in animals to

Table 7.4 Detoxification enzymes of controlling DON contamination

Type of enzyme	Origin	References
Deepoxidase	*Bacillus* sp. LS100 derived from chicken digesta	Li et al. (2011)
Deepoxidase	Bacteria derived from intestines of chicken	Young et al. (2007)
Deepoxidase	A strain of *Bacillus* derived from the intestinaltract of fish	Guan et al. (2009)
Deepoxidase	A mixed microbial culture including six bacterial genera found from soil	Islam et al. (2011)
Deepoxidase	Fecal microbiota derived from intestines of human	Gratz et al. (2013)
Oxidase and epimerase	*Nocardioides* sp. strain WSN05-2 derived from a wheat field	Ikunaga et al. (2011)
Oxidase and epimerase	Genus of *Nocardioides* and *Devosia* derived from field soils and wheat leaves	Sato et al. (2012)
Oxidase and epimerase	*Devosia* mutans 17-2-E-8 isolated from an agricultural soil	He et al. (2015)
UDP-glucosyltransferase	*Arabidopsis thaliana*	Poppenberger et al. (2003)
UDP-glucosyltransferase	*Triticum aestivum* L. cv. Wangshuibai	Lulin et al. (2010)
UDP-glucosyltransferase	Barley	Schisler et al. (2011)
UDP-glucosyltransferase	*Arabidopsis thaliana*	Shin et al. (2012)
UDP-glucosyltransferase	Barley	Li et al. (2015)
Deoxynivalenol hydroxylase	*Sphingomonas* sp. strain KSM1	Ito et al. (2013)
Peroxidase	Rice bran	Feltrin et al. (2017)

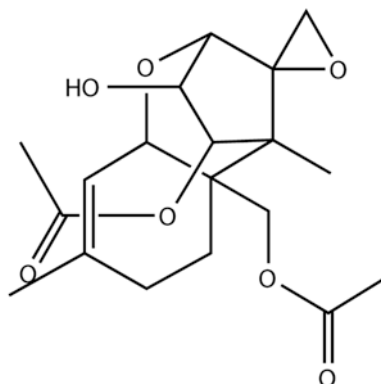**Fig. 7.8** The chemical structure of diacetoxyscirpenol (DAS)

Table 7.5 Detoxification enzymes of controlling diacetoxyscirpenol (DAS) contamination

Enzyme	Producing organism	Final products	References
Deepoxidase	Intestinal microflora in rats, cattle, and swine	Deepoxy MAS, deepoxy scirpentriol	Matsushima et al. (1996)
Deepoxidase	*Mucor racemosus*	Deepoxy scirpentriol	Bocarov-Stancic et al. (2011)
Carboxylesterase	Mouse liver microsomes	15-monoacetoxyscirpendiol, scirpentriol (Triol)	Wu and Marletta (1988)

15-monoacetoxyscirpenol (15-MAS) via C-4 deacetylation and then transformed to scirpentriol (SCP) via C-15 deacetylation (Wu et al. 2010; Pronk et al. 2002; Lautraite et al. 1997). Table 7.5 shows DAS controlling enzyme and enzyme source organism.

7.2.3.5 Nivalenol (NIV)

Nivalenol (NIV) is one of the trichothecene mycotoxins commonly contaminating cereals. This toxin has been associated with the poisoning of animals and humans, and many toxicological studies have been performed on *Fusarium* sp. Fn-2B, a well-known nivalenol producer (Hedman and Pettersson 1996) (Fig. 7.9). The impact of DON and NIV on the increase of *Saccharomyces cerevisiae* strains has been studied. The toxins were added to the growth medium in low and high concentrations. Yeast growth was assessed by the size of dry weight or relative growth, cell number, viability, and conductance change of the growth medium using direct and indirect methods. The inhibitory effect of both DON and NIV on yeast growth was dependent on toxin concentration. Additionally, when the extent of inhibition of yeast growth caused by high concentrations of both toxins was observed, it was subject to a yeast strain, length of incubation, and approach used to investigate yeast growth. The lowest concentrations of mycotoxin causing significant inhibition on
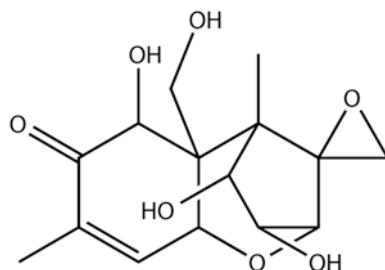
Fig. 7.9 Structure of nivalenol (NIV)

Table 7.6 Detoxification enzymes of controlling NIV contamination

Enzyme	Source of organism	References
UDP-glucosyltransferase, HvUGT13248	Barley	Li et al. (2017)
Cytochrome P450 system (Ddna + Kdx + KdR	*Sphingomonas* sp. strain KSM1	Ito et al. (2013)
Glutathione transferase, microsomal cytochrome P450	Animal liver	Gouze et al. (2007)

the growth of brewing yeasts were 100 μg/mL of DON for the lager strain, 50 μg/ml for the ale strain, and 50 μg/mL of NIV for the ale strain (Pronk ME. et al. 2002). Table 7.6 shows NIV degrading enzymes and its origin organism.

7.2.3.6   Beauvericin (BEA)

Beauvericin (BEA) and enniatins (ENNs) are prominent cyclic hexadepsipeptide mycotoxins primarily manufactured by the fungi of *Fusarium* species such as *F. oxysporum*, *F. avenaceum*, *F. poae*, and *Beauveria bassiana*. They are fundamentally interconnected and comprising of three alternating hydroxyisovaleryl and N-methylamino acid residues. Its molecular formula is C45H57N3O9. According to the scientific opinion on the risks to human and animal health related to the presence of BEA and ENNs in food and feed, 29 naturally take place ENN analogs have been identified but only four ENNs including enniatin A (ENA), A1 (ENA1), B (ENB), and B1 (ENB1) (Han et al. 2019; Maranghi et al. 2018; Wu et al. 2018, 2019). These compounds have antibiotic, insecticidal, and ionophoric properties and different bioactivities (Liuzzi et al. 2017). This study investigated the degradation of BEA by intracellular raw enzymes of four strains of *Saccharomyces cerevisiae*, namely, LO9, YE5, A34, and A17 (Meca et al. 2013). In the Figure 7.10 shows the chemical structure of BEA.
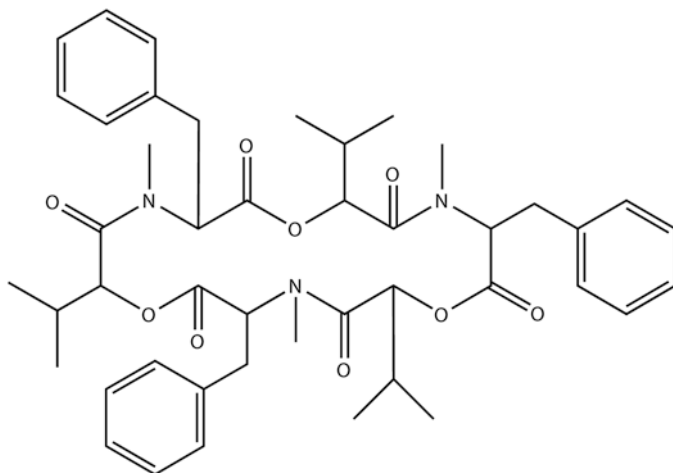
Fig. 7.10 The chemical structure of beauvericin (BEA)

7.3   Source of Degradation Enzymes Against Mycotoxins

7.3.1   *Natural Source of Enzyme*

Except for intact microbes or cell-free enzymatic preparations as feed additives, the expression of the respective genes in genetically manipulated organisms has opened new avenues for the protection of the health of farm animals. Examples of such procedures consist of the genetic engineering of ruminal microorganisms and feeding transgenic corn with reduced mycotoxin production to pigs (Upadhaya et al. 2010).

7.3.2   *Recombinant Fusion Enzyme (Expression, Purification)*

Recombinant fusion proteins are created artificially by recombinant DNA technology for use in biological research or therapeutics. A fusion protein is a recombinant protein created through genetic engineering of a fusion gene consisting of at least two genes. This naturally involves removing the stop codon from a cDNA sequence coding for the first protein, then appending the cDNA sequence of the second protein in the frame through ligation or overlap extension PCR. That DNA sequence will then be expressed by a cell system as a single protein. The protein can be engineered to include the full sequence of both original proteins or only a portion of either fusion enzyme technology, based on fusion protein design, which is frequently used in multifunctional enzyme construction and enzyme proximity control (Huang et al. 2012). The detoxification genes may be cloned and expressed in

A. Genes Fusion	• Target gene
B. Cloning	• Transform to Expression Vector and Expression strain
C. Production	• Over Expression of recombinant protein
D. Purification/Immobilzation	• Protein Purification in His/GST tag protein or Immobilation
E.Cleavage	• Factor Xa used to separate the protein and Tag
F. Elution	• Elution Buffer to eluted the purified protein
G. Direct Application	• Can be used directly or store for future use
H. Incubation	• Toxin +Enzyme
I. Non toxic product	• Biotransformation product analysis by LC-MS
J.Cell cytoxicity Assay	• Final metabolite used to check cell cytotoxicity assay

Fig. 7.11 A schematic outline of enzyme processing and application in the food industry

microorganisms to produce recombinant microorganisms that are suitable in an industrial scale enzyme production and purification (Altalhi 2007). Figure 7.11 shows the schematic outline of enzyme processing and application in the food industry.

As the whole procedure, the industrial production of enzymes from microorganisms generally involves culturing the microorganisms in huge tanks where enzymes are secreted into the fermentation medium as metabolites of microbial activity. And then, the enzymes are extracted, purified, and used as processing aids in the food industry. Purified enzymes are cell-free entities and should not contain any other macromolecules such as DNA residues. There are two common kinds of cells that are used for protein expression, namely, *E. coli* and yeast (Upadhaya et al. 2010).

7.3.3   *Chemical Synthesis*

The chemical synthesis of enzymatic proteins is feasibly completed by chemical ligation, where the key is the chemoselective reaction of unprotected synthetic peptides (so-called chemical ligation).

Notably, native chemical ligation enables the reaction of two unprotected peptides in aqueous solution at neutral pH to form a single product. Full-length synthetic polypeptides are folded to form the defined tertiary structure of the target enzyme, which could be further characterized by analysis of mass spectrometry, NMR, and X-ray crystallography, in addition of assays on biochemical activities (Kent 2003, 2009).

7.4   Basic Enzymatic Characteristics

7.4.1   *Enzyme Activity*

The most important property of one enzyme is the ability to increase the rates of reactions occurring in living organisms, known as "catalytic activity." The enzymatic activity is tediously affected by the factors that disrupt protein structure including temperature and pH value and affect catalysts consisting of reactant or substrate concentration and catalyst or enzyme concentration. The enzymatic activity can be measured by monitoring either the rate at which a substrate disappears or the rate at which a final product forms (Dubey 2018; Butko et al. 2012).

7.4.2   *Enzyme Kinetics*

The kinetics of one enzyme is the branch of enzymology that deals with the factors affecting the rates of enzyme-catalyzed reactions. An enzyme catalyzes the rate of a reaction without changing the equilibrium concentration of the reactants and products. The kinetics are included at two stages: (1) at pre-steady state, also known as the transient state, which monitors the microscopic events along the reaction pathway during the first round of the reaction (prior to enzyme turnover) and (2) at steady state, which monitors multiple rounds or turnovers of an enzymatic reaction (Lee and AJ 2003).

7.4.3   *Enzyme Inhibitor*

As would be anticipated with an enzyme system of such broad-based specificity, competition between ligands for a particular isoform is rife within the P450 family, with competitive inhibition between mutual substrates being a common pharmacological phenomenon (Meighen 2005). Compounds that influence the rates of enzyme-catalyzed reactions are called modulators, moderators, or modifiers. Usually, the effect is to reduce the rate, and this is called inhibition. Sometimes the enzyme reaction is increased, and this is called activation. Accordingly, the compounds are termed inhibitors or activators (Stojan 2005).

7.4.4 Enzyme Immobilization

One enzyme is seriously limited within its phase allowing its re-usability. The lack of purification and efficient recovery is the most critical and challenging aspect, which renders them enormously expensive for industrial costs. Aiming to tackle these problems, magnetic nanoparticles (MNPs) have gained a special place as versatile carriers and supporting matrices for immobilization purposes, owing to the exceptional properties of MNPs, such as larger surface area, larger surface-to-volume ratio, and more mobility, as well as higher mass transference (Bilal et al. 2018).

7.5 What Conditions Affect the Success of the Enzyme for the Degradation of Mycotoxins?

7.5.1 Enzyme Safety

Each enzyme has its own specific mechanism while it could work as usual with its biochemical properties. The enzymes have far fewer side effects and possible unknown reactions than other compounds, supplements, or medications. With the exception for the potential skin and irritating eye effects of some proteases and the well-documented potential for respiratory sensitization in the case of workplace exposure, enzymes, in general, do not produce acute toxicity, dermal sensitization, genotoxicity, or repeated dose oral toxicity. Acute inhalation, reproduction, chronic toxicity, and carcinogenicity are not relevant for enzymes. Several hundred mutagenicity studies have been conducted on bacterial and mammalian cells using a variety of enzymes. No positive findings were observed (Ladics and Sewalt 2018; Spök 2006). In the past, the safety of some food enzymes was assessed by the Scientific Committee on Food (SCF). Since 2003, EFSA has replaced the SCF and is currently undertaking the evaluation of all food enzymes. Only FE for which the proposed uses are considered safe will be on the EU list.

7.5.2 Enzyme Safety Dose Level

Enzymes are approved as safe biological ingredients in the manufacturing of many everyday products. Enzymes are regulated worldwide and are approved as safe biological ingredients in the manufacturing of numerous products such as detergents, textiles, and food. As with other proteins such as pollen or flour, enzymes may cause allergic reactions for people working with them in industrial processes, if not handled correctly. No known toxicity has been demonstrated at any level of enzyme dosing in animal or human beings. As reported, the rats were fed enzymes

equivalent to a human dose of 2500 tablets daily for a short period, and the rats only seemed a little fatigued (Palfey 2005).

7.5.3 Safe Handling of Enzymes

Safe handling of enzymes during the manufacturing process is vital because, if inhaled in their raw state, in the form of dust or aerosols, they may cause respiratory allergy similar to other well-known allergens like pollen, house dust mites, and animal dander. When added to detergent products, enzymes are treated, for example, by encapsulation, making them safe for use.

7.5.4 Difficulties During Mycotoxin Biotransformation

Biodegradation of mycotoxins with microorganisms or enzymes is considered as the best strategy for detoxification of food and feedstuffs. This approach is also pointed as an environmentally friendly approach in contrast to physicochemical techniques of detoxification. Since ruminants are a potential source of microbes or enzymes for mycotoxin biotransformation, isolation of pure culture using enriched media or screening of candidate genes from the metagenomic library seems to be a good strategy. Furthermore, genetic engineering techniques will not only improve the efficiency with which enzymes can be manufactured from these organisms or producing the engineered organism having the target genes, but they also increase their availability, bioavailability. Thus, the use of enzymes or engineered microorganisms as processing aids in the food industry would be proved to have an overall beneficial impact (Upadhaya et al. 2010).

7.5.5 Challenges and Limitations of and During Degradation by Enzymes

The use of enzymes in the food and feed industries is not the new thing, while the enzymes as biocatalysts have been increasingly used in recent years. They are usually applied to reduce the employment of hazardous chemicals, to use mild working conditions, to increase specificity, to speed up a process, or to simply create new products.

Mandatory requirements for large-scale enzyme application in industry are (1) safety, (2) effectiveness, (3) low cost of production and purification for both enzyme and cofactors if needed, and (4) stability to wide ranges of temperature, pH, and organic solvents and thus compatibility to productive processes. Native enzymes

usually do not respond to each unique requirement of a perfect industrial enzyme, while these features can be achieved via molecular engineering and structure-function modifications by mutagenesis. The most important limitation related to the application of degradation enzymes against mycotoxins is the reduced effectiveness of the process due to matrix effects. The physicochemical properties of food, such as the moisture, fat content, acidity, and texture, greatly influence the outcome of detoxification. Moreover, inhibitory compounds might be present in raw materials, and mycotoxins can occur in masked or other modified forms in plants. Their presence would reduce the enzyme catalysis at some degree.

For these cases, the implications might require pretreatments, additional time, and costs. Despite these limitations, the potentialities of the degradation enzymes used in the food and feed industries remain widespread. Their application is versatile since they can be used both in free or immobilized form and easily applied to well-established industrial processes of fermentation, ripening, brewing, or feed manufacturing (Loi et al. 2017).

7.6 Why Is Biotransformation of Mycotoxin by Enzyme More Superior than Other Control Methods?

Importance of biotransformation enzyme against mycotoxins ensuring food safety:

- They can be used as alternatives or even replacements to traditional chemical-based technology, with lower energy consumption and biodegradability.
- They are more specific in their actions than the applicable synthetic chemicals, with the fewer side reactions and waste by-products, resulting in higher-quality purified products.
- Especially for cereal-derived foods and feed, the wide application will minimize crop loss and maximize the levels of ensuring food safety.

7.7 Future of Biotransformation Enzyme Technology

The discovery of novel degradation enzymes for mycotoxins is becoming more interesting and stimulating worldwide.

One big challenge is how to find the applicable degradation enzymes targeting mycotoxin co-occurrence.

7.8 Conclusion

In conclusion, a standard biocontrolling and decontamination technology must have the following criteria: (1) a rapid and well-planned degradation, (2) of a broad spectrum of mycotoxins, (3) into nontoxic end products, (4) by an original nonpathogenic strain or consortium, and (5) under conditions that are relevant for the matrix in which the mycotoxin problem occurs. In order to attain these objectives, we urge to look beyond the disappearance of the mother compound and to explore strange new worlds and seek out new organisms and new metabolic pathways. Several conventional physical and chemical approaches have been used to remove mycotoxins from contaminated grains, but the loss of nutritional values or potential safety problems should not be ignored. Therefore, detoxifying mycotoxins by enzymatic reactions could be a more attractive approach for controlling mycotoxin contamination to the safe levels.

References

Altalhi AD (2007) Plasmid-mediated detoxification of mycotoxin Zearalenone in Pseudomonas Sp. ZEN-1. Am J Biochem Biotechnol 3(3):150–158. https://doi.org/10.3844/ajbbsp.2007.150.158

Bi K, Zhang W, Xiao Z, Zhang D (2018) Characterization, expression and application of a Zearalenone degrading enzyme from Neurospora crassa. AMB Express 8(1):194. https://doi.org/10.1186/s13568-018-0723-z

Bilal M, Zhao Y, Rasheed T, Iqbal HMN (2018) Magnetic nanoparticles as versatile carriers for enzymes immobilization: a review. Int J Biol Macromol 120(Pt B):2530–2544. https://doi.org/10.1016/j.ijbiomac.2018.09.025

Bocarov-Stancic A, Stankovic S, Levic J, Salma N, Pantic V, Barnic S (2011) In vitro degradation of diacetoxyscirpenol and T-2 toxin by use of Mucor racemosus fresen. *f. racemosus* isolate. Zbornik Matice Srpske za Prirodne Nauke 2011(121):51–59. https://doi.org/10.2298/zmspn1121051b

Butko P, Vöpel T, Makhatadze GI (2012) Enzyme activity in the crowded milieu. PLoS One 7(6):e39418. https://doi.org/10.1371/journal.pone.0039418

Carere J, Hassan YI, Lepp D, Zhou T (2018) The enzymatic detoxification of the mycotoxin deoxynivalenol: identification of DepA from the DON epimerization pathway. Microb Biotechnol 11(6):1106–1111. https://doi.org/10.1111/1751-7915.12874

Coward-Kelly G, Chen RR (2007) A window into biocatalysis and biotransformations. Biotechnol Prog 23(1):52–54. https://doi.org/10.1021/bp060358k

Dubey AVA (2018) Enzyme engineering for enzyme activity improvement. Elsevier Incs, Amsterdam

EFSA (2014) Scientific opinion on the safety and efficacy of fumonisin esterase (FUMzyme®) as a technological feed additive for pigs. EFSA J 12(5):3667. https://doi.org/10.2903/j.efsa.2014.3667

Feltrin ACP, Garcia SO, Caldas SS, Primel EG, Badiale-Furlong E, Garda-Buffon J (2017) Characterization and application of the enzyme peroxidase to the degradation of the mycotoxin DON. J Environ Sci Health B 52(10):777–783. https://doi.org/10.1080/03601234.2017.1356672

Frisvad JC, Smedsgaard J, Samson RA, Larsen TO, Thrane U (2007) Fumonisin B2 production by Aspergillus niger. J Agric Food Chem 55(23):9727–9732. https://doi.org/10.1021/jf0718906

Garda-Buffon J, Kupski L, Badiale-Furlong E (2011) Deoxynivalenol (DON) degradation and peroxidase enzyme activity in submerged fermentation. Cienc Tecnol Alime 31(1):198–203. https://doi.org/10.1590/s0101-20612011000100030

Gouze ME, Laffitte J, Pinton P, Dedieux G, Galinier A, Thouvenot JP, Loiseau N, Oswald IP, Galtier P (2007) Effect of subacute oral doses of nivalenol on immune and metabolic defence systems in mice. Vet Res 38(4):635–646. https://doi.org/10.1051/vetres:2007022

Gratz SW, Duncan G, Richardson AJ (2013) The human fecal microbiota metabolizes deoxynivalenol and deoxynivalenol-3-glucoside and may be responsible for urinary deepoxy-deoxynivalenol. Appl Environ Microbiol 79(6):1821–1825. https://doi.org/10.1128/aem.02987-12

Guan S, He J, Young JC, Zhu H, Li X-Z, Ji C, Zhou T (2009) Transformation of trichothecene mycotoxins by microorganisms from fish digesta. Aquaculture 290(3–4):290–295. https://doi.org/10.1016/j.aquaculture.2009.02.037

Han X, Xu W, Zhang J, Xu J, Li F (2019) Co-occurrence of Beauvericin and Enniatins in edible vegetable oil samples, China. Toxins 11(2):100. https://doi.org/10.3390/toxins11020100

Hartinger D, Schwartz H, Hametner C, Schatzmayr G, Haltrich D, Moll WD (2011) Enzyme characteristics of aminotransferase FumI of Sphingopyxis sp. MTA144 for deamination of hydrolyzed fumonisin B(1). Appl Microbiol Biotechnol 91(3):757–768. https://doi.org/10.1007/s00253-011-3248-9

He JW, Bondy GS, Zhou T, Caldwell D, Boland GJ, Scott PM (2015) Toxicology of 3-epi-deoxynivalenol, a deoxynivalenol-transformation product by Devosia mutans 17-2-E-8. Food Chem Toxicol: Int J Published Br Ind Biol Res Assoc 84:250–259. https://doi.org/10.1016/j.fct.2015.09.003

He M, Li Y, Pi F, Ji J, He X, Zhang Y, Sun X (2016) A novel detoxifying agent: using rice husk carriers to immobilize ZENralenone-degrading enzyme from Aspergillus niger FS10. Food Control 68:271–279. https://doi.org/10.1016/j.foodcont.2016.03.042

Hedman R, Pettersson H (1996) Purification and quantification of nivalenol. Mycotoxin Res 12(2):79–90. https://doi.org/10.1007/BF03192266

Heinl S, Hartinger D, Moll WD, Schatzmayr G, Grabherr R (2009) Identification of a fumonisin B1 degrading gene cluster in Sphingomonas spp. MTA144. New Biotechnol 25:S61–S62. https://doi.org/10.1016/j.nbt.2009.06.290

Heinl S, Hartinger D, Thamhesl M, Vekiru E, Krska R, Schatzmayr G, Moll WD, Grabherr R (2010) Degradation of fumonisin B1 by the consecutive action of two bacterial enzymes. J Biotechnol 145(2):120–129. https://doi.org/10.1016/j.jbiotec.2009.11.004

Huang Z, Zhang C, Wu X, Su N, Xing X (2012) Recent progress in fusion enzyme design and applications. Sheng Wu Gong Cheng Xue Bao 28(4):393–409

Huang D, Cui L, Sajid A, Zainab F, Wu Q, Wang X, Yuan Z (2019) The epigenetic mechanisms in Fusarium mycotoxins induced toxicities. Food Chem Toxicol 123:595–601. https://doi.org/10.1016/j.fct.2018.10.059

Ikunaga Y, Sato I, Grond S, Numaziri N, Yoshida S, Yamaya H, Hiradate S, Hasegawa M, Toshima H, Koitabashi M, Ito M, Karlovsky P, Tsushima S (2011) Nocardioides sp. strain WSN05-2, isolated from a wheat field, degrades deoxynivalenol, producing the novel intermediate 3-epi-deoxynivalenol. Appl Microbiol Biotechnol 89(2):419–427. https://doi.org/10.1007/s00253-010-2857-z

Islam R, Zhou T, Christopher Young J, Goodwin PH, Peter Pauls K (2011) Aerobic and anaerobic de-epoxydation of mycotoxin deoxynivalenol by bacteria originating from agricultural soil. World J Microbiol Biotechnol 28(1):7–13. https://doi.org/10.1007/s11274-011-0785-4

Ito M, Sato I, Ishizaka M, Yoshida S, Koitabashi M, Yoshida S, Tsushima S (2013) Bacterial cytochrome P450 system catabolizing the Fusarium toxin deoxynivalenol. Appl Environ Microbiol 79(5):1619–1628. https://doi.org/10.1128/AEM.03227-12

Kent S (2003) Total chemical synthesis of enzymes. J Pept Sci 9(9):574–593. https://doi.org/10.1002/psc.475

Kent SBH (2009) Total chemical synthesis of proteins. Chem Soc Rev 38(2):338–351. https://doi.org/10.1039/b700141j

Ladics GS, Sewalt V (2018) Industrial microbial enzyme safety: what does the weight-of-evidence indicate? Regul Toxicol Pharmacol 98:151–154. https://doi.org/10.1016/j.yrtph.2018.07.016

Lautraite S, Rio B, Guinard J, Parent-Massin D (1997) In vitro effects of diacetoxyscirpenol (DAS) on human and rat granulo-monocytic progenitors. Mycopathologia 140(1):59–64. https://doi.org/10.1023/a:1006833606209

Lee I, AJB (2003) Kinetics. Prog Mater Sci 19:68–109. https://doi.org/10.1016/0079-6425(75)90007-9

Li XZ, Zhu C, de Lange CFM, Zhou T, He J, Yu H, Gong J, Young JC (2011) Efficacy of detoxification of deoxynivalenol-contaminated corn by Bacillus sp. LS100 in reducing the adverse effects of the mycotoxin on swine growth performance. Food Addit Contam A 28(7):894–901. https://doi.org/10.1080/19440049.2011.576402

Li X, Shin S, Heinen S, Dill-Macky R, Berthiller F, Nersesian N, Clemente T, McCormick S, Muehlbauer GJ (2015) Transgenic wheat expressing a barley UDP-glucosyltransferase detoxifies deoxynivalenol and provides high levels of resistance to *Fusarium graminearum*. Mol Plant-Microbe Interact 28(11):1237–1246. https://doi.org/10.1094/MPMI-03-15-0062-R

Li X, Michlmayr H, Schweiger W, Malachova A, Shin S, Huang Y, Dong Y, Wiesenberger G, McCormick S, Lemmens M, Fruhmann P, Hametner C, Berthiller F, Adam G, Muehlbauer GJ (2017) A barley UDP-glucosyltransferase inactivates nivalenol and provides Fusarium Head Blight resistance in transgenic wheat. J Exp Bot 68(9):2187–2197. https://doi.org/10.1093/jxb/erx109

Lin NN, Chen J, Xu B, Wei X, Guo L, Xie JW (2015) The roles of carboxylesterase and CYP isozymes on the in vitro metabolism of T-2 toxin. Mil Med Res 2:13. https://doi.org/10.1186/s40779-015-0041-6

Liuzzi V, Mirabelli V, Cimmarusti M, Haidukowski M, Leslie J, Logrieco A, Caliandro R, Fanelli F, Mulè G (2017) Enniatin and Beauvericin biosynthesis in Fusarium species: production profiles and structural determinant prediction. Toxins 9(2):45. https://doi.org/10.3390/toxins9020045

Loi M, Fanelli F, Liuzzi V, Logrieco A, Mulè G (2017) Mycotoxin biotransformation by native and commercial enzymes: present and future perspectives. Toxins 9(4):111. https://doi.org/10.3390/toxins9040111

Lulin M, Yi S, Aizhong C, Zengjun Q, Liping X, Peidu C, Dajun L, Xiu EW (2010) Molecular cloning and characterization of an up-regulated UDP-glucosyltransferase gene induced by DON from Triticum aestivum L. cv. Wangshuibai. Mol Biol Rep 37(2):785–795. https://doi.org/10.1007/s11033-009-9606-3

Mankevičienė R, Kačergius A, Lugauskas A, Repečkienė J (2006) Mycotoxin contamination in Lithuanian-grown cereal grains and factors determining it. Ekologija 3:21–27

Maranghi F, Tassinari R, Narciso L, Tait S, Rocca CL, Felice GD, Butteroni C, Corinti S, Barletta B, Cordelli E, Pacchierotti F, Eleuteri P, Villani P, Hegarat LL, Fessard V, Reale O (2018) In vivo toxicity and genotoxicity of beauvericin and enniatins. Combined approach to study in vivo toxicity and genotoxicity of mycotoxins beauvericin (BEA) and enniatin B (ENNB). EFSA Supporting Publ 15(5):35. https://doi.org/10.2903/sp.efsa.2018.EN-1406

Masching S, Naehrer K, Schwartz-Zimmermann HE, Sarandan M, Schaumberger S, Dohnal I, Nagl V, Schatzmayr D (2016) Gastrointestinal degradation of Fumonisin B(1) by carboxylesterase FumD prevents Fumonisin induced alteration of sphingolipid metabolism in Turkey and swine. Toxins 8(3):84. https://doi.org/10.3390/toxins8030084

Matsushima T, Okamoto E, Miyagawa E, Matsui Y, Shimizu H, Asano K (1996) Deacetylation of diacetoxyscirpenol to 15-acetoxyscirpenol by rumen bacteria. J Gen Appl Microbiol 42(3):225–234. https://doi.org/10.2323/jgam.42.225

McCormick SP, Price NP, Kurtzman CP (2012) Glucosylation and other biotransformations of T-2 toxin by yeasts of the trichomonascus clade. Appl Environ Microbiol 78(24):8694–8702. https://doi.org/10.1128/AEM.02391-12

Meca G, Ritieni A, Zhou T, Li XZ, Mañes J (2013) Degradation of the minor Fusarium mycotoxin beauvericin by intracellular enzymes of *Saccharomyces cerevisiae*. Food Control 33(2):352–358. https://doi.org/10.1016/j.foodcont.2013.03.035

Medina A, Magan N (2011) Temperature and water activity effects on production of T-2 and HT-2 by *Fusarium langsethiae* strains from north European countries. Food Microbiol 28(3):392–398. https://doi.org/10.1016/j.fm.2010.09.012

Meighen EA (2005) Enzyme structure and function. In: Smiths HJ, Simons C (eds) Enzymes and their inhibition drug development. CRC Press, Boca Raton

Niderkorn V, Boudra H, Morgavi DP (2006) Binding of Fusarium mycotoxins by fermentative bacteria in vitro. J Appl Microbiol 101(4):849–856. https://doi.org/10.1111/j.1365-2672.2006.02958.x

Novozymes AS (2009a) Detoxification of aflatoxin in feed products. European Patent 2252163

Novozymes AS (2009b) Detoxification of feed products. World Patent 2009109607

Palfey BA (2005) Mechanisms. In: Smith HJ, Simons C (eds) Enzymes and their inhibition drug development. CRC Press, Boca Raton

Pioneer H-BI (1985) Fumonisin-detoxifying enzymes. World Patent 1996006175

Poppenberger B, Berthiller F, Lucyshyn D, Sieberer T, Schuhmacher R, Krska R, Kuchler K, Glossl J, Luschnig C, Adam G (2003) Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from Arabidopsis thaliana. J Biol Chem 278(48):47905–47914. https://doi.org/10.1074/jbc.M307552200

Pronk ME., Schotthorst R., H. VE (2002) Toxicology and occurance of nivalenol, fusarenone X, diacetoxyscrirpenol, neosolaniol and 3-and 15-ADON: a review of six tricothescenes. Vasa 1–75

Sato I, Ito M, Ishizaka M, Ikunaga Y, Sato Y, Yoshida S, Koitabashi M, Tsushima S (2012) Thirteen novel deoxynivalenol-degrading bacteria are classified within two genera with distinct degradation mechanisms. FEMS Microbiol Lett 327(2):110–117. https://doi.org/10.1111/j.1574-6968.2011.02461.x

Schisler DA, Slmmger PJ, Boehm MJ, PA P (2011) Co-culture of yeast antagonists of fusarium head blight and their effect on disease development in wheat. Plant Pathol J 10:128–137. https://doi.org/10.3923/ppj.2011.128.137

Scott PM (2012) Recent research on fumonisins: a review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29(2):242–248. https://doi.org/10.1080/19440049.2010.546000

Shephard GS, Snijman PW (1999) Elimination and excretion of a single dose of the mycotoxin fumonisin B2 in a non-human primate. Food Chem Toxicol 37(2–3):111–116. https://doi.org/10.1016/s0278-6915(98)00117-3

Shin S, Torres-Acosta JA, Heinen SJ, McCormick S, Lemmens M, Paris MP, Berthiller F, Adam G, Muehlbauer GJ (2012) Transgenic Arabidopsis thaliana expressing a barley UDP-glucosyltransferase exhibit resistance to the mycotoxin deoxynivalenol. J Exp Bot 63(13):4731–4740. https://doi.org/10.1093/jxb/ers141

Spök A (2006) Safety regulations of food enzymes. Food Technol Biotechnol 44:197–209

Stojan J (2005) Enzyme inhibitors. In: Smith HJ, Simons C (eds) Enzymes and their inhibition drug development. CRC PRESS, London

Takahashi-Ando N, Kimura M, Kakeya H, Osada H, Yamaguchi I (2002) A novel lactonohydrolase responsible for the detoxification of ZENralenone: enzyme purification and gene cloning. Biochem J 365(Pt 1):1–6. https://doi.org/10.1042/BJ20020450

Takahashi-Ando N, Ohsato S, Shibata T, Hamamoto H, Yamaguchi I, Kimura M (2004) Metabolism of ZENralenone by genetically modified organisms expressing the detoxification gene from Clonostachys rosea. Appl Environ Microbiol 70(6):3239–3245. https://doi.org/10.1128/AEM.70.6.3239-3245.2004

Takahashi-Ando N, Tokai T, Hamamoto H, Yamaguchi I, Kimura M (2005) Efficient decontamination of ZENralenone, the mycotoxin of cereal pathogen, by transgenic yeasts through the expression of a synthetic lactonohydrolase gene. Appl Microbiol Biotechnol 67(6):838–844. https://doi.org/10.1007/s00253-004-1816-y

Tian Y, Tan Y, Liu N, Yan Z, Liao Y, Chen J, de Saeger S, Yang H, Zhang Q, Wu A (2016) Detoxification of deoxynivalenol via glycosylation represents novel insights on antagonistic activities of Trichoderma when confronted with *Fusarium graminearum*. Toxins 8(11). https://doi.org/10.3390/toxins8110335

Ueno Y, Nakayama K, Ishii K, Tashiro F, Minoda Y, Omori T, Komagata K (1983) Metabolism of T-2 toxin in Curtobacterium sp. strain 114-2. Appl Environ Microbiol 46(1):120–127

Upadhaya SD, Park MA, Ha JK (2010) Mycotoxins and their biotransformation in the rumen: a review. Asian Australas J Anim Sci 23(9):1250–1260

Vekiru E, Fruhauf S, Hametner C, Schatzmayr G, Krska R, Moll WD, Schuhmacher R (2016) Isolation and characterisation of enzymatic ZENralenone hydrolysis reaction products. World Mycotoxin J 9(3):353–363. https://doi.org/10.3920/wmj2015.2005

Wang M, Yin L, Hu H, Selvaraj JN, Zhou Y, Zhang G (2018) Expression, functional analysis and mutation of a novel neutral ZENralenone-degrading enzyme. Int J Biol Macromol 118(Pt A):1284–1292. https://doi.org/10.1016/j.ijbiomac.2018.06.111

Wu SE, Marletta MA (1988) Carboxylesterase isoenzyme specific deacylation of diacetoxyscirpenol (anguidine). Chem Res Toxicol 1(1):69–73. https://doi.org/10.1021/tx00001a012

Wu Q, Dohnal V, Huang L, Kuča K, Yuan Z (2010) Metabolic pathways of trichothecenes. Drug Metab Rev 42(2):250–267. https://doi.org/10.3109/03602530903125807

Wu Q, Patocka J, Nepovimova E, Kuca K (2018) A review on the synthesis and bioactivity aspects of Beauvericin, a Fusarium mycotoxin. Front Pharmacol 9:1338. https://doi.org/10.3389/fphar.2018.01338

Wu Q, Patocka J, Kuca K (2019) Beauvericin, a Fusarium mycotoxin: anticancer activity, mechanisms, and human exposure risk assessment. Mini Rev Med Chem 19(3):206–214. https://doi.org/10.2174/1389557518666180928161808

Yang L, Tu D, Wang N, Deng Z, Zhan Y, Liu W, Hu Y, Liu T, Tan L, Li Y, Guo S, Wang A (2019) The protective effects of DL-Selenomethionine against T-2/HT-2 toxins-induced cytotoxicity and oxidative stress in broiler hepatocytes. Toxicol In Vitro 54:137–146. https://doi.org/10.1016/j.tiv.2018.09.016

Young JC, Zhou T, Yu H, Zhu H, Gong J (2007) Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food Chem Toxicol: Int J Published Br Ind Biol Res Assoc 45(1):136–143. https://doi.org/10.1016/j.fct.2006.07.028

Yu Y, Qiu L, Wu H, Tang Y, Lai F, Yu Y (2011) Oxidation of ZENralenone by extracellular enzymes from Acinetobacter sp. SM04 into smaller estrogenic products. World J Microbiol Biotechnol 27(11):2675–2681. https://doi.org/10.1007/s11274-011-0741-3

Yu Y, Wu H, Tang Y, Qiu L (2012) Cloning, expression of a peroxiredoxin gene from Acinetobacter sp. SM04 and characterization of its recombinant protein for ZENralenone detoxification. Microbiol Res 167(3):121–126. https://doi.org/10.1016/j.micres.2011.07.004

Yu F-F, Lin X-L, Wang X, Liu H, Yang L, Goldring MB, Lammi MJ, Guo X (2017) Selenium promotes metabolic conversion of T-2 toxin to HT-2 toxin in cultured human chondrocytes. J Trace Elem Med Biol 44:218–224. https://doi.org/10.1016/j.jtemb.2017.08.009